Abstract


Contains reviews of important aspects of the groundnut aflatoxin problem together with annotated bibliographies. Aspects covered include aflatoxicosis in animals and humans, research on aflatoxin contamination of groundnuts, aflatoxins in groundnut and groundnut products, limits and regulations on aflatoxins, methods for aflatoxin analysis, and management of aflatoxin contamination.

Résumé


Resumen


Contiene una recopilación de los problemas más importantes relacionados con las aflatoxinas en el maní, junto con una detallada compilación de datos bibliográficos. Los aspectos considerados incluyen aflatoxicosis en animales y humanos, investigaciones sobre contaminación con aflatoxinas en maní, aflatoxinas en productos obtenidos de maní, límites y reglamentaciones en aflatoxinas, métodos de análisis en aflatoxinas y manejo en caso de contaminación con aflatoxinas.
The Groundnut Aflatoxin Problem
Review and Literature Database

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Aflatoxins can cause serious animal and human health problems and when present in groundnuts reduce their quality and value. They have become a subject for concern in agriculture on a global scale. Many countries have assigned high priority to research to find a solution to aflatoxin contamination of groundnut. This has resulted in rapid proliferation of literature both in conventional and nonconventional forms, and researchers are finding it increasingly difficult to keep track of this information which is essential for their research work. This problem was discussed in the International Workshop on Aflatoxin Contamination of Groundnut held at ICRISAT in October 1987, and it was recommended that ICRISAT should collect and review the literature on the subject and produce a bibliography. The present publication is in response to that workshop recommendation. It provides a comprehensive overview of literature published in the last 30 years and an annotated bibliography. The data collected for this publication are also available in machine-readable form utilizing the Micro CDS/ISIS application.

I compliment the authors for their efforts in bringing out this useful publication and hope it will facilitate research efforts towards solving the aflatoxin problem in groundnut.

Y. L. Nene
Deputy Director General
ICRISAT

This review and bibliography of the groundnut aflatoxin problem should be of particular value to scientists in the developing world who may not have ready access to the special journals and reports in which much of the research on aflatoxins has been documented.

Each aspect of the groundnut aflatoxin problem is reviewed in a separate section, and each review is followed by an annotated bibliography. Citations are arranged numerically in alphabetical order according to the name of the senior author. The same publication may be listed in several different sections, depending upon its contents. The period 1960 to 1990 is covered and 1450 references are given. The authors' original abstracts or summaries are used wherever possible, but some editing has been done to provide consistency of style and economy of space. Where no abstracts or summaries were provided in the original publications/reports, the authors have prepared them. In a publication of this nature omissions are inevitable and the authors would appreciate having these brought to their notice.
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NOTES

1. *Arachis hypogaea* L., the cultivated groundnut (peanut), is referred to in this book as “groundnut”. However, in proper names of organizations e.g., Peanut CRSP or in widely recognized names for groundnut products e.g., peanut butter, the term peanut is used.

2. Different authors have expressed concentrations of aflatoxins in groundnuts and groundnut products in different ways. Initially it was common practice to use parts per million (ppm), later parts per billion (ppb) were used.

   In order to achieve greater uniformity, aflatoxin contents are expressed in micrograms per kilogram (ug kg\(^{-1}\)) or per gram (ug g\(^{-1}\)) or in picograms per gram (pg g\(^{-1}\)).
INTRODUCTION

The aflatoxin problem was first recognized following outbreaks of Turkey 'X' disease in the United Kingdom in 1960. The common factor in the outbreaks was the inclusion of groundnut meal from Brazil in the turkeys' diets. Research revealed that the disease was caused by toxins produced by strains of the fungus *Aspergillus flavus* which had grown in the groundnuts. The toxins were named aflatoxins. Aflatoxins are now known to be hepatotoxic, carcinogenic, and teratogenic in many animal species.

The cultivated groundnut (*Arachis hypogaea* L.) is the most important oilseed in the developing world. It is a valuable source of protein for human and animal nutrition, and provides a high quality cooking oil. The crop is widely grown in tropical and subtropical regions and is important to the economies of several developing countries. Groundnuts are eaten raw, boiled, or roasted, and made into confectionery and snack foods, and are added to soups and other dishes. In the USA, the Philippines, and some other countries groundnuts are commonly processed into peanut butter. In the developing world a significant segment of the groundnut produce goes for oil extraction, while the expeller oilcake is used primarily for animal feed. High protein groundnut flour has been produced to enhance diets in areas where protein malnutrition among children is common.

The aflatoxin contamination problem is of obvious importance as it affects the utilization of, and trade in groundnuts and groundnut products, and affects animal and human health.

The high priority given to the solution of this problem in many countries has resulted in large numbers of research publications and reports on a wide range of subjects involving agriculture, the food and feeds industries, human and animal health, and trade legislation. This literature explosion that has occurred over the past 30 years has made it difficult for an individual scientist to obtain an overview of all areas of the subject and to access relevant information from disciplines other than his own.

In October 1987 an International Workshop on Aflatoxin Contamination of Groundnut was held at ICRISAT to discuss ways of evaluating and managing the aflatoxin contamination problem in groundnuts around the world, and to develop plans for disseminating information useful to researchers, groundnut growers, processors, users, advisory services, and policy makers. A major Workshop recommendation was "to prepare a database on literature on the subject".

After considerable thought we decided to use the computer software package - Mini-Micro CDS\SIS - developed by UNESCO for organizing an annotated bibliography incorporating all available publications both formal and informal. A computerized database and software package has been completed for the period 1960-1990 and may be obtained, together with a User's Manual, from ICRISAT's Information Services. This database provided the foundation for this book which presents in hard copy the annotated bibliography together with reviews on the major aspects of the groundnut aflatoxin problem.

We hope that this publication will be useful to workers in different fields who wish to have an overview of the groundnut aflatoxin problem and an introduction to the available literature on research carried out over the last thirty years.
1. AFLATOXICOSIS FROM INGESTION OF TOXIC GROUNDNUTS AND GROUNDNUT PRODUCTS

1.1 REVIEW

Aflatoxicosis is the disease condition caused by the action of aflatoxin. As it was first recognized and described in farm animals, it is appropriate that we first deal with the condition in several important groups of livestock. We shall then consider clinical and pathological effects of aflatoxin ingestion in laboratory animals and in livestock experiments. Finally, we shall deal with the effects of aflatoxins on primates including man.

1.1.1 Aflatoxicosis in Livestock

Poultry

The aflatoxin story began in 1960 with the report of an outbreak of disease in turkey poults in England. Since the aetiology of the disease was obscure it was called Turkey X disease (Blount 1961). Affected birds lost appetite, became lethargic, and died within 7 days after the onset of symptoms. Livers of diseased turkeys were severely damaged. A similar disease of ducklings and young pheasants was reported from England (Asplin and Carnaghan 1961). A common factor in all disease outbreaks was the inclusion of Brazilian groundnut meal in the affected birds' diets (Blount 1961, Asplin and Carnaghan 1961). Test birds that ingested this groundnut meal developed symptoms typical of Turkey X disease. No known poisonous agent was found in the meal (Blount 1961).

A similar disease of ducklings was reported from Kenya. The ducklings' feed ration contained a groundnut meal produced in eastern Africa, indicating that the problem was not solely associated with Brazilian groundnut meal (Allcroft and Carnaghan 1962). However, there were fewer reports of aflatoxicosis in farm animals from other European countries probably because on the continent groundnut meal is used mainly in the diets of older animals (Allcroft and Carnaghan 1962). The disease in poultry was reported from Spain (Carnaghan and Allcroft 1962), Austria (Kohler and Swaboda 1962), and Hungary (Derzsy et al. 1962).

Several outbreaks of aflatoxicosis in poultry have been reported from India (Char et al. 1982, Choudary and Rao 1982, Gopal et al. 1969, Kishan Rao 1980). In 1962, a heavy mortality occurred among ducklings in Tamil Nadu State (Bhat et al. 1978). The feed used for the ducklings contained groundnut meal with a total aflatoxin content of 6200 μg kg⁻¹. Another outbreak of the disease in ducklings was recorded in Kerala State (Sivadas 1968). Gopal et al. (1969) reported an outbreak of aflatoxicosis in fowls in Kerala State. The disease symptoms included severe and sudden anorexia, loss of weight, staggering gait and convulsive movements. Subacute and chronic cases showed petechial haemorrhage, enteritis, ascites and histopathological changes typical of aflatoxicosis such as yellow liver, fatty changes and biliary epithelial hyperplasia.

Reports of aflatoxicosis in Australia have described acute disease in poultry fed imported groundnut meal (Gardiner and Oldroyd 1965, Hart 1965).

Cole (1986) suggested that cyclopiazonic acid (CPA) could have had a role in the aetiology of Turkey X disease. This is based on the fact that some clinical signs of Turkey X disease described by Blount (1960) and by Siller and Ostler (1961) such as the characteristic posture of affected turkey poults (arched neck, head drawn back, and legs stretched fully backwards) result from an acute dose of CPA and not from an acute dose of aflatoxin. These atypical effects of aflatoxicosis could be explained by the presence of CPA which is commonly produced by A. flavus along with aflatoxins (Cole 1986). It would be interesting to examine the effects of CPA in relation to aflatoxicosis in other animal species.

Several species of farm animals are considered to be particularly susceptible to aflatoxicosis.
Pigs

Loosmore and Harding (1961) first described natural outbreaks of allatoxicosis in pigs associated with ingestion of toxic Brazilian groundnut meal. The carcasses were jaundiced and gross haemorrhage was present in many pans of the body. Experimentally induced allatoxicosis showed that changes in the liver progressed through steatosis, ductule proliferation and pericellular fibrosis to karyomegaly, dissecting fibrosis and finally nodular hyperplasia (Harding et al. 1963). Abrams (1965) stated that seneciosis can easily be confused with allatoxicosis in pigs and cattle. However, this confusion did not appear to exist in pigs since Harding et al. (1964) found the disease in pigs poisoned by Senecio jacobaea (ragwort) differed clinically, anatomically and histologically from that caused by ingestion of allatoxin-contaminated groundnut meal.

Sivadas (1968), in India, reported a disease of pigs in Kerala State. The affected pigs were stunted, and had considerable liver damage. The groundnut cake implicated in the suspected allatoxicosis of the pigs had allatoxin levels estimated at over 20000 μg kg⁻¹. However, this study did not definitively establish allatoxin as the causative factor.

Ketterer et al. (1982) reported five cases of allatoxicosis in pigs in southern Queensland, Australia. Of these, two cases of acute toxicity were caused by feeding groundnut screenings containing high levels of allatoxins (8600 - 44000 μg kg⁻¹). The disease syndrome included severe depression, vomiting, abortion and deaths. Nine pigs died; 2 died within 12 h, another 6 died in the next 12 h and one died a week after ingesting the toxic material. Some of these symptoms, e.g., abortion, were probably not caused by the aflatoxins. Other mycotoxins may have been present raising possibilities of synergistic interactions.

Cattle

Several researchers in the U.K. have reported outbreaks of allatoxicosis in cattle (Loosmore and Markson 1961, dray 1961, Allcroft and Lewis 1963). The lesions were confined mainly to the liver, showing degenerative changes with biliary proliferation and finally leading to diffuse cirrhosis. Allcroft and Lewis (1963) described the effects of experimental feeding of rations containing a highly toxic-groundnut meal to cattle of different ages. The first symptomatic effect of continuous ingestion of toxic groundnut meal in calves (about 6 weeks of age) was a reduction in growth rate followed by unthriftiness, and loss of appetite. After 16 to 25 weeks on the toxic meal, terminal symptoms, characterized by severe tenesmus, occurred 2 to 4 days before death. Post-mortem examination showed fibrosis of the liver, ascites and visceral oedema. Similar lesions were described by Loosmore and Markson (1961).

In India, Sastry et al. (1965) reported outbreaks of aflatoxicosis in Murrah buffaloes in Andhra Pradesh State. This disease was similar to that reported in cattle in the U.K. by Loosmore and Markson (1961). The disease was attributed to the inclusion of groundnut cake (20%) in the animals' feed rations, but no data were provided on aflatoxin contamination.

Groundnut meal in Livestock Feeds

The previously cited publications clearly establish the role of aflatoxin-contaminated groundnut meal in outbreaks of aflatoxicosis in livestock. Groundnut meal has traditionally been an important component of poultry and other livestock feeds both in groundnut-producing and importing countries. The economies of some developing countries, e.g., Senegal, arc strongly dependent on export of groundnuts and groundnut products, and no alternative crop is at present available. However, it should be emphasized that other agricultural commodities used in livestock feeds, e.g., cottonseed and maize, can also contain high levels of aflatoxins and have been implicated in outbreaks of aflatoxicosis.

In several developing countries groundnuts are very important, both for home consumption and as a cash export crop. Every effort should be made to reduce aflatoxin contamination and so maintain trade in groundnuts and groundnut products. Until aflatoxins can be eliminated from groundnut products the feed industry and livestock producers should pay close attention to regulations concerning the inclusion of aflatoxin-contaminated groundnut meal in rations for different animal species and livestock categories. Different species of animals and animals of different ages vary considerably in susceptibility to aflatoxicosis. This has permitted the establishment of codes of practice with regard to permissible levels of aflatoxin in formulation of feeds for particular categories of livestock. Readers can refer to the section on "Regulation and Limits for Aflatoxins" for information on this aspect.

Effects of aflatoxins on animals

Estimates of the susceptibility of most farm and laboratory animals have been derived from feeding aflatoxin-contaminated groundnut meal. Effects of aflatoxins on these animals are discussed from three points of view: (1) acute toxicity associated with ingestion of a lethal dose, (2) subacute toxicity associated with consumption of small amounts of aflatoxin, and (3) carcinogenicity of aflatoxins.

Acute toxicity

Aflatoxins are acutely toxic to most animal species. Many experimental studies and observations on natural outbreaks of aflatoxicosis suggest that ducklings are the species most susceptible to acute poisoning by aflatoxins. The LD₅₀ of a-day-old duckling is approximately 0.3 mg kg⁻¹ bodyweight, considerably lower than LD₅₀'s
for rats and hamsters. Some studies indicate that rainbow trout, rabbit, and
guinea-pig all have LD₅₀ values similar to that of ducklings (Ashley et al. 1964,
Wogan 1966). Single dose LD₅₀ values of aflatoxin B, in various animals have been
obtained by several researchers (Butler 1964, 1966, Wogan 1966, 1968). These
values provide useful index of species susceptibility.

The distinct acute aflatoxicosis in ducklings has been put lo good account by
using this species for bioassay of aflatoxin.

Chickens are much less susceptible than ducklings and turkey poult's. In field
outbreaks of aflatoxicosis, mortality was low and reduced growth rate during the
first few weeks of age was the main clinical effect (Asplin and Carnaghan 1961;
Allcroft and Carnaghan 1963). Carnaghan et al. (1966) confirmed the high tolerance
of Rhode Island Red chicks to the effects of aflatoxin. When the chicks were
continuously fed with a ration containing 15% of a highly toxic meal, there was
only one death and the main effect was retardation of growth rate.

Experimental and field observations have shown that calves from 1 to 6
months are highly susceptible to aflatoxin (Allcroft and Carnaghan 1963, Allcroft
and Lewis 1963, Allcroft 1965). They become more tolerant to aflatoxin with
increased age. Long-term feeding studies have shown that 3- to 4-year-old heifers
became clinically affected after continuous feeding on concentrated rations
containing 20% of a highly toxic groundnut meal, while 8- to 10-year-old cows
under the same conditions showed no adverse clinical effects. In older cows, no
effects on conception or gestation were noted (Allcroft and Carnaghan 1963,
Allcroft 1965).

Pigs from 3 to 12 weeks of age are the most susceptible large farm animals.
Of the mature swine, pregnant sows are the most commonly affected (Harding et al.
1963).

Sheep do not appear to be susceptible to aflatoxin as no outbreaks of
aflatoxicosis have been reported in this species. When 3-month-old sheep were fed
rations containing 20% highly toxic groundnut meal for 3 years they did not show
any obvious clinical effects other than a slight growth retardation. No liver damage
was observed after 2 years on this ration (Allcroft and Carnaghan 1963, Allcroft
and Lewis 1963). Abrams (1965) reported that sheep were not susceptible to amounts
of aflatoxin usually encountered in rations, but were affected when given 2 ounces
of highly toxic groundnut meal containing 60000 ug aflatoxin kg⁻¹ twice weekly for
4 to 6 weeks. The symptoms were not described.

Dogs are susceptible to aflatoxin (Newberne et al. 1966). Holding (1964)
reported that post-mortem examination of a dog that died with acute haemorrhagic
gastroenteritis, fever, and jaundice revealed a friable, bright yellow liver and
haemorrhages in many other organs. Aflatoxin was detected in the food, which
contained groundnut meal.

Rainbow trout are highly susceptible to aflatoxins. Feeding of crude allatoxin
e Extracts to rainbow trout resulted in liver lesions similar to those observed in
ducklings, consisting of massive haemorrhagic necrosis of the liver parenchyma with
little or no bile duct proliferation (Halvcr 1965). Based on these experiments the
LD₁₀ in a 10-day test with a 50 g rainbow trout was 1 to 3 mg of crude allatoxin
kg⁻¹ body weight, which would represent 0.5 to 1 mg of aflatoxin B₁ and G₁ of
body weight. Considerable liver damage was observed when 0.2 mg of pure
aflatoxin kg⁻¹ of body weight was administered.

Guinea-pigs are very susceptible to acute toxicity of aflatoxins (Butler and
Barnes 1963). When fed with 20% toxic groundnut meal, all animals succumbed in 14
to 28 days.

Mice are resistant, to a certain extent, to aflatoxins (Allcroft et al. 1961,
Allcroft and Carnaghan 1962, 1963). Platonow (1964) showed that continued
feeding of toxic groundnut meal to mice for at least 3 months had no deleterious
effects. Levels of aflatoxins B₁ and G₁ in the diet were 4500 ug kg⁻¹, and of
aflatoxins B₂ and G₂ 600 ug kg⁻¹.

Rats are comparatively more resistant to aflatoxin. They were able to survive
short-term feeding experiments with a diet containing as much as 50% of toxic
groundnut meal (Butler and Barnes 1963). However, prolonged feeding of a 20%
toxic meal diet reduced the growth rate of rats and their food intake, and prolonged
feeding beyond a few weeks' time resulted in further liver lesions and hepatomas
(Lancaster et al. 1961). Butler and Barnes (1963) showed that pregnancy increased
the susceptibility of the animals to aflatoxin. When high doses of aflatoxin were
given halfway through pregnancy the litter was stunted and some animals died. The
mothers also exhibited pronounced liver changes.

It is emphasized that the acute toxicity of aflatoxin for any given species of
animal is influenced by such factors as age, sex, breed, condition of animal, and
composition of diet. Therefore, a comparison of LD₅₀ values should be used only
as a rough guide.

Young animals lend to be more sensitive than mature animals. Different
species may metabolize and excrete aflatoxin in different ways and/or at different

In most cases, death occurs within 72 h of the toxin being administered.
Post-mortem examination reveals gross liver damage and occasional haemorrhaging in the intestinal tract and peritoneal cavity.

**Subacute toxicity**

Animals which consume sub-lethal quantities of aflatoxin for several days or weeks develop a subacute toxicity syndrome which commonly includes moderate to severe liver damage. Several types of liver lesions have been observed in different animals (Table 1). Biliary hyperplasia is the lesion most commonly observed in all species except sheep (Wogan 1966). Lethal dietary aflatoxin levels in domestic animals (cattle, swine, turkey, chickens, ducks) range from 0.3 mg kg\(^{-1}\) in the ducklings to 2.2 mg kg\(^{-1}\) in calves (Allcroft 1965). Similar results have been obtained in laboratory animals, including the guinea-pig and rat, which develop toxicity symptoms at 0.7 mg kg\(^{-1}\) and 3-4 mg kg\(^{-1}\), respectively (Butler 1964b, 1966b).

Subacute toxic effects of aflatoxins in monkeys have also been reported (Tulpule et al. 1964). Young Rhesus monkeys (1.5 to 2.0 kg) were fed either 1.0 mg of aflatoxin per day, or 0.5 mg per day for the first 18 days followed by 1.0 mg per day. All animals lost their appetite and died in 14 to 28 days. The principal observations on autopsy were of liver lesions similar to those seen in ducklings and which were suggestive of liver cirrhosis.

The production of liver lesions does not necessarily result in death, and recovery can occur if a wholesome diet is introduced. Factors that influence the acute toxicity of aflatoxin also affect its chronic toxicity.

**Carcinogenicity of aflatoxin**

The chronic effect of aflatoxin which has aroused most interest is the production of liver tumors in fish, ducks, rats and non-human primates. Prolonged administration of the toxin at subacute levels results in the formation of liver tumors which are cancerous. This was observed in early investigations on the feeding of toxic groundnut meal to rats (Lancaster et al. 1961). In these experiments, aflatoxin-contaminated Brazilian groundnut meal (which was highly toxic to poultry) was fed to rats at levels that did not cause acute toxic symptoms. After 24 weeks of feeding, 81% of the rats developed malignant liver tumors. This was confirmed by several other investigators using aflatoxin-contaminated groundnut meals (Butler and Barnes 1963, Newberne et al. 1964, Salmon and Newberne 1963. Schoental 1961). Aflatoxin levels as low as 100 µg kg\(^{-1}\) caused significant incidence of liver tumors in rats when fed for as long as 73 weeks (Salmon and Newberne 1963).

Aflatoxin-contaminated groundnut meals have also been shown to be carcinogenic to ducks and rainbow trout (Carnaghan 1965, Ashley et al. 1965). Some animal species such as mice are relatively resistant to carcinogenic effects of aflatoxin (Platonow 1964, Allcroft 1965).

Several studies with rats have demonstrated a positive correlation between liver tumor incidence and dietary aflatoxin in the range of 60 to 1800 µg kg\(^{-1}\) (Newberne et al. 1964, Wogan 1966). Administration of 1800 µg aflatoxin kg\(^{-1}\) for 370 days resulted in a tumor incidence of more than 90%.

The rainbow trout is considerably more sensitive than the rat to the carcinogenic effects of aflatoxin; this fish develops liver tumors when fed purified diets containing 0.5 to 2.0 µg aflatoxin kg\(^{-1}\) (Ashley et al. 1964, Ashley et al. 1965).

**Clinical and Pathological Effects**

Early clinical signs of aflatoxicosis in livestock are reduction in feed intake, and weight loss, this is often rapidly followed by death. The most important pathological effect is liver damage. Non-specific but characteristic clinical and pathological...
effects can be seen for each species. Several review papers have described in detail the clinical and pathological effects of aflatoxin ingestion in various animal species (Allcroft 1969, Wogan 1966).

The principal lesions occur in the liver, and may be classified as toxic hepatitis. One of the most constant responses to aflatoxin B is bile ductule hyperplasia at the periphery of hepatic lobules. Changes in hepatocytes (vacuolization, fatty change) leading to necrosis, are usually localized in one part of the hepatic lobule, depending on the species (Allcroft 1969, Wogan 1966).

Aflatoxins also impair the effectiveness of native defence mechanisms and immunogenesis. The major effect of aflatoxin is on the cell-mediated immune system as has been shown in poultry with fowl cholera and in swine erysipelas. Aflatoxin ingestion increases susceptibility of poultry to salmonellosis, candidosis, and coccidiosis, and of calves to fascioliasis (Pier et al. 1979).

Role of Nutrition in Aflatoxin Toxicity

Many researchers have investigated the influence of malnutrition on aflatoxin toxicity in various animals. Madhavan et al. (1965) first observed that reduced protein intake significantly increased the susceptibility of monkeys to aflatoxin. Newberne et al. (1966) reported that rats given diets containing low level of protein and exposed to aflatoxin B, over 3 weeks suffered a higher incidence of liver tumors in a shorter period of time than did rats receiving diets containing a normal level of protein. A high-protein diet precluded severe injury/toxicity from aflatoxin (Madhavan and Gopalan 1968).

Foy et al. (1966) has suggested that the high incidence of liver cirrhosis in baboons, and liver carcinoma in human populations in Africa could result from diets containing aflatoxins and deficient in pyridoxine.

Many laboratory studies with rats have indicated that marginal deficiencies of lipotropes may significantly influence the response to aflatoxin (Newberne et al. 1966, Rogers and Newberne 1969). Interactions of vitamin A deficiency with aflatoxin have been investigated by a few workers, but no definite results were obtained (Reddy et al. 1973).

Newberne and Gross (1977) comprehensively reviewed nutritional factors that might influence responses of animals and humans to aflatoxin.

As the situation is by no means clear, it is important that further studies should be made in those least developed countries where malnutrition, infectious diseases and risk of aflatoxicosis coexist.

1.1.2 Aflatoxicosis in Man

During the past two decades, there have been several attempts to correlate the consumption of foodstuffs contaminated with aflatoxins with human diseases. Investigators have relied on natural outbreaks of suspected aflatoxicosis and upon cases of accidental consumption of aflatoxin-contaminated foodstuffs. There have also been extrapolations regarding toxicity and carcinogenicity of aflatoxins from in vitro experiments with primates and other animals.

Association between aflatoxin ingestion and liver cancer

Epidemiological studies have been carried out to see if aflatoxin ingestion might be a factor in the high incidence of liver cancer in some areas of Africa. Asia, and the USA. Several early epidemiological studies in Indonesia, Kenya, Mozambique, South Africa. Swaziland, Thailand, and Uganda found a positive correlation between aflatoxin ingestion by humans and liver cancer incidence (Husaini et al. 1974, Peers et al. 1976, Van Rensburg et al. 1974, Keen and Martin 1971, Shank et al. 1972, Alpert et al. 1969). There appears to be a trend for percentages of aflatoxin-contaminated food samples to be higher at low altitudes than in cooler, high altitude areas. This parallels the trend for liver cancer in Swaziland and Kenya (Keen and Martin 1971, Peers and Linsell 1973). However, it could not definitely be concluded from these studies that aflatoxin caused liver cancer, also there had been no attempt to consider other possible causes of liver cancer. Several researchers have highlighted the strong relationship between hepatitis B virus (HBV) infection and incidence of liver cancer (Beasley et al. 1981, Beasley 1982). All epidemiological studies of aflatoxin and liver cancer conducted in Africa and Asia involved populations subjected to HBV infection.

Subsequent epidemiological studies included data on the prevalence of HBV infection (Astrup et al. 1987, Peers et al. 1987, Van Rensburg et al. 1985, Yeh et al. 1985, 1989). In a repeat study in Swaziland, Peers et al. (1987) found that virtually all the liver cancer cases had been exposed to HBV infection, but aflatoxin exposure was a more important factor in liver cancer than the prevalence of HBV infection. Other studies of populations with endemic HBV infection provided no convincing evidence to support a primary role for aflatoxin in the induction of human liver cancer, although an accessory role to HBV infection for aflatoxin could not be ruled out (Astrup et al. 1987, Van Rensburg et al. 1985, Yeh et al. 1985, 1989). In a recent study in China. Chen et al. (1990) found no association between liver cancer mortality rates and aflatoxin exposure (controlled for HBV infection) but confirmed the strong association of liver cancer mortality with HBV infection. In the USA, a study of liver cancer in relation to aflatoxin ingestion in populations free of HBV infection also ruled out any association between liver cancer and aflatoxin exposure (Stoloff 1983).
Several in vitro experiments with human liver tissue have indicated that it metabolizes aflatoxins in a similar fashion to the livers of species refractory to aflatoxin carcinogenesis (Stoloff 1989).

Stoloff (1989) has given an excellent review of the liver cancer/aflatoxin/HBV relationship. Based on published evidence, he concluded that aflatoxin is not a probable human carcinogen.

**Aflatoxins and occupational diseases among groundnut workers**

Workers engaged in harvesting, shelling, bagging, storage, marketing, and transport of groundnuts may be exposed to aflatoxin through the respiratory route. A chemical engineer involved in sterilizing Brazilian groundnut meal contaminated by *Aspergillus flavus* developed alveolar cell carcinoma and died within a year. Aflatoxin B1 was detected in his lung tissue (Dvorackova et al. 1976). Three cases of pulmonary interstitial fibrosis were reported from Czechoslovakia, of whom two were agricultural workers; aflatoxin (10-54 ug Kg⁻¹) was detected in lung samples of all the workers (Dvorackova and Pichova 1986).

In a Dutch groundnut oil factory workers were exposed to an estimated 0.039-2.5 ug aflatoxin per working week (45 h). An epidemiological study of 55 workers indicated that 11 developed various forms of cancer, mainly of the respiratory tract (Van Nieuwenhuize et al. 1973).

A study conducted 18 years after initial exposure to aflatoxin-contaminated dust did not give a definite indication of cancer risk associated with respiratory exposure to aflatoxin, but it did suggest certain associations warranting further study (Hayes et al. 1984). There was no information on the extent to which dustborne aflatoxin enters the body, nor any explanation of the manner in which aflatoxin could be related to any of the observed cancers.

There can be considerable risk associated with exposure to dust from groundnut and other agricultural commodities contaminated with aflatoxin; however, the exact magnitude of the risk cannot be adequately estimated (Baxter et al. 1981).

**Acute toxicity of aflatoxin in humans**

Acute effects of aflatoxins in humans are well documented. There are two reports of aflatoxicosis in humans involving consumption of maize heavily contaminated with aflatoxins (Krishnamachari et al. 1975 a, b, Ngindu et al. 1982). In one of the incidents, in India, there were 272 hospital admissions with clinical symptoms of aflatoxicosis and a 27% monality. In the second incident, in Kenya, there were 20 hospital admissions with a 60% mortality.

A report (Willis et al. 1980) helps to establish a possible no effect level for aflatoxin B1. In an attempted suicide, a laboratory technician consumed 12 ug kg⁻¹ body weight day⁻¹ over a 2 day period and 6 months later 11 ug kg⁻¹ body weight day⁻¹ over a 14 day period. Outside of transient rash, nausea and headache there were no immediate ill effects, and on a 14 year follow-up the physical examination and blood chemistry, including tests for liver function, gave normal results.

There is certainly a basis for concern about the acute toxicity of aflatoxin in humans from dietary intake of the toxin.

The long term follow up of patients who have survived outbreaks of aflatoxicosis for possible liver cancer should be of considerable importance.

**Sublethal effects of aflatoxins**

In Senegal, several children less than 1 year old each received 70-140 g of groundnut meal per day for 10 months as a treatment for kwashiorkor (Payet et al. 1966). The meal samples were later found to be contaminated with aflatoxin at 500-1000 ug kg⁻¹, providing an aflatoxin intake of 35-140 ug day⁻¹. Two of these children were identified for liver biopsies 4 and 6 years after consumption of the contaminated meal. One child had gross abnormalities in the liver structure, persisting through the sixth year; the other child had minor liver abnormalities at 4 years and recovered to completely normal at 6 years.

**Aflatoxin and Indian childhood cirrhosis**

Ingestion of aflatoxin, viral diseases, and hereditary factors have all been suggested as possible aetiologic agents of Indian childhood cirrhosis (Bhat 1989). Amla et al. (1970, 1971) presented circumstantial evidence to indicate that children exposed to aflatoxin through breast milk and dietary items such as unrefined groundnut oil and parboiled rice may develop cirrhosis. They detected aflatoxin B1 in 7% of urine samples from cirrhotic children. In another study (Amla et al. 1971), they reported that malnourished children in a pediatric ward of a hospital developed liver lesions similar to those observed in Indian childhood cirrhosis after accidentally consuming 30-50 g of groundnut protein flour contaminated with 300 ug kg⁻¹ of aflatoxin for periods ranging from 5 days to 1 month. They also observed similar skeletal muscle changes in cirrhotic children, in children who consumed aflatoxin-contaminated groundnut protein flour, and in rats fed aflatoxin. Other workers have also detected aflatoxins or aflatoxin-like substances in urine samples from cirrhotic children (Yadgiri et al. 1970, Parpia et al. 1972).

It is surprising that childhood cirrhosis has not been found in other countries where aflatoxin contamination of dietary items is fairly high.
Anatoxins and Kwashiorkor

Several epidemiological studies have provided circumstantial evidence for the involvement of aflatoxins in Kwashiorkor, particularly among malnourished children in the humid tropics. In several studies in the Sudan, Nigeria, Ghana, and South Africa, in both autopsied as well as biopsied livers from children with protein energy malnutrition, aflatoxins were detected in Kwashiorkor and marasmic Kwashiorkor, but not marasmus (Coulter et al. 1986, Hendrickse 1984). Groundnuts and peanut butter were the major source of aflatoxins among these populations (Hendrickse et al. 1982). Aflatoxins M. and M$_2$ have also been frequently detected in breast milk and neonatal cord blood of mothers (Coulter et al. 1984, Lamplugh and Hendrickse 1988). Based on these studies it is postulated that Kwashiorkor might result from chronic aflatoxin poisoning (Hendrickse 1984. Pearson 1990). These observations do not establish a cause-effect relationship between aflatoxin ingestion and kwashiorkor. Some typical diagnostic features of kwashiorkor, e.g., oedema, mild pathological damage to liver, and rapid response to dietary therapy, are not characteristic of aflatoxicosis. Some studies have reported that children with kwashiorkor have a reduced ability to metabolize and excrete aflatoxins compared with marasmic and healthy children (Coulter et al. 1986). This could explain the higher levels of anatoxins detected in kwashiorkor children. It appears that aflatoxins are not primarily responsible for kwashiorkor development, but malnourished children may show increased susceptibility to the toxic effects of aflatoxin.

Bhat (1989) critically reviewed the risks to human health associated with consumption of groundnuts and groundnut products contaminated with aflatoxins.

Aflatoxins and Reye’s Syndrome

Several investigators have suggested aflatoxin as an aetiologic agent of Reye’s or Reye’s-like syndromes in children in Thailand, New Zealand, and Czechoslovakia (Becroft and Webster 1972. Dvorackova et al. 1977). This was based on aflatoxins being detected in serum and urine samples from the children suffering from Reye’s syndrome, and the presence of liver lesions. There is as yet no conclusive evidence to show that Reye’s syndrome is directly associated with consumption of aflatoxin-contaminated foodstuffs.

Risks through dietary intake of contaminated groundnuts and groundnut products

Outbreaks of aflatoxicosis in man have been attributed to ingestion of staple foods such as maize highly contaminated with aflatoxins. Although high levels of aflatoxins have been reported in groundnuts and groundnut products these pose less of a hazard to human health than contaminated maize products because of the smaller quantities consumed in most diets. However, dietary intake of groundnuts and/or groundnut products can be substantial in some developing countries, e.g., Mozambique. Senegal, the Sudan. One must consider the possibility of chronic effects of aflatoxin following continuous exposure to relatively low levels of the toxins through ingestion of groundnuts and groundnut products. Studies in several groundnut-producing countries of Africa and Asia have attempted to link aflatoxin ingestion with liver cancer. Although the causal relationship has not yet been definitively established, it is obviously important to reduce dietary intake of aflatoxins. In developed countries there are procedures for monitoring levels of aflatoxins in foodstuffs, but in most developing countries only limited or no protection of this kind is available. Substantial exposure to aflatoxin may result from consumption of contaminated peanut butter, use of unrefined oil, and snack foods from reject trade groundnuts. These risks are much greater in developing than in developed countries. Particular care should be taken to ensure that groundnut products commonly consumed by children should be free from aflatoxin. Protein concentrates used in treatment of malnourished children should be carefully checked to ensure that they are free from aflatoxin.

Although there are some doubts as to the toxic and carcinogenic effects of aflatoxins on man, one should not underestimate their possible hazards to human health, particularly in the presence of nutritional disorders and other diseases.

This paper considers climatic conditions in relation to mycotoxin contamination in different crops in Queensland, Australia. Queensland has a history of recurring drought, and drought stress is an important factor in preharvest aflatoxin contamination of the groundnut and maize crop, particularly in the drier Burnett region. Conversely, while drought depresses yields, dry conditions usually limit the extent of fungal damage to wheat, barley and sorghum. However, the 1983 season was exceptional in that persistent heavy rain produced extensive mold damage to all crops. Mycotoxins produced by *Fusarium* spp. have never been implicated in widespread mycotoxicosis in Queensland, although they may contaminate maize and sorghum in regions with high rainfall during the growing season, such as the tablelands in Far North Queensland. Mycotoxicosis of man is unlikely to occur in Queensland, but mycotoxicosis of livestock occurs occasionally. It is usually associated with feeding materials molded from being stored damp. In addition, cases are likely to occur in drought seasons when feeds are not only scarce, but also likely to be of poorer quality.


The factors influencing aflatoxicosis, clinical signs and gross and microscopical lesions of the condition in fowl, duck, turkey, swine, sheep, monkey, rat, mouse and man, and biochemical changes produced are discussed.


Groundnut oil obtained from Hong Kong markets was frequently contaminated with aflatoxins. A purified diet in which aflatoxin-contaminated market groundnut oil (aflatoxin B, 110 ug kg<sup>-1</sup>) was used as the fat source was given to Sprague-Dawley rats for 22 months from weaning; its estimated aflatoxin B<sub>1</sub> content was 5 to 7 ug kg<sup>-1</sup>. Controls were given a diet of identical composition except that Manzola maize oil (aflatoxin-free) was used. Of 76 rats given aflatoxin, 3 had sarcomat, one in the liver, one in the wall of the colon and one in the subcutaneous tissue of the groin; 18 rats given groundnut oil showed parenchymal liver damage and different degrees of fatty change and one showed premalignant changes in liver cells. Of 90 control rats, none developed malignant tumours. The liver-to-body weight ratios for experimental and control rats were 2.93 and 2.62, respectively. The difference between those values was significant, reflecting the degree of fatty change in the livers of experimental rats compared with that in the controls. Over 90 % of Hong Kong households use groundnut oils for cooking purposes, and these results indicate a possible health hazard in the use of contaminated groundnut oil.


Problems arising from the contamination of groundnut meal with aflatoxin are reviewed. Progress in the detection of related compounds and knowledge of their toxic properties are summarized, with details of acute toxicity on monkeys, and indications of the chronic effects of very small doses on liver and kidneys of children. Transmission of the toxic constituents from animal feeds to cow’s milk and pig liver and kidneys is also reported, although there are no apparent indications of progressive accumulation. Aflatoxin in imported groundnut meal is discussed and the limits imposed by the EEC on feeds of different kinds are tabulated. The practical implications of stringent limits on aflatoxin contents of meal imported into European and other countries are discussed, with particular reference to the danger that more heavily contaminated batches may be retained in the developing countries where the population relies heavily on groundnut meal as a source of protein. The possibility of detoxification with ammonia is briefly discussed, and it is noted that tests with rats indicate that the protein quality if thereby decreased by about 10 %.


This paper briefly reviews mycotoxin research in India. Research on various aspects of the groundnut aflatoxin problem, and on other mycotoxins is highlighted. Aspects discussed include occurrence of aflatoxins in various agricultural commodities, toxicity of aflatoxins to humans, resistance to aflatoxin production in natural substrates, detoxification, and mycology of aflatoxin-producing strains of *Aspergillus flavus*.

This review attempts to trace the connection between the mycology of foodstuffs and the onset of disease due to the toxins that various fungi produce within those foodstuffs. Particular emphasis is placed on the practical side of the problem, especially with reference to the physiological interactions among fungi, the incidence of fungi and their toxins in various substrates, the ecology of mycotoxicosis, and the acute and chronic effects of mycotoxicosis.


The clinical effects of aflatoxin exposure are briefly discussed, and the current US FDA regulations and sampling procedure for control of aflatoxins in groundnuts are discussed. Some quality control procedures to ensure minimal quantities of toxin persisting into the finished product are also outlined.


This paper outlines events leading up to the discovery of aflatoxin, describes briefly research done on the groundnut aflatoxin problem in northern states of Nigeria, and considers the implications of aflatoxin in relation to animal and human health, and discusses measures for elimination of aflatoxin from Nigerian groundnuts, or to at least greatly reduce incidence of aflatoxin.


The important published information on aflatoxin during the period January 1964 to March 1967 is summarized. The topics covered include (a) assay of aflatoxins, (b) toxicology of aflatoxins, (c) biosynthesis of aflatoxins, (d) biochemical and intercellular effects of aflatoxins, (e) metabolism of aflatoxins in animals, (f) incidence, control and detoxification, and (g) implication in man.


This review covers several aspects including a survey of aflatoxin contamination of food grains and of groundnut oil, human and animal health hazards from mycotoxins, techniques for detection and estimation of aflatoxins, factors affecting aflatoxin formation, preventive or control measures.


Part I of this report deals with a survey of mycotoxins contamination in food and its relation to hepatoma in Malaysia. The topics covered include : (a) Biostatistical aspect of liver cancer in Malaysia, (b) A semi-quantitative study on frequency of food intake in Sekinchan, (c) Results of mycological survey of Malaysian foods, and (d) Analysis of mycotoxins in foods in Malaysia. The incidence of liver cancer is higher in Malaysia than in Japan and is highest in the Chinese male population. In the three communities rice, oil, fat and vegetables are the main foods. The Malays frequently eat fresh fish, beans and bawang and use chili kering; the Chinese frequently eat bawang meats, fresh fish and beans; the Indians frequently use milk and its products and dry curry powder. Aspergillus spp. were the major fungal species found, especially in milled rice. Aspergillus candidus was most frequent in rice ( 52 % of samples) and A. niger moderately abundant. A. flavus was isolated from 13-21 % of rice samples and A. fumigatus from 3-21 %. Curry powder was highly contaminated with A. flavus; A. niger, Penicillium citrinum and mucoraceous fungi were also dominant. Aspergillus flavus was found in groundnuts, cereal powders, beans and dried fish and A. niger was widespread in some foodstuffs. No mycotoxins were detected in rice, moldy soybeans or tempeh. Aflatoxin was detected in groundnut samples. Extracts of rice, beans and tempeh samples showed varying toxicity to HeLa cells. All except one groundnut sample showed low toxicity. Of 30 strains of A. flavus isolated, 8 produced aflatoxin, and 3 of 4 strains of A. versicolor produced sterigmatocystin. All 91 strains of A. candidus examined produced terphenyllin but not xanthoasin. Part II, on studies in Thailand, includes (i) Mycological survey on market foods of Thailand, (ii) Distribution of aflatoxin-producing fungi in agricultural soils of Southeast Asia, and (iii) A glimpse into Reye syndrome in Khon Kaen, a north-eastern district of Thailand. The dominant fungi isolated from 36 samples of eight types of foods were : A. candidus, A. flavus, A. niger, Eurotium and Penicillium spp. from rice; A. flavus, A. niger, Mucor and Rhizopus spp. from groundnuts; Fusarium spp. from beans and maize; A. niger from pepper; A. niger and Fusarium spp. from cassava starch; A. flavus and A. niger from chilli, chilli powder, shrimp and shell fish. A. flavus was isolated from 15 of 50 soil samples from Malaysia and 29 of 106 from Thailand. Of these 44 strains, 16 produced aflatoxin.


Current knowledge about mode of action of aflatoxins, their medical and economic importance and prospects for tlicir control are reviewed, considering the following aspects : structure, nomenclature and biosynthesis; mode of action; health risks and legislative regulation; postharvest control (segregation, decontamination, storage);
and preharvest control (pesticides, and biological control by breeding or microbial interactions, particularly repression of aflatoxin synthesis by *Aspergillus flavus* following infection with a virus from *Penicillium chrysogenum").


Mold infection of oil-seed cakes has long been suspected as a possible cause of ill-health among farm animals, but only within the last three years has the problem been closely investigated. Groundnuts may become infected with strains of the fungus *Aspergillus flavus* that produce a group of highly toxic substances known collectively as aflatoxin. This has in the past caused the deaths of large numbers of farm animals, especially young animals. There is, however, no evidence that human beings have suffered, and with recognition of the nature of the hazard, steps can be taken to ensure that there need be no danger from the substance in future.

### 1.2.2 Aflatoxicosis in Livestock


Aflatoxin toxicity problems in farm animals are reviewed. Clinical and pathological effects in animals are described. Metabolism and excretion of aflatoxin in animals are also discussed.


Whole milk of cows given toxic groundnuts as 20 % of the diet, an extract of that milk, and the fraction precipitated by rennet were toxic to ducklings. The degree of toxicity of the milk was related to that of the groundnut given. The toxin was not destroyed by pasteurisation. In 19 samples of National dried full cream and separated milk and proprietary infant foods there was no evidence of toxicity. No toxin was found in the liver of 1 of the cows given toxic groundnut meal or the eggs of pullets given 15 % toxic groundnut meal in their diets.


Biological effects of toxic groundnut meal (meal containing aflatoxin) in various birds and animals are reviewed. Ducklings are the most susceptible to the toxin. They are suitable for bioassay of aflatoxin. Turkey poult are less susceptible, while chickens are comparatively resistant. Among the larger farm animals, pigs are most susceptible. Calves from 1 to 6 months of age are highly susceptible, becoming tolerant with age, and sheep are comparatively resistant.


Cows fed rations containing toxic groundnut meal excreted in the milk a toxic factor having a biological effect in ducklings similar to that caused by aflatoxin. Precipitation of protein fractions of the milk showed that the toxin was present only in the rennet-precipitated casein fraction which also included the fat; none was found in the protein-free filtrate. Its presence was not detected in samples of bulked milk supplies from collection centres in Britain; nor was it found in liver from a cow or a pig, or clotted blood and serum from a cow, or pullet eggs, from animals fed rations containing toxic groundnut meal.


The effects of feeding rations containing 20 % of a highly toxic groundnut meal to calves, first-calving heifers, and older cows are described. The first abnormality in the calves was a reduction in growth rate followed by unthriftiness, and loss of appetite. After 16 to 25 weeks on the toxic diet, terminal symptoms, characterized by severe tenesmus, occurred 2 to 4 days before death. Fibrosis of the liver, ascites and visceral oedema were the most important post-mortem findings. The most notable biochemical changes in the calves were : (1) an increase in serum alkaline phosphatase activity up to the 12th week followed by a decline to normal values during the terminal phase; (2) almost complete absence of vitamin A in the livers at death. The main histological changes in serial-biopsy and post mortem liver specimens consisted of hepatic centro-lobular necrosis, ductal cell hyperplasia and veno-occlusive disease. Loss of condition occurred in the heifers after seven months. No clinical abnormality was noted in the older animals apart from a Jersey cow which developed clinical Johne's disease at calving after 13 months on toxic-groundnut meal. In a group of eight cows a significant fall in milk yield resulted from feeding a concentrate ration containing 15 % of a very highly toxic groundnut meal.

19. Allcroft, R., and Loosmore, R.M. 1963. Toxic effects associated with the

The clinical and pathological effects of a disease in poultry, pigs, cattle and laboratory animals associated with the feeding of toxic batches of groundnut meal are described. The toxic factor is a hepatotoxin and is produced by infection of groundnuts by a toxigenic strain of Aspergillus flavus which has been found in some batches of groundnuts from all major groundnut-producing countries. In cattle, the disease closely resembles seneciosis; in rats, multiple liver tumors, some malignant, have been produced. Cows fed rations containing toxic groundnut meal excrete the toxin in their milk.


Dairy cows were fed diets containing aflatoxin B₁-contaminated groundnut meal and their milk was tested after 4, 5 and 6 days of feeding. Assays of liquid and dried milk samples indicated a simple linear relationship between the amount of aflatoxin B₁ ingested and the concentration of aflatoxin M₁ excreted in the milk (P < 0.001). The lowest aflatoxin B₁ intake to give detectable amounts of aflatoxin M₁ was 0.6-0.9 mg day⁻¹.


A cow of 600 kg liveweight was given a single oral dose (300 mg) of a mixture of aflatoxins (B₁, 44%; G₁, 44%; B₂ 2%), equivalent to 0.5 mg kg⁻¹ bodyweight. The toxins were detected in milk, urine and faeces over a period of 9 days. About 85% of the total aflatoxin was detected in milk and urine in the first 48 hours. No aflatoxin was detected in milk after 4 days and in urine and faeces after 6 days. Only 4.52% of the total dose of 300 mg was detected, the milk containing 0.18%, urine 1.55% and faeces 2.79%. The only form of aflatoxin detected in milk was aflatoxin M₁, and this represented 0.35% of aflatoxin B₁, given to the animal. Aflatoxin M₁ was also the main form found in urine. Aflatoxin G₁ was found in both urine and faeces. Aflatoxin B₁ was present in greatest amounts in faeces. The pattern of excretion was compared with that in a ewe given 1.0 mg of a similar mixture kg⁻¹. The pattern was similar in the 2 species, but excretion of unchanged aflatoxin B₁ was greater in the cow.


A mixture of aflatoxins B₁, G₁, B₂ and G₂, 1 mg kg⁻¹ bodyweight, was given to 3 yearling sheep by stomach tube or by intraperitoneal injection. The sheep were killed 2 h later. Aflatoxin and the "milk toxin" were estimated in liver and kidney and in urine before the toxin was given, after 1 h, and at death. The "milk toxin" was more intense in kidney and urine than the aflatoxins, but the reverse was true for the liver. It is suggested that tests on urine may be useful for investigating ingestion of aflatoxin by man or animals. The name aflatoxin M₁ is proposed for the "milk toxin".


Occurrence of aflatoxicosis in pigs is reported. The disease was associated with feeding toxic groundnut meal.


Outbreaks of a new disease of turkey poults in England are reported. Disease outbreaks were associated with certain feeds. Disease symptoms are described.


Dairy producers were advised against feeding aflatoxin-contaminated feeds to dairy cattle. Aflatoxin toxicity problems in dairy cattle were highlighted. Guidelines for usage of contaminated feeds and for avoiding problems were given.


Examination of chickens after death showed liver damage typical of groundnut poisoning. Birds less than 5 weeks old were more affected than older ones, and their mortality was higher. It was confirmed that diets of all affected chickens contained 5% of Brazilian groundnut meal.


Evidence of the susceptibility of ducklings and chickens to the toxic principle in certain samples of groundnut meal is presented. Ducklings were highly susceptible to the toxic principle in these meals, and it was suggested that they are eminently
suitable for screening suspected samples of groundnut meal and for other experimental work connected with this type of toxicity. A toxic agent was found in certain Brazilian and East African groundnut meals, and evidence is presented which suggests that the toxic principle in these meals is identical. The gross and microscopic lesions in ducklings and chickens fed on toxic groundnut meals are described and the similarities and differences between the lesions in these birds and turkeys and in large animals are discussed.


In a controlled experiment, male Karan Swiss calves (3-4 months old) were given feeds contaminated with aflatoxin at levels of 0.5, 1.0 and 1.5 ug g\(^{-1}\) over a period of 16 weeks. For calves given aflatoxin-contaminated feed, weight gains were significantly reduced from 3rd week onwards. Feeding aflatoxin at levels of 0.26 ug kg\(^{-1}\) did not adversely affect growth.


Aflatoxin toxicity in poultry is described. Effects of ingestion of aflatoxins on young chicks and layers are discussed. Preventive measures for avoiding aflatoxicosis in poultry are given.


Outbreaks of a new disease of turkey poults in England are reported. Disease symptoms are described. Disease outbreaks were associated with certain feeds.


Some comments are given on the implication of rations in turkey X disease. Outbreaks of turkey X disease were not associated with rations containing milo.


This letter emphasizes the importance of labelling of poultry foods in relation to turkey X disease.


This paper is a report upon turkey X disease occurring in the United Kingdom with information on its aetiology.


Aflatoxin B\(_1\) was detected in 23 of 55 feedstuffs known to be either water-damaged or visibly moldy. The highest level of aflatoxin was 700 ug kg\(^{-1}\) of feed, and the mean concentration was 140 |ig kg\(^{-1}\). Of 36 feedstuffs purchased from local manufacturers, only groundnut meal contained aflatoxin B\(_1\), (500 ug kg\(^{-1}\)). Eleven feedstuffs were associated with field outbreaks of animal disease and seven of these contained aflatoxin, but not in sufficient quantity to account for the described disease symptoms.


Various outbreaks of Turkey X disease in ducklings and pheasant chicks in the U.K. during 1961 are described. Indian groundnut meal in the birds' rations was associated with many of these outbreaks. Results of feeding experiments suggested that certain consignments of Indian groundnut meal contained a toxic principle similar to that found in Brazilian and East African samples in 1960. The toxicity of the Indian groundnut meal samples tested was considerably less than any of the toxic Brazilian or East African groundnut meals examined.


This letter highlights the possible hazards of incorporation of toxic groundnut meal in animal feeds.


Literature on the occurrence of cancer in livers of domestic and laboratory animals fed on groundnut meals infected with Aspergillus flavus is reviewed. The possible role of mycotoxins in production of liver tumours in man in Africa and Asia appears to warrant investigation.
Day-old ducklings in groups of six were given two turkey diets which had been associated with outbreaks of Turkey "X" disease. The diets had about 6 % Indian groundnut meal. Other groups were given similar amounts of Indian groundnut meal known to be non-toxic. Those given the toxic meal did not grow well and five in each group died within 5 weeks. Gross and microscopical lesions, similar to those produced by toxic Brazilian and East African groundnut meals, were found. Extracts of the Indian meals in amounts equivalent to 100, 200 and 750 g in 5, 5 and 11 days did not kill day-old ducklings, but liver lesions were found post-mortem.


Heavy mortality in chicks in Chittoor district of Andhra Pradesh State, India, due to aflatoxicosis is reported. Groundnut cake was implicated in the aflatoxicosis; groundnut cake was contaminated with aflatoxin at a level of 3590 ug kg


Broiler chickens were fed a diet containing 2057 and 1323 ug kg


All 16 of a herd of fattening bullocks and heifers from 18 to 22 months of age lost condition when they were given 2 lb Brazilian groundnut meal daily. They ate the meal unwillingly. After 6 weeks 4 were killed, and liver damage typical of groundnut poisoning was found. Even after 6 months without the meal the remaining cattle did not grow well. Liver damage was found in all animals. An extract of the meal produced typical damage in livers of ducklings and guinea pigs.
Experiments were conducted to determine the precise levels of aflatoxin that would produce adverse effects in pigs and cattle in long- and short-term feeding trials. Five groups of pigs and 2 groups of cattle were used in feeding experiments. Feeds were designed to contain equal amounts of groundnut meal but various proportions of control and toxic meal. Acute toxicity did not occur when moderate levels of groundnut meals containing substantial levels of aflatoxin were given to growing and finishing pigs and cattle. Unthriftiness was noted only in pigs. Dietary aflatoxin B<sub>1</sub>, concentrations up to 140 μg kg<sup>-1</sup> were tolerated by growing pigs, and up to 690 μg kg<sup>-1</sup> aflatoxin B<sub>2</sub>, by pigs in the finishing stage, without significant effects on performance or anything exceeding minimal microscopic liver changes. Moderate levels did not appear to affect performance or induce significant liver damage in store or fattening cattle. Post-mortem examination of carcasses, livers and kidneys showed no gross macroscopic changes attributable to toxic groundnut meal in either calves or store cattle. Microscopic liver lesions were present in calves which received 220 and 440 μg kg<sup>-1</sup> aflatoxin B<sub>2</sub>, the incidence of lesions increasing with the increase in toxic groundnut meal. Two of 8 store cattle given the highest level of toxic meal (660 μg kg<sup>-1</sup> aflatoxin B<sub>1</sub>) also showed liver lesions.


Toxin-containing extracts of either toxic groundnut meals or Aspergillus flavus cultures were resolved by thin-layer chromatography into several zones which were fluorescent when viewed in ultraviolet light. The separated fluorescent materials were administered to ducklings. The fraction B<sub>2</sub>, was toxic to ducklings. The combined fractions (B<sub>1</sub>, and B<sub>2</sub>) showed greater toxicity than B<sub>2</sub>, alone, indicating some toxicity also due to B<sub>1</sub>. The B<sub>1</sub>, fraction from mold cultures and B<sub>2</sub>, from extracts of toxic groundnut meals had the same R<sub>r</sub>, values, and identical ultraviolet-absorption spectra. It is concluded that the extracts from cultures of A. flavus contained at least two substances toxic to ducklings.


Thin-layer chromatography of the toxic milk from cows fed on groundnut meal containing aflatoxin revealed a violet-fluorescent spot with an R<sub>r</sub> similar to that of a minor component of an extract of Aspergillus flavus. By chromatography on silica gel columns a fraction which contained this material and induced proliferation of bile duct in ducklings was obtained. Studies showed that the lactating rat could convert aflatoxin B<sub>1</sub>, into the 'milk toxin'.


The Veterinary Research Laboratories report (pages 201-202) an outbreak of aflatoxicosis among 60 bucket fed calves which had been weaned onto a farm-mixed concentrate ration containing groundnut meal imported from the Gambia. Three died after showing nervous signs, blindness and constipation for 4-10 days, while others were dull and unthrifty. Autopsies revealed liver cirrhosis. Analysis revealed high levels of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> in the groundnut meal. This is the first reported outbreak of the disease in the UK since the mid-1960s.


In 1961 two diseases, virus hepatitis and toxic liver damage caused by feeding groundnut meal caused considerable losses of ducks. The preventive inoculation of ducklings on infected premises with hyperimmune serum completely prevented losses caused by virus hepatitis. Food mashings containing groundnut meal imported from Brazil, Africa and India caused heavy losses among young ducks but intoxications of the same origin occurred also among young chickens and turkeys. In young ducklings such a meal of high toxicity caused an acute liver degeneration, but usually the condition was of a subacute or chronic character. Pathologically an inclination to regeneration was characteristic for the condition, but in more adult ducks cirrhotic livers were often encountered. The only possibility of the control of this condition is to stop the feeding of the toxic groundnut meal immediately after loss of appetite was observed. Loss of appetite may usually be observed in such cases several days before the first deaths occurred.


Studies were conducted to determine if low level exposure to aflatoxin from day-old through market age would induce detectable changes in performance, yield, or carcass grade of broiler chickens at processing. In Trial 1, the treatments were control, 0.75, 0.225, and 0.675 μg aflatoxin g<sup>-1</sup> of feed, and in Trial 2 control, 0.3, 0.9, and 2.7 μg toxin g<sup>-1</sup> feed. All aflatoxin dose levels in Trial 1 significantly (P < 0.05) decreased live, dressed, and chilled eviscerated weight, whereas only 2.7 μg g<sup>-1</sup> significantly (P < 0.05) decreased live and dressed weight in Trial 2, with chilled eviscerated weight being significantly (P < 0.05) decreased at 0.3 and 2.7 μg g<sup>-1</sup> aflatoxin in Trial 2. Parts weights and dimension measurements reflected the aflatoxin-induced decrease in dressed weight. Breast yield (%) was significantly (P < 0.05) decreased by aflatoxin while back, wing, drum, and high yields were significantly increased by aflatoxin. No effect of aflatoxin was seen on the incidence of crooked keel, feather follicle infection, breast blisters, or conformation. A hypocardioenidemia and hepatic hyperlipemia were clearly a result of chronic
aflatoxicosis in these broiler chickens. These data demonstrate that the toxicity of aflatoxin is dependent on the environment in which broiler chickens are exposed. In general, reduced growth, poor pigmentation and fatty livers can result from chronic low level aflatoxicosis.


Previous exposure to aflatoxin B, at 0.2 mg kg\(^{-1}\) commercial feed increased susceptibility to and mortality from cecal coccidiosis in chickens but did not interfere with the protection afforded by a coccidiostat. Chickens vaccinated against Marek's disease (MD) and exposed to aflatoxin B, seemed more resistant against challenge exposure to the MD virus than did nonvaccinated control chickens. Vaccinated and nonvaccinated groups of chickens given aflatoxin B, and subsequently exposed to cecal coccidiosis were more susceptible to challenge inoculation with MD virus than were similar groups of chickens not given aflatoxin, as judged from the severity of lesions observed.


Three cows were given for 6 weeks a ration containing groundnut cake (mean aflatoxin B, content 1710 ug kg\(^{-1}\)) and supplying daily 6840 ug aflatoxin B,. Milk contained aflatoxin M, as 6-12 % of aflatoxin ingested. Dried milk given as 30 % of diet to ducklings had no toxic effect in 69 days whereas diets with aflatoxin B, 80, 240 and 280 ug kg\(^{-1}\) supplied by groundnut cake produced liver lesions in 41, 41 and 28 days, respectively, and caused weight loss.


Lactating cows were fed a diet containing aflatoxin B,-contaminated groundnut cakes, and then on cakes treated with ammonia gas. Between the two periods the diet contained noncontaminated soyabean meals. With the nontreated groundnut meal containing aflatoxin B, at 1100 ug kg\(^{-1}\), total excretion of aflatoxin M, was 2.6 % of total ingested aflatoxin B,. With the treated groundnut meal, aflatoxin M, content of milk was below 0.1 ug L\(^{-1}\). In a second experiment 50 lactating cows were fed for 16 montlis on mixed feeds containing 30 % ammoniated groundnut cake containing aflatoxin B, less than 10 ug kg\(^{-1}\). Aflatoxin M, residue in milk was not above 0.1 ug L\(^{-1}\).


In 1964 in Western Australia, aflatoxicosis was diagnosed in broiler flocks fed on rations containing 14 % groundnut meal. Post-mortem examination revealed very pale, sometimes almost white livers and kidneys which were firmer than normal. Aflatoxin B, content of three groundnut meal samples was assayed at 2800, 2200 and 2500 ug kg\(^{-1}\). The pathology in ducklings, chickens and laying hens fed the original ration, or synthetic rations with added groundnut meal, is described. In chickens after 21 days feeding on the toxic ration, pale areas were noticed in skeletal muscle, which were seen to be large areas of necrosis with a diffuse increase in sarcolemmal nuclei. Toxigenic strains of Aspergillus flavus were isolated from the aflatoxin-contaminated samples of groundnut meal.


Outbreaks of Turkey "X" disease have not always been associated with the inclusion of groundnut meal in the diet. It is not considered that listing of ingredients of proprietary foods would serve any practical purpose.


The danger of implicating groundnut meal in all otherwise unexplained liver damage is stressed. Three ducklings sent for examination were all reported as having signs of groundnut poisoning; 1 of them had received no groundnut meal.


It is considered that there is sufficient evidence that recent heavy losses in flocks of turkeys in the United Kingdom were caused by poisoning by Brazilian groundnut meal.


A natural outbreak of aflatoxicosis in dairy cattle is reported. The condition was tentatively diagnosed as aflatoxicosis because of histopathological changes in the liver, and was subsequently confirmed by the presence of aflatoxin in the groundnut meal.
cake fed to the dairy cattle. Feeding extracts of the feed samples to day-old ducklings induced characteristic lesions of aflatoxicosis.


Occurrence of aflatoxicosis in poultry in Mysore State, India, is reported. The disease was first recognised at the Government Poultry Breeding Unit, Hebbal, Bangalore in 1966 wherein 2219 chicks died in one week. Subsequently, several sporadic incidences were found in various poultry farms in the State. The disease was predominant in younger stocks, possibly due to the increased percentage of protein in the form of toxic groundnut cake.


Research on aflatoxins carried out at the National Research Laboratories, Hyderabad (India), is presented. Aflatoxicosis was found in buffaloes and ducks at government farms near Hyderabad. Aflatoxin contamination was found in the livestock feeds. Biological effects of aflatoxins in ducklings and rats are described. Of 60 varieties of groundnut screened for resistance to aflatoxin production by a toxigenic strain of Aspergillus flavus, one variety "U.S. 26" did not support aflatoxin production.


Deaths are reported in calves up to 3 months old which had been given Brazilian groundnut meal in the feed from 3 weeks of age. At first a respiratory disease and lead poisoning was suspected. Several calves died up to a week after withdrawal of groundnut meal from the feed; one calf responded to treatment with vitamin A. The same meal had been given to the dairy herd, without any apparent ill effect.


In an outbreak of poisoning in turkeys the feed had 5% of groundnut meal. The groundnut meal had more than 2000 ug kg⁻¹ aflatoxin B₁, estimated by thin-layer chromatography, and water extracts were poisonous to ducklings. Other samples of groundnut meal imported into Australia had 1600 to 2000 ug kg⁻¹ aflatoxin and a local product had 2700 to 3300 ug kg⁻¹ aflatoxin.


Occurrence of aflatoxicosis in a dog is reported. Postmortem examination of a dog that died with acute haemorrhagic gastroenteritis, fever, and jaundice showed a friable, bright yellow liver. Aflatoxin was found in the food, which contained groundnut meal.


Two cases are reported of poisoning of pigs, the first in 2 store pigs of a group of 6, and the second in 2 sows which refused to eat but otherwise appeared normal. Groundnut meal toxicity was suspected, and lesions in the liver and other organs and tissues of the younger pigs were similar to those described by Loosmore and Harding (1961). The difficulty of diagnosing groundnut poisoning in pigs is stressed.


Ayrshire bull calves in 3 groups of 8 were given weaning meal with no toxic-groundnut meal or with 4 or 8% of a toxic meal with 5000 to 6000 pg kg⁻¹ aflatoxin B₁; all diets had a total of 16% groundnut meal, the balance being made up with non-toxic meal with 20 to 30 pg kg⁻¹ aflatoxin B₁. The diets were given to appetite as meal mixtures to maximum 6 lb per day. Calves got the diets until they were 116 days old when they were killed. For 32 Aberdeen-Angus crossbred bullocks and heifers about 2 years old, the meal mixtures had 0, 4, 8 or 12% toxic groundnut meal. Rations were 9 lb meal daily in the first week rising to 14 lb from 8 to 20 weeks and all the cattle had access to silage and hay. In the diets of calves 4 or 8% toxic meal significantly depressed liveweight gain and intake of feed for 3 months; feed utilisation was impaired. As 4 to 12% of the diet toxic meal had no effect on the performance of fattening cattle. There were enlarged hepatic cell nuclei in the calves, and the incidence of these slight lesions was related to intake of toxic meal; incidence of similar lesions was small in fattening cattle given 12% toxic meal.


In 24 samples of poisonous groundnuts aflatoxin was present at levels of 2000 to 300000 pg kg⁻¹ (mean 66000 pg kg⁻¹). Aspergillus flavus, A. awamori and 4 named species of *Penicillium* were isolated from the samples. Deaths of 10 pigs, 3 cattle, 2 goats and a sheep were investigated. In 8 samples of groundnut hay there was none to 16000 pg kg⁻¹ of aflatoxin (mean 4000 pg kg⁻¹). Only 3 samples had over 2000 pg kg⁻¹. It was thought unlikely that normal groundnut hay would poison animals.
Two samples of groundnut meal were collected from a dairy farm where milk production had fallen to 76% of normal, and many of the cows were in poor condition, with reduced appetites and scouring. Anatoxins were demonstrated at a level which could give about 50 ng kg⁻¹ (dry matter) in the complete diet. In 2 samples of groundnut meal the aflatoxin B₁ levels were 750 and 620, of B₂ 40 and 30, of G, 60 and 50, and of G. up to 10 ng kg⁻¹. Within 3-4 days of withdrawal of the meal, scouring ceased and appetite improved. Milk production rose again, though not to its predicted level.

Five cases of aflatoxicosis in pigs in Southern Queensland are described. One peracute case where aflatoxin concentration of up to 5000 ng aflatoxin B₁ kg⁻¹ was demonstrated in stomach contents was presumed to be caused by consumption of moldy bread. High levels of toxins were also present in the livers. Two cases of acute toxicity were caused by feeding moldy groundnut screenings containing 22000 Mg aflatoxin B₁ kg⁻¹. One case of subacute, and one of chronic toxicity were caused by sorghum grain based rations with lower aflatoxin levels (4640 and 255 ng kg⁻¹). Peracute toxicity caused collapse and deaths within a few hours, acute toxicity caused deaths within 12 h, and subacute toxicity caused deaths after 3 weeks on the toxic rations. Anorexia and ill thrift affecting only growing animals were seen with chronic toxicity. Extensive centrilobular liver necrosis and haemorrhage occurred with peracute toxicity, and in cases of acute poisoning there was hepatic centrilobular cellular infiltration, hepatocyte swelling and bile stasis. With subacute toxicity hepatocyte vacuolation together with bile stasis and bile ductule hyperplasia were seen.

Feeding trials were conducted with swine, beef cattle, dairy cattle and poultry to determine adverse effects, if any, of graded levels of anatoxins in rations. In addition, samples of meat, eggs and milk from these animals were analyzed chemically to determine if aflatoxin was transmitted into these products. In growing-fattening swine, no evidence of toxic effects was observed when the aflatoxin level fed was 233 (ig kg⁻¹ or less. In a swine reproduction experiment, no adverse effects were detected in pigs produced from sows fed 450 ug kg⁻¹ aflatoxin. No toxic effects were observed at levels of 300 ug kg⁻¹ or lower in cross-bred beef steers fed rations containing aflatoxin for 4.5 months. Using recognized chemical methods, no aflatoxin was detected in meat from swine and cattle fed rations containing 800 and 1000 mg kg⁻¹ of aflatoxin, respectively. In dairy cows, weekly intakes of 67 to 200 mg of aflatoxin B₁ per cow produced 70 to 154 ug kg⁻¹ aflatoxin M, in lyophilized milk. Rapid disappearance of aflatoxin M, in the milk took place after withdrawal of aflatoxin from the ration. No adverse effects were discernible in broilers fed from one day to eight weeks of age a ration containing 400 ug kg⁻¹ aflatoxin. Lyophilized meat from broilers fed 1600 ug kg⁻¹ aflatoxin for eight weeks contained no detectable aflatoxin. Striking differences in aflatoxin susceptibility were observed in 17 different breeds and strains of poultry and game birds fed from two to six weeks of age a ration containing 800 ug kg⁻¹ aflatoxin B₁. New Hampshire chicks and turkey poults were highly susceptible to aflatoxin in contrast to the resistance of Barred Rock and Australop chickens and guinea fowls. Hybrid chicks from a New Hampshire-White Leghorn cross were highly resistant to aflatoxin. Eggs and meal from White Leghorn hens fed a ration containing 2700 ug kg⁻¹ aflatoxin contained no detectable aflatoxin.


Aflatoxin toxicity problems in poultry are described. Information on outbreaks of aflatoxicosis in poultry in Hyderabad, India, are presented. Preventive measures for avoiding toxicity problems are suggested.


A group of 5 Kerry crossbred lambs 3 months old at the start were fed for 5 years on hay and a concentrate with 20 % highly toxic groundnut meal, containing 1750 (ig kg⁻¹ aflatoxin during the first 3-5 years, and 1000 (ig kg⁻¹ since then. Lambs born were added to the experimental group. A control group, for the first 3 years, received concentrate with fishmeal and maize gluten supplying protein. In the group given aflatoxin, fertility was lower and growth during the first 18 months was also retarded. One lamb was killed after 5 months and others died during the experiment. Damage to the liver was found in only one, and there were no typical signs of groundnut poisoning. In 2 sheep there were nasal chondromata; the rarity of this disorder and the possibility of its being related to the feed are discussed.


High mortality is reported in young pigs in a herd given Brazilian groundnut meal (17.5 % in the starter pellets and 8.75 % in the sow and weaner meal). Pigs of the
same herd transferred to another farm were not affected. Sows lost appetite and tended to subsist on grass. Liver lesions in acute, subacute and chronic types of poisoning are described. There were some similarities to chronic copper poisoning. There was no difference in toxicity between solvent-and expeller-processed meals. Experimental poisoning was also studied. The suspected diet was given to 2 pigs for 4 weeks and 5 others got a meal with 20 % Brazilian groundnut meal. All developed signs of poisoning, with typical acute or subacute liver damage. Pigs given another commercial meal or 20 % Nigerian groundnut meal were not poisoned.


Over 11 instances of calves dying after eating feeds containing groundnut meals are reported. The meals comprised 10 to 15 % of the feeds given from the age of 3 days onwards; it was estimated that from 40 to 85 lb of the meal was eaten in 6 to 16 weeks. Signs of poisoning developed from 6 weeks to 4 months after the start of feeding. The calves affected were 3 to 9 months old but mainly 4 to 6 months. All test animals which showed clinical signs died, usually within 2 days of severe signs appearing. Clinical signs are described. Typical lesions in the liver were found. On 2 farms concentrates with 15 to 20 % groundnut meal had been given to dairy cows for 3 to 4 months. There were loss of appetite and reduced milk yields, and 3 cows had liver lesions typical of groundnut poisoning. When the meal was withdrawn from the feed there was no further sign of poisoning. One outbreak is reported among store bullocks aged 18 to 22 months, which had received 2 lb of groundnut meal daily for 3 months. In all cases signs were indistinguishable from those of Senecio poisoning. The total dose of the toxic substance seems to be more important than the rate of consumption of the groundnut meal. Susceptibility to poisoning decreases with age.


Acute aflatoxicosis was believed to be the cause of death in 12 of 90 Hereford calves, fed groundnut hay during drought in Queensland. They developed jaundice, photosensitization, diarrhoea, anorexia, and depression. Increase in serum levels of enzymes of hepatic origin and bilirubin content were higher than normal. Haemorrhage, hepatocyte damage and bile duct proliferation were seen in groundnut hay-fed dead calves. The groundnut hay contained up to 2230 ug kg ’ aflatoxin. with most toxin concentrated in the nut-in-shell.


On a commerical rabbit farm with 7000 rabbits in the Kulu valley in Himachal Pradesh State of India, 4000 rabbits died, and mortality was not prevented by antibiotics. In 15 rabbits examined post-mortem, damage to livers similar to that seen in guineapigs poisoned by aflatoxin was noted. The rabbits were fed on green feed to appetite and a pelleted concentrate, with somewhat high moisture content and including 20 % groundnut meal, which was prepared in bulk and stored before use. Aflatoxin was detected in the feed.


After eating moldy groundnuts, 4 pigs and 2 goats died within 24 hours. Investigations showed that the moldy material contained aflatoxin in high concentration produced by the fungus Aspergillus flavus. In various experimental animals, except the rat, it caused acute liver necrosis and generalized haemorrhages.


A high incidence of hepatocellular carcinomas was induced in rats fed aflatoxin-contaminated groundnut meal grown and processed in the United States; the incidence of tumors paralleled the aflatoxin content. Carcinogenicity for rats was reduced progressively by extracting the groundnut meal with petroleum solvent (n-hexane), methanol and chloroform. Widespread bile duct hyperplasia and nodular regeneration of the liver was induced in ductulles fed the toxic groundnut meal for 30 days. Ducks held on experiment for 16 months developed hepatic cirrhosis and hepatomas which appeared nonmalignant histologically. Mice fed toxic groundnut meal for 16 months developed bizarre cell forms throughout the liver and a low incidence (15 %) of hepatomas which appeared to be nonmalignant. It is concluded that aflatoxins were the major contributing factor in liver tumors observed in rats fed groundnut meal in the United States.


Over 13 years, 740 samples of animal feeds were analyzed, about 500 of which were suspected of causing disease in farm animals. Aflatoxin B, was detected in only 13.6 % of the samples. Groundnut meal samples nearly always contained the toxin. Where mycotoxicosis was suspected in dairy cattle, 27 % of the samples proved positive, as compared with 9 % of other dairy feed samples.

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Only 10% of food samples examined during 1973-1975 contained mycotoxins, viz. aflatoxins B, and G, ochratoxin A, sterigmatocystin and zearalenone. Aflatoxin B, (and sometimes G,) was present up to 2000 μg kg⁻¹ in groundnut meal obtained from farms with production disease in cattle. Ill thrift occurred in calves, and milk production was lower. Ochratoxin A was occasionally found in stored barley and it has been suggested that it may cause foetal resorption in farm animals, but experimental studies in pregnant sows did not confirm this when they were fed ochratoxin A or B. Ochratoxin A accumulated in the body tissues of the dam. Zearalenone has been implicated in the aetiology of sprayleg piglets, but only 1 such case was seen among 63 piglets from 7 gilts fed naturally contaminated wheat. However, when such wheat was fed to laying hens, some newly-hatched chicks suffered leg weakness.


Groups of 10 White Leghorn chickens (4 weeks old) were fed for 1, 2, 4 and 6 weeks on diets with 30% toxic groundnut meal, to supply 3100 μg kg⁻¹ aflatoxin in the diet. There was no residue of aflatoxin or its fluorescent metabolites in liver, breast or leg meat of chickens killed at any age.


When an estimated 10-14 pregnant cows in a 68-cow herd were given moldy groundnuts as a supplementary feed, constituting a large proportion of their diet for 4 days, most of them aborted on or soon after the fifth day. The cows were recumbent and unable to rise; in one, there was a low rectal temperature (99 C) and a tremor of the head. All of the cows that aborted were in the third trimester of pregnancy, and all died within 8 days. Biochemical analyses performed on 3 cows revealed hepatic dysfunction, indicated by high values for lactate dehydrogenase, aspartate transaminase and total bilirubin; there was also evidence of mild dehydration; no fetuses were examined. The groundnuts contained 77 μg g⁻¹ of aflatoxin B, which was also found (5 ng g⁻¹) when a liver extract of one cow was analyzed for mycotoxins. Cows fed groundnut hay from the same field as the groundnuts were not affected; the nuts had been kept in the open at freezing temperatures. Abortion preceding fatal mycotoxicosis, as occurred in at least 8 of these cows, is an unusual feature of acute aflatoxicosis.


Four cases are reported of outbreaks of poisoning in ducklings given commercial feeds with 10 or 9% groundnut meal. By tests of fluorescence those meals were found to have 4000 and 2000 μg aflatoxin kg⁻¹, but in one outbreak deaths ceased when another meal without groundnut was given. This meal contained 5000 μg aflatoxin kg⁻¹. The ducklings which died in these outbreaks (more than half of flocks of 400 in 2 of the outbreaks) had lesions in the liver which justified the diagnosis of poisoning by groundnut.


Work on groundnut toxicity to various animals is reviewed. Procedures for isolation and detection of aflatoxins from toxic groundnut meal and fungal cultures are described.


A method for the fractionation of Brazilian groundnut meal is described. All the toxic material, 0.4% by weight of the original, was found in the fraction insoluble in methanol and water, extracted with chloroform and separated with petrol and water. A suspension in water was made so that 1 mL was equivalent in toxicity to 40 g meal. Ducklings were killed in under 24 h by 1 mL of that suspension and it was lethal at lower concentrations. The equivalent of 0.5 g meal caused liver damage. The corresponding fraction from Indian groundnut meal was not toxic. Turkey poults were less susceptible than were ducklings and the mortality and lesions were identical to those in field outbreaks of Turkey "X" disease. It was confirmed that the toxic substance is neither a pyrrolizidine alkaloid nor the N-oxide of such an alkaloid. It may be derived from a micro-organism. A similar toxic substance has since been found in samples of groundnuts from India, Uganda and Tanganyika, French West Africa, Nigeria, the Gambia and Ghana.


The toxic extract of a Brazilian groundnut meal was further purified and a fluorescent method of identification after chromatographic separation was devised. The toxic substance was isolated from a fungus, Aspergillus flavus. When the fungus...
was grown on sterilized groundnuts and fed to ducklings, it resulted in typical diver lesions in ducklings.


A suspected outbreak of groundnut poisoning affecting 24 Murrah buffaloes is described with details of the clinical course, post-mortem lesions and histology of the liver. Investigations eliminated poisonous plants, parasites, bacteria or viruses as agents of the disease. The concentrate mixture contained 20 % groundnut cake. This is the first record of groundnut toxicity in India although frequent cases of liver damage in Murrah buffaloes have been recorded in certain areas. Six buffaloes died in this outbreak.


For 75 days laying hens were given diets containing groundnut cake (GNC), aflatoxin-contaminated GNC (AGNC), or AGNC treated wih 2 % ammonia, 0.1 % gentian violet, 0.5 % propionic acid or defatted with hexanc without or with supplementary copper sulphate or vitamins. Egg production was decreased (p < 0.05) with AGNC. Copper sulphate did not improve egg production but vitamin supplementation increased (p < 0.05) egg production compared with AGNC alone. Treating AGNC with ammonia, gentian violet or propionic acid improved egg production. Defattting AGNC improved (p < 0.05) egg production. Feed intake was not affected by treatment. Average egg weight, feed intake kg⁻¹ egg weight, daily protein intake per hen, protein intake per kg egg weight and percent protein efficiency were affected (p < 0.05) by treatment. Weight (g 100⁻¹ body weight) of liver, heart and pancreas were affected (p < 0.05), but weight of spleen was unaffected by treatment. Liver DM was not affected, but protein content of liver decreased (p < 0.05) accompanied by the increased lipid content with AGNC.


A disease of chickens characterized by variable haemorrhages in several organs including liver, heart, Spleen, kidney, intestine, and other organs and degenerative changes of cells is reported. The blood clotting mechanism is probably defective.


A bile duct carcinoma in a Desi hen is reported. Histopathology of the lesions, particularly of those located in the liver, is described.


Observations on Turkey X disease are presented. Outbreaks of "disease" in turkey poult were associated with commercial ration. Mortality of turkey poult often ceased after a change of feed.


Preliminary observations on Turkey X disease are presented. The authors encountered 45 outbreaks of "disease" in turkey poult associated with high mortality. Birds died in good condition after a short illness and mortality rates ranged from 10 to 70 %. Affected poult were usually about 4 weeks old, but birds 12 to 15 weeks old were sometimes involved. The consistent post-mortem findings were engorgement and congestion of the kidneys. Other lesions often present included enteritis, distention of the gizzard by coarse material, haemorrhages or necrotic foci in the liver and, less commonly, haemorrhages on the pancreas, white flecks on the air sacs and generalized oedema. More than one commercial ration was involved, but mortality often ceased after a change of feed.


Inappetance, apathy and neurological signs were seen in a flock of sheep near Khartoum, Sudan, fed on groundnut oilmeal contaminated with aflatoxins (750 µg kg⁻¹). The gross and microscopic lesions were confined to the liver. The biochemical analysis of the serum was consistent with the presence of liver damage. The presence of aflatoxins in the feeds and tissues of dead sheep supports the view that the condition was due to aflatoxin poisoning.


Observations on Turkey X disease are presented. The most striking post-mortem lesions were generalised oedema with large quantities of fluid in the peritoneal cavity, and in most of the birds, around the coronary of band of the heart. Extensive swelling of the kidneys, the surfaces of which were covered with petechial
haemorrhages was very evident. Enteritis of various parts of the alimentary canal was also a prominent feature.


Two calves less than 3 months old at the start of the experiment were on a diet with 20% non-toxic Indian groundnut meal, and 4 got 20% toxic Brazilian groundnut meal without or with 150,000 IU vitamin A intramuscularly on days 1, 28, 56, 84, 112 and 134. Blood samples were examined for activity of coagulation factors with particular attention to the prothrombin complex. The first blood sample was taken 14 days before the diets were given and others were taken at weekly intervals. The calves given toxic meal without vitamin A were killed, with extreme signs of poisoning, after 16 and 26 weeks, respectively, and one calf given vitamin A was killed with extreme signs at 12 weeks. The other calves survived without sign of disorder for 61 weeks. Clotting time, one-and two-stage prothrombin time tests and prothrombin consumption index indicated impairment of the activity of prothrombin, factors VII and X, and possibly IX. There was some evidence that vitamin A reduced these effects.


A chrome disease characterized by loss of condition, anorexia, apathy and intermittent diarrhoea with tenesmus in a herd of dairy cattle in India was attributed to prolonged feeding with a diet containing groundnut cake in which aflatoxins B2, G1 and G2 were detected. Some animals became comatose and died, lactating animals having the highest mortality. Clinical and post-mortem examination revealed liver damage and ulceration of the abomosum.


Cases of Turkey "X" disease have been seen in chickens, ducks and turkeys given compound feeds containing no groundnut meal.


Occurrence of aflatoxicosis in poultry in India is reported. Guidelines for usage of contaminated feeds and for avoiding the problem are given.


Three consignments (CI-3) of groundnut cake (10 samples in all) were screened for aflatoxin using TLC and fed as 30% of the diet to Murrah buffaloes. Three out of the 10 samples were found to contain 1-3 mg kg⁻¹ aflatoxin B₁. The remainder contained only traces of aflatoxin. Fifty milk samples from these buffaloes were analysed for aflatoxin M₁ (metabolite of B₁): 10 samples came from CI, and 20 each from C2 and C3. Four of CI showed traces of toxin. C2 and 14 C3 were heavily contaminated, with concentration, ranging from traces to 4.8 ug aflatoxin M₁, L¹ milk.


Aflatoxicosis in poultry farming, extraction of aflatoxins from groundnuts, bioconversion of aflatoxins. symptoms and lesions of toxicosis, and prevention and treatment, is discussed.


The influence of varying amounts of aflatoxin B₁ in the groundnut feed (0, 4, 98, 491 ug kg⁻¹) on health and performance of pigs was studied. Only the highest allatoxin concentration exerted a clear negative influence on weight gain, on the values of the aminopyrine respiratory test, and on some clinico-chemical indices. There were remarkable individual differences with that concentration. It was sometimes possible to find aflatoxin residues in muscles and organs.

1.2.3 Aflatoxicosis in Man


Forty-six patients with primary hepatoma were studied in Mulago Hospital, Uganda, over a nine month period. This represented 2.1% of all medical admissions. The age peak was 25 to 45 years and the male to female ratio was 2:1. The predominant clinical picture was abdominal pain and hepatomegaly with a hard, frequently tender mass in the right upper quadrant and rapid clinical deterioration and death within several months. Jaundice, fever, hepatic coma and other signs of active hepatocellular disease were infrequent in contrast to observations in the United States and Europe. An unexplained hypercholesterolemia was noted in
approximately one third of the patients. Bloody ascites occurred in 19% of the patients and was a helpful diagnostic sign. Acute hemoperitoneum occurred in 15% of cases. The clinical diagnosis of hepatoma, prior to histologic confirmation, was correct in 86% of the patients. Although hepatomas from different areas appear to be histologically similar, differences in the clinical and epidemiological features suggest that hepatoma in Uganda differs from the disease described from the United States, possibly due to different causative factors.


Aflatoxins were estimated in 480 food samples stored for consumption between harvests and collected from different parts of Uganda in 1966-67. Among these samples, 29.6% contained detectable amounts of aflatoxins and 3.7% contained more than 1 ug kg$^{-1}$. The frequency of aflatoxin contamination was particularly high in provinces with a high incidence of hepatoma, or where cultural and economic factors favored the ingestion of moldy foods.


Aflatoxin B$_1$ was detected in the urine of 7% of 255 cases of childhood cirrhosis during 1964-1969 and in 25% of breast milk samples from their mothers. The excretion pattern coincided with that of primates injected with C$^{14}$ labelled aflatoxin. Of 25 samples of parboiled rice collected from the homes of patients with cirrhosis, 2 showed both fungal growth and an aflatoxin B$_1$ fluorescent spot on chromatographic examination, and all 25 samples of unrefined groundnut oil showed a similar fluorescent spot. Aflatoxin administered to rats caused muscle fragmentation changes, fibrosis and fatty infiltration characteristic of 56 cases of childhood cirrhosis. Growth retardation was noticed both in 24 cirrhotic children and in the litters of lactating rats fed aflatoxin.


The results of the detection of aflatoxin and its metabolites in the urine and breast milk feeds of both cirrhotic and normal children are presented, and the implication thereof discussed. The possible role of aflatoxin in the aetiology of Indian childhood cirrhosis is further evaluated.


Ten cases of Indian childhood cirrhosis were studied for changes in the striated muscle. A comparative study of clinical factors, liver and muscle biopsy revealed histopathologic changes in the striated muscles which showed loss of muscle sanitation, fatty infiltration and fibrosis. These changes have a direct correlation with the severity of the disease clinically and the histopathologic changes in the liver.


Children suffering from varying degrees of protein-calorie malnutrition were accidentally fed with aflatoxin (300 ug kg$^{-1}$) - contaminated groundnut protein flour for periods of 10 days to 4 weeks. Liver biopsies showed a gradual transition from an increase in central and periportal fat to fibrosis and cirrhosis, which does not usually occur in treated Kwashiorkor. The lesions were identical to those of Indian childhood cirrhosis.


Alarm was expressed at a recent meeting in Rome of the Food and Agriculture Organization of the United Nations about the amount of aflatoxin in groundnuts and other protein supplements. The maximum concentration of aflatoxin which is permitted was laid down in August 1965 by a joint advisory group from the Food and Agriculture Organization and the World Health Organization which took into
account the urgent need to provide extra protein in some parts of the world, and established a level of 30 µg kg⁻¹ of foodstuff. Clearly the group would have preferred a lower figure, but they concluded that the danger of malnutrition was greater than the danger that aflatoxin would produce liver cancer in man.


The possible relationship between aflatoxins and kwashiorkor is reviewed. It is concluded that aflatoxins are not primarily responsible for kwashiorkor, but malnourished children may show increased susceptibility to the toxic effects of aflatoxins.


Data on quality of marketed food in Thailand are reviewed. Groundnut and groundnut products are most frequently contaminated with aflatoxins. A study of chronological and geographical data since 1967 has revealed close correlation between liver cancer incidence and the amount of aflatoxin consumed.


Aflatoxin B, (62 to 4409 pg g⁻¹) was detected in the livers of 20 children in Ghana who had died from kwashiorkor. Aflatoxicol (12-99 pg g⁻¹) was detected in livers of 2 children.


Two major etiological agents, hepatitis B virus and aflatoxin B₁, are considered to be involved in the induction of liver cancer in Africa. A study was conducted in various parts of Kenya with different liver cancer incidence in order to establish the rate of exposure to aflatoxin and the prevalence of hepatitis infections. Of all tested individuals, 12.6 % were positive for aflatoxin exposure as indicated by the urinary excretion of aflatoxin B,guanine. Assuming no annual and seasonal variation, a regional variation in the exposure was observed. The highest rate of aflatoxin exposure was found in Western Highlands and Central Province. The incidence of hepatitis infection nationwide as measured by the presence of the surface antigen was 10.6 %, but a wide regional variation was observed. A multiplicative and additive regression analysis to investigate if hepatitis and aflatoxin exposure had a synergetic effect in the induction of liver cancer was negative. However, a moderate degree of correlation between the exposure to aflatoxin and liver cancer was observed when the study was limited to certain ethnic groups. The study gives additional support to the hypothesis that aflatoxin is a human liver carcinogen.


In Mozambique, there is a high correlation between the incidence of primary liver cancer and the consumption of aflatoxin-contaminated food. Some work has been done to assess and minimize the aflatoxin problem. Institutions such as the Instituto Nacional de Investigacion Veterinaria (INIV) and the Laboratorio Nacional para la Higiene de Agua y Alimentos (LNHAA) are involved in the analysis of food products, for both animals and humans. In 1981, 17 food products, comprising a total of 313 samples were tested and 16 samples were contaminated with aflatoxin B₁, 10 with B₂, 4 with G₁, and 3 with G₂. 87-100 % of the groundnut, beer, rice, and maize samples tested were contaminated. The aflatoxin levels in the groundnut samples ranged from 3 to 5500 µg kg⁻¹, aflatoxin B₁ being the main contaminant. An analysis program is investigating the possible correlation between consumption of contaminated food and the possible presence of aflatoxin M₁ in human breast milk. The possibility of further work involving the INIV, LNHAA, and the Faculdade de Agronomia, Universidade Eduardino Mondlane, Groundnut Improvement Project is being studied to include an agronomic component and formulate practical recommendations for small farmers and traders.


In the USA aflatoxin contamination of certain agricultural crops such as groundnut, corn and cottonseed continues to pose a problem, especially in the south-eastern states where climatic conditions are conducive to growth of aflatoxin-producing fungi. Analysis of airborne dust samples generated from contaminated corn in an agricultural setting has revealed the presence of respirable particles containing aflatoxins. Since aflatoxins are among the most potent of carcinogens, exposure by any route poses a potential hazard. Unfortunately, an adequate assessment of the health hazard resulting from inhalation exposure to aflatoxin- contaminated grain dust is currently impossible due to the almost total lack of data regarding this route
of exposure. However, a prospective analysis of the potential risk has been performed using information concerning (1) the absorption and carcinogenic effects of another potent carcinogen, benzo[a]pyrene, when absorbed onto respirable particles, (2) the metabolic capability of cultured human bronchus towards aflatoxins, (3) the irritant nature of respired organic dusts and (4) potential modifying factors known to influence carcinogenic outcome. Suggestions are given regarding the types of information that are needed in order to adequately assess the risk associated with inhalation of aflatoxin-contaminated grain dust.


The epidemiologic evidence suggesting that hepatitis B virus (HBV) is an etiologic agent for primary hepatocellular carcinoma (PHC) is very strong. It consists of: (i) a strong geographic correlation between HBsAg prevalence and PHC incidence; (ii) multiple case-control studies showing excess HBsAg positivity in PHC patients; (iii) excess HBsAg positivity among mothers compared with fathers of PHC patients, and (iv) a relative risk of 390 among HBsAg carriers in a large general population prospective study in Taiwan. The risk of HBV resulting in the HBsAg carrier state is inversely related to age. Thus, most PHC cases in Taiwan arise from HBV infections which occurred in infancy and childhood.


A prospective general population study of 22707 Chinese men in Taiwan showed that the incidence of primary hepatocellular carcinoma (PHC) among carriers of hepatitis B surface antigen (HBsAg) was much higher than among non-carriers (1158/100 000 vs 5/100 000 during 75000 man-years of follow-up). The relative risk was 223. PHC and cirrhosis accounted for 54.3 % of the 105 deaths among HBsAg carriers but accounted for only 1.5 % of the 202 deaths among non-carriers. These findings support the hypothesis that hepatitis B virus has a primary role in the aetiology of PHC.


The possible involvement of aflatoxins in the syndrome of encephalopathy and fatty degeneration of the viscera was investigated. Liver tissues from two children with Reye's syndrome were analysed for aflatoxins. All liver extracts examined contained aflatoxin B,. These results suggest a further search for aflatoxin-like compounds in the syndrome of encephalopathy and fatty degeneration of the viscera.


Of 78 groundnut samples analyzed in Ivory Coast, 22 contained > 20 ug kg⁻¹ aflatoxin B, and 27 contained > 200 ug kg⁻¹ aflatoxin B, B₂, G, or G₂. At post-mortem, aflatoxins were detected in liver samples from a patient with liver cancer: B, 11.8 ug kg⁻¹; B₂, 1.9 ug kg⁻¹; G₂ 0.1 ug kg⁻¹.


Acute and chronic effects of aflatoxins in man are well documented. The reported outbreaks of aflatoxicosis in man were due to the consumption of staple foods such as maize and not to the consumption of groundnut. Circumstantial evidence has implicated groundnut meal containing aflatoxin as causing Indian childhood cirrhosis. Dietary intake of aflatoxin through groundnut has been implicated in the development of liver cancer in certain parts of the developing world. The incidence of liver cancer associated with the ingestion of aflatoxin from groundnuts is low in developed countries such as the USA. Food consumption surveys in India have indicated that the consumption of nuts (mostly groundnuts) varies from 2 to 35 g per consumption unit per person per day depending on the region and season. Studies carried out in Thailand, the Philippines, and the USA have indicated that the dietary intake of aflatoxins from groundnuts is lower than that from maize. The aflatoxin regulatory actions taken by the European Economic Community (EEC), Japan, and other developed countries on importing groundnuts and groundnut products have resulted in safeguarding exports rather than minimizing health hazards in the developing countries.


This review discusses the major mycotoxins found in food and feeds, approaches towards prevention and control of mycotoxins, and mycotoxicoses in animals and humans in India. Regulations for mycotoxins are also described.

122. Brudzynski, A., Pee, W.van, and Kornaszewski, W. 1977. The occurrence of aflatoxin B, in peanuts, corn and dried cassava sold at the local market in

Of 12 maize samples analyzed, 1 had aflatoxin B$_1$ at over 200 ug kg$^{-1}$, and 33 % of dried cassava samples were contaminated with aflatoxin. Select and low quality groundnuts had 80 and 39 % of samples not contaminated. Of the low quality samples 33 % contained more than 250 ug kg$^{-1}$. Results are related to local incidence of hepatoma and to the high humidity of the climate.


The dietary intakes of 90 confirmed primary liver cancer patients were confirmed against those of 90 age-sex matched controls. The frequency and amounts of food items consumed were calculated into units of aflatoxin load per day using a Philippine table of aflatoxin values of these items. Of the total subjects' aflatoxin load, 51.2 % came from cassava, 20.3 % from maize, 6.8 % from groundnuts and 5.8 % from sweet potato. The mean aflatoxin load per day of patients was found to be 44 % that of the controls. The relative risk of developing primary liver cancer was statistically significant in the following order of rank : cassava, groundnuts, sweet potato, maize, and alcohol. The study demonstrates a strong positive association between the ingestion of increasing levels of aflatoxin and the rising risk of developing primary liver cancer. This effect is synergistically aggravated by alcohol consumption.


For many Filipinos maize forms a large part of the daily diet although regional intake varies widely. The incidence of primary liver carcinoma (PLC) were highly positive. PLC occurred more frequently among younger groups which suggested that a more intensive and frequent toxic insult reduced induction time. Malnutrition was thought to be a contributing factor in PLC but in this study there was no linear correlation between the intake of protein and energy and the occurrence of PLC. Regional intakes of rice, beans, cassava, groundnuts and maize were estimated; intakes of beans and cassava were not correlated with regional PLC while rice and groundnuts had an inverse correlation. The regional maize intake was the only food which was positively correlated with regional PLC. Regional intake was related to climate, practices in harvesting, storage and eating habits. In 1973, when there was a shortage of rice, more maize was eaten and there was an increase in PLC mortality in all regions. Residents of the Bocol region had a high intake of coconut oil and a relatively low PLC incidence despite a moderate intake of highly contaminated maize, suggesting that some component in coconut oil altered the metabolism of aflatoxin in the liver to make it less carcinogenic.


Analyses of 500 samples of food products during 1967-1969 showed that manufactured peanut butter samples were highly contaminated with aflatoxin (mean 500 ug kg$^{-1}$). The authors then examined urine samples from subjects known to have consumed peanut butter. Urine samples were collected before and during consumption of peanut butter; collections were made for 0 to 24 h before and up to 72 h after consumption began. The minimum daily consumption of aflatoxin B$_1$ required to produce detectable levels of M, was 15 ug day$^{-1}$. Of 35 analytical 24 h periods (after consumption began) for the 14 subjects consuming at least the daily minimum, thirty periods were positive for M, and five were equivocal positive because of uncertain interpretations of streaked chromatographic developments. Milk samples collected from 11 mothers known to have consumed peanut butter (3 consumed 4.2 - 8.4 ug day$^{-1}$ and 8 consumed unknown amounts of aflatoxin) were negative for either B, or M,. However, 3 samples of milk from these mothers contained aflatoxin M,. Three of eight urine samples from children who had consumed 11.2 - 15.0 ug peanut butter per day contained aflatoxin. It is suggested that determination of aflatoxin M, in human urine provides a simple method of evaluating aflatoxin ingestion.


Data are presented on the relationship of aflatoxin contamination of food samples (particularly groundnuts and peanut butter) and its ingestion by man. The consumption of peanut butter and maize is possibly linked to high rates of liver cancer in some regions of the Philippines. The presence of aflatoxin M, and the presence of aflatoxin B, in the urine, together with the absence of either B, or M, in faeces in known cases of B, ingestion, indicated considerable metabolism of the ingested B,. These results indicated extensive metabolism by humans and a lower order of toxicity of aflatoxin for man.


The veterinary problem, Turkey X disease, led to the discovery of aflatoxins and to
studies which demonstrated that toxins in the absence of visible molds could produce effects (carcinogenesis and orange damage) far removed in time from the cause. Only a few cases of acute mycotoxicoses in humans have been recorded. To shed some light on the risk to man from ingestion of aflatoxins in his food supply, published data on comparative metabolism, primate studies, inadvertent human incidents, and epidemiology are analyzed and presented in common terms to facilitate intercomparison. Although the data raise some questions, they presently provide the best available basis for estimating risk. The greatest attention has been given to aflatoxins, but other mycotoxins potentially capable of causing damage on chronic ingestion have also been considered. Among them are patulin, penicillic acid, trichotheccenes, luteoskyrin, and citrinin.


When cancer is caused in laboratory animals by a socially-valuable chemical or an unavoidable environmental contaminant, natural or otherwise, the risks to man from exposure to the chemical at very much lower doses must be estimated. The choice of a mathematical model is an important step in this assessment. Several available models are evaluated with respect to aflatoxin, one of the few agents with usable results both from experiments with animals and from surveys in man.


In a region of southern Georgia known for poor school performance, the mothers of the mentally retarded children had diets that differed from the average in terms of foods, but did not differ in terms of critical nutrients. The intake of large amounts of maize, rice, groundnuts and milk (foods potentially high in aflatoxins) were significantly related to mental retardation of children in one county where there were large amounts of aflatoxins in the food supply. No such relation was found in a county with trivial amounts of aflatoxins in the diet.


No association between primary liver cancer mortality rates among Chinese populations and aflatoxin exposure was found. The authors concluded that there was a strong association of primary liver cancer mortality with hepatitis B virus infection.


Aflatoxin contamination of foods was greater in areas of low altitude. Food products most frequently and heavily contaminated included groundnuts (maximum aflatoxin 425 ug kg\(^{-1}\)), cassava (325 ug kg\(^{-1}\)) and maize (148 ug kg\(^{-1}\)). The highest incidence of hepatoma in Burundi coincides with the areas of highest aflatoxin contamination.


Aflatoxin analysis of blood and urine in 584 Sudanese children is reported. The results in 404 malnourished children comprising 34 kwashiorkor, 111 marasmic kwashiorkor and 152 with marasmus were compared with 180 age-matched controls and correlated with clinical findings. Aflatoxin detection rate and mean cocentration were higher in sera of children with kwashiorkor than the other groups. The difference between the detection rate in kwashiorkor and controls was significant (p < 0.05). The aflatoxin detection rate in urine was highest in the marasmic kwashiorkor group and the mean concentration was higher in the marasmic kwashiorkor and marasmic groups than in the kwashiorkor and control groups. There were important differences in the detection of certain aflatoxins between the groups. Aflatoxin was detected in the sera of 16 (11.6 %) kwashiorkor, in 6 (6.1 %) marasmic kwashiorkor, but in none of the controls and only one in marasmus. These differences were highly significant ( p < 0.0001). The ratio of aflatoxin B, to M, was higher in the sera and urines of kwashiorkors than in controls, suggesting that the normal transformation of aflatoxin B, to M, may be impaired in kwashiorkor with consequent increase in transformation of aflatoxin B, to aflatoxicol. It is concluded that the study presents evidence of differences in the metabolism of aflatoxins in children with kwashiorkor compared with children with other kind of malnutrition and normally nourished children and confirms the association between aflatoxins and kwashiorkor.


Aflatoxins were detected in breast milk samples from mothers in the Sudan.

134. Coulter, J.B.S., Suliman, G.I., Lamplugh, S.M., Mukhtar, B.I., and
Aflatoxin analysis of 40 percutaneous needle liver biopsies of 27 children with protein-energy malnutrition and 13 children with miscellaneous liver disease was performed. Aflatoxins B₁ B₂ and aflatoxicol were detected in 5 of the 16 biopsies from kwashiorkor but in none of 11 biopsies from marasmus or marasmic kwashiorkor. Aflatoxins G₁, G₂ and M₂ were detected in 5 of 12 children with chronic liver disease. A very high concentration of aflatoxicol was found in a breast-fed infant with neonatal hepatitis of unknown aetiology.

Three cases of pulmonary interstitial fibrosis, two in agricultural workers and one in a textile worker, are reported. In lung samples of all three patients the presence of aflatoxin B₁ was demonstrated by radioimmunoassay. A possible occupational risk of aflatoxin exposure via the respiratory tract is suggested.

This paper is a report upon two cases of lung cancer in which evidence for presence of aflatoxin in the cancerous tissue is given.

During five years the authors observed 27 children (aged from 3 days to 8 years) who died with symptoms of the encephalitic syndrome with fatty degeneration of the viscera (Reye). According to the morphological changes in the liver and to the clinical course they divided the cases into three groups. In the first group there were 20 children who died within 2-10 days after the first symptoms of the disease appeared. In their livers, diffuse fatty degeneration was found. In the second group there were 3 children who died within 1-2 months after the acute onset of the disease. In their livers, fibrosis with bile duct proliferation and steatosis were found. In the third group, there were 4 children who died within 2-4 months after the first symptoms. Their livers showed cirrhosis. Aflatoxin B₁ was found in the liver specimens of the children in all 3 groups. The source of intoxication in 5 cases was aflatoxin-contaminated milk food. The authors suggest that aflatoxin represents an important factor in the aetiology of this syndrome and a high risk for human health.

Three cases of pulmonary interstitial fibrosis, two in agricultural workers and one in a textile worker, are reported. In lung samples of all three patients the presence of aflatoxin B₁ was demonstrated by radioimmunoassay. A possible occupational risk of aflatoxin exposure via the respiratory tract is suggested.

This paper is a report upon two cases of lung cancer in which evidence for presence of aflatoxin in the cancerous tissue is given.

Total aflatoxin content, measured spectrophotometrically, of crops and spices grown locally was 1600 + 500 ug kg\(^{-1}\) for manihot flour, 400 + 100 for yam flour, 700 + 150 for red pepper, 1400 + 400 for millet, 400 + 100 for soybeans. 1120 + 500 for maize. 500 + 150 for black eye beans. 40 ± to for rice and 1700 + 400 for groundnuts. Results are discussed in relation to the relatively high incidence of cancer in Tropical Africa.


Impaired activity of the liver microsomal mixed-function oxidase (MFO) system is characteristic of protein malnutrition. It explains the accumulation of aflatoxin B, in livers of kwashiorkor victims, whose staple foods are usually contaminated with the toxin. Dietary rehabilitation of such children with high-protein foods not only increases the activity of the liver MFO system but also stimulates DNA replication.


The livers of the pyroxine-deprived baboons showed striking changes including the presence of intracellular fat globules, interstitial fibrosis and marked variations in size and number of liver cell nuclei. The similarity of these changes to those seen in the livers of rats given aflatoxin and in non-malignant tissue from the livers of Africans with primary liver cancer carcinoma is pointed out. and their possible significance in relation to the pathogenesis of cirrhosis and primary liver carcinoma in Africans is discussed.


A study in the Gambia was performed to investigate two important risk factors for hepatocellular carcinoma, hepatitis B virus (HBV) and aflatoxin (AFB). Dietary intake of aflatoxin B, in 20 individuals was determined during a consecutive 8 day period and excretion of AFB-N7-guanine was measured on days 4-8. In addition, on days 1 and 8, blood samples were collected to measure aflatoxin in serum albumin adducts. The individuals chosen were both chronic HBV carriers and non-carriers. In food samples, the primary source of aflatoxin contamination was groundnut sauce with levels of 2-333 ug kg\(^{-1}\). Albumin adducts were determined by two different immunoassays, an ELISA and an RIA, these values ranged from 1.9-399 fmol mg\(^{-1}\) protein and 400-1922 fmol mg\(^{-1}\) protein, respectively. It is suggested that the disparity may be due to different specificities of the antibodies used.


Mortality occurring between 1963 and 1980 in a small cohort (N=71) of Dutch oilpress workers exposed between 1961 and 1969 to aflatoxins primarily via the respiratory route was assessed and compared to that of a similar group of unexposed workers (N=67). For the entire period of study, the observed mortalities of total-cancer and respiratory cancer were higher than expected in the aflatoxin-exposed group. Mortality observed in the comparison group was within the expected range. While two deaths in the exposed group were attributed to non-malignant liver disease, no primary liver tumors were observed. The greatest difference between observed and expected mortality was in the period between 1963 and 1968.


The possible relationship between aflatoxins and kwashiorkor is reviewed. Results of recent work are summarized. Aflatoxins were detected frequently in a variety of commonly eaten foods obtained from local markets in the Sudan. Sixty-eight of 85 samples of raw foods obtained from homes contained aflatoxins. Twenty-six of 57 cooked meals from homes had aflatoxins. Analysis of breast milk samples from 99 Sudanese women showed variable amounts of aflatoxins M\(_1\) and M\(_2\) in about one third of the samples. In a survey of 469 sera and 468 urine samples from children in the Sudan, aflatoxins were detected more often and at higher concentration in sera from children with kwashiorkor than in other malnourished and other control groups. Aflatoxicol was detected in kwashiorkor (12 %) and marasmic-kwashiorkor (6 %) but not in controls and only once in marasmus. Aflatoxins and aflatoxicol were detected in urine of children in all groups. Aflatoxin B, or aflatoxicol was present in all livers from cases of kwashiorkor in a survey of autopsy liver specimens from children in South Africa, Nigeria and Liberia. Aflatoxicol or aflatoxin M, was found in 5 of 6 cases of marasmic kwashiorkor and no aflatoxin was detected in 3 cases of marasmus.

The relationship between aflatoxin contamination of foods and kwashiorkor is discussed in this review of the disease.


Blood and urine samples from 252 children were investigated for their aflatoxin content by high-performance liquid chromatography (HPLC). Aflatoxins were detected more often and at higher concentrations in sera from children with kwashiokor than in other malnourished and control groups. Aflatoxicol was detected in the sera of children with kwashiokor and marasmic kwashiokor but not in the controls and only once in a marasmic child. These differences were significant. Urinary aflatoxin was most often detected in children with kwashiokor but the mean concentration was lower than in the other groups. Aflatoxicol was not detected in urine in any group. It is suggested either that children with kwashiokor have greater exposure to aflatoxins or that their ability to transport or excrete aflatoxins is impaired by the metabolic derangements associated with kwashiokor. Aflatoxins were detected by HPLC in groundnuts (B, 59666 pg g⁻¹, B2 370, G2 23), limed groundnuts (B, 3517, G, 2816. G2 6), chickpeas (B, 876 pg g⁻¹), dried okra (G2 12675 pg g⁻¹) and peanut butter (B, 26300, B2 9720, G, 84500 pg g⁻¹), obtained from local markets.


This report reviews aflatoxin contamination problem in groundnuts and groundnut products in Indonesia. Possible relationship between aflatoxin ingestion and hepatocellular carcinoma in humans is also discussed.


Aflatoxin contents of some Indonesian foods are reviewed. Allatoxin contamination problems in groundnuts and groundnut products are discussed. Possible relationship between dietary aflatoxin and hepatocellular carcinoma in humans is also discussed.


An attempt was made to correlate the presence of aflatoxin in groundnuts with the prevalence of primary liver cancer in Swaziland. Primary liver cancer, which is the commonest malignancy in Swazi males, has an unexpected geographical distribution, mainly in the middleveld and lowveld. The Swazis living in the Southern half of the highveld produced fewer primary liver cancers than the equivalent areas in the northern half. The immigrant Shangaans living in Swaziland have a greater tendency to produce primary liver cancer than have Swazis living in the same environments. Infestation of groundnuts by A. flavus is present in all parts of Swaziland but is proportionately more prevalent in the middleveld and lowveld. These areas have higher average temperatures and lower rainfall averages than the highveld. Aflatoxin was found in 40% of the samples tested, and most of the positive samples came from the middleveld and lowveld. Analysis of the eating habits with regard to groundnuts and of methods of harvesting and storage has shown that in the areas with a higher relative incidence of primary liver cancer the opportunities of ingesting aflatoxin are greater. The circumstantial evidence presented suggests that aflatoxin is probably one of the carcinogenic factors in the causation of primary liver cancer in Swaziland, though this cannot explain the global pattern of primary liver cancer.


Hepatoma seen in 22 men and 3 women at a hospital in the West Nile district of Uganda during 15 years is described. The locations of the patients' villages were plotted on a detailed map, accompanied by maps showing the distribution of groundnut cultivation, population and tribal distribution, and the village distribution of patients with other tumors seen during the same period. The difference in distribution of hepatoma patients compared with all other tumor patients is significant. A statistically significant similarity exists between the distribution of hepatoma patients compared with all other tumor patients is significant. A statistically significant similarity exists between the distribution of groundnut cultivation and the location of the occurrence of hepatoma, but the exact meaning is obscured by the small number of cases and the population distribution of the district. There is also a lack of fungal cultures.


Parts of Western India have experienced outbreaks of hepatitis affecting man and dogs and characterized by jaundice, rapidly developing ascites, portal hypertension, and a high mortality-rate. The disease was associated with the consumption of maize contaminated heavily with Aspergillus flavus. Analysis of contaminated samples showed that affected people might have consumed between 2000 and 6000 ug kg⁻¹ aflatoxin daily over a period of one month. A specimen of liver obtained at
necropsy showed bile duct proliferation and giant cells. The disease appears to be a result of aflatoxicosis.


An outbreak of a disease characterized by jaundice, rapidly developing ascites and portal hypertension, sometimes ending fatally, occurred in over 200 villages of Banswada and Panchamahals districts of Rajasthan and Gujarat. From the epidemiological characters it appeared to be due to a food toxin. Man and dog were afflicted simultaneously. Chemical and biological tests confirmed the presence of high levels of aflatoxin in maize locally grown and consumed by the afflicted tribal population. An estimated intake of about 2000-6000 μg kg⁻¹ of the toxin daily for several weeks appeared to have caused the outbreak. Bile duct proliferation, one of the characteristic features of aflatoxin liver injury was found in the autopsy of liver studied. Unseasonal rains and faulty method of storage of maize seem to have been responsible for the outbreak.


The outbreak of aflatoxicosis in rural populations of many villages in Western States (Rajasthan and Gujarat) of India is reported. The disease was characterized by jaundice, rapidly developing ascites, oedema of the lower limbs, and in a few cases by the development of signs of portal hypertension associated with a high rate of mortality. The disease occurred only among maize eating populations. The disease was ascribed to the consumption of aflatoxin-contaminated maize. This provides direct evidence for incriminating aflatoxin in the causation of acute liver disease with a high mortality rate.


Twenty-four hepatomas were encountered in 6356 autopsies over a period of 17 years. The findings are compared with those cited in the Indian and international literature. Twenty-two (91.83 %) of these hepatomas were associated with cirrhosis. It appeared that more carcinomas of the liver were being encountered in the autopsy room than before.


Between 1974 and 1977 the average level of aflatoxins in peanut butter was 4 ng g⁻¹. The average intake of these foods was 0.4 g kg⁻¹ body weight for children up to 10 years and declined progressively thereafter to < 0.1 g kg⁻¹ bodyweight.


The authors consider possible linkages between aflatoxin ingestion and the occurrence of kwashiorkor in children. Aflatoxins have been identified in livers of children suffering from kwashiorkor. Although suggestive, the data are by no means conclusive.


Aflatoxins were detected in 90 (34 %) of 264 breast milk samples collected from Accra, Ghana. Aflatoxins were also detected in 63 (34%) of the 188 Ghanaian cord blood samples. Blood samples from Nigeria showed aflatoxins in 16 (21 %) of 77 maternal samples and 9(12 %) in 78 cord blood samples (including a set of twins). These findings confirm that newborn infants in Africa are frequently exposed to aflatoxins in breast milk.


Crude aflatoxin mixtures as well as crystallized aflatoxin B, suppressed mitotic division in heteroploid and diploid human embryonic lung cells. This inhibition occurred 4 h after exposure of the cells to the toxin and reached a maximum in 8-12 h. A concentration of 0.01 μg of the toxin could be detected by this method. A sample of 2 μg of TLC pure aflatoxin B, derived from contaminated groundnuts was submitted for assay. A concentration of 0.03 μg produced 51 % reduction in mitosis.


The effect of aflatoxin on cell growth, cell morphology, and the synthesis of DNA was investigated. A heteroploid human embryonic lung cell line, L-132, was used. The cells were cultured in monolayer, using basal medium (Eagle) with Earle's

A total of 36 samples of foods collected during August 1985, December 1985 and March 1986 and served to mentally retarded clients with a high incidence of hepatocellular carcinoma, were analyzed for aflatoxin. Aflatoxin was not detected (< 5 \mu g kg^{-1}) by thin-layer chromatography in 35 food samples containing groundnuts, maize, wheat or milk. One peanut butter sample contained 20 \mu g kg^{-1} aflatoxin. Aflatoxin content of these foods was at or below the level permitted by the Food and Drug Administration. It is concluded that aflatoxin is probably not responsible for liver disease in this population.


Between March and early June 1981, 20 patients with hepatitis, 12 of whom died, were admitted to three hospitals in the Machakos district of Kenya. Two families, from which 8 of 12 sick members died, were eating maize which contained as much as 12000 \mu g kg^{-1} aflatoxin B1. Liver tissue at necropsy contained up to 89 (ig kg^{-1}) aflatoxin. Probably most or all of the hepatitis cases were caused by acute aflatoxin poisoning.


Chronic aflatoxicosis is described and reviewed in depth.


This paper comprehensively reviews nutritional factors that influence responses of animals and humans to aflatoxin. The effects of nutrients on the metabolism and disposition of aflatoxins are also discussed.


Two hundred urine samples from apparently healthy subjects were examined for aflatoxins. Aflatoxin was detected in the urine of 9.5 % of subjects from the lower and 5 % from the upper socioeconomic groups. Liver cancer is also more common in less privileged individuals. Aflatoxin excretion had no relationship with the age or sex of the subjects studied.

Possible carcinogenic effects of mycotoxins in animals and man are reviewed. Aflatoxins are carcinogenic in various animals, producing mainly cancer of the liver, colon and kidney. Epidemiological studies have shown a positive correlation between the average dietary concentrations of aflatoxins in human populations and the incidence of primary liver cancer, but it is far from proven that aflatoxins play a part in the etiology of primary liver cancer. Sterigmatocystin is also carcinogenic in rats. Ochratoxin A has nephrotoxic properties in animals, but these mycotoxins are unlikely to be involved in human cancer.


The role of mycotoxins in human disease is briefly reviewed. The acute effects of the ingestion of aflatoxins and the role that long term exposure may play in human disease are examined in detail. The necessity for cooperation between chemists, veterinarians, physicians and mycologists in the elucidation of the role of the mycotoxins in human disease is stressed.


Twelve of 28 food samples were contaminated with aflatoxin, viz. rice, broken rice, raw grams, almonds, groundnuts, peanut butter, brown beans, white beans, cardamom black, pistachio, maize flour and raw groundnuts. The consumption of foods containing aflatoxins may account for the incidence of liver cancer in Karachi, Pakistan.


Aflatoxin was present at a high level in most common foods stored poorly for long periods in Nigeria (viz. groundnuts, dried fish, groundnut oil, guinea corn (sorghum), palm oil, rice, maize, beans, yams, acha. cassava and garri). It may work synergistically with other carcinogens to produce the high incidence of primary liver cancer seen in men under 40 years.


Earlier hypotheses regarding the cause of liver cancer in Africa fail to explain the epidemiologic pattern of this disease, notably its rarity in Egypt. The hypothesis of mycotoxicosis resulting from spoilage of food by toxic molds fits the distribution better, in that it accounts for the rarity of liver cancer in dry areas, where mold Spoilage is minimal, or in populations that consume a predominantly fresh diet.


Detoxification of groundnut flour with hydrogen-peroxide is feasible and effective in destroying aflatoxin. This treatment is now used commercially, particularly in the preparation of milk substitutes containing groundnut protein. A mixture of phosphine and ammonia can be used as a fungicide for treatment of groundnuts pods immediately after harvest for controlling growth of Aspergillus flavus. Aqueous ethanol can be used effectively to extract about 90 % of aflatoxin from split groundnuts without removing any significant amounts of fat. A study of excretory metabolites of aflatoxin in rats, guinea-pigs and monkeys showed considerable differences in the excretion of aflatoxin M and B between these species. In an exploratory study of the possible role of aflatoxin in infanteile liver cirrhosis about 8 % of the urine samples examined contained 10-50 ugo of aflatoxin B in a 24 h sample. The pattern of muscular damage in cirrhotic children was similar to the muscular damage caused by aflatoxin fed to albino rats.


Suitability of hydrochloric acid, chloroform, methanol, and formalin as urine preservatives for aflatoxin analysis was examined. Both hydrochloric acid and formalin were unsuitable as preservatives. Seven per cent of the urine samples from children contained aflatoxin.


During the period 1958-1960, the acceptability of various groundnut protein concentrates was studied in several MCH centers of Dakar, Senegal. Among the subjects receiving these groundnut protein concentrates, four infants (aged less than one year) were given groundnut protein concentrates at levels ranging from 70 g to 140 g day' for ten months. Samples of these concentrates which had been saved for storage studies, were analyzed for their aflatoxin content. A 1958 groundnut protein concentrate contained 500-1000 u kg' aflatoxin; samples of a 1960 concentrate contained 0 to 100 |lg kg' aflatoxin. Two of these children returned to the hospital for follow-up studies in 1963 and 1965. Clinical and laboratory examinations showed that these children were comparable to other children living in the same environment and who had not been given groundnut protein concentrates. Liver biopsies performed in 1963 and 1965 showed in one case lesions of fibrosis; no signs of primary carcinoma of the liver were found or for that matter no indication of carcinogenic degeneration was present.


The relationship between aflatoxin contamination of foods and kwashiorkor is discussed in this brief review of the disease in Africa.

A study was carried out in Swaziland to assess the relationship between aflatoxin exposure, hepatitis B virus (HBV), and the incidence of liver-cell carcinoma, which is the most commonly occurring malignancy among males in Swaziland. Levels of aflatoxin intake were evaluated in dietary samples from households across the country, and crop samples taken from representative farms. Prevalence of hepatitis B markers was estimated from the serum of blood donors, and liver cancer incidence was recorded for the years 1979-1983 through a national system of cancer registration. Across 4 broad geographic regions, there was a more than 5-fold variation in the estimated daily intake of aflatoxin ranging from 3.1 to 17.5 ug. The proportion of HBV-exposed individuals was very high (86 % in men), but varied relatively little by geographic region; the prevalence of carriers of the surface antigen was 23 % in men, and varied from 21 to 28 %. Liver cancer incidence varied over a 5-fold range, and was strongly associated with estimated levels of aflatoxin. In an analysis involving 10 smaller subregions, aflatoxin exposure emerged as a more important determinant of the variation in liver cancer incidence than the prevalence of hepatitis infection. Aflatoxin estimates from crop samples appeared to be a reasonable surrogate for dietary arrangements. A comparison with dietary aflatoxin levels measured in an earlier survey in Swaziland suggested that programs aimed at reducing contamination levels had had some success.


A study in Swaziland to assess the possible relationship of aflatoxin contamination and the incidence of primary liver cancer is reported. Aflatoxin ingestion levels were determined in food from plate samples collected over a one year period. A significant correlation between the calculated ingested daily dose and the adult male incidence of primary liver cancer in different parts of Swaziland was established. Samples of foodstuffs other than plate samples also reflected the correlation of aflatoxin contamination and liver cancer. This study extends and amplifies the findings of an earlier study in the Murang'a district in Kenya and supports the hypothesis that aflatoxin ingestion is a factor in the genesis of primary liver cancer in Africa.


An association between aflatoxin ingested levels and the liver cancer cases in the Murang'a district of the central province of Kenya is reported.


The autopsy incidence of primary carcinoma of the liver found in the two neighbouring provinces of South India, Andhra Pradesh and Madras was 1.4 and 1.6 % respectively. The incidence of cirrhosis of the liver was also high, forming 6.1 % of the necropsy examinations. This observation, further strengthened by the coexistence of cirrhosis in nearly 80 % of livers with primary cancer, has naturally suggested a relationship between the two conditions, but a careful histological examination of tissues taken from different parts of the cirrhotic livers failed to show a transition of hyperplastic regenerating cells into malignant ones. These findings, along with the occurrence of carcinomatous growth in non-cirrhotic livers fail to establish a direct relationship between the two conditions. It is postulated that there is yet an unidentified factor which is responsible for initiating malignancy and while it can act as a carcinogen on normal cells occasionally, actively regenerating cells in cirrhotic livers are more prone to malignant transformation. Some aspects of the histological patterns of the tumors are dealt with and this shows a high percentage of cholangio hepatomas. Further analysis of the case records revealed that 80 % of the subjects were Andhras who consume hot curries.


The possibility of aflatoxin ingestion being involved in cases of infantile cirrhosis of liver in India is considered.


This paper provides a popular review of the aflatoxin problem in relation to public health.


Aflatoxins were detected in only 1 of the liver specimens of 12 children who presumably died of Reye's syndrome. It is concluded that aflatoxin is not regularly recoverable from cases of Reye's syndrome at a high rate and the proposed aetiological relationship is questioned.

two municipal populations of Thailand. Food and Cosmetics Toxicology 10: 171-179.

The incidence of primary liver cancer was determined in two selected populations in Thailand by direct field investigation. Liver viscerotomy specimens were obtained from 21% of the persons aged 15 years or more who lived and died in the municipal areas of Ratburi, Potharam, Baan Pong and Nakorn Pathom (combined population 99,537) in south-western Thailand, and from 33% of comparable persons in the municipal areas of Songkhla and Haad Yai (combined populations 97,867) in southernmost Thailand. Incidence was six new cases 100,000 people/year in the south-west area and two new cases/100,000/year in the Songhla-Haad Yai area. The available evidence supports the hypothesis that aflatoxin consumption is related to primary liver cancer in Thailand.


Chemical analyses for aflatoxins were performed on autopsy specimens from 23 Thai children who died with acute encephalopathy and tatty degeneration of the viscera (EFDV) and from 15 children and adolescents who died from unrelated causes. The highest levels detected were 93 µg aflatoxin B1 kg⁻¹ in a liver specimen, 123 µg kg⁻¹ in stool, 127 µg kg⁻¹ in stomach and intestinal contents and 8 pg mL⁻¹ in bile. Trace amounts were detected also in brain, kidney and urine. Ailatoxin B2 was indicated, but not confirmed, in 12 specimens of brain, liver, kidney, stool and intestinal contents. A blue fluorescent spot with the chromatographic properties of aflatoxin M1 was found in trace amounts in two urine specimens, and a spot similar to that of aflatoxin B1 was found in another. None of the urine specimens from healthy control children contained any of the anatoxins, but very small amounts of aflatoxin B1 (1-4 pg kg⁻¹ tissue) were demonstrated in some autopsy specimens from 11 of the 15 control subjects.


Aflatoxin consumption through cooked foods was determined by three 2-day surveys over a period of 1 year for 144 randomly selected households in nine villages within three areas of Thailand. The Singburi and Ratburi areas had the highest levels of aflatoxin ingestion, with respective annual means ranging from 73 to 81 and from 45 to 77 ng total aflatoxins kg⁻¹ bodyweight day⁻¹ on a family basis. Intake as high as 1072 ng total aflatoxins kg⁻¹ bodyweight day⁻¹ were recorded for individuals. A verge annual aflatoxin consumption in Songkhla was 10-14 times less than in Singburi. The Singburi dietary load of aflatoxin represents 20-30% of intake values that induce a liver-tumor incidence of nearly 100% in rats following continuous exposure.


Among more than 2000 samples of market foods and foodstuffs collected in Thailand, representing some 170 different human foods, groundnut products were the most frequently and most highly contaminated with aflatoxins. Dried corn, millet, wheat, barley. Job's tear seeds and dried chili peppers also were frequently contaminated, although concentrations were low. The frequency and extent of contamination of Thai foodstuffs showed geographical distributions and followed seasonal trends. Beans of various strains were the chief source of aflatoxin in the Hongkong foodstuffs examined. In general, aflatoxin contamination in Hongkong was less frequent and at lower levels than that in Thailand. Rice, the staple food in both areas, was seldom contaminated and (then only at low levels. The results of this study provided the basis upon which Thai populations were selected to measure directly the amounts of aflatoxins ingested in food.


Primary liver-cell cancer (PLC) mortality ratios, computed from deaths certificate records compiled by the National Center for Health Statistics, for the periods 1968-1971 and 1973-1976 were sorted by race, sex, urbanization, and region. From this sort, rural white males from the Southeast and the "North and West" regions were selected for comparison of mortality ratios and past dietary exposure to aflatoxin. Based on projections of recent aflatoxin contamination information back to the 1910-1960 period, and estimates of corn and groundnut usage from household food consumption surveys relating to that period, the expected average daily ingestion of aflatoxin B1 or each group was calculated (Southeast 13-197 ng kg⁻¹ bodyweight; North and West 0.2-0.3 ng kg⁻¹ bodyweight). An age-adjusted excess PLC mortality ratio was observed for the Southeast population when compared with the "North and West" - 10% excess PLC deaths at all ages, and 6% excess PLC deaths for the 30-49 year age-group-but although the difference was in the expected direction in ration to projected past exposure to aflotoxin, it was far from the manyfold difference that would have been anticipated from experiments with rats and from prior epidemiological studies in Africa and Asia. The remaining major portion of the PLC mortality in the Southeast may be attributed to many unidentified causes for which the two populations that were compared were not controlled, leaving in doubt the validity of any attribution for the excess PLC mortality to
Since the early 1960s, when aflatoxin, the mold-produced contaminant of a number of important food commodities, was found to be a potent hepatocarcinogen for laboratory rats, there has been a sustained search for evidence to support the regulatory presumption that aflatoxin is a probable human carcinogen. The developing laboratory evidence of differences between species in metabolism of aflatoxin and susceptibility to its oncogenic effects indicated that humans were probably refractory to aflatoxin carcinogenesis, but the early epidemiological evidence indicated otherwise. That epidemiological evidence, however, contained flaws so that Working Groups of the International Agency for Research on Cancer (IARC) meeting in 1970, 1976, and 1982, although ignoring the biochemical evidence, did consider the available epidemiological evidence insufficient for a conclusion of human carcinogenicity. During the 1970s and 1980s, studies on the connection between chronic infection with hepatitis B virus (HBV) and primary liver cell cancer (PLC), the expected lesion from aflatoxin exposure, had established a very strong etiological relationship between HBV and PLC. Since all the epidemiological studies of aflatoxin and PLC conducted prior to 1982 had been of populations with endemic HBV infection, and, in addition to other flaws, had not been controlled for this confounding factor, there was a solid basis for their rejection. Most epidemiological studies in the 1980s of aflatoxin and PLC were either in the United States, where HBV-infected groups could be excluded from the study, or, when in areas of chronic HBV infection, attempts were made to include that factor. The study of U.S. populations showed no difference in mortality rates from PLC that could be attributed to aflatoxin exposure. The studies of populations with endemic HBV infection produced no convincing evidence to support a primary role for aflatoxin in the induction of human PLC, although an accessory role to HBV infection for aflatoxin could not be ruled out. However, the epidemiological studies of the HBV/PLC relation indicate that an accessory factor is not an essential condition, a conclusion supported by animal models and a laboratory study that specifically found no interaction between aflatoxin and a hepatitis virus in the duck, a species in which liver cancer can be induced by either agent. It was surprising that an IARC Working Group meeting in 1987 concluded, on the basis of much of this evidence that was available at that time, and citing other studies that appear to be irrelevant to the issue that there was sufficient evidence to consider aflatoxin a probable human carcinogen.


Recent work on mycotoxin contamination of animal and human foods is reviewed and some experimental work on agricultural and biological aspects is reported. Irrespective of growing and harvesting conditions, length of storage affected degree of aflatoxin contamination. In tests with 60 different kinds of groundnuts, including the variety U.S. 26, immune to aflatoxin development but not yet grown in India, the unique resistance of this variety was confirmed. Extensive field experiments showed that it was suitable for cultivation in India. Laboratory tests indicated that Zn, Cu, Co and Cd were required for effective growth and sporulation of the fungus and production of aflatoxin. Feeding experiments with young monekys and with rats confirmed that the level of dietary protein modified the extent of aflatoxin liver damage. Investigations of 16 cases of childhood cirrhosis, however, failed to show conclusively that aflatoxin was responsible.


Of 55 workers in a Dutch groundnut oil factory who had been exposed to an estimated 0.039-22.5 µg aflatoxin week⁻¹, 11 developed various forms of cancer, mainly of the respiratory tract.


Data on the age and sex distribution of primary liver cancer rates in goldminers from the Inhambane district of Mozambique were obtained from a hospital registration program. Annual values for 1964-68 and 1969-71 were 35.5 and 25.4/100000. Occurrence was twice as frequent in males. Aflatoxin was found in 9.3% of 880 random foods, equivalent to a mean consumption of 222.4 ng kg⁻¹ bodyweight. Comparisons with similar studies in areas of lower incidence showed a significant correlation between aflatoxin consumption and liver cancer rates.


Of 55 workers in a Dutch groundnut oil factory who had been exposed to an estimated 0.039-22.5 µg aflatoxin week⁻¹, 11 developed various forms of cancer, mainly of the respiratory tract.
Province of Inhambane, Mozambique, were calculated and together with rates observed in South Africa among mineworkers from the same Province indicated very high incidence in certain districts of Inhambane. Exceptionally high incidences in adolescents and young adults were not sustained at older ages and suggested the existence of a group of highly susceptible persons. A sharp decline in incidence occurred during the period of study. Concurrently with the studies of incidence, 2183 samples of prepared food were randomly collected from 6 districts of Inhambane and from Tahica-Magude, a region of lower hepatocellular carcinoma incidence to the south. A further 623 samples were taken during 1976-1977 in Transkei, much further south, where an even lower incidence had been recorded. The mean aflatoxin dietary intake values for the regions were significantly related to hepatocellular carcinoma rates. Information on aflatoxin B, contamination of prepared food from 5 different countries showed overall a highly significant relation with crude hepatocellular carcinoma rates. In view of the evidence that chronic hepatitis B virus (HBV) infection may be a prerequisite for the development of almost all cases of hepatocellular carcinoma and given the merely moderate prevalence of carrier status which was observed in some high-incidence regions, it is likely that an interaction between HBV and aflatoxin is responsible for the exceptionally high rates in Africa and Asia. Indications from Mozambique suggest that aflatoxin may have a late-stage effect on the development of hepatocellular carcinoma.


In Mozambique, methods of food production, harvesting, storage and preparation were studied to detect points of aflatoxin contamination. Groundnuts, the main dietary source of protein provided the most aflatoxin contamination, traditional agricultural methods promoting fungal infection and growth. Aflatoxin production also occurred in the main carbohydrate sources, cassava and maize, during storage. Western-type foods had particularly low aflatoxin contents. A simple educational program could reduce the incidence of primary liver cancer associated with aflatoxin poisoning; westernisation of some living habits could also reduce the incidence of the disease.


Possibility of aflatoxin ingestion being related to primary liver cancer incidence is considered for a high cancer area of South Africa.

Aflatoxin analyses were made on serum and urine samples from 41 children admitted to a rural hospital in Kenya with kwashiorkor, marasmus, marasmic kwashiorkor or normal nutrition. Aflatoxins were detected most frequently and at highest concentration in the sera of children with kwashiorkor who, conversely, showed aflatoxins least frequently in their urine and in concentrations that were disproportionately low compared with serum/urine aflatoxin levels in other groups. These findings indicate altered aflatoxin metabolism in kwashiorkor and support the hypothesis that there is a special relationship between aflatoxins and kwashiorkor.


In 1966, while working in a U.S. Department of Agriculture research laboratory, a 25-year-old woman attempted suicide twice by ingesting mixtures of aflatoxin containing 15-45 % of aflatoxin B₁. She consumed 5.5 mg of aflatoxin over 2 days and, 6 months later, 35 mg more over 2 weeks. After the first episode, she was admitted to hospital with a transient, non-pruritic, macular rash, nausea, and headache; on the second occasion, she reported nausea only. Both times, physical, radiological, and laboratory examination were normal, except for sulphobromophthalein retentions at 45 min of 9 % and 7 %. Percutaneous liver biopsy on each occasion was normal by light microscopy. On follow-up examination after 14 years, she was found normal.


This paper reviews research on toxicity and carcinogenicity of aflatoxins to animals, and summarizes control measures applied or proposed for protecting human food supplies from aflatoxin contamination. Control measures include use of effective crop handling, processing and storage procedures, segregation of contaminated produce by various sorting and diversion systems, and detoxification.


Of 16 Indian children from 3 months to 2.5 years old with a diagnosis of Indian childhood cirrhosis confirmed histologically, 10 had a compound in their urine which gave a fluorescent spot. The ultraviolet and infrared spectra of aflatoxin B₁
and the fluorescent spot in the urine were quite dissimilar. The unknown fluorescent compound was highly soluble in ethyl ether whereas aflatoxin B₁ was insoluble.


The geographical distribution of primary liver cancer in China is related to contamination of foodstuffs (including groundnuts) with aflatoxins. The warm, humid climate is conducive to contamination of foodstuffs with aflatoxin.


The major risk factor for primary liver cancer was identified as hepatitis virus B infection in certain populations in southeastern China.


Investigations were made into the roles of hepatitis B virus and aflatoxin B₁ in the development of primary hepatocellular carcinoma (PHC) in a cohort of 7917 men aged 25 to 64 yr old in southern Guangxi, China, where the incidence of PHC is among the highest in the world. After accumulating 30188 man-yr of observation, 149 deaths were observed, 76 (51 %) of which were due to PHC. Ninety-one % (69 of 76) of PHC deaths were hepatitis B surface antigen (HBsAg) positive at enrollment into the study in contrast to 23 % of all members of the cohort (RR=38.6). Three of the four patients who died of liver cirrhosis also were HBsAg positive at enrollment. There was no association between HBsAg positivity state and other causes of deaths. Within the cohort, there was a 3.5-fold difference in PHC mortality by place of residence. When estimated aflatoxin B₁ levels in the subpopulations were plotted against the corresponding mortality rates of PHC, a positive and almost perfectly linear relationship was observed. On the other hand, no significant association was observed when the prevalence of HBsAg positivity in the subpopulations was compared with their corresponding rates of PHC mortality.


Maize and groundnut oil (253) samples were collected from 32 households in Fushui County of the Guangxi autonomous region of the People's Republic of China, where high liver cancer incidence has been reported, every day over a period of 1 week and analyzed for aflatoxin B₁. A total of 252 urine samples were collected simultaneously from the residents in the households which had consumed aflatoxin and were analyzed for aflatoxin M₁. A good correlation between dietary aflatoxin B₁ intake and total aflatoxin M₁ excretion in human urine was observed during a 3-day study. Between 1.23 and 2.18 ° of dietary aflatoxin B₁ was present as aflatoxin M₁ in human urine. A good correlation was also found between aflatoxin B₁ concentration in maize and aflatoxin M₁ concentration in human urine. It is suggested that analysis of aflatoxin M₁ in urine by ELISA could be used as an index for human exposure of aflatoxin B₁ in an expensive epidemiological study.


Liver cell cancer is prevalent in many regions of Africa and South-east Asia. The author comments on the possible connection between liver cell cancer and aflatoxins.

1.2.4 Laboratory Studies on Toxicity and Carcinogenicity of Aflatoxin


A primary liver carcinoma occurred in a rhesus monkey given aflatoxin B₁ orally over a period of 6 years. The fact that this tumor rarely arises spontaneously in monkeys, and pathologic changes seen in the livers of other monkeys given aflatoxin B₁ suggested that the neoplasm was induced by this compound.


Three of 42 monkeys, 20 Macaco mulatta, 20 Macaca fascicularis and 2 Cercopithecus aethiops, given aflatoxin B₁ (AFB₁) by mouth, nasogastric tube or intraperitoneally for longer than 2 years developed primary malignant neoplasm of the liver. Liver biopsies made at intervals indicated that neoplasia was preceded by pathological lesions of the liver, including toxic hepatitis, proliferation of pseudotubules and hyperplastic nodules. Serum α-fetoprotein, estimated in one of
Aspergillus flavus strains were obtained from British and domestic sources, and were from wheat. The amounts of toxic substances present were estimated by paper chromatography. Concentrates were obtained by precipitation. One concentrate was reduced. Toxicity tests on ducklings confirmed British findings.


Trout fed Bloor-La Roche extracted cottonseed meal fats developed classical trabecular liver cell neoplasms in five months. One of five cottonseed meals with known agricultural history assayed 300 µg kg⁻¹ aflatoxin B₁, and trout fed this fat showed a high incidence of hepatoma. Trout fed crystalline aflatoxin B₁ and G₁ confirmed previous experiments with these toxins; trabecular hepatoma developed within six months. Small fish-force fed 1, 3, or 5 mg of crude aflatoxin kg⁻¹ bodyweight had from slight focal to extensive liver cell necrosis with copious haemorrhage in severe cases. Extent of these lesions varied directly with the amount of toxin ingested. Preneoplastic or concomitant pathology included generalized cytoplasmic and nuclear vaculation, nuclear pleomorphism and occasional cholangiolar proliferation. Other trout loaded with crystalline aflatoxin B₁ at 0.1, 0.5, 1.0 or 3.0 mg kg⁻¹ bodyweight showed a range of variation in the extent of necrosis and haemorrhage together with accompanying pathology essentially similar to fish fed the crude aflatoxin. Aflatoxin B₁ appeared to be from five to ten times more toxic to trout than the crude aflatoxin.


In rainbow trout fed wheat aflatoxins for 6-9 months (80 µg kg⁻¹ crude aflatoxin), 60 % had classical multinodular hepatoma. Fish force-fed high levels (30-300 µg kg⁻¹ day⁻¹) of thin-layer chromatogram-purified B₁ and G₁ compounds for 5 days died with acute aflatoxicosis at 10-15 days. Tentative LD₅₀ total dose was 1500 µg kg⁻¹.


Rats received in a powdered cube diet 1750 µg kg⁻¹ aflatoxin from toxic groundnut meal. Three rats given aflatoxin for 89 days ultimately developed liver carcinoma after being returned for 300 or more days to stock diet. The carcinogenic dose was not greater than 2500 µg kg⁻¹ per rat. The three rats had multiple trabecular hepatocarcinoma with adenomatous areas. The possible hazard to human health was indicated.

eighteen monkeys were given various doses of chromatographically pure aflatoxin B. Death occurred in one animal receiving 4500 µg kg⁻¹ and in all animals receiving 13500 µg kg⁻¹ or more. Cough, vomiting, diarrhoea and coma were characteristic clinical findings. Changes in the serum included hypoglycaemia, increased nonesterified fatty acids and transaminases and decreased phospholipids, cerebral oedema with neuronal degeneration, bile duct hyperplasia, hepatic cell necrosis, lymphocytosis and marked fatty degeneration of the liver, heart and kidneys were found post-mortem.


biochemical studies were made on liver tissue and blood from ducklings and various seeds of chickens maintained on standard rations containing 500 µg kg⁻¹ aflatoxin B. The results obtained from these birds were compared with those from birds of the same age maintained on groundnut-free mash. Of the various breeds of licken studied only New Hampshires were susceptible to the effects of this level of aflatoxin. In the livers of affected chickens or ducklings, a marked decrease in the activity of certain mitochondrial dehydrogenase and enzymes of the electron transfer chains or oxidative phosphorylation mechanisms was apparent. Mild to moderate anaemia, severe hypoproteinaemia and grossly abnormal plasma protein electrophoretograms were presented by affected birds. Elevations in the activity of liver plasma enzymes, notably those of lactic dehydrogenase, aldolase and citric-oxalacetic or glutamic-pyruvic transaminase, were also noted in these birds, the ability of affected birds maintained on rations containing 500 µg kg⁻¹ aflatoxin B recover rapidly when placed on toxin-free rations was noted.


Aflatoxin B₁ levels greater than 1 mg kg⁻¹ diet had a detrimental effect on mortality, bodyweight gain, food intake and food conversion efficiency. When fed for more than 2 weeks, aflatoxin increased relative liver weight and liver lipid concentration. The effects were less pronounced with vitaminotic A chickens. A synergistic effect on hydropericardium development was observed between aflatoxin and retinol. This effect was not observed when the dietary level of α-tocopherol was increased ten-fold. Liver fatty acid composition was significantly influenced by aflatoxin B₁. The extent of these changes was reduced by the inclusion of additional dietary biotin.


This paper describes the histological changes in the liver produced by a single dose of aflatoxin and contrasts them with the lesions produced in day-old ducklings by other well-studied liver poisons. Day-old Khaki Campbell ducklings given 15 µg aflatoxin orally developed an extensive biliary proliferation in the liver with fatty degeneration of the peripheral parenchyma cells. This lesion reached its maximum in 3 days, and then regressed with repair of the liver parenchyma. A comparable lesion was seen in ducklings given single doses of dimethylnitrosamine and cycasin, but not with other hepatotoxic agents such as carbon tetrachloride, ethionine and thioacetamide. The lesions varied greatly at the same dose level, and it was not possible to estimate the amount of aflatoxin by histological examination of the livers of poisoned birds. Evidence of possible direct action upon the biliary epithelium is given, and it is suggested that aflatoxin may be an alkylating agent.


Acute toxicity of aflatoxin B₁ in rats is reported. The LD₅₀ of aflatoxin B₁ to male rats was estimated as 7.2 mg kg⁻¹ per os and 6 mg kg⁻¹ intraperitoneal and female rats 16 mg kg⁻¹ per os. A periportal zone of liver necrosis developed in 3-4 days. Following this there was a slow recovery so that by 1 month mere was a persistant biliary proliferation and the presence of large hyperchromatic parenchymal cells. Lesions in other organs are described.


Sequential ultrastructural changes were induced in rat liver parenchymal cells by the oral administration of aflatoxin B₁. These were described at intervals of 1, 3 and 6 hours. At 1 h some dilatation of the RER cisternae and dislocation of ribosomes appeared in periportal cells. By 3 hours disruption of the RER was more evident; centrilobular cells remained normal. At 6 hours when the periportal cells exhibited further disruption with alteration in mitochondria, centrilobular parenchymal cells revealed dilatation of RER cisternae and dislocation of ribosomes. At 3 and 6 hours the formation of nucleolar caps was prominent. There appears to be a correlation between inhibition of the protein synthetic mechanism and the disruption of the RER but this does not appear to be related to the nucleolar alterations.

The effects of a single dose of aflatoxin B₁ on guinea-pigs were compared with the effects of feeding groundnut meal containing aflatoxin. The LD₅₀ of aflatoxin B₁ in male and female guinea-pigs was estimated as 1.4 mg kg⁻¹ body weight by interaperitoneal injection. The main hepatic lesion was a centrilobular zone of necrosis developing with biliary proliferation over 2 days. The lesion was healed over a few days and the biliary proliferation rapidly regressed. The pathological changes in other organs are described.


Of six one-year-old rats receiving a diet containing 3000-4000 µg kg⁻¹ aflatoxin, five survived longer than 39 weeks and of these, three showed anaplastic hepatocellular carcinomas. Adenocarcinoma of the rectum or stomach also occurred in two of these three rats. One case each of hepatic carcinoma and carcinosarcoma of the stomach occurred among six young rats which received the diet for three weeks and then returned to a normal diet. Aflatoxin may therefore be a factor in the aetiology of carcinomas in organs other than the liver.


The acute toxicity and carcinogenic activity of aflatoxin and the pathological changes which this toxin induces in laboratory animals are described. The organ primarily affected is the liver but changes are seen in most other organs. The carcinogenicity of aflatoxins has been demonstrated in rats, ducks, and trout. In sheep, mice and guinea-pigs, there is some evidence of carcinogenicity. Guinea-pigs are extremely susceptible to aflatoxin.


Trials were conducted with rats of both sexes of about 100 g bodyweight given diets With from 3500 to 4000 µg kg⁻¹ aflatoxin B₁ supplied by up to 50 % groundnut meal, and with guinea pigs given up to 1600 µg kg⁻¹. With the largest amount of toxic meal rats grew normally for 3 weeks, then growth declined. When the diet was changed to exclude the toxic meal, after 16 weeks growth improved. Other diets were given continuously or intermittently for different periods. There was evidence that some irreversible damage was done to the liver by 12 weeks on the diet with 1400 ug kg⁻¹ aflatoxin. Damage to the liver in different conditions is described and illustrated. The malignant tumors of the liver are compared with those produced by other known carcinogens. Guinea-pigs given 1400 µg kg⁻¹ aflatoxin died, usually within 14 to 28 days. As the dose was reduced the development of clinical and pathological signs was correspondingly retarded. The histological picture was of acute poisoning, and malignant tumors did not develop, probably because the animals died too soon.


Aflatoxin in one quarter of the lethal dose for the non-pregnant female rat was given by mouth to rat at various stages of pregnancy. Animals dosed in early pregnancy were no more severely affected than non-pregnant rats, and there was no evidence of toxic effects on the foetus or placenta other than slight reduction in placental weight at term. Rats dosed on the 16th day, however, had more severe liver injury than non-pregnant rats with increased fatty infiltration and retardation of foetal growth. It is concluded that foetal growth retardation in the latter group of animals occurs secondarily to the toxic effects in the mother and may be due to the maternal liver damage.


Sixteen 7-day-old Khaki Campbell ducklings were fed on a commercial poultry feed free of aflatoxin, and 37 had 0.5 % toxic Brazilian groundnut meal included in the feed. The groundnut meal contained 7000 µg kg⁻¹ aflatoxin B₁. The regression of growth up to 11 weeks did not differ significantly between the groups but those given groundnut meal were significantly lighter at 3, 4 and 5 weeks, and the difference was maintained up to the end of the trial after 14 months. During the first 4 weeks, 19, and in the next 7 months 7 ducks in the group given toxic groundnut meal died; all had damage to liver typical of poisoning by aflatoxin. After 14 months tumours were found in livers of 8 of the 11 survivors of the group. There was no tumor in the 10 survivors of the group given no groundnut meal.


Two groups of weanling female rats were each given a single sublethal dose of aflatoxin by mouth. Almost half the animals in each group developed hepatic tumors. Other chronic changes were found in the liver of a high proportion of the remainder. The mean time of tumor formation was 26 months.
alkaline-phosphatase activity and bilirubin concentration. Aflatoxin B enzymes were released into the serum in the second 24 h of the poisoning, closely

DNA. The toxin inhibits the production of nuclear RNA, probably by preventing the

in the nucleus of the poisoned liver cell and in vitro it was shown to interact with DNA. The toxin inhibits the production of nuclear RNA, probably by preventing the

transcription of DNA by the RNA polymerase. It is proposed that the interaction of the toxin with DNA gives rise to its inhibitory action on mitosis and its necrogenic action.

A comparison was made of the different spectra obtained by causing various aflatoxins (B₁, G₁ and G₂) to interact with calf-thymus DNA. The effect of these toxins on RNA and protein synthesis by rat-liver slices was measured. The extent of their inhibitory action on the synthetic reactions was proportional to the degree of spectral shift obtained with their interaction with DNA. It is proposed that their toxicity depends upon this interaction. It was demonstrated that RNA polymerase of nucleoli isolated from the livers of aflatoxin B₁-poisoned rats was inhibited. This finding is in agreement with the proposed mechanism for the hepatotoxic action of aflatoxin.

Both male and female Cynomolgus (Macaca irus) monkeys survived for 3 years without apparent ill health when fed on diets containing groundnut meal to provide up to 0.36 µg aflatoxin B₁ g⁻¹ diet, and thus supplying a mean daily consumption of up to 2 µg aflatoxin B₁ kg⁻¹ bodyweight. No histological changes attributable to aflatoxicosis were discovered in any of the organs from the monkeys receiving these quantities of aflatoxin. Few monkeys survived for more than a month or two when given a diet containing 1.8 µg aflatoxin B₁ g⁻¹, which provided about 50 (µg aflatoxin B₁ kg⁻¹ bodyweight day⁻¹). No depression in growth rate nor effect on health was noted in these monkeys which survived on diets containing 1.8 (µg aflatoxin B₁, nor in any of the other monkeys. Histological changes were observed in the livers of all monkeys receiving the diet containing 1.8 (µg aflatoxin B₁ g⁻¹ for more than a month or two. but no abnormalities related to aflatoxicosis could be detected in any of the other organs, except for minor changes in the kidneys of two monkeys. No tumors were seen in any of the monkeys, even in those surviving for 3 years on diets providing 1.8 (µg aflatoxin B₁ g⁻¹. Thus, no conclusions can be drawn from this work as to the carcinogenicity of aflatoxin in monkeys (still less in man), because the animals were young and little is known of the duration of exposure required to demonstrate carcinogenicity in this species.

The protein index method, based on Brdicka's Cobalt catalysed polarographic waves, is the most sensitive measure of pathological changes in blood proteins. This technique was applied to plasma from ducklings fed various levels of aflatoxin. The protein indexes were determined and the statistical parameters of the data were calculated. The protein index of the ducklings fed 7 µg of crystalline aflatoxin B, was twice the index value obtained from control birds. The protein index of ducklings fed aflatoxin mold culture, estimated to contain 20 µg aflatoxin B, chemically, was 5 to 6 times the control index. These results correlate with the histological liver data and reflect structural changes in the liver.


The effects on the liver of oral administration of a range of doses of a partially purified mixture of aflatoxins B₁ and G₁ were studied in a group of 64 young male rhesus monkeys. The dosage levels of the toxin used were: 1 mg kg⁻¹ bodyweight daily (group 1); 0.25 mg kg⁻¹ bodyweight twice a week (group II); and 62 µg once a week (group III). The toxin was administered continuously according to this schedule. The animals in groups I and II were further subdivided in order to study the effect of a diet poor in protein on the liver injury induced by aflatoxin. All animals in group I died before the end of the 3rd week of aflatoxin administration; extensive haemorrhagic necrosis of the liver was a characteristic finding. Animals in group II were studied at intervals up to nearly 5 months of aflatoxin administration. Large bizarre hyperchromatic liver cells and bile-duct proliferation appeared around the middle of this period and thereafter progressed in severity. Animals in group III showed comparable changes in a much milder form, which appeared much later and did not then progress significantly up to the end of the 2 year period of observation. No tumors were seen in any of the groups. Protein deficiency did not exert any clear-cut deleterious influence on the liver injury induced by aflatoxin. The investigation emphasises the importance of long-term studies in non-human primates, using low doses of aflatoxins, to elucidate their role in the causation of liver disease (including liver cancer) in man.


Sarcoma or fibrosarcoma developed in 11 rats at the site of subcutaneous injection of repeated doses of aflatoxin. A preparation containing 3.8 % aflatoxin B and 56 % aflatoxin G was injected twice a week in doses of 50 µg for 50 weeks or 500 µg for 8 weeks.


Nine species of fungi isolated from stored domestic groundnuts were grown on sterilized groundnuts and were incorporated into diets fed to ducklings. Symptoms of acute toxicity resulted only after consumption of one of the diets and this one contained material incubated with the fungus Aspergillus flavus.


In an experiment with pigs from 40 to 200 lb live-weight, the effect was studied of inclusion in the diet of groundnut meal at levels of 0, 2.5, 5 and 7.5 % giving concentrations of 2, 140, 280 and 410 µg kg⁻¹ aflatoxin B₁, respectively. Depression of growth rate and feed conversion efficiency occurred at the two highest levels of inclusion at both centres where the experiment was replicated. These effects were detected mainly between 40 and 140 lb live-weight. A significant linear relationship could be represented by regression equations based on aflatoxin B₁ content of feeds for the live-weight ranges of 40 to 140 lb and 40 to 200 lb. The results for both centres could be combined because there was little variation between them. The combined results could be represented also by regression equations. It is suggested that the sets of equations may be of some value in predicting levels at which groundnut meal of defined toxicity may be used for pigs. No clinical signs could be attributed to the levels of toxic groundnut meal used. Carcass abnormalities were not reported and no macroscopic differences between livers were found. Microscopic liver lesions were minimal, except in pigs on diets containing 410 µg kg⁻¹ aflatoxin B₁.


Day-old Muscovy ducklings of mixed sex were divided into 6 groups of 34 or 35 and given for 3 weeks diets in which the control group had 25 % soya oilmeal and the test groups groundnut oilmeal containing 1000 (µg aflatoxin B₁ kg⁻¹. For 4 weeks of the test groups the diet contained also DDT 50 or 150 or phenobarbital 100 or 200 mg kg⁻¹. The diets had 23 % crude protein. Cumulated mortality in 3 weeks was for the controls 6.6 % and for groups with groundnut meal alone or the additives in order as above 45.7, 41.1, 20.5, 14.7 and 14.7 %. Final weights of survivors were 484, 291, 338, 366, 448 and 433 g and weight of liver as proportion of bodyweight was 5.03, 5.97, 6.58, 7.68 and 8.58 %. The mean liver
reserved for vitamin A were 174, 58, 101, 59 and 60 IU g⁻¹. It was concluded that DDT and phenobarbital, especially in the larger dose of the first and the smaller of the second, tended to offset the toxic effects of aflatoxin; their effects were highly significant. The effects on vitamin A reserved might be attributable to the role of vitamin A in enzyme induction.


The induction of tryptophan pyrrolase and tyrosine transaminase in rat liver by hydrocortisone (50 mg kg⁻¹) was significantly inhibited by simultaneous administration of aflatoxin B₁ (1 mg kg⁻¹) in adrenalectomized or intact rats. Inhibition also occurred when the toxin was administered 1 hour after hydrocortisone. However, the induction became intensive to aflatoxin B₁ 3 hours or longer after hydrocortisone administration. In comparative experiments, these effects were shown to be unlike those caused by puromycin but analogous to those of actinomycin D. The latter suggestion was corroborated by the findings that aflatoxin B₁ at an IP dose of 5 mg kg⁻¹ (LD₅₀) resulted in 62 % inhibition and II⁻³ - cytidine incorporation into rat liver nuclear RNA within 30 minutes after administration. The depression of incorporation was accompanied by a 23 % reduction in the nuclear RNA/DNA ratio. In animals killed 90 mm. following aflatoxin B₁ administration, the incorporation of a 30 min. H⁻³ - cytidine pulse was inhibited by 73 % with a concomitant reduction of 28 % in the nuclear RNA/DNA rates. The observed effects on nuclear RNA metabolism may be a primary determinant of the toxicity of aflatoxin B₁.


RNA polymerase activity, incorporation of triitated cytidine into RNA, and RNA/DNA ratios were determined in liver cell nuclei isolated from rats killed at intervals following a single sublethal dose (3 mg kg⁻¹) of aflatoxin B₁. Twelve hours after dosing, cytidine incorporation was decreased to 7 % of control values and me RNA/DNA ratio was suppressed by 29 %. At 24 hours, incorporation was 6.5 % of control, and RNA/DNA ratios had risen to 85 % of control values. By 5 days after dosing, the RNA/DNA ratios had returned to pretreatment levels, but cytidine incorporation was still suppressed by 63 %. The latter inhibition persisted for prolonged periods beyond 5 days. RNA polymerase activity of nuclei isolated from treated animals was inhibited by 32 % at 5 minutes, 75 % at 30 minutes and 78 % at 48 hours.


Effects of various levels of toxic Brazilian groundnut meal on growth and mortality of poult’s and chicks were investigated. The growth rate of poult’s led each level of the meal was markedly reduced and was inversely related to the level of the meal in the birds’ diets. Mortality of the poult’s fed the two higher levels (37 and 55 %) of meal occurred early and by 21st day of the experiment all were dead. Mortality of the poult’s receiving meal at me lowest level (18 %) reached 100 % by me 28th day. Gross observations of the livers from the sacrificed poult’s (at 15th day) indicated severe lesions of the livers from those poult’s receiving groundnut meal. There were no marked differences in the percent mortality of the various groups of chicks. The 4-week weights of the chicks receiving the two higher levels of groundnut meal were significantly (P < 0.01) lower than the other two groups and significantly different from each other. The livers of chicks fed the meal were yellow throughout (on all levels of the meal) while those from controls appeared normal.


The carcinogenic effect of aflatoxin was investigated in the rhesus monkey. The toxin was administered to one male and one female monkey in a sub-acute dose for a period of 5.5 years. In a male monkey, a rapidly growing hepatocellular carcinoma of a giant-cell type developed 2.5 years after the toxin administration had been discontinued. The histopathology of the tumor is described. Observation of the female is continuing.


Rainbow trout fed appropriate levels of 11 chemical carcinogens incorporated into a negative control test ration developed typical adenomatous, ulcerated, or mixed-pattern hepatoma. Occasionally a cholangioma was also observed. Fat carefully extracted from the commercial ration and fed as part of the diet to young trout for 1 year also appeared to be a vector in hepatomegaly. High load tests with crude aflatoxin concenusates showed massive liver damage and multiple haemorrhagic areas throughout the liver, caeca, and other viscera of small trout in a single-dose, 10-day test. Considerable typical liver damage could be observed when only 0.2 mg of the crystalline aflatoxins kg⁻¹ body weight were administered. In a long-term feeding study crude and crystalline aflatoxins B₁ and G₁ extracted,
separated, and purified from contaminated groundnuts or cultured on wheat showed a very high incidence of primary liver cell carcinoma in trout fed only low levels of aflatoxins for 6 months to 1 year. Further current investigations in many laboratories should extend and define the effects of aflatoxicosis in rainbow trout hepatomagenesis.


Young rainbow trout fed 20 µg kg\(^{-1}\) aflatoxin B\(_1\) diet alter 4, 8, 12, and 16 or 20 weeks on control diet developed degrees of hepatomas directly related to size before insult; whereas, control fish fed CTD without toxin failed to exhibit nodules after 20 months on test. Numbers of gross tumors per group varied inversely with age before fish received toxin. Total dose was near constant at 9-10 µg B\(_1\) fish\(^{-1}\). Another study showed total dose at early age spread over 2-20 weeks yielded nearly the same number of tumors at termination. More aflatoxin B\(_1\) increased incidence slightly but when 0.4 µg was ingested during the first four weeks, growth was impaired and acute liver damage was detected. Survivors developed classical hepatoma at 12 or 20 months. In contrast, fish fed 20 µg kg\(^{-1}\) of tetrahydrodesoxy aflatoxin or Wogan's compound 11 had only one or two with tumors alter 20 months. Total dose of each exceeded 10 µg fish\(^{-1}\). Positive controls showed over 90 % with hepatoma at 20 months of 20 µg kg\(^{-1}\) B\(_1\) diet insult and some fish had metastasis to other organs. Coho Salmon fed the same levels of aflatoxin B\(_1\) as positive trout controls failed to develop gross hepatoma.


Landrace x Wessex pigs given feed from a farm where there had been outbreaks of groundnut poisoning developed typical signs, and 20 % toxic groundnut meal in the feed produced the expected lesions. The progress of changes in liver is described. There were sometimes high serum glutamic oxalacetic transaminase and alkaline phosphatase, in liver concentration of fat was increased with vitamin A was less. and ratio of weight of thyroid to bodyweight was low.


Problems arising from the contamination of groundnut meal with aflatoxin are reviewed. Progress in the detection of related compounds and knowledge of their toxic properties are summarized, with details of acute toxicity on monkeys, and indications of the chronic effects of very small doses on liver and kidneys of children. Transmission of the toxic constituents from animal feeds to cow's milk and pig liver and kidneys is also reported, although there are no apparent indications of progressive accumulation. Aflatoxin in imported groundnut meal is discussed and the limits imposed by the EEC on feeds of different kinds are tabulated. The practical implications of stringent limits on aflatoxin contents of meal imported into European and other countries are discussed, with particular reference to the danger that more heavily contaminated batches may be retained in the developing countries where the population relies heavily on groundnut meal as a source of protein. The possibility of detoxification with ammonia is briefly discussed, and it is noted that tests with rats indicate that the protein quality if thereby decreased by about 10 %.


This paper gives a comparative profile of the in vitro hepatic metabolism of aflatoxin B\(_1\) by rat, mouse, duck, monkey, hamster, and human, and introduces certain correlations for predicting human and animal susceptibilities to acute and chronic aflatoxicosis comparing the relative activities of several in vitro metabolic pathways.


Groups of 3 mink were given a basal feed with 2, 15 or 1 % groundnut meal containing 4500 µg kg\(^{-1}\) aflatoxin. Two other groups of 3 were given 0.5 % and one group received no groundnut meal. The mink given 1 % groundnut meal or more died after 24 to 42 days with nonspecific inflammation of the liver, and extensive liver cell changes. Of one group given 0.5 % groundnut meal, 1 mink died after 48 days and the others were killed for examination; all had only slight liver changes. Of the other group, 1 died after 371 days and the others were killed; they had widespread tumors in the liver as well as fatty infiltration.


Experimental feed containing groundnut oilcake contaminated with aflatoxins B\(_1\), B\(_2\) and G\(_1\) was fed once daily to 16 lactating cows to provide daily intakes of 1000, 2000 or 3000 µg aflatoxin. There was no correlation between aflatoxin intake and milk yield or incidence of udder disorders (including mastitis), but contents of
A group of 5 Kerry crossbred lambs 3 months old at the start were fed for 5 years on hay and a concentrate with 20% highly toxic groundnut meal, containing 1750 µg kg\(^{-1}\) aflatoxin during the first 3-5 years, and 1000 µg kg\(^{-1}\) since then. Lambs born were added to the experimental group. A control group, for the first 3 years, got concentrate with fishmeal and maize gluten supplying protein. In the group given aflatoxin, fertility was lower and growth during the first 18 months was also retarded. One lamb was killed after 5 months and others died during the experiment. Damage to the liver was found in only one, and there were no typical signs of groundnut poisoning. In 2 sheep there were nasal chondromata; the rarity of this disorder and the possibility of its being related to the feed are discussed.

High mortality is reported in young pigs in a herd given Brazilian groundnut meal (17.5% in the starter pellets and 8.75% in the sow and weaner meal). Pigs of the same herd transferred to another farm were not affected. Sows lost appetite and tended to subsist on grass. Liver lesions in acute, subacute and chronic types of poisoning are described. There were some similarities to chronic copper poisoning. There was no difference in toxicity between solvent- and expeller-processed meals. Experimental poisoning was also studied. The suspected diet was given to 2 pigs for 4 weeks and 5 others got a meal with 20% Brazilian groundnut meal. All developed signs of poisoning, with typical acute or subacute liver damage. Pigs given another commercial meal or 20% Nigerian groundnut meal were not poisoned.

The effect of prednisolone on aflatoxin liver injury was studied in two experiments on weanling rats kept on diets containing 20% and 5% protein. Rats on the high protein diet were apparently healthy and showed only mild histological changes in the liver when they were given 70 µg of the toxin per day for 20 days. The administration of 1000 µg or 200 µg of prednisolone daily did not alter the picture in any way. Rats on the low protein diet readily developed typical lesions in less than 20 days on the same dose of the toxin. In the presence of the higher dose of prednisolone there was marked inhibition of bile-duct proliferation and fat accumulation. This inhibitory effect was less marked when the smaller dose was given. The clinical signs and mortality due to the toxin did not appear to be influenced by the steroid.

Weanling male rats fed low protein diets (4 % casein) and given 50 µg of aflatoxin daily, developed severe liver lesions characteristic of aflatoxin injury within 20 days. Those fed 20 % casein in the diet had only mild changes in the liver, including vacuolation and typical, possibly precancerous, cells. These were not encountered in the protein-deficient groups.


Three experiments were conducted in which different daily oral doses of aflatoxin were administered to weanling rats for varying periods. In each experiment, the animals were divided into two groups, one (HP) receiving 20 % casein and the other (LP) a 5 % casein in the diet, the former being pair fed to the latter. In all, 30 rats on the HP diet and 12 on the LP diet survived for more than a year. Of the former, 15 (50 %) developed hepatomas or tumors in other organs while the other 15 showed only precancerous lesions in the liver. In contrast, none of the 12 rats on the low-protein diet showed tumors or precancerous lesions of the liver while only one developed a kidney tumor. It is concluded that a low level of dietary protein has an inhibitory effect on aflatoxin carcinogenesis in the rat.


The results of feeding different daily doses of pure aflatoxin to day-old ducklings up to a maximum period of eight weeks are reported. Ducklings given 40 µg to 10 µg of aflatoxin per day died within five days. In addition to the characteristics lesions, a number of them had hepatic infarcts, with or without arterial occlusion. Severe congestion was present in all. Lower doses of the toxin caused milk chronic lesions which were regressive.


Aflatoxin produces liver lesions in different species. Renal changes have been non-specific, such as parenchymal haemorrhages in ducklings given large doses of the toxin, and lipid accumulation in the tubular epithelium along with exudates in Bowman's spaces in monkeys. In this paper the authors describe tubular epithelial reflux resulting from acute aflatoxin poisoning in guinea-pigs.


Studies were undertaken to examine the effect of dietary protein deficiency on the susceptibility of monkeys to aflatoxin injury. Young rhesus monkeys were kept on low and high protein diets and given aflatoxin in two doses in different groups. All monkeys in both dietary groups receiving 500 µg a day of aflatoxin developed fatty liver and biliary fibrosis in 16 to 30 days. Monkeys receiving 100 µg a day on low protein diets behaved similarly, whereas the corresponding high protein group was apparently healthy. It is concluded that dietary protein deficiency markedly enhances the susceptibility of primates to aflatoxin. The implications for the human situation are discussed.


Daily oral administration of high doses of aflatoxin to young monkeys fed a stock diet produced fatty livers and biliary fibrosis in four weeks. The pathological features are described in detail and the implications in experimental and human liver injury discussed.


The consumption of moderately poisonous levels of toxic groundnut meal by turkey poult's induced a degree of tolerance to aflatoxin. Poult's thus "conditioned" gained more than "unconditioned" birds or successively higher levels of aflatoxin, and survived and gained on rations which killed mature turkeys. Ingestion of aflatoxin led to changes in the serum electrophoretic patterns followed in a few weeks by a return to nearly normal patterns. Subsequent increases in aflatoxin intake induced only minimal changes in the serum pattern but the tolerance appeared unrelated to the hepatotoxic effect because extensive pathological changes developed in the liver.


Pathological changes in experimental aflatoxicosis in goat are described. Pathological changes included renal and hepatic degeneration and necrosis. These changes were observed in the pituitary, thyroid and adrenal glands in the aflatoxin fed goats. Considerable reduction in serum vitamin A level was also noted.


In diet of growing pigs, half the protein was from groundnut meal contaminated with aflatoxin. Pigs were given complete diet freely, or the protein portion in the amount eaten by the first group, with the non-protein portion given freely. With separate protein, pigs had aflatoxin B<sub>1</sub>, B<sub>2</sub> and M in liver, gall bladder, heart, muscle and kidney, and developed signs of poisoning. With complete diet there was no sign of poisoning, and no aflatoxin was detected in organs of the one pig killed of that group.


The excretion of aflatoxin components was determined in milk, urine and faeces of a lactating ewe after oral administration of a single dose (1 mg kg<sup>-1</sup>) of a mixture of anatoxins (B<sub>1</sub> 36%; G<sub>1</sub> 52%; B<sub>2</sub> 3%; G<sub>2</sub> 2%). About 90% of the total anatoxin excretion in the milk and urine occurred in the first 48 h. No aflatoxin was detected in milk after 6 days, and none in urine and faeces after 8 and 9 days respectively. Only 8.1% of the total dose was recovered in an identifiable form, the milk containing 0.1%, the urine 6.4% and the faeces 1.6%. Some unchanged anatoxin B<sub>1</sub> was present in the faeces but only trace amounts were found in milk and urine. Aflatoxin M, was excreted in relatively large amounts by all three routes, but mainly in the urine, and was the major form of aflatoxin in milk and faeces. Aflatoxin G, was excreted chiefly in the urine and faeces with only traces in the milk.


When rats were fed groundnut meal (made from groundnuts grown in the USA) for 10-12 months, a high incidence of hepatomas and renal damage was observed. Direct extension of hepatomas to the mesentery and metastasis to lungs occurred in several animals. Ducks fed the toxic meal (which was later found to contain between 100 and 3500 µg kg<sup>-1</sup> aflatoxin) for a month developed liver cell damage and bile duct hyperplasia. Lesions induced in ducklings by administering into the stomach by tube extracts of A. flavus cultures daily for five days were comparable to those induced with toxic groundnut meal.


This paper comprehensively reviews nutritional factors that influence responses of animals and humans to aflatoxin. The effects of nutrients on the metabolism and disposition of aflatoxins are also discussed.


Choline deficiency, partial hepatectomy, repeated biopsy, and iron injections, alone or in combination, did not result in liver tumor induction unless aflatoxin was superimposed at some point during the experiment. Serum protein levels were elevated in rats with tumors induced by aflatoxin-contaminated groundnut meal. It was concluded that cirrhosis was the only form of liver injury among those used in these experiments that influenced neoplasia.


Groundnut meal was extracted with methanol to remove over half the aflatoxin, or again with chloroform to remove all that could be detected, or it was not extracted. Groups of 10, 20 or 25 male Charles-River caesarean-derived rats aged 3 weeks were given freely (until they died) one of 4 diets with casein 7%, groundnut meal 34% and sucrose 32 to 40%, or one of 2 diets with casein 18%, no groundnut, and sucrose about 55%, all with minerals and vitamins. Lard, 12 or 20%, was added to give a total lipid level of 20%. From one diet with the twice-extracted meal and one with casein 18% were omitted the choline chloride that was in the other diets 0.2% for the first week, 0.1% for the second, and the vitamin B<sub>1</sub>2 50 mg per 100 g. Aflatoxin extracted chemically and biologically was for diet with whole meal 1500, with meal extracted once 400, and for other diets under 5 µg kg<sup>-1</sup>. To some of those diets was added urethane (ethyl carbamate) 0.1, 0.2, 0.4 or 0.6%. Liver carcinoma as described in the literature was found in 13 of 20 mice given 400 µg kg<sup>-1</sup>, and in 9 of 10 mice given 1500 µg kg<sup>-1</sup> aflatoxin. Lifespan ranged between groups from 219 to 568 days. The shortest was that of the group given 1500 µg kg<sup>-1</sup> aflatoxin and 0.6% urethane; the lower though still significant incidence of carcinoma in that group might depend on the short lifespan, but in other groups a lower incidence appeared to depend on the giving of both additives together. The effect of aflatoxin and counter effect of urethane were both related to dose. The less
potent carcinogen, urethane, caused malignant lymphoma and vascular lesions of liver and lungs. Choline deficiency caused nodular cirrhosis but no tumors.


This work is a study of the acute effects of aflatoxin B₁ in dogs. The dog has a remarkable susceptibility to aflatoxin B₁ by oral and intraperitoneal routes. Intraperitoneal administration resulted in the shortest survival time and the most profound pathological changes. However, massive single oral doses produced lesions of similar nature and intensity. Oral administration of aflatoxin in small, divided doses, over extended periods proved to be less toxic. The experimentally induced disease resembled hepatitis X in many aspects.


Male White Peking ducklings were given from 32 h old a purified diet with aflatoxin fractions B₁, B₂ or G₁, or were given extracts from infected groundnuts or wheat or from liquid cultures of Aspergillus flavus. Histopathological lesions produced in liver, necrosis of parenchyma and proliferation of bile ductule cells, are described and illustrated. Variations were only in degree and intensity. Each of the crystalline aflatoxins could cause lesions in liver identical with those produced by extracts and cultural extracts containing the toxins; aflatoxin B₁ had the greatest potency. Ducklings given infected groundnuts or wheat as 10 % of the diet, Otherwise of a chick starter mash, had similar lesions but of greater severity.


The short-term effects of dietary modifications on the response of ducklings to aflatoxin were determined. In the presence of aflatoxin, dietary supplements of 4 % of methionine, 1 % of arginine or 0.8 % of lysine, as individual additions, depressed weight gain and decreased mortality. The addition to the diet of 1 % arginine and 0.8 % lysine with, but not without, aflatoxin sharply decreased weight gain and increased mortality. The addition of glutathione or cysteine to the diet as sources of sulfhydral groups had no effect on toxicity. Autoclaving aflatoxin-contaminated groundnut meal decreased toxicity and markedly increased weight gains of ducklings over a 9-day period.


Studies on the effect of aflatoxin B₁ on the blood picture of albino rats and guineapigs revealed a depression in the total RBC and WBC counts, including haemoglobin content of blood. Toxin fed animals exhibited anisocytosis and poikilocytosis. The blood clotting time was prolonged. The implication of these findings with respect to anaemic syndrome in rats and guineapigs is discussed.


The effects of feeding aflatoxin B at a level of 50 µg day⁻¹ animal⁻¹ on the reproductive performance of albino rats was studied. No effect on the oestrous cycle of the adult was observed. The litter size and the birth weight of the young rats was unaffected. However, significant increase in intrauterine foetal resorption in the pregnant rat and significant decrease in the weaning weight of the young rats were observed in the group fed with aflatoxin.


Detoxification of groundnut flour with hydrogen-peroxide is feasible and effective in destroying aflatoxin. This treatment is now used commercially, particularly in the preparation of milk substitutes containing groundnut protein. A mixture of phosphine and ammonia can be used as a fungicide for treatment of groundnuts pods immediately after harvest for controlling growth of Aspergillus flavus. Aqueous ethanol can be used effectively to extract about 90 % of aflatoxin from split groundnuts without removing any significant amounts of fat. A study of excretory metabolites of aflatoxin in rats, guinea-pigs and monkeys showed considerable differences in the excretion of aflatoxin M and B between these species. In an exploratory study of the possible role of aflatoxin in infantile liver cirrhosis about 8 % of the urine samples examined contained 10-50 µg of aflatoxin B in a 24 h sample. The pattern of muscular damage in cirrhotic children was similar to the muscular damage caused by aflatoxin fed to albino rats.

Diets with 15 % toxic groundnut meal were made up and tested at 2 centres. All guinea-pigs given the diets died within 55 days. Histological changes in the liver were typical of groundnut poisoning. A diet used in 1957, containing 15 % toxic-groundnut meal, had caused oedema and deaths in guinea-pigs, and when tested it twice as toxic as the newly formulated feed. In breeding guinea-pigs there was no ill effect when they were given the new diet with hay to appetite. When the hay was restricted to 10 g the stock deteriorated and all guinea-pigs died of oedema disease.


Four groups of six 12-week-old male Friesian calves were fed diets containing 390 to 540 µg kg⁻¹ ochratoxin A, 320 to 500 µg ochratoxin A plus 12 to 13 µg kg⁻¹ aflatoxin B₁, 10 to 13 µg kg⁻¹ anatoxin B₁, or a control diet containing neither toxin. At the end of the 87-day experiment there was no evidence of kidney or liver damage in any group of animals as judged by serial plasma and urine enzyme assays, haematological examination including the assay of blood coagulation factors, gross appearance at autopsy or histopathological assessment at the light microscopic level. Nor was there any significant alteration in serum IgA, IGM, IgG, and IgG₂ levels or in litres to environmental bacterial and viral antigens. Trace amounts of ochratoxin A were detected in kidneys of five of the 12 calves exposed to this toxin, but all 12 kidneys contained residues of the metabolite ochratoxin alpha (less than 5 to 10 µg kg⁻¹). Traces of aflatoxins B₁ and M₁ were detected in the liver of one of the 12 calves exposed to aflatoxin B₁, but nine kidneys contained the metabolite aflatoxin M₁ (less than 0.01 to 0.03 µg kg⁻¹). There was no evidence of interaction between the two toxins.


Groups of 12 male A.D.R.I. mice of 20 to 25 g bodyweight were fed for at least 3 months on diets with no toxic groundnut meal or 15, 30 or 80 %. The meal had aflatoxins B₁ and G₁ (each 4500 µg kg⁻¹), and aflatoxins B₁ and G₂ (each 600 µg kg⁻¹). There was no ill effect on appetite or growth, and no gross pathological signs of poisoning were seen in carcasses at the end of the experiment.


Oxidised pyridine nucleotides were estimated by measuring the extinction coefficient at 260 mµ (F260) of the acid extracts of liver homogenates and serum of chickens during 6 weeks on a diet with 30 % groundnut meal contaminated with aflatoxins. The chickens were White Leghorns of 510 g liveweight at the start in 5 groups each of 6 males and 6 females; groups were killed after 1, 2, 4 and 6 weeks. Serum was tested by iodine agglutination. After 2 weeks on the toxic diet, liver extracts showed a significant depression of F260. The value returned to within the normal range after 4 weeks on the diet and remained constant until the end of the experiment. In serum values were also reduced after 2 weeks and remained low. The iodine agglutination test was negative after 1 and 2 weeks, showed a weak reaction after 4 weeks and a positive reaction after 6 weeks.


Male White Leghorns chickens (4 weeks old) were divided into 3 groups of at least 8 each. Half of each group got a diet with 30 % of toxic groundnut meal for 2, 4 or 6 weeks. The diet contained 3100 µg kg⁻¹ aflatoxin. The other half of each group were given a balanced commercial feed. Urocanase activity was detected in the blood serum of 3 of 9 chickens with signs of poisoning at 2 weeks. By the sixth week 5 of 8 poisoned chickens had urocanase in serum. In liver urocanase per g tissue was uniform throughout the experiment but in total liver its activity increased toward the end of the experiment.


The acute toxicity of aflatoxins B₁, M₁, and M₂ in one-day-old Pekin ducklings was estimated to be 12, 16 and 61.4 µg duckling⁻¹. Single doses of each of the three aflatoxins produced similar liver lesions. Whereas a high dose produced extensive haemorrhagic necrosis with little or no bile-duct proliferation, an intermediate dose induced slight bile-duct proliferation and varying degrees of hepatocellular necrosis and a low dose caused considerable bile-duct proliferation and extensive degenerative changes in liver cells. Renal congestion at low doses of M₁ and M₂ was accompanied by degenerative changes (vacuolation) of the convoluted tubules but frank necrosis was only seen with M₁. Aflatoxin B₁ even in a high dose, induced only slight degenerative changes of the tubules. None of the aflatoxins induced glomerular damage.

A comparative study of aflatoxicosis in crossbred Jersey calves and buffalo calves showed the greater susceptibility of buffalo calves. Clinically, icterus was present in both species. Other features of aflatoxicosis included weight loss, weakness, failure to thrive, ascites. Considerable reduction in erythrocyte sedimentation rate was observed in buffalo calves. Liver was pale yellow, enlarged and fixable with round border.


Weanling albino rats were divided into groups of six males and six females. They were maintained on a vitamin A-free diet, one group being fed ad lib. and the second being pair-fed with the first. Rats in the second group were given an oral supplement of 100 IU vitamin A animal$^{-1}$ day$^{-1}$. At the end of a feeding period of 9 weeks, a crystalline preparation of aflatoxin was administered in a single dose of 3.5 mg kg$^{-1}$ bodyweight. Male rats in the first group showed an increased susceptibility to the toxin as evidenced by rapid mortality and severe liver damage, midzonal in distribution. Their hepatic stores of vitamin A were severely depleted. Vitamin A-supplemented male rats showed only mild hepatic damage to the periportal parenchyma. Liver damage was minimal in female rats of both groups and was unaffected by the amount of vitamin A stored in the liver.


Groups of 9 W.A.G. rats of each sex and of average weight of 168 g were given for 200 days diets with 20, 40 or 80 % crushed groundnuts. Chemical and biological tests with ducklings showed that the nuts had 1000 µg kg$^{-1}$ aflatoxin. Ten male rats got a control diet with 20 % groundnut oilmeal. All rats that ingested groundnuts became ill and many died. Hepatomata were found in some rats in all groups, or there were signs of a precancerous state in the liver. Other lesions were in the bronchi, and in the testes of males which became impotent. Fertility of females was unimpaired. Intake of aflatoxin by the first 2 rats with tumors was 2350 and 1310 µg in 122 and 134 days. The lethal dose of aflatoxin for rats is considered to be 10000 µg per kg bodyweight.


The method of estimating aflatoxin by fluorescence and its extraction by chloroform from samples from Senegal of groundnuts contaminated with Aspergillus flavus are described. The extract was given to groups of Pekin Khaki ducklings (2 days old) to supply different amounts of the toxic substances for up to 7 days. Damage caused in the liver of the ducklings by aflatoxin is described. Most deaths of the birds occurred within 2 days. In the ducks dying early, there was generally necrosis of the liver. In those which died later there were sometimes signs of necrosis, but mainly there was some proliferation of cells. In survivors there was regeneration after initial necrosis. It is suggested that the toxin may contain different substances, one causing necrosis and the other proliferation. Relative toxicity estimated from fluorescence, number of deaths and types and degree of lesions found were not closely related.


Male Fischer rats were fed either a diet severely deficient in lipotropic agents or control diet which induced a borderline lipotrope deficiency. They were given a carcinogenic dose of aflatoxin B$_1$ over a 3-week period and were scarified at intervals of 1 day to 1 year later. A localized hyperplastic response of hepatocytes, measured by thymidine-$^3$H labeling in autoradiographs and by mitotic counts, was found as early as 1 day after administration of the full dose of aflatoxin in the rats fed the control diet. This response persisted up to the time of carcinoma development 6 months to 1 year later and was accompanied by histologic and histochemical abnormalities of the hyperplastic cells. The early hyperplastic response was not found in the livers of the severely deficient rats, but a few hyperplastic nodules developed after 6 months.


Charles River CD rats (4 to 5 weeks old) were given diets in which the chief source of protein was dried lean beef or extracted or commercial groundnut meal, the last from 4 lots of high-quality solvent-process meal. In the first trial, 13 of 59 rats given diets wim 6 or 7.9 % dried beef and 33.3 or 25 % methanol-extracted groundnut meal and 2 of 10 given 34 % groundnut meal alone developed hepatoma, but none of the 50 rats given beef as sole protein. In subsequent work, 64 of 73 rats given commercial meal developed hepatoma; that was not affected by substitution of 7.9 % casein for the beef or of lard for beef fat. In addition, 16 of those 73 rats developed adenomata of kidney tubules, not found in rats on the extracted groundnut meal. The hepatoma was of a type histologically different from that produced by choline deficiency and was not affected by choline intake.
The groundnut meal showed no sign of mold but the possibility of some infection by molds before the meal was prepared was not eliminated. The relatively low incidence of tumors in the first compared with the later trials suggested that the carcinogenic agent was decreased by extraction with hot methanol.


Samples of groundnut meals and decorticated nuts from Nigeria, French West Africa, and the Gambia were examined. Many were previously found toxic in trials with ducklings. Some of the meals were already suspect, being associated with the deaths of calves and sheep, others were not, and among those several were not toxic. Some extracts from decorticated nuts were also toxic. It was suggested that all consignments of groundnuts should be tested before inclusion in livestock feeds.


The toxic principle in certain samples of Brazilian groundnut meal was extracted and concentrated 250 times on a weight basis. This extract produced mortality in ducklings and turkey poults and histological liver lesions identical to those seen in field outbreaks of so-called Turkey "X" disease. The dosing of concentrated extracts to young ducklings gives a rapid and sensitive method for testing the toxicity or otherwise of groundnut meals. The toxic principle is neither a pyrrolizidine alkaloid nor the N-oxide of such an alkaloid. It is suggested that the toxic principle is derived from a microorganism, possibly a fungus. A similar toxic principle was found in some groundnut meals from India, Uganda and Tanganyika, French West Africa, Nigeria, Gambia and Ghana.


The toxic substance present in some Brazilian and African groundnut meals which causes Turkey "X" disease was shown to be even more toxic to ducklings. The signs included loss of appetite, poor growth rate, a tendency to down or feather pulling, liver damage and sometimes purple discoloration of the legs and feet in white-skinned birds over 3 weeks old. Occasionally accompanied by lameness. Not all groundnut meals tested were toxic. Toxic groundnut meal as 10 % of the diet caused death within 6 to 20 days. This interval and the severity of the gross lesions depended on the toxicity of the sample, the amount eaten and the age of the ducklings; younger birds were more susceptible. With chicks, 10 % groundnut meal reduced growth rate but did not cause death; with chickens 45 days old 15 % groundnut meal caused 3 deaths in the first 3 weeks but no more in the rest of the feeding period lasting 9 months.


A batch of guinea pig Diet 18 containing groundnut meal was suspected to be toxic; when fed to rats it induced liver tumours.


Toxic groundnut meals containing aflatoxin when fed to albino rats produced hepatic carcinoma. Morphological alterations in the liver induced by aflatoxin were studied in great detail. The biological picture of primary hepatic carcinoma in rats has been analysed. Probable mode of aflatoxin hepatic carcinogenesis is discussed. Attempts have been made to compare these experimental observations to those seen in naturally occurring primary hepatic carcinoma in man.


In the weanling male rat, a single oral LD2(1) of aflatoxin B1 caused a transient inhibition of leucine incorporation into liver proteins. The brief (6-h) suppression was followed by a prolonged period of stimulation of hepatic protein syntheses which persisted for 3-6 days after dosing. Liver deoxyribonucleic acid metabolism was not altered during this period. Five consecutive daily administrations of sublethal doses of the toxin, each equivalent to 10 % of the LD2(1), caused significant suppression of growth and liver weight in the duckling. Liver glycogen content and glycogenesis were also decreased, whereas lipid content was elevated. Treatment of weanling male rats according to the same dosing schedule resulted in suppression of growth and lowering of food intake, efficiency of food utilization, and liver weight; but liver composition was not significantly altered in this species.


The effect of a single dose of aflatoxin on mouse liver content of glycogen, lipid and protein of mouse liver was studied 2 h after injection of me toxin. No significant changes in these compounds were observed. Enzymes in livers of mice were assayed 2 h and 8 h after aflatoxin treatment. In general, the enzymes of particulate fraction and
isocitrate dehydrogenase in the supernatant showed a significant increase; the remaining enzymes of the supernatant fraction decreased at both time intervals. The response of the activities of aconitase, fumerase and ATP-ase 2 h after injection were the opposite of those at 8 h. The results are explicable in terms of mitochondrial injury of the aflatoxin- treated animals.


The activities of UDP glucose-glycogen transglucosylase, glycogen-phosphorylase, phosphoglucomutase, glucose-6- phosphatase, and combined HMP dehydrogenases were assayed and the incorporation of glucosc-U-14C into liver glycogen in vivo studied in aflatoxin B$_1$-treated and control chicks. A significant decrease in the activities of the first four enzymes and an increase in that of the last one were noted. The decrease in UDP glucose transglucosylase activity was of significant magnitude to account for the depletion of hepatic glycogen, while the catabolic phase of glycogen metabolism was almost unaffected in aflatoxin B$_1$ administered chicks. The increase in combined HMP dehydrogenases may probably be due to an enhanced HMP shunt pathway.


Diagnostic symptoms of Turkey X disease are described. These are based on the presence of histopathological liver lesions.


An outbreak of hepatoma in hatchery rainbow trout led to investigation of the effects of some feedingstuffs on the liver. The control diet was the Colorado diet, of spray-dried skimmed milk 5, delactosed whey 5, white-fish meal 16, solvent-extracted soya meal 16, wheatings 20, dried brewer’s yeast 10, fermentation solubles 10, unextracted liver meal 15, vitamins A and D in oil 2 and salt 1 % by dry weight, with vitamin premixes. In one test diet a solvent-extracted degossypolished cottonseed meal with 41 % protein replaced the soya meal, and to another was added crude toxic groundnut meal with 10000 µg kg$^{-1}$ aflatoxin. The diets were given for 12, 10 and 9 months, respectively, to replicate groups of 300 fish, and some of each group were killed after 6, 9 and 12 months. With the control diet the livers showed no significant pathological change. With the cottonseed meal diet some hyperplasia of bile duct epithelium and cholangitis and hyperplasia of bile duct epithelium in fish 6 months old which had had it for 3 1/2 months, progressing in the next 6 months until cytoplasmic degeneration, cholangioma, hepatoma, hyperchromatism and hypertrophic hepatocytes were common. Mixed cholangioma-hepatoma was rare.


Acute studies of ultrastructural and biochemical abnormalities induced by aflatoxin B$_1$ in the liver of rats and monkeys revealed abnormalities in nuclear fine structure. These were accompanied by decreased cytoplasmic RNA and protein content as well as a fall in nuclear protein levels. The early abnormalities in nuclear ultrastructure resembled those due to actinomycin and other agents which cause nuclear “capping”. The biochemical lesion primarily related to the nucleolar responses, however, requires further elucidation. Mitochondrial function, as determined by P.O ratios, was abnormal in early stages following aflatoxin administration, but it appeared that respiratory activity recovered a simultaneous and complete degree of phosphorylative capacity. The acute hepatic lesions in monkeys, by light microscopy, bore some resemblance to the changes in human liver in acute viral hepatitis. In chronic experiments, nucleolar changes such as those present in acute experiments were not observed. On the other hand, well differentiated hepatocellular carcinomas appeared in a significant number of animals whose livers otherwise showed little architectural abnormality.


Acute ultrastructural and metabolic changes were studied in the liver of rats and monkeys after one dose of aflatoxin. By 1 h, the nucleolus showed formation of nucleolar “caps” similar to those seen with actinomycin and lasiocarpine and followed by dispersion of nucleolar contents. Simultaneously there was proliferation of SER. Lesions became more severe at 48 h when fat droplets were present and accompanied by mitrochondrial degeneration. In non-necrotic cells, these changes reversed by 72 h. A corresponding decrease occurred in the RNA/DNA ratios in the homogenate and in the nuclear fraction. In monekys, while the cytoplasmic lesions were similar, the nucleolus showed unmasking of an additional granular component and other previously undescribed aggregates. In chronic experiments, rats were fed aflatoxin (1000 to 2000 µg kg$^{-1}$) for 30 weeks and studied serially. In contrast to the acute studies, changes were minimal and consisted of proliferation of SER; nuclear changes were not observed. Tumors occurred at 26 weeks in isolated foci. The acute results suggest a similarity in action to lasiocarpine and certain natural carcinogens.

Crystalline aflatoxin B₁, 100 µg in wheat germ oil, was given to 30 Peking ducklings 1 day-old, and 10 similar ducklings got only the wheat germ oil. One bird from each group was killed 1, 2, 4, 8, 16 and 24 h later and the rest after 72 h. Aflatoxin B₁ caused necrosis of the parenchymal cells of liver with focal haemorrhages. There was progressive decrease in activities of succinic dehydrogenase, alkaline phosphatase, adenosine triphosphatase, inosine diphosphatase and thiamine pyrophosphatase during development of the lesions, but an increase in acid phosphatase. Ultrastructure changes in the parenchyma are described. Mode of action of the poison is discussed.


A female rhesus monkey, which had received aflatoxin for a period of over 5 years, partly by intramuscular injection but principally by oral intubation, developed a metastasizing intrahepatic bile-duct carcinoma, which was detected at autopsy 5.25 years after toxin administration was discontinued.


The evidence to incriminate the possible role of microsomal drug metabolism system in aflatoxin toxicity in rat is presented. It is suggested that an effective drug metabolism results in decreased acute toxicity and the products of metabolism are more carcinogenic than the native toxin to the rat liver.


Young rhesus monkeys of 1.5-2.0 kg bodyweight were fed daily 1 mg of aflatoxin (containing 60 % aflatoxin B₁ and nearly 40 % aflatoxin G₁). They appeared unaffected at the end of the second week, when liver biopsies showed only slight changes. Between the 2nd and 4th weeks, all animals developed anorexia and apathy and within two days died in a coma. The liver lesions were similar to those described in ducklings.


Toxicity and carcinogenicity of aflatoxins in various laboratory animals are reviewed. Results of experiments on effects of pure aflatoxins on ducklings and rats are presented. Aflatoxin- contaminated diets are carcinogenic to rats, and aflatoxins in partially purified form or pure aflatoxin B₁, induced hepatomas in rats.


The information reviewed here emphasized the importance of the discovery of aflatoxins. This discovery, arising from astute observations in a seemingly obscure toxicity syndrome in poultry flocks, has led to increasing general attention to the possible significance of toxic mold metabolites as contaminants of foodstuffs and therefore as potential biological agents in problems of animal and human health. In addition, the potency of these compounds as toxic agents and as carcinogens should make them useful and powerful tools in investigations into the mechanism of toxicity and chemical carcinogenesis.


The influence of single oral doses of aflatoxin B₁ on the increase of liver tryptophan pyrrolase six hour following IP injection of 150 mg kg⁻¹ hydrocortisone or 600 mg kg⁻¹ tryptophan was studied in weaning male rats. Animals treated with 1 mg kg⁻¹ toxin and hormone showed no increase in enzyme activity 6 and 12 hours after toxin administration compared to approximately four-fold increase in those receiving hormone only. Although the effect at this dose level was not apparent 5 days later, larger amounts (3 or 5 mg kg⁻¹) of toxin caused inhibition of enzyme induction which persisted for at least 10 days. Animals treated with 5 mg kg⁻¹ toxin and tryptophan showed significant but incomplete inhibition of induction which was apparent after 24 hours and up to 10 days after treatment. These results indicate that the biochemical effects of aflatoxin B₁ include significant alterations in the ability of the liver to respond to hormonal regulation of enzyme activity.


Highly purified aflatoxin B₁ administered to male and female Fischer rats by intubation, or by feeding, induced hepatocellular carcinoma, and other liver lesions. Lesions of other tissues were also observed. Male rats surviving a single LD₅₀ dose (5000 µg kg⁻¹) of the compound developed persistent liver lesions, but no hepatocellular carcinoma, within 69 weeks after dosing. In males dosed with 400 µg administered in 10 equal daily doses, 4/24 animals developed liver cell carcinoma 35-82 weeks after treatment. A similar dose was ineffective in inducing carcinomas in females within an 82-week period, although preneoplastic liver lesions occurred at high incidence. When aflatoxin B₁ was fed continuously at a level of 1000 (ig in the diet, hepatocellular carcinomas were induced in 18/22 males within 35-41 weeks, but did not appear in females until 64 weeks. At a dietary level of 15 µg kg⁻¹, carcinomas were induced in 12/12 males after 68 weeks and in 13/13 females killed after 80 weeks. Feeding of the 1000 µg diet for only the 14 initial days of the
experiment resulted in minimal carcinoma incidence in both sexes 80 weeks after withdrawal.


Aflatoxin B₁ was added to a semi-synthetic diet at levels of 1.5, 15, 50 and 100 µg kg⁻¹. These diets were fed to male Fischer rats, beginning when their body weights were 80 g and continuing for up to 105 wk. A control group received the same diet without aflatoxin. Hepatocellular carcinomas were induced in a high incidence by levels of 50 and 100 µg kg⁻¹, and in a lower incidence by the other dietary levels. Tumors were induced in two of 22 animals fed 1 µg kg⁻¹ and pathological lesions were present in the livers of seven of the remaining survivors. These results suggest that, under these experimental conditions, the sensitivity of the Fischer rat to aflatoxin carcinogenesis is approximately the same as that previously reported for the rainbow trout.


The hepatic metabolism of aflatoxin in different species of farm animals, namely, cow, buffalo, sheep and goat was studied in vitro. A known level of aflatoxin B₁ was incubated with liver slices and concentration of unmetabolised toxin and its metabolite (aflatoxin M₁) were determined. The sheep and goat appeared to metabolise the toxin faster than the cow or the buffalo. These metabolic studies could be correlated with the relative species susceptibility to the toxin.


Human liver tissue culture preparations were exposed to different doses of aflatoxins B₁, G₁ and G₂. The LD₅₀ for aflatoxin B₁ was 1 mg kg⁻¹ 5 mg kg⁻¹ for aflatoxin G₁ and 16 mg kg⁻¹ for aflatoxin G₂. The autoradiography investigations revealed that human embryo liver cells in culture incorporated uridine and thymidine, indicating the ability of the hepatic cells to synthesize both RNA and DNA. The addition of aflatoxin B₁ to the cell culture in low concentrations resulted in an inhibition of both nuclear RNA synthesis and nuclear DNA synthesis by the parenchymal cells. It is concluded that the action of the toxin on the human liver cell is similar to that occurs in the rat liver cell.

2. ASPERGILLUS FLAVUS INFECTION AND AFLATOXIN CONTAMINATION IN GROUNDNUTS

2.1 REVIEW

The groundnut is unusual because flowers are formed and fertilized above the soil with subsequent fruit development in the soil. The subterranean pod is associated with the soil microflora over an extended period of time and thereby facilitating its invasion by fungi and other organisms. In several groundnut-producing countries extensive research has been done on various aspects of the geocarposphere fungal ecology. This research was accelerated by the discovery in 1960 that the fungus Aspergillus flavus could produce a toxic substance (aflatoxin) when growing upon groundnuts. Research institutions in a number of groundnut-producing countries placed a high priority on determining the stage or stages in crop production at which groundnuts are most likely to be invaded by the aflatoxin-producing fungus and became contaminated with aflatoxins.

The literature relevant to the invasion of groundnuts by A. flavus and subsequent aflatoxin contamination before harvest, during postharvest field drying, and during storage is critically reviewed in this section.

2.1.1 Preharvest

Several researchers in the USA and Nigeria studied fungal infection of groundnut fruits from the early stages of fruit development until harvest (Garren 1966, McDonald and Harkness 1964, McDonald 1970a, b). Garren (1966) reported a fairly well defined endogeocarpic myeoflora within the fruit as it developed in the soil, and suggested that a normal succession of soil fungi led to predominantly healthy pods with quiescent fungi. However, Garren concluded that a disturbance of the succession could lead to rotting of fruits. The balance could be disturbed by low calcium content or high organic matter content of the soil with the possibility of subsequent rotting of pods. Garren (1966) regarded A. flavus as a persistent species in the pod shell but of little quantitative importance in undamaged pods. The presence of a ‘natural barrier’ to invasion of undamaged pods was considered by Garren. However, the aspergilli were dominant in Mokwa, Nigeria (McDonald 1970a, b). These differences are probably attributable to environmental variation rather than qualitative differences in the soil mycofloras because most of the shell fungi are commonly present in groundnut soils in widely distributed groundnut production areas of the world (Borut and Joffe 1966, Joffe 1969, McDonald 1970b). These studies and several other investigations conclusively showed that while shells of immature and mature fruits were commonly infected by fungi including A. flavus, very little seed infection occurred at this stage (Jackson 1965, 1968, Ashworth et al. 1965, McDonald and Harkness 1964, Lyle 1966, Austwick and Ayerst 1963, Barnes 1971). Diener et al. (1965) conducted trials in Alabama, Georgia and North Carolina in the USA to determine the extent of shell and seed invasion at harvest in respect of A. flavus and other fungi. In 1963 in Alabama the percentage of seeds and shells invaded by A. flavus increased with time and maturity of the groundnut. In 1964, only low levels of A. flavus were found in seeds and shells in all three states, and low levels were attributed to abundant and well distributed rainfall and moderate temperatures. However, A. flavus was frequently isolated from seed and pods from 10 to 12 fields of different soil types in Israel (Joffe and Borut 1966). In the Gambia (Gilman 1969) A. flavus made up 14% of the fungi infecting seed of mature intact pods at lifting.

After visiting three groundnut-producing countries (The Gambia, Nigeria and Uganda) in Africa, Bampton (1963) reviewed the agricultural aspects of the problem of fungal infection of groundnuts in the field and subsequent aflatoxin production by A. flavus. He recommended procedures that might be used to determine when A. flavus infection of the kernels occurs. In the Gambia it was noticed that no sample taken from me soil showed evidence of fungal infection, with the exception of some plants which were suffering from rosette disease. Some aflatoxin contamination was found in kernels from the diseased plants. In Nigeria, A. flavus infection and aflatoxin contamination were detected in kernels from crops that had been left in the ground for approximately four weeks beyond maturity. Fungus and toxin were also detected in samples from a late-sown experimental crop that had been maintained for the normal length of time. Bampton reported analytical data from only a few of the samples collected in these countries, but complete results were given in confidential reports to the countries concerned. He concluded that A. flavus infection in the soil was unlikely to be of any significance.

McDonald and his coworkers made comprehensive investigations of the field occurrence of A. flavus infection and aflatoxin contamination in several groundnut varieties in Nigeria (McDonald and Harkness 1965, 1967). They conducted several serial harvesting trials at Mokwa and Kano Agricultural Research Stations, Northern Nigeria, in 1962, 1963 and 1964 to investigate the occurrence of aflatoxin in the groundnut crop at harvest, when lifted before, at, and after normal maturity. Crops
harvested at or earlier than the normal time were free from aflatoxin, but late harvesting usually resulted in aflatoxin contamination. The weather, as well as crop age, affected fungal infection and aflatoxin contamination. In the Kano trials there was an increase in the toxicity of the crop at harvest with increase in age. It appeared that the time of appearance of aflatoxin in the Kano crop depended on the occurrence of late-season drought stress (McDonald and Harkness 1967). At Mokwa the long wet season apparently delayed the appearance of aflatoxin in the crop. At Mokwa groundnut is normally harvested before the end of the rains, while in Kano it is harvested either at the end of the rains or later.

Data from Alabama, USA also demonstrated that a much higher percentage of A. flavus invasion occurred in overmature seed and pods than in immature and mature seed and pods from the same plants at harvest (Diener et al. 1965). In India, Mehan et al. (1986) showed that levels of A. flavus and aflatoxin B1 were much higher in seeds from overmature pods of several groundnut genotypes than in seeds from immature and mature pods, especially under drought stress conditions. Seeds become susceptible to A. flavus invasion when seed moisture content is below 30% (Dickens and Pattee 1966, McDonald and Harkness 1967). Drought stress, lowered seed moisture content, over-maturity, and decreased plant vigour in groundnuts are interrelated and moisture related, and these factors contribute to increased susceptibility to A. flavus invasion and aflatoxin contamination (Bampton 1963, McDonald and Harkness 1967, Mehan 1987).

Many investigators have concluded that pods with shells that were damaged while the crop was in the soil were more likely to contain toxic kernels than were pods with undamaged shells (Bampton 1963, Diener et al. 1965, Ashworth and Langley 1964, McDonald and Harkness 1967, Schroeder and Ashworth 1965). Kernels from broken pods had extensive fungal invasion when seed moisture content is below 30% (Dickens and Pattee 1966, McDonald and Harkness 1967). Drought stress, lowered seed moisture content, over-maturity, and decreased plant vigour in groundnuts are interrelated and moisture related, and these factors contribute to increased susceptibility to A. flavus invasion and aflatoxin contamination (Bampton 1963, McDonald and Harkness 1967, Mehan 1987).

A number of soil-inhabiting pests including pod borers, millipedes, mites, white grubs, termites and nematodes have been implicated in A. flavus infection of groundnut pods before harvest. The lesser cornstalk borer (Elasmopalus ligneillus Teller), a common pest of groundnuts in the USA, predisposes groundnut fruit to A. flavus infection (Dickens 1977, Wilson and Lynch 1984). A. flavus propagules may be carried by the insect to ideal infection sites where the kernels are damaged. Kernels from damaged pods often contain very high levels of aflatoxins. Another serious pest of groundnut in the USA, the southern corn rootworm (Diabrotica undecimpunctata howardi Barber), has been associated with increased fungal invasion of groundnut fruit (Porter and Smith 1974). The insect feeding sites on the pod provide portals of entry for A. flavus and other fungi.

Aspergillus flavus invasion and aflatoxin contamination in groundnuts have been widely associated with pod damage in the ground by termites in South Africa (Sellschop 1965), and in Nigeria (McDonald and ABrook 1963, McDonald et al. 1964, Johnson and Gumel 1981). Pod scarring by termites (Microtermes spp. and Odontotermes spp.) is particularly important in facilitating invasion by A. flavus. Additional research is needed to determine if there is a quantifiable relationship between the degree of pod scarring and infection of the kernels by A. flavus.

Several species of mites penetrate groundnut pods, feed on the kernels, and disseminate spores of A. flavus (Aucamp 1969). Several studies have been conducted on the potential involvement of nematodes in the aflatoxin problem in groundnuts in Georgia, USA (Bell et al. 1971, Jackson and Minton 1968, Minton and Doupnik 1969, Minton and Jackson 1967). However, no conclusive relationship between nematode infestation and A. flavus invasion or aflatoxin contamination was established.

Premature death of plants, particularly during pod development and maturity, from root and stem infections by pathogens such as Rhizoctonia solani, Sclerotium rolfsii, and Fusarium spp. increase the chance of seed contamination with aflatoxins (Ashworth and Langley 1964, McDonald 1970, Ahmed et al. 1989). Lesions produced by these pathogenic fungi facilitate invasion of seeds by A. flavus (Ashworth and Langley 1964, Schroeder and Ashworth 1965). Some virus diseases such as groundnut rosette and bud necrosis may also predispose groundnuts to invasion by A. flavus (Bampton 1963).

Rapid invasion of pods by A. flavus in the soil has been widely associated with mechanical damage to pods during field cultivations and at the time of harvest (Ashworth and Langley 1964, Bampton 1963, McDonald and Harkness 1967, Schroeder and Ashworth 1965). Kernels from broken pods had extensive fungal invasion and aflatoxin contamination (McDonald and Harkness 1963, 1964). Schroeder and Ashworth (1965), in the USA, found that kernels from pods with mechanical damage and growth cracks had higher levels of aflatoxin than those with rot and insect injury. Damage to shell or kernel provides an increased probability of rapid and direct invasion of the kernel by A. flavus, which in turn increases the possibility of aflatoxin formation. Damage also increases nutrient availability for rapid growth of A. flavus.

Mechanical injury, growth cracks, and pod damage by soil-inhabiting pests have received considerable attention in research on aflatoxin contamination, but less is known about associations between diseases of groundnut and preharvest aflatoxin contamination of seeds. Information on the incidence of A. flavus and level of aflatoxins in seeds from plants attacked by various pathogens in different groundnut-producing regions is needed.

Preharvest infection of groundnut seeds by A. flavus was previously attributed to depend largely on physical and biological damage to pods (Ashworth and
Late-season drought stress, particularly in the semi-arid tropics, is a major factor associated with aflatoxin contamination (Blaney 1985, Mehan 1987). An association between late season drought stress and increased *A. flavus* invasion and aflatoxin contamination in groundnuts was documented as early as 1965 in South Africa (Sellschop 1965) and has been confirmed by researchers in Nigeria (McDonald and Harkness 1967), in the USA (Dickens et al. 1973, Pettit et al. 1971, Sanders et al. 1981), and in India (Mehan et al. 1986, Mehan et al. 1988). In studies in Texas, USA, the levels of *A. flavus* infection and aflatoxin in groundnuts (cv. Starr) harvested from rainfed and irrigated plots in 1967 and 1969 revealed that drought had a significant influence on preharvest fungal infection and aflatoxin contamination (Pettit et al. 1971). The incidence of *A. flavus* was highest in kernels from rainfed plots in South Texas in 1967 and 1969, years during which moderate and severe droughts occurred. Aflatoxin levels in kernels harvested from rainfed plots 120 and 130 days after sowing averaged from 694 to 10,240 µg kg⁻¹ aflatoxin while either zero or trace amounts of aflatoxin were detected in kernels from irrigated plots. Davidson et al. (1983), in Georgia, USA, highlighted the role of drought stress in accelerating *A. flavus* infection and aflatoxin production in sound mature kernels. Aflatoxin contamination in kernels from three growers’ fields which had no, moderate, and severe drought stress averaged 6, 73, and 444 µg kg⁻¹, respectively (Davidson et al. 1983). However, some studies revealed that drought stress alone was not responsible for aflatoxin contamination since drought-stressed groundnuts were not always contaminated with aflatoxins (Hill et al. 1983, Wilson and Stansell 1983).

Recent reports from extensive research conducted at the USDA controlled environment plots near Dawson, Georgia, USA, provide additional information about the influence of soil temperature and moisture levels on the extent of *A. flavus* infection and aflatoxin contamination in groundnuts (Blankenship et al. 1980, Blankenship et al. 1983, 1984, Blankenship et al. 1989, Cole et al. 1984, Cole et al. 1985, Cole et al. 1989, Sanders et al. 1985). Cole et al. (1985) determined the optimum mean pod-zone soil temperature range to be 28-30.5°C for aflatoxin production in drought conditions during the last 30-50 days of the growing season. Cole and his coworkers reported no aflatoxin contamination in kernels of undamaged pods in plots with adequate irrigation (irrespective of pod-zone soil temperature), or from drought-stressed crops when the mean pod-zone soil temperature during the last 30-50 days before harvest was < 25°C or > 32°C (Sanders et al. 1983, Cole et al. 1985). Their results suggest that groundnuts subjected to drought stress may not be contaminated with aflatoxins unless drought is accompanied by mean pod-zone soil temperatures of 25-31°C during late stages of pod development. Even a small mean temperature change may significantly influence aflatoxin production in drought-stressed groundnuts. These researchers reported high levels of *A. flavus* infection (25-70 %) in different sizes of kernels, even in those from irrigated plots. The high levels of the fungus in kernels of undamaged pods from a well irrigated plot were not explained.

Sanders et al. (1985) conducted studies to determine the duration of end-of-season drought stress necessary for preharvest *A. flavus* invasion and aflatoxin production in groundnuts when mean pod-zone soil temperatures were in the optimum range (28-30.5°C) for aflatoxin development. Their results indicate that more than 20 days, but probably less than 30 days, of drought stress at soil temperatures optimum for aflatoxin development are required for preharvest aflatoxin contamination. Increased duration of drought and temperature stress generally resulted in increased percentages of kernels infected by *A. flavus* (Sanders et al. 1985).

Larger, more mature groundnut kernels require considerably longer drought stress periods to become invaded by *A. flavus* than do smaller, immature kernels (Sanders et al. 1985, Cole et al. 1989).

In some studies, lack of aflatoxin contamination in heavily *A. flavus*-infected kernels from heated irrigated plots (mean geocarposphere temperature of 34.5°C) has been interpreted to mean that adequate irrigation prevents aflatoxin production (Hill et al. 1983). This may be due to the effect of the high or low mean temperature on fungus metabolism rather than adequate irrigation. This is supported by the observations of Cole et al. (1985) and Sanders et al. (1983) who demonstrated that a drought stressed soil temperature mean of 31.3°C was apparently too high for aflatoxin production even with a high incidence of kernel invasion (> 50 %) by *A. flavus*.

Recent studies in the USA have demonstrated that drought and temperature stress conditions in the pod zone and not in the root zone predispose groundnuts to contamination with aflatoxin (Blankenship et al. 1989).

Reduced metabolic activity associated with decreased pod moisture content under drought stress seems to increase susceptibility of groundnuts to *A. flavus* infection. Another possible role of drought stress in preharvest fungal infection could involve suppression of microbial competitors of the aflatoxin-producing fungus by elevating the soil temperature in the pod zone.
Although results of various studies emphasize the importance of late-season drought stress in \textit{A. flavus} infection and aflatoxin contamination, very little is known of the effects of early, mid-season or multiple drought stress periods.

Pod splitting is another factor contributing to aflatoxin contamination. Pods maturing under fluctuating soil moisture conditions during seasons of inadequate or irregular rainfall, are prone to pod splitting. Seed in split pods are frequently invaded by \textit{A. flavus} and subsequently become contaminated with aflatoxins (Graham 1982).

Some research has been done on possible effects of calcium content of pods and seeds on preharvest aflatoxin contamination, but no definite relationship has been established (Cole et al. 1985, Wilson et al. 1985). If a relationship exists it could be a complex one because there is an interaction between drought and calcium deficiency.

Limited research has shown that the incidence of \textit{A. flavus} infection and aflatoxin contamination is likely to be much higher in groundnuts planted on light sandy and red sandy loam soils than in groundnuts planted on Vertisols (Graham 1982, Mehan et al. unpublished). This appears to be related mainly to the water potential and aeration in the soils; light sandy and red sandy loam soils have lower water-holding capacity and groundnuts grown on these soils are more prone to drought stress than those grown on Vertisols that have higher water-holding capacity. Light sandy and red sandy loam soils appeal to favor rapid proliferation of the aflatoxigenic fungus, especially under conditions of low water potential at which the activity of other microorganisms is minimal. More intensive studies are needed to determine interactions between moisture stress and \textit{A. flavus} invasion of groundnuts in Vertisols, and to determine the effects of other soil types on preharvest aflatoxin contamination of groundnuts.

It is well established that \textit{A. flavus} invasion can occur in soil during pod development and maturation; the fungus directly penetrates the pod wall or enters passages created by pod pests and disease/lesions. However, the exact mode of infection of groundnut fruit has not been fully elucidated. Some researchers in the USA (Lindsey 1970, Wells et al. 1972, Styler et al. 1983) have suggested that \textit{A. flavus} may invade the flowers, travel down the pegs and become established in the developing seed. However, recent studies in Australia (Pitt 1989) have failed to establish a definite link between flower and peg invasion, and between peg and fruit invasion. In comparative studies of the invasion of flowers, aerial pegs, and kernels by wild-type and mutant strains of \textit{A. flavus} or \textit{A. parasiticus}, Cole et al. (1986) in the USA have conclusively proven that preharvest \textit{A. flavus} infection and subsequent aflatoxin contamination originates mainly from the soil. They presented the following evidence in support of soil invasion as opposed to aerial invasion: (i) a greater percentage invasion of kernels rather than flowers or aerial pegs by either wild-type \textit{A. flavus} or mutants; (ii) significant invasion by an \textit{A. parasiticus} color mutant occurred only in groundnut from soil supplemented with the mutant, whereas adjacent plants in close proximity but in nontreated soil were invaded only by endemic strains of \textit{A. flavus} or \textit{A. parasiticus}; (iii) aflatoxin data from drought stressed, visibly intact groundnut kernels showed that samples from soil not supplemented with a mutant strain contained a preponderance of aflatoxins Bs (from wild-type \textit{A. flavus}) whereas adjacent samples from mutant-supplemented soil contained a preponderance of aflatoxins Bs plus Gs (from wild-type and mutant \textit{A. parasiticus}); and (iv) data from two air samplings showed no propagules of \textit{A. flavus} or \textit{A. parasiticus} in the air near the experimental site.

More research is needed to answer the important question “can flower and aerial peg invasion lead to significant invasion of groundnut fruit by \textit{A. flavus}?”; and, “can this occur under both normal and drought stress situations?” If would be interesting to determine if the fungus could become systemic in the groundnut plant.

### 2.1.2 During Postharvest Drying

At harvest, mature pods contain a complex of microorganisms, the endogeocarpic microflora, which includes \textit{A. flavus} (Garren et al. 1969). When these infested groundnut pods are lifted from the soil to be cured and dried, they are subjected to rapidly changing environmental conditions which cause shifts in the dominant and sub-dominant fungal species on and within the pods. The terms curing and drying have been defined as two distinct phases of change in groundnut composition following lifting (Blatchford and Hall 1963a). Curing of groundnuts is generally considered to occur after lifting during the period when the groundnuts are still attached to the haulms. It has been hypothesized that during curing several chemical and physical changes occur which influence kernel quality. The hypothesis is based on reported differences in seed germination, nutritional and taste qualities which develop during curing. Pods dried off the haulms are generally of reduced quality. The term “curing” thus relates to those as yet unidentified processes which terminate when the plants become dry or the groundnut pods are removed from partially dried haulms. The term “drying” is used to describe all phases of moisture removal from groundnuts, including the moisture lost during curing and after removal from the haulms. At lifting time, moisture levels in pods and kernels from living plants range from as high as 50 % to approximately 20 %, depending upon soil conditions. The most important environmental factor which influences the endogeocarpic microflora during curing and drying is pod and kernel moisture. When high moisture groundnuts are lifted and cured/dried in windrows there may be considerable invasion of seeds by \textit{A. flavus} and other fungi already established in the shell (McDonald and Harkness 1963), McDonald and A Brook 1963, Burrell et al. 1964, Bampton 1963, Jackson 1965, Gilman 1969, Troeger et al. 1970). This is
encouraged if drying is slow and seeds are in the very susceptible range of 12-30% moisture content for extended periods. In warm, wet weather the drying time is extended and the risk of aflatoxin contamination is increased (Jackson 1967b). A rain shortly after lifting is not particularly harmful, but a rain alters the groundnuts are partially dried, followed by poor drying, is likely to result in aflatoxin contamination (Troeger et al. 1970). Rains in the evening may keep the groundnuts wet all night, thus providing fungi with the needed moisture. Rains early in the morning are less likely to slow down drying and accelerate mold growth, because of effective daytime drying.

Several investigations of sun-drying methods in relation to moisture content of groundnuts have been done in two areas of Nigeria (Burrell et al. 1964, McDonald et al. 1964, McDonald and Harkness 1965). Groundnuts were harvested during the wet season in one area and during the dry season at the other location. In the wet area, where large differences occurred in the rate of drying between the various treatments, it was found that the slower rate of drying was associated with a higher incidence of A. flavus and other fungi and a higher incidence of toxic samples. When the pods were separated from the haulms shortly after lifting and then sun-dried in thin layers on matting with protection from rain, the drying rate was fast and pods were free of aflatoxin. Treatments in which the pods were field dried on the haulms did not give a toxin-free crop and the majority of the produce was of very low quality. In the area where the crop was lifted under dry conditions, no incidence of aflatoxin contamination was reported. In inverted windrows, the risk of aflatoxin contamination is probably less for groundnuts in inverted windrows than for those held in dryers without proper ventilation (Dickens 1977). Adequate procedures have been developed to prevent molding of sound intact groundnuts when dried in inverted windrows. Aflatoxin was present in the sun-dried material, whereas material artificially dried shortly after harvest was free of toxin.

Windrowing of lifted plants is the standard procedure for groundnuts produced in the United States (Dickens 1977). A digger-shaker inverter implement is used to dig the plants, shake soil from the roots and pods, and invert the plants. After several days, the partially dried groundnuts are removed from the haulms with a combine. They are then piled about 15 meters deep in dryer wagons with perforated floors and dried, by forcing heated air up through the pods, to achieve an average moisture content of less than 10% (wet basis). If the combine damages the pods, the groundnuts become more susceptible to subsequent mold damage than groundnuts in sound pods (Dickens and Khalsa 1967). Precautions should be taken to protect groundnuts from rain while they are in combine baskets, dryer wagons, or other containers in the field, and during transport to the dryer. A layer or batch of wet groundnuts in a dryer may mold before drying.

When drying capacity is inadequate, groundnuts should be left in the windrows rather than combined and held for drying. Even during periods of rain, the risk of aflatoxin contamination is probably less for groundnuts in inverted windrows than for those held in dryers without proper ventilation (Dickens 1977). Adequate procedures have been developed to prevent molding of sound intact groundnuts during bulk drying (Dickens and Pattee 1966).

### 2.1.3 During Storage

The number of ecological studies of storage fungi involving quantitative mold counts of populations is limited (Dierens 1960, Schroeder and Boiller 1971, Borut and Joffe 1966, Joffe 1969, Moubasher et al. 1980). In Alabama, USA, quantitative data on the mycoflora of seed from farmers' stock groundnuts stored in 26 farm-size bins were related to initial moisture, seed damage, type of bin, and length of storage. Species of the A. glaucus group, A. flavus group, and P. citrinum were dominant in the mycoflora of the seed of farmers' stock groundnuts stored for 8-56 months. The number and kind of fungi associated with seed stored for 1-6 months were determined (Welty and Cooper 1969). Initially, A. repens was isolated from 54% of the seed, Penicillium spp. from 80% and A. flavus from 7%. After 2-3 months of storage, the percentage of seed with A. repens and Penicillium spp. dropped to about...
10% and then gradually increased to 40% after 6 months. \textit{A. flavus} remained at 7% for the first 3 months, but after 4-6 months it was isolated from 18% of the seed.

In Israel, \textit{A. niger} was the most common species in 114 stored groundnut samples from the 1963 and 1964 crops (Borut and Joffe 1966). \textit{A. niger} occurred in 97.4% of the samples, while \textit{A. flavus} was detected in 78.4% of the 1963 samples and in 63.5% of the 1964 samples. However, \textit{A. niger} occurred in large numbers (40% of the total colonies) in 114 samples, whereas \textit{A. flavus} made up only 5.7% of the mycoflora of stored seed. \textit{A. niger} was the dominant species in the mycoflora of 419 samples of stored seed examined over a 5-year period in Israel (Joffe 1969), and \textit{A. flavus} was present in relatively small quantities in comparison with \textit{A. niger}. The high incidence of \textit{A. niger} in stored seed that were disinfected with mercuric chloride was considered to be attributable to selective removal of antagonists (Joffe 1968).

In Egypt, groundnut seed were adjusted to 8.5, 13.5, 17.5, and 21% moisture levels and stored for 6 months at 5, 15, 28, and 45°C (Moubasher et al. 1980). \textit{A. fumigatus} was the dominant fungus followed by \textit{A. flavus}, \textit{A. niger}, \textit{A. terreus}, and \textit{P. funiculosum}.

The main factors influencing the growth of \textit{A. flavus} and other storage fungi in groundnuts are moisture (relative humidity), temperature, time, and gaseous composition of the atmosphere. High mycofloral counts have been associated more often with high initial moisture contents of groundnuts going into storage than with any other factor (Diener 1960). Austwick and Ayerst (1963) studied the growth of \textit{A. flavus} and \textit{A. chevalieri} of the \textit{A. glaucus} group at different relative humidities and temperatures; they were unable to grow at less than 80% RH equilibrium. The literature on the influence of moisture, temperature, and other factors on the growth of \textit{A. flavus} in groundnuts has been comprehensively reviewed by Diener and Davis (1977).

\textit{A. flavus} infection and aflatoxin contamination may increase in groundnuts during storage until their moisture content drops below 9%. Increases in moisture from rewetting in storage or exposure to high humidity for extended periods of time result in rapid invasion by the aflatoxigenic \textit{A. flavus} with consequent aflatoxin contamination. Natural accumulation of carbon dioxide (CO$_2$) and decreased levels of oxygen (O$_2$) in closed storage reduce mycofloral development (Landers et al. 1967, Jackson and Press 1967). Low temperatures and uniform moisture distribution reduce mold growth and insect activity. Aeration is necessary to reduce aflatoxin contamination during storage. High relative humidity and temperatures, rain water leakage, condensation, and insect infestation are all important factors that contribute to aflatoxin contamination of groundnuts in storage (Diener and Davis 1977).

### 2.1.4 Aflatoxin-Producing Potential of Aspergillus flavus Isolates from Groundnuts

Various investigators have determined the aflatoxin-producing potential in groundnuts and nutrient media of isolates of \textit{A. flavus} obtained from groundnuts grown in various geographical areas. A number of researchers have qualitatively determined aflatoxin production on natural and nutrient media (Wallbridge 1963, Austwick and Ayerst 1963, Sreenivasamurthy et al. 1965, Rao et al. 1965, Borut and Joffe 1965, 1966). Austwick and Ayerst (1963) found that 52% of a selection of \textit{A. flavus} isolates from groundnuts from several African countries were toxin producers. Investigators in India (Rao et al. 1965, Sreenivasamurthy et al. 1965) reported that less than 6% of 179 isolates formed aflatoxin. In Israel, Borut and Joffe (1965, 1966) reported that 71.2% of 330 \textit{A. flavus} isolates from groundnut soils and kernels produced aflatoxin. In Egypt, El-Khadem et al. (1975) found over 50% of \textit{A. flavus} isolates from groundnut shells and seeds to be aflatoxin producers.

Many investigators have made quantitative determinations of aflatoxin producing ability of \textit{A. flavus} isolates. Diener and Davis (1966) reported aflatoxin production on groundnuts and in a nutrient solution from about 86% of a number of \textit{A. flavus} strains isolated from several natural substrates. Taber and Schroeder (1967) assayed 78 samples of Spanish groundnuts from nine geographical areas in Texas, USA, for aflatoxin and isolated 213 \textit{A. flavus} isolates, 107 of which were toxin producers. In Israel, Joffe (1970) reported that 89.6% of 1626 \textit{A. flavus} isolates from groundnut kernels (1084), rhizosphere (30) and geocarposphere (11), and from groundnut soils (501) were aflatoxigenic. These data indicated a remarkably uniform and high frequency of toxicity among such isolates, ranging in soils from 81.6% to 90%, in fresh kernels from 84.6% to 100%, and in stored kernels from 78.2% to 97.7%. A summary of data from investigations in the U.K., Holland, India, South Africa, and the USA indicated that 58% of 1390 isolates of the \textit{A. flavus} group were aflatoxin producers (Diener and Davis 1969). This included 330 isolates from Israel (Borut and Joffe 1965, 235 (71.2%) of which produced some aflatoxin. Some other workers have reported a very high frequency of aflatoxigenic isolates from groundnut kernels (Chen et al. 1988, Hasegawa et al. 1987, Sripathomswat and Thasnakorn 1981). Hasegawa et al. (1987) reported that 91.5% of 47 isolates of \textit{A. flavus} (from groundnuts imported into Japan) were aflatoxin producers. Chen et al. (1988) found that all \textit{A. flavus} and \textit{A. parasiticus} isolates from raw groundnut kernels from several areas of Taiwan were aflatoxigenic.

Isolates of \textit{A. flavus} and \textit{A. parasiticus} vary widely in the amount of aflatoxin produced on groundnuts, and in their capacity to produce different aflatoxins. Some isolates produce no aflatoxins (Codner et al. 1963, Diener and Davis 1966, Taber and Schroeder 1967). In the UK, Codner et al. (1963) tested 5 isolates of \textit{A. flavus}...
and one isolate of *A. parasiticus* for aflatoxin-producing potential on groundnuts. The *A. parasiticus* isolate produced 265 µg g\(^{-1}\) of a mixture of all four anatoxins, while *A. flavus* isolates produced from 14-162 µg g\(^{-1}\). In Texas, aflatoxin B\(_1\) production by 213 *Aflavus-oryzae* isolates from Spanish groundnuts ranged from 0 to 349 µg g\(^{-1}\) on a groundnut substrate (Taber and Schroeder 1967). In general, some isolates produced 8 to 10 times more aflatoxin B\(_1\) than B\(_2\); no isolate producing aflatoxins G\(_1\) or G\(_2\) was found. Isolates collected from groundnuts in seven southern states varied in aflatoxin B\(_1\) production from 0.07 to 17 µg g\(^{-1}\) in groundnuts (Diener and Davis 1969). This indicated that 8.4% isolates were probably *A. parasiticus*, which commonly produces all four aflatoxins (Diener and Davis 1977, Ogundero 1987). About 7% of the isolates produced less than 1 µg g\(^{-1}\), 70% produced from 1 to 100-125 µg g\(^{-1}\), and 23% produced more than 100-125 µg g\(^{-1}\) (Doupnik 1969). Table 2 summarizes data on aflatoxigenic isolates of *A. flavus* reported by various investigators.

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Country</th>
<th>Investigators</th>
<th>Substrate</th>
<th>No. of isolates Tested</th>
<th>Aflatoxigenic isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundnuts UK</td>
<td>Austwick and Ayerst (1963)</td>
<td>Groundnut</td>
<td>59</td>
<td>11</td>
<td>18.6</td>
</tr>
<tr>
<td>Groundnuts UK</td>
<td>Codner et al. (1963)</td>
<td>Groundnut</td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>Groundnuts C.C. UK</td>
<td>Wallbridge (1963)</td>
<td>N M</td>
<td>43</td>
<td>32</td>
<td>74.4</td>
</tr>
<tr>
<td>Groundnuts USA</td>
<td>Armbrrecht et al. (1963)</td>
<td>N M</td>
<td>10</td>
<td>7</td>
<td>70.0</td>
</tr>
<tr>
<td>Groundnuts USA</td>
<td>Diener and Davis (1966)</td>
<td>Groundnut</td>
<td>26</td>
<td>25</td>
<td>96.1</td>
</tr>
<tr>
<td>Groundnuts USA</td>
<td>Taber and Schroeder (1967)</td>
<td>Groundnut, rice</td>
<td>213</td>
<td>107</td>
<td>50.2</td>
</tr>
<tr>
<td>Groundnuts USA</td>
<td>Doupnik (1969)</td>
<td>N M</td>
<td>244</td>
<td>161</td>
<td>66.0</td>
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<tr>
<td>Groundnuts, Israel soils</td>
<td>Borut and Joffe (1965)</td>
<td>Groundnut</td>
<td>330</td>
<td>235</td>
<td>71.2</td>
</tr>
<tr>
<td>Groundnuts, Israel soils</td>
<td>Joffe (1969)</td>
<td>N M</td>
<td>1626</td>
<td>1463</td>
<td>90.0</td>
</tr>
<tr>
<td>Groundnuts, India groundnut meal</td>
<td>Sreenivasamurthy et al. (1965)</td>
<td>Groundnut</td>
<td>150</td>
<td>4</td>
<td>2.6</td>
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<tr>
<td>Groundnuts India</td>
<td>Rao et al. (1965)</td>
<td>Groundnut</td>
<td>29</td>
<td>6</td>
<td>20.7</td>
</tr>
<tr>
<td>Groundnuts India</td>
<td>Kang (1970)</td>
<td>N M</td>
<td>21</td>
<td>18</td>
<td>85.7</td>
</tr>
<tr>
<td>Groundnuts India</td>
<td>Subrahmanyam and Rao (1974)</td>
<td>N M</td>
<td>240</td>
<td>72</td>
<td>30.0</td>
</tr>
<tr>
<td>Groundnut seedlings India</td>
<td>Mehan (1979 unpublished data)</td>
<td>N M</td>
<td>17</td>
<td>16</td>
<td>94.1</td>
</tr>
<tr>
<td>Groundnuts Japan</td>
<td>Haseaawa et al. (1987)</td>
<td>Groundnut meal</td>
<td>47</td>
<td>43</td>
<td>91.5</td>
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<tr>
<td>Groundnuts France</td>
<td>Lafont and Lafont (1971)</td>
<td>Groundnut</td>
<td>26</td>
<td>26</td>
<td>100</td>
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<tr>
<td>Groundnuts Nigeria</td>
<td>Ogundero (1987)</td>
<td>Groundnut</td>
<td>10(^4)</td>
<td>6</td>
<td>60</td>
</tr>
</tbody>
</table>

\(^a\) NM = Nutrient medium

\(^b\) C.C. = Culture collection

\(^c\) From infected cotyledons of 'aflaroot' affected seedlings

\(^d\) Tour isolates of *A. parasiticus* and 6 of *A. flavus*; all 4 isolates of *A. parasiticus* produced aflatoxins
2.2 BIBLIOGRAPHY

2.2.1 ASPERGILLUS FLAVUS INFECTION AND AFLATOXIN CONTAMINATION OF GROUNDNUTS


This paper discusses causes of aflatoxin contamination and methods of prevention and segregation to control the aflatoxin problem in farmers’ stock groundnuts from the time the groundnuts are growing in the field until they are marketed, stored, and shelled.


This paper reviews seed infection by Aspergillus flavus and other fungi, and aflatoxin contamination of groundnuts from the time the groundnuts are growing in the field until they are marketed, stored and shelled. Causes of aflatoxin contamination and methods to control the aflatoxin problem are discussed.


Of the mycotoxins that have played important roles in human and animal health in the last century, aflatoxin is the most significant threat in modern agriculture. The aflatoxin-producing fungi, Aspergillus flavus and A. parasiticus, are omnipresent in soils and air throughout the world. Groundnuts, corn, and cottonseed and their products are constantly monitored for aflatoxin contamination. When environmental conditions are favorable for growth of the fungus, A. flavus may rapidly invade and contaminate groundnuts with aflatoxin in the field before harvest and after harvest during curing/drying in the windrow. Drought stress has been the factor most frequently correlated with aflatoxin contamination in groundnuts before harvest. Control of aflatoxin in groundnuts in the field can probably be accomplished most successfully with well-managed irrigation practices that prevent drought stress. The use of rotations, fungicides, and soil insecticides has not been demonstrated to control or greatly reduce pod invasion by A. flavus and/or aflatoxin contamination in the field or windrow. Breeding for resistance to A. flavus invasion and/or aflatoxin formation is a sound but long-term approach to control. Inversion of groundnut pods in the windrows reduces the probability of aflatoxin contamination during curing. However, groundnuts contaminated before harvest must be diverted from the human food chain by inspection or eliminated by sorting and other techniques during processing. Aflatoxin removal or inactivation by chemical treatments is feasible, but the product may be suitable for animal feeds and not for human consumption. Aflatoxin appears to be the only mycotoxin problem in groundnuts of any consequence at this time.


Groundnut samples, collected soon after harvest, from different districts in the irrigated region (Central Sudan) were free from aflatoxins. Samples collected from the rainfed region (Western Sudan) showed incidence of aflatoxin contamination ranging from 100 % samples in El Hamdi to only 10 % samples in Casgeal. Damaged pods were highly contaminated with Aspergillus flavus and aflatoxins. Sound intact pods had lower fungal contamination and were almost free of aflatoxins. Groundnut products (groundnut paste, red and grey roasted pods) collected from Khartoum North (Bahri) had higher levels of aflatoxin than those collected from Khartoum and Umdorman. Grey and red roasted pods showed higher levels of aflatoxins than the groundnut paste. None of the three varieties (MH383, Barbenton, Ashford) of groundnut tested was completely resistant to A. flavus contamination and aflatoxin production. A temperature of 30°C and 86.3 % relative humidity were the optimum conditions for both A. flavus growth and aflatoxin production in groundnuts.


The presence of aflatoxin in groundnuts was studied at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, 90 % contained aflatoxin. Aflatoxin B₁ at >1000 μg kg⁻¹ was found in 90 % of the samples, the level rising from the time of sale to milling, then falling after oil extraction.

314. Fonseca, H. 1976b. [Study of aflatoxin in groundnut from harvest to processing in the Santa Adelia region, Sao Paulo.]. Estudo da aflatoxina no
amendoim. da colheita a industrializacao, na regiao de Santa Adelia. A.P. Anais da Escola Superior de Agricultura "Luiz de Queiroz" 33: 385-393.

The presence of aflatoxin in groundnuts was examined at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, all but one contained aflatoxin. Aflatoxin B1 levels were very high in 31.6 %, and high in 55 % of the samples. Aflatoxin levels fell from the time of sale to milling. Five samples had >10,000 µg kg⁻¹ aflatoxin.


The presence of aflatoxin in groundnuts was examined at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, all but one contained aflatoxin. Aflatoxin levels were very high in 38.3 %, and high in 51.7 % of the samples. Aflatoxin levels rose from the time of sale to milling, then fell. Five samples had > 10,000 µg kg⁻¹ aflatoxin.


In the present work the occurrence of aflatoxin in groundnut in the region of Matao, Sao Paulo, was investigated at three stages from harvest to processing: (a) at the time the grower sells it to the oil mill; (b) during its storage prior to milling; and (c) after oil extraction (groundnut flour). At each stage two collections of 10 samples each were made. The conclusions were: (1) the majority of the samples (85 %) were toxic; (2) the toxicity level of samples, in terms of aflatoxin B₁, grew from stage (a) to stage (c) giving average values of 60 pg kg⁻¹ at the first and 1220 pg kg⁻¹ at the second stage. The mean value was 440 pg kg⁻¹; (3) good drying practices and proper storage can minimize aflatoxin levels and even prevent its occurrence; and (4) aflatoxin is already present when groundnuts are delivered to the mill.


Surveys were carried out for two years to determine the natural occurrence of mycotoxins (aflatoxin, ochratoxin and zearalenone) in some common Brazilian foods including raw and salted roasted groundnuts, pacoca (a very popular groundnut candy), peanut butter, maize, soybean, cured cheese, salami and copa (cured and dried pork). Eight characteristic regions, Vale do Paraiba, Mogiana, Paulista Velha, Araraquarense, Noroeste, Paulista Nova. Sorocabana and Vale do Ribcira/Litoral Sul, were surveyed, and 742 samples were analyzed. Estimation of toxins was made by thin-layer chromatography (TLC) by comparison with standards. Six samples of corn (4.7 %) were contaminated with aflatoxin, ranging from 41 to 2000 µg kg⁻¹ of aflatoxin B₁; 81 samples of pacoca (63.3 %), ranging from < 20 to 1187 µg kg⁻¹; 80 samples of peanut butter (62.5 %), from < 20 to 275 pg kg⁻¹; 17 samples of raw groundnuts (17.7 %), from 19 to 3125 µg kg⁻¹; and 9 samples of roasted salted groundnuts (9.4 %) from < 30 to 4250 µg kg⁻¹. From the first to the second year, the percentage of contaminated samples increased slightly for raw groundnuts (from 15.6 to 18.8 %) and decreased for pacoca (67.2 to 59.4 %) for roasted salted groundnuts (12.5 to 7.8 %), corn (6.2 to 3.1 %), and for peanut butter (84.4 to 40.6 %). The average content of aflatoxin of the contaminated samples increased for raw groundnuts from 166.7 to 641.7 pg kg⁻¹ and roasted salted groundnuts (410 to 980 pg kg⁻¹) and decreased for pacoca (165.6 to 108.8 pg kg⁻¹), peanut butter (91.3 to 50.4 µg kg⁻¹), and corn (853.3 to 700 µg kg⁻¹).


This review traces the connection between the mycology of foodstuffs and the onset of disease due to the toxins that various fungi produce within those foodstuffs. Particular emphasis is placed on the practical aspects, especially with reference to the physiological interactions among fungi, the incidence of fungi and fungal toxins in various substrates, the ecology of mycotoxin formation, and the acute and chronic effects of mycotoxicosis.


A chronological review is presented of research into the toxicity of groundnuts in Northern Nigeria caused by aflatoxin. Most results obtained up to 1963 have already been published. Investigations in 1964-65 included varietal resistance trials, studies of the mycoflora of groundnut roots and fruits, and trials on times and methods of lifting, methods of crop drying, and decortication in connection with the moisture content of the crop. Though it seems possible to develop varieties resistant to Aspergillus flavus, the standard varieties form the most resistant material at present available. An appendix by D. Halliday summarizes research on aflatoxin carried out by the Nigerian Stored Products Research Institute; 2 further appendices contain
recommendations to farmers on the avoidance of aflatoxin contamination, on the basis of conclusions drawn from the experimental work.


Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95% of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that *A. flavus* infection of kernels and aflatoxin production occurred in both zones. The condition of the shell had a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by *A. flavus*, whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20% for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were found to be free from fungal infection at lifting, infection by *A. flavus* not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of *A. flavus* in groundnut.


This paper outlines events leading up to the discovery of aflatoxin, describes briefly research done on the groundnut aflatoxin problem in the northern states of Nigeria, considers the implications of aflatoxin in relation to animal and human health, and discusses measures for elimination of aflatoxin from Nigerian groundnuts, or to at least greatly reduce incidence of aflatoxin.


The status of the global aflatoxin problem is reviewed with special reference to African groundnut producing countries, and research needs are highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus *Aspergillus flavus*.


Cultural and preharvest conditions and early postharvest conditions which influence the vulnerability of groundnuts to seed invasion by *Aspergillus flavus* and to aflatoxin contamination are discussed. Management practices, including chemical control and genetic resistance are considered.


The important published information on aflatoxin during the period January 1964 to March 1967 is summarized. The topics covered include (a) assay of aflatoxins, (b) toxicology of aflatoxins, (c) biosynthesis of aflatoxins, (d) biochemical and intercellular effects of aflatoxins, (e) metabolism of aflatoxins in animals, (f) incidence, control and detoxification, and (g) implication in man.


Twenty-six groundnut oil samples (12 unrefined, 7 refined and 7 from ration shops) were collected from the local market and assessed for *Aspergillus flavus* contamination. Three unrefined and three ration oil samples contained the fungus. Five of these six isolates produced aflatoxins in a nutrient medium. One isolate from unrefined groundnut oil was not toxigenic. When the ability of all isolates to produce aflatoxin in sterile groundnut oil was tested, the isolates did not produce any toxin during a 3-month period.


The toxic extract of Brazilian groundnut meal was further purified and a fluorescent method of identification after chromatographic separation was devised. The toxic substance was isolated from a fungus, *Aspergillus flavus*. When the fungus was grown on sterilized groundnuts and fed to ducklings, it resulted in typical liver lesions in ducklings.
History of control of aflatoxin levels in foods, especially groundnut products, in the USA is surveyed, and data presented show changes in aflatoxin contamination from 1967 to 1978 for raw shelled groundnuts, consumer groundnut products, almonds, pecans, and walnuts, and imported Brazil and pistachio nuts. The effect of establishment of an FDA action level of 0.5 ng aflatoxin M1 mL⁻¹ milk on aflatoxin concentration in market milk is discussed. Aflatoxin contamination of food is effectively controlled in the USA, with the possible exception of some cornmeal and corn grits products locally made and consumed in rural South Eastern USA.

2.2.2 Preharvest


The effects of irrigation regimes and date of harvesting on preharvest infection by Aspergillus flavus and aflatoxin contamination of seed of four commercial and two other groundnut cultivars were studied. Groundnuts watered at 1-, 2-, and 3-week intervals and harvested at the normal time, and at 1 week before, and 1 week after, were free from A. flavus infection and aflatoxin contamination. Aspergillus flavus infected a low percentage (2.7 - 7 %) of groundnuts left in the soil for 6 weeks after harvest. However, no aflatoxin contamination was detected. Wilt diseases and insect damage, mainly by white grubs and termites, predisposed seeds to preharvest A. flavus infection (56.4-69.8 %) and aflatoxin contamination (18-21 µg kg⁻¹). Groundnuts stored for 3 months in a well-ventilated room with an average temperature of 15°C were infected by A. flavus at a low level, but had no aflatoxin contamination. Infection increased with time in storage. Groundnuts harvested 1 week before maturity were not affected by A. flavus infection of seeds in storage, and there were no differences among genotypes. Groundnuts stacked in sacks at shelling sites were sampled; 4 % were contaminated with aflatoxin, the average level being 11 µg kg⁻¹. Fifteen per cent of the samples from oil mill sites were contaminated, the average aflatoxin content being 20 (ig kg⁻¹). Groundnuts left in the soil for 2-3 weeks after harvest in trials on the Gezira and Rahad irrigation schemes had 12 % of contaminated samples, with an average aflatoxin content of 10 µg kg⁻¹. This produce is usually allocated for local processing.


Results of 10 field experiments indicated that Rhizoctonia solani, alone and in combination with insect larvae, caused 87 %, and Seterotium rolfsii and unidentified agents 13 %, of the preharvest pod damage in Spanish groundnuts in Texas, USA. Kernel damage and the entry of fungi such as Aspergillus niger and A. flavus into the kernels was associated with the breakdown in pod structure, primarily by R. solani.


Aflatoxins were absent from freshly harvested groundnuts although Aspergillus flavus infected most of the kernels from pods with visible openings. Microbial competition, governed by kernel moisture, limited aflatoxin content of kernels. The toxins were subjected to microbial breakdown, but the amount of toxin destruction was governed by initial aflatoxin concentration.


Field surveys in northern Transvaal, the Republic of South Africa, showed large numbers of Astigmatid mites associated with the subterranean parts of the groundnut plants. Mites were usually heavily contaminated with spores of species of the fungus Aspergillus. Under suitable conditions, contaminated mites may enter the groundnut kernels, causing proliferation of the fungus and production of aflatoxin.


This article was written as an introduction to a series of research papers on the agricultural aspects of the problem of infection of groundnuts and subsequent aflatoxin production by Aspergillus flavus. It briefly reviews the problem in the widest sense, and then deals with the preliminary steps required to determine the point of entry of the fungus into the groundnut and subsequent development of toxicity.


Starr and Argentine groundnut cultivars were grown in soil fungicide-treated
Illustrations to show preharvest seed infection by *Aspergillus flavus*.
irrigated and non-irrigated plots in a 2 year study designed to determine possible-effects of management practices on invasion of developing pods and seeds by *Aspergillus flavus* and other soil fungi. Many genera and species of fungi were isolated from whole pods and half shells throughout each season, fungi were seldom isolated from kernels. No significant differences in populations between fungicide treatments, irrigation, or cultivars were found. Significant shifts in frequency of isolation of dominant genera (*Fusarium* and *Penicillium*) occurred during 1965 and 1966. *Trichoderma viride*, a minor component of the population at the first sampling in 1966, became a sub-dominant member by the last sampling. The reverse was true with *A. niger*. Several species of fungi, not previously isolated from groundnut pods, were found in pods in this study: *Actinomucor elegans*, *A. sclerotiorum*, *Sordidum fumicola*, *S. humana*, and *Sporormia australis*.


The role of groundnut growers, shellers and manufacturers of consumer groundnut products, together with USDA and FDA, played in forming a joint task force to develop a program of research into incidence, causes and control of aflatoxin contamination of groundnuts and groundnut products is discussed.


Studies were conducted to determine the effects of *Meloidogyne arenaria* and curing time on pod infection by *Aspergillus flavus*. Groundnuts of the cultivar Argentine were grown in field microplots containing methyl bromide-treated soil inoculated with either *A. flavus*, *M. arenaria*, or *A. flavus + M. arenaria*. Noninoculated microplots served as controls. Each treatment was replicated six times. At maturity the plants were dug, the pods rated for root knot galling, and one-third of the pods harvested. The remaining pods were left attached to the plants and placed on a wire-mesh greenhouse bench to cure. Additional samples were made after 5 or 12 days of curing. After each sampling, 25 two-seeded pods from each replicate were assayed for fungi. The remaining pods were dried for subsequent aflatoxin analyses. Pods from *M. arenaria*-inoculated plants were heavily galled, but the incidence of *A. flavus* and total fungi was not affected. The length of the curing time did not affect the incidence of *A. flavus* or total fungi. Only in pods from *A. flavus*-inoculated plants was the incidence of *A. flavus* increased. Aflatoxin contamination was not affected by any of the treatments. *M. arenaria* damage to groundnut pods did not affect *A. flavus* infection.


The effects of *Meloidogyne arenaria*, *Aspergillus flavus*, and curing time on infection of groundnut pods by *A. flavus* were studied. Plants were grown in field microplots of fumigated soil inoculated with either *A. flavus*, *M. arenaria*, or *A. flavus + M. arenaria*. Pods from *M. arenaria* inoculated plants were heavily galled, but the incidence of *A. flavus* and total fungi was not affected. Length of curing time also did not affect the incidence of *A. flavus* or total fungi. Only in pods from *A. flavus*-inoculated plants was the incidence of *A. flavus* increased. Aflatoxin contamination was not affected by any of the treatments. It was concluded that *M. arenaria* damage to groundnut pods did not affect *A. flavus* infection.


This paper considers climatic conditions in relation to mycotoxin contamination in different crops in Queensland, Australia. Queensland has a history of recurring drought, and drought stress is an important factor in preharvest aflatoxin contamination of the groundnut and maize crop, particularly in the drier Burnett region. Conversely, while drought depresses yields, dry conditions usually limit the extent of fungal damage to wheat, barley and sorghum. However, the 1983 season was exceptional because persistent heavy rain resulted in extensive mold damage to all crops. Mycotoxins produced by *Fusarium* spp. had not been implicated in widespread mycotoxicosis in Queensland, although they may contaminate maize and sorghum in regions with high rainfall during the growing season, e.g., the tablelands in Far North Queensland. Mycotoxicosis of man seems extremely unlikely to occur in Queensland, but mycotoxicosis of livestock occurs occasionally. It is usually associated with moldy feed that is stored in moist conditions. In addition, cases are likely to occur in drought stress seasons when feeds are scarce, and of low quality.


Six 18 ft x 40 ft plots with automatic mechanized roof systems for rainfall control were constructed. The 6-ft-deep artificial soil profiles in the plots were positively drained and protected from lateral soil moisture movement. Soil physical property data were measured and collected automatically. The facility was designed to provide absolute moisture control so that factors and relationships affecting *Aspergillus flavus* invasion of groundnuts in the field could be studied.
Six 66.92 sq m environmental control plots with automatic, movable shelters were designed and built at the National Peanut Research Laboratory, Dawson, Georgia, for the study of *Aspergillus flavus* invasion of groundnuts during drought stress. The shelters move over the plots at the onset of rainfall and off the plots when rainfall ceases. Plots were fitted with apparatus which allows soil temperature manipulation in the fruiting zone of groundnuts. Each plot has a separate shelter-mounted irrigation system. During operation, soil temperature and moisture data of the plots are collected automatically with a microprocessor-based, digital data-acquisition system.

Florunner groundnuts in research plots were subjected to 5 soil temperature and moisture treatment regimes resulting in *A. flavus* infection and subsequent aflatoxin contamination in drought-stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Mycopathologia 85: 69-74.


In 1980, an environmental control plot facility was designed and built at the National Peanut Research Laboratory, Dawson, Georgia, to study the preharvest invasion of groundnuts by *Aspergillus flavus* and subsequent aflatoxin production. Requirements for the planned research included the ability to induce drought and to manipulate soil temperature. Initially, the facility consisted of six, 12.2-m long x 5.5-m wide x 18 m deep, isolated plots with electric-motor-powered roofs for rainfall exclusion as required. Geocarposphere temperature manipulation was accomplished with thermostatically controlled, electrically heated cables; and cooling coils supplied with chilled water. Environmental data were collected using a microprocessor-based, digital data acquisition system that recorded conditions every 2 h during experiments. The facility was recently expanded to investigate the potential of the separate roles of plant stress and pod stress in aflatoxin contamination using two ancillary plots in which pod and root locations in the soil are separated and independently controlled. A micro-computer-based temperature control/alarm system was designed and installed to replace manual controls for soil temperature manipulation. The functional performance of the facility was adequate to provide a wide variety of required environmental conditions for research.

There was a low incidence of aflatoxin contamination in the 1962/63 Southern Rhodesian groundnut crop (50-250 µg kg⁻¹ aflatoxin B₁), whereas with the 1963/64 crop, an appreciable number of samples were contaminated with more than 250 µg kg⁻¹ aflatoxin B₁. This incidence occurred before marketing and was not aggravated by storage conditions used in Rhodesia. Although fungal infection might occur before harvesting, the major portion of aflatoxin contamination occurred in the period between lifting of the crop and delivery of the shelled groundnuts to the Grain Marketing Board. There was no evidence that the distribution of contamination was dependant upon the rainfall in the harvesting period. There appeared to be a higher level of aflatoxin B₁ contamination in areas with high air temperatures (65°F and above) in the harvesting period. The aflatoxin research program for the 1965 crop is described.
Samples of groundnuts were collected in the field prior to harvest, immediately after harvest, and from farmers' stock storage warehouses at widely separated points in Southwest Georgia, Alabama and North Florida. Samples were analyzed for aflatoxin contamination. Some groundnuts showing no visible hull damage (mechanical or insect damage) were analyzed just as pods came from the ground, and the kernels contained high levels of aflatoxin, but no mold was apparent, even when examined under a microscope. Extremely dry weather during the latter part of the growing season allowed the groundnuts to dry to the moisture level which was conducive for aflatoxin production in the field. Some groundnuts were dug, inverted and sprayed immediately with fungicides. Low levels of aflatoxin were present at digging and aflatoxin increased with exposure in the windrow even though they were sprayed with fungicides.

The allatoxin problem in groundnuts was first associated with poor windrow drying conditions and, to a lesser extent, improper storage due to condensation and/or roof leakage. Recent research has resulted in a re-evaluation of the etiology of the allatoxin contamination of groundnuts. Preharvest contamination is thought to be the most significant source of contamination with storage contamination being a less significant problem. The probable sequence of events leading up to preharvest contamination of groundnuts is outlined. This is based on detailed studies carried out over a number of years.

Groundnuts (cv. Florunner) grown three consecutive years in environmental control plots were subjected to various soil temperature and moisture treatment regimes resulting in Aspergillus flavus infection and subsequent aflatoxin contamination in some of the drought-stressed groundnuts. Treatments imposed beginning 85-100 days after sowing were drought, drought with heated soil, and drought with cooled soil treatments. The incidence of A. flavus in drought-stressed, unshelled, sound mature kernels (SMK) decreased with decreases in the mean 5 cm deep soil temperature. The incidence of A. flavus was greater in inedible categories and in damaged kernels than in sound mature kernels. The mean, threshold, geocarposphere temperature required for allatoxin development during the latter part of the groundnut growth cycle was between 26.3°C and 29.6°C with a mean geocarposphere temperature of 31.3°C, i.e., too high for aflatoxin contamination.

Previous experiments established the optimum conditions of temperature and moisture for preharvest aflatoxin contamination of groundnuts. The optimum conditions are a mean temperature in the geocarposphere of 29.5-30°C with a moisture level of between 40-60 bars. Visibly-undamaged groundnuts subjected to these stress conditions during the last 45-50 days of the growing season were highly contaminated with aflatoxin at harvest. The objective for CY 1983 studies was to determine the length of stress period required for preharvest contamination of groundnuts. Stress conditions were imposed 20, 30, 40 and 50 days before harvest. A stress period of 20 days before harvest was not sufficient to cause contamination. Groundnuts subjected to stress conditions for 30, 40 and 50 days were contaminated, therefore, a threshold stress period for preharvest aflatoxin contamination of groundnuts was 20-30 days before harvest.

In 1984, studies were conducted to evaluate the role of calcium in controlling preharvest aflatoxin contamination of groundnuts using the environmental control plots at Dawson, Georgia, USA. Florunner groundnuts were grown under soil environmental stress conditions optimum for preharvest aflatoxin contamination. Calcium treatments were 0, 58 and 230 lbs acre⁻¹ calcium as CaSO₄ added to pretreatment levels. The levels of calcium in kernels from the 0 and 230 lbs treatment levels were significantly different, reflecting a response to added calcium in spite of relatively high pretreatment levels of soil calcium. There were no significant differences in calcium levels of kernels between the 0 and 58 lbs calcium treatments. No significant relationship between aflatoxin contamination and kernel calcium levels was found. The authors concluded that application and uptake of calcium by the groundnut seed was not a viable method to prevent preharvest aflatoxin contamination.
of corn kernels and peanut pods. Developments in Industrial Microbiology 23: 229-236.

Invasion of groundnuts and corn by Aspergillus flavus and A. parasiticus and contamination with aflatoxin occur primarily under drought-stress conditions. However, the elevated soil (groundnut) and ambient temperatures (corn) are extremely important factors. In the case of groundnuts, irrigation may provide effective management by reducing soil temperatures in the geocarposphere. This same protection apparently is not available for corn since irrigation may not be adequate to sufficiently alter ambient temperatures sufficiently.


A comparison of the invasion of flowers, aerial pegs, and groundnut kernels by wild-type and mutant strains of Aspergillus flavus and A. parasiticus along with aflatoxin analyses of kernels from different drought treatments have supported the hypothesis that preharvest contamination with aflatoxin originates mainly from the soil. Evidence in support of soil invasion as opposed to aerial invasion was the following: (i) A greater percentage of invasion of kernels rather than flower or aerial pegs by either wild-type A. flavus or mutants, (ii) Significant invasion by an A. parasiticus color mutant occurred only in groundnuts from soil supplemented with the mutant, whereas adjacent plants in close proximity but in untreated soil were only invaded by wild-type A. flavus or A. parasiticus, (iii) Aflatoxin data from drought-stressed, visibly undamaged groundnut kernels showed that samples from soil not supplemented with a mutant strain contained a preponderance of aflatoxin Bs (from wild-type A. flavus) whereas adjacent samples from mutant-supplemented soil contained a preponderance of aflatoxins Bs plus Gs (from wild-type and mutant A. parasiticus), (iv) Preliminary data from two air samplings showed an absence of propagules of A. flavus or A. parasiticus in air around the experimental site.


Two new rapid methods that use monoclonal antibodies to detect aflatoxins in groundnuts were tested. The Quantitox is an enzyme immunoassay using microtitre wells and the Aflatest is based on monoclonal antibodies absorbed onto an affinity column. It was confirmed that aflatoxin contamination occurs preharvest and that storage under low-moisture conditions prevents additional toxin development. There were differences in susceptibility of local cultivars to aflatoxin development. The Aflatest procedure was useful to rapidly screen groundnut samples for aflatoxin contamination, but there were problems with the Quantitox method. Aflatoxin contamination occurred in the field prior to harvest and was not aggravated by storage conditions at Cleveland Depot during 1987. There was a 19.23 % incidence of aflatoxin contamination in the samples taken in early 1987, but it was not related to the farm management system (communal, small-scale commercial, and large-scale commercial) or any particular pest or disease.


Environmental conditions necessary for preharvest aflatoxin contamination of visibly sound groundnuts are reviewed on the basis of studies conducted at Dawson, Georgia. USA during six consecutive crop years using six environmentally controlled plots. The role of temperature and moisture in preharvest aflatoxin contamination of groundnuts was established. Preventive measures, including the use of so-called 'resistant varieties', calcium nutrition, and irrigation, were evaluated using environmentally controlled plots. The studies showed that groundnuts do not become contaminated with aflatoxins in the absence of severe and prolonged drought stress in spite of infection levels of up to 80 % by the aflatoxin-producing fungi, Aspergillus flavus and A. parasiticus. Also, larger, more mature groundnut kernels require considerably more drought stress to become contaminated than do smaller, immature kernels. Phytoalexin-based resistance can readily explain the resistance in immature kernels, but it does not explain the broader-based resistance observed in larger, more mature kernels. Studies during 1983 supported the hypothesis that preharvest contamination with aflatoxin originates mainly from the soil and not from the air via floral invasion.


Apparently undamaged groundnuts grown under environmental stress in the form of drought and heat become contaminated with Aspergillus flavus and aflatoxin in the soil prior to harvest. The upper mean temperature limit for aflatoxin contamination in undamaged groundnut kernels grown under drought stress during the latter 4-6 weeks of the growing season was between 29.6 - 31.3°C. The lower limit was between 25.7 - 26.3°C. Groundnuts grown under drought stress with a mean geocarposphere temperature of 29.6°C were highly contaminated while those at 31.3°C were not contaminated. Likewise, those grown under drought stress with
a mean geocarposphere temperature of 25.7°C were not contaminated while those subjected to a mean geocarposphere temperature of 26°C resulted in some categories becoming contaminated. Increasing the mean temperature to 29.6°C increased the amount of contamination.


Two runner type groundnut cultivars. Sunbelt Runner and Florunner, identified by a laboratory method as having large differences in seed resistance to colonization by Aspergillus flavus, were evaluated for resistance to natural seed infection by A. flavus and subsequent aflatoxin contamination. Groundnuts were grown on three nonirrigated farms during 1980 using two planting dates and three harvest dates for each cultivar. Groundnuts grown on two farms experienced moderate to severe drought stress and both cultivars contained high levels of aflatoxin. Groundnuts on the third farm had adequate rainfall and contained only very low levels of aflatoxin. Sunbelt Runner (reported to be resistant to A. flavus colonization of seeds) had no advantage over Florunner (reported to have only moderate resistance to seed colonization) in respect of levels of A. flavus and subsequent aflatoxin contamination under field conditions. Levels of A. flavus infection and aflatoxin contamination were related primarily to environmental conditions, especially drought stress, during pod maturation. These results show that the current laboratory assay method for selecting resistant lines should be carefully reassessed.


This paper discusses causes of aflatoxin contamination and methods of prevention and segregation to control the aflatoxin problem in fanners’ stock groundnuts from the time the groundnuts are growing in the field until they are marketed, stored, and shelled.


The geographical distribution of rainfall and of farms which produced segregation-3 groundnuts in North Carolina suggest that drought after groundnuts are formed but prior to harvest is conducive to infection by Aspergillus flavus before harvest. Damage from the lesser cornstalk borer (LCB) may also favor infection. However, many drought-area fields infested with LCB did not produce segregation-3 groundnuts. Aspergillus flavus growth and aflatoxin contamination probably occurred before harvest. Some groundnuts which contained visible A. flavus growth and high concentrations of aflatoxin were found on freshly-dug plants in two fields. Typical LCB damage was found on some pods in 96% of the samples taken from lots of segregation-3 groundnuts. The incidence of kernels with visible A. flavus growth (AFK) appeared to be related to pod condition. There were 9.4, 7.6, 2.5, 0.0 and 0.8 AFK kg⁻¹ of kernels from LSK, insect- damaged pods, mechanically-damaged pods, sound-mature pods and other pods, respectively. Kernels from LCB-damaged pods apparently had a much higher incidence of AFK than did kernels from pods with any other type of damage. Kernels with no visible damage that passed over a 15/64 inch slotted screen (R 15) contained an average of 149 µg kg⁻¹ aflatoxin. The portion that consisted of all damaged kernels in the samples and all kernels that passed through the screen contained an average of 10,018 µg kg⁻¹ aflatoxin. Even the R15 kernels from only the sound pods contained an average of 29 µg kg⁻¹ aflatoxin. Kernels from pods not damaged by LCB often contained aflatoxin.


Groundnuts were hand-picked from plants or ground immediately after digging and separated into immature, mature, and overmature-damaged categories. Groundnuts within each category were separated on the basis of pod color, appearance, and
location on the plant. Microfloral analysis showed that *Aspergillus flavus* was present only in the overmature-damaged groundnuts (100 spores g\(^{-1}\) of kernels). Two per cent of the mature and 8 % of the overmature-damaged kernels were infected with *A. flavus*.


The epidemiology of aflatoxin contamination in groundnuts, maize and cottonseed, the life cycle of the aflatoxin-producing fungus *Aspergillus flavus*, from sources of primary and secondary inoculum, the mode of entry of *A. flavus* into the plant, seed infection, and aflatoxin formation in the seed is reviewed.


This review discusses factors influencing production of aflatoxin in groundnuts including: the aflatoxin-producing fungus *Aspergillus flavus*, the substrate, relative humidity, moisture, temperature and time, aeration, the pod, and damage to the kernel.


The extent of pod (shell) and kernel invasion by *Aspergillus flavus* and other fungi by digging time was determined in groundnut fields in Alabama, Georgia, and North Carolina. Groundnut fruits were classified into maturity, damage, and discoloration categories. Pods were surface-sterilized, shells and kernels plated on Czapek-Dox agar medium containing 20 % sucrose, and incubated at 26-30°C for 4 to 7 days. Data from Alabama in 1963 showed that the percentage of kernels and shells invaded by *A. flavus* increased with time and with maturity of the groundnuts. In 1964, low levels of *A. flavus* were found in kernels and shells in all three States. Low infections were attributed to abundant and well-distributed rainfall and moderate temperatures.


Cultivar Florunner groundnuts were grown in an environmental control plot at the National Peanut Research Laboratory, Dawson, USA. Plants were subjected to late-season drought stress under conditions favoring preharvest aflatoxin contamination. Individual plants were harvested during the drought period, and single-kernel analyses were performed for aflatoxin contamination. An association between aflatoxin-contaminated kernels and certain individual plants was observed. Of the 1340 kernels analyzed from 44 plants, only 3.5 % had > 10 µg kg\(^{-1}\) aflatoxin, but 87.2 % of those kernels came from 9.1 % of the plants. Over half of the contaminated kernels (> 10 µg kg\(^{-1}\)) came from one plant. Of the kernels that contained > 10 µg kg\(^{-1}\) aflatoxin, 42.6 % were from yellow 2 hull-scare maturity stage pods, and 78.8 % were confined to the immature yellow 1, yellow 2, and orange A stages combined. Only 17 % were from the mature brown and black maturity stages. There was not a strong relationship between aflatoxin contamination and the location of the kernel in the pod. Both kernels were contaminated in 45.5 % of the pods containing kernels with > 10 µg kg\(^{-1}\) aflatoxin. In 21.1 % only one apical kernel was contaminated and in 15.2 % contamination was confined to the basal kernel. One-kernel pods accounted for 18.2 % of the contaminated kernels.


Since 1962, a research team in Rhodesia has studied the factors that may affect the incidence and level of aflatoxin contamination of groundnuts and ways of dealing with the aflatoxin problem. This report gives a brief account of the research projects and discusses pertinent results. Among these is the possibility of predicting levels of aflatoxin contamination of the crop resulting from different meteorological, seasonal and cultural factors.


In the Gezira, the Sudan, field experiments showed that *Aspergillus flavus* and other fungi such as *Penicillium* spp. and *Fusarium* spp., were always present in kernels from broken groundnut pods. On watering before harvest, the kernel moisture content immediately after lifting was 58.7 % for the cultivar Ashford, and 46 % for the cultivar Barberton, but after 7 days in the windrow the moisture content dropped to 4.7 and 3.8 %, respectively. Groundnuts dried immediately after harvest for at least 7 days were considered to be free from aflatoxin.


Groundnut samples, collected soon after harvest, from different districts in the
irrigated region (Central Sudan) were free from aflatoxins. Samples collected from the rainfed region (Western Sudan) showed incidence of aflatoxin contamination ranging from 100% samples in El Hamdi to only 10% samples in Casgeal. Damaged pods were highly contaminated with \textit{Aspergillus flavus} and aflatoxins. Sound intact pods had lower fungal contamination and were almost free of aflatoxins. Groundnut products (groundnut paste, red and grey roasted pods) collected from Khartoum North (Bahri) had higher levels of aflatoxin than those collected from Khartoum and Um Dorman. Grey and red roasted pods showed higher levels of aflatoxins than the groundnut paste. None of the three varieties (MH383, Barberton, Ashford) of groundnut tested was completely resistant to \textit{A. flavus} contamination and aflatoxin production. A temperature of 30°C and 86.3% relative humidity were the optimum conditions for both \textit{A. flavus} growth and aflatoxin production in groundnuts.


In Virginia (USA), \textit{Trichoderma viride} appears to be dominant, and \textit{Penicillium} spp. sub-dominants, in the climax endogeocarpic community of sound and rotting pods, \textit{Aspergillus flavus} and \textit{A. niger} being minor but persistent and potentially dangerous. Pathogenicity tests suggest a stage in fruit growth when factors as yet unknown weaken the natural resistance of the pod to invasion by rot fungi so that it becomes susceptible to several efficient saprophytes from the geocarposphere. \textit{Pythium myriotylum} was indicated as the prime pod-rot pathogen and \textit{Rhizoctonia solani} as sporadically important.


Field infections of groundnuts and corn by the toxigenic \textit{Aspergillus flavus} were studied in plots of an irrigation study. April-September rainfall in the study area was 40% of normal. Prior to the 1980 harvests the hidden ("unseen") \textit{A. flavus} infections in freshly dug groundnuts - i.e. pockets of infection from which the mold proliferates when groundnuts are not handled properly - varied from none to 15%. On 10/2/80 this hidden infection was 0.75% in irrigated groundnuts and 3.5% in non-irrigated groundnuts. Visible infections (the hallmark of "Seg.3") had never been reported at digging in Virginia before 1980, but in 1980 visible \textit{A. flavus} infections were found on many windrowed groundnuts (pods) within a few hours after digging in the non-irrigated plots. Corn taken directly from the field to the laboratory had much more \textit{A. flavus} infection if it came from non-irrigated plots, but careful handling of the irrigated corn was indicated. Hot, dry weather induced \textit{A. flavus} infection in the irrigated corn. The 1980 droughts' effects on aflatoxin formation in corn and on aflatoxin potentials in groundnuts increased the area's 1980 economic woes.


The mycotoxin potential of a plant product can be gauged by seeking answers to two or three questions basic to the mycotoxin problem, namely, 'Which of the fungi invading the product can be toxigenic ?', and 'How can they become established in the product?' Both questions relate to the quite new science of microbial synecology. In contrast, the third basic question ('What factors are necessary for toxin production by a given toxigenic fungus?') relates to the much older but more complex science of microbial autecology. Fruits of groundnuts are completely hypogeic in their development, and there seems to be a successional invasion of them by soil-borne or 'field' fungi with the result that sound mature fruits have a dormant or quiescent endogeocarpic microflora community. Results of co-operative research in six groundnut producing states (U.S.A.) and at the University of Minnesota, interpreted in the light of present-day groundnut harvesting practices in the U.S.A., justify the following conclusions: (1) \textit{Aspergillus flavus} can be toxigenic under field conditions and is a serious problem. (2) Many other fungi from the groundnut endogeocarpic community are toxigenic under laboratory conditions. (3) Until the conditions under which these fungi may become toxigenic are better known, their presence in lifted groundnuts constitutes a real mycotoxin potential. (4) The genera posing the greatest threat are, in estimated order of importance, \textit{Penicillium}, \textit{Aspergillus} (primarily because of \textit{A. flavus}), \textit{Alternaria}, and \textit{Fusarium}. (5) Much care must be exercised with groundnuts after lifting, to minimise opportunity for proliferation of such fungi. This care must be exercised at least until we determine the extent to which such fungi might be expected to produce toxins in groundnuts on the farm and during subsequent handling.


Studies in the Gambia showed that mature kernels inside visibly intact pods of the groundnut varieties 'Senegal' and 'G.O.M.B.' were frequently associated with the fungus \textit{Macrophomina phaseolina} before and directly after harvest. Some mature pods were occasionally contaminated with the fungus \textit{Aspergillus flavus} at lifting. The degree of invasion by these fungi was influenced, among other things, by soil moisture, shell and kernel maturity, correct lifting techniques, and the intensity of sun drying. Postharvest invasion increased with higher rainfall intensities. In general, invasion patterns were independent of varieties.

370. Goarin, P., and Goarin, S. 1970. (Contribution to the knowledge of
Research conducted mainly in Senegal on the significance of aflatoxin contamination of groundnut, methods of analysis, and control measures are reviewed. Contrary to the general assumption that high aflatoxin contents are associated with wetting of crop produce, in northern Senegal it was found that aflatoxin contamination was attributed to failure of crops to attain physiological maturity during dry years. Less arid production area, where infection is less, improved crop drying and possibly grading of produce should allow the production of acceptable edible or confectionery groundnuts and even groundnut meals. In general, the use of varieties better adapted to the environmental conditions, and careful harvesting, will keep the aflatoxin contents of groundnut and oil-cakes at an acceptable level.


The problem of aflatoxin contamination, which was serious in the Queensland groundnut industry in drought years, is reviewed with special reference to factors influencing aflatoxin formation including moisture stress, insect damage, and cultivar resistance. Control measures included crop hygiene, resistant varieties, avoiding moisture stress, harvesting the crop at optimum maturity, avoiding pest and mechanical injury, drying rapidly and segregating contaminated batches of groundnuts.


Under greenhouse conditions, pegs of Virginia Bunch 46-2 groundnut plants were introduced into small pots containing a nonsterile loamy line sand artificially infested with washed conidia of a clone of Aspergillus flavus isolated from groundnut fruit. Dilution plate analysis indicated the inner 0.5 mm layer of geocarposphere soil of pegs and mature fruits had A. flavus populations slightly different from nongeocarposphere soils. In contrast, the population of total fungi was greater, and the populations of bacteria and actinomycetes were much greater, in geocarposphere soils. Microscopic observation of the inner 0.5 mm soil layers indicated that no germination of A. flavus conidia occurred in peg geocarposphere soil, and trace germination occurred in geocarposphere soil for plants maintained in a growth chamber at 30°C. But A. flavus conidia germinated readily in soil adjacent to pods after 16 h at 30°C and 35°C when a 4- to 6- sq mm area of pod surface was superficially injured and inoculated with infested soil. Dry conidia applied to aerial portions of pegs in the greenhouse germinated at a low percentage. These results are discussed in relation to the exogenous carbon and nitrogen requirements for spore germination.


Selective media were used to isolate Aspergillus flavus and fungi of the A. niger group from aerial groundnut pegs in the field during 1973,1974, and 1975 and from groundnut flowers in 1974 and 1975. Aspergillus flavus was isolated from about 7 % of washed groundnut flowers in both years, but isolation frequency from terminal portions of washed aerial pegs and surface-sterilized aerial pegs over the same period was lower (1.5 % and 0.3 %, respectively). In 1973, A. flavus was isolated from 0.2 % of surface-sterilized aerial pegs. Isolation frequency of antagonists of A. flavus in the A. niger group was lower than A. flavus on flowers and similar on aerial pegs. Low levels of colonization of groundnut fruits by A. flavus via flower and aerial peg colonization appears possible under Virginia field conditions.


A preliminary survey of the incidence of aflatoxin in the main groundnut production areas of the Sudan is described, correlating the extent of infection with Aspergillus flavus and other kernel fungi with the aflatoxin content of the kernels. In general, the rainfed samples had a higher incidence of kernel contamination than those from the irrigated areas. The majority of the groundnut samples from the irrigated areas were aflatoxin-free, whereas in those from the rainfed areas none was free, and 48 % were classified low-medium (<50 to 50-250 µg kg⁻¹ aflatoxin), while 52 % were in the high to very high category (250-1000 µg kg⁻¹ to > 1000 µg kg⁻¹ aflatoxin).


Groundnut is an important crop in the Sudan, both in rainfed areas where end-of-season droughts are common, and under irrigation in the Gezira. Quality factors are important in determining acceptability and market price. Aflatoxin contamination is a problem in the rainfed crop. Aflatoxin levels are important in determining quality grade and price. The groundnut marketing system is described. A detoxification plant is under construction and will be used to detoxify groundnut cake and meal.
Results showed that 84 % of groundnut pegs contained fungi before entering the soil; shell invasion remained high (90-100 %) throughout the season and seed invasion rose to 82 %, declining at harvest. The most common shell fungi were *Penicillium*, *Fusarium*, and *Phoma* spp., and seed fungi *Penicillium* and *Gliocladium* spp. Groundnut fungi can be ecologically grouped into an aerial flora, terrestrial flora, and species colonizing fruits above the soil and in the soil.

Intensive studies of the mycoflora of "Dixie Spanish" groundnuts grown under different combinations of tillage, organic matter, and previous crop revealed no consistent reduction in either total fungi, *Aspergillus* spp., *A. niger*, *A. flavus*, *Penicillium* spp., or *Fusarium* spp. for any of the 16 combinations studied. The level of fungal invasion in apparently sound groundnut shells and seeds was consistently high, but the species composition of this flora changed as the season progressed. *Penicillium* was the most common genus, being isolated from over half of the shells sampled. The level in seeds was lower, but in both shells and seeds there were more *Penicillium* spp. in fruits in the soil than in fruits from stacks. *Aspergillus* spp. occurred at lower levels than *Penicillium*, but they also decreased in fruits in the stack. Of particular interest is that at all three collecting dates the level of *Aspergillus* isolated from seeds was at least twice that from shells. The lower frequency of certain fungi in fruits from stacks is undoubtedly related to the lower moisture level in the drying fruits. Other fungi, especially *Fusarium* spp., increased sharply in the dried tissues so that the overall level of invasion remained high. This may be of considerable practical importance since certain species of *Fusarium* produce toxins.

Current information is reviewed, with special reference to work on the mycoflora of groundnuts and pecans and factors which favor invasion of seeds by *Aspergillus flavus* and the production of aflatoxin.

Cultivar Florunner groundnuts were grown for 145 days in experimental plots in 1980. Different treatment regimes, imposed 94 days after sowing were irrigated (I); irrigated with heated soil (III); drought-stressed (D); and drought-stressed with cooled soil (DC). Soil temperature and moisture tension at 2", 12" and 24" below the surface were measured throughout the growing season. At harvest, the incidence of the *Aspergillus flavus* group within kernels and aflatoxin concentration were increased by any kind of damage for all treatments. In sound mature kernels (SMK) colonization by the *A. flavus* group was greatest with treatment D (75 % kernels colonized), least in I (7 %) and DC (11 %) and intermediate for III (26 %). Aflatoxin was absent from or negligible (<1 µg kg$^{-1}$) in SMK with 1, 11f or DC treatments but there were 244 µg kg$^{-1}$ aflatoxin in D treated SMK. The proportion of immature and damaged kernels at harvest was increased by drought-stress and decreased by irrigation but little affected by temperature. More aflatoxin was found in immature than mature sound kernels. Extensive colonization of SMK by the *A. flavus* group, and subsequent aflatoxin production, was favored by hot, dry conditions when most associated microorganisms did not grow. Elevated temperature alone or drought stress alone did not cause aflatoxin contamination in SMK. When the ratio of SMK colonized by *A.flavus* compared to *A. niger* was > 19:1 there was aflatoxin contamination, but no aflatoxin if this ratio was < 9:1. Irrigation is recommended to prevent aflatoxin contamination in groundnuts.
The mycoflora in soil clinging to dry pods of groundnuts (cv. Argentine) was changes of sterile water. Of the nine principal fungi, Aspergillus niger, A. flavus, A. parasiticus, Sclerotium bataticola, and Rhizopus spp. occurred in large numbers and either increased or slowly decreased in numbers in subsequent dilutions. In some instances variations in numbers followed trends in percentages of soil and organic particles less than 0.016 mm in size. When dry pods with this known mycoflora were allowed to hydrate over a 6-day period at 26°C, 32°C, or 38°C, there was extensive pod penetration and kernel infection by A. niger, A. flavus, S. bataticola and Rhizopus spp., but not by other fungi. The degrees of A. flavus and A. niger infection increased with increasing temperatures.

The fungal associations that occurred on groundnut fruit of the Argentine, Early Runner, and Florigiant cultivars were studied during a two-year period by sampling pods from three experiments at various intervals before and after maturity. No significant differences were found among cultivars, in estimated numbers of all fungi found on pod surfaces, or in kernels. The dominant fungi were Penicillium spp. (P. funiculosum, P. rubrum, and P. citrinum) and Aspergillus spp. (A. niger, A. terreus, A. flavus). Fusarium was a sub-dominant genus. A succession of well-defined communities was not recognized, although the densities of the dominant fungi fluctuated and Rhizopus became prominent alter maturity. Immature kernels from pods removed from the soil had a variable endocarpic community dominated by Aspergilli and Penicillia. A post-maturity community succeeded the first community and was evident at 15 days after maturity. It was characterized by the dominance of Aspergilli, Penicillia, Sclerotium and Rhizopus. The endocarpic community that developed in the windrow was characterized by the same fungi found in the post-maturity community. The windrow community was dominated by Fusarium, Rhizopus and Sclerotium. Rhizoctonia, Trichoderma, Nigrospora, and Curvularia were sub-dominant genera.

A study conducted in groundnut in the U.S.A. in 1965 indicated a relationship between the presence of lesion nematodes (Pratylenchus brachyurus) and the numbers of fungi in the shells. In 1966, however, such a relationship was not ascertained, either for the shells or for the kernels. The results of the 1967 microplot experiment indicated that, although total numbers of all fungi in kernels at maturity were increased by the presence of lesion nematodes, the numbers of Aspergillus flavus and A. niger in the kernels were not changed.

The mycoflora of groundnut kernels was studied over a 5 year period in Israel. A total of 132 samples of fresh, and 419 samples of stored kernels were examined. A list of 173 species isolated from these kernels is given, comprising 109 from fresh, and 153 from stored kernels. In three of the four years in which a comparative study of fresh and stored kernels was done, the number of fungal colonies from the former exceeded that from the latter by about 25 %. Aspergillus flavus usually occurred only in small quantities. Aspergillus niger was the most abundant fungus. On stored kernels it was even more predominant than on fresh kernels. Penicillium funiculosum and P. rubrum also occurred frequently.


Aflatoxin was found in up to 6.4 % of fresh groundnut kernels and in up to 32 % of stored kernels, although the intensity of toxicity was always very low (up to 125 µg kg⁻¹). About 90 % of 1626 Aspergillus flavus isolates from groundnut kernels, rhizospheres, and geocarpospheres produced aflatoxin in culture. In quantitative tests with 750 isolates, 60 % of the isolates produced > 25 µg g⁻¹ aflatoxin.


Soil mycoflora in 12 groundnut fields of different edaphic and climatic conditions throughout Israel was studied. The greatest number of fungi gm⁻¹ of soil was found in the Huleh peat location (217,000), whereas other locations gave lower counts (20,000-80,000). No significant differences occurred on the four collection trips. A list of the 95 species that were isolated from soils is given. Those that occurred in at least 6 fields were: Mucor racemosus, Rhizopus oryzae, Aspergillus amstelodami, A. flavus, A. nidulans, A. niger, A. sulphureus, A. ustus, A. versicolor, A. wentii, Penicillium funiculosum, P. lilacinum, Cephalosporium curtipes, Fusarium equiseti, F. oxysporum, F. solani, Hormodendrum nigrescens, Myrothecium verrucaria, Paecilomyces flavescens, Trichoderma lignorum and Rhizoctonia spp. The others were found in fewer locations. The mycoflora of freshly collected kernels from the same fields consisted of only 34 species. Those found in at least 6 samples were: Rhizopus oryzae, A. flavus, A. niger, P. funiculosum, P. rubrum, F. oxysporum and F. solani. Species that were common in soils and kernels also occurred on the groundnut shells.


During the first six months of storage Aspergillus niger was the dominant fungus in samples of groundnut kernels from the 1965-1966 and 1966-1967 crops on all types of soil. A. flavus and Fusarium spp. were present in only small amounts, whereas prevalence of Penicillium spp. declined in early stages of storage. Aspergillus niger appeared to suppress other fungi in the kernel mycoflora. The numbers of A. niger colonies in the geocarposphere and within groundnut kernels were positively related, but for other fungi no relationship was observed.


In Israel, the effects of crop sequence and soil types on the mycoflora of groundnut kernels was investigated over 3 years in 81 fields. Some of the fields were previously fallowed, and others were continuously cropped with or without groundnuts. The general mycoflora of kernels was most dominant in fields previously planted to groundnuts. In the cropped fields, whether groundnuts were included in the previous crops or not, Aspergillus niger accounted for 50-60 % of the total mycoflora. Penicillium funiculosum and P. rubrum were considerably more prevalent on previously fallowed fields. Prevalence of A. flavus was always low and was not influenced by crop sequences. Total kernel mycofloras were consistently higher on medium and heavy soil than on other soils.


The incidence of termite-scarified groundnut pods and resulting kernel contamination were studied in field and market samples in northern Nigeria. Scarification was caused by Microtermes lepidus and was restricted to the more mature pods. In the field, scarification was much higher (40.9-87.9 % of the pods) in the dead stands where the tap root had been invaded by Microtermes than in healthy stands (7.9-31.6 %). In the market samples, the number of scarified pods rarely exceeded 5 %; 85-91 % of the kernels from scarified pods were infected, the dominant fungi being Macrophomina phaseolina (37-61 %) and Fusarium spp. (8-26 %). Less than 5 % of the kernels were infected by Aspergillus spp. The fungi carried by the termites bore little relation to kernel infecting fungi. Of the kernels from undamaged pods collected from dead stands, 67 % were infected, indicating that, compared with plant death, scarification was of secondary importance in inducing fungal...
infection. The quality of infected planting seed was not altered when the seed was dressed with Fernassan D (a mixture of lindane and thiram).


This review emphasized the major pitfalls in the detection of mycotoxins that occur before harvest, with reference to variation in toxins in forages and silages, aflatoxin in groundnuts, cottonseed and maize, and trichothecces and zearalenone in maize. Variations attributable to interaction with other feed components and detection hazards in presumptive tests are also discussed.


Two varieties of groundnut (Tennessee Red and Virginia Bunch 46-2) were grown under gnotobiotic conditions, and the pods were inoculated with a conidial suspension of the aflatoxin-producing fungus Aspergillus flavus. No evidence of pathogenicity to groundnut plants or pod rot symptoms was observed. A. flavus penetrated the shell tissue consistently, but was limited in its seed invasion to the testa. Invasion and colonization of the embryos by A. flavus appeared to be limited.


In Israel, nonsterilized soil was infested with Aspergillus flavus before sowing four varieties of groundnut. Seed infection by this fungus did not increase as compared with the controls (no-infestation). A depressing effect on various other fungi and inverse relationships between fungi in the soil were noted, as well as differences in susceptibility among varieties.


Studies were conducted to determine mycoflora of groundnuts in Alabama, Georgia, North Carolina. Oklahoma, Texas and Virginia states. Aspergillus flavus was isolated infrequently from pegs, shells, and kernels. Species of Fusarium, Penicillium, Rhizopus, and Curvularia were predominant fungi isolated from pegs, while species of Rhizoctonia, Fusarium, Rhizopus, and Penicillium predominated among those isolated from shells and kernels. Soil type, location, and groundnut cultivar had no apparent effect on frequency and number of fungi isolated. Assays of kernel and shell samples obtained 3 weeks prior to harvest showed considerable levels of aflatoxin. No appreciable differences in aflatoxin occurred in samples taken from the top and bottom of the windrow during the curing process.


Research carried out at the Gampela research Station in Burkina Faso during 1986 and 1987 indicated that pod scarification and penetration by termites were enhanced by late harvest, pod damage was greater on plants where the tap root had been invaded and the plants killed by termites. Millipede damage was significantly greater in plants harvested at 70 and 90 days than for plants harvested at 125 days. Delayed harvest enhanced Aspergillus flavus invasion of groundnut pods and kernels. Insecticide application for the control reduced insect damage and yields were significantly greater and increased with longer growing time. In the evaluation of groundnut cultivars for resistance to millipedes and termites, millipede damage was significantly greater on QH 243. The lowest millipede damage was recorded for NC Ac 2240 and NC Ac 343. Banga, a local cultivar, showed least termite damage to plants and pods, while maintaining acceptable yields.


During 1983, studies were conducted on the relationship between lesser cornstalk borer, Elasmopala pus lignosellus (Zeller), damage to groundnut pods in the field, and the incidence of Aspergillus flavus. Florunner groundnuts were sown on April 8, April 27, May 18, and June 10. Groundnut pods were sampled on September 1, 8, and 15, and separated into undamaged, externally damaged, and pod penetrated classes according to lesser cornstalk borer feeding damage. The pods and kernels were assayed for A. flavus and A. niger. The incidence of A. flavus on the pods and kernels was significantly greater on pods that were penetrated by lesser cornstalk borers.

A committee to coordinate action on the problem of aflatoxin contamination in Nigeria was formed in 1961 with representatives from four ministries, i.e., the Institute for Agricultural Research (IAR), Zaria, the Nigerian Stored Products Research Institute (NSPRI), and the Northern Nigerian Marketing Board. This Committee was given the responsibility of assessing the extent of the aflatoxin problem in groundnut in the country and of initiating and coordinating all actions leading towards elimination of aflatoxin. IAR investigated the aflatoxin contamination of the groundnut crop up to the stage where the produce was sold by farmers, while NSPRI studied the problem from the time of storage until produce was exported or consumed. IAR investigated the time of invasion of groundnut kernels by *Aspergillus flavus*, and when, and under what conditions it produced aflatoxin. An interplay of temperature, relative humidity, drought, and erratic rainfall patterns, and maturity of the crop at lifting affected invasion by *A. flavus* and aflatoxin contamination of groundnut in the field and store. In the wetter areas of the Southern Guinea Savanna which have long rainy seasons, aflatoxin contamination of groundnuts is mainly a postharvest problem, while in the major groundnut growing areas that lie in the drier Northern Guinea and Sudan Savanna the problem is largely preharvest. Insect infestations and wetting of stored groundnuts increase aflatoxin contamination. Research information from IAR and NSPRI still provides the basis for recommendations on the handling of groundnuts to either minimize or prevent aflatoxin contamination. Vegetable oil and feed mill companies routinely submit their groundnut and other feed materials for aflatoxin analysis as there is great awareness among the companies, people, and government of Nigeria of the dangers posed by aflatoxin to poultry, livestock, and humans. Nigeria is a consignatory to the African Groundnut Council’s resolution to export only groundnuts with an aflatoxin content that does not exceed the maximum permissible limit of 20 μg kg⁻¹ set by the European Economic Community. None of the commercially grown groundnut cultivars in Nigeria is resistant to *A. flavus* invasion and aflatoxin contamination of seeds. Breeding materials from both domestic and exotic sources are being screened for resistance while other improved management practices are being used or researched.


Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95% of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that *A. flavus* infection of kernels and aflatoxin production occurred in both zones. The condition of the shell had a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by *A. flavus*, whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20% for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were free from fungal infection at lifting, infection by *A. flavus* not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of *A. flavus* in groundnut.


In trials at Mokwa, Nigeria, fruits of the long-season groundnut variety Samaru 38 were tested at intervals during development for fungal infection of the shell and of the seed. The shells were infected by fungi at an early stage in development, but infection of seeds did not occur until later. The dominant fungi in the endogeocarpic mycoflora were *Fusarium* spp., and *Macrophomina phaseoli*; other common species included *Aspergillus* spp., *Botryodiplodia theobromae*, *Penicillium* spp. and *Rhizopus* spp. Dominant fungi in seeds were *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp.


In trials at Mokwa, Nigeria, shells and seeds of the long-season groundnut variety Samaru 38 were examined for fungal infection. Samples were taken at normal lifting time, during windrow drying, after sun-drying on mats, and after becoming over-mature in the ground. At normal maturity the shells were commonly infected, although very few seeds were invaded. Fruits lifted when mature and dried in windrows showed an increase in invasion of seeds. Seeds from fruits picked shortly after lifting, then sun-dried on mats, showed little increase in fungal infection during drying. When fruits were left in the soil after reaching maturity, there was a gradual increase of fungal invasion of the seeds. *Macrophomina phaseoli* and *Fusarium* spp. were the dominant fungi in shells; they were also dominant in seeds of over-mature and windrowed-dried fruit. *Aspergillus* spp. were not abundant in seeds or seeds but were frequently present, the toxigenic *Aspergillus flavus* being the most common.
With the discovery of aflatoxin in groundnuts from many producing countries preliminary investigations were made on the 1961 crop in Nigeria. Results of the toxin tests showed that none of the varieties sampled was more resistant to toxin formation than another and that toxic samples appeared with equal frequency in both Northern and Riverain Provinces. Within each provincial group individual sites varied considerably for toxicity of samples and this may have been partially associated with different post-harvest drying and storage procedures. The fungal contamination tests showed no evidence of varietal resistance but did indicate considerably more contamination in samples from the Riverain Provinces than in samples from the Northern Provinces. Condition of the shell markedly influenced the degree of contamination of the kernels and, as for toxicity, there were considerable differences between individual sites for contamination with *Aspergillus flavus* and other fungi.

Investigations on aflatoxin in groundnuts were carried out during 1962 at Mokwa and five other localities in the Riverain Provinces of Northern Nigeria. The results indicated that: (1) *Aspergillus flavus* grew much more successfully on moribund or dead groundnut tissue than it did on living tissue. (2) There was virtually no toxicity in the crop at harvest, the exceptional cases being from broken pods. (3) Contamination of kernels with *A. flavus* and appearance of the toxin did not occur until at least five days after harvest. (4) Kernels from broken pods were more likely to be contaminated with *A. flavus* and to be toxic than were kernels from undamaged pods. (5) Toxicity of the crop varied from year to year at specific localities.

Trials were conducted at Mokwa Agricultural Research Station, Northern Nigeria, in 1963 to test various natural methods for postharvest drying of groundnuts. Samples of groundnuts were taken from the range of treatments at intervals from harvest until the end of drying, and these were tested for aflatoxin content and kernel contamination with *Aspergillus flavus* and other fungi. Large differences in rates of drying occurred between the various treatments and, in general, the slower the rate of drying the higher the kernel contamination with *A. flavus* and other fungi and the higher the incidence of toxic samples. When pods were hand-picked from the haulms and sun-dried in a layer, with protection from rain, the rate of drying was high and a toxin-free crop resulted. None of the treatments in which the pods were dried on the haulms in the field resulted in a toxin-free crop.

Serial harvesting trials were carried out at Mokwa and Kano Agricultural Research Stations, Northern Nigeria, in 1963 and 1964 to investigate me occurrence of aflatoxin in the groundnut crop at lifting, when harvested before, at, and after the normal time. This work formed part of the programme of work on the toxicity of the groundnut crop in Northern Nigeria. Crops harvested either at or earlier than the normal time were free from aflatoxin, but late harvesting usually resulted in some toxicity. The weather, as well as the age of the crop, had some effect on toxicity, wet weather delayed the appearance of aflatoxin. Pods with shells damaged while the crop was in the ground were more likely to contain toxic kernels than were pods with undamaged shells.

Trials were carried out at Kano Agricultural Research Station, Northern Nigeria in 1963 to test various natural methods for postharvest drying of groundnuts. Samples of groundnuts were taken from the different treatments at intervals from harvest until the end of drying and tested for aflatoxin content and kernel contamination with *A. flavus* and other fungi. There was very little difference in rate of drying between the various treatments, and no effect on toxicity or fungal contamination was observed. Some kernel samples were found to be toxic at harvest and this was associated with both pod damage and kernel moisture content.

Trials were conducted at Mokwa Agricultural Research Station, Northern Nigeria, in 1963 to test various natural methods for postharvest drying of groundnuts. Samples of groundnuts were taken from the range of treatments at intervals from harvest until the end of drying, and these were tested for aflatoxin content and kernel contamination with *Aspergillus flavus* and other fungi. Large differences in rates of drying occurred between the various treatments and, in general, the slower the rate of drying the higher the kernel contamination with *A. flavus* and other fungi and the higher the incidence of toxic samples. When pods were hand-picked from the haulms and sun-dried in a layer, with protection from rain, the rate of drying was high and a toxin-free crop resulted. None of the treatments in which the pods were dried on the haulms in the field resulted in a toxin-free crop.
Several groundnut genotypes reported as resistant, susceptible or highly susceptible to *in vitro* colonization of rehydrated, mature, stored, undamaged seed by *Aspergillus flavus* (IVSCAF) were tested for natural seed infection by *A. flavus* and other fungi in two or more replicated field trials at 1CRISAT Center, Patancheru, India, in 1979-1984. Undamaged pods were sampled before maturity, at optimum maturity (normal harvest) and when over-mature (late harvest) and seed examined for infection by *A. flavus* and other fungi. In the 1983 and 1984 rainy and 1983/84 postrainy seasons, only four genotypes (one resistant and three susceptible) were tested, and seed were also tested for aflatoxin content. In all seasons the genotypes reported as IVSCAF-resistant had significantly lower levels of seed infection with *A. flavus* and other fungi than did genotypes reported as IVSCAF-susceptible. Genotypic differences in levels of seed infection by *A. flavus* were consistent over seasons. The resistant cultivar J 11 had a significantly lower aflatoxin content than the other three IVSCAF-susceptible genotypes tested in the 1983 and 1984 rainy and 1983/84 postrainy seasons. Drought stress in the 1984 season apparently increased susceptibility to seed infection by *A. flavus* and other fungi, and to aflatoxin contamination, in all genotypes. Seed infection by *A. flavus* and other fungi, and aflatoxin contamination increased with increasing maturity of pods, indicating the importance of lifting the groundnut crop at optimum maturity.


Drought stress during late stages of pod maturation in an irrigated groundnut crop during the postrainy season significantly increased the level of seed infection by *A. flavus*. A line-source sprinkler irrigation system imposing a drought-stress gradient was used for field screening of groundnut genotypes for resistance to seed infection by *A. flavus*. A significant, positive, linear relationship was found between water deficit (drought intensity) and seed infection in groundnut genotypes. Genotypic differences for seed infection by *A. flavus* were evident at all levels of drought stress, but, under the more severe drought stress conditions, the genotypes resistant to *A. flavus* had low but positive levels of seed infection giving improved statistical precision.


Rainfed groundnut production in Queensland, Australia, is often severely affected by aflatoxin contamination. The Queensland Department of Primary Industries (QDP1) provides extension and research services to groundnut producers, and has addressed this problem in a variety of ways since becoming aware of it. Extension activates have attempted to improve producers' understanding of the causes of aflatoxin formation in groundnuts and of the management methods available at the farm level and at the shelling plant. Scientific support has been made available to: assist the groundnut industry establish its own quality-control facilities; help define some of the local factors important in aflatoxin development; conduct an Australian site for the International Groundnut *Aspergillus flavus* nursery; and to collaborate with industry, the Commonwealth Scientific and Industrial Research Organization (CSIRO), and the Australian National University (ANU) in aflatoxin research activities. The Australian Centre for International Agricultural Research (AC1AR) funded groundnut project in Indonesia conducted in collaboration by scientists from QDP1 and the Agency for Agricultural Research and Development (AARD), in future, consider the inclusion of research on production aspects of aflatoxin contamination.


'Argentine', 'Early Runner' and 'Florigiant' groundnut cultivars: were grown in methyl bromide-treated soil in field microplots inoculated with: (1) *Aspergillus flavus* or (ii) *A. flavus* + *Meloidogyne hapla*. Nematode infection produced heavy root galling and light pod galling equally on all cultivars. *A. flavus*, *A. niger*, *Cephalosporium* spp., *Colletotrichum* spp., *Curvularia* spp., *Fusarium* spp., *Penicillium* spp. and *Trichoderma viride* were isolated from shells and kernels. A significantly greater incidence and density of *A. flavus* was obtained from kernels of plants inoculated with both organisms than from kernels of plants receiving only the fungus. Differences were not significant, however, for incidence and density of *A. flavus* in shells or for the total of all fungal propagules in shells and kernels. Shells of 'Early Runner' contained significantly greater incidence and density of *A. flavus* than the other two cultivars; also, kernels of this cultivar contained more fungal propagules than kernels of 'Argentine'. A significantly larger number of total fungi was isolated from kernels of 'Argentine' than from 'Florigiant'. Anatoxins occurred in only two shell samples and were not detected in kernels.


Groundnut plants grown in steam-heated soil were inoculated with the root-knot nematode *Meloidogyne arenaria*. Inoculation with the nematode, resulting in severe galling of pods and pegs, increased the incidence of shell infection by *Aspergillus flavus*, *A. niger*, and *Sclerocium baticola*, and increased the infection of the kernels by *A. niger*. The random occurrence of trace amounts of aflatoxins among
Groundnut plants were subjected to drought and temperature stress for various periods and the seeds from these plants were separated into Jumbo, Medium and No. 1 market size categories and analyzed for soluble and total carbohydrates, alpha amino nitrogen, total protein and oil. Results showed that soluble and total carbohydrate content of the seed increased due to drought and temperature stress, with Jumbo and Medium sizes showing the highest increase. It is suggested that drought and temperature stresses caused increased accumulation and/or synthesis of carbohydrates and certain polypeptides and thus may enhance seed invasion by Aspergillus flavus and aflatoxin production.


Seven Virginia groundnut varieties, viz. S 230, Kadiri I, Kadiri 3, Karad 4-11, M13, GAUG 10 and TMV 10 were grown in four locations (Chintamani, Jalgaon, Khargaon, and Junagadh) in India in the rainy season of 1982. After harvest the pods were received at Junagadh during February-March 1983. Pods were shelled and aflatoxin analyses carried out on seeds with a thin-layer chromatography (TLC) method. Aflatoxin B₁ was found in all the groundnut varieties (0.8 to 65.8 µg kg⁻¹). Aflatoxins B₂ and G₁ were found in a few samples from some locations. Among varieties, S 230 had the highest level of aflatoxins at all the locations (49.8 to 85.9 µg kg⁻¹) followed by GUAG 10 (15 to 88.3 µg kg⁻¹) and Kadiri 3 (17.8 to 106.7 µg kg⁻¹). Karad 4-11 had low levels of aflatoxins at all four locations (1.6 to 30.3 µg kg⁻¹) followed by Kadiri 1 (4 to 52 µg kg⁻¹). M13 and TMV 10 had moderate levels of aflatoxins. Among the locations, Junagadh and Chintamani samples contained higher levels of aflatoxins. Unfavorable postharvest drying conditions at Junagadh and Chintamani probably resulted in higher levels of aflatoxins at these locations. The varieties with more than 3 % phenols in the seed testa generally contained low levels of aflatoxins. The varieties with protein content higher than 25 % contained more aflatoxins, while those having about 22 % proteins contained less aflatoxins except TMV 10 which also had less total phenols. Sugar and oil content did not appear to have any effect on the aflatoxin levels.


The incidence of Aspergillus flavus and aflatoxin in groundnuts was greater in the hotter, lower altitude areas. A. flavus and the toxin were found primarily in broken pods, but they occasionally occurred in apparently undamaged pods. No significant correlation was found between drying treatments and incidence of the fungus in kernels. There was no correlation between superficial shell and kernel infection as long as the shell remained intact.


An improved nondestructive technique for accurately detecting the extent to which kernels are damaged by molds and aflatoxin has been under study. A helium-neon laser emitting light at 6550 A wavelength with horizontal and vertical polarized components was first standardized on a known background target. The laser was then directed on the groundnut kernel surface and the back scattering amplitude of both polarizations recorded along with a computer calculated ration of the amplitudes. Preliminary results indicate that the amplitudes detected for pickout kernels are 30 to 40 % below the amplitude recorded for sound mature healthy kernels. The amplitudes for Aspergillus flavus inoculated kernels were 15 to 20 % below those recorded for the sound mature kernels. The polarization ratio for sound mature healthy groundnut kernels deviated only slightly from unity. With mold damaged kernels examined along the long axis the horizontal wave component decreased so that the polarization ratio ranged from 0.60 to 0.80. Evidence indicates that the amplitude of the reflected laser beam correlates with the surface features of the kernels while the polarization ratio provides information on the internal composition of the kernels.


Dielectric characteristics of good sound mature kernels and damaged kernels of groundnut were investigated over a frequency range of 20 KHz to 20 MHz. Groundnuts were placed in a test capacitor and measurements were made on a HP 4342A Q meter. Equivalent moisture contents were maintained using humidity chambers as determined by the oven dry method. Preliminary results indicate that the real part of the dielectric constant (permittivity) is essentially independent of mold contamination level whereas the imaginary pan (alt. loss tangent) is strongly dependent upon contamination level. The simultaneous determination based upon these dielectric characteristics, provides a means of detecting varying degrees of mold and mycotoxin damage.

Groundnuts (cultivar Starr) grown rainfed under drought stress conditions had markedly higher levels of Aspergillus flavus infection and aflatoxin contamination of kernels before digging than had groundnuts grown under irrigation, in field trials at Yoakum and Stephenville, USA, in the 1967, 1968 and 1969 seasons. Levels of seed infection and aflatoxin contamination were considerably higher in 1967 and 1969 than in 1968. A. flavus infection and aflatoxin contamination levels were higher in drought-stressed groundnuts produced at Yoakum than in groundnuts produced at Stephenville. In 1969 some samples of freshly-dug groundnuts from irrigated plots had high levels of A. flavus infection (16-59 %), but no aflatoxin was detected in these samples. Absence of aflatoxin in these samples is attributed to high seed moisture levels and to low soil temperatures (10 to 21° C). Several fungicides applied to the soil and foliage did not affect fungal infection and aflatoxin contamination of groundnuts.


This paper deals with three groundnut diseases in West Africa that affect yield and quality of this crop. The infection of pods and seeds by the aflatoxin-producing fungus Aspergillus flavus is linked to climatic factors. Field infection is increased by drought at the end of the growing cycle. Rosette is a virus disease transmitted by an aphid. Its development and propagation are directly related to those of the insect, which in turn result from well-defined climatic conditions, notably temperature and humidity. Groundnut rust, a fairly recent but fast-developing disease in West Africa, is caused by the fungus Puccinia arachidis. The fungus has, however, a short viability period for its development in tropical climate, the spread ofuredospores, their release, and dissemination by the wind and the conditions for infection are presented. A good knowledge of the agroclimatic environment that affects these diseases, well defined for certain parameters, facilitates assessment of risks and of the need to take protective measures. It also cuts down the loss caused by these diseases through appropriate cropping methods, so that the pathogens do not encounter satisfactory conditions for their development on the plants. This is, however, an interim solution that awaits the use of resistant varieties, the only really effective method of control that could be employed by all farmers.


Anatoxins have been a serious problem in Australian groundnuts in the past decade. With the aid of government and industrial funding, the Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Food Research has carried out an ongoing project for most of this period, with emphasis on studies under commercial field conditions. Research has been primarily concerned with understanding the variables that influence the invasion of groundnuts by Aspergillus flavus and A. parasiticus, and the subsequent production of aflatoxins. Factors studied include: levels of A. flavus in soils, environmental factors, farm management practices affecting A. flavus invasion, and the influence of drying and storage procedures on aflatoxin development. Most groundnut soils in Kingaroy contain detectable levels of A. flavus, while surrounding virgin soils usually do not. Levels of A. flavus in groundnut soils vary widely, from less than 10^5 spores g^-1 to as high as 10^6 spores g^-1, high levels are much more likely to lead to invasion. Some fields contained consistently high levels over several years. The A. flavus/A. parasiticus ratio also varies widely from farm to farm, and may influence invasion and toxin production. Investigations have shown that invasion of groundnuts by A. flavus takes place before groundnuts are harvested. Invasion will not occur subsequently, aflatoxin is not produced, even under the least effective drying procedures. In all but exceptionally dry seasons little aflatoxin is produced while groundnuts are in the ground, i.e., most aflatoxin is produced postharvest. Under the most favorable conditions, groundnuts require 6 to 10 days to dry in the field after harvest, a period sufficient for aflatoxin to reach unacceptable levels. Field drying cannot be sufficiently rapid, even in dry seasons, to ensure aflatoxin-free nuts at intake to shellers. The perceived importance of preharvest invasion as the necessary condition for the production of unacceptable aflatoxin levels has led to attempts to predict aflatoxin levels at shelling intake from A. flavus levels at harvest. Success rates have been encouraging but are not yet of practical utility.


In Virginia, U.S.A., the endogeocarpic microflora of groundnuts was studied over a 3-year period. The density of fungi in the pods decreased and that of bacteria increased as the pods matured. On the basis of the isolation density, the isolated fungi were divided into dominant and sub-dominant groups. Shells were more susceptible to fungal infection than seed. The dominant genera of the isolated fungi were Penicillium, Trichoderma, Chaetomium, and Fusarium. The isolation density of Aspergillus flavus, a sub-dominant fungus, was only 3 %. The isolation density
of *A. flavus* from shells remained constant during the growing season, but it increased for isolations from seed as the season progressed.


The endocarpic microorganisms of groundnut fruit dried in either a random windrow (plants left as they fell from the digger) or an inverted windrow (plants inverted to expose fruit to sunlight) were different from that of freshly dug fruit. *Chactomium, Penicillium, Trichoderma, Rhizoctonia,* and *Fusarium* were dominant fungi associated with shells (pericarp) of freshly dug fruit. The dominant fungi of shells of windrowed fruit included *Chactomium, Rhizoctonia, Fusarium, Sclerotium,* and *Alternaria.* Seeds of freshly dug fruit were dominated by *Fenicillium* and *Aspergillus.* The only dominant species in seed of windrowed fruit was *Fenicillium.* Microorganisms were isolated from shells and seed of freshly dug fruit at a frequency of 79 % and 52 %, respectively. The percentage of infection was reduced by drying in the field. This was particularly true of the inverted windrows. The proportion of shells and seed infected with a microorganism was reduced 13 % and 36 % respectively, after field drying for 5 to 7 days in random and inverted windrows. Microorganisms were isolated much more frequently from shell pieces (73 %) than from seed (36 %).


Field-grown groundnut fruit injured by the feeding of southern corn rootworm larvae were more susceptible to fungal colonization than noninjured fruit. Seed from injured fruit were colonized by fungi at a much greater frequency than seed from noninjured fruit. However, colonization by the toxigenic fungus *Aspergillus flavus* was not affected. In greenhouse tests, pod breakdown, an important in-soil rot of groundnut fruit caused by *Pythium myriotylum* was greatly enhanced by the presence of rootworm larvae. Under high inoculum densities of *P. myriotylum* and abnormally high rootworm populations the incidence of pod breakdown was almost twice that observed when only the fungus was present. Rootworm population densities influenced the severity of pod breakdown; severity increased as rootworm populations increased. The data suggest that insect feeding sites could provide portals of entrance into the groundnut fruit for many fungi, including *P. myriotylum.*


Differences in infection of seed by *Aspergillus flavus* were noted when comparisons were made between seed from sound or damaged groundnut fruit with different moisture contents. Seed from pods with visible damage (shell damage detected visually) and invisible damage (shell damage detected by a staining technique) were infected more frequently by *A. flavus* than those from sound fruit (no visible or invisible damage). Seed from invisibly damaged fruit were infected almost as rapidly as seed from visibly damaged fruits. The isolation frequency of *A. flavus* from invisibly and visibly damaged fruit was 23 % and 26 %, respectively. *A. flavus* was isolated at a frequency of 8 % from nondamaged fruit. After incubation at a temperature and relative humidity conducive to the rapid proliferation of *Aspergillus* spp. for a period of 24 to 48 hours, *A. flavus* was isolated just as rapidly from seeds from invisibly damaged fruit as from visibly damaged fruit. Although the isolation frequency of *A. flavus* from seed from sound fruit increased with time, the frequency did not approach that obtained from seed from damaged fruit. Before incubation, the isolation frequency of *A. flavus* from seed from non-inoculated fruit with moisture contents of 10 %, 30 % and 50 % was 4 %, 7 % and 10 %, respectively. Following incubation the isolation frequency of *A. flavus* from seed was greater in partially dried fruit than in fruit with a high moisture content. The isolation frequency of *A. flavus* from seed from partially dried fruit with a moisture content of 10 % and 30 % was 20 % and 25 %, respectively, following incubation. *A. flavus* was isolated less frequently (12 %) from seed from fruit with a 50 % moisture content, probably due to competition with other fungi during incubation.


Seeds from groundnut pods exhibiting microscopic shell damage, only detected with a staining technique, were as susceptible to colonization by *Aspergillus flavus* as seed from pods with visible damage. The purpose of this study was to determine the relationship between incubation time, pod moisture, shell damage and groundnut seed colonization by *A. flavus* and to develop a mathematical expression to represent this relationship. It appeared that pod resistance to fungal colonization was greatly reduced when protective physical barriers were breached. Therefore, practices that minimize microscopic damage should be developed to aid in minimizing seed colonization by undesirable fungi.


The results of surveys in the Philippines have shown that farm level aflatoxin
significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 µg kg⁻¹ aflatoxin. During windrowing, aflatoxin levels increased at the rate of 1.5 µg kg⁻¹ day⁻¹. In farm storage aflatoxin contamination continued to increase at the rate of 1 µg kg⁻¹ day⁻¹. Aflatoxin contamination was significantly higher during the main cropping season than the second cropping season. At the traders’ level, groundnut samples taken from various middlemen contained 35 µg kg⁻¹ aflatoxin. On the other hand, samples taken from the wholesalers’ newly procured groundnuts contained 188 µg kg⁻¹ aflatoxin. Groundnuts that had been in wholesalers’ warehouse for more than 3 months contained 275 µg kg⁻¹ aflatoxin. At the processors’ level, raw groundnuts for confectionery use (roasted and fried) contained 7.73 ug aflatoxin kg⁻¹, groundnuts intended for peanut butter contained 17.13 µg kg⁻¹, and rejected groundnuts had 120.6 ìg kg⁻¹. Aflatoxin contamination could start during harvest. Aflatoxin content increased to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.


Groundnut samples were collected from test fields and farmers’ fields at harvest during the rainy season and from kernels sold in a market. Aspergillus flavus infection was only detected in the samples collected from the market. It is suggested that infection of groundnuts occurs during postharvest drying.


Groundnut stem and pod temperatures of plants growing in irrigated, drought, drought-heated soil, and drought-cooled soil treatments were determined near the end of the growing season. Mean soil temperatures of the treatments during this period were 21.5, 25.5, 30 and 20°C, respectively. Groundnut stem temperatures in all drought treatments reached a maximum of ca. 40°C and for 6-7 h each day were as much as 10°C warmer than irrigated groundnut stems. Pod temperatures in drought-heated soil and drought treatments were ca. 34°C and 30°C, respectively, for several hours each day. As pod temperatures approached the optimum for A. flavus growth (ca. 35°C), the proportion of kernels infected and aflatoxin concentration increased. Increased plant temperature without accompanying pod temperature increases (drought-cooled soil) resulted in infection percentages and aflatoxin concentrations only slightly higher than those of the irrigated groundnuts.


Data compiled over 4 years showed that 5 cm of soil at a mean of 29.4°C and severe drought 40-50 days before harvest resulted in Aspergillus flavus infection and aflatoxin contamination in preharvest groundnuts. Four genotypes, which were rated as resistant to seed colonization by A. flavus in a laboratory screening assay, and 2 other genotypes were grown in plots and subjected to the above stress conditions 98 days after sowing. Tests of dried groundnuts harvested 143 days after sowing showed that nuts of all the genotypes contained aflatoxin and were extensively infected by A. flavus.


Posharvest quality of groundnut is influenced by the particular set of environmental and cultural practices that influence physiology and maturation. Groundnut composition, although related to environment, changes dramatically as groundnuts mature. There is biochemical basis for inferior quality in immature groundnut. Drought stress and soil temperature influence maturation rate and thus have an indirect effect on postharvest quality. Aspergillus flavus invasion and aflatoxin contamination in groundnuts are related to drought stress, soil temperature, and maturity. Small, immature seed are more likely to be contaminated with A. flavus than larger, mature seed. The biochemical composition, fungal contamination, and the tendency toward higher moisture content complicate storage of immature seed. Each of these factors predisposes immature seed to rapid quality deterioration in storage. Agrometeorological studies must include an awareness of the interrelationships of environment, maturity, and postharvest quality.


In 1982 Florunner groundnuts were grown in experimental plots to evaluate the effect of various drought soil temperatures on aflatoxin production in preharvest groundnuts. Drought and soil temperature regulation were initiated 90 days after sowing. Mean soil temperatures under the groundnut rows in the various treatments were 31.7°C, 29.9°C, 27.7°C, 26.3°C, 24.7°C and 25.6°C (irrigated control). At harvest, no aflatoxin was found in groundnuts from the control or 31.7°C plot and concentrations of aflatoxin decreased with decreasing temperature for other drought...
treatments. At 24.7°C only the other edible size category contained aflatoxin (20 µg kg⁻¹). *Aspergillus flavus* group fungi were found in groundnuts from each plot.


Previous experiments have established that late-season water deficit conditions and 28-30.5°C in the geocarposphere are optimum for preharvest aflatoxin contamination of Horunner groundnuts. Visibly-undamaged groundnuts from plants exposed to these stress conditions during the last 40-45 days before harvest were highly contaminated with aflatoxin at harvest. The objective of this study was to determine the duration of water and soil temperature stress required for extensive preharvest invasion by *Aspergillus flavus* and contamination of groundnuts with aflatoxin. Stress conditions were imposed 20, 30, 40 and 50 days before harvest. Incidence of *A. flavus* was greatest in edible groundnuts from 30, 40, and 50 day stress treatments. A stress period of 20 days before harvest did not result in aflatoxin contamination of edible groundnuts by *A. flavus*. Groundnuts subjected to defined temperature and water deficit stress conditions for 30, 40, and 50 days became contaminated. Therefore, a threshold stress period for preharvest aflatoxin contamination of groundnuts by *A. flavus* was more than 20, and possibly less than 30 days before harvest.


Cultivar Florunner groundnuts were grown in experimental plots with soil moisture and soil temperature modified during the last third of the growing period to produce drought, drought with cooled soil, irrigated and irrigated with heated soil treatments. Twice each week, beginning 97 days after sowing, random samples were harvested and maturities of individual pods were determined without destroying pod integrity. The nature and quantity of the microflora associated with the pods and kernels were subsequently assessed. Drought and lower soil temperature resulted in maturity distributions containing higher proportions of immature pods. On groundnuts with no visible damage to the pod or kernel, colonization by *Aspergillus flavus* was more frequent in immature than mature kernels. Drought stress increased the incidence of *A. flavus* and irrigation decreased it, except when soil temperatures were modified. *A. flavus* infection was greatly increased at all maturity levels by pod damage.


Nondamaged and damaged groundnut kernels from broken and perforated pods were analyzed to determine their relative importance as sources of aflatoxins. Apparently damage-free kernels (accounting for 48.5% of the total kernels) contained between 500 and 1000 µg kg⁻¹ aflatoxins. Damaged kernels, on the other hand, contained 2000 µg kg⁻¹ or more aflatoxins. Subsequently, kernels from mechanically broken, rot- and insect-perforated, and growth-cracked pods were assayed separately for aflatoxin content. Results indicated that kernels from pods with growth cracks are more subject than kernels from pods with other types of damage to aflatoxin development.


Groundnuts, cottonseed, rice and sorghum from Texas, USA, were sampled over a 3-year period. Aflatoxins were present each year in groundnut and cottonseed and in 2 of 3 years in rice and sorghum. The *Aspergillus flavus* group fungi were much more prevalent in groundnut and rice than in cottonseed and sorghum. Of the isolates of the *A. flavus* group, 96 % from groundnut, 79 % from cottonseed. 49 % from sorghum and 35 % from rice produced aflatoxins. The average toxin production of isolates was much less for rice than for the others. More than 90 % of all isolates of the *A. flavus* group were identified as the species *A. flavus*. *A. parasiticus* was isolated from all crops. Only *A. parasiticus* isolates produced aflatoxin G as well as B.


Environmental and biological factors responsible for aflatoxin contamination of the South African groundnuts are discussed. During the 1963/64 growing season, maturing groundnuts in the North-Western Transvaal areas were damaged by certain species of termites; this predisposed groundnuts to seed invasion by *Aspergillus flavus*. Protracted droughts followed by the late rains in these areas were also conducive to infection of groundnuts by the aflatoxigenic fungus. Most of the infection of groundnuts by the fungus was believed to occur in the groundnut before harvest.


Flowering groundnuts (cv. Florunner) in pots were placed in a growth chamber with
14 hours of light, 80-90 % humidity, and a temperature of 29°C. Freshly opened
flowers were inoculated by dusting the stamens and style with a camel's hair brush
conveying spores of A. flavus cultures isolated from groundnuts. Flowers were
collected in 70 % ethanold immediately before and after inoculation and after periods
of one to eight days. The styles were removed from the flowers and placed in a
mixture of malachite green, acid fuchsin, lactophenol and glycerol. No spores were
observed on styles of uninoculated flowers, whereas styles from inoculated flowers
were covered with large numbers of spores. Some germination had taken place 24
hours post inoculation (PI) but by 48 hours PI, many spores had germinated and
hyphae were observed growing over the surface of the stigma and pollen grains.
Some of the hyphae entered the style through the stigma and ramified in the styler
tissue proximal to the stigma, until some hyphae had grown down to the top of the
ovary. In some flowers, after as little as two days PI, conidiophores bearing spores
were observed on the anthers and distal portions of the filaments, thus providing a
secondary source of inoculum. Research is being undertaken to determine if the
ovary and later, the fruit harbor the fungus.

citrinin in groundnut (Arachis hypogaea L.) at harvest in relation to pod condition
and kernel moisture content. Current Science 43(22): 707-710.

Groundnut pods were collected from fields on the day of harvest in November 1972,
graded into undamaged and damaged pods and seed moisture contents determined.
High levels of aflatoxin were found in seeds with low moisture (< 30 %), which
occurred under rainfed conditions and low levels or no toxins in those with high
moisture content (under irrigation). In all instances damaged seeds were found to
contain the toxins. Seed moisture content and pod damage appeared to be the major
governing factors for fungal infection and toxin accumulation before harvest. The
accumulation of a yellow pigment in some seeds, especially in damaged pods, was
noticed and it was identified as citrinin. Only Aspergillus flavus isolates were found to
produce aflatoxins while isolates of Penicillium citrinum, P. jenseni and A.
terreus produced citrinin.

and aflatoxin accumulation before harvest. Proceedings of the Indian Academy of
Sciences 85(6):432-443.

Shells and kernels of groundnut were examined in plants from 60 days old to
harvest in the rainy (Kharif) and postrainy (Rabi) seasons. Most were free of fungal
infection at first but nearly all shells were infected by harvest time. There was no
aflatoxin in kernels from undamaged pods from Rabi season, but aflatoxins (118-128
µg kg⁻¹) were found in samples from rainfed plots in Kharif season. In kernels from
damaged pods, higher levels of aflatoxin were detected in samples from rainfed
plots (780-6700 µg kg⁻¹) than from irrigated plots (820-1840 µg kg⁻¹) in Kharif
season. Low levels of aflatoxins (82-180 µg kg⁻¹) were found in damaged pods from
Rabi season.

439. Vidal (Jaona, G., and Zenteno Zevada, M. 1982. The mycoflora of
groundnuts (Arachis hypogaea L.) destined for human consumption. Anales del
Instituto de Biologia Universidad Nacional Autonoma de Mexico, Botanica 47-53:
229-238.

Groundnut samples from Mexico FD, and several localities in Jalisco, Oaxaca and
Guerrero were tested using malt salt agar (6 % NaCl. MSA) and mall acar (MA)
media for Aspergillus flavus. MSA was more favorable for the development of
mycoflora than MA. Groundnuts from Mexico FD showed both high and low
levels of fungi and those retaining the tests were more susceptible to fungal
colonization. Since A. glaucus was present in all the tests and A. flavus in few
samples and in low quantity, the relative humidity (RH) equilibrium of the fruits
was deduced to be less than 75 %.

440. Waliyar, F., and Roquebert, M.F. 1979. [Mycoflora of groundnut pods and
seeds in Senegal]. Mycoflore des gousses et des graines d'arachide au Senegal.

Fungi isolated from groundnut pods and seeds are listed. Aspergillus, particularly
A. flavus, Penicillium and Fusarium spp. occurred most commonly.

441. Waliyar, F., and Zambettakis, Ch. 1979. [Study of the mycoflora of
groundnut pods and seeds in Senegal]. Etude de al mycoflore des gousses et des

About 50 species of fungi were isolated from groundnut pods and seeds at harvest.
Aspergillus, Penicillium, Fusarium spp., and Mucorales occurred most commonly;
A. flavus was predominant. There were no significant differences in the mycoflora
over the two successive years of experimentation. A few pathogenic species
(Fusarium solani and Rhizotonia solani) were found only rarely and did not appear
to present a threat to groundnuts in Senegal.

gnotobiotically grown peanuts by Aspergillus flavus and selected interacting fungi.
Phytopathology 62:1238- 1242.

Groundnut plants (cv. Tennessee Red) were grown under gnotobiotic conditions in
the presence of Trichoderma viride, Penicillium funiculosum, and the
aflatoxin-producing Aspergillus flavus. These fungi singly or in combinations
colonized aerial and subterranean parts of groundnut plants; however, colonization
by T. viride was restricted primarily to subterranean tissues. Immature pods, mature
pericarp, and testae, to a lesser extent, were susceptible. Embryos showed limited invasion. Colonization of immature and mature pericarps by A.\textit{flavus} was reduced in the presence of T. \textit{viride}. \textit{Penicillium funiculosum} not only nullified this antagonistic effect, but also appeared to stimulate colonization of mature groundnut pericarps and testae by A.\textit{flavus}.


Soil populations of the \textit{Aspergillus flavus} group fungi gradually increased in two groundnut fields during the 1973 growing season. In one field, populations increased from approximately 50 propagules g\(^{-1}\) of dry soil in June to about 225 propagules g\(^{-1}\) in October. In another field the increase was from 10 in June to 25 propagules g\(^{-1}\) in October. Less than 0.1 % of groundnut kernels in windrows had \textit{A. flavus} infection when collected daily from digging to combining (0-7 days). However, the day after combining and drying to 10-12 % moisture \textit{A. flavus} was isolated from 15-25 % of the kernels. Recovery of \textit{A. flavus} from pods by washing with sterile water demonstrated that there were enough propagules on the pod surface to allow this infection. The number of propagules on the surface did not increase from digging to combining in either field. Even though there was a low \textit{A. flavus} kernel infection at harvest, 2 to 15 µg kg\(^{-1}\) aflatoxins were found in 21 of the 37 samples collected from digging to combining (0-7 days). The aflatoxins must have been produced by early infections by the \textit{A. flavus} group fungi that did not persist or were not isolated by the technique used.


In order to determine whether there is a detectable background level of aflatoxins in sound mature groundnuts at the time they are dug, samples were collected from each of the 4 replications in 1973, 1974, 1975 and 1976, in the USA. Each year 5 lb samples of sound groundnuts from each plot were shelled, hand-sorted to remove damaged and immature kernels, ground, and samples analysed for aflatoxin using the AOAC method. Level of \textit{Aspergillus flavus} infection was : 1973, 0.9 % of 8000 seeds ; 1974, 1.4 % of 3000 seeds ; and 1975 and 1976, 0.6 % of 2000 seeds. Quality control measures have been instituted in the US to ensure that unavoidable aflatoxins in consumer groundnuts and groundnut products do not exceed 20 µg kg\(^{-1}\). However, it was found that aflatoxin contamination at a low level (trace to 50 (µg kg\(^{-1}\)) can occur before the groundnuts arc dug. The data indicate that low level contamination is not related to high level of \textit{A. flavus} infection or to current production practices. Low level of aflatoxin contamination of groundnuts may be endemic and current sorting procedures may not be effective in removing unblemished contaminated groundnuts.


Lesser cornstalk borer (LCB) larvae were fed groundnut pods, maturity stage 2-6, as described by Lynch (1984). In one half of the laboratory test the LCB larvae were infested with a color mutant of \textit{Aspergillus parasiticus}; the remaining larvae were not infested. After 10 days the groundnuts were sorted according to damage category and the surviving larvae were recovered. Groundnut hulls, kernels and larvae were placed on 10 % malt salt medium and incubated at 30°C for 6 days before observation. Infestation of the larvae with \textit{A. parasiticus} decreased LCB damage but did not influence larval survival. LCB damage was stage related. Kernels from penetrated pods contained more green \textit{A. flavus} group and \textit{A. parasiticus} than kernels from pods with no damage or external damage. Damage by LCB did not affect the incidence of fungi recovered from hulls. \textit{Aspergillus flavus} was recovered from uninfested more often than infested treatments. \textit{Aspergillus parasiticus} was recovered more often from kernels in stage 3 than those in 2, 4, and 5. Stage 6 kernels had the least \textit{A. parasiticus}. These results show that LCB larvae can be vectors of \textit{A. parasiticus} and that kernels in penetrated pods are often colonized.


Florunner and Florigiant groundnuts were grown in 1974, 1975, 1976 and 1977 and inoculated with \textit{Aspergillus parasiticus} 30 days after sowing. Four replicates were grown in plots for 140 to 145 days under rainfall controlled shelters with six irrigation treatments: (1) wet from 0-140, (2) dry from day 36-70, (3) dry from day 71-105, (4) dry from day 106-140, (5) dry from day 36-105, (6) dry from day 71-140. Aflatoxin concentrations from Florunner groundnuts showed significant differences among treatments (P=0.01) in 1974 and 1976 but not in 1975 or 1977. In 1974 and 1976, Florunner sound mature kernels had significantly more aflatoxin in treatments 4 and 6 than in other treatments. Aflatoxin concentrations from Florigiant treatments 4 and 6 were significantly greater (P=0.01) than other treatments in 1974 and 1975, but not in 1977. No data were taken in 1976 for Florigiant groundnuts. Water stress during the last 35 or 70 days of the season affected aflatoxin contamination of sound mature kernels in three of the four years on one or both cultivars. Because of year to year variation, drought stress alone will not account for high levels of field aflatoxin contamination. However, in all
treatments with irrigation during the last 35 days of the season no significantly high levels of aflatoxin contamination occurred in any year or cultivar.


Effects of irrigation and drought stress treatments on preharvest aflatoxin contamination of groundnuts were investigated in the 1974, 1975, 1976 and 1977 seasons. Two groundnut cultivars, Florunner and Florigiant, were grown under rainfall controlled shelters with six irrigation treatments: (1) full irrigation throughout the growing season, (2) no irrigation from 36 to 70 days after sowing (DAS), (3) no irrigation from 71 to 105 DAS, (4) no irrigation from 106 to 145 DAS, (5) no irrigation from 36 to 105 DAS, and (6) no irrigation from 71 to 145 DAS. Groundnut plants in each replicated plot were inoculated by sprinkling them with a spore suspension of aflatoxigenic isolate (NRRL 2999) of Aspergillus parasiticus. Significant differences in aflatoxin contents of Florunner groundnuts were attributable to the irrigation treatments in 1974 and 1976 but not in 1975 and 1977. In 1974 and 1976, sound mature kernels of Florunner from the treatments 4 and 6 had significantly higher levels of aflatoxins than had the kernels from other treatments. Aflatoxin contamination levels in sound mature kernels of cultivar Florigiant from treatments 4 and 6 in 1975 and from treatment 6 in 1974 were significantly greater than in sound mature kernels from other treatments. Such differences in aflatoxin contamination were not evident in 1977. Drought stress during the last 40 to 70 days of the season favored aflatoxin contamination in three of the four seasons in one or both cultivars. Because of season to season variation, drought stress was not alone responsible for preharvest aflatoxin contamination. In some seasons, other environmental factors interacted with drought stress to either favor or inhibit aflatoxin contamination. No aflatoxin was detected in seeds of the test cultivars from treatments where irrigation was applied during the last 40 days of the season.


The effects of gypsum, irrigation and inoculation with Aspergillus parasiticus (NRRL 2999) on mycoflora and aflatoxin contamination of groundnuts were examined. Two rows of groundnut plants in each plot were inoculated by sprinkling them with a spore suspension of the A. parasiticus isolate. Two irrigation regimes and four gypsum rates were tested. Soil samples were collected three times during the season and at harvest to monitor populations of A. flavus group fungi (A. parasiticus and A. flavus). Groundnut pods were collected at harvest for P, K, Ca, Mg, aflatoxin and mycoflora analyses. No aflatoxins were found in groundnuts from any treatment. Populations of A. flavus group fungi were significantly higher in soil from inoculated plots for the first two sampling dates only; A. parasiticus apparently did not persist in the soil throughout the growing season. More kernels were infected with the A. flavus group fungi and other fungi in plots with no gypsum treatments than in plots with gypsum treatments. The relationship between calcium nutrition and A. flavus infection in groundnuts may be important in preharvest aflatoxin contamination.

2.2.3 During Postharvest Field Drying


This article was written as an introduction to a series of research papers on the agricultural aspects of the problem of infection of groundnuts and subsequent aflatoxin production by Aspergillus flavus. It briefly reviews the problem in the widest sense, and then deals with the preliminary steps required to determine the point of entry of the fungus into the groundnut and subsequent development of toxicity.


This paper reports results of research at Oklahoma State University directed toward control of mold development on high moisture groundnut pods by storage in anaerobic and fungitoxic gases at two temperatures during 1968 and 1969. Storage of pods in N\textsubscript{2} or CO\textsubscript{2} to prevent mold growth and aflatoxin production was tested. Nondried (freshly harvested) and partially dried pods were inoculated with an aflatoxin-producing strain of Aspergillus flavus and stored in chambers held at 3 and 24°C. During 1968 and 1969, undiluted N\textsubscript{2} and CO\textsubscript{2} were used as lest anaerobic storage gases. During 1969, a mixture of 5 % SG\textsubscript{2} and 95 % N\textsubscript{2} by volume was also tested. Compressed air was used as a check gas each year. The gases were fed through the test chambers for 32 days. Spaced samples were assayed for quality, moisture, mycoflora, and aflatoxins. All air-treated pods at 24°C soon became covered with mycelium of species of Fusarium, Rhizopus, and Mucor, but the moldy, partially dried pods soon became overgrown with A. flavus. In both years, mold development was greatly delayed by CO\textsubscript{2} or N\textsubscript{2}. CO\textsubscript{2} prevented mold development and aflatoxin contamination, the treatment caused serious off flavors in both raw and roasted kernels from the treated pods. Oil
extracted from SO$_2$-treated kernels was very dark and had an odour distinctly different from normal. Moldy pods had a fermentation odour. High levels of aflatoxins occurred in kernels from pods stored in air at ambient room temperature; levels increased with time. Higher levels of aflatoxins occurred in high moisture kernels than in those from partially dried pods. All CO$_2$, N$_2$ and SO$_2$ treatments produced aflatoxin-free kernels. Molds were isolated from all visibly mold-free CO$_2$ and N$_2$ treated pods.


Molds develop on high-moisture groundnuts when these are improperly dried or held in bulk. Storage of pods in N$_2$ or CO$_2$ to prevent mold growth and aflatoxin production was tested. Nondried and partially dried pods were inoculated with an aflatoxin-producing strain of *Aspergillus flavus* and stored in chambers held at 3 and 24°C. Air, N$_2$ and CO$_2$ were metered through replicated chambers. Spaced samples were assayed for quality, moisture, mycoflora, and aflatoxins. All air-treated pods at 24°C soon became covered with mycelium of species of *Fusarium*, *Rhizopus*, and *Mucor*, but the moldy, partially dried pods soon became overgrown with *A. flavus*. Mold development was greatly delayed by CO$_2$ or N$_2$. CO$_2$ prevented mold development for over 2 weeks at 24°C while pods held 36 days at 3°C in CO$_2$ had their original mold-free appearance and a near-normal odour. Moldy pods had a fermentation odour. High levels of aflatoxins occurred in kernels from pods stored in air at 24°C; levels increased with time. Higher levels of aflatoxin occurred in nondried pods than in partially dried pods. CO$_2$ and N$_2$ treatments produced aflatoxin-free kernels. Molds were isolated from all pod samples regardless of treatment. CO$_2$ and N$_2$ were fungistatic.


*Fusarium* spp., *Penicillium* spp., and *Alternaria tenuis* were the dominant fungi in harvested groundnut pods in 1965 in Oklahoma, USA. *Fusarium* spp., *Trichoderma viride*, and mucoraceous species (*Rhizopus* sp. and *Mucor* sp.) were dominant in 1966. *Aspergillus flavus* was rarely isolated either year. Populations of the fungi from machine-compounded pods were not significantly different from those of hand-picked pods except for mucoraceous species, which were greater in combine-harvested pods. Aflatoxins were found in all samples from the 1965 season, but only in a few in the 1966 season. Aflatoxins were not correlated with presence of *A. flavus*, but were correlated with isolations of *A. tenuis*.


Studies were conducted to determine the effects of *Meloidogyne arenaria* and curing time on pod infection by *Aspergillus flavus*. Groundnuts of the cultivar Argentine were grown in field microplots containing methyl bromide-treated soil inoculated with either *A. flavus*, *M. arenaria*, or *A. flavus* + *M. arenaria*. Noninoculated microplots served as controls. Each treatment was replicated six times. At maturity the plants were dug, the pods rated for root knot galling, and one-third of the pods harvested. The remaining pods were left attached to the plants and placed on a wire-mesh greenhouse bench to cure. Additional samples were made after 5 or 12 days of curing. After each sampling, 25 two-seeded pods from each replicate were assayed for fungi. The remaining pods were dried for subsequent aflatoxin analyses. Pods from *M. arenaria-inoculated* plants were heavily galled, but the incidence of *A. flavus* and total fungi was not affected. The length of the curing time did not affect the incidence of *A. flavus* or total fungi. Only in pods from *A. flavus*-inoculated plants was the incidence of *A. flavus* increased. Aflatoxin contamination was not affected by any of the treatments. *M. arenaria* damage to groundnut pods did not affect *A. flavus* infection.


The effects of *Meloidogyne arenaria*, *Aspergillus flavus*, and curing time on infection of groundnut pods by *A. flavus* were studied. Plants were grown in field microplots of fumigated soil inoculated with either *A. flavus*, *M. arenaria*, or *A. flavus* + *M. arenaria*. Pods from *M. arenaria-inoculated* plants were heavily galled, but the incidence of *A. flavus* and total fungi was not affected. Length of curing time also did not affect the incidence of *A. flavus* or total fungi. Only in pods from *A. flavus*-inoculated plants was the incidence of *A. flavus* increased. Aflatoxin contamination was not affected by any of the treatments. It was concluded that *M. arenaria* damage to groundnut pods did not affect *A. flavus* infection.


Natural groundnut drying methods used by farmers in several countries are described. The necessity of adequate drying to prevent the development of the aflatoxin-producing fungus *Aspergillus flavus* in seeds is emphasized.

Information on the artificial drying of groundnuts, which is of special importance in preventing the development of the fungus *Aspergillus flavus* is summarized. It is recommended that, before drying, the nuts should be cured on the plant in windrows and should then be threshed rather than remain on the haulms. Reference is made to the extensive experimental work carried out in the USA. Various types of dryers used there and in Australia, Israel, Nigeria and Tanzania are described with special reference to batch dryers. In the tropics, where ambient temperatures can be above 29°C, the use of forced air without additional heat may be sufficient for drying groundnuts.


Samples of groundnuts were collected in the field prior to harvest, immediately after harvest and from farmers stock storage warehouses at widely separated points in Southwest Georgia, Alabama and North Florida. Samples were analyzed for aflatoxin contamination. Some groundnuts showing no visible hull damage (mechanical or insect damage) were analyzed just as pods came from the ground, and the kernels contained high levels of aflatoxin, but no mold was apparent, even when examined under the microscope. Extremely dry weather during the latter pan of the growing season allowed the groundnuts to dry to the moisture level which was conducive for aflatoxin production in the field. Some groundnuts were dug, inverted and sprayed immediately with fungicides. Low levels of aflatoxin were present at digging and aflatoxin increased with exposure in the windrow even though they were sprayed with fungicides.


Studies on the effects of plant orientation in windrows were conducted in North Carolina, USA. Groundnuts on inverted plants dried much more rapidly than those on plants in random windrows. Both plant orientation and moisture content at time of combining affected pod damage during combining, the amount of kernel damage caused by subsequent shelling operations and seed germination. Apical kernels were more subject to mechanical damage during combining than basal kernels. In groundnuts inoculated with *Aspergillus flavus*, kernels in broken pods and shelled kernels were more often contaminated with aflatoxin during bulk curing than were groundnuts in sound pods.


Effects of time, temperature and moisture on the production of aflatoxin in groundnuts inoculated with an aflatoxin-producing strain of *Aspergillus flavus* were studied. Moisture contents between 15 and 30% were conducive to aflatoxin production. At least 48 and over 100 hours from the time of inoculation were required for aflatoxin to develop in kernels at 32°C and 21°C, respectively, at all moisture levels. Groundnut curing usually involves partial drying in windrows and subsequent drying in bulk curing bins. Unless considerable mold growth occurs before the start of bulk curing, aflatoxin is not likely to develop during the curing process if recommended practices are followed. In cool weather, even prolonged
periods in the windrows at high moisture contents did not cause aflatoxin development in groundnuts previously inoculated with *A. flavus* isolates.


Freshly dug Early Runner and Florigiant groundnuts were surface-disinfected, inoculated with an aflatoxin-producing isolate of *Aspergillus flavus*, and incubated for 3 weeks in eight 10 ft³ environmental chambers at 10-45 ± 0.5°C and relative humidities (RH) of 70-98 ± 1 %. Groundnuts were sampled after 7 and 21 days of incubation and determinations of kernel moisture, aflatoxin, and free fatty acid content were made. In 1965 at 30°C, aflatoxin formed in Early Runner kernels in 21 days at 92 and 98 % RH, whereas in Florigiant kernels it formed at 87-98 % RH. At 98 % RH, aflatoxin developed at 20-40°C in both varieties, but no aflatoxin developed at 15 and 45°C. In 1966 at 30°C, aflatoxin formed in Early Runner kernels at RH as low as 85 % and in Florigiant as low as 87 %. At 98 % RH, aflatoxin was produced at 20-35°C in both varieties, with low levels occurring at 40°C in Early Runner and at 15°C in Florigiant. Growth of *A. flavus* was generally correlated with aflatoxin production except at high temperatures of 40-45°C. Free fatty acid formation was generally correlated with the growth of *A. flavus*, but not with aflatoxin production.


In Alabama (USA), healthy pods of two groundnut varieties collected from freshly dug-up plants were surface-sterilized and inoculated with spores of an aflatoxin-producing strain of *Aspergillus flavus*. Subsequent incubation for 21 days at 30°C resulted in aflatoxin production in the Early Runner variety when the relative humidity exceeded 83 %, in Florigianit when it exceeded 85 %. Incubation in near-saturated air resulted in aflatoxin formation at temperatures ranging from 12.5 to 42.5°C. Growth of the fungus was generally correlated with aflatoxin formation except at temperatures of 43-45°C when the mycelium was able to grow but did not produce the toxin. Pod shell and seed tests were found to form temporary barriers to penetration by the fungus.


Since 1962, a research team in Rhodesia has studied the factors that may affect the incidence and level of aflatoxin contamination of groundnuts and ways of dealing with the aflatoxin problem. This report gives a brief account of the research projects and discusses pertinent results. Among these is the possibility of predicting levels of aflatoxin contamination of the crop resulting from different meteorological, seasonal and cultural factors.


In the Gezira, the Sudan, field experiments showed that *Aspergillus flavus* and other fungi such as *Penicillium* spp. and *Fusarium* spp., were always present in kernels from broken groundnut pods. On watering before harvest, the kernel moisture content immediately after lifting was 58.7 % for the cultivar Ashford. and 46 % for the cultivar Barberton, but after 7 days in the windrow the moisture content dropped to 4.7 and 3.8 %, respectively. Groundnuts dried immediately after harvest for at least 7 days were considered to be free from aflatoxin.


After six months in storage, approximately 15 % of windrow-cured (rapid) and approximately 40 % of stack-cured (slow) seed of the Virginia type groundnut (cv. Florigiant) were infected with *Aspergillus flavus*, whereas with stack curing in the Spanish types Spancross and Starr only approximately 4 and 5 % of the seed, respectively, were infected. The more rapid curing and the tightly adhering pericarp of the small-seeded Spanish-type fruit appears to inhibit invasion by the fungus.


Studies in the Gambia showed that mature kernels inside visibly intact pods of the groundnut varieties ‘Senegal’ and ‘G.O.M.B.’ were frequently associated with the fungus *Macrophomina phaseolina* before and directly after harvest. Some mature pods were contaminated with the fungus *Aspergillus flavus* at lifting. The degree of invasion by these fungi was influenced, among other things, by soil moisture, shell and kernel maturity, correct lifting techniques, and the intensity of sun drying. Postharvest invasion increased with higher rainfall intensities. In general, invasion patterns were independent of varieties.


A preliminary survey of the incidence of aflatoxin in the main groundnut production areas of the Sudan is described, correlating the extent of infection with *Aspergillus*
flavus and other kernel fungi with the aflatoxin content of the kernels. In general, the rainfed samples had a higher incidence of kernel contamination than those from the irrigated areas. The majority of the groundnut samples from the irrigated areas were aflatoxin-free, whereas in those from the rainfed areas none was free, and 48% were classified low-medium (<50 to 250 µg kg⁻¹ aflatoxin), while 52% were in the high to very high category (250-1000 µg kg⁻¹ to > 1000 µg kg⁻¹ aflatoxin).


Two experiments were conducted on Tifton loamy sand in Georgia (USA) with a view to studying the growth of Aspergillus flavus and other fungi in windrowed groundnuts. A. flavus invaded groundnut kernels to a slight extent during excellent drying conditions. Other fungi were more abundant. Aflatoxin was absent in kernels and shells from windrowed-groundnuts. Kernels from groundnuts windrowed during cooler weather were rarely invaded by Aspergillus spp. Kernels from pods cured against the soil surface were not invaded to a greater extent than those from pods cured in the air. A. flavus was present in the pod surface mycoflora in both experiments.


Mature groundnuts (cv. Early Runner), with a moisture content of 45 to 50% (“green”) or 18 to 25% (“semidry”), were harvested by combining or by hand. Drying rates in the laboratory were then controlled to achieve rapid drying (2 days at 29-35°C, continuous air flow) or slow drying (11 days at 27-35°C, intermittent air flow) until pods reached 8% moisture. Fungal populations on outer surfaces of pods were estimated by a wash-dilution plate method and surface-sterilized kernels were cultured directly. Aflatoxin content of kernel samples was also determined. Mean numbers of total pod surface and kernel fungi were not affected by harvesting method, but slow drying led to significantly larger numbers than rapid drying. Aspergillus flavus was more abundant in kernels from combine-harvested than from hand-harvested pods and more abundant on slowly dried than on rapidly dried pods. Mean aflatoxin B₁ content of slowly dried “green” groundnuts was 1780 µg kg⁻¹ for combine-harvested pods, compared with 140 µg kg⁻¹ for hand-harvested pods. For slowly dried “semidry” groundnuts, combined samples had 1160 µg kg⁻¹ compared with 140 µg kg⁻¹ for hand-harvested samples. Aflatoxin B₁ was absent or found only in trace amounts in rapidly dried groundnuts.


Harvesting methods and drying conditions were studied in relation to fungal invasion and aflatoxin content of groundnut kernels using freshly harvested and partially dried groundnuts. In 1965 and 1966, pods of the groundnut cultivar Early Runner were taken from field plantings near Tifton and Plains, Georgia, USA. At maturity, the plants were mechanically lifted, shaken, and windrowed. Alternate windrows were harvested mechanically with a combine operating at an approximate picking-teeth speed of 300 ft min⁻¹, or manually. In 1965, fresh, mature pods were harvested and placed in driers immediately after windrowing. In other experiments, pods that had dried in the windrows for 2-3 days (semidry) were harvested by the same methods and placed in driers. Combine- and hand-harvested pods from each replication were divided and placed separately in single layers in wire-bottom drier trays. Rapid drying was achieved by using continuous air flow at 29-35°C for 1-2 days. In 1965, green pods required 11 days and semidry pods 7 days to dry to 8% moisture content. Aspergillus flavus was more abundant in kernels from all slowly dried samples, especially the combine-harvested samples in 1965. In 1966, no difference was found in the incidence of A. flavus in kernels harvested by the two methods. The frequency of A. flavus was generally related to aflatoxin content of kernels. An approximate tenfold difference in aflatoxins levels between harvesting methods was found in both 1965 experiments. The difference in 1966 was much less pronounced. Rapidly dried groundnuts were largely free from aflatoxins and A. flavus. The brief windrow periods did not greatly affect aflatoxin content in semidry groundnuts.


A committee to coordinate action on the problem of aflatoxin contamination in Nigeria was formed in 1961 with representatives from four ministries, i.e., the Institute for Agricultural Research (IAR), Zaria. the Nigerian Stored Products Research Institute (NSPRI), and the Northern Nigerian Marketing Board. This Committee was given the responsibility of assessing the extent of the aflatoxin problem in groundnut in the country and of initiating and coordinating all actions leading towards elimination of aflatoxin. IAR investigated the aflatoxin contamination of the groundnut crop up to the stage where the produce was sold by farmers, while NSPRI studied the problem from the time of storage until produce was exported or consumed. IAR investigated the time of invasion of groundnut kernels by Aspergillus flavus, and when, and under what conditions it produced
Groundnuts are produced in two distinct zones in Nigeria: the dry northern zone and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that A. flavus infection of kernels and aflatoxin production occurred in both zones. The condition of the shell was found to have a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by A. flavus, whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20% for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were found to be free from fungal infection at lifting, infection by A. flavus not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of A. flavus in groundnut.


Investigations were carried out at Samam in 1967 in which windrow-dried pods of the groundnut variety Samaru 38 were artificially wetted and the development of kernel infection by fungi studied. One week after windrow-drying, the dried plants with pods still attached were built into two "kiriga" of 3 ft high by 6 ft in diameter, and to one of these the equivalent of 0.75 in. of rain was applied. On the following day a further 0.5 in. of water was applied. Pods sampled from the wetted "kiriga" showed an increase in kernel fungal infection to 33.5% within 24 h of wetting and the level of infection increased to 67.3% after three days. The initial increase was due to invasion of kernels by Fusarium spp. and Aspergillus flavus, but the final increase was due largely to heavy infection of kernels by Macrophomina phaseolina. Undamaged pods from the dry "kiriga" (control) showed no increase in kernel fungal infection.


Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95% of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone the crop is normally harvested after the rains have ended, but in the riverain zone...
variety Samaru 38 showed that there were considerable differences in kernel infection with *Aspergillus flavus* and other fungi between samples taken at different stages in the drying processes but no aflatoxin was found in any of the samples tested.


With the discovery of aflatoxin in groundnuts from many producing countries preliminary investigations were made on the 1961 crop in Nigeria. Results of the toxin tests showed that none of the varieties sampled was more resistant to toxin formation than another and that toxic samples appeared with equal frequency in both Northern and Riverain Provinces. Within each provincial group individual sites varied considerably for toxicity of samples and this may have been due in part to different post-harvest drying and storage procedures. The fungal contamination tests showed no evidence of varietal resistance but did indicate considerably more contamination in samples from the Riverain Provinces than in samples from the Northern Provinces. Condition of the shell was found to affect markedly the degree of contamination of the kernels and, as for toxicity, there were considerable differences between individual sites for contamination with *Aspergillus flavus* and other fungi.


Investigations on aflatoxin in groundnuts were carried out during 1962 at Mokwa and five other localities in the Riverain Provinces of Northern Nigeria. The results indicated that: (1) *Aspergillus flavus* grew much more successfully on moribund or dead groundnut tissue than it did on living tissue. (2) There was virtually no toxicity in the crop at harvest, the exceptional cases being from broken pods. (3) Contamination of kernels with *A. flavus* and appearance of the toxin did not occur until at least five days after harvest. (4) Kernels from broken pods were more likely to be contaminated with *A. flavus* and to be toxic than were kernels from undamaged pods. (5) Toxicity of the crop varied from year to year at specific localities.


Trials were carried out at Mokwa Agricultural Research Station, Northern Nigeria, in 1963 to test various natural methods for postharvest drying of groundnuts. Samples of groundnuts were taken from the range of treatments at intervals from harvest until the end of drying, and these were tested for aflatoxin content and kernel contamination with *Aspergillus flavus* and other fungi. Large differences in rates of drying occurred between the various treatments and, in general, the slower the rate of drying the higher was kernel contamination with *A. flavus* and other fungi and the higher the incidence of toxic samples. When pods were hand-picked from the haulms and sun-dried in a layer, with protection from rain, the rate of drying was high and a toxin-free crop resulted. None of the treatments in which the pods were dried on the haulms in the field gave a toxin-free crop.


Trials were carried out at Kano Agricultural Research Station, Northern Nigeria in 1963 to test various natural methods for postharvest drying of groundnuts. Samples of groundnuts were taken from the different treatments at intervals from harvest until the end of drying and these were tested for aflatoxin content and kernel contamination with *A. flavus* and other fungi. There was very little difference in rate of drying between the various treatments and no effect on toxicity or fungal contamination was observed. Some kernel samples were found to be toxic at harvest and this was associated with pod damage and with kernel moisture content.


Mycological analysis of postharvest groundnut samples was carried out. Various soil-fungi were found to infect the pods; most of them grew through the shell, but not all reached the kernels. The most common were *Aspergillus flavus* and *A. niger*. Where they are treated in oil mills, the originally heavily contaminated kernels are processed through some heat treatments which progressively eliminate all fungal contamination. Nevertheless, recontamination occurs as soon as the pellets are cooled, and a large mold development occurs in the usual conditions of storage. The cakes used to feed cattle are often highly contaminated. Detoxification of cakes by ammoniation destroys the aflatoxin and also has a fungicidal action against many molds, especially *A. flavus*.


Mycological analysis of postharvest groundnut samples was carried out. Various soil-fungi were found to infect the pods; most of them grew through the shell, but not all reached the kernels. The most common were *Aspergillus flavus* and *A. niger*. 
Where they are treated in oil mills, the originally heavily contaminated kernels are processed through some heat treatments which progressively eliminate all fungal contamination. Nevertheless, recontamination occurs as soon as the pellets are cooled, and a large mold development occurs in the usual conditions of storage. The cakes used to feed cattle are often highly contaminated. Detoxification of cakes by ammoniation destroys the aflatoxin but also has a fungicidal action against many molds, especially Aspergillus flavus.


Seven Virginia groundnut varieties, viz. S 230, Kadiri 1, Kadiri 3, Karad 4-11, M13, GAUG 10 and TMV 10 were grown in four locations (Chintamani, Jalgaon, Khargaon, and Junagadh) in India in the rainy season of 1982. After harvest the pods were received at Junagadh during February-March 1983. Pods were shelled and aflatoxin analyses carried out on seeds with a thin-layer chromatography (TEC) method. Aflatoxin B1 was found in all the groundnut varieties (0.8 to 65.8 µg kg⁻¹). Aflatoxins B2 and G1 were found in a few samples from some locations. Among varieties, S 230 had the highest level of aflatoxins at all the locations (49.8 to 85.9 µg kg⁻¹) followed by GUAG 10 (15 to 88.3 µg kg⁻¹) and Kadiri 3 (17.8 to 106.7 µg kg⁻¹). Karad 4-11 had low levels of aflatoxins at all four locations (1.6 to 30.3 µg kg⁻¹) followed by Kadiri 1 (4 to 52 µg kg⁻¹) aflatoxins. M13 and TMV 10 had moderate levels of aflatoxins. Among the locations, Junagadh and Chintamani samples contained higher levels of aflatoxins. Unfavorable postharvest drying conditions at Junagadh and Chintamani probably resulted in higher levels of aflatoxins at these locations. The varieties with more than 3 % phenols in the seed tests generally contained low levels of aflatoxins. The varieties with protein content higher than 25 % contained more aflatoxins, while those having about 22 % proteins contained less aflatoxins except TMV 10 which also had less total phenols. Sugar and oil content did not appear to have any effect on the aflatoxin levels.


The incidence of Aspergillus flavus and aflatoxin in groundnuts was greater in the hotter, lower altitude areas. A. flavus and the toxin were found primarily in broken pods, but they occasionally occurred in apparently undamaged pods. No significant correlation was found between drying treatments and incidence of the fungus in kernels. There was no correlation between superficial shell and kernel infection as long as the shell remained intact.


A critical evaluation of different methods of harvesting and curing groundnuts was conducted with respect to the incidence of aflatoxin content and overall groundnut quality. Groundnuts (cv. Early Runner) were harvested in bulk according to both the conventional method and according to a New Concept Method. Those groundnuts harvested by the conventional procedure were either subjected to field drying and curing or were dried by artificial means in bins using forced air. The New Concept Method involved deliberately allowing the groundnuts to remain in the soil about one month beyond the ideal harvest date, followed by cutting and removing the vines two days prior to digging. The freshly dug kernels were then immediately subjected to artificial curing, employing the same procedure as used for one lot of control groundnuts. Aspergillus flavus was found in damaged nut samples obtained by the new harvesting method. Heavy mold colonization was noted on many kernels which remained in the soil. The percentage of rejected (damaged) kernels from the new harvesting method was about four times that experienced with the two control samples after conventional harvesting, those field cured and those artificially cured.

Many of the kernels remaining in the soil after the ideal harvest date showed serious hull deterioration and this contributed to loss of natural protection against mold spoilage. A significant increase in aflatoxin content was associated with the increase in mold damage. The artificially cured groundnuts were superior in quality characteristics than those field cured, following conventional harvesting. The mature and damage-free groundnuts, obtained by the New Concept Method, were scored higher in good groundnut flavor after roasting than the control groundnuts obtained by the other two methods. This was attained with no change of direct practical significance in fatty acid composition. However, the advantage of superior flavor of good groundnuts obtained by the New Concept Method was wiped out by the high incidence of mold contamination in the overall crop, associated with increased aflatoxin content (30 to 40 times higher). The standard harvesting procedure, with properly controlled artificial curing thereafter, still offers the best way to obtain quality groundnuts with the least aflatoxin contamination.


Accumulation of aflatoxin in Spanish groundnut kernel samples from different geographical areas in Texas during 1966, as detected by thin-layer chromatographic method, was relatively low. Analysis of samples obtained from growers using artificial drying equipment (forced and supplemental heat), when windrow conditions were unfavorable for rapid drying, suggests that this practice reduces the possibility of aflatoxin accumulation. In general, groundnuts harvested from land
planted to groundnuts the previous year were more highly infected with fungi and contained more aflatoxin than groundnuts grown on land planted with rye, oats, melons, or potatoes the previous year. Aflatoxin incidence tended to decrease from south to north Texas. These findings verify previous research observations that moist tropical climates are conducive to fungal infection and aflatoxin accumulation. Detection of aflatoxin in sound mature kernels (kernels screened for minimal size) indicates that the practice of screening for removal of small immature kernels and removal of obviously damaged kernels does not completely eliminate aflatoxin accumulation.


Groundnuts were dug from field plots at Yoakum and Stephenville, Texas, during the years 1967 through 1969, and were subjected to the following treatments: field-dried in inverted (pods in the upright position) and random windrows for different time periods; cured on the vines with forced air; combined at kernel moisture levels above 25% and either flash-dried or stored at 10°C and/or dried at different air flow rates with and without supplemental heat. Following each treatment replicated, 100-kernel samples were surface-sterilized and plated on rose bengal-streptomycin agar. The degree of fungal invasion increased when the average drying rate was less than 0.12% per hour. This rate was influenced by the air velocity, heavy dew and shower frequency, air temperature, and relative humidity. The best drying treatment was on the vines in bins with forced air, and the second best was where 25 CFM of air were passed through several different volumes of bulk groundnuts. Groundnuts from the inverted windrows were less severely damaged by fungi. Groundnut pods in contact with the soil surface beneath the random windrows were more heavily invaded. High-moisture groundnuts held in bulk containers over 18-24 hours without adequate aeration, and those stored at 10°C for over 48 hours were heavily invaded.


Fungal and bacterial damage to groundnuts during windrow-curing was determined by: visual examination of pods and kernels, noting the degree of kernel infection; and determination of germination percentages. Groundnuts removed from the field at digging time and cured on the vines under cover were superior in quality. Infection and physical damage to windrow-cured groundnuts by fungi and bacteria were found to be related to inoculum potential of specific fungal species, degree of pod damage before and during harvest, pod location within the windrows, and climatic conditions during curing. Groundnuts which were cured on the soil surface or inside the windrow during shower periods were more severely infected with bacteria and fungi compared to those cured in the upper part of the windrow. Groundnuts from inverted windrows dried more uniformly under adverse drying conditions and were less severely infected with fungi. Drying groundnuts within the field in random or inverted windrows under high temperatures and/or low humidities caused an increased level of sound splits when shelled. There was an inverse relationship between bacterial infection and percent germination.


Aflatoxins have been a serious problem in Australian groundnuts in the past decade. With the aid of government and industrial funding, the Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Food Research has carried out an ongoing project for most of this period, with emphasis on studies under commercial field conditions. Research has been primarily concerned with understanding the variables that influence the invasion of groundnuts by Aspergillus flavus and A. parasiticus, and the subsequent production of aflatoxins. Factors studied include: levels of A. flavus in soils, environmental factors, farm management practices affecting A. flavus invasion, and the influence of drying and storage procedures on aflatoxin development. Most groundnut soils in Kingaroy contain detectable levels of A. flavus, while surrounding virgin soils usually do not. Levels of A. flavus in groundnut soils vary widely, from less than $10^3$ spores g$^{-1}$ to as high as $10^5$ spores g$^{-1}$ : high levels are much more likely to lead to invasion. Some fields contained consistently high levels over several years. The A.flavus/A. parasiticus ratio also varies widely from farm to farm, and may influence invasion and toxin production. Investigations have shown that invasion of groundnuts by A. flavus takes place before groundnuts are harvested. Invasion will not occur subsequently, aflatoxin is not produced, even under the least effective drying procedures. In all but exceptionally dry seasons little aflatoxin is produced while groundnuts are in the ground, i.e., most aflatoxin is produced postharvest. Under the most favorable conditions, groundnuts require 6 to 10 days to dry in the field after harvest, a period sufficiently long for aflatoxin to reach unacceptable levels. Field drying cannot be sufficiently rapid, even in dry seasons, to ensure aflatoxin-free nuts at intake to shellers. The perceived importance of preharvest invasion as the necessary condition for the production of unacceptable aflatoxin levels has led to attempts to predict aflatoxin levels at shelling intake from A. flavus levels at harvest. Success rates have been encouraging but are not yet of practical utility.


The endocarpic microorganisms of groundnut fruit dried in either a random windrow (plants left as they fell from the digger) or an inverted windrow (plants inverted to expose fruit to sunlight) were different from that of freshly dug fruit. *Chaetomium*, *Penicillium*, *Trichoderma*, *Rhizoctonia*, and *Fusarium* were dominant fungi associated with shells (pericarp) of freshly dug fruit. The dominant fungi of shells of windrowed fruit included *Chaetomium*, *Rhizoctonia*, *Fusarium*, *Sclerotium*, and *Alltmania*. Seeds of freshly dug fruit were dominated by *Penicillium* and *Aspergillus*. The only dominant species in seed of windrowed fruit was *Penicillium*. Microorganisms were isolated from shells and seed of freshly dug fruit at a frequency of 79 % and 52 %, respectively. The percentage of infection was reduced by drying in the field. This was particularly true of the inverted windrows. The proportion of shells and seed infected with a microorganism was reduced 13 % and 36 % respectively, after field drying for 5 to 7 days in random and inverted windrows. Microorganisms were isolated much more frequently from shell pieces (73 %) than from seed (36 %).


Fewer fungi were isolated from groundnut (cv. Virginia 61-R) pods that were windrowed in the up position (44.4 %) than from the down windrow (52.4 %). *Fusarium* spp., *Trichoderma* spp., *Rhizoctonia* spp., *Epicoccum* spp. and *Botrytis* spp. were isolated more frequently from the down windrow than from the up windrow. However, *Chaetomium* spp., *Thielavia* spp., and *Alternaria* spp. were isolated more frequently from the up windrow. More fungi were isolated from pods that were windrowed for 12 days (59.8 %) than those windrowed for 4 days (36.8 %). *Fusarium* spp., *Trichoderma* spp., *Epicoccum* spp., *Phoma* spp., *Botrytis* spp. and *Alternaria* spp. were isolated more frequently after 12 days than after 4 days. Others including *Chaetomium* spp., *Thielavia* spp. and *Rhizoctonia* spp. were isolated more frequently after 4 days exposure. Incubation for 5 days after combining had little effect on the pod microflora. The isolation density of *Aspergillus flavus* was low (3.7 %) although pods were inoculated with this fungus immediately after digging. *A. flavus* was isolated more frequently from seed (4.9 %) than from pieces of shell (2.4 %). The isolation density of *A. flavus* from pods (shell and seed) windrowed in the down and in the up position was 4.5 % and 2.9 %, respectively. Isolates of *A. flavus* were obtained almost twice as readily from pods exposed for 4 days as from pods exposed for 12 days. Plating immediately after combining, or 5 days after combining, had little effect on the isolation frequency of *A. flavus*.


Artificially infested groundnut fruit, field-dried in random windrows (fruit covered with foliage and not exposed to sunlight), yielded more isolates of *Aspergillus flavus* than fruit dried in inverted windrows (fruit resting on top of the foliage and exposed to direct sunlight). Windrow-dried, small (15 mm long), whole immature groundnut fruits were invaded more often by *A. flavus* after artificial infestation than were pieces of shell and seed of larger (30 mm long) immature and mature fruit. The isolation frequency of *A. flavus* from artificially infested mature fruit was about that of untreated mature fruit. Isolates of *A. flavus* were obtained more readily from shell pieces and seed from infested fruit after a 4-day exposure period in either windrow than after a 12-day exposure period. Periods of adverse drying conditions enhanced aflatoxin production in seed of mature windrow-dried fruit.


Differences in infection of seed by *Aspergillus flavus* were noted when comparisons were made between seed from sound or damaged groundnut fruit with different moisture contents. Seed from pods with visible damage (shell damage detected visually) and invisible damage (shell damage detected by a staining technique) were infected more frequently by *A. flavus* than those from sound fruit (no visible or invisible damage). Seed from invisibly damaged fruit were infected almost as rapidly as seed from visibly damaged fruits. The isolation frequency of *A. flavus* from invisibly and visibly damaged fruit was 23 % and 26 %, respectively. *A. flavus* was isolated at a frequency of 8 % from nondamaged fruit. After incubation at a temperature and relative humidity conducive to the rapid proliferation of *Aspergillus* spp. for a period of 24 to 48 hours, *A. flavus* was isolated just as rapidly from seeds from invisibly damaged fruit as from visibly damaged fruit. Although the isolation frequency of *A. flavus* from seed from sound fruit increased with time, the frequency did not approach that obtained from seed from damaged fruit. Before incubation, the isolation frequency of *A. flavus* from seed from non-inoculated fruit with moisture contents of 10 %, 30 % and 50 % was 4 %, 7 % and 10 %, respectively. Following incubation the isolation frequency of *A. flavus* from seed was greater in partially dried fruit than in fruit with a high moisture content. The isolation frequency of *A. flavus* from seed from partially dried fruit with a moisture content of 10 % and 30 % was 20 % and 25 %, respectively, following incubation.
A. flavus was isolated less frequently (12%) from seed from fruit with a 50% moisture content, probably due to competition with other fungi during incubation.


The results of surveys in the Philippines have shown that farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 µg kg⁻¹ aflatoxin. During windrowing, aflatoxin levels increased at the rate of 1.5 µg kg⁻¹ per day. In farm storage aflatoxin contamination continued to increase at the rate of 1 µg kg⁻¹ per day. Aflatoxin contamination was significantly higher during the main cropping season than the second cropping season. At the traders’ level, groundnut samples taken from various middlemen contained 35 µg kg⁻¹ aflatoxin. On the other hand, samples taken from the wholesalers’ newly procured groundnuts contained 188 µg kg⁻¹ aflatoxin. Groundnuts that had been in wholesalers’ warehouse for more than 3 months contained 275 µg kg⁻¹ aflatoxin. At the processors’ level, raw groundnuts for confectionery use (roasted and fried) contained 7.73 µg aflatoxin kg⁻¹, groundnuts intended for peanut butter contained 17.13 µg kg⁻¹, and rejected groundnuts had 120.6 µg kg⁻¹. Aflatoxin contamination could start during harvest. Aflatoxin content increased to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.


Groundnut samples were collected from test fields and farmers’ fields at harvest during the rainy season and from kernels sold in a market. Aspergillus flavus infection was only detected in the samples collected from the market. It is suggested that infection of groundnuts occurs during postharvest drying.


Postharvest quality of groundnut is influenced by the particular set of environmental and cultural practices that influence physiology and maturation. Groundnut composition, although related to environment, changes dramatically as groundnuts mature. There is biochemical basis for inferior quality in immature groundnut. Drought stress and soil temperature influence maturation rate and thus have an indirect effect on postharvest quality. Aspergillus flavus invasion and aflatoxin contamination in groundnuts are related to drought stress, soil temperature, and maturity. Small, immature seed are more likely to be contaminated with A. flavus than larger, mature seed. The biochemical composition, fungal contamination, and the tendency toward higher moisture content complicate storage of immature seed. Each of these factors predisposes immature seed to rapid quality deterioration in storage. Agrometeorological studies must include an awareness of the interrelationships of environment, maturity, and postharvest quality.


Groundnuts were either stacked undried (Stack I) or field-dried to a safe moisture content immediately after harvesting and then stacked (Stack II). The stacks (6 x 3 x 2.5 m) were built on a floor covered with paddy hay. At day 0, 30 and 60, kernels from stack I contained, respectively, 48, 20.5 and 7 percentage moisture; corresponding moisture content for stack II was 8.6.9 and 6.8%. At day 0, 30 and 60, the % of kernels from stack I infected with fungi was, respectively 40, 100 and 72%; corresponding levels of fungal infection in stack II were 48, 28 and 25%. Among the fungi, Aspergillus flavus, A. niger, Fusarium spp. and Macrophomina phaseoli predominated. Those kernels exhibiting visible microbial damage had an unpleasant rancid taste and all yielded M. phaseoli.


Experiments with three groundnut varieties, conducted in the USA, indicated that green or partially dried groundnuts, held with no air flow for 24 hours, were highly susceptible to aflatoxin contamination. Low air flow through the groundnuts did not suppress aflatoxin production. Groundnuts held under N₂ or CO₂ atmospheres developed less aflatoxin, but produced a highly offensive odour. Groundnuts with initial moisture contents below 30% developed considerably more aflatoxin after having been rewetted and placed in high humidity chambers for five days than groundnuts with initial moisture content above 30%. The variety Starr Spanish had considerably more aflatoxin than the varieties Early Runner and Florigiant.
Immature groundnuts were less susceptible to aflatoxin contamination. Prompt drying is the most effective practice for eliminating aflatoxin contamination of groundnuts.


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Fungi isolated from groundnut pods and seeds are listed. Aspergillus, particularly A. flavus, Penicillium, and Fusarium spp. occurred most commonly.


Soil populations of the Aspergillus flavus group fungi gradually increased in two groundnut fields during the 1973 growing season. In one field, populations increased from approximately 50 propagules g⁻¹ of dry soil in June to about 225 propagules g⁻¹ in October. In another field the increase was from 10 in June to 25 propagules g⁻¹ in October. Less man 0.1% of groundnut kernels in windrows had A. flavus infection when collected daily from digging to combining (0-7 days). However, the day after combining and drying to 10-12% moisture A. flavus was isolated from 15-25% of the kernels. Recovery of A. flavus from pods by washing with sterile water demonstrated that there were enough propagules on the pod surface to allow this infection. The number of propagules on the surface did not increase from digging to combining in either field. Even though there was a low A. flavus kernel infection at harvest, 2 to 15 µg kg⁻¹ aflatoxins were found in 21 of the 37 samples collected from digging to combining (0-7 days). The aflatoxins must have been produced by early infections by the A. flavus group fungi that did not persist or were not isolated by the technique used.

2.2.4 In Storage


Fungi isolated from 53 groundnut samples from three production areas (with different soils and irrigation systems) are listed. Aspergillus niger and A. flavus were isolated from all samples, the former in 29-60% and the latter in 4-52% of kernels. Rhizopus spp., Penicillium spp. and Macrophomina phaseolina were fairly prominent in Gezira samples. A. terreus, A. nidulans and Fusarium spp. were minor components. Further development of fungal growth was checked by the low moisture content of kernels and by favorable postharvest climatic and storage conditions.


The percentages of lipids and free fatty acids did not change in non-inoculated groundnuts during storage for 5 days at 28°C, but increased in groundnuts inoculated with Aspergillus and Rhizopus spp. Fatty acids did not fluctuate significantly over the storage period, except that percentages of palmitic and stearic acids tended to decrease, while percentages of oleic and linoleic acids increased. Arachidic, eicosenoic, behenic and lignoceric acids levels remained constant.


The contents of thiamin, riboflavin and niacin decreased when stored groundnuts were infected by Aspergillus and Rhizopus spp.

Research in Nigeria from 1948 to 1982 has identified the factors that determine quality in Nigerian groundnuts. These include, physical state of groundnuts, their moisture content, and storage conditions with emphasis on temperature, humidity, and infestations by pests, rodents and fungi. Procedures for ensuring purchase of only good quality produce and measures for prevention of damage to the groundnuts during storage are reviewed with particular emphasis on pest control. The problem of aflatoxin contamination of groundnuts is discussed. Recommendations to growers, and those concerned with storage and transport of groundnuts on methods of preventing aflatoxin contamination are summarized.


The effects of irrigation regimes and date of harvesting on preharvest infection by Aspergillus flavus and aflatoxin contamination of seed of four commercial and two other groundnut cultivars were studied. Groundnuts watered at 1-, 2-, and 3-week intervals and harvested at the normal time, and at 1 week before, and 1 week after were free from A. flavus infection and aflatoxin contamination. Aspergillus flavus infected a low percentage (2.7-7 %) of groundnuts left in the soil for 6 weeks after harvest. However, no aflatoxin contamination was detected. Will diseases and insect damage, mainly by white grubs and termites, predisposed seeds to preharvest A. flavus infection (56.4-69.8 %) and aflatoxin contamination (18-21 µg kg⁻¹). Groundnuts stored for 3 months in a well-ventilated room with an average temperature of 15°C were infected by A. flavus at a low level, but had no aflatoxin contamination. Infection increased with time in storage. Groundnuts harvested 1 week before maturity were not affected by A. flavus infection of seeds in storage, and there were no differences among genotypes. Groundnuts stacked in sacks at shelling sites were sampled; 4 % were contaminated with aflatoxin. the average level being 11 µg kg⁻¹. Fifteen % of the samples from oil mill sites were kept under three different conditions in commercial warehouses. During the storage period environmental temperature, relative humidity (RH), water activity, and free fatty acids of the samples, aflatoxin levels and fungal flora were determined at monthly intervals. Samples stored from fall to winter showed no significant accumulation of aflatoxin even though Aspergillus including A. flavus and Fusarium spp. were present. The level of free fatty acids remained unchanged for five months. During summer to fall, groundnut samples contained detectable levels of aflatoxins. However, the variation of aflatoxin levels was small under these conditions after six months of storage. Seasonal changes in RH caused a decrease in fungal species including A. flavus. Accumulation of free fatty acids was observed during storage at relatively high temperature. Laboratory studies of aflatoxin production on raw shelled groundnuts were also carried out. Groundnuts were inoculated with an aflatoxigenic strain of A. parasiticus (ATCC 15517) and incubated under various conditions [0, 15, 30 or 60 days at temperature/RH levels of 15°C/65 %, 25°C/79 %, 25°C/100 %]. Aflatoxins were found after incubation at 100 % RH and 25°C for 2 months. However, growth of the aflatoxigenic fungi and the production of aflatoxins were not observed during storage for the same period at RH <79 % at 25°C.


Sound mature groundnuts at kernel moisture levels of 48.9, 38.3, 36.9, 32.8, 25.4, 12.8, and 5.7 % were inoculated with an aflatoxin-producing Aspergillus flavus and incubated under compressed air. nitrogen, 2-aminobutane, and a mixture of 87 % CO₂ and 13 % N₂ at 25°C. Fungal growth and aflatoxin production were determined after 9 days. Growth occurred under compressed air at all moisture levels, but was not evident under mixed gas at 12.8 % moisture and below. Growth of A. flavus was completely inhibited by the nitrogen gas; consequently no aflatoxin was produced. Fusarium species grew under the nitrogen atmosphere, and these kernels developed an offensive odor at all moisture levels. Less growth occurred on kernels stored under 2- aminobutane or washed with a solution of 2-aminobutane; however, the kernels readily absorbed this compound and became dark and odoriferous. These results indicate that nitrogen gas and 2-aminobutane are undesirable for use in preventing fungal growth on groundnuts while they are held in storage. A mixed gas containing a desirable ratio of CO₂, N₂, and O₂ appears to be more desirable than natural air for use as a storage gas.


Various samples of groundnuts and groundnut meals imported into the UK from six countries (Brazil, Nigeria, Uganda, South Africa, the Gambia, and Zimbabwe) were.

Two bulk samples of imported raw shelled groundnuts naturally contaminated with aflatoxigenic Aspergillus flavus were kept under three different conditions in storage for the same period at RH <79 % at 25°C.
examine for microflora and toxicity. The microscopical examination of 29 meal samples showed hyphae in the cotyledon cells of 19, of which 16 were toxic. The proportions of sound, damaged and discolored kernels in the samples were also determined. The proportion of sound kernels was generally high in all samples, and even in the highly toxic sample V.3827, 78% of the kernels were of this type. Microscopical and cultural examination of 61 sound kernels (undamaged kernels with white flesh cotyledons) showed only three (4.9%) to be invaded by fungi. The toxicity of 11 samples of these nuts was tested in ducklings and no liver lesions were observed from eight. The remaining three also included damaged and discolored kernels. Fungi were frequently isolated from kernels with yellow, orange, buff brown or black flesh (74.4% infected). Aspergillus flavus was dominant in the mycoflora followed by Phoma spp. and A. tamarii. Nine of 19 isolates of A. flavus tested, 9 were toxin-producers.


This paper reports results of research at Oklahoma State University on control of mold development on high moisture groundnut pods by storage in anaerobic and fungitoxic gases at two temperatures during 1968 and 1969. Storage of pods in N2 or CO2 to prevent mold growth and aflatoxin production was tested. Nondried and partially dried pods were inoculated with an aflatoxin-producing strain of Aspergillus flavus and stored in chambers held at 3 and 24°C. Air, N2 and CO2 were metered through replicated chambers. Spaced samples were assayed for quality, moisture, mycoflora, and aflatoxins. All air-treated pods at 24°C soon became covered with mycelium of species of Fusarium, Rhizopus, and Mucor, but the moldy, partially dried pods soon became overgrown with A. flavus. Mold development was greatly delayed by CO2 or N2. CO2 prevented mold development for over 2 weeks at 24°C while pods held 36 days at 3°C in CO2 had their original mold-free appearance and a near-normal odor. Moldy pods had a fermentation odor. High levels of aflatoxins occurred in kernels from pods stored in air at 24°C; levels increased with time. Higher levels of aflatoxin occurred in nondried than in partially dried kernels. CO2 and N2 treatments produced aflatoxin-free kernels. Molds were isolated from all visibly mold-free CO2 and N2 treated pods.


Molds develop on high-moisture groundnuts when these are improperly dried or held in bulk. Storage of pods in N2 or CO2 to prevent mold growth and aflatoxin production was tested. Nondried and partially dried pods were inoculated with an aflatoxin-producing strain of Aspergillus flavus and stored in chambers held at 3 and 24°C. Air, N2 and CO2 were metered through replicated chambers. Spaced samples were assayed for quality, moisture, mycoflora, and aflatoxins. All air-treated pods at 24°C soon became covered with mycelium of species of Fusarium, Rhizopus, and Mucor, but the moldy, partially dried pods soon became overgrown with A. flavus. Mold development was greatly delayed by CO2 or N2. CO2 prevented mold development for over 2 weeks at 24°C while pods held 36 days at 3°C in CO2 had their original mold-free appearance and a near-normal odor. Moldy pods had a fermentation odor. High levels of aflatoxins occurred in kernels from pods stored in air at 24°C; levels increased with time. Higher levels of aflatoxin occurred in nondried pods than in partially dried pods. CO2 and N2 treatments produced aflatoxin-free kernels. Molds were isolated from all pod samples regardless of treatment. CO2 and N2 were fungistatic.


Fungal contamination of 10 samples of groundnuts sold in the 1988 warm rainy season in Reunion Island was examined. Aspergillus niger was the most frequent and abundant species in surface and internal mycoflora, followed by A. glaucus group. Predominant species on seeds were the A. glaucus group, Syncephalastrum sp., A. niger and Rhizopus sp. (15.8, 6, 5.6 and 3.6% of seeds infected, respectively). A. flavus was isolated from all samples; 8 were aflatoxigenic (7 seemed to be highly toxigenic). All groundnut samples were negative for aflatoxins.


Samples containing excess amounts of aflatoxin, normal and defatted groundnut meal, raw and roasted peanut butter, were kept exposed to air at 50% relative humidity and 73°F for up to two years. The aflatoxin contents were not significantly affected by time or storage temperature.

The problem of mold growth and aflatoxin contamination in commodities transported over long distances from cultivation regions to consumption centres is discussed. If the contamination occurs during transit, often no insurance coverage for the risk is available. Because of different methods of sampling followed in the exporting and importing countries, it is often difficult to define the exact responsibility of the development of aflatoxin as having taken place during transit. The statistics of the export of aflatoxin high-risk commodities like red pepper (chillies), cottonseed and groundnut extractions clearly, for the last decade, demonstrate the extent of loss suffered by the exporting countries because of aflatoxins. The problem of mold damage and mycotoxin contamination can be minimized by improving facilities for storage at port and transit points and on ships.


Fifty-one samples of stored groundnut kernels of the 1963 crop and 63 of the 1964, both from different qualities, varieties and regions in Israel, were tested for fungal infection, and for their moisture content and toxicity. Under storage conditions during the seasons relatively low moisture content of the kernels was maintained, always below 9%. The 71 identified fungal species were similar to those found on groundnut kernels from other countries. Aspergillus niger was the most common species, which appeared in large numbers in 97.4% of the samples. The relative levels of Rhizoctonia and Rhizopus increased when the samples were exposed to humidity prior to storage. The average number of fungal colonies per sample, the percentage of samples infected with A. flavus and the percentage of A. flavus in the total mycoflora rose as storage time increased and especially after shelling of pods. A. flavus was detected in 78.4% of the 1963 samples and 63.5% of the 1964 samples. The percentages of toxic samples for each year were 20% and 12.7% respectively. It is assumed that storage conditions in Israel during these years were not conducive to the production of aflatoxins.


Fungi isolated from 55 oilseed samples (20 mustard, 10 sunflower, 4 soybean, 4 groundnut, 6 linseed, 5 safflower, 4 sesame and 2 niger) included Aspergillus fumigatus, A. flavus, A. niger, Fusarium spp. and Penicillium spp.


In summer (June-Aug) and winter (Dec-Feb) 1985-1986, 25 and 51 samples of raw groundnut kernels were obtained from retail stores distributed in Chiayi, Yunlin and Tainan areas of Taiwan. Moisture content, germination, mold count and aflatoxin-producing molds were assayed. Average moisture contents (7.83-8.17%) did not differ significantly between summer and winter samples. Mold counts of summer samples were much higher than those of winter samples (2.45 vs 1.31 x 10^6 cfu g^-1). Percentage samples with Aspergillus flavus parasiticus agar (AFPA) positive colonies in summer and winter samples were 24 and 7.8%, respectively. The germination percentage of the former was lower than that of the latter. All AFPA colonies were confirmed as toxin producers. Mold counts were not linearly dependent upon moisture contents for either sample. No significant difference was noted between mold counts on AFPA and potato-glucose agar media.


Sound inshell runner-type groundnuts, manually damaged inshell groundnuts, shells, sound kernels deskinne kernels and skins were stored in separate flasks under an atmospheric relative humidity of 100% at 28°C. After 5 days, water was absorbed at levels of 1.2, 1.7, 3.9, 0.9, 1 and 9.5 g 100 g^-1 dry material, respectively. Surface disinfested components were inoculated with spores of an aflatoxin-producing isolate of Aspergillus parasiticus (NRRL 2999) and incubated under the same conditions. The time required for visible growth of the fungus was 8, 6, 4, 12, 10 and 3 days, respectively. The time for appearance of the conidiophores was 14, 10, 6, 16, 13 and 6 days. After a 3-week incubation period, aflatoxin levels in groundnut components were 111.4, 159.1, 4.4, 58.7, 99 and 1.5 μg kg^-1, respectively.


The fungi most frequently isolated from samples of foods collected during 1976-77 were Absidia, Aspergillus (mostly A. eandidus, A. flavus and A. glaucus), Mucor, Paecilomyces, Penicillium and yeasts. Mycotoxins found in 1976 were ochratoxin in wheat, stigmatoxystein and ochratoxin in barley, and aflatoxin in groundnut kernels and pellets. In 1977, ochratoxin and zearalenone were found in wheat,

Unshelled seeds of groundnuts ( cvs. GAUG-1, J 11, and JL 24) stored at 62, 76, 85 or 93 % relative humidity (RM) suffered greater invasion by Aspergillus niger, A. flavus and particularly by Rhizopus sp. at higher RH. At 62 % R11 seed viability was not reduced. Storage at 85 % RH for 120 days resulted in complete loss of viability, while with 93 % RH for 90 days the highest germination percentage was 5 in cultivar J 11.


In quantitative determinations of mycoflora, farmer stock groundnuts from 26 farm-size bins of several types of construction differed in initial moisture, damage, type of bin, and length of time in storage. High mycofloral counts were associated more often with high initial moistures of groundnut kernels than with any other factor. The predominant fungi were several species of Aspergillus glaucus group, A. tamarii, and Penicillium citrinum. Of the A. glaucus group, A. ruber, A. repens, and A. restrictus occurred more frequently and in greater numbers than did A. chevalieri and A. amstelodami. Other species occurring frequently were A. candidus, Cladosporium sp., Torula sacchari, and P. funiculosum. Storage fungi, found in large populations on stored groundnuts, probably contribute to deterioration as demonstrated with other kinds of stored seed.


Samples of farmers’ stock groundnuts were collected from 6 locations at weekly intervals for 5 weeks during the harvesting season. Seventeen of the 228 samples collected contained aflatoxins. Aflatoxins were present in 2.5 % of the Segregation I (highest quality). 12.1 % of the Segregation II, and 25.7 % of the Segregation III samples, and averaged 22, 264, and 324 µg kg⁻¹ total aflatoxin respectively. A significant relationship was thus found between the presence and concentration of aflatoxins and grading factors. Samples with a high visible damage values were more likely to contain atoxins than were samples with high invisible damage (rancidity, mold or decay) values. The weekly incidence of aflatoxins was related to the climatological conditions during the 14-day period prior to the date of collection. Mycoflora studies of 73 selected samples showed a relationship between the mean number of fungi per sample and grading factors; the mean number increased as quality decreased. These findings support previous observations that factors which influence groundnut quality also influence the incidence of aflatoxins in farmers' stock groundnuts.


Samples of farmers' stock groundnuts were collected from six locations at weekly intervals for five weeks during the 1967 and 1968 harvesting seasons. Seventeen of 228 samples collected in 1967 and 23 of 356 samples collected in 1968 contained aflatoxins. In 1967, 2.5 % of Segregation I, 12.1 % of Segregation II, and 25.7 % of Segregation III samples contained aflatoxins and averaged 22, 264, and 324 µg kg⁻¹ total aflatoxins respectively In 1968, 3.2 % of Segregation I, 6.3 % of Segregation II, and 11.9 % of Segregation III samples contained aflatoxins and averaged 28, 61, and 583 µg kg⁻¹ total aflatoxins respectively. Thus, in both years, significant relationships were found between the presence and concentration of aflatoxins and grading factors. Weekly incidences of aflatoxin contaminated samples were related to climatological conditions during the 14-day period to the collection dates in both years. Mycofloral studies of 73 selected samples in 1967 and of all samples in 1968 showed a relationship between the mean number of fungi/sample, grading factors and aflatoxin contamination. These findings support previous observations that factors which influence groundnut quality also influence aflatoxin contamination in farmers' stock groundnuts.


A survey of groundnuts from Egypt showed 19.5 % of unshelled and 49.0 % of shelled samples to contain low levels of aflatoxins B₁, B₂, G₁ and G₂; only B₁ was detected in 3.5 % samples of roasted groundnuts. However, 60 % of Aspergillus flavus strains from Egypt produced one or more aflatoxin, and 40 % of toxigenic strains produced high quantities (5000-20000 µg kg⁻¹) of B aflatoxins; the levels found in groundnuts were therefore lower than expected. Studies on the effects of storage temperature and RH on groundnut mycoflora and aflatoxin development showed an RH of 95 % to be optimum for aflatoxin production; since the RH in Egypt is lower than this, this was considered a factor contributing to the low levels encountered. Inoculation of Egyptian groundnuts (cv. Giza 1) with different strains of A. flavus showed the shells to act as a barrier towards invasion; this was...
considered the main reason for low aflatoxin production. Consequently, it is considered imperative to discard groundnuts with cracked shells before storage.


Aspergillus flavus, A. niger, Alternaria, Fusarium, Helminthosporium, Mucor, Penicillium, Rhizoctonia, Rhizopus and Sclerotium spp. were isolated from groundnut shells, whereas A. flavus, A. niger, Fusarium, Macrophomina, Penicillium, Rhizoctonia and Sclerotium spp. were the most frequent isolates from kernels. Over 50 % of A. flavus strains produced aflatoxins.


Sixty-four species and two varieties belonging to 19 genera of fungi were isolated from 40 groundnut seed samples collected from different places in Egypt by using a dilution plate method on glucose-Czapek’s medium. The most frequent genera were Aspergillus (21 species and 2 varieties), Penicillium (16 species) and Fusarium (6 species). A. flavus, A. fumigatus, A. niger, P. chrysogenum and F. oxysporum were the most common fungal species. Forty-seven % of the samples were toxic to brine shrimp (Anemia salina) larvae. Groundnut samples were contaminated with aflatoxins (11 samples), T-2 toxin (7 samples), diacetoxyscirpenol (3 samples), zearalenone (one sample) and citrinin (one sample). The authors believe that this is the first report of the natural occurrence of zearalenone and trichothecenes in groundnuts.


Comparisons were made of the quiescent mycofloral communities in mature, cured groundnut pods from the crops grown in Virginia, USA, with those grown in Puerto Rico. Few fungi were found in seed of undamaged pods from Virginia (VA) and Puerto Rico (PR). After hydration, fungi were found in 95 % of seed in discolored shells and in 40 % and 85 %, respectively, of VA and PR seed in unblemished shells. Ten species or genera of fungi were characteristic of one or more of the eight endocarpic communities. Shell communities were more complex. Among the characteristic forms were five toxigenic types and three groundnut pathogens. The PR and VA communities each had three exclusively characteristic forms. Only two forms seemed to invade seeds from shells during hydration. Aspergillus flavus was rare in PR samples, and in VA samples was not found before hydration, A. flavus was a characteristic form of VA unblemished shells and seed. Though no other fungus was found in more than 20 % of VA seed, A. flavus was found in up to 30 % and 50 %, respectively, of seed from unblemished and discolored shells. Possibly it was suppressed in discolored VA shells by competition from Fusarium spp. and Rhizopus stolonifer.


After six months in storage, approximately 15 % of windrowed-cured (rapid) and approximately 40 % of stack-cured (slow) seed of the Virginia type groundnut (cv. Florigiant) was infected with Aspergillus flavus, whereas with stack curing in the Spanish types Spancross and Starr only approximately 4 and 5 % of the seed, respectively, was infected. The more rapid curing and the tightly adhering pericarp of the small-seeded spanish-type fruit appears to inhibit invasion by the fungus.


When seeds of 40 species of leguminosae were examined by the PDA and the blotter method, Aspergillus clavatus, A. flavus, A. fumigatus, A. giganteus, A. niger and A. oryzae were the most common fungi. Rhizopus spp. were found only on groundnut, pigeonpea, Lablab niger (L. purpureus) and cowpea.


The occurrence of aflatoxin-producing strains at Aspergillus flavus was surveyed in retail samples of five kinds of edible nuts (mostly tree nuts) and compared with 10 samples of groundnuts. All samples had been imported from various countries in 1981 and 1983. Aspergillus flavus was present in 128 (46.5 %) of 275 tested kernels of the edible nuts, and 49.6 % of the groundnuts. A total of 91 isolates of A. flavus were selected and examined for their aflatoxin-producing ability; of 44 isolates of A. flavus from other edible nuts. 37 (84.1 %) were shown to produce aflatoxin on defatted groundnut meal medium, while 43 of 47 isolates (91.5 %) of A. flavus from groundnuts were positive. Most aflatoxigenic isolates from the edible nuts were identified as A. flavus subsp. flavus var. flavus, and four isolates of these, which were isolated from Hawaiian macadamia nut, produced higher levels of aflatoxins G1 and G2 than those of aflatoxins B1 and B2. Most isolates from
groundnuts imported from USA produced aflatoxins B and G and were identified as *A. flavus* subsp. *parasiticus* var. *parasiticus*. This survey revealed that imported edible nuts including almond, chickpea, hazelnut, macadamia nut and pistachio were significantly infected with aflatoxigenic *A. flavus*.


Unshelled and shelled groundnuts with initial moisture contents of 7.5 and 6 %, respectively, were stored for 12 months in glass containers at temperatures of 4° and 27°C; the containers were weekly flushed with air, CO₂ or N₂. The composition of pod surface and kernel mycoflora was not affected by any treatment, the number of propagules remained unchanged at 4° and decreased at 27°C. The data indicated that CO₂ and N₂ may depress aflatoxin production during storage though these atmospheres did not suppress the fungus *Aspergillus flavus*, and that storage of shelled groundnuts for 1 year may involve hazards of deterioration and aflatoxin increase.


The mycoflora was determined for fresh and stored groundnut kernels removed from disinfested pods, and for kernels from similar pods but disinfested after their removal. Eighty-three fungal species were found on all these kernels, with 35 species (including *Aspergillus flavus*) from surface-sterilized kernels. Forty-six of the species did not appear to have been recorded previously on groundnut pods or kernels. Although the number of colonies yielded by most species was predictably larger on kernels without surface-sterilization, four of the more common species were more frequent after disinfestation: *Aspergillus versicolor*, *Fusarium solam*, and *Penicillium rubrum* on fresh kernels, and *A. niger* on stored kernels. The high incidence of *A. niger* in stored kernels that were disinfested. as compared with those that were not, is taken to indicate a selective removal of antagonists of this species by mercurial disinfestation.


The mycoflora of groundnut kernels was studied over a 5 year period in Israel. A total of 132 samples of fresh, and 419 samples of stored kernels were examined. A list of 173 species isolated from these kernels is given, comprising 109 from fresh, and 153 from stored kernels. In three of the four years in which a comparative study of fresh and stored kernels was done, the number of fungal colonies from the former exceeded that from the latter by about 25 %. *Aspergillus flavus* usually occurred only in small quantities. *Aspergillus niger* was the most abundant fungus. On stored kernels it was even more predominant than on fresh kernels. *Penicillium funiculorum* and *P. rubrum* also occurred frequently.


Aflatoxin was found in up to 6.4 % of fresh groundnut kernels and in up to 32 % of stored kernels, although the intensity of toxicity was always very low (up to 125 μg kg⁻¹). About 90 % of 1626 *Aspergillus flavus* isolates from groundnut kernels, rhizospheres, and geocarpospheres produced aflatoxin in culture. In quantitative tests with 750 isolates, 60% of the isolates produced > 25 μg g⁻¹ aflatoxin.


During the first six months of storage *Aspergillus niger* was the dominant fungus in samples of groundnut kernels from the 1965-1966 and 1966-1967 crops on all types of soil. *A. flavus* and *Fusarium* spp. were present in only small amounts, whereas prevalence of *Penicillium* spp. declined in early stages of storage. *Aspergillus niger* appeared to suppress other fungi in the kernel mycoflora. The numbers of *A. niger* colonies in the geocarposphere and within groundnut kernels were positively related, but for other fungi no relationship was observed.


Twenty species of fungi were isolated from pods and seeds of stored groundnuts collected from different areas of Tamil Nadu. Germination was not significantly affected by most of the fungi except *Aspergillus niger* and *Rhizoctonia bataticola* which reduced germination by about 30 % after storage for 6 months. Root development was reduced by 93 to 95 % by *A. flavus* and *A. niger*, shoot development was most reduced (72 %) by *Rhizoctonia bataticola*. The most predominant fungus on the groundnut seeds, *Rhizopus nigricans*, was not pathogenic. Germination was considerably lower when seeds infected with *R. bataticola*, *A. flavus* and *Cladosporium herbarum* were stored at 95 % relative humidity than at 32.3 % relative humidity. TMTD was more effective than captan or Ceresan in maintaining seed viability. Groundnut seeds stored in jute bags for 3 or 6 months had lower germination values than those stored in wooden boxes, paper or polythene bags.
Llewellyn, G.C., O'Rear, C.E., Moll, M.B., and Dashek, W.V. 1986. Aflatoxin contamination of peanuts grown in Virginia for the crop-years 1974-1982. Monthly aflatoxin levels in groundnut lots brought to market, for September and the aflatoxin levels in postharvest groundnuts. Aflatoxin levels varied with the month of the year in a cyclic fashion, with peaks in May and November. The temperature and the rainfall level were also related to aflatoxin levels in shelled groundnuts.


Sound, mature kernels of the groundnut cultivar Early Runner were inoculated with Aspergillus flavus and incubated under various concentrations of carbon dioxide (CO\(_2\)), nitrogen (N\(_2\)), and oxygen (O\(_2\)) at high moisture levels for 2 weeks at 30°C and 6 weeks at 15°C. Observations were made on growth and sporulation of the fungus and assays were conducted on infected kernels to determine the concentrations of aflatoxins and free fatty acids. No reduction in growth and sporulation of A. flavus occurred when the CO\(_2\) concentration was increased from 0.03% (air) to 20%. Fungus growth and sporulation were reduced with each 20% increase in CO\(_2\) from 20% to 90%. No growth occurred in 100% CO\(_2\). No apparent decrease in growth or sporulation occurred when O\(_2\) was reduced from 20% cent to 50%, regardless of the CO\(_2\) concentration. However, striking reductions occurred when O\(_2\) was reduced from 5 to 1% with 0, 20, or 80% CO\(_2\). Aflatoxin production decreased with increasing concentrations of CO\(_2\) from 0.03 to 100%. In general, reducing the O\(_2\) concentration decreased amounts of aflatoxin. Sizeable decreases in aflatoxin production occurred when O\(_2\) was reduced from 5 to 1% in combination with 0, 20, or 80% CO\(_2\). Aflatoxin production was lower in groundnuts stored at 15°C under 20% CO\(_2\) for 6 weeks when O\(_2\) was reduced from 20% to 5%. Aflatoxin was low in groundnuts stored at 15°C for 6 weeks under high concentrations of CO\(_2\). Free fatty acid formation closely paralleled growth, sporulation, and aflatoxin production by the fungus. Striking decreases in percentage of free fatty acids occurred when O\(_2\) was reduced from 5 to 1%.


Monthly aflatoxin levels in groundnut lots brought to market, for September 1968-June 1974, were compared to certain monthly environmental characteristics. Graphical and linear regression analyses were employed both separately and in tandem to determine whether a relationship exists between the environmental data and the aflatoxin levels in postharvest groundnuts. Aflatoxin levels varied with the month of the year in a cyclic fashion, with peaks in May and November. The temperature and the rainfall level were also related to aflatoxin levels in shelled groundnuts.


An eight year (1974-1982) investigation assessing the relationships between air temperature (AT), precipitation (P) and aflatoxin (AFT) contamination of stored groundnuts is reported. The mean percentages of nuts exhibiting various AFT levels for the 8 years (July-June, 1000 samples year\(^{-1}\)) were: 80 \pm 21, 10 \pm 7, 3 \pm 3, 4 + 6 and 3 + 6 for 0-4, 5-15, 16-25, 26-100 and 100 µg \(\text{kg}^{-1}\), respectively. The mean percentage for pooled period USA/FDA regulations was 8 ± with 1980/1981 a high (46 %) contamination year. Statistical correlations for either linear (Pearson's) or non-linear (Spearman’s) relationships suggested that: (1) the % AFTs 15 µg \(\text{kg}^{-1}\) was not related to either AT or P in either a linear or non-linear manner; (2) total AFT levels were related non-linearly to AT but exactly how was not evident; (3) the environmental variable most influencing AFT levels was AT the month that the sample was obtained; and (4) as AT increased, there was an associated decrease in total AFT levels and vice versa.


To establish those environmental conditions which promote the growth of aflatoxin-producing Aspergillus spp. on groundnuts, a 4-year (1982-1986) investigation was undertaken to examine possible relationships between air temperature (AT), precipitation (P) and aflatoxin contamination of stored groundnuts. The mean percentage of nuts possessing various aflatoxin levels for the years 1982-1986 (July-June) were 74.2- 88.0 for 0-4 µg kg\(^{-1}\), 6.3-14.9 for 5-15 µg kg\(^{-1}\), 2.4-5.9 for 16- 25 µg kg\(^{-1}\), 2.3-6.4 for 26-100 µg kg\(^{-1}\), and 0-4.7 for >100 µg kg\(^{-1}\). The mean percentage for the years which exceeded USDA/FDA regulations were 7.1 (1982-83), 7.6 (1983-84), 11.6 (1984-85) and 17.0 (1985-86). Examination of the mean percentage >15 µg kg\(^{-1}\) for each month during these four years revealed that the following months fell within that range: September, November, December, January, February and May (1982-83); July. October, April and June (1983-84); August and June (1984-85); and July, April and May (1985-86). Comparisons of pooled-aflatoxin levels, rainfall and temperature over four years suggested a ‘better lit’ between mean monthly P and mean % aflatoxin > 15 µg kg\(^{-1}\), than between the latter and mean monthly air temperature. However, application of a predictor equation indicated a correlation between aflatoxin levels and monthly air temperature.

Mycological analysis of postharvest groundnut samples was carried out. Various soil-fungi were found to infect the pods; most of them grew through the shell, but not all reached the kernels. The most common were Aspergillus flavus and A. niger. Where they are treated in oil mills, the originally heavily contaminated kernels are processed through some heat treatments which progressively eliminate all fungal contamination. Nevertheless, recontamination occurs as soon as the pellets are cooled, and a large mold development occurs in the usual conditions of storage. The cakes used to feed cattle are often highly contaminated. Detoxification of cakes by ammoniation destroys the aflatoxin and also has a fungicidal action against many molds, especially A. flavus.


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In groundnut seeds adjusted to various levels of moisture content (8.5, 13.5, 17.5 and 21 % dry weight basis) and stored for 6 months at 5, 15, 28 and 45°C, the total count of fungi (recovered at 28°C) increased regularly and the germinability declined with the rise in moisture content and with lengthening of storage period. At 5, 15 and 28°C, Aspergillus fumigatus was the most dominant species followed by A. flavus, A. niger, A. terreus, Penicillium sp., Pyrenocheata sp. and Scopulariopsis brevicaulis. The degree of dominance of each species depended on conditions of storage and length of storage.


Among the fungi isolated from 80 seed and 40 shell samples of groundnuts (from different places) were 16 Aspergillus spp., 14 Penicillium spp., 2 Mucor, 2 Rhizopus spp., 3 Fusarium spp., 2 Gibberella spp., 2 Gliocladium spp., 2 Drechslera spp. and 16 other species. Aspergillus niger, A. fumigatus, A. flavus, A. terreus, A. ochraceous and P. funicullosus were generally the most common.


There was a 20-fold variation in the aflatoxin content of groundnut cake stored in sacks for about 3 years. The two batches with highest contents had ranges of 630 to 17630 and 1560 to 28440 µg kg⁻¹. Up to 60 % of fungal strains produced aflatoxin. Isolated Aspergillus flavus strains produced aflatoxin when grown on moist wheat at 24°C.


Aflatoxin was present at a high level in most common foods stored poorly for long periods in Nigeria (viz. groundnuts, dried fish, groundnut oil, guinea corn (sorghum), palm oil, rice, maize, beans, yams, acha, cassava and garri). It may work synergistically with other carcinogens to produce the high incidence of primary liver cancer seen in men under 40 years of age.


In a survey of groundnut samples from markets at 6 locations, 20 per cent of the samples contained aflatoxin. Aspergillus, Penicillium and Rhizopus spp. predominated among the fungi encountered.

Groundnut storage problems were studied in the Cote d’Ivoire during 1985 and 1986, from three different aspects: insect pests, fungal infection, and aflatoxin contamination. Sampling was done in all parts of the groundnut-growing areas. Selected from a large population of traditional farmers (only manual practices), 164 local stocks were sampled three times throughout the storage season, in December 1985, and in February and April 1986. Other samples were taken at the same time from different town and village markets. Several parameters such as pest damage (penetrated and/or scarified pods, non-damaged pods), fungal infection, germination of seeds, and aflatoxin contamination levels were studied. Generally, the market samples were less contaminated than the samples from the local stocks. With a few exceptions the stocks studied were always contaminated with detectable aflatoxin levels. Nine % of all the local stocks had more than 250 µg kg⁻¹ of total aflatoxins, but 65 % of the samples never reached the European Economic Community (EEC) norm of 10 µg kg⁻¹ of aflatoxin (against 4 % and 82 %, respectively for related market samples. Highly significant correlations were found between aflatoxin contamination and different parameters such as scarified, penetrated and broken pods, time in storage and quantity of remaining groundnut stocks, prior to sampling. Corresponding regressions could explain more than 25 % of the whole variance (from a particular study of 75 local storage areas).


Groundnut storage problems were studied in Côte d’Ivoire during two successive storage periods (1985/86 and 1986/87) and from three different aspects: insect pests; mold damage; and contamination with aflatoxins. Samples were taken periodically from traditional producers’ fields throughout the groundnut growing areas of the survey and from town and village markets. Generally, locally stored samples were a little less infected than samples from markets. With few exceptions, all the locally sampled material was contaminated with measurable levels of aflatoxin. Over the 2-year survey period, 7.9 % of the 434 local stocks examined exceeded the toxicity level threshold of 250 µg kg⁻¹, with 4.4 % above 1000 µg kg⁻¹. It was also found that 73 % of these samples were above the European Economic Community (EEC) safety level of 10 µg kg⁻¹. Significant correlations were found between aflatoxin contamination and different storage and meteorological variables. These included physical characteristics and age of the pods, and the influence of the prevailing atmospheric conditions.


In the Philippines, where rice is harvested by hand, there is frequently a delay between harvesting and threshing, and grain with up to 30 % moisture content may be left in the field for lengthy periods prior to storage. This leads to the development of discoloration, bad odours, and ultimately to molds and mycotoxins. Reports are given on a survey recently concluded in which samples of paddy, groundnuts, soyabeans and copra were collected and examined for molds. The principal fungus recorded was Aspergillus flavus. The importance of mycotoxins and their effect on man and animals is discussed, with special reference to aflatoxin, and its occurrence as recorded during the survey.


The results of surveys in the Philippines have shown that farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 µg kg⁻¹ aflatoxin. During windrowing, aflatoxin levels increased at the rate of 1.5 µg kg⁻¹ day⁻¹. In farm storage aflatoxin contamination continued to increase at the rate of 1 µg kg⁻¹ day⁻¹. Aflatoxin contamination was signifiandy higher during the main cropping season than the second cropping season. At the traders’ level, groundnut samples taken from various middlemen contained 35 µg kg⁻¹ aflatoxin. On the other hand, samples taken from the wholesalers’ newly procured groundnuts contained 188 µg kg⁻¹ aflatoxin. Groundnuts that had been in wholesalers’ warehouses for more than 3 months contained 275 µg kg⁻¹ aflatoxin. At the processors’ level, raw groundnuts for confectionery use (roasted and fried) contained 7.73 ug aflatoxin kg⁻¹, groundnuts intended for peanut butter contained 17.13 µg kg⁻¹, and rejected groundnuts had 120.6 µg kg⁻¹. Aflatoxin contamination could start during harvest. Aflatoxin content increased to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.


Part I of this report deals with a survey of mycotoxins contamination in food and its relation to hepatoma in Malaysia. The topics covered include: (a) Biostatistical aspect of liver cancer in Malaysia (b) A semi-quantitative study on frequency of
food intake in Sekinchan, (c) Results of mycological survey of Malaysian foods, and (d) Analysis of mycotoxins in foods in Malaysia. The incidence of liver cancer is higher in Malaysia than in Japan and is highest in the Chinese male population. In the three communities rice, oil, fat and vegetables are the main foods. The Malays frequently eat fresh fish, beans and bawang and use chili kering; the Chinese frequently eat bawang meats, fresh fish and beans; the Indians frequently use milk and its products and dry curry powder. Aspergillus spp. were the major fungal species found, especially in milled rice. Aspergillus candidus was most frequent in rice (52 % of samples) and A. niger moderately abundant. A. flavus was isolated from 13-21 % of rice samples and A. fumigatus from 3-21 %. Curry powder was highly contaminated with A. flavus; A. niger, Penicillium citrinum and mucoraceous fungi were also dominant. Aspergillus flavus was found in groundnuts, cereal powders, beans and dried fish and A. niger was widespread in some foodstuffs. No mycotoxins were detected in rice, moldy soybeans or tempah. Aflatoxin was detected in groundnut samples. Extracts of rice, beans and tempah samples showed varying toxicity to HeLa cells. All except one groundnut sample showed low toxicity. Of 30 strains of A. flavus isolated, 8 produced aflatoxin, and 3 of 4 strains of A. versicolor produced sterigmatocystin. All 91 strains of A. candidus examined produced terphenyllin but not xanthoascin. Part 11, on studies in Thailand, includes (i) Mycological survey on market foods of Thailand, (ii) Distribution of aflatoxin-producing fungi in agricultural soils of Southeast Asia, and (iii) A glimpse into Reyes syndrome in Khon Kaen, a north-eastern district of Thailand. The dominant fungi isolated from 36 samples of eight types of foods were: A. candidus, A. flavus, A. niger, Eurotium and Penicillium spp. from rice; A. flavus, A. niger, Mucor and Rhizopus spp. from groundnuts; Fusarium spp. from beans and maize; A. niger from pepper; A. niger and Fusarium spp. from cassava starch; A. flavus and A. niger from chilli, chilli powder, shrimp and shell fish. A. flavus was isolated from 15 of 50 soil samples from Malaysia and 29 of 106 from Thailand. Of these 44 strains, 16 produced aflatoxin.


Farmers’ stock groundnuts with sound pods and with damaged pods were inoculated in two separate tests with spores of Aspergillus flavus; an aflatoxin-producing strain (P-70-511) and a white-spored mutant strain (AF-2) capable of producing little or no aflatoxin. After one week incubation in a relative humidity of 90 % at 25º C, A. flavus was recovered from surface-sterilized shelled kernels as follows: (i) inoculated with P-70-511, 10 % of kernels from sound pods and 62 % of kernels from damaged pods, (ii) inoculated with AF-2, 6 % of kernels from sound pods and 78 % of kernels from damaged pods. Tests of A. flavus isolates from (i) and (ii) indicated that nearly all strains were similar to corresponding parent cultures.

Species of the Aspergillus glaucus group became prevalent after 2 weeks in storage. Penicillium spp. became a significant segment of the mycoflora after 4 weeks. Aflatoxins were detected in (i) after 2 weeks. Levels of the toxins remained low until 4 weeks, then reached 70 µg kg⁻¹ in sound pods and over 7000 µg kg⁻¹ in damaged pods. In (ii), aflatoxin B₁ was detected in groundnuts from damaged pods after 1 week at a level of 4 µg kg⁻¹. After 2 weeks, 21 µg kg⁻¹ were detected in groundnuts from sound pods compared to a trace level from broken pods. The moisture content of the groundnuts varied from 13.0 to 14.9 % in 8 determinations made after 3 and 5 weeks, respectively.


All the 33 samples of dried shrimps, shrimp paste and raw groundnut kernels were contaminated with fungi. Aspergillus and Penicillium spp. were the predominant isolates from dried shrimps and raw groundnut kernels but no Aspergillus spp. were present in peanut butter or shrimp paste samples. No aflatoxins were detected in the food samples although some were visibly moldy and some had high mold counts. The occurrence of aflatoxin-producing strains of A. flavus in dried shrimps and raw groundnut kernels warrants further investigation of these foods and their products as potentially significant sources of aflatoxins.


Soldiers (columns of moldy groundnuts) and samples of the groundnuts surrounding the soldiers were gathered from several warehouses in which farmers stock groundnuts were stored. The sound mature kernels and sound split kernels as well as loose shelled kernels from each soldier and sample were analyzed using the minicolumn method to determine aflatoxin contents. Two lots of groundnuts, one officially graded Segregation 1 and one officially graded Segregation 3, were used to create artificial soldiers in the warehouse by soaking samples before storage. After a 90-day storage period the samples were removed, shelled, and aflatoxin determinations were made. Results showed that moisture accumulations in farmers stock groundnuts from roof leaks and condensation drips create ideal conditions for A. flavus growth and aflatoxin production.

Developed less aflatoxin, but produced a highly offensive odour. Groundnuts with initial moisture content above 30\% developed considerably more aflatoxin than the varieties Early Runner and Florigiant. Immature groundnuts were less susceptible to aflatoxin contamination. Prompt drying is the most effective practice for eliminating aflatoxin contamination of groundnuts.


Experiments with three groundnut varieties, conducted in the USA, indicated that green or partially dried groundnuts, held with no air flow for 24 hours, were highly susceptible to aflatoxin contamination. Low air flow through the groundnuts did not suppress aflatoxin production. Groundnuts held under N\textsubscript{2} or CO\textsubscript{2} atmospheres developed less aflatoxin, but produced a highly offensive odour. Groundnuts with initial moisture contents below 30\% developed considerably more aflatoxin after having been rewetted and placed in high humidity chambers for five days than


In Punjab, India, samples of groundnuts were collected from farmers' fields and from stores of grain markets to evaluate susceptibility of aflatoxins to different moisture contents in kernels, in relation to methods of postharvest handling of groundnuts. The moisture contents of kernels were kept under laboratory conditions at five levels ranging from 10 to 16\% with corresponding relative humidities (RH) ranging from 82 to 95\%. Groundnut samples from farmers' fields were more vigorous than those from stores of grain markets indicating poor storage conditions. There was a definite correlation between ambient RH, moisture content of kernels and aflatoxin contamination. Aflatoxins developed at high RH (>82\%) and corresponding moisture contents of kernels (>10\%). However, aflatoxins (B\textsubscript{1}, B\textsubscript{2}, G\textsubscript{1} and G\textsubscript{2}) were present in varied proportions at different moisture contents of kernels.


Aflatoxin B\textsubscript{1} (at 10-1120 \(\mu g\) kg\(^{-1}\)) was detected in 22 of 29 samples of groundnut from commercial sources. Aspergillus \textit{flavus} was isolated from 11 of the aflatoxin-contaminated samples.


Experiments with three groundnut varieties, conducted in the USA, indicated that green or partially dried groundnuts, held with no air flow for 24 hours, were highly susceptible to aflatoxin contamination. Low air flow through the groundnuts did not suppress aflatoxin production. Groundnuts held under N\textsubscript{2} or CO\textsubscript{2} atmospheres developed less aflatoxin, but produced a highly offensive odour. Groundnuts with initial moisture contents below 30\% developed considerably more aflatoxin after having been rewetted and placed in high humidity chambers for five days than


In a study of four Virginia and four Spanish groundnut varieties at 2 sites, preharvest pod damage, by insects or microorganisms, led to increased aflatoxin contamination during storage. Virginia varieties were more easily damaged than Spanish varieties at both sites. Of 350 varieties and lines screened for reaction to seed colonization by \textit{Aspergillus flavus} in laboratory tests, 16 proved resistant. The commercial cultivars Tainan 9, Tainan 10, Tainung 4 (all Spanish types) and Penghu 2 (Virginia type) were highly susceptible.


\textit{Aspergillus flavus}, A. \textit{niger}, Penicillium \textit{citrinum}, P. \textit{cylopium}, P. \textit{juniculosum}, P. \textit{paraherquei}, Fusarium and \textit{Rhizopus} spp. were isolated from groundnut samples in Papua New Guinea and \textit{A. flavus}, \textit{A. terreus}, \textit{A. niger}, \textit{Fusarium}, \textit{Nectria} and \textit{Mucor} spp. were isolated from maize. The \textit{A. flavus} isolates produced varying amounts of aflatoxins B\textsubscript{1}, B\textsubscript{2}, G\textsubscript{1} and G\textsubscript{2}. The fungi isolated from milled rice from Malaysia.
were *A. candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. versicolor*, *Chaetomium globosum*, *Eurotium*, *Fusarium*, *Mucorales* and *P. citrinum*. Other food samples yielded *A. clavatus*, *A. flavus*, *A. niger*, *A. versicolor*, *P. citrinum*, *P. meleagrinum*, *Fusarium* spp. and *Mucorales*. *A. flavus* was also isolated from soil samples from Malaysia and Thailand.


A number of feeds and feeding stuffs, along with the postharvest factors predisposing them to aflatoxin contamination are outlined. Based on field reports, the nutritional and physiopathological implications of aflatoxins as an environmental pollutant and feed contaminant in Nigeria are discussed. Viewed from the context of the positive response of the farming population to the call for local sourcing of a substantial proportion of industrial raw materials (backward integration) by various governments, the economic implications of postharvest deterioration of large tonnages of food crops (now being experienced nation-wide) are highlighted. Various ameliorative measures (both short and long term) are suggested to minimize both postharvest deterioration and the imminence of the scourge of aflatoxicosis, especially in the poultry industry.


Groundnut samples from Mexico FD, and several localities in Jalisco, Oaxaca and Guerrero were tested using malt salt agar (6 % NaCl, MSA) and malt agar (MA) media for *Aspergillus flavus*. MSA was more favorable for the development of the mycoflora than MA. Groundnuts from Mexico FD showed both high and low numbers of fungi and those retaining the tests were more susceptible to fungal colonization. Since *Aspergillus glaucus* was present in all the tests and *A. flavus* in few samples and in low quantity, the RH equilibrium of the fruits was deduced to be less than 75 %. Although the proportion of *A. flavus* involved in the colonization was small, the use of tests to detect mycotoxin levels is recommended.


Comparisons were made on the storage fungi present on groundnut seed samples stored for 6 months at 22-28°C and at different moisture contents. At 4.5 % *Aspergillus* species remained constant but *Penicillium* spp. decreased; at 8.9 % *A. flavus*, *A. repens*, and *P. spp*. decreased but *A. amstelodami* and *A. ruber* increased; and at 11.5 % all seed yielded *A. ruber* after 4 months but other fungi decreased to 0-2 %. In a sample in which the moisture content increased slowly from 4 to 28 % the dominant fungi changed monthly; optimum moisture contents were: for *A. ruber* 12-15 %, *A. repens* 18.5 %, *A. flavus* and *P. spp*. 20 %, and *A. amstelodami* 20-28 %. At > 18 % *Fusarium* spp. increased very rapidly.


Shelled groundnuts remoistened to 16.7 % were stored for four weeks at about 27°C in air (0.03 % CO₂, 21 % O₂, 78 % N₂) and in three modified atmospheres: (1) 13.6 % CO₂, 0.3 % CO, 0.6 % O₂, 84.7 % N₂; (2) 12.2 % CO₂, 3.1 % CO, 0.3 % O₂, 83.5 % N₂; and (3) 13.6 % CO₂, 0.1 % CO, 1.5 % O₂, 83.9 % N₂. Aflatoxins, % free fatty acids, and % seed infection by the *Aspergillus flavus* group were determined weekly. Groundnuts in air and in the atmosphere containing 1.5 % O₂ accumulated high levels of aflatoxin in 1 and 2 weeks, respectively. In the other two controlled atmospheres aflatoxin B₁ did not exceed 21 fig kg⁻¹ and the % of free fatty acids increased only slightly. None of the treatments eliminated seed infection by the *A. flavus* group. After 4 weeks groundnuts in all treatments had visible fungal growth on seeds.


Groundnuts with 16.7 % moisture stored for 4 weeks in air and a modified atmosphere with 15 % O₂ were contaminated with high levels of aflatoxins. Groundnuts in modified atmospheres with 0.6 % and 0.3 % O₂ had 21 µg kg⁻¹ aflatoxin B₁ or less. *Aspergillus flavus* survived in all treatments. When containers of freshly harvested uninoculated U.S. No.2, IRN no. 4-02-931 maize with 18.8 % moisture were purged with 14.3 % CO₂, 0.5 % O₂ and 85.2 % N₂, scaled and stored for 36 and 109 days at 25°C, *A. flavus* and *Fusarium moniliforme* survived and anatoxin contamination was < 15 µg kg⁻¹. The 5-week product was compared with 18.8 % moisture maize treated with 0.8 % propionic acid and with dried maize in feeding experiments. The modified atmosphere maize and dried maize diets were preferred.

Pilot-scale experiments were designed to determine if long-term storage of groundnuts is practical in modified atmospheres with minimal deterioration through molding, aflatoxin contamination, and insect infestation, without use of refrigerator or pesticides. Two lots of U.S. No. 1 groundnuts (248 kg) were stored at 26 + 2°C for 1 year in 1978-1979 and in 1980-1981, in atmospheres either of 60 % CO₂ simulated burner gas, 99 % N₂ or in refrigerated and non-refrigerated ambient air. In 2 larger tests, the CO₂ atmosphere was used with 1996 and 6451 kg of groundnuts in different non-refrigerated bins. When the CO₂ was not recirculated in the 1996 kg bin the humidity and moisture increased and moisture migration occurred. The groundnuts at the top molded, predominantly with yeast species and *Penicillium roqueforti*. Species of the genus *Eurotium* (*Aspergillus glaucus* group), the *A. flavus* group, and various *Penicillium* spp. were also isolated. These mycofloral changes were different from those seen in ambient air or refrigerated storage when moisture increases occurred. No molding and only minor change in the microflora was observed in the bin with 6451 kg of groundnuts with CO₂ recirculation and humidity control. Only minor changes in the microflora occurred in the control treatments. No aflatoxins accumulated in any treatment.


Harvested shelled and unshelled groundnuts of genotypes PI 337409 and PI 337394F, previously reported to be resistant to invasion and colonization by *Aspergillus* spp. of the *A. flavus* group, were held under conditions of high humidity and the aflatoxin content that developed was compared with that in similarly stored nuts of PI 343360, susceptible to *A. flavus*, and the aflatoxin content that developed was compared with that in similarly stored nuts of PI 337394F, previously reported to be resistant to invasion and colonization by *Aspergillus* spp. All genotypes developed appreciable amounts of aflatoxin after 9-10 days' storage at 87-95 % RH and 23-26°C. Sound nuts of PI 337394F also developed aflatoxin after 9 days of 80 + 2 % RH (the lowest RH) and 23°C. It is concluded that genotypes invaded or colonized with difficulty in the field may be readily infected in storage under conditions of high RH and temperature favorable to colonization by the fungus.

2.2.5 In Transit


The problem of mold growth and aflatoxin contamination in commodities transported over long distances from cultivation regions to consumption centres is discussed. If the contamination occurs during transit, often no insurance coverage for the risk is available. Because of different methods of sampling followed in the exporting and importing countries, it is often difficult to define the exact responsibility of the development of aflatoxin as having taken place during transit. The statistics of the export of aflatoxin high-risk commodities like red pepper (chilies), cottonseed and groundnut extractions clearly, for the last decade, demonstrate me extent of loss suffered by the exporting countries because of aflatoxins. The problem of mold damage and mycotoxin contamination can be minimized by improving facilities for storage at port and transit points and on ships.

### 2.2.6 Aflatoxin-Producing Potential of *Aspergillus flavus* Isolates from Groundnuts


*Aspergillus flavus* strains were obtained from British and domestic sources, and were cultured on natural substrates. Aflatoxin was extracted from groundnut meal and from wheat. The amounts of toxic substances present were estimated by paper chromatography. Concentrates were obtained by precipitation. One concentrate was reduced. Toxicity tests on ducklings confirmed British findings.


Various samples of groundnuts and groundnut meals imported into the UK from six countries (Brazil, Nigeria, Uganda, South Africa, the Gambia, and Zimbabwe) were examined for microflora and toxicity. The microscopical examination of 29 meal samples showed hyphae in the cotyledon cells of 19, of which 16 were toxic. The proportions of sound, damaged and discolored kernels in the samples were also determined. The proportion of sound kernels was generally high in all samples, and even in the highly toxic sample V.3827. 78 % of the kernels were of this type. Microscopical and cultural examination of 61 sound kernels (undamaged kernels with white flesh cotyledons) showed only three (4.9 %) to be invaded by fungi. The toxicity of 11 samples of these nuts was tested in ducklings and no liver lesions were observed from eight. The remaining three also included damaged and discolored kernels. Fungi were frequently isolated from kernels with yellow, orange, buff brown or black flesh (74.4 % infected). *Aspergillus flavus* was dominant in the
mycoflora followed by *Phoma* spp. and *A. tamarii*. Nine of 19 isolates of *A. flavus* tested, 9 were toxin-producers.


Fungal contamination of 10 samples of groundnuts sold in the 1988 warm rainy season in Reunion Island was examined. *Aspergillus flavus* was the most frequent and abundant species in surface and internal mycoflora, followed by *A. glaucus* group. Predominant species on seeds were the *A. glaus* group, *Syncephalastrum* sp., *A. niger* and *Rhizopus* sp. (15.8, 6.0, 5.6 and 3.6 % of seeds infected, respectively). *A. flavus* was isolated from all samples; 8 were aflatoxigenic (7 seemed to be highly toxigenic). All groundnut samples were negative for aflatoxins.


Two experiments were conducted for the determination of aflatoxin production by strains of *Aspergillus flavus* isolated from Vietnamese yellow maize and groundnut oil cakes. Strains were inoculated onto sterilized (irradiated) maize, wheat and feed mixture (Experiment 1). The *A. flavus* strain isolated from groundnut oil cakes was inoculated onto unirradiated maize and feed mixture (Experiment 2). The moistened and dry samples of inoculated feeds were stored for 50 days at 20°C and 30°C. The feed samples were analyzed for aflatoxin B1 before the experiments and then after 10, 20, 30, 40 and 50 days. The feed samples were microbiologically examined before the experiments and then after 30 and 50 days. *A. flavus* grew more intensively in the moistened samples (about 20 °C moisture) and produced much more aflatoxin B1 than in dry samples. The strain of *A. flavus* isolated from groundnut oil cakes produced more aflatoxin B1. Most feed samples attained peak production of toxin between 20 and 40 days in storage, depending on moisture and temperature (max 520.6 mg kg⁻¹). The growth and aflatoxin production ability of *A. flavus* was lower in the mixture of fungi obtained from unirradiated feeds.


Research was done in Israel on the occurrence of aflatoxin-producing fungi in soils and in groundnut kernels. *Aspergillus flavus* was prevalent in low levels in soils of the groundnut fields in Israel. *Aspergillus flavus* infection of groundnut kernels increased with storage time. 80 % of 55 stored groundnut samples tested from 1963 crop and 64 % of 63 stored samples from 1964 crop were infected with the fungus while only 20 % of the 1963 crop and 12.7 % of the 1964 crop were contaminated with aflatoxin. 71.2 % of 330 *A. flavus* isolates tested were toxigenic. The percentage of aflatoxigenic strains among soil isolates was higher than that of the kernel isolates.


In summer (June-Aug) and winter (Dec-Feb) 1985-1986, 25 and 51 samples of raw groundnut kernels were obtained from retail stores distributed in Chiai, Yunlin and Tainan areas of Taiwan. Moisture content, germination, mold count and aflatoxin-producing molds were assayed. Average moisture contents (7.83-8.17 %) did not differ significantly between summer and winter samples. Mold counts of summer samples were much higher than those of winter samples (2.45 vs 1.31 x 10⁸ cfu g⁻¹). Percentage of samples with *Aspergillus flavus* parasiticus agar (AFPA) positive colonies in summer and winter samples were 24 and 7.8 %, respectively. The germination percentage of the former was lower than that of the latter. All AFPA colonies were confirmed as toxin producers. Mold counts were not linearly dependent upon moisture contents for either sample. No significant difference was noted between mold counts on AFPA and potato-glucose agar media.


By growing *Aspergillus parasiticus* (C.M.I. 15957) under controlled conditions on sterilized groundnuts, an average of 265 mg kg⁻¹ aflatoxin was produced. Other strains from the *Aspergillus flavus-oryzae* group tested gave lower yields of aflatoxin and one such strain produced aflatoxin from which certain normal components were absent. The aflatoxin produced on sterilized groundnuts by any particular strain of *A. flavus-oryzae* was shown by thin-layer chromatography to contain the same major components as were produced by that strain on unsterilized whole groundnuts.


Strains of *Aspergillus flavus* obtained from groundnuts, corn, and other seed and food materials or from other research workers were tested for aflatoxin-producing potential on groundnut substrate and on an artificial medium. Strains varied widely in the amount of aflatoxins produced; the proportion of aflatoxins B₁ and G₁ varied with the strain.

Species of the *Aspergillus flavus* group were isolated from groundnuts, corn, feed, and other sources. Also, isolates of *A. flavus* from groundnuts, cereals, soybeans, and other crops were obtained from other investigators. Isolates were screened for aflatoxin production on groundnuts and in a nutrient solution. About 80% of the *A. flavus* isolates produced aflatoxin to some degree. Ninety % of the isolates produced primarily aflatoxin B₁, whereas about 10% produced both aflatoxins B₁ and G₁. Optimal temperature for aflatoxin B₁ production by *A. flavus* on groundnuts and in nutrient solution was 25°C for an incubation period of 7 to 9 days. Optimal temperature range for aflatoxin production by *A. parasiticus* on both media was 25 to 30°C. Aflatoxin levels were high throughout the 7- and 21-day incubation periods. The proportion of aflatoxin B₁ to aflatoxin G₁ varied with the temperature.


This paper comprehensively reviews aflatoxin production by *Aspergillus flavus* isolates from various agricultural commodities, and the factors affecting aflatoxin production on natural substrates in nature, in vitro, and in synthetic and semi-synthetic media by the fungus.


This review discusses factors influencing production of aflatoxin in groundnuts including: the aflatoxin-producing fungus *Aspergillus flavus*, the substrate, relative humidity, moisture, temperature, time, aeration, the pod, and damage to the kernel.

583. Diener, U.L., Davis, N.D., Salmon, W.D., and Prickett, C.O. 1963. Toxin producing Aspergillus isolated from domestic peanuts. Science 142: 1491-1492. Nine species of fungi isolated from stored domestic groundnuts were grown on sterilized groundnuts and were incorporated into diets fed to ducklings. Symptoms of acute toxicity resulted only after consumption of one of the diets and this one contained material incubated with the fungus, *Aspergillus flavus*.


Isolates within the *Aspergillus flavus* group obtained from Spanish, Runner, and Virginia-type groundnuts, were screened for aflatoxin production in a culture medium. Of 244 isolates, 161 (66%) produced aflatoxins; 30 isolates produced only aflatoxin B₁, 50 produced aflatoxins B₁ and B₂, 25 produced aflatoxins B₁ and G₁, 7 produced aflatoxins B₁, B₂, and G₁, 36 produced aflatoxins B₁, G₁ and G₂, 13 produced all four aflatoxins. Quantitatively, 16 yielded less than 1 µg g⁻¹ total aflatoxins, 16 yielded from 1-10 µg g⁻¹, 48 yielded from 10-100 µg g⁻¹, 77 yielded from 100-500 µg g⁻¹, and 4 yielded over 500 µg g⁻¹. Eighty-five % of the lower yielding isolates (less than 100 µg g⁻¹) produced only aflatoxins Bs, whereas 85 % of the higher yielding isolates produced both aflatoxins Bs andGs. In general, the isolates produced 30-40 times more B₁ than B₂ or G₂, and 2 times more B₁ than G₁. Aflatoxin production was not related to the source of an isolate or to its ability to sporulate on the test medium.


A survey of groundnuts from Egypt showed 19.5 % of unshelled and 49.0 % of shelled samples to contain low levels of aflatoxins B₁, B₂, G₁ and G₂; only B₁ was detected in 3.5 % samples of roasted groundnuts. However, 60 % of *Aspergillus flavus* strains from Egypt produced one or more aflatoxin, and 40 % of toxigenic strains produced high quantities (5000-20000 µg kg⁻¹) of B aflatoxins; the levels found in groundnuts were therefore lower than expected. Studies on the effects of storage temperature and RH on groundnut mycoflora and aflatoxin development showed an RH of 95 % to be optimum for aflatoxin production; since the RH in Egypt is lower than this, this was considered a factor contributing to the low levels encountered. Inoculation of Egyptian groundnuts (cv. Giza 1) with different strains of *A. flavus* showed the shells to act as a barrier towards invasion; this was considered the main reason for low aflatoxin production. Consequently, it is considered imperative to discard groundnuts with cracked shells before storage.


*Aspergillus flavus*, *A. niger*, *Altcriariia*, *Fusarium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Rhizoctonia*, *Rhizopus* and *Sclerotium* spp. were isolated from groundnut shells, whereas *A. flavus*, *A. niger*, *Fusarium*, *Macrophomina*, *Penicillium*, *Rhizoctonia* and *Sclerotium* spp. were the most frequent isolates from kernels. Over 50% of *A. flavus* strains produced aflatoxins.


Rice was the best substrate for aflatoxin production with from 0.036 to 15 µg g⁻¹, produced depending on the original source of the *Aspergillus flavus* culture. The
other substrates were maize (traces to 3 µg g⁻¹), groundnut (none to 0.75 µg g⁻¹), copra (none to 1.35 µg g⁻¹) and soybean (none to 0.75 µg g⁻¹).


Of a total of 107 cultures of Aspergillus flavus. A, oryzae var. effusus, A, oryzae, A. parasiticus and A. ochraceus from groundnuts 33 (31 %) (32 A. flavus and 1 A. oryzae) produced aflatoxin, four only in the culture medium and two only in the mycelium. Toxin production was much greater in the mycelium than in the culture medium.


Production of aflatoxin by Aspergillus flavus isolates from groundnut pods, kernels and soils from the Marathwada area ranged from 131 to 1240 µg kg⁻¹ okra seeds. Of 40 isolates tested, 2 isolates (AF-28 and AF-29) were most toxigenic. There was variation in the sensitivity of isolates to fungicides; AF-29 was resistant. Under selection pressure of carbendazim the population of the resistant strain was increased. At equal proportions (50:50) 100 % survival was noted from second passage isolates only. The same was the case with thiophanate methyl. Survival ability of the resistant strain among the population of geocarposphere fungi under fungiclidal pressure was also maximum. The resistant strain, however, could not compete with A. fumigatus under carbendazim pressure from the 4th passage at equal proportions in me mixture. Soil type, cultivation pattern of groundnut and soils from the Marathwada area ranged from 131 to 1240 µg kg⁻¹ okra seeds. Of 40 isolates tested, 2 isolates (AF-28 and AF-29) were most toxigenic. There was variation in the sensitivity of isolates to fungicides; AF-29 was resistant. Under selection pressure of carbendazim the population of the resistant strain was increased. At equal proportions (50:50) 100 % survival was noted from second passage isolates only. The same was the case with thiophanate methyl. Survival ability of the resistant strain among the population of geocarposphere fungi under fungiclidal pressure was also maximum. The resistant strain, however, could not compete with A. fumigatus under carbendazim pressure from the 4th passage at equal proportions in me mixture. Soil type, cultivation pattern of groundnut and soils from the Marathwada area ranged from 131 to 1240 µg kg⁻¹ okra seeds. Of 40 isolates tested, 2 isolates (AF-28 and AF-29) were most toxigenic. There was variation in the sensitivity of isolates to fungicides; AF-29 was resistant. Under selection pressure of carbendazim the population of the resistant strain was increased. At equal proportions (50:50) 100 % survival was noted from second passage isolates only. The same was the case with thiophanate methyl. Survival ability of the resistant strain among the population of geocarposphere fungi under fungiclidal pressure was also maximum. The resistant strain, however, could not compete with A. fumigatus under carbendazim pressure from the 4th passage at equal proportions in me mixture. Soil type, cultivation pattern of groundnut and irrigation all related to the distribution of toxigenic strains. It is concluded that chemical management with fungicides in the field is possible, but at the same time prediction for development of resistance can be provided.


The occurrence of aflatoxin-producing strains of Aspergillus flavus was surveyed in retail samples of five kinds of edible nuts (mostly tree nuts) and compared with 10 samples of groundnuts. All samples were imported from various countries in 1981 and 1983. Aspergillus flavus was present in 128 (46.5 %) of 275 tested kernels of the edible nuts, and 49.6 % of the groundnuts. A total of 91 isolates of A. flavus were selected and examined for their aflatoxin-producing ability; of 44 isolates of A. flavus from other edible nuts, 37 (84.1 %) produced aflatoxin on defatted groundnut meal medium, while 43 of 47 isolates (91.5 %) of A. flavus from groundnuts were positive. Most aflatoxigenic isolates from the edible nuts were identified as A. flavus subsp. flavus var. flavus, and four isolates of these, which were isolated from Hawaiian macadamia nut, produced higher levels of aflatoxins G₁ and G₂ than those of aflatoxins B₁ and B₂. Most isolates from groundnuts imported from USA produced aflatoxins B and G and were identified as A. flavus subsp. parasiticus var. parasiticus. This survey revealed that imported edible nuts including almond, chickpea, hazelnut, macadamia nut and pistachio were significantly infected with aflatoxigenic A. flavus.


This paper briefly reviews mycotoxin research in India. Research on various aspects of the groundnut aflatoxin problem, and on oilier mycotoxins is highlighted. Aspects discussed include occurrence of aflatoxins in various agricultural commodities, toxicity of aflatoxins to humans, resistance to aflatoxin production in natural substrates, detoxification, and mycology of aflatoxin-producing strains of Aspergillus flavus.


The natural occurrence of aflatoxin in agricultural commodities and factors affecting aflatoxin production in nature are reviewed. Aflatoxin production by strains of Aspergillus flavus-oryzae is also discussed. Research on aflatoxins at the Northern Regional Research Laboratory (NRRL), USA, is presented.


A total of 33 soil samples from five different establishments for processing imported groundnuts, cocoa, coconuts, coffeebeans and similar imported foodstuffs, were examined. The soil samples were placed directly on Sabouraud's glucose agar with 7.5 % of NaCl. Of 619 strains of Aspergillus flavus, 312 were positive on APA medium (for aflatoxin producing Aspergillus spp.) and 304 were negative. A total of eight strains were found to produce aflatoxin B₁. The risk of contamination of foodstuffs from this source in Central Europe is very low.

A population of 1,626 isolates of *Aspergillus flavus* obtained from groundnut kernels and from soil of groundnut fields in various regions of Israel were quantitatively and qualitatively tested for their potential to produce aflatoxin. About 90% of the isolates produced aflatoxin. Most of the aflatoxicogenic isolates produced anatoxins B₁ and B₂, but only 8.4% produced aflatoxins B₁, B₂, G₁ and G₂. The quantitative tests, performed only on 750 isolates, showed that some isolates produced as much as 150 mg kg⁻¹ aflatoxin B₁, while others produced 50 mg kg⁻¹ only. However, more than 60% of the isolates produced aflatoxin in excess of 25 mg kg⁻¹. This indicates the enormous potential range of aflatoxin production by the isolates.


Aflatoxin was found in up to 6.4% of fresh groundnut kernels and in up to 32% of stored kernels, although the intensity of toxicity was always very low (up to 125 µg g⁻¹). About 90% of 1626 *Aspergillus flavus* isolates obtained from groundnut kernels, rhizospheres, and geocarpospheres produced aflatoxin in culture. In quantitative tests with 750 isolates, 60% of the isolates produced > 25 µg g⁻¹ aflatoxin.


Pathogenesis of groundnut by *Aspergillus flavus* was investigated. Aflatoxicogenic isolates of *A. flavus* were pathogenic to groundnut seedlings and produced the characteristic disease syndrome of aflaroot. Injury to the cotyledons was considered essential for infection by *A. flavus*. The nontoxicogenic isolates of *A. flavus* tested were not pathogenic to groundnut. Biochemical changes in the diseased and healthy groundnut seedlings are described. The culture filtrates of toxigenic isolates of *A. flavus* had deleterious effects on germination of seeds of some crop plants and produced chlorosis in the cotyledonary leaves of seedlings of *Abelmoschus esculentus*. Of 21 isolates of *A. flavus* from groundnuts tested, 18 were aflatoxicogenic. All the aflatoxicogenic isolates produced only aflatoxin B₁.


The aflatoxin production of 124 isolates of *Aspergillus flavus* was studied. Among these isolates two principal groups were characterized: one with a high toxigenesis, the other with low or no toxigenesis. The percentage of highly toxigenic isolates was higher among *A. flavus* from groundnuts than *A. flavus* from wheat, maize, and animal feedstuffs.


The formation of aflatoxins by nine strains of *Aspergillus flavus* isolated from moldy maize naturally contaminated with both aflatoxins and aflatoxicol and by two strains of *A. parasiticus* was investigated. All of the aflatoxin-producing *A. flavus* and *A. parasiticus* strains produced both aflatoxins and aflatoxicols. The accumulation of aflatoxins and aflatoxicols in maize grits, polished rice and groundnuts infected with an aflatoxin-producing strain of *A. flavus* was investigated. In each substrate, the greatest amount of aflatoxin was detected after incubation for 6 to 10 days, followed by a rapid increase in aflatoxins A and B.


There was a 20-fold variation in the aflatoxin content of groundnut cake stored in sacks for about 3 years. The two batches with highest contents had ranges of 630 to 17630 and 1560 to 28440 µg kg⁻¹. Up to 60% of fungal strains produced aflatoxin. *Aspergillus flavus* strains produced aflatoxin on moist wheat at 24°C.


The abilities of 4 isolates of *Aspergillus parasiticus* and 6 of *A. flavus* to produce aflatoxins on Nigerian groundnuts at incubation temperatures of 10-35°C were studied. While all *A. parasiticus* isolates produced aflatoxins B₁, B₂, G₁ and G₂, only aflatoxins B₁ and B₂ were produced by 2 isolates of *A. flavus*. The optimum temperature for aflatoxin production by the 2 species was 30°C; there was negligible production at 10°C.


Of 288 samples of groundnuts from six coastal districts of Andhra Pradesh State,
India, it was possible to isolate strains of *Aspergillus flavus* from 29. Of the 29 strains 6 produced aflatoxin B₁ when cultured, as estimated by thin-layer chromatography (TLC) and in tests with ducklings. Aflatoxin contamination was found in 36 samples. Toxic samples were more prevalent in some districts than others. Samples commonly included 6 to 14 % of pods with shell damage, but this damage was not related to toxicity.


Twenty-six groundnut oil samples (12 unrefined, 7 refined and 7 from ration shops) were collected from the local market and assessed for *Aspergillus flavus* contamination. Three unrefined and three ration oil samples contained the fungus. Five of these six isolates produced aflatoxins in a nutrient medium. One isolate from unrefined groundnut oil was not toxigenic. When the ability of all isolates to produce aflatoxin in sterile groundnut oil was tested, the isolates did not produce any toxin during a 3-month period.


The quantity and quality of aflatoxins produced by two *Aspergillus flavus-oryzae* isolates from rough rice and Spanish groundnut were compared with a known, highly toxigenic *A. flavus* strain. Total aflatoxin yields on sterile groundnut, rough rice, and shredded wheat after 18 days at 30° ranged from 4000 to 892,000 µg kg⁻¹. In shake cultures growing on 4% suspensions of these substrates, yields ranged from 17000 to 426,000 µg kg⁻¹. Total aflatoxin yields and the proportion of the four primary aflatoxins reflected interactions between strains, substrates, and methods of culture. In shake cultures, from 52 to 80% of the aflatoxins were located in the mycelium. Conidia collected from a shredded wheat substrate after 18 days were found to contain aflatoxins at concentrations ranging from 700 to 56,100 µg kg⁻¹.


Of the nearly 150 isolates of *Aspergillus flavus* from groundnuts tested, only four produced aflatoxin B₁. These isolates differed from the type strain of *A. flavus* M001 (obtained from the Tropical Products Institute, London) in some morphological characteristics. Of the several salt solutions tested, 1 % CaCl₂ was found most promising in extracting aflatoxin from the contaminated groundnut cake. In the preparation of the protein isolates, precipitation of the protein in CaCl₂ solution helped to remove nearly 80 % of the toxin from the protein. Certain apparently healthy groundnuts showed bright bluish-violet fluorescence under ultraviolet light. Extracts of such groundnuts on thin-layer chromatography (TLC) plates showed the fluorophor at the same Rf as that of aflatoxin B₁. A simple solvent system of benzene : cyclohexane : acetic acid (3:5:2, v/v/v) on a paper chromatogram was as good for resolutions of the aflatoxin complex as the alumina TLC with chloroform and methanol as a solvent.


Aflatoxin-producing fungi were found in fermented foods and beverages: fermented rice (kaomak), soybean sauce (taotjo), peanut butter, soy sauce (shoyu), Thai red and white wine, and rice sugar wine. These foods were extracted directly and tested for aflatoxins by thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC). Four strains of aflatoxin-producing fungi were isolated from peanut butter, taotjo, and shoyu. Direct extracts of 10 % of the peanut butter samples tested and 5 % of the kaomak contained high levels of aflatoxins.


A total of 79 *Aspergillus flavus* strains isolated from samples of swine, cattle and poultry feed, groundnut and soya meals, wheat, barley, rye, ground pea. dry cracover sausage, cowshed ceiling and laboratory air and 6 control strains were tested for aflatoxin production using thin-layer chromatography (TLC) and UV detection methods. Of 19 aflatoxin-positive strains, 13 were isolated from feedstuffs (19 % swine feeds, 10 % poultry feeds, 43 % groundnut meal and 29 % wheat). Aflatoxin production was very low at 0.08 µg g⁻¹, low at 0.12-0.32 µg g⁻¹ and high at 18.7-21.8 µg g⁻¹. In strains from feedstuffs, aflatoxin production was very low in 54 %, low in 31 % and high in 15 % of strains.


Groundnut pods were collected from fields on the day of harvest in November 1972, graded into undamaged and damaged pods and seed moisture contents determined. High levels of aflatoxin were found in seeds with low moisture (< 30 %), which occurred under rainfed conditions and low levels or no toxins in those with high moisture content (under irrigation). In all instances damaged seeds contained the toxins. Seed moisture content and pod damage appeared to be the major factors.
contributing to fungal infection and toxin accumulation before harvest. The accumulation of a yellow pigment in some seeds, especially in damaged pods, was identified as citrinin. Only *Aspergillus flavus* isolates produced aflatoxins while isolates of *Penicillium citrinum*, *P. jenseni* and *A. terreus* produced citrinin.


> Of 240 isolates of *Aspergillus flavus* isolated from groundnut samples, only 72 produced anatoxins. Sixteen isolates produced only aflatoxin B₁, 8 isolates produced aflatoxins B₁ and G₁ and others produced aflatoxins B₁, G₁, B₂, and G₂. Aflatoxin production was higher when groundnut, copra and castor were used as substrates, whereas it was progressively lower in sunflower, safflower, niger seed and gingelly, respectively.


The influence of temperature and relative humidity (RH) on biodeterioration of groundnut fodder and aflatoxin production by *Aspergillus flavus* was investigated. Although aflatoxin production was observed at all incubation temperatures tested, its production was maximum at 25°C. The amount of aflatoxin production then decreased significantly with increase in incubation temperature. When a marginal increase in total protein and ash content was recorded, cellulose, lignin, starch and total nitrogen content decreased significantly due to *A. flavus* infestation. This was maximum at 25°C and decreased both with an increase or decrease of incubation temperature. Groundnut fodder showed significant weight loss due to *A. flavus* infestation and this was maximum at 25°C. Relative humidity of more or equal to 90 % was conducive for aflatoxin production. *A. flavus* could produce aflatoxins (trace amounts) even at 30 % RH.


Seventy-eight samples of farmers' stock groundnuts, representing groundnuts grown in nine different geographical areas in Texas during 1964, were assayed for aflatoxin and examined for associated microflora. Only two samples contained more than 50 µg kg⁻¹ aflatoxin. Infestation by members of the *Aspergillus flavus-oryzae* group varied from 35 to 100 % of the kernels per area and from 1 to 100 % of the kernels per sample. 213 isolates of *A. flavus* were examined for their abilities for aflatoxin production on groundnut and rice substrates. Aflatoxin production by individual isolates ranged from 0 to 349.1 µg kg⁻¹ on a groundnut substrate. In general, the isolates produced 8 to 10 times more aflatoxin B₁ than aflatoxin B₂, and no isolate produced aflatoxins G₁ or G₂.


Of 41 isolates from groundnut samples, 16 were *Aspergillus flavus*, 10 of which produced aflatoxins. Of 27 isolates from soil samples five were *A. flavus*, two of which produced aflatoxins.


An aflatoxin-producing isolate of *Aspergillus flavus* was a consistent producer of aflatoxin on all substrates which supported the growth of *A. flavus*. In competition with six other selected molds, this isolate was dominant over all but a species of *Penicillium*. Aflatoxin production was directly related to *A. flavus* growth irrespective of substrate or competition.
3. BIOCHEMICAL CHANGES IN GROUNDNUTS AFTER COLONIZATION BY THE ASPERGILLUS FLAVUS GROUP OF FUNGI

3.1 REVIEW

The importance of aflatoxin contamination of groundnuts when colonized by Aspergillus flavus group fungi is discussed in other sections. Here we focus upon the biochemical changes that occur when these fungi colonize groundnuts and groundnut products.

Much of the research on biochemical changes induced in groundnuts by either A. flavus or A. parasiticus has been done in the USA (Diener and Davis 1977, Pattee and Sessoms 1967, Cherry et al. 1972, Cherry et al. 1975, Cherry et al. 1978, Deshpande and Pancholy 1979). In infected groundnuts there is reduction of dry matter and oil content, increase in fatty acids, and deterioration of seed quality and nutritive value (Diener 1973).

Pattee and Sessoms (1967) showed a positive relationship between growth of A. flavus and rapid increase in free fatty acid (FFA) in groundnut kernels. The decrease in oil content was accompanied by a hydrolytic rancidity (high FFA) that made groundnuts unacceptable for utilization in edible products (Farag et al. 1980).

Studies conducted by Cherry and coworkers (Cherry et al. 1975, Cherry et al. 1978) have shown that infection and colonization of groundnut kernels by A. flavus and A. parasiticus cause degradation of the major storage proteins to small molecular weight components and quantitative depletion of the small protein components. Changes in enzyme activity and quantity of ether-soluble oil, and reduction in content of the basic amino acids (lysine, histidine, and arginine) were also reported (Cherry et al. 1978, Cherry et al. 1972, Cherry and Beuchat 1976). In artificial inoculation studies. Deshpande and Pancholy (1979) showed that A. flavus-resistant genotypes (PI 337394F and PI337409) underwent less biochemical change than A. flavus-susceptible cultivars. Other reports indicated that decreases in vitamins (thiamin, riboflavin and niacin) in groundnuts are associated with infection and colonization by A. flavus (Abdel-Rahman 1983). A. flavus infestation and aflatoxin contamination also affects protein quality of groundnut meal (Ostrowski-Meissner 1984).

The ability of the aflatoxigenic fungi to metabolize groundnut carbohydrates, proteins and oil and to reduce quality of kernels has been clearly demonstrated in laboratory experiments. But there is limited information on the effects of A. flavus and other fungi, individually or collectively, on quality of groundnut kernels when stored under suboptimal conditions for extended periods of time. In commercial storage, insects, fungi, humidity and temperature contribute to seed deterioration. Therefore, it may be difficult to determine the specific effects of A. flavus group fungi. Fortunately, storage practices recommended for control of A. flavus infection and colonization are generally effective in minimizing deterioration associated with Other factors.

It is obvious that seed viability is affected by physical damage of the embryo and cotyledons by the invading fungal mycelium and that biochemical changes of the stored carbohydrates, proteins and oil reduce the value of these nutrients to the embryo and young seedling. In some cases the mycelium of the fungus remains viable when the seed is sown and may contribute to either seed rot or seedling disease (aflaroot).
3.2 BIBLIOGRAPHY

3.2.1 BIOCHEMICAL CHANGES IN GROUNDNUTS AFTER COLONIZATION BY THE ASPERGILLUS FLAVUS GROUP OF FUNGI


The percentages of lipids and free fatty acids did not change in non-inoculated groundnuts during storage for 5 days at 28°C, but increased in groundnuts inoculated with Aspergillus and Rhizopus spp. Fatty acids did not fluctuate significantly over the storage period, except that percentages of palmitic and stearic acids tended to decrease, while percentages of oleic and linoleic acids increased. Arachidic, eicosenoic, behenic and lignoceric acids levels remained constant.


The contents of thiamin, riboflavin and niacin decreased when stored groundnuts were infected by Aspergillus and Rhizopus spp.


Groundnut meal was fermented with 9 different fungi at 28°C for periods of time ranging up to 98 h. Qualitative changes in groundnut protein resulting from fungal proteolysis were followed electrophoretically using polyacrylamide gel. During fermentation the large molecular weight globulins observed in gel patterns of non-fermented samples were hydrolyzed to smaller components. The extent of peptone formation varied with the fungal strain used for fermentation. Total amino acid composition and free amino acid content of the ferments were determined. Only small changes in the total amino acid profile of groundnuts occurred upon fermentation; the percentage of amino acids as free and the proportions of specific amino acids within this fraction varied greatly among the ferments as well as between ferments and non-fermented controls.


Quantitative protein assays showed that groundnut seeds inoculated with Aspergillus parasiticus or A. oryzae and incubated for time intervals ranging up to 18 days showed decreases in percentages of proteins during the early stages (from 0 to 4-6 days) of the incubation period. During longer incubation periods, fungus-infected seeds showed a progressive decrease in soluble protein that leveled off at the later stages of colonization by A. parasiticus.


In this review biochemical changes that occur in fungus-infected groundnut seeds are discussed with particular reference to degradation of proteins in groundnut seeds infected with Aspergillus parasiticus.


Percentage oil or other diethyl ether soluble material was determined in lyophilized ground whole seeds and in buffer (sodium phosphate: pH 7.9, 1-0.01) soluble and insoluble extracts of high quality Florunner groundnuts not inoculated or inoculated with Aspergillus parasiticus, Aspergillus oryzae, Rhizopus oligosporus, or Neurospora silophila and held for various time intervals ranging up to 18 days. During the test period, percentage ether extractable material increased in buffer soluble fractions of groundnut infected with the fungi and decreased in insoluble preparations. The trends of these quantitative changes at various test intervals were similar for seeds infected with A. parasiticus and R. oligosporus. Likewise, percentages were similar for seed infected with N. silophila and A. oryzae. The percentage ether soluble material in whole seeds infected with these fungi either decreased slightly or did not change during the test periods. Data suggest that techniques developed to separate oil and protein fractions from high quality groundnuts using aqueous extraction processes may not be suitable for isolating these components from molded or partially decomposed seeds.


Buffered extracts of groundnut seeds infected with 4 aflatoxigenic and 5 nonaflatoxigenic strains of Aspergillus flavus were examined electrophoretically for soluble proteins and selected enzymes. Quantitatively, soluble proteins in extracts of infected seeds were significantly lower after 4 days than those in control seeds; however, the number of low molecular weight proteins in infected seeds increased.
Enzyme patterns of extracts from seeds infected with aflatoxicogenic *A. flavus* did not differ distinctively from patterns of seeds infected with nonaflatoxicogenic strains. Esterase, leucine, aminopeptidase, gluconate and alcohol dehydrogenase, and alkaline and acid phosphatase patterns in extracts could be distinguished between infected and control seeds.


Dehulled and testa-free seeds from 15 groundnut cultivars in the USA were all colonized by *Aspergillus parasiticus* when kept in moist chambers at 30°C. Gel electrophoretic patterns of proteins and seven enzymes of colonized seeds, cotyledons and axial tissues showed the same sequence of changes from 0 to 5 days after inoculation. The changes were distinct from a standard profile of healthy kernels. During this period high molecular weight globuline degraded to smaller components, proteins decreased quantitatively, enzymes changed in composition, and aflatoxins were formed. Many of the new isozymes in colonized groundnuts were also observed in zymograms from extracts of fungal tissue only, collected from the exterior surface of seeds. Transformations such as the rapid catabolism of groundnut proteins coincided with changes in isozyme patterns during colonization by *A. parasiticus*.


Gel electrophoretic studies were employed to develop "standard" gel patterns of total proteins and enzymes from crude extracts of individual seeds of a commercial groundnut cultivar (Virginia market type; Virginia 56R) for use in comparative biochemical investigations. Changes in these "standard" patterns due to growth of *Aspergillus parasiticus* on the groundnuts were easily detected. Within two to three days after inoculation of groundnuts with the fungus, large molecular weight proteins in the upper half of the gels rapidly decreased. At the same time, many new, small molecular weight proteins appeared in the lower half of the gels. After 5 days of fungal development, most of the small molecular weight proteins were difficult to detect in the electrophoretic patterns. Simultaneously with these changes in the total protein patterns, new and more complex zymograms were observed for several enzymes compared to their "standard" patterns. Examination of these enzymes in the fungal tissue collected from the external surfaces of groundnuts, or grown separately in Czapek's solution, indicated that most of the new isoenzymes in contaminated seed extracts were derived from the invading mold. The implications of these changes from the "standard" protein and enzyme patterns of groundnuts and their relation to the development of *A. parasiticus* on these seeds are discussed.


Protein and amino acid composition of groundnuts inoculated with *Aspergillus parasiticus* were compared with those of noninfected seeds during an 18-day test period to determine metabolic changes within this interrelationship. The levels of buffer soluble proteins of infected groundnuts decreased rapidly to quantities much lower than those of noninfected seeds shortly after inoculation. Simultaneously the levels of insoluble proteins increased to quantities greater than those contained in soluble fractions. Gel electrophoresis of soluble extracts from inoculated groundnuts showed that proteins were hydrolyzed to many small molecular weight components, which eventually dissappeared as fungal growth progressed. A corresponding increase in quantity of most free amino acids was observed shortly after inoculation. Major changes in free amino acid content coincided with substantial alterations of proteins in both soluble and insoluble fractions. These data suggest that inoculation with *A. parasiticus* initiated a sequence of events whereby proteins were hydrolyzed first to small polypeptides and/or insoluble components, then to free amino acids. After extended periods of infection, levels of free amino acids varied from day to day, suggesting that differential utilization of these components by the fungus was taking place. Quantities of total amino acids in whole seeds and soluble and insoluble fractions were different for noninoculated and inoculated groundnuts. Distinct differences were particularly noted among samples of these three fractions of inoculated seeds. Differences in total amino acid contents apparently reflect qualitative and quantitative changes in proteins and/or polypeptides present in various fractions examined during the infection period.


Seeds of six commercially grown groundnut cultivars (Altika, Dixie Runner, Early Bunch, Florigiant, Florunner and NC-Fla 14) and four lines were inoculated with an aflatoxicogenic strain of *Aspergillus flavus*, and incubated at 37° C and > 90% relative humidity for 4, 7, or 14 days. After the incubation period, groundnuts were analyzed for oil, protein, free fatty acids, changes in amino acid composition, and aflatoxin production. Seeds of these cultivars/lines had varying degrees of resistance to colonization by the fungus. Early Bunch, NC-Fla 14, and UF 77412 were highly susceptible, while Altika, Dixie Runner and Florigiant showed moderate resistance.
and Florunner, PI 337409 and PI 337394F showed high resistance to A. flavus colonization. The significant biochemical changes in groundnuts resulting from A. flavus colonization included: reduction of oil and protein content, rapid increase in free fatty acids, aflatoxin production, and changes in the amino acid composition. There was a significant decrease in the basic amino acids, an increase in glutamic acid and no change in aromatic amino acids.


Seeds from three commercially grown groundnut cultivars and two "resistant" genotypes had varying degrees of resistance to colonization by the fungus Aspergillus flavus. Groundnut genotypes PI 337409 and PI 337394F had significantly higher resistance to colonization than other cultivars. Some of the biochemical changes in groundnuts resulting from A. flavus infection included: reduction of oil and protein content, rapid increase in free fatty acids and changes in the amino acid composition.


This paper reviews seed infection by Aspergillus flavus and other fungi, and aflatoxin contamination of groundnuts from the time the groundnuts are growing in the field until they are marketed, stored and shelled. Causes of aflatoxin contamination and methods to control the aflatoxin problem are discussed.


This review discusses factors influencing production of aflatoxin in groundnuts including: the aflatoxin-producing fungus Aspergillus flavus, the substrate, relative humidity, moisture, temperature and time, aeration, the pod, and damage to the kernel.


Sterilized and nonsterilized wheat kernels, soybean seeds, sesame seeds, groundnuts and faba beans were infected by an aflatoxigenic strain of Aspergillus parasiticus. The chemical composition, aflatoxin content and fatty acid patterns of the seeds were determined. Aflatoxins B₁, B₂, G₁ and G₂ were detected, and the amounts of aflatoxins B₁ and G₁ were greater than those of B₂ and G₂. When infected by the fungus, sterilized seeds contained greater amounts of aflatoxins than did nonsterilized seeds. The highest and lowest toxicity indices were recorded for sterilized wheat and soybeans, respectively. Sesame, groundnut and soybeans exhibited intermediate toxicity indices. In every instance the amounts of aflatoxins produced were related significantly to the carbohydrate and lipid: protein ratio, and not to the polyunsaturated fatty acids of the seeds.


Some physical and chemical properties were studied in groundnut, cottonseed and corn germ oils extracted from seeds inoculated with A. flavus and from healthy seeds. Results, shown graphically (UV visible and IR spectra) and in a table (refractive index, color, acid, saponification, iodine and peroxidase values), revealed serious deterioration in corn oil due to fungal infection, with great rise in the Lovibond red color and in peroxidase value. Similar but less pronounced changes were observed in the other 2 oils. All 3 oils showed greatly increased acid value. These results cast doubt on industrial use of oil extracted from infected seeds for food processing. Spectrophotometric data were examined for possible elucidation of chemical changes in the oils from infected seeds.


The effect of aflatoxin on the protein quality of groundnut meal and fish meal was measured. Total protein efficiency, protein efficiency ratio (PER), and net protein utilization, examination of the histopathology of the liver, ileal digestion of amino acids and plasma amino acids concentration were used as biochemical methods. In one trial, aflatoxin-free groundnut meal was compared with infected groundnut meal at graded levels of toxin when fed to chickens and ducklings. In another trial, various mixtures of groundnut meal and fish meal at a constant aflatoxin level (280 µg kg⁻¹) were given to compare the effects of aflatoxin on proteins of differing quality. Ducks were more sensitive to the toxin than chickens, as indicated by deterioration of protein quality, and the effects on growth and the histological appearance of the liver were magnified on diets of low quality groundnut meal, but not of high quality fish meal. Contamination of groundnut meal resulted in progressive increase in DBC and, to a lesser extent, in DC-PER, while EAAI and CS were not affected. The
importance of these findings lies in the problem of mold contamination of animal feedstuffs in humid, tropical conditions which may affect the sensitive animals, and may not be detected by chemical methods of measuring protein quality or by bioassay on chickens, if the levels of contamination are low.


The protein quality of groundnut oil meal either aflatoxin-free or contaminated with aflatoxins was evaluated using chemical score and predicted discriminant computed protein efficiency ratio (DC PER) or protein efficiency ratio (PER), net protein utilization (NPU), total protein efficiency (TPE), plasma total (PTAA) and essential (PEAA) amino acids, and nitrogen retention with both chickens and ducks. When ducklings were used because of their high sensitivity to aflatoxins, results were much lower with the contaminated than with the aflatoxin-free groundnut meal. Bioassays on chickens and chemical assays were insensitive in predicting an increased utilization of dietary proteins by ducks given the meal contaminated with aflatoxins. In the humid tropics where most stored foods are infested with Aspergillus flavus, a reduction in the utilization of protein from foods contaminated with the fungus may be expected for organisms sensitive to aflatoxins. Such a reduction may not be detected if chemical assays or bioassays with test animals of low sensitivity to aflatoxins are used.


The influence of fungal growth, under standardized conditions, on fat acidity in large-seeded Virginia-type groundnuts inoculated with Aspergillus flavus, and relationships between fat acidity and aflatoxin were studied. Fat acidity increased quadratically and was highly correlated with visible fungal growth. A lag in aflatoxin production in relation to fat acidity increase was noted; fat acidity reached 60 mg KOH per 100 g kernels before aflatoxin became detectable. This relationship suggests that a rapid method of determining fat acidity might be used to screen groundnut samples for the possible presence of aflatoxin.

632. Szerszen, J.B., and Pettit, R.E. 1990. Changes in isozyme patterns of buffer-extractable cotyledonary proteins from Aspergillus flavus and A. parasiticus infected groundnut kernels from five cultivars grown under drought stress and normal irrigation were assayed electrophoretically by means of microprocessor-controlled IEF-PAGE (pl 3-9) and discontinuous native-PAGE (gradient 8-25). Drought stress was imposed 100 days after planting until harvest. Testa-free viable kernels, hydrated previously to 25 % of moisture, were inoculated with conidia of the aspergilli (7x10^6 conidia mL^-1), and sampled every 6 h during 72 h of incubation (dark, 32°C, 95 % RH). Total protein profiles of non-infected cotyledons from drought-stressed and irrigated plants were identical. Both fungi caused qualitative and quantitative changes of ADH, ACPH, ALPH, EST, LAP, PER, 6-PGD. MDH. and G-6PD within 12-72 h of incubation Aspergillus-infected cotyledons from drought-stressed plants exhibited differences in banding patterns and activities of ACPH, ALPH, EST, MDH. and G-6 PD, when compared to infected cotyledons from plants grown under irrigation. Drought-stressed cultivar TX 79836 showed the most isozyme changes among cultivars tested. Drought stress can predispose viable groundnut kernels to altered enzymatic reactions occurring during early stages of infection by Aspergillus flavus group spp.


Aspergillus niger and A. flavus caused spoilage of stored groundnut and brought about reduction in quality and quantity of oil during pathogenesis. Postharvest treatment with propionic acid and sodium metabisulphite as spray and dip were effective against infection by these fungi.
4. FACTORS AFFECTING PRODUCTION OF AFLATOXIN IN GROUNDNUTS IN THE LABORATORY

4.1 REVIEW

In the early years of research on aflatoxins there was much interest in determining what natural substrates were capable of supporting aflatoxin production when colonized artificially or naturally by toxigenic strains of *Aspergillus flavus* and/or *A. parasiticus*. There was also an interest in producing aflatoxins for experimental purposes.

Many natural substrates have been used to produce large quantities of aflatoxin in the laboratory. Typically, natural substrates are rehydrated, sterilized, inoculated with strains of the fungi *Aspergillus flavus*, and/or *A. parasiticus*, and then incubated at 25°C-30°C for 5-14 days. Aflatoxins have been produced by the aflatoxigenic fungi when grown on every major cereal, grain, and oil-seed (Hesseltine et al. 1966, Diener and Davis 1966, Diener and Davis 1977). Crushed groundnuts, whole groundnut kernels, groundnut germ and groundnut meal support substantial amounts of aflatoxins by aflatoxin-producing isolates of *A. flavus* and/or *A. parasiticus*. Various investigators obtained high levels of aflatoxin production by culturing *A. flavus* isolates of varying toxin-producing abilities on groundnut substrates (Table 3). Differences in aflatoxin production on uniform substrates are attributed to such factors as fungal strain, temperature, moisture in the substrate and/or surrounding relative humidity, aeration, length of the incubation period, and the method of aflatoxin analysis (Hesseltine et al. 1966, Diener and Davis 1977).

Relative Humidity and Moisture

Important factors in growth and aflatoxin production by *A. flavus* are moisture content and relative humidity (RH) equilibrium of the substrate (Austwick and Ayerst 1963). Diener and Davis (1967) found that a relative humidity of 85 ± 1% at 30°C for 21 days was the limiting condition for aflatoxin production in heat-killed, sound- and broken- mature groundnut kernels, immature kernels, and kernels from unshelled Early Runner groundnuts. Aflatoxin production in living kernels from freshly dug, surface-sterilized Early Runner pods showed a similar limiting RH. Dickens and Pattee (1966) found that aflatoxin developed in 2 days at moisture contents between 15 and 30% at 32.2°C and in 4 days at moisture contents between 20 and 31% at 21.1°C in freshly dug groundnuts.

Temperature and Time

It is difficult to separate the effects of temperature and time from those of RH and moisture. Minimal and maximal temperatures for growth are affected by moisture, oxygen concentration, availability of nutrients, and other factors (Diener and Davis 1977). Diener and Davis (1977) reported minimum temperature for growth of *A. flavus* to be 6-8°C, optimum 36-38°C, and maximum 44-46°C.

Optimum production of aflatoxin by *A. flavus* on sterilized groundnuts can be obtained by incubation at 30°C for 5-7 days, at 25°C for 7-9 days, or at 20°C for 11-13 days (Diener and Davis 1966). Research by Schroeder and Hein (1967) supports these results.

In critical studies, Diener and Davis (1967) found that the lower limiting temperature for aflatoxin production by *A. flavus* was 13°C ± 1°C for a 21-day incubation at 98 ± 1% RH. The upper limiting temperature was 41.5°C ± 1.5°C. Minimum time for aflatoxin production under optimal conditions is two and a half days after inoculation.

Temperature also influences the proportion of aflatoxin B1 to G1 produced by *A. flavus* in groundnuts and in a semisynthetic medium (Diener and Davis 1966; Schroeder and Hein 1967).

Aeration

The proportions of anaerobic gases present influence growth, sporulation, and aflatoxin formation by *A. flavus* in groundnuts (Landers et al. 1967, Sanders et al. 1968). Landers ct al. (1967) observed no visible change in *A. flavus* growth and sporulation when CO2 concentration was increased from 0.03% (concentration in air) to 20%; however, aflatoxin formation was reduced by 75%. Aflatoxin production decreased proportionately with an increase in CO2 levels ranging from 20 to 80%, and aflatoxin was not produced at 100% CO2. In general, reducing the O2 concentration decreased aflatoxin production, but the largest decreases occurred when O2 was reduced from 5 to 1% with 0, 20, or 80% CO2.

Proportions of N2 to O2 as high as 99% - 1% did not inhibit fungus growth, as evidenced by production of aflatoxin (Landers ct al. 1967). Sanders et al. (1968) found that *A. flavus* could produce aflatoxin at 25°C under 99%; RH in an atmosphere containing 20% CO2, but when temperature was reduced to 17°C and RH to 86-92% aflatoxin production was inhibited. Similarly, aflatoxin production was inhibited at 25°C by 60% CO2 at 86 and 92% RH and by 40% CO2 at 86% RH, but not at a higher RH (92%) and temperature (30°C). The importance of aeration...
to aflatoxin production in natural substrates was previously demonstrated by Hesseltine et al. (1966), who recorded 3- to 100-fold increases in aflatoxin $B_1$ with shake cultures when compared with stationary cultures of the fungus cultured on corn, groundnut, rice, sorghum, soybean, and wheat.

Most studies of in vitro aflatoxin production in natural substrates have involved the use of autoclaved seeds. This is logical in terms of eliminating problems from resident seed microflora which could interfere with aflatoxin production by artificial inoculations with toxigenic fungi. It is emphasized that the autoclaving of natural substrates can induce biochemical changes in the substrates that may either enhance or decrease aflatoxin by the toxigenic cultures. It is desirable to utilize healthy natural substrates in studies on aflatoxin production.

Table 3. Maximum aflatoxin production by different isolates of *Aspergillus flavus* and *A. parasiticus*

<table>
<thead>
<tr>
<th>Investigator(s)</th>
<th>Substrate</th>
<th>Fungus isolate</th>
<th>Aflatoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codner et al. (1963)</td>
<td>Groundnut</td>
<td><em>A. parasiticus</em></td>
<td>894</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 15957</td>
<td></td>
</tr>
<tr>
<td>Vogel et al. (1965)</td>
<td>Crushed groundnut</td>
<td><em>A. flavus</em></td>
<td>1000(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- NRRL 3000</td>
<td></td>
</tr>
<tr>
<td>Cucullu et al. (1966)</td>
<td>Groundnut germ</td>
<td>Unknown</td>
<td>4000</td>
</tr>
<tr>
<td>Schroeder (1966)</td>
<td>Spanish groundnut</td>
<td><em>A. parasiticus</em></td>
<td>650</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 64-R8</td>
<td></td>
</tr>
<tr>
<td>Boiler and Schroeder (1966)</td>
<td>Spanish groundnut</td>
<td><em>A. flavus</em></td>
<td>894</td>
</tr>
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<td></td>
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<td>- F262</td>
<td></td>
</tr>
<tr>
<td>Diener and Davis (1966)</td>
<td>Groundnut</td>
<td><em>A. parasiticus</em></td>
<td>364</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Ala-6</td>
<td></td>
</tr>
<tr>
<td>Taber and Schroeder (1967)</td>
<td>Spanish groundnut</td>
<td><em>A. flavus</em></td>
<td>363(^b)</td>
</tr>
<tr>
<td>Schroeder and Hein (1967)</td>
<td>Groundnut</td>
<td><em>A. flavus</em> it 10</td>
<td>680</td>
</tr>
<tr>
<td>Hesseltine et al. (1968)</td>
<td>Groundnut</td>
<td><em>A. flavus</em></td>
<td>488</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- NRRL 3145</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) aflatoxin $B_1$ only; \(^b\) aflatoxins $B_1$ and $B_2$
Investigations were made into growth and competitive behavior of six isolates of *Aspergillus flavus* with different aflatoxin-producing potentials, when inoculated onto fumigated, rehydrated groundnut pods (cv. Starr). Inoculated pods were placed in shallow pans in a controlled environment of 99% relative humidity at 27°C in the dark for 7 days. Six replicate batches of 100 pods were inoculated with each isolate or pair of isolates. There were no differences in invasive potential between the isolates. Double-isolate inoculations of 'nil' - 'high', 'low' - 'high', and 'medium' - 'medium' pairs of isolates produced lower amounts of aflatoxins in kernels than single-isolate inoculations of the two 'high' isolates and of the higher 'medium' isolate. Results indicated that one isolate did not suppress completely infection by another. However, competition for infection between isolates in double-isolate inoculations was not determined directly by re-isolation of each isolate because there were insufficient diagnostic characteristics.

Effects of time, temperature and moisture on the production of aflatoxin in groundnuts inoculated with an aflatoxin-producing strain of *Aspergillus flavus* were studied. Moisture contents between 15 and 30% were conducive to aflatoxin production. At least 48 and over 100 hours from the time of inoculation were required for aflatoxin to develop in kernels at 32°C and 21°C, respectively, at all moisture levels. Groundnut curing usually involves partial drying in windrows and subsequent drying in bulk curing bins. Unless considerable mold growth occurs before the start of bulk curing, aflatoxin is not likely to develop during the curing process if recommended practices are followed. In cool weather, even prolonged periods in the windrows at high moisture contents did not cause aflatoxin development in groundnuts previously inoculated with *A. flavus* isolates.

### 4.2 BIBLIOGRAPHY

#### 4.2.1 FACTORS AFFECTING PRODUCTION OF AFLATOXIN IN GROUNDNUTS IN THE LABORATORY


Species of the *Aspergillus flavus* group were isolated from groundnuts, corn, feed, and other sources. Also, isolates of *A. flavus* from groundnuts, cereals, soybeans, and other crops were obtained from other investigators. Isolates were screened for aflatoxin production on groundnuts and in a nutrient solution. About 80% of the *A. flavus* isolates produced aflatoxin to some degree. Ninety per cent of the isolates produced primarily aflatoxin *B*₁, whereas about 10% produced both aflatoxins *B*₁ and *G*₁. Optimal temperature for aflatoxin *B*₁ production by *A. flavus* on groundnuts and in nutrient solution was 25°C for an incubation period of 7 to 9 days. Optimal temperature range for aflatoxin production by *A. parasiticus* on both media was 25°C to 30°C. Aflatoxin levels were high throughout the 7- and 21-day incubation periods. The proportion of aflatoxin B₁ to aflatoxin G₁ varied with the temperature.


Sound mature kernels, immature kernels, and unshelled groundnuts (cv. Early Runner) were heat-treated and inoculated with spores of an aflatoxigenic strain of *Aspergillus flavus*, and incubated at 97-99% relative humidity (RH) at different temperatures ranging from 5 to 55°C and also at 30°C with RH ranging from 55 to 99%. Samples were removed after 7 and 21 days and assayed for aflatoxin, free fatty acids, and kernel moisture content. The limiting RH for aflatoxin production was 85 ± 1% RH for 21 days at 30°C. The limiting low temperature for visible growth and aflatoxin production by the fungus was 13 ± PC for 21 days at 97-99% RH. Damaged kernels, however, developed some aflatoxin in 21 days at 12°C. The maximum temperature for aflatoxin production was 41.5 ± 1.5°C for 21 days at 97-99% RH. Fungus growth and sporulation at 43°C were equal to that at 40°C. But no aflatoxin was produced. Moisture content of immature kernels was higher at equilibrium with the same RH than the moisture content of sound mature kernels, damaged kernels, or kernels from unshelled groundnuts. There appeared to be no proportional quantitative correlation between synthesis of aflatoxin and production of free fatty acids in heat-treated groundnuts, but no aflatoxin was produced without a simultaneous increase in free fatty acids.


This paper comprehensively reviews aflatoxin production by *Aspergillus flavus*.
isolates from various agricultural commodities, and the factors affecting aflatoxin production on natural substrates in nature, in vitro, and in synthetic and semi-synthetic media by the fungus.


The influence of temperature, relative humidity (RH), nature of the substrate, atmospheric gases, and other factors on growth and aflatoxin production by 
Aspergillus flavus in groundnuts (cv. Early Runner) was investigated under controlled environments. Sound or broken mature kernels, immature kernels, and unshelled groundnuts were inoculated with spores of an aflatoxicogenic strain of Aspergillus flavus and incubated at 98 ± 1% RH at temperatures ranging from 10 to 45°C, and also 30 ± 0.5°C in RH ranging from 70 to 99%. The substrate was heat-treated (for 12-14 h at 99% RH and 85°C). cured groundnuts, or surface-sterilized pods of freshly dug groundnuts, or nonsterile cured groundnuts.

Surface-sterilized, sound mature kernels were used in studies with atmospheric gases. Samples were removed after 7, 21, 42, or 84 days and assayed for aflatoxin, free fatty acids, and kernel moisture content. The limiting RH for aflatoxin production was 83 ± 1% or higher at 30°C, varying with the substrate and length of the incubation period. The lower limiting temperature was 11-12 ± 1°C, whereas the upper limiting temperature was 40.5 ± 0.5°C at 98 ± 1% RH. Groundnuts in shell were slightly less susceptible to invasion by the fungus and to aflatoxin formation than were shelled or heat-treated groundnuts. Aflatoxin production in sound mature kernels decreased with increasing concentrations of CO₂ from 0.03 to 100%. Reducing O₂ concentrations generally reduced aflatoxin production. Notable decreases in aflatoxin production resulted when O₂ was reduced from 5 to 1% in combination with 0, 20, or 80% CO₂. Lowering temperature or RH below optimal levels also reduced aflatoxin production. Free fatty acid formation paralleled fungus growth rather than aflatoxin formation.


This review discusses factors influencing production of aflatoxin in groundnuts including the aflatoxin-producing fungus Aspergillus flavus, the substrate, relative humidity, moisture, temperature and time, aeration, the pod, and damage to the kernel.


Sterilized and nonsterilized wheat kernels, soybean seeds, sesame seeds, groundnuts and faba beans were infected by an aflatoxicogenic strain of Aspergillus parasiticus. The chemical composition, aflatoxin content and fatty acid patterns of the seeds were determined. Aflatoxins B₁, B₂, G₁ and G₂ were detected, and the amounts of aflatoxins B₁ and G₁ were greater than those of B₂ and G₂. Sterilized seeds infected by the fungus contained greater amounts of aflatoxins than nonsterilized seeds. The highest and lowest toxicity indices were recorded for sterilized wheat and soybeans, respectively. Sesame, groundnut and soybeans exhibited intermediate toxicity indices. In every instance the amounts of aflatoxins produced were related significantly to the carbohydrate and lipid : protein ratio, and not to the polyunsaturated fatty acids of the seeds.


This paper briefly reviews the natural occurrence of aflatoxin in agricultural commodities and factors affecting aflatoxin production in nature. Aflatoxin production by strains of Aspergillus flavus-oryzae is also discussed. Research on aflatoxins at the Northern Regional Research Laboratory (NRRL), USA, is presented.


Sound, mature kernels of the groundnut cultivar Early Runner were inoculated with Aspergillus flavus and incubated under various concentrations of carbon dioxide (CO₂), nitrogen (N₂), and oxygen (O₂) at high moisture levels for 2 weeks at 30° C and 6 weeks at 15° C. Observations were made on growth and sporulation of the fungus and assays were conducted on infected kernels to determine the concentrations of aflatoxins and free fatty acids. No reduction in growth and sporulation of A. flavus occurred when the CO₂ concentration was increased from 0.03% (air) to 20%. Fungus growth and sporulation were reduced with each 20% increase in CO₂ from 20% to 80%. No growth occurred in 100% CO₂. No apparent decrease in growth or sporulation occurred when O₂ was reduced from 20% to 50%, regardless of the CO₂ concentration. However, striking reductions occurred when O₂ was reduced from 5 to 1% with 0, 20, or 80% CO₂. Aflatoxin production decreased with increasing concentrations of CO₂ from 0.03 to 100%. In general, reducing the O₂ concentration decreased amounts of aflatoxin. Sizeable decreases in aflatoxin production occurred when O₂ was reduced from 5 to 1% in combination with 0, 20, or 80% CO₂. Aflatoxin production was lower in groundnuts stored at 15°C under 20% CO₂ for 6 weeks when O₂ was reduced from 20% to 5%. Aflatoxin was low in groundnuts stored at 15°C for 6 weeks under high concentrations of CO₂. Free
fatty acid formation closely paralleled growth, sporulation, and aflatoxin production by the fungus. Striking decreases in percentage of free fatty acids occurred when O\textsubscript{2} was reduced from 5 to 1%.


Production of aflatoxins in mixed feeds at 22, 28 and 37°C was studied in relation to various moisture contents. Growth of Aspergillus parasiticus was not observed in meals with a moisture content of 15%. When incubated at 22°C and 19.4% moisture content, aflatoxin was detected on the fourth day. When incubated at 28°C and 29.3% moisture content, 113 µg g\textsuperscript{-1} of aflatoxin was produced within 4 days. The ratio of aflatoxin B\textsubscript{1} to aflatoxin G\textsubscript{1} increased as the temperature increased.


The production of total aflatoxins (B\textsubscript{1}, B\textsubscript{2}, M\textsubscript{1} and M\textsubscript{2}) in ten tropical foodstuffs (with and without zinc enrichment) inoculated with Aspergillus flavus strain NRRL 3251 was examined to determine the effect of zinc on aflatoxin production. Production of aflatoxin was not linearly correlated with zinc levels of the food substrates. The data presented indicate that optimal zinc requirement for maximal aflatoxin production was substrate specific. Aflatoxin production was high on groundnut, melon, and plantain without zinc enrichment. These results indicated a poor correlation between levels of aflatoxin produced and the zinc content of the foodstuffs.


The abilities of 4 isolates of Aspergillus parasiticus and 6 of A. flavus to produce aflatoxins on Nigerian groundnuts at incubation temperatures of 10-35°C were studied. While all A. parasiticus isolates produced aflatoxins B\textsubscript{1}, B\textsubscript{2}, G\textsubscript{1} and G\textsubscript{2}, only aflatoxins B\textsubscript{1} and B\textsubscript{2} were produced by 2 of the isolates of A. flavus. The optimum temperature for aflatoxin production by the 2 species was 30°C; there was negligible production at 10°C.


Physical (moisture, temperature, water activity, oxygen concentration, incubation time, presence of other microbes) and biochemical (carbon source, sugars available) factors affecting aflatoxin production on food by Aspergillus flavus and A. parasiticus are reviewed.


Effects of atmospheric gases on the production of aflatoxin in groundnuts inoculated with an aflatoxin-producing strain of Aspergillus flavus were studied. Sizeable decreases in aflatoxin production occurred when O\textsubscript{2} was reduced from 21 to 3%.


The relationship between fungal growth and aflatoxin production by Aspergillus parasiticus was investigated. Thirteen types of powdered plant material were used, including nuts, beans, roots and seeds, classified into three groups according to chemical composition, viz., (i) high glucide or starch content (5 types), (ii) high lipid content (5 types) and (iii) high protein content (3 types). The mold grew actively on (i) and (ii). Aflatoxin production was particularly active on (ii); maximum total aflatoxin content (µg g\textsuperscript{-1}) for (i), (ii) and (iii) was in the range 0.1-172, 4.4-1223 and 0.9-10.2 respectively. However, aflatoxin production in castor seeds (group ii) was low despite rapid fungal growth, suggesting that some constituent(s) of castor seeds may inhibit aflatoxin production. Graphs plotting substrate consumption against aflatoxin production for rice, castor seeds, pine nuts, groundnuts and soybeans are included.


Effects of carbon dioxide (CO\textsubscript{2}) in combination with reduced relative humidities (RH) and temperatures on growth and aflatoxin production by Aspergillus flavus in groundnuts were investigated. Sound mature kernels of the variety Early Runner were surface-disinfested, inoculated with A. flavus, and incubated at various temperatures, RH, and CO\textsubscript{2} concentrations. Visible growth, aflaioxin production, and free fatty acids (FFA) formation by A. flavus was inhibited at approximately 86% RH by 20% CO\textsubscript{2} at 17°C and by 60 and 40% CO\textsubscript{2} at 25°C. Aflatoxin and FFA levels decreased as RH decreased from approximately 99% to 86%. At constant temperature, increase in CO\textsubscript{2} concentration caused decrease in aflatoxin and FFA.
and, at a given CO₂ concentration, lowering the temperature decreased aflatoxin and FFA levels.


Two aflatoxin-producing isolates of *Aspergillus Jlavus* were grown for 5 days on Won media at 3.7, 13, 18, 29, 35, 41, 46, and 52°C. Maximal production of aflatoxin occurred at 24°C. Maximal growth of *A. flavus* isolates occurred at 29 and 35°C. The ratio of the production of aflatoxin B₁ to aflatoxin G₁ varied with temperature. Aflatoxin production was not related to growth rate of *A. flavus*; one isolate at 4°C, at almost maximal growth of *A. Jlavus*, produced no aflatoxins. At 5 days, no aflatoxins were produced at temperatures lower than 18°C or higher than 35°C. Color of chloroform-extracts appeared to be directly correlated with aflatoxin concentrations. *A. Jlavus* isolates grown at 2, 7, and 41°C for 12 weeks produced no aflatoxins. At 13°C, both isolated produced aflatoxins in 3 weeks, and one isolate produced increasing amounts with time. The second isolate produced increasing amounts through 6 weeks, but at 12 weeks lower concentrations of aflatoxins were recovered than at 6 weeks.


The quantity and quality of aflatoxins produced by two *Aspergillus Jlavus oryzae* isolates from rough rice and Spanish groundnut were compared with a known, highly toxigenic *A. Jlavus* strain. Total aflatoxin yields on sterile groundnut, rough rice, and shredded wheat after 18 days at 30°C ranged from 4000 to 162,000 µg kg⁻¹. In shake cultures growing on 4% suspensions of these substrates, yields ranged from 17000 to 426,000 µg kg⁻¹. Total aflatoxin yields and the proportion of the four primary aflatoxins reflected interactions between strains, substrates, and methods of culture. In shake cultures from 52 to 80% of the aflatoxins were located in the mycelium. Conidia collected from a shredded wheat substrate after 18 days were found to contain aflatoxins at concentrations ranging from 700 to 56,100 µg kg⁻¹.


The production or accumulation of aflatoxins in vitro by four isolates of *Aspergillus Jlavus* on three substrates (acid-delinted cottonseed, groundnut, and rough rice) was studied in relation to temperature in the range of 10 to 40°C. Within the first 10 days after incubation, the optimal temperature range for aflatoxin production was between 20 and 35°C. Only small amounts of the toxins were produced at 10 and 40°C. Within the optimal temperature range, the time required for toxin production and for significant accumulation decreased as the temperature increased. More aflatoxin G was produced or accumulated in relation to aflatoxin B at low temperatures (within the optimal range), and aflatoxins G were metabolized rapidly at the higher temperatures.


An aflatoxin-producing isolate of *Aspergillus Jlavus* was found to be a consistent producer of aflatoxin on all substrates which supported the growth of the mold. In competition with six other selected molds, this isolate was dominant over all but a species of *Penicillium*. Aflatoxin production was directly related to *A. Jlavus* growth irrespective of substrate or competition.


Groundnut, corn and soybean seed were separately inoculated with 14 isolates of *Aspergillus Jlavus* and *A. parasiticus*. The seeds were hand sorted to remove all visibly damaged seeds and were fumigated under vacuum with 2.2% cyano (methylmercury) guanidine at 37°C for 48-96 h. All fumigated seed had a minimum of 95% germination and a maximum of 5% residual contamination by fungi and bacteria. Corn and groundnut samples (100 g per flask) were rehydrated to 28% moisture and separately inoculated with all isolates; soybean samples (100 g per flask) were rehydrated to 28% moisture and separately inoculated with four *A. flavus* and two *A. parasiticus* isolates. Samples were inoculated for 10 days at 30°C and analyzed for aflatoxins. Aspergillus parasiticus isolates produced aflatoxins B₁, B₂, G₁, and G₂, while *A. Jlavus* isolates produced aflatoxins B₁ and B₂. Mean B₁ production for 12 isolates was 34 mg kg⁻¹ in groundnut seed and 3.6 mg kg⁻¹ in corn seed. Two *A. Jlavus* isolates produced 3.8 to 5.4 mg kg⁻¹ B₁ in groundnut seed, and 2.2 mg kg⁻¹ in corn seed. Overall, the mean B₁ production was about 10 times higher on groundnut seed than on corn seed. However, more G₁ was produced on soybean seed than B₁. The isolate and the substrate are apparent limiting factors in aflatoxin production. Groundnut seed accumulated more aflatoxin than corn or soybean seed when inoculated with the same isolates and incubated under similar conditions.
5. AFLATOXINS IN GROUNDNUTS AND GROUNDNUT PRODUCTS

5.1 REVIEW

Recognition of the aflatoxin problem in 1960 led to a number of country surveys to determine the incidence of aflatoxins in agricultural products and imported products. Special emphasis was placed on contamination of groundnut kernels and groundnut products and a considerable amount of information was collected. Some of these data have been published and will be quoted in this review, but there are many unpublished data in government and commercial archives that could be of interest but may be difficult to access. One problem encountered when trying to obtain an accurate record of the extent and degree of aflatoxin contamination of groundnuts and groundnut products is the variation in efficiency of sampling and in the sensitivity and applicability of the analytical procedures. In some countries only a few samples have been collected from farms or from trade groundnuts, whereas in others there has been regular monitoring of groundnuts and groundnut products from the farm and final consumer products.

In this section of the review we have arranged the information on aflatoxins in groundnuts and groundnut products according to regions and countries. This was necessary because some publications contained data important to both exporters and importers. In the database the publications are grouped according to specific products.

Aflatoxins in Groundnuts and Groundnut Products in Africa

Aflatoxins have been reported in groundnuts and groundnut products in only one country of North Africa - Egypt; in five countries in East Africa - Burundi, Ethiopia, Kenya, the Sudan and Uganda; in six countries in West Africa - the Gambia, Ghana, Cote d'Ivoire, Niger, Nigeria and Senegal; and from seven in Southern Africa - Malawi, Mozambique, Swaziland, Zaire, Zambia, Zimbabwe, and the Republic of South Africa.

Egypt

In Egypt, the crop is cultivated in the governorates of Assuit, El-Behera, El-Minia, El-Sharkitt, Giza, Sohage, and Ismailia. Approximately 40 % of the national production (25000 t) is exported, and the remainder is consumed locally as confectionery nuts, edible oil, peanut butter, and animal feeds.

Only two studies have reported aflatoxins in some groundnut samples. Girgis et al. (1977) found low levels of aflatoxin (3-12 µg kg⁻¹) in 2 of six samples of groundnuts analyzed. Abdel-Hamid (1985) reported aflatoxin contamination in 44 % of 95 samples of various foods and feedstuffs, including groundnuts, from various parts of Egypt; groundnuts from Ismailia showed the highest contamination (400 µg kg⁻¹ aflatoxin B₁).

Burundi

Constant et al. (1984) examined the geographical distribution of aflatoxins in human foods in Burundi. Food products most frequently and heavily contaminated included groundnuts (maximum aflatoxin content 425 µg kg⁻¹), cassava (325 µg kg⁻¹) and maize (148 µg kg⁻¹); aflatoxin contamination was most common in the low altitude production areas.

Ethiopia

A single report from Ethiopia gave mean levels of aflatoxin B₁ of 34.7 and 105 µg kg⁻¹ in samples of groundnuts and peanut butter, respectively (Besrat and Gebre 1981).

Kenya

In a foods and feeds survey conducted in Nairobi, only one sample of raw groundnuts contained aflatoxin in the amount of 1050 µg kg⁻¹ (Muraguri et al. 1981). Other nuts and oil samples were free of aflatoxin.

The Sudan

Groundnut is an important crop in the Sudan, with production of over 1 million tons of in-shell nuts. Of the estimated 1 million ha of groundnuts grown in the Sudan, about 200,000 ha are grown in the irrigated Gezira region along the Nile south of Khartoum, and 800,000 ha are grown in the Western rainfed region.

Several investigators conducted surveys for aflatoxin levels in groundnuts and groundnut products (Habish et al. 1971, Elamin et al. 1988, Ahmed et al. 1989, Singh et al. 1989). In a 1969-70 survey, samples from the rainfed groundnut production areas of the Sudan were collected from one of the principal auction markets about six weeks after the beginning of the harvest. Samples from the...
irrigated areas were taken from the field about two weeks after the beginning of the harvest. Of 110 samples from the irrigated areas, 9 contained anatoxin; levels in five samples were below 50 µg kg\(^{-1}\), one contained 50 to 250 µg kg\(^{-1}\) and three contained > 1000 (µg kg\(^{-1}\). In contrast, aflatoxin was detected in 62 of the 63 samples collected from the rainfed areas (Kosti and El Obeid); levels in 24 samples were less than 50 µg kg\(^{-1}\), 27 contained between 50 and 1000 µg kg\(^{-1}\), and 11 had levels > 1000 (µg kg\(^{-1}\) (Habish et al. 1971). The high incidence of contamination in the rainfed samples was attributed to poor drying during the six or seven days immediately following harvest. Postharvest washing to remove soil from groundnuts grown under irrigation in heavy clay soils was identified as a cause for aflatoxin contamination (Habish et al. 1971).

Elamin et al. (1988) reported that the samples collected from the rainfed region (Western Sudan) showed incidence of aflatoxin contamination ranging from 100% of samples in El Hamdi (mean aflatoxin level < 10 µg kg\(^{-1}\)) to only 10 % in Casgeal (mean aflatoxin level < 1 µg kg\(^{-1}\)). None of the 100 samples collected from the irrigated region (Central Sudan) was contaminated with aflatoxin. They also found that groundnut products (groundnut paste, red and grey roasted pods) collected from Khartoum North (Bahri) had higher levels of aflatoxin (6-15 µg kg\(^{-1}\)) than those collected from Khartoum and Umdorman (< 5 µg kg\(^{-1}\)). Grey and red roasted pods showed higher levels of aflatoxins than did the groundnut paste.

Surveys were also conducted in the Gezira and Rahad schemes to determine the level of aflatoxin contamination in irrigated groundnuts (Ahmed et al. 1989). No aflatoxin was detected in the 500 samples collected from groundnuts heaped in the traditional manner. Of the 200 samples collected from sacks stacked at shelling sites in Central Sudan, only 4 % were contaminated, with the average aflatoxin content was 11 µg kg\(^{-1}\). Of 120 samples from oil mills’ stores, 15 % were contaminated with an average aflatoxin content of 20 µg kg\(^{-1}\) (Ahmed et al. 1989).

In 1984 a survey was conducted in a rainfed area (El Obeid) and an irrigated area (Wad Medani) of the Sudan, to evaluate farmers’ stock groundnuts for aflatoxin contamination (Singh et al. 1989). None of the samples collected from farm households in the regions contained more than 20 µg kg\(^{-1}\) of total anatoxins. However, samples collected during 1983 and 1984 from markets in Khartoum and Wad Medani contained anatoxins up to 945 µg kg\(^{-1}\) in raw groundnuts, and up to 994 µg kg\(^{-1}\) in groundnut paste (Singh et al. 1989). A possible reason for the high levels of aflatoxins in these groundnuts and groundnut paste appears to be the use of groundnuts gleaned after the main harvest, a common practice in the Sudan. The gleaned groundnuts are consumed by farm families or sold in the markets (Singh et al. 1989). Paste prepared from carefully sorted and cleaned groundnuts had a lower level of aflatoxins (19 µg kg\(^{-1}\)) than the commonly sold groundnut paste (Singh et al. 1989).

The occurrence of aflatoxins in Sudanese food items, including groundnuts, is a recognized problem. A study conducted by the Liverpool School of Tropical Medicine, UK, indicated that groundnuts contained aflatoxin B\(_1\) up to a level of 59666 µg kg\(^{-1}\), B\(_2\) to 370 µg kg\(^{-1}\) and G2 to 23 µg kg\(^{-1}\). Peanut butter contained aflatoxin B\(_1\) at up to 28300 µg kg\(^{-1}\), G1 to 84500 µg kg\(^{-1}\) and B2 to 9720 µg kg\(^{-1}\) (Hendrickse et al. 1982).

Studies reported the occurrence of all four aflatoxins in Sudanese groundnuts, but levels of aflatoxin B\(_1\) were generally higher than those of B\(_2\), G1, and G2.

Uganda

Groundnut is the most important legume crop in Uganda, with production levels ranging from 70,000 to 200,000 metric tons. Groundnuts are produced mainly on light sandy loams, but appreciable amounts are also produced on clay loams. They are mainly consumed locally.

In a survey conducted in 1966-1967, samples of foods were collected from randomly selected homes and markets from all parts of Uganda. Of 152 samples of groundnuts analyzed, 18 % contained aflatoxin, 8 % had low levels (< 100 µg kg\(^{-1}\)), 5 % had levels in the range of 100 to 1000 µg kg\(^{-1}\), and 5 % had levels > 1000 µg kg\(^{-1}\) (Alpert et al. 1971). This study did not relate the incidence of aflatoxin in groundnuts to climatic conditions or cultural practices, but the incidence of aflatoxin contamination in foods was generally highest in two provinces, one of which had a dry climate.

West Africa

Ghana

In a study of groundnuts in Ghana (Mintali and Hunter 1978), 80 samples of groundnut, representing both the Northern and Volta Region types, collected from markets in and around Accra were tested for aflatoxin contamination. The Volta type groundnuts contained significantly higher levels of aflatoxin (10-216 µg kg\(^{-1}\)), mean 62 (µg kg\(^{-1}\)) than the Northern nuts (3-133 µg kg\(^{-1}\); mean 27 µg kg\(^{-1}\)). The high incidence and levels of aflatoxin in the Volta type groundnuts was attributed to improper drying during harvest in the Volta region.

Cote d'Ivoire

Surveys have been conducted in Cote d'Ivoire to determine the level of aflatoxin contamination in groundnuts during two successive storage periods (1985/86 and
Groundnut is a very important food and cash crop, especially in the northern part of Nigeria (Manzo and Misari 1989). The crop is grown in two main zones: (1) the northern zone with a drier climate, an annual rainfall of 750 to 900 mm, and a rainy season from June to September; and (2) the riverain zone with a humid climate, and annual rainfall of 1000 to 1250 mm, and a rainy season from April to November.

Many studies have determined levels of aflatoxin in Nigerian groundnuts and groundnut products (Crowther 1973, McDonald 1976, Nwokolo and Okonkwo 1978, Abalaka and Elegbede 1982, Ajulo and Opadokun 1974).

Researchers at the Nigerian Stored Products Research Institute (NSPRI) have carried out regular surveys of groundnuts supplied to the Kano oil mills, and also of groundnut cake and other products. They found that over the years 1962-1968 levels of aflatoxin in cake were low, averaging 250 µg kg\(^{-1}\) with a range of 50-1000 µg kg\(^{-1}\) (Halliday and Kazaure 1967, McDonald 1976).

Samples of groundnuts for aflatoxin analyses were collected in 1973 from major storage sites, from lots delivered to oil mills, and from vendors in the Kano city market (Crowther 1973). Aflatoxin levels of 35 samples taken from storage sites ranged from 50 to 200 µg kg\(^{-1}\) (mean, 130 µg kg\(^{-1}\)). The range in levels in 51 samples from groundnuts delivered to oil mills was 50 to 1000 pg kg\(^{-1}\) (mean, 150 µg kg\(^{-1}\)). Seven samples purchased from vendors had levels ranging from 50 to 60 µg kg\(^{-1}\).

Handpicked selected groundnuts (HPS) from Kano State rarely contain more than 50 µg kg\(^{-1}\) aflatoxin. The occurrence of a number of highly toxic samples of HPS nuts in consignments tested by the NSPRI in Kano in 1971 led to that Institute, in cooperation with the Institute for Agricultural Research (1AR), carrying out a survey of groundnuts held by Licensed Buying Agents and the Northern States Marketing Board. Of 98 samples collected from sites within live of the northern states, 94 were contaminated with aflatoxin. Fifty-four per cent of the samples had levels of aflatoxin in excess of 30 µg kg\(^{-1}\) (McDonald 1976). This bad situation in 1971 was attributed to the sudden and early ending of the rains in that year. It was noted that many farmers left their crops in the ground long after the rains had ended, a practice known to favor aflatoxin development.

In the riverain area, however, HPS groundnuts in 1973 contained > 70 µg kg\(^{-1}\) aflatoxin. Rejected nuts contained about 400 µg kg\(^{-1}\) aflatoxin and were sold in the local market (Crowther 1973).

Groundnut cake from 5 oil mills in Kano were also sampled and analyzed. Aflatoxin levels were between 270 and 400 µg kg\(^{-1}\) and averaged 380 µg kg\(^{-1}\), excluding one sample that had > 1000 µg kg\(^{-1}\). This high level was in a sample from a mill which received groundnuts from the riverain zone. In comparison, analyses of groundnut cake from these oil mills in 1966 showed an average aflatoxin content of 250 pg kg\(^{-1}\) and a range from 10 to 1000 µg kg\(^{-1}\) (Crowther 1973). Nwokolo and Okonkwo (1978) reported aflatoxin B₁ in groundnut cake at levels between 600 and 1100 µg kg\(^{-1}\), and in crude groundnut oil from 200 to 500 pg kg\(^{-1}\).

Investigations by Abalaka and Elegebde (1982) revealed that edible oils including groundnut oil were significantly contaminated with aflatoxins.

Recently, Akano and Atanda (1990) reported aflatoxin levels in groundnut cake products sampled from four markets (Shasha, Bodija, Dugbe and Oje) in Ibadan, Oyo State. All samples tested during April-November 1988 contained aflatoxin; levels of aflatoxin B₁ ranged from 20 to 455 µg kg\(^{-1}\).

**Senegal**

Groundnut is the major cash crop in Senegal, and represents from 1/3 to 1/2 of the Senegalese exports. Over 1 million tons are produced annually; most of the crop produce is crushed for oil and nearly all of the groundnut cake is exported for animal feed. Most of the groundnuts are grown in the West-Central part of the country north of the Gambia, with some grown in the north, east and southern areas. Rainfall averages from 900 mm in the south to 400 mm in the north of this area. Senegal has a hot, generally dry climate. Rainfall occurs between July and early October and dry weather prevails for the remainder of the year. Groundnuts are harvested during the first week of October.

Aflatoxin contamination of groundnut is a major problem in Senegal. The
significance of the problem in relation to public health and to the future of the export trade has been recognized.

Aflatoxin content of groundnut cake from Zigninchor ranged from 70 to 350 µg kg\(^{-1}\) in 1970; from 140 to 700 µg kg\(^{-1}\) in 1971; from 70 to 140 µg kg\(^{-1}\) in 1973 and from 140 to 750 µg kg\(^{-1}\) in 1973 (Report de Mission dans les Regions du Sine-Saloume et de la Casamance. 1975. Institute de Technologie Alimentaire, Senegal).

Analyses of 46 crude oil samples collected from the groundnut basin (Kaolack and Gossas areas) revealed that 78% of samples contained aflatoxin B\(_1\). The average aflatoxin concentration of the samples from Kaolack and Gossas was 1.5 µg kg\(^{-1}\) (maximum 4 µg kg\(^{-1}\)) and 9 µg kg\(^{-1}\) (maximum 35 µg kg\(^{-1}\)), respectively (Pettit 1989).

Southern Africa

Malawi

Groundnut is an important cash and food crop in Malawi. The main groundnut cultivars in Malawi are long-duration, large-seeded confectionery types. They are grown in the plateau areas (>1000 m altitude) which are cool and moist in the growing season. Some cultivars are cultivated for oil extraction in the medium altitude areas (<500-1000 m altitude) along the lake shore where it is hot and humid with erratic rainfall in the growing season. In the main groundnut-producing areas, there is no rain after April and the dry weather favors rapid drying of pods.

The Agricultural Development and Marketing Corporation (ADMARC) is the main marketing organization for all agricultural produce in Malawi. ADMARC buys shelled confectionery groundnuts and in-shell oil nuts from farmers. Malawi exports 25000 to 40000 tons of HPS nuts every year to the U.K., Germany, Holland, and South Africa. ADMARC monitors aflatoxin levels in all confectionery export consignments. Generally, Malawean HPS groundnuts contain low levels of aflatoxin (<5 µg kg\(^{-1}\)), chiefly because the groundnuts are hand-graded.

Some 38000-60000 tons of nuts are crushed for oil every year; 2280-4800 tons are processed as groundnut cake meal. Anatoxins (73-300 µg kg\(^{-1}\)) occur in oil nuts (Personal communication-Mr. Mkandawire).

Mozambique

Groundnuts are produced in Mozambique for local consumption and as a cash crop for export

In a survey of foods conducted in the Inhambane district, groundnuts provided the main source of dietary protein but also contained the main source of aflatoxin in the diet. Analysis of 153 samples of groundnuts collected from households revealed a mean aflatoxin level of 1036 µg kg\(^{-1}\). In-shell groundnuts contained less aflatoxin than shelled nuts. The average level in the in-shell nuts was 233 µg kg\(^{-1}\), whereas the shelled nuts contained an average of 1838 µg kg\(^{-1}\). However, aflatoxin levels in prepared foods were relatively low as compared with those found in stored groundnuts, suggesting a considerable degree of selection by the housewife prior to the preparation of food (Van Rensburg et al. 1975).

In 1981, 17 food products, comprising a total of 313 samples, were tested and it was found that 16 samples were contaminated with aflatoxin B\(_1\), 10 with B\(_2\), 4 with G\(_1\), and 3 with G\(_2\). It was found that 87-100% of the groundnut, beer, rice, and maize samples tested were contaminated. The aflatoxin contamination levels in the groundnut samples ranged from 3 to 5500 µg kg\(^{-1}\), aflatoxin B\(_1\) being the main contaminant (Casadei et al. 1982).

Swaziland

Groundnut is an important source of dietary protein in Swaziland (Peers et al. 1976).

In an epidemiological study of primary liver cancer, samples of food and prepared meals were collected from homes in all parts of Swaziland during 1972 to 1973 and analyzed for aflatoxin (Peers et al. 1976). Of 93 samples of groundnuts, 11 contained aflatoxin. Groundnuts stored for five or six months contained about live limes as much aflatoxin as those tested shortly after entering storage.

Zaire

Only a single report is available and this indicated aflatoxin levels in excess of 250 µg kg\(^{-1}\) in low quality nuts (Brudzynski et al. 1977).

Zambia

Groundnut is an important food and cash crop in Zambia. Most of the groundnuts are consumed locally, the quantity exported fluctuating considerably. Very little of the crop is used for edible oil. Monitoring kernels for aflatoxin contamination has been limited to export quality control.

Quality control of export materials is handled by the Eastern Co-operative Union (ECU) Limited, Chipata, a parastatal organization handling all Zambian groundnut kernel exports. From 1977/78 to 1985/86, a total of 28410 samples were analyzed;
a mean of 6.3 % samples had aflatoxin levels of > 5 µg kg\(^{-1}\) (Kannaiyan et al 1989).

**Zimbabwe**

Groundnuts are a controlled product in the country and must be sold through the Grain Marketing Board (GMB). Estimated deliveries to the Board are 20000 tons annually, of which some 5000 tons are exported annually as confectionery nuts, and about 2000 tons of confectionery nuts are consumed in the country. The remainder of the delivered nuts are crushed for oil.

Investigations of aflatoxin contamination of groundnuts were started in 1962 following the discovery by the Veterinary Research Laboratories of two cases of aflatoxicosis in poultry (Bushnell 1965). There was a low incidence of aflatoxin contamination in the 1962/63 Southern Zimbabwean groundnut crop (50-250 µg kg\(^{-1}\) aflatoxin B\(_1\)), whereas with the 1963/64 crop, an appreciable number of samples were contaminated with > 250 µg kg\(^{-1}\) aflatoxin B\(_1\). Since then, results of a 10-year survey have been published (du Toit 1977). From 1964/65 and 1973/74, a total of 4667 samples were analyzed; a mean of 67 % samples had aflatoxin levels ranging from 5-50 µg kg\(^{-1}\) (38 % samples), 50-250 µg kg\(^{-1}\) (20 %), 250-1000 µg kg\(^{-1}\) (8 %) and > 1000 µg kg\(^{-1}\) (1 %). The levels of aflatoxins in groundnuts varied considerably from season to season and were highest in the 1972-73 season and lowest in the 1966-67 season. In general, aflatoxins B\(_1\) and G\(_1\) were found in contaminated samples; contamination with aflatoxin G\(_2\) was lower than that for B\(_1\) in practically all cases, but showed similar seasonal variations (du Toit 1977). Some interesting points were raised enabling the prediction of aflatoxin contamination in the Zimbabwean groundnut crop from consideration of differing climatic conditions and agronomic practices. Aflatoxin contamination probably occurred prior to harvest. In seasons where end-of-season rainfall was low, aflatoxin contamination was high, while more favorable growing seasons had lower levels. Variations of aflatoxin levels in groundnuts from individual GMB depots were observed. GMB depots drawing their groundnuts from the cooler, high-rainfall areas had groundnuts with less aflatoxin contamination than those serving the hotter and drier areas (du Toit 1977).

Siwela and Caley (1989) reported results of surveys carried out from 1982/83 to 1986/87. Aflatoxins were analyzed in groundnuts stored for either local or export sales. Four hundred and forty-one samples of seven groundnut varieties were collected for analysis. Sixty-eight per cent of the samples had total levels of aflatoxins B\(_1\) and G\(_1\) of up to 25 µg kg\(^{-1}\). In the 1986/87 groundnut crop, the Flamingo and Makulu Red varieties, which constitute the bulk of export sales, had up to 25 µg kg\(^{-1}\) aflatoxin in 79 % of the samples, plus aflatoxin G\(_1\) in 67 % of the samples.

**South Africa**

A survey over the years 1963-1964 of groundnuts and groundnut products from various parts of the Republic of South Africa showed that the samples obtained from the North-Western Transvaal (Bushveld areas) were the most heavily contaminated with aflatoxin (aflatoxin content > 2000 µg kg\(^{-1}\)). The samples from the Northern Cape Province, Orange Free State and Natal, except for 16 samples with 100-2000 µg kg\(^{-1}\), were free of aflatoxin (Sellschop et al. 1965). A high degree of aflatoxin contamination in the groundnut samples from the North-Western Transvaal was attributed mainly to the damage caused to the maturing groundnut pods by termites, and to protracted droughts followed by late rains. Eleven of 16 groundnut cake samples (implicated in livestock deaths) contained > 2000 µg kg\(^{-1}\) aflatoxin. Seventeen of 101 peanut butter samples tested contained aflatoxin; 12 had aflatoxin contents of between 100 and 500 µg kg\(^{-1}\) and 5 had 25 µg kg\(^{-1}\) (Sellschop et al. 1965).

A study reported in 1988 of groundnuts and peanut butter samples collected from supermarkets revealed only low levels of aflatoxin (up to 10 µg kg\(^{-1}\)) in about 30 % of the samples (Lotter and Krohm 1988).

**Aflatoxins in Groundnuts and Groundnut Products in Asia**

Countries in Asia that have reported detection of aflatoxins in groundnuts and groundnut products are India, Indonesia, Malaysia, Pakistan, the People's Republic of China, the Philippines, Taiwan and Thailand.

**India**

India is the world's largest producer of groundnut, with an annual production of between 6 and 7 million metric tons. Most of the groundnuts produced are consumed locally, with only 5% being exported. Groundnuts are the primary source of vegetable oil. Only 1.5% of the production is consumed directly as confectionery and other products.

Limited surveys have been conducted to determine aflatoxin levels in groundnuts and groundnut products in several groundnut-producing states of India. A study reported in 1965 of groundnut samples collected from six coastal districts of Andhra Pradesh State showed that 36 (12.5%) of the 288 samples were contaminated with aflatoxin (Rao et al. 1965). Toxic samples were more prevalent in some districts than others. Samples commonly included 6 to 14% of pods with shell damage, but this condition was not related to toxicity. Aflatoxin levels ranging from 1000 to 5000 µg kg\(^{-1}\) were found.
A 1965-1967 survey of groundnuts and groundnut cake samples obtained from districts of Gujarat, Andhra Pradesh and Tamil Nadu States showed that 20-40% of the groundnuts and 82% of the cakes contained fairly high levels of aflatoxin (Anonymous 1967).

Nearly 50% of 500 samples of groundnuts collected in a 1967-68 survey of the West Coast crop contained aflatoxin B1 at levels between 100 and 250 µg kg⁻¹ (Wagle 1970).

In a survey of foods and animal feeds conducted in Tamil Nadu, aflatoxins were detected in 4 of 91 samples of groundnut candies and in 28 of 134 samples of groundnut cake (Neelakantan et al. 1981).

Nagaraj and Kumar (1986) reported aflatoxin levels in kernel samples of seven Virginia groundnut cultivars collected from the 1982 rainy season crops grown at four locations, viz., Chintamani, Jalgaon, Khargon and Junagadh. All cultivars were contaminated, with levels of aflatoxin B₁ ranging from 0.8 to 65.8 µg kg⁻¹. Junagadh and Chintamani samples had the highest levels of aflatoxin, probably because of unfavorable postharvest drying conditions.

In Uttar Pradesh State, most samples of raw and roasted groundnuts tested were toxic, levels of aflatoxin B₁ ranging from 33-440 µg kg⁻¹ in raw groundnuts and from 10-85 µg kg⁻¹ in roasted groundnuts (Singh et al. 1982). Only 5 of 26 samples of groundnuts collected from local markets in Maharashtra State showed aflatoxin contamination (Patil and Shinde 1985). A study of groundnut samples collected from Ahmednagar city and nearby villages revealed that 75 samples were contaminated with aflatoxin B₁. Among the contaminated samples, 37 had aflatoxin B₁ in excess of 120 µg kg⁻¹ (Kshemkalyani and Patel 1988).

Various studies have reported the occurrence of aflatoxins in commercial groundnut cake used for cattle feed in Andhra Pradesh, Gujarat, Tamil Nadu and Punjab States (Fulsoundar and Shukla 1978, Ghowande et al. 1989, Nusrath and Nahdi 1983, Nahdi and Nusrath 1985, Choudary and Rao 1982, Balasubramanian 1985. Patel et al. 1981, Pal et al. 1979, Reddy et al. 1986, Phutela and Kabra 1979). In a survey of feeds conducted in Tamil Nadu, aflatoxin B₁ was detected in 66% of 101 samples. Of the 13 feed ingredients analyzed, only groundnut oilcake contained aflatoxin B₁ and that at levels between 330 and 2670 µg kg⁻¹ (Balasubramanian 1985). Aflatoxins (1400-3600 µg kg⁻¹) have been reported to occur in groundnut cake samples implicated in aflatoxicosis in livestock and poultry birds (Choudary and Rao 1982).

A few studies have highlighted the occurrence of aflatoxins in unrefined groundnut oil samples in the states of Karnataka, Uttar Pradesh and Andhra Pradesh (Dwarakanath et al. 1969, Amla et al. 1974, Peers and Linsell 1973, Sreenivasamurthy 1975. Pal et al. 1979, Giridhar and Krishnamurty 1977); levels of aflatoxins were in the range of 20-400 µg kg⁻¹ (Dwarakanath et al. 1969, Amla et al. 1974). Some samples contained > 2000 µg kg⁻¹ (Sreenivasamurthy 1975, Giridhar and Krishnamurty 1977). Nearly 70% of the contaminated oil samples from Uttar Pradesh contained in excess of 2000 µg kg⁻¹ aflatoxin B₁ (Pal et al. 1979). Refined groundnut oil or hydrogenated oil samples did not contain any aflatoxins.

**Indonesia**

Groundnut is a major food legume in Indonesia, with annual estimated production of 0.5 million tons of unshelled nuts from 550000 ha. Although Indonesia exports some of its groundnuts, most are used domestically, primarily for human consumption as boiled nuts or in other food products. There is also a delicacy called "oncom" made by fermenting groundnut presscake (bungkil), a by-product of groundnuts that have been pressed to extract the oil (Machmud 1989).

Surveys for aflatoxins in groundnuts and groundnut products were initiated in 1970. Of the marketable groundnuts tested, around 70% were contaminated with aflatoxin at levels from 40 to 4100 µg kg⁻¹ (Muhilal et al. 1971). Interestingly, groundnut samples obtained from distributors or subdistributors contained virtually no aflatoxin, while those obtained from the retailers contained considerable levels of aflatoxin. It was also reported that almost all the groundnuts and groundnut products (groundnut oil, "oncom", and peanut butter) sampled from markets, stores, and food manufacturers contained aflatoxins (from trace amounts to over 1000 µg kg⁻¹), except in fried groundnuts in which aflatoxin was not detectable. Manufacturers of groundnut food products tend to use the lower grades of groundnuts for products in which the appearance of the kernels is not important.

Analysis of groundnuts exported in 1975/76 showed some consignments of exported kernels (990 tons) to contain aflatoxin at over 5 µg kg⁻¹ (Muhilal and Nurjadi 1977). Most Indonesian groundnut exports are to the Netherlands; those containing more than 5 µg kg⁻¹ of aflatoxin B₁ are not acceptable.

The humid tropical conditions of Indonesia and the prevailing agricultural practices favor molding of foodstuffs, and groundnuts are commonly infested by A. flavus, the mold responsible for aflatoxin production (Husaini et al. 1974).

**The People's Republic of China**

During 1973-77, 1689 samples of groundnut kernels and 1172 samples of groundnut oil from 24 provinces were analyzed for contamination with aflatoxin B₁ (PHI.
The percentages of samples contaminated were 26.3% for kernels and 47.3% for oil. The percentage of contaminated samples decreased with increase in latitude, being high in southern China (41.7% in groundnuts samples; 68.1% in groundnut oil samples), moderate in the Yangtze valley, and negligible in northern China (no contamination in groundnuts; 4.7% in groundnut oil samples) (PHI 1983, Daren 1989). In southern China, the warm and humid weather favors postharvest aflatoxin contamination. Daren (1989) reported aflatoxin B$_1$ contamination in various samples of stored groundnuts from several provinces, with levels of aflatoxin in the range of 3-500 µg kg$^{-1}$.

**Malaysia**

High levels of aflatoxin in samples of groundnuts and peanut butter have been reported (Mat and Nazarifah 1986). Of 16 samples of groundnut oil (9 of refined oil and 7 of unrefined oil) from local markets analyzed, five samples of unrefined oil contained aflatoxin at levels of 8-16 µg kg$^{-1}$ (Chong and Beng 1965).

**Nepal**

Only limited surveys have been carried out to determine aflatoxin levels in groundnuts. During 1980-1986, 764 samples of various food commodities from hills and Terai region were analyzed for aflatoxin contamination. One hundred and forty-eight samples of different feed ingredients from different Feed Industries of Kathmandu area were also analyzed. Of 67 samples of groundnuts analyzed, 2 were heavily contaminated with aflatoxin. Peanut butter samples (31.7%) were also contaminated with aflatoxin (Karmacharya 1984, 1988). Maize and groundnut cake, used as feed ingredients, were mostly contaminated.

**Pakistan**

A few researchers have conducted surveys for aflatoxin contamination in groundnuts and groundnut products (Begum et al. 1985, Shah et al. 1981, Sheikh et al. 1983). In a survey of various foodstuffs, Shall et al. (1981) found aflatoxins in one of 16 samples of roasted groundnuts tested (total aflatoxin content 800 µg kg$^{-1}$). Begum et al. (1985) reported that all samples of roasted groundnuts obtained from local markets in Khuzdar were contaminated with aflatoxins. Many samples from local markets in Lahore also showed aflatoxin contamination (aflatoxin content 200-800 µg kg$^{-1}$). None of the samples from Quetta, Murree, and Rawalpindi had any detectable aflatoxin contamination.

The Pakistan Council of Scientific and Industrial Research (PCS1R) Central Laboratories, Karachi, reported aflatoxin levels of up to 300 µg kg$^{-1}$ in 150 samples of groundnuts collected from various parts of Sind and Baluchistan provinces (Rana 1989).

**Philippines**

Groundnuts are grown on a small scale throughout the Philippines, but the primary production area is in northern Luzon in the Cagayan and Isabelle provinces. There are about 90000 ha grown annually with a total production of 45 000 tons. Over 90% of the production is used as human food, mainly in the form of snack items and peanut butter.

Surveys for aflatoxins in groundnuts and groundnut products were initiated in 1968 by the Food and Nutrition Research Institute (FNRI), since then, there has been regular monitoring. All processed groundnuts sampled in the Philippines contained levels of aflatoxin ranging from trace amounts to more than 1000 µg kg$^{-1}$ (Santamaria et al. 1985). Results of the 1985 surveys showed that the farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season (Quitco et al. 1989). At harvest, groundnuts contained, on average, 3.16 µg kg$^{-1}$ aflatoxin. Groundnut samples taken from middlemen contained an average of 35 µg kg$^{-1}$ aflatoxin, but samples from the wholesalers’ newly procured groundnuts contained an average of 188 µg kg$^{-1}$ aflatoxin. Groundnut samples stored in warehouses for more than 3 months contained 275 µg kg$^{-1}$ aflatoxin. At the processors’ level, raw materials for confectionery groundnuts (roasted and fried) contained 7.73 µg kg$^{-1}$ aflatoxin while those intended for peanut butter contained 17.13 µg kg$^{-1}$, and rejected groundnuts had 120 µg kg$^{-1}$ (Quitco et al. 1989).

**Taiwan**

In a survey of foods for aflatoxin in 1966, samples of groundnuts from stores of eight oil mills surveyed contained aflatoxin B$_1$ levels in the range of 40 to 430 µg kg$^{-1}$. Groundnut cake samples collected from 4 of 12 oil mills contained aflatoxin B$_1$ at levels of from 80 to 290 µg kg$^{-1}$ (Tung and Ling 1968).

**Thailand**

Groundnut is an important crop in Thailand, with an annual production of around 137000 tons. The major growing areas are in the North and Central Plains and in the Northeast. Groundnuts are utilized in various types of confectionery products and for oil. Oil cake is used for animal feed.

Aflatoxin levels in groundnuts were determined in a survey of all Thai foods during a 2-year period from 1967 to 1969. Food samples were collected from over...
100 towns and villages during both rainy and dry seasons. Of 219 samples of groundnuts analyzed, aflatoxin was detected in 49% of samples at an average level of 1530 µg kg\(^{-1}\). The highest level was 12300 µg kg\(^{-1}\). Contamination was most frequent in samples collected in the rainy season, when the average concentration of total aflatoxins in all groundnut samples was almost twice the level observed in the dry season (Shank et al. 1972).

Several other studies have reported aflatoxin contamination (aflatoxin levels 10-1120 µg kg\(^{-1}\)) in samples of groundnuts and peanut butter from commercial sources (Thasnakom 1976, Sripathomswat and Thasnakorn 1981, Sommartya et al. 1988, Imwidthaya et al. 1987).

### Israel

Groundnut is an important crop in Israel where approximately 30 000 metric tons of groundnuts are produced annually.

Only limited surveys have been conducted to determine aflatoxin levels in groundnuts. During 1964-1967, 186 samples of freshly harvested groundnuts and 419 samples of stored kernels were analyzed for aflatoxin contamination (Joffe 1970). Only 5 samples of fresh kernels contained aflatoxin, while 56 samples of stored kernels were contaminated with aflatoxin; levels of aflatoxin were low in most contaminated samples. Only 6 samples (5 of stored kernels and one of fresh kernels) contained aflatoxin levels in excess of 50 µg kg\(^{-1}\) (range 60-125 µg kg\(^{-1}\)).

### Jordan

Only a single report is available and this indicated aflatoxin levels in the range of 98-1056 µg kg\(^{-1}\) in only 3 of 40 groundnut samples analyzed (Jarrar et al. 1983).

### Tunisia

Of 65 samples of groundnuts analyzed, only 4 contained aflatoxin and these had levels between 6 and 46 µg kg\(^{-1}\) (Boutrif et al. 1977).

### Japan

There is little mention of aflatoxin contamination of locally produced groundnuts. Most concern has been given to aflatoxin contamination of imported groundnuts and groundnut products.

In a survey of commercial foods conducted in Tokyo during 1982-1986, 3 samples of peanut butter were contaminated with aflatoxin (Tabata and Kamimura 1988).

### Aflatoxins in Groundnuts and Groundnut Products in South America

Two countries, Brazil and Argentina, have reported analyses of groundnut and groundnut products for aflatoxin.

#### Argentina

Groundnut is an important food and cash crop in Argentina. The main groundnut-growing area is in Cordoba province. About 300 000 metric tons of shelled groundnuts are produced annually. About 75% of the crop is used for edible oil production.

Only a single report is available and this indicated aflatoxin levels in excess of 50 µg kg\(^{-1}\) in groundnut samples from Cordoba markets (Jodral 1974).

#### Brazil

Groundnut is an important crop in Brazil where approximately 500 000 tons of groundnuts are produced annually. The principal groundnut-growing area is in the state of Sao Paulo. Most of the crop is used for edible oil production. About 10% of the produce is consumed as roasted and salted nuts.

Several surveys have been conducted to determine aflatoxin levels in groundnuts and groundnut products in Brazil. Very high levels (> 1000 µg kg\(^{-1}\)) and incidences (60-90%) of aflatoxin contamination in groundnuts and groundnut products (groundnut cake, groundnut bran and groundnut flour) have been reported (Fonseca 1968, 1976 a, b, c, Fonseca et al. 1983, Sabino et al. 1982, Scussel and Rodriguez-Amaya 1985, Sabino et al. 1989). Some samples contained > 10000 µg kg\(^{-1}\) (Fonseca 1976b, c).

Sabino (1989) reported aflatoxin levels in groundnuts from the rainy season and the dry season crops in the West, Northwest and Northeast regions of Sao Paulo State. The survey of groundnuts from the rainy season crop in West Sao Paulo State showed that 61.2% of 152 samples were contaminated with aflatoxin; 9% samples contained levels above 5000 µg kg\(^{-1}\) of aflatoxins B\(_1\) and G\(_1\), and the average level of total aflatoxins in all contaminated samples was 2391 µg kg\(^{-1}\). Analysis of 111 samples from northeast Sao Paulo State showed that 44% were contaminated with aflatoxin, 7% contained > 5000 µg kg\(^{-1}\) aflatoxins B\(_1\) and G\(_1\), and the average level
of total aflatoxins in all these contaminated samples was 2664 µg kg\(^{-1}\). Of 50 samples from northwest Sao Paulo State, 60% were contaminated with aflatoxin; 10% of samples contained > 5000 µg kg\(^{-1}\) aflatoxins, and the average level of total aflatoxins in all the contaminated samples was 1971 µg kg\(^{-1}\).

Of 83 samples from the dry season crops, 23% had aflatoxin levels of more than 30 µg kg\(^{-1}\). However, a few samples from West Sao Paulo State contained high levels of aflatoxins (991-33500 µg kg\(^{-1}\)).

High levels of aflatoxins in the Brazilian groundnuts are attributed to poor postharvest drying conditions.

### Aflatoxins in Groundnuts and Groundnut Products in North America

The United States and Mexico are the principal producers of groundnuts in North America. Reports on aflatoxin incidence are available only from the USA.

#### USA

Groundnut is an important food and cash crop in the USA. The crop is grown in three principal regions of the USA - the Southeast, the Southwest and the Virginia-North Carolina region. Total production is over 2 million tons (in-shell groundnuts). About 300000 tons of shelled nuts are exported. Major domestic use is as human food.

Soon after aflatoxin contamination was discovered in the domestic crop, a program was initiated to control the aflatoxin content in marketed groundnuts. A system of analyses and certification was developed by the U.S. Department of Agriculture (USDA), the Food and Drug Administration (FDA) and the groundnut industry which provides comprehensive data on the crop as farmers’ stock groundnuts (in shell) or as shelled nuts.

Eadie and O’Rear (1967) reported that 6.1% of groundnut samples from the 1964-1965 Virginia-North Carolina crop contained aflatoxin, and 23 of 51 samples of peanut butter were contaminated (Will) aflatoxin. Contaminated lots of groundnuts were reduced to 5.3% in 1965-1966 and to 4.4% in 1966-1967; no peanut butter sample was contaminated in 1965-1966.

Taber and Schroeder (1967) found aflatoxin in farmer’ stock groundnuts throughout Texas, but levels of aflatoxin rarely exceeded 50 µg kg\(^{-1}\).

Pettit and Taber (1968) reported results of aflatoxin analyses of 334 groundnut samples from 155 individual farms in South, East and North Texas. Twenty-eight per cent of samples were contaminated with aflatoxin, but only 2.7% of these contained levels of aflatoxin > 30 µg kg\(^{-1}\). None of the North Texas samples contained significant levels of aflatoxin. Aflatoxin accumulation was highest in kernels from those areas which had near tropical environmental conditions during the harvest period.

Doupinik (1969) found aflatoxins in 17 of 228 samples of farmers’ stock groundnuts collected from six locations in Georgia State. Aflatoxins were detected in 2.5% of Segregation I groundnuts (highest quality groundnuts), 12.1% of Segregation II, and 25.7% of Segregation 111 samples, and averaged 22, 264, and 324 µg kg\(^{-1}\) total aflatoxin respectively, indicating a strong relationship between levels of aflatoxin and grading factors.

Stoloff (1980) has provided comprehensive data on aflatoxin contamination levels in the US groundnuts over the years 1967-1978, indicating a general improvement over time in the occurrence of aflatoxin contamination, presumably attributable to improved agronomic, warehousing, and inspection practices.

Aflatoxin levels in peanut butters (various national brands of peanut butter) and confectionery products containing groundnuts were determined in surveys conducted in the 3-year period 1982-1984 (Gilbert and Shepherd 1985). Most of the samples tested contained aflatoxin but at levels well below the voluntary guideline limit for total aflatoxin (30 µg kg\(^{-1}\)). Only a few samples contained high levels of aflatoxin (318-345 µg kg\(^{-1}\)).

#### Aflatoxins in Groundnuts and Groundnut Products in Central America

Groundnut production is relatively small in Central America where approximately 100000 tons of nuts (in-shell) are produced annually.

Analyses for aflatoxin have been reported from Guatemala (Campos and Olszyna-Marzys 1979). In a survey of farmers’ groundnuts conducted in Jamaica and St. Vincent, 8 of 160 samples had aflatoxin contamination ranging from 8 to 7526 µg kg\(^{-1}\) (Singh et al. 1989). Four of the toxic samples were from the St. Elizabeth area of Jamaica, and four from growing areas adjacent to Kingstown, St. Vincent. Analyses of nine groundnut products collected from markets in St. Vincent in 1984 revealed high levels of aflatoxins (97-469 µg kg\(^{-1}\)) in 4 products, viz., roasted groundnuts, salted and unsalted groundnuts, and peanut butter (Singh et al. 1989).

Chang-Yen and Felmine (1987), in Trinidad, reported only low levels of
aflatoxin (< 5 µg kg\(^{-1}\)) in one of 64 bulk samples of raw groundnuts and peanut butter tested.

**Aflatoxins in Groundnuts and Groundnut Products in Australasia**

**Australia**

Groundnut is an important food and cash crop in Australia. The crop is mainly grown in the state of Queensland. In Queensland, the traditional area for groundnut production is the Burnet region, northwest of Brisbane. A second major area is the Atherton Tableland, west of Cairns in the far northern part of the state. Most groundnuts are grown under rainfed conditions. Total production is over 50,000 metric tons. Most of the produce is used for confectionery and table purposes. A small quantity of oil is produced from groundnuts unfit for these purposes.

Surveys for aflatoxins in groundnuts were initiated in 1979 (Pitt 1989). High levels of aflatoxin (45-1680 µg kg\(^{-1}\)) in groundnuts produced in the Burnett region were reported in 1980 and 1981 (Pitt 1989). Similar levels of aflatoxin occurred in groundnuts sampled from several farms in the Burnett region in 1986 (Pitt 1989).

Aflatoxins (500-220000 µg kg\(^{-1}\)) have been reported in groundnut meals implicated in aflatoxicosis in livestock (Bryden et al. 1980, Connole et al. 1981, Ketterer et al. 1982, McKenzie et al. 1981).

**Aflatoxins in Groundnuts and Groundnut Products in Europe**

Production of groundnuts in Europe is very limited, small areas being planted to the crop in some mediterranean countries, Hungary and the U.S.S.R. Most attention has been given to aflatoxin contamination of imported groundnuts and groundnut products. Countries in Europe with reported detection of aflatoxins in imported groundnuts and groundnut products are Czechoslovakia, Finland, Federal Republic of Germany, German Democratic Republic, France, Norway, Spain, Sweden, the U.K., U.S.S.R. and Yugoslavia (Fukal et al. 1987, Korpinnen 1971, Pensala et al. 1977, Seibold and Ruch 1977, Yndestad and Underdal 1975, Sanchis et al. 1986, Josefsson et al. 1975, Jewers 1982, Tutelyan el al. 1989, Haberle et al. 1978). All European countries have regulations on the acceptable limits for aflatoxin in groundnuts and groundnut products. Papers relating to regulatory aspects of aflatoxins in trade are discussed in a separate section.

Most of the samples of imported groundnuts and groundnut products analyzed in the Federal Republic of Germany, Spain, Sweden, and Yugoslavia contained aflatoxin levels at lower than the permissible level (5 µg kg\(^{-1}\)). A few samples of groundnuts imported into Finland, Norway, Spain, U.S.S.R., and Yugoslavia contained fairly high levels of aflatoxin (50-3650 µg kg\(^{-1}\)) (Korpinnen 1971, Pensala et al. 1977, Haberle et al. 1978, Yndestad and Underdal 1975, Sanchis et al. 1986, Tutelyan el al. 1989).

Testing of groundnut imports into the U.K. from South America and Africa for toxic substances was carried out to determine the cause of the “Turkey X” disease outbreaks of 1960. This led to the discovery of aflatoxins. Since then, analyses of imported groundnuts and groundnut products has been a regular practice in the U.K.

The U.K. surveys showed significant levels of aflatoxin in a number of samples of imported groundnuts (Jewers 1982). Thirty-one of 159 samples examined in the period 1977-1978 had aflatoxin levels in excess of 30 µg kg\(^{-1}\) (the maximum permitted level in the U.K.). This emphasized the need for regular surveillance by importers and processors of edible nuts for the presence of aflatoxin.

High levels of aflatoxins in some imported groundnut meals have been reported (Harvey 1980).

**Concluding Remarks**

Because the incidence of aflatoxin contamination is closely associated with weather conditions, considerable emphasis has been given to effects of rainfall and temperature. Data from regions where aflatoxin contamination has been found could possibly be useful in predicting aflatoxin formation in areas where surveys have not been conducted. Although aflatoxin incidence in groundnuts has been reported from all regions of the world where the crop is grown, only a few satisfactory monitoring and surveillance activities have been conducted. The mycotoxin problem is obviously not limited to these countries, and the incidence and levels of contamination is likely to be even higher in countries that have neglected this problem. It is fortunate that several important groundnut-producing countries have recognized the problem and are supporting research and monitoring/control activities aimed at mitigating the problem. It is significant that several countries are working towards acceptance of only aflatoxin-free products.
5.2 BIBLIOGRAPHY

5.2.1 AFLATOXINS IN GROUNDNUTS AND GROUNDNUT PRODUCTS


All samples of groundnut kernels, groundnut and cottonseed pellets and groundnut and cottonseed oils (crude and refined) screened contained aflatoxins B$_1$, B$_2$, G$_1$ and G$_2$. Aflatoxin B$_1$ was measured quantitatively by thin-layer chromatography and was present in the samples at 9-860 µg kg$^{-1}$.


From 1965 to 1967, a survey of aflatoxin contamination in groundnuts and groundnut cake was carried out in three districts each of Gujarat, Andhra Pradesh and Tamil Nadu states. About 600 samples were analyzed for aflatoxin contamination. Results showed that 20-40 % of the groundnuts and 82 % of samples of the cake contained fairly high levels of aflatoxin. The contamination was greater in samples collected from Andhra Pradesh and Tamil Nadu states than in samples collected from Gujarat state.


A survey carried out during 1972-73 in Nigeria on the aflatoxin problem in groundnuts and groundnut products has shown that the basic need is to reduce the levels of toxin in the groundnut crop. Present extension procedures giving advice to farmers on aflatoxin control are too diffuse and it is suggested that a film be produced dealing with the problem and its control and shown incentives must be worked out to recompense the farmer for carrying out these recommendations. In order to check on the efficacy of these extension and incentive processes, it is suggested that the Nigerian Stored Products Research Institute, Kano should improve their aflatoxin testing facility.


Samples of foods and foodstuffs purchased from local markets around Zaria, Nigeria, were investigated for aflatoxin content. The findings indicated that a large number of them were susceptible to contamination by aflatoxin since they could support both the growth of Aspergillus flavus and the production of aflatoxin by aflatoxin-producing strains of the fungus. The most susceptible foods were groundnut based foods, rice meals and cereal based foods such as koko and dan wake. The need for routine screening of certain local foods to determine the actual level of aflatoxin in them is suggested.


The aflatoxins B and G contents of 264 samples of groundnut meal, bran and flour from 40 oil-extracting mills were determined. All the samples were contaminated, with aflatoxin B$_1$ content being >1000 µg kg$^{-1}$ in 89.78 % of the samples. Allatoxin levels were much higher in samples from 'wet weather harvests' in March and May than from 'dry weather harvests' in July and September. In one region of the Sao Paulo State, aflatoxin G level was high, possibly indicating a different strain of Aspergillus flavus or a high zinc content in the soil enabling the fungus to produce more aflatoxins.


The presence of aflatoxin in groundnuts was studied at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, 90 % contained aflatoxin. Aflatoxin B$_1$ at >1000 µg kg$^{-1}$ was found in 90 % of the samples, the level rising from the time of sale to milling, then falling after oil extraction.


The presence of aflatoxin in groundnuts was examined at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, all but one contained aflatoxin. Aflatoxin B$_1$ levels were very...
high in 31.6 %, and high in 55 % of the samples. Aflatoxin levels fell from the time of sale to milling. Five samples had >10 000 µg kg\(^{-1}\).


The presence of aflatoxin in groundnuts was examined at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, all but one contained aflatoxin. Aflatoxin levels were very high in 38.3%, and high in 51.7% of the samples. Aflatoxin levels rose from the time of sale till milling, then fell. Five samples had >10 000 µg kg\(^{-1}\) aflatoxin.


Surveys were carried out for two years to determine the natural occurrence of mycotoxins (aflatoxin, ochratoxin and zearalenone) in some common Brazilian foods including raw and salted roasted groundnuts, pacoca (a very popular groundnut candy), peanut butter, maize, soybean, cured cheese, salami and copa (cured and dried pork). Eight characteristic regions. Vale do Paraiba, Mogiana, Paulista Velha, Araraquarense, Noroeste, Paulista Nova, Sorocabana and Vale do Ribeira/Litoral Sul, were surveyed, and 742 samples were analyzed. Estimation of toxins was made by thin-layer chromatography (TLC) by comparison with standards. Six samples of corn (4.7 %) were contaminated with aflatoxin. ranging from 41 to 2000 (µg kg\(^{-1}\)) of aflatoxin B\(_1\); 81 samples of pacoca (63.3 %), ranging from < 20 to 1187 µg kg\(^{-1}\); 80 samples of peanut butter (62.5 %), from < 20 to 275 µg kg\(^{-1}\); 17 samples of raw groundnuts (17.7 %), from 19 to 3125 µg kg\(^{-1}\); and 9 samples of roasted salted groundnuts (9.4 %) from < 30 to 4250 µg kg\(^{-1}\). From the first to the second year, the percentage of contaminated samples increased slightly for raw groundnuts (from 15.6 to 18.8 %) and decreased for pacoca (67.2 to 59.4 %) for roasted salted groundnuts (12.5 to 7.8 %), corn (6.2 to 3.1 %), and for peanut butter (84.4 to 40.6 %). The average content of aflatoxin of the contaminated samples increased for raw groundnuts (from 166.7 to 641.7 µg kg\(^{-1}\)) and roasted salted groundnuts (410 to 980 µg kg\(^{-1}\)) and decreased for pacoca (165.6 to 108.8 µg kg\(^{-1}\)), peanut butter (91.3 to 50.4 µg kg\(^{-1}\)), and corn (853.3 to 700 µg kg\(^{-1}\)).


A preliminary survey of peanut butter in 1982 indicated that 31 of 32 samples of major national brand-named products contained aflatoxin B\(_1\) up to 10 µg kg\(^{-1}\), and that 59 % of those were below the limit of detection (2 µg kg\(^{-1}\)). Of 25 peanut butter samples from specialist 'Health Food' outlets, 64 % contained up to 10 µg kg\(^{-1}\), and the remainder contained 16-318 µg kg\(^{-1}\), with one sample having 345 µg kg\(^{-1}\) aflatoxin. Surveys of 'Health Food' products in 1983 and 1984 confirmed that manufacturers were still experiencing some difficulty in complying with the 30 (µg kg\(^{-1}\) voluntary guideline limit for total aflatoxin. In 1984. 228 retail samples of nuts and nut confectionery products comprising groundnuts (shelled, unshelled, roasted and salted), mixed nuts, almonds (both unblanched and ground), Brazil nuts (in shell), hazelnuts (in shell), chocolate-coated groundnuts, peanut brittle and coconut ice were examined. Results showed that 74 % of the samples contained aflatoxin B\(_1\) at up to 0.5 µg kg\(^{-1}\), and 3.1 %, mainly groundnuts and Brazil nuts, exceeded the guideline tolerance for total aflatoxin. Total aflatoxin was greatest in unshelled groundnuts, 4920 µg kg\(^{-1}\), and in a composite sample of visibly molded Brazil nuts, 17926 µg kg\(^{-1}\).


Blood and urine samples from 252 children were investigated for their aflatoxin content by high-performance liquid chromatography (HPLC). Aflatoxins were detected more often and at higher concentrations in sera from children with kwashiorkor than in other malnourished and control groups. Aflatoxicol was detected in the sera of children with kwashiorkor but not in the controls and only once in a marasmic child. These differences were significant. Urinary aflatoxin was most often detected in children with kwashiorkor but the mean concentration was lower than in the other groups. Aflatoxicol was not detected in urine in any group. It is suggested either that children with kwashiorkor have greater exposure to aflatoxins or that their ability to transport and excrete aflatoxins is impaired by the metabolic derangements associated with kwashiorkor. Aflatoxins were detected by HPLC in groundnuts (B\(_1\), 59666 µg g\(^{-1}\), B\(_2\) 370, G\(_2\) 23), limed groundnuts (B\(_1\), 3517, G\(_1\), 2816, G\(_2\), 8), chickpeas (B\(_1\), 876 µg g\(^{-1}\), dried okra (G\(_2\) 12675 µg g\(^{-1}\)) and peanut butter (B\(_1\), 26300, B\(_2\) 9720, G\(_1\), 84500 µg g\(^{-1}\), obtained from local markets.

This report reviews aflatoxin contamination problem in groundnuts and groundnut products in Indonesia. Possible relationship between aflatoxin ingestion and hepatocellular carcinoma in humans is also discussed.


Aflatoxin contents of some Indonesian foods are reviewed. Aflatoxin contamination problems in groundnuts and groundnut products are discussed. Possible relationship between dietary aflatoxin and hepatocellular carcinoma in humans is also discussed.


Samples of cereals and cereal products (150) were collected from various markets in the Bangkok area from July 1983 to April 1985. These included 40 samples of rice products, 50 samples of groundnuts and groundnut products, 20 samples of soybean and soybean products, 20 samples of other beans, and 20 samples of maize. Forty-three samples of groundnuts and groundnut products were examined. Seventy-five % of groundnut samples were contaminated with aflatoxin, and 38 % contained more than 100 µg kg\(^{-1}\).


This paper reviews the worldwide levels and occurrence of mycotoxins in various commodities since 1976. Comparatively few countries have lowered the acceptable levels for aflatoxins in susceptible commodities. However, intensified efforts are needed to establish control of aflatoxin levels in global food supply, particularly in groundnuts, tree nuts, com, and animal feeds. Extensive deoxynivalenol (DON) contamination of grains, especially wheat, was demonstrated. Co-contamination of grains by Fusarium toxins, especially DON and nivalenol, with zearalenone to a lesser extent, was reported. However, more information on co-occurrence of Fusarium toxins in cereals should be developed. When contamination of feeds by ochratoxin A was significant, this toxin occurred in swine kidney and smoked meats in high levels. On the basis of occurrence and/or toxicity, patulin and penicillalic acid contamination of foods does not appear to be of real concern. More recent developments suggest, however, that expanded monitoring studies of Alternaria toxins, moniliformis citrinin, cyclopiazonic acid, penitremin A, and ergol alkaloids are indicated.


Groundnut products imported into Denmark for feed manufacturing were investigated for aflatoxin contents. Fifty-two samples were analyzed for aflatoxin contamination using two methods: thin-layer chromatography (TLC) and a minicolumn method. A few selected samples were subjected to confirmatory tests including U.V. spectrophotography and bioassay (duckling test). The investigations showed that 86.5 % of the samples were contaminated with aflatoxin, and 82.7 % contained a content of total aflatoxin > 100 µg kg\(^{-1}\). The highest toxin content was found in sample no. 20, with a total content of 3465 µg kg\(^{-1}\) and 2520 µg kg\(^{-1}\) aflatoxin B1. Aflatoxin occurred in all kinds of groundnut products (meal, expellers, and cakes and whole nuts). Aflatoxin was detected in batches from each of the 10 groundnut exporting countries (Argentina, Brazil, Congo, Ghana, Indonesia, Kenya, Nigeria, Senegal, the Sudan, and Uganda). All four aflatoxins (B1, B2, G1, and G2) were only detected in 38 % of the contaminated samples. In the present investigations the sensitivity of the Holaday minicolumn test was in the range of 100-200 µg kg\(^{-1}\).


Groundnut products and copra products imported into Finland for feed manufacturing as well as for other purposes, were investigated for aflatoxin content. Twenty-four samples of groundnut products, two samples of feedstuff containing groundnut, and 16 samples of copra products were analyzed using a thin-layer chromatography (TLC) method. Seventy-five % of the groundnut samples were contaminated with aflatoxin, and 38 % contained more than 10 µg kg\(^{-1}\). The
highest toxin content was found in sample No. 18, with aflatoxin B₁ content of 222 µg kg⁻¹ out of a total content of 4,056 µg kg⁻¹. Twenty-three % of the contaminated groundnut samples contained all four aflatoxins B₁, B₂, G₁ and G₂. Among the copra samples, 62.5 % were contaminated, with a comparatively lower aflatoxin content. The highest toxin content was found in sample No. 8, which contained 100 µg kg⁻¹. Aflatoxins G₁ and G₂ were not detected in the copra samples.


Aspergillus flavus and other molds were isolated from 900 food samples tested. Aflatoxin was found in groundnut, groundnut products, nuts and their raw products (marzipan).


Of the 1000 food samples examined, aflatoxins were found in groundnut products, maize products, almond products and nuts. Of 316 samples of groundnut, 35 (111%) contained aflatoxins; 23 (7.3%) had high levels (> 10 µg kg⁻¹ aflatoxin B₁ or > 20 µg kg⁻¹ total aflatoxin).


A survey of unrefined groundnut oil, kernels, and peanut butter for aflatoxin contamination was carried out during 1966-1967. Twenty-seven samples of unrefined oil from oil mills located in the Yunglin county and 23 unrefined oil from the markets in Taipei were analyzed. Few samples of groundnut kernels and cake were obtained from the same oil mills. Various kinds of peanut butter were purchased from markets in Taipei. Both the incidence and levels of aflatoxin contamination were as follows: peanut butter > groundnut cake > kernels > oil. Only very low levels of aflatoxin were found in unrefined oil. High levels of aflatoxin were found in peanut butter.


A total of 36 samples of foods collected during August 1985, December 1985 and March 1986 and served to mentally retarded clients with a high incidence of hepatocellular carcinoma, were analyzed for aflatoxin. Aflatoxin was not detected (< 5 µg kg⁻¹) by thin-layer chromatography in 35 food samples containing groundnuts, maize, wheat or milk. One peanut butter sample contained 20 µg kg⁻¹ aflatoxin. Aflatoxin content of these foods was at or below the level permitted by the Food and Drug Administration. It is concluded that aflatoxin is probably not responsible for liver disease in this population.


Aflatoxin research in Indonesia was initiated in 1969. Of the marketable groundnuts tested, 60-80 per cent were contaminated with aflatoxin at levels from 40 to 4100 µg kg⁻¹ seeds; retail groundnuts being the most highly contaminated. Processing raw groundnut seeds into other products, such as peanut butter and fermented groundnut presscake significantly reduced aflatoxin contamination. Clinical studies suggested a positive correlation between aflatoxin ingestion and human hepatic cancer. More research is needed on the role of preharvest fungal infection on postharvest aflatoxin contamination, the control of storage contamination, and on fungi x groundnut x environment interactions favoring aflatoxin production.


Aflatoxin contamination in live agricultural commodities namely groundnut, dried cocoa beans, copra, pepper (black and white) and paddy and rice was determined. Shelled groundnuts and their products such as peanut butter and satay gravy were highly susceptible to aflatoxin contamination, but the "menglembu" type groundnut was free of aflatoxin. Copra, dried cocoa beans and pepper (black and white) were the other highly susceptible commodities, but stored paddy and rice were safe but a few samples contained aflatoxin. The extent of contamination in several other commodities is also mentioned and general control methods are discussed.


This paper outlines events leading up to the discovery of aflatoxin, describes briefly research done on the groundnut aflatoxin problem in the northern states of Nigeria. considers the implications of aflatoxin in relation to animal and human health, and
discusses measures for elimination of aflatoxin from Nigerian groundnuts, or to at least greatly reduce incidence of aflatoxin.


Samples of groundnuts and groundnut products obtained from distributors and retailers in different markets in different areas of Bogor. Nine of 16 samples of groundnuts contained aflatoxins at levels from 40 to 4500 µg kg⁻¹. Groundnut samples obtained from distributors or sub-distributors were free of aflatoxins or contained only low levels of aflatoxin. While those obtained from the retailers contained substantial levels of aflatoxins. Six of seven samples of black 'oncom', made of groundnut presscake, contained aflatoxins at levels from traces to 1800 µg kg⁻¹. Two samples of groundnut oil tested contained aflatoxins at levels of 1000 and 1270 µg kg⁻¹. Fried groundnuts prepared in various ways were free of aflatoxins. Groundnut presscake samples contained aflatoxins ranging from traces to 760 (µg kg⁻¹). One peanut butter sample was negative for aflatoxin and the other one contained 40 µg kg⁻¹ aflatoxin.


Aflatoxin contamination of various foodstuffs in Indonesia is reviewed. Aflatoxins were determined in various samples of groundnuts and groundnut products obtained in 1970 and 1976 from collectors, sub-distributors and retailers in different markets in Bogor, West Java, Indonesia. Groundnut samples obtained from collectors or sub-distributors were free of aflatoxins or contained only traces of aflatoxins, but those obtained from the retailers contained considerable levels of aflatoxins (traces to 4 100 µg kg⁻¹). However, levels of aflatoxins were lower in retail groundnuts in 1970 (o to 67 µg kg⁻¹) than in 1970. Aflatoxins were also detected in samples of sweetened groundnut cake (average aflatoxin B₁ 170 µg kg⁻¹; aflatoxin G₂ 83 µg kg⁻¹), groundnut sauce (average aflatoxin B₁ 83 µg kg⁻¹; G₂ 49 µg kg⁻¹), and peanut butter (average B₁ 13 µg kg⁻¹) samples. Fried groundnuts were free of aflatoxin. Aflatoxins were also detected in some consignments of exported groundnut kernels (990 tons) at a level of > 5 µg kg⁻¹. Only a few samples of rice, corn, spices and cassava were contaminated with aflatoxins.


The results of a survey to detect aflatoxins in foods and food products, and in feeds and feed ingredients in the Tamil Nadu region of India are presented. Aflatoxins were detected in 4 of 91 samples of groundnut candies, 1 of 18 dried date samples, 2 of 67 scented supan, 1 of 54 broken processed arecanut, 6 of 37 lime pickles. 4 of 43 dried vegetables, 1 of 6 apples, 2 of 6 dried coconut, 2 of 30 samples of cottonseed, 28 of 134 groundnut oil cake samples, 4 of 83 gingelly oil cake samples, 3 of 96 coconut oil cake samples, 7 of 38 animal feeds, 19 of 60 poultry feeds and 4 of 10 oilseed cake mixtures.


 Twelve of 28 food samples were contaminated with aflatoxin, viz. rice, broken rice, raw grams, almonds, groundnuts, peanut butter, brown beans, white beans, cardamom black, pistachio, maize flour and raw groundnuts. The consumption of foods containing aflatoxins may account for the incidence of liver cancer in Karachi, Pakistan.


Samples of nuts and nut products examined on entry to Finland revealed 4.2 % contamination with aflatoxin, of which 97 % involved groundnuts. Aflatoxins B₁, and B₂ predominated, and aflatoxins G₁ and G₂ were found only in combination with aflatoxins B₁ and B₂. Levels of aflatoxin < 5 µg - 1000 µg kg⁻¹ were found. Sliced and crushed nuts had the highest levels of aflatoxins. The producer's and consumer's risks are assessed, sampling methods are reviewed and reaffirmation is made of the acceptable quality level of 5 µg aflatoxin kg⁻¹ nuts.


From 1973 to 1977, a general survey of aflatoxin contamination in foodstuffs was carried out in 24 provinces. More than 14 000 samples of maize, groundnut, rice, wheat, and various legumes were analyzed for aflatoxin B₁ contamination. The contamination in groundnut samples was 26.3% and in groundnut oil samples 47.3%. The contamination in groundnut samples was high in south-central China, moderate in east and northwest China, and negligible in northeast China. The percentage of aflatoxin- contaminated samples gradually decreased as latitude increased.

Aflatoxin B$_1$ was determined in samples of groundnuts and their products, wheat, corn and manioc flour, collected in commercial establishments in Belo Horizonte in 1983, with a thin-layer chromatography (TLC) method. Aflatoxin B$_1$ was detected in 44 % of the samples of groundnut and groundnut products; aflatoxin levels were above the tolerance level permitted by Brazilian legislation (30 µg kg$^{-1}$ aflatoxins B$_1$+G$_1$).


Of 32 foods (for human consumption) and 130 rations (for animals) analyzed for aflatoxin, 20 foods and 104 rations contained aflatoxin. Besides groundnut flour and other groundnut products, manioc flour was highly contaminated with aflatoxin. Sodium bisulfite (1:10000) prevented the development of Aspergillus flavus in the groundnut flour.


This review covers several aspects including a survey of aflatoxin contamination of food grains and of groundnut oil, human and animal health hazards from mycotoxins, techniques for detection and estimation of aflatoxins, factors affecting aflatoxin formation, preventive or control measures.


Aflatoxin B$_1$ was detected in 50 samples of groundnut paste and groundnut sweet bars for sale in various parts of the state of Sao Paulo. Aflatoxin levels ranged from 10 to 278 µg kg$^{-1}$.


Aflatoxins were determined in 1374 samples of groundnuts and groundnut products sold for consumption in Sao Paulo city. Aflatoxins were detected in 576 samples and the levels of aflatoxin contamination varied from year to year. Of these samples, 68.75% had > 30 µg kg$^{-1}$, the maximum permitted by Brazilian legislation.


Samples of groundnuts and groundnut products (raw shelled and unshelled groundnuts, fried salted groundnuts with or without skin, soygroundnut, sugar-coated colored groundnuts, chocolate- coated groundnuts, peanut butter, ground groundnut bar and candied groundnut bar), purchased at random from different supermarkets and smaller stores during the period from the second semester of 1980 to the first sememster of 1982, were analyzed for aflatoxin with the Romer method. Of the 241 samples analyzed. 128 had aflatoxins, and 92 of these contained levels above the tolerance limit permitted by Brazilian legislation (30 µg kg$^{-1}$ aflatoxins B$_1$+G$_1$). Ground groundnut bar, raw shelled groundnuts, soygroundnut and fried salted groundnuts with the skin had greater contamination levels with 61 %, 55 % and 42 % respectively of their samples with aflatoxin levels above the limit. The highest levels of aflatoxins B$_1$ and G$_1$ detected were 1 282 and 476 µg kg$^{-1}$ for the ground groundnut bar, 1 904 and 69 µg kg$^{-1}$ for raw shelled groundnuts and 1 026 and 366 µg kg$^{-1}$ for soygroundnut, respectively.


Of 60 samples of groundnut-free feed compounds for dairy cows, only one contained more than 20 µg kg$^{-1}$ aflatoxin, the maximum level permitted under new Federal German regulations. In feed compounds including groundnut products, however, average aflatoxin levels far exceeded the maximum permitted level. The new regulations are intended to prevent aflatoxin contamination of milk and thus possible human health hazards.


Distribution and degree of occurrence of aflatoxins in various samples of groundnuts and groundnut products in the Republic of South Africa are described. Over 1000 samples of groundnuts and groundnut products were examined during 1963 and another 1000 samples of producers’ grades were investigated during 1964. The samples obtained from the North- Western Transvaal, so-called Bushveld areas,
were most seriously contaminated with aflatoxin; all of the 75 samples containing > 2000 µg kg\(^{-1}\) aflatoxin were from the receiving depots in the North-Western Transvaal. The samples from the Northern Cape Province, Orange Free State and Natal, except for 5 samples with 500-2000 µg kg\(^{-1}\) and 11 samples with 100-500 µg kg\(^{-1}\), were free from aflatoxin. Similar results were found in the 1964 survey for aflatoxin contamination; however, only 23 samples from the North-Western Transvaal areas contained > 2000 µg kg\(^{-1}\). High degree of anatoxin contamination in the groundnut samples from the North-Western Transvaal was ascribed mainly to (1) the damage caused to the maturing groundnut pods and kernels by termites, and (2) protracted droughts followed by late rains. Lower or inferior grade groundnuts contained higher levels of aflatoxins than the higher or superior ones. No aflatoxins were detected in any of the samples of hand-picked selected (HPS) kernels examined in 1963 and 1964. Groundnut cake samples implicated in livestock deaths were also examined for aflatoxin contamination. Eleven of the 16 samples of this groundnut cake contained > 2000 µg kg\(^{-1}\) anatoxin. Of 101 peanut butter samples tested, 17 were contaminated with aflatoxin. Of these 17 samples, 12 had an aflatoxin contents of 100-500 µg kg\(^{-1}\) and 5 had up to 25 µg kg\(^{-1}\).


All of 33 samples of dried shrimps paste, peanut butter and raw groundnuts were contaminated with fungi. Aspergillus and Penicillium species were the predominant types in dried shrimps and raw groundnuts, but no Aspergillus spp. were present in peanut butter or shrimp paste samples. Among 81 Aspergillus isolates obtained from dried shrimps and raw groundnuts, 10 were A. flavus/A. parasiticus, of which 5 were potential aflatoxin- producing A. flavus strains. No aflatoxins were detected in the food samples, but some were moldy and some had high mold counts.


Based on analyses using the Velasco and the Tropical Development Research Institute (TDRI) methods, none of the samples collected from farm households in two regions of Sudan; a rainfed area (EL Obeid), and an irrigated area (Wad Medani) for the crop year 1983/84. contained more than 15 µg kg\(^{-1}\) of aflatoxin. However, samples collected from the markets in Khartoum and Wad Medani contained up to 945 µg kg\(^{-1}\) in raw groundnuts, up to 517 µg kg\(^{-1}\) in roasted groundnuts, and up to 994 µg kg\(^{-1}\) in groundnut paste. Groundnut paste prepared after a careful sorting and cleaning had only 19 µg aflatoxin kg\(^{-1}\). Analyses of 145 samples in Jamaica and St. Vincent in 1984 indicated only eight samples containing more than 20 µg kg\(^{-1}\) of aflatoxins. Roasted groundnuts and peanut butter samples collected from markets in Jamaica and Trinidad did not contain detectable amounts of aflatoxins. However, groundnut products collected from St. Vincent had very high levels of aflatoxin varying from 1 to 469 µg kg\(^{-1}\).


The incidence, distribution, and disposition of products containing aflatoxins are discussed. Susceptible commodities include groundnuts, com, sorghum, rye, wheat, rice, barley, oats, soybeans, pecans, walnuts, almonds, filberts and figs. Anatoxins in groundnuts and com are discussed in detail.


This report of the Working Party on Naturally Occurring Toxicants in Food. Sub-Group on Mycotoxins, describes surveillance for mycotoxins since 1979. During the survey aflatoxin content in milk decreased; 75 and 4 % of bulk-dried milk samples contained aflatoxins in 1977-79 and 1981-83, respectively. Aflatoxins were also detected in nuts and nut products, particularly in groundnuts. Ochratoxin was detected in trace amounts in pigs’ kidney and nuts. During 1982, 44, 24 and 16 % of samples of UK- grown barley, wheat and oats contained deoxynivalenol between 20 and 100 µg kg\(^{-1}\) but only 4 % of all UK-grown cereals between 1980 and 1982 contained deoxynivalenol at more than 100 µg kg\(^{-1}\). Legislative control of mycotoxins in the U.K. food supply is summarized. A maximum permitted limit of 10 µg kg\(^{-1}\) for total aflatoxins (B\(_1\), B\(_2\), G\(_1\) and G\(_2\)) in nuts and nut products is proposed.


Analysis was carried out on 401 samples of locally-consumed groundnut and groundnut products including peanut butter, groundnut cake, tried or roasted groundnuts, and oil, using a high pressure liquid chromatography (HPLC) method. The incidence of aflatoxin contaminated samples was 23.4 %. Aflatoxin B\(_1\) was found in all positive samples.

Data generated from compliance programmes on aflatoxins in foods and feeds, enforced by the FDA, are summarized for the fiscal year 1986. Commodities sampled included groundnuts and groundnut products, maize and maize products, tree nuts, cottonseed, milk, spices, manufactured products and miscellaneous foods and feeds. Correlations were highest between aflatoxin contamination and geographical areas for maize/maize products and cottonseed/cottonseed meal. Higher incidences of aflatoxin contamination in maize and maize products designated for human consumption were observed in samples collected in the southeastern states (32 and 28 %, respectively). A higher incidence of contamination was observed in maize designated for animal feed from Arkansas-Texas (74 %) than from the southeastern states (47 %). Only 3 % of feed maize from corn belt states contained detectable aflatoxins. All aflatoxin-contaminated cottonseed was collected in the Arizona-California area; 80 % of cottonseed meal analyzed from this area also contained detectable levels of aflatoxins. No aflatoxin M1 was detected in any of the 182 samples of fluid milk and milk products examined. It is concluded that the percentage of samples that contain measurable levels of aflatoxins is expected to vary with commodities from year to year and the 1986 information can be used as a baseline for comparison to determine the effectiveness of control efforts exerted by the food and feed industries.


A survey of the aflatoxin content in some food products available on the market in Norway is reported. During autumn 1973, samples of Brazil nuts, groundnuts, peanut butter, hazelnuts, walnuts, mixed nuts, cocoa, cocoa products and dried milk were bought from stores in the Oslo area. In addition, samples of Brazil nuts and groundnuts were taken from import stores. Aflatoxins were detected in 6 of 34 samples of bought groundnuts, 1 of 3 of sorted and pooled groundnuts, 1 of 16 mixed nuts, 1 of 14 walnuts, 1 of 3 cashew nuts, 18 of 27 Brazil nuts and 1 of 40 cocoa products. Levels of aflatoxins B1, B2, G1 and G2 were 4200, 1600, 3100 and 600 µg kg\(^{-1}\), respectively, for one sample of Brazil nuts and 400, 50, 350 and 30 µg kg\(^{-1}\) for a sample of groundnuts. Other aflatoxin levels were generally much lower. No aflatoxin was detected in peanut butter, hazelnuts and dried milk.

5.2.2 Groundnut kernels


Of 95 samples of various feedstuffs, which were tested for aflatoxins B1, B2, G1 and G2 by thin-layer chromatography (TLC), 44.2 % were positive (including maize, rice crack, rice germ, rice germ cake, rice bran, wheat bran, cotton seed, cotton seed cake, and groundnut and mixed feed for broilers, egg production, calf fattening and milk production). Most of the samples (90.4 %) were contaminated with <100 µg kg\(^{-1}\) total anatoxins. Groundnuts from "Ismailia" had the highest contamination (400 µg kg\(^{-1}\) aflatoxin B1) and the ratio of aflatoxins in kernels and shells was 1:7. Soybeans were contaminated with 5 µg kg\(^{-1}\) aflatoxin B1, while horse beans and fish meal were not contaminated. Aflatoxin B1 was present in only 76.1 % of the positive samples, and the concentration ratio of aflatoxins B2:G1:B1 was 1:2.3:22.4.


Aflatoxins were detected in some cereal and leguminous seeds (including maize, wheat, haricot bean, broad bean, lupin, groundnuts and cowpea) collected from 2 cities in the Sudan (Wad Medani and Khartoum). Levels of aflatoxins in these seeds were below the reported hazard threshold. Studies on the effect of seed grading on aflatoxin contamination of 4 crops (dura, maize, haricot bean and broad bean) showed that sound intact seeds contained low or no aflatoxins, compared with significantly higher levels detectable in damaged and moldy seeds. Effect of temperature on production of 4 aflatoxin components (B1, B2, G1 and G2) was studied in 2 isolates of Aspergillus flavus (MS III from maize and HCB 33 from haricot bean). Optimum temperature was 30°C for MS III and 20°C for HCB 33. Large amounts of aflatoxins B1, B2 and G1 were produced by the isolates, while aflatoxin G2 was produced only by HCB 33 at optimum temperature.


The effects of irrigation regimes and date of harvesting on preharvest infection by Aspergillus flavus and aflatoxin contamination of seed of four commercial and two other groundnut cultivars were studied. Groundnuts watered at 1-, 2-, and 3-week intervals and harvested at the normal time, and at 1 week before, and 1 week after,
were free from *A. flavus* infection and aflatoxin contamination. *Aspergillus flavus* infected a low percentage (2.7-7%) of groundnuts left in the soil for 6 weeks after harvest. However, no aflatoxin contamination was detected. Wilt diseases and insect damage, mainly by white grubs and termites, predisposed seeds to preharvest *A. flavus* infection (56.4-69.8%) and aflatoxin contamination (18-21 µg kg⁻¹). Groundnuts stored for 3 months in a well-ventilated room with an average temperature of 15°C were infected by *A. flavus* at a low level, but had no aflatoxin contamination. Infection increased with time in storage. Groundnuts harvested 1 week before maturity were not affected by *A. flavus* infection of seeds in storage, and there were no differences among genotypes. Groundnuts stacked in sacks at shelling sites were sampled; 4% were contaminated with aflatoxin, the average level being 11 µg kg⁻¹. Fifteen per cent of the samples from oil mill sites were contaminated, the average aflatoxin content being 20 µg kg⁻¹. Groundnuts left in the soil for 2-3 weeks after harvest in trials on the Gezira and Rahad irrigation schemes had 12% of contaminated samples, with an average aflatoxin content of 10 µg kg⁻¹. This produce is usually allocated for local processing.


Existing data indicated that 10-20% of the corn tortillas sold in Mexico City are contaminated with aflatoxins. A large part of imported corn is also contaminated with aflatoxins. Results of surveys of Mexican foods from 1979-1981 found the frequency of aflatoxin contamination of groundnuts, processed groundnuts, beans, sorghum and corn to be 47, 7-31, 5, 3 and 1%, respectively.


Ninety-two samples of grain and common feed ingredients were analyzed in Australia using thin-layer chromatography (TLC) and a rapid minicolumn screening method. Three samples contained aflatoxins; a sorghum grain sample containing 30 µg kg⁻¹ aflatoxin B₁, and two samples of moldy groundnuts containing 2500 µg kg⁻¹ aflatoxin B₁ and 400 µg kg⁻¹ aflatoxin B₂, and 200 µg kg⁻¹ B₁ and 38 µg kg⁻¹ B₂, respectively. A non-destructive detection technique involving scanning the grain with a UV lamp and examination of fluorescence is reported.


Aflatoxins were estimated in 480 food samples stored for consumption between harvests and collected from different parts of Uganda in 1966-67. Among these samples, 29.6% contained detectable amounts of aflatoxins and 3.7% contained more than 1 µg kg⁻¹. The frequency of aflatoxin contamination was particularly high in provinces with a high incidence of hepatoma, or where cultural and economic factors favored the ingestion of moldy foods.


In Mozambique, there is a high correlation between the incidence of primary liver cancer and the consumption of aflatoxin-contaminated food. Some work has been done to assess and minimize the aflatoxin problem. Institutions such as the Instituto Nacional de Investigacion Veterinaria (INIV) and the Laboratorio Nacional para la Higiene de Agua y Alimentos (LNHAA) are involved in the analysis of food products, for both animals and humans. In 1981, 17 food products, comprising a total of 313 samples were tested and 16 samples were contaminated with aflatoxin B₁. 10 with B₂, 4 with G₁, and 3 with G₂. 87-100% of the groundnut, beer, rice, and maize samples tested were contaminated. The aflatoxin levels in the groundnut samples ranged from 3 to 5500 µg kg⁻¹, aflatoxin B₁ being the main contaminant. An analysis program is investigating the possible correlation between consumption of contaminated food and the possible presence of aflatoxin M, in human breast milk. The possibility of further work involving the INIV, LNHAA, and the Faculdade de Agronomia, Universidade Edurado Mondlane, Groundnut Improvement Project is being studied to include an agronomic component and formulate practical recommendations for small farmers and traders.


Groundnut samples collected from different areas in Pakistan were tested for aflatoxin contamination. Romer’s method was used for the analysis of aflatoxins. Fifteen per cent of the groundnut samples from Lahore markets were contaminated with aflatoxins, while all samples from Khuzdar showed aflatoxin contamination. Among the contaminated samples, levels of aflatoxins (B₁ and B₂) ranged from 80-800 µg kg⁻¹, except for one sample that had 24 µg kg⁻¹. Raw groundnut samples did not have any aflatoxins.

Injera, a fermented, pancake-shaped food prepared from *Eragrostis tef*, showed no aflatoxin contamination as commonly prepared and handled; fermentation of the dough or storage of injera for prolonged periods did not increase aflatoxin B₁ contamination. Preparations of red pepper powder and its paste showed some aflatoxin contamination (mean 32 µg kg⁻¹ for powder, 1 paste sample had 102.2 pg kg⁻¹ aflatoxin B₁). Samples of groundnuts and peanut butter had aflatoxin B₁ at mean values of 34.7 and 105 µg kg⁻¹, respectively.


Of 78 groundnut samples analyzed in Ivory Coast, 22 contained > 20 pg kg⁻¹ aflatoxin B₁, and 27 contained > 200 µg kg⁻¹ aflatoxins B₁ B₂ G₁ or G₂. At post-mortem, aflatoxins were detected in liver samples from a patient with liver cancer: B₁ 11.8 µg kg⁻¹; B₂ 1.9 µg kg⁻¹; G₂ 0.1 µg kg⁻¹.


A group of 60 samples from aflatoxin-contaminated groundnuts was provided by the Federal-State Inspection Service from six widely separated grading points in Southwest Georgia. Another group of 28 samples was collected from various warehouses in Georgia, Alabama, and Florida. Each sample of groundnuts was shelled and the kernels divided into four subsamples of different sizes. Standard slotted-hole grading screens having either 20/64-, 18/64-, or 16/64-inch width slots were used to make the size separations. Analysis of the subsamples for aflatoxin showed that 85 % of the subsamples in the group of 60 samples contained measurable amounts of aflatoxin and the kernels that fell through the 18/64-inch screen and rode the 16/64-inch screen had a significantly higher average concentration of aflatoxin than the other kernels. Aflatoxin at > 20 µg kg⁻¹ was detected in 65 % of the subsamples in the 28-sample set. The smaller size kernels contained higher levels and had more frequent occurrence of aflatoxin than the larger size kernels.


Research was done in Israel on the occurrence of aflatoxin-producing fungi in soils and in groundnut kernels. *Aspergillus flavus* was prevalent in low levels in soils of the groundnut fields in Israel. *Aspergillus flavus* infection of groundnut kernels increased with storage time. 80 % of 55 stored groundnut samples tested from 1963 crop and 64 % of 63 stored samples from 1964 crop were infected with the fungus while only 20 % of the 1963 crop and 12.7 % of the 1964 crop were contaminated with aflatoxin. 71.2 % of 330 A. flavus isolates tested were toxigenic. The percentage of aflatoxigenic strains among soil isolates was higher than that of the kernel isolates.


1076 samples of different commodities taken from stores and dealers throughout the country were screened for aflatoxin contamination. Results showed that 13 samples were contaminated with aflatoxin, with 3 of 38 corn samples containing 48-62 pg B₁ kg⁻¹, and 8-22 µg G₁ kg⁻¹, 2 of 6 corn samples 30-35 µg B₁ kg⁻¹, 1 of 19 pistachio nut samples 22 µg B₁ kg⁻¹, 1 of 35 pea samples 25 pg B₁ and 42 pg G₁ kg⁻¹, 2 of 248 cheese samples 1 µg B₁ and 6,2,10,6 µg M₁ kg⁻¹ and 4 of 65 groundnut samples 6-46 µg B₁ and 0.38 µg G₁ kg⁻¹. No positive samples were found in wheat, barley, lentils, almonds, sesame and sunflower seeds, bean and milk (45 samples).


Of 12 maize samples analyzed, 1 had aflatoxin B₁ at over 200 µg kg⁻¹, and 33 % of dried cassava samples were contaminated with aflatoxin. Select and low quality groundnuts had 80 and 39 % of samples not contaminated. Of the low quality samples 33 % contained more than 250 µg kg⁻¹. Results are related to local incidence of hepatoma and to the high humidity of the climate.


In 2 surveys during 1985 and 1986 highest incidence of anatoxin B₁, B₂, G₁ and G₂, contamination in 422 samples of unshelled and shelled groundnuts, pistachio nuts and brazil nuts from different regions of Spain was in unshelled groundnuts (9.1 and 10.6 % of samples in the 2 surveys). Incidence in shelled groundnuts was 4.9 and 4.0 %, respectively. No aflatoxins were detected in pistachio nuts. The highest value, 2478 µg kg⁻¹ was in an unshelled groundnut sample.

There was a low incidence of aflatoxin contamination in the 1962/63 Southern Rhodesian groundnut crop (50-250 µg kg⁻¹ aflatoxin B₁), whereas with the 1963/64 crop, an appreciable number of samples were contaminated with more than 250 µg kg⁻¹ aflatoxin B₁. This incidence occurred before marketing and was not aggravated by storage conditions used in Rhodesia. Although fungal infection might occur before harvesting, the major portion of aflatoxin contamination occurred in the period between lilleting of the crop and delivery of the shelled groundnuts to the Grain Marketing Board. There was no evidence that the distribution of contamination was dependant upon the rainfall in the harvesting period. There appeared to be a higher level of aflatoxin B₁ contamination in areas with high air temperatures (65°F and above) in the harvesting period. The aflatoxin research program for the 1965 crop is described.


Seventeen per cent of 264 samples (of maize, rice, cottonseed, beans, groundnuts, coffee, cocoa, wheat, sorghum, sesame and other foods) were contaminated with aflatoxins; 8 % exceeded the 20 µg kg⁻¹ level. The highest level (240 µg kg⁻¹) was in silo-stored yellow maize from a hot and humid area. This maize sample showed visible fungal contamination. Aflatoxin B₁ alone or in combination with other aflatoxins was found in all positive samples. Aflatoxin G, was found in maize, groundnut, rice, beans and meat meal, while aflatoxin G₂ was found in rice and beans.


Aflatoxin contamination levels in groundnut samples collected in Mozambique ranged from 3 to 5500 µg kg⁻¹.


Anatoxins B₁, B₂, G₁ and G₂ were determined in bulk samples of raw groundnuts, peanut butter, wheat flour, and chicken, pig and dairy feeds. Samples were taken in accordance with an established sampling plan, and dry samples were subsampled with the Dickens Subsampling Mill. A minicolumn procedure was used to screen 64 samples, eight of which were further analysed by thin-layer and high-performance liquid chromatography. Of the eight samples, only one contained aflatoxin G₁ (2 µg kg⁻¹).


Aflatoxin contamination of foods was found to be greater in areas of low altitude. Food products most frequently and heavily contaminated included groundnuts (maximum aflatoxin 425 µg kg⁻¹), cassava (325 µg kg⁻¹) and maize (148 µg kg⁻¹). The highest incidence of hepatoma in Burundi coincides with the areas of highest aflatoxin contamination.


During 1973-1977, 1689 samples of groundnut kernels and 1172 samples of groundnut oil from 24 provinces of the People's Republic of China were analyzed for contamination with aflatoxin B₁. The percentages of samples containing the toxin were 26.3 for kernels and 47.3 for oil. Across the country as a whole the percentage of contaminated samples decreased with increase in latitude, being high in southern China, moderate in the Yangtze valley, and low in northern China. The key to prevention of aflatoxin contamination was rapid drying of groundnuts to below 10 % moisture content. Screening of groundnut germplasm for resistance to Aspergillus flavus is in progress.


Of 18 samples of groundnuts analyzed, 5 contained higher levels of aflatoxin G than aflatoxin B₁. One sample of roasted nuts contained aflatoxins (21 to 26 µg kg⁻¹) with about 20 times as much G as B aflatoxins.


Samples of farmers' stock groundnuts were collected from 6 locations at weekly intervals for 5 weeks during the harvesting season. Seventeen of the 228 samples
collected contained aflatoxins. Aflatoxins were present in 2.5 % of the Segregation I (highest quality), 12.1 % of the Segregation II, and 25.7 % of the Segregation III samples, and averaged 22, 264, and 324 µg kg$^{-1}$ total aflatoxin respectively. A significant relationship was thus found between the presence and concentration of aflatoxins and grading factors. Samples with high visible damage values were more likely to contain aflatoxins than were samples with high invisible damage (rancidity, mold or decay) values. The weekly incidence of aflatoxins was related to the climatological conditions during the 14-day period prior to the date of collection. Mycofloral studies of 73 selected samples showed a relationship between the mean number of fungi per sample and grading factors; the mean number increased as quality decreased. These findings support previous observations that factors which influence groundnut quality also influence the incidence of aflatoxins in farmers' stock groundnuts.


Samples of farmers' stock groundnuts were collected from six locations at weekly intervals for five weeks during the 1967 and 1968 harvesting seasons. Seventeen of 228 samples collected in 1967 and 23 of 356 samples collected in 1968 contained aflatoxins. In 1967, 2.5 % of Segregation I, 12.1 % of Segregation II, and 25.7 % of Segregation III samples contained aflatoxins and averaged 22, 264, and 324 µg kg$^{-1}$ total aflatoxin respectively. In 1968, 3.2 % of Segregation I, 6.3 % of Segregation II, and 11.9 % of Segregation III samples contained aflatoxins and averaged 28, 61, and 583 µg kg$^{-1}$ total aflatoxin respectively. Thus, in both years, significant relationships were found between the presence and concentration of aflatoxins and grading factors. Weekly incidences of aflatoxin contaminated samples were related to climatological conditions during the 14-day period prior to the collection dates in both years. Mycofloral studies of 73 selected samples in 1967 and of all samples in 1968 showed a relationship between the mean number of fungi/sample, grading factors and aflatoxin contamination. These findings support previous observations that factors which influence groundnut quality also influence aflatoxin contamination in farmers' stock groundnuts.


Aflatoxin levels in groundnut samples from the Virginia-North Carolina crop were determined in a survey carried out in the 3-year period 1964-1967. About 6% of samples from the 1964-1965 crop contained aflatoxin, and 23 of 51 samples of peanut butter were contaminated with aflatoxin. Contaminated lots of groundnuts were reduced to 5.3% in 1965-1966 and to 4.4% in 1966-1967. No peanut butter sample was contaminated in 1965-1966.


Groundnut samples, collected soon after harvest, from different districts in the irrigated region (Central Sudan) were free from aflatoxins. Samples collected from the rainfed region (Western Sudan) showed incidences of aflatoxin contamination ranging from 100 % samples in El Hamdi to only 10% samples in Casgeal. Damaged pods were highly contaminated with Aspergillus flavus and aflatoxins. Sound intact pods had lower fungal contamination and were almost free of aflatoxins. Groundnut products (groundnut paste, red and grey roasted pods) collected from Khartoum North (Bahri) had higher levels of aflatoxin than those collected from Khartoum and Umdorman. Grey and red roasted pods showed higher levels of aflatoxins than the groundnut paste. None of the three varieties (MH383, Barberton, Ashford) of groundnut tested was completely resistant to A. flavus contamination and aflatoxin production. A temperature of 30°C and 86.3 % relative humidity were the optimum conditions for both A. flavus growth and aflatoxin production in groundnuts.


A survey of groundnuts from Egypt showed 19.5 % of unshelled and 49.0 % of shelled samples to contain low levels of aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$; only B$_1$ was detected in 3.5 % samples of roasted groundnuts. However, 60 % of Aspergillus flavus strains from Egypt produced one or more aflatoxin, and 40 % of toxigenic strains produced high quantities (5000-20000 µg kg$^{-1}$) of B aflatoxins; the levels found in groundnuts were therefore lower than expected. Studies on the effects of storage temperature and RH on groundnut mycoflora and aflatoxin development showed an RH of 95 % to be optimum for aflatoxin production; since the RH in Egypt is lower than this, this was considered a factor contributing to the low levels encountered. Inoculation of Egyptian groundnuts (cv. Giza 1) with different strains of A. flavus showed the shells to act as a barrier towards invasion; this was considered the main reason for low aflatoxin production. Consequently, it is considered imperative to discard groundnuts with cracked shells before storage.


Total aflatoxin content, measured spectrophotometrically, of crops and spices grown locally was 1600 ± 500 µg kg$^{-1}$ for manihot flour, 4 0 0 1 0 0 for yam flour, 700±
Three samples of groundnut oils obtained from local markets in Hong Kong were tested for mutagenicity using the Salmonella/microsome test system, in the presence of microsomes, dimethylsulphoxide (DMSO) extracts of the samples were mutagenic to Salmonella typhimurium strains TA 98 and TA 100, but the mutagenic activity of the oils decreased after they had been repeatedly cooked. However, when the extracts were pre-incubated with the bacteria (TA 100) and microsomes, the mutagenic activity of the cooked oils was greater than that of the uncooked oil, suggesting the possible contamination of the cooked oils by N-nitrosamines. Mutagenic activity was also detected in random samples of seven other brands of uncooked oils obtained from local markets. By absorption spectrophotometry, aflatoxin B$_1$ levels in 3 of these oils were in the range 98-150 µg kg$^{-1}$. DMSO-extracts of 10 samples of poor-grade market groundnuts were also mutagenic and the groundnuts contained 95-1055 µg kg$^{-1}$ aflatoxin B$_1$.

Four samples of groundnut oils obtained from local markets in Hong Kong were tested for mutagenicity using the Salmonella/microsome test system, in the presence of microsomes, dimethylsulphoxide (DMSO) extracts of the samples were mutagenic to Salmonella typhimurium strains TA 98 and TA 100, but the mutagenic activity of the oils decreased after they had been repeatedly cooked. However, when the extracts were pre-incubated with the bacteria (TA 100) and microsomes, the mutagenic activity of the cooked oils was greater than that of the uncooked oil, suggesting the possible contamination of the cooked oils by N-nitrosamines. Mutagenic activity was also detected in random samples of seven other brands of uncooked oils obtained from local markets. By absorption spectrophotometry, aflatoxin B$_1$ levels in 3 of these oils were in the range 98-150 µg kg$^{-1}$. DMSO-extracts of 10 samples of poor-grade market groundnuts were also mutagenic and the groundnuts contained 95-1055 µg kg$^{-1}$ aflatoxin B$_1$.


Surveys were carried out for two years to determine the natural occurrence of mycotoxins (aflatoxin, ochratoxin and zearealenone) in some common Brazilian foods including raw and salted roasted groundnuts, pacoca (a very popular groundnut candy), peanut butter, maize, soybean, cured cheese, salami and copa (cured and dried pork). Eight characteristic regions, Vale do Paraiba. Mogiana, Paulista Velha, Araquariense, Noroeste, Paulista Nova, Sorocabana and Vale do Ribeira/Litoral Sul, were surveyed, and 742 samples were analyzed. Estimation of toxins was made by thin-layer chromatography (TLC) by comparison with standards. Six samples of corn (4.7 %) were contaminated with aflatoxin, ranging from 41 to 2000 µg kg$^{-1}$ of aflatoxin B$_1$; 81 samples of pacoca (63.3 %), ranging from < 20 to 1187 µg kg$^{-1}$; 80 samples of peanut butter (62.5 %), from < 20 to 275 µg kg$^{-1}$; 17 samples of raw groundnuts (17.7 %), from 19 to 3125 µg kg$^{-1}$; and 9 samples of roasted salted groundnuts (9.4 %) from < 30 to 4250 µg kg$^{-1}$. From the first to the second year, the percentage of contaminated samples increased slightly for raw groundnuts (from 15.6 to 18.8 %) and decreased for pacoca (67.2 to 59.4 %) for roasted salted groundnuts (12.5 to 7.8 %), corn (6.2 to 3.1 %), and for peanut butter (84.4 to 40.6 %). The average content of aflatoxin of the contaminated samples increased for raw groundnuts (from 166.7 to 641.7 µg kg$^{-1}$) and roasted salted groundnuts (410 to 980 µg kg$^{-1}$) and decreased for pacoca (165.6 to 108.8 µg kg$^{-1}$). peanut butter (91.3 to 50.4 µg kg$^{-1}$), and corn (853.3 to 700 µg kg$^{-1}$).


The concentrations of aflatoxins B$_1$, B$_2$, G$_1$, G$_2$ and M$_1$, ochratoxin A, patulin and byssosphilamic acid in a variety of foods were determined by fluorescence spectral analysis. In groundnuts concentrations of up to 1600 pg aflatoxin kg$^{-1}$ were found. Of 198 apparently uncontaminated milk samples, aflatoxin B$_1$ was detected in 1. and aflatoxin M$_1$ was identified in 4 of 60 milk samples examined. No aflatoxin M$_1$ was detected in 22 milk product samples analyzed. Of 49 moldy cereal samples, 2 contained ochratoxin A but no aflatoxins were detected. Both samples of raw coffee examined contained ochratoxin A. Patulin was detected in 19 of 110 samples of fruits and fruit products, in particular commercial apple juice. Byssosphilamic acid was not identified in fruits or fruit products. The toxicological implications of mycotoxins in foodstuffs and the possible ways in which levels in foods could be reduced are discussed.


Results of 492 analyses for aflatoxin in raw shelled groundnuts imported into Czechoslovakia during 1982-1984 are presented. In most (55.3%) of the samples no aflatoxin was detectable by the analytical method used (sensitivity limit of radioimmunochemical method 0.8 µg kg$^{-1}$). Further analyses showed that 239 of 410 samples of roasted groundnuts contained aflatoxin below the detection limit. Only 1.9% of samples had aflatoxin present at more than 5 µg kg$^{-1}$. The highest concentrations of aflatoxin were in a raw sample (202.1 µg kg$^{-1}$) and in a roasted sample (32.6 µg kg$^{-1}$).


A study employing the Best Foods (BF) method was carried out for a period of six
years to determine the aflatoxin content in groundnuts, peanut butter and other foodstuffs. The study was divided into three groups. In Group 1 a total of 3928 samples were analyzed; 1679 samples of raw groundnuts, 2092 samples of peanut butter, and 157 samples of processed groundnuts; 5.8%, 0.1%, and 1.3% of the samples analyzed in these groups contained a total aflatoxin content over 25 µg kg⁻¹, respectively. Group II consisted of oilier nuts: cashew, almonds, walnuts, pecans, filberts, Brazil nuts, and pistachios, of which 641 samples were analyzed. Two per cent contained aflatoxin over 25 µg kg⁻¹. Group III, other foodstuffs, consisted of sunflower seeds, cocoa beans, beans, corn, mushrooms, and coffee beans. The total number of samples analyzed were 244 and only 0.4% contained aflatoxin at over 25 µg kg⁻¹. On the basis of the samples analyzed it is evident that in the past six years the incidence of aflatoxin occurring at levels of less than 5 µg kg⁻¹ in groundnuts and groundnut products has been 90.1% of the samples analyzed. In the other nuts tested, 91.7% of the samples contained less than 5 µg kg⁻¹ of aflatoxin. The test data of samples of foodstufs other than nuts showed that 95.9% of the samples contained aflatoxin at a level of less than 5 µg kg⁻¹.


A study using the ‘Best Foods’ (BF) method was done over 6 years to determine aflatoxin contents in groundnuts, peanut butter and other foods. Results showed that of 1679 samples of raw groundnuts, 98 (5.8%) contained > 25 µg kg⁻¹ total aflatoxins, 89.8% < 15 µg kg⁻¹. Of 157 samples of processed nuts, 2 (1.3%) contained > 25 µg kg⁻¹ and the rest < 15 µg kg⁻¹. Of 2092 samples of peanut butter, 3 (0.14%) contained > 25 µg kg⁻¹ and 12 (0.57%) contained 15-25 µg kg⁻¹. Of 120 samples of cashews, 10 (8.3%) contained > 15 µg kg⁻¹ as well as 1 of 78 samples of almonds, 2 (1.3%) of 156 samples of walnuts, 10 (5.7%) of 175 samples of pistachios. None of the samples of pecans, filberts and Brazil nuts tested contained aflatoxin. Of 244 samples of other foods, only one sample each of sunflower seeds and corn contained > 15 µg kg⁻¹. None of the samples of cocoa beans, beans, mushrooms tested had aflatoxin contamination. Test data of foods other than nuts had aflatoxin levels of < 5 µg kg⁻¹ in 95.9% of samples.


Of 100 imported groundnut samples examined, 27 contained aflatoxin B₁ at 5-800 fig kg⁻¹. Aspergillus flavus, A. niger and some mucoraceae were isolated from both aflatoxin-positive and aflatoxin-negative samples.


A preliminary survey of peanut butter in 1982 indicated that 31 of 32 samples of major national brand-name products contained aflatoxin B₁ at up to 10 µg kg⁻¹, and that 59% of those were below the limit of detection (2 µg kg⁻¹). Of 25 peanut butter samples from specialist ‘Health Food’ outlets, 64% contained up to 10 µg kg⁻¹, and the remainder contained 16-318 µg kg⁻¹, with one sample having 345 µg kg⁻¹ aflatoxin. Surveys of ‘Health Food’ products in 1983 and 1984 confirmed that manufacturers were still experiencing some difficulty in complying with the 30 µg kg⁻¹ voluntary guideline limit for total aflatoxin. In 1984, 228 retail samples of nuts and nut confectionery products comprising groundnuts (shelled, unshelled, roasted and salted), mixed nuts, almonds (both unblanched and ground). Brazil nuts (in shell), hazelnuts (in shell), chocolate-coated groundnuts, peanut brittle and coconut ice were examined. Results showed that 74% of the samples contained aflatoxin B₁ at up to 0.5 µg kg⁻¹, and 3.1%, mainly groundnuts and Brazil nuts, exceeded the guideline tolerance for total aflatoxin. Total aflatoxin was greatest in unshelled groundnuts, 4920 µg kg⁻¹, and in a composite sample of visibly molded Brazil nuts, 17926 µg kg⁻¹.


Six samples each of wheat, maize, lentils, beans, fenugreek, groundnuts and cottonseed cake from various areas of Egypt were analyzed for aflatoxins at the time of collection and after 12 months in storage. Aflatoxin was found at low levels (3-12 µg kg⁻¹ total aflatoxins) in 14 of 42 samples (1 each of maize, lentils and beans, 2 of groundnuts, 3 of fenugreek and 6 of cottonseed cake).


Aflatoxins were estimated in 295 samples of groundnuts, coffee, barley, wheat and walnuts imported into Yugoslavia. Levels were from 1 to 25 µg kg⁻¹ (mean 3.16 µg kg⁻¹). Only 20% of samples had more than 5 µg kg⁻¹ aflatoxin.

A preliminary survey of the incidence of aflatoxin in the main groundnut production areas of the Sudan is described, correlating the extent of infection with Aspergillus flavus and other kernel fungi with the aflatoxin content of the kernels. In general, the rainfed samples had a higher incidence of kernel contamination than those from the irrigated areas. The majority of the groundnut samples from the irrigated areas were aflatoxin-free, whereas in those from the rainfed areas none was free, and 48 % were classified low-medium (<50 to 50-250 µg kg⁻¹ aflatoxin), while 52 % were in the high to very high category (250-1000 µg kg⁻¹ to > 1000 µg kg⁻¹ aflatoxin).


Of 50 raw groundnut samples analyzed, 3 were contaminated with allatoxin. Testing of fungi isolated from samples showed that 37.5 % of the isolates of Aspergillus flavus were capable of producing aflatoxins in vitro. None of the samples was resistant to the production of aflatoxin following inoculation with aflatoxin-producing isolates.


Of 19 food commodities (63 samples) analyzed for aflatoxin, 15 commodities including wheat, legumes, nuts, dried fruits, vegetables, cheese and some local popular foods showed no contamination. Aflatoxins were detected in groundnuts, dried figs, lentils and the dairy product koshk. The level of aflatoxin was highest in dried figs with aflatoxin B₁ at 11.8 µg kg⁻¹.


Aflatoxin at up to 200 µg kg⁻¹ was detected in 16-20 % of imported groundnut samples analyzed during 1976-1978. In 1979 a highly contaminated Egyptian groundnut lot raised the positivity to > 80 %, with a maximum contamination of 1000 µg kg⁻¹. The efficiency of recovery was 75-90 % with Pons’ method and 60-71 % by the Beljaars method. Aflatoxins were not detected in samples of Hungarian flour and vegetables for soup. In an examination of samples of milk from large-scale dairy farms, aflatoxin M₁ at 0.06-0.08 µg/L was detected in 2 of 20 samples in 1977 and 2 of 30 samples in 1978. The aflatoxin B₁ content of groundnut-base feed at one farm was 60 µg kg⁻¹. The 30 milk samples analyzed in 1979 did not contain aflatoxin. In 1977 one of 20 powdered milk samples examined contained aflatoxin M₁ at 200 µg kg⁻¹. Egg samples examined during 1978-79 contained no aflatoxin.

The methods used in milk and egg analyses had extraction efficiencies of 80-90% and 75-80 %. respectively.


Samples of cereals and cereal products (150) were collected from various markets in the Bangkok area from July 1983 to April 1985. These included 40 samples of rice products, 50 samples of groundnuts and groundnut products, 20 samples of soybean and soybean products, 20 samples of other beans, and 20 samples of maize. Forty-three samples of groundnuts and groundnut products contained aflatoxins (aflatoxin B₁ at 40-780 µg kg⁻¹ and aflatoxin G₁, at 10-160 µg kg⁻¹). Aspergillus flavus and A. niger were isolated from these samples. A total of 40% of a fermented soybean product (tso-chiew) contained aflatoxins (aflatoxin B₁ 20 µg kg⁻¹ and aflatoxin G₁, 20-100 µg kg⁻¹); 20 % of salted bean curd derived from soybean contained aflatoxins (aflatoxin B₁ at 210 µg kg⁻¹ and aflatoxin G₁ at 110-270 µg kg⁻¹); 40% of soybean sauce samples contained aflatoxin B₁ (20-170 µg kg⁻¹) and aflatoxin G₁ (40 µg kg⁻¹); 10 % of rice samples contained aflatoxin B₁ (10 µg kg⁻¹) and aflatoxin G₁ (20 µg kg⁻¹). Bacteria found included only Bacillus spp. According to the standard safety level for aflatoxin offered by WHO, groundnut, fermented soybean, salted bean curd and maize are not safe for consumption but rice is safe. This research project also investigated the ability of A. niger to produce aflatoxins in glutinous rice and groundnut and revealed that A. niger could produce both aflatoxins B₁ and G₁ in these substrates.


Of 430 samples of 22 different foods collected in Jordan between January 1978 and January 1979, 5 were contaminated with aflatoxin, 3 of 40 groundnut samples, 1 of 50 rice samples, and 1 of 15 samples of dried milk. Groundnuts contained aflatoxin B₁ at 98-1056 µg kg⁻¹, rice contained aflatoxin B₁ at 29 µg kg⁻¹ and dried milk contained aflatoxin M₁ at 15 µg kg⁻¹. Aflatoxigenic fungi isolated from the food samples were mainly Aspergillus flavus, but some A. parasiticus was also detected. Some soil samples collected from 20 different areas of Jordan contained aflatoxigenic fungi.


The methodology for conducting a survey of mycotoxin contamination of foods and feedstuffs, including sampling, analysis and treatment of data obtained is presented.
Results of a survey of the aflatoxin content of a small proportion of groundnuts from the 1977/78 season imported into the U.K. are given. Thirty-one of the 159 samples exceeded the maximum permitted level of aflatoxin in raw groundnuts (30 µg kg\(^{-1}\)). The highest level of aflatoxin contamination was in samples from the Gambia and India (> 400 µg kg\(^{-1}\) in 1 sample each). Results of a survey of the aflatoxin M\(_1\) content of samples of producer-retailer milk in the U.K. commissioned in 1977 by the Ministry of Agriculture, Fisheries and Food are presented. The survey showed that 11.9 % of the milk samples tested contained > 0.1 µg L\(^{-1}\) aflatoxin M\(_1\) (the limit permitted by European Community Regulations). A parallel study of random samples of dairy feed indicated that 8 % contained > 30 µg kg\(^{-1}\) aflatoxin B\(_1\). In another study the average level of aflatoxin in a 12 ton batch of groundnut cake obtained from a farmer in the U.K. was 1540 µg kg\(^{-1}\), which would result in feedstuffs containing aflatoxin well in excess of the permitted level.


Samples of groundnuts from Cordoba market contained aflatoxins B\(_2\) and M\(_1\). Groundnuts containing 50 µg kg\(^{-1}\) aflatoxin B\(_1\) were considered unfit for human consumption.


Aflatoxin was found in up to 6.4% of fresh groundnut kernels and in up to 32% of stored kernels, although the intensity of toxicity was always very low (up to 125 µg kg\(^{-1}\)). About 90% of 1626 Aspergillus flavus isolates from groundnut kernels, rhizospheres, and geocarpospheres produced aflatoxin in culture. In quantitative tests with 750 isolates, 60% of the isolates produced > 25 µg g\(^{-1}\) aflatoxin.


The total number of molds and of species of fluorescent Aspergillus and Penicillium varied widely in samples of rye and wheat flour. The fluorescent strains formed ochratoxin. Aflatoxin was found in certain nuts, especially Brazil nuts and groundnuts, imported into Sweden.


In Zambia, groundnut kernels for export are routinely monitored for aflatoxin contamination. Since 1979, 6.3 % of the 28 410 samples analyzed had contamination levels of more than 5 µg aflatoxin kg\(^{-1}\). A 2-year study with promising varieties revealed the variability of Aspergillus flavus seed infection. Seed treatment with Benlate\((R)\)/Labilit\((R)\) at 3 g kg\(^{-1}\) seed controlled A. flavus in groundnut seed and improved crop stand.


Four hundred and sixty five samples of various food commodities and 94 samples of different feeds and feed ingredients were analyzed for aflatoxin contamination. These samples were collected from different parts of Nepal. Mostly maize and groundnut samples were contaminated with aflatoxins B\(_2\) and M\(_2\). Of 101 samples, 10 samples of maize used as food were contaminated with aflatoxin at levels > 30 µg kg\(^{-1}\), while only 2 of 67 samples of groundnuts were highly contaminated. Among feed samples, 25 of 58 poultry feed samples were highly contaminated. Maize and groundnut cake were mostly contaminated used as feed ingredients. The other commodities used as foods and feeds had aflatoxin levels well within the sale limit (30 µg kg\(^{-1}\)).


Studies on aflatoxin contamination in various food samples of Nepal were carried out during the period 1980-1986. About 764 samples of various food commodities from hills and Terai region were analyzed for aflatoxin contamination. One hundred and forty-eight samples of different feed ingredients from different Feed Industries of Kathmandu were also analyzed. Of the total number of respective samples analyzed, 19 % of maize, 29.5 % of maize Hour, 17.9 % to 33.8 % of groundnuts, 31.7 % of peanut butter and 9.4 % of wheat flour were contaminated with aflatoxin, but tolerance limit (30 µg kg\(^{-1}\)) exceeded in 4.5 % to 15.9 % of these samples. Rainy season is the most prone period for aflatoxin contamination. In case of feed, about 50 % of poultry feed, 26.7 % of cattle feed and 20 % of pig feed samples were contaminated with aflatoxins. Twenty % of the poultry feed samples had aflatoxins above the tolerance limit (10 to 50 µg kg\(^{-1}\) depending upon the age of the animals).

Data from analysis of 188 groundnut samples imported into the U.K. during 1982-1984 showed that most samples (140/188; 74%) had aflatoxin content < 5 µg kg⁻¹.


Fifty-four samples of Meju (a naturally inoculated soybean substrate for soy sauce and paste fermentation), 125 samples of Doenjang (a Korean style fermented soybean paste), both produced at the household level, and 31 samples of groundnuts were collected from 8 major cities of South Korea and subjected to aflatoxin assay by the AOAC method. Results showed that frequencies of occurrence of aflatoxins in Meju, Doenjang, and groundnuts were 7.4% (4 out of 54), 8.8% (11 out of 125) and 0 (none out of 31) respectively. A Doenjang sample from Busan had the highest content of aflatoxins; concentrations of aflatoxin B₁, B₂, G₁ and G₂ were 66, 13, 0 and 5 µg kg⁻¹, respectively, while in other samples only aflatoxin G₂ was detected. Aflatoxin B₁, isolated from the Doenjang sample from Busan, was confirmed by TLC, derivative formation and chicken embryo bioassay.


Of the 182 samples of groundnuts and other nuts examined, aflatoxin was detected in 6 samples of groundnuts (all of poor quality) at levels of 2-50 µg kg⁻¹.


A thin-layer chromatographic (TLC) method for semi-quantitative determination of aflatoxin B₁ in foodstuffs, suitable for routine analysis, is described. The application of the method to the analysis of groundnut kernels is discussed. Of groundnut samples imported into Germany in 1970-1971, 10% of samples had aflatoxin. Measures for improving health control of imported groundnuts are discussed.


Groundnuts stored and sold in Ahmednagar city and nearby villages were analyzed, 75 of samples had aflatoxin B₁. Among the contaminated samples, 37 had aflatoxin B₁ at over 120 µg kg⁻¹. Three samples had aflatoxins B₁ and B₂. Storage in open containers with other grains (at 70-80% relative humidity) was observed in most of the shops.


Of 1000 food samples examined, aflatoxins were found in groundnut products, maize products, almond products and nuts. Of 316 samples of groundnut, 35 (11.1%) contained aflatoxins; 23 (7.3%) had high levels (> 10 µg kg⁻¹ aflatoxin B₁ or > 20 µg kg⁻¹ total aflatoxin).


The content of aflatoxin was estimated in groundnuts sold for human consumption in Uganda. About 15% of the samples examined contained > 1000 µg kg⁻¹ aflatoxin B₁ and 2.5% contained > 10000 µg kg⁻¹. The level of aflatoxin contamination seemed to be highest at the end of the rains and before the new crops were harvested.


During 1985 and 1986, samples of sorghum beer, sorghum cereal, groundnuts, peanut butter and maize meal, purchased from supermarkets, were analyzed for aflatoxins using HPLC and fluorescence detection methods. Of the samples analyzed during 1985, approximately one-third were contaminated with aflatoxins, although none were above the legal limit of 10 µg kg⁻¹ aflatoxins. In 1986, the numbers of contaminated samples increased, but the level of contamination remained low, with only one sample exceeding the legal maximum.

Of 33 groundnut samples analysed, 50 % of Fijian samples were contaminated with aflatoxin and 9 % of Tongon samples contained aflatoxin. Local copra, cassava and corn samples examined were also contaminated with aflatoxin with corn contaminated at a high level.


Samples of groundnut imported into Japan during 1972-1981 were investigated for aflatoxin content. Of 2612 samples of Virginia type groundnuts examined, 13 contained aflatoxin B₁ (12 contained < 10 µg kg⁻¹ and one > 10 µg kg⁻¹ aflatoxin B₁). Of 9412 samples of Spanish-Valencia type groundnuts examined, 474 contained aflatoxin B₁ (310 contained < 10 µg kg⁻¹ and 164 > 10 µg kg⁻¹ aflatoxin B₁). Samples containing > 10 µg kg⁻¹ were from Brazil, the Sudan, Indonesia, India, Paraguay, and the USA.


A committee to coordinate action on the problem of aflatoxin contamination in Nigeria was formed in 1961 with representatives from four ministries, i.e., the Institute for Agricultural Research (IAR), Zaria, the Nigerian Stored Products Research Institute (NSPRI), and the Northern Nigerian Marketing Board. This Committee was given the responsibility of assessing the extent of the aflatoxin problem in groundnut in the country and of initiating and coordinating all actions leading towards elimination of aflatoxin. IAR investigated the aflatoxin contamination of the groundnut crop up to the stage where trie produce was sold by farmers, while NSPRI studied the problem from the time of storage until produce was exported or consumed. IAR investigated the time of invasion of groundnut kernels by Aspergillus flavus, and when, and under what conditions it produced aflatoxin. An interplay of temperature, relative humidity, drought, and erratic rainfall patterns, and maturity of the crop at lifting affected invasion by A. flavus and aflatoxin contamination of groundnut in the field and store. In the wetter areas of the Southern Guinea Savanna which have long rainy seasons, aflatoxin contamination of groundnuts is mainly a postharvest problem, while in the major groundnut growing areas that lie in the drier Northern Guinea and Sudan Savanna the problem is largely preharvest. Insect infestations and wetting of stored groundnuts increase aflatoxin contamination. Research information from IAR and NSPRI still provides the basis for recommendations on the handling of groundnuts to either minimize or prevent aflatoxin contamination. Vegetable oil and feed mill companies routinely submit their groundnut and other feed materials for aflatoxin analysis as there is great awareness among the companies, people, and government of Nigeria of the dangers posed by aflatoxin to poultry, livestock, and humans. Nigeria is a consignatory to the African Groundnut Council’s resolution to export only groundnuts with an aflatoxin content that does not exceed the maximum permissible limit of 20 µg kg⁻¹ set by the European Economic Community. None of the commercially grown groundnut cultivars in Nigeria is resistant to A. flavus invasion and aflatoxin contamination of seeds. Breeding materials from both domestic and exotic sources are being screened for resistance while other improved management practices are being used or researched.


Groundnuts (3 samples), bananas (2), millet (3), maize flour (10), sorghum (2), cassava flour (13), sweet potatoes (6), potatoes (3) and germinated sorghum (6) were examined. Aflatoxins were detected in 19 samples; 5 had more than 1 µg g⁻¹ and a sample of sweet potato had 17 µg g⁻¹. Values were generally greatest in the samples with highest water contents. Most fungi were of Aspergillus spp. and A. flavus was most common. Studies in vitro showed that A. flavus was not the only species to synthesize aflatoxin.


Aflatoxin contamination in five agricultural commodities namely groundnut, dried cocoa beans, copra, pepper (black and white) and paddy and rice was determined. Shelled groundnuts and their products such as peanut butter and satay gravy were highly susceptible to aflatoxin contamination, but the "menglembu" type groundnut was free of aflatoxin. Copra, dried cocoa beans and pepper (black and white) were the other highly susceptible commodities, but stored paddy and rice were safe but a few samples contained aflatoxin. The extent of contamination in several other commodities is also mentioned and general control methods are discussed.


Eighty samples of groundnut, representing both the Northern and Volta Region types, were obtained from markets in and around Accra, Ghana, and analyzed for aflatoxin. Sample size was 0.5 kg. Mean levels found for each type (with ranges in
Groundnuts obtained from distributors were aflatoxin-free, while about 30% of those bought from small retailers in the market were contaminated with aflatoxin (7 to 2000 µg/kg aflatoxin B₁). All samples of rice, green grain and soybean tested were aflatoxin-free. Eleven of 20 samples of corn were contaminated with aflatoxin, with average values of 25.8 µg kg⁻¹ of aflatoxin B₁, and 24.8 µg kg⁻¹ of aflatoxin G₁. One sample of moldy candle nut had 426 µg kg⁻¹ of aflatoxin. The results of this limited survey on aflatoxins in nuts and grains have indicated that groundnut and corn are the commodities which are most susceptible to contamination with aflatoxin. Guidance on postharvest technology should be given to the farmers, distributors, and retailers concerning proper drying and storage of the crops to minimize mold growth.


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A total of 195 samples of human food and 12 samples of animal feed were analyzed for aflatoxins. Aflatoxins were detected in 40 of the human food samples, mainly maize and maize products. Most of the contaminated samples contained < 100 µg kg⁻¹ aflatoxin, but 1 maize flour sample from a gunny bag in a shop contained 140 µg kg⁻¹ aflatoxin. Contamination in wheat flour did not exceed 25 µg kg⁻¹ aflatoxin, and for rice, the few samples tested contained only trace amounts of aflatoxin. Except for 1 sample of raw groundnuts, containing 1050 µg kg⁻¹ aflatoxin, other nuts and oils were relatively free of contamination.


Seven Virginia groundnut varieties, viz. S 230, Kadiri 1, Kadiri 3, Karad 4-11, M₁₃, GAUG 10 and TMV 10 were grown in four locations (Chimamani, Jalgaon, Khargaon, and Junagadh) in India in the rainy season of 1982. After harvest the pods were received at Junagadh during February-March 1983. Pods were shelled and aflatoxin analyses carried out on seeds with a thin-layer chromatography (TLC) method. Aflatoxin B₁ was found in all the groundnut varieties (0.8 to 65.8 µg kg⁻¹). Aflatoxins B₂ and G₁ were found in a few samples from some locations. Among varieties, S 230 had the highest level of aflatoxins at all the locations (49.8 to 85.9 µg kg⁻¹) followed by GAUG 10 (15 to 88.3 µg kg⁻¹) and Kadiri 3 (17.8 to 106.7 µg kg⁻¹). Karad 4-11 had low levels of aflatoxins at all four locations (1.6 to 30.3 µg kg⁻¹) followed by Kadiri 1 (4 to 52 µg kg⁻¹). M₁₃ and TMV 10 had moderate levels of aflatoxins. Among the locations, Junagadh and Chintamani samples contained higher levels of aflatoxins. Unfavorable postharvest drying conditions at Junagadh and Chintamani probably resulted in higher levels of aflatoxins at these locations. The varieties with more than 3% phenols in the seed testa generally contained low levels of aflatoxins. The varieties with protein content higher than 25% contained more aflatoxins, while those having about 22% proteins contained less aflatoxins except TMV 10 which also had less total phenols. Sugar and oil content did not appear to have any effect on the aflatoxin levels.


The present investigation is the first definitive study of the distribution of aflatoxins in a wet-milling process of raw groundnuts. The results show that the majority of aflatoxins originally present in the groundnuts remained in the solid fractions, particularly the protein fractions, during wet-milling. In the protein concentrate preparation, the concentrates carried 81-89% of the total aflatoxin; crude oil, 5-8%; and whey fraction, 3-14%. In the case of protein isolate preparation, 51-56% of the total toxin remained with the isolates, 22-26% with the residue, 11-17% with the whey, and 7-8% with the crude oil. Distribution of aflatoxins in the preparation of protein isolates from defatted groundnut meal showed that 55-65% of the total toxin originally present in the meal remained with the protein isolates, 20-28% with the residue, and 10-20% with the whey fraction. Changes in extraction pHs for the preparation of protein isolates either from raw groundnuts or defatted meal did not alter the distribution pattern. A new approach based upon the charge-transfer (electron acceptor-donor) complex formation is suggested to shift this aflatoxin distribution from protein products to disposable whey or residue fraction from the processing of raw groundnuts and defatted meal for protein products.
A study of major foods in Nigeria showed that groundnuts and dried fish were highly contaminated with aflatoxins (900 µg kg⁻¹ in groundnuts; 600-700 µg kg⁻¹ in dried fish) whereas various grains, millets, guinea corn and rice were moderately contaminated (300-150 µg kg⁻¹). Low risk foods such as yams became contaminated with aflatoxin after storage, particularly when wrapped in cellophane or even in newspaper. Immersion of foods in a dilute (1 %) solution of NaCl 16.5 % and sodium hypochlorite 1 % (Milton) reduced the level of aflatoxin by almost 50 %. Exposure to direct sunlight caused a marked decline (30-16 %) in aflatoxin levels of foods.


A survey was carried out to determine the extent of aflatoxin contamination of groundnuts in Maharashtra. Aflatoxin contamination was detected in 5 of 26 samples of groundnuts collected from six markets. Species of Aspergillus, Rhizopus and Penicillium were predominant in the seed samples.


A study in Swaziland to assess the possible relationship of aflatoxin contamination and the incidence of primary liver cancer is reported. Aflatoxin ingestion levels were determined in food from plate samples collected over a one year period. A significant correlation between the calculated ingested daily dose and the adult male incidence of primary liver cancer in different parts of Swaziland was established. Samples of foodstuffs other than plate samples also reflected the correlation of aflatoxin contamination and liver cancer. This study extends and amplifies the findings of an earlier study in the Murang's district in Kenya and supports the hypothesis that aflatoxin ingestion is a factor in the genesis of primary liver cancer in Africa.


Levels of aflatoxin in Spanish groundnut kernel samples from different geographical areas in Texas during 1966 were low. Of the 334 samples tested, 239 (71.6 %) contained no aflatoxin and only 2.7 % contained > 30 µg kg⁻¹ and 25.8 % contained trace-29 µg kg⁻¹. Analysis of samples obtained from growers using artificial drying equipment (forced air and supplemental heat), when windrow conditions were unfavorable for rapid drying, suggests that this practice reduces the possibility of aflatoxin accumulation. In general, groundnuts harvested from land planted to groundnuts the previous year were more highly infected by fungi and contained more aflatoxin than groundnuts grown on land planted to rye, oats, melons, or potatoes the previous year. Aflatoxin incidence tended to decrease from south to north Texas. Detection of aflatoxin in sound mature kernels (kernels screened for minimal size) indicates that the practice of screening for removal of small immature kernels and removal of obviously damaged kernels does not completely eliminate aflatoxin contamination.


Aflatoxins have been a serious problem in Australian groundnuts in the past decade. With the aid of government and industrial funding, the Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Food Research has carried out an ongoing project for most of this period, with emphasis on studies under commercial field conditions. Research has been primarily concerned with understanding the variables that influence the invasion of groundnuts by Aspergillus flavus and A. parasiticus, and the subsequent production of aflatoxins. Factors studied include : levels of A. flavus in soils, environmental factors, farm management practices affecting A. flavus invasion, and the influence of drying and storage procedures on aflatoxin development. Most groundnut soils in Kingaroy contain detectable levels of A. flavus, while surrounding virgin soils usually do not. Levels of A. flavus in groundnut soils vary widely, from less than 10² spores g⁻¹ to as high as 10⁵ spores g⁻¹; high levels are much more likely to lead to invasion. Some fields contained consistently high levels over several years. The A. flavus/A. parasiticus ratio also varies widely from farm to farm, and may influence invasion and toxin production. Investigations have shown that invasion of groundnuts by A. flavus takes place before groundnuts are harvested. Invasion will not occur subsequently, aflatoxin is not produced, even under the least effective drying procedures. In all but exceptionally dry seasons little aflatoxin is produced while groundnuts are in the ground, i.e., most aflatoxin is produced postharvest. Under the most favorable conditions, groundnuts require 6 to 10 days to dry in the field after harvest, a period sufficiently long for aflatoxin to reach unacceptable levels. Field drying cannot be sufficiently rapid, even in dry seasons, to ensure aflatoxin-free nuts at intake to shellers. The perceived importance of preharvest invasion as the
necessary condition for the production of unacceptable aflatoxin levels has led to attempts to predict aflatoxin levels at shelling intake from *A. flavus* levels at harvest. Success rates have been encouraging but arc not yet of practical utility.


Groundnut storage problems were studied in Cote d'Ivoire during two successive storage periods (1985/86 and 1986/87) and from three different aspects: insect pests; mold damage; and contamination with aflatoxins. Samples were taken periodically from traditional producers' fields throughout the groundnut growing areas of the survey and from town and village markets. Generally, locally stored samples were a little less infected than samples from markets. With few exceptions, all the locally sampled material was contaminated with measurable levels of aflatoxin. Over the 2-year survey period, 7.9 % of the 434 local stocks examined exceeded the toxicity level threshold of 250 µg kg
-1
, with 4.4 % above 1000 µg kg
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. It was also found that 73 % of these samples were above the European Economic Community (EEC) safety level of 10 µg kg
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. Significant correlations were found between aflatoxin contamination and different storage and meteorological variables. These included physical characteristics and age of the pods, and the influence of the prevailing atmospheric conditions.


Aflatoxin B1 was determined in samples of groundnuts and their products, wheat, corn and manioc flour, collected in commercial establishments in Belo Horizonte in 1983, using a thin-layer chromatography (TLC) method. Aflatoxin B1 was detected in 44 % of the samples of groundnut and their products; aflatoxin levels were above the tolerance level permitted by Brazilian legislation (30 µg kg
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 aflatoxins B1+G1).


The results of surveys in the Philippines have shown that farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 µg kg
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 aflatoxin. During windrowing, aflatoxin levels increased at the rate of 15 µg kg
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 day
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. In farm storage aflatoxin contamination continued to increase at the rate of 1 µg kg
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 day
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. Aflatoxin contamination was significantly higher during the main cropping season than the second cropping season. At me traders' level, groundnut samples taken from various middlemen contained 35 µg kg
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 aflatoxin. On the other hand, samples taken from the wholesalers' newly procured groundnuts contained 188 µg kg
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 aflatoxin. Groundnuts that had been in wholesalers' warehouses for more than 3 months contained 275 µg kg
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 aflatoxin. At the processors' level, raw groundnuts for confectionery use (roasted and fried) contained 7.73 µg aflatoxin kg
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, groundnuts intended for peanut butter contained 17.13 µg kg
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, and rejected groundnuts had 120.6 µg kg
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. Aflatoxin contamination could start during harvest. Aflatoxin content increased to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.


Groundnut samples from various parts of Pakistan were analyzed for aflatoxin content, and no fresh samples contained the toxin. However, 6-15 % of the roasted groundnuts from areas other than Khuzdar were contaminated. The aflatoxin content of the contaminated samples varied from 24 to 800 µg kg
-1
. All the tested samples of roasted groundnuts from Khuzdar were contaminated with aflatoxins.


Of 288 samples of groundnuts from six coastal districts of Andhra Pradesh State, India, it was possible to isolate strains of *Aspergillus flavus* from 29. Of the 29 strains 6 produced aflatoxin B1 when cultured, as estimated by thin-layer chromatography (TLC) and in tests with ducklings. Aflatoxin contamination was found in 36 samples. Toxic samples were more prevalent in some districts than others. Samples commonly included 6 to 14 % of pods with shell damage, but this damage was not related to toxicity.

Analyses of groundnut samples collected in the surveys of rainy season (313 samples) and dry season (83 samples) crops in the State of Sao Paulo showed that on an average 48-74 % of the samples collected from the west and northeast regions contained 5-22500 µg aflatoxin B1 kg⁻¹. This reconfirmed the trend and level of occurrence of aflatoxins in groundnut in Brazil and showed that a mycotoxic problem exists. Suggestions and recommendations were made to the relevant authorities as a result of the survey.


A survey was carried out to obtain data on the occurrence of aflatoxins and aflatoxigenic mold contamination of foods in Spain. 338 samples of cereal grains, mixed feeds, edible nuts, wheat flour for bread-making, biscuits, sliced bread, soybeans and breakfast cereals were analyzed. Aflatoxins were detected in 4 of 27 samples of mixed feeds at levels below 5 µg kg⁻¹; one sample of groundnuts was contaminated with 120 µg aflatoxin B1 kg⁻¹ and 22 µg aflatoxin B2 kg⁻¹. Aflatoxins B1 and B2 were also detected in a lot of whole maize flour, averaging 8 µg kg⁻¹ and 3 µg kg⁻¹ respectively. All of 288 samples tested showed fungal contamination to varying extent. Corn samples were most frequently contaminated with Aspergillus flavus (54.5 %). Isolates of A. flavus from corn samples also showed a higher proportion of aflatoxigenic strains (17.2 %) as compared with those from other sources.


The degree of aflatoxin contamination in groundnut hulls (shells) used in cattle feed was determined by analyzing inoculated hand-shelled hulls and hulls from groundnuts known to contain aflatoxin. Hulls adjusted to 20 % moisture, inoculated with Aspergillus flavus and incubated for 7 days at 25°C supported growth of the fungus but not aflaioxin production. Groundnuts from 20 selected lots with visible A. flavus contamination contained 13-353 µg kg⁻¹ aflatoxin. The machine-shelled hulls from these lots were analyzed and 3 lots contained no detectable aflatoxin, 13 lots contained 4-88 µg kg⁻¹ and 4 lots contained 116 µg kg⁻¹. Aflatoxin concentrations of 53-87 µg kg⁻¹ were detected in hulls when groundnuts containing relatively high levels of aflatoxin (up to 26800 µg kg⁻¹ in damaged kernels) were carefully machine-shelled. Hulls from the same samples obtained by hand-shelling contained no detectable aflatoxin. Separating hulls over a 4.76 mm screen appeared to provide a means of removal of most aflatoxin-containing particles in groundnut hulls.


Raw agricultural crop produce and their by-products collected from different areas of the Philippines contained varying amounts of aflatoxin and degrees of Aspergillus flavus infection. Some of the test samples showed no visible signs of A. flavus, but contained high levels of aflatoxin. Conversely, other samples yielded the fungus in agar plates but contained little or no aflatoxin. Corn grown in the Visayas and Mindanao had more aflatoxin than those grown in Northern Luzon. Dried groundnuts from the Visayas and Mindanao generally contained low levels of aflatoxin. Sorted out groundnuts with wrinkled kernels unfit for commercial processing, however, had as much as 144 µg kg⁻¹ aflatoxin. The processes in copra-making like drying and storage predispose the coconut meat to infection by A. flavus and other micro-organisms. However, a finished product, copra pellet, contained no aflatoxin after treatment with hexane.


The occurrence of aflatoxins and aflatoxigenic fungi in staple Ugandan food crops and poultry feed derived from these foodstuffs was investigated. Fifty-four samples of maize, groundnuts, soybean and poultry feed were analyzed for aflatoxigenic fungi, Aspergillus flavus and A. parasiticus, and 25 of the samples were also screened for aflatoxins B₁ and G₁, zearalenone, sterigmatocystin, ochratoxin A, citrinin, vomitoxin and diacetoxyscirpenol. Aflatoxigenic A. flavus/A. parasiticus was detected from the majority of maize (77 %), groundnuts (36 % human food and 83.3 % animal feed) and poultry feed (66.6 %), but not from soybean samples. Two of the 25 samples (maize and poultry feed) contained detectable levels of aflatoxin B₁ (20 µg kg⁻¹). Zearalenone (3 samples) and vomitoxin (2) were detected in maize.


Distribution and degree of occurrence of aflatoxins in various samples of groundnuts and groundnut products in the Republic of South Africa are described. Over 1000 samples of groundnuts and groundnut products were examined during 1963 and another 1000 samples of producers' grades were investigated during 1964. The samples obtained from the North-Western Transvaal, or so-called Bushveld areas, were most seriously contaminated with aflatoxin; all of the 75 samples containing > 2000 µg kg⁻¹ aflatoxin were from the receiving depots in the North-Western
Transvaal. The samples from the Northern Cape Province, Orange Free State and Natal, except for 5 samples with 500-2000 µg kg\(^{-1}\) and 11 samples with 100-500 µg kg\(^{-1}\), were free from aflatoxin. Similar results were found in the 1964 survey for aflatoxin contamination; however, only 23 samples from the North-Western Transvaal area contained > 2000 µg kg\(^{-1}\). High degree of aflatoxin contamination in the groundnut samples from the North-Western Transvaal was ascribed mainly to (1) the damage caused to the maturing groundnut pods and kernels by termites, and (2) protracted droughts followed by late rains. Lower or inferior grade groundnuts contained higher levels of aflatoxins than the higher or superior ones. No aflatoxins were detected in any of the samples of hand-picked selected (HPS) kernels examined in 1963 and 1964. Groundnut cake samples implicated in livestock deaths were also examined for aflatoxin contamination. Eleven of the 16 samples of this groundnut cake contained > 2000 µg kg\(^{-1}\) aflatoxin. Of 101 peanut butter samples tested, 17 were contaminated with aflatoxin. Of these 17 samples, 12 had an aflatoxin contents of 100-500 µg kg\(^{-1}\) and 5 had up to 25 µg kg\(^{-1}\).


Two of 97 food samples (roasted groundnuts and maize) contained aflatoxin at 800 and 500 µg kg\(^{-1}\), respectively. Aflatoxin was also detected in 13 of 74 feed samples (5 poultry feed samples at 5-1000 µg kg\(^{-1}\), 1 sample of cottonseed meal at 8 µg kg\(^{-1}\) and 7 samples of maize gluten at 450-1140 µg kg\(^{-1}\)).


Cereals, pulses, nuts and other food and feed ingredients from different regions of Pakistan were screened for aflatoxin contamination. Samples of wheat, rice, pulses and beans showed no aflatoxin contamination although toxigenic strains of *Aspergillus flavus* were present in some samples. Aflatoxins B\(_1\) and B\(_2\) were present in maize and maize products at up to 800 µg kg\(^{-1}\) and in groundnuts, pistachio nuts and walnuts in the range 400-800 µg kg\(^{-1}\). Poultry feed and its ingredients contained 8-1140 µg kg\(^{-1}\) aflatoxin.


Of the 125 samples of foodgrains and oilseeds analyzed, aflatoxin B\(_1\) was found in 23.2% of samples. Among the 15 samples of each foodgrain analyzed, aflatoxin B\(_1\) was found in 2 samples of rice (20 and 50 µg kg\(^{-1}\)), 6 samples of sorghum (22.8-550 µg kg\(^{-1}\)), 10 samples of raw groundnuts (33-440 µg kg\(^{-1}\)), 5 samples of roasted groundnuts (10-85 µg kg\(^{-1}\)), and in 6 samples of maize (15-680 µg kg\(^{-1}\)).


Aflatoxins were analyzed in groundnuts stored for either local or export sales in the period 1982/83 to 1986/87. Of the 441 samples of seven groundnut varieties analyzed, 68% had total concentrations of aflatoxins B\(_1\) and G\(_1\) of up to 25 µg kg\(^{-1}\). In me 1986/87 groundnut crop, the Flamingo and Makulu Red varieties, which constitute the bulk of export sales, had up to 25 µg kg\(^{-1}\) aflatoxin B\(_1\) in 79% of the samples, plus aflatoxin G\(_1\) in 67% of the samples. Overall, the Egret variety was the most susceptible to aflatoxin contamination during this period.


Groundnut kernels collected from north-eastern plantations in Kalasin Province, Thailand, in August 1987 were roasted, split open and the seed coat, cotyledons and embryos were analyzed for aflatoxins by a Velasco aflatoxin meter. Results indicated accumulation of aflatoxin in embryos (274 µg kg\(^{-1}\)) and ground cotyledons (275 µg kg\(^{-1}\)). Of various food preservatives studied, sodium bisulfite was the best detoxification agent in ground groundnuts, with effective dose as low as 100 mg kg\(^{-1}\).


Numerous samples of foods, animal tissues, faeces, Lepidoptera, and edible and poisonous fungi were analysed for aflatoxins by radioimmunoassay. Among the foods, high aflatoxin concentrations were recorded in: groundnuts (> 40 µg kg\(^{-1}\)), and mold-contaminated grapefruit juice (33.1 µg kg\(^{-1}\)). The other samples (cheese, currant juice, coffee, sweets, bread, maize, oats, wheat, barley) had low aflatoxin concentration (< 10 µg kg\(^{-1}\)). Aflatoxin concentrations in the fungi ranged from 0 to 7.3 µg kg\(^{-1}\).


Causes of the high aflatoxin levels in foods, in particular in groundnuts and
groundnut oil, are discussed. A survey of 22 market samples of unrefined groundnut oil showed aflatoxin levels ranging from 0 to 2600 µg kg\(^{-1}\). With the increase in export of handpicked seeds, the quantity of aflatoxin-contaminated seeds getting crushed for edible oil seems to have increased considerably in recent years.


Groundnut kernels, barley, rice, peanut butter, soybeans, soybean oil, sunflower seed oil, wheal Hour, soya flour, wholemeal flour, pearl barley and whole millet samples were analyzed for aflatoxin. Aflatoxin B\(_1\) was detected only in two groundnut samples (at 5 and 6 µg kg\(^{-1}\)).


The extraction and cleanup steps of the AOAC minicolumn technique were modified to provide an inexpensive, rapid screening procedure for aflatoxins in groundnuts and groundnut products. A total of 52 samples of groundnuts and groundnut products were collected from shops and markets in Campinas, Brazil, and were analysed for aflatoxins by the old and the modified methods. The results by both methods were the same: 28 samples were negative, four contained <20 µg kg\(^{-1}\) aflatoxin, 12 contained 20-50 µg kg\(^{-1}\), three had 50-100 µg kg\(^{-1}\) and five had >100 µg kg\(^{-1}\). The detection limit in both minicolumn methods was 10 µg kg\(^{-1}\).


Seventy-eight samples of farmers' stock groundnuts, representing groundnuts grown in nine different geographical areas in Texas during 1964, were assayed for aflatoxin and examined for associated microflora. Only two samples contained more than 50 µg kg\(^{-1}\) aflatoxin. Infestation by members of the Aspergillus flavus-oryzae group varied from 35 to 100 % of the kernels per area and from 1 to 100 % of the kernels per sample. 213 isolates of A. flavus were examined for their abilities for aflatoxin production on groundnut and rice substrates. Aflatoxin production by individual isolates ranged from 0 to 349.1 µg kg\(^{-1}\) on a groundnut substrate. In general, the isolates produced 8 to 10 times more aflatoxin B\(_1\) than aflatoxin B\(_2\), and no isolate producing aflatoxins G\(_1\) or G\(_2\) was found.


Aflatoxin B\(_1\) (at 10-1120 µg kg\(^{-1}\)) was detected in 22 of 29 samples of groundnut from commercial sources. Aspergillus flavus was isolated from 11 of the aflatoxin-contaminated samples.


The examination of foods for mycotoxins in the German Democratic Republic is discussed, with particular reference to aflatoxins in groundnuts, and resulting aflatoxins M\(_1\) and M\(_2\) in milk from cows fed contaminated groundnut meal. Sampling procedures for imported groundnuts are described and the official methods for screening for aflatoxin content and quantitative determination of aflatoxins in groundnuts are summarized. Contaminated groundnuts are used for producing groundnut oil. 23% of groundnut samples analyzed contained aflatoxins (mainly B\(_1\) and G\(_1\)). It is recommended that sampling be extended to cover other foods and other mycotoxins, and that animal feed should be sampled as a preventive measure against contamination of milk and meat.


Laboratory studies of soybeans, groundnuts, and their mixtures with various legumes (lentils, dried garden peas, Bengal gram (chick pea), green peas, red peas and broad beans showed that Aspergillus flavus and aflatoxins B\(_1\) and G\(_1\) were present in soybeans and groundnuts, but not in the other legumes. Growth of A. flavus and aflatoxin formation was inhibited in mixtures of other legumes with soybeans, but promoted in mixtures of groundnuts with soybeans, suggesting that soybeans should be included in the national food monitoring programme.


A highly sensitive normal-phase HPLC method was developed to study the occurrence of aflatoxins in Soviet imported and domestic foodstuffs. The detection limit was 0.1 µg kg\(^{-1}\) and the coefficients of variation were 11 % and 8.5 % at contamination levels of 10 and 100 µg kg\(^{-1}\) aflatoxin B\(_1\), respectively. A survey of the occurrence of aflatoxins B\(_1\), B\(_2\), G\(_1\) and G\(_2\) in domestic and imported cereals and nuts (totalling 4532 samples) collected during 1985-87, showed that 26.9 % of imported groundnuts, 2.2 % of maize and 28.3 % of cottonseed were contaminated by aflatoxins at levels exceeding the maximum tolerance level established in the USSR (5 µg kg\(^{-1}\) for aflatoxin B\(_1\) in foodstuffs of all types excluding baby foods), maximum concentrations were 3650, 600 and 153 µg kg\(^{-1}\), respectively.
An improved normal phase HPLC method was developed to study the occurrence of aflatoxins B₁, B₂, G₁, G₂ and M₁ in domestic and imported foods. Ether-methanol-water (95:4:1) mobile phase and fluorometric detector with silica gel packed flow cell were used. The detection limit of the method was 0.1 µg kg⁻¹ for aflatoxin B₁, coefficients of variation were 11 and 8.5 % at contamination levels 10 and 10 µg kg⁻¹ of aflatoxin B₁, respectively. Recoveries of added aflatoxins B₁, B₂, G₁, and G₂ for maize ranged from 78 to 88 %. This method allowed the determination of aflatoxins B₁, B₂, G₁, G₂ and M₁, B₃a, M₂, as well as other aflatoxin metabolites. The method was used in monitoring aflatoxin contamination of foods, the first stage of which is a preliminary screening of samples by TLC (the detection limit is 1 µg kg⁻¹ for aflatoxin B₁). A survey of the occurrence of aflatoxins B₁, B₂, G₁, G₂ in Soviet domestic and imported cereals, nuts, beans and oilseeds harvested in 1985-87 (> 4300 samples) as well as aflatoxin M₁ in domestic dairy products (> 250 samples) was carried out using HPLC and TLC methods. It was shown that 26.9 % of imported groundnuts, 2.8 % of maize and 6.2 % of barley were contaminated with aflatoxins at levels exceeding the maximum tolerated level established in the USSR (5 µg kg⁻¹ for aflatoxin B₁ in foods of all kinds excluding baby foods). Maximum concentrations were 3600, 155 and 8 µg kg⁻¹, respectively. As much as 28.3 % of domestic cottonseed samples, which were chosen for analysis due to toxic effects on animals, were also contaminated with aflatoxins.


In Mozambique, methods of food production, harvesting, storage and preparation were studied to detect points of aflatoxin contamination. Groundnuts, the main dietary source of protein, provided the most aflatoxin contamination, traditional agricultural methods promoting fungal infection and growth. Aflatoxin production also occurred in the main carbohydrate sources, cassava and maize, during storage. Western-type foods had particularly low aflatoxin contents. A simple educational program could reduce the incidence of primary liver cancer associated with aflatoxin poisoning; westernisation of some living habits could also reduce the incidence of the disease.


5.2.3 Groundnut oil


Studies by the Nigerian Stored Products Research Institute showed that while refined groundnut oil had negligible aflatoxin content, crude groundnut oils had traces of aflatoxin present.

Aflatoxin B1 was detected in the urine of 7% of 255 cases of childhood cirrhosis during 1964-1969 and in 25% of breast milk samples from their mothers. The excretion pattern coincided with that of primates injected with C14-labelled anatoxin. Of 25 samples of parboiled rice collected from the homes of patients with cirrhosis, 2 showed both fungal growth and an aflatoxin B1 fluorescent spot on chromatographic examination, and all 25 samples of unrefined-groundnut oil showed a similar fluorescent spot. Aflatoxin administered to rats caused muscle fragmentation changes, fibrosis and fatty infiltration characteristic of 56 cases of childhood cirrhosis. Growth retardation was noticed both in 24 cirrhotic children and in the livers of lactating rats fed with aflatoxin.


Sixteen samples of groundnut oil (9 refined and 7 unrefined) purchased from local markets were analyzed for aflatoxins. Five samples of unrefined oil contained 8-16 µg kg\(^{-1}\) aflatoxin B1. Aflatoxin G1 was also present in approximately the same concentration range.


During 1973-1977, 1689 samples of groundnut kernels and 1172 samples of groundnut oil from 24 provinces of the People's Republic of China were analyzed for contamination with aflatoxin B1. The percentages of samples containing the toxin were 26.3 for kernels and 47.3 for oil. Across the country as a whole the percentage of contaminated samples decreased with increase in latitude, being high in southern China, moderate in the Yangtze valley, and low in northern China. The key to prevention of aflatoxin contamination was rapid drying of groundnuts to below 10% moisture content. Screening of groundnut germplasm for resistance to Aspergillus flavus is in progress.


Sprague-Dawley rats for 22 months from weaning, its estimated aflatoxin B1 content was 5 to 7 µg kg\(^{-1}\). Controls were given a diet of identical composition except that Manzola maize oil (aflatoxin-free) was used. Of 76 rats given aflatoxin, 3 had sarcomat, one in the liver, one in the wall of the colon and one in the subcutaneous tissue of the groin; 18 rats given groundnut oil showed parenchymal liver damage and different degrees of fatty change and one showed pre-malignant changes in liver cells. Of 90 control rats, none developed malignant tumours. The liver-to-body weight ratios for experimental and control rats were 2.93 and 2.62, respectively. The difference between those values was significant, reflecting the degree of fatty change in the livers of experimental rats compared with that in the controls. Over 90% of Hong Kong households use groundnut oils for cooking purposes, and these results indicate a possible health hazard in the use of contaminated groundnut oil.


Groundnut oils obtained from Hong Kong markets were frequently contaminated with aflatoxins. A purified diet in which aflatoxin-contaminated market groundnut oil (aflatoxin B1 110 µg kg\(^{-1}\)) was used as the fat source was given to Sprague-Dawley rats for 22 months from weaning, its estimated aflatoxin B1 content was 5 to 7 µg kg\(^{-1}\). Controls were given a diet of identical composition except that Manzola maize oil (aflatoxin-free) was used. Of 76 rats given aflatoxin, 3 had sarcoma, one in the liver, one in the wall of the colon and one in the subcutaneous tissue of the groin; 18 rats given groundnut oil showed parenchymal liver damage and different degrees of fatty change and one showed pre-malignant changes in liver cells. Of 90 control rats, none developed malignant tumours. The liver-to-body weight ratios for experimental and control rats were 2.93 and 2.62, respectively. The difference between those values was significant, reflecting the degree of fatty change in the livers of experimental rats compared with that in the controls. Over 90% of Hong Kong households use groundnut oils for cooking purposes, and these results indicate a possible health hazard in the use of contaminated groundnut oil.


Four samples of groundnut oils obtained from local markets in Hong Kong were tested for mutagenicity using the Salmonella/microsomal test system. In the presence of microsomes, dimethylsulphoxide (DMSO) extracts of the samples were mutagenic to Salmonella typhimurium strains TA 98 and TA 100, but the mutagenic activity of the oils decreased after they had been repeatedly cooked. However, when the
The extent of aflatoxin contamination in groundnut oil, groundnut cake and hydrogenated oil in the market of Hapur, Uttar Pradesh, India, was investigated. Twenty-seven samples of groundnut oil, 15 were contaminated with aflatoxin B₁ at 2-270 µg kg⁻¹. Of the 69 groundnut oils, 15 were contaminated with aflatoxin B₁ at 3-175 µg kg⁻¹, of which seven also contained aflatoxin B₂. Of the 16 other oils, only one, a coconut oil, contained 3 µg kg⁻¹ aflatoxin B₁. Detection of aflatoxin B₁ in artificially contaminated maize oil was only apparent at 250°C and above. Using naturally contaminated groundnut oil (175 µg kg⁻¹ aflatoxin) the results were essentially the same and confirmed that destruction of aflatoxin B₁ is likely to occur at normal frying temperature if cooking is done indoors.

### References


The present investigation is the first definitive study of the fate of aflatoxins in vegetable oils undergoing processing. Crude oils, obtained by solvent extraction or by hydraulic pressing of ground moldy groundnuts (not suitable for human consumption), contained only small fractions of the aflatoxin originally present in the groundnuts; the meals retained the bulk of the aflatoxin. Conventional alkali refining and washing of the oils reduced aflatoxin content to a range of 10 to 14 µg kg⁻¹. The subsequent bleaching operations essentially eliminated aflatoxin from the oils; the concentrations after this process being less than 1 µg kg⁻¹. The previous results were confirmed using corn oils obtained from corn germ deliberately contaminated in the laboratory with Aspergillus flavus. The nonfluorescing forms of aflatoxins, capable of being produced during the alkali refining operations, are also absent from the refined vegetable oils; these aflatoxin derivatives are readily converted to their original form on acidification and are thereby measurable by fluorescence, if present.


Sixty-nine groundnut oils and 16 other cooking oils on sale in Indian villages were analyzed for aflatoxin contamination. Groundnut and groundnut meal samples (24) were also analyzed and three were positive for aflatoxin B₁ at 2-270 µg kg⁻¹. Of the 69 groundnut oils, 15 were contaminated with aflatoxin B₁ at 3-175 µg kg⁻¹, of which seven also contained aflatoxin B₂. Of the 16 other oils, only one, a coconut oil, contained 3 µg kg⁻¹ aflatoxin B₁. Destruction of aflatoxin B₁ in artificially contaminated maize oil was only apparent at 250°C and above. Using naturally contaminated groundnut oil (175 µg kg⁻¹ aflatoxin) the results were essentially the same and confirmed that destruction of aflatoxin B₁ is likely to occur at normal frying temperature if cooking is done indoors.


Causes of the high aflatoxin levels in foods, in particular in groundnuts and groundnut oil, are discussed. A survey of 22 market samples of unrefined groundnut oil showed aflatoxin levels ranging from 0 to 2600 µg kg⁻¹. With the increase in export of handpicked seeds, the quantity of infected seeds crushed for edible oil seems to have increased considerably in recent years.
5.2.4 Groundnut cake


Groundnut cake (Kulikuli) purchased from four major markets in Ibadan, Oyo State, Nigeria, during April-November 1988 were analyzed for aflatoxin B₁ and associated mycoflora. In all but 2 of the samples aflatoxin B₁ levels were between 20 and 455 µg kg⁻¹. Mold counts were low (1.0 - 4.41 x 100 colonies g⁻¹). Eight mold species were isolated. Of these, Aspergillus niger, Paecilomyces variotii, A. flavus and Fusarium moniliforme dominated. It is concluded that groundnut cake on sale in Ibadan markets is unacceptable for animal feed rations and human consumption and there is a need for some form of quality control and decontamination before usage.


Aflatoxin B₁ was detected in 66 % of 101 samples of feeds and feed ingredients at up to 2670 µg kg⁻¹. Of the 13 feed ingredients analyzed, only groundnut oilcake contained aflatoxin B₁ (330-2670 µg kg⁻¹).


The presence of aflatoxin B₁ was examined in 67 samples of feeds (18 kinds), 8 of protein concentrates (3 kinds), 22 of cattle feed mixtures (11 kinds), 16 of pig feed mixtures (8 kinds), 10 of poultry feed mixtures (8 kinds), 4 of sheep feed mixtures (2 kinds), 2 of feed mixtures for game and herbivorous animals in zoological gardens (2 kinds), 15 of fish feed mixtures (4 kinds), 2 of biofactor supplements (2 kinds) and 7 of non-traditional feeds containing varying amounts of pig excrement. Four kinds of currently-used foodstuffs were also tested (semi-fine flour, hulled rice, groundnuts, walnuts). Aflatoxin B₁ was detected in 26 samples of groundnut cake imported from India, 2 of laying hen protein concentrate, 4 of breeding pig concentrate, 2 of supplement feed mixture for lamb fattening, 1 of meat-bone meal, 1 of supplement feed mixture for lactating sows, 2 of protein supplement feed mixture for dairy cows, 1 of concentrate for cattle fattening mixture, 1 of protein concentrate for mixture with fat for broiler fattening, 1 of rape groats, 1 of cocoa shells and 1 of glycine supplement feed mixture for cattle fattening.


Aflatoxins exceeding the maximum permitted limits were detected in groundnut, cottonseed, cocos and palm kernel cakes. Soya products, however, were practically free of aflatoxin. Nearly all the tested samples of mixed feeds for ruminants, which contained a large proportion of oil-seed cakes, contained aflatoxin.


During 1986 and 1987, mold infestation and aflatoxin B₁ occurrence were examined in 52 samples of Vietnamese feeds. Aspergillus, Rhizopus and Penicillium spp. were the most prevalent fungi in the feeds, with Aspergillus spp. predominating. Aspergillus flavus was isolated from 78 % of samples. The range of aflatoxin B₁ in the feeds was between 2 and 640 µg kg⁻¹ in 1986 and between 5 and 41 µg kg⁻¹ in 1987. The highest incidence of aflatoxin B₁ in the 1986 feed samples was found in maize, groundnut oil cake and feed mixtures for chicks. In 1987, the levels of aflatoxin B₁ in the feeds were lower, but the frequency of positive samples was higher than in 1986. However, in 1987, only 14 samples were analyzed for aflatoxin in comparison with 38 samples in 1986.


Aflatoxin B₁ was detected in 23 of 55 feedstuffs known to be either water damaged or visibly moldy. The highest level of aflatoxin was 700 µg kg⁻¹ of feed and the mean concentration was 140 µg kg⁻¹. Of 36 feedstuffs purchased from local manufacturers, only groundnut meal contained aflatoxin B₁ (500 µg kg⁻¹). Eleven feedstuffs were associated with field outbreaks of animal disease and seven of these contained aflatoxin, but not in sufficient quantity to account for the described disease symptoms.


Suspected samples of Polish grain and imported seed meals obtained from 1970-1976 were analyzed for aflatoxin. Of 21 samples of wheat 1 contained aflatoxin B₁ (8.4 µg kg⁻¹). Of 24 samples of barley 1 contained 3 µg kg⁻¹ aflatoxin B₁. All 14 samples of groundnut meal had aflatoxin B₁ (about 1000 µg kg⁻¹). Ochratoxin was detected in 2 of 11 samples of rye and in 1 of 5 samples of wheat.
with levels 25 and 5 µg kg\(^{-1}\), respectively. Of 5 samples of sorghum from Argentine 2 had ochratoxin (25 µg kg\(^{-1}\)).


Of 150 samples of groundnut cake awaiting shipment in Bombay, 11 harboured Aspergillus flavus and 20 A. flavus var. oryzae, A. fumigatus, A. niger, Penicillium sp., Rhizopus sp. and Mucor sp. Of stored groundnuts, 5 % of the undamaged white and 57 % of the damaged and discolored ones were contaminated; none of the white nuts yielded A. flavus, compared with 26 % of discolored nuts. Thin-layer chromatography of extracts of isolates and of cake showed that only two strains of A. flavus were fluorescent and hence toxic. Groundnut cake medium was suitable for the growth of A. flavus and production of aflatoxin.


Cyanotic combs, loss of appetite and listlessness, with a fall in egg production from 80 to 20 % and 100 % mortality, occurred in poultry farms in and around Chittoor (Andhra Pradesh), India. Post-mortem examination revealed hydropericardium, ascites and large, friable haemorrhagic livers, some of which had ruptured. Aflatoxins (1400-3600 µg kg\(^{-1}\)) were found in samples of maize and groundnut cake fed to the birds during the outbreak. The crops used for the feed had been harvested in heavy rain.


Aflatoxins were detected in 67 feed samples. 45 of which were groundnut meals and by-products. Aflatoxins were produced by 49 % of 200 isolates of Aspergillus flavus cultured on maize meal. Ochratoxin A was detected in 1 of 25 feed samples analyzed. Ochratoxin A was produced by 28 % of 47 A. ochraceus isolates and zearalenone by 2 Fusarium equiseti isolates.


Analysis of 93 samples of extracted oilseed residues showed mean aflatoxin concentration (max. in parentheses) of 33 µg kg\(^{-1}\) (80) in coconut, 15 (30) in palm kernel, 63 (1 sample only) in Babassu, 26 (75) in cottonseed, < 5 (5) in sunflower, < 5 (<5) in linseed and 5 (20) in maize germ; no detectable aflatoxin was found in sesame, rapeseed and wheat germ residues. These results contrast with a mean 530 MB kg\(^{-1}\) in groundnuts. Inferences with the determination by fluorescent compounds with similar R\(_f\) values on TLC as listed by Gedek & Kablau, only rarely cause errors during routine analysis due to improvements in the method.


Of 104 feed samples examined chromatographically, 14 contained aflatoxins, the highest level (1920 µg kg\(^{-1}\)) being in a sample of groundnut from India. Aflatoxin was also found in samples of feed mixtures for pig fattening, pregnant and lactating sows and boars, levels ranging from 50-350 µg kg\(^{-1}\) of feed. Almost all samples of organoleptically altered feed, taken from metal containers outside the animal houses, contained aflatoxin.


All 48 samples of groundnut flour from crops in two seasons contained 100-20000 µg kg\(^{-1}\) aflatoxin. the level being higher (average 4340 µg kg\(^{-1}\)) in the March-May in the rainy season than in July-September in the dry season (average 1830 µg kg\(^{-1}\)). Only 8.33 % of the material tested could be used as animal fodder. A weak, non-significant correlation was found between aflatoxins B and G.


All 116 samples of groundnut flour from crops in two seasons contained 100-20000 µg kg\(^{-1}\) aflatoxin, the level being higher (5500 µg kg\(^{-1}\)) in the rainy season than in the dry season (1760 µg kg\(^{-1}\)). Only 10.35 % of the material was fit for animal consumption. A weak, statistically significant correlation was found between the production of aflatoxins B and G.

All 44 samples of groundnut flour from crops in two seasons contained 100-10000 µg kg⁻¹ aflatoxin, the level being higher (average 3780 µg kg⁻¹) in the rainy season than in the dry season (average 1740 µg kg⁻¹). Only 4.54 % of the material tested could be used as animal fodder. There was practically no correlation between the production of aflatoxins B and G.


All 56 samples of groundnuts from 9 mills produced from crops in the rainy season (March-May) and the dry season (July-Sep) contained aflatoxin (100-20000 µg kg⁻¹), levels being higher in the rainy season (average 4550 µg kg⁻¹) than in the dry season (2400 µg kg⁻¹). Only 16.07 % of the material could be used for admixturing in feedstuffs. In six samples the level of aflatoxin G was higher than that of aflatoxin B. A statistically significant positive correlation was found between the production of aflatoxin B and G.


Of 86 samples of groundnut meal, imported into Norway during 1968 to 1973, 19.8 % had < 100, 41.8 % had 100 to 500, 26.8 % had 500 to 1000 and 11.6 % had > 1000 µg aflatoxins B₁ and B₂ kg⁻¹. There were marked differences between countries of origin but small variations between years.


Of 133 feed samples (groundnuts, cottonseed, kardi, salseed, soybean, sunflower, sheria, ambadi and rubberseed) analyzed. 42 % of samples contained aflatoxins and aflatoxin-like substances. Each class of feed, e.g. cakes, cereal grains, cereal by-products and similar samples together with compounded samples contained aflatoxins. Aflatoxin B₁ was present in practically all contaminated samples while aflatoxins B₂ and G₁ were observed in some of them; aflatoxin G₂ was absent from all samples except soybean cake.


Aflatoxin B₁ was detected at 80-2000 µg kg⁻¹ (mean 798 µg kg⁻¹) in imported groundnut meal and at 10-175 µg kg⁻¹ in 56 % of animal feed mixtures examined. Of 22 samples of milk from state-owned farms, 11 were contaminated with aflatoxin M₁ at 0.01-0.25 µg L⁻¹. Aflatoxins were not found in market milk samples, probably because of mixing samples from various purveyors.


The potential of Aspergillus flavus isolates to produce aflatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings of groundnut were studied. Isolates NRRL 3000 and V3734/10 produced high levels of aflatoxins in culture. Culture filtrates from the isolates and from NRCGAFA were most toxic to seeds and seedlings. Commercial cultivars. advanced breeding lines, and wild Arachis species were screened for resistance to in vitro colonization of seeds by A. flavus isolates, and to aflatoxin production. Genotypes CGC 2, 1-4, CGC 7. S230, derivatives of S230 x PI 337394F. Latur 33 x PI 337394F, and the wild species, Arachis cardenasii and A. duranensis were resistant to seed colonization by A. flavus. All genotypes of groundnut and three wild Arachis species supported high production of aflatoxins by NRRL 3000, but only trace levels were produced in A. cardenasii and A. duranensis. Aflatoxins were found (range of 27-146 µg kg⁻¹) in commercial groundnut cake and in de-oiled cake. Moisture intake capacity, levels of seed coat phenols, and protein content of seeds were considered to influence aflatoxin contamination levels. Soaking seeds in various organic and inorganic substances was found to influence the degree of seed colonization by A. flavus and of aflatoxin production in in vitro inoculation tests.

Work carried out during 1962-67 at the Nigerian Stored Products Research Institute on aflatoxin levels in groundnut cake is reported. A total of 895 samples of groundnut cake from oil mills in Kano were analysed. Most samples had aflatoxin contents of less than 50 or 50-250 µg kg⁻¹, and that only 2 samples had aflatoxin contents in excess of 1000 µg kg⁻¹. The number of samples containing 250-1000 µg kg⁻¹ aflatoxin was substantial in the 1963-64 and 1964-65 seasons, almost negligible in the 1965-66 season and nil in the 1966-67 season.


There is concern among farmers' leaders and consumers in the UK about high levels of aflatoxin in some imported groundnut oilmeal. The 1976 Fertilizer and Feedingstuffs Regulations specify maximum aflatoxin concentration in groundnut oilmeal sold for animal feed as 50 µg kg⁻¹, and in 'whole feedingstuffs' for dairy cattle and calves as 10 µg kg⁻¹. However, groundnut oilmeal sold as a feedstuff has been found with aflatoxin levels as high as 900 µg kg⁻¹. Farmers are recommended to ascertain whether compound feeds contain groundnut, and to get a certificate showing aflatoxin content of groundnut for inclusion in home-mixed feed. There are no UK recommendations for maximum aflatoxin in milk, but Milk Marketing Board officials are satisfied that current levels are safe. A feed with the statutory maximum aflatoxin B₁ content could result in about 100 µg kg⁻¹ of aflatoxin in milk, and an average concentration of 0.25 µg kg⁻¹ in cheese or dried milk. A supplementary article reports the experience of one dairy herd where moldy compound feed with aflatoxin content of 600 µg kg⁻¹ was associated with loss of condition and coordination, fall in milk yield and increased incidence of mastitis. Pound-sterling 6700 has been received by the fanners as compensation from the feed firm involved.


Problems arising from the contamination of groundnut meal with aflatoxin are reviewed. Progress in the detection of related compounds and knowledge of their toxic properties are summarized, with details of acute toxicity on monkeys, and indications of the chronic effects of very small doses on liver and kidneys of children. Transmission of the toxic constituents from animal feeds to cow's milk and pig liver and kidneys is also reported, although there are no apparent indications of progressive accumulation. Aflatoxin in imported groundnut meal is discussed and the limits imposed by the EEC on feeds of different kinds are tabulated. The practical implications of stringent limits on aflatoxin contents of meal imported into European and other countries are discussed, with particular reference to the danger that more heavily contaminated batches may be retained in the developing countries where the population relies heavily on groundnut meal as a source of protein. The possibility of detoxification with ammonia is briefly discussed, and it is noted that tests with rats indicate that the protein quality is thereby decreased by about 10 %.


Aflatoxin at up to 200 µg kg⁻¹ was detected in 16-20 % of imported groundnut samples analyzed during 1976-1978. In 1979 a highly contaminated Egyptian groundnut lot raised the positivity to > 80 %, with a maximum contamination of 1000 µg kg⁻¹. The efficiency of recovery was 75-90 % with Pons' method and 60-71 % by the Beljaars method. Aflatoxins were not detected in samples of Hungarian flour and vegetables for soup. In an examination of samples of milk from large-scale dairy farms, aflatoxin M₁ at 0.06-0.08 µg L⁻¹ was detected in 2 of 20 samples in 1977 and 2 of 30 samples in 1978. The aflatoxin B₁ content of groundnut-base feed at a farm was 60 µg kg⁻¹. The 30 milk samples analyzed in 1979 did not contain aflatoxin. In 1977 one of 20 powdered milk samples examined contained aflatoxin M₁ at 200 µg kg⁻¹. Egg samples examined during 1978-79 contained no aflatoxin. The methods used in milk and egg analyses had extraction efficiencies of 80-90% and 75-80 %, respectively.


The aflatoxin B₁ content of samples of various feedstuffs, including 52 samples of yellow maize, 50 of groundnut cake, 39 of starter feed, 34 of layer feed, 22 of grower feed, 22 of fish meal, 20 of rice bran, 16 of wheat bran, 5 of mustard oilcake, 4 of guar meal and 2 of meat meal, collected during 1985, was estimated with the Pons' method. Feed samples were found to be contaminated with aflatoxin B₁ as follows: maize (50 % of samples contaminated at 0-1000 µg kg⁻¹; average 200 µg kg⁻¹), groundnut cake (62 %, average 300 µg kg⁻¹), starter feed (38 %, average 140 µg kg⁻¹), layer feed (44 %, average 160 µg kg⁻¹), grower feed (36 %, average 120 µg kg⁻¹). All other samples were free from aflatoxin.


Two samples of groundnut meal were collected from a dairy farm where milk production had fallen to 76 % of normal, and many of the cows were in poor condition, with reduced appetites and scouring. Aflatoxins were demonstrated at a level which could give about 50 µg kg⁻¹ (dry matter) in the complete diet. In 2
samples of groundnut meal the aflatoxin B\(_1\) levels were 750 and 620, of B\(_2\) 40 and 30, of G\(_1\) 60 and 50, and of G\(_2\) up to 10 µg kg\(^{-1}\). Within 3-4 days of withdrawal of the meal, scouring ceased and appetite improved. Milk production rose again, though not to its predicted level.


Four hundred and sixty-five samples of various food commodities and 94 samples of different feeds and feed ingredients were analyzed for aflatoxin contamination. These samples were collected from different parts of Nepal. Mostly maize and groundnut samples were contaminated with aflatoxins B\(_1\) and B\(_2\). Of 101 samples, 10 samples of maize used as food were contaminated with aflatoxin at levels > 30 µg kg\(^{-1}\), while only 2 of 67 samples of groundnuts were highly contaminated. Among feed samples, 25 of 58 poultry feed samples were highly contaminated. Maize and groundnut cake were mostly contaminated used as feed ingredients. The other commodities used as foods and feeds had aflatoxin levels well within the safe limit (30 µg kg\(^{-1}\)).


Five cases of aflatoxicosis in pigs in Southern Queensland are described. One peracute case where aflatoxin concentration of up to 5000 µg aflatoxin B\(_1\) kg\(^{-1}\) was demonstrated in stomach contents was presumed to be caused by consumption of moldy bread. High levels of toxins were also present in the livers. Two cases of acute toxicity were caused by feeding moldy groundnut screenings containing 22000 µg aflatoxin B\(_1\) kg\(^{-1}\). One case of subacute, and one of chronic toxicity were caused by sorghum grain based rations with lower aflatoxin levels (4640 and 255 µg kg\(^{-1}\)). Peracute toxicity caused collapse and deaths within a few hours, acute toxicity caused deaths within 12 h, and subacute toxicity caused deaths after 3 weeks on the toxic rations. Anorexia and ill thrift affecting only growing animals were seen with chronic toxicity. Extensive centrilobular liver necrosis and haemorrhage occurred with peracute toxicity, and in cases of acute poisoning there was hepatic centrilobular cellular infiltration, hepatocyte swelling and bile stasis. With subacute toxicity hepatocyte vacuolation together with bile stasis and bile ductule hyperplasia were seen.


A survey of 43 oil factories in the State of Sao Paulo, Brazil, revealed that due to the high aflatoxin levels only 3.2 % of the groundnut meal samples and 3 % of the cake samples could be utilized as animal feed.


Of 87 samples (maize meal, groundnut meal, palm meal, and cottonseed meal) analyzed, 12 were contaminated with aflatoxins. The most heavily contaminated sample was maize which contained > 1000 µg kg\(^{-1}\) aflatoxin B\(_1\). Of 56 isolates of *Aspergillus* spp. isolated from the substrates, 32 were of *A. flavus*, 11 strains of which produced aflatoxin B\(_1\) or B\(_1\) and B\(_2\). One strain isolated from rice bran produced 880 µg g\(^{-1}\) aflatoxin B\(_1\).


Of 29 different feedstuffs tested, aflatoxin B, was detected in mixed feed No. 1 and 2 with incidence of 46 % and 36 %, respectively. In the constituents of mixed feed No. 1, aflatoxin B\(_1\) incidence ranged from 20 to 100 % and from 40 to 100 % in those of mixed feed No.2. Groundnut cake showed the maximum percentage of incidence. Among other simple feeds, higher levels of aflatoxin B\(_1\) were detected in cottonseed-cake followed by coconut-cake, green gram, black gram, chickpeas and wheat. No contamination of rice bran was found. Ailatoxin G\(_1\) was found in trace amounts in Murukool (constituent of mixed feed No. 2) and in groundnut cake (simple feeds). Zearalenone was detected in mixed feeds No. 1 and 2 and also in maize meal, maize-oil cake, sunflower cake and maize of feed No. 2. In simple feeds, low levels of zearalenone were detected in chickpeas and wheat. Neosolaniol was detected in mixed feed No. 1 and groundnut cake (simple feed) with a low incidence of 10 % in each case. Low levels of penicillic acid and patulin were detected in legume mixture of feed No. 2 and maize meal of feed No. 1, respectively.

The present investigation is the first definitive study of the distribution of aflatoxins in a wet-milling process of raw groundnuts. The results show that the majority of aflatoxins originally present in the groundnuts remained in the solid fractions, particularly the protein fractions, during wet-milling. In the protein concentrate preparation, the concentrates carried 81-89% of the total aflatoxin; crude oil, 5-8%; and whey fraction, 3-14%. In the case of protein isolate preparation, 51-56% of the total toxin remained with the isolates, 22-26% with the residue, 11-17% with the whey, and 7-8% with the crude oil. Distribution of aflatoxins in the preparation of protein isolates from defatted groundnut meal showed that 55-65% of the total toxin originally present in the meal remained with the protein isolates, 20-28% with the residue, and 10-20% with the whey fraction. Changes in extraction pHs for the preparation of protein isolates either from raw groundnuts or defatted meal did not alter the distribution pattern. A new approach based upon the charge-transfer (electron acceptor-donor) complex formation is suggested to shift this aflatoxin distribution from protein products to disposable whey or residue fraction from the processing of raw groundnuts and defatted meal for protein products.


Aflatoxin B₁ was detected in all simple and mixed feeds and from most of the ingredients tested. The maximum amount of aflatoxin B₁ was found in groundnut cake and cottonseed cake. Aflatoxin B₂ was found in oil cakes of sunflower and maize, and also in husk. Considerable quantities of ochratoxin A and sterigmatocystin were observed in samples of sunflower cake and green gram, respectively.


The extent of aflatoxin contamination in groundnut oil, groundnut cake and hydrogenated oil sold in the market of Hapur, Uttar Pradesh, India, was investigated. Nearly 66.7% of the total samples of groundnut oil contained aflatoxin. Of these, 70% contained as much as 2660 µg kg⁻¹ aflatoxin B₁. Of the groundnut cake samples, 70% were contaminated with 1135-2250 µg kg⁻¹. Refined oil or Vanaspathi did not contain any aflatoxin.


Aflatoxins were estimated in samples of 19 different types of oilcake, 4 cereals, 2 compounded feeds and three other products. 80% of the samples of cottonseed cake and 64% of groundnut cake contained 200-520 µg kg⁻¹ aflatoxin B₁. Anatoxins B₂ and G, were also found in some samples, but in lower concentrations than B₁. More than half of the cereals tested contained aflatoxin B₁ as did 14 of 20 samples of poultry feed and all of 4 samples of cattle feed. None of the samples contained more than the permissible limit (taken as 1000 µg kg⁻¹) of the toxins.


Only 10% of food samples examined during 1973-1975 contained mycotoxins, viz. aflatoxins B₁ and G₁, ochratoxin A, sterigmatocystin and zearalenone. Aflatoxin B₁ (and sometimes G₁) was present up to 2000 µg kg⁻¹ in groundnut meal obtained from farms with production disease in cattle. Ill thrift occurred in calves and milk production was lower. Ochratoxin A was occasionally found in stored barley and it has been suggested that it may cause foetal resorption in farm animals, but experimental studies in pregnant sows did not confirm this when they were fed ochratoxin A or B. Ochratoxin A accumulated in the body tissues of the dam. Zearalenone has been implicated in the aetiology of sprayleg piglets, but only 1 such case was seen among 63 piglets from 7 gilts fed naturally contaminated wheat. However, when such wheat was fed to laying hens, some newly-hatched chicks suffered leg weakness.


Over 13 years, 740 samples of animal feeds were analyzed, about 500 of which were suspected of causing disease in farm animals. Aflatoxin B₁ was detected in only 13.6% of the samples. Groundnut meal samples nearly always contained the toxin. Where mycotoxicosis was suspected in dairy cattle, 27% of the samples proved positive, as compared with 9% of other dairy feed samples.


Aflatoxin levels in groundnut cake, in groundnut flour, and in Arlac are reported. The mean aflatoxin content of groundnut cake available in Nigeria for animal consumption was established as 340 µg kg⁻¹ (range 100 µg > 800 µg kg⁻¹ in 148 samples) by taking periodic samples from the consignments supplied to Vom and to the Piggeries at Minna. Aflatoxin levels in groundnut flour (59 analytical
samples) ranged from < 80 pg to > 250 µg kg\(^{-1}\). Aflatoxin levels in groundnut flour and Arlac were lowered to about 20 µg kg\(^{-1}\) by following improved practices of hygiene, storage conditions, initial hand-sorting of nuts, and by utilization of better grade nuts for production of groundnut flour and Arlac.


Twenty-six groundnut cake and 13 cottonseed cake samples from Ludhiana, Punjab, India, were analyzed for aflatoxin contamination. Fifty per cent of the groundnut cake samples and 30.7 % of cottonseed cake samples were contaminated with aflatoxin. Aflatoxin B\(_1\) was present in all the contaminated samples whereas aflatoxins B\(_2\) and G\(_2\) were found in 2 and 10, respectively. Aflatoxin G\(_2\) was not present in any of the samples. The levels of aflatoxin B\(_1\) ranged from 16-2000 and 40-500 µg kg\(^{-1}\) in groundnut and cottonseed cake samples, respectively, while the levels of the other aflatoxins were < 1 µg kg\(^{-1}\).


A survey on aflatoxin contamination of some poultry feed ingredients, conducted in Andhra Pradesh during winter of 1981-82, indicated that groundnut cake (GNC), in general, contained more total aflatoxin (587 pg kg\(^{-1}\)) than did maize (71 µg kg\(^{-1}\)), pearl millet (38 pg kg\(^{-1}\)), broken rice (43 µg kg\(^{-1}\)) and rice polish (23 µg kg\(^{-1}\)). The GNC samples collected from the coastal region had more aflatoxin (974 µg kg\(^{-1}\)) than those from Rayalaseema region (573 µg kg\(^{-1}\)), and Telengana region (215 µg kg\(^{-1}\)). Maize from Rayalaseema, the coast and Telengana had aflatoxin 99, 62 and 53 µg kg\(^{-1}\), respectively. No consistent trend was observed in the other feedingstuffs. The storage of samples for 2 months significantly increased aflatoxin content in GNC and maize but not in other ingredients.


Samples of groundnut cake, maize, pearl millet, broken rice and rice polish were collected from two representative districts for each of the 3 regions of Andhra Pradesh, India, over a 30-day period. The standard blotter method was used to detect fungal infestation in maize, pearl millet and broken rice. Fungal contaminants from groundnut cake and rice polish were detected by the Czapek's agar plate method. Samples of feed ingredients were analyzed for aflatoxin soon after collection. The predominant fungi infesting these feeds were *Aspergillus flavus*, *A. niger*, *Fusarium, Penicillium, Rhizopus, Curvularia, Drechslera* and *Alternaria*. The percentage incidence of colonies of *A.flavus* ranged from 9 to 12 in maize, 14.7 to 22 in pearl millet and 11 to 12 in broken rice. Most positive samples of *A.flavus* infestation were groundnut cake. The levels of aflatoxin in maize were higher than those found in other feed samples, and it suggested that this might be due to the greater surface area of the maize grain.


The degree of aflatoxin contamination in groundnut hulls (shells) used in cattle feed was determined by analyzing inoculated hand-shelled hulls and hulls from groundnuts known to contain aflatoxin. Hulls adjusted to 20 % moisture, inoculated with *Aspergillus flavus* and incubated for 7 days at 25°C supported growth of the fungus but not aflatoxin production. Groundnuts from 20 selected lots with visible *A.flavus* contamination contained 13-353 µg kg\(^{-1}\) aflatoxin. The machine-shelled hulls from these lots were analyzed and 3 lots contained no detectable aflatoxin, 13 lots contained 4-88 µg kg\(^{-1}\) and 4 lots contained 116 µg kg\(^{-1}\). Aflatoxin concentrations of 53-87 µg kg\(^{-1}\) were detected in hulls when groundnuts containing relatively high levels of aflatoxin (up to 26800 µg kg\(^{-1}\) in damaged kernels) were carefully machine-shelled. Hulls from the same samples obtained by hand-shelling contained no detectable aflatoxin. Separating hulls over a 4.76 mm screen appeared to provide a mean of removal of most aflatoxin-containing particles in groundnut hulls.


A high pressure liquid chromatography (HPLC) technique is described for detection of mycotoxins (aflatoxins, zearalenone, vomitoxin, and ochratoxin A). The detection limit for toxins was: aflatoxins (B\(_1\), B\(_2\), G\(_1\), and G\(_2\)) 2 µg kg\(^{-1}\), zearalenone 1 µg kg\(^{-1}\), vomitoxin 25 µg kg\(^{-1}\), and ochratoxin A 5 µg kg\(^{-1}\). Of groundnut products, mainly groundnut meal, 87 % were contaminated (maximum 11620 µg kg\(^{-1}\) total aflatoxin). In Southeast Styria a high rate of vomitoxin contamination was found in maize (80 %, average 761 µg kg\(^{-1}\)), zearalenone was found in 47 % of samples tested (average concentration 145 µg kg\(^{-1}\)), 23.6 % of 83 stored grain samples contained ochratoxin A at low concentration.
A total of 234 samples of poultry feed from Andhra Pradesh and their ingredients were examined for the presence of aflatoxins. Among the feed ingredients analyzed, groundnut cake was found to be the only major feed component affected by aflatoxin. The overall incidence of aflatoxin was 30.76 % in feedstuffs. The predominant fungi contaminating these feeds were *Aspergillus flavus*, *A. niger*, *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp., and *Curvularia* spp.


A total of 127 samples of animal and poultry feeds were collected from 15 sources in India, ground, sieved and dried. Aflatoxin B₁ was estimated by chromatography. About 35 % of samples were contaminated with aflatoxin B₁, and concentrations were particularly high in groundnut cake and cottonseed cake samples.


Of 14 groundnut cake samples collected before the rainy season 8 contained aflatoxin B₁ (mean 350 µg kg⁻¹), 17 of 19 samples collected during and after the rainy season had 470 µg kg⁻¹, 3 in each of 4 samples of cattle feed, poultry mash and pig grower mash had 140, 240 and 170 µg kg⁻¹, 2 of 4 samples of linseed meal had 50 µg kg⁻¹ and 2 of 10 maize samples 260 µg kg⁻¹. Aflatoxin B₂ or G₁ was also detected in some of the groundnut cake samples. Four samples of fish meal, 4 of wheat bran and one of berscem hay did not contain any aflatoxin.


In 130 samples of groundnut meal and 680 samples of feed mixtures examined during 1974-1976, aflatoxin B₁ levels varied from 0 to 1280 µg kg⁻¹ in groundnut meal and from 0 to 200 µg kg⁻¹ in feed mixtures. Annual fluctuations in aflatoxin B₁ levels might be influenced by weather.


Of 366 samples of groundnut oilmeal from India and Brazil. 90 % of samples contained < 50 colonies g⁻¹ of *Aspergillus flavus* and 78 % of samples contained < 100 µg kg⁻¹ aflatoxin B₁. It is concluded that there is a correlation between contamination of groundnut oilmeal by *A. flavus* and aflatoxin.


The Japanese Government ordinance specifies groundnut meal as the specific feed which should not contain > 1000 µg kg⁻¹ aflatoxin. Aflatoxin levels in imported groundnut meals from India are given. Levels of aflatoxins B₁ ranged from 69 to > 26000 µg kg⁻¹ in different groundnut meals. It is assumed that only a small number of highly contaminated consignments (bags) would be the cause of disqualification of the lot.


Of 36 samples of groundnut oil cake examined in 1971, 17 (47.2 %) contained aflatoxin. Eleven of these 17 contaminated samples contained up to 100 µg aflatoxin kg⁻¹ (mainly aflatoxin B₁) and 6 > 100 µg kg⁻¹. Of 74 samples examined in 1972, 66 (89.2 %) were contaminated; 13 contained up to 100 µg kg⁻¹ and 53 > 100 µg kg⁻¹ aflatoxin.

### 5.2.5 Peanut butter


Injera, a fermented, pancake-shaped food prepared from *Eragrostis tef*, showed no aflatoxin contamination as commonly prepared and handled, but fermentation of the dough or storage of injera for prolonged periods did not increase aflatoxin B₁ contamination. Preparations of red pepper powder and its paste showed some aflatoxin contamination (mean 32 µg kg⁻¹ for powder. 1 paste sample had 102.2 µg kg⁻¹ aflatoxin B₁). Samples of groundnuts and peanut butter had aflatoxin B₁ at mean values of 34.7 and 105 µg kg⁻¹, respectively.


Aflatoxins B₁, B₂, G₁ and G₂ were determined in bulk samples of raw groundnuts, peanut butter, wheat flour, and chicken, pig and dairy feeds. Samples were taken
in accordance with an established sampling plan, and dry samples were subsampled with the Dickens Subsampling Mill. A minicolumn procedure was used to screen 64 samples, eight of which were further analysed by thin-layer and high-performance liquid chromatography. Of the eight samples, only one contained aflatoxin G₁ (2 µg kg⁻¹).


Aflatoxin levels in groundnut samples from the Virginia-North Carolina crop were determined in a survey carried out in the 3-year period 1964-1967. About 6% of samples from the 1964-1965 crop contained aflatoxin, and 23 of 51 samples of peanut butter were contaminated with aflatoxin. Contaminated lots of groundnuts were reduced to 5.3% in 1965-1966 and to 4.4% in 1966-1967. No peanut butter sample was contaminated in 1965-1966.


Surveys were carried out for two years to determine the natural occurrence of mycotoxins (aflatoxin, ochratoxin and zearalenone) in some common Brazilian foods such as raw and salted roasted groundnuts, pacoca (a very popular groundnut candy), peanut butter, maize, soybean, cured cheese and pork. Eight characteristic regions. Vale do Paraiba. Mogiana. Paulista Velha, Araraquarense, Noroeste, Paulista Nova. Sorocabana and Vale do Ribeira/Litoral Sul, were surveyed and 742 samples were analyzed. Estimation of toxins was made by thin-layer chromatography (IXC) by comparison with standards. Six samples of corn (4.7 %) were contaminated with aflatoxin, ranging from 41 to 2000 µg kg⁻¹ of aflatoxin B₁; 81 samples of pacoca (63.3 %), ranging from < 20 to 1187 µg kg⁻¹; 80 samples of peanut butter (62.5 %), from < 20 to 275 µg kg⁻¹; 17 samples of raw groundnuts (17.7 %), from 19 to 3125 fig kg⁻¹; and 9 samples of roasted salted groundnuts (9.4 %) from < 30 to 4250 µg kg⁻¹. From the first to the second year, the percentage of contaminated samples increased slightly for raw groundnuts (from 15.6 to 18.8 %) and decreased for pacoca (67.2 to 59.4 %) for roasted salted groundnuts (12.5 to 7.8 %), corn (6.2 to 3.1 %), and for peanut butter (84.4 to 40.6 %). The average content of aflatoxin of the contaminated samples increased for raw groundnuts (from 166.7 to 641.7 µg kg⁻¹) and roasted salted groundnuts (410 to 980 µg kg⁻¹) and decreased for pacoca (165.6 to 108.8 µg kg⁻¹), peanut butter (91.3 to 50.4 µg kg⁻¹), and corn (853.3 to 700 µg kg⁻¹).


A study employing the Best Foods (BF) method was carried out for a period of six years to determine the aflatoxin content in groundnuts, peanut butter and other foodstuffs. The study was divided into three groups. In Group I a total of 3,928 samples were analyzed; 1,679 samples of raw groundnuts, 2,092 samples of peanut butter, and 157 samples of processed groundnuts. 5.8 %, 0.1 %, and 1.3 % of the samples analyzed in these groups contained a total aflatoxin content over 25 µg kg⁻¹, respectively. Group II consisted of other nuts: cashew, almonds, walnuts, pecans, filberts, Brazil nuts, and pistachios, of which 641 samples were analyzed. Two per cent contained aflatoxin over 25 µg kg⁻¹. Group III, other foodstuffs, consisted of sunflower seeds, cocoa beans, beans, corn, mushrooms, and coffee beans. The total number of samples analyzed were 244 and only 0.4 % contained aflatoxin at over 25 µg kg⁻¹. On the basis of the samples analyzed it is evident that in the past six years the incidence of aflatoxin occurring at levels of less than 5 µg kg⁻¹ in groundnuts and groundnut products has been 90.1 % of the samples analyzed. In the other nuts tested, 91.7 % of the samples contained less than 5 µg kg⁻¹ of aflatoxin. The test data of samples of foodstuffs other than nuts showed that 95.9 % of the samples contained aflatoxin at a level of less than 5 µg kg⁻¹.


A study using the 'Best Foods' (BF) method was done over 6 years to determine aflatoxin contents in groundnuts, peanut butter and other foods. Results showed that of 1679 samples of raw groundnuts, 98 (5.8%) contained > 25 µg kg⁻¹ total anatoxins. 89.8 % < 15 µg kg⁻¹. Of 157 samples of processed nuts. 2 (1.3%) contained > 25 µg kg⁻¹, and the rest < 15 µg kg⁻¹. Of 2092 samples of peanut butter, 3 (0.14%) contained > 25 µg kg⁻¹ and 12 (0.57%) contained 15-25 µg kg⁻¹. Of 120 samples of cashews, 10 (8.3%) contained > 15 µg kg⁻¹ as well as 1 of 78 samples of almonds, 2 (1.3%) of 156 samples of walnuts, 10 (5.7 %) of 175 samples of pistachios. None of the samples of pecans, filberts and Brazilnuts tested contained aflatoxin. Of 244 samples of other foods, only one sample each of sunflower seeds and corn contained > 15 µg kg⁻¹. None of the samples of cocoa beans, beans, mushrooms tested had aflatoxin contamination. Test data of foods other than nuts had aflatoxin levels of < 5 µg kg⁻¹ in 95.9% of samples.


A preliminary survey of peanut butter in 1982 indicated that 31 of 32 samples of major national brand-named products contained aflatoxin B$_1$ at up to 10 ng kg$^{-1}$, and that 59 % of those were below the limit of detection (2 µg kg$^{-1}$). Of 25 peanut butters from specialist ‘Health Food’ outlets, 64 % contained up to 10 µg kg$^{-1}$, and the remainder contained 16-318 µg kg$^{-1}$, with one sample having 345 µg kg$^{-1}$ aflatoxin. Surveys of ‘Health Food’ products in 1983 and 1984 confirmed that manufacturers were still experiencing some difficulty in complying with the 30 µg kg$^{-1}$ voluntary guideline limit for total aflatoxin. In 1984, 228 retail samples of nuts and nut confectionery products comprising groundnuts (shelled, unshelled, roasted and salted), mixed nuts, almonds (both unblanched and ground), Brazil nuts (in-shell), hazelnuts (in shell), chocolate-coated groundnuts, peanut brittle and coconut ice were examined. Results showed that 74 % of the samples contained aflatoxin B$_1$ at up to 0.5 µg kg$^{-1}$, and 3.1 %, mainly groundnuts and Brazil nuts, exceeded the guideline tolerance for total aflatoxin. Total aflatoxin was greatest in unshelled groundnuts, 4920 µg kg$^{-1}$, and in a composite sample of visibly molded Brazil nuts, 17926 µg kg$^{-1}$.


Studies on aflatoxin contamination in various food samples of Nepal were carried out during the period 1980-1986. About 764 samples of various food commodities from hills and Terai region were analyzed for aflatoxin contamination. One hundred and forty-eight samples of different feed ingredients from different Feed Industries of Kathmandu were also analyzed. Of the total number of respective samples analyzed, 19 % of maize, 29.5 % of maize flour, 17.9 % to 33.8 % of groundnuts, 31.7 % of peanut butter and 9.4 % of wheat flour were contaminated with aflatoxin, but tolerance limit (30 µg kg$^{-1}$) exceeded in 4.5 % to 15.9 % of these samples. Rainy season is the most prone period for aflatoxin contamination. In case of feed, about 50 % of poultry feed, 26.7 % of cattle feed and 20 % of pig feed samples were contaminated with aflatoxins. Twenty % of the poultry feed samples had aflatoxins above the tolerance limit (10 to 50 µg kg$^{-1}$ depending upon the age of the animals).


Between 1974 and 1977 the average level of aflatoxins in peanut butter was 4 ng g$^{-1}$. The average intake of these foods was 0.4 g kg$^{-1}$ bodyweight for children up to 10 years and declined progressively thereafter to < 0.1 g kg$^{-1}$ bodyweight.


A total of 36 samples of foods collected during August 1985, December 1985 and March 1986 and served to mentally retarded clients with a high incidence of hepatocellular carcinoma, were analyzed for aflatoxin. Aflatoxin was not detected (< 5 µg kg$^{-1}$) by thin-layer chromatography in 35 food samples containing groundnuts, maize, wheat or milk. One peanut butter sample contained 20 µg kg$^{-1}$ aflatoxin. Aflatoxin content of these foods was at or below the level permitted by the Food and Drug Administration. It is concluded that aflatoxin is probably not responsible for liver disease in this population.


During 1985 and 1986, samples of sorghum beer, sorghum cereal, groundnuts, peanut butter and maize meal, purchased from supermarkets, were analyzed for aflatoxins using HPLC and fluorescence detection methods. Of the samples analyzed during 1985, approximately one-third were contaminated with aflatoxins, although none were above the legal limit of 10 µg kg$^{-1}$ aflatoxins. In 1986, the numbers of contaminated samples increased, but the level of contamination remained low, with only one sample exceeding the legal maximum.


Aflatoxin contamination in five agricultural commodities namely groundnut, dried cocoa beans, copra, pepper (black and white) and paddy and rice was determined. Shelled groundnuts and their products such as peanut butter and satay gravy were highly susceptible to aflatoxin contamination, but the "menglembu" type groundnut was free of aflatoxin. Copra, dried cocoa beans and pepper (black and white) were the other highly susceptible commodities, but stored paddy and rice were safe but a few samples contained aflatoxin. The extent of contamination in several other commodities is also mentioned and general control methods are discussed.

A survey was carried out in 19X6 for the occurrence of aflatoxin $B_1$ in peanut butters (129 samples) obtained from specialist Health Food outlets. The results showed that 6.2 % of the samples contained > 10 µg kg$^{-1}$ of aflatoxin, 8 % contained between 2.5 and 10 µg kg$^{-1}$, and in the remainder (86 %) aflatoxin could not be detected at a limit of 2.5 µg kg$^{-1}$. These results show a lower contamination by aflatoxin than found in these products in previous surveys (1982-1984). An aflatoxin $B_1$-specific enzyme-linked immunosorbent assay (ELISA) was employed for the first time in these analyses; and to make an assessment of its performance positive aflatoxin results, together with a random selection of those below the ELISA limit of detection, were additionally analyzed by conventional extraction and clean-up followed by HPLC. The ELISA technique offered a significant improvement in speed of analysis over conventional approaches, enabling a six-fold increase in sample throughput as compared with that required for conventional analysis.


A simple procedure was devised for the routine screening of aflatoxin $B_1$ in peanut butter using enzyme-linked immunosorbent assay (ELISA). Peanut butter samples (5g) were spiked with aflatoxin $B_1$ and extracted by blending with 25 ml of 55 % methanol and 10 ml of hexane. The extract was filtered and aqueous filtrate analyzed by a direct competitive ELISA. Recovery of aflatoxin $B_1$ added to peanut butter samples ranged from 85 to 112 %, with an average coefficient of variation of 18.4 %. Using this procedure, only three of 63 samples of peanut butter had detectable levels (>5.0 µg kg$^{-1}$) of anatoxin $B_1$.


A simple procedure was devised for the routine screening of aflatoxin $B_1$ in groundnuts and peanut butter using enzyme-linked immunosorbent assay (ELISA). Samples of peanut butter were spiked with aflatoxin $B_1$ and extracted by blending with 25 ml of 55 % methanol and 10 ml of hexane. The extract was filtered and aqueous filtrate analyzed by a direct competitive ELISA. Recovery of aflatoxin $B_1$ added to peanut butter samples ranged from 85-112 %, with a mean recovery of 97 %. Coefficient of variation between test wells in the assay was 18.4 %. Only 3 of 63 commercial samples of peanut butter showed detectable aflatoxin $B_1$ (>5 ng g$^{-1}$) with this procedure.


Distribution and degree of occurrence of anatoxins in various samples of groundnuts and groundnut products in the Republic of South Africa are described. Over 1000 samples of groundnuts and groundnut products were examined during 1963 and another 1000 samples of producers' grades were investigated during 1964. The samples obtained from the North-Western Transvaal, or so-called Bushveld areas, were most seriously contaminated with aflatoxin; all of the 75 samples containing > 2000 µg kg$^{-1}$ aflatoxin were from the receiving depots in the North-Western Transvaal. The samples from the Northern Cape Province, Orange Free State and Natal, except for 5 samples with 500-2000 µg kg$^{-1}$ and 11 samples with 100-500 µg kg$^{-1}$, were free from aflatoxin. Similar results were found in the 1964 survey for aflatoxin contamination; however, only 23 samples from the North-Western Transvaal areas contained > 2000 µg kg$^{-1}$. High degree of aflatoxin contamination in the groundnut samples from the North-Western Transvaal was ascribed mainly to (1) the damage caused to the maturing groundnut pods and kernels by termites, and (2) protracted droughts followed by late rains. Lower or inferior grade groundnuts contained higher levels of aflatoxins than the higher or superior ones. No aflatoxins were detected in any of the samples of hand-picked selected (HPS) kernels examined in 1963 and 1964. Groundnut cake samples implicated in livestock deaths were also examined for aflatoxin contamination. Eleven of the 16 samples of this groundnut cake contained > 2000 ng kg$^{-1}$ aflatoxin. Of 101 peanut butter samples tested, 17 were contaminated with aflatoxin. Of these 17 samples, 12 had an aflatoxin contents of 100-500 µg kg$^{-1}$ and 5 had up to 25 µg kg$^{-1}$.


Aflatoxin-producing fungi were found in fermented foods and beverages: fermented rice (kaomak), soybean sauce (taotjo), peanut butter, soy sauce (shoyu), Thai red and white wine, and rice sugar wine. These foods were extracted directly and tested for aflatoxins by thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC). Four strains of aflatoxin-producing fungi were isolated from peanut butter, taotjo, and shoyu. Direct extracts of 10 % of the peanut butter samples tested and 5 % of the kaomak tested contained high levels of aflatoxins.

The aflatoxin contamination in Tokyo’s commercial foods and foodstuffs during 1982-1986 was investigated. A high incidence of contamination was found in buckwheat (23 of 134 samples positive for aflatoxin) and coix seed (38 of 159 positive), although the aflatoxin levels were relatively low. Of 464 samples of nuts and their products tested, aflatoxin was detected in 3 samples of peanut butter and one of sesame. One sample of butter bean was found to be heavily contaminated with aflatoxins (aflatoxin B\textsubscript{1}: 254 µg kg\textsuperscript{-1}; aflatoxin B\textsubscript{2}: 8.5 µg kg\textsuperscript{-1}). When spices were analyzed, a high incidence (60 %) and level of aflatoxin was found in nutmeg from Indonesia. A high incidence of aflatoxin was also found in white pepper, but not in black pepper.


A survey of the aflatoxin content in some food products available on the market in Norway is reported. During autumn 1973, samples of Brazil nuts, groundnuts, peanut butter, hazelnuts, walnuts, mixed nuts, cocoa, cocoa products and dried milk were bought from stores in the Oslo area. In addition, samples of Brazil nuts and groundnuts were taken from import stores. Aflatoxins were detected in 6 of 34 samples of bought groundnuts, 1 of 3 of sorted and pooled groundnuts, 1 of 16 mixed nuts, 1 of 14 walnuts, 1 of 3 cashew nuts. 18 of 27 Brazil nuts and 1 of 40 cocoa products, levels of aflatoxins B\textsubscript{1}, B\textsubscript{2}, G\textsubscript{1} and G\textsubscript{2} were 4200, 1600, 3100 and 600 µg kg\textsuperscript{-1}, respectively, for one sample of Brazil nuts and 400, 50, 350 and 30 µg kg\textsuperscript{-1} for a sample of groundnuts. Other aflatoxin levels were generally much lower. No aflatoxin was detected in peanut butter, hazelnuts and dried milk.

5.2.6 Groundnut protein concentrate


Groundnut cake (‘Kulikuli’) samples purchased from four major markets in Ibadan. Oyo State, Nigeria, during April-November 1988 were analyzed for aflatoxin B\textsubscript{1} and associated mycoflora. In all but two of the samples aflatoxin B\textsubscript{1} levels were between 20 and 455 µg kg\textsuperscript{-1}. Mold counts were low (1.0 x 10\textsuperscript{2} - 4.40 x 10\textsuperscript{2} colonies g\textsuperscript{-1}). Eight mold species were isolated. Of these Aspergillus niger, Paecilomyces variotii, Aspergillus flavus and Fusarium moniliforme dominated in decreasing sequential order. The results show that groundnut cake on sale in Ibadan markets is unacceptable for animal feed rations and human consumption and there is a need for some form of quality control and decontamination before usage.


Children suffering from varying degrees of protein-calorie malnutrition had accidentally consumed aflatoxin-contaminated, low fat. commercially produced groundnut protein flour for periods ranging from 5 days to 4 weeks. The hepatic lesions showed a gradual transition from an increase in central and periportal fat to fibrosis and cirrhosis, which does not usually occur in treated Kwashiorkor. The lesions were identical to those of Indian childhood cirrhosis.


Children suffering from varying degrees of protein calorie malnutrition were accidentally fed with aflatoxin (300 µg kg\textsuperscript{-1}) - contaminated groundnut protein flour for periods of 10 days to 4 weeks. Liver biopsies showed a gradual transition from an increase in central and periportal fat of the liver, to formation of fatty cysts, to fibrosis and cirrhosis.


The present investigation is the first definitive study of the distribution of aflatoxins in a wet-milling process of raw groundnuts. The results show that the majority of aflatoxins originally present in the groundnuts remained in the solid fractions, particularly the protein fractions, during wet-milling. In the protein concentrate preparation, the concentrates carried 81-89 % of the total aflatoxin; crude oil, 5-8 %; and whey fraction, 3-14 %. In the case of protein isolate preparation, 51-56 % of the total toxin remained with the isolates, 22-26 % with the residue, 11-17 % with the whey, and 7-8 % with the crude oil. Distribution of aflatoxins in the preparation of protein isolates from defatted groundnut meal showed that 55-65 % of the total toxin originally present in the meal remained with the protein isolates, 20 28 % with the residue, and 10-20 % with the whey fraction. Changes in extraction pHs for the preparation of protein isolates either from raw groundnuts or defatted meal did not alter the distribution pattern. A new approach based upon the charge- transfer (electron acceptor-donor) complex formation is suggested to shift this aflatoxin distribution from protein products to disposable whey or residue fraction from the processing of raw groundnuts and defatted meal for protein products.
Aflatoxin levels in groundnut cake, in groundnut Hour, and in Arlac are reported. The mean aflatoxin content of groundnut cake available in Nigeria for animal consumption was established as 340 µg kg\(^{-1}\) (range 100 µg - > 800 µg kg\(^{-1}\) in 148 samples) by taking periodic samples from the consignments supplied to Vom and to the Piggeries at Minna. Aflatoxin levels in groundnut flour (59 analytical samples) ranged from < 80 µg to > 250 µg kg\(^{-1}\). Aflatoxin levels in groundnut flour and Arlac were lowered to about 20 µg kg\(^{-1}\) by following improved practices of hygiene, storage conditions, initial hand-sorting of nuts, and by utilization of better grade nuts for production of groundnut flour and Arlac.

With the assistance of the laboratories of six major soybean protein producers, simulated commercial protein concentrate and isolate processes were applied to aflatoxin-contaminated groundnut and cottonseed flours to determine the fate of the aflatoxins. Processes for producing concentrate by washing the flour at the protein isoelectric point (pH 4.5) were ineffective in separating the aflatoxin from products, but a process using an aqueous alcohol wash accomplished in 90% reduction in the aflatoxin level of the concentrate compared to the flour. Processes for producing isolate by protein dissolution in alkali and its recovering by precipitation at the isoelectric point resulted in some aflatoxin loss but an increase in the aflatoxin concentration associated with the isolate compared to the original flour. However, a process for applying carbon adsorbent to the alkaline solution accomplished a 90% reduction in the aflatoxin level of the isolate.

### 5.2.7 Groundnut Hay


Acute aflatoxicosis was believed to be the cause of death in 12 of 90 Hereford calves, fed groundnut hay during drought in Queensland. They developed jaundice, photosensitization, diarrhoea, anorexia, and depression. Increase in serum levels of enzymes of hepatic origin and bilirubin content were higher than normal. Haemorrhage, hepatocyte damage and bile duct proliferation were seen in groundnut hay-fed dead calves. The groundnut hay contained up to 2230 µg kg\(^{-1}\) aflatoxin, with most toxin concentrated in the nut-in-shell.


When an estimated 10-14 pregnant cows in a 68-cow herd were given moldy groundnuts as a supplementary feed, constituting a large proportion of their diet for 4 days, most of them aborted on or soon after the fifth day. The cows were recumbent and unable to rise; in one, there was a low rectal temperature (99°F) and a tremor of the head. All of the cows that aborted were in the third trimester of pregnancy, and all died within 8 days. Biochemical analyses performed on 3 cows revealed hepatic dysfunction, indicated by high values for lactate dehydrogenase, aspartate transaminase and total bilirubin; there was also evidence of mild dehydration; no fetuses were examined. The groundnuts contained 77 µg g\(^{-1}\) of aflatoxin B\(_1\), which was also found (5 µg g\(^{-1}\)) when a liver extract of one cow was analyzed for mycotoxins. Cows fed groundnut hay from the same field as the groundnuts were not affected; the nuts had been kept in the open at freezing temperatures. Abortion preceding fatal mycotoxicosis, as occurred in at least 8 of these cows, is an unusual feature of acute aflatoxicosis.


The degree of aflatoxin contamination in groundnut hulls used as cattle feed was determined by analyzing inoculated hand-shelled hulls and hulls from groundnuts known to contain aflatoxin. Hulls adjusted to 20 % moisture, inoculated with Aspergillus flavus and incubated for 7 days at 25°C supported growth of the fungus but not aflatoxin production. Groundnuts from 20 selected lots with visible A. flavus contamination contained 13-353 µg kg\(^{-1}\) aflatoxin. The machine-shelled hulls from these lots were analyzed and 3 lots contained no detectable aflatoxin, 13 lots contained 4-88 µg kg\(^{-1}\) and 4 lots contained 116 µg kg\(^{-1}\). Aflatoxin concentrations of 53-87 µg kg\(^{-1}\) were detected in hulls when groundnuts containing relatively high levels of aflatoxin (up to 26800 fig kg\(^{-1}\) in damaged kernels) were carefully machine-shelled. Hulls from the same samples obtained by hand-shelling contained no detectable aflatoxin. Separating hulls over a 4.76 mm screen appeared to provide a means of removal of most aflatoxin-containing particles in groundnut hulls.
6. LIMITS AND REGULATIONS

6.1 REVIEW

It is imperative that food contaminated with a hazardous substance is considered unsafe for human consumption. Therefore all food laws, which prohibit trade in adulterated food, consider food contaminated with a hazardous substance as adulterated food. In many countries, this general legislation has been translated into specific regulations that impose tolerable limits on specific mycotoxins (e.g., aflatoxins) in foods.

After the discovery of the acute and chronic toxicity of aflatoxins in the early 1960s, many countries decided to introduce regulations to control the levels of the toxins in foods for human and animals. In the 1970s, some 18 countries had regulations or guidelines which prescribed maximum permissible levels of aflatoxins in foods and/or feeds (Stoloff 1977). Because of increasing awareness of the risk of aflatoxin contamination of foods and feeds, there has been increased enforcement of regulations and legislations on permissible levels of aflatoxins. Aflatoxin regulations have been changed, expanded or created in various countries. At present some 50 countries impose aflatoxin regulations and most countries have a separate maximum acceptable level for food and feed (van Egmond 1989). Regulated commodities vary from country to country, ranging from all foods and feeds to an individual commodity. Groundnuts and groundnut cake are among the most frequently regulated commodities.

The permitted level of aflatoxin in a commodity varies widely depending on the country and the foodstuffs and also whether the foodstuff is destined for human or animal consumption. For example, the maximum permitted level of aflatoxin in foods for human consumption varies from zero to 50 µg kg\(^{-1}\) in those countries with existing regulations (van Egmond 1989). There has been a tendency for regulations to become increasingly stringent as methods of aflatoxin detection have improved (Stoloff 1977, van Egmond 1989).

Aflatoxin Limits in Groundnuts and Groundnut Products for Humans

Many groundnut importing countries have placed limits on the levels of aflatoxins permissible in groundnuts and groundnut products (Table 4). A few countries including Cuba, Dominican Republic, Malaysia, and Portugal have a zero tolerance limit. Several countries including Belgium, Luxembourg, The Netherlands, Norway, Surinam. Sweden. Switzerland, and the USSR have set a reasonable limit of 5 µg aflatoxin(s) kg\(^{-1}\), while most other countries have set “practical limits” of 10 to 30 µg kg\(^{-1}\) (either aflatoxin B\(_1\) or aflatoxins B\(_1\), B\(_2\), G\(_1\), and G\(_2\)). Some countries consider only aflatoxin B\(_1\), while others use the total of aflatoxins B\(_1\), B\(_2\), G\(_1\), and G\(_2\). Therefore, aflatoxin B\(_1\) is the most common toxic and carcinogenic aflatoxin.

The U.K. has a guideline limit of 30 µg kg\(^{-1}\) (total aflatoxins) for imported groundnuts and groundnut products (Jewers 1982). The USA has a guideline limit of 25 µg kg\(^{-1}\) (total aflatoxins) in raw groundnuts, and 20 µg kg\(^{-1}\) in edible nuts and nut products (Stoloff 1977).

In 1966 the WHO/FAO/UNICEF Protein Advisory Group (PAG) set a limit of 30 µg kg\(^{-1}\) for groundnut protein supplements for children. India initially adopted the PAG recommendation and set a guideline limit of 30 µg kg\(^{-1}\) for edible groundnut flour. Later it was impossible to meet this standard; therefore a limit of 60 µg kg\(^{-1}\) (aflatoxin B\(_1\)) was set (Anonymous 1979).

Countries dependent on export of aflatoxin-susceptible commodities e.g., groundnuts, are obliged to establish export limits that meet importers’ requirements. This leads to economic loss if the requirements are unnecessarily strict. Where a local food is also an export item, exportation of the most wholesome food may lead to local consumption of more contaminated food part, thereby increasing the risk of toxic effects in the indigenous population.

Aflatoxin Limits in Animal Feeds

Currently, some 35 countries regulate aflatoxins in animal feedstuffs (van Egmond 1989). The European Economic Community (EEC) has given directives on the maximum permissible levels of aflatoxin in groundnuts and groundnut cake imported into the EEC member countries. The first directive 74/63 became effective 11 February 1974 (EEC’s official Journal no. L-38, page 31). The maximum permissible levels ranged from 20 µg kg\(^{-1}\) (in whole feedingstuffs for pigs and poultry except piglets and chicks) to 50 µg kg\(^{-1}\) (in straight feedingstuffs and whole feedingstuffs for cattle, sheep and goats except dairy cattle, calves, lambs). The EEC limits on aflatoxins in animal feeds were set after the monitoring program of milk in the U.K. showed that many milk samples contained aflatoxin M\(_1\). Groundnuts and cottonseed (raw materials used in dairy feeds) were regarded as the sources of aflatoxin contamination (Jewers 1982, 1988). Consequently, in 1982, the U.K. Government introduced a ban on imports of groundnuts and cottonseed. However, this was soon changed to a ban on imports of groundnuts/meal when contaminated...
with aflatoxin exceeding 50 µg kg⁻¹ (Cappuccio 1989). The EEC regulations varied in the actual limits on aflatoxins in animal feeds. Some countries e.g., France allowed the importation of groundnut meal containing 700 µg kg⁻¹ of aflatoxin B₁ on the condition that it went immediately to feed manufacturers who incorporate the contaminated feedstuffs into animal feeds at an amount that does not exceed the legal limit. The U.K. has a 50 µg kg⁻¹ limit for imported groundnut cake.

The 1974 directive was tightened in 1984 when the tolerance for aflatoxin B₁ in complementary feedstuffs for dairy cattle was reduced from 20 to 10 µg kg⁻¹ (Cappuccio 1989, U.K. Ministry of Agriculture, Fisheries and Food 1989). This was in accordance with the trend in Western European countries to establish tolerances for aflatoxin M₁ at a level of 0.05 µg L⁻¹ milk. A recent development in EEC legislation is the introduction of a tolerance for aflatoxin B₁ in feedstuffs ingredients at 200 µg kg⁻¹ (van Egmond 1989). Some EC countries and countries in other parts of the world have already specified tolerances for some feedstuff ingredients (Table 4). It is possible that EC regulations for aflatoxins in animal feedstuffs will be more stringent in the near future.

### Concluding Remarks

Differences in maximum permissible levels of aflatoxin(s) in groundnuts and groundnut products vary widely among countries with regulations on aflatoxins. It is imperative to follow reasonably uniform regulations and limits on aflatoxins in groundnuts and groundnut products in order to prevent any unnecessary damage to the exporting countries/agencies and to freedom of international trade in oilseeds. Efforts for uniformity of aflatoxin regulations should be supported by knowledge about the rationale for the decisions that resulted in the enforcement of current regulations in various countries. International organizations including FAO, WHO, IUPAC, and EEC are currently attempting to develop uniform aflatoxin regulations for various foods and feeds. The permissible levels of aflatoxin(s) in foods and feeds must be based on the current knowledge on the toxicity of the toxins to humans and animals and on the methods available for their detection.

### Table 4. Maximum permissible aflatoxin levels in foods and feeds in various countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Commodity</th>
<th>Aflatoxin limit (µg kg⁻¹)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
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</tr>
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<td>50</td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>all foods</td>
<td>5a</td>
<td>see EEC</td>
</tr>
<tr>
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<td>50a</td>
<td>Export control</td>
</tr>
<tr>
<td>Canada</td>
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<td>15b</td>
<td>Control under hazard</td>
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<td>5</td>
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</tr>
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</tr>
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<tr>
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<td>complete feedingstuffs</td>
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</tr>
<tr>
<td></td>
<td>(not for dairy cattle, calves, lambs)</td>
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<td>(for pigs and poultry)</td>
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EEC includes Belgium, Denmark, France, Germany, Greece, Ireland, Italy, Luxembourg, The Netherlands, and United Kingdom.
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<thead>
<tr>
<th>Country</th>
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<th>Aflatoxin limit (µg kg⁻¹)</th>
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*Based on Stoloff (1977), Schuller et al. (1982), van Egmond (1989).
ᵃAflatoxin B₁,ᵇTotal Aflatoxins, ᶜAflatoxin M₁

EEC = European Economic Community
6.2 BIBLIOIOGRAPHY

6.2.1 LIMITS AND REGULATIONS


Alarm was expressed at a recent meeting in Rome of the Food and Agriculture Organization of the United Nations about the amount of aflatoxin in groundnuts and other protein supplements. The maximum concentration of aflatoxin which is permitted was laid down in August 1965 by a joint advisory group from the Food and Agriculture Organization and the World Health Organization which took into account the urgent need to provide extra protein in some parts of the world, and established a level of 30 µg kg⁻¹ of foodstuff. Clearly the group would have preferred a lower figure, but felt that the danger of malnutrition was greater than the danger that aflatoxin would produce liver cancer in man.


Aflatoxins. their occurrence, chemistry and toxicology, and regulatory standards for aflatoxin levels in Australian foods, particularly groundnuts, are discussed.


This paper very briefly reviews some analytical methods and bioassays for aflatoxins in groundnuts and groundnut products. Regulatory aspects are also discussed, and the actions of the Food and Drug Administration (FDA) of the US are highlighted.


New European legislation on aflatoxin content of six raw materials for animal feeds will increase the cost of using those materials as quality control costs rise. The legislation is too recent to determine yet if usage of the regulated materials has been affected or if feed manufacturers are looking for alternate nonregulated materials. There are data indicating the level of aflatoxin in imported raw materials is lower, perhaps indicating closer control at the point of origin. The trade is hoping an approved method can be developed to detoxify aflatoxin contaminated materials.


In member states of the African Groundnut Council (AGC) groundnuts are an important traditional and economic crop. For the past 25 years, the problem of aflatoxin has confronted the groundnut industry and AGC. Aflatoxin is a serious constraint to exports particularly in groundnut cake and meal destined for the traditional western European markets. The AGC launched an aflatoxin control program in 1975. Phases I and II have been completed with financial aid from the European Economic Community (EEC) and the United Nations Development Programme (UNDP) and with technical assistance from the Food and Agriculture Organization of the United Nations (FAO). Staff have been trained to survey and monitor aflatoxin in fields and laboratories, and laboratories for aflatoxin analysis have been established and equipped. Two pilot detoxification plants have been constructed to supplement cultural control measures. The AGC monitors contacts with RFC representatives and exporters of groundnut products on relevant legislation and standards and their application.


The cost-effectiveness of adopting aflatoxin tolerance levels of 15, 10 and 5 µg kg⁻¹ for groundnuts and groundnut products was assessed. Estimates of the annual cost to manufacturers of monitoring and controlling aflatoxin levels at the current 20 µg kg⁻¹ action level, and estimates of the projected increase in costs of establishing lower tolerances were elicited from producers by a questionnaire. Exposures to groundnut products were derived from the HANES I survey and from groundnut production statistics. The risk of liver cancer at each tolerance level was estimated using both epidemiological and extrapolated experimental data assuming that exposure would be reduced in direct proportion to the decrease in the tolerance. It was found that the 15 µg kg⁻¹ tolerance would cost $ 60 000 per cancer death averted (range $ 20000 - $ 700000) and is therefore relatively cost-effective. The marginal costs per life saved for both the 10 µg kg⁻¹ and 5 µg kg⁻¹ levels were found to be $ 1.7 million (range $ 0.6 million - $ 11.4 million) and $ 1.6 million (range $ 0.6 million - $ 31.1 million), respectively. Conclusions on the optimal regulatory approach should be guided by comparisons of these figures with corresponding cost-effectiveness ratios for alternative regulatory uses of national resources in the interests of public health.


This review discusses the major groups of mycotoxins found in food and feeds, methods for their analysis and the regulatory action taken by some countries for maximum permissible levels of mycotoxins in food and feeds.
There is concern among farmers’ leaders and consumers in the UK about high levels of aflatoxin in some imported groundnut oilmeal. The 1976 Fertilizer and Feedingstuffs Regulations specify maximum aflatoxin concentration in groundnut oilmeal sold for animal feed as 50 µg kg\(^{-1}\), and in ‘whole feedingstuffs’ for dairy cattle and calves as 10 µg kg\(^{-1}\). However, groundnut oilmeal sold as a feedstuff has been found with aflatoxin levels as high as 900 µg kg\(^{-1}\). Farmers are recommended to ascertain whether compound feeds contain groundnut, and to get a certificate showing aflatoxin content of groundnut for inclusion in home-mixed feed. There are no UK recommendations for maximum aflatoxin in milk, but Milk Marketing Board officials are satisfied that current levels are safe. A feed with the statutory maximum aflatoxin B\(_1\) content could result in about 100 µg kg\(^{-1}\) of aflatoxin in milk, and an average concentration of 0.25 µg kg\(^{-1}\) in cheese or dried milk. A supplementary article reports the experience of one dairy herd where moldy compound feed with aflatoxin content of 600 µg kg\(^{-1}\) was associated with loss of condition and coordination, fall in milk yield and increased incidence of mastitis. Pound-sterling 6700 has been received by the farmers as compensation from the feed firm involved.

Many agricultural commodities can be contaminated with aflatoxins. Increasing concern about the effects of aflatoxins on the health of humans and animals has resulted in worldwide regulatory measures limiting the level of aflatoxin contamination that is acceptable in foods and feeds. International agreement on maximum limits for aflatoxin contamination in internationally traded products, and on methods of sampling and analysis, would better protect the consumer and facilitate international trade. It would obviate arbitrary regulatory measures. Improved agricultural practices, quick postharvest drying, proper storage and transport should help to reduce the incidence of aflatoxin contamination, especially if this could be coupled with marketing practices that offer a premium for quality.
and poultry producers to ensure mycotoxins do not enter the food chain, and this may necessitate them introducing quality control procedures even though a legal framework for such testing is not in place. Utilization of highly contaminated raw materials presents a major problem. Blending of highly contaminated and noncontaminated raw materials is not advisable, as most mixing techniques involving unground material are likely to lead to pockets of highly contaminated material which could have a disastrous effect on poultry production. An alternative strategy would be to decontaminate the highly contaminated raw material or the feed prior to the addition of vitamins and other additives. This approach is being investigated in a project in Pakistan.


Nutrient, mineral, trace elements and vitamin contents in groundnuts are discussed. Particular emphasis is given to storage problems connected with fungal contamination by Aspergillus flavus which is widespread in consignments of groundnuts, necessitating strict legislation on aflatoxin B₁ levels, and limiting its use in concentrates for lactating cows, calves, piglets and young chickens. Shelled groundnuts with low aflatoxin levels are used at 10-20% in mixed cattle feeds, 3-5% in pig and poultry feeds, due to low lysine content. Partially shelled groundnuts can only be used in mixed cattle feeds up to 5 or 10%. Unshelled nuts should not be used in any quality mixed feeds.


Problems relating to the determination of acceptable levels of additives and contaminants in foodstuffs are discussed with examples, including the occurrence of aflatoxins in milk. Since the use of DDT in cattle farming has been prohibited in the Netherlands, the DDT content of milk has been greatly reduced, e.g., in the Alkmaar region only 2 out of a total of 1153 milk samples analysed during 1972-1975 contained > 125 ppm DDT/DDE in fat, vs. 29 out of 555 samples in 1970. As aflatoxin M, has been shown to be carcinogenic, efforts are being made to prevent its occurrence in milk by minimizing the amount of aflatoxins in mixed fodder. Dutch legislation specifies that aflatoxins must not be present in detectable amounts in groundnuts and their products.


The clinical effects of aflatoxin exposure are briefly described, and the current US FDA regulations and sampling procedure for control of aflatoxins in groundnuts are discussed. Some quality control procedures to ensure that only minimal quantities of toxin persist into the finished product are outlined.


The United States Federal laws and regulations concerned with mold/aflatoxin contamination of foods and feeds are described. International regulations and tolerance levels for aflatoxins in groundnuts and groundnut products are briefly reviewed.


Current knowledge about mode of action of aflatoxins, their medical and economic importance and prospects for their control are reviewed, considering the following aspects : structure, nomenclature and biosynthesis; mode of action; health risks and legislative regulation; postharvest control (segregation, decontamination, storage); and preharvest control (pesticides, and biological control by breeding or microbial interactions, particularly repression of aflatoxin synthesis by Aspergillus flavus following infection with a virus from Penicillium chrysogenum).


Limits and regulations on aflatoxins in agricultural commodities including groundnuts and groundnut products in various countries are described. Problems in enacting regulations on aflatoxins in foods and feeds are highlighted.


Samples of groundnuts and groundnut products (raw shelled and unshelled groundnuts, fried salted groundnuts with or without skin, soygroundnut, sugar-coated
colored groundnuts, chocolate-coated groundnuts, peanut butter, ground groundnut bar and candied groundnut bar), purchased at random from different supermarkets and smaller stores during the period from the second semester of 1980 to the first semester of 1982, were analyzed for aflatoxin using the Romer method. Of the 241 samples analyzed, 128 had aflatoxins, and 92 of these contained levels above the tolerance limit permitted by Brazilian legislation (30 μg kg⁻¹ aflatoxins B₁+G₁). Ground groundnut bar, raw shelled groundnuts, soygroundnut and fried salted groundnuts with the skin had greater contamination levels with 61%, 55% and 42% respectively of their samples having aflatoxin levels above the limit. The highest levels of aflatoxins B₁ and G₁ detected were 1282 and 476 μg kg⁻¹ for the ground groundnut bar, 1904 and 69 μg kg⁻¹ for raw shelled groundnuts and 1026 and 366 μg kg⁻¹ for soygroundnut, respectively.


Of 60 samples of groundnut-free feed compounds for dairy cows, only one contained more than 20 μg kg⁻¹ aflatoxin, the maximum level permitted under new Federal German regulations. In feed compounds including groundnut products, however, average aflatoxin levels far exceeded the maximum permitted level. The new regulations are intended to prevent aflatoxin contamination of milk and thus possible hazards to human health.


In this review the occurrence of aflatoxins in food crops, toxicity of aflatoxins to animals, analytical methods, and regulations for aflatoxins in foods and feeds are briefly described.


The regulation of aflatoxin in foods is part of the risk management process that in its initial stages in the period 1964-1969 required considerable speculation to supplement the meager knowledge that formed the basis of the risk assessment. The regulatory stance of most governments is still based on that original speculation, even though a risk assessment using current knowledge does not support the assumptions that were used. The original assumption that aflatoxin might be a potent hepatocarcinogen for humans can not be supported by me considerable data on aflatoxin that has since evolved. But there is clear evidence mat humans can suffer acute liver damage from aflatoxin ingestion. By considering the regulation between the dose levels at which acute effects are manifest, and the frequency distribution of the contamination levels in the ingested foods, a maximum tolerated level can be established that best protects the consumers' health, pocketbook, and food choices.


This article explains the background and previous regulatory steps that led to current legislation on aflatoxin. The European Economic Community's regulations on aflatoxins in groundnuts and groundnut meal and other feeds are described.


A worldwide enquiry was undertaken in 1986-87 to obtain up-to-date information about mycotoxins legislation in as many countries of the world as possible. Together with some additional data collected in 1981, information is now available about planned, proposed, existing legislation, or the absence of legislation, in 66 countries. Details of tolerances, legal bases, responsible authorities, prescribed methods of sampling and analysis, and disposition of commodities containing inadmissible amounts of mycotoxins are given. The information concerns aflatoxins in foodstuffs, aflatoxin M, in dairy products, aflatoxins in animal feedstuffs, and other mycotoxins in food- and feedstuffs. In comparison with the situation in 1981, limits and regulations for mycotoxins have been expanded in 1987 with more countries having legislation (proposed or passed) on the subject, more products, and more mycotoxins covered by this legislation. The differences between tolerances in various countries are sometimes quite large, which make harmonization of mycotoxin regulations highly desirable.


The following regulation to food safety is presented: Decision M (77) 5 of 2 June 1977 of the Committee of Ministers of the Benelux Economic Union concerning the application of Benelux reference methods of analysis with respect to aflatoxin in groundnuts and products derived therefrom. The Government of each of me three Benelux countries is to submit a report to the Committee of Ministers on the measures taken for the enforcement of this decision.
A selection of Belgian health laws and regulations is presented including the following which relate to food hygiene: Crown Order of 29 July 1977 amending Annexes 2 (Regenerated cellulose film) and 3 (Plastics materials and articles) of the Crown Order of 12 September 1972 concerning the manufacture and use of, and trade in, articles and materials intended to come into contact with foodstuffs and substances used as food; and Crown Order of 16 September 1977 establishing the reference method of analysis applicable to aflatoxin in groundnuts and products derived therefrom, is made in pursuance of the Law of 24 January 1977 on the health of consumers with regard to foodstuffs and other products and Decision M (77) 5 of 2 June 1977 of the Committee of Ministers of the Benelux Economic Union.
7. AFLATOXIN ANALYSIS IN GROUNDNUTS AND GROUNDNUT PRODUCTS

7.1 REVIEW

7.1.1 Sampling and Sample Preparation Procedures

Sampling is a very important step in the analysis for aflatoxin in groundnuts, and contributes significantly to its variability. This fact stems from the highly skewed nature of the distribution of aflatoxin in groundnuts (Cucullu et al. 1966, Whitaker and Wiser 1969, Coker 1984). A major problem in sampling is that the toxicity often resides in only a few contaminated kernels, and there is extreme variation in the level of aflatoxin among contaminated kernels (Whitaker et al. 1972, Coker 1989). The problem of sampling shelled groundnuts for aflatoxin has been extensively investigated in the USA and the U.K. (Whitaker and Wiser 1969, Whitaker et al. 1972, Whitaker et al. 1974, Coker 1989). These studies have shown that the highly localized aflatoxin contamination of groundnuts can be represented by the negative binomial distribution. The negative binomial probability function describes a situation with high probabilities of zero level counts and low probabilities of very high level counts. The negative binomial distribution pattern can be obtained if individual kernels in a given lot are analyzed for aflatoxin. A similar pattern can be obtained if large numbers of random sub-samples are analyzed. Thus, sampling accuracy can be maximized by taking large representative samples composed of numerous sub-samples (Whitaker et al. 1972, Coker 1984).

Several statistical models have been suggested for describing the distribution of aflatoxin in groundnuts. Whitaker et al. (1974) used the negative binomial distribution to develop a method to evaluate sampling plans used to estimate the levels of aflatoxin in shelled groundnuts. Waibel (1977) suggested an alternative statistical model, the compound Poisson-gamma distribution. This model and the negative binomial distribution have been shown to differ markedly from each other for large samples as a consequence of the statistical law known as the Central Limit Theorem (Knutti and Schlatter 1982). Brown (1984) examined the suitability of the negative binomial distribution in describing aflatoxin levels in 15 batches of Virginia Bunch and seven batches of Spanish groundnuts. He considered that an appealing aspect of this model is the property that enables distribution results to be inferred for different sample sizes. However, the substantial variability due to sub-sampling and actual analysis was thought to undermine the value of this property. In addition, the negative binomial model did not treat the large number of zero counts in a satisfactory manner. Consequently, Brown employed an alternative model which assumed a normal (Gaussian) distribution for the log of the non-zero aflatoxin levels. Jewers et al. (1986) and Jewers (1987) consider the Weibull distribution to be a suitable model for describing the distribution of aflatoxin in groundnuts.

Sampling Plans

Considerable efforts have been expended on the development of sampling plans for groundnut quality control programs in the USA and U.K. Whitaker et al. (1974) summarized the procedure for developing sampling plans, and evaluated four sampling plans used in the USA during the period 1969-1972 to control aflatoxin content in shelled groundnuts. Two statistically sound sampling plans have been developed based on the negative binomial distribution of aflatoxin in groundnuts and the aflatoxin tolerance limits established (Whitaker and Dickens 1979, Coker 1984). These are the USDA sampling plan (Whitaker and Dickens 1979) currently in use in the USA, and the so-called TPI Plan (Coker 1984) used in the U.K. These plans are used to monitor aflatoxin levels in raw groundnut kernels. Both plans require the collection of a representative large sample, composed of at least 100 incremental samples, which is then divided into three portions of equal weight. The main difference between the plans is the weight of the original sample; the TPI plan requires collection of a 10.5 kg sample, whereas the USDA plan utilizes a much larger sample of 66 kg. The objective of the USDA plan is to accept all batches of groundnuts that contain less than 25 µg kg\(^{-1}\) of aflatoxin, and reject all batches in which this level is exceeded. The TPI plan is designed to detect those batches of groundnuts that contain more than 30 µg kg\(^{-1}\), this being the maximum level of aflatoxin permitted in edible groundnuts imported into the U.K.

Development and application of sampling plans for quality control purposes require guidelines by Government authorities or Codex authorities in any country, or an agreement between countries/agencies regarding the maximum level of aflatoxin permitted, definitions of good (acceptable) and of bad (rejectable) lots, and a statement as to acceptable consumer and producer risks (Schuller et al. 1976). In the absence of this information, selection of any sampling plan will be arbitrary. Several countries, e.g., Canada and Australia, have adopted the modified USDA sampling plans for quality control purposes. Other sampling plans include those suggested by Brown (1984) for groundnuts, and Waltking (1980) for peanut butter.

The quality control of in-shell groundnuts is especially difficult. Samples cannot be collected from sacks by simple spear sampling, and the collected samples must either be decorticated prior to analysis, or the analysis result adjusted for the mass of the shells (Coker 1989). Limited work has been done on the distribution of aflatoxin in in-shell groundnuts, groundnut cake, groundnut meal, and peanut butter (Coker 1989, Waltking 1980). Sampling protocols based on statistical grounds need to be devised for these products.
Assessment of the efficiency of a sampling plan is best achieved through determining the operating characteristics of the plan. This requires a knowledge of the consumer and producer risks associated with the plan. The consumer risk is an assessment of the likelihood of accepting a batch of groundnuts whose aflatoxin content exceeds the maximum level imposed for the toxin, and the producer risk is an assessment of the likelihood of rejecting a batch which contains less toxin than the statutory maximum level. Coker (1984) and Jewers (1987) have discussed in detail these risks associated with the USDA and TP1 sampling plans. An ideal operating characteristic would be one which accepts all batches containing less than the statutory maximum level of the toxin, and rejects all the batches that equal or exceed this level, i.e., the consumer and producer risks would be zero. However, this is unlikely to happen in reality. Attempts to reduce the consumer risk by adopting a more rigorous and expensive sampling can only be achieved by increasing the producer risk (Jewers 1987).

Some importing countries use aflatoxin testing plans with accept levels lower than those of most groundnut exporting countries. For example, the U.S. aflatoxin testing plan for shelled groundnuts (kernels) has been designed with a final accept level of 25 µg kg\(^{-1}\) total aflatoxin while the accept level of a testing plan used in The Netherlands (a groundnut-importing country) is 5 µg kg\(^{-1}\) aflatoxin B\(_1\) or 10 µg kg\(^{-1}\) total aflatoxin (Whitaker and Dickens 1989). In such cases, some lots accepted by the US testing plan are likely to be rejected by the testing plan of an importing country allowing a lower accept level than 25 µg kg\(^{-1}\). The Netherlands testing plan uses four 2-kg samples and only 50-g sub-sample per sample. Whitaker and Dickens (1989) developed computer models to determine the effects of decreasing the final accept level of the U.S. testing plan on the number of lots accepted and rejected in the United States and me number of exported lots accepted and rejected by The Netherlands testing plan. Decreasing the accept level of the U.S. testing plan from 25 to 5 µg kg\(^{-1}\) increased the number of lots rejected in the U.S. by 371 % while reducing the number of export lots rejected by 51 % (Whitaker and Dickens 1989). These results indicate the high cost of applying more stringent testing plans to improve the acceptance of exported groundnuts. We have used the example of the USA as groundnut exporting country, the situation would be even more of a problem for most developing countries that export groundnuts.

Methods for Analysis of Anatoxins

Three types of assay have been developed for me detection of aflatoxins - biological, chemical and immunochemical. Early methods (biological assays) for detection of aflatoxins relied upon the reaction of the test animals to ingestion of contaminated products. Biological assays are qualitative or at best semiquantitative, and are often non-specific. They are too time consuming for routine analysis. However, biological assays such as the duckling test are useful confirmatory tests.

Assessment of the aflatoxin content of a lot. The proportion of error due to sampling becomes greater at lower aflatoxin levels. The model of Whitaker and Wiser (1969) appears to be a valuable tool for selecting and comparing sampling plans.

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Proper grinding and subdivision of the sample is obviously essential before actual aflatoxin analysis can be initiated. Ideally, a subsampling mill should simultaneously grind and subdivide the sample, and that developed by Dickens and Satterwhite (1969) is suitable for mis purpose. Alternatively, me sample may be ground and subdivided in separate operations. Coker (1984) has described several subsampling devices. Rotary sample dividers such as "spinning riffles" and the "cascade sampler" are capable of producing several representative sub-samples which can be useful in aflatoxin analysis (Coker 1984). The size of the sub-sample may vary, depending upon the method of analysis, but for most cases it ranges from 20 to 100 g. A sub-sample size of 50 g is used in most methods and appears to be the best to obtain both solvent economy and a representative sample. The "slurry technique" proposed by Velasco and Morris (1976) is a useful means of reducing sample size.

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An experimental study of the relationships of sampling, subsampling, and analytical variances as a function of the size of sample and sub-sample and number of analyses has been described by Whitaker et al. (1974). For the test procedure used in this study, sampling constituted by far me largest single source of error, followed by subsampling, and then by analysis. Tiemstra (1969) also concluded that sampling was the critical variable in determining the aflatoxin content of a lot. The proportion of error due to sampling becomes greater at lower aflatoxin levels. The model of Whitaker and Wiser (1969) appears to be a valuable tool for selecting and comparing sampling plans.

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Following isolation, purification and characterization of the aflatoxins, physicochemical assay methods were soon developed. Chemical and immunochemical assays are suitable for routine analyses, e.g., quality control and survey programs. They are invariably quicker, cheaper, more specific, and more reproducible, than biological assays, and often more sensitive.

Various quantification techniques were developed for the estimation of aflatoxins
including thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and fluorometric methods. Some of the TLC methods developed in the 1960s are still widely used. In the 1970s, various applications of HPLC were developed, and as they were more sensitive than the TLC methods, they became popular for analysis when a high degree of accuracy was required. Rapidity of analysis is sometimes more important than absolute accuracy, and the minicolumn methods were evolved with this in mind. In the late 1970s and 1980s, enzyme-linked immunosorbent assays (ELISA) were developed for aflatoxin analysis and are now replacing the minicolumn methods. From the wide range of aflatoxin analysis methods now available, it should be possible to choose method(s) suitable for specific purposes.

In this section we critically review various chemical and immunochemical methods for analysis of aflatoxins in groundnuts and groundnut products. Biological assays are discussed in a separate section.

### 7.1.2 Analytical Methods

A large number of physicochemical methods have been developed for the determination of aflatoxins in agricultural commodities including groundnuts and groundnut products (Stoloff 1972, Schuller et al. 1976. Coker 1984). Many of them are minor modifications of basic methods, adapted to specific commodities or problems, differing in respect of solvents used to extract the anatoxins, and in techniques for estimating the intensity of fluorescence. However, all analytical methods for aflatoxins basically involve the same steps: sampling, extraction, cleanup, separation, and quantitation. Observations by Sargeant et al. (1963) that, (i) aflatoxins are much more soluble in chloroform than in hydrophilic solvents, and (ii) the isolated toxins fluoresce brightly when exposed to longwave ultraviolet light have provided the basis for most of the extraction/separation and quantification procedures developed for aflatoxins. Early methods of extracting aflatoxins from groundnut products used exhaustive techniques in which the samples were extracted for several hours in a Soxhlet extractor using methanol as the solvent. Defatung and lipid removal were considered necessary before extraction. Problems in these systems were loss of the toxins during prolonged extraction, and occurrence of polar lipids, pigments, and other impurities. The remedy lay in the use of rapidly penetrating solvents, coupled with high shear-rate mixers which would reduce substrate particle size and move the solvent rapidly across each particle. The solvents used were methanol: water (Nesheim et al. 1964), then acetone: water (Pons et al. 1966) and later acetonitrile: water (Yin 1969). The ratio of organic solvent to water was adjusted to achieve the best balance between solvent penetration, extraction of interfering materials and subsequent physical separation from the substrate. In a later development water was used as the extracting solvent (Lee 1965), extraction and liquid/liquid partitioning being performed simultaneously. The trick was to remove the aflatoxins from the water quickly by partitioning to an immiscible solvent, chloroform, in which aflatoxins are more soluble. In a similar development, methanol: water/hexane was used to extract and de-fat in one step (Nesheim et al. 1964). Both the chloroform: water and the methanol: water/hexane systems contain a good fat solvent, making them particularly effective with high-fat substrates, e.g., groundnuts. In almost all the proposed methods, one of the following solvent systems is now used for extracting aflatoxins: methanol: water (55:45, v/v), acetone: water (85:15, v/v), acetonitrile: water (9:1, v/v), chloroform: water (250:25, v/v). These methods were based on the recognition that aqueous solvents were needed to penetrate hydrophilic tissues in order to effectively extract the toxins.

Purification and clean-up of aflatoxin extracts can be accomplished by liquid-liquid partitioning, followed by partitioning of impurities and their removal using column chromatography. Partitioning between solvents can occur during extraction as is the case with the solvent mixture of chloroform and water. When other aqueous solvents such as methanol-water and acetone-water are used, the toxins are partitioned into the chloroform layer after extraction. In these situations some prior concentration of the aqueous phase may be needed. Also, a prior cleanup step involving precipitation of interfering materials using lead acetate may be necessary. Lead acetate precipitation removes plant pigments, lipids, fatty acids and other unknown materials which may cause streaking on TLC plates (Pons et al. 1966). Column chromatography may also be used to effect partitioning of aflatoxins from one solvent to another and thereby purify the extract. Columns packed with silica gel, cellulose, acidic alumina, or Florisil may be used to clean and purify an extract. The sample extract is usually added to the column in chloroform or another appropriate solvent, and then washed with one or more solvents in which the toxins are insoluble or less soluble than the impurities.

After removal of impurities the toxins are eluted from the column using a solvent in which the toxin is soluble. The toxin solution can then be collected, concentrated, and examined for quantity of toxin present.

### 7.1.3 Thin-Layer Chromatography (TLC) Methods

The aflatoxins were first isolated as fluorescent spots seen under longwave ultraviolet light on paper chromatograms (Coomes and Sanders 1963). At this time TLC was emerging as an effective tool and most of the subsequent methods for aflatoxin analysis depended upon this system. TLC separation of aflatoxins has provided the basis for extremely sensitive analytical methods. The use of silica gel (Kieselgel G) coated TLC plates for the resolution of the four aflatoxins B₁, B₂, G₁, and G₂ was introduced by de longh et al. (1964) who used chloroform: methanol (98:2, v/v) for development. Subsequently, various procedures incorporated the use of TLC plates coated with Kieselgel G and several chloroform: methanol
development solvents (Pons and Goldblatt 1969). Improvement in the resolution of individual aflatoxins on silica gel coaled plates was achieved by using chloroform: acetone mixtures for TLC development (Pons et al. 1966). It should be emphasized that R, values and the degree of aflatoxin resolution are markedly influenced by variations in gel preparations, relative humidity, and other variables. Hence the need to use authentic aflatoxin standards chromatographed under the same conditions as the unknown.

The ability to segregate the aflatoxins from other interfering compounds, on the TLC plate, imparts a reasonable level of selectivity and sensitivity to TLC quantification methods. Two-dimensional TLC provides an especially powerful clean-up, but it is very time-consuming as only one sample can be accommodated on a single plate. The use of alumina-backed TLC plates has facilitated the simultaneous 'two-dimensional' clean-up of multiple samples (Coker 1984). Some confirmatory tests also involve the use of TLC. The major disadvantages of TLC are the slow speed and the lack of precision (variation between plates, expressed as coefficient of variation). Investigations on the precision and accuracy of fluorodensitometric estimations of aflatoxins suggest that the technique is a precise and accurate analytical tool, and superior to visual estimation (Pons et al. 1966, Coker 1984). However, fluorodensitometry, while a good research tool, is expensive, time-consuming, and not always practical for routine sample analysis. Several publications describe different types of fluorodensitometers (Pons et al. 1966, Beljaars and Fabry 1972, Pons 1971, Schuller et al. 1976). The application of TLC methods to the quantification of aflatoxins has been extensively reviewed by Schuller et al. (1976) and Coker (1984).

Several collaborative studies have been organized by the AOAC and AOCS to monitor the efficiency of TLC-based procedures developed for aflatoxin analysis in groundnuts and groundnut products (Eppley et al. 1968, Waltking 1970, Stack 1974, Jemmal 1973). The AOAC (1984) recommends the Contamination Branch (CB) and the Best Foods (BF) methods for aflatoxin analysis in groundnuts and groundnut products. The CB method is the standard by which other methods are judged. It is an excellent TLC method, but it has two major disadvantages: (i) it is expensive since it uses large amounts of expensive solvents, and these also create a disposal problem, and (ii) the major solvent used is chloroform and this can be a hazard to workers. The CB method simultaneously extracts the sample with water and chloroform, transfers the lipids and aflatoxins to a silica gel column, selectively elutes the fat with hexane and the pigments and other impurities with absolute ether, and finally elutes aflatoxins from the column with 3% methanol in chloroform. The BF method is faster and more economical of solvents but provides a poorer cleanup. It simultaneously extracts and defats the sample with a 2-phase system, aqueous methanol-hexane, and then partitions the aflatoxins from the aqueous phase into chloroform. After concentration of the aflatoxins in the chloroform by evaporation, both methods separate the aflatoxins by TLC and quantitate them by fluorometry on a TLC plate by visual or densitometry comparison with aflatoxin standards.

Several check sample programs have been organized to monitor the efficiency of TLC-based procedures (CB, BF, Pons and EEC) used for aflatoxin analysis in raw groundnut meal, de-oiled groundnut meal, and peanut butter (Coon et al. 1972, 1973, Friesen et al. 1980, Friesen and Garren 1982). High CVs obtained reflected lack of precision in most cases. It must be emphasized that since these studies did not specify the method of analysis to be used, they are not comparable with collaborative studies of the methods. They reflect the efficiency of the numerous participating laboratories (of widely different levels of expertise) rather than the efficiency of the methods. These programs provide laboratories around the world with a basis for judging their analytical competence and need for quality control.

The International Mycotoxin Check Sample Program, organized by the International Agency for Research on Cancer, has provided interesting statistical comparisons of the CB, BF, and EEC methods used for the analysis of naturally contaminated samples of raw groundnut meal and de-oiled groundnut meal (Friesen and Garren 1982). Over 30 laboratories participated in the program using the CB and BF methods while 10 laboratories used the EEC method. The CV varied with the method, the sample and the aflatoxin concentration. No significant differences were found among means for laboratories using these three methods for the analysis of raw groundnut meal (containing approximately 250 µg kg⁻¹ aflatoxin) for both aflatoxins B₁ and B₂. The laboratories which used the CB method found consistently higher levels of aflatoxins B₁ and B₂ than did laboratories using the BF and EEC methods. The BF method gave higher CV values (53 and 66% for B₁ and B₂, respectively) compared to the CB (48 and 49%) and EEC (37 and 39%) methods. For the assay of deoiled groundnut meal (containing approximately 70 µg kg⁻¹ total aflatoxin), the laboratories which used the BF method consistently reported significantly lower levels of aflatoxins B₁, B₂, G₁, and G₂ (approximately half of the levels) than did laboratories using the CB and EEC methods. The BF method was associated with CVs of 98, 52, 108 and 136% for B₁, B₂, G₁, and G₂, respectively. The CB method gave CVs of 60, 51, 74 and 79% for B₁ and B₂, G₁, and G₂, respectively, while the EEC method afforded CVs of 38, 33, 76 and 50% for B₁, B₂, G₁, and G₂, respectively.

The errors associated with the assay of aflatoxin in raw groundnuts by the BF method have been discussed by Whitaker and Dickens (1981). The TLC quantification step has been reported to be the major source of error (Coker 1984). Interestingly, the Smalley Check Sample Program includes the analysis of standard solutions of the aflatoxins thus facilitating the assessment of the precision of the quantification step alone. The apparent lack of precision (reported CVs of 40–81%) demonstrates the important contribution made by the quantification step to the overall efficiency of the assay. More collaborative studies involving only the
The determination of aflatoxins in vegetable oils, including groundnut oil, is usually based on partition between two immiscible solvents. Aflatoxin is extracted from the oil into a polar solvent and subsequently partitioned into chloroform (Parker and Melnick 1966, Robertson et al. 1965). Although the recoveries are acceptable, these methods are time consuming, require large quantities of solvents, and are subject to occurrence of troublesome emulsions. Miller et al. (1985) developed a simple method for determination of aflatoxins in vegetable oils which is suitable for TLC and LC quantitation. The oil sample, dissolved in hexane, is applied to a silica column and washed with ether, toluene, and chloroform; aflatoxins are then eluted from the column with chloroform: methanol (97:3, v/v). Aflatoxins in chloroform are quantitated using the two-dimensional TLC.

Several multi-mycotoxin screening methods have been developed that are capable of detecting two or more toxins in agricultural commodities including groundnuts and animal feedingstuffs (Vorster 1969, Eppley 1968, Wilson et al. 1976, Roberts and Patterson 1975, Roberts et al. 1981, Howell and Taylor 1981). Although these methods follow the basic pattern described above they vary considerably in solvents or mixtures of solvents used, in clean-up steps, and in the number and nature of toxins that can be detected. TLC has been the most widely used method for the determination of mycotoxins in the multi-mycotoxin screening methods.

**Confirmatory Tests**

Despite the use of clean-up techniques, some compounds which behave like and so may be confused with aflatoxins may still be present on TLC plates. In order to eliminate false-positives, the identity of the mycotoxin in positive samples has to be confirmed.

Confirmatory tests can be carried out directly on a TLC plate. They are generally based on the formation of a derivative which has different properties, e.g., color of fluorescence and polarity from the presumptive mycotoxin. Both mycotoxin standard and suspected sample are subjected to the same derivatization reaction. Consequently, in positive samples a derivative from the mycotoxin should appear, identical to the derivative from the mycotoxin standard. The procedures for the confirmation of aflatoxin B1 were originally developed by Przybylski (1975) and Verhulsdonk (1977), and they have been adopted as official methods by AOAC. In both methods, aflatoxin B1 is derivatized under acid conditions on the TLC plate into its hemiacetal aflatoxin B2a, which has a blue fluorescence at a lower Rf than B1. In the method of Przybylski this is achieved by superimposing trifluoroacetic-

acid (TFA) directly onto the extract spot before development. After reaction, the plate is developed and examined under UV light for the presence of the blue fluorescent spot of B2a, which can be recognized with the help of the B, standard, spotted on the same plate, which underwent the same procedure. As an additional confirmation, sulphuric acid (50%) is sprayed on another part of the plate where unreacted aliquots of extract and B, standard were developed. The sulphuric acid spray changes the fluorescence of aflatoxin from blue to yellow. This test only confirms the absence of aflatoxin, i.e., spots which do not turn yellow are definitely not aflatoxin, whereas many materials other than aflatoxin may give a yellow spot with sulphuric acid.

In the case of very "dirty" sample extracts it may be difficult to observe the hemiacetal of B1 (B2a) due to heavy background fluorescence, and the two-dimensional method of Verhulsdonk in which the so-called separation-reaction-separation technique is carried out should then be the method of choice. Separation is first made in one direction and the plate sprayed with hydrochloric acid. After reaction, separation is carried out in the second direction, under exactly identical conditions. The reaction of hydrochloric acid with aflatoxin B1 leads to the formation of a hemiacetal B2a, which has a specific Rf value, lower than that of B1. This is recognized after subsequent chromatography in the second direction. Both methods are effective for confirmation of the identity of aflatoxin G1.

**7.1.4 High Performance Thin-Layer Chromatography (HPTLC) Methods**

The application of HPTLC to the quantification of aflatoxins has been reviewed by Coker (1984). The lack of precision associated with TLC procedures arises from the introduction of possible errors during the sample application, plate development and plate interpretation steps. HPTLC procedures improve the precision by automating the sample application and plate interpretation steps, by improving the uniformity of the adsorbent layer on the TLC plate and by developing the plate under carefully controlled conditions. A fully automated sample applicator is commercially available. It is important that the spots are accurately positioned if an automated densitometer/scanner is used for quantification of aflatoxins. This technique can be used successfully in conjunction with a computing integrator to complete the automation of the plate interpretation step. Concentrations of aflatoxin as low as 5 pg (B1, G1 and M1) can be detected using HPTLC procedures. Researchers at the Overseas Development Natural Resources Institute (ODNRI) have been able to detect 30 pg of aflatoxin B1 in groundnuts using HPTLC procedures (Coker 1984); the reproducibility of the quantification was represented by the CV of 3% at an aflatoxin level of 5 μg kg⁻¹. Kamimura et al. (1985) described a simple HPTLC method which compared favorably with the CB method.
The advent of HPTLC procedures may result in renewed interest in TLC as an efficient quantification technique for aflatoxins, ideally suited to the analyses of large numbers of samples.

### 7.1.5 High Performance Liquid Chromatography (HPLC) Methods

The development of highly automated HPLC systems has afforded very precise, selective and sensitive quantification techniques for aflatoxin analysis. HPLC methods have been developed using both normal and reverse phase systems in conjunction with UV absorption and fluorescence detection techniques. Most normal phase separations have been performed on 5-10 µm silica gel columns using several multicomponent elution solvents. Some researchers e.g., Hurst and Toomey (1978), have experienced difficulties in reproducing the separation of the aflatoxins. It is difficult to maintain the water saturation of some organic solvents such as chloroform and methylene chloride, and the silica gel column may also absorb excessive water over a period of time. Consequently, several reverse phase methods have been developed where the composition of the elution solvent is more easily reproduced. Most reverse phase separations have been performed on 5 and 10 µm octadecyl (C18) columns using water, methanol and acetonitrile as elution solvents; the normal elution order is G1, B1, G2, and B2.

Reverse-phase HPLC separations of aflatoxins are more widely used than normal-phase separations. However, the fluorescence intensities of aflatoxins B1 and G1 are diminished in reverse-phase solvent mixtures so the derivatives of B1 and G1 are generally prepared before injection. The derivatives of B1 and G1 are not stable in methanol, which should be used with caution, especially in injection solvent. Acetonitrile-water mixtures do not degrade B1 and G1 rapidly and are preferred to the methanol:water mobile phases. The methods developed by DeVries and Chang (1982) and Tarter et al. (1984) use trifluoroacetic acid (TFA) derivatization and compare favorably with other methods. The hemiacetal derivatives of B1 and G1 are highly fluorescent and may be produced by pre-column treatment of the parent aflatoxins with TFA. Reverse phase HPLC with derivative formation facilitates the detection down to 5 pg of aflatoxins B1, B2, G1, and G2.

Davis and Diener (1980) developed a reverse-phase method with fluorescence detection of an iodine derivative of aflatoxin B1, and this led to the development of post-column derivatization methods which utilize the interactions between the aflatoxins and iodine. Thiel et al. (1986) have used post-column iodination to determine aflatoxins in peanut butter in conjunction with reverse-phase HPLC. A 3 µm C18 column was used with 0.01 M potassium dihydrogen phosphate:acetonitrile:methanol (39:9:7, v/v/v) and a 10670 x 0.34 µm reaction coil. The optimum mobile phase and reagent flow rates were 0.6 and 0.3 mL min⁻¹ respectively. Shepherd and Gilbert (1984) have investigated the conditions needed for the postcolumn iodination reaction to enhance fluorescence of aflatoxins B1 and G1. They have successfully applied this technique to a survey of aflatoxin in edible nuts, including groundnuts. This method has considerable potential (Coker and Jones 1988).

The sensitivity of fluorescence detection methods can be further enhanced by packing the detector flow cell with silica gel. using normal phase solvents. Panalaks and Scott (1977) developed a silica-gel-packed flow cell for fluorometric detection of aflatoxins in peanut butter. Sub-nanogram quantities of the aflatoxins could be detected using samples of spiked peanut butter. Other workers have also applied this technique to the assay of peanut butter by normal phase HPLC (Francis et al. 1982, Tarter et al. 1984). The major disadvantage of the packed cell appears to be lack of stability. The cell needs to be repacked often as the detection signal weakens with time. The recent emphasis on the reverse phase separations of the aflatoxins, coupled with the efficiency and convenience of post-column derivatization with iodine, reflects diminished interest in the silica-gel-packed flow-cell technique (Coker and Jones 1988).

Gilbert and Shepherd (1985) have used both normal and reverse phase HPLC in a survey of aflatoxin in groundnuts and their products. Sample extraction and clean-up was performed using a modified version of the AOAC CB method which involved extraction with chloroform-water, separation of the crude extract by centrifugation, and clean-up on a silica gel column. The final peanut butter extracts were dissolved in benzene-acetonitrile (98:2, V/V), while the groundnut and the groundnut-confectionery extracts were dissolved in water-acetonitrile (1:1, V/V). Normal phase HPLC was used for the assay of peanut butter using a 5 µm silica gel column and a non-quenching mobile phase of benzene-acetonitrile-formic acid, 90% (83:12:15, V/V/V). The excitation and emission wavelengths of the fluorescence detector were 360 and 425 nm respectively. Detection limits of 2 µg kg⁻¹ were reported for the individual aflatoxins. Edible nuts and nut-confectionery products were assayed by the reverse phase HPLC, using post-column iodination. A detection limit of 0.5 µg kg⁻¹ for each aflatoxin was reported.

Tarter et al. (1984) found that the application of a modified CB clean-up method to peanut butter resulted in oily extracts. Hence, they extracted their samples with aqueous methanol and sequentially partitioned the crude extract against hexane and dichloromethane. The final organic layer was dried with anhydrous sodium sulphate and subjected to a silica gel column clean-up. The resultant extract was treated with TFA to convert the aflatoxins B1 and G1 to their hemiacetal derivatives. Reverse phase HPLC was employed using a 10 µm C18 column with water-acetonitrile-methanol (70:17:17, V/V/V) as the mobile phase in conjunction with fluorescence detection. A detection limit of 0.3 µg kg⁻¹ was reported for each...
of the aflatoxins. Hurst et al. (1984) have used reverse phase HPLC in combination with TFA derealization to determine the aflatoxins in raw groundnuts. The samples were extracted with aqueous acetone in the presence of cupric carbonate. The resultant filtrate was defatted by partitioning against hexane, and the aflatoxins were then extracted into dichloromethane. The clean-up step was completed using a silica gel cartridge.

No collaborative studies of these HPLC methods have been reported. A few check sample programs have involved the use of HPLC methods for the estimation of aflatoxins in peanut butter and de-oiled groundnut meal (Friesen et al. 1980, Friesen and Garren 1982). High CVs (49-66%) obtained reflected poor interlaboratory precision of the HPLC determination of aflatoxins.

These HPLC methods involve the use of complex extraction and clean-up steps. It is emphasized that the development of assay methods which exhibit a high interlaboratory precision will only occur when the extraction and clean-up procedures have been considerably simplified. Two recent developments - (i) the development of bonded-phase clean-up cartridges, and (ii) the development of automated liquid-handling equipment should assist in attaining these objectives (Coker and Jones 1988). A comprehensive review of HPLC-based methods for the determination of mycotoxins has been given by Coker and Jones (1988).

7.1.6 Minicolumn Methods

For convenience in large scale screening for the detection of aflatoxins, minicolumn methods have considerable advantages. Compared with TLC methods the minicolumn methods are rapid, less expensive, and simpler to use. They are qualitative in their capacity to detect aflatoxins, but cannot accurately distinguish and quantitate the individual aflatoxins (B₁, B₂, G₁, and G₂).

The first rapid screening procedure using a minicolumn for aflatoxin detection in groundnuts was reported in 1968 (Holaday 1968); since then, improved procedures have been widely used to detect aflatoxin in groundnuts (Davis et al. 1980. Holaday 1976. Holaday and Barnes 1973. Holaday and Lansden 1975, Romer 1975). Minicolumns are typically 20 cm long glass tubes with an internal diameter of approximately 6 mm. They may be packed with either a single adsorbent or a combination of several adsorbents. The toxin, after adsorption onto the minicolumn, is detected by observing its natural fluorescence under UV light. The state of the art for minicolumn chromatography has been comprehensively reviewed by Holaday (1981).

The methods developed by Davis et al. (1980). Holaday (1976). and Holaday and Lansden (1975) are rapid screening procedures for detecting aflatoxin in groundnuts. Only a few collaborative studies have been performed to evaluate the efficiency of these methods in detecting aflatoxins in groundnuts (Shotwell and Holaday 1981). The Holaday-Velasco method has been adopted official first action for groundnuts (Official Methods of Analysis, 1980). This method combines the speed and simplicity of the Holaday extraction and clean-up with the sensitivity of the minicolumn originally described by Velasco. The detection limits by these methods range from 5 to 10 µg kg⁻¹ total aflatoxins.

The minicolumn method of Romer (1975) has also been adopted by the AOAC for aflatoxin in groundnuts and groundnut products, cottonseed products, corn, edible nuts, and mixed seeds (Stoloff 1980). This method is widely used as a screening test for aflatoxin in groundnuts. Briefly, aflatoxins are extracted with acetone : water (85:15, V/V), and interferences are removed by adding cupric carbonate and ferric chloride gel. The aflatoxins are then extracted from the aqueous phase with chloroform and the chloroform extract is washed with a basic aqueous solution to effect further purification. The chloroform extract is applied to the top of a minicolumn containing successive layers of neutral alumina (top), silica gel, and Florisil (bottom), will) calcium sulfate drier at both ends. The column is developed with chloroform : acetone (9.1, V/V), trapping the aflatoxins as a tight band at the top of the Florisil layer where they can be detected by their blue fluorescence under UV light. The fluorescence of the band under UV light can be measured directly by inserting the developed minicolumn into the fluorotoxinimeter which may be calibrated to give a direct read-out of the total aflatoxins (in µg kg⁻¹) in the sample. The fluorotoxinimeter is a rapid, reasonably priced quantification system that merits greater use.

Reference columns are prepared using chloroform extracts from a non-contaminated sample spiked with a suitable ratio and level of aflatoxins. Packed sample and reference minicolumns are commercially available (source: Myco-lab Co., P.O. Box 321, St. Louis, MO 63017. USA).

An advantage of this screening method is that the remaining chloroform extract is sufficiently clean to be used for a TLC presumptive lest should the screening test prove to be positive. Groundnut oil can also be analyzed by the Romer method using a simple modification of the extraction procedure. The Romer's method has been found to extract considerably lower amounts of aflatoxin B₁ from groundnuts compared with the BF and CB procedures (Mehan et al. 1985). The two alkali treatment steps using NaOH and KOH during clean-up procedures probably result in low extraction efficiency of Romer's method.

The Romer screening method has been subjected to a collaborative study (Romer and Campbell 1976). Considering only the four groundnut and groundnut product samples used, 94% of the participating laboratories correctly identified the negative samples, 60% the 5 µg kg⁻¹ samples, 95% the 10 µg kg⁻¹, and 100% the 20 µg kg⁻¹.
Coker (1984) has briefly discussed factors that can affect the chromatographic behaviour of the aflatoxin band in the minicolumn, especially when analyzing groundnuts for aflatoxins. The use of acetone-water and methanol-water extraction solvents can result in the presence of significant levels of acetone/methanol in the final 'chloroform' extract and these can interfere with the reproducibility of the aflatoxin band. Although the aflatoxin appears as a discrete band on the minicolumn, a certain proportion is invariably washed from the column during the elution step. The degree of this leaching of the aflatoxin depends on the variety of groundnut and/or its source, presumably because of variations in the composition of the groundnut constituents (Coker 1984).

Madhyastha and Bhat (1984) developed a minicolumn confirmation method for aflatoxins. They confirmed the identity of aflatoxins on the developed minicolumn by applying 20% H$_2$SO$_4$, 20% HCl, or TFA in 20% HNO$_3$. All acids changed the fluorescence from blue to yellow, with the TFA in 20% HNO$_3$ having the lowest detection limit.

### 7.1.7 Immunochemical Methods

In recent years, several simple, sensitive and specific radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) of aflatoxins have been developed, using polyclonal and monoclonal antibodies against the mycotoxins. ELISA in particular has emerged as one of the major immunoassays for aflatoxins in groundnuts and groundnut products (El-Nakib et al. 1981, Chu 1984, Morgan et al. 1986). ELISA technique is well suited to large scale screening for the detection of aflatoxins; it is more rapid, more sensitive, and simpler to use than conventional analytical procedures. ELISA does not require extensive extraction and clean-up steps. Several commercial ELISA-applied aflatoxin assay kits, using highly specific monoclonal antibodies, are currently being marketed. Such kits are easily adaptable for analyzing large numbers of samples, and are directly applicable to assaying methanol-water extracts of a wide range of agricultural commodities. The recent commercial success of ELISA kits shows that the value of this technology is being increasingly recognized.

#### Antibody Production

Aflatoxins are not antigenic and must be conjugated to a protein (this is referred to as hapten) before immunization. Bovine serum albumin (BSA) is the most commonly employed carrier protein. Conjugation of a mycotoxin to a protein is complicated by the functional groups present in the molecule. Aflatoxins lack a reactive group, and a reactive carboxyl or other group must be introduced to the toxin molecule. Methods for preparation of mycotoxin conjugates have been developed, and specific antibodies against aflatoxin B$_1$ and B$_2$A have been produced (Chu et al. 1977, Chu and Ueno 1977, Gaur et al. 1981, Pestka and Chu 1984, Morgan et al. 1986). For instance, the aflatoxins B$_2$A or B$_1$ dihydrodiol are converted to their dialdehydic phenolate ion forms (Gaur et al. 1981, Pestka and Chu 1984) and B$_1$ to the putative 8,9-epoxide (Groopman et al. 1988) so they can react directly with a carrier protein. Alternatively, functional moieties such as carboxymethyloxime or hemisuccinate groups can be introduced, and the derivative can be conjugated to a protein by carbodiimide, mixed anhydride, or hydroxysuccinimide methods (Chu and Ueno 1977).

The most frequently used method for production of polyclonal antibodies is multiple-site immunization of rabbits with the hapten, using a mixture of hapten and complete Freund's adjuvant (Chu and Ueno 1977). Antibodies having sufficient titer are generally obtained 5 to 7 wk after the initial immunization. Booster injections are essential if the titers are low (Gaur et al. 1981). Polyclonal antisera are less expensive than monoclonals and often contain a subpopulation of high affinity antibodies (Pestka 1988).

The specificity of an antibody is determined primarily by the antigens used in its production. Before performing any immunoassay, the specificity of an antibody preparation must be tested thoroughly by a competitive binding assay utilizing an appropriate antigen. When rabbits are immunized with aflatoxin, conjugated to a protein utilizing cyclopentane portion of the molecule, such as the carboxymethyloxime of aflatoxin B$_1$ (Chu and Ueno 1977), the antibodies generally recognize the dihydrofuran portion of the molecule. When conjugates are prepared utilizing the dihydrofuran portion of the aflatoxin molecule, such as aflatoxin B$_2$A (Gaur et al. 1981), the antibody recognizes the cyclopentane ring. Since such diversity in antibody specificity exists, different antibody preparations should be used for analysis of different aflatoxin metabolites (Chu 1984). An alternative approach to polyclonals is the development of stable hybridoma cell lines which secrete reagent-quality monoclonal antibodies to aflatoxins (Pestka 1988). This is achieved by isolating splenic lymphocytes from an immunized mouse with hapten and fusing these with a myeloma cell line. Following a series of selection and screening steps, an "immortalized" clone that consistently produces antibodies of desired affinity, specificity, and performance characteristics can be isolated. Monoclonal antibodies give more reproducible results than polyclonal antibodies. Nevertheless their production is very costly, time-consuming, and requires sophisticated facilities.

In earlier studies polyclonal antibodies were used but more recently monoclonal antibodies have been used in ELISA for the assay of aflatoxins (Groopman et al. 1988, Sun et al. 1983).
The effectiveness of the antibodies used in different immunological tests is dependent upon their specificity and titers. Specificity provides information on cross-reactivity of antibody with different toxin analogs (quality). High titered antibodies are economical and are likely to give more reproducible results. The antibody specificity is measured by a competition binding assay with different structurally related analogs, while the titers are measured by the amount of a specific marker ligand bound to the antibody. Chu (1989) has discussed in detail the specificity of antibodies for aflatoxins prepared by different approaches.

**Immunoaassays**

Three immunoassays - RIA, ELISA, and affinity binding assay, have been developed for the analysis of mycotoxins in various foods and feeds. These have been used for the analysis of aflatoxin in groundnuts and groundnut products. Both RIA and ELISA techniques are based on the competition between the free aflatoxin in the sample extract and the labelled aflatoxin in the assay system for the specific sites of antibody molecules. In the RIA a radioactive aflatoxin is used, whereas a toxin-enzyme conjugate is used in the ELISA. The affinity binding assay involves the use of an antibody column that specifically traps the aflatoxin in the column (Sun and Chu 1977). The toxin can then be eluted from the column for subsequent analysis.

**Radioimmunoassays (RIA)**

The RIA procedure involves simultaneous incubation of the sample extract or known standard with a specific antibody, and a constant amount of labelled toxin. Free toxin and bound toxin are then separated by an appropriate technique, and the radioactivity in these fractions is then determined (Chu 1984). The toxin concentration of the unknown sample is determined by comparing the results with a standard curve established by plotting the ratio of radioactivities in the bound fraction and free fraction vs $\log_{10}$ concentration of non-labeled standard toxin. Analysis of aflatoxin B$_1$ has been achieved by the double antibody technique (Langone and Van Vunakis 1976). RIA can detect 0.25-0.50 ng of purified aflatoxin per assay in a standard preparation. The lower limit for aflatoxin detection in food or feed samples is from 2 to 5 µg kg$^{-1}$.

Radioimmunoassays have the disadvantages that (i) RIA requires labeling of the toxins with tritium, which is difficult and expensive, (ii) radioactive waste disposal is a problem, and a scintillation counter is expensive, and (iii) only a small number of samples can be screened in a short period of time. For these reasons interest in RIA has diminished.

**Enzyme-Linked Immunosorbent Assays (ELISA)**

Two types of ELISA have been used for the analysis of aflatoxins - (i) direct ELISA, and (ii) indirect ELISA. Both are heterogeneous competitive assays which involve the separation of free (unreacted) toxin in liquid phase from the bound toxin in solid-phase. Direct competitive ELISA involves the use of aflatoxin-enzyme conjugate, whereas indirect ELISA involves the use of a protein-aflatoxin conjugate and secondary antibody such as goat anti-rabbit IgG to which an enzyme has been conjugated. Although horseradish peroxidase (HRP) is the most commonly used enzyme for conjugation, other enzymes such as alkaline phosphatase have been used (Chu 1984, Anjaiah et al. 1989). Of several methods used in the conjugation of mycotoxin to HRP, water soluble carbodiimide appears to be the least likely to denature proteins.

**Direct Competitive ELISA**

In this assay, specific antibody is first coated to a solid phase such as a microliter plate (Chu 1984). The sample extract or standard toxin is generally incubated simultaneously with enzyme conjugate or separately incubated in two steps. After appropriate washings, the amount of enzyme bound to the plate is determined by incubation with a specific substrate solution. The resulting color is then measured spectrophotometrically or by visual comparison with standards. Because this assay is based on competition for antibody binding sites, free toxin concentration is inversely related to antibody-bound enzyme conjugate.

Several direct competitive ELISA methods have been reported for the analysis of aflatoxin in groundnuts and groundnut products (El-Nakib et al. 1981. Chu et al. 1987, Ram et al. 1986, Ueno 1985). In earlier studies these assays took rather a long time to complete, and had large CVs for each sample (Chu 1986). Recently, Chu et al. (1987) have developed a new ELISA protocol that takes approximately an hour to complete quantitative analysis of aflatoxin B$_1$ in groundnuts and maize. In this assay, samples are extracted with 70% methanol in water containing 1% dimethylformamide (1 g of groundnuts in 5 mL of extraction solvent), diluted with assay buffer (sodium-phosphate buffer, pH 7.2, 0.01 mol L$^{-1}$ with 0.15 mol L$^{-1}$ of NaCl) to a final concentration of 3.5% methanol and then directly subjected to the following ELISA procedure: (a) 50 µL of diluted sample or aflaiaxin standard is added to each well of a ELISA plate followed by 50 µL of aflatoxin-HRP conjugate, and the plate is then incubated at room temperature (25-28°C) for 30 min, (b) the wells are washed with 300 µL of PBS-tween 3 times, (c) 100 µL of enzyme substrate is added, followed by incubation at room temperature for 10 min; and (d) 100 µL of stopping reagent is added and absorbance measured at 490 nm. High analytical recovery (95.4%) of aflatoxin B$_1$ added to peanut butter has been reported by this method (Chu et al. 1987). The interwell and interassay CV has been
reported to be 10% or less at the 20 µg kg\(^{-1}\) level and above. This protocol has been used in the analysis of naturally occurring aflatoxins in groundnuts. Excellent agreement for aflatoxin levels in over 30 samples of naturally contaminated maize, mixed feed and groundnuts have been found between the ELISA results, and the results obtained from different laboratories using TLC or other analytical methods (Chu 1989). Improvement in precision has been reported when an additional extraction step with hexane was incorporated into the protocol before ELISA (Chu 1989). A similar protocol with longer incubation time for the analysis of aflatoxin B\(1\) in groundnut products was described by Ram et al. (1986) who included a defatting procedure for the peanut butter samples before HLISA. Amounts of aflatoxin B\(1\) as low as 0.01 µg kg\(^{-1}\) in peanut butter could be determined (Ueno 1985).

The use of various forms of ELISA as screening methods for anatoxins has increased rapidly (Chu et al. 1987, Mortimer et al. 1987). One method using microtiter plates was collaboratively studied (Park et al. 1989a, Park et al. 1989b), and was adopted official first action by AOAC for screening for aflatoxin B\(1\) in cotton seed and mixed feed (Changes in Official Methods of Analysis 1989). This method has also received interim official first action approval for aflatoxin B in corn and groundnut products (Park et al. 1989). A similar method has been adopted official first action for screening for aflatoxins (B\(1\), B\(2\), G\(1\), and G\(2\)) at ≥ 20 ng g\(^{-1}\) in corn and raw groundnuts (Trucksess et al. 1989).

**Indirect ELISA**

In the indirect ELISA, an aflatoxin-protein conjugate is coated to the microplate. The plate is then incubated with specific rabbit antibody in the presence or absence of the homologous mycotoxin. The amount of antibody bound to the plate coated with mycotoxin-protein conjugate is then determined by reaction with commercially available goat anti-rabbit IgG conjugated to an enzyme followed by addition of a suitable substrate. In this assay, toxin in the sample and toxin in the solid-phase compete for the same binding site with the specific antibody in the solution. Indirect ELISA has been used for the analysis of aflatoxins in groundnut products, using different proteins as carriers for the conjugation of aflatoxin (Fan and Chu 1984, Morgan et al. 1986). Morgan et al. (1986) used an indirect ELISA for the analysis of 18 peanut butter samples obtained from retail stores and found that the aflatoxin levels ranged from less than 1 µg kg\(^{-1}\) in eight samples to as high as 775 µg kg\(^{-1}\) in one sample. In this procedure, aflatoxin-protein conjugate (Keyhole limpet haemocyanin (KLH)- aflatoxin B\(1\) conjugate) is coated onto the microtiter plate (Nunc Immunoplate 1). Sample or standard aflatoxin B\(1\) is added to the wells followed by an aliquot of anti-aflatoxin antibody. The amount of antibody bound to the solid phase is detected by the addition of goat anti-rabbit IgG-conjugated to alkaline phosphatase followed by reaction with p-nitrophenoxy phosphate to give a colored product. The aflatoxin B\(1\) content of the sample is determined by reference to a standard curve.

The sensitivity of the indirect ELISA is comparable to that of the direct ELISA. Because only small amounts of antibody are required for the indirect ELISA, this method has been used frequently for monitoring the antibody titers of hybridoma culture fluids for the screening of monoclonal antibody-producing cells (Groopman et al. 1988) in addition to toxin analysis.

Of the two types of ELISA, direct ELISA is usually preferred for analytical purposes because it utilizes a single conjugated protein, requires one less incubation step and one less washing step, and exhibits substantially less variability than the indirect ELISA (Pestka 1988). The improvement and extent of use of ELISA procedures for detecting aflatoxins depend on the availability of specific antibodies. It is also important to develop simple, effective extraction procedures specially suitable for ELISA.

Although several solid phases, such as polystyrene, polyvinyl, irradiated ELISA plates, tubes, and Terasaki plates have been used for ELISA, the microtiter plates are preferred because of the availability of automated throughput systems.

**Commercial ELISA-Kits**

Several companies in the UK, France, Japan, and USA have produced ELISA-applied aflatoxin assay kits on a commercial basis for routine use as a food safety and quality control tool in analytical laboratories. The commercially available ELISA kits for quantitative analysis of aflatoxin(s) in groundnuts are described in Table 5. Only the "Biokits" uses indirect ELISA procedure, while all other kits involve direct competitive ELISA. Commercial ELISA kits for aflatoxin B\(1\) have been reported to perform favorably in routine groundnut analysis in some comparative studies (Wilson et al. 1987, Cole et al. 1987). In another study, the sensitivity of the kits used was reported to be higher than that of all other methods (Goto and Manabe 1988). Patey et al. (1989) evaluated three commercial ELISA kits (Quantitox, Aflasure, and Biokits) for analysis of aflatoxin in peanut butter samples in a collaborative study involving 16 United Kingdom analytical laboratories. The Biokits and Aflasure kits were reported to give higher mean values of aflatoxins present than the levels obtained by liquid chromatography analysis. The Biokits appeared more precise than the other two kits. The relative amounts of the different aflatoxins must be taken into consideration when the performance of one kit is compared with that of another since the aflatoxin specificity of each kit is different. For instance, the Biokits assays concentrations of aflatoxins B\(1\), B\(2\), G\(1\), and G\(2\), the Aflasure kit assays aflatoxins B\(1\) and B\(2\), and the Quantitox kit assays aflatoxin B\(1\) alone.
Rapid Immunoscreen Tests

Some of the ELISA procedures have been designed as screening methods with the objective of determining the aflatoxin level ≥ 20 µg kg⁻¹ (e.g., Agri-Screen test for aflatoxin B₁ - ELISA kit from the Neogen Corporation, 620 Lesher Place, Lansing, Michigan 48912-1509, USA). These methods are designed for use in situations that require short analysis time, simple operation, and relatively low cost.

Another approach is to immobilize the antibody on a paper disk mounted in a plastic card (Immunoassay Quick-card Test) (Cole 1987, Goto and Manabe 1988). The principle of the reaction in this assay format is similar to that of direct ELISA. Experimentally, sample extracts (a few drops) are first applied to the test spot (paper disk) in the plastic card, then aflatoxin-enzyme conjugate is applied, and finally the substrate solution is added. The absence of color at the spot indicates the sample contains aflatoxin as compared with the negative control spot where a bright blue color appears. Cole et al. (1987) and Dorner and Cole (1989) have found this method a convenient way to screen for aflatoxin in groundnuts. Nevertheless this method is not suitable for quantitative estimation of aflatoxins.

Affinity Column Immunoassay

The application of affinity column chromatography for immunoassay of aflatoxins was first reported by Sun and Chu (1977). and recent advances in instrumentation have led to its increasing adoption (Chu 1989). In practice, aflatoxin extracted from the sample is subjected to a disposable affinity column containing anti-aflatoxin Sepharosegel. After washing, aflatoxin is removed from the column with methanol and its fluorescence determined. The affinity column merely serves as a separation and concentration tool for the analysis. A company in the U.K. (May and Baker Diagnostics Ltd.) is currently marketing an affinity column-based ELISA kit (Aflatest P) for determining aflatoxins in groundnuts.

The high cost of commercially available ELISA kits may limit their use in analytical laboratories in developing countries. The major application for ELISA procedures at present appears to be screening for aflatoxin below a predetermined level. More development is required before ELISA techniques can be generally adopted for critical quantification. Though some studies have shown that results obtained from ELISA are comparable to TLC and HPLC methods, more comparative studies are required. Methods need to be developed that will determine B₁, B₂, G₁, and G₂ individually or collectively.

### Table 5. Commercially available ELISA kits for analysis of aflatoxin(s) in groundnuts

<table>
<thead>
<tr>
<th>Test/character</th>
<th>Antibody</th>
<th>Specificity</th>
<th>Detection</th>
<th>Quantification</th>
<th>Extraction solvent</th>
<th>System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitox (U.K.)</td>
<td>Monoclonal</td>
<td>Aflatoxin B₁</td>
<td>450 nm absorption</td>
<td>2-30 µg kg⁻¹</td>
<td>Methanol : water (55:45, V/V)</td>
<td>microtitre plate</td>
</tr>
<tr>
<td>Afla-check Kit (Japan)</td>
<td>Monoclonal</td>
<td>Aflatoxin B₁</td>
<td>492 nm absorption</td>
<td>2-40 µg kg⁻¹</td>
<td>Methanol : water (55:45, V/V)</td>
<td>microtitre plate</td>
</tr>
<tr>
<td>Transia Kit (France)</td>
<td>Monoclonal</td>
<td>Allatoxins B₁, B₂, G₁, and G₂</td>
<td>410 nm absorption</td>
<td>1-30 µg kg⁻¹</td>
<td>Methanol : water (80:20, V/V)</td>
<td>microtitre plate</td>
</tr>
<tr>
<td>Aflasure B Kit (U.K.)</td>
<td>Monoclonal</td>
<td>Aflatoxin B₁ and B₂</td>
<td>450 nm absorption</td>
<td>2-200 µg kg⁻¹</td>
<td>Acetonitrile : water (60:40, V/V)</td>
<td>microtitre plate</td>
</tr>
<tr>
<td>Biokits (U.K.)</td>
<td>Monoclonal</td>
<td>Allatoxins B₁, B₂, G₁, and G₂</td>
<td>414 nm absorption</td>
<td>2-200 µg kg⁻¹</td>
<td>Acetonitrile : water (50:50, V/V)</td>
<td>microtitre plate</td>
</tr>
</tbody>
</table>

1. May & Baker Diagnostics Ltd., 187 George Street. Glasgow G1 1YT, U.K.
3. TRANS1A, 8 rue Saint Jean de Dieu - 69007 Lyon. France.
5. Thames Genelink Ltd., Deeside, Clwyd CH5 2NT. U.K.
7.1.8 Biological Methods

Tests developed for the bioassay of aflatoxins have utilized bacteria, brine shrimp, chick embryos, day-old ducklings, and albinism in several crop plants. Bioassays for aflatoxins are frequently carried out as confirmatory tests following chemical identification.

1. The Day-old Duckling Bioassay

Toxicity to day-old ducklings is the most widely utilized bioassay technique for aflatoxins in various commodities. It has been used since the outbreak of “Turkey X” disease first highlighted the aflatoxin problem (Aspin and Camaghan 1961). In standardizing this test, solutions of aflatoxins of known concentration may be administered as single doses directly into the stomach of the duckling, or they can be added to the diet in single doses or spread over time, usually for 5 days. This assay is unique in that the specific response of bile duct proliferation occurs within a few days of administering a single dose of aflatoxin (Butler 1964). The minimum concentration of aflatoxin B1 required to produce bile duct proliferation is 0.04 mg Kg⁻¹ (Wogan 1966). Aflatoxins are usually administered in propylene glycol, but can also be given in dimethyl formamide, methanol or in wheat germ oil.

2. The Chick Embryo Bioassay

The development of the chick embryo technique as a definitive bioassay for aflatoxin was reported by Verrett et al. (1964). Fertile eggs from in-bred White Leghorn hens are injected with the toxin prior to incubation. The air cell location is marked and the surface is sterilized by swabbing with alcohol prior to drilling a hole (about 5 mm in diameter) into the shell covering the center of the air cell. The toxin solution is injected into the air cell and deposited onto the inner egg membrane. The hole is then sealed with a small piece of adhesive cellophane tape. The eggs are candled daily from the fourth day of incubation; all non-viable embryos are removed. Death of the embryo is counted as a positive test. The major disadvantage is that the embryos, while being very sensitive to aflatoxins, are not specific in their response and may be killed by several other compounds.

The chick embryo test is simple to perform, is relatively inexpensive, and is very sensitive. The assay is reproducible and typical lesions are observed in the embryos with subacute levels of aflatoxin B1 (less than 0.1 µg egg⁻¹). The major disadvantage of this test is that the embryos, while being very sensitive to aflatoxins, are not specific in their response and may be killed by several other compounds.

3. Guinea pig Bioassay

The guinea pig is highly susceptible to aflatoxin toxicity. Butler (1966) reported an LD₅₀ of aflatoxin B1 at a concentration of 1.4 mg Kg⁻¹ body weight with a 95% confidence limit of 1.05-1.8 mg Kg⁻¹. This sensitivity is very similar to that of ducklings. The main hepatic lesion reported in guinea pig is also similar to liver lesions in the duck, with biliary proliferation 3-4 days after injection of the toxin.

4. The Brine Shrimp Bioassay

Several workers have reported the brine shrimp bioassay as a convenient test for aflatoxin (Brown et al. 1968, Tanaka et al. 1975, 1979, 1982). In the brine shrimp bioassay, a suspension of newly-hatched brine shrimps is placed in a small petri plate and the total number of shrimps present is counted. A solution of the toxin is then added, and the plate incubated at high humidity for 24-48 h at 30° C. The percentage of shrimps killed is then determined. The test is non-specific and is a good general bioassay for toxic plant constituents.

Microbiological Assay Techniques

Bacillus megaterium and B. stearothermophilus have been used for detecting and assaying aflatoxins (Clements 1968, Reiss 1975). In the B. megaterium assay, the inoculum of the bacterium is uniformly distributed in the tryptone-yeast-glucose (TYG) agar medium. Paper discs containing toxin solutions are placed on the surface of the medium and plates are incubated at 37° C and then checked at intervals for production of inhibition zones around the discs. The inhibition zones indicate the presence of aflatoxin.

B. stearothermophilus is used in a rapid confirmatory test for aflatoxin B1. In this bioassay, the bacterial culture is placed in tubes containing toxin solutions. The cultures are then transferred to tubes of dextrose tryptone broth containing bromocresol purple as pH indicator and incubated at 55° C in the dark for 24-48 h. Change in color of the medium from purple to yellow indicates the absence of aflatoxin. No color change in the nutrient medium means that the toxin is present. Aflatoxin levels as low as 0.01 µg can be detected (Reiss 1975).
Plant Bioassays

Aflatoxins elicit biochemical responses in plant tissues similar to those produced in animal systems. Several plant bioassays have been developed for aflatoxins (Schoental and White 1965, Kang 1970, Mehan and Chohan 1974). Schoental and White (1965) reported a bioassay of aflatoxin based on 'Virescence' or 'albinism' in seedlings of watercress (Lepidium sativum L.). Kang developed a bioassay based on inhibition of germination of okra seeds (Abelmoschus esculentum L.) or chlorophyll inhibition in the cotyledonal leaves depending on the concentration of aflatoxin. This bioassay is very simple and can be used for screening A. flavus isolates for ability to produce aflatoxins, and also for detecting aflatoxins in various commodities (Kang 1970, Mehan and Chohan 1973). Two other bioassays for aflatoxin based on chlorosis and albinism in cotyledonal and primary leaves of Raphanus sativus and Sorghum vulgare seedlings have been reported (Mehan and Chohan 1974).

The role of bioassays in routine laboratory screening of aflatoxins in agricultural commodities appears to have been under-valued. This may have been due to the rapid development of the physicochemical and immunochemical tests which tend to give more specific information as to the particular toxin present.

7.2 BIBLIOGRAPHY

7.2.1 AFLATOXIN ANALYSIS IN GROUNDNUTS AND GROUNDNUT PRODUCTS


The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.


This paper gives a brief background of the occurrence and chemistry of mycotoxins (aflatoxins, ochratoxin A, citrinin, patulin, zearalenone, and trichothecenes) in foods and feeds. The importance of sampling, sample preparation and assay techniques in the analysis of the mycotoxins in various agricultural commodities is discussed. High performance liquid chromatography (HPLC) methods for the analysis of mycotoxins are discussed. Future developments in the HPLC analysis of mycotoxins are anticipated.


Procedures are discussed for sampling groundnuts and groundnut products and preparing those samples for aflatoxin analysis. Analytical methods available for the analysis of foods and feedstuffs for aflatoxin are given.


This review discusses the major groups of mycotoxins found in food and feeds.
methods for their analysis and the regulatory action taken by some countries for maximum permissible levels of mycotoxins in food and feeds.


This report reviews some of the analytical methods available for the analysis of foods and feedstuffs for aflatoxin, particularly those which are suitable for use in laboratories in developing countries. Full details of procedures for sampling, extraction, separation and estimation of aflatoxins are given. Methods for the determination of the concentration and purity of aflatoxin standards and the recommendations for the bulk sampling of commodities for aflatoxin analysis are also given.


This paper reviews the sampling plans and collaboratively studied methods of analysis for aflatoxins in foods and feeds. Aflatoxins are the only food contaminants being monitored routinely on an international scale with methods operating at the order of a magnitude of 10 µg kg⁻¹. At this level, methods of analysis which can achieve coefficients of variation of 30-40 % with recoveries of 70 % or greater in interlaboratory collaborative studies can be considered eligible for reference status. In most cases, sample reproducibility is the variable limiting the reliability of methods of analysis. The inherent uncertainty of the identity of chromatographically separated entities requires the application of confirmatory tests to verify that the characteristics measured result from the presence of aflatoxin. The methods are also inoperable without a verification of the identity, purity, and concentration of the reference standards used. Screening methods which reliably eliminate negative samples from further consideration are indispensable for the practical operation of monitoring programs.


Developments in methodology for analysis of mycotoxins in foods are reviewed, covering aflatoxins, Alternaria toxins, citrinin, cyclopiazonic acid, ergot alkaloids, ochratoxins, penicillic acid, sterigmatocystin, trichotheccenes, and zearalenone. As well as papers on methodology, oilier recent papers of interest on health significance and occurrence of individual mycotoxins are covered.

7.2.2 Sampling and Sample Preparation Procedures


Fungal species that produce aflatoxins (Aspergillus flavus and Aspergillus parasiticus) are common in field soils, and invasion of groundnut kernels by these fungi frequently takes place during pod development. In environments where conditions are suboptimal, entire kernels may not become invaded by these fungi, however, some colonization of testa and outer layers of cotyledons may occur. If mycotoxin-producing fungi become established in these external kernel layers, aflatoxin is most likely to be concentrated in the same areas. A technique was developed for sampling only the testa and outer cotyledary tissue of raw groundnuts. This involved abrading of kernels up to 4 % of their external tissue. Fungi established in this tissue could be assessed by plating groundnut particles on selective media plates. Aflatoxin levels were measured in abraded particles and chopped whole kernels from the same groundnut samples. Toxin levels in whole kernels samples were 9.2 % of levels measured in panicles abraded from external kernel tissue. When aflatoxin concentration was less than 5 µg kg⁻¹ in whole kernels, it was often found to be as high as 50 µg kg⁻¹ in external tissue of the kernels. This technique allows measurement of low aflatoxin levels in groundnut lots.


The suitability of the negative binomial distribution in describing aflatoxin levels in compositied samples of groundnuts was studied for two varieties of groundnut grown in Kingaroy, Queensland, Australia during the 1981-1982 season. The model was inadequate and an alternative is suggested : the parameters of this model were fitted to the current data and to previously published data for comparisons. Results obtained have implications in the choice of practical acceptance of sampling plans which are discussed in terms of the current Australian testing level.


Effective mycotoxin survey and monitoring programmes are discussed. The US aflatoxin monitoring plan for groundnut processing includes import, export and manufacturing quality control points of testing, sampling, sample preparation, and types of test, e.g., screening and multidetection chemical and confirmation tests, are discussed.
This paper reviews the work carried out at the Overseas Development Natural Resources Institute (ODNRI), London, on the development of efficient sampling, sample preparation, aflatoxin analysis, and chemical detoxification procedures. The control of aflatoxin in groundnut products requires a combination of quality control and decontamination/detoxification procedures. The use of selected mathematical models to describe the distribution of aflatoxin in groundnut kernels, roasted groundnuts, peanut butter, and groundnut cake has been investigated for developing statistically sound sampling plans for these commodities. A subsampling mill has been developed, in collaboration with a UK company, which enables representative, comminuted subsamples to be rapidly produced from large samples of groundnut kernels. Methods have been developed for the accurate analysis of the aflatoxin content of groundnut products using bonded-phase clean-up procedures in combination with high performance liquid chromatography (HPLC) and high performance thin-layer chromatography (HPTLC) quantification methods. The application of enzyme-linked immunosorbent assay (ELISA) methods to the analysis of aflatoxin in peanut butter has also been extensively examined. A procedure for the detoxification of groundnut cake using ammonia gas at high temperatures and moderate pressures has been developed.

Traditional means of sampling and sample preparation of agricultural crops and foodstuffs are generally not adequate for mycotoxin analyses. The main reason for this is that mycotoxin contamination is usually of a heterogeneous nature and this presents problems in the preparation of a homogenous sample for analysis. Sampling plans, sampling equipment, and sample preparation are discussed.

Methods have been developed, in collaboration with a UK company, which enables representative, comminuted subsamples to be rapidly produced from large samples of groundnut kernels. Models to describe the distribution of aflatoxin in groundnut kernels, roasted groundnuts, peanut butter, and groundnut cake have been investigated for developing statistically sound sampling plans for these commodities. A subsampling mill has been developed, in collaboration with a UK company, which enables representative, comminuted subsamples to be rapidly produced from large samples of groundnut kernels. Methods have been developed for the accurate analysis of the aflatoxin content of groundnut products using bonded-phase clean-up procedures in combination with high performance liquid chromatography (HPLC) and high performance thin-layer chromatography (HPTLC) quantification methods. The application of enzyme-linked immunosorbent assay (ELISA) methods to the analysis of aflatoxin in peanut butter has also been extensively examined. A procedure for the detoxification of groundnut cake using ammonia gas at high temperatures and moderate pressures has been developed.

A micro method, adapted from the aqueous acetone procedure proposed by Pons and Goldblatt for the determination of aflatoxins in individual peanuts and peanut sections, was developed to permit accurate determination of aflatoxins in individual groundnut kernels and kernel sections. Use of this procedure permitted the topographic distribution of aflatoxins within individual kernels to be mapped and indicated that the toxins were not uniformly distributed within contaminated kernels, even when the kernel contained a high level of aflatoxins. Although wrinkling or discoloration sometimes indicated that a kernel was contaminated, this type of physical damage was not found to be a reliable indication of aflatoxin content. Also it was noted that a few apparently sound and mature kernels contained high levels of aflatoxins.
This report examines and summarizes current knowledge regarding mycotoxins, surveys, sampling techniques, conditions conducive to post-collection production of mycotoxins in grain samples, and analytical methods for mycotoxin analysis. Priority attention is given to samples of corn suspected of containing aflatoxin. The report includes recommendations where deemed appropriate by the AdHoc Work Group.

A subsampling mill designed to simultaneously comminute and subsample large samples of granular material is described for use by the groundnut industry to prepare groundnut samples for aflatoxin analyses. During operation, the cylindrical screen is fastened to a circular platform beneath the blades. The central blades hammer the groundnut kernels against the cylindrical screen until the fragments are small enough to penetrate the 1/8 inch mesh. Most of the comminuted material then falls to waste but a small portion (5% by weight) passes through each of the two openings and into the sub-sample collection chute.

Ten 50 lb pneumatic samples from 17 loads of farmers stock groundnuts were collected during the 1987 harvest season in Georgia. Grade samples were run over a 24/64 belt screen. Loose-shelled kernels (LSK) and small pods that fell through the belt screen were separated, pods were shelled, and the kernels were screened over a 16/64 and 14/64 slotted screen. Pods that rode the 24/64 belt screen were similarly shelled and screened, and in addition, the damaged kernels were removed. The resulting components were analyzed separately for aflatoxin by high performance liquid chromatography (HPLC). From the grade samples, the LSK, other kernels (OK), and damaged kernels were combined and the sound mature kernels (SMK) and sound splits (SS) were combined. These two grade components were analyzed separately for aflatoxin by UPLC. Results showed the distribution of aflatoxin within each component and provided variance data to determine sample sizes needed to detect specified levels of aflatoxin in each respective component. Results also provided additional information needed for finalizing the design of a pilot study to determine the performance of the belt screen separator in removing foreign material and poor quality groundnuts prior to marketing of farmers stock groundnuts.

Procedures are discussed for sampling groundnuts and groundnut products and preparing those samples for aflatoxin analysis. Analytical methods available for the analysis of foods and feedingstuffs for aflatoxin are given.

Sampling and methods for reducing a laboratory sample to a test sample are discussed, with particular emphasis on sampling groundnuts for aflatoxin analysis as a practical example. The only way to control the total error in the analysis of this heterogeneous product is to take and to analyze many and large samples.
The distribution of aflatoxins in groundnuts has been extensively studied and plans have been developed in the USA and the UK for monitoring the levels of aflatoxin in this commodity. Attempts have been made to assess the sampling plans using various statistical models. In determining the aflatoxin levels in bulk commodities errors can arise at several stages, for example, in sampling, sample preparation, and analysis. These errors and the attempts which are being made to limit their effects are discussed. Earlier workers have applied the negative binomial and compound poisson-gamma distribution to the distribution of anatoxins in edible groundnuts, but with limited success. Data for aflatoxin levels in different batches of groundnuts and groundnut cake were found to give a better fit over a wider range using the Weibull distribution than the other distributions.


This report reviews some of the analytical methods available for the analysis of foods and feedingstuffs for aflatoxin, particularly those which are suitable for use in laboratories in developing countries. Full details of procedures for sampling, extraction, separation and estimation of aflatoxins are given. Methods for the determination of the concentration and purity of aflatoxin standards and the recommendations for the bulk sampling of commodities for aflatoxin analysis are also given.


Problems of analysis of whole groundnuts for aflatoxin are discussed. A proposed sampling plan is based on a limit for rejection of 1 µg kg\(^{-1}\) with a producer risk of 1 % and a consumer risk of 1 % for accepting a lot with 5 µg kg\(^{-1}\). Three samples of 10000 kernels are analysed for aflatoxin in the first step. If no decision can be reached another sample of 10000 kernels has to be analysed.


Statistical distribution models are considered for investigation into the effect of the number of groundnut kernels sampled on the effectiveness of the detection of aflatoxin contamination. It is shown by the analysis of 368 samples of 1 to 10000 kernels, from the same lot of nuts, that the negative binomial distribution represents a good statistical model. Different sampling plans are considered, and it is concluded that samples of many kilograms must be analyzed to ensure a low risk of a wrong decision both to the consumer and to the producer.


A technical paper by Whitaker and Dickens [Journal of the Association of Official Analytical Chemists (1989) 72 : 644-648] on aflatoxin testing plans that discusses (without a literature reference) a testing plan used in the Netherlands is examined. It is noted that this testing plan has never been in operation. The current situation in the Netherlands with respect to legislation and sampling plans on aflatoxin, which has fairly important consequences for the results of the simulation study of Whitaker and Dickens, is presented. It is shown that the percentage of rejected US-exported lots in the Netherlands would increase from 16 to 27 % based on the actual testing plan in the Netherlands. The need for international harmonization of testing and the role of Codex Alimentarius is also emphasized.


The clinical effects of aflatoxin exposure are briefly described, and the current US FDA regulations and sampling procedure for control of aflatoxins in groundnuts arc discussed. Some quality control procedures to ensure that only minimal quantities of toxin persist into the finished product are outlined.


The primary goal of a sampling plan for natural toxins, i.e., mycotoxins and seafood toxins, is to obtain a sample that accurately represents the concentrations of individual components of a given lot. Factors affecting the ability of the sampling plan to accomplish this goal include : (1) nature of the analyte of interest, (2) distribution of the analyte throughout the lot, (3) physical characteristics of the product, (4) accessibility of the product to random representative sampling, (5) sampling procedure, and (6) size of the sample. Sampling plans are composed of three distinct components : (a) sampling, (b) sample preparation, and (c) analysis. Normally, sampling contributes the largest relative error while analysis comprises the least. Automatic, continuous stream samples provide the most representative samples for commodities such as nuts, cottonseed, and cereal grains. Good sample
preparation equipment is currently available for these commodities; the use of this equipment to obtain a representative test sample is discussed.


A sampling plan for groundnuts must provide a high level of protection for the consumer and reasonable assurance to the processor that lots of good groundnuts are not rejected by the testing program. Samples consisting of replicate detection in groundnut sub-samples were considered statistically. Results of statistical analysis indicated that the normal model fitted the data quite well and definitely better than the log normal model.


This paper reviews the sampling plans and collaboratively studied methods of analysis for aflatoxins in foods and feeds. Anatoxins are the only food contaminants being monitored routinely on an international scale with methods operating at the order of a magnitude of 10 µg kg⁻¹. At this level, methods of analysis which can achieve coefficients of variation of 30-40 % with recoveries of 70 % or greater in interlaboratory collaborative studies can be considered eligible for reference status. In most cases, sample reproducibility is the variable limiting the reliability of methods of analysis. The inherent uncertainty of the identity of chromatographically separated entities requires the application of confirmatory tests to verify that the characteristics measured result from the presence of aflatoxin. The methods are also inoperable without a verification of the identity, purity, and concentration of the reference standards used. Screening methods which reliably eliminate negative samples from further consideration are indispensable for the practical operation of monitoring programs.


Cases have been reported of individual groundnuts, cottonseeds or Brazil nuts so highly contaminated with aflatoxin that, for a 50 g portion to be representative of the whole, the sample preparation procedures should grind each unit to a large number of particles and distribute them uniformly throughout the sample. Assuming uniform contamination of the individual kernel, each 50 g sample should contain 1/100 of that kernel. Even though these extreme cases may be encountered only infrequently, the more usual situation still presents difficulties because of great variability in individual kernel contamination. However, if the extreme can be handled, one can expect to handle the more usual situation. Equipement and procedures to achieve this distribution goal are described. The equipement studied includes a food chopper (Hobart), a nut mill (Thomas Mills), a disc mill (Bauer), a hammer mill (Fitzpatrick Model D comminuting machine), a hammer mill designed specifically for groundnut samples (Dicken's subsampling mill), a Polytron homogenizer (Bronwill Scientific), a vertical cutter-mixer (Hobart), and a sample splitter (Jones riffle). Commodities examined were shelled groundnuts and in-shell Brazil nuts, walnuts, pecans and almonds. Communion and mixing effectiveness were determined by particle size analysis, by distribution of kernels made radioactive by neutron activation and by aflatoxin analysis of naturally contaminated products. From the results it is concluded that the ultimate in sample uniformity can be achieved with a disc mill, solvent addition to obtain a fluid system and mixing and grinding of the fluid with a dispersion mixer-grinder. A practical uniformity can be achieved in a vertical cutter-mixer with less expenditure of time and effort for the commodities studied.


A procedure was devised for preparing lot samples of mycotoxin-contaminated nut meats so that a representative analytical sample may be removed. The sample is rapidly reduced to coarse size. A relatively large portion (about 1/10 of total sample) of subsample is then split out and further comminuted to a fine particle size with the aid of a fat solvent (meat solvent, w/v, 3:2). The analytical sample is removed from this mixture. The procedure was tested with shelled almonds and shelled walnuts using radioactive nuts to simulate mycotoxin contamination and provide a simple, precise measure of the contaminated nut meat distribution. The pooled coefficient of variation was 18 % for the subsamples and 4.4 % for the analytical samples. Considering the dilution factors used (1.50 and 2.14 contaminated nuts 10⁴ nuts) and the low degree of reliability of the lot sample, the sample preparation methods tested appear to be practical and reliable.


One lot of groundnuts known to contain aflatoxin was extensively sampled to study the sources of variability. A nested design was used where sections (50 bag units), subsamples and analytical variation were the variables studied. Sample size was the most critical factor in characterizing this lot. Variability from section to section was not significant indicating random distribution of the toxin.
Three 20 lb samples were taken on a number of lots from the 1968 crop, each of which was subdivided into two equivalent subsamples. The aflatoxin was determined in each of these subsamples. The results indicated that all the significant variation came from the subsamples, further enforcing the thesis that sample size is the critical factor in variability, and not lot inhomogeneity. Analyses of 550 lots from the 1967 crop where triple samples and analyses were available indicated that the magnitude of the variability was a little greater than was found on the experimental lot. Using the pooled standard deviation of the 1967 crop data, operating characteristic curves were plotted to demonstrate the improvement that can be expected by increasing the sample size.


A method based on the use of water slurries was developed for aflatoxin analysis. This procedure enables aliquots, from kilogram size samples, to be analyzed without an increase in solvent or reagent costs. Groundnuts, peanut butter, groundnut meal, cottonseed, cottonseed meal, copra and maize were successfully analyzed by this slurry technique. Slurries were prepared in a 1-gal blender and sample weight to water volume ratios were 1:1.5 for peanut butter, 1:2.25 for whole seeds, 1:4 for commercial meals and 1:5 for copra. Extraction of 100-g aliquots of slurry with 200 mL of acetone resulted in aflatoxin yields comparable to those obtained by extraction of the dry products by the standard method. The precision of slurry analysis for the products, expressed as % coefficient of variation, ranged from 2.6 to 7.8 compared to 5.7 to 20.8 for analyses by the standard procedure. Determinations were made by fluorometric measurements of aflatoxin absorbed on the florisil layer in a minicolumn.


Problems of sampling of groundnuts for aflatoxin determination are discussed, with reference to the 1976 legislation setting tolerances for aflatoxins in foods. Problems include the relative rarity of contaminated nuts (e.g., 1 in 10000), and the considerable variation in aflatoxin concentration in contaminated nuts. A statistical study on optimization of sample size is described. Tables of data are given showing the likelihood of negative results with sample of 60 g-60 kg with samples of groundnuts with contaminated : sound nut ratios of 1:4000, 1:10000, and 1:30000, and the probability of various aflatoxin concentrations in various sample sizes, as a proportion of the aflatoxin concentration in the total batch. A sample size > 5 kg is recommended for random checks by groundnut processors. Random sampling of commercially available groundnuts by official quality control organizations is also discussed.


Procedures are discussed for sampling peanut butter and preparing those samples for aflatoxin analysis. Special emphasis is placed on sampling the product from shipping pallets and comminuting chunk style peanut butter in order to reduce the variability in the analysis associated with the nonuniform distribution of aflatoxin in the product. The slurry method of preparation is a convenient means of obtaining a sample which is representative of a non-homogeneous product.


A multiple sampling plan, called attribute sampling, is discussed as a method to determine if the mean level of aflatoxin in a lot of shelled groundnuts exceeded a predetermined critical level. Assuming that the distribution of aflatoxin in a lot of groundnuts may be approximated by the negative binomial distribution, the method of computing an operating characteristic curve for a multiple sampling plan is described. From the operating characteristic curve, a measure of both the consumer's and processor's risk can be evaluated. The operating characteristic curve for the sampling plan to be used by the groundnut industry starting in fall 1969 is described.


Methodology for use in evaluation of aflatoxin testing programs to estimate the aflatoxin concentration in lots of granular material is presented. A computer model is used to simulate sampling, subsampling, and analytical errors in aflatoxin testing programs. The negative binomial function is used to estimate the distribution of sample concentrations taken from contaminated lots and the distribution of subsample concentrations taken from contaminated samples. The normal distribution is used to predict the distribution of analytical results about the subsample concentration. Monte Carlo solution techniques are employed to account for conditional probabilities that arise from use of multiple samples, subsamples, or analyses in testing programs. Operating characteristic curves, computed by the
model, arc used to evaluate the processors’ risk, the consumers’ risk, and the costs associated with aflatoxin testing programs. The aflatoxin testing programs used for shelled groundnuts in the United States are described and evaluated.


Methodology is described for the design and evaluation of testing programs to estimate aflatoxin concentrations in lots of granular foodstuffs. Use of operating characteristic curves and of the prior distribution of lot concentrations for comparing and evaluating processor and consumer risks related to testing programs are demonstrated. Operating characteristic curves, computed from a system of equations that accounts for errors in sampling, subsampling, and analysis are developed for the 1976 groundnut aflatoxin testing program in the United States. Estimated are given of aflatoxin concentration in lots accepted and rejected by the testing program.


In reply to a previous paper [Koe, W.J.de, and Defize, P.R. - Journal of the Association of Official Analytical Chemists (1990) 73 : 809-811], methods used to evaluate aflatoxin testing plans for shelled groundnuts are discussed.


The distribution of shelled groundnut lots according to their true aflatoxin concentrations is different from the distribution of aflatoxin assays made on those lots. This difference is due to assay errors associated with sampling, subsampling and analysis. A method was developed to estimate the distribution of lots according to their true aflatoxin concentration based on aflatoxin assays. Estimates of the distribution of the 1973, 1974, and 1975 groundnut lots were computed from their aflatoxin assays which were recorded by the Peanut Administrative Committee (PAC). Fifty-five % of the PAC assays for the three crop years were less than 3 µg kg⁻¹ aflatoxin, whereas the estimated lot distribution indicated that only 48 % of the lots were less than 3 µg kg⁻¹. On the other hand, only 94 % of the assays compared to an estimated 96 % of the lots were less than 25 µg kg⁻¹.


Under provisions of a USDA Marketing Agreement, an aflatoxin control program for groundnuts produced in the United States is administered by the Peanut Administrative Committee (PAC) composed of groundnut growers and shellers. The PAC requires aflatoxin tests on all commercial lots of shelled groundnuts. The present PAC testing program initiated in 1975 was evaluated for Crop A which averaged 9.5 µg kg⁻¹ aflatoxin and for Crop B which averaged 5.2 µg kg⁻¹ aflatoxin. For Crop A and B, respectively, 7.3 % and 2.0 % of the lots were rejected. The accepted lots contained an average of 7.5 µg kg⁻¹ for Crop A and 4.7 µg kg⁻¹ for Crop B. A correct decision (accept a lot with < 25 µg kg⁻¹ aflatoxin and reject a lot with > 25 µg kg⁻¹ aflatoxin) was made 95 % of the time for Crop A and 98 % of the time for Crop B.


The present aflatoxin testing plan used in the United States for shelled groundnuts was designed with a final accept level of 25 µg kg⁻¹ total aflatoxin. Some of the importers of US groundnuts use aflatoxin testing plans with accept levels lower than the 25 µg kg⁻¹ used in the US. For example, the accept level of a testing plan used in The Netherlands is 5 µg kg⁻¹ B₁, or 10 µg kg⁻¹ total aflatoxin. Computer models were developed to simulate the testing of groundnut lots with the US and The Netherlands testing plans. The model was used to determine the effects of decreasing the final accept level of the US testing program on the number of lots accepted and rejected in the US and the number of exported lots accepted and rejected when using The Netherlands testing plan. Decreasing the final accept level of the US testing program from 25 to 5 µg kg⁻¹ increased the number of lots rejected in the US by 371 % while reducing the number of exported lots rejected by 51 %. For every additional 8.3 lots rejected in the US, one less export lot will be rejected.


The 1987 United States aflatoxin testing plan for shelled groundnuts was designed with a final accept level of 25 µg kg⁻¹ total aflatoxin. Some of the importers of U.S. groundnuts use aflatoxin testing plans with accept levels lower than 25 µg kg⁻¹. For example, the accept level of a testing plan used in The Netherlands is 5 µg kg⁻¹ B₁, or 10 µg kg⁻¹ total aflatoxin. Whenever export lots are re-tested for aflatoxin by an importing country, some lots accepted in the United States will be rejected by the importing country’s aflatoxin testing plan. Computer models were developed to determine the effects of decreasing the final accept level of the U.S. testing plan on the number of lots accepted and rejected in the United States and the number of
exported lots accepted and rejected by the Netherlands testing plan. Decreasing the final accept level of the U.S. testing plan from 25 to 5 μg kg⁻¹ increased the number of lots rejected in the United States by 371 % while reducing the number of exported lots rejected by 51 %. For every additional 8.3 lots rejected in the United States, one less export lot will be rejected.


The negative binomial probability function was used to model the distribution of sample aflatoxin test results when replicated grade samples from fanners stock groundnuts were analysed by thin-layer chromatography and minicolumn methods. The Poisson probability function was used to model the distribution of the number of kernels with visible Aspergillus flavus growth found in replicated grade samples of farmers stock groundnuts when the visible A. flavus method was used. The probabilities of accepting a lot of farmers stock groundnuts with given aflatoxin concentrations when using a 465-g grade sample and two different accept/reject levels were predicted with the models and compared to observed acceptance probabilities computed from previously published data for each of the three methods. The comparisons showed good agreement between the predicted acceptance probabilities and the observed acceptance probabilities.


The negative binomial equation was used to simulate the distribution of sample aflatoxin test results when replicated grade samples from farmers stock groundnuts were analyzed by TLC and minicolumn methods. The Poisson equation was used to simulate the distribution of samples according to the number of kernels with visible Aspergillus flavus growth found in replicated grade samples from farmers stock groundnuts when the visible A. flavus method was used. The probability of accepting a lot of farmers stock groundnuts with a given aflatoxin concentration when using a 465-g grade sample and four different accept/reject levels were predicted with the models and compared to observed acceptance probabilities for each of the three methods. Comparisons between predicted acceptance probabilities and observed acceptance probabilities from a previous study were good for each method at each accept/reject level.


Suitability of the negative binomial distribution for use in estimating the probabilities associated with sampling lots of shelled groundnuts for aflatoxin analysis was studied. Large samples, called "mini-lots", were drawn from 164 lots of shelled groundnuts contaminated with aflatoxin. These mini-lots were subdivided into 10 lb samples which were analyzed for aflatoxin. Variance of the sample means about the mean, M, of the mini-lots from which the samples were taken was determined. These variances were then used to compute the % non-contaminated groundnuts, F(o), in the mini-lots by means of the equation for the negative binomial distribution. The relationship between F(o) and lot mean M was found to be described by the regression equation: F(o) = 99.983 - 0.003 M. The observed distribution of 10 sample means from each of 11 mini-lots were compared to the negative binomial distribution by means of the Kolmogorov-Smirnov test. The null hypothesis that each of the 11 observed distributions were negative binomial was not rejected at the 95 % confidence level.


Using 12-pound samples, 280-g subsamples, the Waltking method of analysis, the densitometric procedures, the sampling, subsampling, and analytical variances associated with aflatoxin test procedures were estimated. Regression analysis indicated that each of the above variance components is a function of the concentration of aflatoxin in the population being tested. Results, for the test procedures given above, showed that sampling constitutes the greatest single source of error, followed by subsampling and analysis. Functional relationships are presented to determine the sampling, subsampling, and analytical variance for any size sample, subsample, and number of analyses.


Using 12 lb samples, 280 g subsamples, the Waltking method of analysis, and densitometric procedures, the sampling, subsampling, and analytical variances associated with aflatoxin test procedures were estimated. Regression analysis indicated that each of the above variance components is function of the concentration of aflatoxin in the population being tested. Results, for the test procedures given above, showed that sampling constitutes the greatest single source
of error, followed by subsampling and analysis. Functional relationships are presented to determine the sampling, subsampling, and analytical variance for any size sample, subsample, and number of analyses.


Suitability of the negative binomial distribution for use in estimating the probabilities associated with sampling lots of shelled groundnuts for aflatoxin analysis was studied. Large samples, called "mini-lots", were drawn from 29 lots of shelled groundnuts contaminated with aflatoxin. These mini-lots were subdivided into 12 lb samples which were analyzed for aflatoxin. The mean and variance of these aflatoxin determinations for each mini-lot were determined. The shape parameter K and the mean aflatoxin concentration m were estimated for each mini-lot. A regression analysis indicated the functional relationship between K and m to be

\[ K = (2.0866 + 2.3898m) \times 10^{-6} \]

The observed distribution of sample concentrations from each of the 29 mini-lots was compared to the negative binomial distribution by means of the Kölmogorov-Smirnov test. The null hypothesis that each of the true unknown distribution functions was negative binomial was not rejected at the 5% significance level for all 29 comparisons.


Methodology for use in the design and evaluation of sampling plans to estimate aflatoxin concentrations in lots of shelled groundnuts is presented. Use of the operating characteristic curve for comparing and evaluating processor and consumer risks related to various sampling plans and application of the negative binomial distribution to estimate probabilities associated with sampling lots of shelled groundnuts for aflatoxin concentration are discussed. Operating characteristic curves are developed for two different single-sample plans, an attribute multiple sample plan, and the plan presently used by the groundnut industry to estimate aflatoxin concentrations in commercial lots of shelled groundnuts. An estimated prior distribution of lots according to aflatoxin concentration is used to predict, among others, such values as the % of all lots tested that will be accepted by the sampling plans and the average aflatoxin concentration in the accepted lots. All four of the sampling plans described in the paper are compared on the basis of values such as these. Other factors to be considered in critical evaluation and selection of sampling plans for estimating aflatoxin concentrations in commercial lots of shelled groundnuts are discussed.


A computer model that accounts for sampling, subsampling, and analytical variability was developed to simulate aflatoxin testing programs. Monte Carlo solution techniques were employed to account for conditional probabilities that arise from multiple samples, subsamples, and/or analyses being used in testing programs. The aflatoxin testing program to be used on the 1974 groundnut crop was evaluated by the use of the described model.


The design and evaluation of sampling plans for estimating aflatoxin contamination in groundnuts are discussed. A method to predict the number of good lots that will be rejected and the number of bad lots that will be accepted by a given testing program is presented. Four sampling plans used by the groundnut industry in the United States are evaluated using the described methodology.


Effect of sample size on sampling accuracy was studied in relation to estimate the aflatoxin levels in groundnuts. The negative binomial distribution was used to simulate the actual distribution of aflatoxin since it allowed for a high probability of zero counts along with small probabilities of large counts. Using both the Monte Carlo technique and a direct computation method, the effect of sample size on sampling accuracy is quantitatively described.


A sampling method according to the binomial distribution rule was intended to be established as a simple system for the qualitative evaluation of aflatoxin contamination in groundnuts. A sampling method is described.
ANALYTICAL METHODS

7.2.3 Thin-Layer Chromatography (TLC) Methods


Ninety-two samples of grain and common feed ingredients were analyzed in Australia using thin-layer chromatography (TLC) and a rapid minicolumn screening method. Three samples contained aflatoxins; a sorghum grain sample containing 30 µg kg⁻¹ aflatoxin B₁, and two samples of moldy groundnuts containing 2500 µg kg⁻¹ aflatoxin B₁ and 400 µg kg⁻¹ aflatoxin B₂, and 200 µg kg⁻¹ B₁ and 38 µg kg⁻¹ B₂, respectively. A non-destructive detection technique involving scanning the grain with a UV lamp and examination of fluorescence is reported.


Collaborative studies with various laboratories were organized to test the efficiency of various methods, but the results revealed a disturbing frequency of underestimation. The 'best food' (BF) method in conjunction with a personnel training program is recommended.


Irradiation of aflatoxins B₁ and G₁ with UV light (principal wavelength 365 mJ) converts both compounds to new fluorescent photoproducts which have much lower Rf values than aflatoxin B₁ and G₁ when chromatographed on silica gel thin layer plates. Photoproducts of aflatoxin B₁ form much faster on a silica gel surface than in methanol solution. Photoconversion of aflatoxin B₁ is shown to alter fluorescence comparison assays and identification tests. The principal photoproduct developed from aflatoxin B₁ is significantly less toxic than the parent aflatoxin.


Three confirmatory tests have been devised to identify aflatoxin B₁. Portions of the isolated toxin are treated with formic acid-thiacyl chloride, acetic acid-thiacyl chloride, and trifluoroacetic acid, respectively, and aliquots of the three fluorescent reaction products are spotted on thin-layer chromatography (TLC) plates. Standards treated with each of three reagents, plus an untreated standard, are spotted on the same plate, and after development the spots are compared under ultraviolet light.


Analytical methods based on thin-layer chromatography are described for detection and estimation of aflatoxins in agricultural commodities including groundnuts and groundnut products. Full details of official procedures for sampling, extraction, separation and estimation of aflatoxins are given.


This paper very briefly reviews some analytical methods and bioassays for aflatoxins in groundnuts and groundnut products. Regulatory aspects are also discussed, and the actions of the Food and Drug Administration (FDA) of the US are highlighted.


The suitability of five screening methods for the detection of aflatoxin B₁ in 10 different feeds, feed concentrates and complete feed mixtures was compared. Aflatoxin B₁ was found in samples of groundnut cake and protein concentrates for laying hens and breeding pigs. No aflatoxins were detected in soya, sunflower, maize, fish meal, table trout iced, carp stock feed, table carp feed and duckling fattening mixture.


A rapid method for aflatoxin analysis in groundnut and its products is described. Aflatoxin-containing food samples in 80% methanol were dialysed against 20% aqueous methanol as dialysing solvent. The toxin in the dialysate was estimated by direct extraction with chloroform and quantified by a visual fluorescence extinction method using a long wave UV light. The percentage of aflatoxin dialysed was linear up to 60 min and then decreased gradually. Temperature did not influence the toxin yield. Advantages of the method are: least interference with fluorescence; clean chromatograms; less changes of thermal degradation of compounds; elimination of usual cleanup procedures; and selective separation of the toxin from common interfering substances.
Quantitative fluorodensitometric measurement of aflatoxin B₁. Comparison of B₁ measurements in spiked and naturally contaminated samples. The precision limit of visual comparison procedures used in aflatoxin assay methods can be no better than + 20 % for a single observation and, under operating conditions, it is probably close to ± 28 %. Fluorodensitometric procedures described in the literature can improve this precision to ± 9 % for the average of multiple observations. A possible source of inaccuracy in the published densitometric procedures is pointed out and a method using internal standards to avoid the inaccuracy is presented. Tested with spiked extracts of "clean" peanut butter, the procedure gave an average 101 ± 3 % recovery of added aflatoxin B₁ and 89 ± 6 % recovery of aflatoxin B₂ with a precision for individual assays equal to the precisions reported for the other procedures. The major source of error was shown by elimination to reside in the thin-layer chromatography and aflatoxin instability.


The procedure for measurements of standard aflatoxin B₁ spots on thin-layer chromatographic (TLC) plates by flying-spot densitometer is described. Three different types of silica gel layers were spotted with standard aliquots of aflatoxin B₁ covering a range of 0.4-20 ng. Silica gel H plates were developed with a mixture of chloroform-trichloroethylene-n-amyl alcohol-formic acid (80+15+4+1), while silica gel G and MN-G-HR plates were developed with a mixture of chloroform-acetone (90+10). TLC plates were then scanned with a reflectance flying-spot densitometer. Sharp and defined separation patterns for aflatoxins B₁, B₂, G₁, and G₂ were recorded on H and MN-G-HR plates, while unsatisfactory separation was found on G plates. The relationship between peak area and concentration was linear for H and MN-G-HR plates, but not for G plates. Variations of 15-20 % were calculated among the fluorescence of aflatoxin B₁ spots between plates with same sorbent layers. Coefficients of variation (CVs) of 5-7 % for single densitometric measurements on one plate under normal conditions were calculated for a series of 3 or 4 aflatoxin B₁ spots ranging from 1 to 4 ng on H and MN-G-HR plates, whereas CVs of 11-12 % were recorded for G plates. Results are compared with those of previous investigators.


Peanut butter extracts and samples spiked with 5-40 µg aflatoxin B₁ kg⁻¹ were analyzed, together with naturally contaminated groundnut products, by three extraction procedures: the official Dutch method (KB), the Liem et al. method (methanol) and the IUPAC method. The latter procedure was selected as a reference method since it has international application. KB extracts were separated on silica gel G plates with a mixture of chloroform-acetone (90+10), whereas IUPAC extracts were separated similarly on MN-G-HR plates. Methanol extracts were resolved on silica gel H plates, using chloroform-trichloroethylene-n-amyl alcohol-formic acid (80+15+4+1) as the developing solvent. After development, plates were scanned with a reflectance flying-spot densitometer. With such techniques, average recoveries for spiked peanut butter extracts ranged from 99 to 105 %, with variation values of 11-12%. Recovery values of 69 % (KB method) and 84 % (methanol and IUPAC methods) were obtained for spiked peanut butter samples. Coefficients of variation ranged from 13 to 15 % for fluorodensitometric measurements. In naturally contaminated groundnuts and groundnut products, precision values were 13.6 % for fluorodensitometric measurements compared to 36 % for visual estimations. Both the methanol and IUPAC methods yield extracts suitable for densitometric analysis after spotting on TLC plates; the analytical results are comparable. Extracts from the KB method contained more interfering fluorescent material than the other two methods.


A collaborative study was carried out by 20 laboratories in The Netherlands, representing governmental and industrial institutes, on the determination of aflatoxin B₁ in peanut butter extracts. Blank peanut butter extracts prepared according to the proposed official Dutch method were spiked with aflatoxin B₁, representing contamination levels of 0, 3, 6, and 12 µg kg⁻¹. Sample extracts and standards were spotted on silica gel G thin-layer chromatographic (TLC) plates by the antidiagonal spot application technique. Spotted plates were developed by two-dimensional TLC with diethyl ether:methanol:water (94.4:5.1:5.1) in a lined tank in the first direction and with chloroform:acetone (90:10) in an unlined tank in the second direction. Separated aflatoxin B₁ spots from sample and standard developed in both directions were free from background interference. The quantities of aflatoxin B₁ present in the sample were determined by visual comparison of the fluorescent intensities of samples and standard B₁ spots. For this procedure the variability of measurements
within and between laboratories was statistically investigated: 80-90% of the complete results given by the participants were correct for the blank and spiked samples (contamination level of 12 µg kg\(^{-1}\)). For contamination levels of 3 and 6 µg kg\(^{-1}\), an approximate coefficient of variation of 35% was calculated from within- and between-laboratory results. Results obtained in this investigation were compared with those found by other investigators who used the one-dimensional TLC technique. It is concluded that, with the antidiagonal procedure, small amounts of aflatoxin B\(_1\) (3 µg kg\(^{-1}\)) may be detected.


The method of Velasco based on separation of aflatoxins on a Florisil/silica gel/alumina minicolumn and UV fluorometric detection, using a Velasco Fluorotoxin Meter, was compared with visual detection after separation on the minicolumns, and thin-layer chromatographic (TLC) determination. Trials were conducted on spiked samples of groundnuts, soybean and sunflower presscakes, chickpeas, beans, almonds, wheat, maize, barley, cassava, silage, and a feed mixture. Severe interference by other constituents of most of the food or feed samples studied was observed with Velasco's method. The filter fluorometer system did not allow differentiation of interfering impurity bands having maximum fluorescence wavelengths close to those of aflatoxins but differing in colour; visual evaluation of the column permitted differentiation of these. The Velasco minicolumn/UV fluorometry method gives a total aflatoxin concentration rather than values for individual aflatoxins, whereas TLC gives data for concentration of individual aflatoxins. It is concluded that TLC techniques are likely to give better results than Velasco's method.


A semi-quantitative method capable of detecting 0.006 µg of aflatoxin B\(_1\) in groundnuts and groundnut products, especially groundnut meals, by thin-layer chromatography (TLC), is described.


A second collaborative study was carried out on the determination of aflatoxin in peanut butter under the sponsorship of the Aflatoxin Methodology Working Group. Thirteen collaborators, representing industrial, independent, and government laboratories, analyzed 12 peanut butter samples containing known amounts of aflatoxin at levels of 10 and 110 µg kg\(^{-1}\) of peanut butter. Two samples of naturally contaminated peanut butter containing aflatoxins B\(_1\) and B\(_2\) were also analyzed. The study was designed to estimate the accuracy and precision of the method, both within laboratories and between laboratories. Some samples and collaborators were common to both this study and an international study sponsored by the IUPAC. Results of the two studies have been compared. The method was recommended for adoption as official, first action.


A method of acetylation of aflatoxins B\(_1\) and G\(_1\) before identification is described. Extracts containing aflatoxin B, and/or G, are spotted on a silica gel TLC plate and overspotted with acetic anhydride and HCl. After reaction, the plate is developed and the Rf values of reacted aflatoxin B, and/or G, compared with those of authentic standards treated similarly. The procedure has been successfully applied to a variety of samples such as groundnuts, pistachios, hazelnuts and almonds.


The difference between the CB and BF methods in extracting aflatoxins from groundnut products was studied. The CB method gave 60, 121, 35, and 22% higher results for aflatoxins B\(_1\), B\(_2\), G\(_1\), and G\(_2\), respectively for four samples of groundnut meal and six samples of peanut butter tested. Both reverse-phase liquid chromatography and thin-layer chromatography were used to quantitate the extracted aflatoxins.


Polaroid color print recordings were made of aflatoxins separated by thin-layer chromatography (TLC) and revealed by fluorescence in ultraviolet light.


The influence of the chloroform-methanol solvent system on the fluorescence of...
aflatoxins B₁, B₂, G₁, and G₂ was investigated. Fluorescence intensity is markedly affected by solvent composition. The formation of excited state dimers and ionization are proposed to explain the effects observed.


An assay method for aflatoxin B₁ in cottonseed meal and groundnut seed meal with a sensitivity limit of < 0.02 µg kg⁻¹ is described. The sample is extracted with aqueous methanol in a Waring blender. The extract is purified by lead acetate precipitation, followed by partition chromatography on Celite, and assayed by comparison of fluorescence intensity with aflatoxin B₁ standards on silica gel G-HR thin-layer chromatograms. This method eliminated potential interference from a "new blue spot" found in cottonseed meal extracts.


A simple, rapid enzyme-linked immunosorbent assay (ELISA) was used to evaluate the performance of each step (extraction, filtration, solvent partition and silica gel column chromatography) of a solvent-efficient thin-layer chromatography (TLC) method undergoing interlaboratory collaborative study for the determination of aflatoxin B₁ in maize, raw groundnuts and peanut butter. The apparent mean recoveries using the ELISA method were approximately 30-50 % higher than those using the TLC method if only the amount of aflatoxin B₁ added to the samples was used in the calculations. After the cross reaction of the antibody with other aflatoxins added to the samples was considered, the amounts recovered approached the levels of the aflatoxins added in all the three commodities tested. With no cleanup treatment, ELISA recoveries at aflatoxin B₁ levels >7.5 ng g⁻¹ were 84, 79 and 103 % for maize, raw groundnuts, and peanut butter, respectively. With each cleanup step in the TLC method, ELISA detected a progressive decrease in recovery from 150.5 to 105.3 % (before correction for the presence of other aflatoxins) or from 93.5 to 65.4 % (after correction for other aflatoxins) of aflatoxin B₁ added to samples. It is concluded that cleanup treatments are not necessary in the ELISA and that when large amounts of other aflatoxins are present, an understanding of the cross-reactivity of antibody with other aflatoxins in the ELISA is essential for final interpretation of the data.


The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.


Paper-chromatography and thin-layer chromatography methods were compared for detection and estimation of aflatoxin in groundnuts and groundnut meals. The lowest weight of pure aflatoxin B₁ that gave an observable fluorescence on Whatman No. 1 filter-paper after development in the solvent system described by Coomes and Sanders (1963) was determined as 0.1 µg while it was 0.003 µg on alumina chromatographic plates (740 ±10 µ thick), under the same excitation conditions (Y - 365 mµ). Work on extracts of groundnuts, using Kieselgel G chromatographic plates, indicated that more satisfactory estimates of known aflatoxin levels were feasible by using the technique of diluting the extract until no fluorescence was observed on the plates. However, over-estimates of the toxin are possible with certain samples.


A sensitive, high-resolution thin-layer chromatographic (TLC) method is described for determining aflatoxin B₁ in groundnut kernels, groundnut meals and peanut butter by fluorescence. Kieselgel G is used as adsorbent and is shown to offer advantages over alumina. Aflatoxin levels are determined by a dilution technique.


This report reviews some of the analytical methods available for the analysis of groundnuts and groundnut products for aflatoxin B₁. Full details of recommended procedures for sampling, extraction, separation and estimation of aflatoxin B₁ are given.

A method based on paper-chromatography for the detection and semi-quantitative determination of aflatoxin B₁ is described. The correlation between the analytical results by this method and the biological response in ducklings based on the ten groundnut samples tested appeared satisfactory.


An International Aflatoxin Check Sample Committee was formed in 1971 to establish a check sample program of commodities or materials with aflatoxin contamination, which should be available to laboratories throughout the world. A set of four aflatoxin-contaminated groundnut samples was prepared and sent to 150 participating laboratories. Sufficient data were obtained from this study to permit a statistical comparison of three of the four AOAC methods for analysis of aflatoxins in groundnuts and groundnut products. The analysis showed significantly higher means for the CB method than for the BF or Pons method for two of the samples examined.


In the second International aflatoxin check sample series, a single peanut butter sample was submitted to 152 laboratories throughout the world. Sufficient data were obtained from 117 responding laboratories to permit a statistical comparison of the BF, CB, and Pons methods for aflatoxin determinations. No significant differences in mean values were observed. One quarter of the participating laboratories were from countries other than Canada and the United States.


Aflatoxin B₁ reacts with 2,4-dinitrophenylhydrazine (DNPH) to form a colored aflatoxin dinitrophenylhydrazone. This derivative can be prepared in situ on thin-layer chromatograms of crude extracts by spraying them with a DNPH reagent solution. The use of this reagent spray provides a simple method whereby the identity of a fluorescent chromatographic spot suspected of being aflatoxin B₁ can be confirmed rapidly in situ.


Aflatoxins B₁ and B₂ contain a reactive carbonyl group in their cyclopentenone rings, which can take part in several classical carbonyl reactions. Procedures are given for forming the oximes and 2,4-dinitrophenylhydrazones of the B toxins. Lacking the carbonyl group, aflatoxins G₁ and G₂ are nonreactive. The formation of the aflatoxin derivatives is proposed as confirmatory tests for aflatoxins B₁ and B₂. These procedures can also be used to remove traces of the B toxins from preparations of the G toxins.


A reliable method for separation of individual aflatoxins from crude extracts is described, based on the use of selectively deactivated adsorbents. Activated Silic AR, Mallinckrodt type CC-4, permitted recovery of 87% of the aflatoxin B₁ contained in a crude extract. Methods of purifying the remaining aflatoxin G are also described. Using these methods, working quantities of the major aflatoxins, B₁ and G₁, can be obtained.


Chemical methods of analysis for the extraction, cleanup and determination of aflatoxins from animal feedingstuffs and foods are described. The advantages and disadvantages of thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC) are discussed. Immunoassays are briefly discussed. Methods for other mycotoxins such as ochratoxin, patulin, and the trichothecenes are also outlined.


A micro method, adapted from the aqueous acetone procedure proposed by Pons and Goldblatt for the determination of aflatoxins in cottonseed products, was developed to permit accurate determination of aflatoxins in individual groundnut kernels and kernel sections. Use of this procedure permitted the topographic distribution of
aflatoxins within individual kernels to be mapped and indicated that the toxins were not uniformly distributed within contaminated kernels, even when the kernel contained a high level of aflatoxins. Although wrinkling or discoloration sometimes indicated that a kernel was contaminated, this type of physical damage was not found to be a reliable indication of aflatoxin content. Also it was noted that a few apparently sound and mature kernels contained high levels of aflatoxins.


An accurate and sensitive procedure is proposed for estimating aflatoxins in both alkaline and acidulated soapstocks. Sample suspensions in aqueous acetone are adjusted to pH 3 with hydrochloric acid, extracted in a high speed blender, treated with lead acetate and partitioned into chloroform. After silica gel cleanup, aflatoxins in purified extracts are estimated by thin-layer chromatography. The use of acetone and lead acetate together apparently catalyzes the relactonization of aflatoxins B
\(^1\) and G
\(^1\) and leads to essentially quantitative recovery of aflatoxin B
\(^1\) and somewhat lower recovery of G
\(^1\) added to alkaline or acidulated soapstock.


A spectrofluorometric method is described for quantitative determination of aflatoxins B
\(^1\), B
\(^2\), G
\(^1\) and G
\(^2\) in groundnuts. Aflatoxins were extracted with an 80:20 mixture of acetone and water. The extracts were purified with a 20 % solution of lead acetate and then on an aluminium oxide column. After chromatographic separation of the aflatoxins on a thin-layer plate, the gel areas fluorescing in UV light were eluted with ethanol. The intensity of the fluorescence was measured with an Aminco-Bowman spectrofluorimeter. The content of aflatoxins was determined on the basis of standard curves in ethanol. The detectability of aflatoxins B
\(^1\), B
\(^2\), G
\(^1\), and G
\(^2\) was respectively 5.0, 2.0, 3.0, and 0.5 µg kg
\(^{-1}\).


Standard grade samples (16) from each of 20 selected minilots were used to evaluate three methods for detecting minilots of farmers stock groundnuts with unacceptable levels of aflatoxin. A visual, a minicolumn and a modified thin-layer chromatography (TLC) method were used to compare analytical results, variation, and probability of acceptance for minilots having mean aflatoxin levels ranging from 8 to 255 µg kg
\(^{-1}\). Mean values obtained by each of the three methods increased linearly with mean aflatoxin levels of the minilots and variation for each method as determined by the variance and coefficient of variation (CV) was very large. The CV for all three methods decreased as aflatoxin level increased. Overall performances of the methods were similar in accepting and rejecting these minilots on the basis of the 1.8 kg grade samples. The greatest difference in the three methods occurred at the zero acceptance level where the modified TLC, minicolumn and visual methods rejected 97, 98 and 88 %, respectively, of the minilots with more than 60 µg kg
\(^{-1}\) aflatoxin. At this acceptance level the TLC, minicolumn and visual methods also rejected 55, 50 and 30 %, respectively, of the minilots with < 30 µg kg
\(^{-1}\) aflatoxin.


Groundnuts were screened for aflatoxin using a rapid, inexpensive fluorometric method. Groundnuts were ground and extracted with methanol, and the extract was treated with acidified zinc- acetate-sodium chloride solution, filtered and diluted with water. Fluorescence of the extracts was compared with that from aflatoxin-free control groundnuts. Test samples (160) of several varieties and grades of groundnuts, obtained from storage and several commercial sources, were screened for the presence of aflatoxin. One hundred thirty-five samples (84 %) were identified by this method as aflatoxin positive (> 15 µg kg
\(^{-1}\)) or aflatoxin negative (< 15 µg kg
\(^{-1}\)). Although 22 samples (13.6 %) were incorrectly labeled as aflatoxin positive, most of these showed evidence of the presence of mold metabolites other than aflatoxin. Three samples (1.8 %) were incorrectly labeled as aflatoxin negative when they actually contained 20, 33 and 34 µg kg
\(^{-1}\) aflatoxin.


A method is described for the separation and preparation of aqueous solutions of aflatoxins pure enough for experimentation with most biological systems. It is simple and rapid and has been used successfully for preparing solutions of aflatoxins G
\(^2\) and C
\(^{14}\)-labeled B
\(^1\). The toxicity of aqueous solutions of aflatoxin B
\(^1\), prepared as described, was confirmed by a bioassay using fertile chicken eggs. The method has the advantage over other procedures in that the final product is dissolved in water and may be directly utilized in experiments involving most biological systems.

Several methods have been developed to analyze groundnuts for aflatoxin by using thin-layer chromatography (TLC); solvent solutions used to extract aflatoxin from groundnuts also extract measurable quantities of other compounds such as oils, fats, sugars, and protein. The volume of these extracted compounds causes error in measuring the proportion of the solvent solution analyzed for aflatoxin. Also, because the cleanup procedures for some methods are inadequate, the volume of some of these extracted compounds also causes error in measuring the proportion of the extracted aflatoxin placed on TLC plates. These two errors cause underestimation of aflatoxin concentrations by approximately 11, 14, and 5 % for the CB method, the modified version of the BF method generally used for raw groundnuts, and a water slurry method, respectively. The correction specified by the CB method for fats in the extraction solvent reduces the approximate error for the CB method from 11 to 1 %.

The official AOAC Method III for aflatoxins in groundnuts and groundnut products (Best Foods or BF method) has been adapted to a larger sample size (500 g), approaching the sample handling capacity of the 1 kg official AOAC Method I (FDA Contaminants Branch or CB method). The modified procedure (NY method) was used in place of the 1 kg CB method for obtaining quantitative assay results and for isolation of pure aflatoxin B₁ for derealization and bioassay. Two samples of naturally contaminated unshelled pistachio nuts and four groundnut product samples were analyzed using the modified NY method and the results were compared with quantitative assays using official methods. Results were in excellent agreement with the official methods. The thin-layer chromatographically pure aflatoxin B₁ recovered using the modified procedure on various products was 39 to 57 % of that calculated to be present in the samples, a favorable recovery compared to the 1 kg CB method (usually about 50 %). All derealizations carried out were successful (3 samples) and all biological confirmations were successful (2 samples were tested by the chick embryo bioassay for aflatoxin B₁ toxicity).
The results of two intercomparison exercises involving some 20 European laboratories who applied a wide variety of analytical methods to determine aflatoxin B$_1$ levels in groundnut meal are presented. It is shown that the major source of error and discrepancy is connected with incomplete extraction and/or losses during cleanup and that, provided correction for recovery/background interference is made, many methods can achieve acceptable accuracy. Sources of error and their control are discussed, and essential details of the methods used are presented. It is concluded that analytical quality assurance is more important than the use of standarized methods when a high degree of accuracy and comparability are required.


The method described involves extract purification by two-dimensional thin-layer chromatography and quantitative determination by fluorescence. Using this technique, aflatoxin B$_1$ was detected in 24 of 145 food samples examined. Maximum aflatoxin content was in maize and groundnuts. Maize samples also contained aflatoxins B$_2$, G$_1$ and G$_2$.


A procedure in which the direct extraction of a water-wetted sample by chloroform is combined with the use of a silica gel column for defatting and cleanup has been devised for obtaining clean quantitative extracts of aflatoxins from 50-g and 1-kg groundnut product samples. The low level of interference permits the detection and estimation of as little as 1 µg aflatoxins kg$^{-1}$ of groundnut product sample.


A rapid and sensitive procedure has been developed for detecting aflatoxin, ochratoxin, and zearalenone. This procedure uses a water-chloroform extraction combined with sequential elution of the mycotoxins from a silica gel column. Applicability to a wide variety of products was demonstrated by analysis of 31 different commodities. Three commodities (cottonseed, green coffee, and capsicum peppers) contained serious interferences for all three toxins.


A collaborative study of the CB procedure for aflatoxin in groundnut products was carried out on naturally contaminated peanut butter and groundnut meal and on peanut butter spiked with aflatoxins B$_1$ and G$_1$. Part of the study included preparatory isolation of the aflatoxin B$_1$ found in the naturally contaminated samples and confirmation of the identity by both chemical and biological tests. The results from 13 collaborators demonstrated both between- and within-laboratory precision and accuracy equal to the official, first action procedure; advantages in speed and convenience were noted. The preparatory separation was easily accomplished with clear chemical and biological proof of satisfactory isolation by seven of the nine participants in this phase of the study.


The Joint AOAC-AOCS Aflatoxin Committee, first proposed in 1964 and an active committee in 1965, coordinates methods development in the two societies. The history and accomplishments of this committee are outlined and areas of interest of the AOAC and the AOCS are defined. The results of the following actions have been taken: AOAC has adopted two methods for groundnuts and groundnut products and a chemical derivative confirmation test as official final action and a method for cottonseed as official first action; the AOCS has adopted one of the above methods for groundnuts and groundnut products as a tentative method. The AOAC Associate Referee topics for aflatoxins are listed and the current status of research under these topics is described.


Three aflatoxin-contaminated samples (raw groundnut meal, deoiled groundnut meal, and yellow corn meal) were analyzed by 121 laboratories in 31 countries. Sufficient data were obtained to permit a statistical comparison of the performance of laboratories using the BF, CB, and EEC methods and those using high performance liquid chromatography (HPLC) for quantitation. No significant differences were found between means for laboratories using these four methods for the analysis of raw groundnut meal or yellow corn meal. However, for deoiled groundnut meal,
means were significantly different for laboratories using BF method compared with the CB or EEC methods for $B_1$ and $B_2$, and for laboratories using the CB method compared with HPLC methods for $G_2$.


Three aflatoxin-contaminated samples, raw groundnut meal, finished peanut butter, and white corn meal, were analyzed by 139 laboratories in 34 countries. Sufficient data were obtained to permit a statistical comparison of the performance of laboratories using the BF, CB, and Pons methods and those using high performance liquid chromatography for quantification. A raw groundnut meal sample showed no significant differences among means for laboratories using the four methods, and a white corn meal sample showed only one such significant differences; however, a finished peanut butter sample containing less than 10 ($\mu$g total aflatoxins kg$^{-1}$ showed 10 significant differences among means for laboratories using the four methods considered.


Methods used in the German Democratic Republic for detecting specific aflatoxins in routine inspection of foods are discussed. Some moldy foods (cherries, strawberry jam, grapes and baby foods) contained fluorescent compounds which were not aflatoxins, but absence of aflatoxins was only demonstrated by UV or fluorescence spectroscopy. The reagent 'Echtblausalz-B' was found useful for spraying TLC plates, as specific color compounds are formed with aflatoxins. Elution of fluorescent spots from TLC plates followed by UV spectroscopy can detect 0.01 $\mu$g aflatoxin kg$^{-1}$. Fluorescence spectrophotometers can record spectra directly from TLC plates, and direct fluorodensitometric quantification is possible. Recovery of aflatoxin $B_1$ is 89 %, standard deviation 6.4 % with a variation coefficient of 7.3 %. Similar methods can be applied to ochratoxins. Aflatoxin contents of oranges and groundnuts determined during routine investigation of spontaneously molded foods are given.


A modified method for analysis of aflatoxins in peanut butter is described. Samples are first treated with a mixture of pentane:hexane (4:1) to remove oil, and aflatoxins are then extracted with methanol in a Soxhlet extraction apparatus. The methanolic solution (yellow solution) is filtered through glass wool and washed with the pentane:hexane mixture to remove residual oil. The precipitate is filtered off and the methanolic solution is evaporated to dryness, the residue is dispersed in distilled water and then saturated with sodium chloride. After deflating, aflatoxins are partitioned into chloroform for cleanup with column chromatography. Aflatoxins are eluted with chloroform:methanol (95:5, v/v), the eluate is evaporated to dryness, and the residue is then dissolved in chloroform. Aflatoxins are determined by thin-layer chromatography.


Methods for aflatoxin analysis using thin-layer chromatography (TLC) and fluorescence detection were developed in the 1960s and are still widely used. In the late 1970s, several applications of high performance liquid chromatography (HPLC) were developed and as they were generally more sensitive than the TLC methods, they are now popular for aflatoxin analysis when a high degree of accuracy is required. For some test purposes convenience and rapidity of analysis are more important than accuracy, and the bright greenish yellow fluorescence (BGYF) and minicolumn methods were evolved with this in mind. Recently several enzyme-linked immunosorbent assay (ELISA) system kits for aflatoxin analysis have been developed and some of them found suitable for the kind of testing now carried out using the BGYF and minicolumn methods. Gas chromatography can also be used for aflatoxin analysis under certain conditions. From the range of aflatoxin analysis methods now available it should be possible to choose methods suitable for specific purposes.


A thin-layer chromatography (TLC) cleanup development with benzene : hexane (3:1) effectively removed lipids and some contaminants from mixtures of mycotoxins in corn oil, olive oil, groundnut oil, soybean oil, and seed extracts. A second development in the same direction as the first, using toluene : ethyl acetate : formic acid (6:3:1) or benzene : acetic acid (9:1), separated the mycotoxins. Satisfactory separation was achieved for commercial oils spiked with sterigmatocystin, zearalenone, ochratoxins A, B, and C, and aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$. This technique permits detection of 5 $\mu$g kg$^{-1}$ aflatoxin $B_1$ in corn.
Four closely related aflatoxins B1, B2, G1, and G2 were isolated and characterized and their inter-relationships studied. The molecular formulae of the aflatoxins were established from elementary analyses and mass spectrometric determinations. The infra-red and ultraviolet absorption spectra of the four aflatoxins were very similar and indicated that all four compounds were closely related. Aflatoxins B2 and G2 were far less toxic than B1 and G1.

A rapid analytical method for aflatoxin in groundnuts and groundnut products is described. Aflatoxins are extracted with an acetone:hexane:water solvent mixture (60:35:5. v/v/v). The extract is filtered and the solvent evaporated, the residue is dissolved in tetrahydrofuran, and then transferred to a Florisil-packed chromatographic column. Lipids and other extraneous materials are eluted together with tetrahydrofuran, and the aflatoxins are eluted with acetone. After evaporation of acetone, the aflatoxin residue is taken up in chloroform. Aflatoxins are then determined by thin-layer chromatography.

A sensitive, reliable, and economical method for the determination of six mycotoxins in mixed feeds is described. The feed is extracted with chloroform-water and the extract is cleaned up using a disposable Sep-Pak silica cartridge. The procedure requires less time (15 min from sample extraction to extract preparation) and less solvent (approximately one-tenth) compared with conventional methods and is suitable for a fast, economical screen. Additional cleanup procedures, involving dialysis or extraction into base, are described for samples containing high levels of interfering compounds. Thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) with fluorescence detection are described for identification and estimation of mycotoxins. The method has been applied to a wide range of mixed feeds, including laboratory animal diets and raw materials. The limit of detection is 1 µg kg\(^{-1}\) for all mycotoxins measured by 11PLC.

A method is described for simple and rapid detection of aflatoxins in corn, buckwheat, groundnuts and cheese. Aflatoxins were extracted with a chloroform-water solvent and were purified by a florisil column chromatographic procedure. Column eluates were concentrated and spotted on a high performance thin-layer chromatography (HPTLC) plate, which was then developed in chloroform-acetone (9:1) and/or ether:methanol:water (94:4.5:1.5) or chloroform:isopropanol:acetone (85:5:10). Aflatoxins were quantitated by densitometry. The minimum detectable aflatoxin concentration (µg kg\(^{-1}\)) in various test materials were 0.2 B1, 0.1 B2, 0.2 G1, 0.1 G2, and 0.1 M1. Recoveries of the aflatoxins added to corn, groundnuts, and cheese samples at 10-30 µg kg\(^{-1}\) were > 69 % (aflatoxin G2) and averaged 91 % B1, 89 % B2, 91 % G1, and 78 % G2, and 92 % M1. The simple method described was compared with the AOAC CB, AOAC BF, and AOAC milk and cheese methods. These methods were applied to corn, groundnuts, and cheese composites spiked with known amounts of aflatoxins, and

In order to develop a method for the determination of aflatoxins in groundnuts, groundnut products, cereals and cereal products, a comparative study was made of some existing procedures (GB, TPI, BF, Cclite, Pons). Collaborative studies using these procedures were undertaken. The CB procedure was selected on account of the quality of the extracts obtained, the low level of background interference and the relative precision and simplicity of the required equipment. Taking into account the frequently high levels of aflatoxin B, (around 1 ppm) in groundnuts and the quality of silica gel and chloroform available in France, some minor changes have been incorporated into the CB procedure: the chloroform used for the column is washed and dried before use, the volume of elution is increased and an ether: methanol: water (96:3:1) mixture is used as a developing system.
to naturally contaminated buckwheat and cheese. Recoveries were much lower for the BF method compared with the simple method and the CB method.


Five analytical methods, viz. AOAC "BF" and "Celite", the T.P.I. method, a Dutch method and a Polish method were compared for determination of aflatoxins in groundnut meals. The T.P.I. method was considered optional. The best separation of aflatoxin on thin-layer chromatographic (TLC) plates was achieved with chloroform-acetone (90+10) as the developing solvent.


Aflatoxin B1 at 560-1550 µg kg⁻¹ was determined by thin-layer chromatography and spectrophotometry in all of 10 batches of groundnuts from Hong Kong. There was good correlation between presence of aflatoxin B1 and mutagenic activity of extracts, demonstrated by using the Ames test.


A technique is described, involving thin-layer chromatography and fluorescence spectrophotometry, for the estimation of aflatoxins. Using this technique, aflatoxin B1 was detected in imported cashewnuts and groundnuts at 2.6-6.8 µg kg⁻¹.


A thin-layer chromatographic (TIX) method for semi-quantitative determination of aflatoxin B1 in foodstuffs, suitable for routine analysis, is described. The application of the method to the analysis of groundnut kernels is discussed. Of groundnut samples imported into Germany in 1970-1971, 10% of samples had aflatoxin.

Measures for improving health control of imported groundnuts are discussed.


A rapid extraction method is described for determining aflatoxin in groundnut meal. It involves the extraction of the toxin from the ground meal by chloroform in the presence of water at room temperatures. A determination can be carried out on a de-fatted meal in less than 1 h. The toxin is determined by using thin-layer chromatography. The method worked equally well with groundnut kernels that had been de-fatted by the normal procedure, i.e., with light petroleum or hexane.


A minicolumn method is described for determination of aflatoxin B1 in groundnuts. The method allowed detection of aflatoxin B1 at more than or equal to 1 µg kg⁻¹. The thin-layer chromatography (TLC) method was used for semiquantitative visual determination of the toxin; mean recoveries for the values more than or equal to 2.5 µg kg⁻¹ were 76-87 %.


A convenient miniassay for aflatoxin is described for two strains of Aspergillus flavus (NRRL 6539 and 5565) and one strain of A. parasiticus (NRRL 3240). Cultures were grown for 3-10 days in 10 mL of a coconut extract medium. Sensitivity of the assay as measured by photofluorometry (365 nm max. excitation; 445 nm max. emission) was of the order of 0.01 µM (3.12 ng mL⁻¹) for aflatoxin B1 dissolved in aqueous iodine (0.26 mM). High pressure liquid chromatography (HPLC), monitored by fluorometric analysis of aflatoxin B1 standard and selected culture filtrates, was used to confirm the sensitivity of the assay and gave an indication of the specificity for iodine-enhanced fluorescence of aflatoxin in the coconut extract medium. Thin-layer chromatography (TLC) was used to further confirm aflatoxin litres and specificity for enhancement of aflatoxins B1 and G1 in culture filtrates. The method provides a semiquantitative assay for aflatoxin in fermentation cultures allowing for genetic selection and scale-up for critical examination of toxigenic potential of A. flavus and A. parasiticus strains growing in a coconut-based medium. The potential for the development of the method as a
A substitute for direct assay of aflatoxin from contaminated field samples or other substrates is considered.


A simple and rapid aflatoxin extraction procedure, based on a direct extraction introduced by Pons and single liquid-liquid extraction proposed by Waltking, is described for groundnuts and groundnut products. Methanol is used as the extraction solvent. Thin-layer chromatography (TLC), using the system described by Engstrom, gave satisfactory results. The method is suitable for aflatoxin B₁ levels of 8 µg kg⁻¹ or higher in peanut butter and roasted groundnut products. Aflatoxin B₁ levels down to 3 µg kg⁻¹ can be detected in raw groundnuts and groundnut shavings.


Problems relating to the determination of acceptable levels of additives and contaminants in foodstuffs are discussed with examples, including the occurrence of aflatoxins in milk. Since the use of DDT in cattle farming has been prohibited in the Netherlands, the DDT content of milk has been greatly reduced, e.g., in the Alkmaar region only 2 out of a total of 1153 milk samples analysed during 1972-1975 contained > 1.25 ppm DDT/DDE in fat, vs. 29 out of 555 samples in 1970. As aflatoxin M₁ has been shown to be carcinogenic, efforts are being made to prevent its occurrence in milk by minimizing the amount of aflatoxins in mixed fodder. Dutch legislation specifies that aflatoxins must not be present in detectable amounts in groundnuts and their products.


A quantitative method for the estimation of four aflatoxins, based on chromatographic separations and selective destruction of aflatoxins B₁ and G₁ by nitric acid, has been developed. To enhance the specificity of the method, a sample blank is determined and is used to correct the values for non-specific interfering substances.


The American Oil Chemists' Society Smalley Check Sample Program offers ongoing check sample series for determination of aflatoxins in groundnut meal, cottonseed meal, and corn meal. Laboratories participating in this program represent a worldwide cross section of industry, regulatory, and commercial laboratories. Each annual series consists of seven samples including a solution of an unknown mixture of aflatoxins B₁, B₂, G₁, and G₂ for direct spotting and quantitation. Participants analyses of the solution of aflatoxins resulted in coefficients of variation generally higher than those of any sample. Comparison of four years of results for analysis using BF and CB methods of analysis for groundnut meal samples with aflatoxin B₁ levels ranging from 2 to 56 ng g⁻¹ resulted in higher aflatoxin B₁ results for the CB method. The number of participants reporting results by HPLC method is small; however, their results compare closely with those using TLC methods.


The International Smalley Aflatoxin Check Sample Program of the American Oil Chemists' Society has offered check sample series for aflatoxins in groundnut meal, cottonseed meal, and corn meal since 1976, and an aflatoxin M in raw milk series since 1980. This paper provides the computed mean of all analysts' results and between-laboratory precision for each of the samples in each of the check sample series distributed in 1980-81 and 1981-82. In addition, a comparison is made of the relative measurement and analytical accuracy of those analysts who have participated in the groundnut meal series for at least four years and in the cottonseed and corn meal series since their inception (six years). For this comparison, each analyst's result for each sample was calculated as a % of the mean for all analysts for that sample; these values were then averaged for each analyst over all the meal samples in all the series for each meal type in which the analyst had participated, to obtain an overall measure of analytical accuracy. A similar calculation was made using the reported results for the defined solution of aflatoxins included in each series, to obtain an overall measure of measurement accuracy. An evaluation of the meal series results for the past two seasons shows an overall within-laboratory precision in the range reported for the collaborative studies by which the methods were validated; the between-laboratory precision, although improved over past years, is still far from the collaborative study range. The precision data for the aflatoxin solution included in each series indicate this bias could be related, in large part, to the reference standards used. The extended period evaluation of analysts' performance shows no apparent correlation between measurement and analytical accuracy except for a general positive trend for those analysts using the BF method.
for aflatoxins in groundnut meal. A comparison of the accuracy of the BF and CB methods for aflatoxins in groundnut meal shows no significant difference between results by the two methods on the basis of the extended period evaluation, in contrast to the generally higher results for the CB method in the past two seasons' evaluation. The scatter in the average analysts' analytical accuracy is essentially the same, regardless of the method used.


Operational and statistical evaluations are presented for two Smalley Subcommittee series check sample programs for mycotoxin-contaminated commodities, i.e. collaborative programs on deoiled aflatoxin-contaminated groundnut meal and cottonseed meal series. Methods of analysis were restricted to the AOAC 'CB' or the 'BF' methods for groundnut products and the Pons procedure for cottonseed products.


Six different methods of extraction were compared for estimation of aflatoxin B1 in groundnuts. Partially ground groundnut kernels were autoclaved and inoculated with an aflatoxigenic isolate of Aspergillus flavus. After 8 days of incubation at 22-25°C, extraction of aflatoxin B1 from the substrate was done according to different methods used. Aflatoxin B1 was determined using thin-layer chromatography (TLC). Marked differences were found in the amounts of aflatoxin B1 extractable by different methods. Of all the methods tested that of Leim and Beljaars (1970) proved the best for extracting maximum amount of aflatoxin in short time.


Aflatoxin extraction methods namely Best Food (BF), Contamination Branch (CB), Pons' and Romer's and analytical methods namely thin-layer chromatography (TLC), spectrophotometry and minicolumn techniques, in different combinations, were compared for their efficiency for determining aflatoxin B1 occurring naturally in groundnuts and also for estimating aflatoxin B1 levels in groundnut meal samples spiked with known quantities of the toxin. The BF and Pons' methods gave better efficiency than the other two methods. The spectrophotometric method was more effective than the TLC and minicolumn techniques. The BF method was relatively less expensive and less time consuming than the other extraction methods. The Pons' method was convenient for handling larger numbers of samples, especially in the absence of centrifuge facilities required for the BF method.


A simple method for determination of aflatoxins in vegetable oils is described. The method was successfully applied to both crude and degummed oils. The oil sample, dissolved in hexane, was applied to a silica column and washed with ether, toluene and chloroform; aflatoxins were eluted from the column with chloroform : methanol (97:3). As quantitated by thin-layer chromatography (TLC) and liquid chromatography, the oil> analyzed contained aflatoxin B1 at levels of 5-200 µg kg⁻¹. Recoveries of aflatoxin B1 standards added to aflatoxin-free oils were between 89.5 and 93.5%, with coefficient of variation of 6.3-8.0%.


Chloroform is an extraction agent and solvent which is frequently used for aflatoxin analysis. But this may involve a considerable carcinogenic potential. Therefore, tests were carried out to replace chloroform by a harmless solvent. The modified method of Seitz and Mohr (Seitz, L.M., and Mohr. H.E. 1974. Cereal Chemistry 51: 487-491) using methylene chloride was used for the determination of aflatoxin levels in food products. The recovery rates and reproducibility of the results were within a range as known from previously applied methods.


A method is described for determining the aflatoxins, particularly aflatoxin B1, based on the intensity of the ultraviolet absorption at 363 mµ, after purification by thin-layer chromatography. This procedure has been applied to groundnut meal and crude aflatoxin isolated from cultures of certain strains of Aspergillus flavus and A. parasiticus. This method has only been applied to meals of high toxicity and is not applicable, in its present form, to meals of low or medium toxicity. The extraction and decomposition of aflatoxin B1 have also been investigated.


A rapid procedure for aflatoxin (developed by Neshcim et al. 1964) was collaboratively tested in 15 laboratories on samples of groundnuts, groundnut meal, and peanut butter. For a semiquantitative method the results were in good agreement. It is recommended that the method be further studied.


Satisfactory resolution of the four common aflatoxins, $B_1$, $B_2$, $G_1$, and $G_2$, on thin layer chromatograms has been a recurring problem. The most frequently observed cause of poor resolution and tailing of spots in the chromatograms was the variable properties of the commercial silica gel-calcium sulfate adsorbent preparations. Variations in quality were observed even from one container to the next within single lots produced by individual manufacturers. Other variables which affected the chromatography to some degree included adsorbent particle size, concentration and nature of the calcium sulfate binder, silica gel layer thickness and moisture content, vapor phase composition in the developing chamber and the solvent used for development.


A rapid method for detecting aflatoxins in peanut butter is described. The sample is comminuted in a waring blender for 2 minutes with a mixture of methanol:water:hexane. The resulting slurry is centrifuged or filtered, and an aliquot is mixed with diatomaceous earth and transfanned to a chromatographic column. Lipids and other extraneous materials are eluted with hexane, and the aflatoxins are then eluted with a chloroform:hexane mixture. The solvent is removed, and the residue is dissolved in chloroform and chromatographed on silica gel thin-layer chromatographic (TLC) plates. The aflaioxins are determined on the plates by means of their fluorescent characteristics under ultraviolet light.


A method for determination of aflatoxins in groundnut and groundnut products is described. Aflatoxins ($B_1$, $B_2$, $G_1$, and $G_2$) are extracted from the ground groundnut sample by water and chloroform. After filtration the extract is purified by column chromatography using hexane and anhydrous ether, and the components are eluted with methanol-chloroform (3:97, v/v). Separation and quantification are by thin-layer chromatography (TLC). Involving use of internal standards. Detection limit is in the range of 1-5 µg kg$^{-1}$, with a coefficient of variation of 20-30 % by visual quantification, or approximately 5 % using a densitometry technique.


An interlaboratory study of a negative ion chemical ionization mass spectrometric (MS) confirmation procedure for aflatoxin B$_1$ was conducted in laboratories in the USA, UK and Federal Republic of Germany. Twelve partially purified, dry film extracts from naturally and artificially contaminated roasted groundnuts, cottonseed, and ginger root containing various quantities of aflatoxin B$_1$ were distributed to the participating laboratories. The extracts required additional cleanup before MS analysis, using either an acidic alumina column and preparative thin-layer chromatography (TLC) or a two-dimensional TLC procedure. Recovery of aflatoxin B$_1$ was influenced by degree of recovery of the sample from acidic alumina and/or TLC plate and incomplete elution of aflatoxin B$_1$ from silica gel. Factors affecting MS confirmation included the purity and recovery of aflatoxin and MS instrument sensitivity. Aflatoxin B$_1$ identity was confirmed in 19.5, 90.9, and 100 % of samples containing $<5$, 5-10, $>10$ ng aflatoxin B$_1$, g$^{-1}$ product, respectively, by solid probe introduction using full mass scans. The MS method has been adopted as official first action.


The present investigation is the first definitive study of the fate of the aflatoxins in vegetable oils undergoing processing. Crude oils, obtained by solvent extraction or by hydraulic pressing of ground moldy groundnuts (not suitable for human consumption), contained only small fractions of the aflatoxin originally present in the groundnuts; the meals retained the bulk of the aflatoxin. Conventional alkali refining and washing of the oils reduced aflatoxin content to a range of 10 to 14 µg kg$^{-1}$. The subsequent bleaching operations essentially eliminated aflatoxin from the oils; the concentrations were now less than 1 µg kg$^{-1}$. The above results were confirmed using corn oils obtained from corn germ deliberately contaminated in the laboratory with Aspergillus flavus. The nonfluorescing forms of aflatoxins, capable of being produced during the alkali refining operations, are also absent from the refined vegetable oils, these aflatoxin derivatives are readily converted to their original form on acidification and thereby measurable by fluorescence, if present.
A revised method has been developed for the formation of aflatoxin B₁ derivatives for chemical confirmation. The method involves treating the aflatoxin-containing extract with concentrated HCl and water to yield the water adduct and with concentrated HCl and acetic anhydride to yield the epimeric acetates. The method is considered simpler to perform and produces fewer side reactions than the AOAC official final action method.


A reflectance fluorodensitometer employing illumination of chromatograms with longwave UV light at 45 degree angles to the plate surface and measurement of reflected fluorescence at 90 degree was found to be suitable for measuring aflatoxins on silica gel-coated thin-layer plates. The relationship of peak area vs. concentration was linear for 1-20 ng aflatoxins B₁ and G₁/spot. Degradation of aflatoxins was slight. Five repetitive scans of the same chromatogram containing 5 ng each of B₁ and G₁ reduced the recorded areas an average of 1 % per scan. Consecutive scans of 8 identical standard chromatograms containing 5 ng each of B₁ and G₁ and 1.5 ng each of B₂ and G₂ showed a reproducibility, as measured by coefficients of variation, of + 4-5 % (B₁ and G₁) and + 5-9 % (B₂ and G₂), representing the combined errors of standard application, TLC development, and scanning. Analysis of aflatoxins in purified sample extracts from six contaminated oilseed meals, 3-500 µg aflatoxins kg⁻¹, in which the same TLC plates were scanned by a transmission densitometer and the reflectance densitometer yielded essentially equivalent values.


A rapid and simple method, originally developed for detecting aflatoxin contamination in cottonseed products, was modified for application to a variety of agricultural products. The modified method involves rapid blender extraction of the sample with aqueous acetonitrile, treatment of an aliquot of the filtrate with lead acetate solution to remove interfering pigments, and rapid partition of aflatoxins in the treated extract into benzene to effect an 8-fold concentration of the aflatoxins. A portion of the benzene extract is adsorbed by capillary attraction onto the bottom of a small column (4 mm x 20 cm) filled with zones of acidic alumina and silica gel and the column is allowed to develop 5 min in chloroform-acetonitrile-2-propanol (93+5+2). Aflatoxin is detected as a sharp blue fluorescent band about 1 cm above the alumina zone when the developed column is viewed under longwave UV light. The total analysis time is about 20 min. The

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A semi-mechanized method of analysis for aflatoxins in groundnuts is described which enable samples from every ton of nuts used to be analyzed, and increases the accuracy of determinations which is limited at present by sampling errors. The technique is a modification of the AOAC BF method with changes to some of the reagents, elimination of centrifugation, replacement of some of the apparatus with disposable items, and reduction to a semi- micro scale. The complete process is outlined with reference to a schematic diagram. The method, termed 'Aflamatic'.
allows an analyst to perform up to 100 analyses in 8 h, has a detection limit of 2-3
µg kg\(^{-1}\) aflatoxin B\(_1\) and a precision of + 20 %.

method of estimating aflatoxin B\(_1\) in corn, groundnut and groundnut cake. Journal
of Food Science and Technology, India 24(2): 90-91.

Pons' method for estimation of aflatoxin B\(_1\), was modified by eliminating the column
clean-up step and by introducing additional thin-layer chromatography (TLC) with
ether and hexane (1:1). Estimation of aflatoxin B\(_1\) in contaminated groundnut meal
by this method compared well with the standard method.

economical method for determination of aflatoxin and ochratoxin in animal
feedstuffs. Journal of the Association of Official Analytical Chemists 64(4):
961-963.

A quantitative procedure widely used in European Economic Community (EEC)
countries has been successfully scaled down to produce a rapid mediod for
determination of aflatoxin B\(_1\) (and other aflatoxins) in animal feeds. Without
modification, the method may be used for simultaneous ochratoxin A determination
in simple feeds, but a slightly different extraction procedure is required for
compound feeds. Validity of the method has been demonstrated by comparison with
the full EEC procedure for aflatoxin B\(_1\) and the Neshchim method for ochratoxin A.
Analyses may be completed within 2 h and there is a considerable savings in
materials over the two reference methods. The procedure is also less hazardous
because volumes of toxic extract are small, and the operator is exposed to minimum
solvent vapor.

in mixed animal feedstuffs, using a novel membrane cleanup procedure. Journal

A multimycotoxin thin-layer chromatographic screening method is described which
is applicable to most animal feedstuffs. Interference from nonspecific lipid, pigment,
and other coagglutinogens of simple and mixed feeds is reduced to a minimum by using
a membrane cleanup step. Aflatoxins B\(_1\), B\(_2\), G\(_1\), and G\(_2\), citrinin,
diacetzoxyscirpenol, ochratoxin A, patulin, penitrem A, sterigmatocystin, T-2 toxin,
and zearalenone may be reliably detected. The sensitivity of the method is generally
low for mixed feeds but even so aflatoxin can be detected at a level of 3 µg kg\(^{-1}\) and
ochratoxin A at 80 µg kg\(^{-1}\). While the basic method is less sensitive for
sterigmatocystin (330 µg kg\(^{-1}\)), patulin (600 µg kg\(^{-1}\)), zearalenone (1000 µg kg\(^{-1}\)),
and the trichothecenes (1000-4000 µg kg\(^{-1}\)), it may be adapted so as to reduce the
above detection limits when the presence of these toxins is suspected. Lower levels
may be detected in extracts of simple feeds.

Assay of aflatoxin in peanuts and peanut products using acetone-hexane-water for

A method for analysis of aflatoxins in groundnut products is described. The method
involves extraction of aflatoxin from the sample with a homogeneous acetone:hexane:water solvent mixture followed by purification of the extract by
phasic extraction of the aflatoxin with aqueous sodium chloride and then with
chloroform. The purified chloroform extract is analyzed by thin-layer chromatography by comparison of the intensity of fluorescence of any aflatoxin
with the intensity of known standard. The aflatoxin analyses of groundnuts were
found very variable due to sampling, and this variability was greatly reduced by
finely grinding and thoroughly mixing 2 kg sample before removal of an aliquot for
assay. The mediod is sensitive to approximately 2 µg kg\(^{-1}\).

individual aflatoxins B\(_1\), B\(_2\), G\(_1\), and G\(_2\) standards in benzene and chloroform
299-302.

Aflatoxins B\(_1\), B\(_2\), G\(_1\), and G\(_2\) standards were stored in benzene and chloroform
solutions for one year at 28 and -18°C. Storage at either temperature produced only
minor changes in molar absorptivity; the decreases ranged from 0.5 to 2.9 % and
averaged 1.9 % at 28°C and ranged from 0.3 to 1.4 % and averaged 1 % at -18°C.
Solution fluorescence KQ showed a greater decrease; it ranged from 8 to 22 % and
averaged 15 % at 28°C and ranged from 7 to 19 % and averaged 14 % at -18°C.
Thin-layer chromatography of standards stored for one year showed a single spot
for each aflatoxin and the absence of any other fluorescent derivatives.


Aflatoxins deposited as dry films in glass vials are adsorbed to the surface and are
not completely recovered by the addition of solvent. The concentrations of solutions
prepared from dry films must be determined by UV absorptivity measurements.

purity of aflatoxin standards. Journal of the Association of Official Analytical
The concentration of solutions of aflatoxin is determined by ultraviolet absorbance measurement and the purity is determined by examination of developed 50 ng spots of the aflatoxins on thin-layer chromatography (TLC) plates. Results from three collaborative studies demonstrated the effectiveness of the method. This procedure should be used each time solutions of aflatoxins are prepared for use in quantitative analysis. The method is recommended for adoption as official first action.


An analyst should have the capability of determining the concentration and purity of any reference standard before he uses the standard in analysis and should be aware of the period over which he can rely on the concentration and purity of the standards. A method for aflatoxin standard solutions has been developed based on UV absorption measurements and thin-layer chromatography (TLC) examination. The method has been described in detail in a report (1) of the Associate Referee for Mycotoxin standards of the Association of Official Analytical Chemists (AOAC). This report includes a detailed justification for the criteria employed and recommends adoption of the method as official, first action. The recommendation, which was accepted, was based on a history of successful use for over a year by the U.S. Food and Drug Administration (FDA) District Laboratories, good precision and accuracy when used to calibrate standards employed in two separate AOAC collaborative studies of aflatoxin assay procedures, and partial results from this IUPAC sponsored collaborative study. The IUPAC sponsored study has now been completed and is herein reported in detail.


Measurements of molar absorptivities in methanol were carried out by two laboratories on samples of aflatoxins B1 and G1 prepared and purified independently in four laboratories and on samples of aflatoxins B2 and G2 prepared and purified independently in three laboratories. Molar absorptivities of pure aflatoxins B1, B2, G1, and G2 in benzene-acetonitrile (98:2) were determined in two laboratories. With the exception of aflatoxin G2, no significant difference between aflatoxin samples could be demonstrated. Molar absorptivity values and absorbance ratios for each aflatoxin, based on these data, are given. Statistical 95% confidence limits were established for a single determination of molar absorptivity. A statistical study was made of the components of variance which contribute to the accuracy of the determination of molar absorptivity. A comparison of the fluorescence intensities of spots of various aflatoxin preparations, developed on silica-gel coated thin-layer plates, with the amount of aflatoxin in each spotting solution as determined by absorbance measurements, demonstrated the validity of using absorbance and fluorescence comparison measurements for aflatoxin quantitation.


A screening method is described for detecting total aflatoxins (B1 + B2 + G1 + G2) in mixed feeds, grains, nuts, and fruit products in samples containing 5-15 µg kg⁻¹. In addition, the presence of aflatoxins in positive samples can be confirmed and the toxins can be quantitatively measured, using the same extract as that used for screening. In the screening method, aflatoxins are extracted with acetone:water (85:15), and interferences are removed by adding cupric carbonate and ferric chloride gel. The aflatoxins are extracted from the aqueous phase with chloroform and the chloroform extract is washed with a basic aqueous solution. A Velasco-type minicolumn is used to further purify the extract and capture the aflatoxins in a tight band. The screening method has been successfully applied to 24 different agricultural commodities. Quantitative TLC was also performed with extracts of each of these commodities. An average recovery of 94% B1, 108% B2, 130% G1, and 103% G2 was obtained compared to the official final action AOAC method for cottonseed products. Within-laboratory coefficients of variation of 10-15% were obtained for each of the aflatoxins and total aflatoxins in a sample of groundnut meal naturally contaminated with 11 µg B1 + 3 µg B2 + 11 µg G1 + 5 µg G2 kg⁻¹.


A rapid confirmatory method for aflatoxins B1 and B2 has been developed. The extract used for thin-layer chromatography quantitation is rapidly cleaned up by elution through a silica gel Sep-PAK cartridge and then analyzed by gas chromatography/mass spectrometry/selected ion monitoring at 3000 resolution using a bonded-phase fused silica capillary column with on-column injection. Limits of detection for aflatoxins B1 and B2 in groundnut samples were 0.1 µg kg⁻¹.


The toxic extract of Brazilian groundnut meal was further purified and a fluorescent method of identification after chromatographic separation was devised. The toxic
substance was isolated from a fungus, Aspergillus flavus. When the fungus was grown on sterilized groundnuts and fed to ducklings, it resulted in typical liver lesions in ducklings.


This paper reviews the sampling plans and collaboratively studied methods of analysis for aflatoxins in foods and feeds. Aflatoxins are the only food contaminants being monitored routinely on an international scale with methods operating at the order of a magnitude of 10 µg kg⁻¹. At this level, methods of analysis which can achieve coefficients of variation of 30-40 % with recoveries of 70 % or greater in interlaboratory collaborative studies can be considered eligible for reference status. In most cases, sample reproducibility is the variable limiting the reliability of methods of analysis. The inherent uncertainty of the identity of chromatographically separated entities requires the application of confirmatory tests to verify that the characteristics measured result from the presence of aflatoxin. The methods are also inoperable without a verification of the identity, purity, and concentration of the reference standards used. Screening methods which reliably eliminate negative samples from further consideration are indispensable for the practical operation of monitoring programs.


A procedure is described to distinguish aflatoxin from non-specific fluorescent compounds. The method is based on the poor mobility of aflatoxin on thin-layer chromatography (TLC) plates with diethyl ether as developing solvent and photo-decomposition of aflatoxins by UV light into slow moving and fast moving compounds on TLC. Two tests, namely, preliminary development of the chromatograph with ether, and exposure of the spots to UV light prior to development with the methanol-chloroform solvent can be adopted as tests complementary to each other for distinguishing aflatoxin B₁ from other non-specific fluorescent compounds.


The Tropical Products Institute (TPI) method was used to estimate aflatoxins in 45 samples of groundnut. There were 15 non-toxic or slightly toxic, 25 moderately toxic and 5 highly toxic samples. Aflatoxin was estimated by a modification of TPI method. In 230 samples of groundnut cake from India levels of aflatoxin ranged from 5-2000 µg kg⁻¹. The modified method was suitable for analysis of aflatoxin in groundnut cake.


Of the nearly 150 isolates of Aspergillus flavus from groundnuts tested, only four produced aflatoxin B₁. These isolates differed from the type strain of A. flavus M001 (obtained from the Tropical Products Institute, London) in certain morphological characteristics. Of the several salt solutions tested, 1 % CaCl₂ was found most promising in extracting aflatoxin from the contaminated groundnut cake. In the preparation of the protein isolates, precipitation of the protein in CaCl₂ solution helped in removing nearly 80 % of the toxin from the protein. Certain apparently healthy groundnuts showed bright bluish-violet fluorescence under ultraviolet light. Extracts of such groundnuts on thin-layer chromatography (TLC) plates showed the fluorophor at the same Rf as that of aflatoxin B₁. A simple solvent system of benzene : cyclohexane : acetic acid (3:5:2, v/v/v) was found to give on paper chromatogram as good resolutions of the aflatoxin complex as found on alumina TLC with chloroform and methanol as solvent.


A collaborative study was designed and carried out to test the accuracy and precision, at low levels of contamination, of AOAC methods I and III, for the determination of aflatoxins in peanut butter. Ten test samples and one practice sample of each peanut butter were analyzed by each method. Two test samples were naturally contaminated with about 2 µg aflatoxin B₁ kg⁻¹. The eight spiked samples (duplicate samples for each level) had 0, 2, 4, and 8 µg B₁, added kg⁻¹ and aflatoxin B₂ was added at one-fourth the aflatoxin B₁ level. The seven collaborators were instructed to analyze the samples according to methods I and III except for minor modifications necessary to handle the low levels of aflatoxins in the sample extracts and to quantitate the aflatoxin content both visually and densitometrically. The results of the study indicated that the analyses were able to determine the aflatoxins in the 2-10 µg kg⁻¹ range with either method although method I gave better accuracy and precision and had less interferences than method III.
The chemical method for confirmation of the identity of aflatoxin by derivative formation directly on the thin-layer chromatographic (TLC) plate was studied collaboratively by eight participants. Aflatoxin B<sub>1</sub> was confirmed in 17 of 17 sample extracts representing 15 µg aflatoxin B<sub>1</sub> kg<sup>-1</sup> peanut butter, in 13 of 16 extracts representing 5 µg kg<sup>-1</sup>, and in none of the seven aflatoxin-free extracts. Collaborators commented that the method was easily performed and gave good results. The method has been adopted as official first action.

A modification of the Andrellos procedure for identification of aflatoxin B<sub>1</sub> was studied collaboratively in 19 laboratories. The procedure, based on the altered chromatographic behaviour of the aflatoxin after reaction with trifluoroacetic acid, formic acid/thionyl chloride, and glacial acetic acid/thionyl chloride, was modified by an improved silica gel column cleanup and a clearer definition of sources of difficulty. Each collaborator examined three extract samples: two naturally contaminated with 5 µg aflatoxin B<sub>1</sub>/sample, and one aflatoxin-free extract to which an aflatoxin B<sub>2</sub> artifact was added. No false identifications were made. Sixteen laboratories obtained reasonably good results with the trifluoroacetic acid and formic acid/thionyl chloride reagents. Twelve laboratories obtained reasonably good results with the acetic acid/thionyl chloride reagent but there was general difficulty with a side reaction assumed to be caused by inability to maintain anhydrous conditions. The method was recommended for adoption as official first action.

A revised method for the preparation of aflatoxin B<sub>1</sub> derivatives for chemical confirmation has been compared with the AOAC official final action method in collaborative study, using TLC- pure aflatoxin B<sub>1</sub> isolated by the individual collaborators from peanut butter, groundnut meal, and corn. The revised method was simpler, shorter, and more reliable than the official method. It is recommended for adoption as an official first action alternative to the preparation and observation portions of the official final action method.

This paper critically reviews analytical methods for mycotoxins in agricultural commodities including groundnuts and groundnut products with emphasis on thin-layer chromatographic (TLC) and high performance liquid chromatographic (HPLC) based-methods. Procedures for confirmation of aflatoxins are also discussed. Screening methods including minicolumn techniques are briefly reviewed.

A thin-layer chromatography procedure for improved resolution of aflatoxin is described. This procedure eliminates potential interference from a fluorescent material found in extracts of groundnuts with red testa. Improved resolution of aflatoxins was obtained by developing thin-layer chromatography plates at 40 F and using 9 % methanol in chloroform as the developing solvent.

Water was added to solvent systems for thin-layer chromatography of aflatoxins to achieve more reproducible results in laboratories where temperature and relative humidity vary. Resolution of aflatoxins also improved. Increments of water were added to solvent systems composed of acetone-chloroform (10+90, 12+88, and 15+85, v/v). As the water concentration was increased, separation of aflatoxins B<sub>2</sub> and G<sub>1</sub> improved. These two aflatoxins are usually the most difficult to resolve in these solvent systems. Separations were the best with water-acetone-chloroform (1.5+12+88, v/v/v). Water added to methanol-chloroform (3+97, v/v) improved resolution of this system but not enough for quantitative analysis, or at times, qualitative analysis. The solvent system water-methanol-ether (1+3+96, v/v/v) separated aflatoxins as well as water-acetone-chloroform (1.5+12+88, v/v/v).
A simple, systematic analytical method for multiple mycotoxins was developed for detecting 14 mycotoxins: aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$, sterigmatocystin, T-2 toxin, diacetoxyscirpenol, neosolaniol, fusarenon X, zearalenone, ochratoxin A, citrinin, luteoskyrin, and rugulosin. These mycotoxins were extracted with 20 % H$_2$SO$_4$ - 4 % KCl-acetonitrile (2+20+178), defatted with isooctane, and transferred to DC-Fertigplatten Kieselgel for the solid layer and chloroform-methanol-water-90 % formic acid (90:10:1:1) as the developing solvent. The recovery percentage of each mycotoxin from polished and unpolished rice, maize and groundnuts is given.


A simple, rapid, and solvent-efficient method for determining aflatoxins in corn and peanut butter is described. Aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$ were extracted from a 50-g sample with 200 mL methanol-water (85+15). A portion of the extract was diluted with 10 % NaCl solution to a final concentration of 50 % methanol, and then defatted with hexane. The aflatoxins were partitioned into chloroform. The chloroform solution was evaporated, and the residue was placed on a 0.5 g disposable silica gel column. The column was washed with 3 mL each of hexane, ethyl ether, and methylene chloride. Aflatoxins were eluted with 6 mL chloroform-acetone (9+1). The solvent was removed by evaporation on a steam bath, and the aflatoxins were determined using thin-layer chromatography (TLC) with silica gel column chromatography/mass spectrometry. Journal of the Association of Official Analytical Chemists 67(5): 973-975.

A simple, rapid, and solvent-efficient method for determining aflatoxins in corn and peanut butter is described. Aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$ were extracted from a 50-g sample with 200 mL methanol-water (85+15). A portion of the extract was diluted with 10 % NaCl solution to a final concentration of 50 % methanol, and then defatted with hexane. The aflatoxins were partitioned into chloroform. The chloroform solution was evaporated, and the residue was placed on a 0.5 g disposable silica gel column. The column was washed with 3 mL each of hexane, ethyl ether, and methylene chloride. Aflatoxins were eluted with 6 mL chloroform-acetone (9+1). The solvent was removed by evaporation on a steam bath, and the aflatoxins were determined using thin-layer chromatography (TLC) with silica gel plates and a chloroform-acetone (9+1) developing solvent. Overall average recovery of aflatoxin B$_1$ from corn was 82 %, and the limit of detection was 2 ng g$^{-1}$. For mass spectrometric (MS) confirmation, aflatoxin B$_1$ in the extract from a 3 g sample (20 ng g$^{-1}$) was purified by TLC and applied by direct column injection at 40°C into
a 6 m fused silica capillary gas chromatographic column. The column was connected directly to the ion source. After injection, the temperature was rapidly raised to 250°C, and the purified extract was analyzed by negative ion chemical ionization MS.


Three different methods were compared for the determination of total aflatoxins in corn and groundnuts naturally contaminated with aflatoxins and in corn, groundnuts, cottonseed, peanut butter, and poultry feed spiked with aflatoxins B1, B2, and G1. The three methods were an enzyme-linked immunosorbent assay (F.LISA) screening test, a monoclonal antibody-affinity column-solid phase separation method, and the AOAC official thin-layer chromatography (TLC) methods for all except poultry feed, for which Shannon's TLC method for mixed feed was used. The ELISA test is designed to provide only positive results for total aflatoxins at > 20 ng g−1 or negative results at < 20 ng g−1. The affinity column separation is coupled with either bromination solution fluorometry to estimate total aflatoxins or liquid chromatography (LC) to quantitate individual aflatoxins. Fluorodensitometry was used to determine aflatoxins in commodities analyzed by the TLC methods. The LC and TLC results were in good agreement for all the analyses. The results for the affinity column using bromination solution fluorometry were similar except those for cottonseed, which were about 60% higher. The ELISA screening method correctly identified naturally contaminated corn and groundnut positive samples. No false positives were found for controls. The correct response for spiked corn, raw groundnuts, peanut butter, and cottonseed at > 20 ng aflatoxins g−1 was about 90%. The correct response for spiked poultry feed at > 20 ng aflatoxins g−1 was about 50%.


Results from two 1980 experiments were used to compare the performance of the visual, minicolumn and thin-layer chromatography (TLC) methods in detecting loads of farmers stock groundnuts with aflatoxin. The first experiment was conducted to establish variability of the methods under ideal conditions. The second experiment was conducted to evaluate the performance of the three methods under commercial conditions. Date and empirical models were used to explore potential improvements in detecting low-level contaminated lots. The minicolumn and TLC methods were very sensitive and provided consistent measurements. Operation characteristic curves developed by using empirical models (Logistic and Gompertz) were compared to statistical distribution functions used by Whitaker and coworkers. Assuming a desired probability of acceptance of 15%, both the minicolumn and TLC methods would accept lots with 60 µg kg−1 aflatoxin, while the visual methods would accept lots with 150 µg kg−1 aflatoxin. In crop years similar to 1980, the currently used method would not segregate groundnuts to meet the 15 µg kg−1 (total) tolerance levels and would provide excessive sheller risk. Because of the objectivity, precision and higher sensitivity, the minicolumn and TLC have potential for improving the detection of contaminated loads of farmers stock groundnuts.


A method based on the use of water slurries was developed for aflatoxin analysis. This procedure enables aliquots, from kilogram size samples, to be analyzed without an increase in solvent or reagent costs. Groundnuts, peanut butter, groundnut meal, cottonseed, cottonseed meal, copra and maize were successfully analyzed by this slurry technique. Slurries were prepared in a 1-gal blender and sample weight to water volume ratios were 1:1.5 for peanut butter, 1:2.25 for whole seeds, 1:4 for commercial meals and 1:5 for copra. Extraction of 100-g aliquots of slurry with 200 mL of acetone resulted in aflatoxin yields comparable to those obtained by extraction of the dry products by the standard method. The precision of slurry analysis for the products, expressed as % coefficient of variation, ranged from 2.6 to 7.8 compared to 5.7 to 20.8 for analyses by the standard procedure. Determinations were made by fluorometric measurements of aflatoxin absorbed on the florisil layer in a minicolumn.


Confirmation reactions of aflatoxins B1 and G1 are described. The techniques involve the preparation of hemiacetal and epimeric acetates of the toxins. The hemiacets of B1 and G1 have a lower Rf value in comparison with the epimeric acetates. All the reaction products have lower Rf value than their original substances. Confirmation tests for aflatoxin directly on thin-layer plates are usefully performed in any laboratory without much experience.


A method is proposed for the analysis of groundnut samples for three mycotoxins, aflatoxin, ochratoxin and sterigmatocystin, by suitable treatment of a single sample
extract. Based on the objective evaluation of thin-layer chromatography (TLC) of the extract, results can be reproduced with an accuracy of + 20%. The method is considered to be satisfactory for the purposes of a field survey when the determination of the approximate level of mycotoxin contamination of cereals and groundnuts in the shortest possible time is of prime importance. Problems encountered with samples that have high oil contents or that are darkly pigmented are dealt with by suitable modifications of the method.


A collaborative study directly comparing the proposed BF method with the two AOAC official first action methods for aflatoxin in groundnut products (commonly known as the CB and the Celite methods) was carried out on both naturally contaminated groundnuts and specially prepared peanut butters, as well as commercial peanut butter and groundnut meal to which known amounts of the aflatoxins were added. Included in the study were: comparisons of a proposed variation in the CB method; a study of silica gels used for the clean-up step of the CB method; comparison of the visual and instrumental evaluation of the thin-layer chromatography (TLC) plates; and a spectrophotometric procedure for calibration of the standards. Statistical evaluation of the results indicated that no advantage was obtained through the proposed revision in the CB method nor in the use of a common silica gel lot for the clean-up column. The standard calibration method was shown to be both accurate and precise and instrumental evaluations of the TLC plates were more precise than visual determinations. In addition to being faster and more convenient and requiring less reagents, the BF method was equal to the official methods with respect to between- and within-laboratory precision and accuracy.


A practical, short cut, sensitive method for more rapidly determining aflatoxin in groundnuts and groundnut products has been described. Through reductions in solvent volumes, utilization of pressure filtration for clarification, and substitution of liquid : liquid extraction for a lengthy column cleanup, equivalent results are possible in less than one half the time required for the current official procedures. Sensitivity, precision and accuracy are comparable to the current methods for raw nuts and peanut butter. Using this method, one can analyze a ground sample of groundnuts within a period of less than 90 min and one analyst can assay more than 16 samples within an 8 h working day.


Under provisions of a USDA Marketing Agreement, an aflatoxin control program for groundnuts produced in the United States is administered by the Peanut Administrative Committee (PAC) composed of groundnut growers and shellers. The PAC requires aflatoxin tests on all commercial lots of shelled groundnuts. The present PAC testing program initiated in 1975 was evaluated for Crop A which averaged 9.5 µg kg⁻¹ aflatoxin and for Crop B which averaged 5.2 µg kg⁻¹ aflatoxin. For Crop A and B, respectively, 7.3% and 2.0% of the lots were rejected. The accepted lots contained an average of 7.5 µg kg⁻¹ for Crop A and 4.7 µg kg⁻¹ for Crop B. A correct decision (accept a lot with < 25 µg kg⁻¹ aflatoxin and reject a lot with > 25 µg kg⁻¹ aflatoxin) was made 95% of the time for Crop A and 98% of the time for Crop B.


Estimates were made of the errors associated with the extraction, cleanup, drying, and quantification steps of the analytical procedure used by the Food Safety and Quality Service to test groundnuts for aflatoxin. An analysis of variance indicated that the errors associated with the extraction, cleanup, and drying steps were each negligible and that the quantification step was the major source of analytical error. The error associated with the quantification step (coefficient of variation average 18.6%) probably comes from three sources (1) differences in fluorescence among replicated spots on a TLC plate (spot to spot error), (2) differences in fluorescence among spots on different TLC plates (spot to spot plus plate to plate error), and (3) errors in measuring the fluorescence of the spots (reading error). The fluorescence spots on the TLC plates were read densitometrically.


Four lots of raw shelled groundnuts, naturally contaminated with aflatoxin, were each ground into a paste. Sixty-four 50-g samples were removed from three of the lots and twenty 50-g samples were removed from the fourth lot. For each lot, aflatoxin was extracted from half of the samples by the AOAC Method 1 (CB) and from the remaining half by the AOAC Method II (BF). The four lots averaged 52, 115, 215, and 402 µg kg⁻¹ total aflatoxin when measured by the CB method. On the average across the four lots, the BF method extracted 26, 25, 22 and 18% less aflatoxin B₁, B₂, G₁, and G₂, respectively, than the CB method.
Four lots of raw shelled groundnuts, naturally contaminated with aflatoxin, were each ground into a paste. Sixty-four 50-g samples were removed from three of the lots and forty 50-g samples were removed from the fourth lot. For each lot, aflatoxin was extracted from half of the samples by the AOAC Method I (CB) and from the remaining half by the AOAC Method II (BF). The four lots averaged 57.8, 127.6, 238.5, and 447.1 µg kg\(^{-1}\) total aflatoxin when measured by the CB method. On the average across the four lots, the BF method extracted 26, 25, 22, and 18 % less aflatoxin B\(_1\), B\(_2\), G\(_1\), and G\(_2\), respectively, than the CB method.

This study estimated the efficacy of the visual A. flavus (VAF), minicolumn (MCL), and thin-layer chromatography (TLC) methods to detect farmers’ stock groundnuts which contained aflatoxin. Aflatoxin tests on grade samples from each of 2300 lots of farmers’ stock groundnuts were used to estimate the distribution of farmers’ stock lots according to their aflatoxin concentration (lot distribution). This lot distribution (with an average aflatoxin concentration of 59.5 µg kg\(^{-1}\)) was incorporated into each of the 3 computer models that simulate the testing of farmers’ stock groundnuts for aflatoxin when the VAF, MCL, and TLC methods are used. The number of lots accepted and the average aflatoxin concentration (AA) in the accepted lots was predicted. Results indicate that when a given percentage of the lots are accepted, lots accepted by the VAF method have less aflatoxin than those lots accepted by either the MCL or TLC methods. When the present visual method was used to test the above lot distribution, 75.8 % of the lots tested were accepted and the AA in the accepted and rejected lots were 4.1 and 232.8 µg kg\(^{-1}\), respectively.

The negative binomial equation was used to simulate the distribution of sample aflatoxin test results when replicated grade samples from farmers stock groundnuts were analyzed by TLC and minicolumn methods. The Poisson equation was used to simulate the distribution of samples according to the number of kernels with visible Aspergillus flavus growth found in replicated grade samples from farmers stock groundnuts when the visible A. flavus method was used. The probability of accepting a lot of farmers stock groundnuts with a given aflatoxin concentration when using a 465-g grade sample and four different accept/reject levels were predicted with the models and compared to observed acceptance probabilities for each of the three methods. Comparisons between predicted acceptance probabilities and observed acceptance probabilities from a previous study were good for each method at each accept/reject level.

Aflatoxin B\(_1\) was extracted by a water slurry process using methanol concentrations of 55, 60, 65, and 70 % in water and solvent: groundnut ratios of 3, 4, 5, and 6 mL g\(^{-1}\). Results failed to show that methanol concentration had an effect on amount of B\(_1\) extracted; however, the amount of B\(_1\) extracted increased with an increase in solvent: groundnut ratio. Aflatoxin B\(_1\) was also extracted by the official AOAC method II, using methanol concentrations of 55, 60, 65, and 70 % in water and solvent: groundnut ratios of 2, 3, 4, and 5 mL g\(^{-1}\). Results showed that the amount of aflatoxin B\(_1\) extracted increased with % methanol at low solvent: groundnut ratios but not at high ratios. Also, the amount of aflatoxin B\(_1\) extracted increased with solvent: groundnut ratios at all methanol concentrations.


The negative binomial probability function was used to model the distribution of sample aflatoxin test results when replicated grade samples from farmers stock groundnuts were analysed by thin-layer chromatography and minicolumn methods. The Poisson probability function was used to model the distribution of the number of kernels with visible Aspergillus flavus growth found in replicated grade samples from farmers stock groundnuts when the visible A. flavus method was used. The probabilities of accepting a lot of farmers stock groundnuts with given aflatoxin concentrations when using a 465-g grade sample and two different accept/reject levels were predicted with the models and compared to observed acceptance probabilities computed from previously published data for each of the three methods. The comparisons showed good agreement between the predicted acceptance probabilities and the observed acceptance probabilities.


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The amount of aflatoxin extracted from raw groundnuts by using the water-slurry modification of AOAC method II was determined for 49 different combinations of methanol concentration and solvent : groundnut ratio. Results indicate that the amount of aflatoxins B<sub>1</sub> and B<sub>2</sub> extracted from raw groundnuts is a function of both methanol concentration and solvent : groundnut ratio. A cubic equation was developed, using regression techniques to describe the combined effects. From the functional relationship, the predicted methanol concentration and solvent:groundnut ratio that extracts the most aflatoxin B<sub>1</sub> was computed to be 60 % and 10.8 mL solvent g<sup>-1</sup> groundnuts, respectively. This combination extracted 12.1 % more aflatoxin than did AOAC method II.


A water slurry method in which 1100-g of comminuted groundnuts were blended with 1500 ml of tap water for 3 min in a blender and the aflatoxin in a 130-g portion of the slurry was extracted by solvent according to methods similar to those used in Method II of AOAC was compared to the method presently used by the Food Safety and Quality Service (FSQS), USDA. The proposed water slurry method required only 180 mL and 60 mL per sample, respectively, of methanol and hexane compared to the 1650 and 1000 mL, respectively, required by the FSQS method. Blending comminuted groundnuts with water reduced the average particle size and distributed the contaminated particles throughout the slurry. Ninety-four % of the blended particles passed a sieve with 149-µ openings compared to only 66 % of the unblended product. Variance among analyses with the FSQS method did not differ significantly from the variance among analyses with the slurry method. However, analyses with the slurry method averaged 16 % more aflatoxin than with the FSQS method.


A microcomputer was interfaced to an instrument (spotmeter) previously designed to measure the fluorescent intensity of aflatoxin spots on thin layer chromatography (TLC) plates. Software was developed that uses a cubic regression equation to describe the relationships between the spotmeter readings and the known quantities of aflatoxin in standard spots on TLC plates. The regression technique also provides methods to detect spotting and/or measurement errors. Based on the regression equation and measurements of sample extract spots on the same TLC plate, the system computes and records the amount of aflatoxin in the sample extract spots and the concentration of aflatoxin that was in the extracted sample. The percent error associated with computed amounts of aflatoxin in sample extract spots is affected by the amount of aflatoxin in the sample extract and standard spots on the plate. The average percent error ranged from 14.9 % for a 2.6 ng spot to 4.1 % for a 13 ng spot.


An improved cleanup procedure for analysis of aflatoxins in various agricultural commodities is described. The cleanup procedure consists of an initial thin-layer chromatography (TLC) development of the sample extract with methyl acetate as the solvent The Rf values of the impurities and aflatoxins vary in this solvent from those in the methanol-chloroform system; thus, the aflatoxins are readily separated from interfering fluorescent compounds. This method has given good results on a wide variety of agricultural materials such as feed, grains, nuts, and vegetables. In a relatively clean material such as wheat, aflatoxins can be detected at concentrations of 10 µg kg<sup>-1</sup>, while in alfalfa, which contains many interfering fluorescent compounds, the lower limit is about 40 µg kg<sup>-1</sup>.


This review includes discussion of safety, sampling, standards, presumptive and screening methods, quantitative methods, including thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC) and immunochemical methods, and selection of analytical approach in relation to the detection and estimation of aflatoxins in maize and groundnuts.


Three immunochemical and three chemical methods were compared for aflatoxin analysis, using fifty naturally contaminated lots of raw shelled groundnuts. The USDA/AMS analyzed all lots using the water slurry modification of the Best Foods (BF) method. The lots were chosen so mat aflatoxin levels ranged from 0 to 100 µg kg<sup>-1</sup>. The Federal-Suites Inspection Service modified Dickens mill was used to collect special 10 lb, subsamples from commercial lots. Each 10 lb sample was divided, and duplicate analytical subsamples for each method were prepared to make
each subsample as representative as possible. The Aflatest-P mycotoxin testing system uses a monoclonal based affinity column and was provided by Cambridge Naremo, Springfield, MO. The ELISA methods used were Agrichek aflatoxin test provided by the Agri Tech Systems, Inc., Portland, ME, and the Agri-Screen test supplied by Neogen Corporation, Lansing, MI. The chemical methods used were the water-slurry method, CB method and an HPLC method using normal phase separation and a silica gel packed cell with fluorescence detection following the CB extraction step. The overall mean from all analyses and methods was 19 g kg⁻¹ with a range of 0 to 100 µg kg⁻¹ total aflatoxins; the coefficient of variation (CV) was 36 %. Analysis of variance showed no significant differences between methods. The methods contributed little to variation, but samples contributed to 56 % and subsamples to 35 % of the variation. All methods showed significant relationships (P< 0.01) with regression using the water slurry method as the dependent variable. The R values ranged from 0.66 to 0.83. All methods performed well and were comparable. Any one of the immunochemical methods could be routinely used by trained personnel to perform aflatoxin analyses.


The method uses 0.5 N phosphoric acid-chloroform (1 + 10) in the initial extraction; the extract is divided and eluted from two columns to provide a quantitative thin-layer chromatographic (TLC) method for aflatoxin and ochratoxin in maize and dried beans. Aflatoxin and zearalenone are eluted from 1 column and ochratoxin, penicillic acid and citrinin from the other. Ochratoxin A recoveries were low (50 %) in groundnuts. Zearalenone, penicillic acid and citrinin were quantitatively recovered from maize and beans; zearalenone and penicillic acid were recovered from groundnuts but citrinin was not.


When certain strains of Aspergillus flavus are grown on substrates for the production of aflatoxins, many yellow to orange pigments may also be produced which can be extracted along with the aflatoxins. These pigments must be removed before chromatography for several reasons. They absorb light in the spectral region of the aflatoxin fluorescence and can thereby obscure the progress of fluorescent zones on columns; they cause some degree of streaking on thin-layer chromatographic (TLC) plates. The pigments may be easily separated from aflatoxins by briefly swirling the chloroform extracts containing 1 % ethyl alcohol with insoluble basic green copper carbonate which absorbs the pigments or forms complexes with them. Filtration of the copper-treated solution through a thin layer of Celit 545 yields a nearly colorless filtrate. The copper reagent is useful for quantitative analytical procedures within the limits of error imposed by visual comparison of thin-layer spots. It is effective in removing pigments from extracts of A. flavus cultures grown on substrates of wheat, corn, groundnuts, rice, straw, and alfalfa.


An extraction procedure for extracting aflatoxins from contaminated groundnuts is described which does not require extensive cleanup of the original extract to obtain the desired sensitivity. Aflatoxins are extracted with acetonitrile:water (9:1, v/v) and hexane. The extracts are filtered, acetonitrile-water layer is removed and evaporated to dryness. The residue is dissolved in benzene for spotting on silica gel thin-layer chromatographic (TLC) plates for detection and quantification of aflatoxins.

### 7.2.4 High Performance Thin-Layer Chromatography (HPTLC) Methods


The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.


Initial results are presented of a study of the determination of aflatoxins B₁, B₂, G₁ and G₂ in foods and feeds by high-performance thin-layer chromatography (HPTLC) on either glass plates (20 cm x 10 cm) or aluminium sheets (20 cm x 20 cm) coated with silica gel 60. Samples were applied as solution in benzene-
acetonitrile (49:1) and development was in the absence of light with a mobile phase of chloroform-xylene-acetone (6:3:1) in two 20-min stages. Spots were quantified by densitometric scanning at 366 nm (monochromator) or 360 nm (filter) vs. an external standard containing the analytes. The more expensive monochromatic densitometer was required for accurate results, with 30-fold increased sensitivity for aflatoxin B$_1$.


A method is described for simple and rapid detection of aflatoxins in corn, buckwheat, groundnuts and cheese. Aflatoxins were extracted with a chloroform-water solvent and were purified by a florisil column chromatographic procedure. Column eluates were concentrated and spotted on a high performance thin-layer chromatography (HPTLC) plate, which was then developed in chloroform:acetone (9:1) and/or ether:methanol:water (94:4.5:1.5) or chloroform:isopropanol:acetone (85:5:10). Aflatoxins were quantitated by densitometry. The minimum detectable aflatoxin concentration (µg kg$^{-1}$) in various test materials were 0.2 B$_1$, 0.1 B$_2$, 0.2 G$_1$, 0.1 G$_2$, and 0.1 M$_1$. Recoveries of the aflatoxins added to com, groundnuts, and cheese samples at 10-30 µg kg$^{-1}$ were > 69 % (aflatoxin G$_2$) and averaged 91 % B$_1$, 89 % B$_2$, 91 % G$_1$, and 78 % G$_2$, and 92 % M$_1$. The simple method described was compared with the AOAC CB, AOAC BF, and AOAC milk and cheese methods. These methods were applied to corn, groundnuts, and cheese composites spiked with known amounts of aflatoxins, and to naturally contaminated buckwheat and cheese. Recoveries were much lower for the BF method compared with the simple method and the CB method.


Liquid chromatography (LC) and high performance thin-layer chromatography (HPTLC) techniques were compared for determination of aflatoxins in peanut butter. With respect to precision, accuracy, sensitivity, recovery, and linearity of response, HPTLC appeared to be equivalent to LC. These points illustrate the viability of HPTLC as an alternative technique in the determination of aflatoxin.

7.2.5 High Pressure Liquid Chromatography (HPLC) Methods


The following detectors were compared: a fixed wavelength (254 nm) photometer, a multiwavelength filter photometer, a combination absorbance-fluorescence photometer, and a variable wavelength spectrophotometer. Advantages and disadvantages of each detector are discussed as well as their abilities to function either as all-purpose or specific detectors. The detection of vitamin A alcohol in milk extract using a multi-wavelength filter photometer operating at 254 and 334 nm, and of aflatoxin B$_1$ in peanut butter extract at 254 and 365 nm are discussed, as well as the detection of riboflavin in a noodles and beef dinner using an absorbance-fluorescence detector. Chromatograms are given.


The various high performance liquid chromatographic techniques for the detection of aflatoxins in foodstuffs were examined. The need for resolution, selectivity, and sensitivity is emphasized and examples from the literature are used to illustrate this need. Examples include both normal and reversed-phase techniques. Sample cleanup, detection methods, chromatographic conditions, and pre- and post-column derivatization methods are discussed.


It was demonstrated that solvent mixtures commonly used for sample and standard dissolution in the reversed-phase HPLC determination of aflatoxins in agricultural products can lead to degradation of the toxins. Exposure to light in non-HAc containing solvents led to the most rapid and extensive degradation. Even when stored at -18°C, MeOH : H$_2$O resulted in toxin degradation. Since samples and standards may remain in autosamplers for many hours and cooling of autosamplers is not usually feasible, it is noted that it is important to utilize solvents which result in minimal degradation. If the toxin solutions can be protected from light, either MeCN: H$_2$O : HAc (50:50:0.5) or MeOH: H$_2$O : HAc (50:50:0.5) were shown to result in little degradation of any of the toxins. If light cannot be easily excluded, MeCN: H$_2$O : HAc (50:50:0.5) provided greatest stability. All four of the studied
A method for determining aflatoxins by high pressure liquid chromatography (HPLC) with fluorescence detection after CB extraction and cleanup has been applied to various foods. Recoveries at 1-15 µg kg⁻¹ levels from green coffee and peanut butter were 72-85 and 74-104 %, respectively. Precision of the method has been tested for peanut butter. Other products to which the method has been successfully applied included tree nuts, seeds, grains, chocolate-covered-peanut butter candy, and roasted, salted-in-shell groundnuts. High levels of aflatoxins found in several samples of nuts by this method have been verified by the official thin-layer chromatographic (TLC) method. The advantages of this HPLC method are speed, precision, sensitivity, selectivity, and immediate chemical confirmation of aflatoxins B₁ and G₁. None of the products analyzed required special cleanup procedures.

A simple quick method for estimating aflatoxin in press cakes was developed, using the chloroform extract without purification. The method has a sensitivity of 1 ng with good repeatability. Analysis of aflatoxins by high performance liquid chromatography (HPLC) with spectrophotometric estimation at 362 nm was more sensitive and accurate than current thin-layer chromatography (TLC) and fluorescence methods. The method can also be used for other mycotoxins such as ochratoxin, zearalenone, patulin, aflatoxin M (in milk), and sterigmatocystin.

Two methods for determining aflatoxins in peanut butter, one using normal phase and the other reverse phase liquid chromatography (LC), were studied by 8 and 10 collaborators, respectively. Fluorescence detection was used for the determinative step in both methods. For reverse phase LC, aflatoxins B₁ and G₁ were converted to B₂a and G₂a; for normal phase LC, a silica gel-packed flow cell was placed in the irradiating light path of the detector. The samples included spiked and naturally contaminated peanut butter with total aflatoxin levels from about 5 to 20 ng g⁻¹ and controls in a balanced pair design. For the normal phase LC method, recoveries of B₁, B₂, G₁, and G₂ from spiked samples averaged 79, 92, 74, and 88 %, respectively; for the reverse phase method, the recoveries were 103, 104, 89, and 163 %. For the normal phase LC method, pooled repeatabilities were 20, 23, 28, and 17 % for B₁, B₂, G₁, and G₂, respectively; for the reverse phase method, the repeatabilities were 19, 22, 38, and 31 %. For normal phase method, pooled reproducibilities were 34, 33, 39, and 34 % for B₁, B₂, G₁, and G₂ respectively; for the reverse phase method, the reproducibilities were 32, 46, 51, and 52 %. Both methods show an improved limit of detection and better within-laboratory precision over current AOAC methods; however, between-laboratory precision is no better, and the reverse phase method shows evidence of interferences being measured. For these reasons and because of no benefits of present value, neither method was submitted as official first action.

A technique is described involving a combination of Styrigel and Florisil columns for determination of aflatoxins. Two switching values between the columns reduce the analysis time and exposure to solvents, Florisil is modified with oxalic acid, allowing recovery of aflatoxins at levels below 500 pg. The method is useful for determining aflatoxins in corn and groundnut meal samples.

Afflatoxins B₁, B₂, G₁ and G₂ can be easily and rapidly detected in aqueous solutions using an affinity chromatography column coupled to a monoclonal antibody specific for the toxin molecules. A method for their detection, based on this procedure, is described. Water : memanol extracts of aflatoxin-free food were spiked with aflatoxins, diluted with water and passed through the affinity matrix. The monoclonal antibody is bound to the aflatoxins, which can then be released using a small volume of methanol. This concentrated and separated the aflatoxin present in solutions. As little as 5 ng aflatoxin can be seen if the methanol eluate is passed over a small florisil tip under UV light, while 0.5 ng can be detected if the eluate is analysed by HPLC. This system can be used to analyse aflatoxins in contaminated


samples by spot testing (>5 ng) or as a means of clean-up for quantitative analysis at subnanograin levels. Advantages of this immunological assay in relation to other immunoassays and traditional methods are discussed.


A liquid chromatographic method using on-line sample cleanup, reverse flow analytical column loading, gradient elution, and post-column derivatization with iodine permits direct, rapid determination of aflatoxins B₁, B₂, G₁, and G₂, as well as ochratoxin A and zearalenone. Limits of quantitation are 5 µg kg⁻¹ for the aflatoxins and ochratoxin A and 30 µg kg⁻¹ for zearalenone. This procedure performs well as a multimycotoxin screen for cereal grains and oilseeds, with more limited success in complete animal feeds.


The difference between the CB and BF methods in extracting aflatoxins from groundnut products was studied. The CB method gave 60, 121, 35, and 22 % higher results for aflatoxins B₁, B₂, G₁, and G₂, respectively for four samples of groundnut meal and six samples of peanut butter tested. Both reverse-phase liquid chromatography and thin-layer chromatography were used to quantitate the extracted aflatoxins.


The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.


This paper gives a brief background of the occurrence and chemistry of mycotoxins (aflatoxins, ochratoxin A, citrinin, patulin, zearalenone, and trichothecenes) in foods and feeds. The importance of sampling, sample preparation and assay techniques in the analysis of the mycotoxins in various agricultural commodities is discussed. High performance liquid chromatography (HPLC) methods for the analysis of mycotoxins are discussed. Future developments in the HPLC analysis of mycotoxins are anticipated.


Grade samples from 152 lots of farmers stock groundnuts were analysed for aflatoxin by an enzyme-linked immunosorbent assay (ELISA) rapid screening test and high-pressure liquid chromatography (HPLC) and the results were compared with those of the visual inspection method used by the Federal State Inspection Service (FSIS) in Georgia. The results showed 41 % of the grade samples with visible Aspergillus flavus (Segregation 3) contained < 20 µg kg⁻¹ aflatoxin when analysed by both ELISA and HPLC methods; 18.7 % of Segregation groundnuts (no visual contamination) contained 26-2542 µg kg⁻¹ aflatoxin. The results of ELISA and HPLC agreed in 98.6 % of the composite lot analyses with the detection of 20 µg kg⁻¹ or greater. However, the ELISA rapid screening test failed to give positive tests 12 of 13 times when the aflatoxin content was 20-43 µg kg⁻¹ in the component samples.


The potential of high-performance liquid chromatography as a technique for separating aflatoxins B₁, B₂, G₁, G₂, B₂a, Q₁, M₁, P₁, aflatoxicol, and a degradation product of aflatoxin B₁, 2,3- dihydrodiol, has been assessed. A microparticulate silica adsorption column used with a 1:1 chloroform-dichloromethane eluant provided good resolution of aflatoxins B₁, B₂, G₁, and G₂ but the addition of 1 % propan-2-ol was necessary for the elution of aflatoxins M₁ and Q₁. By selecting appropriate solvent mixtures, good resolution of all of the aflatoxins studied was obtained using columns containing an octadecyl (C₁₈) reversed-phase bonded to a microparticulate support. Details are given for resolving : (1) aflatoxins B₁, B₂, G₁, and G₂ using a 5 % tetrahydrofuran-15 % dimethylformamide in water eluant and (2) aflatoxins B₁, B₂a, Q₁, M₁, P₁, aflatoxicol, and a product of aflatoxin B₁,
2,3-dihydrodiol treated with Tris-bu’lter, using either 15 % dimethylfornamide in water or 10 % tetrahydrofuran in water as eluant.


Chemical methods of analysis for the extraction, cleanup and determination of aflatoxins from animal feedingstuffs and foods are described. The advantages and disadvantages of thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC) are discussed. Immunoassays are briefly discussed. Methods for other mycotoxins such as ochratoxin, patulin, and the trichothecenes are also outlined.


The iodine derivative of aflatoxin B₁ was determined by reverse phase high pressure liquid chromatography with fluorescence detection. Aqueous solutions of aflatoxin B₁ were chromatographed on a Bondapak C18 column with methanol-water (66:34) before and after treatment with iodine. A 4.5-min shift in retention time and a 25-fold increase in fluorescence resulted from the reaction.


A method is described for the rapid determination of aflatoxins in corn and groundnut samples by high pressure liquid chromatography. The method was compared with the current CB method. For seven samples of corn and 14 samples of groundnut meal and peanut butter, the correlation between methods was 0.991, and no significant differences were noted between methods for aflatoxins, using the students’ t-test at 15.7 % x-risk.


The use of high-pressure liquid chromatographic separation in conjunction with laser-induced fluorescence detection permits the analysis of trace fluorescent species at new limits of sensitivity. This technique was applied to aflatoxins B₁, B₂, G₁, and G₂ which were linearly quantitated to 7.5 x 10⁻⁶ g. The procedure consists of forming more fluorescent aflatoxin derivatives, eluting the ’aflatoxins from a reverse-phase column, focusing the 325-nanometer output of a helium-cadmium ion laser into a suspended droplet of the eluent, and measuring the resulting fluorescence using phase-sensitive detection.


A method is described for rapid cleanup followed by reverse-phase liquid chromatography (LC) quantitation of aflatoxins in raw groundnuts. A modified minicolumn cleanup is used for sample preparation, and preliminary estimation of aflatoxin content by minicolumn can be made so that highly contaminated samples can be diluted before LC analysis. Use of minicolumn cleanup eliminates the need for further column or cartridge cleanup. Sensitive quantitation is achieved using a phenyl column, mobile phase of water/tetrahydrofuran (80+20, v/v), and postcolumn derivatization with water-saturated iodine followed by fluorescence detection. Recoveries of aflatoxins B₁, B₂, G₁, and G₂ from groundnut meal spiked at three levels ranged from 71.7 to 88.3 % (average 80 %), with coefficients of variation from 2.7 to 10.4 %.


Two enzyme-linked immunosorbent assay (ELISA) rapid screening tests were compared to high performance liquid chromatographic (HPLC) analyses of 100 raw groundnut extracts for the determination of aflatoxins. Identical extracts, all contaminated between 0 and 70 µg kg⁻¹, were analyzed in duplicate by the Afla-10 cup test, the EZ-screen quick card test, and an HPLC method. The screening tests had detection thresholds of 10 µg kg⁻¹ and 20 µg kg⁻¹, respectively. Both assays were accurate compared to HPLC when samples were negative for aflatoxins or contained aflatoxins above their respective detection thresholds. Errors that did occur were associated with samples that contained aflatoxins at concentrations just below their detection thresholds. The cup test identified as > 10 µg kg⁻¹ 9 % of samples that were in the 5-10 µg kg⁻¹ range as determined by HPLC. The card test identified as > 20 µg kg⁻¹ 53 % of samples that fell in the 10-20 µg kg⁻¹ range by HPLC. Of the samples that were outside the range of 5-10 µg kg⁻¹, the cup test was accurate with both replications compared to HPLC 97 % of the time. Similarly, the card test results (both replications) were comparable to HPLC in 91 % of samples outside the 10-20 µg kg⁻¹ range.
A high pressure liquid chromatographic method has been developed for determining aflatoxins B₁, B₂, G₁, and G₂ in peanut butter. The method is based on extraction with acidified aqueous methanol, partition of the aflatoxins into methylene chloride, and purification of the extract on a 2 g silica gel column. The extracted aflatoxins are resolved on a microparticulate (10 µm) porous silica gel column in Ca 10 min with a water-washed chloroform-cyclohexane-acetonitrile solvent that contains 2 % isopropanol. The fluorescence detection system determines aflatoxins B₁, B₂, G₁ and G₂ at low levels, i.e., 0.25 µg kg⁻¹ B₁, 0.5 µg kg⁻¹ G₁, and 0.2 µg kg⁻¹ B₂ and G₂. Multiple assays of five samples of naturally contaminated peanut butters containing total aflatoxins (B₁+B₂+G₁+G₂) at levels of 1, 2, 3, 9 and 17 µg kg⁻¹ gave intralaboratory coefficients of variation of 7, 4, 4, 11, and 3 %, respectively. Samples spiked at levels of 5, 9, and 17 µg kg⁻¹ total aflatoxins showed recoveries of 79, 81, and 81 %, respectively.

Three aflatoxin-contaminated samples (raw groundnut meal, deoiled groundnut meal, and yellow corn meal) were analyzed by 121 laboratories in 31 countries. Sufficient data were obtained to permit a statistical comparison of the performance of laboratories using the BF, CB, and EEC methods and those using high performance liquid chromatography (HPLC) for quantitation. No significant differences were found between means for laboratories using these four methods for the analysis of raw groundnut meal or yellow corn meal. However, for deoiled groundnut meal, means were significantly different for laboratories using BF method compared with the CB or EEC methods for B₁ and B₂, and for laboratories using the CB method compared with HPLC methods for G₂.

Three aflatoxin-contaminated samples, raw groundnut meal, deoiled groundnut meal, and yellow corn meal, were analyzed by 121 laboratories in 31 countries. Sufficient data were obtained to permit a statistical comparison of the performance of laboratories using the BF, CB, and EEC methods and those using high performance liquid chromatography for quantitation. A raw groundnut meal sample showed no significant differences among means for laboratories using the four methods, and a white corn meal sample showed only one such significant differences; however, a finished peanut butter sample containing less than 10 µg total aflatoxins kg⁻¹ showed no significant differences among means for laboratories using the four methods. The finished peanut butter sample showed no significant differences among means for laboratories using the four methods.
10 significant differences among means for laboratories using the four methods considered.


A method for complete and quantitative separation of the naturally occurring aflatoxins using a commercial high-pressure liquid chromatography, which is nearly as sensitive as TLC methods, is reported. Retention times were extremely reproducible and each run took < 10 min. The method should be applicable to the quantitative assay of anatoxins in foods.


A preliminary survey of peanut butter in 1982 indicated that 31 of 32 samples of major national brand-named products contained aflatoxin B₁ up to 10 µg kg⁻¹, and that 59 % of those were below the limit of detection (2 µg kg⁻¹). Of 25 peanut butter samples from specialist ‘Health Food’ outlets, 64 % contained 10 µg kg⁻¹ and the remainder contained 16-318 µg kg⁻¹, with one sample having 345 µg kg⁻¹ aflatoxin. Surveys of ‘Health Food’ products in 1983 and 1984 confirmed that manufacturers were still experiencing some difficulty in complying with the 30 µg kg⁻¹ voluntary guideline limit for total aflatoxin. In 1984, 228 retail samples of nuts and nut confectionery products comprising groundnuts (shelled, unshelled, roasted and salted), mixed nuts, almonds (both unblanched and ground), Brazil nuts (in shell), hazelnuts (in shell), chocolate-coated groundnuts, peanut brittle and coconut ice were examined. Results showed that 74 % of the samples contained aflatoxin B₁ at up to 0.5 µg kg⁻¹, and 3.1 %, mainly groundnuts and Brazil nuts, exceeded the guideline tolerance for total aflatoxin. Total aflatoxin was greatest in unshelled groundnuts, 4920 µg kg⁻¹, and in a composite sample of visibly molded Brazil nuts, 17926 µg kg⁻¹.


Methods for aflatoxin analysis using thin-layer chromatography (TLC) and fluorescence detection were developed in the 1960s and are still widely used. In the late 1970s, several applications of high performance liquid chromatography (HPLC) were developed and as they were generally more sensitive than the TLC methods, they are now popular for aflatoxin analysis when a high degree of accuracy is required. For some test purposes convenience and rapidity of analysis are more important than accuracy, and the bright greenish yellow fluorescence (BGYF) and minicolumn methods were evolved with this in mind. Recently several enzyme-linked immunosorbent assay (ELISA) system kits for aflatoxin analysis have been developed and some of them found suitable for the kind of testing now carried out using the BGYF and minicolumn methods. Gas chromatography can also be used for aflatoxin analysis under certain conditions. From the range of aflatoxin analysis methods now available it should be possible to choose methods suitable for specific purposes.


Aflatoxins B₁, B₂, G₁ and G₂ were dissolved in benzene-acetonitrile (49:1) and a portion of the solution was injected on to a column (15 m x 0.25 mm) coated with DB-5 (0.25 µm) and operated with temperature programming from 50 to 300 at 15 or 20 degree min⁻¹. He as carrier gas and FID; confirmation of identities was achieved by 70-eV MS. Calibration graphs were rectilinear from 1 to 50 ng and the coefficients of variation (at the 2 to 4 ng level) for aflatoxins B₁, B₂, G₁, and G₂ were 3.2, 4.3, 6.0, and 3.8 %, respectively (n=7); the detection limit was 1 ng for each.


A sensitive, reliable, and economical method for the determination of six mycotoxins in mixed feeds is described. The feed is extracted with chloroform-water and the extract is cleaned up using a disposable Sep-Pak silica cartridge. The procedure requires less time (15 min from sample extraction to extract preparation) and less solvent (approximately one-tenth) compared with conventional methods and is suitable for a fast, economical screen. Additional cleanup procedures, involving dialysis or extraction into base, are described for samples containing high levels of interfering compounds. Thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) with fluorescence detection are described for identification and estimation of mycotoxins. The method has been applied to a wide range of mixed feeds, including laboratory animal diets and raw materials. The limit of detection is 1 µg kg⁻¹ for all mycotoxins measured by HPLC.
A rapid method, economic in solvent usage, for determination of aflatoxins in raw groundnuts is described. Aflatoxins are extracted with aqueous acetone and interfering compounds precipitated with CuCO₃. After defatting, aflatoxins are partitioned into dichloromethane for cleanup with silica Sep-pak which eliminates other interfering compounds. Aflatoxins are then eluted with chloroform : ethanol (95:5). The eluate is dried, derivatized with trifluoroacetic acid and analysed by high-pressure liquid chromatography (HPLC) on a Spherisorb ODS column with a mobile phase of water/acetonitrile/methanol (62:18:18, v/v/v), using a fluorometer detector. Limits of detection are 2-5 ng g⁻¹ total aflatoxin. Coefficients of variation were < 8 % and recoveries of individual aflatoxins were in the range 89-103 %. There is negligible background interference, and detection takes 25-30 min.


A method for the determination of aflatoxins in groundnut extracts using disposable bonded-phase columns for sample cleanup is described. The only solvents used for extraction and cleanup were methanol and water. Results indicated that the method is accurate and precise with lower limits of detection of 100 pg total aflatoxins and linearity from this limit to 100 times that level with a regression coefficient of 0.99. An additional attribute was that the time to prepare a sample for analysis was about 20 min.


A high pressure liquid chromatography (HPLC) method is described for determination of the 4 major aflatoxins, B₁, B₂, G₁, and G₂ in groundnut products (sweetened and unsweetened peanut butter, ground groundnuts). The aflatoxins were extracted by adapting a procedure developed by Pons, and quantitated utilizing a new 5 µm reverse-phase column with NaCl/acetonitrile/methanol mobile phase (3+1+1). The 5 µm column achieved baseline resolution of each of the 4 aflatoxins. Retention times and peak heights were reproducible. The procedure was successfully applied to several types of groundnut products, which is a decided advantage over the current CB and BF extraction methods. Additionally, it can be used for sweetened groundnut matrixes with no interferences in the chromatography. The total time required for sample preparation and aflatoxin detection is < 1.5 h.


A method for post-column derivatization of aflatoxins with iodine is described. The method was found to be cost effective and yielded reproducible results, a linear response over at least two orders of magnitude and detection limits of about 1 µg kg⁻¹, both for standard solutions and for peanut butter samples.


The aflatoxin B₁ content of various foodstuffs from Indonesia, Philippines and Japan was determined by high-performance liquid chromatography (HPLC) using a micro-Porasil column and a micro-Bondapak column after the conversion of aflatoxin B₁ to its water adduct aflatoxin B₁-. Aflatoxin B₁ levels in foods from Indonesia and Philippines were generally higher than in foods from Japan, especially in groundnuts, cassava and maize; levels were significantly less in raw products than in processed products.


A method for determination of aflatoxins in groundnut meals by high performance liquid chromatography (HPLC) was investigated. After extraction and purification according to the AOAC CB method, an extract is injected on a Corasil 11 column and eluted by n-hexane/chloroform/ethanol (50:49.7:0.3). Detection is performed with an UV detector. The method determines aflatoxins B₁, B₂, G₁, and G₂. The detection limit for aflatoxin B₁ is 50 µg kg⁻¹ using a 254-mm UV detector, or less with more effective detectors (absorption at 350-360 nm).


A method of aflatoxin analysis by high performance liquid chromatography (HPLC) was investigated and used for the routine control of imported groundnut meal. The optimal resolution of aflatoxins B₁, B₂, G₁, and G₂ was obtained with a Corasil 11 column and a mobile phase composition : n-hexane:chloroform:ethanol (50:49.5:0.5, v/v/v). The column dimensions were 50 x 0.2 cm; pressure 15-30 arm; flow rate 1.2 mL min⁻¹. Retention volume for aflatoxin B₁ was about 12 mL, and retention time...
10 min. Reproducibility of retention volume was examined and the coefficients of variation for aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> were 0.76-0.91 % . Detection was performed by UV-254 detector. The detection limit was about 30 µg kg<sup>-1</sup>. Linearity of detection was tested and found to be satisfactory in the range of 100-2500 ng aflatoxin B<sub>1</sub> injected onto the column. Recovery of aflatoxin B<sub>1</sub> added to the noncontaminated samples in the range 100-2000 µg kg<sup>-1</sup> was 95.2-118 % (average 105.4 %). Precision of the method was examined at the levels of 200 and 500 lg kg<sup>-1</sup>; coefficients of variation were 4.99 and 2.59 %, respectively. The HPLC method and a standard thin-layer chromatography (TLC) method were tested on 30 commercial samples of naturally contaminated groundnut meal. The application of paired statistical analysis did not show significant differences between the methods for aflatoxin levels.


Column chromatographic separation of aflatoxins from groundnuts on adsorption columns or an Sephadex was attempted. Chromatography on Sephadex G-25 gave reasonably good resolution of aflatoxins B<sub>1</sub> and B<sub>2</sub>, but aflatoxin B<sub>2a</sub> was present in some fractions and was absent before chromatography. Large-diameter silica gel columns gave unsatisfactory results, but good resolution of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> was obtained by chromatography on 50 x 0.15 cm silica gel (200/240 mean ASTM) columns using chloroform/carbon tetrachloride (1+2) as eluent, or on Corasil II columns using chloroform/n-hexane/acetonitrile (80+20+0.3, v/v/v) as eluent. High inlet pressure could be used to increase flow rate.


A minicolumn method was modified as a simple, efficient cleanup procedure for use with high pressure liquid chromatography (HPLC) for detection of aflatoxins in groundnuts, rice and corn. The procedure, which utilizes a heavy metal salt precipitation combined with a short alumina filtration under vacuum, can be easily adapted to various sample sizes and provides adequate cleanup for use with both micro-particle and pellicular solid support columns.


A high-pressure liquid chromatographic (HPLC) technique was used to study the recovery of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> from extracts of maize and groundnuts spiked with 16.4 and 32.8 ng of each toxin. Average recovery of each toxin was 101.7 %, with an average deviation of (+) 2.6 % from 100 %. The technique was improved by addition of a flow injection analysis step. The integrated method combined the advantages of flow injection analysis, namely rapidity, simplicity and economy, with the separation capability of HPLC.


Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> were quantitatively detected by high performance liquid chromatography using a 12 ul flow-cell in the fluorimetric detector and as mobile phase, a toluene system instead of a chloroform, dichloromethane or methanol system. Various kinds of columns and mobile phases were tested, and fine mutual separation of all 4 aflatoxins without quenching their fluorescence was achieved by using a silica gel column and toluene/ethyl acetate/formic acid/methanol (89.0:7.5:2.0:1.5). The relationship between the fluorescence peak area and the amount injected was linear in the range 0.3-120 ng. This method, as applied to food (rice, corn, groundnut meal) and feed extracts, is sensitive at the 10-20 µg kg<sup>-1</sup>, levels of the 4 kinds of aflatoxins.


Aminicolumn method was modified as a simple, efficient cleanup procedure for use with high pressure liquid chromatography (HPLC) for detection of aflatoxins in groundnuts, rice and corn. The procedure, which utilizes a heavy metal salt precipitation combined with a short alumina filtration under vacuum, can be easily adapted to various sample sizes and provides adequate cleanup for use with both micro-particle and pellicular solid support columns.

The American Oil Chemists' Society Smalley Check Sample Program offers ongoing check sample series for determination of aflatoxins in groundnut meal, cottonseed meal, and corn meal. Laboratories participating in this program represent a worldwide cross section of industry, regulatory, and commercial laboratories.
Aflatoxins were extracted from groundnuts using methyl cyanide and chloroform, or from milk using chloroform, and estimated by high performance liquid chromatography using chloroform : benzene : methyl cyanide : ethanol (225:75:10:25) on a Lichrosorb SI 60 column using a Shimadzu RF-500 LC detector. The lower limit of detection was 10 µg kg$^{-1}$ for aflatoxin B$_2$ and M$_1$ and 0.1 µg kg$^{-1}$ for G$_1$ and G$_2$. No further purification of samples from milk was required prior to estimation of aflatoxin M$_1$.


Aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$ were quantitated by high pressure liquid chromatography (HPLC) on a 5 µm Lichrosorb column, using a silica-gel packed flowcell in the fluorometric detector. The relationship between peak height and the amount injected was linear only up to about 2 ng but showed a linear log-log relationship. Methods for constructing and packing the flowcell are given. A guard column and venting valve were used to minimize deterioration of the analytical column and adsorbent-packed flowcell. The method was applied to a peanut butter extract. Although with the cleanup procedure used, the life expectancy of the flowcell is limited.


An interlaboratory study of a negative ion chemical ionization mass spectrometric (MS) confirmation procedure for aflatoxin B$_1$ was conducted in laboratories in the USA, UK and Federal Republic of Germany. Twelve partially purified, dry film extracts from naturally and artificially contaminated roasted groundnuts, cottonseed, and ginger root containing various quantities of aflatoxin B$_1$ were distributed to the participating laboratories. The extracts required additional cleanup before MS analysis, using either an acidic alumina column and preparative thin-layer chromatography (TLC) or a two-dimensional TLC procedure. Recovery of aflatoxin B$_1$ was influenced by degree of recovery of the sample from acidic alumina and/or TLC plate and incomplete elution of aflatoxin B$_1$ from silica gel. Factors affecting MS confirmation included the purity and recovery of aflatoxin and MS instrument sensitivity. Aflatoxin B$_1$ identity was confirmed in 19.5, 90.9, and 100 % of samples containing < 5, 5-10, > 10 ng aflatoxin B$_1$ g$^{-1}$ product, respectively, by solid probe introduction using full mass scans. The MS method has been adopted as official first action.


A collaborative study of a liquid chromatographic method for the determination of aflatoxins B$_1$, B$_2$, G$_1$ and G$_2$ was conducted in laboratories located in the United States, Canada, South Africa and Switzerland. Twenty-one artificially contaminated raw groundnuts, peanut butter and maize samples containing varying amounts of aflatoxins B$_1$, B$_2$, G$_1$ and G$_2$ were distributed to participating laboratories. The test portion was extracted with methanol-0.1 N HCl (4+1), filtered, defatted with hexane and then partitioned with methylene chloride. The concentrated extract was passed through a silica gel column. Aflatoxins B$_1$ and G$_1$ were derivatized with trifluoroacetic acid and the individual aflatoxins were determined by reverse-phase liquid chromatography with fluorescence detection. Statistical analysis of the data was performed to determine or confirm outliers and to compute repeatability and reproducibility of the method. For maize, relative standard deviations for repeatability (RSDr) for aflatoxin B$_1$ ranged from 27.2 to 8.3 % for contamination levels from 5 to 50 ng g$^{-1}$. For raw groundnuts and peanut butter, RSDr values for aflatoxin B$_1$ were 35.0 to 41.2 % and 11.2 to 19.1 %, respectively, for contamination levels from 5 to 25 ng g$^{-1}$. RSDr values for aflatoxins B$_2$, G$_1$ and G$_2$ were similar. Relative standard deviations for reproducibility (RSDR) for aflatoxin B$_1$ ranged from 15.8 to 38.4 %, 24.4 to 33.4 % and 43.9 to 54.0 % for maize, peanut butter and raw groundnuts, respectively. It is reported that the method has been adopted official first action for the determination of aflatoxins B$_1$, B$_2$, G$_1$ and G$_2$ in peanut butter and maize at concentration more or =13 ng total aflatoxins g$^{-1}$. 

Six laboratories analysed portions of the same aqueous acetonitrile extracts of 3 peanut butters for aflatoxin concentration by an HPLC procedure (using immunoffainity column clean-up) and an ELISA procedure. The extracts were from a nominal “blank” peanut butter, a peanut butter naturally contaminated with aflatoxins (mostly B1) and from a “blank” peanut butter to which equal amounts of aflatoxins B1, B2, G1, and G2 standards had been added. Similar results for the HPLC and ELISA procedures were obtained for the blank (means 2.8 and 4.9 µg kg\(^{-1}\), respectively) and naturally contaminated (means 26.0 and 25.9 µg kg\(^{-1}\), respectively) peanut butters. However, the results by ELISA (mean 16.7 µg kg\(^{-1}\)) for the spiked peanut butter was much lower than that obtained by HPLC (mean 28.3 µg kg\(^{-1}\)).


Aflatoxins were completely resolved as sharp peaks in the order B1-B2-G1-G2 by high-pressure liquid chromatography (HPLC) on a small particle (10 µm) porous silica gel column in 7-13 min (B1 through G2) by a water-saturated chloroform-cyclohexane-acetonitrile elution solvent (25:7.5:1.0), with detection by UV absorbance at 360 nm. The relationship between peak height and amount injected was linear over a 5-400 ng range for each aflatoxin. Both retention times and peak heights were highly reproducible, multiple injections of mixed standards giving coefficients of variation of 1.0-1.4 % (retention time) and 1.6-2.8 % (peak height) for the four aflatoxins. Detection was highly sensitive, with mean peak height, mm ng\(^{-1}\). of 7.1 (B1), 6.4 (B2), 4.5 (G1), and 4.1 (G2), allowing detection of 1-2 ng of each aflatoxin.


A precise and sensitive high pressure liquid chromatographic method is described for determining aflatoxins B1, B2, G1, and G2 in all types of groundnut products. The method is based on acidified aqueous methanol extraction, partition of aflatoxins into dichloromethane, and purification of the extract on a 2-g silica gel column. Aflatoxins in the purified extract are completely resolved on a microparticulate (10 µm) porous silica gel column in approximately 10 min with a water-saturated chloroform-cyclohexane-acetonitrile solvent. The preferred detection system. B1 and B2 by ultraviolet absorbance at 360-365 nm and G1 and G2 by fluorescence, allows accurate and sensitive detection of all four aflatoxins at levels as low as 0.3 - 1.0 µg kg\(^{-1}\). Repetitive assay of three samples of naturally contaminated peanut butter containing total aflatoxins (B1+B2+G1+G2) at levels of 5, 10, and 15 µg kg\(^{-1}\) gave within-laboratory coefficients of variation of 11.5, and 5 %, respectively.


A rapid confirmatory method for aflatoxins B1 and B2 has been developed. The extract used for thin-layer chromatography quantitation is rapidly cleaned up by elution through a silica gel Sep-PAK cartridge and then analyzed by gas chromatography/mass spectrometry/selected ion monitoring at 3000 resolution using a bonded-phase fused silica capillary column with on-column injection. Limits of detection for aflatoxins B1 and B2 in groundnut samples were 0.1 µg kg\(^{-1}\).


The method used for determination of aflatoxins is described. Aflatoxins were extracted, chromatographed on reverse phase column and detected by UV at 350 nm. Recovery was 92-106 %. In 10 samples of groundnut flour tested, the limit of detection was 2 µg kg\(^{-1}\).


A new method for the rapid detection of aflatoxins (i.e., SAM) was compared with a method of high pressure liquid chromatography (HPLC) method. Fifty groundnut samples were analyzed in this study. For the SAM method, groundnut samples (40 g) were ground and extracted with methanol : water (80:20). Aliquots (5 mL) of the methanolic phases were added to 5 mL of toluene. Aliquots (0.5 mL) of the toluene phases were added to the top of SAM detectors. Following elution with toluene : chloroform : acetone (95:20:5, v/v/v) all samples were analyzed for aflatoxins with longwave ultraviolet light (365 nm). Groundnut samples (20 g) were ground for HPLC analysis and extracted with acetone : water (75:25, v/v). Fats were removed with hexanc, and the aflatoxins were extracted with chloroform and analyzed by normal phase HPLC. No differences was observed between the two methods. All samples positive by HPLC were positive by SAM, and all samples negative by HPLC were negative by SAM. The percent recovery and the stability of aflatoxin B1 in the toluene phase in the SAM assay were determined. Aliquots (5 mL) of the extraction solvent were "spiked" with aflatoxin B1 at levels of 5500, 550, 220, and 55 ng. Aflatoxin B1 was partitioned with the toluene phase and analyzed by HPLC. The percent recovery of aflatoxin B1 was 70 % (a recovery of 90 % is routinely attained when chloroform...
A high pressure liquid chromatography (HPLC) technique is described for detection of mycotoxins (aflatoxins, zearalenone, vomitoxin, and ochratoxin A). The detection limit for toxins was: aflatoxins (B₁, B₂, G₁, and G₂) 2 µg kg⁻¹, zearalenone 1 µg kg⁻¹, vomitoxin 25 µg kg⁻¹, and ochratoxin A 5 µg kg⁻¹. Of groundnut products, mainly groundnut meal, 87% were contaminated (maximum 11620 µg kg⁻¹ total aflatoxin). In Southeast Styria a high rate of vomitoxin contamination was found in maize (80%, average 761 µg kg⁻¹), zearalenone was found in 47% of samples tested (average concentration 145 µg kg⁻¹), 23.6% of 83 stored grain samples contained ochratoxin A at low concentration.

Partial resolution of aflatoxins B₁, B₂, G₁, G₂, and P₁ was achieved by high-speed liquid chromatography (HSLC) on a porous layer silica adsorbent, using chloroform-isooctane as the eluting solvent and a 254 nm UV monitor for detection. The resolution was somewhat less than, although comparable with, that obtained by thin-layer chromatography (TLC), using Adsorbosil-1 adsorbent and fluorodensitometric detection. The HSLC response to aflatoxins B₁ and G₁ was linear in the 400-3000 ng range, allowing application of the technique to the quantitative analysis of B₁ and G₁ in crude extracts of Aspergillus parasiticus cultures. The coefficients of variation were 4.2% for B₁ and 23.2% for G₁ in a series of four replicate injections. The advantages and limitations of the technique for quantitative analysis and isolation are compared with those of more conventional chromatographic methods.
while the presence of aflatoxin Q, could be confirmed in a similar manner to aflatoxins B, and G. The sensitivity of the procedure compares favorably with the most sensitive chromatographic procedures available while the technique is also highly reproducible.


Liquid chromatography (LC) and high performance thin-layer chromatography (HPTLC) techniques were compared for determination of aflatoxins in peanut butter. With respect to precision, accuracy, sensitivity, recovery, and linearity of response, HPTLC appeared to be equivalent to LC. These points illustrate the viability of HPTLC as an alternative technique in the determination of aflatoxin.


A highly sensitive normal-phase HPLC method was developed to study the occurrence of aflatoxins in Soviet imported and domestic foodstuffs. The detection limit was 0.1 µg kg⁻¹ and the coefficients of variation were 11 % and 8.5 % at contamination levels of 10 and 100 µg kg⁻¹ aflatoxin B₁, respectively. A survey of the occurrence of aflatoxins B₁, B₂, G₁, and G₂ in domestic and imported cereals and nuts (totalling 4532 samples) collected during 1985-87, showed that 26.9 % of imported groundnuts, 2.8 % of maize and 6.2 % of barley were contaminated with aflatoxins at levels exceeding the maximum tolerated level established in the USSR (5 µg kg⁻¹ for aflatoxin B₁, in foods of all kinds excluding baby foods). Maximum concentrations were 3600, 155 and 8 µg kg⁻¹, respectively. As much as 28.3 % of domestic cottonseed samples, which were chosen for analysis due to toxic effects on animals, were also contaminated with aflatoxins.


Positive fast atom bombardment tandem mass spectrometry is demonstrated to be an effective technique for determination of crude aflatoxins and sterigmatocystin-related compounds. The molecular ion was selected by the first system and bombarded to produce characteristic daughter ions that could be used to identify mycotoxins in mixtures and with the same molecular weight.


Analysis was carried out on 401 samples of locally-consumed groundnut and groundnut products including peanut butter, groundnut cake, fried or roasted groundnuts and oil, using a high pressure liquid chromatography (HPLC) method. The incidence of aflatoxin contaminated samples was 23.4 %. Aflatoxin B₁ was found in all positive samples.


This review includes discussion of safety, sampling, standards, presumptive and screening methods, quantitative methods, including thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC) and immunochemical methods, and selection of analytical approach in relation to the detection and estimation of aflatoxins in maize and groundnuts.
Three immunochemical and three chemical methods were compared for aflatoxin analysis, using fifty naturally contaminated lots of raw shelled groundnuts. The USDA/AMS analyzed all lots using the water slurry modification of the Best Foods (BF) method. The lots were chosen so that aflatoxin levels ranged from 0 to 100 µg kg$^{-1}$. The Federal-States Inspection Service modified Dickens mill was used to collect special 10 lb, subsamples from commercial lots. Each 10 lb sample was divided, and duplicate analytical subsamples for each method were prepared to make each subsample as representative as possible. The Aflatrest-P mycotoxin testing system uses a monoclonal based affinity column and was provided by Cambridge Naremcno, Springfield, MO. The ELISA methods used were Agrichek aflatoxin test provided by the Agri Tech Systems, Inc., Portland, ME, and the Agri-Screen test supplied by Neogen Corporation, Lansing, MI. The chemical methods used were the water-slurry method, CB method and an HPLC method using normal phase separation and a silica gel packed cell with fluorescence detection following the CB extraction step. The overall mean from all analyses and methods was 19 µg kg$^{-1}$ with a range of 0 to 100 µg kg$^{-1}$ total aflatoxins; the coefficient of variation (CV) was 36 %. Analysis of variance showed no significant differences between methods. The methods contributed little to variation, but samples contributed to 56 % and subsamples to 35 % of the variation. All methods showed significant relationships ($P< 0.01$) with regression using the water slurry method as the dependent variable. The R values ranged from 0.66 to 0.83. All methods performed well and were comparable. Any one of the immunochemical methods could be routinely used by trained personnel to perform aflatoxin analyses.

### 7.2.6 Minicolumn Methods


Ninety-two samples of grain and common feed ingredients were analyzed in Australia using thin-layer chromatography (TLC) and a rapid minicolumn screening method. Three samples were found to contain aflatoxins; a sorghum grain sample containing 30 µg kg$^{-1}$ aflatoxin $B_1$, and two samples of moldy groundnuts containing 2500 µg kg$^{-1}$ aflatoxin $B_1$ and 400 µg kg$^{-1}$ aflatoxin $B_2$, and 200 µg kg$^{-1}$ $B_1$ and 38 µg kg$^{-1}$ $B_2$, respectively. A non-destructive detection technique involving scanning the grain with a UV lamp and examination of fluorescence is reported.


The method of Velasco based on separation of aflatoxins on a Florisil/silica gel/alumina minicolumn and UV fluorometric detection, using a Velasco Fluorotoxin Meter, was compared with visual detection after separation on the minicolumns, and thin-layer chromatographic (TLC) determination. Trials were conducted on spiked samples of groundnuts, soybean and sunflower presscakes. chickpeas, beans, almonds, wheat, maize, barley, cassava, silage, and a feed mixture. Severe interference by other constituents of most of the food or feed samples studied was observed with Velasco's method. The filter fluorometer system did not allow differentiation of interfering impurity bands having maximum fluorescence wavelengths close to those of aflatoxins but differing in colour; visual evaluation of the column permitted differentiation of these. The Velasco minicolumn/UV fluorometry method gives a total aflatoxin concentration rather than values for individual aflatoxins, whereas TLC gives data for concentration of individual aflatoxins. It is concluded that TLC techniques are likely to give better results than Velasco's method.


The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunossay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.


Standard grade samples (16) from each of 20 selected minilots were used to evaluate three methods for detecting minilots of farmers stock groundnuts with unacceptable levels of aflatoxin. A visual, a minicolumn and a modified thin-layer chromatography (TLC) method were used to compare analytical results, variation, and probability of acceptance for minilots having mean aflatoxin levels ranging from
minicolumn methods were evolved with this in mind. Recently several methods for aflatoxin analysis using thin-layer chromatography (TLC) and fluorescence detection were developed in the 1960s and are still widely used. In the late 1970s, several applications of high performance liquid chromatography (HPLC) were developed and as they were generally more sensitive than the TLC methods, they are now popular for aflatoxin analysis when a high degree of accuracy is required. For some test purposes convenience and rapidity of analysis are more important than accuracy, and the bright greenish yellow fluorescence (BGYF) and minicolumn methods were evolved with this in mind. Recently several enzyme-linked immunosorbent assay (ELISA) system kits for aflatoxin analysis have been developed and some of them found suitable for the kind of testing now carried out using the BGYF and minicolumn methods. Gas chromatography can also be used for aflatoxin analysis under certain conditions. From the range of aflatoxin analysis methods now available it should be possible to choose methods suitable for specific purposes.


Groundnuts were screened for aflatoxin using a rapid, inexpensive fluorometric method. Groundnuts were ground and extracted with methanol, and the extract was treated with acidified zinc-acetate-sodium chloride solution, filtered and diluted with water. Fluorescence of the extracts was compared with that from aflatoxin-free control groundnuts. Test samples (160) of several varieties and grades of groundnuts, obtained from storage and several commercial sources, were screened for the presence of aflatoxin. One hundred thirty-five samples (84 %) were identified by this method as aflatoxin positive (> 15 µg kg$$^{-1}$$) or aflatoxin negative (< 15 µg kg$$^{-1}$$). Although 22 samples (13.6 %) were incorrectly labeled as aflatoxin positive, most of these showed evidence of the presence of mold metabolites other than aflatoxin. Three samples (1.8 %) were incorrectly labeled as aflatoxin negative when they actually contained 20, 33 and 34 µg kg$$^{-1}$$ aflatoxin.


Methods for aflatoxin analysis using thin-layer chromatography (TLC) and fluorescence detection were developed in the 1960s and are still widely used. In the late 1970s, several applications of high performance liquid chromatography (HPLC) were developed and as they were generally more sensitive than the TLC methods, they are now popular for aflatoxin analysis when a high degree of accuracy is required. For some test purposes convenience and rapidity of analysis are more important than accuracy, and the bright greenish yellow fluorescence (BGYF) and minicolumn methods were evolved with this in mind. Recently several enzyme-linked immunosorbent assay (ELISA) system kits for aflatoxin analysis have been developed and some of them found suitable for the kind of testing now carried out using the BGYF and minicolumn methods. Gas chromatography can also be used for aflatoxin analysis under certain conditions. From the range of aflatoxin analysis methods now available it should be possible to choose methods suitable for specific purposes.


A rapid and simple minicolumn procedure for detecting and quantifying aflatoxin in groundnuts is described. Minicolumns are prepared by filling a length of 4-mm diameter glass tubing with silica gel to a depth of about 4.5 cm. The minicolumn is developed in a chloroform-methanol extract of a groundnut sample. If aflatoxin is present, a blue fluorescent band at the lower end of the column is observed when the column is exposed to long-wave ultraviolet light. Sensitivity is in the order of 5 µg kg$$^{-1}$$ and an assay can be completed in 15-25 min. Some degree of quantification is possible by comparison with columns developed in extracts with known aflatoxin contents.


A rapid, economical minicolumn procedure for screening aflatoxins and ochratoxin A in a wide range of products (wheat, barley, sorghum, corn, rice, rye and groundnuts) is presented. The technique includes high speed blending of the sample with aqueous methanol, purification of the extract with a solution of zinc sulphate and phosphotungstic acid, partitioning in benzene, and minicolumn chromatography. Sensitivities of 4 µg kg$$^{-1}$$ for the aflatoxins and 20 µg kg$$^{-1}$$ for ochratoxin A could be achieved; and the simplicity of the method, which includes the use of disposable plastics and glass items, makes it practical for field or in-plant applications.


It was recognized in the early 1960s that a rapid screening method for aflatoxin was needed. Holaday first proposed the minicolumn chromatography method as a rapid screening method in 1968. Since that time, many improvements have been made in this method. The latest minicolumn method has a limit of detection of 5 µg kg$$^{-1}$$ and can be completed in 5-7 min. The minicolumn technique has been expanded to include screening commodities other than groundnuts for aflatoxin, as well as for other mycotoxins including ochratoxin, zearalenone and aflatoxin M$_1$. 

8 to 255 µg kg$$^{-1}$$. Mean values obtained by each of the three methods increased linearly with mean aflatoxin levels of the minilots and variation for each method as determined by the variance and coefficient of variation (CV) was very large. The CV for all three methods decreased as aflatoxin level increased. Overall performances of the methods were similar in accepting and rejecting these minilots on the basis of the 18 kg grade samples. The greatest difference in the three methods occurred at the zero acceptance level where the modified TLC, minicolumn and visual methods rejected 97, 98 and 88 %, respectively, of the minilots with more than 60 µg kg$$^{-1}$$ aflatoxin. At this acceptance level the TLC, minicolumn and visual methods also rejected 55, 50 and 30 %, respectively, of the minilots with < 30 µg kg$$^{-1}$$ aflatoxin.

An improved minicolumn procedure for detecting aflatoxins in groundnuts and groundnut products is described. The technique has a sensitivity of about 3-5 µg kg⁻¹ and can be completed in approximately 15 min. This can be used on a number of commodities. Proximate quantitation of aflatoxins is also possible.


An improved qualitative minicolumn procedure for screening a number of products, e.g. peanut butter, groundnut meal, corn, rice, and cottonseed meal for aflatoxin is described. High-speed blending of the sample with aqueous methanol followed by purification with zinc acetate and re-extraction with benzene before subjecting to minicolumn chromatography is a simple, economical, and rapid method for aflatoxin detection. Sensitivities of 2 µg kg⁻¹ can be achieved and the use of disposable plastics and glass items makes the method practical for field or in-plant application.


An improved procedure for detecting aflatoxin in peanut butter and oilier agricultural commodities has been developed. Heretofore, considerable difficulty was encountered using the millipicolum for detecting aflatoxin in peanut butter. This new procedure overcomes the difficulty by using alumina and florisil as packing materials in the column and by salting out highly fluorescent pigments in the extract before it is placed on the column. Time for an analysis is about 8-9 minutes. Sensitivity as low as 2-3 µg kg⁻¹ is possible.


A minicolumn method is described for determination of aflatoxin B₁ in groundnuts. The method allowed detection of aflatoxin B₁ at more than or equal to 1 µg kg⁻¹. The thin-layer chromatography (TLC) method was used for semiquantitative visual determination of the toxin; mean recoveries for the values more than or equal to 2.5 µg kg⁻¹ were 76-87 %.


The minicolumn proposed by Holaday and Lansden was developed with standard aflatoxin solution and also with the extracts of com, rice, wheat, cottonseed, groundnut cake and black pepper; each having different levels of aflatoxins. One-half mL each of 2, 4-dinitrophenylhydrazine, p-anisaldehyde, 20% H₂SO₄, 20% HC1 and trifluoroacetic acid (TFA) with 25% HNO₃, which were used for confirming aflatoxins on TLC, were applied to the developed column. Among these, all the three acid reagents changed the blue fluorescence of aflatoxins to yellow and thus were found to be satisfactory confirmatory tests. The TFA with 25% HNO₃ had the lowest detection limit (5 µg kg⁻¹).


Aflatoxin extraction methods namely Best Food (BF), Contamination Branch (CB), Pons' and Romer's and analytical methods namely thin-layer chromatography (TLC), spectrophotometric and minicolumn techniques, in different combinations, were compared for their efficiency for determining aflatoxin B₁ occurring naturally in groundnuts and also for estimating aflatoxin B₁ levels in groundnut meal samples spiked with known quantities of the toxin. The BF and Pons' methods gave better efficiency than the other two methods. The spectrophotometric method was more effective than the TLC and minicolumn techniques. The BF method was relatively less expensive and less time consuming than the other extraction methods. The Pons' method was convenient for handling larger numbers of samples, especially in the absence of centrifuge facilities required for the BF method.


A new mycotoxin detection procedure termed "Selectively Absorbed Mycotoxins (SAM)" has been developed. It is a modification of the Holaday-Velasco Minicolumn procedure for aflatoxin screening. With the SAM assay procedure, mycotoxins are extracted into a methanol-water solvent. Toluene is mixed with the
extract to form a two-phase solution where aflatoxins and zearalenone partition into the upper toluene phase, thus separating them from potential interfering compounds. The toluene sample is passed through a SAM-Aflatoxin Zearalenone (SAM-AZ) tube which removes other interfering compounds in a pre-absorption layer, and selectively absorbs any aflatoxin and/or zearalenone at specific bands in the tip of the tube. Each positive band glows with an obvious blue color under long wave ultraviolet light. The sensitivity of the assay is designed to provide a YES/NO screen at designated levels of aflatoxin and zearalenone. Detoxification of aflatoxin-contaminated groundnut products has been accomplished by the addition of a high-affinity sorbent (hydrated sodium calcium aluminosilicate) when added at 0.5 % by weight. Aluminas, silicas, and aluminosilicates were evaluated for their ability to absorb aflatoxin from groundnut oil and aqueous solution. Sorbents such as boehmite alumina, synthetic xerolite and muscovite silica sorbed less than 50 % of the aflatoxin present. Novo Sil (hydrated sodium calcium aluminosilicate, HSCAS), Pyran RG 140 pyrophyllite, and Filtrol (acid-activated bleaching earth), sorbed 85 % or more of the aflatoxin. When HSCAS was added to chicken feed containing 7.5 mg aflatoxin, the toxicity was reduced so that broiler and leghorn chicks appeared normal in their growth and their livers were normal. The safety and effectiveness of the detoxification procedure was measured with the Ames assay.


A screening method is described for detecting total aflatoxins ($B_1 + B_2 + G_1 + G_2$) in mixed feeds, grains, nuts, and fruit products in samples containing 5-15 µg kg$^{-1}$. In addition, the presence of aflatoxins in positive samples can be confirmed and the toxins can be quantitatively measured, using the same extract as that used for screening. In the screening method, aflatoxins are extracted with acetone:water (85:15), and interferences are removed by adding cupric carbonate and ferric chloride gel. The aflatoxins are extracted from the aqueous phase with chloroform and the chloroform extract is washed with a basic aqueous solution. A Velasco-type minicolumn is used to further purify the extract and capture the aflatoxins in a tight band. The screening method has been successfully applied to 24 different agricultural commodities. Quantitative TLC was also performed with extracts of each of these commodities. An average recovery of 94 % $B_1$, 108 % $B_2$, 130 % $G_1$, and 103 % $G_2$ was obtained compared to the official final action AOAC method for cottonseed products. Within- laboratory coefficients of variation of 10-15 % were obtained for each of the aflatoxins and total aflatoxins in a sample of groundnut meal naturally contaminated with 11 µg $B_1 + 3$ µg $B_2 + 11$ µg $G_1 + 5$ µg $G_2$/kg.


A screening method for aflatoxins was collaboratively tested on 11 different agricultural and food products: white and yellow corn, groundnuts, peanut butter, pistachio nuts, groundnut meal, cottonseed meal, chicken, pig and turkey starter rations, and dairy cattle feed. The method involves a rapid extraction and cleanup procedure followed by the detection of total aflatoxins ($B_1 + B_2 + G_1 + G_2$) as a fluorescent band on the florisor layer of a Velasco-type minicolumn. The results of 32 collaborators from 10 countries are presented. Samples containing 0, 5, 10, 15, 20 and 25 µg aflatoxins kg$^{-1}$ were analysed. 84 % of the negative samples and 89 % of the samples containing 10-25 µg total aflatoxins/kg were correctly identified. This method has been adopted as official first action for the detection of aflatoxins in corn, groundnuts, peanut butter, groundnut meal, cottonseed meal, mixed feeds, and pistachio nuts.


A new method for the rapid detection of aflatoxins (i.e., SAM) was compared with a method of high pressure liquid chromatography (HPLC) method. Fifty groundnut samples were analyzed in this study. For the SAM method, groundnut samples (40 g) were ground and extracted with methanol: water (80:20). Aliquots (5 mL) of the methanolic phases were added to 5 mL of water and then partitioned with 3 mL of toluene. Aliquots (0.5 mL) of the toluene phases were added to the top of SAM detectors. Following elution with toluene: chloroform: acetone (95:20:5, v/v/v) all samples were analyzed for aflatoxins with longwave ultraviolet light (365 nm). Groundnut samples (20 g) were ground for HPLC analysis and extracted with acetone: water (75:25, v/v). Fats were removed with hexane, and the aflatoxins were extracted with chloroform and analyzed by normal phase HPLC. No differences was observed between the two methods. All samples positive by HPLC were positive by SAM, and all samples negative by HPLC were negative by SAM. The percent recovery and the stability of aflatoxin $B_1$ in the toluene phase in the SAM assay were determined. Aliquots (5 mL) of the extraction solvent were "spiked" with aflatoxin $B_1$ at levels of 5500, 550, 220, and 55 ng. Aflatoxin $B_1$ was partitioned with the toluene phase and analyzed by HPLC. The percent recovery of aflatoxin $B_1$ was 70 % (a recovery of 90 % is routinely attained when chloroform is used to extract the methanolic phase). A correction factor was determined for standards of aflatoxin $B_1$ in toluene. Aflatoxin $B_1$ was found stable in the toluene phase of SAM. Simple and rapid screening tests such as SAM for the detection of aflatoxins in groundnuts may greatly facilitate prevention through effective...
monitoring programs that allow for the diversion of contaminated crops and animal feeds.


Aflatoxins in food and feeds are detected simply, rapidly and reliably by using a 5-mL glass syringe packed (from the bottom) with 3 mm of anhydrous Na₂SO₄, 5 mm of silica gel, 1 mm of Florisil and 4 mm of anhydrous Na₂SO₄. The column is activated at 110°C for 1 to 2 hr. A sample extract (2 mL) is mixed for 1 min with 400 mg of neutral Al₂O₃, and 1 mL of extract is applied to the packed syringe, followed by 3 mL of hexane-CHCl₃-THF (7:2:1). The solvent is rapidly drained under pressure, and the syringe is examined under 365 nm radiation. A blue fluorescent band at the silica-Florisil interface indicates the presence of aflatoxins; this can be confirmed by standard chemical tests, preferably by reaction with trifluoroacetic acid-H₂SO₄. Semi-quantitative determination of aflatoxins is achieved by comparing the intensity of fluorescence with that for a standard. Depending on which confirmatory test is used, the detection limit is 10 to 50 µg kg⁻¹.


The Holaday-Velasco method and a modified Holaday method were compared for anatoxin analysis in raw groundnuts. The former method combines the speed and simplicity of the Holaday extraction and cleanup with the sensitivity of the minicolumn originally described by Velasco. The combination method has been approved by the AOAC and the AACC for determining aflatoxin in com. The Holaday method was modified by substituting toluene for benzene in the solvent partition, and methylene chloride for chloroform in the minicolumn development to eliminate use of hazardous solvents. The neutral alumina in the Holaday minicolumn was changed from activity V to activity III to provide a more stable column. At aflatoxin levels in raw groundnuts of 13-20 ng g⁻¹, the presence of aflatoxin was missed by the modified Holaday method in 13 of 42 reported. There were no misses in this contamination range by the Holaday-Velasco method. There were no misses by either method with samples containing > 20 ng total aflatoxins g⁻¹. Analysis of non-contaminated raw groundnuts by the modified Holaday method resulted in two false positives of 14 reports; the Holaday-Velasco method produced no false positive reports from 15 analyses of non-contaminated groundnuts. The Holaday-Velasco method was adopted as official first action for groundnuts.


The extraction and cleanup steps of the AOAC minicolumn technique were modified to provide an inexpensive, rapid screening procedure for aflatoxins in groundnuts and groundnut products. A total of 52 samples of groundnuts and groundnut products were collected from shops and markets in Campinas, Brazil, and were analysed for aflatoxins by the old and the modified methods. The results by both methods were the same: 28 samples were negative, four contained <20 µg kg⁻¹ aflatoxin, 12 contained 20-50 µg kg⁻¹, three had 50-100 µg kg⁻¹ and five had >100 µg kg⁻¹. The detection limit in both minicolumn methods was 10 µg kg⁻¹.


Results from two 1980 experiments were used to compare the performance of the visual, minicolumn and thin-layer chromatography (TLC) methods in detecting loads of fanners stock groundnuts with aflatoxin. The first experiment was conducted to establish variability of the methods under ideal conditions. The second experiment was conducted to evaluate the performance of the three methods under commercial conditions. Date and empirical models were used to explore potential improvements in detecting low-level contaminated lots. The minicolumn and TLC methods were very sensitive and provided consistent measurements. Operation characteristic curves developed by using empirical models (Logistic and Gompertz) were compared to statistical distribution functions used by Whitaker and coworkers. Assuming a desired probability of acceptance of 15%, both the minicolumn and TLC methods would accept lots with 60 µg kg⁻¹ aflatoxin, while the visual methods would accept lots with 150 µg kg⁻¹ aflatoxin. In crop years similar to 1980, the currently used method would not segregate groundnuts to meet the 15 µg kg⁻¹ (total) tolerance levels and would provide excessive sheller risk. Because of the objectivity, precision and higher sensitivity, the minicolumn and TLC have potential for improving the detection of contaminated loads of farmers stock groundnuts.


A method based on the use of water slurries was developed for aflatoxin analysis. This procedure enables aliquots, from kilogram size samples, to be analyzed without an increase in solvent or reagent costs. Groundnuts, peanut butter, groundnut meal, cottonseed, cottonseed meal, copra and maize were successfully analyzed by this slurry technique. Slurries were prepared in a 1-gal blender and sample weight to water volume ratios were 1:1.5 for peanut butter, 1:2.25 for whole seeds, 1:4 for
commercial meals and 1.5 for copra. Extraction of 100-g aliquots of slurry with 200 mL of acetone resulted in aflatoxin yields comparable to those obtained by extraction of the dry products by the standard method. The precision of slurry analysis for the products, expressed as % coefficient of variation, ranged from 2.6 to 7.8 compared to 5.7 to 20.8 for analyses by the standard procedure. Determinations were made by fluorometric measurements of aflatoxin absorbed on the florisil layer in a minicolumn.


This study estimated the efficacy of the visual A. flavus (VAF), minicolumn (MCL), and thin-layer chromatography (TLC) methods to detect farmers’ stock groundnuts which contained aflatoxin. Aflatoxin tests on grade samples from each of 2300 lots of fanners' stock groundnuts were used to estimate the distribution of farmers’ stock lots according to their aflatoxin concentration (lot distribution). This lot distribution (with an average aflatoxin concentration of 59.5 µg kg\(^{-1}\)) was incorporated into each of the 3 computer models that simulate the testing of farmers’ stock groundnuts for aflatoxin when the VAF, MCL, and TLC methods are used. The number of lots accepted and the average aflatoxin concentration (AA) in the accepted lots was predicted. Results indicate that when a given percentage of the lots are accepted, lots accepted by the VAF method have less aflatoxin than those lots accepted by either the MCL or TLC methods. When the present visual method was used to test the above lot distribution, 75.8 % of the lots tested were accepted and the AA in the accepted and rejected lots were 4.1 and 232.8 µg kg\(^{-1}\), respectively.


The negative binomial probability function was used to model the distribution of sample aflatoxin test results when replicated grade samples from farmers stock groundnuts were analysed by thin-layer chromatography and minicolumn methods. The Poisson probability function was used to model the distribution of the number of kernels with visible Aspergillus flavus growth found in replicated grade samples of farmers stock groundnuts when the visible A. flavus method was used. The probabilities of accepting a lot of farmers stock groundnuts with given aflatoxin concentrations when using a 465-g grade sample and two different accept/reject levels were predicted with the models and compared to observed acceptance probabilities computed from previously published data for each of the three methods. The comparisons showed good agreement between the predicted acceptance probabilities and the observed acceptance probabilities.

### 7.2.7 Immunochemical Methods


The commercially available hapten, aflatoxin-B\(_1\)-oxime-bovine serum albumin (hapten-BSA), was used to produce an antiserum in rabbits. The same hapten was coupled with alkaline phosphatase (hapten-BSA-ALP) and used in the competitive direct enzyme-linked immunosorbent assay (ELISA) for the detection of aflatoxin B\(_1\). Aflatoxin B\(_1\) was extracted in aqueous methanol from naturally contaminated or spiked groundnut seed samples. Wells of a polystyrene microtitre plate were coated with the antiserum, the plates were washed in PBS-Tween, aflatoxin B, standards or groundnut samples extracts, and hapten-BSA-ALP conjugate were added and the plates incubated. The plates were again washed, and the amount of conjugate bound to the antibody was determined after addition of the substrate, p-nitrophenylphosphate. The hapten-BSA-ALP conjugate has advantages in stability, simplicity of preparation, and high specificity, over the conventional toxin-enzyme conjugate in direct competitive ELISA. The assay method is more rapid and less expensive than the physico-chemical methods of aflatoxin analysis and it can detect levels of aflatoxin B\(_1\) as low as 50 picograms.


An enzyme-linked immunosorbent assay (ELISA) technique is described for determining aflatoxin B\(_1\) in contaminated groundnut meal. The technique showed a 100 pg g\(^{-1}\) sensitivity and a recovery rate from spiked samples with higher concentrations of c. 90 %.

Aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ can be easily and rapidly detected in aqueous solutions using an affinity chromatography column coupled to a monoclonal antibody specific for the toxin molecules. A method for their detection, based on this procedure, is described. Water : methanol extracts of aflatoxin-free food were spiked with aflatoxins, diluted with water and passed through the affinity matrix. The monoclonal antibody is bound to the aflatoxins, which can then be released using a small volume of methanol. This concentrated and separated the aflatoxin present in solutions. As little as 5 ng aflatoxin can be seen if the methanol eluate is passed over a small Florisil tip under UV light, while 0.5 ng can be detected if the eluate is analysed by HPLC. This system can be used to analyse aflatoxins in contaminated samples by spot testing (>5 ng) or as a means of clean-up for quantitative analyses at subnanogram levels. Advantages of this immunological assay in relation to other immunoassays and traditional methods are discussed.


A monoclonal antibody (mAb) has been produced to aflatoxin $B_1$ after immunization of mice and fusion of sensitised spleen cells with myeloma cancer cells. The mice were immunized with aflatoxin $B_1$-oxime-protein conjugate. Positive mAbs were screened using an indirect ELISA specific for aflatoxin $B_1$. The selected mAb was then developed in direct competitive ELISA and immunoaffinity column chromatography methods for aflatoxin detection in foods and feeds. Both assays are rapid, sensitive, specific and require only the minimum of sample preparation. Both immunological assays have now been commercialised and are produced in convenient ready-made kit formats.


A simple procedure is described for the routine immunochemical analysis of aflatoxin $B_1$ in groundnut kernels, peanut butter and maize. The specificity, affinity and sensitivity of the monoclonal antibody employed is such that minimal sample preparation is required. The enzyme immunoassay (EIA) has a sensitivity for standard aflatoxin $B_1$ of 0.2 ng mL$^{-1}$ with working range up to 30 ng mL$^{-1}$ and is not significantly affected by matrix interference of samples. Essential protocol features are: blending substrate with methanol : water, filtering blended sample, and analysis of filtrate by EIA after dilution with buffer. Average recoveries of aflatoxin $B_1$ spiked samples at levels of $6-400 \mu g \text{kg}^{-1}$ were 90-112.5 %. Using laboratory prepared samples contaminated with Aspergillus flavus there was high positive correlation ($r=0.97$) when EIA results were compared with thin-layer chromatography (TLC) techniques.


A commercial immunoaffinity column (EASI-EXTRACT) for the routine assay of aflatoxins in groundnuts was compared with the official first action AOAC CB method. For the CB method, 82.0 and 84.1% of aflatoxin $B_1$ was recovered from the 10 and 50 $\mu g \text{kg}^{-1}$ spiked groundnut samples, respectively. For the immunoaffinity procedure, the recoveries were 93 and 95.5%, respectively. These values were significantly ($P < 0.05$) higher than the CB method. There was also considerable time-saving using the EASI-EXTRACT method.


During the past few years, several laboratories have prepared specific antibodies against aflatoxins $B_1$, $M_2$, $B_2a$ and $Q_1$, ochratoxin A, T-2 toxin, and zearalenone. With the availability of these antibodies, specific, simple and sensitive radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) procedures for monitoring mycotoxins and their metabolites in foods, feeds and body fluids have been developed. In this review details are given for the preparation of the antibodies and the application of RIA and ELISA to determine aflatoxins $B_1$, and $M_1$, ochratoxin A and T-2 toxin in corn, groundnut, milk and other biological fluids. The sensitivity of ELISA for analysis of these mycotoxins in foods varied from 0.1 $\mu g \text{L}^{-1}$ for aflatoxin $M_1$ in milk to 5 $\mu g \text{kg}^{-1}$ of aflatoxin $B_1$ in groundnuts. The advantages of ELISA for monitoring mycotoxins in foods and feeds are discussed. In addition, a description of recent progress on simplified cleanup procedures which may increase the sensitivity of immunoassays is presented.


This paper reviews methods for production of specific antibodies against mycotoxins, and the applications of radioimmunoassay (RIA) and an enzyme-linked immunosorbent assay (ELISA) for analysis of several mycotoxins in various agricultural commodities.
With the availability of specific monoclonal and polyclonal antibodies against mycotoxins in recent years, simple, sensitive and specific radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) of mycotoxins have been developed. The sensitivities of RIA were in the range of 0.1-0.5 ng and of ELISA 2.5-25 picograms/assay. Simple and quick immunoassay protocols (ELISA) for monitoring aflatoxin B₁ in groundnuts and groundnut products, that require less than 1h to complete, have been developed and successfully tested in naturally contaminated groundnut samples at levels about 5 to 10 µg kg⁻¹. In addition, antibodies against mycotoxins have been used as an immunohistochemical tool to monitor mycotoxins in tissues and for the preparation of immunoaffinity columns that were then used, either for aflatoxin determination in groundnuts, or as a cleanup tool for aflatoxin analysis. Details of the recent progress on the production of the antibodies, antibody specificity, and the advantages and disadvantages of different immunoassays, as well as problems associated with immunochemical research on mycotoxins, are reviewed. Emphasis is centered on the immunoassays of aflatoxin in groundnut products.


An improved enzyme-linked immunosorbent assay (ELISA) for aflatoxin B₁ in cornmeal and peanut butter was developed. Aflatoxin B₁ in cornmeal and peanut butter samples was extracted with 70 % methanol in water containing dimethylformamide diluted with assay buffer to a final concentration of 7 % methanol, and directly subjected to an ELISA procedure that took less than 1h for quantitative analysis and less than 30 min. for screening tests. Analytical recoveries for 5-100 ng g⁻¹ B₁ added to the cornmeal and peanut butter were 91 and 95.4 %, respectively. The interwell and interassay coefficient of variation was 10 % or less at the 20 ng g⁻¹ level and above. Agreement for B₁ levels in more than 30 naturally contaminated corn, mixed feed, and peanut butter samples was excellent between the ELISA data and the data obtained from different independent laboratories using thin-layer chromatography (TLC) or other analytical methods.


A method is described for the preparation and purification of aflatoxin B₁-1-(O-carboxymethyl) oxime from aflatoxin B₁. The overall yield was about 73-83%. The new aflatoxin B₁, derivative was characterized by mass, ultraviolet, infrared, and nuclear magnetic resonance spectral analyses, and was nontoxic to 8-day-old chicken embryos when tested at a concentration of 3.48 µg egg⁻¹.


A simple, rapid enzyme-linked immunosorbent assay (ELISA) was used to evaluate the performance of each step (extraction, filtration, solvent partition and silica gel column chromatography) of a solvent-efficient thin-layer chromatography (TLC) method undergoing interlaboratory collaborative study for the determination of aflatoxin B₁ in maize, raw groundnuts and peanut butter. The apparent mean recoveries using the ELISA method were approximately 30-50 % higher than those using the TLC method if only the amount of aflatoxin B₁ added to the samples was used in the calculations. After the cross reaction of the antibody with other aflatoxins added to the samples was considered, the amounts recovered approached the levels of the aflatoxins added in all the three commodities tested. With no cleanup treatment, ELISA recoveries at aflatoxin B₁ levels >7.5 ng g⁻¹ were 84, 79 and 103 % for maize, raw groundnuts, and peanut butter, respectively. With each cleanup step in the TLC method, ELISA detected a progressive decrease in recovery from 150.5 to 105.3 % (before correction for the presence of other aflatoxins) or from 93.5 to 65.4 % (after correction for other aflatoxins) of aflatoxin B₁ added to samples. It is concluded that cleanup treatments are not necessary in the ELISA and that when large amounts of other aflatoxins are present, an understanding of the cross-reactivity of antibody with other aflatoxins in the ELISA is essential for final interpretation of the data.


Antibody against aflatoxin G₁ (AFG₁) was obtained from rabbits after immunizing the animals with AFG₁, hemiacetal (AFG₂a) conjugated to bovine serum albumin. A direct heterogenous ELISA in which AFG₂a was conjugated to horseradish peroxidase was used for monitoring the antibody titers and for toxin detection.
Competitive ELISA assay revealed that the antibody was most specific for AFG$_{2a}$ and least for AFB$_2$. The relative cross-reactivity of this antiserum with aflatoxins G$_{2a}$, G$_2$, G$_1$, M$_1$, B$_1$, and B$_2$ was found to be 1, 7, 13, 47, 48 and 63%, respectively. The lower detection limits for detection of AFG$_1$ after derivatizing to AFG$_{2a}$ was around 15-25 pg assay$^1$.


Antibody against aflatoxin B$_1$ was obtained after one multiple-site injection of bovine serum albumin-aflatoxin B$_1$ conjugate into rabbits. The antibody had greatest binding efficiency for aflatoxin B$_1$, less efficiency for B$_2$, G$_1$, and Q$_1$, and least for aflatoxicol, G$_2$, and M$_1$. Sterigmatocystin, coumarin, and 4-hydroxycoumarin did not give a cross-reaction with the antibody. The sensitivity of the binding assay for detection of aflatoxin B$_1$ is in the range of 0.2 to 2.0 ng 0.5 mL$^{-1}$ sample. Detailed methods for the preparation of the conjugate, production of immune serum, and methods for antibody titer determination are described.


The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.


Grade samples from 152 lots of farmers stock groundnuts were analysed for aflatoxin by an enzyme-linked immunosorbent assay (ELISA) rapid screening test and high-pressure liquid chromatography (HPLC) and the results were compared with those of the visual inspection method used by the Federal State Inspection Service (FSIS) in Georgia. The results showed 41% of the grade samples with visible Aspergillus flavus (Segregation 3) contained < 20 µg kg$^{-1}$ aflatoxin when analysed by both ELISA and IIPLC methods; 18.7% of Segregation groundnuts (no visual contamination) contained 26-2542 µg kg$^{-1}$ aflatoxin. The results of ELISA and HPLC agreed in 98.6% of the composite lot analyses with the detection of 20 µg kg$^{-1}$ or greater. However, the ELISA rapid screening test failed to give positive tests 12 of 13 times when the aflatoxin content was 20-43 µg kg$^{-1}$ in the component samples.


An immunoassay quick-card test was evaluated on farmers' stock groundnuts relative to the criteria of speed, reproducibility, sensitivity, and economic consideration. The test was conducted at a buying point laboratory by two-technical personnel. Fifty-two 25-lb samples of farmers' stock groundnuts were collected by pneumatic probe from 26, 4-5 ton groundnut wagons (2 samples load$^{-1}$) representing live fields that experienced some drought stress. Each sample was processed by the Federal State Inspection Service in the same fashion as regular grade samples resulting in five grade categories for each sample (sound mature kernels (SMK), sound splits, other kernels, loose-shelled kernels and damaged kernels). The results of the immunoassay were compared to high performance liquid chromatography (HPLC) analyses of the same sample extracts. The official thin-layer chromatographic (TLC) analysis was only conducted on the SMK category due to limiting amounts of other sample categories. The immunoassay test was set up to detect 0-20 µg kg$^{-1}$, 20-100 µg kg$^{-1}$, and > 100 µg kg$^{-1}$ aflatoxin. Of the 520 analyses, 12 were in wide disagreement between the immunoassay and HPLC. Errors resulting from the immunoassay were possible in six of the 12 discrepancies. The test, using two personnel, one to weigh, extract and filter samples, and the other to actually conduct the test, achieved a rate of 30 analyses h$^{-1}$. The rate was achieved doing 20 analyses at a time.


Two new rapid methods that use monoclonal antibodies to detect aflatoxins in groundnuts were tested. The Quantitox is an enzyme immunoassay using microtitre wells and the aflatest is based on monoclonal antibodies absorbed onto an affinity column. It was confirmed that aflatoxin contamination occurs preharvest and that storage under low-moisture conditions prevents additional toxin development. There were differences in susceptibility of local cultivars to aflatoxin development. The aflatest procedure was useful to rapidly screen groundnut samples for aflatoxin.
contamination, but there were problems with the Quanritox method. Aflatoxin contamination occurred in the field prior to harvest and was not aggravated by storage conditions at Cleveland Depot during 1987. There was a 19.23 % incidence of aflatoxin contamination in the samples taken in early 1987, but it was not related to the farm management system (communal, small-scale commercial, and large-scale commercial) or any particular pest or disease.


Chemical methods of analysis for the extraction, cleanup and determination of aflatoxins from animal feedstuffs and foods are described. The advantages and disadvantages of thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC) are discussed. Immunoassays are briefly discussed. Methods for other mycotoxins such as ochratoxin, patulin, and the trichothecenes are also outlined.


The recoveries of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> from groundnut meal spiked at 3 levels ranged from 71.7 to 88.3 % (average 80 %) with coefficients of variations from 2.7 to 10.4 %, using the technique described.


Two enzyme-linked immunosorbent assay (ELISA) rapid screening tests were compared to high performance liquid chromatographic (HPLC) analyses of 100 raw groundnut extracts for the determination of aflatoxins. Identical extracts, all contaminated between 0 and 70 µg kg<sup>-1</sup>, were analyzed in duplicate by the Afla-10 cup test, the EZ-screen quick card test, and an HPLC method. The screening tests had detection thresholds of 10 µg kg<sup>-1</sup> and 20 µg kg<sup>-1</sup>, respectively. Both assays were accurate compared to HPLC when samples were negative for aflatoxins or contained aflatoxins above their respective detection thresholds. Errors that did occur were associated with samples that contained aflatoxins at concentrations just below their detection thresholds. The cup test identified as > 10 µg kg<sup>-1</sup> 9 % of samples that were in the 5-10 µg kg<sup>-1</sup> range as determined by HPLC. The card test identified as > 20 µg kg<sup>-1</sup> 53 % of samples that fell in the 10-20 µg kg<sup>-1</sup> range by HPLC. Of the samples that were outside the range of 5-10 µg kg<sup>-1</sup>, the cup test was accurate with both replications compared to HPLC 97 % of the time. Similarly, the card test results (both replications) were comparable to HPLC in 91 % of samples outside the 10-20 µg kg<sup>-1</sup> range.


A study was conducted to evaluate the performance of two enzyme-linked immunosorbent assays (ELISA) for rapidly screening samples of groundnuts for the presence of aflatoxin. The EZ-Screen Quick Card Test and Alfa-10 Cup Test were compared with liquid chromatography in duplicate analysis of common extracts of groundnut contaminated in the range of 0-70 ng g<sup>-1</sup>. Each assay properly identified 95 % of the samples containing no detectable aflatoxin as negative and >97 % of samples containing >10 ng g<sup>-1</sup> aflatoxin as positive. The card test, which had a 20 µg g<sup>-1</sup> detection threshold, identified as positive 32 of 34 samples in the 11-20 µg kg<sup>-1</sup> range. This indicates that the test card test might actually have a detection threshold closer to 10 µg kg<sup>-1</sup>. Most of the errors associated with the assays occurred on the samples containing <10 ng g<sup>-1</sup> aflatoxins. The cup and card tests identified 76 and 67 % of the samples, respectively, as negative, in the range of 4-10 ng g<sup>-1</sup>. For the samples either negative or positive contaminated above their detection thresholds for the assays, the methods are well suited for use as rapid screening tests.


Determination of aflatoxin B<sub>1</sub> in corn, wheat, and peanut butter by an enzyme-linked immunosorbent assay (ELISA) and a solid phase radio immunoassay (RIA) were compared. Samples spiked with 2.9-43.2 ng g<sup>-1</sup> B<sub>1</sub> were subjected to the AOAC extraction procedure. The extracts were concentrated, redissolved in methanol, diluted in phosphate-buffered saline with Tween 20, and directly analysed for B<sub>1</sub> by either ELISA or RIA. At >5.8 ng g<sup>-1</sup> recoveries for B<sub>1</sub> in corn, wheat, and peanut butter samples were 80.0, 86.6 and 94.8 by ELISA and 61.0, 93.3 and 110.0 % by RIA, respectively. Recoveries >120 % were obtained for wheat and peanut butter samples spiked with 2.9 ng g<sup>-1</sup> aflatoxin B<sub>1</sub> by the RIA method but not by the ELISA. Overall results indicated that ELISA gave more consistent data, lower s.d. and lower coefficients of variation than did RIA. Analysis of three samples naturally contaminated with the aflatoxins revealed that the ELISA data were comparable to those obtained by other established chemical methods.

The method described involves coating aflatoxin B<sub>1</sub> polycline conjugate on microtitre plates as immunized antigen, followed by incubation with free toxin standard or sample extract and anti-aflatoxin antibody from rabbits. The amount of antibody bound to the solid phase was determined by subsequent incubation with a secondary antibody conjugated with an enzyme (goat anti-rabbit IgG-horseradish peroxidase conjugate) and reaction with the chromogenic substrate. Anatoxins were extracted from peanut butter and maize meal and dissolved in assay buffer for ELISA. Using the technique 79.5-98.6 % and 68-97 % of aflatoxin B<sub>1</sub> added in the range 5-40 µg kg<sup>-1</sup> to the maize meal and peanut butter were recovered, respectively. The specificity for aflatoxin B<sub>1</sub> as that obtained from the direct ELISA, with an additional advantage that much less antibody was required for the assay.


An essential requirement for the immunoassy of aflatoxin in a food extract is the complete solubilization of the toxin in an aqueous buffer pipetted in the radioimmunoassay procedure. One means of achieving this is by diluting methanol or acetone extract in buffer. However, organic solvent can affect the antigen-antibody interaction. This study determined the effect of acetone and methanol contents in an aqueous casein-buffer solution pipetted with aflatoxin B<sub>1</sub> in the radioimmunoassay procedure on some parameters of radioimmunochemical detection of aflatoxin B<sub>1</sub>. These organic solvents lower the antiserum litre and the relative zero specific binding, and at higher concentration worsen the detection limit and the accuracy of radioimmunoassay. However, in radioimmunoassay of food extracts containing very low levels of aflatoxin, it could be advantageous to add the extract volume to an organic solvent concentration of 60 %.


A simple and sensitive radioimmunoassay [see Journal of Radioanalytical Nuclear Chemistry Letters (1986) 108:259-268 and (1987) 109:383-391] was applied to detection of aflatoxin B<sub>1</sub> in spiked food samples. Before the radioimmunoassay procedure was performed a considerable amount of interfering substances was removed from crude chloroform extracts using Sep-Pak cartridge clean-up. This made possible the detection of 0.1, 0.5, 0.5, 0.1 and 1.0 µg of aflatoxin B<sub>1</sub>/kg in wheat, corn, groundnuts, muscle and liver, respectively. The % recoveries for aflatoxin B<sub>1</sub> were 77-128 % in the range of detection limit to 10.0 µg kg<sup>-1</sup>. If crude chloroform extracts were not cleaned up on Sep-Pak cartridges, false positive results prevented detection of aflatoxin B<sub>1</sub> at concentrations of <1.0 µg kg<sup>-1</sup>.


Two methods of aflatoxin analysis are described both of which use monoclonal antibodies for immunoconcentration for aflatoxin detection. The methods were used to detect aflatoxins in spiked milk and peanut butter.


The specificity and sensitivity of antiserum elicited from rabbits against aflatoxin B<sub>2a</sub>-bovine serum albumin conjugates were characterized with radio immunosorbent assay (RIA) and an enzyme-linked immunosorbent assay (ELISA). Aflatoxin B<sub>1</sub> was first converted to aflatoxin B<sub>2a</sub> and then conjugated to bovine serum albumin and horseradish peroxidase by the Teducutive alkylation method. The antiserum was developed in New-Zealand While rabbits by multiple-site injection with aflatoxin B<sub>2a</sub>-bovine serum albumin conjugate. Antibody titer were determined by both RIA and ELISA. Competitive RIAs with various aflatoxin analogs indicated that the antiserum was most reactive with aflatoxin B<sub>1</sub> and slightly cross-reactive with aflatoxins B<sub>2a</sub>, B<sub>2</sub>, and M<sub>1</sub>. Competitive ELISAs showed the antiserum to be equally specific for aflatoxins B<sub>2s</sub> and B<sub>1</sub> and less reactive with aflatoxins B<sub>3</sub> and M<sub>1</sub>. The relative sensitivities of RIA and ELISA for aflatoxin B<sub>1</sub> quantitation were 100 and 10 pg per assay, respectively.


A simple sample preparation method was developed for immunological aflatoxin analysis of maize. Maize and mixed feeds were extracted 2.5 times (v/w) with 80 % methanol. Recovery of aflatoxins using this solvent was high and adequate for further immunological analysis. Using samples extracted by this method, fice commercially available aflatoxin analysis kits: Aflatest, TD 100, Afla 10 Cup, Aflacheck UBE and Agriscreen were compared to determine their accuracy and practicality. The accuracy of these immunological methods was variable especially
with certain mixed feeds. However, these methods were considered as simple for aflatoxin analysis.


Three types of enzyme immunoassay kits were tested for their ability to detect and/or quantitate aflatoxins. The E1A Kit (Japan), which uses 96 well micro-plates was suitable for the analysis of a large number of samples, had some difficulties regarding sample recovery and antibody specificity to aflatoxin B₁. Using the E-Z screen (USA), aflatoxins were detected rapidly, but could not be assayed quantitatively. The Aflatest 10 kit (U.K.), which uses a small Florisil chip for aflatoxin detection was suitable for semiquantitative analysis of aflatoxins but unfortunately the fluorescence of the chip occasionally masked the fluorescence of the aflatoxins, making quantitation difficult. It was concluded that there is a large possibility for the practical application of these kits for aflatoxin analysis.


Methods for aflatoxin analysis using thin-layer chromatography (TLC) and fluorescence detection were developed in the 1960s and are still widely used. In the late 1970s, several applications of high performance liquid chromatography (HPLC) were developed and as they were generally more sensitive than the TLC methods, they are now popular for aflatoxin analysis when a high degree of accuracy is required. For some test purposes convenience and rapidity of analysis are more important than accuracy, and the bright greenish yellow fluorescence (BGYF) and minicolumn methods were evolved with this in mind. Recently several enzyme-linked immunosorbent assay (ELISA) system kits for aflatoxin analysis have been developed and some of them found suitable for the kind of testing now carried out using the BGYF and minicolumn methods. Gas chromatography can also be used for aflatoxin analysis under certain conditions. From the range of aflatoxin analysis methods now available it should be possible to choose methods suitable for specific purposes.


Monoclonal antibody technology has been used to produce antibodies that recognize aflatoxins in order to develop noninvasive methods in conduction with other chemical analytical techniques to monitor human exposure to the environmental carcinogens. These methods require the ability to quantitate aflatoxins and their metabolites, including DNA and protein adducts, in readily accessible compartments such as serum and urine. The techniques permit efficient analysis of many samples in a relatively short time. Also, these monoclonal antibody affinity columns have been extremely useful for rapid isolation of aflatoxins from food and grain samples, as well as aflatoxin M₁ from milk. Monoclonal antibody affinity methods are nondestructive to the aflatoxin molecule, so the sample aliquot can be used for confirmation. The use of monoclonal antibody preparative affinity columns represents a major, substantive breakthrough for analytical chemists and will be a generally applicable technology for isolation of many different substances.


An improved ELISA combined with monoclonal antibody (MAb) and one-step extraction method is described for the estimation of aflatoxin B₁ in groundnut products. AFB₁ was converted to AFB₁-oxime and then conjugated with bovine serum albumin (BSA). Spleen cells from mice immunized with AFB₁-BSA conjugates were fused with myeloma cells. After double selection with AFB₁-ovalbumin (OVA) and carbodiimide-modified OVA, 5 stable hybridoma cells secreting anti-AFB₁ MAbs (AFl, AF2, AF3, AF4 and AF5) were cloned. Using three anti-AFB₁, MAbs, the indirect competitive ELISA (cELISA) was developed with alkaline phosphatase (ALP)-labelled sheep anti-mouse IgG as marker and the direct cELISA with AFB₁-oxime horseradish peroxidase (POD) as marker. The minimum detectable limits of the indirect cELISA with AFl, AF2, AF3, AF4 and AF5 were 5, 5, 5 and 50 pg of standard AFB₁, per assay, respectively, and those of the direct cELISA with AFl, AF3, AF4 and AF5 were 2.5, 5, 25 and 100 pg of standard AFB₁, assay⁻¹, respectively. The cross reactivity of each toxin with these MAbs in the indirect cELISA was as follows : (a) AF1 and AF2 were reactive with AFB2 as well as AFB₁, weakly with AFG2>AFLG> aflatoxicol II (COL II) > aflatoxicol I (COL 1) and less weakly with other aflatoxins; (b) AF3 and AF4 were reactive with COL II as well as AFB₁, weakly with COL I > AFQ, and less weakly with others; (c) AF5 was reactive with AFQ, as well as AFB₁, weakly with COL II > AFG2 > COL I and less weakly with others. The 60 % aqueous methanol extracts of oil-roasted blanched groundnuts ("peanut butter"), naturally contaminated with AFB₁, were assayed by the direct cELISA without further purification. The direct cELISA with the most sensitive MAb AFl was able to determine 1 ng of AFB₁ g⁻¹ sample.

The specificities of antisera produced by rabbits injected with aflatoxin B\(_1\) or an analog were studied with respect to aflatoxins B\(_2\), B\(_{2a}\), G\(_1\), G\(_2\), Q\(_1\) and P\(_1\). Radioimmunoassays which can detect levels of 0.06 ng of aflatoxin B\(_1\) were used to analyze serum, urine and crude extracts of maize and peanut butter supplemented with aflatoxin. In the foodstuffs 1 µg aflatoxin kg\(^{-1}\) could be measured. The immunoassay was at least as sensitive and specific as any other available analytical methods, but did not require purification of samples before analysis.


An enzyme-linked immunosorbent assay (ELISA) permitted the detection of less than 10 pg of aflatoxin B\(_1\) mL\(^{-1}\). The antitoxin was most specific for aflatoxins B\(_1\) and B\(_{2a}\), and least specific for aflatoxin G\(_1\). The enzyme-linked immunosorbent assay for aflatoxin B\(_1\) is of value for studying in vivo aflatoxin formation during experimental aspergillosis and as a diagnostic aid in cases of suspected aflatoxicosis.


High specificity, high titre antisera to aflatoxins B\(_1\) and G\(_1\) have been produced by immunisation of rabbits with a bovine serum albumin-aflatoxin B\(_1\) conjugate. The antiserum has been used to set up an indirect, double antibody microtitration plate ELISA with a limit of detection of 0.1 pg toxin well\(^{-1}\). The assay has been validated for application to peanut butter, and requires minimal sample preparation before assay, is technically simple and has high throughput.


A survey was carried out in 1986 for the occurrence of aflatoxin B\(_1\) in peanut butters (129 samples) obtained from specialist Health Food outlets. The results showed that 6.2 % of the samples contained > 10 µg kg\(^{-1}\) of aflatoxin, 8 % contained between 2.5 and 10 µg kg\(^{-1}\); and in the remainder (86 %) aflatoxin could not be detected at a limit of 2.5 µg kg\(^{-1}\). These results show a lower contamination by aflatoxin than found in these products in previous surveys (1982-1984). An aflatoxin B\(_1\)-specific enzyme-linked immunosorbent assay (ELISA) was employed for the first time in these analyses; and to make an assessment of its performance positive aflatoxin results, together with a random selection of those below the ELISA limit of detection, were additionally analyzed by conventional extraction and clean-up followed by HPLC. The ELISA technique offered a significant improvement in speed of analysis over conventional approaches, enabling a six-fold increase in sample throughput compared to that required for conventional analysis, together with other advantages.


Fourteen laboratories in the UK participated in a collaborative trial of a commercially available ELISA test kit for the detection of aflatoxin B\(_1\) in peanut butter. Each laboratory carried out four replicate analyses of each of six individual samples. Collaborators received a control, noncontaminated sample, together with samples prepared by blending naturally-contaminated and control material to give target levels of 8, 25 and 75 µg kg\(^{-1}\) aflatoxin B\(_1\). Two of these samples (8 and 25 µg kg\(^{-1}\)) were supplied as undisclosed duplicates. The repeatabilities of the assay ranged from 6.2 to 16.7 µg kg\(^{-1}\). The reproducibilities for aflatoxin B\(_1\) concentration in naturally contaminated samples ranged from 3.6 to 18.7 µg kg\(^{-1}\) using noncontaminated peanut butter as a reference blank. Modifications to the format of the commercial kit were recommended as a result of the collaborative trial.


The technique of immunochemical analysis, including the principles involved, is described and various types of assay are discussed. Application of these methods to the analysis of foods for residues of pesticides, mycotoxins, contaminants, drug residues, and natural constituents is reviewed. Compared with the conventional methods, immunoassays offer similar detection limits and greatly simplified sample preparation procedures.


A joint AOAC/IUPAC interlaboratory study of an enzyme-linked immunosorbent screening assay (ELISA) for aflatoxins was conducted in laboratories in Canada,
France, Japan, South Africa, Switzerland, The Netherlands, Tunisia, and the United States. Twenty-eight samples of raw and roasted groundnuts, corn, whole cottonseed, cottonseed meal, ammoniated cottonseed meal, and poultry feed containing various quantities of natural aflatoxins, and supplemented when appropriate with aflatoxin $B_1$, were distributed to participating laboratories for testing. The assay is based on competition between an enzyme-conjugated aflatoxin $B_1$ and (free) aflatoxins in the test sample for aflatoxin-specific antibodies coated onto the microtiter wells. After a wash step to remove all unbound aflatoxins, a substrate added to each well is catalyzed from colorless to a green solution by any bound enzyme-conjugated aflatoxin $B_1$ present. The intensity of the color decreases as the amount of free aflatoxin $B_1$ in the test sample increases. Overall correlation was good between ELISA and thin-layer chromatography (TLC) results for cottonseed products and mixed feed. Variable results were reported for com and groundnut product samples. Although some positive samples (>15 ng $g^{-1}$) of cottonseed products and mixed feed were reported to contain <15 ng $g^{-1}$ by visual determination, a review of data for absorbance measurements showed that the contamination level was close to the >15 ng $g^{-1}$ standard and would not have been reported as negative under routine screening. Variation in ELISA results may have been due to several factors such as lack of homogeneity of the aflatoxin contamination in the samples (prestudy TLC analysis samples were collected randomly from a pool of subsamples) interferences that resulted from incomplete removal of hexane during the filtration step, and antibody strips at or past their expiration date. The ELISA method has been adopted as official first action as a screening method to determine the presence or absence of aflatoxin $B_1$ at a concentration of >15 ng $g^{-1}$ in cottonseed products and mixed feed.


A joint AOAC/IUPAC interlaboratory study of an enzyme-linked immunosorbent screening assay (ELISA) for anatoxins was conducted in laboratories in Canada, France, Japan, The Netherlands, Switzerland, Tunisia, and The USA. Twelve raw and roasted groundnut and com portions containing various concentrations of natural anatoxins and supplemented when appropriate with anatoxin $B_1$ were distributed to participating laboratories for testing. The assay is based on competition between an enzyme-conjugated aflatoxin $B_1$ and (free) aflatoxins in the test sample for aflatoxin-specific antibodies coated onto microtiter wells. After a wash step to remove all unbound aflatoxins, a substrate added to each well is catalyzed from colorless to a blue solution by any bound enzyme-conjugated aflatoxin $B_1$ present. The intensity of the color decreases as the amount of free aflatoxin $B_1$ in the test sample increases. Final determination of aflatoxin concentrations can be made by either visual comparison with standard solutions or spectrophotometric comparisons (at 650 nm) to knowns. Overall correlation was good between ELISA and thin-layer chromatographic results for corn and roasted groundnut products, with 93 and 98 % correct responses for visual and instrumental determinations, respectively. For instrumental determinations of aflatoxin in com and roasted groundnuts in the <20 ng $g^{-1}$ range, the relative standard deviations for repeatability (RSDR) were 14.9 and 41.4 %, respectively, and the relative standard deviations for reproducibility (RSDR) were 45.7 and 43.5 %. For instrumental determination of >20 ng $g^{-1}$, the respective RSDR and RSDR values were 19.4 and 52.7 for com and 23.3 and 23.3 % for roasted groundnuts. For visual determinations in the <20 ng $g^{-1}$ range, the respective RSDR and RSDR values for com were 38.5 and 60.7 % and for roasted groundnuts 73.7 and 73.7 %. The respective RSDR and RSDR values for determinations of >20 ng $g^{-1}$ for com were 13.5 and 59.5 % and 24.3 and 57.3 % for roasted groundnuts. It is concluded that the ELISA method is approved interim official first action as a screening method to determine the presence or absence of aflatoxin $B_1$ at a concentration of >20 ng $g^{-1}$ in com and roasted groundnuts.


Sixteen United Kingdom analytical laboratories participated in an evaluation of three commercially available enzyme-linked immunosorbent assay (ELISA) kits for analysis of aflatoxin in peanut butter. Each laboratory was sent three sets of 10 randomly numbered samples of peanut butter. Each set consisted of five pairs of undisclosed duplicates. Four of the sets of duplicates were naturally contaminated butters with "target" aflatoxin values (estimated by liquid chromatography) between 8 and 81 µg kg$^{-1}$. The fifth pair was a blank peanut butter containing approximately 3 µg kg$^{-1}$ of total aflatoxins. A statistical treatment of the results of the studies is presented, together with discussion of the relative merits of the different kits.


Six laboratories analysed portions of the same aqueous acetonitrile extracts of 3 peanut butters for aflatoxin concentration by an HPLC procedure (using immunoaffinity column clean-up) and an ELISA procedure. The extracts were from a nominal "blank" peanut butter, a peanut butter naturally contaminated with anatoxins (mostly B1) and from a "blank" peanut butter to which equal amounts of aflatoxins B1, B2, G1 and G2 standards had been added. Similar results for the HPLC
and ELISA procedures were obtained for the blank (means 2.8 and 4.9 µg kg\(^{-1}\), respectively) and naturally contaminated (means 26.0 and 25.9 µg kg\(^{-1}\), respectively) peanut butters. However, the results by ELISA (mean 16.7 µg kg\(^{-1}\)) for the spiked peanut butter was much lower than that obtained by HPLC (mean 28.3 µg kg\(^{-1}\)).


Ten United Kingdom laboratories participated in an evaluation of an immunoaffinity column sample preparation procedure used to prepare aflatoxin B\(_1\) containing extracts obtained from peanut butters contaminated with aflatoxins. Each laboratory was sent 7 randomly numbered samples of roasted peanut butter which included 2 sets of undisclosed triplicates. These 2 peanut butters were naturally contaminated with aflatoxin B\(_1\) at levels of about 12 and 35 µg kg\(^{-1}\). The other sample was a nominal blank peanut butter containing approximately 2 µg kg\(^{-1}\) aflatoxin B\(_1\) which was also employed by participants for recovery experiments. Participating laboratories were instructed to follow a protocol regarding the use of the immunoaffinity columns for extract preparation, but were allowed a free choice of instrumental technique for quantification of aflatoxin levels. Mean recovery for spikes was 72%. Coefficients of variation for the results from the 10 participants for the 2 contaminated roasted peanut butters were, respectively, 45% (on a mean of 13.6 µg kg\(^{-1}\)) and 36% (on a mean of 37.2 µg kg\(^{-1}\)).


Mycotoxins are a chemically diverse group of fungal secondary metabolites with a wide range of toxic effects. Conventional thin-layer and instrumental methods of mycotoxin analysis are time-consuming and make routine safety and quality control screening of these compounds in agricultural commodities difficult. As an alternative, specific polyclonal and monoclonal antibodies have been produced against mycotoxin-protein conjugates and used in sensitive radio immunoassays (RIA) and enzyme-linked immunosorbent assays (ELISAs). One of the simplest ELISA approaches involves competition for a solid-phase antibody between a mycotoxin-enzyme conjugate and nonconjugated mycotoxin in the sample extract. ELISAs have been developed for aflatoxins B\(_1\) and M\(_1\), zearalenone, T-2 toxin, and deoxynivalenol, which are highly specific, rapid (10 min), easily adaptable for analyzing large numbers of samples, and directly applicable to assaying methanol-water extracts of a wide range of foods. Several commercial mycotoxin ELISAs using this approach (most typically for aflatoxin B\(_1\)) are currently being marketed. Since ELISAs will be used in a large part by personnel with limited technical expertise, individual kits must be critically evaluated by analytical chemists for suggested sampling procedures, efficiency of extraction, cross-reactivity, mycotoxin recovery, assay reproducibility, and product shelf-life prior to routine use in food safety and quality control screening.


A specific antibody for 2, 3-dihydro-2,3-dihydroxy aflatoxin B\(_1\) (AFB\(_1\)-diol) was prepared, and its reactivity was characterized for the major aflatoxin B\(_1\) (AFB\(_1\)) metabolites. Reductive alkylation was used to conjugate AFB\(_1\)-diol to ethylenediamine-modified bovine serum albumin (EDA-BSA) and horseradish peroxidase for use as an immunogen and an enzyme-linked immunosorbent assay (ELISA) marker, respectively. High reactant ratios, 1:5 and 1:10, for AFB\(_1\)-diol-EDA-BSA (wt/wt) resulted in precipitated conjugates which were poorly immunogenic. However, a soluble conjugate obtained by using a 1:25 ratio of AFB\(_1\)-diol to EDA-BSA could be used for obtaining high titer AFB\(_1\)-diol rabbit antibody within 10 weeks. Competitive ELISAs revealed that the AFB\(_1\)-diol antibody detected as little as 1 pmol of AFB\(_1\)-diol per assay. Cross reactivity of AFB\(_1\)-diol antibody in the competitive ELISA with AF analogs was as follows: AFB\(_1\)-diol, 100 %; AFB\(_1\), 200 %; AFM\(_1\), 130 %; AFB\(_{2a}\), 100 %; AFG\(_1\), 6 %; AFG\(_2\), 4 %; aflatoxicol, 20 %; AFQ\(_1\), 2 %; AFB\(_1\)-modified DNA, 32 %; and 2, 3-dihydro-2-(N7- guanyl)-3-hydroxy AFB\(_1\), 0.6 %. These data indicated that the cyclopentanone and methoxy moieties of the AF molecule were the primary epitopes for the AFB\(_1\)-diol antibody. The AFB\(_1\)-diol competitive ELISA was subject to substantial interference by human, rat, and mouse serum albumins but not by BSA, Tris, human immunoglobulin G, or lysozyme. By using a noncompetitive, indirect ELISA with an AFB\(_1\)-modified DNA solid phase, a modification level of one AFB, residue for 200000 nucleotides could be determined.


A specific microtest plate enzyme-immunoassay has been developed for the rapid quantitation of aflatoxin B\(_1\) at levels as low as 25 pg assay\(^{-1}\). Multiple-site injection of rabbits with an aflatoxin B\(_1\)-carboxymethylxime-bovine serum albumin conjugate was used for the production of hyperimmune sera. Dilutions of the purified antibody were air dried onto microplates previously treated with bovine serum albumin and glutaraldehyde and then incubated with an aflatoxin B\(_1\)-carboxymethylxime-horseradish peroxidase conjugate. The amount of enzyme bound to antibody was determined by monitoring the change in absorbance at 414
nm after the addition of a substrate solution consisting of hydrogen peroxide and 2,2-azino-di-3-ethyl-benzthiazoline-6-sulfonate. Antibody titers determined in this manner closely correlated with those determined by radioimmunoassay. Competition assays as performed by incubation of different aflatoxin analogs with the peroxidase conjugate showed that aflatoxins B₁ and B₂ and aflatoxicol caused the most inhibition of conjugate binding to antibody. Aflatoxins G₁ and G₂ inhibited the conjugate binding to a lesser degree, whereas anatoxins M₁ and B₂, had no effect on the assay.


A simple procedure was devised for the routine screening of aflatoxin B₁ in peanut butter using enzyme-linked immunosorbent assay (ELISA). Peanut butter samples (5g) were spiked with aflatoxin B₁ and extracted by blending with 25 mL of 55% methanol and 10 mL of hexane. The extract was filtered and aqueous filtrate analyzed by a indirect competitive ELISA. Recovery of aflatoxin B₁ added to peanut butter samples ranged from 85 to 112%, with an average coefficient of variation of 18.4%. Using this procedure, only three of 63 samples of peanut butter had detectable levels (>5.0 µg kg⁻¹) of aflatoxin B₁.


A simple procedure was devised for the routine screening of aflatoxin B₁ in groundnuts and peanut butter using enzyme-linked immunosorbent assay (ELISA). Samples of peanut butter were spiked with aflatoxin B₁ and extracted by blending with 25 mL of 55% methanol and 10 mL of hexane. The extract was filtered and aqueous filtrate analyzed by a direct competitive ELISA. Recovery of aflatoxin B₁ added to peanut butter samples ranged from 85-112%, with a mean coefficient of variation of 97%. Coefficient of variation between test wells in the assay was 18.4%. Using this procedure, only 3 of 63 commercial samples of peanut butter showed detectable aflatoxin B₁ (>5 ng g⁻¹).


Cross-reactions and resulting nonspecific binding of substances with structures resembling aflatoxins (derivatives of coumarin, and cinnamonic and benzoic acids, etc.) were investigated. The concentrations of these substances causing erroneously high or false positive values in radioimmunoassay were determined. One µg aflatoxin B₁ kg⁻¹ sample may be simulated by the occurrence of 5 g coumarin, 10 g caffie acid, 16 g chlorogenic acid, or 15 g vanillin kg⁻¹ fodder or food sample.


A microtitre-based competitive enzyme immunoassay (EIA) earlier developed was adapted to a membrane-based EIA to show the feasibility of such a test in qualitative/semi-quantitative determination of aflatoxin B₁ (AFB₁) requiring no instrumentation. Anti-AFB₁ antibody was immobilized on 8 mm Immobilon TM membrane discs at different protein concentrations. Standard curves were prepared by direct competition between AFB₁, in standards and AFB₁-peroxidase conjugate for binding to specific antibody immobilized to membrane discs. Sensitivity of the membrane-based assay could be controlled to a desired level by adjusting the immobilized antibody on the solid support; decreasing the immobilized antibody concentration on the discs increased the sensitivity of the assay within a certain limit. A visual cut-off in color could be established at a level of 20 ng mL⁻¹ AFB₁, with an appropriate amount of antibody on the membrane. Preliminary results for attaching the membrane to a solid support to develop a dipstick EIA test is discussed.


A solid-phase radioimmunoassay (RIA) for aflatoxin B₁ was developed. This method involved the incubation of aflatoxin B₁ both labelled and unlabelled, with immunoglobulin (IgG)-sepharose gel which was prepared by conjugation of the IgG highly specific to aflatoxin B₁ with CNBr-activated sepharose gel, followed by a filtration step. The binding capacity was determined by counting the radioactivity in the filtrate. Studies with different aflatoxin B₁ analogs revealed that the IgG-gel bound most effectively with B₁. Binding of aflatoxin B₂, G₁, G₂, and aflatoxicol to the IgG-gel was less effective in comparison with the IgG before coupling. Between 0.5-5.0 ng assay⁻¹, the displacement of radioactivity from gel was directly proportional to the amount of aflatoxin B₁ present. Using a simple extraction procedure without cleanup step, the recovery yields for aflatoxin B₁ in the contaminated corn or wheat at levels of 5 ng g⁻¹ or above were 60%.

A direct competitive enzyme-linked immunosorbent assay (ELISA) screening method for aflatoxins at 20 ng g\(^{-1}\) was studied by 12 collaborators. Test samples of peanut butter were extracted by blending with methanol-water-hexane (55:45:100) and heating the test extracts on a steam bath; test samples of other commodities were extracted by blending with methanol-water (80:20). All the test extracts were filtered and the filtrates were diluted with buffer to a final methanol concentration of <30 %, each diluted extract was applied to a plate containing a filter with immobilized polyclonal antibodies specific to aflatoxins B\(_1\), B\(_2\), and G\(_1\). Aflatoxin-B\(_1\)-peroxidase conjugate was added, the cup was washed with water, and a mixture of hydrogen peroxide and tetramethylbenzidine was added. The test sample was judged to contain >20 ng aflatoxins g\(^{-1}\) when, after exactly 1 min, no color was observed without the filter; when a blue or a grey color developed, the test sample was judged to contain <20 ng aflatoxin g\(^{-1}\). All collaborators correctly identified naturally contaminated corn and raw peanut positive test samples. No false positives were found for controls containing <2 ng aflatoxins g\(^{-1}\). The correct response for positive test samples spiked at levels of 10, 20, and >30 ng aflatoxins g\(^{-1}\) (the ratio of B\(_1\):B\(_2\):G\(_1\) was 10:1:3) were 52, 86, and 96 %, respectively. The method, which is rapid and simple has been adopted official first action for screening for aflatoxins at 20 ng g\(^{-1}\) in cottonseed and peanut butter and for aflatoxins at >30 ng g\(^{-1}\) in corn and groundnuts. Positive test samples may require reanalysis by an official, quantitative method.


Three different methods were compared for the determination of total aflatoxins in corn and groundnuts naturally contaminated with aflatoxins and in corn, groundnuts, cottonseed, peanut butter, and poultry feed spiked with aflatoxins B\(_1\), B\(_2\), and G\(_1\). The three methods were an enzyme-linked immunosorbent assay (ELISA) screening test, a monoclonal antibody-affinity column- solid phase separation method, and the AOAC official thin-layer chromatography (TLC) methods for all except poultry feed, for which Shannon’s TLC method for mixed feed was used. The ELISA test is designed to provide only positive results for total aflatoxins at > 20 ng g\(^{-1}\) or negative results at < 20 ng g\(^{-1}\). The affinity column separation is coupled with either bromination solution fluorometry to estimate total aflatoxins or liquid chromatography (LC) to quantify individual aflatoxins. Fluorodensitometry was used to determine aflatoxins in commodities analyzed by the TLC methods. The LC and TLC results were in good agreement for all the analyses. The results for the affinity column using bromination solution fluorometry were similar except those for cottonseed, which were about 60 % higher. The ELISA screening method correctly identified naturally contaminated corn and groundnut positive samples. No false positives were found for controls. The correct response for spiked corn, raw groundnuts, peanut butter, and cottonseed at > 20 ng aflatoxins g\(^{-1}\) was about 90 %. The correct response for spiked poultry feed at > 20 ng aflatoxins g\(^{-1}\) was about 50 %.


From a single aflatoxin B\(_1\) oxime-bovine serum albumin conjugate, polyclonal and monoclonal antibody preparations were produced. The four rabbit polyclonal antisera were specific for aflatoxin B\(_1\) in a microtitration plate enzyme-linked immunosorbent assay. The monoclonal antibodies showed a wide range of differing specificities, recognizing, for example, aflatoxins B\(_1\), B\(_2\), G\(_1\) and G\(_2\); B\(_1\) and B\(_2\); B\(_1\) and G\(_1\); and G\(_1\) alone. No antibody preparations reacted with aflatoxin M\(_1\). The significance of these results to the strategy of anti-aflatoxin antibody production for use in quantitative enzyme immunoassays is discussed.


ELISAs for the determination of aflatoxins B\(_1\) and G\(_1\) in peanut butter and of sterigmatocystin in barley are described briefly.


This review includes discussion of safety, sampling, standards, presumptive and screening methods, quantitative methods, including thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC) and immunochemical methods, and selection of analytical approach in relation to the detection and estimation of aflatoxins in maize and groundnuts.

Three immunochemical and three chemical methods were compared for aflatoxin analysis, using fifty naturally contaminated lots of raw shelled groundnuts. The USDA/AMS analyzed all lots using the water slurry modification of the Best Foods (BF) method. The lots were chosen so that aflatoxin levels ranged from 0 to 100 µg kg\(^{-1}\). The Federal-States Inspection Service modified Dickens mill was used to collect special 10 lb, subsamples from commercial lots. 10 lb sample was divided, and duplicate analytical subsamples for each method were prepared to make each subsample as representative as possible. The Aflatesl-P mycotoxin testing system uses a monoclonal based affinity column and was provided by Cambridge Naremo, Springfield, MO. The ELISA methods used were Agrichek aflatoxin test provided by the Agri Tech Systems, Inc., Portland, ME, and the Agri-Screen test supplied by Neogen Corporation, Lansing, MI. The chemical methods used were the water-slurry method, CB method and an HPLC method using normal phase separation and a silica gel packed cell with fluorescence detection following the CB extraction step. The overall mean from all analyses and methods was 19 µg kg\(^{-1}\) with a range of 0 to 100 µg kg\(^{-1}\) total aflatoxins; the coefficient of variation (CV) was 36%. Analysis of variance showed no significant differences between methods. The methods contributed little to variation, but samples contributed to 56% and subsamples to 35% of the variation. All methods showed significant relationships (P< 0.01) with regression using the water slurry method as the dependent variable. The R values ranged from 0.66 to 0.83. All methods performed well and were comparable. Any one of the immunochemical methods could be routinely used by trained personnel to perform aflatoxin analyses.


Recent developments in the use of ELISA kits for the rapid detection of aflatoxin in corn, nuts, peanut butter and cottonseed are discussed and compared with other detection methods (TLC, UV and minicolumn screening). Advantages of ELISA are outlined with reference to ease of use, detection limits and cost.

### 7.2.8 Biological Methods


Aflatoxin B\(_1\) is acutely toxic to embryos and larvae of Zebra fish, Brachydanio rerio (Hamilton-Buchanan). These organisms are sensitive to sub-microgram quantities of the toxin and can be used as bioassay test organisms. The larval response is easier to measure than embryos, response, and a standard dosage-mortality curve for the larvae can be developed in 33 h. As an aflatoxin bioassay test organism, the larvae of zebra fish have several advantages over other test organisms, including ducklings, embryos, and tissue cultures. They require no special skill or equipment and can be obtained easily in the laboratory throughout the year.


The effects of 13 fungal toxins (and one derivative) on the larvae of zebra fish, Brachydanio rerio, were investigated. The most toxic were sterigmatocystin, gliotoxin, and aflatoxin B\(_1\), which were lethal at concentrations of less than 1 µg mL\(^{-1}\). Allatoxins B\(_2\), G\(_1\), and G\(_2\), stemphone, diacetoxyscirpenol, ochratoxin A, asperpoxin, and patulin also showed toxicity to the larvae, while aflatoxin B\(_1\), hemiacetal, penicillic acid, and griseofulvin were non-toxic at the levels tested. Specific morphological effects were noted with sterigmatocystin, aflatoxins, and stemphone. The usefulness of zebra fish larvae as bioassay test organisms for fungal toxins is discussed.


Biological effects of toxic groundnut meal (meal containing aflatoxin) in various birds and animals are reviewed. Ducks are the most susceptible to the toxin. They are suitable for bioassay of aflatoxin. Turkey poult s are less susceptible, while chikens are comparatively resistant. Among the larger farm animals, pigs are most susceptible. Calves from one to six months of age are highly susceptible, becoming tolerant with age, and sheep are comparatively resistant.


A toxic substance was found in the chloroform extract of Brazilian groundnut meal. This extract from 10 g meal was lethal to ducklings. Signs of poisoning were similar to those of Senecio poisoning of poultry, but the isolated poison was not a pyrrolizidine alkaloid or the N-oxide of such an alkaloid.

The Peking White duckling was found suitable for bioassay of aflatoxin. Aflatoxin caused liver damage in the birds 1-2 days after oral administration. The LD50 of the aflatoxin sample A16A was 1.40 mg kg⁻¹. The lower limit of liver damage detection after a single dose was 0.1 mg kg⁻¹; it could be reduced by a factor of 10 with multiple dosage. The multiple dosage protocol is suitable for qualitative screening, but an LD₅₀ determination is recommended for quantitative measurements. It is recommended that mycological, chemical, and toxicological results be considered in a qualitative interpretation of aflatoxin poisoning.


Evidence is presented of the susceptibility of ducklings and chickens to the toxic principle in certain samples of groundnut meal. Ducklings were highly susceptible to the toxic principle in these meals, and it was suggested that they are eminently suitable for screening suspected samples of groundnut meal and for other experimental work connected with this type of toxicity. A toxic agent was found in certain Brazilian and East African groundnut meals, and evidence is presented which suggests that the toxic principle in these meals is identical. The gross and microscopic lesions in ducklings and chickens fed on toxic groundnut meals are described and the similarities and differences between the lesions in these birds and turkeys and in large animals are discussed.


This paper very briefly reviews some analytical methods and bioassays for aflatoxins in groundnuts and groundnut products. Regulatory aspects are also discussed, and the actions of the Food and Drug Administration (FDA) of the US are highlighted.


Twenty-two isolates of Aspergillus flavus from seeds of groundnut, maize, cotton, rice and wheat were screened for production of aflatoxins on YES+salt medium by thin-layer chromatography (TLC) and the okra seedling chlorosis test. To develop a rapid bioassay, seeds of different crop plants and weed plants were soaked in culture filtrates of a toxigenic isolate from maize and in pure aflatoxin B₁ (50 ppm). Chlorosis and albinism of the primary leaves occurred in six species. The effects were most pronounced in sorghum, radish and okra and these could be used to screen aflatoxicogenic strains of A. flavus.


Following the outbreaks of Turkey "X" disease in turkey poults in Britain in 1960, workers have reported the suitability of ducklings as biological assays for toxic principle in groundnut meals. This note reports that mere may be a difference in response whether one uses White Penine ducklings or Khaki Campbell ducklings. Both these ducklings were compared for sensitivity to the toxic principle. The authors concluded that Khaki Campbell ducklings were more sensitive to aflatoxin (toxic principle) than the White Penine ducklings, and as such should be used for biological assays until a more sensitive breed of species is known.


Temperature-dose relationships with aflatoxin B₁ were studied, using the brine shrimp, Artemia salina. Increase in temperature resulted in increased sensitivity by the brine shrimp to aflatoxin. Optimum sensitivity occurred at 37.5°C. Positive results were obtained at 0.5 µg aflatoxin B₁ mL⁻¹ artificial sea water with a mortality of over 60 %. Greater than 90 % mortality occurred at dose levels of 1 µg mL⁻¹ and above. The test can be conducted in 24 h.


Bioassay methods for mycotoxins are reviewed. An outline of the range of bioassay methods is given and the role of cytotoxicity tests in particular is emphasized.


The growth of Bacillus megaterium from spores is inhibited by a variety of toxic fungal metabolites and related compounds. Most of the mycotoxins possessing a carbonyl function conjugated with a double bond system, such as α, β-unsaturated lactones, pyrones, and quinones, were inhibitory. The notable exception was rubratoxin B₁. Of a number of 3-substituted 4-hydroxycoumarins tested, only those possessing a 3 substituent terminated by a large group were highly inhibitory. A number of compounds which act as uncouplers of oxidative phosphorylation were found inhibitory. It is suggested that, among others, toxic fungal metabolites which possess a β-unsaturated carbonyl system or which act as uncoupling agents of oxidative phosphorylation may be detected by the use of Bacillus spores.
This paper describes the histological changes in the liver produced by a single dose of aflatoxin and contrasts them with the lesions produced in day-old ducklings by other well-studied liver poisons. Day-old Khaki Campbell ducklings given 15 µg aflatoxin by mouth developed an extensive biliary proliferation in the liver with fatty degeneration of the peripheral parenchyma cells. This lesion reached its maximum in 3 days, and men regressed with repair of the liver parenchyma. A comparable lesion was seen in ducklings given single doses of dimethyl nitrosamine and cycasin, but not with other hepatotoxic agents such as carbon tetrachloride, ethionine and thioacetamide. The lesions varied greatly at the same dose level, and it was not possible to estimate the amount of aflatoxin by histological examination of the livers of poisoned birds. Evidence of possible direct action upon the biliary epithelium is given, and it is suggested that aflatoxin may be an alkylating agent.

The effects of single dose of aflatoxin B1 on guinea-pigs were compared with the effects of feeding groundnut meal containing aflatoxin. The LD50 of aflatoxin B1 in male and female guinea-pigs was estimated as 1.4 mg kg⁻¹ bodyweight by interaperitoneal injection. The main hepatic lesion was a centrilobular zone of necrosis developing with biliary proliferation over 2 days. The lesion was healed over a few days and the biliary proliferation rapidly regressed. The pathological changes in other organs are described.

Day-old ducklings in groups of six were given two turkey diets which had been associated with outbreaks of Turkey "X" disease. The diets had about 6 % Indian groundnut meal. Other groups were given similar amounts of Indian groundnut meal known to be non-toxic. Those given the toxic meal did not grow well and five in each group died within 5 weeks. The lesions varied greatly at the same dose level, and it was not possible to estimate the amount of aflatoxin by histological examination of the livers of poisoned birds. Evidence of possible direct action upon the biliary epithelium is given, and it is suggested that aflatoxin may be an alkylating agent.

Aflatoxin was estimated with Bacillus megaterium. Of 133 samples of cattle feed tested, 56 were positive for aflatoxin. Some samples positive by thin-layer chromatography (TLC) were negative in inhibiting growth of B. megaterium. Evidence of possible direct action upon the biliary epithelium is given, and it is suggested that aflatoxin may be an alkylating agent.

This paper comprehensively reviews the available bioassays for aflatoxins. Both genetic and nongenetic effects of aflatoxin toxicity in microorganisms, animals, and birds are discussed. Guidelines for formulating aflatoxin for biological studies are given.

Twenty-one isolates of Aspergillus flavus obtained from cotton, maize and wheat were screened for their ability to produce aflatoxins in two liquid media. Of these, 16 isolates were aflatoxigenic and produced only aflatoxin B1 as assessed by bioassay on okra seedlings and thin-layer chromatographic (TLC) method. For screening isolates of A. flavus for aflatoxin formation, 0.7 % YES + Salt medium was found good as also for obtaining high yields of the toxin. Isolates of A. flavus
produced aflatoxin B₁ ranging from 0.85 to 17.2 mg 50 mL⁻¹. Maximum yield of aflatoxin was obtained when rice was used as the substrate in case of toxigenic isolates L-27 and C-9, and on maize in isolate M-11.


The effects of aflatoxin containing filtrates from aflatoxin producer and non-producer isolates of Aspergillus flavus on seed germination and seedlings of different crop plants were reported, producer isolate M-11 and non-producer isolate M-7 of A. flavus were used; the isolates were grown separately on 0.7 % YHS + salt liquid medium at 25 + 1°C for 14 days and the cultures were filtered and autoclaved. The effects of filtrates from the isolates were observed on seed germination of different crop plants. The soaking of seeds of different crop plants in the culture filtrates of M-11 for different durations inhibited seed germination of many crop plants tested. In some cases the toxic effects were observed on the germinating seedlings too. Seedlings of Raphanus sativus and Sorghum vulgare showed chlorosis and albinism in the cotyledonal and primary leaves, respectively. These plants can be used as bioassays for aflatoxin.


Spores of Bacillus stearothermophilus in standardized spore strips are pretreated with solutions of the mycotoxins aflatoxin B₁, polonin, rubratoxin B, and diacetoxyscirpenol and subsequently incubated in a nutrient solution containing bromocresol purple as pH indicator. After 16.5 h of incubation the color of the indicator medium inoculated with non-treated spore strips of B. stearothermophilus changes from purple to yellow; no color change occurs in the indicator medium inoculated with spore strips treated 15 min with 0.01 µg of any of the mycotoxins mL⁻¹ during a 60 h incubation.


Samples of groundnut meals and decorticated groundnuts from Nigeria, French West Africa, and the Gambia were examined. Many were toxic in trials were ducklings. Some of the meals were already suspect, being associated with the deaths of calves and sheep, others were not. and among those several were not toxic. Some extracts from decorticated groundnuts were also toxic.


The toxic principle in certain samples of Brazilian groundnut meal was extracted and concentrated 250 times on a weight basis. This extract produced mortality in ducklings and turkey poult and histological liver lesions identical to those seen in field outbreaks of so-called Turkey "X" disease. The dosing of concentrated extracts to young ducklings gives a rapid and sensitive method for testing the toxicity or otherwise of groundnut meals. The toxic principle is neither a pyrrolizidine alkaloid nor the N-oxide of such an alkaloid. It is suggested that the toxic principle is derived from a microorganism, possibly a fungus. A similar toxic principle was found in some groundnut meals from India, Uganda and Tanganyika, French West Africa, Nigeria, Gambia and Ghana.


The toxic substance present in some Brazilian and African groundnut meals which causes Turkey "X" disease was shown to be even more toxic to ducklings. The signs included loss of appetite, poor growth rate, a tendency to down or feather pulling, liver damage and sometime purple discoloration of the legs and feet in white-skinned birds over 3 weeks old, occasionally accompanied by lameness. Not all groundnut meals tested were toxic. Toxic groundnut meal as 10 % of the diet caused death within 6 to 20 days. This interval and the severity of the gross lesions depended on the toxicity of the sample, the amount eaten and the age of the ducklings; younger birds were more susceptible. With chicks, 10 % groundnut meal reduced growth rate but did not cause death; with chickens 45 days old 15 % groundnut meal caused 3 deaths in the first 3 weeks but no more in the rest of the feeding period lasting 9 months.


Studies were carried out to investigate whether aflatoxins were the factors responsible for the inhibition of chlorophyll synthesis in plants. Crude extracts were prepared from two groundnut meal samples. One meal was known to be toxic to animals and to contain aflatoxins, and the other was a non-toxic meal. These extracts were tested on the rapidly germinating seeds of cress (Lepidium sativum). The leaves of the seedlings that had the toxic extract (5 mg mL⁻¹ corresponding to approximately 0.5 g of meal) appeared yellowish, while those which had the same concentration of the non-toxic extract, or water, were bright green. The tests were repeated using various concentrations of aflatoxins, coumarin and several other...
fungal metabolites. Aflatoxins, like coumarin, inhibited the germination of seeds in concentration of 25 µg mL^{-1} but its effect on the color of leaves was more marked. Complete absence of the green color occurred in the presence of 10 µg mL^{-1} of aflatoxin. The striking effect of aflatoxins in inhibiting the green color of cress seedlings suggests that this effect could serve as the bioassay for aflatoxin in foodstuffs.


In order to develop a low cost and simple biological method for the detection of mycotoxins, the properties of Moina macrocopa, a kind of animal plankton in fresh water, were studied. City water was treated with charcoal in order to remove chlorine and this treated water was used for rearing the plankton. Vital yeast for bread-making was used as feed for this plankton. The temperature for rearing M. macrocopa was set between 22-24°C and the temperature for biological test was set at 20°C. The survival rate of M. macrocopa was found to be high for pH values ranging from 6.5 to 8.5 based on pH's tests using a mixture of water (2 parts) and Britton-Robinson buffer (1 part). The effects of salinity and organic matters of M. macrocopa which appear to be harmless were examined. When the concentration was below 0.23 % in case of sodium chloride, 0.45 % in case of sodium glutamate, and 2.0 % in case of glucose, these chemicals did not appear to affect the survival rate of the plankton directly. Based on the results of experiments on the survival rate of M. macrocopa using solvents such as acetone, methanol, ethanol and dimethyl sulfoxide (DMSO), DMSO was found to be the least toxic. Therefore DMSO could be used to dissolve mycotoxins into the rearing water of plankton. Vital yeast for bread-making was used as feed for this plankton. The temperature for rearing M. macrocopa was set between 22-24°C and the temperature for biological test was set at 20°C. The survival rate of M. macrocopa was found to be high for pH values ranging from 6.5 to 8.5 based on pH's tests using a mixture of water (2 parts) and Britton-Robinson buffer (1 part). The effects of salinity and organic matters of M. macrocopa which appear to be harmless were examined. When the concentration was below 0.23 % in case of sodium chloride, 0.45 % in case of sodium glutamate, and 2.0 % in case of glucose, these chemicals did not appear to affect the survival rate of the plankton directly. Based on the results of experiments on the survival rate of M. macrocopa using solvents such as acetone, methanol, ethanol and dimethyl sulfoxide (DMSO), DMSO was found to be the least toxic. Therefore DMSO could be used to dissolve mycotoxins into the rearing water of plankton. Of the 17 metallic compounds tested, the toxicity levels of potassium dichromate, potassium chromate, cupric sulfate, and mercuric chloride were comparatively high. At toxic level, the pH of the solutions of each of the metallic compounds was measured ; pH was low and that itself could have influenced the survival rate of brine shrimp in the case of chromium potassium sulfate, ferrous sulfate, ferric sulfate and ferric chloride. The simultaneous administration of half the median Tolerance Limit (TLm) of each of the six mycotoxins and one of seven metallic compounds to the brine shrimp was carried out. A weak synergistic effect was observed with the administration of ochratoxin A and zinc sulfate while comparatively strong synergistic effects were observed with the administration of ochratoxin A and mercuric chloride, and aflatoxin B_{1} and mercuric chloride. The toxicity level was weakened by the simultaneous administration of sterigmatocystin and cupric sulfate. The mean values of the toxicity levels of ochratoxin A when administered simultaneously with cadmium chloride, or potassium chromate, or potassium dichromate, and sterigmatocystin when administered simultaneously with mercuric chloride were lower than the toxicity value of 1/2 TLm of each mycotoxin or metallic compound although the value varied widely. In the case of other mycotoxins and metallic compounds, the levels of toxicity were cumulative.


The synergistic effects of mycotoxins to brine shrimp were examined. Aflatoxin B_{1}, diacetoxyscirpenol, fusarenon-X, ochratoxin A, sterigmatocystin and T-2 toxin were used as the mycotoxins. When aflatoxin B_{1} and T-2 toxin were administered to the brine shrimp, the mortality was between 20 and 30 % at 1/2 median Tolerance Limit (TLm). The mortality increased almost linearly up to TLrn. When diacetoxyscirpenol, fusarenon-X and sterigmatocystin were administered, the mortality was less than 20 % at 1/2 TLm, then increased rapidly up to TLm. In the case of ochratoxin A, the mortality was more than 30 % at 1/2 TLm, then the mortality increased slowly up to TLm. When 1/2 TLm of two mycotoxins are to be administered at one time and if the mortality exceeds 60 %, it is regarded as synergistic effect. The synergistic effect was observed at the administration of fusarenon-X and ochratoxin. In other cases the toxicities were considered, not synergistic, but additive.


Biological tests carried out on the brine shrimp using metallic compounds and simultaneous administration of mycotoxins and metallic compounds. The survival rate of brine shrimp was high at pH 6.1 to 9.0, when examined at various pH's using a mixture of artificial sea water (2 parts) and Britton-Robinson buffer (1 part). Cl and SO_{4}^{2-} were found less toxic to the brine shrimp compared to the metallic ions. Of the 17 metallic compounds tested, the toxicity levels of potassium chromate, potassium dichromate, cupric sulfate, and mercuric chloride were comparatively high. At toxic level, the pH of the solutions of each of the metallic compounds was measured ; pH was low and that itself could have influenced the survival rate of brine shrimp in the case of chromium potassium sulfate, ferrous sulfate, ferric sulfate and ferric chloride. The simultaneous administration of half the median Tolerance Limit (TLm) of each of the six mycotoxins and one of seven metallic compounds to the brine shrimp was carried out. A weak synergistic effect was observed with the administration of ochratoxin A and zinc sulfate while comparatively strong synergistic effects were observed with the administration of ochratoxin A and mercuric chloride, and aflatoxin B_{1} and mercuric chloride. The toxicity level was weakened by the simultaneous administration of sterigmatocystin and cupric sulfate. The mean values of the toxicity levels of ochratoxin A when administered simultaneously with cadmium chloride, or potassium chromate, or potassium dichromate, and sterigmatocystin when administered simultaneously with mercuric chloride were lower than the toxicity value of 1/2 TLm of each mycotoxin or metallic compound although the value varied widely. In the case of other mycotoxins and metallic compounds, the levels of toxicity were cumulative.

Several conditions to rear the brine shrimp were examined, and sensitivity of the brine shrimp to mycotoxins was examined. Temperature-dose relationships with methanol on the brine shrimp were tested in "Kuroshio" artificial sea water. When 3 % methanol was used, the viability was high at 10 to 20°C. Of six artificial sea waters examined, Lyman and Fleming's artificial sea water proved good for hatching and rearing. The optimum temperature for rearing was 20°C while it was 30°C for hatching the brine shrimp. When the viability of the brine shrimp on solvents was tested, dimethylsulfoxide (DMSA) and propylene glycol were harmless to the brine shrimp. So DMSO was used to dissolve mycotoxins into artificial sea water. T-2 toxin, sterigmatocystin, diacetoxyscirpenol, aflatoxin B1, and aflatoxin G1 were very toxic to the brine shrimp. This method may be used for screening these mycotoxins.


Aflatoxin B1 inhibits cell cleavage in fertilized mollusk eggs without preventing fertilization or nuclear division. The reaction is very dramatic and positive since fertilized eggs in the presence of aflatoxin are multinuclear, whereas the controls have multiplied to multicellular larvae. The bioassay requires a minimum of technique and training and is sensitive to concentrations of 0.05 µg mL⁻¹. Since the reaction is observed with the aid of a low-powered microscope (70 X), the concentration of aflatoxin required can be reduced considerably below 0.05 µg. The test required two to four hours for completion when observing egg division and 18 h for swimming larvae.


The possibility of using the chicken embryo as a test organism for the assay of aflatoxin toxicity was investigated. The injection of test solutions was made before incubation, in fertile white Leghorn eggs, by either of 2 routes: yolk or air cell. The development of the embryos was observed for the full 21-day incubation period. The injection of solutions of pure aflatoxins B1 and G1 and of extracts of aflatoxin-producing mold cultures indicated that the chicken embryo was sensitive to these compounds. A dose-response was exhibited in that the toxicity of the samples was related to the mortality at the time of hatching. Extracts of aflatoxin-free groundnut products were nontoxic to the chicken embryo. The addition of aflatoxin B1 to such uncontaminated extracts produced the expected toxicity in the embryos. The injection of extracts from aflatoxin-contaminated groundnut products resulted in a toxic response that correlated well with that obtained by injection of pure aflatoxin B1 solutions at the same dose levels, and in most cases the chemical analysis was confirmed. The presence of aflatoxins G1, B2, and G2 had no apparent effect on the toxicity due to aflatoxin B1, at the levels at which they occurred in the particular samples tested. The separation of aflatoxin B1 from contaminated extracts by thin-layer chromatography, and its subsequent elution from the plates and injection into the eggs, confirmed that the toxicity of these extracts was due primarily to their aflatoxin B1 content.
8. MANAGEMENT OF ASPERGILLUS FLAVUS INFECTI ON AND AFLATOXIN CONTAMINATION OF GROUNDNUT

8.1 REVIEW

For management of A. flavus infection and aflatoxin contamination of groundnuts both preventative and curative procedures may be necessary. Aflatoxin management therefore starts in the farmer’s field, continues through crop produce handling, marketing, storage, and processing, and ends with the consumer.

In general, cultural practices and use of crop protection chemicals are preventative in nature as are provision of effective storage procedures and care during transportation and processing. Curative measures concentrate on isolation and segregation of toxic groundnuts and various systems of detoxification. Varietal resistance is a desirable component of any integrated aflatoxin management system.

8.1.1 Cultural Control

To be effective, cultural control of aflatoxin contamination of groundnuts must take into consideration all the varied environmental and agronomic factors that influence pod and seed infection by the aflatoxin-producing fungi, and aflatoxin production. These factors can vary considerably from one location to another, and between seasons in the same location. Some environments may be particularly favorable to fungal infection and subsequent aflatoxin contamination of groundnuts, and this may even raise the question as to whether or not the crop should be grown in such places. However, for most situations it should be possible to devise cultural practices that can greatly reduce or even eliminate aflatoxin contamination from the saleable crop produce. Aflatoxin contamination may occur preharvest, postharvest or during both periods. The extent to which the aflatoxin problem occurs in one or other of the two periods is largely influenced by weather conditions. Where drought occurs late in the growing season aflatoxin contamination may occur prior to lifting of the crop. Where rainfall is well distributed and late season drought does not occur, wet and humid conditions postharvest may lead to fungal infection and aflatoxin contamination.

Growing groundnuts continuously on the same land may lead to a build-up of high populations of A. flavus and/or A. parasiticus in the soil, which in turn increases the probability of seed infection and aflatoxin contamination (Joffe and Lisker 1970, Pettit and Taber 1968, Subrahmanyam and Rao 1974). Limited research has been done on effects of crop rotations on fungal infection and aflatoxin contamination in groundnuts (Pettit and Taber 1968, Joffe and Lisker 1970, Griffin et al., 1981). In hot and arid environments, populations of A. flavus may be very high, and crop rotations may have little influence on the fungal activity. Cropping systems in some regions involve varied cultivation and fertilizer practices that individually or taken together may affect survival or build-up of populations of the toxigenic fungi. Evidence is accumulating that groundnuts grown in different soil types may have significantly different levels of seed infection by A. flavus/A. parasiticus (Graham 1982, Mehan et al. unpublished data). Light sandy soils and Alfisols favor rapid proliferation of the toxigenic fungi, particularly under dry conditions. Vertisols have high water-holding capacity and this may be partly responsible for the lower than average levels of aflatoxin contamination in groundnuts grown on such heavy black soils.

Some research has been done on possible effects of calcium on preharvest aflatoxin contamination of groundnuts. Davidson et al. (1983) reported that application of gypsum to a soil in Georgia, USA, reduced aflatoxin contamination, but Cole et al. (1985) and Wilson et al. (1985) did not observe any such effects. The groundnut pod obtains its calcium requirements from the surrounding soil and this may be difficult under drought conditions. This indicates possible interrelationships of drought, available calcium, pod development and A. flavus infection.

Choice of groundnut cultivar can be important. A cultivar should fit a particular growing season and mature at the end of the rains so that postharvest field drying can be done under favorable conditions (McDonald 1969, Mehan 1987). It is undesirable that a variety should suffer from drought stress during pod maturation and some compromise may have to be effected between harvesting under dry conditions and avoidance of drought stress by using short-duration cultivars that mature before the rains have ended. Also, planting dates may be adjusted so that the crop matures at the end of the rainy season and post-harvest conditions favor rapid and effective drying of the crop. Care should be taken to avoid drought stress during late stages of pod maturation. Optimum plant populations should be established bearing in mind that too high a population may lead to severe drought stress where rainfall is suboptimal in a growing season (Graham 1982). Excessive weed growth may also deplete available soil moisture, and effective weed control by use of herbicides, or cultivations is advisable. Care should be taken during cultivations to avoid damage to pegs and pods (McDonald 1969). Cultivations and crop protection practices that lower the incidence of soil insects, mites and nematodes should help in reducing aflatoxin contamination (Sellschop 1965).
Irrigation to ensure adequate soil moisture during the last 4-6 weeks of crop growth should prevent preharvest aflatoxin contamination of groundnuts (Sanders et al. 1986, Wilson and Stansell 1983). This may be achieved by growing a completely irrigated crop or by applying supplementary irrigation to a basically rainfed crop.

Individual plants that die from attack by pests and diseases should be lifted separately as their produce is likely to contain aflatoxin (McDonald 1969). It is very important to harvest the crop at optimum maturity, as excessive numbers of over-mature or very immature pods at harvest can be reflected in high levels of aflatoxin in the produce (McDonald 1969).

Where groundnuts are already invaded by aflatoxigenic strains of A. flavus/A. parasiticus at time of harvest, there may be a serious build-up of aflatoxin reduced seed infection by A. flavus. Application of fungicides to freshly-lifted groundnut pods has been tried but with no definitive results in terms of their effects on subsequent fungal invasion and aflatoxin contamination of seeds (Jackson 1967a, b, Fonseca et al. 1976). However, Bell and Doupnik (1971, 1972) found that aflatoxin contamination of windrowed groundnuts could be substantially reduced by treatment with Manzatc, Benlate, or Botran. Madaan and Chohan (1978) reported prevention of A. flavus invasion of seeds by spraying freshly-lifted groundnut pods with propionic acid, sorbic acid, or chlorothalonil. No fungicide, combinations of fungicides, or other chemical treatments have been adopted for practical control of A. flavus infection and subsequent aflatoxin contamination of groundnuts in the field.

Biocides applied to the soil may have direct or indirect effects on the populations of the toxigenic fungi. For instance, they may affect other components of the soil microflora and fauna and stimulate biological control systems. Such interactions are likely to be complex and difficult to unravel. Similarly, addition of organic substances to soils, e.g., green-manure, crop residues, farmyard manure, is likely to have complex effects upon the soil microflora.

There are a number of reports (Ghewande and Nagaraj 1987, Bean et al. 1971, Bean and Rambo 1975, Premlata Singh and Sinha 1986) of the effects of various chemicals in preventing or inhibiting growth of A. flavus/A. parasiticus and aflatoxin production in vitro but their practical application is doubtful.

8.1.2 Chemical Control

Several attempts have been made to control or reduce A. flavus infection of pods and seeds by applying fungicides to soil, to groundnut foliage or to freshly-lifted groundnut pods (Jackson 1967a, b, Bell and Doupnik 1971, Pettit et al. 1971, Madaan and Chohan 1978).

Soil fumigation during land preparation has been tried to reduce populations of A. flavus developing around groundnut plants, but numbers of fungi on pod surfaces at maturity were not affected by use of vapam sodium-N-methylthio-carbamate or Vorlex or DD (1,2-dichloropropene) (Jackson 1967a). But Mixon et al. (1984) found that PCNB-fensulfothion or CGA 64250 reduced seed infection by A. flavus. Application of fungicides to freshly-lifted groundnut pods has been tried but with no definitive results in terms of their effects on subsequent fungal invasion and aflatoxin contamination of seeds (Jackson 1967a, b, Fonseca et al. 1976). However, Bell and Doupnik (1971, 1972) found that aflatoxin contamination of windrowed groundnuts could be substantially reduced by treatment with Manzatc, Benlate, or Botran. Madaan and Chohan (1978) reported prevention of A. flavus invasion of seeds by spraying freshly-lifted groundnut pods with propionic acid, sorbic acid, or chlorothalonil. No fungicide, combinations of fungicides, or other chemical treatments have been adopted for practical control of A. flavus infection and subsequent aflatoxin contamination of groundnuts in the field.

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8.1.3 Biological Control

Aspergillus flavus is frequently found associated with several other fungi in groundnut pods and seeds (Hanlin 1970). Based on such observations it has been hypothesised that interactions between fungi as they compete for the substrate might, under favorable environmental conditions, restrict invasion of groundnuts by A. flavus and/or contamination with aflatoxin. Microbial competition or microbial breakdown may be responsible for lower levels of aflatoxin in parasite-damaged pods than in seed from mechanically broken pods. It has been reported that several fungi can break down aflatoxin in groundnuts and in aflatoxin-containing liquid culture media; A. niger and R. solani appeared to limit the development of A. flavus and aflatoxin production in the substrate (Diener 1973). In Israel, Joffe (1969) observed that a large number of viable propagules of A. niger in the geocarposphere and moderate invasion of seeds by this species was associated with very limited invasion of seeds by A. flavus, Fusarium solani, and Penicillium spp. Frequent invasion of seeds by A. niger has been suggested to be important in preventing the development of A. flavus (Joffe 1969). Aspergillus niger has been shown to inhibit growth of A. flavus and aflatoxin production in vitro (Burnett et al. 1972). Presence of normal
endogeocarpic mycoflora, including A. niger and M. phaselogina. appears to inhibit invasion of groundnuts by A. flavus. This notion is supported by the studies of Lindsey (1970) who found that A. flavus penetrated and colonized a high percentage of shells of living, attached, immature and mature pods under gnotobiotic conditions.

**8.1.4 Genetic Resistance to Aspergillus flavus Invasion and Aflatoxin Production**

The aflatoxin problem could be solved if a groundnut cultivar could be identified or bred that was immune to seed infection by the aflatoxin-producing fungi or, once infected, did not support aflatoxin production. Initially the aflatoxin problem in groundnut was linked more to the postharvest period than to the period of pod development in the soil, and interest was focussed mainly on groundnut products such as meal and cake. Thus early work tended to concentrate on resistance to aflatoxin production.

An absolute evaluation of genotypic resistance to aflatoxin contamination can only be obtained by comparing the aflatoxin contents of seeds of the different genotypes. However, it is easier to screen for resistance to A. flavus seed infection and this may be used to provide an indication of possible resistance to aflatoxin contamination. But it should be borne in mind that not all strains have similar aflatoxin-producing ability, and that A. flavus strains may interact with host genotypes to influence rate of aflatoxin production.

**8.1.5 Resistance to Aflatoxin Production**

Rao and Tulpule (1967) first reported varietal resistance in groundnut to aflatoxin production. In laboratory inoculation tests they found that the cultivar US 26 (PI 246388) did not support aflatoxin production when seeds were colonized by aflatoxin-producing strains of A. flavus. This finding was not confirmed by other workers, but did stimulate research on possible varietal resistance to aflatoxin production (Doupnik 1969. Doupnik and Bell 1969, Nagarajan and Bhat 1973, Tulpule et al. 1977, Aujla et al. 1978, Kiran Kalia et al. 1988). To test cultivars for their ability to support aflatoxin production most researchers autoclaved the groundnut seeds before inoculation with aflatoxigenic strains of A. flavus and/or A. parasiticus. However, any reaction measured on autoclaved or otherwise killed seeds can have only limited relevance to genetic resistance. A laboratory method to screen live groundnuts for resistance to aflatoxin production was used at ICRISAT (Mehan and McDonald 1980) to test 502 genotypes. None was totally resistant to aflatoxin production but highly significant differences in aflatoxin production were found (Mehan et al. 1986). Recently, two wild Arachis species, A. cardenasi and A. duranensis, have been reported to support production of only trace levels of aflatoxins (Ghewande et al. 1989). These Arachis species were also found highly resistant in vitro seed colonization by A. flavus. It is important that these findings should be confirmed and wild Arachis species accessions tested should be fully specified. A limited search has been made for groundnut genotypes that do not support, or support only very low levels of aflatoxin production following seed infection by aflatoxigenic strains of A. flavus/A. parasiticus. In most cases aflatoxin production tests have been carried out using only one or two aflatoxin- producing isolates of A. flavus and/or A. parasiticus. As interactions may occur between genotypes and aflatoxin-producing fungal isolates, it is obviously important to use several highly aflatoxigenic strains of A. flavus and A. parasiticus to establish low aflatoxin production supporting ability of the groundnut genotypes. Promising germplasm should be made available to all interested laboratories for validation of resistances to aflatoxin production.

**8.1.6 Resistance to Aspergillus flavus Infection**

The existence of seed resistance to infection by A. flavus/A. parasiticus was a logical assumption considering that seeds with damaged testae are more easily and rapidly invaded by fungi than are seeds with intact testae, and colored testae confer greater resistance to invasion by A. flavus than do white or variegated testae (Carter 1973).

Mixon and Rogers (1973a) first suggested that use of groundnut cultivars resistant to seed invasion and colonization by the aflatoxin-producing fungi could be an effective means of preventing aflatoxin contamination. They developed a laboratory inoculation method for screening groundnut genotypes for resistance to A. flavus/A. parasiticus invasion and colonization of rehydrated, mature, sound, stored seeds. Essentially, tests are carried out on sound, mature seeds from undamaged, hand-shelled pods that have been dried and stored for at least one month. Seeds are surface-sterilized, hydrated and then inoculated with a conidial suspension of an aflatoxigenic strain of A. flavus or A. parasiticus.

Mixon and Rogers (1973a) reported that two Valencia type genotypes, PI 337394F and PI 337409, had high levels of resistance to in vitro seed colonization by A. flavus and A. parasiticus. Six more breeding lines (GFA 1, GFA 2, AR 1, AR 2, AR 3, and AR 4) were later reported resistant (Mixon 1986). Other workers have used Mixon and Rogers’ method, or modifications of it, in screening genotypes for resistance to seed colonization by aflatoxin-producing strains of A. flavus and A. parasiticus (LaPrade 1973, Bartz et al. 1978, Zambettakis et al. 1981, Mehan and McDonald 1980, Tsai and Yeh 1985, Pua and Medalla 1986). The methods have on the whole given comparable
Resistant to A. flavus invasion and colonization of rehydrated, stored, dried seeds has relevance when aflatoxin contamination is largely postharvest. But significant invasion of undamaged groundnut pods by A. flavus and subsequent aflatoxin contamination can occur before harvest (Davidson et al. 1983, Mehan et al. 1986). In recent years there has been considerable research into possible genetic resistance in groundnuts to seed infection and aflatoxin contamination in the field (Davidson et al. 1983, Mehan and McDonald 1984a, b, Kisyombe et al. 1985, Mehan et al. 1986). In the first place, it was important to establish the relevance of in vitro "seed colonization" resistance to A. flavus invasion of developing pods in the field. A few studies (Mixon and Rogers 1973a, Mixon 1983a. b. Mixon 1986) indicated that the genotypes. PI 337394F, PI 337409, GAF 1 and GFA 2, resistant to in vitro seed colonization by A. flavus (IVSCAF-resistant), showed considerably lower levels of natural seed infection with A. flavus and of aflatoxin contamination than the susceptible (IVSCAF-susceptible) genotypes Florunner and PI 331326. In these studies, observations on natural seed infection were made primarily to determine the "initial" levels of A. flavus A. parasiticus infection that could possibly interfere with the laboratory seed inoculation tests for resistance to seed colonization. The natural seed infection was estimated from occurrence of sporulating colonies of A. flavus on rehydrated, nontreated (no inoculation) seeds.

Davidson et al. (1983) found no significant differences at harvest in A. flavus infection or in aflatoxin contamination of seed between the cultivars Sunbelt Runner and Florunner, reported to be resistant and moderately susceptible respectively to in vitro seed colonization by A. flavus and A. parasiticus. Blankenship et al. (1985) reported that four genotypes (A 72118 (GFA 1), A 7404 (AR 3). UF 77316 and UF 791041) resistant to seed colonization, and the cultivar Florunner, grown under late season drought stress, were all highly susceptible to aflatoxin contamination. Other workers (Zambettakis et al. 1977, Zambettakis et al. 1981, Mehan et al. 1987) have reported several IVSCAF-resistant genotypes to be field resistant to A. Jlavus seed infection. Of 37 IVSCAF-resistant genotypes, only 10 (PI 337394F, PI 337409, UF 71513, Ah 7223, J 11, Var. 27, 55-437, 73-30, Monir 240-30. and RMP 12) have been tested for resistance to seed infection by A. Jlavus in the field (Zambettakis et al. 1981. Kisyombe et al. 1985, Mehan et al. 1987), and only three of them (PI 337409, PI 337394F, and J 11) have been evaluated in more than one location. Cultivar J 11 has been found resistant to seed infection in multilocational field trials in India and in the USA (Mehan et al. 1987, Kisyombe et al. 1985); PI 337409 showed resistance in Senegal (Zambettakis et al. 1981) but was susceptible in the USA (Kisyombe et al. 1985).

It should not be assumed that all IVSCAF-resistant genotypes will have resistance to seed infection in the field, or all IVSCAF-susceptible genotypes will show susceptibility to field infection by A. flavus A. parasiticus (Mehan et al. 1987). The IVSCAF-resistant genotypes Var. 27, Monir 240-30. and RMP 12 have shown susceptibility to A. Jlavus seed infection in the field while some IVSCAF-susceptible genotypes such as Lampang and Exotic 6 have been found to have low levels of seed infection in the field (Mehan et al. 1987, Kisyombe et al. 1985).

Certain conditions should be met when screening groundnuts for resistance to seed infection by the aflatoxigenic fungi and/or aflatoxin production under field conditions. First, only undamaged pods should be analyzed as any kind of damage is likely to over-ride resistances. Second, environmental conditions such as temperature and moisture stress during pod maturation are important since A. Jlavus is a weak pathogen and its ability to invade undamaged pods and seeds is strongly influenced by environmental conditions. Third, the soil should contain aflatoxin-producing strains of A. flavus and A. parasiticus since some strains of these fungi may not be capable of producing aflatoxin. Therefore, to ensure uniform, high levels of seed infection and aflatoxin contamination, screening trials should be sited on a light sandy soil with high populations of A. flavus A. parasiticus. A test site where late season drought stress is of common occurrence would be most effective. Otherwise, the screening might have to be carried out on early or late sown crops or on irrigated dry season groundnut crops where control of soil moisture during late stages of pod development can be assured.

8.1.7 Mechanisms of Resistance to Aspergillus flavus Colonization and Infection

In all reported cases of resistance to seed colonization by the aflatoxigenic fungi the protective role of the seed testa has been emphasized (Dieckert and Dieckert 1977, LaPrade et al. 1973, Mehan et al. 1983, Mixon and Rogers 1975, Zambettakis et al. 1977). Resistance depends upon the testa being complete and undamaged. Most research points to the testa resistance being physical, and it has
been correlated with thickness, density of ‘pallisade cell’ layers, absence of fissures and cavities, etc. (LaPrade et al. 1973, LaPrade and Bartz, 1972, Zambettakis 1975, Zambettakis and Bockele-Morvan, 1976). Permeability could also be an important factor as presence of wax layers on the testa of A. flavus-resistant genotypes has been noted (Camara 1977; LaPrade 1973). Fungistatic phenolic compounds have been found in testae and may have a role in resistance. Some studies (Amaya et al. 1977; Amaya et al. 1980) have shown that testae of IVSCAF-resistant genotypes contained significantly lower levels of certain amino and carbohydrate compounds than testae of IVSCAF-susceptible genotypes. Resistance could result from combinations of physical, chemical and biological factors operating in the testa rather than being due to a specific mechanism.

The groundnut shell has logically been considered a barrier to penetration by A. flavus, as seeds from pods with damaged shells are more frequently contaminated with aflatoxin than those from undamaged pods (McDonald and Harkness 1967). The resistance of the groundnut fruit to A. flavus invasion appears to be associated with certain structural and biochemical characters of the pod and seed, and there is a possibility that genotypes may have differential effects upon the populations of A. flavus in the geocarposphere (Nahdi 1989).

8.1.8 Resistance Breeding

Breeding for resistance has relied on information obtained in the assessment of A. flavus infection in harvested, mature, dried seeds, as the laboratory screening test can readily be used to compare progenies for resistance (Mixon 1979, 1981, Rao et al. 1989). Several breeding lines with resistance to A. flavus colonization of seeds comparable to that of the resistance sources and with greater yield potential have been bred (Mixon 1986, Rao et al. 1989). However, when dealing specifically with resistance to natural seed infection in the field, resistance mechanisms may operate at the pod surface, within the shell, at the seed surface and within the testa/cotyledons. This makes it difficult for breeders to specify particular resistance traits to aim for. There appear to be different genes in conferring resistance to seed colonization, preharvest seed infection, and aflatoxin production by aflatoxin-producing fungi (Utomo et al. 1990).

Use of resistant varieties should be considered as part of an integrated aflatoxin management program incorporating cultural and crop handling procedures appropriate to different agroecological situations. Cultivars resistant to fungus invasion in the soil would be particularly desirable for semi-arid regions where preharvest aflatoxin contamination is a serious problem. The existence of significant resistance in the commercial cultivars J 11 and 55-437 could be immediately useful in minimizing aflatoxin contamination in some environments.

Resistance to A. flavus infection is also important for maintaining seed quality for planting as the fungus causes seed rots and aflaroot seedling disease.

8.1.9 Segregation and Decontamination

Groundnuts intended for human consumption may contain levels of aflatoxin in excess of the acceptable limit. But, in a toxic sample not all seeds are contaminated, and in many cases toxicity resides in a very small number of toxic seeds (Dickens 1977). Provided that seeds on which aflatoxigenic fungi have grown can be distinguished from apparently healthy seeds, it should be possible to recognize and remove the toxic seeds. This is the basis for procedures that have been developed and utilized in the groundnut industry in several countries (Dickens 1977, 1983, Tiemstra 1977, Read 1989). The methods are based on such characteristics as color and kernel size and can be applied to raw kernels or to cotyledons after blanching. Separation of toxic seeds may be done by hand-picking or by the use of electronic color-sorting equipment (Tiemstra 1977). Using these methods it is possible to significantly reduce aflatoxin levels in contaminated lots of groundnuts (Tiemstra 1977).

Sorting of raw kernels by visual examination may not be effective when healthy-appearing kernels have concealed damage (i.e., mold growth between cotyledons but no mold damage evident on the seed surface) (Dickens and Whitaker 1975). This does not apply when scanning blanched kernels and emphasizes the need for such examinations at this later stage in processing. The most effective way to remove off-color, suspect kernels is by means of electronic color sorting. Kernels that differ substantially in color (i.e., are darker, or lighter, or molded) from the standard for the particular cultivar or cultivars being examined, should be rejected. Blanching followed by photoelectric color sorting and hand-picking effectively reduces aflatoxin contamination. Tiemstra (1977) has discussed in detail the use of electronic color sorters in removing contaminated kernels from blanched groundnuts. In processes where sorting is done after roasting and blanching, the darker and nonblanched kernels may also be removed. One of the best methods of detecting suspect kernels is by partially drying them to about 2 to 3% moisture content, and then blanching (Tiemstra 1977). The cotyledons of contaminated kernels will darken perceptibly whereas sound cotyledons remain white. Sorting at this stage is more effective than after roasting since the roasting process can produce a range of color in sound kernels that obscures the off-colors of suspect kernels. Unfortunately, groundnuts treated in this way have very limited shelf life and should be used immediately.

In the USA, segregation of aflatoxin-contaminated groundnuts has been very successful (Tiemstra 1977, Dickens 1983). Contaminated lots are diverted for oil extraction and non-food uses (Dickens 1983). While such an approach can
be recommended for developed countries such as the USA and Australia, safeguards need to be established if segregation of contaminated groundnut lots is to be used in less developed countries where the reject groundnuts could find their way into local markets.

It is appreciated that while the primary need is to reduce aflatoxin levels by removal of toxic kernels, methods for inspection, separation and diversion should be efficient so that only contaminated kernels are removed and there is no wastage of non-contaminated kernels.

The potential exists to improve electronic sorting equipment. Polarized laser beams may be used to detect roughening and shrivelling of seed surfaces due to mold damage (Pettit and Chan 1980). It may be possible to detect hidden mold damage by measuring dielectric properties of groundnut seeds (Pettit and Geiger 1981).

### 8.1.10 Chemical Detoxification

Where removal and segregation of toxic kernels cannot be effectively carried out, or is only partially successful, there still remains the possibility of inactivating or destroying the aflatoxins in groundnuts or groundnut products by chemical and/or physical treatments. Chemical treatments, normally referred to as detoxification, should be technically and economically viable, and should meet the criteria listed by the FAO/WHO/UNEP Conference on Mycotoxins held in Nairobi, Kenya (1977). These criteria are that the process (a) destroys or inactivates the toxin, (b) does not produce or leave toxic or carcinogenic residues in the finished product, (c) destroys fungal spores and mycelia that could later proliferate and produce the toxin, (d) preserves the nutritive value and acceptability of the product, and (e) does not significantly alter important technological properties of the product. Similar requirements have been established by the U.S. Food and Drug Administration (FDA); however, the FDA requires additional data on the impact of the process on the environment (Park et al. 1988). The International Union of Pure and Applied Chemistry Food Chemistry Commission has developed guidelines for safety evaluation of aflatoxin-contaminated feed (Jemmali 1982).

The polar solvents used in the solvent extraction methods for processing groundnuts remove most aflatoxins from the extracted oil. However, in the case of small-scale village level processing some aflatoxin may be left in the crude oil. Further processing of oil using alkali treatments (i.e., alkali refining) and filtration can largely remove this residual toxicity from the oil (Parker and Melnick 1966). Aflatoxin in oil may also be significantly reduced by exposing the contaminated oil to bright sunlight (Shantha and Sreenivasamurthy 1975, Shantha 1989). In some rural parts of India, unrefined, locally-expressed groundnut oil is preferred to refined oil because of its lower cost and preferred taste (Shantha and Sreenivasamurthy 1975). Extraction of such crude oil with 10% aqueous sodium chloride (1:4) at 80°C for 30 minutes removes almost 85% of contaminating aflatoxin (Shantha and Sreenivasamurthy 1975). It is generally agreed that most of the aflatoxin present in groundnuts remains in the cake after the oil is removed, contamination levels in the cake being almost double the levels in the raw kernels. There have been reports that the high temperatures that are reached during mechanical crushing of groundnuts might destroy some of the toxin, but considerable quantities are left in the cake.

Various chemicals have been tested for destruction of aflatoxins in groundnut cake and groundnut protein isolates: methoxymethane (Aibara and Yano 1977), formaldehyde (Codifer et al. 1976. Mann et al. 1969, Ochomogo 1979), calcium hydroxide (Codifer et al. 1976, Giddey et al. 1977), ethylene oxide (Degesch 1978), sodium hydroxide (Dollear et al. 1968, Mann et al. 1969), methylamine (Dollear et al. 1968. Mann et al. 1969), chlorine (Sreenivasamurthy et al. 1967), sodium chloride (Farah et al. 1983), sodium hypochlorite (Natarajan et al. 1975, Ochomogo 1979, Rhee et al. 1977), isopropyl alcohol (Rayner and Dollear 1968, Ochomogo 1979), hydrogen peroxide (Srinivasamurthy et al. 1967, Rhee et al. 1977), and ammonia (Viroben et al. 1983. Coker et al. 1985a, Coker 1989, Conkerton et al. 1980). The most promising chemical detoxification procedure is that using ammonia; this was first demonstrated by Masri et al. (1969). and Gardener et al. (1971) reported on its development and application on a pilot plant scale. Several patented ammonification techniques are undergoing commercial tests (Coker 1989, Coker et al. 1985). Three pilot plant scale processes for ammoniation of groundnut cake and meal have been developed: the Lesieur process (Lesieur 1977), the Extechnik process (Extraktiotechnik) and the TDRI process (Coker et al. 1985). The Lesieur process was in operation in Senegal for some time but it has now been superseded by an ammonia/formaldehyde process (Coker et al. 1985). A pilot plant using the Extechnik process has been installed in the Sudan (Coker et al. 1985). The African Groundnut Council and its Member States actively support aflatoxin detoxification programs (FAO 1985). France, Ireland, The Netherlands, and Germany import ammonia-treated groundnut meal for animal feeds (Park et al. 1988). The TDRI process uses ammonia gas at high temperature but moderate pressure and a pilot plant has given satisfactory performance, reducing aflatoxin levels in groundnut cake by > 95%. Some loss of cystine and lysine occurs during the ammoniation process (Coker et al. 1982).

Treatment with ammonia produces complex reactions and only a small percentage of reaction products have been fully investigated (Cucullu et al. 1976).
Nutritional and toxicological evaluation of the ammoniated groundnut cake produced by these ammoniation processes has been carried out on a number of animal species (Viroben et al. 1978, DeLort-Laval et al. 1980, Frayssinet et al. 1976, Viroben et al. 1983). International acceptance of the ammoniation process will be delayed until safety of the treated products can be guaranteed (Coker et al. 1985a). Park et al. (1988) have presented a comprehensive review of the ammonia detoxification process and outlined current applications and regulatory status of the ammonia process for reducing aflatoxin levels in animal feeds.

**8.1.11 Microbial Detoxification**

Many microorganisms, including fungi, actinomycetes, bacteria and algae, have been tested for ability to destroy or transform aflatoxin (Ciegler et al. 1966, Lillegard et al. 1967a, 1971, Mann and Rehm 1976, Cole and Kirksey 1971, Nout 1989). A bacterium, Flavobacterium aurantiacum, has been found to remove aflatoxin from solutions and preparations of peanut milk (Ciegler et al. 1966, Hao and Brackett 1988, Hao et al. 1989). But there could be problems in adopting this technology for commercial detoxification of groundnuts and groundnut products.

**8.1.12 Physical Detoxification**

Aflatoxins are stable up to their melting point of around 250° C (Feuell 1966). While dry heating has not been particularly effective, heating moist meal or autoclaving groundnuts has been found to reduce aflatoxin content (Coomes et al. 1966). Not all aflatoxins react equally to heating, for example aflatoxin B₁ is heat stable, but aflatoxin G₁ can be destroyed by heat (Reegner 1967). Prolonged heating may adversely affect the quality of the protein or availability of lysine (Woodham and Dawson 1966). Roasting has been reported in some cases to reduce aflatoxin levels, but in no case has total destruction been achieved (Lee et al. 1968, Coker 1989).

Neither ultraviolet light nor gamma irradiation reduces aflatoxin levels in groundnut meal (Feuell 1966, Shantha 1989). However, several studies have shown that sunlight can be very effective in destruction of aflatoxin in oil (Shantha and Sreenivasamurthy 1975, 1977, Shantha 1989). Aflatoxin-contaminated unrefined groundnut oil in glass containers was subjected to direct sunlight (approximately 50,000 lux) and within one hour the aflatoxin was almost completely destroyed (Shantha and Sreenivasamurthy 1980, Shantha 1989). The safety and shelf life of the sunlight-exposed oil have been confirmed (Shantha 1989). Large-scale trials have not been done. The kind of container used could be critical as the sunlight has to penetrate to and within the oil. Sunlight has not proved to be effective for destruction of aflatoxin in contaminated groundnut seeds and cake (Shantha and Sreenivasamurthy 1981), as the toxin is bound to the protein molecules in these substrates and there are obvious problems of light penetration.

Aflatoxin in crude groundnut oil remains in finely suspended form and can easily be separated by filtration. Special filter pads have been developed by Basappa and Sreenivasamurthy (1979) which can easily be adopted in oil mills to remove aflatoxin from crude oil. This appears to be a simple approach to the problem of aflatoxin in unrefined groundnut oil.

Several types of clays can bind aflatoxin from oil (Miller et al. 1985, Pettit et al. 1990). As a follow up to this, hydrated sodium calcium aluminosilicate (HSCAS) was added to aflatoxin contaminated chicken feed and this reduced the toxicity of the feed to poultry (Pettit et al. 1990).

**8.1.13 Control in Storage/Transit**

Storage of groundnuts under clean, dry conditions with low kernel moisture content (about 8%) and at low temperature, and with protection from insect infestation should avoid molding of groundnuts and consequent risk of aflatoxin contamination (Dickens 1977). However, trade groundnuts are often transported and stored in open containers and permeable bags and so are at risk. Various ways in which groundnuts may be wetted and absorb moisture have been highlighted. These include direct wetting by rainfall and by leakage through covers, and through seepage of ground water and rising damp, and by direct absorption of moisture from humid atmosphere, this being particularly important when the temperature of stored material falls below ambient temperature (Dickens 1977. Smith 1989). Moisture may also be generated in stored groundnuts from respiration of pests (insects and rodents). Any measure that reduces the risk of such rewetting of groundnuts will be effective in reducing risk of aflatoxin contamination.

As considerable quantities of groundnuts are shipped from tropical areas to temperate zones there does exist a problem of condensation in ship holds. Use of sealed containers could reduce this problem. The problem of mold damage and aflatoxin contamination can be further minimized by improving facilities for storage at port and transit points as well as on ships (Bhat 1988).
8.2 BIBLIOGRAPHY

8.2.1 MANAGEMENT OF ASPERGILLUS FLAVUS INFECTION AND AFLATOXIN CONTAMINATION OF GROUNDNUT


Research in Nigeria from 1948 to 1982 has identified the factors that determine quality in Nigerian groundnuts. These include, physical state of groundnuts, their moisture content, and storage conditions with emphasis on temperature, humidity, and infestations by pests, rodents and mold fungi. Procedures for ensuring purchase of only good quality produce and measures for prevention of damage to the groundnuts during storage are reviewed with particular emphasis on control of pests. The problem of aflatoxin contamination of groundnuts is discussed. Recommendations to growers, and those concerned with storage and transport of groundnuts on methods of preventing aflatoxin contamination are summarized.


The role of groundnut growers, shellers and manufacturers of consumer groundnut products, together with USDA and FDA, played in forming a joint task force to develop a program of research into incidence, causes and control of aflatoxin contamination of groundnuts and groundnut products is discussed.


This paper discusses several physical methods for detoxification of aflatoxin-contaminated groundnuts, groundnut oil, maize, cottonseed, and rice. Removing discolored seeds by handpicking or by electronic color sorting devices is efficient for reducing aflatoxin content in groundnuts, maize and cottonseed. The possibility of using air classification to separate aflatoxin-contaminated groundnuts and cottonseed has been explored and found to be a useful tool. Milling of contaminated brown rice has been found to reduce aflatoxin in the polished rice. Roasting, frying, boiling, baking and cooking of contaminated groundnuts have been found to inactivate aflatoxin to a certain extent. Detoxification of groundnut oil by exposure to bright sunlight has proved useful. Decontamination of groundnut oil is also possible by the use of filterpads in the processing line of oil mills. Washing the oil with sodium chloride solution is another promising method for removal of aflatoxin. These detoxification/decontamination procedures are discussed with special reference to their economic and technical feasibility.


In this review the occurrence of aflatoxin in food crops and the magnitude of the problem, aflatoxin production (by Aspergillus flavus and A. parasiticus) under different conditions with special reference to pre-harvest contamination and the management of aflatoxin contamination are discussed.


This paper gives a brief background to the mycotoxin problems in major food crops including maize and groundnut. Problems in implementing prevention and control of mycotoxin contamination with special emphasis on aflatoxins are outlined. Several measures for prevention are discussed, including use of appropriate cultural practices, e.g., irrigation, crop drying, storage, chemical control, and use of resistant cultivars. Need for education and extension activities is emphasized. Need for regulatory programs to ensure quality of produce for export and for local consumption is stressed. Surveillance studies are needed for all regions.


This report on aflatoxin control in groundnut production and processing reviews available information on: (1) preventive measures; (2) contamination of groundnut oil and press-cake; and (3) detoxification methods. Basically, there are two possible solutions of the aflatoxin problem, viz. prevention of invasion of groundnuts by the toxigenic fungus Aspergillus flavus, and elimination of aflatoxins from the contaminated groundnuts.
Possibilities for reducing the aflatoxin contamination of groundnuts include the selection of varieties resistant to Aspergillus flavus.

Specific recommendations are made as to which aflatoxin control measures developing countries could introduce for producing groundnuts with an acceptable low aflatoxin content. Tanzania was used as the reference country for a study of the relevant agricultural conditions. Topics discussed included the production and handling of groundnuts, drying and storage, control, quality regulations and processing, preservation of groundnuts and use of insecticides and fumigation, detoxification, and economic aspects.

This is a review of work done at the Punjab Agricultural University on Aspergillus flavus infection of groundnut seeds and seedlings. Issues discussed include the physiology and pathology of aflatoxin production by A. flavus, a bioassay technique for detection of aflatoxin-producing strains of the fungus, varietal response for aflatoxin production, field control of A. flavus infection and postharvest technology for aflatoxin control.

This paper briefly discusses various aspects of the groundnut aflatoxin problem. The aspects discussed include, susceptibility of major food crops to aflatoxin contamination, the acute and chronic effects of aflatoxins, the natural occurrence of aflatoxicosis, and the metabolic fate of the anatoxins. Various approaches to the reduction of aflatoxin contamination are outlined, including appropriate cultural practices, chemical control, sampling and analysis together with detoxification procedures. Areas of research which need to be investigated in the future are briefly discussed.

In Nigeria groundnut is sold as unsorted kernels, groundnut cake, oil, hand picked selected (HPS) kernels, and groundnut flour. All these products may contain aflatoxin. Interdisciplinary committee meeting on behalf of the Ministries of Natural Resources of the six northern states of Nigeria recommended to growers, and to those concerned with purchase, storage, transport and processing of groundnuts various procedures for reducing aflatoxin contamination.

The African Groundnut Council (AGC) aimed at promoting production, consumption and international trade of groundnuts in its six member states (The Gambia, Mali, Niger, Nigeria, Senegal and Sudan) operates through its 3 bodies (a council of ministers, a council of representatives and a permanent executive secretariat), and had several installations in member states either wholly or partially financed by this organization. Several research projects including one on aflatoxins and another a regional Variety Trial are executed in collaboration with each member state and with the technical/financial assistance of national and international institutions. The AGC organizes various forms of training for scientists, conducts conferences, publishes on-going research and statistical data, and standardizes the price and sales policies in member states.
This paper discusses causes of aflatoxin contamination and methods of prevention due to infection by the fungus Aspergillus flavus as influenced by environmental conditions and postharvest treatments. Control measures recommended in Senegal include: elimination of plants prematurely dried in the field before harvesting; rapid drying of the lilted plants, first in small heaps or windrows and then in stacks; breaking-up of stacks if these become wetted by late rains; reduction of toxicity of the product by elimination of all perforated pods and all discolored kernels.

Approaches to solution of the aflatoxin problem in groundnut are discussed. None of the approaches is perfect. A progressive aflatoxin control program by all segments of the groundnut industry is necessary to achieve a final solution to the aflatoxin problem.

The control of Aspergillus flavus infection of groundnuts and contamination with aflatoxin is discussed in relation to seed infection, detection of infection/contamination and divergence of contaminated groundnuts to non-food uses. Sampling and aflatoxin analysis protocols for use at various stages in groundnut production and processing are described.

Under provisions of a USDA Marketing Agreement, an aflatoxin control programme for groundnuts produced in the United States is administered by the Peanut Administrative Committee composed of peanut (groundnut) growers and sellers. Regulations of this committee contain provisions about the quality of groundnuts acquired from farmers, storage of unshelled groundnuts, aflatoxin testing, quality and disposition of processed lots, and indemnification of handlers for losses caused by lots which contain over 25 μg Kg⁻¹ aflatoxin. Effects of the control programme on aflatoxin concentration in peanut products are discussed.

Many years of study have shown that preharvest aflatoxin contamination of groundnuts is preventable when groundnuts arc provided with adequate moisture (through rainfall or irrigation) during the pod maturation period. However, since all groundnuts cannot be grown under such conditions, other strategies to prevent contamination arc being investigated. Two of those discussed include: (1) prevention of aflatoxigenic fungal invasion by development of groundnut varieties resistant to invasion and use of biocompetitive agents to exclude aflatoxin-producing fungi from the soil; and (2) drought-tolerant varieties, enhancement of phytoalexin-based as well as other natural defense mechanisms, and incorporation of resistance characteristics through genetic manipulation.

Ten 50 lb pneumatic samples from 17 loads of farmers stock groundnuts were collected during the 1987 harvest season in Georgia. Grade samples were run over a 24/64 belt screen. Loose- shelled kernels (LSK) and small pods that fell through the belt screen were separated, pods were shelled, and the kernels were screened over a 16/64 and 14/64 slotted screen. Pods that rode the 24/64 belt screen were similarly shelled and screened, and in addition, the damaged kernels were removed. The resulting components were analyzed separately for aflatoxin by high performance liquid chromatography (HPLC). From the grade samples, the LSK, other kernels (OK), and damaged kernels were combined and the sound mature kernels (SMK) and sound splits (SS) were combined. These two grade components were analyzed separately for aflatoxin by HPLC. Results showed the distribution of aflatoxin within each component and provided variance data to determine sample sizes needed to detect specified levels of aflatoxin in each respective component. Results also provided additional information needed for finalizing the design of a pilot study to determine the performance of the belt screen separator in removing
This paper reviews the problem of aflatoxin contamination in groundnut and other agricultural commodities and discusses preventive and control measures. Prevention is the first and best approach and preventive measures should be taken at all stages of cultivation, harvest, transportation, storage and processing. Physical separation of contaminated seed has proved feasible in some cases. Effective removal of aflatoxins by extraction with polar solvents has been accomplished. Several chemicals such as ammonia, methylamine, sodium hydroxide, hydrogen peroxide and ozone have been used with success to substantially reduce, inactivate or destroy aflatoxins in contaminated oilseed meals, but some reduction in protein quality occurs during most of the treatments.


This paper briefly discusses various aspects of the groundnut aflatoxin problem. The aspects discussed include, analytical methods and chemistry of aflatoxins, the acute and chronic effects of aflatoxins, and the natural occurrence of aflatoxicosis. Various approaches to the prevention/reduction of aflatoxin contamination of agricultural commodities are outlined, including cultural and crop handling practices, inactivation and detoxification procedures.


Approaches to solution of the aflatoxin problem in groundnut are discussed. Prevention of aflatoxin contamination is the best approach and preventive measures should be taken at all stages of cultivation, harvest, transportation, storage and processing. If preventive measures have failed or are not used, aflatoxin-contaminated groundnuts can still be salvaged by mechanical removal of contaminated seed, by extraction with polar solvents, or by destruction of aflatoxins with appropriate chemicals. Heat is relatively ineffective for destruction of aflatoxin although normal roasting, as of groundnuts for the preparation of peanut butter, results in considerable reduction in aflatoxin content. Treatment with Flavobacterium aurantiacum removes aflatoxin and may be useful for beverages. Oxidizing agents readily destroy aflatoxin, and treatment with hydrogen peroxide may be useful. Aflatoxins that may be present in crude oil are effectively removed in conventional refining operations. Treatment of defatted oilseed meals with ammonia can reduce aflatoxin content to very low or nondetectable levels with only slight reduction in protein quality.


To the extent feasible, the best approach to contain aflatoxin contamination is prevention. Good farm management practice is essential. This includes use of sound, fungus-free, viable seed, proper fertilization, adequate control of insects and diseases, and harvesting at maturity with equipment properly adjusted and operated to avoid damaging the crop and picking up excessive leaves, trash, and dirt. Special attention should be given to diverting aflatoxin-containing lots from food and feed channels as early as possible in the marketing chain to avoid contamination of uncontaminated lots. Provision of clean, dry, adequately cooled and ventilated storage is important, and good sanitation is essential to minimize mold contamination during storage and processing. Genetic approaches which result in resistance to elaboration of aflatoxins and use of preservatives or mold inhibitors may be helpful in specific situations. When prevention fails, removal or destruction by physical, chemical, or other means must be considered. Removal of contaminated seeds or kernels may be achieved by hand or electronic sorting as is done with groundnuts or pneumatic sorting of Brazil nuts. Aflatoxin may be removed during processing, as in alkali refining of crude oils obtained from contaminated oilseeds, or by extraction of contaminated oilseed meals with polar organic solvents. Cells of Flavobacterium aurantiacum have been reported to completely remove aflatoxin in liquid media, including milk. A host of chemicals, especially oxidizing and reducing agents and acids and bases, have been screened as reagents for destroying aflatoxin in contaminated feeds. Several processes have been patented.


The best approach to contain aflatoxin contamination is prevention. Good farm management practices are essential. This includes use of sound, fungus-free, viable seed, proper fertilization, control of insects and diseases, prevention of lodging, and harvesting practices that avoid damaging the crop and picking up excessive leaves, trash, and dirt. Special attention should be given to diverting aflatoxin-containing lots from food and feed channels as early as possible in the marketing chain. Clean, dry, adequately cooled and ventilated storage and good sanitation are essential to minimize mold contamination. Genetic approaches and use of mold inhibitors may be helpful. When prevention fails, hand or electronic sorting can remove contaminated seeds. Aflatoxin may be removed during processing by alkali refining of crude oils or by extraction of contaminated oilseed meals with polar organic solvents. Some aflatoxin is destroyed or degraded during normal preparation of some foods. Many chemicals, especially oxidizing and reducing agents and acids and bases, have been screened as reagents for destroying aflatoxin in contaminated feeds. Several processes have been patented. In the USA aflatoxin in copra has been destroyed on a commercial scale by hot aqueous calcium hydroxide and in cottonseed meal by treatment with ammonia under pressure.


In the United States, the effort to prevent the entry of mycotoxins into foodstuffs is a cooperative one between industry and government. This effort is concentrated mainly on preventing aflatoxin-contaminated lots of groundnuts from entering food marketing channels. The US groundnut industry has significantly contributed to this objective by the adoption of a code of good practices for purchasing, handling, storage, and processing of groundnuts. Strict limits are placed on the grades and qualities of farmers' stocks of unshelled groundnuts that can go into food channels, and even stricter limits on shelled groundnuts that can be sold for edible use. These classifications or "segregations" are principally based on the percentage of damaged kernels (Aspergillus flavus-infested kernels) in the loads of groundnuts as delivered by farmers and in the outturn from the shelling plants as determined by the official inspection service.


The problem of aflatoxin contamination, which was serious in the Queensland groundnut industry in drought years, is reviewed with special reference to factors influencing aflatoxin formation including moisture stress, insect damage, and cultivar resistance. Control measures included crop hygiene, resistant varieties, avoiding moisture stress, harvesting the crop at optimum maturity, avoiding pest and mechanical injury, drying rapidly and segregating contaminated batches of groundnuts.


Groundnut is an important crop in the Sudan, both in rainfed areas where end-of-season droughts are common, and under irrigation in the Gezira. Quality factors are important in determining acceptability and market price. Aflatoxin
contamination is a problem in the rainfed crop. Aflatoxin levels are important in determining quality grade and price. The groundnut marketing system is described. A detoxification plant is under construction and will be used to detoxify groundnut cake and meal.


Cultivation, harvesting, threshing, sorting, storage and transportation of groundnuts are briefly discussed. Problems due to contamination of groundnuts with aflatoxins are considered, with reference to aflatoxin concentrations in groundnuts from various countries. Methods for manual sorting of groundnuts to eliminate broken and discolored kernels (which have a relatively high incidence of contamination with aflatoxins) are briefly discussed, together with sampling plans. An electronic device for sorting of groundnuts on the basis of their color relative to standard color sheets is described. Use of this device permits a 90 % reduction in aflatoxin content of the batch. 8-18 % of kernels are rejected; approximately 85 % of these are in fact free from aflatoxins.


The results of aflatoxin assays of foodstuffs including groundnuts are presented. Conditions for controlling aflatoxins in stored products and for preventing mold growth and aflatoxin production are discussed. Storage conditions for certain raw materials are outlined.


Problems adherent to the cultivation and storage of food crops in tropical Africa are reviewed in relation to the development of mycotoxins in the stored produce due to fungal activity. Attention is paid in particular to the production and storage of groundnuts, and to the studies conducted in northern Nigeria over the period 1961-64 on contamination of groundnut pods and kernels by Aspergillus flavus causing the occurrence of aflatoxin. Recommendations given to reduce the incidence of aflatoxin in groundnuts in that region mainly comprise early harvesting, avoidance of damaging the pods during harvest, removal of damaged or molded pods and kernels, rapid and effective drying of produce, and storage under adequately dry conditions.


Methods are recommended for the harvesting, curing, drying, transport and storage in warehouses or markets of groundnuts to minimize the development of aflatoxin by growth of the mold Aspergillus flavus.


Methods are recommended for the control of aflatoxin during the buying of groundnuts, their storage, and their processing to oilcake or edible flours, and during the treatment of raw groundnut oil prior to edible use. Guidelines are given for selection of groundnut kernels/pods as regards chemical characteristics for food and feed categories.


To prevent the losses resulting from the presence of mycotoxins in poultry feed the EEC has introduced regulations limiting the level of aflatoxin in poultry feeds, and has set maximum levels for aflatoxin in six major raw materials used in animal feed production: babassu, copra, cottonseed, groundnut, maize, and palm kernels. However, the sampling, sample preparation and analytical methodologies required for the monitoring of aflatoxin in these commodities are not available at present, and further work is required to ensure that the heterogeneous distribution of aflatoxin in a commodity is taken into account when new quality control procedures are developed. At present no limits have been set for the other mycotoxins known to produce adverse effects in poultry. Care must be taken by animal feeds processors and poultry producers to ensure mycotoxins do not enter the food chain, and this may necessitate them introducing quality control procedures even though a legal framework for such testing is not in place. Utilization of highly contaminated raw materials presents a major problem. Blending of highly contaminated and noncontaminated raw materials is not advisable, as most mixing techniques involving unground material are likely to lead to pockets of highly contaminated material which could have a disastrous effect on poultry production. An alternative strategy would be to decontaminate the highly contaminated raw material or the feed
prior to the addition of vitamins and other additives. This approach is being investigated in a project in Pakistan.


The nature of the aflatoxin problem and the significance of the aflatoxin contamination of feeds is discussed from both the animal production and the public health aspects. Procedures that can be adopted to minimize aflatoxin contamination of products and detoxification of aflatoxin-contaminated material are reviewed. Legislative and quality control measures which regulate the use of contaminated material and the world situation regarding the incidence of aflatoxin in feedstuffs and feed-materials are discussed.


This paper describes testing, removal, and segregation of aflatoxin-contaminated groundnuts in relation to the USA groundnut industry. Sorting and removal of discolored, mold-damaged groundnuts can greatly reduce aflatoxin levels in raw groundnuts. These selective procedures have been very effective in the USA so that very few lots exceed the acceptance level of 25 µg kg\(^{-1}\) aflatoxin.


In Malawi the rains start in October and end in April so that long-duration groundnut cultivars are harvested under dry conditions after the rains. The dry conditions favor rapid postharvest drying of groundnuts thus limiting seed infection by Aspergillus flavus and A. parasiticus and aflatoxin contamination. However, certain practices used by small holder farmers to process groundnuts in readiness for sale provide conditions that favor the rapid development of the toxigenic fungi and aflatoxin contamination of groundnuts. These practices include moistening groundnut pods in order to soften the shell for ease of handshelling. The Agricultural Development and Marketing Corporation (ADMARC) purchases shelled and graded groundnuts from small holder farmers, and electronically sorts and tests the groundnuts for aflatoxin contamination at the Liwonde Groundnut Factory. The process of handshelling and handgrading of groundnuts by smallholder farmers, followed by re-grading, and aflatoxin testing of the groundnuts has earned Malawi a reputation as a source of high-quality groundnuts for the confectionery trade.


A committee to coordinate action on the problem of aflatoxin contamination in Nigeria was formed in 1981 with representatives from four ministries, i.e., the Institute for Agricultural Research (IAR), Zaria, the Nigerian Stored Products Research Institute (NSPRI), and the Northern Nigerian Marketing Board. This Committee was given the responsibility of assessing the extent of the aflatoxin problem in groundnut in the country and of initiating and coordinating all actions leading towards its elimination. IAR investigated the aflatoxin contamination of the groundnut crop up to the stage where the produce was sold by farmers, while NSPRI studied the problem from the time of storage until produce was exported or consumed. IAR investigated the time of invasion of groundnut kernels by Aspergillus flavus, and when, and under what conditions it produced aflatoxin. An interplay of temperature, relative humidity, drought, erratic rainfall patterns, and maturity of the crop at lifting was found to affect invasion by A. flavus and aflatoxin contamination of groundnut in the field and store. In the wetter areas of the Southern Guinea Savanna which have long rainy seasons, aflatoxin contamination of groundnuts is mainly a postharvest problem, while in the major groundnut growing areas that lie in the drier Northern Guinea and Sudan Savanna the problem is largely preharvest. Insect infestations and wetting of stored groundnuts increase aflatoxin contamination. Research information from IAR and NSPRI still provides the basis for recommendations on the handling of groundnuts to either minimize or prevent aflatoxin contamination. Vegetable oil and feed mill companies routinely submit their groundnut and other feed materials for aflatoxin analysis as there is great awareness among the companies, people, and government of Nigeria of the dangers posed by aflatoxin to poultry, livestock, and humans. Nigeria is a consignatory to the African Groundnut Council’s resolution to export only groundnuts with an aflatoxin content that does not exceed the maximum permissible limit of 20 µg kg\(^{-1}\) set by the European Economic Community. None of the commercially grown groundnut cultivars in Nigeria is resistant to A. flavus invasion and aflatoxin contamination of seeds. Breeding materials from both domestic and exotic sources are being screened for resistance while other improved management practices are being used or researched.

Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95% of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that A. flavus infection of kernels and aflatoxin production occurred in both zones. The condition of the shell had a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by A. flavus, whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20% for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were free from fungal infection at lifting, infection by A. flavus not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of A. flavus in groundnut.


This paper outlines events leading up to the discovery of aflatoxin, describes briefly research done on the groundnut aflatoxin problem in the northern states of Nigeria, considers the implications of aflatoxin in relation to animal and human health, and discusses measures for elimination of aflatoxin from Nigerian groundnuts, or to at least greatly reduce incidence of aflatoxin.


This paper reviews the problem of aflatoxin contamination in groundnuts and underlines some of the areas where additional research is most needed. Possible preventive and control measures are also discussed with special reference to conditions in the semi-arid tropics.


The status of the global aflatoxin problem is reviewed with special reference to African groundnut producing countries, and research needs are highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus Aspergillus flavus.


This paper reviews the problem of aflatoxin contamination in groundnuts and discusses possible preventive and control measures. The possible use of genetic resistance to the aflatoxin-producing fungus Aspergillus flavus is considered. Reports of natural occurrence of mycotoxins in groundnuts are reviewed.


The possible use of genetic resistance to seed invasion by Aspergillus flavus and to aflatoxin production is considered and some research data presented. Many species of fungi have been found associated with groundnut seeds and several are known to be capable of producing mycotoxins on suitable substrates. Reports of natural occurrence of mycotoxins in groundnuts are reviewed, and the natural occurrence of citrinin and zearalenone reported. Preliminary data on mycotoxin production by fungi isolated from groundnuts are presented.


Cultural and preharvest conditions and early postharvest conditions which influence the vulnerability of groundnuts to seed invasion by Aspergillus flavus and to aflatoxin contamination are discussed. Management practices, including chemical control and genetic resistance are considered.


The global problem of aflatoxin is being pursued by the Peanut Collaborative Research Support Program (Peanut-CRSP) through: (1) development of cultivars resistant to seed invasion by aflatoxin-producing fungi; (2) cultural practices to minimize insect damage which facilitates fungal invasion; (3) detoxification of contaminated nuts and their products; and (4) separation of contaminated nuts. The dimensions of the problem appear to indicate that a substantial portion of the crop must be sorted out to eliminate aflatoxin. Progress in each of these areas gives promise of the elimination of aflatoxin from food-grade groundnuts.


The code of practices for purchasing, handling, sorting, processing, and testing of groundnuts is described. Adherence to these voluntary practices should ensure groundnuts and groundnut products being of good quality and meeting required limits for aflatoxin content.


The code of practices for groundnut produc the groundnut industry. A recently developed belt screen was utilized to evaluate quality improvements in moisture, foreign material, and aflatoxin content. When farmers' stock groundnuts were cleaned before drying, four drying wagon loads from each of seven farm fields were evaluated. Two loads were screened over a belt screen which had a 10.3 mm gap between belts and two loads were left unscreened. Fall- through weight of the screened loads ranged from 2-26% (weight) of the loads. One screened and one unscreened load were artificially dried at 8.3°C rise above ambient and the other loads were dried at a 16.7°C rise above ambient. Approximately 100 pounds of shelled seed were sized. Groundnuts from three of the seven locations contained detectable levels of aflatoxin. All size categories of groundnuts from the three locations contained aflatoxin regardless of whether or not they had been screened and regardless of whether pods rode on or fell through the screen. Jumbo, medium, and No. 1 size groundnuts from pods riding the screen were hand-picked to remove damaged seed and, with one exception, aflatoxin levels were less than 2.5 µg kg⁻¹ in subsequent analyses.

Retention of aflatoxin-contaminated groundnuts from the edible market is of high priority in the groundnut industry. A recently developed belt screen was utilized to evaluate quality improvements in moisture, foreign material, and aflatoxin content when farmers' stock groundnuts were cleaned before drying. Four drying wagon loads from each of seven farm fields were evaluated. Two loads were screened over a belt screen which had a 10.3 mm gap between belts and two loads were left unscreened. Fall-through weight of the screened loads ranged from 2-26% (weight) of the loads. One screened and one unscreened load were artificially dried at 8.3°C rise above ambient and the other loads were dried at a 16.7°C rise above ambient. Approximately 100 pounds of shelled seed were sized. Groundnuts from three of the seven locations contained detectable levels of aflatoxin. All size categories of groundnuts from the three locations contained aflatoxin regardless of whether or not they had been screened and regardless of whether pods rode on or fell through the screen. Jumbo, medium, and No. 1 size groundnuts from pods riding the screen were hand-picked to remove damaged seed and, with one exception, aflatoxin levels were less than 2.5 µg kg⁻¹ in subsequent analyses.


The important published information on aflatoxin during the period January 1964 to March 1967 is summarized. The topics covered include (a) assay of aflatoxins, (b) toxicology of aflatoxins, (c) biosynthesis of aflatoxins, (d) biochemical and intercellular effects of aflatoxins, (e) metabolism of aflatoxins in animals, (f) incidence, control and detoxification, and (g) implication in man.


Removal of aflatoxin-contaminated groundnuts from the edible market is of high priority in the groundnut industry. A recently developed belt screen was utilized to evaluate quality improvements in moisture, foreign material, and aflatoxin content when farmers' stock groundnuts were cleaned before drying. Four drying wagon loads from each of seven farm fields were evaluated. Two loads were screened over a belt screen which had a 10.3 mm gap between belts and two loads were left unscreened. Fall-through weight of the screened loads ranged from 2-26% (weight) of the loads. One screened and one unscreened load were artificially dried at 8.3°C rise above ambient and the other loads were dried at a 16.7°C rise above ambient. Approximately 100 pounds of shelled seed were sized. Groundnuts from three of the seven locations contained detectable levels of aflatoxin. All size categories of groundnuts from the three locations contained aflatoxin regardless of whether or not they had been screened and regardless of whether pods rode on or fell through the screen. Jumbo, medium, and No. 1 size groundnuts from pods riding the screen were hand-picked to remove damaged seed and, with one exception, aflatoxin levels were less than 2.5 µg kg⁻¹ in subsequent analyses.
Current knowledge about mode of action of aflatoxins, their medical and economic importance and prospects for their control are reviewed, considering the following aspects: structure, nomenclature and biosynthesis; mode of action; health risks and legislative regulation; postharvest control (segregation, decontamination, storage); and preharvest control (pesticides, and biological control by breeding or microbial interactions, particularly repression of aflatoxin synthesis by Aspergillus flavus following infection with a virus from Penicillium chrysogenum).

History of control of aflatoxin levels in foods, especially groundnut products, in the USA is surveyed, and data presented show changes in aflatoxin contamination from 1967 to 1978 for raw shelled groundnuts, consumer groundnut products, almonds, pecans and walnuts, and imported Brazil and pistachio nuts. The effect of establishment of an FDA action level of 0.5 ng aflatoxin M1 mL\(^{-1}\) milk on aflatoxin concentration in market milk is discussed. Aflatoxin contamination of food is effectively controlled in the USA, with the possible exception of some commeeal and corn grits products locally made and consumed in rural South Eastern USA.

Testing, removal, and segregation of aflatoxin-contaminated groundnuts is described in relation to the USA groundnut industry. Checking at all stages from growers to consumers is necessary. Sorting and removal of discolored groundnuts can reduce aflatoxin levels in raw groundnuts by 16%. Reduction may be improved if sorting is done after roasting and blanching. About one third of the aflatoxin is destroyed during roasting. Raw groundnuts containing 25 µg kg\(^{-1}\) of aflatoxin will, as a finished product, contain approximately 8 µg kg\(^{-1}\) of aflatoxin. Selective procedures in the USA have been very effective so that few lots exceed the acceptance level of 25 µg kg\(^{-1}\). The average aflatoxin content of groundnut products sold in the USA is less than 2 µg kg\(^{-1}\).

The problem of mycotoxins in farm commodities is reviewed. Concerning groundnuts, it is stressed that controlling pod insects and diseases, careful harvesting and postharvest drying to prevent damage to pods and ensure rapid drying of seeds should help in reducing risk of aflatoxin contamination. Drying the crop produce to below 8% seed moisture content will inhibit colonization of seeds by aflatoxin-producing fungi.

A general account is given of the aflatoxin contamination problems in stored groundnuts. Brief accounts are given of the natural occurrence of aflatoxins. Aspergillus flavus causes aflaroot disease of groundnuts; the aflatoxigenic fungus being seedborne results in infected seeds becoming rancid. Of 18 cultivars listed as having resistance to seed infection by A. flavus, only J 11 is widely grown in India. Regulations concerning trade in affected commodities are discussed. Preventive and curative measures for controlling aflatoxin contamination are listed.

This paper reviews research on toxicity and carcinogenicity of aflatoxins to animals, and summarizes control measures applied or proposed for protecting human food supplies from aflatoxin contamination. Control measures include use of effective crop handling, processing and storage procedures, segregation of contaminated produce by various sorting and diversion systems, and detoxification.

8.2.2 Cultural Control

Quality of groundnut depends on the effectiveness of the curing, drying, storage, and decortication processes. It is important to harvest at optimum maturity as late harvest may result in seed infection with Aspergillus flavus and aflatoxin contamination. In the USA, almost all commercially grown groundnuts are mechanically harvested, artificially dried and mechanically handled and shelled. They are therefore unlikely to become contaminated with aflatoxin during these processes. In less developed countries groundnuts are harvested and processed manually and are often stored under conditions favorable to mold growth.

Natural methods used by farmers in several countries for drying groundnuts are described. The necessity of adequate drying to prevent the development of the aflatoxin-producing mold Aspergillus flavus in seeds is emphasized.


Information on the artificial drying of groundnuts, which is of special importance in preventing the development of the mold Aspergillus flavus is summarized. It is recommended that, before drying, the nuts should be cured on the plant in windrows and should then be threshed rather than left on the haulms. Reference is made to the extensive experimental work carried out in the USA; various types of dryers used there and in Australia, Israel, Nigeria and Tanzania are described with special reference to batch dryers. In the tropics, where ambient temperatures can be above 29°C, the use of forced air without additional heat may be sufficient for drying groundnuts.


Investigations of sun-drying methods in relation to moisture content of groundnuts were carried out in two areas in Northern Nigeria. Groundnuts are harvested during the wet season in one area and during the dry season in the other. In the wet area, of the several sun-drying methods tried, the only one which was satisfactory was to pick the nuts from the haulms as soon as practicable after lifting and to expose them to the sun on materials, such as matting, which could be carried under cover at night and during rain. In the dry area, a variety of sun-drying methods were used and in all cases the groundnuts dried rapidly.


In 1984, studies were conducted to evaluate the role of calcium in controlling preharvest aflatoxin contamination of groundnuts using the environmental control plots at Dawson, Georgia, USA. Florunner groundnuts were grown under soil environmental stress conditions optimum for preharvest aflatoxin contamination. Calcium treatments were 0, 64.7, 256.7 kg ha⁻¹ calcium as CaSO₄ added to pretreatment levels. The levels of calcium in the kernels from the 0 and 256.7 kg treatment levels were significantly different, reflecting a response to added calcium in spite of relatively high pretreatment levels of soil calcium. There were no significant differences in calcium levels of kernels between the 0 and 64.7 kg calcium treatments. No significant relationship between aflatoxin contamination and kernel calcium levels was found. The authors concluded that application and uptake of calcium by the groundnut seed was not a viable method to prevent preharvest aflatoxin contamination.


In field trials in Georgia, USA, in 1981-82 Florunner groundnuts were grown without irrigation in rows 90 cm apart or in paired rows 15 or 25 cm apart with 90 cm between pairs. Gypsum application (1120 kg ha⁻¹) at flowering was compared with no gypsum, and East-West orientation of rows was compared with North-South orientation. Soil temperature was lower, and seed yields and germination percentages higher, in groundnuts grown with North-South orientation. Close spacing of rows gave lower soil temperatures during crop growth, and seeds produced had higher germination percentages, but there was no effect on yield. Soil moisture content was highest in close rows with North-South orientation. Gypsum application increased seed germination from 71.2 to 83.8 % in 1981 and from 83.2 to 86.8 % in 1982 and reduced aflatoxin concentration by 40 %.


Studies on the effects of groundnut plant orientation in windrows were conducted in North Carolina, USA. Groundnuts on inverted plants dried much more rapidly than did those on plants randomly oriented in windrows. Both plant orientation and moisture content at time of combining affected pod damage during combining, seed germination, and the degree of kernel damage caused by subsequent shelling operations. Apical kernels were more subject to mechanical damage during combining man basal kernels. In groundnuts inoculated with Aspergillus flavus, kernels in broken pods and shelled kernels were more often contaminated with aflatoxin during bulk curing man were groundnuts in sound pods.

The problem of aflatoxin contamination, which was serious in the Queensland groundnut industry in drought years, is reviewed with special reference to factors influencing aflatoxin formation including moisture stress, insect damage, and cultivar resistance. Control measures included crop hygiene, resistant varieties, avoiding moisture stress, harvesting the crop at optimum maturity, avoiding pest and mechanical injury, drying rapidly and segregating contaminated batches of groundnuts.


The effects of various crop sequences of maize, soybean, groundnut, green manuring with sorghum sudangrass, fallow, and minimum-tillage soybean cropping on Aspergillus flavus group populations in soil were examined in field plots on a farm in Southampton County, Virginia, from 1975 to 1979. Plantings of maize in 1975 and groundnut in 1976 were associated with significant increases in the population of A. flavus group in soil in following years, compared with the fallow treatment. Populations increased primarily in the lower half of the plough layer following maize planting. Other crop sequences did not significantly affect A. flavus group populations.


In Israel, the effects of crop sequence and soil types on the mycoflora of groundnuts were examined over three years in 81 fields. Some fields were previously fallowed, others continuously cropped with or without groundnuts. The mycoflora of the groundnut kernels from fields previously sown with groundnuts was more abundant than the mycoflora of kernels from fallow fields and from fields cropped without groundnuts. In the cropped fields, whether groundnuts were included in the previous crops or not, Aspergillus niger made up 50-60 % of the total mycoflora. Penicillium funiculosum and P. rubrum were considerably more prevalent in groundnuts produced in previously fallowed fields. Incidence of A. flavus was always low and it was not influenced by any crop sequence. Total kernel mycofloras were consistently higher on medium and heavy soils than on light soils.


Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95 % of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that A. flavus infection of kernels and aflatoxin production occurred in both zones. The condition of the shell had a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by A. flavus, whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20 % for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were found to be free from fungal infection at lifting, infection by A. flavus not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of A. flavus in groundnut.


The status of the global aflatoxin problem is reviewed with special reference to African groundnut producing countries, and research needs are highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus Aspergillus flavus.


Levels of aflatoxin in Spanish groundnut kernel samples from different geographical areas in Texas during 1966 were low. Of the 334 samples tested, 239 (71.6 %) contained no aflatoxin and only 2.7 % contained > 30 µg kg⁻¹ and 25.8 % contained trace-29 µg kg⁻¹. Analysis of samples obtained from growers using artificial drying equipment (forced air and supplemental heat), when windrow conditions were unfavorable for rapid drying, suggests that this practice reduces the possibility of aflatoxin accumulation. In general, groundnuts harvested from land planted to groundnuts the previous year were more highly infected by fungi and contained more aflatoxin than groundnuts grown on land planted to rye, oats, melons, or potatoes the previous year. Aflatoxin incidence tended to decrease from south to
north Texas. Detection of aflatoxin in sound mature kernels (kernels screened for minimal size) indicates that the practice of screening for removal of small immature kernels and removal of obviously damaged kernels does not completely eliminate aflatoxin contamination.


Groundnuts (cultivar Starr) grown rainfed under drought stress conditions had markedly higher levels of Aspergillus flavus infection and aflatoxin contamination of kernels before digging than had groundnuts grown under irrigation, in field trials at Yoakum and Stephenville, USA. in the 1967, 1968 and 1969 seasons. Levels of seed infection and aflatoxin contamination were considerably higher in 1967 and 1969 than in 1968. A. flavus infection and aflatoxin contamination levels were higher in drought-stressed groundnuts produced at Yoakum than in less stressed groundnuts produced at Stephenville. In 1969 some samples of freshly-dug groundnuts from irrigated plots had high levels of A. flavus infection (16-59 %), but no aflatoxin was detected in these samples. Absence of aflatoxin in these samples is attributed to high seed moisture levels and to low soil temperatures (10 to 21°C). Several fungicides applied to the soil and foliage did not affect fungal infection and aflatoxin contamination of groundnuts.


Postharvest quality of groundnut is influenced by the particular set of environmental and cultural practices that influence physiology and maturation. Groundnut composition, although related to environment, changes dramatically as groundnuts mature. There is biochemical basis for inferior quality in immature groundnut. Drought stress and soil temperature influence maturation rate and thus have an indirect effect on postharvest quality. Aspergillus flavus invasion and aflatoxin contamination in groundnuts are related to drought stress, soil temperature, and maturity. Small, immature seed are more likely to be contaminated with A. flavus than larger, mature seed. The biochemical composition, fungal contamination, and the tendency toward higher moisture content complicate storage of immature seed. Each of these factors predisposes immature seed to rapid quality deterioration in storage. Agrometeorological studies must include an awareness of the interrelationships of environment, maturity, and postharvest quality.


Environmental and biological factors responsible for aflatoxin contamination of the South African groundnuts are discussed. During the 1963/64 growing season, maturing groundnuts in the North-Western Transvaal areas were damaged by certain species of termites; this predisposed groundnuts to seed invasion by Aspergillus flavus. Protracted droughts followed by the late rains in these areas were also conducive to infection of groundnuts by the aflatoxigenic fungus. Most of the infection of the fungus by the fungus was believed to occur in the groundnut before harvest.


The effect of the previous season's crop (vegetables, rice or groundnut) on populations of Aspergillus flavus and other fungi in the soil, rhizosphere and geocarposphere of groundnuts and of shells and seeds at various stages of development, and on aflatoxin contamination at harvest was investigated. A. flavus populations in the rhizosphere and geocarposphere were high where groundnut was the previous crop and infection of shells and seeds were also high. Aflatoxin levels were higher in seeds from damaged pods than in seeds from undamaged pods.


Florunner and Florigiant groundnuts were grown in 1974, 1975, 1976 and 1977 and inoculated with Aspergillus parasiticus 30 days after sowing. Four replicates were grown in plots for 140 to 145 days under rainfall controlled shelters with six irrigation treatments: (1) wet from 0-140, (2) dry from day 36-70, (3) dry from day 71-105, (4) dry from day 106-140, (5) dry from day 36-105, (6) dry from day 71-140. Aflatoxin concentrations from Florunner groundnuts showed significant differences among treatments (P=0.01) in 1974 and 1976 but not in 1975 or 1977. In 1974 and 1976, Florunner sound mature kernels had significantly more aflatoxin in treatments 4 and 6 than in other treatments. Aflatoxin concentrations from Florigiant treatments 4 and 6 were significantly greater (P=0.01) than other treatments in 1974 and 1975, but not in 1977. No data were taken in 1976 for Florigiant groundnuts. Water stress during the last 35 or 70 days of the season
Effects of irrigation and drought stress treatments on preharvest aflatoxin contamination of groundnuts were investigated in the 1974, 1975, 1976 and 1977 seasons. Two groundnut cultivars, Florunner and Florigiant, were grown under rainfall controlled shelters with six irrigation treatments: (1) full irrigation throughout the growing season, (2) no irrigation from 36 to 70 days after sowing (DAS). (3) no irrigation from 71 to 105 DAS, (4) no irrigation from 106 to 145 DAS, (5) no irrigation from 36 to 105 DAS and (6) no irrigation from 71 to 145 DAS. Groundnut plants in each replicated plot were inoculated by sprinkling them with a spore suspension of an aflatoxigenic isolate (NRRL 2999) of Aspergillus parasiticus. Significant differences in anatoxin contents of Florunner groundnuts from treatments 4 and 6 had significantly higher levels of aflatoxins than had the kernels from other treatments. Aflatoxin contamination levels in sound mature kernels of cultivar Florigiant from treatments 4 and 6 in 1975 and from treatment 6 in 1974 were significantly greater than in sound mature kernels from other treatments. Such differences in aflatoxin contamination were not evident in 1977. In 1974 and 1976, sound mature kernels of Florunner from the treatments 4 and 6 had significantly higher levels of aflatoxins than had the kernels from other treatments. Aflatoxin contamination levels in sound mature kernels of cultivar Florigiant from treatments 4 and 6 in 1975 and from treatment 6 in 1974 were significantly greater than in sound mature kernels from other treatments. Such differences in aflatoxin contamination were not evident in 1977. Drought stress during the last 40 to 75 days of the season favored aflatoxin contamination in three of the four years in one or both cultivars. Because of season to season variation, drought stress was not alone responsible for preharvest aflatoxin contamination. In some seasons, other environmental factors interacted with drought stress to either favor or inhibit aflatoxin contamination. No aflatoxin was detected in seeds of the test cultivars from treatments where irrigation was applied during the last 40 days of the season.


Field experiments were conducted in the 1984, 1985, 1986 and 1987 seasons on a calcium deficient Lakeland sand at Tifton, Georgia, USA. Florunner groundnuts were planted in 1984 and 1987 while NC 7 was planted in 1985 and 1986. Irrigation and non-irrigation treatments were the whole plots, split-plots were rates of gypsum corresponding to 0, 112, 224 and 336 kg ha⁻¹ of added calcium. Split-plots were inoculated or not by sprinkling a spore suspension of Aspergillus parasiticus (NRRL 2999) over the plants at early bloom. Soil populations of the A. flavus group fungi were monitored four times each year. Harvested hulls and kernels were plated to assess A. flavus incidence. Aflatoxin contents of kernels were determined using high-pressure liquid chromatography (HPLC). Gypsum applications increased yield, value, per cent sound mature kernels (SMK) and reduced damage in all years. A. parasiticus inoculation decreased yield of Florunner but not NC 7. Inoculation increased soil populations of the A. flavus group fungi. Both irrigation and gypsum decreased hull and kernel infection by A. flavus. Aflatoxin contamination was infrequently observed and occurred in a random manner.


The effects of gypsum, irrigation, and inoculation with Aspergillus parasiticus (NRRL 2999) on mycoflora and aflatoxin contamination of groundnuts were examined. Two rows of groundnut plants in each plot were inoculated by sprinkling them with a spore suspension of the A. parasiticus isolate. Two irrigation regimes and four gypsum rates were tested. Soil samples were collected three times during the season and at harvest to monitor populations of A. flavus group fungi (A. parasiticus and A. flavus). Groundnut pods were collected at harvest for P, K, Ca, Mg, aflatoxin and mycoflora analyses. No aflatoxins were found in groundnuts from any treatment. Populations of A. flavus group fungi were significantly higher in soil from inoculated plots for the first two sampling dates only; A. parasiticus apparently did not persist in the soil throughout the growing season. More kernels were infected with the A. flavus group fungi and other fungi in plots with no gypsum treatments than in plots with gypsum treatments. The relationship between calcium nutrition and A. flavus infection in groundnuts may be important in preharvest aflatoxin contamination.

8.2.3 Chemical Control


Direct addition of 200 ppm of butylated hydroxyanisole (BHA) inhibited growth of some species of Penicillium. Aspergillus, or Geotrichum in a glucose salts broth (GSB), and of Aspergillus flavus and P. expansum in potato dextrose agar (PDA) or applesauce. In processed cheese spread, direct addition of 400 ppm of BHA was
required to inhibit growth of A. flavus or P. expansion, while the concentration required for inhibition of A. flavus, by a BHA emulsion sprayed on the surface was 150-200 ppm. BHA was effective in GSB over a wide range of pH values and incubation temperature against A. flavus at a concentration of 200 ppm. A combination of 150 ppm BHA + 0.2 % potassium sorbate gave total inhibition of growth of A. flavus in GSB.


The effects of the systemic fungicides tridemorph, fenpropimorph and fenarimol (at 240, 250 and 0.75 µg mL⁻¹ respectively) on growth and aflatoxins B₁ and G₁ production by Aspergillus parasiticus (NRRL 3145) was studied in a chemically defined medium. All three fungicides inhibited growth and simultaneously decreased aflatoxin production. The ratio of aflatoxin B₁ to G₁ produced remained relatively unchanged in the presence of tridemorph (the NRRL 3145 strain characteristically produces more G₁ than B₁). In the absence of fenpropimorph the ratio of B₁ to G₁ was more pronounced than in the presence of fenarimol (considered to be an inhibitor of cytochrome P₄₅₀).


Red ginseng saponin (0.36 %) inhibited mycelial growth, sporulation and aflatoxin production by Aspergillus parasiticus in a culture medium during 9 days at 28°C. The mold caused no change in pH of the medium when it contained red ginseng saponin or ginseng tea (9 %). Most ginseng products supported mycelial growth and production of aflatoxin B₁, but inhibited production of aflatoxin G₁. However, when compared to the control, aflatoxin production by A. parasiticus was reduced by most of the ginseng products tested. Ginseng tea (9 %) resulted in a higher index of maximum accumulation of aflatoxins per interval of mold growth than occurred in the control. Red ginseng was more effective than white ginseng for inhibiting mold growth and aflatoxin production.


Effects of several herbal substrates on growth and aflatoxin production by Aspergillus parasiticus were studied. The mold was grown in the presence of the selected herbal extracts: burdock, cromwell, honeysuckle, licorice, and ginger. Two % of each herb was used in an enriched medium which was inoculated with spores of the aflatoxigenic strain of A. parasiticus and incubated at 28°C for 9 days. Mycelial growth was inhibited by honeysuckle and no sporulation occurred in the presence of burdock and honeysuckle. Burdock, cromwell, ginger and licorice enhanced mycelial growth over that in the control. All the herbs inhibited accumulation of aflatoxins B₁ and G₁, especially extracts of honeysuckle flower and root-stem, which inhibited both mycelial growth and aflatoxin production. In the presence of licorice, loss of aflatoxin from the medium during later stages of incubation was greatest.


This study was conducted to determine the potential of carrot root to support Aspergillus parasiticus growth and aflatoxin production. Raw carrot tissue did not support the germination of A. parasiticus spores. Autoclaved carrot tissue supported germination, growth, sporulation, and aflatoxin production by A. parasiticus. There was no measurable difference in water activity before or after autoclaving the carrot tissue. There was an increase in water extractable carbohydrate and protein as a result of autoclaving the carrot tissue. Chloroform extracts of carrot tissue contained a compound that inhibited differentiation and aflatoxin production by A. parasiticus in both synthetic and semisynthetic media. The inhibitor was optimally active within a pH range 3.5 - 4.0. The minimum inhibitory concentration of the extract at pH 4.5 and at 28°C in minimal salts medium containing 6 % glucose and 17 x 10⁻³ A. parasiticus spores was 3.84 g equivalent weights of tissue mL⁻¹ assay medium.


Effects of the volatile fraction of carrot seed oil (VCSO) and its components on growth and aflatoxin production by Aspergillus parasiticus were studied. Geraniol, citral and terpineol prevented growth and aflatoxin production. VCSO inhibited growth and prevented aflatoxin production. Limonene and terpinene did not affect growth but inhibited aflatoxin production. VCSO, limonene and terpinene reduced growth rate, measured by incorporation of [³⁵S] amino acids into trychloracetic acid insoluble protein. Addition of VCSO at any time up to 5 days reduced aflatoxin accumulation in the culture medium at 7 days.


Investigations were undertaken into the effects of subinhibitory concentrations of
food preservatives on mycotoxin production. Methyl-p-hydroxybenzoate, propyl-p-hydroxybenzoate, benzoic acid and sorbic acid stimulated aflatoxin production by Aspergillus flavus. No stimulation was observed with propionic acid. Fusarium acuminatum grown on maize meal supplemented with 0.025-0.05 % sorbic acid produced more T-2 toxin than was produced without this chemical. It is assumed that attack of the krebs cycle by preservatives leads to high amounts of acetyl coenzyme A which is essential for aflatoxin B1 and T-2 toxin synthesis.


Aspergillus flavus was grown at 22, 28, and 35°C in a culture medium containing different concentrations of dimethyl sulfoxide (DMSO). Mycelial growth and aflatoxin production were monitored. Optimum temperature for aflatoxin production in the medium was 35°C after 1 week, 28°C after 2 weeks and 22°C after 4 and 6 weeks. Addition of DMSO (5,000 ppm) to the medium influenced aflatoxin concentration; either increasing or decreasing concentration depending upon temperature and periods of incubation.


Aflatoxin production by Aspergillus flavus was reduced by dimethyl sulfoxide (DMSO) being added to culture media. Mycelial growth of A. flavus on inoculated shelled or unshelled groundnuts was reduced when they had been treated with 5, 10 or 20 % DMSO. Chick embryo bioassays were also carried out to confirm the inhibitory effect on aflatoxin production of DMSO.


The influence of pyridazinone herbicides on aflatoxin production by Aspergillus flavus and A. parasiticus was studied in liquid media. Mycelia production was not affected by 20, 40, or 60 μg of herbicide mL⁻¹; however, aflatoxin production by A. parasiticus was higher in media with herbicide, whereas A. flavus produced lower aflatoxin levels in the presence of herbicides.


The effects of various chemicals on seed infection by Aspergillus flavus and aflatoxin contamination of windrowed groundnuts (cultivar Starr) were studied. Aqueous solutions or suspensions of 24 chemicals were applied to pods immediately after harvest, and the plants were covered with polyethylene film (PEF). After 24 h the PEF was removed, pods were inoculated with an aqueous spore suspension of an aflatoxigenic strain (NRRL 2999) of A. flavus, and the PEF replaced. After 6-day incubation, pods were sampled and seeds assayed for infection with A. flavus and for aflatoxins. Chemicals that proved effective in reducing A. flavus infection and aflatoxin contamination were p-aminobenzoic acid (PABA) + dimethylsulfoxide (DMSO), Bordeaux 8-8-100D, and Captafol - DMSO. Seed infection of freshly-dug groundnuts averaged 15 %, and aflatoxin contamination 21 μg kg⁻¹. Aflatoxin contamination was not correlated with A. flavus seed infection levels.


The efficacy of chemicals for controlling Aspergillus flavus infection and aflatoxin contamination of groundnuts (cultivar Starr) on windrowed plants in the field was examined. Plants were dug and inverted in the windrows 135 days after planting. Approximately 6.4 mm of water was then applied to the plot area by overhead irrigation. After irrigation, aqueous solutions or suspensions of 27 chemicals (fungicides and some industrial chemicals) were applied to pods in each of four replicated plots. Then, the plants were covered with Tri-Pli (T) white, opaque moisture barrier, which was sealed around the edges with moist soil. After 24 h, the Tri-Pli was removed, pods were inoculated with spore suspension of an aflatoxin-producing isolate of A. flavus (NRRL 2999), and the Tri-Pli was replaced and sealed with moist soil. Both inoculated and noninoculated windrows treated with water only were maintained as controls. After six days of incubation, pod samples were collected and kernels from each replicate were assayed for infection by A. flavus and aflatoxins. Results indicated that aflatoxin contamination of windrowed groundnuts could be substantially reduced by chemicals. Manzate, Benlate, and Botran treated pods had no detectable aflatoxins. A. flavus, however, was recovered from kernels from these treatments.


Experiments were conducted to determine if two food preservatives, potassium sorbate and sodium benzoate, had a synergistic effect with heat on inactivation of conidia of Aspergillus flavus and Penicillium puberulum and vegetative cells of Geotrichum candidum. Investigations were also made to determine if heated conidia
had increased sensitivity to preservatives in a recovery medium. As the pH of heating menstrua was decreased from 7.0 to 2.5, the rates of inactivation of molds were increased. Conidia were not as adversely affected by acid pH as were vegetative cells. At 50 ppm, potassium sorbate caused a significant increase in the rate of thermal inactivation of A. flavus and G. candidum, while 100 ppm had a significant effect on P. puberulum. Sodium benzoate caused significant decreases in decimal reduction times of A. flavus and P. puberulum when present at a concentration of 50 ppm in heating media. Viable heated conidia of A. flavus and P. puberulum had increased sensitivity to potassium sorbate and sodium benzoate, indicating heat injury. However, the relative effects of the preservatives on colony formation in recovery agar were reversed from those noted in heating media, i.e., at comparable concentration potassium sorbate was more effective than sodium benzoate for inhibiting colony formation.


Groundnuts (cvs. Tifspan and Florunner) were treated with selected foliar fungicides for control of leafspots. Groundnuts were harvested on three different dates and analysed for aflatoxin, oil and protein content, and degree of maturity. No significant differences in aflatoxin levels were associated with either fungicide treatments or harvest dates. However, significant differences were found in the protein and oil contents of the groundnut kernels, depending upon the fungicide treatment. Delayed maturity was caused by specific fungicide treatments.


The effect of neem leaf extract on Aspergillus parasiticus growth and aflatoxin synthesis was studied. The extracts were prepared either by blending 50 g (wet weight) of fresh leaves in 1L of 10 mM potassium phosphate (pH 7.0) or by boiling the leaves in the buffer. The extracts were added to a culture medium at 1, 5, 10 and 20 % concentrations prior to inoculation. The neem leaf extracts did not affect fungal growth but completely inhibited (> 98 %) aflatoxin synthesis. The inhibitory effect was somewhat diminished (60-70 % inhibition) in the heated leaf extracts. The volatile components of the extracts were stripped with air onto Tenax tubes and analyzed using capillary gas chromatography/mass spectrometry. The major volatile present, 3-methyl 2-buten-1-ol, was nearly 400-fold greater in the blended extract than in the heated extract. The observed inhibition is probably at the level of the regulation of the synthesis of the secondary metabolic enzymes, because once the secondary metabolism was initiated, the inhibitory effect of the neem leaf constituents was lost.


Soaking seeds of rice, wheat, maize, groundnut and mustard in 500 ppm aqueous ferulic acid solution for 3 h inhibited aflatoxin production in them by Aspergillus parasiticus. Seed germination was not affected by the treatment.


Seeds of rice (var. Sita), wheat (var. S308), maize (var. Ganga 2), groundnut (var. Ak 12-24), and mustard (var. BR 13) were soaked for 2 h in 500 ppm O-vanillin solution. Seeds were then inoculated with an aflatoxin-producing strain of Aspergillus parasiticus. The seed treatment successfully checked aflatoxin production without having any adverse effect on seed germination.


Fungicides used for control of southern stem rot (Sclerotium rolfsii) and limb and pod rot (Rhizoctonia solani) have also been found to reduce fungal damage affecting seed quality. Starting in 1988, the fungicides, terbutrazole, flutolanil, and diniconazole were applied to groundnuts in addition to standard foliar sprays for leafspot control with chlorothalonil. Kernels harvested from these plots were evaluated for infection by Aspergillus spp., other soil-borne fungi, and aflatoxins. In 1988, groundnuts from irrigated plots, that were treated with these fungicides, showed no significant differences in incidence of fungal invasion. However, groundnuts from plots treated with each of these fungicides had lower aflatoxin contamination than plots treated with only chlorothalonil. In 1989, similar studies were conducted in non-irrigated plots. Groundnuts from plots treated with each of these fungicides had lower fungal infection and flutolanil-treated groundnuts had lower aflatoxin contamination than groundnuts from non-treated (control) plots. In both years, the use of these fungicides resulted in higher yields and improved crop value.

The effects of caffeine and theophylline on growth and aflatoxin B, production by Aspergillus parasiticus (NRRL 2999) were studied in AMY medium at pH 4.5. Caffeine levels of 0.5, 1.0 and 2.0 mg mL\(^{-1}\) decreased aflatoxin production by 86, 96 and 100 %, respectively. Theophylline levels of 2.0, 4.0 and 8.0 mg mL\(^{-1}\) were tested, but only the highest concentration was inhibitory, decreasing aflatoxin production by 54 %. Inhibition of growth was noted, but did not completely account for the reduction in aflatoxin production. The data help explain why aflatoxins are not usually reported from caffeine-containing commodities.


Thymol concentrations equal to or greater than 500 µg mL\(^{-1}\) completely inhibited the growth of Aspergillus parasiticus, while lower concentrations of the flavor compound caused either partial or transitory growth inhibition. Aflatoxin production was also inhibited to a degree equal to or lesser than that of growth.


Growth and aflatoxin production by selected strains of Aspergillus flavus and A. parasiticus in the presence of potassium sorbate in yeast-extract sucrose broth were studied. Potassium sorbate at 0.05, 0.10 and 0.15 % delayed or prevented spor germination and initiation of growth, and slowed growth of these fungi at 12°C. Increasing the concentration of sorbate resulted in a decrease in total mycelial weight. Potassium sorbate also greatly reduced or prevented production of aflatoxin B, by A. parasiticus and A. flavus for up to 70 days at 12°C. At 0.10 and 0.15 % concentrations of sorbate, aflatoxin production was essentially prevented. At 0.05 % sorbate, aflatoxin production was greatly decreased in A. flavus over the control, but only slightly decreased in A. parasiticus.


The effects of cinnamon oil, clove oil, cinnamic aldehyde and eugenol on growth and aflatoxin production by Aspergillus parasiticus were studied using yeast-extract sucrose broth as the substrate. All four substances inhibited mold growth and aflatoxin production. Cinnamon and clove oils were inhibitory at 200-250 ppm, cinnamic aldehyde at 150 ppm and eugenol at 125 ppm. Since cinnamic aldehyde and eugenol are the respective major components of cinnamon and clove oils, it was concluded that these are the major active antifungal ingredients of these two essential oils. The inhibitory effect of these substances was judged to be inhibition of growth rather than of aflatoxin production. Given sufficient time, cultures which were inhibited initially, but which subsequently grew, produced aflatoxin levels equivalent to control cultures. Levels of the oils above 250 ppm and of cinnamic aldehyde and eugenol above 250 ppm completely inhibited mold growth, or supported only a small amount of growth that never reached secondary metabolism and never produced aflatoxin during the time of this study.


A study was conducted to determine the effects of sorbic acid and several derivatives of fatty acids (amides, aminimides, and monoglycerides) on aflatoxigenic cultures of Aspergillus flavus and A. parasiticus. A synthetic medium was inoculated with spores of the aflatoxigenic fungi, incubated for 48 h at 27°C, and then supplemented with sorbic acid and fatty acid derivatives. Cultures were then incubated for an additional five days. Mycelial mats were dried, weighed, and analysed for lipid and mineral content. Aflatoxins were also quantitated. Cerulenin (8 µg mL\(^{-1}\)) was the most effective fatty acid derivative examined, reducing mycelial growth by 37 % and completely inhibiting extracellular accumulation of aflatoxins. Other derivatives, in decreasing order of effectiveness included M-20 (an aminimide), lauribic and lauricidin. Inhibition by sorbic acid was nonspecific, affecting both mycelial growth and extracellular aflatoxin accumulation to the same extent. Utilization of fatty acid derivatives for determining mechanisms of aflatoxin accumulation and lipid biosynthesis appears promising.


A study was conducted to determine the effect of o-nitrobenzoate, p-aminobenzoate, benzoic acid, ethyl benzoate, methyl benzoate, salicylic acid, trans-cinnamic acid, trans-cinnamaldehyde, ferulic acid, aspirin, and anthranilic acid on growth and aflatoxin release in Aspergillus flavus (NRRL 3145) and A. parasiticus (NRRL 3240). Inhibition of mycelial growth and aflatoxin production by various concentrations of these aromatic compounds indicates the possibility of their use as fungicides. However, further investigation is needed to determine the possible toxic effects of any residues. At concentrations of 2.5 and 5.0 mg 25 mL\(^{-1}\) medium, methyl benzoate and ethyl benzoate were the most effective in reducing both mycelial growth and aflatoxin production.

The effects of some chemicals (p-aminobenzoic acid (PABA), potassium sulfite, and potassium fluoride) on growth and aflatoxin production by Aspergillus parasiticus (ATCC 15517) were studied in a culture medium. PABA inhibited aflatoxin production at all concentrations used, and, at higher concentrations, inhibited growth as well. Also, aflatoxin production was reduced up to 50% in groundnuts that had been treated with PABA solutions. Potassium sulfite inhibited aflatoxin production without significant effects on the growth of the fungus. Potassium fluoride inhibited aflatoxin production only at very high concentration used.


Groundnut shells contain luteolin, eriodictyol, and 5,7-dihydroxycromone. These flavonoid-related compounds, and a mixture of them, were tested to determine whether they would affect aflatoxin production. Broth medium (50 mL) was amended with 0.01, 0.02 and 0.06 mg mL\(^{-1}\) of the individual and mixed compounds, inoculated with 0.1 mL of a spore suspension (1 x 10^6 conidia mL\(^{-1}\)) of an aflatoxigenic isolate of Aspergillus parasiticus and incubated at 27°C. At 4, 7, 11 and 14 days after inoculation, the mycelium was removed, dried and weighed. The medium was extracted and tested for aflatoxin. No differences in mycelial weight were observed among the controls and amended cultures. However, each individual compound, at all concentrations, depressed aflatoxin production as compared with the controls. The mixture of compounds was the most effective on a percentage basis in reducing aflatoxin concentration.


Of 24 dyes tested for their inhibitory effects on growth, sporulation, and aflatoxin production by an aflatoxigenic isolate (NRRL 2999) of Aspergillus flavus in a liquid medium, four (brilliant green, malachite green, gentian violet, and crystal violet) significantly inhibited growth, sporulation, and aflatoxin production at 100 ppm. These four dyes completely inhibited growth at 500 ppm.


Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Mycelia of Aspergillus parasiticus can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of A. parasiticus, amount of mycelium, temperature, pH and concentration of aflatoxin. Adsorbents, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin can be degraded by fermenting yeasts and rubratoxin can be degraded by the mycelium of Penicillium rubrum.


Bisulfite reacted with aflatoxins B\(_1\) and G\(_1\) resulting in their loss of fluorescence. This was a first order reaction whose rate depended on bisulfite concentration. Aflatoxin G\(_1\) reacted more rapidly with bisulfite than did aflatoxin B\(_1\). In the presence of 0.035 M potassium acid phthalate - NaOH buffer (pH 5.5) + 13% (v/v) methanol at 25°C, the reaction rate constant for degradation of aflatoxin G\(_1\) was 2.23 x 10^8 h\(^{-1}\) and that for aflatoxin B\(_1\) was 1.87 x 10^9 h\(^{-1}\) when 50 mL of reaction mixture contained 1.60 g of K\(_2\)SO\(_3\). Besides bisulfite concentration, temperature influenced reaction rates. The Q\(_{10}\) for the bisulfite - aflatoxin reaction was approximately 2 while activation energies for degrading aflatoxin B\(_1\) and aflatoxin G\(_1\) were 13.1 and 12.6 Kcal mol\(^{-1}\), respectively. Data suggest that treating foods with 50-500 ppm of SO\(_3\) probably would not effectively degrade appreciable amounts of aflatoxin. Treating foods with 2000 ppm of SO\(_3\) or more, and increasing the temperature, might reduce aflatoxin to an acceptable level.


Citric acid retarded degradation of aflatoxins B\(_1\) and G\(_1\) by bisulfite. Methanol also retarded degradation of aflatoxins by bisulfite. Presence of citric acid and various concentrations of methanol also reduced rates at which free bisulfite concentration changed. From these observations, known effects of methanol and probable effects of citric acid on bisulfite oxidation, it is suggested that degradation of aflatoxin by bisulfite is dependent on bisulfite oxidation. Most of the degradation product(s) were in the water soluble phase, indicating that a structural modification of aflatoxin occurred.

This investigation determined growth and aflatoxin production by Aspergillus parasiticus when selected insecticides, i.e., naled (NL), dichlorvos (DC), Landrin (LD), pyrethrum (PY), Sevin (SV), malathion (ML), Diazinon (DZ), methoxychlor and piperonyl butoxide, were incorporated into liquid medium, and identified some factors contributing to insecticide inhibition of aflatoxin production. NL at 100 ppm completely inhibited production of aflatoxins B₁, B₂, G₁ and G₂ and growth of A. parasiticus. DC, LD, PY, SV, ML and DZ at concentration of 100 ppm significantly inhibited production of aflatoxins, but at 10 ppm inhibition was found only with NL, DC, SV, LD and PY. Growth of the fungus was inhibited by 28.9, 18.9, 15.7 and 100 % by 100 ppm of DC, LD, SV and NL, respectively. Stimulation of growth occurred when DZ was added to cultures.


Effects of a range of organophosphorus and various other compounds on production of aflatoxin by Aspergillus flavus were investigated. Five organophosphorus compounds. Chlormephos, Ciodrin, Naled, Phosdrin and Trichlorphon, at concentrations of 20 and 100 µg mL⁻¹ of liquid medium were found to have activity similar to dichlorvos, in that they inhibited aflatoxin production and caused formation of several anthraquinone pigments. Two of these pigments have not previously been described; one was named Versicol whilst the other was its acetate derivative. A rationale is presented for the required elements of structure that are necessary for an organophosphorus compound to have dichlorvos-type activity. Two unrelated compounds, ammonium nitrate and tridecanone were also found to elicit dichlorvos-type activity. It is likely that tridecanone or its breakdown products competitively inhibit enzymes involved in aflatoxin biosynthesis. It is possible that this inhibition effect explains the inhibition of aflatoxin production in lipid-rich commodities infected by A. flavus


The effect of sodium chloride on growth and aflatoxin production by an aflatoxin-producing strain of Aspergillus parasiticus NRRL 2999 was studied in glucose-yeast-salt broth. The culture was incubated at 13 or 28°C. Increasing the concentration of sodium chloride (2 to 10 %) reduced accumulation of aflatoxin and also induced a lag in growth of the culture. At 13°C, the mold produced small amounts of aflatoxin after an extended lag phase. Sodium chloride was markedly more inhibitory at 13 than at 28°C.


Effects of some essential oils from herbs and spices on growth and aflatoxin production by Aspergillus parasiticus were studied. Gas-liquid chromatography was used to determine me essential oil compositions of thyme, cumin, clove, caraway, rosemary, and sage. The basic components of these oils were thymol, cumin aldehyde, eugenol, carvone, borneol and thujone, respectively. The essential oils caused complete inhibition of both mycelial growth and aflatoxin production. The effectiveness followed the sequence: thyme > cumin > clove > caraway > rosemary > sage. The major components of the essential oils produced an inhibitory effect at minimum inhibitory concentrations equal to those obtained with the oils.


Aflatoxin production by Aspergillus parasiticus in a synthetic medium was significantly increased in the presence of 10, 20, or 30 ppm of growth regulator indole-3-acetic acid (IAA) or 10 ppm (but not 20 or 30 ppm) of gibberelllic acid (GA) ; mycelial growth was increased in the presence of 10 ppm of GA and decreased in the presence of 20 or 30 ppm of IAA or GA. The herbicide Trefflan [trifluralin] at 5, 10 and 20 ppm had a stimulatory effect upon growth and aflatoxin production, whereas the herbicide Stomp [pendimethalin] had the reverse effect at 10 and 20 ppm, and Gramoxone [paraquat] at 5 ppm increased and at 20 ppm decreased aflatoxin production. Malathion at 5 ppm stimulated aflatoxin production, but malathion at 10 and 20 ppm and actellic [pirimiphos-methyl] and guthion [azinphos-methyl], at 5, 10 and 20 ppm, decreased both growth and aflatoxin production, effectiveness being in the order guthion > actellic > malathion. At the recommended application rates, all products, except IAA and trifluralin, were inhibitory to growth and aflatoxin production.


The possibility of chemical control of Aspergillus flavus to prevent the production of aflatoxin in groundnuts by spraying fungicides to the freshly-dug pods in me windrows was studied. Four fungicides, viz., ferbam, thiram, sodium orthophenylphenate and captan were used in experiments run for 4 years in
different regions. When the harvest was carried out in rainy periods, the fungicides seemed to be inefficient, and when rains were absent the weather condition by itself seemed to inhibit the growth of A. flavus and so prevented the occurrence of aflatoxin. Nevertheless, more studies on the subject are suggested.


The effect of rock salts, sodium chloride, propionic acid, NCP.75, plant products-asafetida, turmeric powder and aqueous leaf extracts of Azadirachta indica, Lawsonia alba, Pongamia glabra and Tridax procumbens on seed colonization and aflatoxin production by Aspergillus flavus was studied in two Spanish bunch groundnut varieties (J 11 and JL 24). All these treatments inhibited in vitro seed colonization and aflatoxin production to varying degrees. Inhibition of seed colonization with chemicals, plant products and aqueous leaf extracts ranged from 17 to 96 %, 27 to 100 % and 8 to 75 %, while inhibition of aflatoxin production ranged from 14 to 74 %, 42 to 71 %, and 6 to 64 %, respectively. In general, salts (20 g L$^{-1}$), propionic acid (10 mL L$^{-1}$), asafetida (pure 1 g L$^{-1}$ and impure 20 g L$^{-1}$), and Azadirachta indica aqueous leaf extract ( 20 g L$^{-1}$) proved better in inhibiting aflatoxin production in both the varieties than other chemicals, plant products and aqueous leaf extracts tested.


The potential of Aspergillus flavus isolates to produce anatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings of the genus were studied. Isolates NRRL 3000 and V3734/K produced high levels of aflatoxins in culture. Culture filtrates from the isolates and from NRRCGAFA were most toxic to seeds and seedlings. Commercial cultivars, advanced breeding lines, and wild Arachis species were screened for resistance to in vitro colonization of seeds by A. flavus isolates, and to aflatoxin production. Genotypes CGC 2, 1-4, CGC 7, S230, derivatives of S230 x PI 337394F, Latur 33 x PI 337394F. and the wild species. Arachis cardenasii and A. duranensis were resistant to seed colonization by A. flavus. All genotypes of groundnut and three wild Arachis species supported high production of aflatoxins by NRRL 3000, but only trace levels were produced in A. cardenasii and A. duranensis. Aflatoxins were found (range of 27-146 µg Kg$^{-1}$) in commercial groundnut cake and in de-oiled cake. Moisture intake capacity, levels of seed coat phenols, and protein content of seeds were considered to influence aflatoxin contamination levels. Soaking seeds in various organic and inorganic substances was found to influence the degree of seed colonization by A. flavus and of aflatoxin production in in vitro inoculation tests.


The effects of 13 kinds of powdered herbal drugs and seven kinds of commercial dry condiments on the growth and toxin production of Aspergillus parasiticus, A. flavus, A. ochraceus, and A. versicolor were studied by introducing these substances/drugs into culture media. Of the twenty substances tested, cinnamon bark completely inhibited the fungal growth, while the others only inhibited the toxin production. The inhibitors were easily extracted from the substances/drugs with solvents such as hot water, chloroform, or ethanol. The extracts from coptis, philodendron bark, mustard, green tea leaves, and zanthoxylum completely inhibited aflatoxin production by A. parasiticus, however, they had little or no inhibitory effect against A. flavus.


The inhibitory effects of 29 commercial powdered spices on growth and toxin production of three species of toxigenic Aspergillus were investigated by introducing these materials into culture media. Three spices, namely, cloves, star anise seeds, and allspice, completely inhibited growth of toxigenic strains of A. flavus, A. versicolor and A. ochraceous, whereas most of the other spices inhibited only the toxin production (aflatoxin, ochratoxin and sterigmatocystin). Eugenol extracted from cloves and thymol from thyme caused complete inhibition of the growth of A. flavus and A. versicolor at 0.4 mg mL$^{-1}$ or less. At a concentration of 2 mg mL$^{-1}$, anethol extracted from star anise seeds inhibited the growth of all the toxigenic strains of Aspergillus spp.


Effects of temperature, pH, water activity, and nine antifungal agents on growth of Aspergillus flavus and A. parasiticus were studied using Sabouraud-dextrose agar and corn as the substrates. Maximal growth of the two molds occurred at 33°C, the highest temperature used, pH of 5.0 and water activity of 0.99. At 15°C, growth was observed at water activity of 0.95 but not 0.90. Slight growth was observed at water
activity of 0.85 at 27°C and 33°C. All antifungal agents (Botran, Orthocide, Folyram 80, Topsy-M, Thiram. Imazalil, sodium propionate, sodium sulfite and DDVP) showed antifungal activity, but Imazalil and DDVP were the most effective at the lowest concentrations. Activity of the antifungal agents increased as the water activity was decreased.


Dichlorvos (dimethyl 2,2-dichlorovinyl phosphate) was found to possess a strong inhibitory effect on aflatoxin biosynthesis by Aspergillus parasiticus ATCC 15517. At 10 ppm it inhibited 90% of aflatoxin production in three types of liquid media without affecting the fungal growth. When the actively synthesizing cultures were supplemented with 10 ppm of dichlorovos and [1-14C]acetate, a reduced amount of aflatoxin B1 was produced which contained almost no label from the acetate, suggesting that dicholorvos inhibits an early step in the secondary metabolic pathway for aflatoxin biosynthesis. Experiments with other organophosphorus insecticides showed that dichlorvos was particularly inhibitive to the biosynthesis of aflatoxin.


In 1966 and 1967 field experiments in Tifton, Georgia, Vapam was used at rates of 115 and 260 mL per 100 feet of row and Vorlex at 115 mL per 100 feet of row as pre-planting treatments for groundnuts. DD was included as a nematicide check treatment. Use of soil fumigants did not increase yield, reduce pod mycoflora, or indirectly suppress the formation of aflatoxins in Argentine groundnuts.


The possibility of chemical control of fungi invading pods and seeds of groundnuts by spraying fungicides onto the freshly-dug pods in the windrows was studied. Fungicides were applied 12 h after lifting groundnuts (cultivar Early Runner). Fungicides applied were: (i) Difolatan - 0.67 kg; (ii) triphenyltin hydroxide (DuTer) - 0.67 kg; (iii) Tri-basic copper sulfate (TBCS) - 0.67 kg; (iv) tetrachloroisophthalonitrile (DAC 2787) - 0.67 kg; (v) Captan - 0.89 kg; (vi) sodium propionate - 1.78 kg and 3.57 kg; (vii) sulfur - 3.57 kg and (viii) tap water (control). Difolatan and TBCS reduced development of pod surface fungi. Kernels from these treatments also had fewer fungi than the nontreated controls. Aflatoxin contents of kernels from slowly dried pods, which had been treated previously with various fungicides in the windrow, were not related closely to observed efficacy of fungicides in controlling pod surface fungi or fungi from kernels.


In Zambia, groundnut kernels meant for export are routinely monitored for aflatoxin contamination. Since 1979, 6.3% of the 28410 samples analyzed had contamination levels of more than 5 µg aflatoxin kg⁻¹. A 2-year study with promising varieties revealed the variability of Aspergillus flavus seed infection. Seed treatment with Benlate(R)/Labilite(R) at 3 g kg⁻¹ seed was found to control A. flavus in groundnut seed and can improve crop stand.


Growth of Aspergillus flavus and Trichoderma viride on agar media was inhibited around embryos of green groundnut seeds but not around those of cured, mature seeds, intact seeds or testae; both colonized intact seeds and testae. Substances inhibitory to the fungi were extracted with acetone from cotyledons of freshly harvested seeds. Four compounds inhibitory to A. flavus were detected in crude acetone extracts; three of these had phenolic properties. The inhibitory compounds in groundnut cotyledons may be factors in protecting the embryo from fungal infection.


When mature, freshly-harvested groundnut pods were sprayed with propionic acid (5 %), sorbic acid (0.1 %) or chlorothalonil (0.15 %), invasion by Aspergillus flavus and the consequent formation of aflatoxins in the kernels was prevented.


Growth and aflatoxin production by Aspergillus parasiticus (NRRL 2999) in black
Six sucrose esters substituted to different degrees with a mixture of fatty acids (palmitic and stearic) were examined for antimicrobial properties. Growth and acid production of several lactic acid bacteria and growth of Saccharomyces cerevisiae were not inhibited by 0.2 % of the sucrose esters in the test medium. Antimycotic activity was detected against several mold species of Aspergillus, Penicillium, Cladosporium, and Alternaria. The least substituted sucrose ester was the most active in reducing mold growth. Reduction of mold growth ranged from 37 to 91 % with this ester at a 1 % concentration. Inhibitory activity did not appear to be influenced by changes in pH. Aflatoxin production by A. parasiticus was not affected by 0.1 % sucrose ester.


The effects of sucrose esterified with a mixture of palmitic and stearic acids (commonly referred to as sucrose ester) in combination with cinnamon, potassium sorbate, or calcium propionate on growth and aflatoxin production by Aspergillus parasiticus were studied in broths at two pH values. Cinnamon in combination with sucrose ester did not result in additive or synergistic inhibitory effects on growth or aflatoxin production. At pH 4.0, subinhibitory levels of cinnamon were stimulatory toward growth and antagonistic to inhibition of growth by sucrose ester. Complete inhibition of growth and aflatoxin production was observed with a cinnamon level of 1.0 %, alone and in combination with sucrose ester. Low levels (0.1 %) of calcium propionate or potassium sorbate combined with sucrose ester did not enhance inhibition of growth or aflatoxin production. A synergistic effect on inhibition of growth was observed with high levels of propionate or sorbate in combination with sucrose ester, while aflatoxin production remained relatively unaffected. However, subinhibitory levels of propionate resulted in a 10-fold increase of aflatoxin production and a shift in the ratio of aflatoxin B₁ to G₁ from 1:1 to 1:8. Subinhibitory levels of sorbate also caused a stimulation of aflatoxin production during the latter stages of incubation, though to a lesser degree than propionate.


Growth of Aspergillus flavus (ATCC 15546) and its aflatoxin production were completely inhibited in groundnuts and maize treated with acid mixtures of benzoic : propionic, benzoic : sorbic and propionic : sorbic at 0.2:0.1, 0.2:0.3 and 0.1:0.5 %, respectively. When the chemicals were mixed with feed given to male Wistar rats, there was no decrease in growth of the rats. Aqueous and alcoholic extracts from the treated seeds showed neither toxicity upon development of chick embryo nor mutagenicity using Ames bacterial test.


Chemical and biological soil amendments were investigated for effects on seed infection by fungi of the Aspergillus flavus group (A. flavus and A. parasiticus) and, in certain instances, on aflatoxin contamination of one or more genotypes of groundnut. During 1976, 1977, 1981, and 1982, granular and/or liquid pesticide formulations and Trichoderma harzianum were applied either as soil amendments or as postharvest liquid applications to groundnut pods. In 1981 and 1982, soil treatments also were applied to plots treated with surface applications of 0, 673, and 1345 kg of gypsum hectare⁻¹. Genotypes grown with chemicals applied to either the soil (both alone and in certain combinations) or to the pods of these genotypes varied in infection of the seed by the A. flavus group fungi. Gypsum applications reduced the percentage of seed infected by these fungi. Applications of gypsum also enhanced the control of seed infection in plots treated with T. harzianum, PCNB-fensulfothion, or CGA 64250, but treatment and genotype interactions were noted. No aflatoxin was detected in groundnuts harvested from gypsum-treated plots, but it was occasionally found in groundnuts from the non-gypsum treatments resulting in a highly significant treatment x genotype interaction.


Growth of Aspergillus flavus and its aflatoxin production were completely inhibited in groundnuts and maize treated with acid mixtures of benzoic : propionic, benzoic : sorbic and propionic : sorbic at 0.2:0.1, 0.2:0.3 and 0.1:0.5 %, respectively. When the chemicals were mixed with feed given to male Wistar rats, there was no decrease in growth of the rats. Aqueous and alcoholic extracts from the treated seeds showed neither toxicity upon development of chick embryo nor mutagenicity using Ames bacterial test.

Unshelled Spanish groundnuts contaminated with aflatoxin were shelled and dried with heated air, liquid nitrogen, \( \text{H}_2\text{O}_2 \), \( \text{HCl} \), sodium oleate and water spray. After passing through a whole nut blancher the aflatoxin content and percentages of blanched and whole kernels were determined. Blanched percentages were significantly higher for sodium oleate, water spray, liquid nitrogen and \( \text{H}_2\text{O}_2 \) than for \( \text{HCl} \) or heat treatments. The heat treatments produced the highest percentage of whole kernels. In most tests, the non-blanched kernels had higher levels of aflatoxin than had those that blanched fully. The treatments which were most effective in producing low levels of aflatoxin in the blanched kernels were \( \text{H}_2\text{O}_2 \), water spray and \( \text{HCl} \).


Groundnuts (cultivar Starr) grown rainfed under drought stress conditions had markedly higher levels of Aspergillus flavus infection and aflatoxin contamination of kernels before digging than had groundnuts grown under irrigation, in field trials at Yoakum and Stephenville, USA, in the 1967, 1968 and 1969 seasons. Levels of seed infection and aflatoxin contamination were considerably higher in 1967 and 1969 than in 1968. A. flavus infection and aflatoxin contamination levels were higher in drought-stressed groundnuts produced at Yoakum than in groundnuts produced at Stephenville. In 1969 some samples of freshly-dug groundnuts from irrigated plots had high levels of A. flavus infection (16-59 %), but no aflatoxin was detected in these samples. Absence of aflatoxin in these samples is attributed to high seed moisture levels and to low soil temperatures (10 to 21°C). Several fungicides applied to the soil and foliage did not affect fungal infection and aflatoxin contamination of groundnuts.


Of 22 plant extracts screened, aqueous extracts of Adiantum sp., Euphorbia hirta, Gynandropsis pentaphylla, Justicia gendarussa and Thuja orientalis significantly inhibited aflatoxin production by Aspergillus parasiticus on agricultural commodities, including rice, wheat, maize and groundnut.


The effect of dimethyl sulfoxide (DMSO) on the production of aflatoxin by Aspergillus flavus (ATCC 2221) in groundnuts was determined. Fifty g of dry groundnuts (cultivar Early Runner) were soaked for 30 min in distilled water containing 0.0, 0.6, 1.2, 2.5, 5.0, 10.0 or 20 % DMSO. After soaking, they were autoclaved and inoculated with a spore suspension of A. flavus. The cultures were incubated for 7 days at 24°C, and aflatoxins were extracted. Toxin production in cultures containing 0.6 and 1.2 % DMSO was comparable to the controls. However, at concentrations of 2.5 % and above, aflatoxin production decreased rapidly. At 20 % DMSO, little or no fungal growth was detected. These results were verified by a bioassay using chick embryos.


Treatment of groundnut seeds (cultivar Early Runner) with 2.5 % or higher concentration of dimethyl sulfoxide (DMSO) prior to inoculation with the aflatoxin-producing fungus Aspergillus flavus caused an inhibition of normal conidial pigmentation and 62-64 % inhibition of aflatoxin production.


In laboratory tests, wheat, maize, rice and groundnuts were treated with various concentrations of dichlorvos either before or after infection with Aspergillus flavus. Treatment with dichlorvos at concentration of 20 ppm before infection prevented aflatoxin production on wheat. However, production of aflatoxin occurred when the dichlorvos concentration before infection was less than 20 ppm, when the treatment followed infection, and when the substrate was maize, rice or groundnut.


Inhibition of mold growth and mycotoxin production by use of simple chemicals, antibiotics and natural plant products was reviewed, especially considering treatment of foods. Topics discussed included effects of organic acids, antibiotics, herbs and spices, essential oils, insecticides and fumigants, phenolic antioxidants, methylxanthines, and chlorine.


This review paper summarizes information on effects of commonly used food
additives or preservatives and chemicals on growth and aflatoxin production by Aspergillus parasiticus and A. flavus. Growth and aflatoxin production by toxigenic aspergilli are partially or completely inhibited by the undissociated form of acetic, benzoic, citric, lactic, propionic, and sorbic acids. Salts such as sodium chloride, potassium chloride, and sodium nitrate, at low concentrations, can enhance aflatoxin production. At higher concentrations they become inhibitory, but marked inhibition requires amounts of the salts greater than are commonly used in foods. Phenolic antioxidants, sometimes added to foods to prevent oxidative deterioration, also are inhibitory to toxigenic aspergilli. Other inhibitory agents include certain insecticides, methylxanthines (caffeine and theophylline and components of some herbs, spices and other plants.


Aflatoxin production by Aspergillus flavus and A. parasiticus was greatly reduced in vitro in the presence of the insecticide dichlorvos. Reductions in the production of aflatoxins were accompanied by the appearance of an orange pigment. Spectral analyses of the pigment and of its methylated and acetylated derivatives indicated the compound to be versiconal acetate (IV). The data suggest that IV is an intermediate in the metabolic cycle that may terminate in the production of aflatoxin or of the versicolorins, or both. Dichlorvos apparently inhibits biosynthesis of the difurano ring structure common to the aflatoxins and the versicolorins.


Aflatoxin biogenesis by Aspergillus parasiticus was studied in a culture medium. Sub-inhibitory concentrations of the fungicides stimulated biosynthesis of all the four aflatoxins B₁, B₂, G₁, and G₂ in culture. At a higher concentration (2.4 μg kg⁻¹) of the fungicides, the fungus lost the ability to form a mycelial mat. Instead, abnormal growth in the form of giant cells and a poorly differentiated mycelium was observed in such cultures, which failed to produce aflatoxin.


Aflatoxin production was observed in the 2-kg lots of groundnuts and maize that were stored for 90 days at ambient temperatures 28-30°C and R.H. 100% after infection with an aflatoxin-producing strain of Aspergillus parasiticus (NRRL 3145). Treatment of the samples with an aqueous solution of 2-chloro ethylphosphonic acid (ethephon) prevented aflatoxin formation in both commodities, whereas the nontreated lots supported aflatoxin production.


Ethyl acetate extracts prepared from potatoes exposed to a sprout-inhibiting dose (10 Krad) of gamma irradiation were tested for inhibitory activity towards Aspergillus flavus and A. parasiticus. The treatment did not adversely affect the naturally occurring compounds which inhibit growth of these aflatoxin-producing fungi and which were still evident 4 weeks after irradiation following storage at 15°C.


Various extracts of onion were tested for their inhibitory activity against the growth of the aflatoxigenic fungi, Aspergillus flavus and A. parasiticus. Ether extract and lachrymatory factor (LF), which has been earlier identified as thiopropanal-s-oxide, were found to have potent antifungal activity. Steam-distilled onion oil, which is devoid of LF, was not as potent as ether extract and LF. Its major component, dipropyl disulfide was ineffective as a fungal inhibitor. Ethyl acetate extract containing phenolics was also ineffective. Exposure of onions to gamma-irradiation at a sprout-inhibiting dose (6 krad) did not alter the inhibitory potency of the onion extracts which, however, appeared to be heat-labile.

Effects of selected antifungal compounds on growth and aflatoxin production by the aflatoxin-producing fungi, Aspergillus flavus and A. parasiticus, in a liquid medium were studied. Of the antifungal agents evaluated, propionic acid and crystal violet were the most effective in retarding mold growth. Propionic acid was fungicidal at concentrations > 3.0 µg mL⁻¹ whereas crystal violet exhibited a mold retarding activity at levels ≥ 2.0 µg mL⁻¹. Crystal violet retarded the growth rate of the mold during the initial stages of growth; however, this retardation was overcome after 10 days of incubation. Crystal violet also inhibited aflatoxin production and sporulation of A. parasiticus. A survey involving 12 toxigenic isolates of A. parasiticus and A. flavus indicated that these species vary markedly in susceptibility to crystal violet.


A study was conducted to evaluate growth of Aspergillus parasiticus on white potato. White potatoes did not support the growth of A. parasiticus (NRRL 2999) unless they were previously heated to above 60°C. The mold grew well on autoclaved potatoes and produced, on an average, 8 µg g⁻¹ total aflatoxins (mainly B₁ and G₂) at 27°C and 95-97 % relative humidity in 20 days. When soluble potato starch was substituted for sucrose in yeast-extract sucrose (YES) medium, the maximum growth of A. parasiticus was 80 % of that observed in YES medium, but total aflatoxin production was only 0.5-1 % of the production in YES medium. Chlorogenic acid and solamine, at levels normally found in the white potato, did not show appreciable fungitoxic activity towards A. parasiticus in vitro. Caffeic acid partially inhibited the growth of A. parasiticus and the production of aflatoxins at 0.01 M concentration in YES medium. A compound with high inhibitory activity towards A. parasiticus was isolated from the potatoes. The compound is extractable with ethyl acetate. From its behavior on TLC systems, its ultraviolet spectrum and its reactions with chromagenic spray reagents, it is inferred that the compound is phenolic in nature. It appears to be a hydroxy-cinnamic acid derivative structurally similar to caffeic acid but lacking the ortho-dihydroxy structure of caffeic acid.

1474. Tango, J.S., and Tela, R. 1971. [Control of Aspergillus flavus in peanuts during drying period.]. Controle de Aspergillus flavus en amendoim durante o período de secagem. Coletanea do Instituto de Tecnologia de Alimentos (Brazil) 4: 83-90.

Aflatoxin production by a toxigenic strain of Aspergillus flavus was greatly reduced by benzoic acid and sodium benzoate in synthetic media. The reduction was accompanied by the appearance of a yellow pigment. Spectral analyses partially characterized this pigment as closely related to an acetyl derivative of a versicolor-type compound. A cell-free extract prepared from A. flavus grown in synthetic media was active in converting this yellow compound into aflatoxin B₁ in the presence of reduced nicotinamide adenine dinucleotide phosphate at 25°C (pH 7.4). In the presence of benzoic acid and its salt or autoclaved cell-free extract, conversion of yellow compound to aflatoxin B₁ was prevented. These results suggest that the yellow compound is an intermediate in the secondary metabolic cycle involved in aflatoxin B₁ production. Benzoic acid, sodium benzoate, or autoclaving the cell-free extract appear to block or denature an enzymatic step later in the biosynthetic pathway of aflatoxin B₁.
Aspergillus flavus (two aflatoxigenic strains and one non-toxigenic strain). With only parasiticus were investigated. In a defined medium, benzoic acid (2 and 3 mg mL\(^{-1}\)) and salicylic acid (20 mg g\(^{-1}\)) inhibited mycelial growth and aflatoxin production by Aspergillus flavus in groundnut. At their effective concentrations these aromatic compounds were non-toxic to 1-day-old chicks. Chicks fed aflatoxin-contaminated diets experienced depressed body and liver weights and their livers manifested various pathological changes. Groundnut samples supplemented with various concentrations of these aromatic compounds did not give a foul odour.

Effects of several chemicals on growth and aflatoxin production by Aspergillus parasiticus were investigated. In a defined medium, benzoic acid (2 and 3 mg mL\(^{-1}\)), cinnamon (1 mg mL\(^{-1}\)), and sodium acetate (5 mg mL\(^{-1}\)) were fungitoxic. Benzoic acid (0.5 and 1 mg mL\(^{-1}\)), chlorox (5 µg mL\(^{-1}\)), and dimethyl sulfoxide (5 µL mL\(^{-1}\)) did not affect dry weight or mycelial pigmentation. Sodium benzoate (1, 2, 4 and 8 mg mL\(^{-1}\)) added after two days’ growth inhibited aflatoxin production in two defined culture media. The authors were unable to confirm previously published reports that an uncharacterized yellow pigment accumulated with benzoate-inhibition of aflatoxin production.

Benzoic acid (10 mg g\(^{-1}\)), sodium benzoate (24 mg g\(^{-1}\)), ethyl-p-aminobenzoate (10 mg g\(^{-1}\)), and salicylic acid (20 mg g\(^{-1}\)) inhibited mycelial growth and aflatoxin production by Aspergillus parasiticus on groundnuts. With only parasiticus were investigated. In a defined medium, benzoic acid (2 and 3 mg mL\(^{-1}\)) and salicylic acid (20 mg g\(^{-1}\)) inhibited mycelial growth and aflatoxin production by Aspergillus flavus in groundnut. At their effective concentrations these aromatic compounds were non-toxic to 1-day-old chicks. Chicks fed aflatoxin-contaminated diets experienced depressed body and liver weights and their livers manifested various pathological changes. Groundnut samples supplemented with various concentrations of these aromatic compounds did not give a foul odour.

Studies were made to determine if the melanin pathway reductase inhibitor, chlobenthiazone, inhibits aflatoxin synthesis in Aspergillus spp. A. flavus and A. parasiticus were grown in shake cultures containing up to 8 µg mL\(^{-1}\) chlobenthiazone. This compound had a strong inhibitory effect on the accumulation of aflatoxins B\(_1\) and B\(_2\) in cultures of both fungi. At 8 µg mL\(^{-1}\) it caused a 24 % decrease in the mycelial dry weight of both fungi. Levels of aflatoxin B\(_1\), in cultures of A. flavus were decreased by 90 and 99 % at 1 and 4 µg mL\(^{-1}\) chlobenthiazone, respectively. Levels of aflatoxin B\(_1\) in cultures of A. parasiticus were decreased by 64, 81. and 86 % at 1, 4, and 8 µg mL\(^{-1}\) chlobenthiazone, respectively.

β-ionone inhibited mycelial growth and sporulation of Aspergillus flavus and A. parasiticus on potato dextrose agar medium. The fungal colonies were restricted, remained buff-colored and had little or no sporulation. No sporulation occurred at levels of 5 µl or above of β-ionone even after 4 week’s incubation. Concentrations of 100 µl and above of β-ionone L\(^{-1}\) in liquid cultures of A. parasiticus (NRRL 2999) inhibited aflatoxin production.

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aflatoxins. But fungal cells were lysed more readily by Clorox than by NaOCl. Mycelia older than 8 days lysed more readily than younger ones. Most conidia survived concentrations below $1.4 \times 10^{-3}$ M. The lowest effective concentration for a 2-hr treatment was $8.8 \times 10^{-3}$ M which is well below the Clorox concentration recommended for routine laboratory decontamination of aflatoxins. Mice and rats injected with aflatoxins and aflatoxins incompletely destroyed by Clorox died within 72 hr and had typical liver and kidney damage caused by aflatoxins. However, animals injected with NaOCl or Clorox or Clorox-destroyed aflatoxin extracts survived and showed no obvious liver or kidney damage.


Dichlorvos (dimethyl 2,2-dichlorovinyl phosphate) inhibits the biosynthesis of aflatoxin by Aspergillus parasiticus. Cultures treated with dichlorvos excrete an orange pigment which can be converted into aflatoxin B$_1$ by the nontreated mycelia. The orange pigment was partially identified as an acetyl derivative of a versiconal-type compound. In the presence of dichlorvos, sterigmatocystin is converted into aflatoxin B$_1$ without being interfered, but averufin is converted into the orange pigment instead of aflatoxin B$_1$. Therefore, dichlorvos appears to block an enzymatic step in the aflatoxin biosynthetic pathway, which lies beyond averufin but before sterigmatocystin, at the formation of the orange pigment.

8.2.4 Biological Control


A metabolite produced by Aspergillus niger grown on groundnut kernels inhibited the production of aflatoxins by Aspergillus flavus. A. niger was incubated on autoclaved groundnuts at room temperature for 3, 6, 9 and 14 days, then the cultures were re-autoclaved and inoculated with A. flavus, incubated at 24°C for 14 days and then the aflatoxins were extracted. The concentration of aflatoxin produced decreased with increasing length of incubation with A. niger, those incubated for 14 days with A. niger had only 12 % as much aflatoxins as the controls (without incubation with A. niger). When A. flavus was grown on a 9-day-old culture filtrate of A. niger no aflatoxins were detected and mycelial growth was inhibited Germination of conidia of A. flavus was also retarded in the filtrates although the final percentage germination was the same as in the controls.


This paper reviews seed infection by Aspergillus flavus and other fungi, and aflatoxin contamination of groundnuts from the time the groundnuts are growing in the field until they are marketed, stored and shelled. Causes of aflatoxin contamination and methods to control the aflatoxin problem are discussed.


A three year study was conducted to evaluate the use of a biocompetitive agent as an effective management strategy for preharvest aflatoxin contamination. The strategy involved the incorporation of a non-aflatoxin producing strain of Aspergillus parasiticus into the soil of an environmental control plot facility. The agent was tested by subjecting groundnuts to ideal conditions for preharvest aflatoxin contamination and comparing the effects with non-treated controls. The biocompetitive agent maintained a dominance over the wild, toxigenic strains of A. flavuslparasiticus for the three year period with no further addition of fungal propagules after the first year. This treatment also resulted in a significant reduction in aflatoxin in edible grade groundnuts compared to non-treated controls. Results from the first year showed that control, non-treated groundnuts averaged 522 µg kg$^{-1}$ aflatoxin, while biocontrol treated groundnuts averaged 11 µg kg$^{-1}$. The second year, controls contained 96 µg kg$^{-1}$ compared to 1.1 µg kg$^{-1}$ in treated groundnuts. The third year controls had 241 µg kg$^{-1}$ and treated groundnuts 40 µg kg$^{-1}$. Also of significance, soil populations of the biocompetitive agent were not higher than populations of wild strains of A. flavuslparasiticus that were present in untreated groundnut soils subjected to late-season drought stress. This is an important ecological consideration related to ultimate implementation of this strategy.


Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Mycelia of Aspergillus parasiticus can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of A. parasiticus, amount of mycelium, temperature, pH and concentration of aflatoxin. Adsorbants, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin can be degraded by fermenting yeasts and rubratoxin can be degraded by the mycelium of Penicillium rubrum.
Streptococcus lactis is known to produce an extracellular compound which inhibits the production of aflatoxin by Aspergillus flavus. Attempts made to identify the compound revealed that it appears to be a low molecular weight (<500) phosphoglyco-lipid containing an aromatic ring structure. No mutagenic or carcinogenic activity was detected by the Ames test extracts of A. flavus grown in mixed cultures with S. lactis.

Relationships between Aspergillus flavus, A. niger, Penicillium funiculosum, P. rubrum, and Fusarium solani were studied in plate culture with 234 samples of freshly lifted and stored groundnuts obtained from the 1965 and 1966 seasons’ crops in different parts of Israel. Pronounced antagonism was noted between A. flavus and A. niger, and rather lower level antagonism between each of these species and P. funiculosum, P. rubrum, and F. solani.

Two varieties of groundnut (Tennessee Red and Virginia Bunch 46-2) were grown under gnotobiotic conditions, and the pods were inoculated with a conidial suspension of the aflatoxin-producing fungus Aspergillus flavus. No evidence of pathogenicity to groundnut plants or pod rot symptoms was observed. A. flavus penetrated the shell tissue consistently, but was limited in its seed invasion to the testa. Invasion and colonization of the embryos by A. flavus appeared to be limited.

Twenty-three groundnut genotypes were evaluated for resistance to seed colonization by Aspergillus parasiticus and aflatoxin production when incubated under high relative humidity. Tannin-containing extracts from seed testae and cotyledons of these genotypes were prepared and tested for their effect on growth of A. parasiticus and aflatoxin production. Seed colonization was low (<30 per cent) in the genotypes Toalson X UF 73-4022 (selections TX-798731 and TX-798736), A72118, 55-437, PI 337409 and Florunner. Genotypes with low levels of seed colonization also had low aflatoxin contamination. Higher levels of tannins were detected in testae.
Seed lots could give different results unless the date of digging, methods of curing, and production location were the same. Some apparent shifts in susceptibility of seed to fungal colonization were quite extreme. One lot of stackpole-cured cultivar ‘Altika’ had 12% of seed colonized whereas a window-cured seed lot, dug on the same day from the same plot had 77% of seed colonized. No particular change in the harvesting procedure was consistently associated with increases or decreases in apparent susceptibility. Based on tests of all seed lots of 15 commonly grown cultivars during the years 1971-1974, ‘Florunner’ was the most tolerant and ‘Tifspan’ the most susceptible to seed colonization. The three resistant lines PI 337394F, PI 337409 and UF 71513 had markedly lower percentages of seed colonized man had the other cultivars.


Seven groundnut genotypes were tested in greenhouse and microplot experiments during 2 consecutive years to determine peg colonization by Aspergillus flavus and to determine the effect of 2 drought stress treatments on the susceptibility of shells and kernels to Aspergillus colonization and aflatoxin contamination. Moisture tensions within the soil and temperature of the soil and air were monitored during these experiments. Colonization of pegs was inconsistent among genotypes and between years. In general, low soil moisture tension enhanced colonization of shells and kernels. Shells of most genotypes were highly colonized after harvest from each moisture regime. Kernels of all genotypes were more susceptible to A. flavus and A. parasiticus colonization under both long and short drought stress conditions compared with non-stressed conditions. However, no significant differences were obtained in the degree of Aspergillus infection of kernels from PI 337409, Starr and J 11 in the greenhouse experiments. Under microplot conditions, a comparison of several genotypes revealed that kernels of genotypes TX811956 and TX798736 (short stress treatments) contained significantly lower Aspergillus infection and kernels of PI 337409 and TX811956 TX798736 contained significantly less aflatoxin.


Hand-shelled seeds of various groundnut genotypes cured and dried in different ways were assayed for seed colonization by Aspergillus parasiticus in Florida in the years 1971-1974. The assay involved exposing groundnut seed at 20-30% moisture content to conidia of A. parasiticus in petri plates and incubating at 25°C. After 1 week, the percentage of the seeds with sporulating colonies of the test fungus was determined. Typically, individual lines or cultivars were evaluated on the basis of the average of three plates. However, second or third assays of the same seed lots were done on 45 occasions during the 4-year period. Repeat assays yielded data similar to those from the original assay. But assays of specific lines from different seed lots could give different results unless the date of digging, methods of curing and production location were the same. Some apparent shifts in susceptibility of seed to fungal colonization were quite extreme. One lot of stackpole-cured cultivar ‘Altika’ had 12% of seed colonized whereas a window-cured seed lot, dug on the same day from the same plot had 77% of seed colonized. No particular change in the harvesting procedure was consistently associated with increases or decreases in apparent susceptibility. Based on tests of all seed lots of 15 commonly grown cultivars during the years 1971-1974, ‘Florunner’ was the most tolerant and ‘Tifspan’ the most susceptible to seed colonization. The three resistant lines PI 337394F, PI 337409 and UF 71513 had markedly lower percentages of seed colonized man had the other cultivars.


Four peanut genotypes, selected as resistant to invasion by Aspergillus flavus in laboratory screening with rehydrated, stored seed and the cultivar Florunner were subjected to preharvest drought and temperature conditions conducive to A. flavus invasion and aflatoxin contamination. Preharvest aflatoxin contamination of peanuts has been previously correlated with geocarposphere temperature and moisture conditions during drought. All genotypes were highly contaminated with aflatoxin. This study indicates that a critical assessment should be made of the value of using the current laboratory method to select germplasm for resistance to A. flavus invasion and assuming resistance to aflatoxin contamination under field conditions.


Possibilities for reducing the aflatoxin contamination of groundnut include the selection of varieties resistant to Aspergillus flavus.


The effects of damaging the testa and the application of seed dressings were examined in field trials on several short-and long-season cultivars of groundnut with differendy-pigmented testa at Samaru, Nigeria, in 1967. There was a high correlation between the number of seedlings which emerged and the resistance or susceptibility of the seed to invasion by the fungus Aspergillus flavus, as assessed by laboratory tests. When the testa was not damaged the emergence of white

(23.9-97.2 mg g\(^{-1}\)) compared with cotyledons (0.17-0.82 mg g\(^{-1}\)). Some of the methanol-extracted and water-soluble tannin extracts from testae and cotyledons, when incorporated into yeast extract sucrose liquid medium (100 mg L\(^{-1}\)), significantly inhibited growth of A. parasiticus and aflatoxin production. There was no overall correlation between genotypes and the influence of tannin extracts on A. parasiticus growth and aflatoxin production.

(susceptible) seed was only 50 % while that of colored (resistant) seed was between 95 and 98 %. Damage to the testa greatly reduced emergence. The application of seed dressing increased the emergence of susceptible seed and also restored the emergence of damaged seeds to the level of undamaged seeds. The effect of the complete removal of the testa was not counteracted by seed dressing; naked seeds, with and without dressing, gave 10 % emergence. The importance of pigment in the testa, the condition of the seed and the effects of seed dressing are discussed.


Two runner type groundnut cultivars, Sunbelt Runner and Florunner, identified by a laboratory method as having large differences in seed resistance to colonization by Aspergillus flavus, were evaluated for resistance to natural seed infection by A. flavus and subsequent aflatoxin contamination. Groundnuts were grown on three nonirrigated farms during 1980 using two planting dates and three harvest dates for each cultivar. Groundnuts grown on two farms experienced moderate to severe drought stress and both cultivars contained high levels of aflatoxin. Groundnuts on the third farm had adequate rainfall and contained only very low levels of aflatoxin. Sunbelt Runner (reported to be resistant to A. flavus colonization of seeds) had no advantage over Florunner (reported to have only moderate resistance to seed colonization) in respect of levels of A. flavus infection and aflatoxin contamination under field conditions. Levels of A. flavus infection and aflatoxin contamination were related primarily to environmental conditions, especially drought stress, during pod maturation. These results show that the current laboratory assay method for selecting resistant lines should be carefully reassessed.


Two runner type groundnut cultivars, "Sunbelt Runner" and "Florunner", were compared under differing field conditions for natural seed infection by Aspergillus flavus and aflatoxin contamination. Laboratory tests had shown marked differences in seed resistance to colonization by A. flavus. Groundnuts were grown on three nonirrigated farms during 1980 using two planting dates and three harvesting dates for each cultivar. Groundnuts grown on two farms experienced moderate to severe drought stress and both cultivars contained high levels of aflatoxin. Groundnuts on the third farm received adequate rainfall and contained only very low levels of aflatoxin. Sunbelt Runner (reported to be resistant to A. flavus colonization of seeds) had no advantage over Florunner (reported to have moderate resistance to seed colonization) in respect of levels of A. flavus infection and subsequent aflatoxin contamination under field conditions. Levels of A. flavus infection and aflatoxin contamination were related primarily to environmental conditions, especially drought stress, during pod maturation. These results show that the current laboratory assay method for selecting resistant lines should be carefully reassessed.


Twenty-eight groundnut genotypes were tested for resistance to seed colonization by Aspergillus flavus and for aflatoxin content. Of these, J 11, TMV 12 and Ah 7223, in general, were found to have less seed colonization. Ten cross derivatives, involving J 11 as pollen parent in some cases, were also tested for seed colonization. Genotypes OGO-2 and 1-4 had seed colonization of 8-9 %. Seed treatment with solid sodium chloride at 2 % level inhibited seed colonization considerably. Aflatoxin content was found to vary among genotypes. Those which had more than 3 % phenols were found to contain less aflatoxins. Fumigation with ammonia was found to be highly effective in detoxification of aflatoxins (68 %). NaOH spray (2 %) also reduced aflatoxins by about 70 %. Fumigation with burning cowdung fumes and sun drying for one day were found effective in reducing aflatoxin (16-25 %).


The potential of Aspergillus flavus isolates to produce aflatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings of groundnut were studied. Isolates NRRL 3000 and V3734/10 produced high levels of aflatoxins in culture. Culture filtrates from the isolates and from NRCGAFA were most toxic to seeds and seedlings. Commercial cultivars, advanced breeding lines, and wild Arachis species were screened for resistance to in vitro colonization of seeds by A. flavus isolates, and to aflatoxin production. Genotypes CGC 2, 1-4, CGC 7, S230, derivatives of S230 x PI 337394F, Latur 33 x PI 337394F, and the wild species, Arachis cardenasii and A. duranensis were resistant to seed colonization by A. flavus. All genotypes of groundnut and three wild Arachis species supported high production of aflatoxins by NRRL 3000, but only trace levels were produced in A. cardenasii and A. duranensis. Aflatoxins were found (range of 27-146 μg kg⁻¹) in
Commercial groundnut cake and in de-oiled cake. Moisture intake capacity, levels of seed coat phenols, and protein content of seeds were considered to influence aflatoxin contamination levels. Soaking seeds in various organic and inorganic substances was found to influence the degree of seed colonization by *A. flavus* and of aflatoxin production in *in vitro* inoculation tests.


Fourteen groundnut genotypes were evaluated for resistance to *Aspergillus parasiticus* infection in 1983 and 1984 in rain-shaded field microplots where water stress conditions were simulated, and in unshaded microplots under normal rainfall conditions. A brown color mutant of *A. parasiticus* (ATCC 24690) was grown on cracked corn and the colonized corn was spread on the soil in each microplot. Infection of groundnut pegs, pods, kernels and tap and fibrous roots was enhanced by low soil moisture. Infection of kernels differed among genotypes; all other tissues were found to be infected at moderate to high frequency and no genotypic differences were observed. Genotypes J11 and Lampang were characterized as resistant to *A. parasiticus* under both dry and moist field conditions. Although percentage infection of kernels varied with genotype, ranking of genotypes reported to have drought resistance was consistent under both dry and moist field conditions. Thirty-four genotypes, including those tested in field microplots, were also evaluated for resistance to seed colonization by *A. parasiticus* in the laboratory. Genotypes J11 and PI 337409 were highly resistant. Except for J11, there was no correlation between genotype rankings for resistance to seed colonization and resistance to seed infection under field conditions.


The percentage of intact groundnut pods with sporulating colonies of *Aspergillus flavus* varied widely following inoculation of pods of different groundnut breeding lines and incubation at 25°C for 14 days. The pods had been windrow-cured for 2-3 days and then artificially dried in a forced air drier at 35°C. Pod moisture content was adjusted to 25 % at the time of inoculation. Although 0-100 % of the pods of the different lines were colonized, only 0-40 % of the seeds within those pods were invaded. The presence of *A. flavus* colonies on the surface of intact pods was not correlated with the presence of infected seeds within these pods. In addition, some apparently noncolonized pods contained infected seeds. With similar shell colonization, groundnut lines with tolerance to seed colonization had fewer seeds colonized than had the more susceptible lines. With similar seed susceptibility, those lines with lower shell colonization also had fewer seeds invaded. Differences in shell susceptibility appeared to be due to the presence of antagonistic microflora.


Pods and seeds from 17 groundnut genotypes were separately tested for resistance to colonization by *Aspergillus parasiticus* after each of two successive growing seasons. In each year's tests, pods of three genotypes remained completely free from colonies of the test fungus, whereas all the pods of two genotypes in the first year and of three genotypes in the second year had at least one colony. The percentages of inoculated pods and seed colonized by *A. parasiticus* were inversely correlated (r = -0.5 and -0.6 in tests 1 and 2, respectively). The shell of the intact pod seemed to provide an effective barrier to *A. parasiticus*. The quantity of aflatoxin B1 in seeds of four genotypes was correlated (r = 0.89) with the percentage of pods with surface colonies at 21 days after inoculation. The latter values also were correlated (r = 0.89 and 0.94) with the percentage of seeds that had been penetrated and those with surface colonies, respectively. Resistance of pods to *A. parasiticus*, however, may not be a true plant resistance, since genotypes that were relatively resistant one year were susceptible the next, and vice versa. Colonies arising from natural infections occurring before the pods were artificially inoculated accounted for only three of the seven significant changes in the percentage of pods with colonies between the first and second tests.


Hand-shelled, intact seeds of 165 groundnut breeding lines were assayed for seed colonization by three isolates of *Aspergillus flavus* (NRRL 3794, NRRL 2999, and one Florida isolate). Significant differences in tolerance to colonization by the fungus were noted between the lines. Seed coats of the tolerant lines were not as permeable as those of the susceptible lines. An intact testa was required for tolerance and appeared to act as a mechanical barrier to penetration by the fungus. Seeds of tolerant lines appeared to possess more wax-like accumulations on the testa than did susceptible lines. Several preconditioning factors that fluctuate under growing and storage conditions were found to affect the tolerance expressed by two breeding lines and the moderately tolerant Florunner variety. Tolerance was lost after 1 year of storage of shelled groundnuts compared to non-shelled groundnuts of the same tolerant genotypes.

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Hand-shelled, intact seeds of 165 groundnut breeding lines were assayed for seed colonization by three isolates of *Aspergillus flavus* (NRRL 3794, NRRL 2999, and one Florida isolate). Significant differences in tolerance to seed colonization were noted between the lines. No differences in tolerance were found when seed coats were punctured with a needle or abraded with carborundum before inoculation. When intact seeds were soaked in an aqueous solution of 1.0 % 2,3,5-triphenyl-2H-tetrazolium chloride (TZC), a red stain occurred in the cotyledons of the susceptible lines, but not in those of the tolerant lines, indicating that the seed coats of the tolerant lines were not as permeable as those of the susceptible lines. Aqueous extracts of intact seeds of both tolerant and susceptible lines stimulated germination of *Aspergillus flavus* conidia when compared to conidia incubated in distilled water. Diethyl ether extracts of intact seeds of tolerant groundnut lines did not inhibit germination of conidia. Thus, an intact testa was required for tolerance and appeared to function as a mechanical barrier to penetration by the fungus.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has comprehensive responsibility for research on its mandate crops of sorghum, pearl millet, pigeonpea, chickpea, and groundnut. However, priority has been given to field problems and only limited research has been done on postharvest problems. The highest priority project with important postharvest and storage aspects is the aflatoxin problem in groundnut. The research emphasis at ICRISAT has been placed on utilization of genetic resistance to develop groundnut cultivars with pods or seeds which the fungus cannot invade, or which if invaded do not support aflatoxin production. A number of breeding lines and cultivars have been identified which have seeds with good resistance to invasion by *A. flavus*. All genotypes so far tested have supported production of aflatoxins but some have given much slower rates of toxin accumulation than have others.

The aflatoxin contamination problem in groundnut is reviewed in detail, with special reference to African groundnut producing countries, and research needs are highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus *Aspergillus flavus*.

Research in several countries into evaluation of responses of groundnuts to seed infection and colonization by *Aspergillus flavus* and/or aflatoxin production is reviewed, and progress made in this field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is summarized. Several laboratory and field screening procedures have been developed to screen groundnuts for resistance to *A. flavus* infection and/or aflatoxin production. Research on the effects of environmental factors on pod and seed invasion by *A. flavus* has provided information useful in the development of field screening methods. For instance, imposed drought stress has been used to improve large-scale field screening of groundnut genotypes for resistance to preharvest infection of seeds by *A. flavus*. Several genotypes were found resistant to infection, and some of them were also resistant to in vitro seed colonization by *A. flavus* in laboratory inoculation tests. Two genotypes supported only very low levels of aflatoxin B₁ production when seeds were colonized by an aflatoxin-producing strain of *A. flavus*. The usefulness of these resistances in management of aflatoxin contamination is discussed.

Twenty-one groundnut genotypes reported resistant and susceptible to in vitro seed colonization by *Aspergillus flavus* were tested for field resistance to seed infection, particularly preharvest infection and for aflatoxin contamination: report of work done during May 1988-April 1989. Paris, France: Institut de Recherches pour les Huiles et Oleagineux. 76 pp.

The stams of the global aflatoxin problem is reviewed with special reference to African groundnut producing countries, and research needs are highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus *Aspergillus flavus*.


genotypes. Low levels of infection (1-3 %) found in the cultivar 55-437 in all the northern groundnut growing regions of Senegal indicated its stable resistance to field infection of seed by *A. flavus*.


This paper describes laboratory procedures to screen groundnuts for resistance to seed infection and colonization by *Aspergillus flavus*, and to aflatoxin production. Some methods for aflatoxin analysis are also outlined.


The possible use of genetic resistance to seed invasion by *Aspergillus flavus* and to aflatoxin production is considered and some research data presented. Many species of fungi have been found associated with groundnut seeds and several are known to be capable of producing mycotoxins on suitable substrates. Reports of natural occurrence of mycotoxins in groundnuts are reviewed, and the natural occurrence of citrinin and zearalenone reported. Preliminary data on mycotoxins production by fungi isolated from groundnuts are presented.


Aflatoxin contamination of groundnut is a serious problem in most groundnut producing countries and as such is given high priority by the Groundnut Improvement Program of ICRISAT. Since 1979 the emphasis has been on selecting cultivars resistant to seed invasion and colonization by toxigenic suains of *Aspergillus flavus*, and/or to aflatoxin production following invasion by the fungus. Of 850 germplasm lines screened for reaction to seed invasion and colonization by *A. flavus* in laboratory tests, eight proved resistant. Three of these lines (PI 337394F, PI 337409 and UP 71513) had been reported resistant in the USA but the other five were new sources of resistance. Several groundnut cultivars with aflatoxin have been screened for seed resistance in the field, both under natural conditions and with the inoculum of the fungus added to the soil in the pod zone. Some cultivars with resistance to seed colonization also showed resistance to seed invasion by *A. flavus*. None of the cultivars tested was completely resistant to aflatoxin production but significant cultivar differences occurred in the amounts of aflatoxin produced in seeds inoculated with a toxigenic strain of *A. flavus*.


Nine groundnut genotypes were tested for resistance to seed colonization by five different strains of *Aspergillus flavus*. These genotypes, and the cultivar J 11 which has been shown to be resistant to seed colonization by *A. flavus*, were also checked for production of aflatoxin following infection of scarified, surface-sterilized seeds by three aflatoxigenic strains of *A. flavus*. The genotypes PI 337394F and PI 337409 showed significantly less seed colonization and internal invasion than the other genotypes. The *A. flavus* strains differed significantly from one another in their ability to colonize seeds and produce internal infection. Strain NRRL 3000 was the least effective. Of the three strains used in the aflatoxin production tests. AF 8-3-2A produced the highest levels of aflatoxin B₁ on all genotypes while AFS-3 produced the least; NRRL 3000 being intermediate in this respect. Aflatoxin G₁ was produced on all genotypes by NRRL 3000, and on J 11 by AF 8-3-2A. There was no obvious correlation between seed resistance to *A. flavus* colonization and aflatoxin production when seeds were infected. Significantly higher amounts of aflatoxin B₁ were produced in the two genotypes resistant to *A. flavus* colonization than in the highly susceptible genotype FESR-11-P11-B2-B1.


*Aspergillus flavus* colonization levels on 10 groundnut genotypes were significantly higher on seed from the 1979/80 and 1980/81 postrainy season crops than on seed from the 1979 and 1980 rainy season crops. Sixty-four groundnut genotypes were tested for resistance to seed colonization by *A. flavus* in relation to crop season, location (fields) and period of windrow drying. Seed colonization levels on the genotypes were higher on seed from the 1980/81 postrainy season crops than on seed from the 1981 rainy season crops. Windrow drying treatment for 48 h in the postrainy season resulted in significantly higher percentages of seed colonized compared to the 24 h treatment. Significant interactions occurred between genotypes and locations (fields) in both the rainy and postrainy seasons. Levels of seed colonization by *A. flavus* can be influenced by growing season, crop location, and postharvest drying treatment.

Groundnut cultivars with seed resistant to invasion by *Aspergillus flavus* were grown in replicated field trials at ICRISAT Center, Patancheru, India, during 1981 to 1984. Addition of inoculum of an aflatoxigenic strain of *A. flavus* to the pod-zone soil increased preharvest *A. flavus* seed infection and aflatoxin contamination. Levels of aflatoxin B1 contamination were positively correlated with percentage of seed infected by *A. flavus*, irrespective of whether or not inoculum was added to the soil. Genotypic differences for seed infection by *A. flavus* and for aflatoxin contamination were about the same in both control and inoculum treated plants. Levels of seed infection by other soil fungi were not significantly influenced by addition of *A. flavus* inoculum to the pod-zone soil. Most of the genotypes included as resistant to in vitro seed colonization by *A. flavus* of rehydrated, mature, undamaged, stored seed, also showed resistance to invasion by the fungus in the field.


Several groundnut genotypes reported as resistant, susceptible or highly susceptible to in vitro colonization of rehydrated, mature, stored, undamaged seed by *Aspergillus flavus* (IVSCAF) were tested for natural seed infection by *A. flavus* and other fungi in two or more replicated field trials at ICRISAT Center, Patancheru, India, in 1979-1984. Undamaged pods were sampled before maturity, at optimum maturity (normal harvest) and when over-mature (late harvest) and seed examined for infection by *A. flavus* and other fungi. In the 1983 and 1984 rainy and 1983/84 postrainy seasons, only four genotypes (one resistant and three susceptible) were tested, and seed were also tested for aflatoxin content. In all seasons the genotypes reported as IVSCAF-resistant had significantly lower levels of seed infection with *A. flavus* and other fungi than did genotypes reported as IVSCAF-susceptible. Genotypic differences in levels of seed infection by *A. flavus* were consistent over seasons. The resistant cultivar J 11 had a significantly lower aflatoxin content than the other three IVSCAF-susceptible genotypes tested in the 1983 and 1984 rainy and 1983/84 postrainy seasons. Drought stress in the 1984 season apparently increased susceptibility to seed infection by *A. flavus* and other fungi, and to aflatoxin contamination, in all genotypes. Seed infection by *A. flavus* and other fungi, and aflatoxin contamination increased with increasing maturity of pods, indicating the importance of lifting the groundnut crop at optimum maturity.


Drought stress during late stages of pod maturation in an irrigated groundnut crop during the postrainy season significantly increased the level of seed infection by *A. flavus*. A line-source sprinkler irrigation system imposing a drought-stress gradient...
was used for field screening of groundnut genotypes for resistance to seed infection by *A. flavus*. A significant, positive, linear relationship was found between water deficit (drought intensity) and seed infection in groundnut genotypes. Genotypic differences for seed infection by *A. flavus* were evident at all levels of drought stress, but, under the more severe drought stress conditions, the genotypes resistant to *A. flavus* had low but positive levels of seed infection giving improved statistical precision.


Screening, selection and breeding procedures for increasing the resistance of groundnut varieties to aflatoxin-producing strains of *Aspergillus flavus* are reviewed and discussed. This review includes consideration of sources and nature of resistance, reaction of genotypes to seed colonization, variation among fungal isolates of *A. flavus*, and factors associated with the interaction of aflatoxin-producing strains of the fungus and seed of groundnut genotypes. The pros and cons of breeding an improved groundnut variety with greater resistance to aflatoxin contamination are presented.


The potential for using genotypes identified to be resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus* for breeding agronomically suitable groundnut cultivars with resistance to the toxigenic fungi is discussed. Factors affecting seed colonization in groundnuts are discussed and factors associated with resistance to colonization of the groundnut seed tests are reviewed.


Evidence for the possible development of groundnut cultivars with favourable agronomic characteristics and resistance to aflatoxin-producing strains of *Aspergillus flavus* is presented. In studies of F$_2$ and F$_3$ progenies from crosses of resistant and susceptible genotypes data on the frequency distribution and least square estimates of genetic effects showed the possibility of selecting for resistance to seed colonization by the fungus. Yield, value and seed quality data for seven advanced

lines developed by using pedigree selection from crosses showed that potentially favorable groundnut cultivars can be developed.


In laboratory studies, six groundnut genotypes grown at three locations were tested for the effect of pod and seed inoculation methods on seed colonization by *Aspergillus parasiticus* and/or incidental prior infection in the field or storage by *A. flavus* or *A. parasiticus*. The genotypes had been identified as having varying levels of resistance to seed colonization by *A. parasiticus* in laboratory tests. Noninoculated seed (incubated like the inoculated samples) was considerably less colonized. Pod inoculation resulted in a lower subsequent seed colonization of the more susceptible genotypes when compared to seed inoculation (genotype x method interaction). For all three methods, seed colonization was consistently less for the resistant genotypes than for the 'Florunner' cultivar or the highly susceptible check PI 343419. There was a location x genotype interaction but the resistant genotypes were colonized less frequently than susceptible genotypes. Pod inoculation resulted in a noticeable reduction in seed colonization of the more susceptible genotypes when compared to seed inoculation (genotype x method interaction). Genotypes resistant to seed colonization by *A. parasiticus* were best identified by seed inoculation.


In laboratory studies, six groundnut genotypes grown at three locations were tested for the effect of pod and seed inoculation methods on seed colonization by *Aspergillus parasiticus* and/or incidental prior infection in the field or storage by *A. flavus* or *A. parasiticus*. The genotypes had been identified in laboratory tests as having varying levels of resistance to seed colonization by *A. parasiticus*. Pod inoculation resulted in noticeably less seed colonization of the more susceptible genotypes compared to inoculated seed (genotype x method interaction). Noninoculated seed incubated similarly to the inoculated samples exhibited considerably less colonization. For all three methods, seed colonization was consistently less for the resistant genotypes than for the 'Florunner' cultivar or the highly susceptible check PI 343419. A location x genotype interaction resulted from the difference in the magnitude of % colonization but the resistant genotypes were colonized less frequently than were susceptible genotypes. Genotypes resistant to seed colonization by *A. parasiticus* could be best identified by seed inoculation.
Reducing aflatoxin contamination in peanut genotypes by selection and breeding. Journal of the American Oil Chemists’ Society 58: 961A-966A.

The potential for developing agronomically suitable cultivars using groundnut genotypes that exhibit resistance to seed colonization by aflatoxin-producing strains of *Aspergillus flavus* is discussed. The practical implications of developing resistant cultivars are presented in data for yield, value, and seed quality for six advanced groundnut lines that were developed by selection from crosses.

Two groundnut lines GFA-1 and GFA-2 are resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus*. GFA-1 was derived from a 1972 cross between lines A137 and A5-5, while GFA-2 was derived from a 1972 cross between line A137 and the cultivar Florunner. Both these lines were derived from one to four single-plant selections made within each F2 through F10 progeny-row generation. Seed and pod yields over a three-year period were equal to or greater than those of Florunner. Average seed colonization of both lines by aflatoxin-producing strains of *A. flavus* and/or *A. parasiticus* was equal to or less than that shown by the resistant control (PI 337409) and much less than that of the Florunner control. These lines have growth habit, testa and flower colors, and plant height similar to that of Florunner. Both are runner market types.

Four groundnut lines AR-1, AR-2, AR-3, and AR-4 are resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus*. These lines are semi-upright in growth habit. AR-1 was derived from a cross between Florunner and a selection from PI 337409, an *A. flavus*-resistant line. AR-2 was derived from a cross between an experimental line A5-5 and a selection from PI 337409. AR-3 was derived from a cross between a selection from PI 337432 and Tifspan, while AR-4 was derived from a cross between PI 337394F (*A. flavus*-resistant line) and Florunner. All four lines have for several years shown greater resistance to aflatoxin-producing strains of *A. flavus* and *A. parasiticus* than have standard cultivars and hundreds of germplasm accessions and breeding lines. The yield potential of these Ones is too low for their practical use as commercial cultivars.

In laboratory inoculation tests, four groundnut lines were identified as resistant to seed colonization by aflatoxin-producing strains of *Aspergillus parasiticus*. Although their yields were poor, it is suggested that their resistance could be used in breeding.

Studies were conducted to determine the potential for reducing aflatoxin contamination using recently released groundnut genotypes found to be resistant to seed invasion by aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus*. When rehydrated groundnut seeds inoculated with *A. parasiticus* were incubated under conditions favoring infection, the resistant genotypes GFA-1, GFA-2, AR-1, AR-2, AR-3, and AR-4 were more resistant than the commercial cultivars, ‘Florunner’ and ‘Sunbelt Runner’, in successive years of evaluating. Aflatoxin contamination of seed in the field was markedly less in the resistant genotypes than in the commercial cultivars. Pod yields of GFA-1 and GFA-2 were similar to those of the commercial cultivars. Use of such resistant genotypes could reduce aflatoxin contamination of seed immediately following harvest or in short term storage when environmental conditions are conducive to infection by *A. flavus* and *A. parasiticus*.

Sources of resistance or tolerance to various diseases and pests of groundnut are listed. Groundnut genotypes have been reported to be resistant or tolerant to bacterial wilt, early leaf spot, late leaf spot, rust, Cylindrocladium black rot, pod breakdown, stem rot, Diplodia collar rot, Verticillium wilt, and Sclerotinia blight. Seventeen genotypes are listed as resistant to in vitro seed colonization by aflatoxin-producing strains of *Aspergillus flavus* or *Aspergillus parasiticus*. Several sources of resistance to bud necrosis, clump and rosette virus diseases are also given. Some genotypes resistant to the major insect pests or nematode diseases of groundnut are presented.

A laboratory procedure for screening groundnut germplasm for resistance to *A. flavus* has been developed: seeds are rehydrated then inoculated with a spore suspension of the fungus and incubated for 7 days, after which seed colonization can be assessed. Of 1406 accessions, selections and varieties tested by this method, two germplasm accessions, PI 3373941 and PI 337409, were resistant to two aflatoxin-producing strains of the fungus. Seed with flesh seedcoat color from PI
337394 had an average of 5 % seed colonization for hand-picked and hand-shelled samples evaluated at optimum seed maturity in five separate evaluations during 4 years. Also, seed colonization for samples of PI 337409 averaged 9 % for similar evaluation. For two years, comparable checks of two susceptible accessions, PI 331326 and PI 343419, had an average of 92 % and 91 % seed colonization, respectively, and 'Argentine', 'Florunner', and 'Wilco I' had an average of 34 %, 39 %, and 30 % respectively.


Two groundnut accessions averaged less than 5 % seed colonization by aflatoxin-producing strains of *Aspergillus flavus* following laboratory inoculation of samples at optimum seed maturity and incubating under conditions highly conducive to fungal development. Comparable checks of two susceptible accessions averaged 89 % and 91 % seed colonization, and Florunner, Goldin I and Argentine varieties averaged 22 %, 23 %, and 38 %, respectively. Three maturity separations of the resistant accessions indicated that sound mature seed were less susceptible to *A. flavus* than immature and overmature seed. Seed from the two resistant accessions and a susceptible check harvested at four 2-week intervals beginning near optimum maturity revealed that delayed harvest increased the incidence of infection, but the resistant selections were considerably more tolerant to delayed harvest than the susceptible check.


Laboratory evaluation of groundnut accessions and varieties for their reaction to two strains of *Aspergillus flavus* revealed two accessions (PI 337394F and PI 337409) with a high degree of resistance to the fungus.


The effects of initially adjusted seed moisture, incubation and storage time, seed maturity, harvest time and seed handling on seed colonization by *Aspergillus flavus* of the two groundnut genotypes PI 337394F and PI 337409 (resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus*), and the susceptible genotype PI 331326 were studied. Seed colonization in PI 337394F was greater at 20 % adjusted seed moisture than at 25 % seed moisture. Colonization was least at 15 and 30 % adjusted moisture. *Aspergillus flavus* colonized a low percentage (17 %) of the cotyledons of PI 337409 after 48 hours of incubation, whereas 100 % of the cotyledons of PI 331326 were colonized. Colonization of PI 337394F seed with intact seed coats increased with each increase in storage time from 0 to 6 and 12 weeks, and for each increase in temperature from 5 to 20 and 35°C. Seed colonization in the immature and overmature seed of both resistant genotypes was greater than in the mature seed. Mature seed of the resistant genotypes were colonized at a low level, with no difference for seed harvested at 4 successive 2-week intervals, whereas PI 331326 had greater colonization for each successive harvest date. Seed coat abrasion, soaking for 5 min. in a H_2SO_4 solution, machine picking or machine-shelling increased colonization of seed over check treatments. Seed colonization of PI 331326 was greater than that of the resistant genotypes under all conditions.


Two groundnut genotypes PI 337394F (Reg. No. GP3) and PI 337409 (Reg. No. GP4) are resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus*. Both lines are of the Valencia botanical type and were found in a collection brought from South America in 1968. The original population of PI 337394 was a mixture of genotypes with seed testa color ranging from light pink to purple. A true-breeding genotype with light pink or flesh testa was designated PI 337394.


Environmental factors influence degree of groundnut seed infection by *Aspergillus flavus* and other fungi. This complicates resistance screening over seasons and locations as levels of infection can vary considerably within a genotype. Statistical methods were used to separate genotypes into different resistance/susceptibility categories and to ensure a stable basis for comparisons of control and test entries across environments. This approach was also adopted to compare the degree and distribution of resistance in Spanish and Valencia groundnuts. The establishment of such procedures would facilitate interpretation of screening data from different environments.

Four groundnut genotypes, two resistant (J 11 and PI 337394F) and two susceptible (TMV 2 and EC 76446 (292)) to in vitro seed colonization by *Aspergillus flavus*, were grown in field trials at ICRISAT Center in the 1984 and 1985 rainy seasons. Geocarposphere mycobiota were examined and significant quantitative and qualitative differences were observed between genotypes. Populations of *A. flavus* were markedly higher in the geocarpospheres of the susceptible genotypes than in those of the resistant genotypes. Genotypes were also evaluated for levels of seed infection by *A. flavus* at harvest. The *flavus*-susceptible genotypes had significantly higher levels of seed infection than the *A. flavus*-resistant genotypes. In a greenhouse experiment pod exudates were collected from potted plants. Exudates from the two resistant genotypes inhibited in vitro germination of *A. flavus* conidia.

Factors determining resistance to aflatoxin contamination are reviewed. Possible factors influencing resistance in groundnut pods and seeds to invasion by *Aspergillus flavus* and to aflatoxin production are considered. A range of groundnut genotypes were examined for presence of inhibitory compounds in testae and cotyledons and considerable variation was found. Tannin-like compounds found in seed testae of some genotypes inhibited *A. flavus* growth and aflatoxin production. Polypeptide contents of seeds varied between genotypes susceptible and resistant to seed colonization by *A. flavus*.

Results of screening of various groundnut genotypes for resistance to seed colonization by *Aspergillus flavus* are presented. Four genotypes (ACC 63, CES 48-30, Celebes, and UPL PN 4) are reported resistant to seed colonization by *A. flavus*.

Progress worldwide in breeding groundnuts resistant to seed colonization by *Aspergillus flavus* and aflatoxin contamination is summarized, and research at ICRISAT is described. Resistance to *A. flavus* infection may occur at various levels, but efforts to breed for resistance have concentrated on the utilization of the resistance in the testae of mature seeds. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), genotypes identified as resistant to in vitro seed colonization by *A. flavus* have been crossed with susceptible cultivars of good agronomic character, and several breeding lines with stable resistance to seed colonization and with acceptable yield and quality have been produced. The genetics of inheritance of testa resistance is discussed. The need for breeders to incorporate other resistance traits is stressed, and future prospects for breeding for resistance to aflatoxin production are discussed.

Data compiled over 4 years showed that 5 cm of soil at a mean of 29.4°C and severe drought 40-50 days before harvest resulted in *Aspergillus flavus* infection and aflatoxin contamination in preharvest groundnuts. Four genotypes, which were rated as resistant to seed colonization by *A. flavus* in a laboratory screening assay, and 2 other genotypes were grown in plots and subjected to the above stress conditions 98 days after sowing. Tests of dried groundnuts harvested 143 days after sowing showed that nuts of all the genotypes contained aflatoxin and were extensively infected by *A. flavus*.
Seeds of three groundnut cultivars (Flori Spanish 334A, Georgia 119-20 and Local Gilan Iran) obtained from the 1974 and 1975 crops of the Seed and Plant Improvement Institute, Karaj, Iran, were stored under conditions conducive to mold attack. When these cultivars were analysed for aflatoxin contamination (using a minicolumn and thin-layer chromatography methods), seeds of Mori Spanish 334A and Georgia 119-20 were contaminated, but the cultivar Local Gilan Iran showed no contamination. This interesting observation should be followed up.

In a study of four Virginia and four Spanish groundnut varieties at 2 sites, preharvest pod damage, by insects or microorganisms, led to increased aflatoxin contamination during storage. Virginia varieties were more easily damaged than Spanish varieties at both sites. Of 350 varieties and lines screened for reaction to seed colonization by Aspergillus flavus in laboratory tests, 16 proved resistant. The commercial cultivars Tainan 9, Tainan 10, Tainung 4 (all Spanish types) and Penghu 2 (Virginia type) were highly susceptible.

Differences in resistance to seed colonization by Aspergillus flavus were observed in 36 groundnut varieties, which could be divided into four classes on this basis. In conditions of drought, some varieties usually susceptible were classified as resistant, although A. flavus-resistant varieties remained the most resistant.

During the 1989 rainy season, 25 lines, including germplasm of advanced Aspergillus flavus-resistant breeding lines and some cultivars from West Africa, were tested in three locations (Sadore, Bengou and Maradi) in Niger. More than 50 % of seed were infected by A. flavus at Sadore. Average seed infection depended on the location (25 % at Sadore, 13 % at Bengou and 13 % at Maradi). Significant differences between genotypes were found. Genotypes 55-437 and J 11 were the least infected lines. Among the 1CR1SAT advanced A. flavus-resistant breeding lines ICGV 87107, ICGV 87094 and ICGV 87110 were the least infected. At the same locations trials were conducted to estimate the yield and plant losses from seedling diseases of groundnut, using 2 fungicides to control seedling diseases. Seeds were treated with thiran or corvet CM at the rate of 3 g kg⁻¹ seed. The percentage of unprotected plants that died after emergence in the non- fungicide treatment ranged from 19-43 + 3.2 %. Seed treated with fungicide produced higher yields than the untreated seed. ICGS 11 showed a high percentage of plant losses in all three locations in the untreated plots. There were no significant differences between the two fungicides for these traits.

Some 40 groundnut genotypes were screened for field resistance to seed infection by Aspergillus flavus in trials at Bambe and Darou in Senegal during the period 1977-1980. Significant varietal differences were observed at harvest for levels of natural seed infection by A. flavus. Field resistance to A. flavus in genotypes were positively correlated with previously measured resistance to seed colonization by A. flavus in laboratory inoculation tests. The commercially grown cultivar 55-437 had high levels of resistance to A. flavus in both field and laboratory screening, while two other cultivars (73-30 and 73-33) also grown in Senegal had moderate levels of resistance. Genotypes with seed resistance to A. flavus had a lower proportion of A. flavus in their rhizosphere mycoflora than had genotypes susceptible to seed infection by the fungus.

Experiments were carried out on 24 varieties of groundnut for their reaction to pod and seed infection by Aspergillus flavus in the field in Darou, Senegal, and for seed colonization in artificial inoculation tests in the laboratory in Paris. The varietal differences observed in seed colonization in inoculation tests and in infection of pods and seeds in the soil before harvest appeared to be linked to the shell and seed coat structure. Drought at the end of the growth cycle was a contributing factor to increased infection.

Results of studies on groundnut hybrids selected to restrict infestation by Aspergillus flavus.]. Resultats de recherches sur les hybrides
A brief survey of breeding groundnut for resistance to *Aspergillus flavus* is presented. It is noted that marked varietal differences exist and that the extent of seed infection in natural field conditions was correlated with that obtained using in vitro inoculation. Varieties bred using resistant material from India, Senegal and the USA gave good results in Senegal; they included 55-437, 73-30, 57-422 and 73-33.


In field trials at Darou, Senegal in 1975. 33 groundnut varieties were tested under drought conditions favourable for infection by *Aspergillus flavus*. Varieties were sown on two dates (normal sowing date, late sowing date) and were harvested at maturity and 14 days before maturity. Significant differences were found between varieties for pod infection (0.8-8 %) and for seed infection (0.4-4 %) by *A. flavus*. Among the 10 varieties least infected were PI 337394F and PI 337409 (previously reported resistant to seed colonization by *A. flavus* in artificial inoculation tests in the laboratory), Florunner and seven varieties selected in Senegal for resistance to drought. In laboratory inoculation tests, varieties showing field resistance in general showed resistance to seed colonization by the fungus. However, the results varied considerably for certain varieties depending on the sowing and harvesting dates.


Seven trials, each on some 40 varieties of groundnut at 2 locations (Bambey and Darou) in Senegal, were carried out in 1976-1980. Natural seed infection by *Aspergillus flavus* in the field varied with varieties, and was strongly correlated to seed colonization in in vitro inoculation tests, indicating the value of the latter method in selecting resistant varieties. A Senegalese variety of proven agronomic value, 55-437, showed resistance similar to that of the resistant control genotype PI 337409. Two other varieties, 73-30 and 73-33, were considered tolerant to *A. flavus*.

### 8.2.6 Mechanisms of Resistance to *Aspergillus flavus*

**Colonization and Infection**


The testae of three *Aspergillus flavus*-resistant (PI 337394F, UF 734022, PI 337409) and three susceptible (UF 73515, PI 331326, PI 343419) lines of groundnut were analyzed for soluble amino compounds and carbohydrates. Water-soluble nitrogenous compounds were found within the testae of resistant lines in significantly lower concentrations (17.09-28.09 µmol g\(^{-1}\)) than those in testae of susceptible lines (33.94-65.28 µmol g\(^{-1}\)). Arginine, glycine, lysine, ammonia, methionine, and aspartic acid contents were most strongly correlated with susceptibility. These results suggest that the nonavailability of readily soluble, small molecular weight amino compounds on or within the testa matrix may play a role in the mechanism of *A. flavus* resistance exhibited by some groundnut lines.


In 18 groundnut genotypes of known resistance or susceptibility to *Aspergillus flavus* grown in the USA or in Puerto Rico, the total content of soluble amino compounds in the testa was directly correlated with susceptibility except in two susceptible lines, UF 71513 and Tifspan, which had a low content. In a further study of 12 genotypes, including UF 71513 and Tifspan. susceptibility was associated with a low content of acid-hydrolysable arabinose in the testa.


Of 23 genotypes evaluated in a humidity chamber, Florunner, PI 337409, 55-437 and Texas 7 were the most resistant, with low infection by *Aspergillus parasiticus* and low aflatoxin contamination. Levels of tannin compounds in seed coats and cotyledons differed among genotypes, with levels being higher in seed coats. Some compounds significantly decreased the growth of *A. parasiticus* and inhibited the production of aflatoxin.

Twenty-three groundnut genotypes were evaluated for resistance to seed colonization by Aspergillus parasiticus and aflatoxin production when incubated under high relative humidity. Tannin-containing extracts from seed testae and cotyledons of these genotypes were prepared and tested for their effect on growth of A. parasiticus and aflatoxin production. Seed colonization was low (< 30 %) in the genotypes Toalson X UF 73-4022 (selections TX-798731 and TX-798736), A72118, 55-437, PI 337409 and Florunner. Genotypes with low levels of seed colonization also had low anatoxin contamination. Higher levels of tannins were detected in testae (23.9-97.2 mg g⁻¹) compared with cotyledons (0.17-0.82 mg g⁻¹). Some of the methanol-extracted and water-soluble tannin extracts from testae and cotyledons, when incorporated into yeast extract sucrose liquid medium (100 mg L⁻¹), significantly inhibited growth of A. parasiticus and aflatoxin production. There was no overall correlation between genotypes and the influence of tannin extracts on A. parasiticus growth and aflatoxin production.


Several tannin-like compounds were tested in a liquid nutrient medium at concentrations of 100, 500, and 1000 mg L⁻¹ to determine their influence on growth of Aspergillus parasiticus and aflatoxin production. Fungal growth inhibition was greatest using tannic acid, catechol, and methyl catechol. Aflatoxin production was significantly decreased by methyl catechol, naringenine, umbelliferone, and hydrobenzoic acid. Ferulic acid caused significant growth inhibition at concentration of 1000 mg L⁻¹. Tannin-like compounds extracted from groundnut seed coats also inhibited fungal growth and aflatoxin production.


Phenolic acids were extracted from seed coats of twenty-three groundnut genotypes with an acetone-water solution. The acetone was evaporated and the extract partitioned in ethyl acetate. This phase was roto-evaporated to dryness at 35-40° C, redissolved in methanol, filtered, and injected into a C18 column with a 10 um particle size. Phenolic acids were detected at a wavelength of 254 nm. Twelve different compounds were separated. Comparisons with known compounds revealed a preliminary identification of protoatechnic acid, genetisic acid, catechin, methyl catechin, epicatechin and p-coumaric acid. Correlation analysis of the ability of the initial testae extracts to inhibit Aspergillus flavus and A. parasiticus growth and aflatoxin production in liquid culture media revealed R² values which ranged from 0.36 to 0.89. Testae extracts from seed of genotypes which contained relatively high levels of phenolic acids caused the least inhibition of fungal growth and aflatoxin production. Dark testae color was negatively correlated with kernel resistance and aflatoxin production. The concentration of specific phenolic acids was variable among genotypes and was related to testae color, market type, and selections within a genotype. Some positive correlations were obtained with specific phenolic acids and inhibition of fungal growth and aflatoxin formation in extracts from groundnut genotypes classified in the Virginia market type.


After soaking the seeds of four varieties of groundnut in deionized water for periods ranging from 5 to 60 min, the water from soaking cultivar 28-206 had the highest electrical conductivity and that from Florunner the least, showing that these varieties had respectively the most and least permeable testae. It is suggested that these measurements could constitute a rapid test of seed resistance to penetration by Aspergillus flavus.


Environmental conditions necessary for preharvest aflatoxin contamination of visibly sound groundnuts are reviewed on the basis of studies conducted at Dawson, Georgia, USA during six consecutive crop years using six environmentally controlled plots. The role of temperature and moisture in preharvest aflatoxin contamination of groundnuts was established. Preventive measures, including the use of so-called 'resistant varieties', calcium nutrition, and irrigation, were evaluated using environmentally controlled plots. The studies showed that groundnuts do not become contaminated with aflatoxins in the absence of severe and prolonged drought stress in spite of infection levels of up to 80 % by the aflatoxin-producing fungi, Aspergillus flavus and A. parasiticus. Also, larger, more mature groundnut
The ultrastructures of seed coats of mature seeds of a n
resistant and susceptible genotypes are described. The groundnut seed coat may
more tightly packed than in the susceptible genotypes PI 343360 and PI 343326. It
is suggested that the structure of the testa is genetically determined.


Acetone extracts of a variety of groundnut were shown by Lindsey and Turner (1975) to inhibit the growth of Aspergillus flavus. They identified one of the inhibitory substances as 5,7-dimethoxyisoflavone. This present work with the use of standards and high performance liquid chromatography shows that a huge number of groundnut genotypes contain not the dimethoxy compound but 5,7-dihydroxyisoflavone. Twenty genotypes were screened for resistance to A. flavus in the laboratory. The resistance of these genotypes and their correlation to 5,7-dihydroxyisoflavone content are discussed. The fungal inhibition characteristics of the dihydroxyisoflavone are described.


The groundnut genotypes PI 337394F and PI 337409, resistant to seed colonization by Aspergillus parasiticus, had thinner testae in which the collapsed cell walls were more tightly packed than in the susceptible genotypes PI 343360 and PI 343326. It is suggested that the structure of the testa is genetically determined.


The ultrastructures of seed coats of mature seeds of an Aspergillus flavus-resistant genotype, PI 337394F and of a susceptible genotype, PI 343326 were studied. Observations were made on thin sections of epoxy-embedded samples by transmission electron microscopy and the ultrastructures of the seed coats of the resistant and susceptible genotypes are described. The groundnut seed coat may serve as a structural barrier to the hyphae of the aflatoxigenic fungus A. flavus.


Samples of Florunner groundnuts were collected throughout a period of late-season drought stress with mean geocarposphere temperature of 29 and 25°C, and determinations of maturity, kernel water activity (aw), percentage moisture, capacity for phytoalexin production and aflatoxin contamination were made. Results showed an association between the loss of the capacity of kernels to produce phytoalexins and the appearance of aflatoxin contamination. Kernel water activity (aw) appeared to be the most important factor controlling the capacity of kernels to produce phytoalexins. Mature groundnuts possessed additional resistance to contamination that could not be attributed solely to phytoalexin production. Kernel moisture loss was accelerated in the 29° treatment compared with the 25°C treatment, and data indicated that the higher soil temperature also favored growth and aflatoxin production by Aspergillus flavus in groundnuts susceptible to contamination.


Seed coat leachates from some of the 36 groundnut cultivars were inhibitory to spore germination of Aspergillus niger and A. flavus and others were stimulatory. Maximum inhibition of A. niger (89.74 %) and A. flavus (74.47 %) was observed in leachates of Jyoti and TMV-12, respectively. The inhibition and/or stimulation is attributed to the presence of chemicals in seed coats which may effect invasion of the seed by Aspergillus spp.


Thirteen groundnut genotypes, eight resistant and five susceptible to in vitro seed colonization by Aspergillus flavus were grown in replicated trials at three locations in Andhra Pradesh, India. Seed coats of cured, mature seeds of these genotypes were analyzed for polyphenols using different methods. No significant correlation was observed between seed colonization and polyphenol content.

Seeds from two *Aspergillus flavus* resistant and three *A. flavus*-susceptible genotypes grown in 1972 and 1973 in Puerto Rico and in Texas in 1973, were tested for water uptake and drying characteristics. Growing season and location significantly affected rate and total water uptake of the cured seeds. The rate and total water uptake were greater for seeds from Puerto Rico than those from Texas. Seeds of the two *A. flavus*-resistant genotypes and Florunner had slower rates of water uptake and dried out at a faster rate than two of the *A. flavus*-susceptible genotypes. Lipid and protein contents of seeds were not correlated with water uptake or drying characteristics, but varied with growing season and site.


The percentage of intact groundnut pods with sporulating colonies of *Aspergillus flavus* varied widely following inoculation of pods of different groundnut breeding lines and incubation at 25°C for 14 days. The pods had been windrow-cured for 2-3 days and then artificially dried in a forced air drier at 35°C. Pod moisture content was adjusted to 25% at the time of inoculation. Although 0-100% of the pods of the different lines were colonized, only 0-40% of the seeds within those pods were invaded. The presence of *A. flavus* colonies on the surface of intact pods was not correlated with the presence of infected seeds within these pods. In addition, some apparently noncolonized pods contained infected seeds. With similar shell colonization, groundnut lines with tolerance to seed colonization had fewer seeds colonized than had the more susceptible lines. With similar seed susceptibility, those lines with lower shell colonization also had fewer seeds invaded. Differences in shell susceptibility appeared to be due to the presence of antagonistic microflora.


Various fractions of polyphenols were isolated from groundnut seedcoats and hulls and were assayed for their fungistatic properties on *Aspergillus parasiticus* NRRL 2999. The fractions exerted different degrees of inhibition. The fractions were also assayed for their ability to inhibit aflatoxin production. Isolation and partial characterization of the polyphenol fractions were performed.


Hand-shelled, intact seeds of 165 groundnut breeding lines were assayed for seed colonization by three isolates of *Aspergillus flavus* (NRRL 3794, NRRL 2999, and one Florida isolate). Significant differences in tolerance to colonization by the fungus were noted among the lines. Seed coats of the tolerant lines were not as permeable as those of the susceptible lines. An intact testa was required for tolerance and appeared to act as a mechanical barrier to penetration by the fungus. Seeds of tolerant lines appeared to possess more wax-like accumulations on the testa than did susceptible lines. Several preconditioning factors that fluctuate under growing and storage conditions were found to affect the tolerance expressed by two breeding lines and the moderately tolerant Florunner variety. Tolerance was lost after 1 year of storage of shelled groundnuts compared to non-shelled groundnuts of the same tolerant genotypes.


Hand-shelled, intact seeds of 165 groundnut breeding lines were assayed for seed colonization by three isolates of *Aspergillus flavus* (NRRL 3794, NRRL 2999, and one Florida isolate). Significant differences in tolerance to seed colonization were noted between the lines. No differences in tolerance were found when seed coats were punctured with a needle or abraded with carborundum before inoculation. When intact seeds were soaked in an aqueous solution of 1.0 % 2,3,5-triphenyl-2H-tetrazolium chloride (TZC), a red stain occurred in the cotyledons of the susceptible lines, but not in those of the tolerant lines, indicating that the seed coats of the tolerant lines were not as permeable as those of the susceptible lines. Aqueous extracts of intact seeds of both tolerant and susceptible lines stimulated germination of *Aspergillus flavus* conidia when compared to conidia incubated in distilled water. Diethyl ether extracts of intact seeds of tolerant groundnut lines did not inhibit germination of conidia. Thus, an intact testa was required for tolerance and appeared to function as a mechanical barrier to penetration by the fungus.


Wax-like accumulations were noted in scanning electron micrographs on the testae of dried groundnut seeds. Seeds of a breeding line resistant to colonization by *Aspergillus flavus* (NRRL 2999) appeared to possess more of the wax-like accumulations than did several lines that were highly susceptible to colonization by...
the fungus. Extraction of waxes and lipids from intact seeds with a chloroform-methanol (2:1, v/v) mixture, for up to five minutes increased the susceptibility of the seeds without reducing their germination. Conidial germination was slightly stimulated when a conidial suspension of A. flavus was placed on the dried solvent residue after extraction of intact resistant groundnut seed. It appears that the wax-like accumulations help prevent A. flavus from penetrating the intact seed coat.


Serial harvesting trials were carried out at Mokwa and Kano Agricultural Research Stations, Northern Nigeria, in 1963 and 1964 to investigate the occurrence of aflatoxin in the groundnut crop at lifting, when harvested before, at, and after the normal time. This work formed part of the programme of work on the toxicity of the groundnut crop in Northern Nigeria. Crops harvested either at or earlier than the normal time were free from aflatoxin, but late harvesting usually resulted in some toxicity. The weather, as well as the age of the crop, had some effect on toxicity; wet weather delayed the appearance of aflatoxin. Pods with shells damaged while the crop was in the ground were more likely to contain toxic kernels than were pods with undamaged shells.


Aspergillus flavus colonization levels on 10 groundnut genotypes were significantly higher on seed from the 1979/80 and 1980/81 postrainy season crops than on seed from the 1979 and 1980 rainy season crops. Sixty-four groundnut genotypes were tested for resistance to seed colonization by A. flavus in relation to crop season, location (fields) and period of windrow drying. Seed colonization levels on the genotypes were higher on seed from the 1980/81 postrainy season crops than on seed from the 1981 rainy season crops. Windrow drying treatment for 48 h in the postrainy season resulted in significantly higher percentages of seed colonized compared to the 24 h treatment. Significant interactions occurred between genotypes and locations (fields) in both the rainy and postrainy seasons. Levels of seed colonization by A. flavus can be influenced by growing season, crop location, and postharvest drying treatment.


The effects of initially adjusted seed moisture, incubation and storage time, seed maturity, harvest time and seed handling on seed colonization by Aspergillus flavus of the two groundnut genotypes PI 337394F and PI 337409 (resistant to seed colonization by aflatoxin-producing strains of Aspergillus flavus), and the susceptible genotype PI 331326 were studied. Seed colonization in PI 337394F was greater at 20% adjusted seed moisture than at 25% seed moisture. Colonization was least at 15 and 30% adjusted moisture. Aspergillus flavus colonized a low percentage (17%) of the cotyledons of PI 337409 after 48 hours of incubation, whereas 100% of the cotyledons of PI 331326 were colonized. Colonization of PI 337394F seed with intact seed coats increased with each increase in storage time from 0 to 6 and 12 weeks, and for each increase in temperature from 5 to 20 and 35°C. Seed colonization in the immature and overmature seed of both resistant genotypes was greater than in the mature seed. Mature seed of the resistant genotypes were colonized at a low level, with no difference for seed harvested at 4 successive 2-week intervals, whereas PI 331326 had greater colonization for each successive harvest date. Seed coat abrasion, soaking for 5 min. in a H2SO4 solution, machine picking or machine-shelling increased colonization of seed over check treatments. Seed colonization of PI 331326 was greater than that of the resistant genotypes under all conditions.


Seed coat tannins caused radial growth inhibition of Aspergillus parasiticus, on agar plates, after 52 h, and only minor differences were noted between the six groundnut genotypes resistant and susceptible to A. flavus seed colonization. Growth inhibition by seed coat extracts or seed coat tannins showed considerable seed coat genotype interaction in relation to seed colonization by the fungus.


Four groundnut genotypes, two resistant (J11 and PI 337394F) and two susceptible (TMV 2 and EC 76446 (292)) to in vitro seed colonization by Aspergillus flavus, were grown in field trials at ICRISAT Center in the 1984 and 1985 rainy seasons. Geocarposphere mycoflorae were examined and significant quantitative and
Flavus conidia. Parasticus, seed colonization by A. flavus.

Polypeptide contents of seeds varied between genotypes susceptible and resistant to A. flavus seed tests. Genotypes inhibited cotyledons and considerable variation was found. Tannin-like compounds found in Aspergillus flavus parasiticus, Rhizoctonia solani, microscopy. An attempt was made to correlate structural differences with variations in resistance to fungal invasion with particular reference to Sclerotium rolfsii, Rhizoctonia solani, and Aspergillus flavus. Fungal hyphae were restricted to the imafasscular parenchyma and parenchyma in the exocarp tissue of intact pods. Physical injuries which disrupted the sclerenchyma mantle provided avenues for penetration of hyphal strands into the parenchyma and gave access to the kernels. Sclerenchyma layers were more developed in mature pods of Tammun 74 and TP 1025 than in the more susceptible Florunner cultivar.


Factors determining resistance to aflatoxin contamination are reviewed. Possible factors influencing resistance in groundnut pods and seeds to invasion by Aspergillus flavus and to aflatoxin production are considered. A range of groundnut genotypes were examined for presence of inhibitory compounds in testae and cotyledons and considerable variation was found. Tannin-like compounds found in seed testae of some genotypes inhibited A. flavus growth and aflatoxin production. Polypeptide contents of seeds varied between genotypes susceptible and resistant to seed colonization by A. flavus.


Pod structures in three groundnut genotypes were examined by scanning electron microscopy. An attempt was made to correlate structural differences with variations in resistance to fungal invasion with particular reference to Sclerotium rolfsii, Rhizoctonia solani, and Aspergillus flavus. Fungal hyphae were restricted to the imafasscular parenchyma and parenchyma in the exocarp tissue of intact pods. Physical injuries which disrupted the sclerenchyma mantle provided avenues for penetration of hyphal strands into the parenchyma and gave access to the kernels. Sclerenchyma layers were more developed in mature pods of Tammun 74 and TP 1025 than in the more susceptible Florunner cultivar.


Pods and seed coats of groundnut genotypes resistant and susceptible to seed colonization by Aspergillus flavus were examined histochemically with the light microscope and structurally with the transmission and scanning electron microscopes. Significant biochemical and structural differences were associated with an ability to restrict fungal penetration. Seed coats of the A. flavus-resistant genotypes PI 337394 and PI 337409 had a more even deposition of wax on their surfaces and more compact arrangement of palisade-like cells than had the A. flavus-susceptible genotypes tested. The resistant genotypes had smaller hila than the susceptible cultivars. Pods of genotype PI 337409 stained heavily for tannin and callose, and pods of Florunner (moderately resistant to A. flavus) stained heavily for tannin.


Tannin concentrations in the seed coats of six groundnut varieties grown in seven geographical locations for 2 years were determined. Tannin content within variety varied significantly between locations. Levels among varieties at a location were also variable. Tannin concentrations for a specific variety at a specific location in 1975 and 1976 were compared and 60 % of the comparisons differed significantly. These data indicate that any resistance to Aspergillus parasiticus colonization associated with groundnut seed coat tannin concentration would probably vary somewhat with production location and year.


The relationship between tannin content of mature, intact, cured groundnut seed and % seed colonization by Aspergillus parasiticus was examined. Tannin content in 9 groundnut cultivars, 7 of which were grown in both Tifton, Georgia, and Puerto Rico, was significantly correlated with % seed colonization. For data expressed as mg tannin g-1 intact seed and mg tannin g-1 seed coat, correlation coefficients with % seed colonization were 0.74 and 0.76, respectively. Seedcoat tannin, methanol-extracted, water-soluble material from groundnut seed coats, was tested in vitro for effects on growth of A. parasiticus. As concentrations of tannins were increased to 7.5 %, inhibition of fungal growth increased linearly to 88 %, a concentration of 20 % produced over 96 % inhibition.

The ultrastructural analysis of the sequence of penetration of *Aspergillus flavus* mycelium into the groundnut seed coat after artificial inoculation revealed that the fungus acted on the protective layer of the testa as from the 4th day after surface inoculation. On the 5th and 6th days it reached the central cavity of the epidermal cell. Most of the epidermal and parenchymatous cells were invaded on the 7th day. Some hyphae re-emerged on the outside of the testa on the 8th day to form characteristic fructifications of A. flavus; others formed similar fructifications in the intercotyledonary space.


Experiments were carried out on 24 varieties of groundnut for their reaction to pod and seed infection by *Aspergillus flavus* in the field in Darou, Senegal, and for seed colonization in artificial inoculation tests in the laboratory in Paris. The varietal differences observed in seed colonization in inoculation tests and in infection of pods and seeds in the soil before harvest appeared to be linked to the shell and seed coat structure. Drought at the end of the growth cycle was a contributing factor to increased infection.


In a study of 36 groundnut genotypes, seed infection or seed colonization by *Aspergillus flavus* was dependent on the genotype. Factors implicated in resistance to fungal penetration included a high proportion of sclerenchyma in the pods and particularly the structure of the seed testa.


Thirty-six groundnut genotypes were tested for resistance to *Aspergillus flavus* seed infection in the field in Senegal, and for resistance to seed colonization in vitro inoculation tests in the laboratory. Seed testae were examined under the light microscope and by scanning electron microscopy. Great differences in structure were found in the seed segment (seed testa) and these appeared to be related to fungal penetration. Certain structural features might obstruct seed invasion by the fungus.

The groundnut seed coat plays an important part in resistance to seed invasion and colonization by *Aspergillus flavus*. An examination of the surface of the seed coat by scanning electron microscope and of transverse sections by light microscope was carried out on 36 genotypes of groundnut. Great differences in structure were found in the seed coat and these appeared to be related to fungal penetration. These genotypes could be classified according to certain characteristics which may affect resistance to fungal penetration: thickness of the wax layer, junction between the epidermal cells, thickness of cell walls, presence of cracks or detachment of the epidermal foundation.

In field trials at Darou, Senegal in 1975, 33 groundnut varieties were tested under drought conditions favourable for infection by *Aspergillus flavus*. Varieties were sown on two dates (normal sowing date, late sowing date) and were harvested at maturity and 14 days before maturity. Significant differences were found between varieties for pod infection (0.8-8 %) and for seed infection (0.4-4 %) by *A. flavus*. Among the 10 varieties least infected were PI 337394F and PI 337409 (previously reported resistant to seed colonization by *A. flavus* in artificial inoculation tests in the laboratory), Florunner, and seven varieties selected in Senegal for resistance to drought. In laboratory inoculation tests, varieties showing field resistance in general showed resistance to seed colonization by the fungus. However, the results varied considerably for certain varieties depending on the sowing and harvesting dates.

### 8.2.7 Resistance to Aflatoxin Production


In field trials at Darou, Senegal in 1975, 33 groundnut varieties were tested under drought conditions favourable for infection by *Aspergillus flavus*. Varieties were sown on two dates (normal sowing date, late sowing date) and were harvested at maturity and 14 days before maturity. Significant differences were found between varieties for pod infection (0.8-8 %) and for seed infection (0.4-4 %) by *A. flavus*. Among the 10 varieties least infected were PI 337394F and PI 337409 (previously reported resistant to seed colonization by *A. flavus* in artificial inoculation tests in the laboratory), Florunner, and seven varieties selected in Senegal for resistance to drought. In laboratory inoculation tests, varieties showing field resistance in general showed resistance to seed colonization by the fungus. However, the results varied considerably for certain varieties depending on the sowing and harvesting dates.


Thirty-seven cultivars of groundnut were tested for their ability to support aflatoxin production following inoculation of seeds with an aflatoxigenic isolate of *Aspergillus flavus*. One hundred genotypes were tested for resistance to aflaroot disease, when their seeds were artificially inoculated with the aflatoxigenic fungus. All cultivars tested supported substantial levels of aflatoxin production. None of the genotypes showed resistance to the aflatoxigenic isolate of the fungus. Only two genotypes, U4-7-2 and U2-1-14, were moderately resistant to aflaroot disease.


Screening of 36 groundnut varieties, seven maize varieties, and five varieties each of soyabean and sunflower, using several aflatoxin-producing isolates of *Aspergillus flavus* and *A. parasiticus*, showed that there were varietal differences in resistance to aflatoxin production. The groundnut variety J 11 supported the least toxin production even by the most aflatoxigenic isolates of *A. flavus* and *A. parasiticus*. The low toxin-producing attribute of this variety was confirmed in further tests. The scope of the genetic resistance approach for minimising aflatoxin contamination in agricultural commodities is discussed.


Seeds of 15 selected groundnut genotypes were evaluated for production of aflatoxins following inoculation with an aflatoxigenic strain of *Aspergillus flavus*. The data after 8 days of incubation at 28 + 1°C showed aflatoxin production in all genotypes. The cultivar J 11 supported the lowest amount of aflatoxin production (2658 µg kg⁻¹) followed by Robut 33-1 (6558 µg kg⁻¹), var. 27 (7030 µg kg⁻¹) and Faizpur (8262 µg kg⁻¹) whereas high amounts of aflatoxins were supported by OG 43-4-1 (23956 µg kg⁻¹) followed by TMV2 (22746 µg kg⁻¹) and GAUG-10 (21102 fig kg⁻¹). The rest of the cultivars supported production of aflatoxins in the range 10011-18906 µg kg⁻¹. It is emphasized that J 11 is a released cultivar and can be adopted in areas where aflatoxin contamination of groundnut is a serious problem.


Florunner groundnuts were grown in environmental control plots, and 103 days after sowing (DAS) the following treatments were imposed: irrigated, drought with mean 2 in. soil temperature of 29°C (optimum for aflatoxin contamination) and drought with mean 2 in. soil temperature of 25°C (less conducive for aflatoxin contamination). Beginning at 114 DAS (11 treatment days) samples of groundnuts were taken at weekly intervals from the three treatments. Groundnuts were hand-picked and classified into maturity stages by the Hull-Scrape method. Water activity (Aw), moisture, capacity for phytoalexin production, and aflatoxin...
contamination were measured in groundnuts from five maturity stages (yellow 1, yellow 2, orange, brown, black). The objectives of the study were to determine (1) what role, if any, stilbene phytoalexins have in natural resistance of groundnuts to aflatoxin contamination, and (2) the role of elevated soil temperature in aflatoxin contamination of groundnuts subjected to late-season drought stress. Results showed that kernels from the irrigated treatment maintained high Aw, high capacity for phytoalexin production, and essentially no aflatoxin contamination throughout the study. As the drought period progressed in the other two treatments, kernel Aw decreased, phytoalexin production ceased, and aflatoxin contamination appeared. This rate of change was faster in the 29°C treatment than in the 25°C treatment, indicating that the primary role of elevated soil temperature is in the rate at which groundnuts become susceptible to *Aspergillus flavus* infection and aflatoxin contamination. Evidence for phytoalexin involvement in natural resistance of groundnuts to aflatoxin contamination was strong. Regardless of maturity, there was no significant aflatoxin contamination of groundnuts until the ability to produce phytoalexins was lost as a result of decreased water activity.


Four groundnut genotypes (PI 268893, PI 295170, PI 246388 and Starr) were tested for their ability to support aflatoxin production following inoculation of seeds with two aflatoxin-producing isolates of *Aspergillus flavus*. The inoculated seed samples were incubated for 7 days at 25°C, and then analyzed for aflatoxins. All four genotypes supported substantial levels of aflatoxin production. The genotype PI 246388 (US 26) was earlier reported to be resistant to aflatoxin production.


Twenty groundnut breeding lines were tested for their ability to support aflatoxin production following inoculation of seeds with an aflatoxin-producing isolate of *Aspergillus flavus*. The inoculated seed samples were incubated for 7 days at room temperature (25-27°C), and then analyzed for aflatoxins. All lines supported substantial aflatoxin production.


The potential of *Aspergillus flavus* isolates to produce aflatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings of groundnut were studied. Isolates NRR 3000 and V3734/10 produced high levels of aflatoxins in culture. Culture filtrates from the isolates and from NRCGAFA were most toxic to seeds and seedlings. Commercial cultivars, advanced breeding lines, and wild *Arachis* species were screened for resistance to in vitro colonization of seeds by *A. flavus* isolates, and to aflatoxin production. Genotypes CGC 2, 1-4, CGC 7, S230, derivatives of S230 x PI 337394F, Latur 33 x PI 337394F. and the wild species. *Arachis cardenasi* and *A. duranensis* were resistant to seed colonization by *A. flavus*. All genotypes of groundnut and three wild *Arachis* species supported high production of aflatoxins by NRR 3000, but only trace levels were produced in *A. cardenasi* and *A. duranensis*. Aflatoxins were found (range of 27-146 µg kg⁻¹) in commercial groundnut cake and in de-oiled cake. Moisture intake capacity, levels of seed coal phenols, and protein content of seeds were considered to influence aflatoxin contamination levels. Soaking seeds in various organic and inorganic substances was found to influence the degree of seed colonization by *A. flavus* and of aflatoxin production in *in vitro* inoculation tests.


Research on aflatoxins carried out at the National Research Laboratories, Hyderabad (India), is presented. Aflatoxicosis was found in buffaloes and ducks at government farms near Hyderabad. Aflatoxin contamination was found in the livestock feeds. Biological effects of aflatoxins in ducklings and rats are described. Of 60 varieties of groundnut screened for resistance to aflatoxin production by a toxigenic strain of Aspergillus flavus, one variety "U.S. 26" did not support aflatoxin production.


Fifty-three varieties of groundnut were tested for their ability to support aflatoxin production following inoculation of autoclaved seeds with an aflatoxigenic isolate of *Aspergillus flavus*. All varieties tested supported production of aflatoxins B₁ and B₂. High-yielding lines supported high levels of aflatoxin production. The cultivar J 11, earlier reported to have resistance to aflatoxin production, supported moderate levels of aflatoxin production. The line OG 35-1 showed the least aflatoxin production, but had a low yield potential.

Two genotypes supported only very low levels of aflatoxin production in groundnuts. CRISAT has placed on utilization of genetic resistance to develop groundnut cultivars with pods or seeds on which the fungus cannot invade, or which if invaded do not support aflatoxin production. A number of breeding lines and cultivars have been identified which have seeds with good resistance to invasion by *A. flavus*. All genotypes so far tested have supported production of aflatoxins but some have given much slower rates of toxin accumulation than have others.


Research in several countries into evaluation of responses of groundnuts to seed infection and colonization by *Aspergillus flavus* and aflatoxin production is reviewed, and progress made in this field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is summarized. Several laboratory and field screening procedures have been developed to screen groundnuts for resistance to *A. flavus* infection and aflatoxin production. Research on the effects of environmental factors on pod and seed invasion by *A. flavus* has provided information useful in the development of field screening methods. For instance, imposed drought stress has been used to improve large-scale field screening of groundnut genotypes for resistance to preharvest infection of seeds by *A. flavus*. Several genotypes were found resistant to infection, and some of them were also resistant to in vitro seed colonization by *A. flavus* in laboratory inoculation tests. Two genotypes supported only very low levels of aflatoxin B1 production when seeds were colonized by an aflatoxin-producing strain of *A. flavus*. The usefulness of these resistances in management of aflatoxin contamination is discussed.


This paper describes laboratory procedures to screen groundnuts for resistance to seed infection and colonization by *Aspergillus flavus*, and to aflatoxin production. Some methods for aflatoxin analysis are also outlined.


The use of groundnut cultivars resistant to seed invasion and colonization by *Aspergillus flavus* is a possible means of preventing or reducing aflatoxin contamination. Such resistance was identified in several cultivars, one of which was the released commercial Indian cultivar J 11. No direct relationship was found between resistance to seed colonization by *A. flavus* and the quantity of aflatoxins produced when seeds were colonized by toxigenic strains of the fungus. Some of the cultivars with seed resistance to *A. flavus* colonization have been found resistant to fungal pod rot.


Aflatoxin contamination of groundnut is a serious problem in most groundnut producing countries and as such is given high priority by the Groundnut Improvement Program of ICRISAT. Since 1979 the emphasis has been on selecting cultivars resistant to seed invasion and colonization by toxigenic strains of *Aspergillus flavus*, and aflatoxin production following invasion by the fungus. Of 850 germplasm lines screened for reaction to seed invasion and colonization by *A. flavus* in laboratory tests, eight proved resistant. Three of these lines (PI 337394F, PI 337409 and UF 71513) had been reported resistant in the USA but the other five were new sources of resistance. Several groundnut cultivars have been screened for resistance to seed invasion by *A. flavus* in the field, both under natural conditions and with the inoculum of the fungus added to the soil in the pod zone. Some cultivars with resistance to seed colonization also showed resistance to seed invasion by *A. flavus*. None of the cultivars tested was completely resistant to aflatoxin production but significant cultivar differences occurred in the amounts of aflatoxin produced in seeds inoculated with a toxigenic strain of *A. flavus*.


Nine groundnut genotypes were tested for resistance to seed colonization by five
different strains of *Aspergillus flavus*. These genotypes, and the cultivar J 11 which has been shown to be resistant to seed colonization by *A. flavus*, were also checked for production of aflatoxin following infection of scarified, surface-sterilized seeds by three aflatoxigenic strains of *A. flavus*. The genotypes PI 337394F and PI 337409 showed significantly less seed colonization and internal invasion than the oilier genotypes. The *A. flavus* strains differed significantly from one another in their ability to colonize seeds and produce internal infection. Strain NRRL 3000 was the least effective. Of the three strains used in the aflatoxin production tests, AF 8-3-2A produced the highest levels of aflatoxin B1 on all genotypes while AF8-3 produced the least; NRRL 3000 being intermediate in this respect. Aflatoxin G, was produced on all genotypes by NRRL 3000, and on J 11 by AF 8-3-2A. There was no obvious correlation between seed resistance to *A. flavus* colonization and aflatoxin production when seeds were infected. Significantly higher amounts of aflatoxin B1 were produced in the two genotypes resistant to *A. flavus* colonization than in the highly susceptible genotype FESR-11-P11-B2-B1.


Rehydrated, mature, undamaged seed of 502 groundnut genotypes were scarified, inoculated with an aflatoxigenic strain of *Aspergillus flavus*, and tested for aflatoxin B1 production after incubation at 25°C for 10 days. All genotypes supported production of aflatoxin B1 but significant genotypic differences in levels of aflatoxin B1 were found. Genotypes U 4-7-5 and VRR 245 supported the lowest levels of aflatoxin B1 (<10 µg g<sup>-1</sup> seed), whereas the commonly grown Indian cultivar TMV 2 supported production of aflatoxin B1 at levels of over 150 µg g<sup>-1</sup> seed. Eight genotypes with low, moderate or high capacity to support aflatoxin B1 production were further tested using seed from one rainy season crop, and two irrigated postrainy season crops. Genotypic differences in levels of aflatoxin B1 production were consistent over seasons. Production levels were slightly lower in seed from the rainy season crop than in seed from the two postrainy season crops.


Tests with the groundnut varieties TMV 2 and US 26, three isolates of *Aspergillus flavus* (producing aflatoxins B<sub>1</sub> and B<sub>2</sub>) and two isolates of *A. parasiticus* (producing aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>) showed that aflatoxin production in groundnut depended both on the host variety and on the species or strain of the fungus.


Twenty-one groundnut genotypes obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, and the United States Department of Agriculture (USD A), and one cultivar (Giza 4) from Egypt included as a local susceptible control, were tested for their ability to support aflatoxin production. All the genotypes supported production of aflatoxins B<sub>1</sub> and B<sub>2</sub>, although the amount produced differed among genotypes. The lowest level of total aflatoxin production being 19180 µg kg<sup>-1</sup> seed in genotype Ah 7223, and the highest 44290 µg kg<sup>-1</sup> seed in cultivar Giza 4.


Factors determining resistance to aflatoxin contamination are reviewed. Possible factors influencing resistance in groundnut pods and seeds to invasion by *Aspergillus flavus* and to aflatoxin production are considered. A range of groundnut genotypes were examined for presence of inhibitory compounds in testae and cotyledons and considerable variation was found. Tannin-like compounds found in seed testae of some genotypes inhibited *A. flavus* growth and aflatoxin production. Polypeptide contents of seeds varied between genotypes susceptible and those resistant to seed colonization by *A. flavus*.


Seeds of 13 maize and six groundnut varieties were inoculated with a spore suspension of *Aspergillus parasiticus*. Wide variations were observed between varieties of both crops for fungal growth, assessed by glucosamine content, and aflatoxin production. Aflatoxin production per unit growth of the fungus differed between varieties, and maize varieties with soft endosperm, such as Shakti, supported greater fungal growth than hard-endosperm varieties like composite H3, although aflatoxin production did not differ. Both fungal growth and aflatoxin production increased linearly with incubation time up to 7 days, but there was no correlation between growth rate and aflatoxin production in the varieties used.
indicating that some varieties are less suitable for aflatoxin production than others, irrespective of how well they support fungal growth.


Sixty varieties of groundnut were tested for their ability to support aflatoxin production following inoculation of seeds with an aflatoxin-producing strain of Aspergillus flavus. All varieties except US 26 supported production of aflatoxin. Resistance to aflatoxin production in US 26 was confirmed in further tests.


Varietal differences in aflatoxin production by Aspergillus flavus and A. parasiticus were studied in 78 varieties of groundnut, 38 varieties of maize, five varieties of soybean, and five varieties of sunflower. Varieties supporting minimal aflatoxin production were identified.


During 1988 and 1989, preliminary screening trials were conducted under two rainout shelters to determine possible differential aflatoxin production in four groundnut genotypes (Florunner, Sunbelt Runner, Tifrun and Tifton-8). In 1988, the rainout shelters failed due to old sensors malfunctioning during critical rainy periods. Thus, this extra moisture eliminated aflatoxin and any test results. However, in 1989 after repairs the shelters performed as expected, and drought stress was severe and uniform. Significant genotypic differences were obtained in one shelter but not another. Overall, Tifton-8 had a significantly lower aflatoxin content than Tifrun and Florunner; Sunbelt Runner was intermediate. These data strongly suggest that differences do exist among certain groundnut genotypes for aflatoxin production.

8.2.8 Breeding for Resistance to Aspergillus flavus Infection and Colonization


Screening, selection and breeding procedures for increasing the resistance of groundnut varieties to aflatoxin-producing strains of Aspergillus flavus are reviewed and discussed. This review includes consideration of sources and nature of resistance, reaction of genotypes to seed colonization, variation among fungal isolates of A. flavus, and factors associated with the interaction of aflatoxin-producing strains of the fungus and seed of groundnut genotypes. The pros and cons of breeding an improved groundnut variety with greater resistance to aflatoxin contamination are presented.


The potential for using genotypes identified to be resistant to seed colonization by aflatoxin-producing strains of Aspergillus flavus and A. parasiticus for breeding agronomically suitable groundnut cultivars with resistance to the toxigenic fungi is discussed. Factors affecting seed colonization in groundnuts are discussed and factors associated with resistance to colonization of the groundnut seed tests are reviewed.


Evidence for the possible development of groundnut cultivars with favourable agronomic characteristics and resistance to aflatoxin-producing strains of Aspergillus flavus is presented, in studies of F2 and F3 progenies from crosses of resistant and susceptible genotypes data on the frequency distribution and least square estimates of genetic effects showed the possibility of selecting for resistance to seed colonization by the fungus. Yield, value and seed quality data for seven advanced lines developed by using pedigree selection from crosses showed that potentially favorable groundnut cultivars may be developed.

by selection and breeding. Journal of the American Oil Chemists' Society 58: 961A-966A.

The potential for developing agronomically suitable cultivars using groundnut genotypes that exhibit resistance to seed colonization by aflatoxin-producing strains of Aspergillus flavus is discussed. The practical implications of developing resistant cultivars are presented in data for yield, value and seed quality for six advanced groundnut lines that were developed by breeding and selection from crosses.


A groundnut breeding programme is outlined, of which the major aims are high yields, resistance to drought, Aspergillus flavus, Puccinia arachidis, Cercospora arachidicolac, Cercospora personata and rosette virus, and high technological qualities. The breeding methods briefly discussed include mass selection, pedigree selection, the bulk method, single seed descent and multifamilies (a composite of isogenic families).


Progress worldwide in breeding groundnuts resistant to seed colonization by Aspergillus flavus and aflatoxin contamination is summarized, and research at ICRISAT is described. Resistance to A. flavus infection may occur at various levels, but efforts to breed for resistance have concentrated on the utilization of the resistance in the testae of mature seeds. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), genotypes identified as resistant to in vitro seed colonization by A. flavus have been crossed with susceptible cultivars of good agronomic character, and several breeding lines with stable resistance to seed colonization and with acceptable yield and quality have been produced. The genetics of inheritance of testa resistance is discussed. The need for breeders to incorporate other resistance traits is stressed, and future prospects for breeding for resistance to aflatoxin production are discussed.


The heritability of three mechanisms of resistance to aflatoxin including seed colonization resistance (SCR), aflatoxin production resistance (APR), and preharvest infection resistance (PIR), as well as phenotypic correlations among the mechanisms, was estimated. Forty-five F2-derived F6 families of two crosses, AR-4/NC 7 and GFA-2/NC 7, were evaluated using a randomized complete block design with three replications. To evaluate SCR, sterilized rehydrated seeds were inoculated by a spore suspension of Aspergillus parasiticus strain NRRL 2999 (5 x 10^3 conidia mL^-1). Percent seed colonization was recorded after 8 days of incubation at 25°C. To evaluate APR, seed coat was removed from five seeds which were then sterilized, rehydrated, inoculated, and incubated for 4 days at 29°C. Samples were analyzed using an HPLC to determine aflatoxin production. Plants of each family were evaluated for PIR in the greenhouse and inoculated at 40 days after sowing (DAS). Drought stress was imposed at 60 DAS to induce A. parasiticus infection. Seeds from each plant were harvested, dried, and plated onto malt salt agar, and percent infection was recorded after 8 days. In cross AR-4/NC 7, heritability estimates of SCR, APR, and PIR were 0.55 ± 0.23, 0.20 ± 0.25, and 0.27 ± 0.25, with family means ranging from 7-95 %, 7.95-43.62 mg kg^-1, and 0-100 %, respectively. In cross GFA-2/NC 7, heritability estimates of SCR, APR, and PIR were 0.63 ± 0.21, 0.47 ± 0.22, and 0.33 ± 0.26, with family means ranging from 25-98 %, 14.73-69.62 mg kg^-1, and 0-100 %, respectively. The means of the three traits in cross AR-4/NC 7 were generally lower than those of GFA-2/NC 7. In both crosses, there was no significant correlation among the three mechanisms of resistance, indicating that the three mechanisms are controlled by different genes. Selections from cross GFA-2/NC 7 should produce a number of progenies with resistance than selections using AR-4/NC 7.

8.2.9 Segregation and Decontamination


The occurrence of aflatoxin in the Australian groundnut crop is mainly due to late season drought stress. The Australian Peanut Marketing Board uses a minicolumn test to segregate aflatoxin- contaminated (aflatoxin > 16 µg kg^-1) farmers' stock groundnuts at the point of delivery. To check the effectiveness of this segregation, an investigation was made to quantify the incidence of aflatoxin in groundnuts at several major points in the deshelling, grading and blanching operations. The aflatoxin "positive" segregation lots contained aflatoxin levels eight times higher than the aflatoxin "negative" lots. Seventy-one % of the aflatoxin through the
Shellers was concentrated into the oil milling kernels, which comprised 16 % of the total kernels. Oil kernels from "negative" and "positive" stock averaged 48 µg kg⁻¹ and 253 µg kg⁻¹ aflatoxin respectively. It appears that in excess of 50 % of the aflatoxin in the product which was roasted and blanched was lost and/or degraded in the operation.


Approximately 7 tonnes of Segregation 3 official grade check samples from farmers' stock groundnuts marketed in 1980 were cleaned and shelled in the USDA pilot shelling plant. Large samples were removed at 28 different points in the shelling process within the plant. The portion of material that was removed from each point was blended and divided into four samples, ground, blended and subsampled. The subsamples were analyzed for aflatoxin using minicolumn chromatography. Pod damage and pod strength were directly correlated with aflatoxin levels while pod size, seed density, and pod terminal velocity were inversely correlated with aflatoxin levels. Use of these findings in designing farmers' stock cleaning and shelling systems are discussed.


In a trial in 1972, 8 samples of unshelled groundnuts from Mali, Niger and Senegal were sorted manually. Intact pods averaged 73 % of the total of all samples and contained 24 % of the total aflatoxin content. Pods damaged by millipedes were only 4.3 % of the total, but contained 40 % of the total aflatoxin content. In a similar trial in Senegal, 4 samples of shelled seeds were sorted manually, using the criteria employed for confectionery groundnuts, into intact seeds, broken seeds, seeds which had lost their testa, (a) abnormally-colored seeds and (b) seeds showing growth of Aspergillus flavus. Almost all the total aflatoxin content was in (a) and (b). The results are discussed in relation to development of mechanical/electronic methods of sorting pods and seeds to reduce aflatoxin content.


Aflatoxin contamination of groundnut kernels from francophone West Africa is largely confined to those from pods which become physically damaged by pests before harvesting, or during harvesting. Trials are described in which it is shown that a significant reduction in overall aflatoxin contamination of unshelled groundnuts can be achieved by the removal of defective pods by hand or pneumatic sorting. The removal of abnormal kernels from shelled groundnuts by mechanical or hand sorting also greatly reduces overall aflatoxin contamination.


This paper very briefly reviews several methods for decontamination and detoxification of aflatoxin-contaminated foods and feeds. The utilization of ammonia gas has been extensively investigated and commercial facilities exist for the detoxification of groundnut cake by ammoniation.


Data from two different belt screening studies have shown that belt screening reduced aflatoxin in all three groundnut types. A further reduction was achieved when oil stock and damaged kernels were removed. The amount of reduction for each step depended on the distribution of aflatoxin contamination in a farmers' stock load. When the loose shelled kernels contribute a significant amount of the aflatoxin in a farmers’ stock load, the belt screen will be very effective for aflatoxin reduction. When the oil stock and damage contribute more of the aflatoxin in a farmers' stock load, the belt screen will be less effective. Ideally, both risk components need to be removed for efficient aflatoxin reduction.


A 20-tonne lot of Segregation 3 groundnuts grown in 1979 was shelled in the USDA pilot shelling plant and 50 to 250 kg of groundnut samples were removed at each of 42 different locations throughout the plant. The groundnut sample mat was removed from each location was blended, divided into four samples, ground, blended, subsampled, and the subsamples analyzed for aflatoxin. Aflatoxin contamination was directly related to the stage of shelling, resistance to shelling, and inversely related to seed thickness and specific gravity. Use of these findings in designing shelling and processing plants is discussed.
Selective harvesting may help reduce segregation-3 peanuts. Southern Peanut Farmer 12: 3.


This paper discusses causes of aflatoxin contamination and methods of prevention and segregation to control the aflatoxin problem in farmers' stock groundnuts from the time the groundnuts are growing in the field until they are marketed, stored, and shelled.


The availability of mycotoxin-free food products depends upon a successful program of mycotoxin prevention and control. Such a program must be designed according to the growing, harvesting, drying, storing, transporting and processing methods employed for each food commodity. The aflatoxin prevention and control program for groundnuts is broader in scope and better organized than any other mycotoxin control program employed in the United States. Many features of this program may be used for mycotoxins in other commodities. Rapid aflatoxin detection methods are employed when groundnuts are marketed from the farm to avoid mixing lots of groundnuts containing high concentrations of aflatoxin with those lots which contain either low concentrations or no aflatoxin. Lots with high concentrations of aflatoxin are diverted to non-food uses. Sorting techniques and other processes are utilized to remove aflatoxin-contaminated kernels from those lots of groundnuts intended for food use. An extensive aflatoxin sampling and testing program is used to prevent marketing for food processing those lots of shelled groundnuts which test more than 25 µg kg\(^{-1}\) aflatoxin. The finished product is then tested for aflatoxin by the Food processor. Although less well developed than the program for groundnuts, aflatoxin prevention and/or control methods are also employed for corn, cottonseed, almonds, pistachio nuts, Brazil nuts, animal feeds, milk and other commodities.


In the USA, determination of visible Aspergillus flavus growth on groundnut kernels has been reported to be an effective method for detecting aflatoxin contamination and for classification of farmers' stock groundnuts. Lots containing A. flavus on kernels are designated "Segregation-3" groundnuts and are diverted to non-food use, except for the oil which is aflatoxin-free. Lots not found to contain kernels with visible A. flavus and with low levels of damage are designated "Segregation-1" groundnuts. This article discusses the method and the results of the tests to determine the efficacy of the method for determining lots of farmers' stock groundnuts with high concentrations of aflatoxin.


Samples (89.3 kg) from 40 commercial lots of shelled groundnuts each containing an average concentration of 48 µg kg\(^{-1}\) aflatoxin were sorted, using an electronic color sorter, 3 to 5 times and subsequently discolored kernels were removed by hand. Prediction equations indicated that cumulative removal of 2, 4, 6, 8 and 10 % of the kernels from each sample by electronic sorting would remove an average of 16, 28, 37, 45, and 51 % of the aflatoxin content respectively. Electronic sorting became less selective for aflatoxin-contaminated kernels during each additional sorting operation. Careful hand picking of discolored kernels was far more selective for aflatoxin-contaminated kernels than electronic color sorting. An average 72 % of the aflatoxin was in kernels that were removed by electronic sorting and subsequent hand picking. The efficacy of aflatoxin removal with electronic sorting varied considerably among the lots, indicating that each lot should be pretreated to determine if aflatoxin can be effectively removed before the expense of electronic color sorting is incurred.


Samples from 17 loads of farmers’ stock groundnuts suspected of containing aflatoxin were screened over a belt cleaner, shelled and sorted into grade components. Tests showed that removal of loose shelled kernels (LSK) and small pods by belt screening reduced aflatoxin levels by an average of 35 %. Belt screening removed 97 % by weight of the LSK, but only 4 % of the sound mature kernels and sound splits (SMK+SS). Further removal of other edibles (OE), oil stock (OS), LSK and damaged kernels (DM) from the groundnuts riding over (OVERS) the belt screen reduced aflatoxin levels from an average of 110.7 µg kg\(^{-1}\) in the unscreened load to 3.8 µg kg\(^{-1}\) in SMK+SS. The OE, OS, LSK and DK were removed from the OVERS through the use of slotted screens and by sorting.

Samples of groundnuts (cv Florunner) were randomly drawn from a commercial color sorter reject stream, to increase the chances of acquiring aflatoxin contaminated samples. Two hundred samples from each size range (jumbo, medium and No. 1) were utilized to map the density distributions of aflatoxin contaminated groundnuts. A water displacement technique developed in the Food Science and Technology laboratory. Georgia Agricultural Experiment Station, that practically eliminates water absorption by the nuts, was used to determine individual kernel densities. A two parameter logistic function was found to be most appropriate for modelling the density data. To facilitate widespread practical applicability, predictive models were developed for the cumulative density distribution of each size range. The same kernels were split and studied for internal discoloration and/or fluorescence under long range UV light, these being indicators of possible contamination. The suspect kernels were individually quantitated for aflatoxins by reverse phase high performance liquid chromatography. This data would allow correlation of aflatoxin levels with density on an individual kernel basis, minimizing the extreme variability associated with sampling for aflatoxin contamination. The predictive models developed in this study could be used in the design and assessment of density based separation techniques for aflatoxin contaminated groundnuts from non-contaminated groundnuts.


Legal requirements concerning freedom of groundnuts from allatoxin in the Federal Republic of Germany are briefly considered. A study on batches of groundnuts from various countries, studied in 1976/77. showed that the percentage of allatoxin-free samples were: China, 98 % of 77 samples: Gambia, 50 % of 6 samples: India, 50 % of 16 samples: Sudan, 16 % of 104 samples: South Africa, 98 % of 399 samples: and USA, 90 % of 102 samples. Aflatoxin concentrations were highest in the Sudanese samples (<300 µg kg⁻¹). A description is given of an optical/electronic sorting system for separation of discolored kernels. The kernels pass in front of a screen coloured to match normal kernels: an optical system detects kernels which differ in color from the screen. These kernels (which have a much higher than normal likelihood of aflatoxin contamination) are then automatically separated. Throughput of a 12-channel apparatus is 2 t h⁻¹: mean aflatoxin content of the selected normal kernels is reduced by 90-95 % as compared to the unsorted material.


A patented process to separate aflatoxin-contaminated groundnuts from uncontaminated groundnuts is discussed. Contaminated groundnut lots taken through the process, from incoming raw groundnuts to peanut butter in the jar, are analytically profiled by data from three analytical procedures: (1) aflatest affinity column chromatography, (2) thin-layer chromatography (TLC), and (3) high performance liquid chromatography (HPLC) with post-column iodine derivatization.


Disclosed is a method for separating mycotoxin-contaminated grains, kernels, seeds and nuts from uncontaminated whole grain seeds, whole or split kernel nuts and seeds to obtain a substantially uncontaminated supply of these foods. The separate contaminated source can be further processed to lower the mycotoxin contamination. The process involves the separation of the mycotoxin or aflatoxin-containing materials by floating the aflatoxin-contaminated foods in a liquid having a specific gravity of from about 0.9 to about 1.2. A highly preferred process uses dynamic flotation.


A water floatation method was used to study the distribution of aflatoxin relative to kernel density in naturally contaminated samples of shelled farmers' stock groundnuts. Five-hundred gram samples of visibly undamaged, contaminated groundnuts were added to 2000 mL of tapwater, and approximately 15-30 % of the kernels rose to the surface as buoyant kernels. These buoyant kernels contained an average of 95 plus % of the total sample aflatoxin content. Buoyant kernels, when examined internally, all had a hollow space or "lumen" inside the kernel between the two cotyledons. Data showed an association between aflatoxin content, kernel lumen volume, and the propensity of kernels to float. The lumen may provide a reservoir of air for flotation, fungal growth, and aflatoxin production. The positive association between the presence of a lumen and aflatoxin contamination may provide a possible resistance strategy, if the presence or absence of a lumen is genetically controlled or if it can be manipulated physiologically.


A water floatation method was tried to reduce aflatoxin concentrations in naturally
contaminated samples of shelled farmers' stock groundnuts. Five-hundred-gram samples of contaminated groundnuts were added to 2000 mL of tap water with approximately 55-15 % of the kernels rising to the surface as "floaters". These floaters, when analyzed, contained 80-90 % of the total sample aflatoxin content and their removal resulted in considerable cleanup of the samples. However, there were some limitations. Splits, small whole kernels (< 16/64 in.), and balds did not follow the same "float-sink" pattern of aflatoxin reduction. The method seems best suited for aflatoxin reduction in jumbo, medium, and No. 1 sized kernels.


The role of the manufacturer is to assure that groundnut products meet consumer requirements for both product performance and product wholesomeness. Aflatoxin measurements on shelled groundnut lots do not correlate well with aflatoxin levels in peanut butter made from the lots. However, removing defects (loose shelled kernels) can reduce aflatoxin levels in peanut butter and improves the correlation between aflatoxin levels in raw groundnuts and in peanut butter. Most of the defects identified are best isolated at the farmers' stock and shelling plant levels of the production chains, although manufacturers have influence on many sources of aflatoxin through blanching and related systems.


A detailed discussion of electronic color sorting of groundnuts, pecans and almonds is presented, particularly in relation to aflatoxin sampling and reduction, preparing for further processing, and evaluating commercial sorters for speed and accuracy.


The clinical effects of aflatoxin exposure are briefly described, and the current US FDA regulations and sampling procedure for control of aflatoxins in groundnuts are discussed. Some quality control procedures to ensure that only minimal quantities of toxin persist into the finished product are outlined.


Optical electronic sorters are being used in Senegal to reduce the high incidence of aflatoxin in groundnuts, and thereby raise the quality and value of the residual cake following oil extraction. Tests set up to discriminate between acceptable nuts and those containing Aspergillus flavus confirmed that the machine could achieve the critical level of 50 µg kg⁻¹ aflatoxin Bl. Once the oil has been extracted from the nuts, the remaining meal represents 50% of the input and contains virtually all the contaminants, raising its aflatoxin content to 100 µg kg⁻¹. This level is considered safe for mixing with other ingredients to form cattle cake containing a maximum of 20 µg kg⁻¹ aflatoxin. The machine has been developed in order to recognize not only dark nuts but also unwholesome shades of yellow, pink, brown and green from areas of acceptable white meat. Provision is also made for removing dust produced during shelling.


Removal of the skin or testa from dried groundnuts provides a superior color contrast when sorting a white, dried kernel in comparison to redskin or roasted groundnuts. The low temperature heat from blanching produces a bloom effect on the major and minor damage resulting in a significant increase of me damaged kernels that can be rejected and removed by electronic sorting. Groundnut lots designated for blanching by PAC-USDA must not exceed an average of 10 µg kg⁻¹ aflatoxin alter blanching. Shelf life is not changed as a result of the blanching process.


A field trial was conducted into the separation of aflatoxin- contaminated groundnuts from sound kernels using air separation, size grading and color sorting equipment. With the use of air separation and color sorting equipment, it was possible to remove infected from non-infected kernels almost completely.


An improved nondestructive technique for accurately detecting the extent to which kernels are damaged by molds and aflatoxin has been under study. A helium-neon laser emitting light at 6550 A wavelength with horizontal and vertical polarized components was first standardized on a known background target. The laser was then directed on the groundnut kernel surface and the back scattering amplitude of both polarizations recorded along with a computer calculated ration of the amplitudes. Preliminary results indicate that the amplitudes detected for pickout kernels are 30 to 40 % below the amplitude recorded for sound mature healthy kernels. The
amplitudes for *Aspergillus flavus* inoculated kernels were 15 to 20 % below those recorded for the sound mature kernels. The polarization ratio for sound mature healthy groundnut kernels deviated only slightly from unity. With mold damaged kernels examined along the long axis the horizontal wave component decreased so that the polarization ratio ranged from 0.60 to 0.80. Evidence indicates that the amplitude of the reflected laser beam correlates with the surface features of the kernels while the polarization ratio provides information on the internal composition of the kernels.


Dielectric characteristics of good sound mature kernels and damaged kernels of groundnut were investigated over a frequency range of 20 KHz to 20 MHz. Groundnuts were placed in a test capacitor and measurements were made on a HP 4342A Q meter. Equivalent moisture contents were maintained using humidity chambers as determined by the oven dry method. Preliminary results indicate that the real part of the dielectric constant (permittivity) is essentially independent of mold contamination level whereas the imaginary part (loss tangent) is strongly dependent upon contamination level. The simultaneous determination based upon these dielectric characteristics, provides a means of detecting varying degrees of mold and mycotoxin damage.


Several methods of decontamination and detoxification of groundnuts and groundnut cake are reviewed. Aflatoxin levels can be reduced by segregation and decontamination of groundnuts at shelling and after shelling. Influences of different stages in refining of oil on elimination of aflatoxin are described. Ammoniation of contaminated cake seems to be appropriate at industrial level.


The Australian groundnut crop is significantly affected by aflatoxin contamination in some years because of preharvest drought stress. By a process of selective segregation and sorting, aflatoxin-contaminated kernels are removed from the contaminated lots to reduce aflatoxin levels to an acceptable regulatory limit (15 µg kg⁻¹). This sorting is made possible by the characteristic discoloration of groundnut flesh caused by *Aspergillus* spp. growth and the small percentage of aflatoxin-containing kernels. The variance contributions of sampling, sample preparation, and analysis are quoted. Even with very high standards of sampling and analysis, uncertainty in aflatoxin control is significant.


Groundnuts are sampled by shellers when delivered by the grower and tested for moisture and aflatoxin content. Aflatoxin positive loads are processed separately by procedures which include color sorting and hand picking to reach the required 15 µg kg⁻¹ limit. Moisture is controlled below the level which allows aflatoxin production.


Testing, removal, and segregation of aflatoxin-contaminated groundnuts is described in relation to the USA groundnut industry. Checking at all stages from growers to consumers is necessary. Sorting and removal of discolored groundnuts can reduce aflatoxin levels in raw groundnuts by 16 %. Reduction may be improved if sorting is done after roasting and blanching. About one third of the aflatoxin is destroyed during roasting. Raw groundnuts containing 25 µg kg⁻¹ of aflatoxin will, as a finished product, contain approximately 8 µg kg⁻¹ of aflatoxin. Selective procedures in the USA have been very effective so that few lots exceed the acceptance level of 25 µg kg⁻¹. The average aflatoxin content of groundnut products sold in the USA is less than 2 µg kg⁻¹.

8.2.10 Chemical Detoxification


Present measures for prevention or reduction of aflatoxin levels in groundnuts are described and a suggestion is made that during solvent extraction of groundnut meal, the solvent, hexane, should carry dissolved ammonia gas, which is known to destroy
Chlorine dose related study at 10 min exposure indicated that even the treatment of aflatoxin B$_1$ and two of them were identified as 2,3-dichloro aflatoxin B$_1$ destroying aflatoxin B to 60 to 75%. Time course study of this treatment (100 µg aflatoxin B$_1$ with 7.5 mg of chlorine) showed that about 75% destruction. During the treatment process, at least three new fluorescent reaction products were produced with 15 mg chlorine gas at standard temperature and pressure. The extract can be used for preparation of groundnut oil, as aflatoxin is inactivated during refining, and methoxymethane is easily removed from the defatted product because of its low boiling point. The following compositions were obtained for raw and methoxymethane-treated groundnut samples, respectively: crude fat 54.8 and 0.1%, crude protein 19.7 and 54.7%, and total aflatoxins 1000 and 2.25-3.25 ppb (2.25-3.25 µg kg$^{-1}$). Duckling feeding tests showed no toxic effects of methoxymethane-treated groundnut powder.

A method is described for removal of aflatoxin from groundnuts by extraction with methoxymethane (dimethylether) at room temperature in an autoclave or in pressure-tight columns. Contaminated groundnuts are pulverized to pass through a 12 mesh screen, and extracted in stainless steel columns at approximately 6 kg cm$^{-2}$ pressure. The following compositions were obtained for raw and methoxymethane-treated groundnut samples, respectively: crude fat 54.8 and 0.1%, crude protein 19.7 and 54.7%, and total aflatoxins 1000 and 2.25-3.25 ppb (2.25-3.25 µg kg$^{-1}$). Duckling feeding tests showed no toxic effects of methoxymethane-treated groundnut powder.

The effects of oven and microwave roasting on aflatoxin contaminated groundnuts and the effect of chlorine treatment on aflatoxin B$_1$ detoxification were studied. In artificially contaminated groundnuts, oven roasting for 30 min at 150°C or microwave roasting for 8.5 min at 0.7 KW were equally effective in destroying 30 to 45% of aflatoxin B$_1$. In naturally contaminated groundnuts, both oven and microwave roastings were equally effective in destroying 48 to 61% of aflatoxin B$_1$, and 32 to 40% of aflatoxin G$_1$. Chlorine gas treatment was very effective in destroying aflatoxin B$_1$. Time course study of this treatment (100 µg aflatoxin B$_1$ with 15 mg chlorine gas at standard temperature and pressure) showed that about 60 to 75% of the toxin was destroyed within 10 min of exposure. During the treatment process, at least three new fluorescent reaction products were produced and two of them were identified as 2,3-dichloro aflatoxin B$_1$ and 2,3-dihydroxy aflatoxin B$_1$ (diol). Use of radio-labelled aflatoxin B$_1$ confirmed these results. Chlorine-dose related study at 10 min exposure indicated that even the treatment of 100 µg of aflatoxin B$_1$ with 7.5 mg of chlorine caused about 75% destruction. Preliminary mutagenicity study using the Ames Salmonella assay indicated that the mutagenic activity of the 10-min treated sample in the presence of rat liver S-9 mix can be reduced to about 10% of that of the untreated control. The results indicated that low energy microwave roasting is not an efficient method to remove aflatoxin B$_1$ from contaminated groundnut samples, while chlorine gas could be an effective agent in reducing aflatoxin toxicity.


A process is described for removal of aflatoxin from groundnuts, cereals, and oilseeds by extraction with methoxymethane (dimethylether) which may optionally be mixed with water, avoiding denaturation of proteins.

Chemical methods investigated for detoxifying aflatoxins in foods and feeds. Pages 58-67 in Mycotoxins and other Fungal Related Food Problems (Rodricks, J.V., ed.) Advances in Chemistry Series No. 149, American Chemical Society, Washington, DC, USA.

Refined edible vegetable oils are free of aflatoxins because the alkaline washes and bleaching agents used in the oil processing are among the chemical systems that remove or destroy aflatoxin. Currently, ammonia in conjunction with elevated temperatures or pressures, or both, as well as elevated moisture levels offers the best way to detoxify agricultural seed commodities for feed. Research at the Northern Regional Research Laboratory, Illinois, has shown that ammoniation of contaminated
whole corn reduces aflatoxin B₁ to a chemically nondetectable level and that the ammoniation products are nontoxic to ducklings and chickens. Using radio labelled aflatoxin B₁ to spike white corn meals it was shown that ammoniation at ambient temperature induced the covalent binding of aflatoxin B₁ or B₂ degradative products primarily to corn proteins and water-soluble components.


The nutritional value of a groundnut cake detoxified by ammoniation (25 µg kg⁻¹ of aflatoxin B₁ after treatment compared to 600 µg kg⁻¹ prior to it) was estimated in developing rat. The cake included at the rate of 20 % in the diet had about 63 % nitrogen. The control diet consisted of a groundnut cake having a standard nutritional value with the aflatoxin B₁ content less than 25 µg kg⁻¹. In ad-libitum feeding, no significant differences were found between the cumulative weights obtained during 4 weeks. In case of weekly weights, the detoxified cake caused a reduction of 17 % in the fourth week. No differences were obtained in consumption indexes during the experimental period. The urea and alkaline phosphatases content in the blood plasmas did not vary during the fourth week, while the weekly and cumulative protein efficiency coefficient (PEC) was reduced from 15 and 6 % respectively in the detoxified cake.


It is a report of a study group that went to Senegal to examine possibilities of detoxifying groundnut cake. Detoxification of aflatoxin-contaminated groundnut cake is feasible. It is suggested that chemical treatment (formaldehyde + ammonia) should be used on all groundnut cakes to ensure that aflatoxin levels in the cake are below 100 µg kg⁻¹. Cultural and storage practices to minimize aflatoxin contamination are also suggested.


Methods of decontamination and detoxification of aflatoxin-contaminated agricultural commodities are reviewed. Aspects discussed include physical removal/segregation of contaminated materials, inactivation, and chemical detoxification.


Groundnut meal contaminated with aflatoxins (600 µg kg⁻¹) was treated with formaldehyde alone and in combination with calcium hydroxide in a bench-scale reactor, operated both sealed and at atmospheric pressure. In general, addition of calcium hydroxide to formaldehyde caused greater inactivation of the aflatoxins than did formaldehyde alone. With the reactor sealed and 25 % moisture in the meal, treatments for 1 h with 0.5 % and 1 % formaldehyde plus 2 % calcium hydroxide yielded products having 3 and 1 µg kg⁻¹ aflatoxins, respectively, whereas reflux at atmospheric pressure with 20 % meal moisture and 1 h treatment with 1 % calcium hydroxide yielded a product with 5 µg kg⁻¹ aflatoxins.


This paper reviews the work carried out at the Overseas Development Natural Resources Institute (ODNR1), London, on the development of efficient sampling, sample preparation, aflatoxin analysis, and chemical detoxification procedures. The control of aflatoxin in groundnut products requires a combination of quality control and decontamination/detoxification procedures. The use of selected mathematical models to describe the distribution of aflatoxin in groundnut kernels, roasted groundnuts, peanut butter, and groundnut cake has been investigated for developing statistically sound sampling plans for these commodities. A subsampling mill has been developed, in collaboration with a UK company, which enables representative, comminuted subsamples to be rapidly produced from large samples of groundnut kernels. Methods have been developed for the accurate analysis of the aflatoxin content of groundnut products using bonded-phase clean-up procedures in combination with high performance liquid chromatography (HPLC) and high performance thin-layer chromatography (HPTLC) quantification methods. The application of enzyme-linked immunosorbent assay (ELISA) methods to the analysis of aflatoxin in peanut butter has also been extensively examined. A procedure for the detoxification of groundnut cake using ammonia gas at high temperatures and moderate pressures has been developed.


This paper reviews the effects on trade of legislation restricting the levels of aflatoxin in cereals and oilseed cakes used in the preparation of animal feeds, and
the processes which have been developed to destroy the aflatoxin in situ by means of ammonia gas. The acute and chronic effects of aflatoxin in animals are outlined, and the way in which these data have been used to set upper limits for the level of aflatoxin in commodities is described. Methods available for controlling the amounts of aflatoxin in raw materials are briefly discussed, and the processes for the ammination of maize, cottonseed and groundnut cake are summarized. The toxicological data available for the various ammoniated products is considered. It is concluded that ammoniation provides an effective way to reduce the aflatoxin content of contaminated raw materials, but that universal acceptance of the products will only be forthcoming when adequate toxicological data have been obtained to show that the transformation products of aflatoxin present no hazard to animals or man.


This paper very briefly reviews several methods for decontamination and detoxification of aflatoxin-contaminated foods and feeds. The utilization of ammonia gas has been extensively investigated and commercial facilities exist for the detoxification of groundnut cake by ammoniation.


A process for detoxifying an agricultural product, particularly groundnut cake, contaminated with aflatoxin is outlined. The process comprises treating, in an insulated reaction vessel, the contaminated product with steam until the product is moistened to a level of from 12 to 18 %, especially from 15 to 17 % (by weight) of water, treating the moistened product with gaseous ammonia until the level of ammonia in the mixture is from 2 to 5 %, especially about 3 % (by weight) and the temperature of the mixture in the reaction vessel is between 110 to 150°C, especially in the range 115 to 125°C. This process leads to about 90 % destruction of aflatoxin in groundnut cake.


Groundnut and cottonseed meals prepared from seeds inoculated with aflatoxin-producing Aspergillus parasiticus were treated in a laboratory ammoniator under three conditions of heat and pressure. Detoxification of the meal was similar for all treatments. Differences between the nonammoniated meals and those treated under the mild conditions of heat and pressure were negligible in respect of nitrogen solubility, methionine, lysine, available lysine, sugars, and immunochemical and gel-electrophoretic protein patterns. Meals ammoniated under the harsher conditions of heat and pressure showed reductions in methionine, lysine and available lysine contents and in free sugars as well as changes in nitrogen solubility and gel-electrophoretic patterns.


This paper describes the isolation and characterization of a second major ammoniation product from a model system in which aflatoxin B1 reacted with ammonium hydroxide under elevated temperature and pressure. The compound (product) was identified as dihydro-4-hydroxy-6-methoxyfurol2,3-b]benzofuran, molecular weight 206, melting point 145-146 C, Y max (MeOH) 205, 227 (sh), 269, and 278 (sh) nm with 35000. 8130, 730, and 560. respectively. It is a nonfluorescent phenol which is similar in structure to aflatoxin D1, but lacks the cyclopentenone ring.


A process is described for treatment of moldy foods (groundnut) to neutralize toxic-products, such as aflatoxins, which involves treatment with ethylene oxide and/or methyl formate in a moist medium.


It has been reported that gaseous ammonia at less than 100°C (Viroben et al. 1978) or monomethylamine (Giddey et al. 1977) reduces the level of aflatoxin in contaminated groundnut oilmeal. To test this a 22 % protein diet of maize and groundnut oilmeal with added lysine and threonine was given to chickens in an experiment similar to the protein efficiency test. The ammonia treatment at about 1, 2, or 3 atmospheres pressure did not have any significant effect on feed intake of 22-49 day-old chickens, daily gain or efficiency of feed conversion (FCE) for chickens given groundnut oilmeal with aflatoxin B1 at 0.98 or 1.14 mg kg−1. Mono-methylamine treatment of the diet with aflatoxin at 0.98 mg kg−1 given to chickens from 10 to 24 days old, significantly decreased average daily gain from 13.2 to 7.8 g, daily DM intake from 38.2 to 27.7 g, and gain g−1 feed intake from 0.35 to 0.28 g. In another experiment chickens were given a semi-purified diet with
groundnut oilmeal as the only source of protein, plus amino acids (including methionine at 0.01, 0.2, or 0.3 % of diet). Addition of methionine increased the daily gain and FCE; FCE was highly correlated with the daily intake of sulphur amino acids when plotted on a logarithmic scale (r = 0.983). There was no decrease in availability of amino acids from the treatments under comparison, all differences being explained by the lower cystine content and the lower acceptance of the treated products. It is concluded that rations should be formulated after allowance is made for the non-protein nitrogen added during the treatment of the groundnut oilmeal and that allowance should also be made for cystine present in the groundnut oilmeal. It is recommended that the levels of ammonia nitrogen in diets for broilers, laying hens and fattening pigs should not exceed 0.15, 0.3 and 0.1 %, respectively. After treatment with monoethylamine, the maximum levels should be one-third of these values.


Methods of decontamination and detoxification of aflatoxin- contaminated groundnuts, cottonseed and their products are reviewed. Topics discussed include physical separation of contaminated nuts and seeds, removal of aflatoxin by solvent extraction, heat inactivation, microbial degradation, and chemical detoxification of contaminated commodities. Criteria for detoxification are also reviewed.


Several methods of decontamination and detoxification of agricultural commodities including groundnuts and groundnut meal are reviewed. Research results on detoxification of aflatoxin- contaminated groundnut and cottonseed meals with ammonia are presented. Ammoniation of contaminated meal appears to be effective in greatly reducing aflatoxin levels. Aflatoxin removal by solvent extraction is also discussed.


Treatments of aflatoxin-contaminated groundnut meal with ammonia, methylamine, sodium hydroxide and ozone were effective in either destroying aflatoxins or greatly reducing aflatoxin levels as indicated both by aflatoxin analysis, by TLC, and feeding experiments with ducklings and rats. Extraction with acetone-water (90:10, v/v) proved most effective in removing all aflatoxins from the contaminated meal. The treated groundnut meals had lower protein efficiency ratios than the original meals.


Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Mycelia of Aspergillus parasiticus can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of A. parasiticus, amount of mycelium, temperature, pH, and concentration of aflatoxin. Adsorbants, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin is stable at low pH values but not in the presence of large amounts of vitamin C or bisulfite. Patulin can be degraded by actively fermenting yeasts and rubratoxin can be degraded by the mycelium of Penicillium rubrum.


Aflatoxin B1 was mixed with eleven concentrations of sodium hydroxide, sodium hypochlorite and ammonium hydroxide. Aflatoxin was quantified by fluorometric determination and toxicity of aflatoxin treated with NaOH and NH4OH was evaluated by the brine shrimp assay. Detoxified aflatoxin B1 was then screened for mutagenicity using the Salmonella/mammalian microsome mutagenicity test (Ames test). Sodium hydroxide, sodium hypochlorite and ammonium hydroxide reduced fluorescence by 92, 96, and 94 %, respectively, at concentrations of 25, 11, and 875 mg 50 g⁻¹. A high negative correlation was observed between decrease in fluorescence and increase in survival of brine shrimp (r = 0.88) for aflatoxin treated with NaOH and NH4OH. Equivalent amounts of aflatoxin B1 (0.05 µg) and aflatoxin B1 + detoxified B1 (0.05 µg + 0.05 µg, respectively) were not significantly different (P > 0.05) in the number of revertants resulting in the Ames test. Therefore, aflatoxin B1 in the presence of detoxified aflatoxin did not increase in mutagenicity.


Cottonseed and groundnut meals were treated with ozone to destroy or eliminate aflatoxins. High meal moisture contents (cottonseed meal 22 %, groundnut meal 30
No aflatoxins could be detected in salt-boiled groundnuts in contrast to raw, unshelled groundnuts which were all contaminated. The efficiency of sodium chloride in removing aflatoxins was confirmed by cooking laboratory-inoculated batches of raw, unshelled groundnuts in a 5% aqueous sodium chloride solution at 116°C and 0.7 bar for 30 min. Results of 5 batches of groundnuts containing aflatoxin at 19,992-66,320 µg kg⁻¹ showed a removal of aflatoxins from 80% to near 100%.


There was no significant difference between detoxified (with 25% ammonia) groundnut meal and soybean meal with respect to growth and food efficiency. Ducklings fed a diet containing nontreated groundnut meal showed a decrease in body weight compared with the detoxified meal and soybean meal. However, the liver weight increased in that group. Characteristic lesions of aflatoxicosis developed in ducklings fed nontreated groundnut meal which contained 285 µg kg⁻¹ aflatoxin B₁. Lesions were very slight, but present in the liver and kidneys of ducklings fed detoxified groundnut meal, and there were no differences in kidney lesions in the groups fed detoxified and nontreated groundnut meal. Ducklings fed detoxified groundnut meal absorbed 0.4 µg of aflatoxin B₁ during the first 8 days of the experiment, and 8-10 µg over the 4-week period.


Oil extracted from aflatoxin-containing groundnut kernels by crushers or expellers is toxin-free; extraction of residual oil from the press-cake by the usual type of solvent does not remove aflatoxin either. Consequently, press-cake and meal contain practically all the aflatoxin originally present in kernels. Trials to detoxify groundnut meal by radiation, heat, solvents, or reactive chemicals, conducted at the Tropical Products Institute in London, UK, were not very successful. The only treatments that destroyed or removed the toxin - i.e., prolonged wet heating at 120°C, extraction with methanol, and treatment with 10% HCl, SO₂, or Cl₂, are expensive and cause deterioration of the meal. It is concluded that detoxification is not a practical possibility and that the emphasis must be on the prevention of infection by the aflatoxigenic fungus Aspergillus flavus.


This paper briefly presents some findings with regard to the detoxification of aflatoxins. A highly contaminated groundnut meal (containing about 1000 µg kg⁻¹ aflatoxin), when exposed overnight to a 10 per cent chlorine gas atmosphere, was found to have lost about 90% of its initial fluorescence and to have become nontoxic to the chick embryo. NaOCl (5%) solutions are used in disposing of aflatoxin-contaminated materials in laboratories.


Aflatoxin removal from groundnut meal using aqueous ethanol was attempted in a commercial scale experiment. Results showed that 90°GL alcohol removed 87.4% aflatoxin from contaminated groundnut meal after 3 hours of treatment. It was also found that coarse ground meal permitted better aflatoxin extraction than thick pieces and that samples taken from the lower part of the extraction vessel showed better aflatoxin removal than those from the upper part.

The efficacy of detoxification by ammoniation of aflatoxin-contaminated groundnut oil cakes was determined in long-term (18 months) feeding experiments with rats. The aflatoxin content of the cake was reduced very considerably by the pressurized application of ammonia, dropping from 1000 to 140 µg kg⁻¹ at a gas pressure of 2 bar and to 60 µg kg⁻¹ at 3 bar. No reversion was noted during the experiment. The percentage of hepatic tumors obtained was very high for the untreated cakes, but fell sharply with medium treatment and was reduced to zero by the treatment at 3 bar. A satisfactory dose-effect relationship was shown between the residual aflatoxin content of the cakes and the observed incidence of tumours. It is concluded that ammonia treatment is a practical solution to the problem of the carcinogenic potency of contaminated oil cakes.

Lactating cows were fed a diet containing aflatoxin B1-contaminated groundnut cakes, and then on cakes treated with ammonia gas. Between the two periods the diet contained noncontaminated soyabean meals. With the nontreated groundnut meal containing aflatoxin B₁ at 1100 µg kg⁻¹, total excretion of aflatoxin M₁, was 2.6 % of total ingested aflatoxin B₁. With the treated groundnut meal, aflatoxin M₁ content of milk was below 0.1 µg litre⁻¹. In a second experiment 50 lactating cows were fed for 16 months on mixed feeds containing 30 % ammoniated groundnut cake containing aflatoxin B₁ less than 10 µg kg⁻¹. Aflatoxin M₁ residue in milk was not above 0.1 µg litre⁻¹.

Aflatoxin was removed or significantly reduced in cottonseed and groundnut meals by extracting with a tertiary solvent system of 54 % acetone, 44 % hexane, and 2 % water (by weight) or a binary solvent system of 90 % acetone and 10 % water (by weight). The tertiary solvent system simultaneously removed oil and aflatoxin from prepressed cake containing 15 % oil, resulting in residual lipids content of approximately 1 % and aflatoxin levels of < 40 µg kg⁻¹. The binary solvent system reduced the aflatoxin content of prepressed cottonseed and groundnut meals to < 10 µg kg⁻¹ in small scale batch extractions and < 40 µg kg⁻¹ in continuous pilot plant extractions. Both solvent systems definitely offer economically feasible methods for reducing aflatoxins in contaminated cottonseed and groundnut meals to a level of 30 µg kg⁻¹ or below.

Two groups of 50 pullets, each with 5 cockerels, had feed containing 15 % groundnut oilmeal detoxified by the use of alkali. The normal meal contained aflatoxin 1180 µg kg⁻¹, the detoxified meal 120 µg kg⁻¹. The detoxified meal had slightly lower mineral content. During 21 weeks there was no difference in weight or feed intake between the groups on detoxified or nontreated meal. The group having detoxified meal laid more eggs and a greater weight of eggs; the eggs also had a significantly higher hatchability. Chickens produced by pullets given the detoxified meal had a slightly lower growth rate than those from pullets given the contaminated meal.

In pilot plant scale tests, treatment with gaseous ammonia at 2 levels each of moisture content, reaction time, temperature, and ammonia pressure inactivated the aflatoxins (121 µg kg⁻¹) in groundnut meal to a non-detectable level. With a similar treatment, aflatoxins (350 µg kg⁻¹) in cottonseed meal were reduced to 4 µg kg⁻¹. Using large scale equipment the aflatoxin content of cottonseed meal was reduced from 519 µg kg⁻¹ on average to < 5 µg kg⁻¹ under optimum processing conditions. The ammoniation treatment lowered the nitrogen solubility and epsilon amino-free lysine levels of the meal.


Twenty-eight groundnut genotypes were tested for resistance to seed colonization by Aspergillus flavus and for aflatoxin content. Of these, J 11, TMV 12, and Ah 7223, in general, were found to have less seed colonization. Ten cross derivatives, involving J 11 as pollen parent in some cases, were also tested for seed colonization. Genotypes OGO-2 and 1-4 had seed colonization of 8-9 %. Seed treatment with solid sodium chloride at 2 % level inhibited seed colonization considerably.
Aflatoxin content was found to vary among genotypes. Those which had more than 3% phenols were found to contain less aflatoxins. Fumigation with ammonia was found to be highly effective, reducing aflatoxins by 68%. NaOH spray (2%) also reduced aflatoxins by about 70%. Fumigation with burning cowdung fumes and sun drying for one day were found effective in reducing aflatoxin (16-25%).


A method was developed for detoxification of highly aflatoxin-contaminated groundnut meal using the calcium hydroxide/monomethylamine alkaline degradation of aflatoxin.


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An azeotrope of acetone, hexane and water was found as a suitable solvent for removal of aflatoxin from contaminated groundnut meal.


Prevention, removal and inactivation of aflatoxin in groundnut protein concentrates is considered. Prevention is best and may be achieved by careful harvesting, handling, drying and storage of the crop produce. Removal of toxic kernels is also possible and this can be done by handpicking and moldy kernels. Aflatoxin may also be removed by polar solvents, by exposure to moist heat, and by treatment with ammonia.


Methods of detection and elimination of aflatoxins in agricultural commodities are reviewed.


Methods of decontamination and detoxification of aflatoxin-contaminated oilseeds are critically reviewed.


The best approach to contain aflatoxin contamination is prevention. Good farm management practices are essential. This includes use of sound, fungus-free, viable seed, proper fertilization, control of insects and diseases, prevention of lodging, and harvesting practices that avoid damaging the crop and picking up excessive leaves, trash, and dirt. Special attention should be given to diverting aflatoxin-containing lots from food and feed channels as early as possible in the marketing chain. Clean, dry, adequately cooled and ventilated storage and good sanitation are essential to minimize mold contamination. Genetic approaches and use of mold inhibitors may be helpful. When prevention fails, hand or electronic sorting can remove contaminated seeds. Aflatoxin may be removed during processing by alkali refining of crude oils or by extraction of contaminated oilseed meals with polar organic-solvents. Some aflatoxin is destroyed or degraded during normal preparation of some foods. Many chemicals, especially oxidizing and reducing agents and acids and bases, have been screened as reagents for destroying aflatoxin in contaminated feeds. Several processes have been patented. In the USA aflatoxin in copra has been destroyed on a commercial scale by hot aqueous calcium hydroxide and in cottonseed meal by treatment with ammonia under pressure.


Methods of decontamination and detoxification of aflatoxin- contaminated agricultural products are reviewed. Topics discussed include physical removal/segregation of contaminated nuts and seeds, inactivation, and chemical detoxification.
Methods of prevention, elimination and detoxification of ergot, aflatoxin, ochratoxin, zearalenone and T-2 toxin are reviewed. Prevention is the best approach, but when contamination occurs the detection and physical separation of contaminated lots may prevent contamination of larger supplies. Physical separation of ergot-contaminated grain and some aflatoxin-contaminated groundnuts and Brazil nuts was successful. Detoxification of aflatoxin has been studied most extensively; ammonia appears to be the most promising reagent, but processing conditions differ markedly with commodities such as maize, cottonseed and groundnut. Ammonia also appears to be effective for detoxification of zearalenone in maize.


An azeotrope of acetone, hexane and water was found as a good solvent for removal of aflatoxin from contaminated groundnut meal.


Groundnut meals containing an average of 250 µg kg⁻¹ aflatoxin B₁ were processed in a twin-screw extruder in the presence of 0-2.5 % ammonium hydroxide. The aflatoxin B₁ content determined after extrusion was reduced by 23-66 % in the absence, and by 40-87 % in the presence of ammonium hydroxide. When non-extruded groundnut meals were incubated with proteolytic enzymes at acid and/or alkaline pH, and at 37 or 50°C, the aflatoxin content determined by extraction from the dried-enzyme-treated meals was lower than that of the initial meals. It is suggested that extrusion could either cause destruction of aflatoxin molecules or disruption of their binding to groundnut meal constituents.


Aflatoxins in groundnut meals can be removed by treatment with polar solvents or gaseous ammonia. Among several possibilities, economically interesting solutions were chosen in order to propose a commercial plant. Extractions were carried out in a stainless steel apparatus allowing direct extrapolation into a commercial scale de Smet extractor. Numerous extraction series were made with acetone-hexane-water azeotrope and addition of some alkalies. The effect of the number and duration of passes, and the granulometry and the meal-solvent residence time were studied. Commercial conditions resulted in a 50 µg kg⁻¹ level of aflatoxin content. Increase of water and acetone content and introduction of ammonia in the azeotropic mixture allowed reduction of aflatoxin content to 8 µg kg⁻¹. Unfortunately, with a 60 ton day⁻¹ French extractor in a Lesieur plant, the azeotropic mixture was not active enough for removing the toxin to acceptably low levels. Ammoniations were carried out in a Speichim reactor (25 kg Batch⁻¹) in an industrial plant. Hundreds of tests were run with expeller and extraction meals in variable conditions of ammonia pressure, water content, reaction temperature and reaction time. Ammoniation easily allowed reduction to below 50 µg kg⁻¹. The soluble nitrogen content decreased with rigor of working conditions. Amino acid determination showed that lysine degraded by treatment with gaseous ammonia. Water content of meals was the limiting factor. Nutritional tests on rats showed that the food value of the meals was not affected by treatment with polar solvents. Ammoniation, although it gave less rapid growth curves, was judged to be satisfying after zootechnical tests. Examination of organs showed few noxious effects on liver and kidney cells after solvent treatment or ammoniation. In the present state of research, both processes remain possible, ammoniation having a slight advance in technological implications.


A process for detoxifying aflatoxin-contaminated groundnuts is outlined. This process uses the solvent methoxymethane as the removal agent and oil extraction can be carried out simultaneously with the aflatoxin removal process. The solvent used can be removed easily and thoroughly from defatted products because of its low boiling temperature.


A process is given for removing aflatoxins from groundnuts by extraction with ethylene oxide and/or methyl formate. The moisture content of the product to be treated is preferably kept at a maximum of about 16 % by weight. The gas or gas mixture is preferably used in a density of from 1000 to 1500 mg L⁻¹.

Groundnut meal which was spiked with $^{14}$C aflatoxin $B_1$ was ammoniated at 75°C, 35 psig for 30 min in a laboratory reactor, and the distribution of label was measured in subsequent fractions. Only 45-50 % activity was detected in the ammoniated meal after initial air-drying, 8 % was detected in humins remaining after acid hydrolysis, 4-6 % was associated with protein, and 33-36 % was associated with nonprotein residue. This residue had all of the nonreacted aflatoxin $B_1$.


Samples of groundnut and cottonseed meals spiked with pure aflatoxin $B_1$ or inoculated with aflatoxigenic cultures of Aspergillus parasiticus or $A. flavus$ were ammoniated under laboratory conditions closely simulating those used in commercial detoxification. In all experiments, the average content of aflatoxin $B_1$ after processing was similar to that of aflatoxin $D_1$, to which some of the original aflatoxin $B_1$ was converted by ammoniation.


Blanched whole groundnut kernels inoculated with an aflatoxigenic strain of Aspergillus flavus and subjected to continuous shaking during incubation for 40, 46, 64, and 72 h produced groundnuts virtually clear of visible mold and containing four graded levels of total aflatoxins - 130, 260, 2560, and 6300 µg kg$^{-1}$. Although individual kernels differed in aflatoxin content, replicate assays of 50 g aliquots of contaminated whole kernels were subjected to oil and dry roasting under live time-temperature conditions simulating those used in industry to effect a normal roast for high quality groundnuts. Average reduction in aflatoxin content ranged from 45 to 83 %, depending on roasting conditions and the level of aflatoxins in the raw kernels. There was an over-all reduction of 65 % in $B_1$ and 62 % in $G_1$ for oil roasting, and 69 % in $B_1$ and 67 % in $G_1$ for dry roasting. The degree of reduction in aflatoxin content was greatest at the highest aflatoxin contamination levels, for both oil- and dry-roasted groundnuts.


The major product formed from reacting pure aflatoxin $B_1$ with ammonium hydroxide at 100°C under pressure was isolated in crystalline form. The compound, molecular weight 286, ultraviolet absorption, Y max MeOH 227, 324 nm (E 15920. 12440), is nonfluorescent, exhibits phenolic properties, and lacks the lactone group characteristic of aflatoxin $B_1$. Acidification of the compound did not regenerate aflatoxin $B_1$. Calculations made from ultraviolet absorption spectra indicated that the compound comprises about 30 % of the crude ammoniation product. It is postulated that the new product, C$_{16}$H$_{14}$O$_6$, arises from opening the lactone ring of aflatoxin $B_1$ during ammoniation, formation of the ammonium salt of the resultant hydroxy acid, and loss of carbon-dioxide from this B-keto acid. Since this compound arises from decarboxylation the trivial name aflatoxin $D_1$ is proposed.


An industrial process to remove aflatoxin from contaminated groundnut cake using gaseous ammonia is described. Safety and pollution problems are covered along with data on capital investment and running costs.


A process is given for removing aflatoxin from seeds or nuts such as groundnuts by extraction with methoxymethane.


Aflatoxin-contaminated groundnut meal (127 µg kg$^{-1}$) was detoxified by treatment with ammonia gas in a glass desiccator (4L capacity). Aflatoxin $B_1$ concentration was reduced by half by treatment with 13.1 % (w/w) ammonia gas at 20°C for 24 h, and after 144 h the concentration was reduced to 19 µg kg$^{-1}$ (85 % reduction). Chicken embryo bioassay revealed decreasing toxicity with longer treatment time. Treatment with 0.7 % ammonia gas at 20°C for 24 h was sufficient to kill conidia of Aspergillus flavus NRRL 3000. A. ochraceus 0458, and A. versicolor 0056.

Several organic and inorganic reagents were tested for destruction or inactivation of aflatoxins present in groundnut and cottonseed meals. The meals were treated with chemicals in a special laboratory-scale reactor, and were evaluated for aflatoxin contents. In some instances, a larger pilot-plant scale reactor was used for treatments. Ammonia, methyamine, sodium hydroxide and formaldehyde considerably reduced aflatoxin levels in the contaminated meals. Effects of various reaction parameters, including time, temperature and moisture content, on the efficiency of these chemicals are discussed.


Mycological analysis of postharvest groundnut samples was carried out. Various soil-fungi were found to infect the pods; most of them grew through the shell, but not all reached the kernels. The most common were Aspergillus flavus and A. niger. Where they are treated in oil fabrics, the originally heavily contaminated kernels are processed through some thermic treatments which progressively eliminate all fungal contamination. Nevertheless, a recontamination occurs as soon as the pellets are cooled, and a large mold development occurs in the usual conditions of storage. The cakes used to feed cattle are often highly contaminated. Detoxification of cakes by ammoniation destroys the aflatoxin and also has a fungicidal action against many molds, especially A. flavus.


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The efficacy of sodium hypochlorite in destroying aflatoxins in contaminated raw groundnuts and groundnut meal undergoing processing for the preparation of protein isolates was tested. Effects of sodium hypochlorite concentration, reaction pH, temperature, and time were investigated. Treatments with 0.4 % sodium hypochlorite at pH 8 produced groundnut protein isolates with trace amounts of aflatoxins B, and B2 from ground raw groundnuts containing 725 µg kg\(^{-1}\) and 148 µg kg\(^{-1}\) anatoxins B1 and B2 whereas nontreated protein isolates contained 384 µg kg\(^{-1}\) aflatoxin B1 and 76 µg kg\(^{-1}\) aflatoxin B2. At pH 9 or 10, 0.6 % sodium hypochlorite reduced aflatoxin B1 content from 380 µg kg\(^{-1}\) to below detectable levels and aflatoxin B2 content from 52 µg kg\(^{-1}\) to 2 µg kg\(^{-1}\). In defatted groundnut meal containing 136 µg kg\(^{-1}\) aflatoxin B1 and 36 µg kg\(^{-1}\) aflatoxin B2, 0.25 % sodium hypochlorite at pH 8 (0.20 % at pH 9 and 0.15 % at pH 10) reduced both aflatoxins to below detectable levels in protein isolates compared with 75 µg kg\(^{-1}\) aflatoxin B, and 17 µg kg\(^{-1}\) aflatoxin B2 in nontreated protein isolates. Results indicated that both sodium hypochlorite concentration and pH are important factors in inactivation of aflatoxin.


The incidence of aflatoxin in maize and groundnuts in South East Brazil and South East USA was compared. Aflatoxin was found in 45 % of samples in Brazil and in 77 % of samples in the USA, the range of contamination in both countries being >20 - >100 µg kg\(^{-1}\). The Brazilian foods were sold for milling whereas the US ones were not. Attempts to chemically detoxify aflatoxin-contaminated samples were made using hydrogen peroxide, ammonium hydroxide, formaldehyde, sodium hypochlorite and isopropyl alcohol. Results indicated that 1.5 % hydrogen peroxide,
1 % sodium hypochlorite and 75 % isopropyl alcohol significantly reduced aflatoxin contamination.


A study of major foods in Nigeria showed that groundnuts and dried fish were highly contaminated with aflatoxins (900 µg kg⁻¹ in groundnuts; 600-700 µg kg⁻¹ in dried fish) whereas various grains, millets, guinea corn and rice were moderately contaminated (300-150 µg kg⁻¹). Low risk foods such as yams became contaminated with aflatoxin after storage, particularly when wrapped in cellophane or even in newspaper. Immersion of foods in a dilute (1 %) solution of NaCl 16.5 % and sodium hypochlorite 1 % (Milton) reduced the level of aflatoxin by almost 50 %. Exposure to direct sunlight caused a marked decline (30-16 %) in aflatoxin levels of foods.


This paper reviews various studies on ammoniation of aflatoxin contaminated corn, vegetable oils undergoing processing. Crude oils, obtained by solvent extraction or by hydraulic pressing of ground moldy groundnuts (not suitable for human consumption), contained only small fractions of the aflatoxin originally present in the groundnuts; the meals retained the bulk of the aflatoxin. Conventional alkali refining and washing of the oils reduced aflatoxin content to a range of 10 to 14 µg kg⁻¹. The subsequent bleaching operations essentially eliminated aflatoxin from the oils; the concentrations after this process being less than 1 µg kg⁻¹. The above results were confirmed using corn oils obtained from corn germ deliberately contaminated in the laboratory with Aspergillus flavus. The nonfluorescing forms of aflatoxins, capable of being produced during the alkali refining operations, are also absent from the refined vegetable oils; these aflatoxin derivatives are readily converted to their original form on acidification and are thereby measurable by fluorescence, if present.


Research on aflatoxins carried out at the Central Food Technological Research Institute, Mysore (India), is presented. Calcium chloride solution (1 %) removed about 80 % of aflatoxin from contaminated groundnut cake flour. Heating toxic groundnut kernels in water or 0.5 % CaCl₂ solution at 90°C for half an hour removed 90-95 % of the toxin. The results showed that precipitation of proteins with calcium chloride at 6.8 pH helped in avoiding most of the toxin going with the protein fraction. A paper chromatographic method for aflatoxin analysis is reported. Aflatoxin B₁ was found in 20 % samples of breast milk from mothers and in 60 % samples of urine of cirrhotic children.


A method for reclamation of toxic groundnut meal for edible purposes is reported. The method is based on the oxidation of aflatoxin by a suitable oxidising agent. Treated meal has a good appearance with good organoleptic quality. Nutritive value of the protein is not altered. Some extraction procedures and effects of aflatoxin on rats are reported.


Inactivation of aflatoxin B₁ was studied by using gamma radiation and hydrogen peroxide. A 100-krad dose of gamma radiation was sufficient to inactivate 50 µg of aflatoxin B₁ in the presence of 5 % hydrogen peroxide ; 400 krad was required for total degradation of 100 µg of aflatoxin in the same system. Degradation of aflatoxin B₁ was confirmed by high pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC). Ames microsomal mutagenicity tests showed loss of aflatoxin activity. This method of inactivation also reduced the toxin levels in artificially contaminated groundnuts.


Unshelled Spanish groundnuts contaminated with aflatoxin were shelled and treated with forced heated air, liquid nitrogen, H₂O₂, HCl, sodium oleate and water spray. After passing through a whole nut blancher the aflatoxin content and percentages of blanched and whole kernels were determined. Blanching percentages were significantly higher for sodium oleate, water spray, liquid nitrogen and H₂O₂ than for HCl or heat treatments. The heat treatments produced the highest percentage of whole kernels. In most tests, the non-blanched kernels had higher levels of aflatoxin than had those that blanched fully. The treatments which were most effective in producing low levels of aflatoxin in the blanched kernels were H₂O₂, water spray and HCl.


In groundnut meal treated with 4 % calcium hydroxide and 0.5 % paraformaldehyde in an autoclave at 2 atmospheres for 20 min, aflatoxin B₁ decreased from 401 to 29.5 10 µg kg⁻¹. In two trials lasting 10 and 22 days, aflatoxin M₁ was less in milk of two dairy cows given diets containing detoxified meal than in milk of cows given aflatoxin B₁ contaminated nontreated meal. The percentage of aflatoxin M₁ in milk compared with aflatoxin B₁ intake was similar with both treated and nontreated feeds.


A method to remove aflatoxins from contaminated groundnuts is described. Partly defatted groundnuts were used to prepare a flour which contained per 100 g protein 45.5 g, fat 25 g and 500 Kcal. The use of the groundnut flour in diets for man is discussed.


Several methods at decontamination and detoxification of groundnuts and groundnut cake are reviewed. Aflatoxin levels can be reduced by segregation and decontamination of groundnuts at shelling and after shelling. Influences of different stages in refining of oil on elimination of aflatoxin are described. Ammoniation of contaminated cake seems to be appropriate at industrial level.


In spite of preventative efforts, aflatoxin contamination of groundnuts is often unavoidable. Removal by extraction and by many chemicals has been tried. In 1979, an ammoniation semi-continuous plant (100 t day⁻¹) was installed by the French Lesieur Co. at the oil mill of Dakar (Senegal) with the financial support of the European Fund of Development (FED). Improvements to the detoxification process have been made by Sonacos combining formaldehyde as antibinding agent and ammonia. The capacity is now 600 t day⁻¹, and 3 other plants have been built. The treated groundnut meal has good nutritional quality. In France, a detoxification plant built in Brittany using a similar process has a capacity of 500 t day⁻¹ and gives similar results.


Aqueous isopropanol was found to be an effective solvent for removal of aflatoxins from contaminated cottonseed and groundnut meals. Extraction with six passes of 80 % aqueous isopropanol at 60°C resulted in complete removal of aflatoxins in both meals, as analysed by thin-layer chromatography. Under similar extraction conditions, the isopropanol-water azeotrope, 88 % isopropanol by weight, removed 88 % of the total aflatoxins in groundnut meal, a reduction from 82 to 10 µg kg⁻¹ and 79 % of the total aflatoxins in cottonseed meal, a reduction from 214 to 46 µg kg⁻¹. Lower temperatures were less effective with both solvent systems.


Cottonseed and groundnut meals containing aflatoxins were extracted with 95.6 % and 90 % aqueous ethanol at 75°C to lower the level of aflatoxins. These solvents
removed 93-96 % of the aflatoxins in the cottonseed meal and 96-98 % of the aflatoxins in groundnut meal.


Experiments were conducted to study the efficacy of some oxidizing or other reactive chemicals for destruction of aflatoxins in consultation with the aqueous extraction process for the production of groundnut protein concentrates and/or isolates directly from aflatoxin-contaminated raw groundnuts. The chemicals tested included acetone, isopropyl alcohol, methylvamine, hydrogen peroxide, benzoyl peroxide, ammonia gas, and sodium hypochlorite. Among these chemicals, hydrogen peroxide, benzoyl peroxide, and sodium hypochlorite were effective in destruction of aflatoxins during the aqueous extraction process of contaminated groundnuts. However, the use of benzoyl peroxide may pose some difficulties because it is not readily soluble in the aqueous suspensions. It was concluded that aflatoxins can be effectively destroyed during the aqueous processing of groundnuts by properly utilizing either sodium hypochlorite or hydrogen peroxide to produce either groundnut protein concentrates or isolates.


This paper very briefly reviews several methods used for destruction of aflatoxin in groundnuts and their products. The exposure of the groundnut oil contaminated with aflatoxin to bright sunlight completely destroys the toxin. Aflatoxin is present in finely suspended solids in the oil and can be removed by filtration or by extraction with 10 % sodium chloride. Aflatoxin-contaminated groundnut kernels (0.5 mm thick flakes) can be partially detoxified if exposed to sunlight for 14 hours. To remove aflatoxin from groundnut cake, 50 % alcohol, acetone, methanol, or 1 % calcium chloride can be used. Treating the protein isolate with hydrogen peroxide, and groundnuts in the form of thin flakes or powder with urea and soyaflour with or without formaldehyde can destroy 90 % of their aflatoxin content. The merits and demerits of these processes are discussed.


A method for removing aflatoxin from crude groundnut oil has been developed after studying the efficiency of several extractants such as sodium chloride solution, aqueous acetone, sodium hydroxide and ammonia. Sodium chloride proved to be ideally suitable for removing aflatoxin B1 from crude groundnut oil.


Groundnut kernels collected from north-eastern plantations in Kalasin Province, Thailand, in August 1987 were roasted, split open and the seed coat, cotyledons and embryos were analyzed for aflatoxins by a Velasco aflatoxin meter. Results indicated accumulation of aflatoxin in embryos (274 µg kg⁻¹) and ground cotyledons (275 µg kg⁻¹). Of various food preservatives studied, sodium bisulfite was the best detoxification agent in ground groundnuts, with effective dose as low as 100 mg kg⁻¹.


Of the nearly 150 isolates of Aspergillus flavus from groundnuts tested, only four produced aflatoxin B1. These isolates differed from the type strain of A. flavus M001 (obtained from me Tropical Products Institute, London) in certain morphological characteristics. Of the several salt solutions tested, 1 % CaCl₂ was found most promising in extracting aflatoxin from the contaminated groundnut cake. In the preparation of the protein isolates, precipitation of the protein in CaCl₂ solution helped in removing nearly 80 % of the toxin from the protein. Certain apparently healthy groundnuts showed bright bluish-violet fluorescence under ultraviolet light. Extracts of such groundnuts on thin-layer chromatography (TLC) plates showed the fluorophor at the same Rf as that of aflatoxin B1. A simple solvent system of benzene : cyclohexane : acetic acid (3:5:2, v/v/v) was found to give on paper chromatogram as good resolutions of the aflatoxin complex as found on alumina TLC with chloroform and methanol as solvent.


A new approach to destroy aflatoxin in toxic groundnut meal is described. It involves heat treatment of the meal at 80°C for 30 min with hydrogen peroxide at
a pH of 9.5. The destruction of aflatoxin was confirmed by biological tests, using ducklings and duck embryos.


Detoxification of groundnut oil was successfully accomplished by lowering the aflatoxin content to less than 20 µg kg⁻¹ by treating with Fuller's earth. Stirring the oil with 0.3 % of the earth at 150 rev min⁻¹ for 15 min effectively reduced aflatoxin content from 76 to 7.85 µg kg⁻¹ which is below the Ministry of Public Health Standard safety value requirement of 20 µg kg⁻¹. Chemical and physical properties of the processed oil comply with the food regulations. Two types of Fuller's earth, viz. AAA and Galleon earth, were used for absorbing aflatoxin; there were no significant differences between the types of earth in reducing the toxin. The detoxification process was highly effective and involved simple techniques. Equipment used consisted of a mixing tank, locally fabricated and a filter press, commonly available in a groundnut oil factory. The additional cost of processing (i.e., the cost of Fuller’s earth and electricity) was 37.50 baht t⁻¹ of oil treated.


An attempt was made to detoxify aflatoxin-contaminated groundnut meal for use in compounded rations for ruminants. Detoxification (>99 %) was obtained when the contaminated meal was stored with 5 % ammonia and 20 % water for 10 days in tight plastic bags. Three groups of cows were given, as part of their normal diet, (i) 1 kg groundnut meal containing 2500 µg aflatoxin, (ii) 1 kg of 93 % detoxified groundnut meal containing 175 µg aflatoxin, (iii) 3 kg of 93 % detoxified groundnut meal containing 525 µg aflatoxin, respectively. Aflatoxin M₁ excreted by each cow in groups (i) - (iii), respectively, was 94, 20 and 47 µg cow⁻¹ day⁻¹. There was a reduction of aflatoxin M₁ in milk, due to detoxification, of 79 % with (ii) and 83 % with (iii). Because of the experimental plan it was not understood why the cows fed on detoxified meal excreted a higher percentage of aflatoxin M₁ relative to ingested aflatoxin than cows fed on the aflatoxin-contaminated meal.


The reactions of aflatoxins (B₁, B₂, G₁, and G₂) with a series of selected reagents were observed for possible usefulness in detoxification procedures. Loss of fluorescence and/or change of Rf on TLC were the principal reaction indicators. Chick embryo and tissue culture bioassays were used to provide evidence of detoxification. The reactions appear to be primarily addition and oxidation involving the olefinic double bond of the terminal furan ring and oxidation involving the phenol formed on opening of the lactone ring. Benzoyl peroxide, osmium tetroxide, and 1- /12 react with aflatoxins B₁ and G₁, but not with B₂ and G₂, Ce(NH₂)₂(SO₄)₃, NaOCl, KMnO₄, NaBO₃, and 3 % H₂O₂ + NaBO₂ (1+1) react with all four aflatoxins. Detoxification, after contact with gaseous chlorine, chlorine dioxide, and nitrogen dioxide and after treatment with 5 % NaOCl solution, was confirmed by bioassay.


Various preservatives, such as calcium propionate, sorbic acid, potassium sorbate, dehydroacetic acid, sodium dehydroacetate, benzoic acid, sodium benzoate and isobutyl p-hydroxybenzoate, were examined for their inhibitory effects on the growth and aflatoxin B₁ production of Aspergillus flavus and A. parasiticus in sliced bread coated with groundnut butter. Groundnut butter containing a preservative and 100 conidia of each fungus was coated on the upper surface of a slice of bread, and the slice was incubated at 28°C for 10 days. Calcium propionate and sorbic acid showed the most inhibitory effects.


Treatment of groundnut oilmeal with gaseous ammonia at 2 to 3 bar is fairly easy and rapid. It reduced aflatoxin content of the meal by up to 95 %; the content of nitrogen, particularly non-protein nitrogen increased. There was no adverse effect on digestibility in vitro with pepsin, and the treated meal was more sensitive to proteases. Treatment slightly decreased deamination of protein in an artificial rumen on digestibility in vitro with pepsin, and the treated meal was more sensitive to proteases. Treatment slightly decreased deamination of protein in an artificial rumen. There was no adverse effect on digestibility in vitro with pepsin, and the treated meal was more sensitive to proteases. Treatment slightly decreased deamination of protein in an artificial rumen.

Groundnut meal contaminated with aflatoxin B₁ (at 450 µg kg⁻¹ DM) was treated with an aqueous ammonia solution (approximately 32%) at a rate of 20 kg 100 kg⁻¹ meal. After homogenization for 5 min, the product was packed in plastic sacks and stored for at least 2 wk before being fed to dairy cows. The treated groundnut meal contained aflatoxin B₁ at 4-13.5 µg kg⁻¹ DM, indicating a detoxification of about 98%. Milk from cows fed the contaminated meal contained aflatoxin M₁ at 1-1.8 µg kg⁻¹ on a DM basis, and milk from cows fed the detoxified meal contained only 0.1-0.22 µg kg⁻¹. The detoxified meal was not as acceptable to the cows as the nontreated meal. The amount of aflatoxin M₁ excreted by cows fed the detoxified meal was higher than would be expected from the aflatoxin B₁ intake. The possibility of the detoxification procedure being partly reversible is discussed.


This paper presents the results of an investigation into the possibility of the simultaneous removal of oil and aflatoxin from oilcake by the use of suitable solvents.


A simple method for the detoxification of aflatoxin B₁ in groundnut with ammonium bicarbonate is presented. The effects of ammonium bicarbonate concentrations, treatment time and temperature on aflatoxin detoxification were investigated. Chloroform extracts from the treated samples were assayed by a modified Ames bacterial test which confirmed that the decrease in mutagenicity was related to aflatoxin detoxification.


An investigation into the breakdown of aflatoxins during the hydrolysis of artificially contaminated defatted groundnut oilmeal was conducted using a laboratory scale reactor. The conditions selected emulated a commercial process used to produce a protein hydrolysate used in processed food and soup ingredients. Chromatographic analysis showed that aflatoxins at relatively high concentration (aflatoxin B₁, 321 µg kg⁻¹; B₂, 415 µg kg⁻¹; G₁, 161 µg kg⁻¹; G₂, 15 µg kg⁻¹) were totally destroyed and removed from the product. Residues from extracts were free of mutagenic properties, as monitored by the Ames test.

8.2.11 Microbial Detoxification


Yeasts, molds, bacteria, actinomycetes, algae, and fungal spores were screened for their ability to degrade aflatoxin. Some molds and mold spores partially transformed aflatoxin B₁ to new fluorescing compounds. Only one of the bacteria, Flavobacterium aurantiacum NRRL B-184, removed aflatoxin from solution; both growing and resting cells look up the toxin irreversibly. Toxin-contaminated milk, peanut butter, groundnuts, and corn were completely detoxified, and contaminated soybean was partially detoxified by addition of this organism. Duckling assay showed that detoxification of aflatoxin solution by the bacterium was complete, with no new toxic products being formed.


Rhizopus stolonifer, R. arrhizus, R. oryzae, and R. sp. degraded aflatoxin G₁. An intermediate in the biological degradation was isolated and identified as a previously reported metabolite of Aspergillus flavus (aflatoxin B₁) and A. parasiticus (parasitol). Radioisotope data conclusively demonstrated that this metabolite was derived from aflatoxin G₁ degradation by the Rhizopus spp.


An investigation of aflatoxin B₁ degradation by a Rhizopus arrhizus isolate from Georgia groundnuts and three known Rhizopus species revealed that two fluorescent metabolites of aflatoxin B₁ accumulated during degradation. They were identified by physical, chemical, and spectroscopic data as hydroxylated stereo isomers derived from reduction of the ketone function on the cyclopentane ring of aflatoxin B₁. It was conclusively shown with ¹⁴C-labelled aflatoxin B₁ that these metabolites were derived from aflatoxin B₁. Two additional fluorescent metabolites appeared during purification of the hydroxy isomers. These were identified as ethyl ether derivatives of the hydroxylated compounds and apparently were formed spontaneously from either one or both hydroxy isomers.

Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Mycelia of Aspergillus parasiticus can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of A. parasiticus, amount of mycelium, temperature, pH, and concentration of aflatoxin. Adsorbants, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin is stable at low pH values but not in the presence of large amounts of vitamin C or bisulfite. Patulin can be degraded by actively fermenting yeasts and rubratoxin can be degraded by the mycelium of Penicillium rubrum.


The ability of Flavobacterium aurantiacum to reduce aflatoxin B<sub>1</sub> concentration was determined, by inoculating 10 stationary phase cells into aflatoxin B<sub>1</sub>-contaminated phosphate buffer (PB), non-defatted peanut milk (NDPM) and partially defatted peanut milk (PDPM). Aflatoxin concentration and cell populations of the bacterium were determined periodically throughout incubation at 30°C. After 24 h, aflatoxin B<sub>1</sub> concentration had decreased about 40% in PB, 23% in NDPM and 74% in PDPM. Viable cell count had generally decreased by < 1 log<sub>10</sub> cfu ml<sup>-1</sup>, but increased about 0.8 log 10 units in control PDPM. Aflatoxin B<sub>1</sub> recovery increased about 30% on proteolysis of PDPM; proteolysis had no effect on recovery from NDPM.


The potential for using Flavobacterium aurantiacum to remove aflatoxin B<sub>1</sub> from peanut milk was evaluated. Preliminary experiments revealed that this bacterium grew in both non-defatted peanut milk (NDPM) and partially defatted peanut milk (PDPM). In neither case was the growth inhibited by the presence of aflatoxin B<sub>1</sub>. Other experiments were carried out to assess the ability of resting cells of F. aurantiacum to remove aflatoxin B<sub>1</sub> from phosphate buffer (PB), NDPM, and PDPM. After 24 hr at 30°C, F. aurantiacum decreased aflatoxin B<sub>1</sub> by 40% in PB, 23% in NDPM, and 70% in PDPM. Proteolysis of PDPM before inoculation with the bacterium increased recovery of the toxin by about 30% over non-proteolyzed samples. This increase in recovery of aflatoxin was not observed when NDPM samples were proteolyzed, indicating that some of the toxin may be bound to the peanut protein and not be available for removal by F. aurantiacum.


Removal of aflatoxin B<sub>1</sub> from liquid cultures by resting and growing cells of Flavobacterium aurantiacum NRRL B-184 was studied. Spectrophotometric and thin-layer chromatographic techniques served as aflatoxin assays. Cells grown in the presence of 5 ppm or higher levels of aflatoxin developed aberrant morphological forms. These toxin concentrations partially inhibited growth, and the nature of the inhibition suggested that aflatoxin interfered with cell wall synthesis. Incubation of 1.0 x 10<sup>6</sup> resting cells per milliliter with 7.0 µg ml<sup>-1</sup> of aflatoxin B<sub>1</sub> during a 4 h period facilitated complete toxin removal from a buffered aqueous medium. Autoclaved cells and cell wall preparations could remove a fraction of the aflatoxin of a test system. However, the toxin removed by autoclaved cells and cell walls could be extracted by washing with water but the aflatoxin B<sub>1</sub> removed by intact cells could not be extracted into the liquid phase. The uptake of aflatoxin B<sub>1</sub> by resting cells was sensitive to temperature and pH. Ruptured preparations of F. aurantiacum were not able to remove or modify the aflatoxin in an aqueous solution.


Aflatoxin G<sub>1</sub> was removed from liquid cultures by growing and resting cells of Flavobacterium aurantiacum NRRL B-184. In inoculated culture media containing aflatoxin levels of 7500 µg L<sup>-1</sup> and above, there was a protracted growth lag which was subsequently overcome; then occurred toxin removal, concomitant with growth. Only a few cells showed aberrant morphological forms when cultured in the presence of aflatoxin G<sub>1</sub>. A comparison of the effects of aflatoxin G<sub>1</sub> with aflatoxin B<sub>1</sub> on growth and morphology showed that aflatoxin B<sub>1</sub> was distinctly more toxic. Three hundred and thirty µg of aflatoxin G<sub>1</sub> was removed per 1 x 10<sup>12</sup> resting cells during a 4 h incubation period. Preincubation of resting cells with aflatoxin B<sub>1</sub> did not interfere with subsequent uptake of G<sub>1</sub>.


In liquid cultures growing and stationary phase cells of Flavobacterium aurantiacum NRRL B-184 eliminated aflatoxin M<sub>1</sub>. Toxin concentrations of 15 µg ml<sup>-1</sup> and 37.5 µg ml<sup>-1</sup> interfered with bacterial growth, and at the higher level 4.4 µg M<sub>1</sub> was removed from the growth medium by a milligram (dry weight) of bacteria. Toxin was completely removed from the liquid medium by incubating 5 x 10<sup>10</sup> resting cells.
per millilitre with 8 µg ml⁻¹ of aflatoxin M₁ for 4 h. Attempted recovery of M₁ from cells following incubation of the bacteria with the toxin demonstrated that the M₁ was essentially nonextractable. Bacterial cells also removed aflatoxin M₁ from toxin-contaminated milk.


Degradation of aflatoxin B₁ by Corynebacterium rubrum and by Aspergillus niger was analysed by adding ¹⁴C labelled aflatoxin B₁ to cultures of these microorganisms. Two blue fluorescent compounds, formed by A. niger from aflatoxin B₁, with R₁-values 0.42 and 0.48 (R₁ of aflatoxin B₁ = 0.54) were accumulated and characterized by UV-, fluorescence and mass spectrometry. Based on their properties both products were identified to be aflatoxin R₀. Under the same conditions Mucor ambiguus and Trichoderma viride also produced aflatoxin R₀.


This paper briefly reviews chemical, physical, and biological means of degrading aflatoxins. Research needs in these areas are presented.


Aflatoxin B₁ production by Aspergillus flavus NRRL 3251 was inhibited by Rhizopus sp or Aspergillus niger when grown in culture medium for up to 9 days at 28°C.

1759. Nout, M.J.R. 1989. Effect of Rhizopus and Neurospora spp. on growth of Aspergillus flavus and A. parasiticus and accumulation of aflatoxin in groundnut. Mycological Research 93(4): 518-523. The influence of Rhizopus and Neurospora spp. on growth and aflatoxin B₁, accumulation of Aspergillus flavus and A. parasiticus was investigated. When inoculated simultaneously with Rhizopus or Neurospora spp. on mashed groundnuts, Aspergillus spp. were able to grow, but to a lesser extent and with visually different mycelial development and sporulation behavior. Accumulation of aflatoxin B₁ observed during a 6 wk incubation, averaged 34 % in mixed cultures with Rhizopus spp. and 1.7 % in mixtures with Neurospora spp., as compared with pure cultures of Aspergillus spp. On aqueous groundnut extracts, selected strains of Rhizopus and Neurospora showed the ability to cause an exhaustion, particularly of C compounds, inhibiting Aspergillus growth and aflatoxin B₁ accumulation. There was also evidence for the formation of Rhizopus and Neurospora metabolites inhibiting growth and/or aflatoxin B₁ production. In addition Rhizopus and Neurospora strains degraded aflatoxin B₁.

1760. Teunisson, D.J., and Robertson, J.A. 1967. Degradation of pure aflatoxin by Tetrahymena pyriformis. Applied Microbiology 15(5): 1099-1103. Tetrahymena pyriformis with nutrients, ca. 22 x 10⁶ cells, decreased the concentration of aflatoxin B₁ 58 % in 24 h and 67 % in 48 h. An unknown, bright-blue fluorescent substance was produced, with intensity about one-half that of the un-changed B₁, with an R₁ of 0.52 compared with 0.59 for B₁ and 0.55 for B₂ on a thin-layer chromatography plate, and with an ultraviolet spectrum showing maxima of 253, 261, and 328 μ. In a separate assay, the cells with nutrients did not degrade pure G₁. Starved, washed cells, ca. 11 x 10⁶, decreased the concentration of B₁ 50 % in 10 h, 70 % in 22 h, and 75 % in 30 h, producing the same unknown component. Ethyl alcohol, 1.96 % (v/v), decreased cell populations and size, but the cells remained actively motile in broth plus alcohol for 96 h. In 72 h, neither toxin (ca. 2 ppm) in combination with ethyl alcohol had more inhibitory effect on cell numbers, with or without nutrients, than was produced by alcohol alone. Aflatoxin B₁ had no observed effect on the viability of the starved cells for 30 h or on the nourished cells for 72 h. There was no noticeable effect of G₁ on the starved cells in 30 h or on the nourished cells in 48 h. After 72 h with G₁ plus nutrients, many of the cells were round with blisters, nonmotile, and apparently dead or dying.


8.2.12 Physical Detoxification


The effects of oven and microwave roasting on aflatoxin contaminated groundnuts and the effect of chlorine treatment on aflatoxin B₁ detoxification were studied. In artificially contaminated groundnuts, oven roasting for 30 min at 150°C or microwave roasting for 8.5 min at 0.7 KW were equally effective in destroying 30 to 45 % of aflatoxin B₁. In naturally contaminated groundnuts, both oven and microwave roasting were equally effective in destroying 48 to 61 % of aflatoxin B₁ and 32 to 40 % of aflatoxin G₁. Chlorine gas treatment was very effective in destroying aflatoxin B₁. Time course study of this treatment (100 jig aflatoxin B₁
with 15 mg chlorine gas at standard temperature and pressure) showed that about 60 to 75% of the toxin was destroyed within 10 min of exposure. During the treatment process, at least three new fluorescent reaction products were produced and two of them were identified as 2,3-dichloro aflatoxin \( \text{B}_1 \) and 2,3-dihydroxy aflatoxin \( \text{B}_1 \) (diol). Use of radio-labelled aflatoxin \( \text{B}_1 \) confirmed these results. Chlorine-dose related study at 10 min exposure indicated that even the treatment of 100 \( \mu \text{g} \) of aflatoxin \( \text{B}_1 \) with 7.5 mg of chlorine caused about 75% destruction. Preliminary mutagenicity study using the Ames Salmonella assay indicated that the mutagenic activity of the 10-min treated sample in the presence of rat liver S-9 mix can be reduced to about 10% of that of the untreated control. The results indicated that low energy microwave roasting is not an efficient method to remove aflatoxin \( \text{B}_1 \) from contaminated groundnut samples, while chlorine gas could be an effective agent in reducing aflatoxin toxicity.

### References


Irradiation of aflatoxins \( \text{B}_1 \) and \( \text{G}_1 \) with UV light (principal wavelength 365 \text{nm}) converts both compounds to new fluorescent photoproducts which have much lower \( R_t \) values than aflatoxins \( \text{B}_1 \) and \( \text{G}_1 \) when chromatographed on silica gel thin-layer plates. Photoproducts of aflatoxin \( \text{B}_1 \) form much faster on a silica gel surface than in methanol solution. Photoconversion of aflatoxin \( \text{B}_1 \) is shown to alter fluorescence comparison assays and identification tests. Studies show that the principal photoproduct developed from aflatoxin \( \text{B}_1 \) is sufficiently less toxic than the parent aflatoxin.

1764. Applegate, K.L., and Chipley, J.R. 1974. Effects of \( ^{60} \text{Co} \) gamma irradiation on aflatoxin \( \text{B}_1 \) and \( \text{B}_2 \) production by \text{Aspergillus flavus}. Mycologia LXVI 3: 436-445.

Germination of spores of the aflatoxigenic \text{Aspergillus flavus} NRRL-3145, previously exposed to specific gamma radiation levels, resulted in a greater production of aflatoxins \( \text{B}_1 \) and \( \text{B}_2 \) following inoculation onto a cracked wheat medium or into a synthetic liquid medium than did similarly treated nonirradiated controls. The most notable increases in aflatoxin production occurred from cultures developing from spores having been irradiated with 50, 100, 150, or 200 krad of gamma radiation. Exposure to 400 krad of gamma radiation resulted in complete inhibition of spore germination and consequently negative toxin production. Of the two aflatoxins, the quantities of \( \text{B}_2 \) produced were significantly lower than that of \( \text{B}_1 \) except in synthetic medium where \( \text{B}_2 \) production was comparable to that of \( \text{B}_1 \). Analysis of variance revealed that substrate, time of incubation following the respective irradiation doses, as well as radiation levels, all affected the quantities of aflatoxins produced by the fungus.


The occurrence of aflatoxin in the Australian groundnut crop is mainly due to late season drought stress. The Australian Peanut Marketing Board uses a minicolumn test to segregate aflatoxin-contaminated (aflatoxin > 16 \( \mu \text{g} \) kg\(^{-1} \)) farmers' stock groundnuts at the point of delivery. To check the effectiveness of this segregation, an investigation was made to quantify the incidence of aflatoxin in groundnuts at several major points in the deshelling, grading and blanching operations. The aflatoxin "positive" segregation lots contained aflatoxin levels eight times higher than the aflatoxin "negative" lots. Seventy-one% of the aflatoxin through the shellers was concentrated into the oil milling kernels, which comprised 16% of the total kernels. Oil kernels from "negative" and "positive" stock averaged 48 \( \mu \text{g} \) kg\(^{-1} \) and 253 \( \mu \text{g} \) kg\(^{-1} \) aflatoxin, respectively. It appears that in excess of 50% of the aflatoxin in the product which was roasted and blanched was lost and/or degraded in the operation.


This paper discusses several physical methods for detoxification of aflatoxin-contaminated groundnuts, groundnut oil, maize, cottonseed, and rice. Removing discolored seeds by handpicking or by electronic color sorting devices is efficient for reducing aflatoxin content in groundnuts, maize and cottonseed. The possibility of using air classification to separate aflatoxin-contaminated groundnuts and cottonseed has been explored and found to be a useful tool. Milling of contaminated brown rice has been found to reduce aflatoxin in the polished rice. Roasting, frying, boiling, baking and cooking of contaminated groundnuts have been found to inactivate aflatoxin to a certain extent. Detoxification of groundnut oil by exposure to bright sunlight has proved useful. Decontamination of groundnut oil is also possible by the use of filterpads in the processing line of oil mills. Washing the oil with sodium chloride solution is another promising method for removal of aflatoxin. These detoxification/decontamination procedures are discussed with special reference to their economic and technical feasibility.


Centrifuging at 40,000 g removed 65% of total aflatoxin along with sedimentable cell debris in commercial groundnut oil. Studies suggested that the remainder of the toxin was in a soluble form. Pure aflatoxin added to refined oil was not sedimented. Filtration with membrane filter, sintered glass and filter paper could separate 30-50
% of aflatoxin in the oil. Use of activated Fullers' earth as an adsorbent at 2 % level and centrifugation at 10,000 g sedimented nearly 92.5 % of the aflatoxin.


Attempts were made at laboratory and pilot plant level to remove aflatoxin from groundnut oil by the use of filter pads. The filter beds tried were (i) Celite and salt (1:10), (ii) activated fullers' earth and salt (1:2) and (1:10), and (iii) Kaolinite and salt (1:2). Of these, Kaolinite and salt (1:2) proved best for removing aflatoxin but the rate of filtration was slow. The activated fullers' earth and salt (1:2) proved best in efficiency (96 % aflatoxin removed) and speed. These were further used to remove aflatoxin on pilot scale employing a plate-and-framc filter press, the efficiency ranged from 88-90 % for removal of aflatoxin. The pad could be used to filter 200 kg of oil and the filtration rate was 12 kg oil h⁻¹ and the aflatoxin content in filtered oil was 25 µg kg⁻¹.


Groundnut kernels inoculated with Aspergillus parasiticus conidia and uninoculated kernels were gamma irradiated with 0-15 KGy using¹⁰⁰ Co. Levels of 2.5 and 5.0 KGy were effective in retarding outgrowth of A. parasiticus and reducing the population of natural mold contaminants. However, elimination of these molds was not achieved. When irradiated with doses > 10 KGy, seed germination was inhibited, changes in proteins were observed and oil stabilities decreased. After 4 wk incubation of the inoculated kernels in a humidified condition, aflatoxins produced by surviving A. parasiticus ranged from 69.12 to 13.48 µg g⁻¹ depending upon the original irradiation dose.


This paper reviews the work carried out at the Overseas Development Natural Resources Institute (ODNRI), London, on the development of efficient sampling, sample preparation, aflatoxin analysis, and chemical detoxification procedures. The control of aflatoxin in groundnut products requires a combination of quality control and decontamination/detoxification procedures. The use of selected mathematical models to describe the distribution of aflatoxin in groundnut kernels, roasted groundnuts, peanut butter, and groundnut cake has been investigated for developing statistically sound sampling plans for these commodities. A subsampling mill has been developed, in collaboration with a UK company, which enables representative, comminuted subsamples to be rapidly produced from large samples of groundnut kernels. Methods have been developed for the accurate analysis of the aflatoxin content of groundnut products using bonded-phase clean-up procedures in combination with high performance liquid chromatography (HPLC) and high performance thin-layer chromatography (HPTLC) quantification methods. The application of enzyme-linked immunosorbent assay (ELISA) methods to the analysis of aflatoxin in peanut butter has also been extensively examined. A procedure for the detoxification of groundnut cake using ammonia gas at high temperatures and moderate pressures has been developed.


A process is given for removing aflatoxin from contaminated groundnut meal by autoclaving. Autoclaving at 15 lb. in² (120°C) of wet toxic groundnut meals resulted in a progressive reduction in their aflatoxin B₁ toxicity, with time. Similar experiments involving treatment of pure aflatoxins (either produced by artificial culture or isolated from toxic extracts by preparative-scale, thin-layer chromatography), resulted in the recovery of non-fluorescent products in which the characteristic ultraviolet absorption band of aflatoxin B₁ at 363 µ gave disappeared.


Groundnuts (cultivar Florunner) at moisture contents ranging between 8 % and 22 % (wet weight basis) were shelled and microwave vacuum dried at treatment rates of 4, 8, 16 and 32 times the nominal recommended rate for conventional wagon drying. Electrical energy per unit dry mass supplied to the microwave generators was closely equivalent to the energy content of L.P. gas used in wagon drying. Propensity of microwave treated kernels toward splitting and skin slippage was insignificant when compared to conventional within shell deep bed dried control samples. Significant differences occurred for presence of Aspergillus flavus on surfaces of kernels from the pooled microwave treatment compared to check samples, but differences among the microwave treatments were insignificant in this respect. Aflatoxin was not detected in any microwave or control treatment. Kernel germination decreased with increasing microwave process rate.
Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Mycelia of Aspergillus parasiticus can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of A. parasiticus, amount of mycelium, temperature, pH, and concentration of aflatoxin. Adsorbents, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin is stable at low pH values but not in the presence of large amounts of vitamin C or bisulfite. Patulin can be degraded by actively fermenting yeasts and rubratoxin can be degraded by the mycelium of Penicillium rubrum.


A highly aflatoxin-contaminated groundnut sample was treated to inactivate its toxicity by dry heating (roasting) or autoclaving. Aflatoxins in contaminated groundnuts proved resistant to dry heating, while autoclaving was satisfactory for their destruction. Thin-layer chromatography (TLC), toxicity to chick embryo and inhibition of Bacillus megaterium growth tests were used to evaluate the inactivation of aflatoxins.


Oil extracted from aflatoxin-containing groundnut kernels by crushers or expellers is toxin-free; extraction of residual oil from the press-cake by the usual type of solvent does not remove aflatoxin either. Consequently, press-cake and meal contain practically all the aflatoxin originally present in kernels. Trials to detoxify groundnut meal by radiation, heat, solvents, or reactive chemicals, conducted at the Tropical Products Institute in London, UK, were not very successful. The only treatments that destroyed or removed the toxin - i.e., prolonged wet heating at 120°C, extraction with methanol, and treatment with 10% HCl, SO₂, or Cl₂ - are expensive and cause deterioration of the meal. It is concluded that detoxification is not a practical possibility and that the emphasis must be on the prevention of infection by the aflatoxigenic fungus Aspergillus flavus.


Shelled groundnuts packaged in multi-wall paper bags were r- irradiated at 0-150 krad and stored for 10 months at 27-30°C. Aflatoxin contamination during storage was controlled at 100 and 150 krad. Free fatty acid contents changed slightly at <150 krad, increased 5-6 times at > 150 krad. Thiobarbituric acid values were negative for both irradiated and non-irradiated groundnut samples.


Destruction of aflatoxins in foodstuffs by irradiation was investigated. No significant destruction of aflatoxin in foodstuffs with irradiation was possible. Dry aflatoxins were highly resistant to radiation.


Hydrated sodium calcium aluminosilicate (HSCAS) was added to the diets of growing barrows in 6 treatments of 5 barrows each at the following concentration: 5 g kg⁻¹ HSCAS (0.5 %); 20 g kg⁻¹ HSCAS (2 %); 3 mg kg⁻¹ aflatoxin (AFT); 5 g HSCAS (0.5 %) plus 3 mg AFT kg⁻¹ feed; 20 g HSCAS (2 %) plus 3 mg AFT kg⁻¹ feed; and no HSCAS or AFT (control). Barrows were observed twice daily and were weighed weekly, and blood samples were obtained weekly for haematological and serum biochemical measurements. At the termination of the study, barrows were euthanatized and necropsied. Body weight gains were diminished significantly (P<0.05) by consumption of 3 mg kg⁻¹ AFT, whereas body weight gain in barrows consuming diets containing HSCAS or HSCAS plus AFT did not differ from that in control barrows. Serum enzymatic activities of alkaline phosphatase and Y-glutamyl transferase and prothrombin time were increased in barrows consuming 3 mg AFT kg⁻¹ feed, but not in those consuming HSCAS or HSCAS plus AFT. Aflatoxin alone induced decreased serum concentration of urea nitrogen, albumin, total protein, calcium, phosphorus, cholesterol, and glucose, as well as serum total iron-binding capacity, whereas HSCAS or HSCAS plus AFT did not induce such effects. Hepatic lesions in barrows of the AFT-alone treatment group were characterized as peripheral lobular lipidosis accompanied by perportal and interlobular fibrosis and bile duct hyperplasia. Hepatic lesions were not observed in barrows of the 0.5 % HSCAS plus AFT or 2 % HSCAS plus AFT treatment groups. It is suggested that HSCAS can modulate the toxicity of AFT in growing barrows and may offer a novel approach to the preventive management of aflatoxicosis in animals.

either the double bond of the furan ring or the furan ring itself may be removed from the coumarin portion of the molecule. Furthermore, the lactone ring of the exposure of solutions of aflatoxins B₁ may be removed for high quality groundnuts. Average reduction in aflatoxin content ranged from 45 to 83 %, depending on roasting conditions and the level of aflatoxins in the raw kernels. Some toxins were detected in the roasted portions. When the levels were low in the raw half, toxins were generally not detectable after roasting.


Blanched whole groundnut kernels inoculated with an aflatoxigenic strain of Aspergillus flavus and subjected to continuous shaking during incubation for 40, 46, 64, and 72 h produced groundnuts virtually clear of visible mold and containing four graded levels of total aflatoxins - 130, 260, 2560, and 6300 µg kg⁻¹. Although individual kernels differed in aflatoxin content, replicate assays of 50 g aliquots of contaminated whole kernels were subjected to oil and dry roasting under five time-temperature conditions simulating those used in industry to effect a normal roast for high quality groundnuts. Average reduction in aflatoxin content ranged from 45 to 83 %, depending on roasting conditions and the level of aflatoxins in the raw kernels. There was an over-all reduction of 65 % in B₁ and 62 % in G₁ after one-half h at 150 °C. In most cases the roasted half of each kernel containing aflatoxins, although not deeply colored, was darker than the roasted controls. However, many of the raw halves appeared almost identical to normal groundnuts. Generally, when the level of the toxins was high in the raw kernels, some toxins were detected in the roasted portions. When the levels were low in the raw half, toxins were generally not detectable after roasting.


Changes in UV and infrared absorption spectra were demonstrated upon prolonged exposure of solutions of aflatoxins B₁ and G₁ to long wave UV light. The treated toxins were less toxic to chick embryos and had lower R₁ values on thin-layer chromatograms than the parent compounds. These modified compounds may lack either the double bond of the furan ring or the furan ring itself may be removed from the coumarin portion of the molecule. Furthermore, the lactone ring of the aflatoxins is not opened upon exposure to UV light and is not the reason for decreased toxicity.


Effects of heat treatments on aflatoxins present in a contaminated cottonseed meal were investigated. Temperature, period of heating, and moisture content of the meal were varied in these experiments. Heating at 60 °C and 80 °C did not lead to marked reductions in aflatoxin levels. Definite reductions were obtained at 100°C, greater decreases taking place with increasing periods of heating and increasing moisture content. The lowest level of aflatoxins B₁ and B₂ achieved practically was about 44 µg kg⁻¹ obtained by heating for 120 min at 100°C with a moisture content of 20 %. This represented about 80 % reduction in the 214 µg kg⁻¹ aflatoxins present in the nontreated cottonseed meal. About 34 % reduction in aflatoxins (111 to 73 µg kg⁻¹) was obtained when a contaminated groundnut meal was heated in a similar fashion.


This paper briefly reviews chemical, physical, and biological means of degrading aflatoxins. Research needs in these areas are presented.


Studies on the potential for removal of aflatoxin B₁ from aqueous buffer model solutions by adsorption on fungal mycelium or various clays are described. Treatment with coarse, wet mycelium of non-toxigenic strains of Aspergillus flavus reduced the aflatoxin concentration in the medium to 30-46 % of the initial value; disintegrated mycelium had a greater adsorption effect, reducing aflatoxin concentration to 10-20 % of the initial value. Treatment with clays (18 types tested) considerably reduced the aflatoxin concentration in the supernatant, residual aflatoxin concentration ranging from traces to 30 %; bentonite showed the greatest aflatoxin adsorption capacity of the clays studied. Vermiculite had a greater aflatoxin adsorption capacity in the finely-powdered than the coarse form; particle size had little effect on the adsorption capacity of montmorillonite. Heating at 65°C increased the aflatoxin adsorption capacity of bentonite, montmorillonite and sepiolite, the increase being greatest for sepiolite. It is suggested that it might be possible to remove aflatoxin from contaminated liquid foods by an adsorption process.
Groundnut oil contaminated with aflatoxins was treated with activated or inactivated kaolin at 1, 1.5 and 3 % doses for 15 to 30 min. Satisfactory results were obtained with activated kaolin at 3 % for 15 min at 80°C.

Studies on thermal inactivation duration of heat treatment. Conidia of both strains showed a synergistic effect of combined heat and radiation treatments, although a heat-radiation sequence was more effective than a radiation-heat sequence.

In vitro studies were conducted on conidia of Aspergillus flavus (aflatoxin-producing) and Aspergillus flavus-oryzae (non-toxigenic) strains. These strains differed in resistance to heat and gamma radiation, the toxigenic strain being more resistant to both treatments. Results of tests on dose-modifying factors indicated that composition, temperature and pH of suspending media affected radiation resistance. The size of the initial population and the age of the conidia did not influence the radiation resistance of either strain. Studies on thermal inactivation of the conidia suggested that the temperature employed was more important than the duration of heat treatment. Conidia of both strains showed a synergistic effect of combined heat and radiation treatments, although a heat-radiation sequence was more effective than a radiation-heat sequence.

Inactivation of aflatoxin B1 was studied by using gamma radiation and hydrogen peroxide. A 100-krad dose of gamma radiation was sufficient to inactivate 50 µg of aflatoxin B1 in the presence of 5 % hydrogen peroxide; 400 krad was required for total degradation of 100 µg of aflatoxin in the same system. Degradation of aflatoxin B1 was confirmed by high pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC). Ames microsomal mutagenicity tests showed loss of aflatoxin activity. This method of inactivation also reduced the toxin levels in artificially contaminated groundnuts.

A new mycotoxin detection procedure termed "Selectively Absorbed Mycotoxins (SAM)" has been developed. It is a modification of the Holaday-Velasco Minicolumn procedure for aflatoxin screening. With the SAM assay procedure, mycotoxins are extracted into a methanol-water solvent. Toulene is mixed with the extract to form a two-phase solution where aflatoxins and zearalenone partition into the upper toulene phase, thus separating them from potential interfering compounds. The toulene sample is passed through a SAM-Aflatoxin Zearalenone (SAM-AZ) tube which removes other interfering compounds in a pre-absorption layer, and selectively absorbs any aflatoxin and/or zearalenone at specific bands in the tip of the tube. Each positive band glows with an obvious blue color under long wave ultraviolet light. The sensitivity of the assay is designed to provide a YES/No screen at designated levels of aflatoxin and zearalenone. Detoxification of aflatoxin-contaminated groundnut products has been accomplished by the addition of a high-affinity sorbent (hydrated sodium calcium aluminosilicate) when added at 0.5 % by weight. Aluminas, silicas, and aluminosilicates were evaluated for their ability.

After passing through a whole nut blancher the aflatoxin content and percentages of blanched and whole kernels were determined. Blanching percentages were significantly higher for sodium oleate, water spray, liquid nitrogen and H2O2 than for HCl or heat treatments. The heat treatments produced the highest percentage of whole kernels. In most tests, the non-blanched kernels had higher levels of aflatoxin than had those that blanched fully. The treatments which were most effective in producing low levels of aflatoxin in the blanched kernels were H2O2, water spray and HCl.

Sixty-nine groundnut oils and 16 other cooking oils on sale in Indian villages were analyzed for aflatoxin contamination. Groundnut and groundnut meal samples (24) were also analyzed and three were positive for aflatoxin B1 at 2-270 µg kg-1. Of the 69 groundnut oils, 15 were contaminated with aflatoxin B1 at 3-175 µg kg-1, of which seven also contained aflatoxin B2. Of the 16 other oils, only one, a coconut oil, contained 3 µg kg-1 aflatoxin B1. Destruction of aflatoxin B1 in artificially contaminated maize oil was only apparent at 250°C and above. Using naturally contaminated groundnut oil (175 µg kg-1 aflatoxin) the results were essentially the same and confirmed that destruction of aflatoxin B1 is likely to occur at normal frying temperature if cooking is done indoors.
The effect of irradiation on the ability of some commonly used foods to support aflatoxin production was studied under laboratory conditions. Levels of aflatoxin were significantly higher in some irradiated cereals, millets and root vegetables than in non-irradiated samples of the same commodities.


Groundnuts were inoculated with spore suspension of Aspergillus parasiticus and irradiated with gamma rays from a Co 60 source at 50, 100, 150 and 200 krad. Aflatoxins were detected by thin-layer chromatography. Concentrations of aflatoxins were lowest on groundnuts irradiated at 200 krad.


The solar degradation of anatoxin B1 in foods is discussed. The combination of solvent and radiation, the design of a pilot plant for detoxification of aflatoxin B1 contaminated coconut oil by solar irradiation, and the non-toxicity of solar irradiated coconut oil to ducklings are considered.


Edible oils contaminated with aflatoxin B1 were examined for loss of toxicity on being subjected to solar radiation. Coconut, groundnut, soybean and sesame oils and chloroform and ethyl acetate contaminated with aflatoxin B1 (1µg ml-1) were exposed to radiation from the sun at midday for 30 min at an oil layer thickness of 16 mm. The oils, after exposure, were examined by thin-layer chromatography (TLC) for residual aflatoxin B1 and any new fluorescent compounds. One day old 'veluvi' ducklings were fed with pure aflatoxin B1 (4.7 µg/d) for 7 days. The same amount of aflatoxin B1 in coconut oil was solar-irradiated in a pilot plant and the extract was fed to a second group of ducklings. Control groups of ducklings were fed with extracts from non toxic oil after solar irradiation and the vehicle, propylene glycol, alone. No residual aflatoxin B1 or new fluorescent compounds were observed on TLC after solar irradiation of the oils, whereas both types of compounds were observed with chloroform and ethyl acetate. The pattern of weight gain in the ducklings fed with extracts from toxic oil after solar irradiation was not significantly different (P = 0.05) from those of control groups of ducklings, but was from those fed with pure toxin. The former showed no mortality, as against more than 50 % mortality in the ducklings fed with pure toxin. The ranked mean value for bile duct hyperplasia in the group of ducklings fed with the extracts from toxic oil after irradiation showed no significant differences from that of control groups, but was significantly different from those fed with pure aflatoxin B1 (P = 0.01). It is suggested that irradiation appears to detoxify edible oils contaminated with aflatoxin B1.
This paper very briefly reviews several methods used for destruction of aflatoxin in groundnuts and their products. The exposure of the groundnut oil contaminated with aflatoxin to bright sunlight completely destroys the toxin. Aflatoxin is present in finely suspended solids in the oil and can be removed by filtration or by extraction with 10% sodium chloride. Aflatoxin-contaminated groundnut kernels (0.5 mm thick flakes) can be partially detoxified if exposed to sunlight for 14 hours. To remove aflatoxin from groundnut cake, 50% alcohol, acetone, methanol, or 1% calcium chloride can be used. Treating the protein isolate with hydrogen peroxide, and groundnuts in the form of thin flakes or powder with urea and soyaflour with or without formaldehyde can destroy 90% of their aflatoxin content. The merits and dements of these processes are discussed.

Unrefined groundnut oil containing aflatoxin (>100 µg kg⁻¹) was exposed to bright sunlight, gas-filled tungsten lamp, or long wave UV light. Sunlight destroyed 99% of the aflatoxin present in 15 min, whereas tungsten lamp light and ultraviolet light destroyed 82-85% of aflatoxin in 18 h and 30-40% of aflatoxin in 2 h exposure. The photolysed oil was not toxic to albino rats, and its absorption peak shifted from 700 nm to 280 nm after exposure to sunlight.

Aflatoxin in groundnut oil, altered being degraded by sunlight, was not regenerated during storage for six months of the oil in dark. There was some increase in peroxide value, but the organoleptic quality of the oil remained unchanged during three months of storage.

A study of the effects of the pilot roasting of aflatoxin-contaminated groundnuts showed an average loss, after roasting, of 40-50% of the aflatoxins B₁ and G₁ and 20-40% of the aflatoxins B₂ and G₂. Portions of both raw and roasted groundnuts were stored in paper bags and air-tight glass jars and reassayed for aflatoxins. No significant change in aflatoxin content was found after 3 months storage at 27°C regardless of the container. However, significant reduction of aflatoxin was observed in other aflatoxin-contaminated groundnut products which had been stored at room temperature for >1 year.

A fourteen hour exposure to sunlight destroyed 90% and 77% aflatoxin B₁ added to groundnut flakes with and without fat whereas only 50% of the toxin was destroyed when present as a natural contaminant. Treating the groundnut flakes with 20% urea and 2% soyaflour (a source of urease) at 50% moisture brought about 70% destruction of aflatoxin B₁. In large scale trials, destruction of aflatoxin was about 85%. Treatment with urea did not bring down the protein efficiency ratio (PER) value of the material, which was 1.5 after treatment as against 1.6 in the non-treated groundnuts.

The toxic and mutagenic effects of gamma-irradiated groundnut meal contaminated with aflatoxin B₁ were studied in Salmonella typhimurium strain TM 677. Using forward mutation to 8-azaguanine resistance. After treatment with 5-10 megarad gamma-radiation, the contaminated groundnut meal lost its toxic and mutagenic properties. Irradiation at 0.1-1 megarad removed 75-100% of the toxicity but not mutagenicity.

A study of the effects of the pilot roasting of aflatoxin-contaminated groundnuts showed an average loss, after roasting, of 40-50% of the aflatoxins B₁ and G₁ and 20-40% of the aflatoxins B₂ and G₂. Portions of both raw and roasted groundnuts were stored in paper bags and air-tight glass jars and reassayed for aflatoxins. No significant change in aflatoxin content was found after 3 months storage at 27°C regardless of the container. However, significant reduction of aflatoxin was observed in other aflatoxin-contaminated groundnut products which had been stored at room temperature for >1 year.
The effect of UV light on the fluorescence intensity of aflatoxins in various solvents and on the photo catalyzed addition of alcohols and water to the vinyl ether double bond of aflatoxins B1 and G1 was investigated. Four groups of new aflatoxins, designated as Bm, Bc, Gm, and Gc, were found to result from the UV light-catalyzed aflatoxin-solvent interaction. These compounds were less toxic to chicken embryos than the parent toxins.


Effects of gamma-ray on the destruction of aflatoxin in groundnuts were studied. Forty groundnut samples, collected from markets in different pans of Thailand, were inoculated with a toxigenic strain of Aspergillus flavus. After incubation at room temperature for a certain period of time, the inoculated groundnut samples were irradiated with 6.4 KGy dose. Levels of aflatoxin B₁ in irradiated and non-irradiated samples were determined. There were no significant differences in average levels of aflatoxin B₁ between irradiated and non-irradiated samples. Levels of aflatoxin B₁ in 22 out of 40 samples ranged from 0 to 4834 µg kg⁻¹ and most (42.5 %) had levels above the standard value. Maximum microbial load and aflatoxin level were found on the 9th day of incubation, approximately 1.04 x 10¹⁰ colonies and 1200 µg kg⁻¹ respectively.

8.2.13 Control In Storage/Transit


The problem of mold growth and aflatoxin contamination in the commissaries transported over long distances from the cultivation regions to the consumption centres is discussed. If the contamination occurs during transit, often no insurance coverage for the risk is available. Because of different methods of sampling followed in the exporting and importing countries it is often difficult to define the exact responsibility of the development of aflatoxin as having taken place during transit. The statistics of the export of aflatoxin high-risk commodities like red pepper (chillies), cottonseed and groundnut extractions clearly, for the last decade, demonstrate the extent of loss suffered by the exporting countries because of aflatoxins. The problem of mold damage and mycotoxin contamination can be minimized by improving facilities for storage at port and transit points and on ships.


This paper discusses causes of aflatoxin contamination and methods of prevention and segregation to control the aflatoxin problem in farmers' stock groundnuts from the time the groundnuts are growing in the field until they are marketed, stored, and shelled.


The molding of farmers' stock groundnuts in storage is a problem in certain locations because of inadequate storage facilities. An experiment was designed to test the effectiveness of propionic acid and 'Moldstat' in preventing molding of farmers' stock groundnuts stored in facilities that do not provide adequate protection from rainy weather. Only the highest concentration of propionic acid prevented aflatoxin build-up. 'Moldstat' provided little or no protection from aflatoxin contamination at any concentration. Results of flavor evaluations on samples from the treatments showed that the groundnuts treated with propionic acid were poor in flavor while those treated with 'Moldstat' had about the same flavor as the controls (groundnuts without any treatment). The free fatty acids were significantly lower in the treated samples than in the controls.


Groundnut storage problems were studied in Cote d'Ivoire during 1985 and 1986, from three different aspects; insect pests, fungal infection and aflatoxin contamination. Samples of groundnuts were obtained from 164 farmers' stock three times throughout the storage period : in December 1985, in February and April 1986. Samples were also obtained from different town and village markets. These samples were examined for several parameters such as pest damage (penetrated or scarified pods), fungal infection, aflatoxin contamination, and seed germination. Generally, the market samples were less contaminated with aflatoxin than the samples taken from the farmers' stocks. With a few exceptions the stocks examined were always contaminated with aflatoxin. Nine per cent of the stocks contained aflatoxin levels in excess of 250 µg kg⁻¹. Only four per cent of market samples contained such high levels of aflatoxin. Significant correlations between aflatoxin contamination and scarified/penetrated and broken pods were found.
Aflatoxin contamination of groundnuts during storage can be controlled by good warehousing practices. The most important factor in preventing aflatoxin development in groundnut warehouses is moisture control. Any measure that reduces the risk of rewetting the groundnuts will be effective in aflatoxin control. A properly ventilated structure is needed with a good roof, sidewalls, and floor to prevent water entry. Uniform loading of the warehouse will allow excess heat and moisture to escape and reduce areas favorable for insect infestation which causes heat build-up and moisture accumulation. Frequent checks on warehouse conditions and proper operation of the ventilation system will prevent warehouse conditions from becoming favorable for aflatoxin production.
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