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Aflatoxin producing fungi and its management: A Review

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ABSTRACT

Mycotoxins are a structurally dissimilar group of fungal natural products that are harmful to vertebrate animals or human when they are contaminants of feeds or food. There has been tremendous interest in what climate change scenarios have on crops, toxigenic fungi and aflatoxin contamination. A number of Aspergillus species possess the ability to produce aflatoxins though the main causative agent of contamination globally is Aspergillus flavus. Crop aflatoxin contamination is a complex process that starts in the field due to environmental and biological factors such as host susceptibility, heat and high-temperature, insect damage, and aflatoxin-producing potentials of fungal species. Currently, different types of aflatoxins are known, with aflatoxin B1, B2, G1, and G2 being the most frequent, and aflatoxin B1 is the most toxic and group 1A carcinogen. Understanding the fungi, factors those initiate them may allow development of improved management practices, better allotment of monitoring efforts, and modification of agronomic procedures in anticipation of global climate change. Use of resistant variety, recommended planting date, crop rotation, tillage, avoiding delayed harvesting, chemical, integrated management and biological control agents are the main strategies for the management of toxigenic fungi, also awareness creation for the community play a great role.

INTRODUCTION

The term mycotoxin was named in 1960 as a result of an unusual veterinary catastrophe near London, England, during which roughly 100,000 turkeys died (Blout, 1961). When this unknown turkey X disease was associated with a groundnut *(Arachis hypogea L*.) meal contaminated with secondary metabolites from *Aspergilus flavus* (Bennett and Klich, 2003, Perdoncini M, *et al*., 2019, Joubrane K, *et al*., 2020). Thus, the toxin was coined "aflatoxin" by virtue of its origin from *A. flavus*. Aflatoxins (AF) are produced via a polyketide pathway by various species of *Aspergillus* section Flavi, which includes *A. flavus, A. parasiticus, A. parvisclerotegenus, A. minisclerotigenes* and *A. nomius* (Pleadin *et al*., 2014). Both A. flavus and A. parasiticus are most frequently detected in agricultural products because of their widespread distribution (Frisvad, J, et al., 2019)

Aflatoxins are largely prevalent in major food crops such as maize (Zea mays L.), groundnuts, tree nuts, wheat (*Triticum aestivum L*), sorghum *(Sorghum bicolor L)*, spices, milk and meat products (Iqbal *et al*., 2015). Aflatoxin contamination depends on *Aspergillus* species, growing and storage conditions, management practices employed and other factors (Paterson and Lima 2010). Aflatoxins can accumulate through the food chain posing a serious health concern to humans (Sherif *et al*., 2009). Aflatoxin contamination is common in tropical countries where drought is prevalent throughout the year, in such regions, changes in weather patterns may result in acute aflatoxicoses and deaths (Lewis et al., 2005). Biosynthesis of aflatoxin depends on pH, water activity, insect infestation and temperature (Molina and Gianuzzi 2002). Climate change is anticipated to have a pronounced effect on economically important crops and mycotoxigenic fungal infection and contamination with aflatoxins (Paterson and Lima, 2010). Undeniably, climate change may have impact on the interactions between various mycotoxigenic species and the relative mycotoxin contamination of staple commodities (Magan *et al*., 2011).

In order to minimize the effect of aflatoxin on our commodity, understanding of the factors that predispose the infection of the plant with aflatoxin-producing fungi and the conditions that encourage their formation is crucial (Udomkun et al., 2017). As the presence of aflatoxin in food can be hazardous for human health and signify an enormous economic problem, one has all the reasons to allow for the implementation of new techniques providing for a safe food production. The first step in reducing aflatoxin contamination is through understanding pre- and post-harvest management techniques. Pre-harvest bio control technologies can give us the greatest opportunity to reduce AF production on the spot (Peles, F.*et al*., 2021). This can be possible through proper curing, drying, sorting, storing, physical separation, microbial degradation and different chemical treatments. Therefore, the objective of this review is to summarize on aflatoxin producing fungi and its management strategies.

1. Aflatoxins: Serious Threat to Health and Economy

Aflatoxins are toxic and carcinogenic mycotoxins produced by fungi belonging to Aspergillus section Flavi, primarily A. flavus and A. parasiticus (Amaike and Keller, 2011; Baranyi *et al*., 2013; Cotty *et al*., 1994; Mahuku *et al*., 2019). Different types of aflatoxins have been identified, with aflatoxin B_1, B_2, G_1, G_2, M_1 and M_2 being the most frequent and toxic (Akiama *et al.,* 2001). When ingested, aflatoxin B_1 (AFB_1) is hydroxylated to aflatoxin M_1 (AFM_1) and is secreted in the milk of animals whose feedstuffs have been contaminated by AFB_1 and AFB_2 (Iqbal et al., 2015; Ketney *et al.,* 2017; Serraino *et al.,* 2019). *A. flavus* produces only AFB_1 and AFB_2, whereas *A. parasiticus* produces AFB_1, AFB_2, AFG_1 and AFG_2 (Creppy, 2002). The International Agency for Research on Cancer (IARC) has classified AFB_1, AFB_2, AFG_1 and AFG_2 as Group 1 mutagens, whereas AFM_1 as Group 2B (IARC, 2015). Aflatoxins B_1 and B_2 fluoresce blue, whereas AFG_1 and AFG_2 fluoresce green. Aflatoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive and their presence in food commodities greatly impacts the food and feed industries (Sherif *et al*., 2009; Jalili M, 2015; Peles F *et al.,* 2019; Ráduly Z *et al*., 2020). In particular, co-occurrence of mycotoxins is a major concern because the possible synergetic toxicities are not well known (Palumbo, R, *et al*., 2020)

High dose exposure of AFs on humans result in vomiting, abdominal pain, and even possible death, while small quantities of chronic exposure may lead to liver cancer (Sherif *et al*. 2009). Aflatoxins play a significant role in development of edema in malnourished people as well as in the pathogenesis of kwashiorkor in malnourished children (Coulter et al., 1986). Aflatoxin contamination of human food and animal feed causes severe health and economic risks worldwide, especially in low income countries where the majority of the people consume maize and groundnuts. As a result, most countries set legislation that restricts movement of aflatoxin contaminated commodities (Juan et al., 2012). Safe limit of AFs for human consumption varies from one country to another and ranges from 4 to 30 µg/kg. European Union has set the maximum acceptable limit at 2 µg/kg for AFB_1 and 4 µg/kg for total AFs (EC, 2010) and the US 20 µg/kg (Wu, 2006).

FAO estimates that 25 per cent of the world food crops are affected by mycotoxins each year and constitute a loss at post-harvest (FAO, 1997). In the United States from 1990 to 1996, litigation costs of $34 million from aflatoxin contamination occurred. In 1998, as a consequence of aflatoxin contaminations, maize

farmers lost $40 million (AMCE, 2010). In 2004 annual loss was more than $750 million in Africa due to aflatoxin contamination of agricultural crops. Ultimately, it contributes to increased costs to consumers (AMCE, 2010). A loss due to aflatoxin contamination costs about $100 million per year including $26 million loss in peanut ($69.34/ha). In Japan, AFB_1 was detected in groundnuts imports from 20 of 31 countries, five lots of large type raw shelled and 269 lots of small type raw shelled groundnuts were rejected as having above the regulation level (10 ppb) of AFB_1.

2. Effect of environmental factors on aflatoxin producing fungi and aflatoxin contamination

Warm and humid environments and insect damage promote the growth of fungi and the production of aflatoxins. Atmosphere composition has an immense impact on mould growth, with humidity being the most important variable for their growth (Cotty and Jaime-Garcia, 2007, Valencia-Quintana R, *et al*., 2020). Aflatoxin production is determined by a wide range of substrates due to non-visible spoilage at pre-harvest in the field and post-harvest during storage or processing. Poor storage conditions could also influence mold development and aflatoxin contamination in the stored products (Abdi M, *et al*., 2021). However, high contamination and toxin production occur in poorly stored commodities, because of inadequate moisture and high temperature (Udomkun et al., 2017). Fungal growth can occur over a wider range of temperatures, pH and water activity levels on maize (Aldars-garcía L, 2018). Optimum water activity and temperatures on groundnuts were: 0.94 a_w and 34^oC for growth and 0.99 a_w and 32^oC for AFB_1 production respectively (Table 1). Similarly, in maize temperature ranged from 10 to 43^oC for fungal growth and from 13 to 37^oC for AFB_1 production. Water activity and temperature range vary for toxin production and for mould growth (ICMSF, 1996).

Relative humidity between 83%-88% has been found to be appropriate to influence the mold growth and aflatoxins production (Agriopoulou, 2020). Hotter and drier summers are predicted which may prevent certain fungi from contaminating vegetation due to the lack of humidity (West *et al*., 2012). The germination of the spores is greatly influenced by humidity and temperature, meaning that any change in climate will greatly affect these processes (Doohan *et al*., 2003; Paterson and Lima, 2010). Significant correlations have been reported between agro-ecological zones and aflatoxin levels, with wet and humid climates after longer storage periods increasing aflatoxin risk (Hell *et al*., 2000). Kaaya et al. (2006) disclosed that aflatoxin levels were higher in more humid areas compared to the drier areas in maize samples collected from Uganda and this findings concords with maize samples collected from Nigeria (Atehnkeng *et al*., 2014).

Table 1. Limits of mould growth and aflatoxin production by *A. flavus* and *A. Parasiticus* (ICMSF 1996)

Parameter	*Aspergillus flavus*	*Aspergillus parasiticus*	*Aspergillus flavus*	*Aspergillus parasiticus*	*Aspergillus flavus*	*Aspergillus parasiticus*
Growth	**Minimum**		**Optimum**		**Maximum**	
Temperature (ºC)	10-12	12	33	32	43	42
Water activity	0.8	0.80-0.83	0.98	0.99	>0.99	>0.99
pH	2	2	5-8	5-8	>11	>11
Aflatoxin Production	**Minimum**		**Optimum**		**Maximum**	
Temperature (ºC)	13	12	16-31	25	31-37	40
Water activity	0.82	0.86-0.87	0.95-0.99	0.95	>0.99	>0.99
pH	-	2	-	6	-	>8

When environmental conditions are favorable, wind and insect dispersal of conidia to plants results in colonization, infection and within susceptible hosts, production of aflatoxins (Payne, 2015). Conidia serve as source of inoculum for secondary infections and reservoirs of *A. flavus* for subsequent dispersal to susceptible hosts (Jaime-Garcia and Cotty, 2004). Maize borers on maize, pink bollworm on cotton, lesser corn stalk borer on groundnut and the navel orange worm on pistachio vector aflatoxin-producing fungi resulting in increased aflatoxin contamination (Dowd *e*t al., 2005).

3. Management of aflatoxin producing fungi and aflatoxin contamination

3.1. Cultural Practices

Cultural practices that minimize the occurrence of aflatoxin contamination in the field comprises: timely planting, maintaining optimal plant densities, proper plant nutrition, avoiding drought stress, controlling pests and proper handling during harvesting. Waliyar et al. (2008) reported reduction of *A. flavus* infection and aflatoxin contamination by 50-90% through application

of lime, farm yard manure and cereal crop residues as soil improvement. Delayed the harvested crop in the field prior to storage encourages fungal infection and insect infestation (Udoh et al., 2000). Growths of toxigenic fungi in stored commodities are influenced by moisture and temperature content. Hell et al. (2008) reported that when maize stored for three days, with a moisture content above 13%, aflatoxin contamination increase 10 fold and recommended cereal commodities should dried to a moisture levels of 10–13%. To decrease toxin contamination, technological solutions that assist in decreasing grain moisture quickly have been reviewed by Udomkun et al. (2017). Farmers in lower-income countries store agricultural products in containers usually made from wood, bamboo, mud placed and covered with thatch or metal roofing sheets (Waliyar et al., 2015). Recently, hermetic storage containers such as metal or cement bins have been established as alternatives to traditional storage methods, however, their high costs and difficulties with availability make acceptance by small-scale farms limited (Hell and Mutegi, 2011). Hell et al. (2000) investigated that although polypropylene bags are recently used for grains storage, they are still contaminated by fungal pathogens and aflatoxins especially when those reused bags contain *A. flavus* spores. Williams et al. (2014) point out that the Purdue improved crop storage (PICS) bags effectively suppressed the development of *A. flavus* and minimize aflatoxin contamination in maize in wide range of moisture conditions. Njoroge et al. (2014) recorded less moisture absorbance in grains stored in PICS bags than grains stored in woven polypropylene bags.

3.2. Physical methods

Aflatoxin levels can be minimized in stored products using physical techniques such as color sorting, density flotation, blanching and roasting. Physical sorting of broken and infected grains from the intact commodity reduce aflatoxins levels by 40-80 per cent (Fandohan et al., 2005). Hell et al. (2008) reported that reduction of aflatoxin in cleaned stores as compared to non-cleaned stores. Aflatoxins contamination in pistachio nuts is reduced by 95% through colour sorting (Shakerardekani et al., 2012). However, such physical techniques are usually arduous and ineffective. Computer-based image processing techniques for large-scale screening of fungal and toxin contaminations in food and feed are promising modern techniques. Berardo et al. (2005) quantified fungal infection and mycotoxins produced in maize grain by *Fusarium verticillioides* using Near Infrared Spectroscopy. An additional image based sorting technology has been forwarded by Ozlüoymak (2014), who revealed that approximately 98% of the aflatoxins in contaminated figs were effectively detected and separated by a UV light coupled with colour detection system.

3.3. Chemical methods

The use of chemicals to bind, inactivate or remove aflatoxins has been studied extensively using propionic acid, ammonia, copper sulfate, benzoic acid, urea and citric acid chemicals capable of reacting with aflatoxins (Gowda et al., 2004). Jalili and Jinap (2012) studied the effect of 2% sodium hydrosulphite ($Na_2S_2O_4$) on the reduction of aflatoxins in black pepper and found that a decrease in AFB_1, AFB_2, AFG_1, and AFG_2, without harm to the outer layer of black pepper. Techniques other than the use of chemical sorbents and ammonization have achieved reduction in aflatoxin bioavailability that due to hydrated sodium calcium aluminosilicate binding (Phillips et al., 1988). Since 1988 there are several publications that reveal the use of Hydrated Sodium Calcium Aluminosilicates as adsorbents for mycotoxins *in vivo* and *in vitro*. Ammonization method has been shown to efficiently destroy AFB_1 in cottonseed and cottonseed meal, groundnuts and groundnut meal, and maize (Park and Price, 2001). Although ammonia, sodium bisulfite, and calcium hydroxide treatments are efficient, they do not fulfill food safety requirements (Piva et al., 1995).

3.4. Biological methods

Among numerous research approaches, biological degradation of aflatoxins using microorganisms is one of the striking strategies for the management of these poisonous fungal toxins in food and feed (Shetty and Jespersen, 2006). Several microbes *viz*., bacteria, yeasts, actinomycetes, algae and non-toxigenic strains of *A. flavus* and *A. parasiticus* have been tested to minimize aflatoxin contamination in different crops such as maize (Dorner *et al.,* 1999) and groundnut (Vijayasamundeeswari et al., 2010; Shifa et al., 2016). Bandyopadhyay R *et a.l* (2016); Lewis M *et al.* (2019), Senghor, L.*et al* (2020) demonstrated that bio control non-afla toxigenic strains reduced AF concentrations in treated crops by more than 80% under both field and storage conditions. A toxigenic strain of *A. flavus* in the USA has been approved and marketed as Afla-Guard®. Similarly, in Nigeria atoxigenic strains of *A. flavus* has gained provisional registration (AflaSafe) and confirmed to decrease aflatoxin concentrations up to 99% both *in vitro* and *in vivo* (Atehnkeng et al., 2014). Farzaneh et al. (2012) isolated *Bacillus subtilis* strain UTBSP1 from pistachio nuts and examined for the degradation of AFB_1 and found that *B. subtilis* UTBSP1 significantly reduced AFB_1 by 95 per cent. Several studies showed that inhibition of mycelial growth and reduction of aflatoxin contamination when different crops are treated by *Bacillus subtilis, Pseudomonas solanacearum*, *P. fluorescens* and *Rhodococcus erythropolis (*Nesci et al., 2005; Reddy et al., 2009). Several non-lactic acid bacteria, such as Bacillus spp., Brachybacterium spp., Brevundimonas spp., Cellulosimicrobium spp.,

Enterobacter spp., Escherichia spp., Klebsiella spp., Mycolicibacterium spp., Myxococcus spp., Nocardia spp., Pseudomonas spp., Rhodococcus spp., Streptomyces spp., and Stenotrophomonas spp., can also inhibit the growth and AF production of molds (Peles F *et al*., 2021).

3.5. Plant extracts

Plants produce different secondary metabolites to fight against pathogen attack. Antimicrobial compounds produced by plants are safe for the environment and consumers, and are important to control postharvest diseases. They are classified as generally recognized as safe and have low hazard to the consumers (Tian et al., 2011). Plant extracts have been reported to have antifungal activity against aflatoxins produced by *A. flavus* and *A. parasiticus* (Hajare et al., 2005; Sandosskumar et al., 2007; Velazhahan et al., 2010). Hajare et al. (2005) reported an 80% decrease in total aflatoxin content over the controls after treatment with aqueous extract of *Trachyspermum ammi* seeds. Sandosskumar et al. (2007) incubated AFB_1 with zimmu extract for five days and verified the potential of zimmu extract to degrade aflatoxin and reduce AFB_1 by up to 90 %. In addition, when groundnut was intercropped with zimmu, a significant reduction in the population of *A. flavus* in the soil, kernel infection by *A. flavus* and aflatoxin contamination was observed. Haciseferogullary et al. (2005) evaluated the efficacy of garlic extract at different levels against *A. flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, Penicillium chrysogenum, P. puberulum, P. citrinum, P. corylophilum, Rhizopus stolonifer, Stachybotrys chartarum, Eurotium chevalieri and Emericella nidulans* growth. Eighty four per cent reduction in toxin production occurred at 1% inclusion level.

4. Policy directions

In order to reduce aflatoxin contamination policies should focuses on: (1) improving awareness on sources of aflatoxin contamination along the value chain (farmers, consumers, processors, and traders) and health impacts; (2) implementing appropriate pre- and post-harvest *Aspergillus* fungi control strategies and (3) developing accessible low cost technologies and infrastructure to monitor contamination levels.

CONCLUSIONS

Infection of crops with the fungi in the genus *Aspergillus* both at field and storage conditions leads to aflatoxin contamination in warm and humid areas. Aflatoxin contamination causes stunting in children, immune suppression, liver cancer and death. It is possible to reduce aflatoxin contamination by using management options such as sorting, crop rotation, irradiation, fumigation, chemical, botanical, biological control and improved storage structures to improve health conditions of people, and increase food safety and security. In the future multidisciplinary and inclusive research is vital to evaluate the effect of climate change and the potential benefits of integrated management technologies..

Competing interests

The authors declare that they have no competing interests.

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