



Mitigating Aflatoxin Contamination: A Review on Strategies for Safe Food Crops

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Abstract: Aflatoxin contamination in food and feed is a significant global food safety concern, particularly in sub-Saharan Africa. This review investigates the causes, impact, and control measures related to aflatoxin. The review synthesizes existing literature to provide an overview of aflatoxigenic fungi, favourable growth conditions, factors influencing aflatoxin contamination, and management strategies. Aflatoxin contamination is influenced by factors like climate change, inadequate storage, crop susceptibility, poor agronomic practices, and the prevalence of aflatoxigenic fungi species. The presence of Aflatoxin has significant impacts on both human and animal health, ranging from acute aflatoxicosis to chronic health conditions and fatalities, with economic consequences including trade barriers and yield losses. Effective management strategies encompass physical, chemical, and biological techniques, along with breeding and genetic engineering approaches to mitigate aflatoxin levels. This review underscores the urgent need for integrated strategies to minimise aflatoxin contamination, safeguard public health, and ensure food safety/security, especially in regions vulnerable to aflatoxin outbreaks. The practical implications for policymakers, agricultural stakeholders, and researchers include informed decision-making to reduce aflatoxin levels in food and feed, improve food safety, create awareness, and ensure a stable supply of aflatoxin-free products.

Keywords: Mycotoxin, Aflatoxin contamination, *Aspergillus flavus*, *Aspergillus parasiticus*, Food crops

INTRODUCTION

Aflatoxin contamination in crops is a global threat that compromises the safety of food, feed, and also influences the agricultural economy and crop-dependent small-scale industries. Crops can be contaminated during the process of harvesting, storing, and transporting by the fungi and leads to the productions of several mycotoxins. Mycotoxins are produced by certain fungi as secondary metabolites and aflatoxin is one of them. Aflatoxins are synthesized by many fungi spp. including *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* but *Aspergillus flavus* and *Aspergillus parasiticus* are known to produce the most toxigenic strains of aflatoxins.

There are mainly six different types of aflatoxins Aflatoxins-B1 (AFB1), Aflatoxins-B2 (AFB2), Aflatoxin G1 (AFG1), Aflatoxins G2 (AFG2), Aflatoxin M1 (AFM1), and Aflatoxin M2 (AFM2) (Quadri et al. 2012). Out of these B1, B2, G1, G2 are found in food crops or their products, while M1 (Metabolite of B1) and M2 are found in the animals' by-products such as dairy products.

Aflatoxin B₁ and B₂ are produced by *A. flavus*, while Aflatoxin G₁ and G₂ are synthesized by *A. parasiticus* and largely contaminate a wide range of food commodities including cereals (maize, sorghum, pearl millet, rice, and wheat), oilseeds (peanut, soybean, sunflower, and cotton), spices (chilies, black pepper, turmeric, coriander, and ginger), nuts (almond, Brazil nut, pistachio, walnut, and coconut), yam and various milk products (Rajarajan et al. 2013). *A. flavus* fungus has a green appearance and has the potential to increase its population even under many stressful conditions. The injuries caused by insects and nematode also paved the way for the entry of these fungi (Jeyaramraja et al. 2018). The entry of fungus in crops not only compromises the self-defense of crop plants by the fungal attack but also contaminates the crop seeds which lead the aflatoxin production. The fungal infections affect the crop's growth, yield, and also result in loss of market value.

Aflatoxin B₁ is the most harmful aflatoxin to humans and animals as it is carcinogenic due to its association with hepatocellular carcinoma which leads to liver cancer (Qureshi et al. 2015). Aflatoxins suppress the immune systems of humans and animals by interfering with the fickleness of those cells which are responsible to boost immunity. Large doses of aflatoxins lead to direct death and damage, while small longstanding doses lead to immunologic or nutritional effects, but both types of doses lead to liver cancer due to the accumulation of aflatoxin (Marroquín-Cardona et al. 2014). Children are more prone to the toxicity of Aflatoxin as it increases the risk of early infections by reduced immunization. The carcinogenic nature of aflatoxin is due to its ability to damage DNA either by lipid peroxidation or by oxidation (Zhang et al. 2015). The cytochrome p-450 present in the liver activates AFB₁ which is then converted into AFB₁ 8,9-epoxide. The compound AFB₁ 8,9-epoxide is responsible for various carcinogenic effects (Denissenko et al. 1999). Apart from being carcinogenic, aflatoxin also has negative effects on the kidney, heart, liver, testis, and brain. Aflatoxin was also responsible for the various outbreaks in India and various African countries. The condition of the outbreak is more severe in under-developed and developing countries due to fewer food regulation acts.

To curb the aflatoxins contamination in food crops, countries have imposed different laws regarding the level of these toxins in food crops. The United State Food and Drug Administration (USFDA) imposed strict laws for the aflatoxins level in affected food commodities at 20 ppb (parts per billion) in food and feeds, while 0.5 ppb in milk products European Union (EU) also interpolates the aflatoxin contents level in food commodities in the range of 2-4 ppb (Gurtler & Keller 2019). In India, a maximum limit of 30 µg/kg has been imposed for all food commodities under the Food Safety and Standards Regulations, 2011. However, such laws on Aflatoxins contamination range in various crop plants/food commodities put an extra load of over US\$932 million on the agricultural economy by a reduction in crops growth and yields. Several lakh tones of harvested product go waste every year due to aflatoxin contamination and not fulfilling the export norms.

To minimize the aflatoxins contamination in crop plants, various physical, chemical, and biological methods and various breeding and genetic engineering approach has been used to minimize the toxicity of aflatoxin and reduce its level below the recommended one. This review describes an overview of various aspects of aflatoxigenic fungi, favorable conditions for growth, control measures, detection techniques, and recent reports in various crops.

Favorable conditions for aflatoxin contamination Mycotoxins production depends on the food source, enzymes, and various environmental factors. However, the conditions that are favorable for aflatoxigenic fungi are not always conducive for the production of aflatoxins (Mannaa & Kim 2017). The factors are summarized as:

Physical Factors

Physical factors like pH, light, moisture, temperature, water, relative humidity, and atmospheric gases are responsible for aflatoxin contamination. Aflatoxin-producing molds/Fungi can grow in a wide range of pH (1.7-9.3), but the optimum range of pH is (3-7) (Yoshinari *et al.* 2010). The lower pH ($3 > \text{pH} > 1$) minimizes the fungal growth and a slightly higher pH ($6 > \text{pH} > 3$) promotes both fungal and aflatoxins production (Eshelli *et al.* 2015). Initial pH (pH = 5) promotes AFB (Aflatoxin B) production while higher pH (pH = 7) promotes AFG (Aflatoxin G) production, however, the composition of media in which fungi grow also influence pH (Dalié *et al.* 2010). Fungi growth and aflatoxin production are also affected by the presence of light. Darkness increases aflatoxin production while sunlight inhibits it (Rushing & Selim 2019). High moisture content always favors the aflatoxin contamination because moist conditions are favorable for fungal growth. Relative humidity (85%) is optimal for aflatoxins production, while 95% relative humidity increases aflatoxins production to a considerable level (Ding *et al.* 2015). However, the water level does not have any reported effect on aflatoxin contamination. *Aspergillus flavus* poses an excellent survival capability to grow on a wide range of temperatures ranging from 12 °C to 48 °C, but 28 °C to 37 °C is the optimum temperature range for its growth (Hawkins *et al.* 2005). Aflatoxins production can occur at a wide range of temperature; however, the optimal temperature for aflatoxin production is 25-35 °C (Siciliano *et al.* 2017). Normally at high temperatures, AFB production is high than AFG, but at low temperatures both AFB and AFG production is equal (Matumba *et al.* 2015). Availability of O₂ and CO₂ also influence the aflatoxins productions. Aflatoxins production and fungal growth are inhibited at a higher level of CO₂ and a lower level of O₂ (Mahbobinejhad *et al.* 2019).

Nutritional Factors

Aflatoxin productions are also widely affected by the substrate and various nutritional factors such as carbon, amino acids, nitrogen, lipids, and few trace elements. Substrate rich in carbohydrates supports more production as compare to oil as carbohydrate easily provides carbon which is needed for good fungal growth (Ma *et al.* 2014). Among carbohydrates glucose, ribose, sucrose, xylose, and glycerol acts as excellent substrates, while peptone, lactose, and sorbose were unable to promote aflatoxins production (Liu *et al.* 2016). Nitrogen in the form of nitrite and nitrate also increases the aflatoxin production level by *A. flavus* in various ways (Wang, Han, *et al.* 2017). Lipids also play an important role in aflatoxin production. Aflatoxins biosynthesis in toxigenic fungi leads by lipophilic epoxy fatty acids and fungal growth as well as aflatoxin production induced by ergo-sterol oxidation (Reverberi 2014). Consequently, lipids also act as a substrate to obtain an acyl-CoA starter as well as a signaling molecule. The aflatoxin production and accumulation increase in full-fat substrates as compared to the low-fat substrate. The addition of corn oil in defatted wheat, infected by *A. flavus* promotes aflatoxin production as compare to media without the addition of corn oil (Liu *et al.* 2016). Vitamins, amino acids, and metal ions also

promote aflatoxin production in combination. Amino acids like glycine, glutamate, and alanine along with some bivalent metals like zinc and magnesium promote aflatoxin production (Bolu *et al.* 2014). The aflatoxin production increased by 4, 5, and 19 times with the 20, 50, and 100 (mg/L) concentration of zinc respectively (Liu *et al.*, 2016). Amino acid such as tyrosine promotes aflatoxin production, while it is inhibited by tryptophan (Chang *et al.*, 2015). AFB1 production was supported by arginine, glycine, glutamic acid, and aspartic acid at a concentration of 0.5%.

Biological Factors

Biological factors include fungal species, weeds, and insect injuries. Weeds mostly grow as a competitor and cause plant stress which is associated with aflatoxin production. The amount of aflatoxin production mainly depends on types of fungi; insects wound in the plant cause stress and provide the site for aflatoxigenic fungi for contamination (Kinyungu 2019). Aflatoxin production also depends on the types of strains. *A. flavus* produces fewer aflatoxins as compare to *A. parasiticus* (Manjunath & Mohana 2018). Apart from the abovementioned factors, *A. flavus* is the main species that is mainly responsible for aflatoxin production and crop contamination because it is the most copious molds found in soil and possesses the saprobe character that enables it to grow on many organic nutrient substrates including compost piles, plant debris, cotton, dead insects, stored grains, field crops, animal corpses and animal fodder (Kakde 2012). Pre-harvest contaminations of field crops are common because of the natural existence of *A. flavus* in soil, while postharvest contamination also occurred by *A. flavus* during storage because it spoils the food grains. Due to the lacking of host specificity *A. flavus* contaminate both monocot and dicot seeds (Leger *et al.* 2000).

AFLATOXINS CONTAMINATION IN CROP PLANTS

Aflatoxin contamination occurs in a wide range of regional crops and food commodities. Food and feed like corn, rice, spices, dried fruits, nuts, and figs are mostly contaminated by aflatoxins (Martinez-Miranda *et al.* 2019). The four major aflatoxins like AFB1, AFB2, AFG1, and AFG2 are commonly found in a wide range of food commodities, AFB1 and AFB2 are produced by *A. flavus* while AFG1 and AFG2 are produced by *A. parasiticus*. The post-harvest crops are more likely to be contaminated if the storage conditions are optimum for fungus growth. It was found that about 67.9% of maize samples, 92.9% of millet samples, and 50% of sorghum samples that were obtained from a storage room are contaminated by aflatoxins (Sirma *et al.* 2015).

Methods to detect aflatoxin contamination in crop plants There are many official methods to detect Aflatoxin contamination in crop plants, which are featured by the Association of Official Analytical Chemists (AOAC) (Kumar *et al.* 2017). Amongst them, the most commonly used method is Enzyme-Linked Immunosorbent Assay (ELISA) followed by some chromatographic methods including High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectroscopy (LCMS), and Thin Layer Chromatography (TLC) (Sulyok *et al.* 2015). Wang, Li, *et al.* 2017 developed a highly specific nanobody polyclonal antibody sandwich ELISA for detecting both *A. flavus* and *A. parasiticus* with a minimum detection limit of 1 µg/mL. Unfortunately, there are some limitations associated with the

above-mentioned techniques including their fulsomeness, required high technical skills, and time consumption. Hence, those methods that provide instant results are proved to be useful when considering a large sample. These methods include Polymerase Chain Reaction (PCR), Fluorescence/Near-Infrared Spectroscopy (FS/ NIRS), and Hyper Spectral Imaging (HSI). PCR technique is mainly used for the detection of Aflatoxins producing fungi *A. flavus* at the molecular level (Tao *et al.* 2018). Consequently, genes detected during molecular screening of *A. flavus*, responsible for Aflatoxin biosynthesis were used as a target gene to find out Aflatoxin by using multiplex PCR. Visible/Near-Infrared technique (VNIR) is also used for the detection of toxin contamination. In many parts of North America, a considerable amount of AFB1 was found on the surface of maize kernels when detected through VNIR (Chu *et al.* 2017). However, due to the bad image quality of VNIR, some recent studies focused on the combined use of the HSI technique with chemometric data analysis that resulted in better identification of AFB1 on the surface of maize kernels (Kimuli *et al.* 2018). The advancement in analytical techniques also led to simultaneous detection of aflatoxins with other toxic compounds. Aflatoxins along with zearalenone were simultaneously detected through the use of Time-Resolved Fluorescence Immuno-Chromatographic Assay (TRFICA) (Tang *et al.* 2017) and anti-idiotypic nanobody-Phage Display-mediated Immune-Polymerase Chain Reaction (PD-IPCR) (Ren *et al.* 2019). TRFICA and PD-IPCR provides a detection limit of 0.05 ng/mL and 0.003 ng/mL respectively for aflatoxins. A recent method also detects aflatoxins and fumonisins (a group of mycotoxins derived from *Fusarium* spp.) through Color-encoded Lateral Flow ImmunoAssay (CLFIA) (Di Nardo *et al.* 2019).

Certain nanoparticles-based techniques that use quantum dots (QD), carbon (CBNs), Au/Ag are also used to detect different aflatoxins in crop plants (Xue *et al.* 2019). Furthermore, the use of biosensors for the detection of AFB1 in crop products instead of the chromatographic or spectrophotometric method also gets popularized. The biosensors also permit easy and rapid detection with fewer expenses, minimal sample pretreatment, portability, and on spot identification of aflatoxins by utilizing an electrochemical enzymelinked oligonucleotide array (Rotariu *et al.* 2016; Selvolini *et al.* 2019). Aptamer-based molecular assays are also used for instant detection of aflatoxin in some beverages like wine and certain food crops (Wang *et al.* 2019).

MEASURES TO CONTROL AFLATOXIN CONTAMINATION

Aflatoxin contamination in crops caused a serious threat to production, the food market, health, and economics. Several approaches have been manifested to reduce the aflatoxin contamination in crops which include various physical, chemical, and biological methods.

Physical Methods

Physical methods such as steam under pressure, dry roasting, and other cooking methods are found to be effective in the control or to reduce the aflatoxin contamination in many crops (Peng *et al.* 2018). About 40- 73% reduction in aflatoxin level was also observed by heating the seed samples on 180 °C (Opoku 2013). When groundnut and corn seed was roasted with 30% moisture at 100 °C temperature for 2 h, there is a reduction of aflatoxin by 85% (Leong *et al.* 2010). Roasting also reduced the concentration of AFB1 and AFG1 by

70 and 79% when the seeds are roasted at 150 °C for 15 min (Jalili 2016). An effective reduction in aflatoxin concentration was also observed when the seeds were treated with various radiations such as UV and Infrared radiation (Surekha *et al.* 2015).

Sunlight also plays an important role in the detoxification of AFB₁ in many crops. A reduction in AFB₁ content was found in artificially infected maize (80%) and groundnut (17%) when the seeds are exposed to sunlight for a 10-12 h period (Rushing & Selim 2019). Consequently, gamma radiation exposure was also found to reduce the aflatoxin level (Aquino 2011). Moisture is a significant factor in fungal growth which leads to the production of aflatoxin in crops. Aflatoxigenic fungi growth and aflatoxin production inhibited by drying groundnut to 6.6% and maize to 15% or much lower moisture level within 24-48 h (Awuah & Ellis 2002; Eziah, & Afreh-Nuamah, K. 2015). UV irradiation (220-400 nm) also degrades the aflatoxins particularly AFB₁, AFB₂, and AFM₁ in various crops with a degradation potential ranging from 77 to 99.12% (Diao *et al.* 2015).

Chemical Methods

Several chemicals such as acids, alkalis, oxidizing agents, aldehydes, and several gasses are also proved to mitigate the aflatoxigenic fungal growth and aflatoxin production when used in appropriate quantity (Udomkun *et al.* 2017). Among gasses, ozone was found to be most effective in maximizing the aflatoxin degradation on legumes and cereals by an electrophilic attack on carbon bonds of furan ring (Jalili 2016). However, due to the high-cost factor, ozone treatment is of less use in the post-harvest treatment of crops. Aflatoxin production can also be inhibited by the ammonization process in corn and other food commodities (Karlovsy *et al.* 2016). The positive aspect of the ammoniation process is that high-pressure ammoniation (0.25, 0.5, 1.5, and 2%) minimizes the time required to reduce the aflatoxin production in several crops and food commodities (Temba *et al.* 2016). Certain chemicals such as sodium bisulfite, calcium hydroxide, formaldehyde, sodium hypochlorite, sodium borate, and sorbents also reduce the aflatoxin in many food commodities at a significant level (Carvajal & Castillo 2009). The groundnut cake and poultry feed were detoxified from aflatoxin by treating them with sodium bisulfite (0.5%) and sodium hydroxide (1%) (Bedi & Agarwal 2014). Some food additives are also used for the inhibition of fungal growth and aflatoxin production in combination with some physical factors like temperature and moisture. Treatment of citric acid in conjunction with high temperature and pressure leads to the inhibition of fungal growth as well as aflatoxin production in sorghum (Méndez-Albores *et al.* 2009). Some scientists also observed that some food preservatives such as propionic acid, crystal violet, p-amino benzoic acid, benzoic acid, boric acid, and sodium acetate were also inhibited the *A. flavus* growth and aflatoxin production (Aiko & Mehta 2015). In peanut and maize, AFB₁ production was also inhibited by the treatment of sodium chloride (10%), acetic acid (5%), and propionic acid (5%) (Brožková *et al.* 2015). Even under the favorable conditions for the growth of *A. flavus*, several salts of propionic acid-like calcium, sodium propionates were able to reduce the aflatoxin formation in maize (Hassan *et al.* 2015). In groundnut cake, aflatoxin production by *A. flavus* was also foreclosed by certain weak acids such as citric acid, propionic acid, acetic acid, and sorbic acid at several concentrations like 0.25, 0.5, 0.75, and 1% (Verma *et al.* 2000). Azole fungicides are also used as a tool to control fungus growth and aflatoxin production with prochloraz being more effective than tebuconazole (Mateo *et al.* 2017).

Biological Methods

The aflatoxin contamination in agricultural products and many other food commodities are also minimized by the use of various microorganisms. Biological agents such as bacteria, yeasts, molds, and algae exhibit the different potential to degrade the aflatoxin in the emulative environment. Detoxification of aflatoxins by using biological agents is divided into two procedures named absorption and enzymatic decadence (Jard *et al.* 2011). Aflatoxins can be absorbed by microorganisms directly either by concatenating to their cell wall contents through effectual internalization or congregation (Motawe *et al.* 2014). Aflatoxins can also be absorbed by dead microorganisms; this ability can be helpful for the fabrication of bio-filters in the form of probiotics and found application in fluid decontamination (Mwakinyali *et al.* 2019). Degradation of aflatoxins can also be done by intra or extracellular enzymes; the end products of such enzymatic degradation are mostly water and CO₂ (Aliabadi *et al.* 2013). In a study on testing the efficacy of different microbes' strains, only *Flavobacterium aurantiacum* B-184 was found to be effective enough for aflatoxin degradation (Darsanaki & Miri 2013). However, different bacteria strains were also found to be effective against degrading the AFB₁. The cell-free supernatant of strain *Bacillus velezensis* DY3108 exhibits a strong AFB₁ degradation activity of 91.5% (Shu *et al.* 2018). The thermophilic bacterial strains (*Geobacillus* and *Tepidimicrobium*) also play a major role in AFB₁ degradation when used as a microbial consortium (Wang, Zhao, et al. 2017). Moreover, a significant reduction in *A. flavus* growth was observed in pre-harvest crops by inoculation of antagonistic strains of *Pseudomonas*, *Trichoderma*, *Ralstonia*, *Lactobacilli*, *Burkholderia*, and *Bacillus spp.* (Akocak *et al.* 2015; Yang *et al.* 2017). Surprisingly aflatoxin contamination was also found to be inhibited by using the non-toxigenic strains of *A. flavus* and other molds as a chief controlling agent (Udomkun *et al.* 2017).

CONCLUSION

Aflatoxins contamination in food crops and their derived products have become a serious threat to humans and animal's lives. The problem is severe in developing countries where apart from causing a health hazard, the toxin-contaminated products are also losing economic value in the global food market. Various physical, chemical, biological, and nano-particles based approaches are used for minimizing and management of aflatoxin in food crops. However, researchers are also progressing in the development of fungal resistant varieties through breeding and genetic engineering approaches but their outcome is still a major concern. Hence a combined approach of using resistant varieties along with recommended pre-and post-harvest practices should be followed by farmers and food industries to minimize and degrade the aflatoxin content in food crops and their derived products.

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