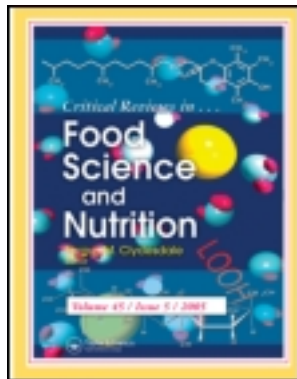


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A Review on Aflatoxin Contamination and Its Implications in the Developing World: A Sub-Saharan African Perspective

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Mycotoxins contamination in some agricultural food commodities seriously impact human and animal health and reduce the commercial value of crops. Mycotoxins are toxic secondary metabolites produced by fungi that contaminate agricultural commodities pre- or postharvest. Africa is one of the continents where environmental, agricultural and storage conditions of food commodities are conducive of Aspergillus fungi infection and aflatoxin biosynthesis. This paper reviews the commodity-wise aetiology and contamination process of aflatoxins and evaluates the potential risk of exposure from common African foods. Possible ways of reducing risk for fungal infection and aflatoxin development that are relevant to the African context. The presented database would be useful as benchmark information for development and prioritization of future research. There is need for more investigations on food quality and safety by making available advanced equipments and analytical methods as well as surveillance and awareness creation in the region.

Keywords Aflatoxins, *Aspergillus flavus*, mycoflora, mycotoxins, decontamination

INTRODUCTION

Agents of Food Spoilage

Many pests and diseases are prevalent on food commodities such as maize, sorghum, millet, barley, yam, and cassava in the field and during storage, including a number of insects. In addition, stored and processed food products carry a wide range of microorganisms that include bacteria, yeasts and filamentous fungi. The population structure of microorganisms depends on field climatic conditions and harvesting processes (Lacey and Magan, 1991). Poor postharvest management can result in rapid deterioration and reduction in food quality, including loss of

nutritional value, loss of products and decreased germinability of seeds (Christensen, 1973; Ominski et al, 1994; Magan et al., 2003). Fungal infestation can lead to discolouration, production of off-odours and can result in mycotoxins (Magan et al., 2003).

Mycotoxins are secondary metabolites produced by fungi that are toxic to humans and animals. The most important toxigenic fungi belong to the genera *Aspergillus*, *Fusarium*, and *Penicillium* (Pitt and Hocking, 1999). The most important mycotoxins worldwide include aflatoxins, fumonisins, deoxynivalenol, zearalenone, and ochratoxins (Pittet, 1998; Pitt, 2000).

Mycotoxins production by fungi is influenced by abiotic and biotic factors. Abiotic factors include environmental factors such as temperature, water availability and gas composition (Magan et al., 2003). Foreign material and debris can also have an impact on both the rate of fungal spoilage and the production of mycotoxins (Atukwase et al., 2009). Biotic factors include variables such as the nature of the substrate, its inherent moisture content, and insect infestation which can contribute to

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increasing fungal population(s) and the subsequent production of mycotoxins (Dowd, 2003).

Mycotoxins and Their Causal Agents

Mycotoxins are produced by toxigenic fungi at both field and storage levels. *Fusarium* and *Penicillium* species (Miller, 1995; Marasas, 1996, 2001) occur predominantly in the field. *Fusarium* species produce a variety of mycotoxins with divergent biological and toxicological effects in humans and animals (Pitt, 2000; Marasas, 2001). Mycotoxins produced by *Fusarium* include fumonisins, deoxynivalenol and zearalenone.

Penicillium and *Aspergillus* species are the most important storage fungi but can also be a problem in the field especially on senescent or stressed plants (Sinha and Sinha, 1992; Miller, 1995). *Penicillium* species produce ochratoxins, citrinin, patulin (Pitt, 2000; Gokmen et al., 2005). *Aspergillus* species produce (principally) aflatoxins, citrinin, patulin (IARC, 1993; Gokmen et al., 2005).

Aflatoxins

Aflatoxins are the most important mycotoxins with regard to occurrence, toxicity, and impact on human health and trade. Aflatoxins are primarily produced by strains of *Aspergillus flavus*, *A. parasiticus*, *A. nomius* (Wilson et al., 2002), *A. pseudotamarii* (Ito et al., 2001), and *A. bombycis* (Peterson et al., 2001). All of these species are found in the soil (Wilson et al., 2002). The four major aflatoxins commonly isolated from foods and feeds are aflatoxins B₁, B₂, G₁, and G₂. *A. flavus* and *A. pseudotamarii* produce only B aflatoxins. They lack the ability to synthesize G aflatoxins due to 0.8- to 1.5-kb deletion in the 28-gene aflatoxin biosynthesis cluster (Ehrlich et al., 2004). *Aspergillus nomius*, *A. bombycis*, and *A. parasiticus* produce all four major aflatoxins. Aflatoxins M₁ and M₂ are hydroxylated metabolites of aflatoxin B₁ and B₂ respectively, and are produced in milk-producing animals (Lanyasunya et al., 2005; Rahimi et al., 2010). Aflatoxin M₁ has been detected in raw milk from cows and water buffaloes in Iran at high concentrations exceeding the maximum tolerance limit of the European Union/Codex Alimentarius Commission (50 ng/L) (Rahimi et al., 2010). El-Nezami et al. (1995) have reported on the presence of aflatoxin M₁ in human breast milk from Victoria, Australia and Thailand. Aflatoxin M₁ was detected at high concentration putting infants at risk of contamination.

Impact of Aflatoxins on Health: Evidence from Africa

Exposure to aflatoxins is widespread in many African countries, and exposure starts before birth (Montesano et al., 1997). Blood tests have shown that a high percentage of West Africans are exposed to aflatoxins. Studies reported by Wild (1996) and carried out in Gambia, Guinea, Nigeria, and Senegal, up to over 98% of subjects tested positive to aflatoxins markers. In Benin 99% of the children had aflatoxin markers in their blood

with some of the highest aflatoxin levels in humans ever observed (Gong et al., 2002). A big part of the world population is chronically exposed to aflatoxins as evident from the presence of aflatoxin M₁ as previously stated (Rahimi et al., 2010) in human breast milk in Ghana, Kenya, Nigeria, Sierra Leone, Sudan, Thailand, the United Arab Emirates, and in umbilical cord blood samples from Ghana, Kenya, Nigeria, and Sierra Leone (Groopman and Kensler, 1999; Bhat and Vasanthi, 2003).

Aflatoxins are hepatocarcinogens and have been classified as class 1 human carcinogen (IARC, 1993). The average daily intake of aflatoxin B₁ in the high-risk area was 184.1 µg. Hepatitis B can act synergistically with aflatoxins to increase the risk of hepatocellular carcinoma (Turner et al., 2000). According to the World Health Organization (WHO) chronic hepatitis B virus infection occurs more frequently (high infection >8%) in developing world including Asia and the Pacific Basin (excluding Japan, Australia, and New Zealand), sub-Sahara Africa, the Amazon Basin, parts of the Middle East, the central Asian Republics, and some countries in Eastern Europe. Whilst the rest of Europe infection rates are below 1% and less than 20% of the population is ever exposed to hepatitis B virus infection (EFSA, 2007). Overall, epidemiological studies of human populations exposed to diets naturally contaminated with aflatoxins revealed an association between the high incidence of liver cancer in Africa and elsewhere and dietary intake of aflatoxins (Turner et al. 2002). Often up to 1 in 10 of the population in sub-Saharan Africa are infected with hepatitis B and C, aflatoxin intake raise the risk of liver cancer by more than ten-fold compared to the exposure of both hepatitis alone (Turner et al., 2003).

Aflatoxins have also been shown to be immunotoxic to both livestock and man. Turner et al. (2003) detected aflatoxins-albumin adducts in 93% of sampled children (6–9 years) in Gambia and provided evidence that immune parameter such as secretory IgA (sIgA) in saliva may be reduced because of high dietary levels of aflatoxins exposure, maximum level for aflatoxin ingestion was established to between 2 and 10 µg/kg (EC, 2006). HIV and esophageal cancer deaths were significantly related to maize consumption in sub-Saharan Africa. HIV infection were minimized (74 compared with 435/100,000 people; odds ratio: 2.41; 95% confidential interval: 1.73' 3.24; $p \leq 0.0001$) by combination of low maize consumption and above-median% Muslim (Williams et al., 2010). The authors concluded that changes to the quality of maize may avoid up to 1000,000 transmissions of HIV annually. There seems also to be an interaction of aflatoxin and HIV on immune suppression, higher levels of aflatoxin B₁-albumin adducts in plasma were associated with a lower percentage of certain leukocyte, aflatoxin may facilitate HIV associated immune hyperactivation and lead to more severe disease (Jiang et al., 2005). These authors demonstrated that participants with high aflatoxin B₁ levels had significantly lower percentages of CD31 and CD191 cells that showed the CD691 activation marker (CD31CD691 and CD191CD691) than participants with low aflatoxin B₁ levels. Also, the percentages of CD81 T cells that contained perforin

or both perforin and granzyme A were significantly lower in participants with high aflatoxin B₁ levels compared with those with low aflatoxin B₁. The same study concluded that these alterations in immunological parameters in participants with high aflatoxin B₁ levels could result in impairments in cellular immunity that could decrease host resistance to infections. In addition, evidence suggests that there may be an interaction between aflatoxins consumption and diseases such as malaria and HIV/AIDS (Gong et al., 2003, 2004; Jolly et al., 2006). Finally, the immuno-suppressive effects of aflatoxins predispose the individual or animal to secondary infections due to other fungi, bacteria and viruses (McLean, 1995).

Higher aflatoxin levels have been found in the blood, urine and livers of children with symptoms of nutritional deficiencies, e.g., kwashiorkor than similar age-matched children (Hendrickse, 1984). Aflatoxin-positive kwashiorkor children showed significantly greater severity of edema, increased number of infections, lower haemoglobin levels and longer duration of hospital stay than aflatoxin-negative kwashiorkor children (Adhikari et al., 1994; Ramjee, 1996). It seems that protein deficiency reduces the capacity of the liver to detoxify aflatoxins, thus aflatoxins may be a contributory factor in increasing the morbidity of children suffering from other disease (Ramjee, 1996). Gong et al. (2002) established that children in Togo and Benin who ate foods contaminated with high-levels of aflatoxins were stunted and underweight, symptoms normally associated with malnutrition. Aflatoxin might be associated with infertility; Uriah et al. (2001) observed aflatoxin levels in blood and semen ranged from 700 to 1393 ng/mL and 60 to 148 ng/mL, in infertile and fertile Nigerian men, respectively.

Looking broadly on the animal kingdom, symptoms of chronic exposure to aflatoxin include decrease in growth rate, lowered milk or egg production, and immuno-suppression. In addition, liver damage is apparent due to the yellow colour that is characteristic of the symptoms of aflatoxicosis jaundice and a swollen gall bladder (Wagacha and Muthomi, 2008). Aflatoxin contaminated feed is detrimental to swine industry (CAST, 1989). Reduced feed intake and feed efficiency has been observed for swine fed contaminated feed (Harvey et al., 1988). Aflatoxin M₁ appears in milk of sows consuming aflatoxin contaminated feeds may affect piglets nursing those sows (Jones et al., 1994). Aflatoxin affects all poultry species. Although it generally takes relatively high levels to cause mortality, intake of low concentration of toxins over a long period of time lead to poor growth, poor feed efficiency, immunosuppression, and sub-optimal production (Jones et al., 1994). Susceptibility to aflatoxin is greatest in young animals, but significant differences exist between species, individuals and sexes with males being more susceptible (Williams et al., 2004). The risk of mycotoxin contamination in dairy feed and milk on smallholder dairy farms in Kenya has been documented (Lanyasunya et al., 2005). In fact, smallholder dairy farmers ignored danger of mycotoxin contamination of dairy feeds possibly leading to animal poisoning. A survey and interviews conducted have shown that livestock get poisoned after consuming contaminated feeds. When levels of

aflatoxin M₁ appearing in milk and other dairy products is more than the Kenyan limit (20 µg/kg), then it becomes a food safety hazard (Lanyasunya et al., 2005).

Exposure to large doses of mixed aflatoxins may cause acute toxicity with lethal effect. The acute toxicity of the most toxic aflatoxin, namely aflatoxin B₁ has been demonstrated to vary very much between animal species. Thus, acute lethal effect is reported to show an oral LD₅₀ value of around 5 mg/kg body weight for the rat but between 0.4 and 0.5 mg/kg b.w. for the rabbit and the cat (ChemIDPlus Lite, 2010). Estimated mixed total doses ingested with food of around 6,000 mg have been reported to cause fatal acute toxicity in adult human beings, whereas exposure to small doses (a total of 2–6 mg) distributed over a prolonged period could lead to cancer (Groopman and Kensler, 1999; Wagacha and Muthomi, 2008). Some of the symptoms of acute toxicity include reduced liver function, disruption of blood clotting mechanism, icterus (jaundice) and a decrease in essential serum proteins that are synthesized by the liver (Wagacha and Muthomi, 2008). Other symptoms of acute to subacute aflatoxicosis are oedema of the lower extremities, abdominal pain, and vomiting. Severe acute liver injury with high morbidity and mortality has been associated with high dose of aflatoxins exposure (Chao et al., 1991). Consumption of aflatoxin-contaminated food can lead to outbreaks of sudden death within a population. Cases of death have been reported in Kenya with 317 cases reported resulting in 125 deaths (Lewis et al., 2005), 15 deaths reported in 2005 and 16 deaths listed in 2006 (Anonymous, 2006). These cases were always in the same drought-prone districts in Kenya.

A study has demonstrated that males were more likely to die from aflatoxicosis despite eating similar quantities of maize as females (Azzi-Baumgartner et al., 2005), suggesting male immune systems are weaker. Similarly a higher liver cancer rate has been observed in male, with a ratio of 3.4 males per female cases (Kirk et al., 2006). Earlier investigation has revealed that ingestion of 2–6 mg/day of aflatoxins for a month can lead to acute hepatitis and death, while symptoms of hepatotoxicity from aflatoxicosis are anorexia, malaise and low-grade fever (Patten, 1981) in addition to vomiting and abdominal pain (Etzel, 2002).

No animal species is resistant to the acute toxic effects of aflatoxins, however, animal species respond differently in their susceptibility to the chronic and acute toxicity of aflatoxins. For most species, the acute lethal dose (LD₅₀) value ranges from 0.5 to 10 mg/kg body weight (Wagacha and Muthomi, 2008). In conclusion, effect of aflatoxin is influenced by environmental factors, exposure level, and duration of exposure beside age, health and nutritional status (FAO, 2000).

Overall, few epidemiological studies have been conducted in Africa, and more studies are needed to fully understand the health impact of these toxigenic and carcinogenic mycotoxin on the population.

Other mycotoxins, such as fumonisins, ochratoxins, gliotoxin, patulin, zearaleone, and trichothecenes including deoxynivalenol, have been reported to modulate the immune system

(Bondy and Pestka, 2000; Meko et al., 2001; Pestka et al., 2004). The co-occurrence of fumonisins and aflatoxin has been shown in maize from Ghana (Kpodo et al., 2000) and Benin (Fandohan et al., 2005b). Further clarification of the immune effects of mycotoxins and multitoxin effects are needed.

AFLATOXINS CONTAMINATION OF FOOD COMMODITIES AND RELATIVE EXPOSURE RISK TO AFRICAN POPULATIONS

The presence of significant levels of aflatoxins has been reported in groundnut, cashew and maize and other crops. Reports on contamination levels in Africa have been partially reviewed by Wagacha and Muthomi (2008). Data collected on aflatoxin risk is rather spotty and isolated; here data is reviewed from potential highest exposure risks to commodities with lower exposure risk in sub-Saharan African populations (Table 1). These data show that there is a need for more investigations focusing on a larger variety of food commodities commonly consumed in sub-Saharan Africa. These disparate reports (Table 1) show the need to determine toxin levels in dried cassava chips with a standardized protocol using modern detection methods so that potentially fluorescence from similar compounds can be excluded. At present, there is little information on the biotic and abiotic conditions influencing *Aspergillus* species and aflatoxin development associated with cassava chips (Gnonlonfin et al., 2008b). The size of the chips and the drying times are important factors that influence the growth of fungi, and have been related to the quality of the chips. Wareing et al. (2001), in their study on dried cassava product in Ghana, reported that fungal growth and mycotoxin development were positively correlated with drying time. A positive correlation was also observed between fungal growth, mycotoxin production, and growing season (Wareing et al. 2001).

Few studies have focused on aflatoxin contamination in beans. Houssou et al. (2009) have shown no aflatoxin contamination of cowpea samples from the four main agroecological zones in Benin, even though *A. flavus* was frequently isolated (Table 1). The authors concluded that cowpea is less susceptible to mycotoxin contamination, probably due to the presence of phytoalexins and glyceollin that inhibit mycotoxin biosynthesis as observed by Song and Karr (1993).

Animals fed with contaminated feeds by aflatoxin B₁ can lead to the occurrence of aflatoxin M₁ in milk and milk products. Lanyasunya et al. (2005) reported the risk of aflatoxin contamination of dairy feed and milk on smallholder dairy farms in Kenya. Assessment of humic acid, oxihumate as an aflatoxin binder in broiler chickens has shown that oxihumate was effective in diminishing the adverse effects caused by aflatoxin on body weight of broilers. Oxihumate also showed protective effects against liver damage, stomach and heart enlargement, as well as some of the hematological and serum biochemical changes associated with aflatoxin toxicity (Van Rensburg et al., 2006). The same authors concluded that oxihumate could

alleviate some of the toxic effects of aflatoxin in growing broilers. Oxihumate might, therefore, prove to be beneficial in the management of aflatoxin-contaminated feedstuffs for poultry when used in combination with other mycotoxin management practices (Van Rensburg et al., 2006).

Very few reports from Africa exist on dairy feed and milk, but since most dairy systems are extensive with little cereal-based feeding, risk of exposure through these products can be judged to be low.

FACTORS INFLUENCING THE GROWTH OF TOXIGENIC FUNGI AND TOXINS DEVELOPMENT UNDER AFRICAN CONDITIONS

Infection of stored products by toxigenic fungi and subsequent contamination by mycotoxins are generally influenced by many factors including fungal populations, environmental conditions (general climate, temperature, and humidity, O₂, CO₂), insect infestation and pre- and postharvest handling, but in most cases there are complex interactions among the different factors. In Africa work on aflatoxin has focused on applied research with very little basic research on fungal populations and even less on the effect of environmental conditions on African *Aspergillus* strains.

Fungal Population Structure and Resultant Aflatoxin Risk

Aspergillus flavus populations are genetically diverse and phenotypic variations have been well documented. Isolates vary considerably in their abilities to produce aflatoxins and colonize plants. They generally can be grouped into two sclerotial morphotypes, L strains and S strains also named *A. flavus* var. *parvisclerotigenus* (Saito and Tsuruta, 1993). L strain isolates produce abundant conidiospores and sclerotia that are usually larger than 400 μ m in diameter, whereas S strain isolates produce fewer conidiospores and numerous sclerotia that are usually smaller. The S strain isolates typically produce higher amounts of aflatoxins than the L strain isolates on the same media. The aflatoxigenic trait of the S strain isolates seems very stable. In contrast, a significant portion of *A. flavus* L strain field isolates do not produce aflatoxins (Chang et al., 2006). Strain S_{BG} is morphologically similar to the S strain of *A. flavus*, but DNAbased phylogenies reveal strain S_{BG} to be a distinct species ancestral to both *A. flavus* and *A. parasiticus* (Ehrlich et al., 2005). The S strain of *A. flavus* is ecologically and physiologically different from other aflatoxin producers (Cotty and Mellon, 2006) and responds to crop rotations and seasons differently than the L strain isolates (Bock et al., 2004; Jaime-Garcia and Cotty, 2004). Analysis of 55 soils samples showed isolation of 85% of *A. flavus* L strain followed by S_{BG} strain (8%) (Donner et al., 2009).

Analysis of maize samples have shown variability in toxigenic and atoxigenic *A. flavus* strains with L strains being most predominant (>90%), while S_{BG} strain was scarce (<3%)

Table 1 Principal food commodity and aflatoxin level of contamination

Food commodity	Country	No samples	Mean level ($\mu\text{g/kg}$)	Range ($\mu\text{g/kg}$)	References
Groundnut cake	Nigeria	–	–	20–455	Akano and Atanda, 1990
Dry roasted groundnut	Nigeria	106	52.4	–	Bankole et al., 2005
Peanut	Botswana	–	–	12–329	Mphande et al., 2004
	Kenya	–	–	0–7525	Mutege et al., 2009
Maize	Nigeria	–	125.6	–	Udoh et al., 2000
	Benin	300	–	2–2500	Hell et al., 2000a
	Zambia	–	–	0.7–108.74	Kankolongo et al., 2009
	Ghana	–	–	20–355	Kpodo et al., 1996
	Kenya	350	–	1–46,400	Lewis et al., 2005; Mwhihia et al., 2008
Fermented maize dough	Tanzania and Republic of Congo	–	–	0.04–120	Manjula et al., 2009
Edible tubers “tiger nuts”	Ghana	–	–	0.7–313	Kpodo et al., 1996
	Nigeria	25	454	–	Adebajo, 1993
Sorghum	Nigeria	–	–	10–120	Bankole and Eseigbe, 1996
Dried yam	Nigeria	–	–	10–80	Salifu, 1981
	Nigeria	–	27.1	–	Bankole and Mabekeje, 2004
Dried cassava	Benin	–	14	2.2–220	Mestres et al., 2004
	Tanzania	–	nd	nd	Muzamila et al., 2000
	Ghana	–	nd	nd	Wareing et al., 2001
	Benin	200	nd	nd	Gnonlonfin et al., 2008a
Cassava products	Tanzania and Republic of Congo	–	–	0.1–13.0	Manjula et al., 2009
Cowpea	Tanzania and Republic of Congo	–	–	0.3–4.4	Manjula et al., 2009
	Benin	–	nd	nd	Houssou et al., 2009
Dried okra	Tanzania	–	–	487.4–1888.7	Seenappa et al., 1983
Dried hot pepper	Benin, Mali and Togo	30	6.0	–	Hell et al., 2009
	Benin, Mali and Togo	30	3.2	–	Hell et al., 2009

nd = not detectable.

aflatoxigenic *A. flavus* strains were more prevalent in the north than in south of Nigeria (Atehnkeng et al., 2008a). Furthermore, different maize samples collected from several districts in Kenya have shown diversity in *A. flavus* population. Indeed, 76% of the *A. flavus* isolates belonged to the S strain and 22% to the L strain (Probst et al., 2010). Incidence of the S strain in soil (61%) was significantly less than in maize (91%) while incidences of L strain were greater in soil than in the commodity (Probst et al., 2010). The S strain was repeatedly isolated from 104 maize samples, consistently produced large amount of aflatoxins in both liquid medium and living maize and were strongly correlated with aflatoxin content (Probst et al., 2007).

The competitiveness of the atoxigenic and toxigenic (La3228) *A. flavus* strains has been proved. Atehnkeng et al. (2008b) reported from their field trial that aflatoxin levels were significantly lower in co-inoculated maize compare with the treatment in which the aflatoxin-producing isolate (La3228) was inoculated alone. These authors concluded on the potential value of these strains as agents for the biocontrol of aflatoxin contamination in maize.

Reviews of biosynthesis of aflatoxin have been compiled by Yu et al. (2002), Yu and Keller (2005) and Bhatnagar et al. (2006), furthermore Holmes et al. (2008) have elucidated some of the compounds and mechanism that might inhibit biosynthesis. Understanding the biosynthetic pathways will help us to develop effective strategies to interrupt the mechanisms in the fungus for producing these toxins.

Fungal Interactions

Interactions among different fungi influence fungal infection and subsequent mycotoxins production. Hill et al. (1983) found that the presence of *A. niger* can inhibit aflatoxin production in groundnuts. *Streptococcus lactis* not only inhibited aflatoxins biosynthesis but also degraded pre-formed toxin (Coallier-Ascah and Idziak, 1985). *Hypopichia burtonii* and *Bacillus amyloliquefaciens*, both frequently isolated from maize seeds, stimulate aflatoxins production by *A. flavus* when grown together on maize and rice previously sterilized by irradiation (Cuero et al., 1987). Harvested maize grains in the tropics already contain mycelium and spores of several fungal species, mainly of the genera *Fusarium*, *Aspergillus*, and *Penicillium* that can come into contact, grow and compete for food if environmental conditions are favourable (Fandohan et al., 2003). Velluti et al. (2000) showed that populations of *F. verticillioides* and *F. proliferatum*, the most important fumonisins producers, are markedly reduced by the presence of *F. graminearum*, and that fumonisin B₁ production can be significantly inhibited. In contrast, Marin et al. (1998) reported that *F. verticillioides* and *F. proliferatum* are generally very competitive and dominant against *A. flavus* and *Penicillium* spp., especially at a_w greater than 0.96. This inhibition can lead to a significant reduction of aflatoxin contamination in infected grains as reported by Zummo and Scott (1992).

Environmental Factors

Aspergillus infection and subsequent aflatoxins contamination of foods in general are associated with warmer and drier climates (Shephard et al., 1996; Hell et al., 2003). Several mathematical models on the factors that influence the growth of *Aspergillus* species and their *in situ* production of aflatoxins have been published (Pitt, 1993; Viquez et al., 1994; Samapundo et al., 2007; Chauhan et al., 2008). However, it has also been reported that at the same location, aflatoxin contamination may differ considerably from one year to another (Hell et al., 2003; Fandohan et al., 2005a). Both Setamou et al. (1997) and Hell et al. (2003) in pre- and postharvest maize respectively found difference in aflatoxin contamination for maize during four consecutive growing seasons in Benin, related to environmental conditions which differed from one growing season to another and varied with management practices. In the same study, higher rates of aflatoxin accumulation per percentage *A. flavus* infection were reported as one moved from the south to the north (Setamou et al., 1997). Similarly, aflatoxin contamination of harvested maize in Uganda has been reported to be affected by agroecological factors. Maize grains obtained from farmers in mid-altitude (moist) zone had the highest aflatoxin percentage contaminated samples (83%) and mean aflatoxin of 9.7 $\mu\text{g}/\text{kg}$ followed by those from the mid-altitude (dry) (70%) with a mean of 7.7 $\mu\text{g}/\text{kg}$, while grains from the highland zone showed lowest contamination (55%) and mean aflatoxin levels of 3.9 $\mu\text{g}/\text{kg}$ (Kaaya et al., 2006). Hell et al. (2000a) identified postharvest practices that were correlated with aflatoxin contamination in Benin, similarly Kaaya et al. (2006) identified positively correlated between leaving maize to dry in the field for more than three weeks, drying maize without husks, drying maize on bare ground, shelling maize by beating, heaping maize on the floor during storage and use of baskets for storage and high toxin levels. Other factors related to increased toxin contamination were intercropping and delayed harvesting (Hell et al., 2003; Kaaya et al., 2006; Atukwase et al., 2009). Growing crops consecutively in the same field year after year increases the risk of toxin contamination (Hennigen et al. 2000). The listed practices are common in Africa, where farmers have few notions on good agricultural practices including crop rotation.

The influences of temperature on the growth of *A. flavus*, *A. parasiticus* and the production of aflatoxins have been investigated in a number of different commodities and on artificial media. Diener and Davis (1967) found that the optimum temperature for aflatoxin production by *A. flavus* was 25°C on groundnuts, while *A. parasiticus* thrived at 25–30°C. The authors also observed a change in the proportions of aflatoxins B₁ and G₁ produced by *A. parasiticus*, with a reduction in aflatoxin G₁ as temperatures increased. Using laboratory media and mathematical modelling Molina and Giannuzzi (2002) found that optimum temperatures for aflatoxin production by *A. parasiticus* were 27.84°C and 27.32°C at pH 5.9 and 5.5, respectively. The optimum temperature for aflatoxin production by *A. bombycis* and *A. nomius* was 25°C (Peterson et al., 2001). Magan et al.

(2003) reported on the growth, mycotoxin production, competitiveness and niche occupation by mycotoxigenic species that are influenced by environmental factors including temperature. Indeed, *F. graminearum* is more competitive than *F. culmorum*, regardless of temperature or water availability. *F. culmorum* is, however, dominant against other grain fungi including *Microdochium nivale* (Magan et al., 2003). In a study carried out in Benin, Setamou et al. (1997) showed higher level of aflatoxins (262.9 µg/kg) in maize samples collected from Southern Guinea Savannah compared to samples from Northern Guinea Savannah (80.6 µg/kg), Sudan Savannah (27.5 µg/kg) and Coastal Savannah (23.3 µg/kg). The Southern Guinea Savannah lies between latitude 7°N and 8°N, and has average relative humidity of 80–85% during the rainy period of the year with maximum temperature of 28–32°C while the Northern Guinea Savannah lies between latitude 8°N and 11°N and has a drier climate with relative humidity only going above 70% during a short period running from July to September and high maximum temperature 28–35°C. However, the Sudan Savannah (latitude 11–12°N) is very dry with low average relative humidity less than 60% and high maximum temperature of 30–42°C. The Coastal Savannah (latitude 6°30'–7° North) has the highest average relative humidity of than 90% most of the year and maximum temperature ranging from 25°C to 35°C. In the same study, higher rate of aflatoxin accumulation per percentage *A. flavus* infection were reported as one moved from the south to the north of Benin, similar relationships have been found in stored maize by Hell et al. (2000a). No detailed studies to relate temperature to toxin development of individual strains from different ecozones has been completed in Africa.

Gqaleni et al. (1997) reported the optimum water activity (a_w) for growth of *A. flavus* as 0.996, with the minimum supporting growth at 0.80–0.82. At higher water activities (0.98–0.99), aflatoxins are produced in greater quantity but toxin production apparently ceases at or near a_w 0.85 (Northolt et al., 1977). Mora and Lacey (1997) reported that high moisture grains (>18%) contained more than 70% of the grains infected with *A. flavus*, with a positive correlation between the rate of infection and aflatoxin development. Moisture content of commodities is directly correlated with resultant toxin contamination. In Africa this relationship has been documented in many commodities like maize (Hell et al., 2000b), cowpea (Houssou et al., 2009) and groundnut (Kaaya et al. 2006). For detailed water activity studies some basic laboratory equipment is necessary that is nearly inexistent in Africa, but to have in-depth knowledge of infection, conditions for infection and toxin development these studies are necessary.

In conclusion, locations with both dry and hot climates have a much higher probability of aflatoxin risk compared with locations having either dry or hot conditions alone. Chauhan et al., (2008) as supported by the earlier published studies of Setamou et al. (1997) and Hell et al., (2003) from Benin showed that the relations are complex between climate and toxin development. Climate influences contamination, in part, by direct effects on the causative fungi. As climate shifts, so do the complex com-

munities of aflatoxin-producing fungi. This includes changes in the quantity of aflatoxin-producers in the environment and alterations to fungal community structure. Fluctuations in climate also influence predisposition of hosts to contamination by altering crop development and by affecting insects that create wounds on which aflatoxin-producers proliferate (Cotty and Jaime-Garcia, 2007).

Favourable temperature and water activity are crucial for mycotoxigenic fungi and mycotoxin production. In general, if the temperature increases in cool or temperate climates, the relevant countries may become more liable to aflatoxins, like already experienced in Italy during recent years (FAO, 2000). In particular, tropical countries may become too inhospitable for conventional fungal growth and mycotoxin production. Countries which can afford to control the environment in storage facilities may be able to avoid postharvest problems including aflatoxin contamination in feed and food but at high additional cost. It also appears to be lack of awareness about the link between food safety and climate change especially in Africa.

Production of aflatoxin by *A. flavus* cultures grown on a medium of groundnuts in oxygen-depleted atmospheres is lower than under normal conditions (Diener and Davis, 1967; Dobson and Sweeney, 1998). Heathcote and Hibbert (1978) reported that a maximum yield of 212 mg of aflatoxin per litre of culture fluid was produced at an aeration rate of 9 L/min, whereas a considerable reduction in aflatoxin occurred at lower aeration rates. This indicates that aflatoxin production is an aerobic process. The effects of various levels of the normal atmospheric gases, carbon dioxide, oxygen, and nitrogen on aflatoxin production under conditions of varying temperature and humidity were investigated by Diener and Davis (1967). It was found that aflatoxin production in sound, mature, groundnut kernels decreased with increasing concentrations of carbon dioxide from 0.03% to 100%. Reducing the oxygen concentration generally decreased aflatoxin production (Diener and Davis, 1967). A significant decrease in mycotoxin production resulted when the oxygen was reduced from 5% to 1% regardless of the carbon dioxide concentration, so that storage under reduced oxygen or in a modified atmosphere might be one of the options to reduce aspergillus development and aflatoxin biosynthesis (Magan and Aldred, 2007). In West Africa a project is being implemented distributing hermetic storage to farmers (Baributsa et al. 2010).

AFLATOXIN MANAGEMENT STRATEGIES APPLICABLE IN AFRICA

As seen in the previous sections exposure to aflatoxin risks in humans in Africa varies by commodity and with factors influencing these commodities. Many developing countries have realized that reducing mycotoxins concentration in foods will not only reduce financial burden on health care but also confer international trade advantages such as exports to the attractive more remunerative markets. Concerning the latter the maximum limits set by the EU and other countries really are bottlenecks

as shown in a report from the European Food Safety Authority (EFSA, 2007).

Factors fundamental to a country's ability to protect its population from mycotoxins, include the political will to address mycotoxins exposure and support capacity for testing commodities, which determines whether requirements can be enforced (Wagacha and Muthomi, 2008). Therefore, it is very important for African countries to take into consideration the prevention of exposure to aflatoxins, possible decontamination and surveillance and monitoring of moulds in contaminated food and feedstuff for effective mycotoxins management (Kabak et al., 2006).

Agricultural Practices

To ensure that foods have the lowest aflatoxins concentration possible, prevention of exposure is required. Good practices during production, harvesting, storage, transportation, marketing, processing and regulation need to be observed. Cultural practices during production including crop rotation, tillage, choice of planting date, and management of irrigation and fertilization can limit infection and subsequent mycotoxins accumulation (Munkvold, 2003; Champeil et al., 2004).

Biological strategies have been developed, such as atoxigenic fungi, which out-compete their closely related strains/species, thus reducing the levels of mycotoxins in the crops. Less toxigenic strain of *A. flavus* that displaces toxigenic strains in the soil through competitive exclusion have been isolated from Nigerian soils (Atehnkeng et al., 2008b) and given approval for test-releases. Such atoxigenic strains of *A. flavus* and *A. parasiticus* upon introduction to soil of developing crops have resulted in aflatoxins contamination in peanuts in the United States ranging from 74.3% to 99.9% of the original seen contamination (Dorner et al., 1998). Postharvest (storage) aflatoxin contamination has even been reduced by 95.9% through field application of nontoxigenic strains of *A. flavus* and *A. parasiticus* (Dorner and Cole, 2002).

Chemical control is another tool. Appropriate use of pesticides during the production process could help in reducing the fungal infection or insect infestation and subsequent mycotoxins contamination. Fungicides such as itraconazole and amphotericin B have been shown to effectively control the aflatoxins producing *Aspergillus* species (Ni and Streett, 2005). Use of fungicides is often not an option in Africa due to economic reasons and growing concerns about environmental and food safety (Ni and Streett, 2005). Lowering of the overall contamination level of a production batch may be achieved by mixing with a non- or less-contaminated batch. This approach is forbidden through legislation in a number of countries such as in the EU (De Koe, 1999). Theoretically, also decontamination, e.g., by treatments with ammonia is a possibility for aflatoxins. Bagley (1979) reports that corn containing aflatoxin can be decontaminated by treatment with gaseous ammonia at atmospheric pressure. Toxicity feeding trials with ducklings, broiler chicks, and trout confirmed that the process inactivates aflatoxin. The process was shown to reduce aflatoxin levels from

1,000 parts per billion (ppb) to within the FDA action level of 20 ppb (Bagley, 1979). Ammoniation is not useful for reducing aflatoxin contamination in food products since they would be unpalatable and in the EU decontamination by chemical means is not allowed for food products (De Koe, 1999). Reasons for not allowing batch mixing or chemical treatments as means of lowering the content of a mycotoxin in foods are multiple. First it may render traceability more difficult in case of problems, secondly a nonhomogenous mixing may still mean that some consumers would face unacceptable high exposure levels, thirdly relying on chemical detoxification may result in unsafe products and unknown compounds may be formed which are either toxic or compromise the approved systems of analysis for control.

Breeding for resistance is one of the most promising long-term strategies for mycotoxin management in Africa. Potential biochemical and genetic resistance markers have been identified in food commodities, especially maize which are being utilized as selectable markers in breeding for resistance of aflatoxin contamination (Wagacha and Muthomi, 2008). Gene clusters housing genes that govern the formation of aflatoxins have been elucidated and are being targeted in strategies to interrupt the biosynthesis of these mycotoxins (Cleveland et al., 2003). To come up with effective strategies to control fungal infection and minimize mycotoxins production in host plants, a better understanding of genetic variability and population structure at the intraspecific level and ability to detect cryptic populations or lineages which might arise that possess significant features in terms of toxins profile or host preferences are necessary (Mulé et al., 2005).

There are also simple management strategies that can significantly reduce toxin levels of crops. Early harvesting reduces fungal infection of crops in the field before harvest. Even though most farmers in Africa are well aware of the need for early harvesting, weather changes, labour constraint, need for cash, threat of thieves, rodents and animals compel farmers to harvest at inappropriate time (Amyot, 1983). For instance, early harvesting and threshing of groundnuts has proven to lower aflatoxins levels and increase the gross returns with 27% as compared to delayed harvesting (Rachaputi et al., 2002).

Management of Insect Infestation

Insect management is an approach in itself, as insect damage influences the extent of mycotoxin contamination. Insects carry spores of mycotoxigenic fungi from plant to the interior of the stalk or kernels and may create infection wounds through their feeding habits (Munkvold, 2003). In consequence proper management of insect pests through appropriate control strategy will reduce mycotoxin contamination. Insects' damage of maize is a good predictor of mycotoxin contamination, and can serve as an early warning. Insects carry the spores from plant surfaces to the interior of the stalk or kernels or create wounds due to the feeding of the larvae on stalks or kernels (Munkvold and Hellminch, 2000). Insect-damaged kernels

provide an opportunity for the fungus to circumvent the natural protection of the integument and establish infection sites in vulnerable interior (St. Leger et al., 2000). Wounding by insects may provide infection courts and allow kernels to dry down to moisture content more favourable for growth of *A. flavus* and aflatoxin production (Wagacha and Muthomi, 2008). Several studies on maize have shown that there is a positive correlation between insect damage and aflatoxin contamination (Bowen and Mack, 1991; Lynch et al., 1991; Hell et al., 2000b). *Aspergillus* spores have also been isolated from the bodies of corn earworm, *Heliothis zea* Boddie (Lepidoptera: Noctuidae), the European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) and the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (McMillian et al., 1990). Lynch and Wilson (1991) reported that insects could act as vectors by transporting fungal spores on their bodies, and contaminating grain as they moved about. The Nitidulidae (*Carpophilus lugubris* Murrey and *C. freemari* Dobson) are important vectors of *A. flavus* on maize (Lussenhop and Wicklow, 1991). These insects consume *A. flavus* spores, without detrimental effect to themselves (Wicklow, 1988).

Feeding by insects breaks the pericarp, rendering grain more vulnerable to invasion by storage fungi (Barry et al., 1992). The metabolic activity of insects can result in increased relative humidity, and thus providing favourable conditions for the growth of *A. flavus* (Mills, 1983). Moisture content of maize was found to increase from 15% to 20% after 30 days when infested with *Sitophilus zeamais*. In addition, significantly more aflatoxin B₁ was found in *S. zeamais* infected maize than in mechanically damaged and control maize that had been inoculated with *A. flavus* (Beti et al., 1995). A survey conducted in Kenya investigated microorganisms present on stem- and cob-borers showed that 7% of the larvae were infected with *Aspergillus* species (Odindo et al., 1989). Studies carried out in Benin have also showed that the cob-borer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) was significantly correlated to *A. flavus* infection and aflatoxin contamination in the field (Setamou et al., 1998; Hell et al., 2000b).

Pre- and Postharvest Operations

Postharvest handling and processing (sorting, washing, dehulling, milling, fermentation, cooking) can increase or reduce fungal infection and mycotoxin production as observed by Fandohan et al. (2005b). Furthermore, rapid drying of agricultural products in order to lower the moisture level is very critical as it creates unfavourable conditions for fungal growth and proliferation, reduces insect infestation and contributes to safe storage over longer periods (Lanyasunya et al., 2005). Studies have demonstrated that drying harvested maize to a moisture content of 15.5% or lower within 24–48 h reduces the risk of fungal growth and subsequent aflatoxin biosynthesis (Hamiton, 2000). When groundnuts were dried to a moisture content of 6.6%, they were free of fungi for 6 months regardless of storage system, whereas at 12% moisture, only jute bags with the plant

Syzigium aromaticum effectively suppressed infection with *A. flavus*. However, when moisture content increased to 18.5%, this method was not effective (Awuah and Ellis, 2002). Turner et al. (2005), in their intervention trial in Guinea, West Africa focused on thorough drying and proper storage of groundnuts in subsistence farm villages and achieved a 60% reduction in the mean of the aflatoxins levels observed in intervention villages. During storage, transportation and marketing, low moisture content should be maintained by avoiding leaking roofs and condensation arising from inadequate ventilation (Wagacha and Muthomi, 2008).

Proper sorting of maize before storage, storage of maize in shelled form, storage of maize in bags, use of improved granary as storage structures, storage of maize above fireplace and use of synthetic pesticides have been reported to reduce aflatoxin development in maize (Kaaya et al., 2006) similarly to results by Hell et al. (2003). Furthermore, sanitation measures such as removal and destruction of debris from previous harvest have been used successfully in the management of mycotoxins. Cleaning stores before loading new produce was correlated with reduced aflatoxins concentration (Hell et al., 2000a).

In addition, processing can be employed for lowering the mycotoxins contamination. A study conducted in Benin by Fandohan et al. (2005b) to determine the fate of aflatoxins and fumonisins through traditional processing of naturally contaminated maize and maize by-products, demonstrated that sorting, winnowing, washing, crushing combined with de-hulling of maize grains were relatively effective in achieving a significant mycotoxin removal. Similarly determining aflatoxins in peanuts showed that a significant proportion (80%) of the toxin is often associated with the small and shrivelled seeds (Davidson et al., 1982) and mouldy and stained peanuts, which can be removed by sorting (Turner et al., 2005).

Substantial amounts of aflatoxins can be removed from grains by immersing them in water and removing the upper floating fraction, as contaminated grains generally have a lower density (Fandohan et al., 2005b). Shetty and Bhat (1999) found that removal of the toxins increases to 86% if salt is added to the water during that process. Likewise, sorting and removal of small, broken and visibly contaminated grains during processing can significantly reduce toxins levels (Charmley and Prelusky, 1995; Doyle, 1998; Fandohan et al., 2005a). In contrast, fermentation of maize does not seem to reduce significantly aflatoxins levels (Kpodo et al., 1996; Shephard et al., 1996; Fandohan et al., 2005a). There are also industrial sorters that can make this process more efficient.

Brera et al. (2006) investigated the effect of industrial processing on the distribution of aflatoxin and zearalenone in corn-milling fractions from conventional and organic corn. For both lots, in the germ, bran and animal feed flour a reduction of both mycotoxins by at least four times from raw material to end products was observed. Dehulling decreased aflatoxin levels by 46.6% (5.5–70%) in maize samples containing 10.7–270 ng/g aflatoxin levels. Soaking muthokoi (a traditional dehulled maize dish) in 0.2%, 0.5% and 1.0% solutions of sodium hypochlorite

or ammonium persulphate for 6 h or 14 h further decreased aflatoxin contents by 28–72% in maize samples containing 107–363 ng/g aflatoxin levels, and boiling muthokoi at 98°C for 150 min in 0.2–1.0% w/v iati decreased aflatoxin contents by 80–93% in samples having 101 ng/g aflatoxin contamination (Mutungi et al., 2008).

When dried to a moisture content of less than 10% proper storage is necessary to prevent reuptake of moisture in cereals, also the elimination of insect activity is necessary since they can convey infection and increase moisture content through respiration. Low temperatures and inert atmosphere can be used to improve storage, but this is not always technical possible and economical (Lanyasunya et al., 2005; Turner et al., 2005).

Many of the above strategies could be applicable to Africa as these are simple and do not imply additional cost. However, training and awareness campaign are needed to inform farmers, traders and processors about the risk of toxin contamination and such campaigns have been successfully implemented in West Africa (James et al., 2007).

Food-based Strategies

Dietary strategies can prevent ingestion or absorption of mycotoxins in prepared foods and feed. To contain the toxic effects of mycotoxins antioxidant compounds (selenium, vitamins, provitamins), food components (phenolic compounds, coumarin, chlorophyll and its derivatives, fructose, aspartame), medicinal herbs and plant extracts, and mineral and biological binding agents (hydrated sodium calcium aluminosilicate, bentonites, zeolites, activated carbons, bacteria, and yeast) can be used (Galvanao et al., 2001). Chemoprevention uses natural or synthetic agents to block, retard, reverse or modulate the carcinogenic process. A number of chemical compounds such as oltipraz and chlorophyllin or dietary intervention like broccoli sprouts and green tea have been tested and found effective in increasing detoxification processes (Kensler et al., 2004) or prevent the production of epoxide that leads to chromosomal damage (Hayes et al., 1998). Enterosorption based on the use of certain clay minerals, such as novasil, which can selectively adsorb mycotoxins tightly enough to prevent their absorption from the gastrointestinal tract has also been evaluated (Wang et al., 2005) and found to be especially useful in binding mycotoxin from contaminated feeds they are presently widely used in animal husbandry especially poultry production (Kubena et al., 1990; Avantaggiato et al., 2005). Clay compounds have also been tested and in a trial in Ghana ingestion of capsules containing a clay compound a significant reduction of the biomarker of aflatoxin exposure was observed (Phillips et al., 2007).

Application of HACCP Systems

It is a structured, systematic approach throughout the commodity system and can be applied from the field to the consumer's plate. To minimize the exposure of people worldwide to mycotoxins—such as aflatoxins—it of paramount importance

that all involved players are able to systematize the analysis and handling of mycotoxin prone crops from the field to the plate. In this respect it is important to note that a number of tools for such a systematisation have been developed. “Hazard Analysis and Critical Control Points” (HACCP) a systematic preventive approach to food safety and pharmaceutical safety addresses physical, chemical, and biological hazards as a means of prevention rather than finished product inspection is to be mentioned (FAO 2001/2003). The basic procedures in Subsaharan Africa would be (a) protection from water, (b) a careful removal of contaminated seeds/grains with fungus, (c) use of good quality seeds/grains to produce powder, etc. More details of the principals of this approach are described in “Manual on the Application of the HACCP System in Mycotoxin Prevention and Control” which includes two aflatoxin cases entitled (1) Yellow maize kernels – South East Asia and (2) Maize-based Animal Feed – South East Asia (FAO 2001/2003). Ainoa-Awua et al. (2007) used Good Management Practices and HACCP to effectively manage the quality and safety of traditional processed kenkey from maize in Ghana.

AFLATOXIN MONITORING AND LEGISLATION

Many countries have legislation with regard to aflatoxins in foods; especially food intended for export. About 100 countries out of which 15 are African, have established regulation to protect consumers from the harmful effects of mycotoxins (Van Egmond, 2002; Barug et al., 2003; Fellingner, 2006). Human foods are allowed to contain 4–30 µg/kg aflatoxin, depending on the country involved (Henry et al., 1999; FDA, 2004). In the United States, the Food and Drugs Administration (FDA) uses an action level of 20 µg/kg as the maximum residue limit of aflatoxins allowed in food for human consumption, except for milk (FAO, 1996; Wu, 2006). The European Union in 1998 enacted aflatoxins tolerance standards of 2 µg/kg aflatoxin B₁ and 4 µg/kg total aflatoxins for human consumption, the strictest in standard worldwide (CEC, 1998; EC, 2006; Wu, 2006). The Codex Alimentarius commission proposed 15 µg/kg of total aflatoxins in food (FAO, 1997).

Specific regulations have been established for different categories of food. The European Union has established the total aflatoxins standard in groundnut subject to further processing at 15 µg/kg (8 µg/kg for aflatoxin B₁), in other nuts and dried fruit subject to further processing at 10 µg/kg (5 µg/kg for aflatoxin B₁) (EC, 2006). The Australian standard for total aflatoxins in groundnut was set at 15 µg/kg (FAO, 1997), while the United States adopted 20 µg/kg as the maximum level of total aflatoxins in various agricultural and food products (FDA, 2004).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) performed in 1997 a quantitative risk assessment by estimation of the population risks from intake of aflatoxins, comparing two hypothetical standards (10 µg/kg and 20 µg/kg). Acknowledging, several limitations and assumptions inherent in this approach, the JECFA concluded in case of a low prevalence

of hepatitis B, that reducing the hypothetical standard from 20 to 10 $\mu\text{g}/\text{kg}$ yields a drop in the estimated population risk of approximately 2 cancers/year per 109 people (and in case of a high prevalence of hepatitis B: 300 cancers/year per 109 people). The Scientific Committee for Food of the EU (SCF) considered this assessment in September 1997 and concluded that it was not possible to assess the degree of uncertainty, arising from these limitations and assumptions, in the quantitative risk assessment and felt therefore that it was premature or SCF to draw definitive conclusions on this issue. The toxicology of the aflatoxins was not questioned by the JECFA, which concluded, "aflatoxins are amongst the most potent mutagenic and carcinogenic substances known" and therefore the SCF concluded that its opinion of 1994 remained valid (EFSA, 2007). Overall, the international standard suggests that products which contain concentration of aflatoxin B₁ as high as 10 $\mu\text{g}/\text{kg}$ would be acceptable for all types of food products, if the total level of aflatoxins does not exceed 15 $\mu\text{g}/\text{kg}$ (Saqib, 2000).

The commission of the European Communities established a limit for aflatoxin M₁ of 50 $\mu\text{g}/\text{kg}$ for milk and a variable limit for cheese, depending on concentration caused by drying process or processing. Furthermore, the Commission stated that even if aflatoxin M₁ is regarded as a less dangerous genotoxic carcinogenic substance than aflatoxin B₁, it is necessary to prevent the presence in milk, and consequently in milk products, intended for human consumption and for young children in particular. The Commission has also set a limit for aflatoxin B₁ of 5 $\mu\text{g}/\text{kg}$ for supplementary feedstuffs for lactating dairy cattle. However, this tolerance level is difficult to observe because the average daily individual intake in a herd should be limited to 40 μg aflatoxin per cow, in order to produce milk with less than 50 μg aflatoxin M₁ per kg (CEC, 1998, 2001, 1991; Veldman et al., 1992; Pietri et al., 2003; EFSA, 2004).

Overall, food safety requirements differ from country to country and can constitute an important barrier for international trade in food products, as their main objective is to protect public health (Broberg, 2009).

AFLATOXIN ANALYSIS

Sampling Procedures

The need to obtain a representative sample deserves particular consideration since a wrong sampling plan can greatly affect the reliability of the measured levels of mycotoxins (Whitaker, 2003). The author gave an example how increasing the sample size can significantly reduce the sampling error. Sampling is one of the most important steps that contribute to the variability of analyses due to the nonhomogeneous nature of aflatoxins distribution in foods and feed (FAO, 2004; Miraglia et al., 2005). The European Union (EC) has put regulation in place that sets the standard sample for analysis at 3×10 kg, which requires that three tests are conducted on a randomly drawn lot of 30 kg. Each sample has to pass the three tests before the shipment can

be allowed to enter the market. In the case of bulk raw nuts the implementation of this procedure is difficult, because aflatoxins are not evenly distributed throughout an entire batch (EC, 2002b). In the United States, FDA requires 3×22 kg laboratory samples to average less than 15 μg total aflatoxins/kg for acceptance (FDA, 2002).

Analytical Methods

In developed countries control of human and animal exposure to mycotoxins is mainly through surveillance and monitoring. These control mechanisms depend on validated analytical methods for the detection and quantification of mycotoxins (Trucksess and Pohland, 2002; Arranz et al., 2004). Many chemical procedures have been developed to identify and measure aflatoxins in various commodities. The basic steps include extraction, lipid removal, cleanup, separation and quantification. Depending on the nature of the commodity, methods can sometimes be simplified by omitting steps. To minimize quantitation errors, two different methods can be used to quantify the same mycotoxin, or collaborative testing methods can be employed, which requires considerable planning in terms of design of the trial, the type of matrix or matrices to be analysed, the level of contamination of the mycotoxin of interest and the numbers of samples (Gilbert, 1988; Trucksess and Pohland, 2002).

For aflatoxins analysis in food commodities, methods used include enzyme-linked immunosorbent assay (ELISA), liquid chromatographic (LC), immunoaffinity column (aflatest), multifunctional column, gas chromatography (GC), thin layer chromatography (TLC), and high performance liquid chromatography (HPLC). The principle behind each method differs as well as the limit of detection (LOD) (Shephard, 2008).

ELISA has been used to determine aflatoxins. Toxin extracts are mixed with enzyme-conjugates and the mixture is placed in wells of antibody-coated microtiter plate. Contaminating sample and enzyme-conjugated compete for binding sites on the antibody. Excess unbound toxin and enzyme-conjugated are washed away. The enzyme substrate is then added to each well and a reaction catalyzed by bound enzyme results in a coloured product that can be then measured. Intensity of colour depends on amount of enzyme-conjugated toxin bound to antibodies present. Colour change can be evaluated visually or by measuring absorbance (OD) (AOAC, 1995; Yu and Chu, 1999; Christensen et al., 2000; Lawrence et al., 2000; Trucksess and Pohland, 2002, Shephard, 2008).

TLC has been used to determine mycotoxins for a long time. The toxins are extracted from the samples and spotted on silica TLC plates and the toxins separated by running the plate in a range of organic solvent mixtures as mobile phase (Trucksess, 2000). The separated toxins can then be visualised by observing under long wavelength UV light (365 nm) or by spraying with specific chemicals to make the toxins spots visible. The toxins can then be quantified by visual or densitometric comparison with standards (AOAC, 2000; Shephard, 2008). TLC with visual

or densitometric estimation has been applied to aflatoxins with reasonable success (AOAC, 1995; AOAC, 2000; Trucksess and Pohland, 2002).

LC is a recent and advance method used worldwide for mycotoxins analyses with reference to aflatoxin (Kim et al., 2000; Goda et al., 2001). The extract can be derivatized with trifluoroacetic acid (TFA) and analyzed by reverse-phase HPLC (Jaimes et al. 2000). The toxins can be detected using either UV or fluorescence detectors or both.

Immunoaffinity columns method is often used nowadays for mycotoxins quantitation. Immunoaffinity columns are available for aflatoxin determination. Samples are extracted with appropriate solvents, filtered, diluted with water before applying to affinity column containing monoclonal antibody specific for the toxin. Aflatoxins are extracted, purified and concentrated on affinity columns. The toxins are then washed off from the columns with appropriate solvents and quantified by TLC or HPLC (AOAC, 1995; Trucksess and Pohland, 2002). Multifunctional columns containing reversed-phase, ion exclusion and ion exchange absorbent have also been developed (Shephard, 2008). For example, aflatoxins can be eluted from the column, derivatized with TFA and quantified by liquid chromatography with fluorescence detection (AOAC, 1995; Trucksess and Pohland, 2002; Shephard, 2008) or by gas chromatography using detectors such as flame ionization (FID), electron capture (ECD), and mass spectrometry (MS and MS/MS) (Shephard, 2008).

HPLC has been extensively used in mycotoxins analysis (Arranz et al., 2004). The polar nature of mycotoxins and their solubility in water and organic solvents such as methanol and acetonitrile suggests that they are amenable to separation on reverse-phase HPLC columns and this has resulted in a diverse array of methods. HPLC is suited for mycotoxin separation and as can be gauged from the compilation of a database of retention times, retention indices, UV absorption maxima and predominant mono-isotopic ions is widely used (Shephard, 2008). HPLC detection has mostly been achieved with UV and fluorescence detectors, although recent successful application of atmospheric pressure ionization techniques has resulted in the development of a range of LC-MS or LC-MS/MS methods capable of very low detection limit (Shephard, 2008). Aflatoxins mixtures can be separated on normal phase silica columns using solvent mixtures consisting of chloroform, acetonitrile, cyclohexane, and ethanol (Sydenham and Shephard, 1996) or reverse-phase column with C18 packing material (Gnonlonfin et al., 2008a; Shephard, 2008). A new HPLC—PHRED (photochemical reactor for enhanced detection) method for the analysis of aflatoxins was developed and validated (Gnonlonfin et al. 2010, in press). An immunoaffinity column was used for clean-up. HPLC with post column derivatization for aflatoxin fluorescence enhancement, and fluorescence determination were used for quantitation of the aflatoxin concentration (Gnonlonfin et al., 2010, in press).

Not all the above described methods are applicable in Africa due to lack of advance equipment required for each specific method. ELISA and TLC methods are the most commonly used in Sub-Saharan African region, may be because these are less

expensive. They can give false positive results since they are less sensitive than the HPLC and HPLC—PHRED. The use of advance methods are scarce in this part of the world. Therefore, there is a need to implement new and advanced methods such as HPLC, HPLC—PHRED and mass spectroscopy for aflatoxin analysis in most countries in Africa, since they are the international accepted methods.

CONCLUSION

Aflatoxins are human carcinogens and actions should focus on the prevention or reduction of all conditions that are conducive to aflatoxin production in feed and foodstuffs in Africa. The prevention of mycotoxin contamination of human foods could have a significant effect on public health especially in low-income countries and deserves significant attention. This would be possible through surveillance and awareness campaign. Food processors or industry should take the lead in these efforts, because it will lead to improved economic sustainability of the industry, enhanced food safety, international trade efforts and improved public health. Also consumers in Africa have to become aware of the risk that mycotoxins pose to their health and in turn they would for all other stakeholders to reduce the risk of exposure. Mycotoxin reduction and control are dependent on the concerted efforts of all actors along the food production chain. Mycotoxin awareness as a public health issue, strengthening laboratory and surveillance capacities, as well as establishing early warning system and training of farmers on the good agricultural and good management practices are key actions.

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