Importance of mycotoxins in food and feed in India

By F WALIYAR, S V REDDY

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India

K SUBRAMANIAM, T Y REDDY

Agricultural Research Center, ANGRAU, DCMS building, Kamala Nagar, Anantapur, Andhra Pradesh, India

K RAMA DEVI

Society for Transformation, Agriculture and Alternatives in Development (STAAD), Plot 1182, Jublee Hills, Hyderabad, Andhra Pradesh, India

P Q CRAUFURD and T R WHEELER

The University of Reading, Cutbush Lane, Shinfield, Reading RG2 9AD, UK

Summary

Various food and feed samples including groundnut seed, maize, sorghum, soybean cake, groundnut cake, cotton cake, poultry feed, buffalo milk, cow milk and milk powders were collected from farmers' field, farmer's stores, oil millers storage, traders' storage, retail shops and supermarkets. More than 2000 samples were analysed by ELISA and most of the commodities with exception of sorghum seed, contained high levels of aflatoxin. Groundnut cake is one of the major cattle feed ingredient in peri-urban area of Hyderabad and >75% of the samples contain >100 μg kg^{-1} aflatoxin leading to high level of aflatoxin M_1 in milk samples. Strategies to reduce aflatoxin levels especially in groundnut with management interventions at pre-harvest, harvest, storage, are discussed.

Key words: India, aflatoxins, milk, groundnut, animal feed, cereals, storage

Introduction

Aflatoxins are toxic, carcinogenic, teratogenic and immunosuppressive substances produced when toxigenic strains of the fungi *Aspergillus flavus* Link. ex Fries and *A. parasiticus* Speare grow on groundnuts, maize, cotton, chilli, and many other agricultural commodities. It is reported that blood tests have shown that very high percentages of the populations of several countries in Asia and Africa have been exposed to aflatoxins. Maize and groundnuts are important in the diet of peoples both in Asia and Africa and are likely to be the main sources of these toxins. Aflatoxins B_1 and G_1 are the forms most commonly produced in groundnut. They are highly toxic among livestock and are being implicated in human diseases. Aflatoxin M_1 (AFM_1) is a major metabolite of Aflatoxin B_1 found in the milk of animals that have consumed contaminated feed with aflatoxin B_1 (Polan *et al.*, 1974). The relatively high levels of primary hepato-cellular carcinoma may reflect interactions between hepatitis B and C (which are related to protein deficiency in children) and aflatoxin. It is not surprising that aflatoxin contamination is the most important quality problem in many commodities worldwide.

Infection of groundnut by *Aspergillus* occurs under both pre-harvest and post-harvest conditions. Pre-harvest infection by *A. flavus* and consequent aflatoxin contamination is more important in the semi-arid tropics, especially when end-of-season drought occurs (Waliyar *et al.*, 1994). There is also evidence that damage to groundnut by soil pests such as termite increases aflatoxin contamination. Post-harvest conditions are also important. Favorable conditions for infection during harvesting and storage may lead to rapid development of the fungi and higher production of the toxin (Mehan *et al.*, 1991). Studies in Africa have shown that groundnut is often stored in the form of pods where insects can easily damage the pods and facilitate penetration of the fungi.

For the purpose of this paper we are reporting the work carried out on importance of aflatoxin in food and feed in the State of Andhra Pradesh in southern India.

Material and Methods

Various food and feed/feed ingredient samples were collected from different sources including farmer field, farmer storage, oil miller storage, trader's storage, super markets and retail shops.

Market samples

During August to December 2002, samples of maize, sorghum, Soybean cake, groundnut cake, groundnut kernel, cotton cake and poultry feed were collected. Each sample consisted of 500 g each and was purchased from retail shops or super markets in and around Hyderabad, Andhra Pradesh, India. Only 100 g was used for aflatoxin extraction.

Storage samples

About 500 g groundnut kernel or groundnut cake sample was taken from each of 70 kg bag stored on farmers, at oil mills or in trader's storage. From each sample a 100 g sub-sample was drawn for aflatoxin analysis.

Farmer's field samples

Groundnut pod samples at the time of harvest (November 2001) were collected from the farmer's fields in two villages (Pampanur and Linganpally) in Ananthapur district and two villages (Valasapally and Nagulakunta of Pileru region) in Chittoor district of Andhra Pradesh. From each one-hectare field 12 samples were collected at uniform distance and each sample was taken from 13 m^2 area. Likewise samples from 55 fields were collected and sub-samples were drawn for aflatoxin analysis.

Survey for groundnut aflatoxin in Ananthapur district

To understand the magnitude of groundnut aflatoxin contamination in rain-fed groundnut, Ananthapur area was selected because this is the largest area in which about 800,000 ha is grown in this district alone, mostly rain-fed groundnut. A survey was conducted covering 250 villages spread over 25 mandals of Ananthapur district. Soon after groundnut harvest (November-December 2001) from each village 1 kg pod sample was drawn from one farmer and sub-samples were used for aflatoxin analysis.

Milk samples

A total of 466 milk samples were collected during 2001-02, from the villages surrounding the city of Hyderabad (peri-urban) and from Ananthapur (rural) area of Andhra Pradesh, India. The samples included 352 raw milk samples (50 ml each) collected from individual buffalos, 50 cow milk samples (50 ml each) from individual cows belonging to a well managed public sector dairy farm, 44 (250 ml each) of commercially available factory sealed milk packets sold in Hyderabad, 10 samples of powdered milk and 10 samples of milk based confectionery sold in retail market in Ananthapur.

Sample preparation for aflatoxin analysis

It is essential to extract the aflatoxin from the seed before the analysis by ELISA. About 100 g of sample was made into powder using a blender and 20 g sub-sample was used for extraction. Then triturated the powder in 70% methanol containing 0.5% K Cl (100 ml for 20 g seed) in a blender until the seed powder is thoroughly ground. Transfer the extract in to conical flask and shake on rotary shaker at 300 rev min^{-1} for 30 minutes. The extract was filtered through Whatman No.41 filter paper and stored at $4^{\circ}C$ till they are used for analysis.

Preparation of Milk Samples for ELISA

To each of the milk sample (usually 15 ml) an equal volume of methanol was added and the mixture was shaken on a rotary shaker for 30 min at 250 rev min^{-1}. Later the mixture was centrifuged at ambient temperature for 10 min at approximately 2000 g and then filtered through Whatman No. 41 filter paper. For samples of powdered milk and confectionery, 10 g was suspended in 100 ml distilled water, heated to about 50°C and homogenised in a Waring blender and then processed as for liquid milk.

Aflatoxin analysis

All the food, feed and milk sample extracts were processed by indirect competitive ELISA as described by Chu *et al.* (1987), Candlish *et al.* (1987), Reddy *et al.* (2001), Reddy *et al.* (2002), and Thirumala-Devi *et al.* (2002). This measures total aflatoxin most of which is aflatoxin B_1.

Results

Importance of aflatoxin in food and feed

To assess the risk of aflatoxin contamination in food and feed, about 216 market samples of maize, sorghum, soybean cake, cotton cake, groundnut cake and groundnut kernel samples were analysed for aflatoxin contamination as shown in Table 1. All the groundnut cake and cotton cake samples were contaminated with toxin ranging from 18 to 1007 $\mu g\ kg^{-1}$ and 11 to 43 $\mu g\ kg^{-1}$, respectively. Forty-three percent of the maize samples were contaminated with toxin with highest aflatoxin level of 806 $\mu g\ kg^{-1}$. More than 31% of the groundnut kernel samples were contaminated with toxin ranging from 11 to 1776 $\mu g\ kg^{-1}$. Eighty nine percent of the soybean samples were contaminated with toxin ranging from 7 to 81 $\mu g\ kg^{-1}$. However, all sorghum samples were free from aflatoxin contamination .

Table 1. *Aflatoxin contamination in market samples*

Sample type	No. of samples	Range	Samples within range (aflatoxin, $\mu g\ kg^{-1}$) 11-30	31-100	>100
Maize	76	0-806	14	12	7
Sorghum seed	8	0-2	0	0	0
Soy bean cake	19	7-81	11	6	0
Groundnut cake	17	18-1007	2	2	13
Groundnut kernels	77	0-1776	6	6	12
Cotton cake	9	11-43	8	1	0
Poultry feed	10	3-34	3	1	0

Importance of aflatoxins in groundnut and its by-products

More than 600 seed samples were collected from Ananthapur and Pileru in Andhra Pradesh and were analysed for aflatoxin contamination as shown in Fig. 1.

Seventy three percent of samples in both locations showed < 10 $\mu g\ kg^{-1}$ aflatoxin. Aflatoxin levels > 500 $\mu g\ kg^{-1}$ were detected in 18% of Pileru samples as against 5% in Ananthapur samples. Although the number of contaminated samples is low in Ananthapur, these samples play a crucial role when they are mixed with uncontaminated seed lots.

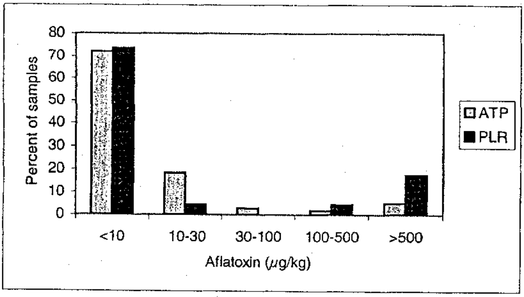

Fig. 1. Distribution percentage for aflatoxin levels in Ananthapur (557) and Pileru (68) samples.

Aflatoxin levels in storage samples

Of the 696 samples tested, 35% of them contained > 10 $\mu g\ kg^{-1}$ aflatoxin as shown in Table 2. About 16% of the samples stored in sacks in farmer storage contain >10 $\mu g\ kg^{-1}$ aflatoxin followed by 27 and 30% samples in oil miller's and trader's storage, respectively. More than 16% of insect damaged kernel samples from storage showed aflatoxin levels > 500 $\mu g\ kg^{-1}$ and this level of aflatoxin contamination is the highest recorded among different post-harvest storage samples. High levels of aflatoxin contamination (> 100 $\mu g\ kg^{-1}$) were detected in >80% of the groundnut cake samples. When animals are fed with highly contaminated groundnut cake their milk also gets contaminated with aflatoxin M_1 (see below).

Table 2. *Aflatoxin levels in storage samples - Ananthapur*

		Range		Samples within range (aflatoxin, μg kg^{-1})	
Sample source/type	No. of samples	10-30	31-100	101-500	> 500
Farmer storage	50	1	4	2	1
Traders storage	229	23	13	27	6
Oil millers storage	233	32	15	8	9
Insect damaged	48	9	2	1	8
Groundnut cake	56	10	0	43	2
Pod with haulms	80	15	7	0	2

Insect damage to groundnut pods in storage not only causes damage to kernels but also enhances aflatoxin contamination. The number of contaminated samples and aflatoxin contamination levels in post-harvest storage samples are alarming.

Survey for groundnut aflatoxin in Ananthapur district

Groundnut pod samples were collected from 250 villages spread over 25 Mandals (local government units) in Ananthapur District in 2001 and analysed for aflatoxin contamination. Using a geographic information system (GIS), we mapped the aflatoxin levels in these mandals (Fig. 2).

Samples from seven Mandals (28%) (D. Hirehal, O. D. Cheruvu, Somendapalli, Puttaparthy, Kalyanadurgam, Singanamala and Nallamada) contained non-permissible levels of aflatoxin of > 48 μg kg^{-1}. Five of the 7 Mandals received < 300 mm rain during the season. These levels of contamination are similar to those we have found in farmers' fields previously.

Aflatoxin in Milk

Our investigation showed that the incidence and level of AFM_1 in milk samples varied from 0.6 to 48 μg litre^{-1} (Table 3). Fifty two percent of buffalo milk samples contained non-permissible level of AFM_1. It was observed that the contamination of AFM_1 was greater (93%) in samples obtained from peri-urban areas than those from rural areas (34%). It is note worthy that 50% incidence was observed in the powdered milk samples intended for infants and 30% in milk-based milk confectionery, although the number of samples tested were insufficient to obtain an accurate picture of the incidence of contamination (Table 3).

Samples of cow's milk were analysed and 34% of samples were found to have had non-permissible levels of aflatoxin.

We analysed 352 raw buffalo milk samples and 53% had higher percentage of samples with non-permissible levels compared to cow milk, which had 34% non-permissible levels of aflatoxin M_1. The high incidence of contamination in milk can be related directly to the contamination of cake (Table 1) and groundnut haulms (Table 2).

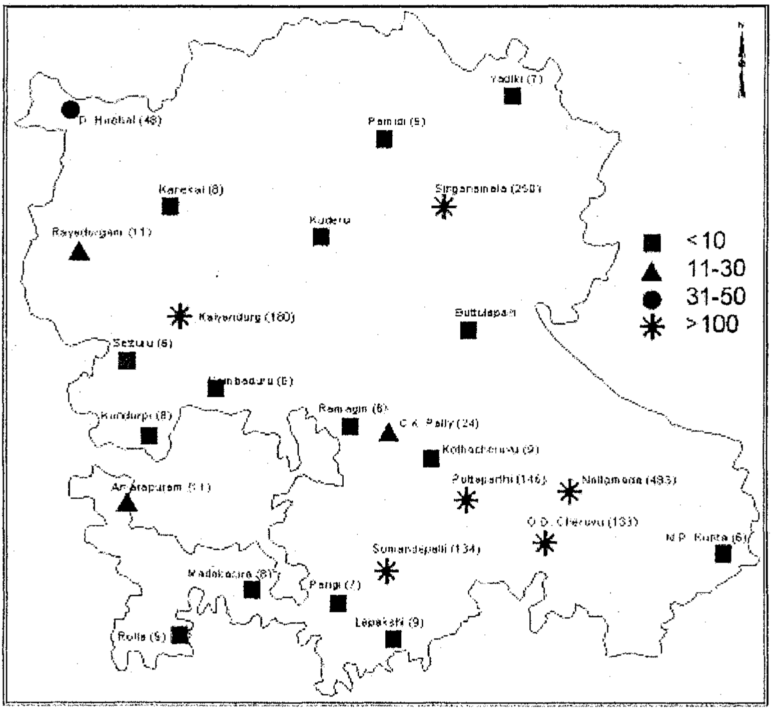

Fig. 2. Aflatoxin contamination (μg/kg) in different mandals of Ananthapur District, Andhra Pradesh.

Discussion

The results from these investigations showed very clearly that the aflatoxin contamination in groundnut was very high in Ananthapur and Chittoor districts of Andhra Pradesh. The major feeds for cattle in peri-urban areas of Hyderabad city are cotton cake, groundnut cake, rice bran and rice straw. We have analysed some of these feed ingredients for aflatoxin content. Some of the groundnut cake samples are contaminated with aflatoxin at levels exceeding 500 μg kg^{-1}. These high levels of contamination in cattle feed contribute to the high levels of AFM_1 in the milk. High incidence of aflatoxins in various ingredients of cattle feeds has been reported from India (Balasubramanian, 1985; Reddy *et al.*, 1984). This can be attributed to prevalence of such optimum climatic factors as temperature and humidity for mold growth.

Our investigations showed that the main source of contamination is the groundnut that has suffered from drought. During our investigation it was clear that Ananthapur district, which is the main growing area for groundnut, is subject to end of season drought and subsequently high levels of aflatoxin are found in farmers' fields, and stores.

Table 3. *Incidence and range of aflatoxin M_1 in milk samples*

Sample type	No. of samples	Samples within range (aflatoxin, $\mu g\ kg^{-1}$)			
		0–0.5	0.6-15	16-30	31-50
Raw milk (peri urban)	116	8	59	37	12
Raw milk (rural)	236	155	74	3	4
Milk packets	44	28	11	5	0
Powered milk*)	10	5	5	0	0
Milk products*	10	7	3	0	0
Cow milk	50	33	17	0	0

*g of dry milk/ml of solution

In post-harvest storage, about 35% of the samples were contaminated with aflatoxins and some of the samples contain high amount of aflatoxin (> 500 $\mu g\ kg^{-1}$). Insect damage to groundnut pod in storage not only causes damage to kernels but also enhances aflatoxin contamination. The number of samples and aflatoxin contamination levels in post-harvest storage samples are alarming, so there is need to develop post-harvest storage devices to eliminate insect damage as well as aflatoxin contamination.

Survey for aflatoxins contamination in Ananthapur district indicated the high level of aflatoxin contamination in some villages is of concern and needs to be addressed. These data will help us to focus more on villages with very high levels of aflatoxin. The main reason for high contamination could be due to severe drought in some of these areas as well as lack of pre- and post-harvest technologies to reduce infection and contamination. Although, there are a number of technologies available, they are not adopted by farmers. Often this is due to lack of awareness; our surveys of farmers have shown that most of the farmers were neither aware of aflatoxin problem nor have market incentives for marketing aflatoxin free groundnuts and are not aware of existing technologies. Since most of the farmers and producers are not aware of aflatoxin contamination a major awareness campaign is being planned.

In order to reduce aflatoxin contamination, there are several steps to be taken. One of the most economic solutions for reducing aflatoxin contamination is development of adapted varieties with resistance to *A. flavus*/aflatoxin. Farmer's participation in varietal selection is being planned.

There is need to develop integrated management approach to combat aflatoxin contamination as well as to increase the groundnut productivity in the semi arid tropic zone. Integration of suitable biological control agent along with resistant or tolerant cultivar with some adaptable cultural practices will be helpful for groundnut production and reducing aflatoxin contamination.

The maximum permissible level of AFM_1 in milk is 0.5 μg litre^{-1}. AFM_1 is relatively stable during pasteurisation, storage and preparation of various dairy products. Therefore AFM_1 contamination poses a significant threat to human health, especially to children, who are the major consumers of milk.

Frequent contamination of AFM_1 in milk and dairy products has lead to the assessment of risk due to liver cancer. Our data clearly show the need for such a risk assessment in India. The results also highlight the importance of surveillance particularly in peri-urban areas for AFM_1 contamination in milk and milk based confectionery. More detailed study is needed to understand the various factors that contribute to high versus low AFM_1 contamination in milk. The AFM_1 estimation methods described and illustrated could form the basis of a low cost risk assessment procedure. Ultimately such surveillance procedures must be linked to technical, policy and institutional interventions that will lead to the reduction of AFM_1 in milk production systems in India.

Post-harvest storage is another important area needs to be addressed as large number of groundnut kernel samples were showing high level of aflatoxins contamination. Groundnut cake (de-oiled) is one of major ingredient in cattle and poultry feeds and > 80% cake samples contain > 100 $\mu g\ kg^{-1}$ aflatoxin. In Ananthapur area farmers use the haulms for cattle feed and high level of aflatoxin (> 500 $\mu g\ kg^{-1}$) was detected in groundnut haulms (the toxin contamination was found in immature pods attached to haulms). There is need to develop strategies to reduce aflatoxin contamination in groundnut-based animal and poultry feeds. The survey of 250 villages covering 25 mandals of Ananthapur indicated high level of aflatoxin in some villages and these villages need to be monitored very closely and management strategies should target these particular villages.

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