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Review

Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa

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Aflatoxins are secondary fungal metabolites that contaminate agricultural commodities and can cause sickness or death in humans and animals. Risk of aflatoxin contamination of food and feed in Africa is increased due to environmental, agronomic and socio-economic factors. Environmental conditions especially high humidity and temperature favour fungal proliferation, but also drought conditions increase risk of aflatoxin contamination. Low-input farming practices compound fungal and aflatoxin contamination of crops. The socio-economic and food security status of the majority of inhabitants of sub-Saharan Africa leaves them few options for choosing low-risk and high quality products. Several technologies have been tested in Africa to reduce aflatoxin risk. Field management practices that increase yields can reduce the risk of aflatoxin development. They include use of resistant varieties, crop rotation, well-timed planting, weed control, pest control especially control of insect pests and avoiding drought and nutritional stress through fertilization and irrigation. Measures to stop the infection process by controlling the aflatoxin causing fungi in the field are achieved through use of pesticides and atoxigenic fungi to competitively displace toxigenic fungi, and timely harvest. Post-harvest interventions that reduce aflatoxin include rapid and proper drying, proper transportation and packaging, sorting, cleaning, drying, smoking, post harvest insect control, and the use of botanicals or synthetic pesticides as storage protectants. Another approach is to reduce the frequent consumption of 'high risk' foods (especially maize and groundnut) by consuming a more varied diet, and diversifying the diet into less risky staples like sorghum and millet. Chemo-preventive measures that can reduce aflatoxin effect include daily consumption of chlorophyllin or oltipraz and incorporating hydrated sodium calcium alumino-silicates into the diet. Reduction and detoxification of aflatoxin is often achieved physically (sorting, physical segregation, flotation etc.), chemically (e.g. calcium hydroxide, ammonia) and microbiologically by incorporating pro-biotics or lactic acid bacteria into the diet. Millers can use blending of less and more contaminated products to reduce the overall risk. There is need for efficient monitoring and surveillance with cost-effective sampling and analytical methods to reduce risk in Africa. Public education and awareness can sensitize the population on aflatoxin risk and its management.

Key words: Aflatoxin, Sub-Saharan Africa, control measures.

INTRODUCTION

Aflatoxins are secondary metabolites primarily produced by the fungi *Aspergillus flavus* Link, *Aspergillus parasiticus* Speare and to a lesser extent *Aspergillus nomius* (Kurtzman et al., 1987; CAST, 2003). Optimal conditions for fungal development are 36 to 38°C, with a high humidity of above 85% (Diener et al., 1987). Suitable conditions for the growth of the fungi and aflatoxin production occur in most areas of Africa and aflatoxin contamination in crops has been reviewed by several authors (Sibanda et al., 1997; Shephard, 2003; Bankole and Adebanjo, 2003; Bankole et al., 2006; Wagacha and Muthomi, 2008). African communities are

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hence exposed to aflatoxin before birth and throughout their lives with serious impact on their health (Williams et al., 2004; Wild and Gong, 2010).

Aflatoxin is the most potent natural carcinogenic substance and has been linked with a higher prevalence of hepatocellular cancer in Africa (Strosnider et al., 2006). There is a high risk of Hepatitis B and Hepatitis C carriers developing liver cancer when they are exposed to aflatoxin (Williams et al., 2004). There have been recent documented outbreaks of acute aflatoxicosis in Kenya (Probst et al., 2007), but chronic exposure to aflatoxin, which has far reaching health effects (Williams et al., 2004), has not been well documented. Moreover, aflatoxins have been linked to immune suppression by Turner et al. (2005) and Jiang et al. (2005). Children in areas of high aflatoxin exposure have been found to have stunted growth (Gong et al., 2004). Aflatoxin contamination has also been linked to micronutrient deficiencies in animals (Williams et al., 2004), but Gong et al. (2004) reported that there was no relationship between aflatoxin-albumin, the biomarker of aflatoxin exposure, and micronutrients.

Aflatoxin contamination in several foodstuffs in Africa has been a recurrent problem (Shephard, 2003). In many parts, maize has become the preferred cereal for food, feed and industrial use, displacing traditional cereals such as sorghum and millets. However, it was found to be significantly more colonized by aflatoxin-producing *Aspergillus* spp. than either sorghum or millet (Bandyopadhyay et al., 2007).

This review paper outlines some of the potential solutions for controlling aflatoxins in Africa that are being developed by researchers either within or from outside Africa. These strategies can be broadly divided into: stopping the infection process (host plant resistance, biocontrol); control of environmental factors (temp, rainfall, relative humidity, evapotranspiration, soil type) including efforts to build predictive models; pre-harvest crop management practices; post harvest management strategies (timely harvesting, proper drying, sorting, proper storage, proper transportation, use of plant extracts and preservatives, good manufacturing practice and finding alternative uses for contaminated grain).

FIRST STRATEGY: STOPPING THE INFECTION PROCESS

Breeding for resistance

Several screening tools have been developed and used to facilitate corn breeding for developing germplasm resistant to fungal growth and/or aflatoxin contamination (Brown et al., 2003). Sources of resistance to *Aspergillus* infection and aflatoxin contamination in corn have been identified, but commercial hybrids have not been developed. This is largely due to the difficulty in finding elite lines that maintain high yields and maintain resistance within multiple environments (Clements and White, 2004). Brown et al. (2001) tested aflatoxin resistance in thirty-six maize inbred lines selected in West and Central Africa for moderate to high resistance to maize ear rot for their resistance to aflatoxin; more than half of the inbred lines accumulated aflatoxin at levels as low as or lower than the resistant U.S. lines. In 2008, six tropical maize germplasm lines with resistance to aflatoxin were registered by the same research group (Menkir et al., 2008) and their distribution to national programs will start soon for the development of locally adapted hybrids.

Similar work has been done on peanuts, and attempts to develop aflatoxin resistant varieties have been carried out (Petit, 1986; Waliyar et al., 1994; Upadhyaya at al., 2004). This has led to the development of elite resistant varieties, which were eventually released as improved germplasm in Niger, Senegal and Burkina Faso (Upadhyaya et al., 2002). However, resistance in peanuts to aflatoxin contamination under all conditions has still not been achieved and breeding efforts continue including the use of microarrays to aid in the identification of genes involved in crop resistance (Guo et al., 2009). Many new strategies that enhance host plant resistance against aflatoxin, involving biotechnologies are being explored and are reviewed for maize by Brown et al. (2003) and for peanut by Guo et al. (2009). These approaches involve the design and production of maize plants that reduce the incidence of fungal infection, restrict the growth of toxigenic fungi or prevent toxin accumulation. They include identification of resistance-associated proteins (RAPs) through proteomics as well as biochemical marker identification (Bhatnagar et al., 2008) and identification of aflatoxin accumulation resistance quantitative trait loci (QTL) and related markers (Warburton et al., 2009). In the long term the identification of compounds that block aflatoxin biosynthesis would significantly enhance aflatoxin control.

Biological control

Another potential means for aflatoxin control is the biocontrol of fungal growth in the field. Numerous organisms have been tested for biological control of aflatoxin contamination including bacteria, yeasts, and non-toxigenic (atoxigenic) strains of the causal organisms (Yin et al., 2008) of which only atoxigenic strains have reached the commercial stage (Dorner, 2009). Biological control of aflatoxin production in crops in the US has been approved by the Environmental Protection Agency and two commercial products based on atoxigenic *Aspergillus flavus* strains are being used (afla-guard® and AF36®), for the prevention of aflatoxin in peanuts, corn and cotton seed (Dorner, 2009). In Africa, atoxigenic strains of *A. flavus* have been identified to competitively exclude toxigenic fungi in the maize and peanut fields.

These strains have been shown to reduce aflatoxin concentrations in both laboratory and field trials by 70 to 99% (Atehnkeng et al., 2008b). A mixture of four atoxigenic strains of *A. flavus* of Nigerian origin has gained provisional registration as AflaSafe® to determine efficacy in on-farm tests. Candidate strains have also been selected for Kenya and Senegal and field trials are currently on-going.

SECOND STRATEGY: CONTROL OF ENVIRONMENTAL FACTORS

To design strategies for the prevention or reduction of aflatoxin, an understanding of the factors that influence the infection of the plant with aflatoxin causing fungi and the conditions that induce their formation is vital. Environmental factors that favor *A. flavus* infection in the field include high soil and/or air temperature, high relative humidity, high rates of evapotranspiration, reduced water availability, drought stress, nitrogen stress and crowding of plants and conditions that aid the dispersal of conidia during silking (CAST, 2003; Klich, 2007). Some of these factors have been included in a model to predict aflatoxin contamination in peanut systems in Mali. Weather and satellite based variables that could be used to indicate aflatoxin presence in peanut were identified (Boken et al., 2008).

Significant correlations exist between agro-ecological zones (AEZ)[1] and aflatoxin levels, with wet and humid climates and drier regions after longer storage periods increasing aflatoxin risk (Hell et al., 2000). Kaaya et al. (2006) observed that aflatoxin levels in Ugandan maize samples were higher in more humid areas compared to the drier areas and similar results were obtained in maize samples from Nigeria (Atehnkeng et al., 2008a). Mutegi et al. (2009) also found peanut samples collected in wetter and humid areas of western Kenya to have higher aflatoxin levels than those in the drier and less humid regions; such trends could be used to elaborate predictive models. Modelling of interactions between host plant and environment during the season can enable quantification of pre-harvest aflatoxin risk and its potential management (Boken et al., 2008). In addition, predictive growth models for fungal and mycotoxin developments are available and have been reviewed by Garcia et al. (2009).

Factors that influence the incidence of fungal infection and subsequent toxin development include invertebrate vectors, grain damage, oxygen and carbon dioxide levels, inoculum load, substrate composition, fungal infection levels, prevalence of toxigenic strains and microbiological interactions. Insects vector fungi and cause damage that allows the fungi to gain access, increasing the chances of aflatoxin contamination, especially when loose-husked maize hybrids are used (Dowd, 2003). High incidence of the insect borer *Mussidia nigrivenella*, was positively correlated with aflatoxin contamination of maize in Benin (Setamou et al., 1998). Storage pests, in particular *Cathartus quadricollis* and *Sitophilus zeamais*, have also been shown to play an important role in the contamination of foods with fungi, especially those that produce toxins (Hell et al., 2003; Lamboni and Hell, 2009).

Third strategy: Pre-harvest crop management strategies

Controlling or reducing infection by regulating the factors that increase the risk of aflatoxin contamination in the field contributes extensively in managing aflatoxin. Management practices that reduce the incidence of aflatoxin contamination in the field include timely planting, maintaining optimal plant densities, proper plant nutrition, avoiding drought stress, controlling other plant pathogens, weeds and insect pests and proper harvesting (Bruns, 2003). In Africa, crops are cultivated under rain fed conditions, with low levels of fertilizer and little pesticide application. These management practices promote *A. flavus* infection in stressed plants. Invariably any action taken to interrupt the probability of silk and kernel infection will reduce aflatoxin contamination (Diener et al., 1987).

Pre-harvest measures that are efficient in reducing aflatoxin levels are the same as those that will enhance yields. Crop rotation and management of crop residues also are important in controlling *A. flavus* infection in the field. Tillage practices, fertilizer application, weed control, late season rainfall, irrigation, wind and pest vectors can all affect the source and level of fungal inoculum, maintaining the disease cycle in maize (Diener et al., 1987). Lime application, use of farm yard manure and cereal crop residues as soil amendments have shown to be effective in reducing *A. flavus* contamination as well as aflatoxin levels by 50-90%, as described by Waliyar et al. (2008). Calcium, which is part of lime, thickens the cell wall and accelerates pod filling, while manure facilitates growth of micro-organisms that suppress soil infections.

Extended field drying of maize could result in serious grain losses during storage (Borgemeister et al., 1998; Kaaya et al., 2006), and as such harvesting immediately after physiological maturity is recommended to combat aflatoxin problems. Kaaya et al. (2006) observed that aflatoxin levels increased by about 4 times by the third week and more than 7 times when maize harvest was delayed for 4 weeks. However, after early harvesting products have to be dried to safe levels to stop fungal growth. Leaving the harvested crop in the field prior to storage promotes fungal infection and insect infestation. This is common practice in Africa often due to labour constraints, and the need to let the crop dry completely prior to harvest (Udoh et al., 2000).

[1] Agroecozones are geographic areas that share similar biophysical characteristics for crop production, such as soil, landscape, and climate.

FOURTH STRATEGY: POST HARVEST CROP MANAGEMENT PRACTICES

Moisture and temperature influence the growth of toxigenic fungi in stored commodities. Aflatoxin contamination can increase 10 fold in a 3-day period, when field harvested maize is stored with high moisture content (Hell et al., 2008). The general recommendation is that harvested commodities should be dried as quickly as possible to safe moisture levels of 10 – 13% for cereals. For peanuts the standard practice is drying of pods in the sun. Often pods are left in the field after uprooting for up to four weeks to partially dry prior to home drying. Achieving this through simple sun-drying under the high humidity conditions of many parts of Africa is difficult. Even, when drying is done in the dry season, it is not completed before loading grains into stores like observed by Mestres et al. (2004) and products can be easily contaminated with aflatoxin. There are several technologies to increase the efficacy of grain drying and reduce the risk of toxin contamination even under low-input conditions. These include the use of drying platforms, drying outside the field and drying on mats (Hell et al., 2008). Technological solutions that could aid in reducing grain moisture rapidly have been reviewed by Lutfy et al. (2008). However, these dryers are not used by farmers in Africa because large capital investments are needed to acquire them. Nonetheless, Gummert et al. (2009) described the very positive effect dryers had on maintaining rice quality and reducing mycotoxin risk in Southeast Asia.

Aflatoxin contamination of foods has further been shown to increase with storage period (Kaaya and Kyamuhangire, 2006). It is compounded in Africa through excessive heat, high humidity, lack of aeration in the stores, and insect and rodent damage resulting in the proliferation and spread of fungal spores. Thus strategies to minimize quantitative and qualitative post harvest losses have been developed (Hell et al., 2008). These improved postharvest technologies have been used successfully to reduce the blood aflatoxin-adducts level in populations in Guinea, where exposure was more than halved 5 months after harvest in individuals from the intervention villages (Turner et al., 2005).

Traditional storage methods in Africa can be divided into two types, namely temporary storage that is mainly used to dry the crop and permanent storage that takes place in the field or on the farm. The latter includes containers made from plant materials (wood, bamboo, thatch) or mud placed on raised platforms and covered with thatch or metal roofing sheet. The stores are constructed to prevent insect and rodent infestation and to prevent moisture from getting into the grains. While new storage technologies such as the use of metal or cement bins by small-scale farmers would serve better, their up-take has been slow due to their high cost. Many farmers nowadays store their grains in bags, especially polypropylene which are not airtight, but there is evidence that this method facilitates fungal contamination and aflatoxin development (Hell et al., 2000; Udoh et al., 2000). Presently there are efforts to market improved hermetic storage bags in Africa, based on triple bagging developed for cowpea which has been or is being tested for other commodities (Ben et al., 2009). Efforts to evaluate effectiveness of this technology in controlling aflatoxin have not been conclusive.

Postharvest contamination of grain can also take place during transportation, as well as marketing. Grain subsequently needs to be well covered and/or aerated during transportation. Storage in appropriate bagging, preferably sisal bags, is necessary to facilitate aeration in transit.

Due to the informal marketing systems that exist in sub-saharan Africa, it is difficult to regulate and/or establish proper systems for handling grain post harvest, especially for small-scale traders. Open air market systems also support spoilage due to weather changes and abrupt rainfall that can wet the grains, as the grains are not covered appropriately (Mutegi et al., unpublished data).

Disinfestation methods: Smoking is an efficient method of reducing moisture content and protecting maize against infestation by fungi. The efficacy of smoking in protecting against insect infestation was found to be high. About 4 to 12% of farmers in the various ecological zones in Nigeria used smoke to preserve their grains, and this practice was found to be correlated with lower aflatoxin levels in farmers' stores (Udoh et al., 2000). Other compounds used for seed fumigation like ethylene oxide and methyl bromide were found to significantly reduce the incidence of fungi including toxigenic species on stored peanuts and melon seeds (Bankole et al., 1996). Among the chemical compounds tested in feeds, propionic acid, sodium propionate, benzoic acid and ammonia were the best anti-fungal compounds, followed by urea and citric acid (Gowda et al., 2004).

Decontamination processes inactivate, destroy or remove the toxin from food, so that it can be used as animal feed. Most decontamination methods are not economically viable at a commercial level, except for ammoniation. This method and its effectiveness for removing toxins has been reviewed (Safamehr, 2008; Nath and Sarma, 2005; Park and Price, 2001).

Past studies have also looked at the use of local plant products for the control of fungi mostly proving their efficacy in-vitro (Hsieh et al., 2001), but these products have not been sufficiently tested for their efficiency in controlling aflatoxin in stored crops. There is need to review the efficacy of the multiple products used by farmers and tested by researchers to get a complete picture about their potential in reducing aflatoxin contamination.

Use of pesticides to control mycotoxins and their efficacy, have been reviewed by D'Mello et al. (1998), but their use by farmers in Africa is not always well practiced and deaths due to pesticide use have been reported. Extension workers should therefore educate farmers on the importance of using recommended chemicals for specific crops at appropriate concentrations and within a safe delay before consumption.

Physical separation and hygiene: Aflatoxin is unevenly distributed in a seed lot and may be concentrated in a very small percentage of the product (Whitaker, 2003). Sorting out of physically damaged and infected grains (known from colorations, odd shapes and size) from the intact commodity can result in 40-80% reduction in aflatoxin levels (Park, 2002; Fandohan et al., 2005; Afolabi et al., 2006). The advantage of this method is that it reduces toxin concentrations to safe levels without the production of toxin degradation products or any reduction in the nutritional value of the food. This could be done manually or by using electronic sorters. Market practices such as grading have also been shown to reduce levels of aflatoxin. Unlike sorting, most of the peanut farmers who graded their peanuts in western Kenya did it for the purpose of determining prices. Nevertheless, nuts graded as low quality had higher levels of aflatoxin compared to those of the highest quality (Mutegi et al., 2007) and were still sold for a lower price in the markets. Similarly, farmers are likely to set aside products that are not marketable for home consumption including feeding of poultry.

Clearing the remains of previous harvests and destroying infested crop residues are basic sanitary measures that are also effective against storage deterioration. Cleaning of stores before loading in the new harvests was correlated with reduction in aflatoxin levels (Hell et al., 2008). Separating heavily damaged ears, that is, those having greater than 10% ear damage also reduces aflatoxin levels in maize (Setamou et al., 1998). Wild hosts, which constitute a major source of infestation for storage pests, should also be removed from the vicinity of stores (Hell et al., 2008).

Reduction through food processing procedures: Sorting can remove a major part of aflatoxin contaminated units, but levels in contaminated commodities may also be reduced through food processing procedures that may involve processes such as washing, wet and dry milling, grain cleaning, dehulling, roasting, baking, frying, nixtamalization and extrusion cooking. These methods and their impact on mycotoxin reduction have been reviewed by Fandohan et al. (2008). The effect of extrusion cooking on mycotoxins in cereals was reviewed by Castells et al. (2005). Dehulling maize grain has been shown to reduce aflatoxin contamination by 92% (Siwela et al., 2005). The effect of nixtamalization in reducing aflatoxin contamination (Park, 2002) has lately been questioned, with Méndez-Albores et al. (2004) reporting that nixtamilization is reversible.

Fermentation can increase the safety of some food products contaminated with aflatoxins. However, the available reports are contradictory, with some showing very efficient reductions in mycotoxins associated with fermentation, whereas others find lesser or no effects. Fandohan et al. (2005) found that processing maize into makume (a solid state fermented maize based product) resulted in 93% reduction of aflatoxin, while reduction levels were 40% for 'owo' which is a non-fermented dry milled maize porridge. The authors identified sorting, winnowing, washing, crushing combined with dehulling of maize grains as the critical aflatoxins reducing steps in the production chain, while fermentation and cooking appeared to have insignificant effect.

Other strategies to reduce the risk of aflatoxin ingestion in Africa are dietary change, chemoprevention, detoxification and vaccination against hepatitis B, which would significantly reduce liver cancer risk (Strosnider et al., 2006; Wild and Gong, 2010).

Clay-based interventions have also been used at a commercial level to bind aflatoxin in animal feed as reviewed by Kabak and Dobson (2009). For example, the use of Novasil® which is a calcium montmorillonite clay, successfully sequestered aflatoxins in the gastrointestinal tract and reduced their availability in animals blood. Their cost effectiveness and long-term safety in rodent studies have led them to be considered as a viable solution in humans (Phillips et al., 2008). In fact Novasil® clay protected inhabitants in a high risk zone in Ghana, significantly reducing toxin levels in their body (Wang et al., 2008).

During processing, quality management systems such as HACCP (Hazard Analysis Critical Control Point) have been recommended as a strong tool for managing mycotoxins along the product chain (Schmale and Munkvold, 2011). Due to the step-by-step monitoring, the system reduces costly end point quality control. Sensitization efforts would also result in a consumer demand for safe products due to increased awareness, and subsequent low consumption of contaminated foods. In West Africa campaigns were highly successful in informing populations about the need for consuming good quality foods and implementation of good management practices (James et al., 2007). This can however be complicated for populations that are food insecure, especially in Sub-Saharan Africa, where high risk foods such as maize constitute the staple diet for majority of people.

Having discussed various options for managing aflatoxin in grain, the avenues for grain contamination are brought to light. Subsequently, integrated approaches to tackling the aflatoxin menace are likely to work better, rather than relying on one approach.

CONCLUSIONS - PERSPECTIVES OF AFLATOXIN RESEARCH IN AFRICA

It is clear that aflatoxin contamination in agricultural crops is widespread in Africa, but food insecurity compounded by drought is a major obstacle to improvements in food safety. Increased pressure on limited food resources and under-nutrition exacerbates the mycotoxin problem by increasing the likelihood of human consumption of contaminated foods and by rendering the population more susceptible to the consequent adverse health effects.

Even though considerable research efforts have been made to control toxin contamination, there are several factors that lead to high aflatoxin risk in Africa:

1) Lack of political commitment to mycotoxin research,
2) Shortage of trained personnel and infrastructure for mycotoxin monitoring and research,
3) Limited awareness on risks at all levels and insufficient knowledge on options to reduce aflatoxin contamination from plough to plate
4) Prevailing climatic conditions favour mycotoxin development, even when management options are in place.

The perspectives of aflatoxin research in Africa can therefore be foreseen as follows:

i. Getting policy makers in the sub-region to recognise that the stimulation of the postharvest sector is an important avenue to increase food production and ensure food safety for the protection of the health of their citizens.
ii. Educating stakeholders on the dangers of commercializing and consuming mouldy foods.
iii. Training personnel at all levels (scientists, technicians, extension agents) in sampling protocols and modern methods of mycotoxin analysis.
iv. Developing infrastructure to accommodate surveillance as well as research on mycotoxins.
v. Conducting food basket surveys for aflatoxin contamination using uniform sampling protocols and modern analytical methods to obtain sound and reliable data on aflatoxin incidence in different food crops, which could then be used to define control strategies.
vi. There should be a co-ordinated and collaborative effort on aflatoxin research in Africa to minimize repetitions so that resources can be focused on identified priority areas, including documenting the impact of aflatoxin on health and economies in Africa.
vii. Developing early warning mechanisms especially in the highly prone areas, in order to avert acute poisoning that leads to fatalities

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