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Food Safety and Security: Aflatoxin A Concern for Human Health

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Abstract

Food safety and security is a basic human need and is considered as vital issue worldwide. Chemical as well as microbial food hazards are the most important concern now-a-days. Among microbial food and feed hazards, mycotoxin attracted world's attention towards fungal invasion to food elements. Among mycotoxins, aflatoxins have been considered as one of the most dangerous contaminant in food and feed. The contaminated food poses a potential health risk to human such as aflatoxicosis and cancer. Aflatoxin is a naturally occurring toxin and one of the strongest carcinogens found in nature. In view of occurrence and toxicity, Aflatoxin B_1 is extremely carcinogenic while others are considered as highly carcinogenic. Epidemiological studies revealed that due to the hazardous nature of aflatoxin, it is considered as a potential source of causing liver cancer in human mostly exposed to fungal contaminated food. The incidence of human liver cancer increased when they ingest fungal contaminated food directly as well as indirectly from animals (milk, meat etc) when fed with contaminated fodder and forage. There is dire need to explore ways to tackle this problem especially in developing countries to reduce the health issues caused by aflatoxins.

INTRODUCTION

The enigma of food and feed contamination by aflatoxin is the current hot issue and it has gained great deal of attention over the last three decades. Aflatoxin is a naturally occurring toxin (Williams *et al.*, 2002) and one of the strongest carcinogens found in nature (Castegnaro and McGregor, 1998). The filamentous fungi that are major producers of aflatoxin are *Aspergillus flavus* (Bankole *et al.*, 2004) and *Aspergillus parasiticus* (Erdogen, 2004). *Aspergillus flavus*, which is ubiquitous, produces B aflatoxins (Samajphati, 1979) while *Aspergillus parasiticus* produces both B and G aflatoxins and has more limited distribution (Garcia-Villanova *et al.*, 2004). The history and discovery of aflatoxin was the incidence with deaths of millions of turkey and farm animals by turkey

'X' disease in 1960 in United Kingdom, the most accepted reason for this disaster was Brazilian peanut feed which was highly infected with *Aspergillus flavus*. The frequent incidence of these toxins in agricultural products have bad impact on the economy of affected regions, especially in developing countries where proper postharvest techniques are not sufficient to prevent mold growth. Aflatoxins being highly toxic, mutagenic, teratogenic as well as carcinogenic compounds that have been considered as causal agent in human hepatic and extra hepatic carcinogenesis (Massey *et al.*, 1995). Li *et al.* (2001) observed the association of aflatoxin contamination of plant foods particularly cereals with liver cancer in China. High contamination rates with aflatoxin in Pakistan also contribute to liver carcinogenesis (Qureshi *et al.*, 1990; Liu and Wu, 2010). Moreover, aflatoxin incidence also associated with kwashiorkor; a disease associated with protein malnutrition in children (Adhikari *et al.*, 1994).

Besides causing health problems to humans, aflatoxins also cause adverse economic effect in which it lowers the yield of food production and fiber crops as well as becoming a major constraint of profitability for food crop producing countries. It has been reported that 25% of the world's food crops are contaminated by mycotoxins every year resulting in significant economic loss for these countries (Lopez, 2002). Livestock as well as poultry producers are the most victim persons due to aflatoxin contaminated feeds which ultimately cause significant economic loss by death of farm animals, destruction of their immune system and reduction in body weight after intake of contaminated feed.

Chemistry of Aflatoxins

Aflatoxins can be classified into two broad groups according to chemical structure which are difurocoumarocyclopentenone series and difurocoumarolactone (Heathcote, 1984). They are highly substituted coumarin derivatives that contain a fused dihydrofuran moiety. There are six major compounds of aflatoxin such as aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_2), aflatoxin G_2 (AFG_2), aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2). The former four are naturally found aflatoxins and the AFM_1 and AFM_2 are produced by biological metabolism of AFB_1 and AFB_2 from contaminated feed used by animals. Aflatoxin B is the aflatoxin which produces a blue color under ultraviolet while Aflatoxin G produces the green color. AFM_1 produces a blue-violet fluorescence while AFM_2 produces a violet fluorescence (Goldblatt, 1969). The G series of aflatoxin differs chemically from B series by the presence of a β-lactone ring instead of a cyclopentanone ring. Also, a double bond that may undergo reduction reaction is found in the form of vinyl ether at the terminal furan ring in AFB_1 and AFG_1 but not in AFB_2 and AFG_2 (Figure 1). However, this small difference in structure at the C-2 and C-3 double bond is associated with a very significant change in activity, whereas AFB_1 and AFG_1 are carcinogenic and considerably more toxic than AFB_2 and AFG_2.

Aflatoxins and Human Health

Aflatoxins have been considered as one of the most dangerous contaminant in food and feed. The contaminated food poses a potential health risk to human (Figure 2) such as aflatoxicosis and cancer (Jeffrey and Williams, 2005). That's why among mycotoxins, aflatoxin have received great deal of attention. These are potent toxin and were considered as human carcinogen by The International Agency for Research on Cancer (IARC) as reported in World Health Organization monograph (WHO, 1987). These mycotoxins are known to cause diseases in man and animals called aflatoxicosis (Eaton and Groopman, 1994).

Liver cancer or Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths worldwide, with prevalence 16–32 times higher in developing countries than in developed countries. Aflatoxin, a contaminant produced by the fungi is a known human liver carcinogen (Liu and Wu, 2010). Figure 3 shows the distribution of HCC cases attributable to aflatoxin in different regions of the world. Human exposure to aflatoxins is principally through ingestion of contaminated foods (Versantroort *et al.*, 2005; Liu and Wu, 2010). In humans, aflatoxins are incriminated source of neonatal jaundice as well as circumstantial evidence to cause perinatal death and reduction in birth weight. Aflatoxins have implicated in series of food poisoning that are associated with serious morbidity with early mortality among young children's (Hendrickse, 1997). Yeh *et al.* (1989) reported that 91% of the deaths in people were observed due to liver cancer caused by regular ingestion of fungal contaminated food and their test also found positive for hepatitis B in Southeast China. In views of occurrence and toxicity, AFB_1 is extremely carcinogenic while others are considered as highly carcinogenic (Carlson, 2000). Liu and Wu (2010) reported that out of 550,000–600,000 new HCC cases worldwide each year, about 25,200–155,000 may be attributable to aflatoxin exposure. Most cases occur in sub-Saharan Africa, Southeast Asia, and China where populations suffer from both high HBV prevalence and largely uncontrolled aflatoxin exposure in food. Although it is impossible to completely eliminate aflatoxin in food, however, its level can be reduced significantly that will ultimately help in reducing liver cancer occurrence worldwide. Therefore, adopting measures to reduce dietary exposure to aflatoxins is crucial for public health.

Factors Affecting Aflatoxin Formation

Series of biotic and abiotic factors are responsible for aflatoxin production but temperature and relative humidity are the most critical. Studies conducted on hazelnuts and pistachios manifested that temperature favorable for aflatoxin production is 25-30ºC and relative humidity 97-99% (Simsek *et al.*, 2002). Water activity, substrate chemical composition storage duration, insect attack as well as moisture contents also take part in aflatoxin contamination to some extent (Sakai *et al.*, 1984 and Schatzki and Ong, 2001). Ross *et al.* (1979) reported that if both temperature (20-38ºC) and moisture (16-24%) are favorable for *Aspergillus flavus*, aflatoxin can be produced within 48 hours but aflatoxin production can occur at low temperatures i.e. 7-12ºC (Steyn and Stander, 2000). The fungus responsible for aflatoxin production is cosmopolitan in nature and there are evidences that aflatoxin may also occur both in pre- and postharvest conditions of cereals, oilseeds, edible nuts and spices (Coker, 1997).

Fungi are a normal component of food micro flora and may be responsible for spoilage and production of mycotoxins (Aziz *et al.*, 1998). Fungus might grow on different foods and feeds under conditions of favorable temperature and relative humidity and produce aflatoxins during postharvest handling, transportation and storage. Aflatoxin residues in contaminated foods with *Aspergillus flavus* remains for a long time even after the death of fungus or disappeared completely in feed stuff. *Aspergillus flavus* can thrive best under range of conditions from hot arid climate to warm humid circumstances. Foods that are particularly susceptible to being contaminated by aflatoxins include groundnuts, maize, spices, cotton seed and tree nuts. Contamination of spices with aflatoxins can cause potential carcinogenic effects if ingested even in small amounts (Roy and Chourasia, 1990).

Aspergillus flavus is widely spread in soil. Moldy grains and nuts are commonly contaminated with the fungus. About half of all known *Aspergillus flavus* strains produce mycotoxins. Aflatoxin production stimulated by moisture and high temperature; at least13 types of aflatoxin are produced by different fungal species but the most potent is aflatoxin B_1. The aflatoxin contamination can take place at any stage of food production from pre-harvest to storage (Wilson and Payne, 1994). Drought stress prior to crop harvest, improper stage of harvest, insect/pest attack, heavy rains at harvest and further poor drying of the crop are the principal factors which induce aflatoxin production (Hell, 2000 and Hawkins *et al.*, 2005). However, the humidity, temperature and aeration during drying and storage remain the most important factors for contamination with aflatoxins. The development of suitable varieties could result in consistent desirable yields and reduce weather-related problems concerning quality such as aflatoxin (Keisling *et al.*, 1999). Crop cultivars are known to have a significant impact on growth of fungi that produce mycotoxins. In general, varieties that are resistant to fungal attacks during the growing season are also less likely to become contaminated with mycotoxins, although mycotoxin problems can still arise during storage (Edwards, 2004).

Sometimes agricultural commodities contaminated with aflatoxin in field before harvest where it is usually conflicted with drought stress (Klich, 1987), even more is the fate of crops stored under circumstances that favor fungal growth. Contamination with *Aspergillus flavus* and subsequent production of aflatoxin during storage is considered as principal hot issue throughout the world (Williams *et al.*, 2004). Development of fungi during storage in a postharvest commodity is determined also by length of time (Lillehoj and Zuber, 1988). The longer the storage time, the greater the possibility of environmental conditions conducive to aflatoxigenisis (Udoh *et al.*, 2000). In storage, the moisture contents of the substrate, temperature as well as relative humidity are very important factors for fungus proliferation and ultimately presented a major risk for aflatoxin production (Nakai *et al.*, 2007). The growth of *Aspergillus* spp. and subsequent aflatoxin production in storage was favored by high relative humidity (> 85%), high temperature (> 25°C) and insect or rodent activity (CAST, 1989).

Prevention and Reduction of Aflatoxins in Agricultural Commodities

- In developing countries prevention of aflatoxins from entering the food chain may not currently be receiving sustainable attention or focus as in developed countries because of different food systems, financial constraints, availability of food policies, levels of food safety education and technological development. Numerous studies worldwide have highlighted different approaches to prevent/reduce the incidence of aflatoxin contamination in agricultural commodities.
- To prevent aflatoxin contamination of commodities in the farm or during storage of farm products, understanding of the prevailing environmental conditions must be considered. Environmental factors that favor *Aspergillus flavus* infection include high soil or air temperature, drought stress, nitrogen stress, crowding of plants and conditions that aid dispersal of conidia (CAST, 1989).
- Early harvesting and adequate drying of crops, cleaning stores before loading new produce as well as physical separation of apparently contaminated cereals from the bulk samples can help in reducing contamination of crops. Thorough drying and proper storage for example: Turner *et al.* (2005) recorded 60% reduction in the mean aflatoxin levels in groundnuts.

- Operations like sorting, winnowing, washing, crushing and dehulling to remove significant amounts of aflatoxins in maize and maize products (Fandohan *et al.*, 2005).
- Application of potential bio-control agents like atoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* which when introduced into the soil of growing crops have been reported to produce 74.3 to 99.9% reduction in aflatoxin in peanuts (Dorner *et al.*, 1998).
- Exposure to sunlight has also been found effective in reducing aflatoxin levels in some food products. For example, Adegoke *et al.* (1996) and Iqbal *et al.* (2011) found that sun drying of pepper (*Capsicum annum*) had some significant effects on aflatoxin levels.
- Detoxification using ozone has been found by some workers to be useful in reducing aflatoxins in food commodities for example de Alencar *et al.* (2012) noted reductions of 30% and 25% for total aflatoxins and aflatoxin B_1 when peanuts were exposed to 21 mg L^{-1} of ozone. Prudent and King (2002) reported a 92% degradation (reduction) in aflatoxin in ozonized contaminated corn.
- Spices and herbs and their bioactive components have been found useful for the reduction of aflatoxins. The bioactive components or volatiles of some plants have been explored in the control of fungal growth and production of aflatoxins, for example, Norton (1999) found that anthocyanins and related flavonoids affect aflatoxin biosynthesis and Juglal *et al.* (2002) found that spice oil was effective in the control of some mycotoxin producing fungi.
- Employing food safety practices like hazard analysis critical control point (HACCP) system can be useful in preventing and reducing aflatoxin contamination in agricultural products.
- Most efforts to address the aflatoxin problem involve analytic detection, government regulation, and diversion of aflatoxin-contaminated commodities from the food supply. Basic research on the biosynthesis and molecular biology of aflatoxins has been a research priority because a full understanding of the fundamental biological processes may yield new control strategies for the abolition of aflatoxin contamination of food crops.

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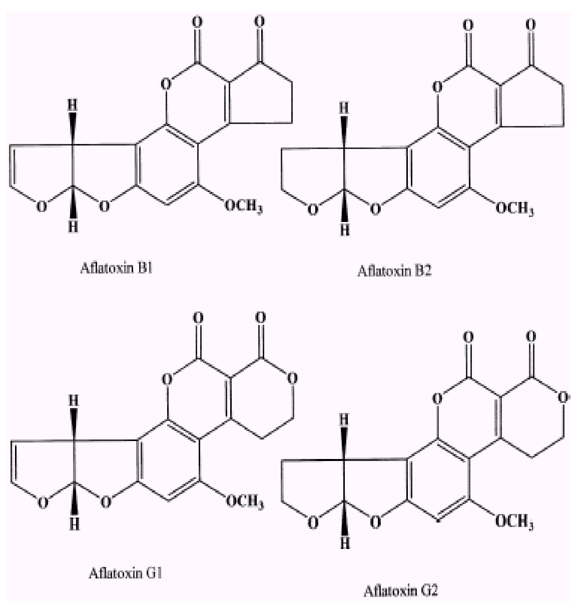

Figure 1: Chemical structures of aflatoxins (Papp *et al.*, 2002).

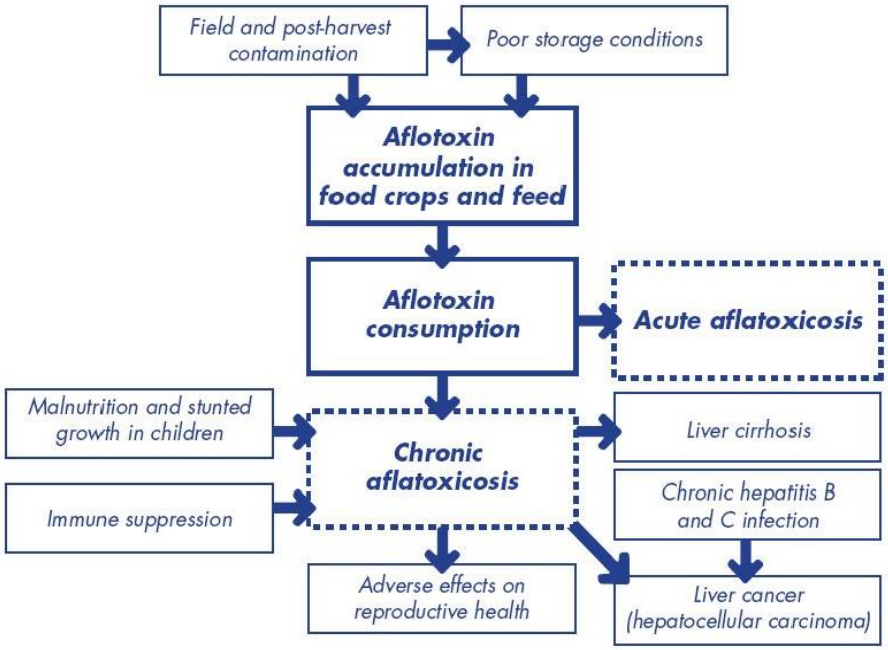

Figure 2. Aflatoxin disease pathways in humans.

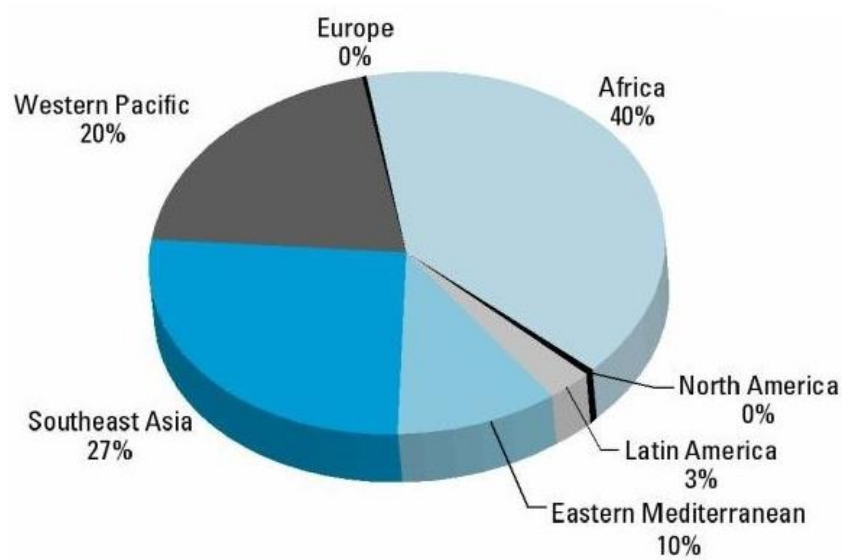

Figure 3: Distribution of HCC cases attributable to aflatoxin in different regions of the world.

Table 1: Maximum levels of aflatoxins in food approved by European Union.

Food	**Maximum aflatoxins levels (µg/kg)**		
	B_1	Sum of B_1, B_2, G_1, G_2	M_1
Groundnuts (peanuts) and other oilseeds, to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs[2], except groundnuts (peanuts) and other oilseeds for crushing for refined vegetable oil production.	8[10]	15[10]	-
Almonds, pistachios and apricot kernels to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs.	12[10]	15[10]	-
Hazelnuts and Brazil nuts, to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs.	8[10]	15[10]	-
Tree nuts, other than the tree nuts listed in 2 and 3, to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs.	5[10]	10[10]	-
Groundnuts (peanuts) and other oilseeds[1] and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs, except crude vegetable oils destined for refining and refined vegetable oils.	2[10]	4[10]	-
Almonds, pistachios and apricot kernels, intended for direct human consumption or use as an ingredient in foodstuffs[2].	8[10]	10[10]	-
Hazelnuts and Brazil nuts, intended for direct human consumption or use as an ingredient in foodstuffs[2].	5[10]	10[10]	-
Tree nuts, other than the tree nuts listed in 6 and 7, and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs.	2[10]	4[10]	-
Dried fruit, other than dried figs, to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs.	51	10	-
Dried fruit, other than dried figs, and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs.	2	4	-
All cereals and all products derived from cereals, including processed cereal products, with the exception of foodstuffs listed in 12, 15 and 17.	2	4	-
Maize and rice to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs.	5	10	-

Raw milk[3], heat-treated milk and milk for the manufacture of milk-based products.	-	-	0.050
Following species of spices: *Capsicum* spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne and paprika) Piper spp. (fruits thereof, including white and black pepper) *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger) *Curcuma longa* (turmeric), mixtures of spices containing one or more of the above-mentioned spices.	5	10	-
Processed cereal-based foods and baby foods for infants and young children[4,5].	0.10	-	-
Infant formulae and follow-on formulae, including infant milk and follow-on milk[6,7].	-	-	0.025
Dietary foods for special medical purposes[8,9] intended specifically for infants.	0.10	-	0.025
Dried figs.	6	10	-

Source: Commission Regulation (EC No. 1881/2006, December 19, 2006)

[1] Oilseeds falling under codes CN 1201, 1202, 1203, 1204, 1205, 1206, 1207 and derived products CN 1208; melon seeds fall under code ex 1207 99.

[2] In case derived/processed products thereof are derived/processed solely or almost solely from the tree nuts concerned, the maximum levels as established for the corresponding tree nuts apply also to the derived/processed products. In other cases, Article 2(1) and 2(2) apply for the derived/processed products.

[3] Foodstuffs listed in this category as defined in Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin (OJL 226, 25.6.2004, p. 22).

[4] Foodstuffs listed in this category as defined in Commission Directive 2006/125/EC of 5 December 2006 on processed cereal- based foods and baby foods for infants and young children (OJL 339, 6.12.2006, p. 16).

[5] The maximum level refers to the dry matter. The dry matter is determined in accordance with Regulation (EC) No 401/2006.

[6] The maximum level refers to the products ready to use (marketed as such or after reconstitution as instructed by the manufacturer).

[7] Foodstuffs listed in this category as defined in Commission Directive 2006/141/EC (OJL 401, 30.12.2006, p. 1).

[8] Foodstuffs listed in this category as defined in Commission Directive 1999/21/EC of 25 March 1999 on dietary foods for special medical purposes (OJL 91, 7.4.1999, p. 29).

[9] The maximum level refers in the case of milk and milk products, to the products ready for use (marketed as such or reconstituted as instructed by the manufacturer) and in the case of products other than milk and milk products, to the dry matter. The dry matter is determined in accordance with Regulation (EC) No 401/2006.

[10] The maximum levels refer to the edible part of groundnuts (peanuts) and tree nuts. If groundnuts (peanuts) and tree nuts 'in shell' are analyzed, it is assumed when calculating the aflatoxin content all the contamination is on the edible part, except in the case of Brazil nuts.