Chapter 5

Strategies for the Prevention and Reduction of Mycotoxins in Developing Countries

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1. Introduction

According to the Fod and Agricultural Organization (FAO) of the United Nations, up to 25% of the world's food crops have been estimated to be significantly contaminated with mycotoxins (WHO, 1999). Significant losses due to mycotoxins and their impact on human and animal health have been linked with national economic implications and all these factors have combined to make mycotoxins important worldwide (Bhat and Vashanti (1999). According to Plancinta *et al.* (1999), surveillance studies showed that world-wide contamination of cereal grains and other feeds with *Fusarium* mycotoxins is a global problem. Thus, in recognition of the global public health importance of food borne diseases and in order to promote economic growth and development, the World Health Organization (WHO) commissioned the Foodborne Disease Burden Epidemiology Reference Group (FERG) to undertake the systematic reviews of some chemicals and toxins like cyanide in cassava, aflatoxin, dioxins and peanut allergens (Hird *et al*. 2009).

In order to understand the mechanisms that can be adopted for controlling mycotoxins, it is essential to have relevant information on the prevailing climatic conditions in the agricultural zones where the crops are being produced. Thus Bhat and Vasanthi (2003) noted that in developing countries of the world, tropical conditions like high temperatures and moisture, monsoons, unseasonal rains during harvest and flash floods can lead to fungal proliferation and production of mycotoxin. In temperate climates, the most important toxins are deoxynivanelol, (DON), zearalenone, diacetoxyscirpenol (DAS), T-2 toxin and fumonisins (Balazs and Schepers, 2007) and in Europe, the most frequent toxigenic fungi are *Aspergillus*, *Penicillium* and *Fusarium* species (Creppy, 2002).

In cereals grown in temperate regions of America, Europe and Asia, *Fusarium* spp have been reported to be the most prevalent toxin-producing fungi (Gajecki, 2002). In Africa and other developing countries, the World Health Organization (WHO, 2006) noted that fumonisins and aflatoxins are likely to be of significance. However, ochratoxin A (OTA) has been reported in food commodities in Africa, for example, Adegoke *et al* (2007) found OTA in *kunu-zaki* (a non-alcoholic beverage); Bonvehi (2004) detected OTA of up to 4 mg/kg –higher than the EU regulatory level- in cocoa powder from Ivory Coast, Guinea, Nigeria and Cameroon. Aroyeun and Adegoke (2007) reported over 50% occurrence of *Aspergillus ochraceus* and *A.niger* in cocoa beans in Nigeria with a corresponding 40-60 ppb OTA concentration.

Consumption of commodities contaminated with mycotoxins according to Bathnagar and Garcia (2001) leads to chronic mycotoxicoses which results in acute poisoning resulting in death. Therefore, as the global occurrence and importance of mycotoxins cannot be overemphasized, methods for preventing and reducing them before entering the food chain must be given continuous attention as 40% of the productivity lost to diseases in developing countries has been associated with diseases exacerbated by aflatoxins (Miller,1996).

2. Foodborne mycotoxins

There are over 300 mycotoxins that have been isolated and characterized (Adegoke, 2004). From the standpoints of health and trade, important mycotoxins are aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisin, T-2 and T-2 like toxins

(trichothecenes) and alternariol. Thomson and Henke (2000) noted that crops in tropical and subtropical areas are more susceptible to mycotoxin contamination than those in temperate zones because the high humidity and temperatures in tropical areas provide optimal conditions for toxin formation. Furthermore, it has been reported that drought conditions can stress plants and render them susceptible to contamination by *Aspergillus* spp (Holbrook *et al*. 2004; Robertson, 2005). With respect to some fruits, under warm and humid weather, fruit rots in blueberries are of concern as post-harvest rot can occur on berries that look fine at harvest but carry fungal spores that can infect and develop in the fruits during storage and processing (Schilder *et al*. 2006). Thus it is important to identify fungal contaminants in fresh fruits because moulds can grow and produce mycotoxins on these commodities (Tournas and Katsoudas, 2005).

3. Factors that affect mycotoxin production

In the food chain, there are some 'time-bound' factors that are important in the production of mycotoxins during pre-harvest and post-harvest handling of agricultural products like:

- **a.** intrinsic factors: moisture content, water activity, substrate type, plant type and nutrient composition;
- **b.** extrinsic factors: climate, temperature, oxygen level;
- **c.** processing factors: drying, blending, addition of preservatives, handling of grains
- **d.** implicit factors: insect interactions, fungal strain, microbiological ecosystem (Magan *et al*. 2004).

4. Prevention and reduction of mycotoxins

Generally, mycotoxin contamination of agricultural products can be prevented using

1. pre-harvest methods:
    - **a.** using resistant varieties;
    - **b.** field management;
    - **c.** use of biological and chemical agents;
    - **d.** harvest management and
2. post-harvest methods:
    - **a.** improved drying methods;
    - **b.** good storage conditions;
    - **c.** use of natural and chemical agents
    - **d.** irradiation (Kabab *et al*. 2006).

The inclusion of sorbent materials in feed or addition of enzymes or microorganisms capable of detoxifying mycotoxins have been reported to be reliable methods for mycotoxin prevention in feeds (Jard *et al*. 2011). However, while bentonite and aluminosilicate clays have been used as binding agents for reducing aflatoxin intoxication in pigs (Schell *et al*. 1993), cattle (Diaz *et al*. 1997) and poultry (Scheideler, 1993) without causing digestive problems when mixed with aflatoxin-contaminated feed, care must be taken as the clays can alter nutritional value by binding trace minerals and vitamins and reducing their bioavailability and even produce dioxins (Devegowda and Castaldo, 2000). Preference for esterified glucomannan (a naturally-occurring organic compound in yeast) over clay in reducing the toxicity of aflatoxin has been reported. Devegowda and Castaldo (2000) found that using glucomannan supplementation at 0.05% of diet of dairy cows that consumed aflatoxin-contaminated feed, there was a reduction of 58% in aflatoxin in the cow's milk. While in developing countries prevention of mycotoxins 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, nonetheless, the following specific mycotoxins can be prevented from developing in agricultural products:

a. Aflatoxins

Aflatoxins may be produced by three species of *Aspergillus-A.flavus, A.parasiticus* and the rare *A.nomius* which according to Creppy (2002) contaminate plants and plant products like peanut, corn, cotton seed and tree nuts (Diener *et al*. 1987; Horn, 2005). According to Holmes *et al.* (2008), aflatoxins can be produced (in addition to *A.flavus*, *A.parasiticus* and *A. nomius*) by *Aspergillus toxicarius* and *A. parvisclerotigens* on crops like corn, peanuts, cotton seeds and coffee beans. Aflatoxin B_1 according to IARC (1993), is the most carcinogenic, mutagenic and teratogenic substance occurring naturally in foods and feeds. There are reports of the association of aflatoxin contamination of plant foods particularly cereals with liver cancer in Africa and China (Bababunmi, 1978; Oettle, 1964; Li *et al*. 2001). 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 favour *Aspergillus flavus* infection include high soil or air temperature, drought stress, nitrogen stress, crowding of plants and conditions that aid dispersal of conidia during silking (CAST, 1989; Robens, 1990). The growth of *A. flavus* and *A. parasiticus* and subsequent aflatoxin production in storage are favoured by high humidity (>85%), high temperature (25 ^{0}C) and insect or rodent activity (CAST, 1989). The most important insects that spread *A.flavus* in postharvest maize are lepidopteran ear borer, *Mussidia nigrivenella, Sitophilus zeamais* and *Carpophilus dimidiatus* (Setamou *et al.* 1998; Hell *et al.* 2000b). Thus, early harvesting and adequate drying of crops can help in reducing contamination of crops. In several developing countries however, early harvesting, unpredictable weather, labour constraint, need for cash, threat of thieves, rodents and other animals compel farmers to harvest at inappropriate time (Amyot, 1983).

With thorough drying and proper storage of groundnuts in Guinea, Turner *et al* (2005) reported that there was a 60% reduction in the mean aflatoxin levels of groundnuts in villages used for the survey. When it is realized that major portion (80%) of aflatoxin is often associated with small and shrivelled (Davidson *et al*. 1982) and mouldy and stained peanut (Fandohan *et al*. 2005; Turner *et al*. 2005), there are reports of possibility of using physical separation of apparently contaminated cereals from the bulk samples. In Benin, West Africa, Fandohan *et al.* (2005) used some unit operations like sorting, winnowing, washing, crushing and dehulling to remove significant amounts of aflatoxins and fumonisins in maize and maize products. Park (2002) also noted the effects of processing on aflatoxin.

Employing food safety practices like hazard analysis critical control point (HACCP) system can be useful in preventing and reducing aflatoxin contamination in agricultural products. Hell *et al*. (2000a) thus noted that cleaning stores before loading new produce correlated with reduced aflatoxin levels. Preharvest HACCP programmes (FAO/IAEA,2001) are available for controlling aflatoxin in corn and coconuts in South east Asia, peanuts, peanut products in Africa, nuts in West Africa, patulin in apple juice and pistachio nuts in South America. While Aldred and Magan (2004) suggested some HACCP approaches for wheat-based commodities, Lopez-Garcia *et al.* (1999) gave some useful guidance for the development of an integrated mycotoxin management.

Application of potential bio-control agents like atoxigenic strains of *Aspergillus flavus* and *A. 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 in the USA (Dorner *et al*. 1998). The ability of dietary factors to counteract the effects of aflatoxins has been studied. Rompelberg *et al*. (1996) noted that phenolic compounds can metabolically enhance aflatoxin B_1 conjugation and elimination. Galvano *et al*. (2001) also noted that food components like fructose, phenolic compounds, coumarins, chlorophyll and food additives like piperine, aspartame, cyproheptadine and allyl sulfides can reduce the toxicity of mycotoxins by decreasing toxin formation and enhancing metabolism. The antioxidant, ethoxyquin has been reported to be effective as a chemo preventive agent against the carcinogenic effects of aflatoxin B_1 in humans (Bammler *et al*. 2000).

Spices and herbs and their bioactive components have been found useful for the reduction of aflatoxins. Olojede *et al*. (1993) found that when *Garcinia kola* was used at 0.32% (w/v), aflatoxin was effectively reduced from 97 to 23 μg/ml. 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.

In other studies conducted in a developing country, processing was found to reduce aflatoxins in cereal pastes boiled for 30 and 60 minutes by 68% and 80.8% respectively (Adegoke, *et al.* 1994). During the processing of cassava bread- a cassava-based product, while the initial raw material had aflatoxin level of 1.91μg /kg, however, after processing, the final concentration of aflatoxin in the final product, cassava bread, was found to be 0.03μg/kg (Adegoke *et al.* 1993). Roasting of peanuts has been reported to have more effect on reducing chemically detectable aflatoxins than boiling (Njapau *et al*. 1996). Scott (1991) noted that fermentation of wheat flour dough reduced detectable aflatoxin by approximately 50% while baking of the dough produced less effect.

Camou-Arriola and Price (1989) found that using 121^{0}C and alkaline treatment of naturally contaminated corn prior to frying resulted in very low levels of chemically detectable aflatoxin. Wet, dry milling processes and heat during cooking processes have been found to be effective in reducing levels of aflatoxin in foods (Scott, 1984). Conway *et al.* (1978); Hale and Wilson (1979) also found significant decreases in aflatoxin content arising from heating and roasting of corn.The use of traditional processing for reducing aflatoxin has also been examined, for example, Hwang *et al.* (2006) found that processing of traditional Korean foods like 'sujebi' (a soup with wheat flakes) and steamed bread caused 71% and 43 % decrease respectively in AFB_1 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. McKenzie *et al*. (1997) found that ozone destroyed aflatoxins B_1 and G_1 in aqueous model systems. Prudent and King (2002) reported a 92 % degradation (reduction) in aflatoxin in ozonized contaminated corn. Exposure to sunlight has also been found effective in reducing aflatoxin levels in some food products, for example, Adegoke *et al*. (1996) found that sun drying of pepper (*Capsicum annum*) had some significant effects on aflatoxin levels.

b. Ochratoxins

Benford *et al.* (2001) noted that *Aspergillus ochraceus, A. carbonarius, A. melleus, A.sclerotium* and *Penicillium verrucosum* are the main producers of OTA. Ochratoxins have been detected in several agricultural products from temperate and tropical zones. Weidenborner (2001) found OTA in cassava flour, cereals, fish, peanuts, dried fruits, wine, eggs, milk coffee and cocoa beans. Cereals, wine, grape juice, coffee and pork are the major sources of human ochratoxin exposure (JECFA, 2001). Aish *et al*. (2004) also noted that ochratoxin A (OTA) is found in wheat, corn and oats having fungal infection and in cheese and meat products of animal consuming ochratoxin-contaminated grains.

Post-harvest measures for preventing OTA from entering the food chain have been documented. Magan and Aldred (2007) suggested the following post-harvest critical control points-that can be equally adopted in developing countries:

1. regular and accurate moisture content measurements;
2. efficient and prompt drying of wet cereal grains for safe moisture levels (maize, 14%; rice, 13-14 %; barley, 14-14.5 % and canola or rapeseed, 7-8 %);
3. infrastructure for quick response including provision for segregation and appropriate transport conditions;
4. appropriate storage conditions at all stages in terms of moisture and temperature control, general maintenance and effective hygiene of storage facilities for prevention of pests and water ingress.

Modifying the gases in atmospheres where cereals are stored can be used in the prevention of OTA production. Paster *et al*. (1983), found that OTA production by *A.ochraceus* was completely inhibited by 30% CO_2. Thus atmospheres greater than 30 % (for example, 30-60 % CO_2) can be used for preventing OTA production during storage or transportation of grains. This technique can be adopted in developing countries.

Using a combination of cleaning, scouring and removal of the bran and offal fraction, Scudamore *et al*. (2003) observed an overall reduction of about 75% of OTA in white bread. Wet- milling, according to Wood (1982), produced 96 % and 49 % reductions of OTA in the germ and grits of corn respectively. The influence of roasting on the reduction of OTA levels has been examined. Van der Stegen *et al*. (2001) noted that although OTA was relatively stable during heat processing, reductions of OTA of up to 90% was found during coffee bean roasting. Nehad *et al.* (2005) found that roasting reduced to 30μg/kg of OTA by 31% and filtering reduced OTA by 72%. Using final coffee temperature of 204 ^{0}C (dark roast), Romani *et al* (2003) obtained reductions of more than 90 % of OTA. Direct removal of damaged coffee has also been found to reduce OTA contamination (CIRAD, http://www.cirad.fr, accessed on 27-03-2012). In Europe, maximum level for OTA in roasted coffee is fixed at 5μg/kg.

In grossly contaminated samples of cocoa beans, the essential oils of some plants can be used to reduce OTA contamination, for example, Aroyeun and Adegoke (2007) used the essential oils of *Aframomum danielli* to reduce OTA levels in spiked cocoa powder and obtained a reduction efficiency of 64-95 %. Aroyeun *et al*. (2011) described the potentials of, *Aframomum danielli* spice in reducing OTA in cocoa powder as the authors found that the powder of *A. danielli* can be used as a biopreservative (maximum concentration of 60,000 ppm) in cocoa powder contaminated with OTA. Adegoke *et al.* (2007) reported that during processing, Daniellin™ completely reduced the OTA level in a non-alcoholic beverage.

c. Fumonisins

Fumonisins are produced by *Fusarium verticillioides* (Sacc.) Nirenberg (=*F.moniliforme* (Sheldon) and the related *F. proliferatum* (Matsushima) Nirenberg. Fumonisins occur in sorghum, asparagus, rice, beer and beans (Creppy, 2002). Fumonisins can also be found on asparagus and garlic (Seefelder *et al*. 2002). In Africa and several other parts of the world, *Fusarium* spp. are very important field fungi of maize as the fungi produce over 100 secondary metabolites that affect adversely human and animal health (Visconti, 2001). In fact, reports have linked maize consumption with high levels of *F. verticillioides* and fumonisins and high incidence of human oesophageal carcinoma in some parts of South Africa and China (Yoshizawa *et al*. 1994; IPCS, 2000). Fumonisins are classified as possible human carcinogens (IARC, 1993).

In developing countries and other countries where fumonisins occur in maize, useful pre- and post-harvest preventive strategies have been suggested by Maga and Aldred (2007):

a. **pre-harvest measures**:
   1. proper selection of maize hybrids, prevention of use of soft kernel hybrids;
   2. no late sowing dates and avoiding high cropping density;
   3. good and balanced fertilization;
   4. avoiding late harvesting;
   5. effective control of pests for example corn borer.

b. **post-harvest measures**:
   1. minimizing periods between harvesting and drying
   2. effective cleaning of maize prior to storage;
   3. efficient drying to less than 14 % moisture content;
   4. effective hygiene and management of silos;
   5. absence of pests in store-pests can provide metabolic water and initiate heating. Insect damage of maize is a good prediction of *Fusarium* mycotoxin contamination which can serve as a warning signal of fumonisin contamination (Avantaggio *et al*. 2002). Furthermore, the spores of *Fusarium* spp. can be carried by insects from plant surfaces to the interior of the stalk or kernels or create infection wounds due to the feeding of insect larvae on stalks or kernels (Munkvold and Hellminch. 2000).
   6. clear specifications and traceability from field to store.

While these pre-and post-harvest techniques for fumonisin management are not difficult to practise in developing countries, use of solar energy has been found useful elsewhere. Ahmad and Ghaffan (2007) in Pakistan noted that soil solarization was useful in reducing the incidence of corn ear rots and consequently fumonisins and aflatoxins in fields and stored corns. Processing can also be used for reducing fumonisins in food commodities as Jackson *et al* (1997) noted that when corn-based batter (for making muffins) was baked at 175^{0}C and 200 ^{0} C for 20 minutes, reductions of 15 % to 30 % respectively were found with increasing losses as the temperature increased. Voss *et al* (2001) found a reduction of up to 80 % in fumonisin in fried corn chips - a reduction which was attributed to nixtamilization and rinsing. Combination of agronomic techniques –seed time, seed density, N fertilization and control of corn borer have been reported to be useful in reducing fumonisin contamination in maize (Blandino *et al*. 2007).

c. Patulin

Food commodities commonly affected by pathogens that produce patulin are apple, grape, pear, apricots and peaches (Speijers, 2004). The most important aspect of handling patulin is by controlling the quality of fruit before it enters the food chain as patulin is not found in intact fruit because it is the damage done to the surface of the fruit which makes the fruit susceptible to infection by *Penicillium* spp (Sewram *et al.* 2000). During the clarification of apple juice and concentrates, Bissessur *et al.* (2001) found patulin reduction of up to 40% and using pasteurization or evaporation at 70-100 ^{0}C, Kadalal and Nas (2003) found a 25 % loss in naturally contaminated apple juice.

5. Conclusions

In reducing mycotoxin accumulation, it must be realized that the concentrations of aflatoxins, deoxynivalenol or fumonisins are greater in symptomatic than in non-symptomatic maize ears or kernels, thus, prevention or reduction in pre-and post-harvest infection is a critical factor (Scott and Zumno 1995; Reid *et al.* 1996; Desjardin *et al*. 1998). While some techniques for the management of mycotoxin contamination may be in practice in developed countries, however, in order to reduce or eliminate rejection of agricultural produce meant for export and at local levels as well as protect consumers from the harmful effects of mycotoxins, developing countries can adopt some of these methods described herein. Harris (1997) also suggested some practical methods for preventing mycotoxin contamination of feeds, for example, keeping grain bins clean and storage at less that 14 % moisture, use of dry feed ingredients that are oxygen-free, fermented or treated with mould growth inhibitors.

For sustained management of mycotoxins along the food chain, the following procedures are recommended:

a. HACCP-compliance: good agronomic practices through transportation and up to consumption;
b. best practices for harvesting, drying and storage of agricultural products coupled with effective insect management;
c. adoption and sustainability of relevant food safety education;
d. careful and systematic enforcement of legislation on food safety.
e. screening in developing countries where fruits and berries are grown and exported for alternariol, alternariol methylether and tenuazonic acid-mycotoxins produced by *Alternaria* spp (Greco *et al*. 2012).
f. adoption and utilization of sensitive and reliable methods for detection of mycotoxins. For public health protection and international trade, sensitive and accurate analytical methods are needed for mycotoxins (Rahmani *et al.* 2009).
g. sustained examination of agricultural commodities under different prevailing climatic conditions.

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