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Aflatoxin contamination of food commodities and their management

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The term aflatoxin was coined in the early 1960s when death of turkey birds was attributed to toxins in groundnut meals imported from South America (Blount, 1961). It has now gained global significance as a result of their deleterious effects on human and animal health and its importance in international trade. They are the most potent carcinogenic, mutagenic and immuno-suppressive agent. Aflatoxins are a group of closely related secondary metabolites of the fungi *Aspergillus flavus* and *A. parasiticus*. *A. flavus* produces aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2) whereas *A. parasiticus* produces aflatoxin B1, B2, G1 and G2. Of these four, AFB1 is most potent toxin (Payne, 1998; Abramson, 1998). AFB1 is produced by *A. flavus, A. parasiticus, A. nomius, A. bombycis, A. ochraceoroseus, A. pseudotamarii* and *Emericella venezuelans,* on a wide range of tropical and subtropical agricultural commodities. Of the various species, *A. flavus* is most widespread and common. It is a saprophyte during most of its life cycle and grows on a variety of substrates including decaying plant and animal debris. The major factors that influence soil populations are temperature and soil moisture. *A. flavus* can grow at temperatures from 12-48ºC and at water potentials as low as –35 Mpa (Klich, 2007). The optimum temperature for growth is 25 to 42ºC. The fungi most commonly occur on groundnut (*Arachis hypogaea*), maize (*Zea mays*), chilli, several tree nuts (pistachio, cashew nuts, Brazil nuts, etc.), figs (*Ficus carica*), etc. Contamination mostly occurs on post-harvest products, stored at high temperatures and high humidity, but also known to occur in the fields before harvest on crops subjected to drought stress (Payne and Brown, 1998).

Economic impact of aflatoxins

The economic impact of aflatoxins derives directly from crop and livestock losses, human health as well as indirectly from the cost of regulatory programs designed to reduce risks to animal and human health. As per the FAO estimates, about 25% of the world's food crops are affected by mycotoxins, of which the most dangerous is aflatoxins (Lopez-Garcia and Park, 1998). Other adverse economic effects of aflatoxins include lowered market potential of food and fiber crops. Keeping in view of these toxins adverse effects on human and animal life, several countries impose strict regulatory limits on their domestic and imported food commodities before they enter into their normal diets.

Prevalence and distribution

Many agricultural commodities including cereals, oilseeds, spices, dry fruits and feeds are contaminated by aflatoxin. Mc Donald and Castle (1996) conducted a survey on aflatoxins in retail herbs and species in U. K. Out of 157 retail samples including curry powders, pepper, cayenne pepper, chilli, paprika, ginger, cinnamon and coriander, 95% of the samples contained < 10 µg/ kg of total aflatoxins and only nine samples had higher levels. Reddy *et al.* (2000) reported the occurrence of aflatoxins in selected cereals and spices. Spices like ginger rhizomes, turmeric, black pepper and coriander were analyzed for the presence of aflatoxin. Reddy *et al.* (2001) assessed the aflatoxin contamination in major chilli growing areas and chilli samples representing all the three grades collected from the wholesale markets of Andhra Pradesh. Chilli Grade 3 samples contained over 25% of discoloured pods and were most contaminated by *Aspergillus flavus*. Ajith Kumar and Naik (2005) conducted survey in Northern Karnataka districts of Gulbarga, Bellary and Raichur to know the incidence and severity of aflatoxin contamination in chilli. The highest incidence of (6.83%) was recorded in Bellary district followed by Raichur (5.29%) and Gulbarga district (4.19%). Waliyar *et al.* (2007) examined the natural occurrence of Aflatoxins (AFB1) produced by *A. flavus* in sorghum and pearl millet grains collected from farmers' field at harvest and storages. Navya *et al.* (2007) studied the incidence of *A. flavus* and aflatoxin on seed samples of groundnut. Among 40 different samples screened, the levels of *A. flavus* ranged from 0 to 72%.

Assessment of population of *Aspergillus flavus*

Aflatoxigenic fungi are common components of soil mycobiota and soil serves as a reservoir for *A. flavus* fungi that produce carcinogenic aflatoxins in agricultural commodities. Aflatoxigenic fungi reside in soil as conidia, sclerotia and hyphae, which act as primary inoculum for directly infecting crops and also invade developing seeds of crops. The effect of corn and peanut cultivation on the soil population of *A. flavus* in soil was examined. Drought stress in corn plants greatly increased the soil population of *A. flavus* from 2000 cfu g^{-1} soil to 6400 cfu g^{-1} of soil (Bruce *et al.*, 1995).

Abbass *et al.* (2004) assessed the spatial variability of soil population of *A. flavus* in Mississippi Delta field under different crops. The highest propagule density of 794 cfu g^{-1} of soil of *A. flavus* was recorded in corn, 251 cfu g^{-1} of soil in cotton and 457 cfu g^{-1} soil in wheat crop. Bruce (2006) studied the relationship between soil density of *A. flavus* and the incidence of peanut colonization. The highest population density of *A. flavus* was recorded in cultivable land (111-1733 cfu g^{-1}) compared to forested (2-19 cfu g^{-1}) and fallow (6-61 cfu g^{-1}) soils and up to 92% seed colonization was observed in peanut seeds.

Reddy *et al.* (2007) examined the effects of cotton-corn rotation and glyphosate use on the soil population of *A. flavus*. The soil population of *A. flavus* ranged from 1.4 to 5.8 X 10^3 (cfu) g^{-1} and the population was significantly greater in glyphosate resistant cultivars. Zablotowicz *et al.* (2007) assessed the density of *A. flavus* propagules and other soil microflora (*Fusarium* spp.) associated with Mississippi Delta soils. Propagule density of

A. flavus ranged from 1.97 to 4.31X 10^3 (cfu) g^{-1} while, the total *Fusaria* ranged from 2.99 to 5.37 X 10^3 (cfu) g^{-1} soil and the frequency of aflatoxin production in isolates ranged from 13 to 81 per cent depending on soil. Naik and Sudha (2009), assessed the distribution of *A. flavus* population present in the soil. Among three districts, Bellary recorded maximum population density (835.90 $cfug^{-1}$ soil) followed by Raichur (677.00 $cfug^{-1}$ soil) and the lowest population density of 501.20 $cfug^{-1}$ soil was recorded in Gulbarga district.

Detection of aflatoxin

Seventy nine pre-packaged samples of 12 different types of spice powders (5 cardamom, 5 cayenne pepper, 8 chilli, 5 cloves, 7 cumin, 5 curry powder, 5 ginger, 5 mustard, 10 nutmerg, 12 paprika, 5 saffron and 7 white pepper) were estimated for the aflatoxin B_1 content by HPLC method. Aflatoxin B1 (AFB_1) was detected in 34 samples, all of the *Cayenne pepper* samples were contaminated with AFB_1 levels ranging from 2-32 µg AFB_1/ kg. Three nutmeg samples contained levels ranging from 6-20 µg/kg. Paprika contained levels of aflatoxin B1 ranging from 1-20µg/kg. Chillies, cumin, curry powder, saffron and white pepper samples had levels ranging from 1-5 µg/kg. (Martin *et al.*, 2001).

Reddy *et al,* (2001) analyzed aflatoxin B_1 (AFB_1) content by an indirect competitive ELISA, for the samples of the three grades of the chilli pod (Grade 1 to 3) and chilli powder. The highest AFB_1 concentration of 969 mg/kg was found in one sample representing grade 3. As much as 9% of the chilli powder contained non-permissible aflatoxin levels. Yellamanda Reddy *et al.* (2001) analyzed the groundnut samples/ kernel and different varieties of groundnut for aflatoxin B_1 production by ELISA method. Very high level of aflatoxin was observed in 9.7% of samples ranging from 35 to 8172 mg/kg. In TMV-2 the widely grown groundnut variety recorded, > 30 mg/kg of aflatoxin contamination and other varieties *viz.*, JL-24, TAG-24, TG-26 and GG-2 had aflatoxin at very low level < 5 mg/kg.

The degree of aflatoxin contamination in different Indonesian food stuffs were determined by ELISA and HPLC methods. Eighty two groundnut products, 12 baby food products and 11 maize products were analyzed for total aflatoxin (AFT) and aflatoxin B_1 (AFB1) using ELISA. Thirty five per cent of the groundnut products were contaminated with aflatoxins ranging from 5 to 87 µg/kg. Eighteen per cent of the maize based products were contaminated with aflatoxin ranging from 5.8 and 12.4 µg/kg from 12 analyzed baby food samples, none of the sample was found to be contaminated with aflatoxin (Razzazi, 2004).

The mycoflora invading chillies (*Capsicum annuum* L.) kept in cold storage was studied and also analysed for the aflatoxin B_1 production by HPLC method. Species of *Aspergillus* was found to be dominant on stored chillies and the samples were contaminated with aflatoxin B_1 to the extent of 5.5 µg/ kg (Kiran *et al.*, 2005).

Fazekas *et al.* (2005) evaluated 91 spice samples (70 ground red pepper, 6 black pepper, 5 white pepper, 5 spice mix and 5 chilli samples) for the presence of aflatoxins B_1, B_2, G_1 and G_2 and Ochratoxin A by HPLC method (High performance liquid

chromatography). Out of 70 ground red pepper samples, 7 of them contained AFB1 concentration exceeding the maximum level of 5 µg/kg (6.1 – 15.7 µg/kg) and one chilli sample exceeded 5 µg/ kg (8.1 µg/kg). Thirty of the 70 ground red pepper samples contained Ochratoxin (OTA), 8 of them in a concentration exceeding the maximum level of 10 µg/kg (10.6 – 66.2 µg/kg), one chilli sample contaminated with Ochratoxin A (2.1 µg/kg).

The kernel samples of discoloured rice cultivars were detected for the presence of aflatoxin B 1 by indirect ELISA. Aflatoxin B_1 contamination was high in rice cultivars of *Cottondorasannalu* and BJ_1 160 –175 µg/kg moderate in *Swathi* and *Vijaya Mahsuri* 33-45 µg/kg and was absent in TKM 9 (Mangala *et al.,* 2006). The production of aflatoxin B_1 in rice cultivars was estimated by indirect competitive ELISA. Aflatoxin B_1 production by *A. flavus* on paddy and milled rice substrates of cultivars were estimated by indirect competitive ELISA. The aflatoxin production on paddy substrate was maximum in cultivar RH 12 (608mg/kg) and was at par (242-270mg/kg) in *Samba Mashuri* and *Ajaya*; it was low in rest of the cultivar. *A. flavus* inoculated on milled grains showed from a minimum of 4018 mg/kg to a maximum of 4655 mg/kg cv. *Ajaya* (Mangala *et al.,* 2007).

The chilli fruit samples from farmers field of Bellary district of Karnataka had higher contamination of 24.64 µg/kg followed by Raichur (7.58 µg/kg) and Gulbarga (2.84 µg/kg) districts. When aflatoxin was detected among various chilli products, only chilli powder contained 23.20 µg/kg of aflatoxin which was above permissible limit. The other indigenous chilli products such as chilli *masala powder, sambar powder, puliogare powder* and *vangibath* did not contain any aflatoxin (Naik and Sudha, 2009).

Management of aflatoxin

Since aflatoxin contamination can occur pre-harvest, post-harvest and in processing and storage conditions, it is necessary to implement various management options at all levels to better manage this problem. Pre-harvest management of the aflatoxin problem in agricultural crops is generally achievable through biological, cultural, chemical control and host plant resistance. Biological control is the most widely used method wherein antagonistic bacteria and fungi are used. An economically viable integrated management strategy involving host plant resistance, amending the soil with lime and organic supplements for enhancing water holding capacity, plant vigor and seed health, use of bio-control agents such as *Trichoderma* spp. and *Pseudomonas* spp. is however an ideal option. It is also important to use timely operations of harvesting and postharvest drying as well as bringing awareness and conducting training courses for disseminating technology to the end-users (Waliyar *et al.,* 2008). Biological control with atoxigenic strains of *A. flavus* and *A. parasiticus* that were applied in different formulations in the preceding cropping season can result in significant reduction (92%) in peanut aflatoxin concentrations. This method was found effective in delivering competitive levels of atoxigenic strains of *A. flavus* and *A. parasiticus* to soil and also in reducing subsequent aflatoxin contamination (Dorner *et al.,* 2003). However, reports indicate that application of atoxigenic *A. flavus* strain alone was found to be more effective than the non-toxigenic

strain of *A. parasiticus*. Combined applications of atoxigenic strains of both *A. flavus* and *A. parasiticus* were also proved to be effective (Dorner and Horn, 2007). The mechanism by which aflatoxin management can be achieved is through competitive exclusion between the strains of these *A. flavus* group of fungi (Chourasia and Sinha, 1994). The conidia of these atoxigenic fungi remained near the soil surface in spite of heavy rains and varying amounts of water through irrigation. Further, it was observed that rainfall could wash the conidia along the furrows and in directions perpendicular to peanut rows up to 100 meters. The retention of conidia of these aflatoxigenic fungi in upper soil layers is vital to reducing aflatoxin contamination of peanuts, maize and cottonseed (Horn *et al.,* 2001).

The use of botanicals or plant products is the safest method for management of aflatoxin. Use of neem leaves and seeds, *Eucalyptus, Pongamia extracts* has been shown to effective against aflatoxin contamination in groundnut (Reddy *et al.,* 2004; Ajith Kumar *et al.*, 2005). Sudha and Naik (2009) reported that neem seed kernel extract (NSKE) and nimbicidin (5%) were effective in reducing the *A. flavus* infection in chilli fruits.

In crops sensitive to aflatoxin, bio-control will have a long lasting solution from the point of view of food safety and health hygiene. The antagonistic nature of *Trichoderma* isolates against *A. flavus* has been shown involving production of volatile and non-volatile antibiotics and hyphal interaction (Srilakshmi *et al.*, 2001). The chilli fruits when treated with bio-agents such as *T. harzianum* and *Pseudomonas fluorescens* ended up in least colonization of 5.17% and 2% respectively as against 38.33% in untreated chilli fruits. (Sudha and Naik, 2011). They further sprayed the bio agent under field conditions 10 days before harvest of chilli crop. The incidence of aflatoxin fungal infection was 2.4 to 2.6 % as against control with 7.4%. Hence, foliar spray of *P. fluorescens* pre-harvest spray has been recommended (Sudha and Naik., 2011). Soil amendment with neem cake has reduced the population of *A. flavus* by 66 to 77% after 90 and 120 days of planting (Sudha and Naik., 2010). Any reduction in population at red ripening stage of chilli crop can bring down the chances of aflatoxin contamination.

Some safe fungicides recommended for managing aflatoxin contamination include mancozeb, thiram, captan, carbendazim and vitavax for seed treatment (Bansal and Sobti, 1990; Sharma and Champawath, 2000; Ajith Kumar *et al.*, 2005). However, their use as a foliar spray is limited in groundnut. But foliar spray of some of these non systemic fungicides like *mancozeb* is recommended as pre harvest spray to manage aflatoxin contamination in chilli.

Chemical control of pre-harvest aflatoxin contamination is through application of gypsum either to soil or seed alone or in combination. Gypsum application results in reduced colonization by *A. flavus* and *A. parasiticus*. Further, gypsum also enhances the control of seed colonization when applied in conjunction with the bioagent. No aflatoxins were detected in peanuts harvested from gypsum-treated plots (Mixon *et al.,* 1994). Although, several management options are available against pre-harvest aflatoxin contamination of peanuts, the field results are not consistent. Toxins continue to enter the food chain persistently since the fungi are ubiquitous in nature. Hence, post-harvest management of aflatoxins is also equally important to avoid or mitigate the problem.

Human and animal exposure to aflatoxins can be reduced by mitigating aflatoxin contamination in food and feed (Turner *et al.*, 2005). However, aflatoxin contamination is a complex problem and is influenced by diverse factors such as cropping practices, climate and socioeconomic background of the people (Waliyar *et al.*, 2005). Aflatoxin contamination of food is severe after long-term crop storage because of excessive heat, humidity; insect and rodent damage resulting in proliferation and spread of fungal spores.

The traditional approach to preventing exposure to aflatoxin has been to ensure that foods consumed have the lowest practical aflatoxin concentrations. In developed countries, this has been achieved for humans largely by regulations that have required low concentrations of the toxin in traded foods. However, this approach has certain limitations and clearly has failed as a control measure for developing countries. In developed countries, where regulations allow higher aflatoxin concentrations in animals, agricultural industries have developed alternative methods like chemoprotection and enterosorption to limit biologically effective exposure without the high cost of preventing contamination (Galvano *et al.*, 2001). This approach has been used extensively and with great success in the animal feeding industry (Rosa *et al.*, 2001).

It is well understood that much of the contamination of commodities with aflatoxin occurs during storage. To prevent aflatoxin production during storage, it is necessary to prevent growth of fungus through adequate drying (<10% moisture), elimination of insect activity that can increase moisture content through condensation of moisture resulting from respiration, low temperature, and inert atmospheres. Conditions needed to prevent aflatoxin contamination are known, but is not always easy to produce them in storage systems in developing countries. Most people in rural areas grow and store their own food; in consequence, most food is stored in small, traditional granaries and there is little investment in the management of ideal storage conditions. Studies of grain quality in such storage structures show a steady increase in the aflatoxin content over time, which reflect the failure to maintain appropriate conditions (Turner *et al.*, 2005).

HACCP (Hazard Analysis Critical Control Point) is in fact a logical plan for all the controls to be implemented to anticipate the problems of food safety. This plan will be specific to the risk. It establishes regular, systematic controls from end to end of the chain of food production. In addition, it provides for corrective action to be implemented if a risk has been identified.

The HACCP method is a well-known and well-established method in the agri-food business, the aim of which is to ensure the risk-free production of food. It is however, little used in the initial stages of agricultural production.

Several approaches can prevent aflatoxin exposure in developing countries. Because much food contamination occurs during post harvest storage, methods to remove nuts or kernels or fruits damaged by fungus before storage and to restrict humidity during storage could reduce fungal growth and toxin production. The possible options for pre and post harvest prevention of aflatoxin contamination and the ICRISAT's management strategy of pre- and post-harvest contamination is given in Table 1 (Waliyar *et al.*, 2005).

Table 1. A few options to reduce pre- and post-harvest contamination in food crops (Waliyar *et al.*, 2005).

Method	Purpose
I. Primary prevention	***To minimize fungal infestation and aflatoxin contamination***
• Cultivation of *Aspergillus flavus* resistant varieties	• Potential for control of fungal invasion and toxin production during crop growth.
• Control of field infection by following appropriate phytosanitary measures to reduce the fungal inoculum	• Limit fungal inoculum in the field
• Seed treatment and application of fungicides	• Limit fungal invasion during crop growth
• Appropriate scheduling for planting, harvest and post harvest	• Avoid drought stress and other a biotic stresses
• Application of soil amendments (gypsum, farmyard manure etc.)	• Enhancing soil nutrient (especially calcium) and water holding capacity, promoting the growth of antagonistic native soil micro biota
• Lowering moisture content of seeds after harvesting and during storage	• Limit fungal invasion and growth during storage
• Preservatives to prevent insect infestation and fungal contamination during storage	• Limit fungal invasion during storage
II. Secondary prevention	***Elimination or limiting the fungal contamination***
• Sorting of contaminated grains, pods and kernels	• Reducing aflatoxin contamination in final product
• Re-drying the harvested produce	• Limit further mold invasion during storage
• Appropriate storage conditions to avoid favorable conditions for mold growth	• Limit further mold invasion during storage
• Detoxification of contaminated product	• Chemical inactivation of aflatoxins through use of detoxification clay, ammonification, electronic sorting of kernels.

Following options have been suggested for management of aflatoxin contamination particularly in chilli (Naik and Sudha, 2009).

- Phyto-sanitary measures to reduce the fungal inoculum.
- Seed treatment in fungicides/bio-agents
- Drying the chilli produce to 9% moisture
- Avoiding drought and insect damage at fruiting stage
- Use of botanical(NSKE) / bio-agent (*Pseudomonads fluorescens*) as pre harvest spray at red ripening stage
- Soil amendment with neem cake and gypsum to reduce the soil population

Conclusion

Cultural practices, especially those followed after hervest, which can lead to minimization of mycotoxin contamination, shuold be investigated. Chill fruits often are wetted by sprinkling with water prior to marketing them. This practice is likley to favour growth of moulds, therefore guidence on post harvest handling food commodities to farmers as-well-as trades can greatly help in minimizing mould growth. We expect farmers to respond to any improved processing methods which can result in a safe and quality product. Additionally, creation of awareness among the consumers about the presence of aflatoxin in food commodities may induce produrcers as well as traders to market mycotoxin free products by use of resistant variety, chemical/botanicals, bioagents and other post harvest treatmets in a judicious way.

Consequently, we must be able to ensure that the risks they represent are reduced to an acceptable level. It is the role of the agri-food industry professionals to maintain a minimal level of risk, as low a level as possible of aflatoxins in products within the limits of what is feasible technologically and practically. On the other hand, public bodies are the only ones with the power of setting and imposing realistic and applicable regulatory limits on the levels of mycotoxins that are acceptable in products. Obviously they have to do this based on analyses provided by research workers.

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