Health impacts of aflatoxin and control of aflatoxigenic fungi

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Article Info	ABSTRACT
Date of Acceptance: 09 February 2020 *Date of Publication:* 06 March 2020 **Keywords** Aflatoxin Aspergillus Bio-control Contamination Fungal metabolites	Aflatoxins are a group of related fungal metabolites primarily produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxin is a global food safety concern with rural subsistence farming communities in developing countries being the populations most at risk of exposure. Aflatoxin contamination is the main food safety problem for field crops produced in tropical and subtropical climate regions where high temperature and humidity promote growth and proliferation of *Aspergillus* spp. Foods and feeds, especially cereal grains, are susceptible to invasion by molds during pre-harvest, processing, transportation, or storage. Aflatoxins impose effects in both economic and health sectors where the crisis leads to death of human beings and animals. The most well established health effect of chronic aflatoxin exposure are hepatocellular carcinoma impaired child growth and immune suppression. To minimize the risk of aflatoxicosis, there are several physical, chemical and biological methods available, but the biological strategies are safest and well effective. Therefore, this review was aimed to show the health impact of aflatoxins and the promising strategies against aflatoxin contamination.

Introduction

Mycotoxins are fungal toxic metabolites produced by particularly by saprophytic molds growing on agricultural products. Some of these mycotoxins, including aflatoxins, are potentially carcinogenic and mutagenic with harmful effects to humans, livestock, and poultry. Aflatoxin was first identified in 1960 following a severe outbreak of a disease called "Turkey X Disease" in the UK that killed over 100,000 turkey birds (Pooja et al., 2015). It is now become a global food safety concern as recognized by the World Health Organization (WHO, 2008), with rural subsistence farming communities in developing countries being the populations most at risk of aflatoxin exposure. Staple foods that are susceptible to aflatoxin contamination, food insecurity, insufficient awareness and lack of enforcement of regulatory limits are some of the contributors to the high level of aflatoxin exposure in these populations (Yun et al., 2016). In many developing countries including Ethiopia, massive investments have been made to raise crop production levels to meet the

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food and nutritional needs of growing populations. Such efforts cannot attain its goals without lying due emphasis on the reduction of postharvest losses which are primarily by aflatoxins and mostly other fungal contaminations. It is common in most developing countries that even if aflatoxins impose great health and economic problem, it has been given little emphasis and has damaged a lot. Therefore, prevention and control strategies have to be primarily focused via environmentally friendly to fight against health and economic risk by aflatoxins (Soad et al., 2018).

Population dynamics of the genus *Aspergillus*

The genus *Aspergillus* which belongs to the phylum Ascomycota includes over 185 known species. Several members of *Aspergillus* section *flavi* produce aflatoxin. These includes *Aspergillus flavus* and *Aspergillus parasiticus,* as well as several less common taxa including *Aspergillus nomius, Aspergillus tamarii, Aspergillus pseudotamarii, Aspergillus minisclerotigenes* and *Aspergillus bombycis* (Matthias, 2009). *Aspergillus* species other than section *flavi* can also produce aflatoxins. For example, *Aspergillus ochraceoroseus* from section *Ochraceoroseus*; SCRR 1468, morphological resembling members of section *Circumdati*, and the Ascomycete *Emericella astellata* and *E. venezuelensis* (*Aspergillus* section *Nidulantes*, also produce aflatoxin (Cary et al., 2005). *Aspergillus flavus* is a ubiquitous and cosmopolitan filamentous fungus which proliferates in a wide range of environmental conditions. It is both a saprophytic and an opportunistic pathogen. *Aspergillus* section *flavi* can survive temperatures ranging from 12°C to 48°C, with the optimal growth from 28°C to 37 °C, with a high humidity of above 80% (Matome et al., 2017).

Life cycle and phases of infection

Aspergillus section *flavi* most abundantly and widely distributed in soil or can be found anywhere such as on many organic nutrient sources like plant debris, tree leaves, decaying wood, animal fodder, cotton, compost piles, dead insects and animal carcasses, outdoor and indoor air environments, stored grains, and even on live humans and animals (Yu et al., 2005). Its life cycle in agricultural fields can be considered as

(1) colonization of plant debris in soil and (2) invasion of seeds and grain in actively growing crop plants (Fig. 1). Soil serves as a reservoir for primary inoculum of *A. flavus* and *A. parasiticus* (Matthias, 2009). *A. parasiticus* is more adapted to a soil environment, being prominent in peanuts, whereas *A. flavus* is more adapted to the aerial and foliar environment, being dominant in corn, cottonseed and tree nuts.
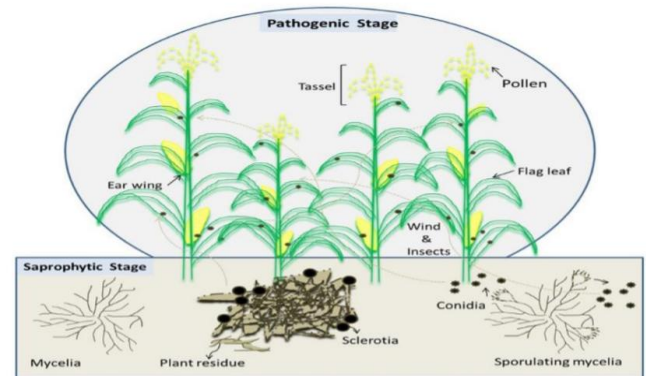

Fig. 1: Life cycle of *A. flavus* in a corn cropping system (Kiran et al., 2014).

Under adverse conditions the fungal mycelium congregates to form resistant structures called sclerotia, pigmented and compacted aggregates of hyphae which are capable of keeping dormant for long period of time (Yu et al., 2005). The fungus overwinters either as mycelium in plant debris and litter on the soil, on insects or as sclerotia in the soil. When the growth conditions are favorable the sclerotia either germinate to produce additional hyphae or produce conidia or can be further dispersed to the soil or air. The fungus mostly exists in the form of mycelium or asexual conidia spores (Matthias, 2009).

A. flavus invades and infects developing seeds in the field before harvest, during harvest and in storage. Aflatoxin contamination can be divided into two distinct phases with the infection of the developing crop in the first phase and increase in contamination after maturation in the second phase (Cotty, 2001). Infections by *A. flavus* and *A. parasiticus* are promoted due to wounding of developing crops by birds, mammals, insects, mechanically or drought stress and elevated temperatures (Fig. 2). Its ability to attack seeds of both monocots and dicots, and to infect seeds produced both above and below the ground,

demonstrates that this fungus has evolved a battery of mechanisms to breach the host's resistance. Conidia of plant, insect, and human derived strains of *A. flavus* rapidly colonize leaves, kernels, and insects injured during inoculation but do not affect uninjured plant or insect material (Yu et al., 2005).
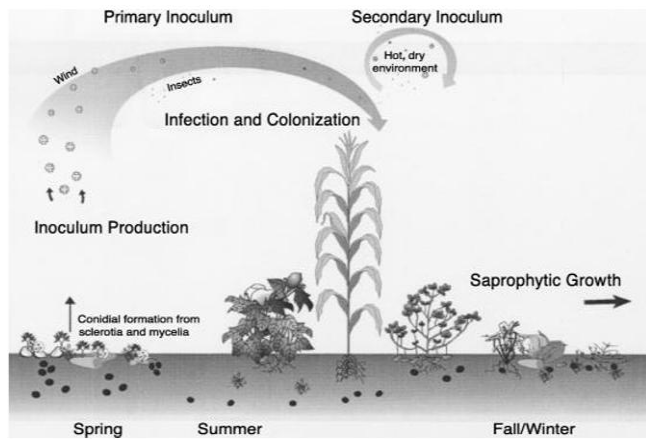

Fig. 2: Diagram of the pre-harvest infection of cotton, corn, and peanuts by *Aspergillus flavus.* Sclerotia and conidia produced by *A. flavus* growing on crop debris and in the soil serve as primary inoculum for young plants in the spring. Later in the growing season, conidia produced on crop debris or on infected plants provide high levels of secondary inoculum when environmental conditions are conducive for disease development (Matthias, 2009).

Biology of aflatoxins

Aflatoxins are a group of related fungal metabolites primarily produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxins, belongs to a group of difuranocoumarins, classified into two broad groups based on their chemical structure as: difurocoumarocyclopentenone and the difuro coumarolactone series. The four major naturally known aflatoxins are AFB1, AFB2, AFG1 and AFG2 (Jef et al., 2015).

The metabolic products of aflatoxins, M1 and M2 are first isolated from milk of lactating animals fed on aflatoxin contaminated grains and the reason why M designation (Bennett and Klich, 2003). Aflatoxins have closely similar structure (Fig. 3) and form a unique group of highly oxygenated heterocyclic compounds. Aflatoxins B2 and G2 were established as the dihydroxy derivatives of B1

and G1 whereas aflatoxin M1 is 4hydroxy aflatoxin B1 and aflatoxin M2 is 4-dihydroxy aflatoxin B2. Of the four major aflatoxins (B1, B2, G1 and G2), G2 occurs in high quantity and less toxic while B1 is the most toxic (Thrasher and Crawley, 2009). Insects and nematode vector infection increases fungal invasion and aflatoxin formation in the seeds (Pooja et al., 2015). Toxic moulds will invade agricultural products during plant growth, storage and processing. Kernels infected with fungal toxins such as aflatoxins may result in feed/food unsafe for consumption by humans or livestock. Aflatoxins have received considerable attention due to their effect in agricultural loss and livestock/ human health. Aflatoxin contamination of crops is a food safety concern throughout the world. The fungus continues to grow and produce toxin in poorly dried grain (Wafaa et al., 2014).
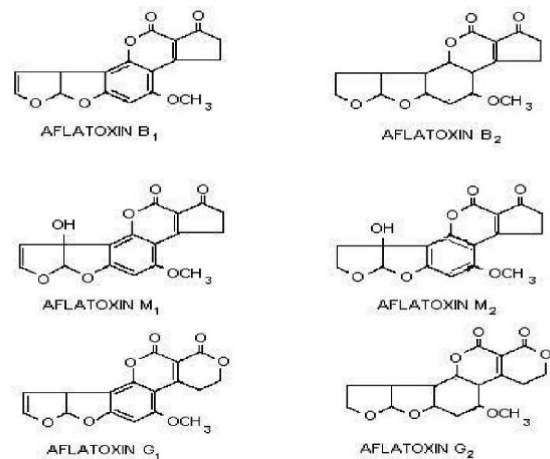

Fig. 3: Structure of Aflatoxins (where the "B" and "G" refer to the blue and green fluorescent colors produced under UV light on thin layer chromatography plates while the subscripts 1 and 2 indicate major and minor compounds, respectively whereas the B designation (B1 and B2) of aflatoxin results from the exhibition of blue fluorescence under UV-light, while the G designation have yellow-green fluorescence of the relevant structures (Sudakin, 2003).

Worldwide aflatoxin exposure

Dietary exposure to aflatoxin can be estimated by contamination level analysis of food samples combined with dietary intake surveys. Although these methods can be effective in estimating aflatoxin exposure levels in large samples, it has many limitations. For instance, dietary intake surveys are subjected to recall bias and social

desirability issues, which could lead to under or over-estimation of exposure (Yun et al., 2016). Biomarkers are considered to be more accurate to measure the degree of individual exposure, as they are objective indicators and key determinants of internal dose and biologically effective dose. The biomarkers for aflatoxin exposure include the aflatoxin-N7-guanine adducts excreted in urine, which reflect the previous day's exposure; AFM1, the hydroxylated metabolite of AFB1, which is found in breast milk and reflects exposure over the previous 24 hours; and the aflatoxin-albumin adduct in plasma or serum, of a half-life of ~2 months, which permits the measurement of more chronic exposure to aflatoxin (Routledge and Gong, 2011). Aflatoxin exposure is not a major issue for developed countries, as there are strictly enforced regulatory limits in place and the diet is more diverse. Aflatoxin albumin is rarely detected in blood samples from populations in these regions. For instance, in a subset of 2051 individuals that participated in the 1999-2000 of National Health and Nutrition Examination Survey, which is a representative cross-sectional survey of the US population, only 1% had detectable levels (≥0.02 µg/L) of AFB1-lysine in their blood (Schleicher et al., 2013). There is high prevalence of aflatoxin exposure in Asian countries. For example a study examining aflatoxin exposure in pregnant women in South Asia using isotope dilution mass spectrometry to measure AFB1-lysine found detectable levels of the biomarker in 94% of blood samples collected from Nepalese pregnant women (n = 141), with levels ranging between 0.45 to 2939.30 pg/mg (Groopman et al., 2014). In the same study, AFB1-lysine was detected in 63/63 (100%) samples collected from pregnant women in their first and third trimester from Bangladesh, as well as in 63/63 (100%) cord blood samples and in 63/63 (100%) infants who were born to the mothers exposed to aflatoxin during pregnancy. In Africa, many reports has been dictating the higher contamination of cereals with aflatoxin and for instance in Ethiopia, aflatoxin contamination in different cereals and commodities and its effect have been investigated by many researchers such as Dereje et al., 2012 (Peanuts in Tigray); Chauhan et al., 2016 (Maize in Gedeo zone); Ezekiel et al., 2018 (Processed Beverages)) and others. Moreover, Urinary aflatoxins have been reported (Abebe et al., 2017).

Impacts of aflatoxin

Aflatoxin contamination is a significant problem worldwide (Assefa and Geremew, 2018) because it is a potent carcinogen and hepatotoxin (Soad et al., 2018) United States federal guidelines for food and feed set a limit of 20 ng /g total aflatoxins, while the European Union guidelines are more strict, with a limit of 1.0 ng /g limit for B1 and a 4.0ng/ g limit for total aflatoxins (Hamed et al., 2006). Aflatoxins are of a great concern and an emerging issue in many countries especially in humid tropics. Aflatoxin contamination is the main food safety problem for field crops produced in tropical and subtropical climate regions where high temperature and humidity promote growth and proliferation of *Aspergillus* spp. The International Agency for Research on Cancer categorized aflatoxins as class I carcinogens, as they are highly poisonous, toxic substances. Thus, exposure to aflatoxin-contaminated commodities imposes a great risk. Biological factors, harvesting, storage and processing conditions and climate change are the most important factors for mycotoxin infectivity (Rashad et al., 2018).

Foods and feeds, especially cereal grains, are susceptible to invasion by molds during pre-harvest, processing, transportation, or storage. Fungal growth is one of the main causes of cereal spoilage. It not only generates great economic losses, but also can cause acute or chronic intoxication to human and animals through the synthesis of mycotoxins. The most fungal toxigenic genera are *Alternaria, Aspergillus, Fusarium* and *Penicillium* (Osweiler, 2000). *Alternaria* and *Fusarium* species are among field fungi, while *Aspergillus* and *Penicillium* species are considered as storage fungi, although these can grow if environmental conditions are favorable (Ines et al., 2018).

The production of mycotoxins in commodities depends on both the environmental conditions and the toxigenic species present. Therefore, good harvesting and processing practices and early detection of mycotoxigenic species are key aspects in any strategy to prevent or reduce mycotoxins in foods. Precise and sensitive detection of the mycotoxigenic species is necessary since even very closely related species may produce a different array of mycotoxins (Sardinas et al., 2011). Cases of

human and animal aflatoxicosis have been reported in Africa as a result of the consumption of food and feed contaminated with aflatoxins (Matome et al., 2017).

Health impact of aflatoxin

Aflatoxins are associated with various diseases; aflatoxicosis in livestock, domestic animals and human worldwide. Aflatoxicosis is resulted from ingestion of food or feed contaminated with aflatoxins. Aflatoxin poisoning is reported from all over the world in almost all domestic and non-domestic animals such as cattle, horse, rabbit, and other primates (Eva et al., 2011. There are four generally accepted dietary aflatoxins; B1, B2, G1 and G2 with toxicity rate B1> G1 > G2> B2 (Ephrem, 2015). The metabolites, M1 and M2, are also found in milk. Aflatoxin M1 (AFM1) has been identified in the milk of dairy cows consuming AFB1-contaminated feeds (Kehinde et al., 2014). *Aspergillus flavus* is associated with many diseases of human, most severe of which is invasive aspergillosis. It also causes diseases in insects as well as in crops (Magoha et al., 2014). Different mycotoxins have also been reported as contaminant of poultry feed, most important of which are aflatoxins (B1, B2, G1, G2) and Ochratoxin A there by induce various diseases (Eva et al., 2011) (Table 1).

Table 1. Major diseases caused by aflatoxicosis in some animal species (Eva et al., 2011).

Animal Species	Disease imposed by Aflatoxins	Symptoms
Horses	**When eating:** Liver damage, centrilobular hepatic necrosis phagocytosed haemosiderin in Kupffer cells, bile-duct hyperplasia, congestion of renal vessels and adrenal cortex. **When inhaling:** Chronic obstructive pulmonary disease (COPD), yellow-brown liver with centrilobular necrosis, icterus hemorrhage, tracheal exudates and brown urine.	**When eating:** Anorexia, icterus, rapid weight loss and dead. **When inhaling:** Chronic cough, nasal discharge, expiratory dyspnoea reduced exercise tolerance, inappetence, depression, fever, tremor, ataxia, cough and dead.
Chickens	Immunosuppression, liver and kidney damage, periportal fatty infiltrations, increase in connective tissue, hemorrhages, susceptibility to opportunistic infectious agents and poor response to vaccination programs.	Low productivity, low growth, low weight, low stance, but no evident clinical symptoms and death.
Swine	Immunosuppression, expression of opportunistic infections, liver swollen, liver congestion, hydrothorax, edematous gall bladder, petechiated and ecchymotic mucosa, extensive centrilobular liver necrosis, hemorrhage, hepatic centrilobular cellular Infiltration, hepatocyte swelling, bile stasis, hepatocyte vacuolation and bile ductile hyperplasia.	Low growth rate, gastrointestinal problems, anoroexia, ill thrift and dead.
Cattle	Fibrosis with biliary proliferation in livers, venocclusive disease increase in connective tissues, degeneration of centrilobular hepatic cells, proliferation of connective tissue, generalized liver damage and immunosuppression.	Icterus, rapid weight loss and dead
Other Animals	Pulmonary edema, generalized liver damage, coagulopathy, capillary fragility, hemorrhage, prolonged clotting times, urine pigmentation, icterus and hepatic injury.	Depression, anorexia, weight loss, bleeding, decline in feed consumption and production, gastro intestinal damage and death.

Aflatoxins are among the most studied groups and apart from producing clinical toxicosis they also reduce the resistance to diseases and interfere with vaccine induced immunity in poultry birds. Agricultural commodities contaminated with toxigenic fungi like *A. flavus* can be injurious for animals and human health. Production of mycotoxins is species specific; therefore, proper identification and characterization of fungi is of prime importance to develop preventive strategies (UNICEF, 2012). Regular monitoring of toxic mycoflora of agricultural based feeds and foods is

an essential pre-requisite for development of strategies to control or prevent mycotoxins exposure of feed animal and human population (Fakruddin et al., 2018).

In Africa, weaning foods are often cereals and legumes of which are susceptible to aflatoxin contamination and as a result children exposure increases during weaning. Aflatoxins have been recently considered as an important sanitary problems because it has been demonstrated that human exposure to mycotoxins may result from consumption of plant derived foods that are contaminated with aflatoxins and their metabolites which can also be present in animal products like milk, meat, visceral organs and eggs or exposure to air and dusts (Kiran et al., 2014).

It has been reported that, because of their low molecular weight, aflatoxins upon ingestion can be rapidly adsorbed in the gastro-intestinal tract through a non-described passive mechanism, and then quickly appear as a metabolite in blood after just 15 minutes and in milk as soon as 12 hours post-feeding (Eva et al., 2011). High exposure of aflatoxin over a relatively short time causes acute aflatoxicosis. Although acute aflatoxicosis occurs on a case by case basis, large outbreaks have been reported in Africa and chronic aflatoxicosis due to low dose aflatoxin exposure over a long period of time is reported more prevalent than acute aflatoxicosis. The most well established health effect of chronic exposure are hepatocellular carcinoma impaired child growth and immune suppression (Azziz Baumgartner et al., 2005) (Fig. 4).
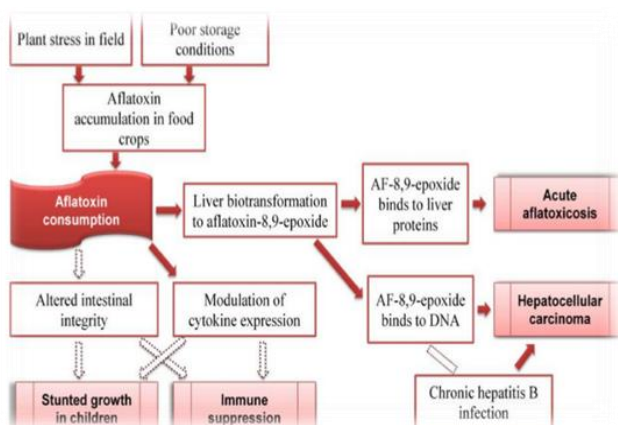

Fig. 4: Aflatoxin disease pathways in human Beings (Azziz Baumgartner et al., 2005).

Hepatocellular carcinoma

Hepatocellular carcinoma is recognized as the sixth most common cancer worldwide, with 83% of cases occurring in less developed countries and the highest incidence rates are observed in Asia and Africa (Bosett et al., 2014). Aflatoxin, owing to its mutagenic and carcinogenic properties, has been classified as a major risk factor, alongside the hepatitis B and C virus. In fact it has been shown that aflatoxin and hepatitis B, which is also highly prevalent in Africa and South Asia, can synergistically interact, resulting in an increased risk of hepatocellular carcinoma (Wu et al., 2009). The P53 gene hotspot mutation at codon 249 is associated with aflatoxin exposure. Villar et al (2011) observed a seasonal variation in levels of the R249S mutation in circulating cell free DNA in the serum of subjects in Gambia that reflected seasonal variations in aflatoxin exposure and markers of HBV infection, an interaction of these risk factors lead to liver cancer. Aflatoxin has also been implicated in the aetiology of other liver diseases including cirrhosis and hepatomegaly. A study in Kenya by Gong et al (2012) reported that the prevalence of hepatomegaly, a firm liver enlargement, increased in children with higher aflatoxin exposure. This proves the fact that liver is the key target organ for aflatoxin toxicity.

Impaired child growth

The first 1000 days of life are critical periods for healthy growth and development and dietary intake during pregnancy plays a key role in the child's future health. In sub-Saharan Africa, malnutrition and impaired child growth are major public health burdens. The impact of aflatoxin on growth impairment at different time points has been investigated as:

Utero exposure

Various studies have been reported that aflatoxin exposure can occur in utero through a transplacental pathway and higher exposure levels in utero have been associated with lower birth weights and stunted child growth (Shuaib et al., 2010). It has been suggested that epigenetic changes, which may occur as a consequence of aflatoxin exposure in utero, is a potential mechanism to explain this relationship. A study by

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Hernandez-Vargas et al (2015) examined the consequences of in utero exposure to aflatoxin on the white blood cell DNA global methylation level in children aged 2-8 months. Differential methylation of genes, including some growth and immune function related genes are associated with AF-alb exposure.

Breast milk exposure

Although breast milk is full of nutritional and immunological components, it is the potential source of aflatoxin exposure for very young infants. AFM1, the hydroxylated metabolite of AFB1, is typically detected in breast milk 12 to 24 hours following ingestion of foods contaminated with AFB1 (Yun et al., 2016). Only a few epidemiological studies have investigated the relationship between AFM1 in breast milk samples and impaired child growth. Magoha et al (2014) examined the relationship between AFM1, measured in breast milk samples of 143 lactating mothers, and growth impairment in their infants under 6 months of age in Northern Tanzania with mean AFM1 exposure concentrations of the infants at months 1, 3 and 5: 11.08±10.13, 11.94±9.69 and 10.91±6.82 ng/kg bodyweight/day, respectively. This highlights the potential for exposure of AFM1 from breast milk contributing to child growth impairment. The WHO recommendation of exclusive breast-feeding until 6 months of age should be encouraged owing to the high nutritional content and immunological properties of breast milk. Furthermore, infants who are exclusively breastfed appear to have lower AF-alb concentrations compared to those partially breastfed and fully weaned (Magoha et al., 2014). It must, therefore, be remembered that prolonged breastfeeding is protective to child health.

Exposure via weaning food

Children of weaning age in developing countries, especially sub-Saharan Africa, are considered a high-risk population group for aflatoxin exposure (Gong et al., 2008). Maize and groundnuts, which are typical constituents of weaning foods, are highly susceptible to aflatoxin contamination (Egal et al., 2005). Level of exposure relative to body weight is higher for children than for adults and the rapid growth that occurs, and the additional nutrients required during this time. It has been reported that stunted growth is highly prevalent in parts of Africa and South Asia. For instance, in East and West Africa near to 42% and 36% respectively and approximately 36% of South-central Asian children aged less than 5 years have stunted growth and the effects have also been investigated in older children (UNICEF, 2012). This is attributed to the consumption of aflatoxin contaminated food and other associated factors.

Potential mechanisms

It has been suggested that aflatoxin exposure may disrupt the insulin-like growth factors (IGF) pathway through liver toxicity (Yun et al., 2016). Other potential mechanisms for the aflatoxin child growth impairment include the immune-suppressive effect of aflatoxin exposure that may increase infection susceptibility, consequently impairing nutritional status through appetite suppression and reduced nutrient absorption (Gong et al., 2008). Smith et al. (2012) postulated that exposure to aflatoxin may promote intestinal damage through protein synthesis inhibition, consequently leading to a reduction in the absorption of essential nutrients and subsequent impaired growth. The reactive aflatoxin-8, 9-epoxide preferentially binds to mitochondrial DNA during hepatocarcinogenesis and causes the disruption of mitochondrial functions and triggers ATP production which is required by various parts of the body. Mitochondrial damage can also leads to mitochondrial diseases and may be responsible for aging process (WHO, 2008) As the mitochondria get diseased, the nucleus detect energetic deficits in its area and attempts to compensate for the ATP shortages by triggering the replication of any nearby mitochondria but unfortunately, the response will promote mitochondrial replication that are causing the local energy deficit hence aggravating the problem (Wallace, 1997). The AFB1 also binds to DNA and cause structural changes to the DNA and leads to gene mutation as well as change in telomere length and the check points in the cell cycle. The binding of AFB1 to DNA at the guanine base in liver cells corrupts the genetic codes which regulate cell growth and thereby lead to tumor formation. Therefore, the reactive aflatoxin-8, 9-epoxide can affect the cell cycle at various stages by disrupting the various check points and furthermore exposure to aflatoxins leads to sperm aberration in mice

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(Ezekiel et al., 2011). Earlier, aflatoxins have been reported to be genotoxic in *in-vivo* sperm head abnormality tests (Fapohunda et al., 2008). This indicates that aflatoxins will result infertility.

Immune suppression

The immunosuppressive effects of aflatoxin, which include reduced antibody production, increased susceptibility to infectious diseases and reduced cell-mediated immunity, have been thoroughly investigated in many animal species (Bondy and Pestka, 2000). In humans the immunosuppressive effects of aflatoxin exposure, however, have not clearly been established, as only a limited number of studies have been conducted. A study conducted in the Gambia (Turner et al., 2003) found a reduction in the secretary Immunoglobulin A antibody in children (aged 6 to 9 years) with detectable AF-alb concentrations blood (n = 432) compared to those with non-detectable levels (n = 32). The sIgA is an important component of the mucosal barrier that protects against infectious diseases and uptake of harmful micro-organisms. This reduced level of sIgA driven by aflatoxin exposure could be a potential mechanism for the impaired child growth that was also observed in this cohort. The high prevalence of aflatoxin exposure coincides geographically with the high prevalence of the human immunodeficiency virus (HIV) in Africa. It has been suggested that the immunosuppressive effects of aflatoxin exposure may accelerate the progression of HIV (there is evidence to show that HIV positive individuals with high aflatoxin exposure, have lower levels of immune markers such as CD4+ T regulatory cells (P = 0.009) and naive CD4 + T cells (P = 0.029), as well as lower percentages of type B lymph cells (P = 0.03), compared to HIV positive individuals with low aflatoxin exposure) (Jiang et al., 2008).

Control of aflatoxins/aflatoxigenic fungi

Chemical and physical control

Wide range of chemicals such as sodium hydroxide, ammonium hydroxide, sodium hypochlorite, hydrogen peroxide and ozone (Tiwari et al., 2010) with different efficacy and selectivity are being used to degrade several types of mycotoxins. The main problems with these chemical and physical methods are their limited range of effect on various mycotoxins and some chemicals may lead to deterioration in animal health. Additionally, it causes a significant reduction in the quality of food products (Gowda et al., 2013). Adsorption is well investigated treatment among physical techniques. Studies related to this field refer to the alleviation of mycotoxicosis by the adsorption of a large molecular weight compound. Animal feed studies have demonstrated that clay additives, such as bentonites (containing smectites such as montmorillonite) minerals, can bind aflatoxins in ingested feed and reduce or eliminate the toxicity (Jaynes and Zarman, 2011). The surface of these minerals can be treated with organic compounds to create surface-modified clays which remove some contaminants more effectively than the untreated clay. These compounds can be applied as mycotoxin sequestering agents in animal feed or as mycotoxin adsorbing agents to seclude the toxic compounds usually *in vitro* and *in vivo* (Merrill et al., 2007).

Aflatoxin can be degraded from food by irradiation of sunlight, γ-radiation, or microwave (Herzallah et al., 2008). Microwave-induced argon plasma can degrade aflatoxin B1, deoxynivalenol and nivalenol in artificial solutions within seconds (Park et al.*,* 2007). On similar line, alkaline electrolyzed water can be a candidate for the removal of aflatoxin from edible plant oils through physico-chemical action at low levels of infection with high efficacy (Fan et al., 2013). Ozone is also one of the most powerful disinfectants and sanitizing agents for aflatoxins. It has been approved as generally recognized as safe which means it can be directly applied as an antimicrobial agent in the food industry (Inan et al., 2007). A most attractive aspect of ozone is that, after reaching its half-life (20-50 min), decomposition products do not represent any hazard for the treated materials (Karaca and Velioglu, 2014). In post-harvest treatment, gaseous and aqueous ozone phases are applied to inactivate bacterial growth, prevent fungal decay, destroy pesticides and chemical residues, control storage pests, and degrade aflatoxins (Patchimaporn et al., 2017). The mechanisms of ozone to inhibit microbial populations in food occur via the progressive oxidation of vital cellular components. It oxidizes polyunsaturated fatty acids or sulfhydryl group and amino acids of enzymes,

peptides, and proteins to shorter molecular fragments. In addition, ozone degrades the cell wall envelope of unsaturated lipids resulting in cell disruption and subsequent leakage of cellular contents (Das et al., 2006). The mechanism of ozone on the degradation of AFB1 and AFG1 involves an electrophilic reaction on the C8-C9 double bond of the furan ring causing the formation of ozonide (Diao et al., 2013). Since there is no C8-C9 double bond in the structure, AF-B2 and AF-G2 are more resistant to ionization than AF-B1 and AF-G1 (Agriopoulou et al., 2016). Even though the efficiency of ozone as a chemical detoxifier is high, a greater concentration is required to kill fungi or contaminated surfaces, while low concentration of ozone and short fumigation time is generally considered necessary in order to preserve product properties like colour, flavor, aroma, and vitamins (Chen et al., 2014). Among the different type of mycotoxins, aflatoxins are widespread in major food crops such as maize, groundnuts, tree nuts, and dried fruits and spices as well as milk and meat products there by inducing great health and economic crisis (Zeinvand-Lorestani et al., 2015). Due to these effects, different countries and international organizations have established Safe limit regulations in order to control aflatoxin contamination in food and feeds and also to prohibit trade of contaminated products (Juan et al., 2012).

The regulations on "acceptable health risk" usually depend on a country's level of economic development, extent of consumption of high-risk crops, and the susceptibility to contamination of crops to be regulated (Kendra and Dyer, 2007). Indeed, the established safe limit of AFs for human consumption ranges 4-30 mg/kg. The EU has set the strictest standards, which establishes that any product for direct human consumption cannot be marketed with a concentration of AFB1 and total aflatoxins greater than 2 mg/kg and 4 mg/kg, respectively (EC, 2010). Likewise, US regulations have specified the maximum acceptable limit for AFs at 20 mg/kg (Wu, 2006). However, if the EU aflatoxin standard is adopted worldwide, lower income countries such as those in Asia and Sub-Saharan Africa will face both economic losses and additional costs related to meeting those standards. This situation requires alternative technologies at pre- and post-harvest levels aimed

to minimize contamination of commercial foods and feeds, at least to ensure that aflatoxin levels remain below safe limits (Prietto et al., 2015). Nowadays, there is an increase in the reported number of possible aflatoxin degrading bioremediators, with microorganisms or enzymes (Giovati et al., 2015). Many species of bacteria and fungi have been shown to enzymatically degrade aflatoxins (Zsolt et al., 2017).

Biological control

Biological control using microbial antagonists either alone or integrated to reduce pesticide inputs has emerged as a promising approach for control of aflatoxins in both pre- and post-harvest systems (Zsolt et al., 2017). Good agricultural practices such as timely planting, maintaining optimal plant densities, avoiding drought stress, providing adequate nutrition and weed control, controlling pathogens and pests, and following proper harvesting practices should be employed to reduce the incidence of aflatoxin contamination in the field (Ehrlich and Cotty, 2004). Crop rotation and residue management also play an important role in controlling *A. flavus* infection in the field (Waliyar et al., 2013). Soil amendments like lime application, use of cereal crop residues, and farm yard manure have also been reported as effective management practices. Several organisms including bacteria, yeasts and non-toxigenic Aspergillus fungi have been tested for their ability to reduce mycotoxin contamination (Dimitrios et al., 2012). Even if the use of biocontrol agents has often been successful in the lab, its commercialization largely depends on whether it can consistently control fungi in different locations and cultivars thereby reduce mycotoxin levels below the legislative limits. The use of microorganisms as biocontrol agents has relatively narrow spectrum of activity as compared to most chemical fungicides. It is a complex process which involves a number of variables. In addition to efficacy towards the targeted plant pathogen, the new biocontrol products must be safe and cost-effective (Kohl et al., 2011). Despite all these problems, a number of successful commercial products have been launched to the market worldwide for mycotoxigenic fungi control, including Aspire (*Candida oleophila* strain 182; Ecogen Inc., Langhorne, PA), BioSave 10 and 11 (*Pseudomonas syringae* strains ESC10 and ESC 11;

Eco Science Corp., Worcester, MA), AF36 (atoxigenic strain of *A. flavus*) (Cotty, 1998), and Afla-Guard (atoxigenic strain of *A. flavus* NRRL 21882) (Dorner and Lamb, 2006). Advances in biological control have been supported by advances in molecular biology, genetic engineering and microbial genomics coupled with the discovery of the broad microbial catabolic potential. *A. flavus* has no known sexual stage and consequently most studies on its genetic variability have been made mainly by characterizing isolates based on vegetative compatibility. Complementary nitrate-non utilizing (nit) mutants are commonly used to identify compatible isolates (Dimitrios et al., 2012). There are numerous reports regarding to the antifungal activities of various lactic acid bacteria, which can act against several *Aspergillus* species and a broad range fungi. During carbon source metabolism, it produces antimicrobial compounds such as lactic acid, acetic acids, hydrogen peroxide, bacteriocins, and low-molecular-weight proteinaceous compounds and they compete with other species by environment acidification the and rapid nutrient depletion (Kabak and Dobson 2009). For example, it is earlier reported the antifungal activity of a *Lactobacillus casei* strain which inhibited both growth and aflatoxin production in *A. parasiticus* (El- Gendy and Marth, 1981). Coallier-Ascah and Idziak (1985) also reported that *Streptococcus lactis* cultures significantly reduced aflatoxin production by *A. flavus* and also antifungal activity from a *Leuconostoc mesenteroides* strain was reported (Suzuki et al., 1991). Another isolate from a silage inoculant of *Lactobacillus casei* pseudoplantarum, has also been reported to inhibit production of AFB1 and AFG1 by 80 and 92%, respectively (Gourama and Bullerman, 1997). Research by Corsetti et al. (1998) reported the antifungal activity of *Lactobacillus sanfrancisco* CB1, caused by the formation of short-chained fatty acids such as caproic acid and inhibited spoilage moulds from the genera Monilia, *Aspergillus, Penicillium,* and *Fusarium*. Moreover, aflatoxin inhibition by metabolites such as benzoic acid, cyclo (Gly-L-leucyl) methylhycantoin, and nevalon actone produced by specific lactic acid bacteria has been reported (Kabak et al.*,* 2006). In addition to the use of biocontrol agents in the field or at post-harvest level, a number of fungal or bacteria cultures have been shown to detoxify AFB1. Fungal taxa such as *Trichoderma, Phoma, Rhizopus,*

Sporotrichum sp. ADA, *Sporotrichum* sp. SF, and *Alternaria* species have been shown to degrade AFB1 by 65 - 99% within 5 days at 28±2°C (Enyiukwu et al., 2014). *Flavobacterium aurantiacum* can also significantly remove AFB1 from a liquid medium and a variety of food products including milk, peanuts, and corn without leaving toxic by-products. *Fusarium aurantiacum* strain NRRL-B-184 is reported to remove 79.9-98.9%, 92.699.8%, and 88.7-100% AFB1 from phosphate-buffered saline, peanuts and red pepper, respectively within 48h (Dimitrios et al., 2012).

One of the recent biological approaches which have proved effective under field conditions is the use of nonaflatoxigenic fungal strains which competitively exclude aflatoxigenic fungi (Zanon et al., 2013; Navya et al., 2017). Afla-Guard® (*A. flavus* strain NRRL 21882) is the first commercialized non-aflatoxigenic strain followed by AF36 (*A. flavus* strain NRRL 18543) and K49 (*A. flavus* strain NRRL 30797) recommended for use in groundnut, maize and cotton production, respectively in USA (Accinelli and Abbas, 2011). Similarly, in different parts of Africa, Afla-safe™, which is a mixture of four native atoxigenic strains, is in use to manage aflatoxins in maize and groundnut production (Probst et al., 2011). Further, bioplastic based granular formulation of non-aflatoxigenic *A. flavus* has been developed and successfully tested under filed conditions as soil and foliar applications (Accinelli et al., 2016).

Phytofungicides

Several edible plant extracts have been reported to have antifungal activity. Essential oils extracted from various plants have been shown to possess significant antifungal properties. The inhibitory effects of plant extracts on aflatoxin synthesis have also been examined well. The extract of *Azadirachta indica* was observed to be a good inhibitor of both growth of *A. flavus* and *A. parasiticus*, and toxin production *in vitro*. The *Ocimum canum* oil exhibits a broad range of activity against fungi, including human pathogens (Pradeep et al., 2003). Phenolic pulp extract of *Dialium guineense* has been proved effective for the prevention of lipid peroxidation, proteins oxidation and DNA fragmentation by AFB1 (Adeleye et al., 2014). Another adsorbent prepared

from bagasse has been proved effective for detoxification of AFB1 in the gastrointestinal tract of chicks and no negative symptoms was reported (Khan and Zahoor, 2014).

Aflatoxin oxidase, an enzyme of *Armillariella tabescens* presents a detoxifying activity towards AFB1. This reaction is dependent on oxygen and hydrogen peroxide producing, which may play the crucial role in detoxification of aflatoxin oxidase (Wu et al., 2015). Laccase enzyme also has proven detoxifying affinity for AFB1 (Zeinvand-Lorestani et al., 2015). Furthermore, *Manganese peroxidase* is an enzyme of *Pleurotus ostreatus* which may detoxify AFB1 according to the enzyme concentration and the incubation period (Mohamed and Aminata, 2016). In an evaluation by Hussain et al. (2012), it has been found that; clove, clove oil, and *Allium* spp. completely inhibit the growth of *Aspergillus flavus* and *A. parasiticus* in laboratory. In Cameroons, Tagne et al. (2008) showed that *Ocimum gratissimum, Cymbopogon citrates* and *Thymus vulgaris* extracts and essential oils retarded the development *of Fusarium verticilliodes* in maize both on-farm and *in-vitro*. According to Thana (2011), betel vine leaf completely inhibited growth and aflatoxin production of *A. flavus* while tobacco extracts (<10% w/v) significantly inhibited the fungus in culture. Neem-derived azadirachtin a norterpenoid is also a well-known aflatoxin inhibitor (Allameh et al., 2011). Neem oil (0.54.0% v/v) in a trial as noted by Da Costa et al (2010) resulted in 95% inhibition of aflatoxin B1 and B2 in vitro but failed to suppress the fungal growth.

Coffee leaves and fruits rich in caffeine alkaloids also showed antifungal activity against species of *Penicillium* and *Aspergillus* which works through inhibition of respiratory systems of the fungus or their glucose uptake necessary for production of acethyl CoA. Aqueous extracts of *Zingiber offcinale* and leaf extracts of *Trignoella faenum-graecum* inhibited aflatoxin production to significant levels while solvent extracts of *Oxalis corniculata* and *Z. offcinale* inhibited both growth and aflatoxin production of A. *flavus* (Reddy et al., 2011). Oil of *Nigella sativum* (1-3%) completely inhibited aflatoxin production while fruit extract of baobab inhibited total aflatoxin production by *A. parasiticus* by 69.1% (El-Nagerabi et al., 2013). Aqueous seed extracts from *Carica papaya* is rich

in the alkaloid carpaine which has high antioxidant activity and reported to be responsible for anti-mycotoxigenic action (Enyiukwu et al., 2014).

Genetic engineering of crop plants for resistance to *Aspergillus* infection

Genetic engineering technology is crucial to develop beneficial plant traits with higher ability to resist attack by insects and pathogens and increasing crop yields (Amalu, 2004). Development of such transgenic varieties is extremely valuable and will be an aid to breeding tools. Genome segments from the pathogenic fungi have been widely used to make transgenic plants varieties for increasing or decreasing the expression of several genes responsible for anti-fungal or anti-toxin activities. The availability of modern and efficient biotechnological tools which aid the evaluation of plant-pathogen protein interactions, genomics and field ecology of the fungus has encouraged researches on transgenic approaches to control invasion of cereals by *Aspergillus* (Enyiukwu et al., 2014). Various antifungal proteins and peptides have been isolated from a wide range of plants and tested for their antifungal activity against several *Aspergillus* species (Rajasekaran et al., 2009). Antifungal proteins and peptides used in genetic engineering of susceptible crop species to against *A. flavus* infection and aflatoxin contamination include defensins, thionins, plant non-specific lipid transfer proteins, knottins, impaiens antimicrobial peptides, ribosome inactivating proteins, lectins, and lectin-like peptides. Research strategies have been developed to control expression of stress-responsive genes through engineering of the regulatory machinery by using transcription factors and gene pyramiding instead of insertion of "single-action" genes (Prasad et al., 2012).

Conclusion

Aflatoxins are potentially carcinogenic and mutagenic with harmful effects to humans, livestock, and poultry. Aflatoxin contamination is a significant problem worldwide because it is a potent carcinogen and hepatotoxin. High exposure of aflatoxin over a relatively short time causes acute aflatoxicosis. The most well established health effect of chronic exposure are hepatocellular

carcinoma impaired child growth and immune suppression. This aflatoxin development can be both prevented and controlled. It can be prevented via good agricultural practices and by the use of appropriate measures and can also be controlled by using physical, chemical and biological measures. The biological method is safer and environmentally friendly which have no side effect if properly applied. More recently, biotechnological tools enable to develop resistant plant varieties and this opens the path of reliable and sustainable aflatoxin detoxification, but not widely utilized in developing countries due to sophisticated technology and limited research. This review recommends countries shall focus on biological detoxification strategies being supported with biotechnological researches.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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