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Review

Major mycotoxins occurrence, prevention and control approaches

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Mycotoxins are secondary metabolites secreted by many fungal species and found in many feeds and foodstuffs of especially in plants during their pre-and post-harvest, transportation, processing and storage and are detected in cereal crops. They are capable of causing disease and death in both humans and livestock and thereby induce great economic crisis. This review aims to examine the occurrence, prevention and control strategies of mycotoxins in Ethiopia; they are beneficial to the public and research institutes. Favorable environmental conditions such as temperature and prompting humidity facilitate fungal growth and mycotoxin development. Members of the fungal genera *Aspergillus, Fusarium,* and *Penicillium* cause frequent and problematic contamination of foods and feeds. Mycotoxin level of sample can be analyzed by sampling, preparation, extraction followed by a cleanup and detection performed by many instrumental and non-instrumental techniques; the molecular analysis is the best and promising approach. In Ethiopia, ochratoxins, fumonisins and aflatoxins frequently occur retarding crop production and livestock productivity; these in turn affect human health and income. To keep this effect dimmed, mycotoxin control and prevention mechanisms have a key role; prevention strategy weighs the overall effect. Moreover, biocontrol activities shall be strongly encouraged and focus has to be given to the aspect of mycotoxin.

Key words: Mycotoxin, aflatoxin, ochratoxin, fumonisin.

INTRODUCTION

Mycotoxins are secondary metabolites (Majeed et al., 2018) produced by a wide variety of filamentous fungi, including species from the genera *Aspergillus, Fusarium, Penicillium, Alternaria* and *Claviceps* that grow under different climatic conditions on agricultural commodities (Marin et al., 2013). Mycotoxins are ubiquitous and contaminate various feedstuffs and agricultural crops and induce a range of harmful effects (Jolly et al., 2011). These metabolites are produced and found in many feeds and foodstuffs especially in plants during their pre- and post-harvest, transportation, processing and storage and are detected in cereal crops (Ezekiel et al., 2014; Juan et al., 2014) and in peanuts (Afolabi et al., 2015). Aflatoxin, ochratoxin, fumonisin, deoxynivalenol and

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zearalenone are all considered the major mycotoxins produced in food and feedstuffs (Wagacha and Muthomi, 2008). Among the dangerous mycotoxins; aflatoxin, ochratoxin A and fumonisins (FB1 and FB2) represent the greatest health risk in tropical Africa (Manjula et al., 2009), Asia (Li et al., 2014) and the rest of the world (Alborch et al., 2012). Mycotoxins are capable of causing disease and death in both humans and livestock (Bennett and Klich, 2003). The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops (Turner et al., 2009). One mold species may produce many different mycotoxins, and several species may produce the same mycotoxin. The spectrum of toxins produced in a commodity largely depends on one or more fungal species/strains contaminating the commodity, type and composition of commodity, environmental conditions, climatic factors, and also handling practices such as pre-harvest agricultural practices, harvesting, drying, storage, and processing (Chilaka et al., 2016; Ogara et al., 2017). The toxic effect of mycotoxins reveals boundary less distribution and harm of health and economic attributes. Therefore, this review aims to examine the occurrence, effect and prevention and control strategies of mycotoxins; they are beneficial to the public and research institutes.

Occurrence and distribution of mycotoxins

Mycotoxins are ubiquitous. They can occur in cereals, cereal products and foods, feeds, animal products and soil. Animal feeds commonly harbor mycotoxins are wheat bran, noug cake, pea hulls and maize grain. Concentrated animal feedstuffs harbor the growth of mycotoxins. Mycotoxins can be transferred from feed to food of animal origin, as this food represents a significant route of exposure for humans. Apart from their toxicological effect in animals, they carry-over through animal derived products, such as meat, milk and eggs and transfer them into the human food chains (Demissie, 2018). Also, they may be distributed in pre-harvest period (time of plant growing), post-harvest during processing, packaging, distribution and storage of food products. Mycotoxin contamination intensity in crop varies geographically (Pereira et al., 2014; Marta and Bedaso, 2016). Conclusively, all crops and cereals which are stored improperly under favorable temperature and prompting humidity for a long time facilitate mold growth and can be subject to mycotoxin contamination (Ahmad and Jae-Hyuk, 2017); no boundary can limit fungal growth and mycotoxin production unless appropriate measures are taken.

Major types of mycotoxins

Mycotoxins contaminate food and feed and affect food security throughout the world, and their effect is higher, especially in low and middle-income countries (Antonio et al., 2018). Researchers have isolated and characterized more than 400 mycotoxin types. The most important and highly toxic mycotoxins include; aflatoxin, ochratoxin A, trichothecenes, zearalenone, fumonisins B1 and B2 (FUMB1, FUMB2), tremorgenic toxins, and ergot alkaloids (Margherita et al., 2012). The major fungi causing frequent and problematic contamination of foods and feeds with mycotoxins are members of the fungal genera *Aspergillus, Fusarium* and *Penicillium* (Ahmad and Jae-Hyuk, 2017)

Aflatoxins

Aflatoxins are poisonous carcinogens which interfere with the immune system and are produced by certain molds (*Aspergillus flavus* and *Aspergillus parasiticus*) (Jef et al., 2015) which grow in soil, decaying vegetation, hay, and grains of primarily found in hot, humid climates, colonizing mostly the aerial parts of plants (Marin et al., 2013). Mostly aflatoxins have related structure (Eaton and Groopman, 1994) (Figure 1). Aflatoxins have earned significant attention because of their deleterious effects on human and livestock health as well as on the international trade of foodstuffs. There are 20 known types of aflatoxins which are mainly classified into aflatoxin B1 (AFB1), B2, G1, G2, M1 and M2 based on structure, chromatographic and fluorescent characteristics (Ephrem, 2015).

AFB1 has higher toxicity and mainly metabolized by liver in to AFB1-8, 9-exo-epoxide and 8, 9-endo-epoxide which bind to DNA to form 8, 9-dihydro-8-(N7-guanyl)-9-hydroxy - (AFB1-N7-Gua) and AFB1-N7-Gua could be converted to two secondary lesion which is an apurinic site where more stable ring is opened. This implies that aflatoxins have an effect on amino acid metabolism. The major human cytochrome P450 (CYP) enzymes involved in aflatoxin metabolism are CYP3A4, 3A5, 3A7, and 1A2 (Marin et al., 2013).

Drought and stress increase aflatoxin spread in the field and can be produced due to insufficient drying of contaminated crops before storage or stored under humid conditions (Jef et al., 2015). Due to their stability to severe processes of roasting, extrusion, baking, and cooking, aflatoxins also induce a great problem in processed foods, such as roasted nuts and bakery products and it can be found alone or simultaneously, as well as co-occurring with other mycotoxins such as OTA (Marin et al., 2013).

Ochratoxins

Ochratoxin A (OTA) was first identified and characterized from fungal cultures in South Africa (Van der Merwe et

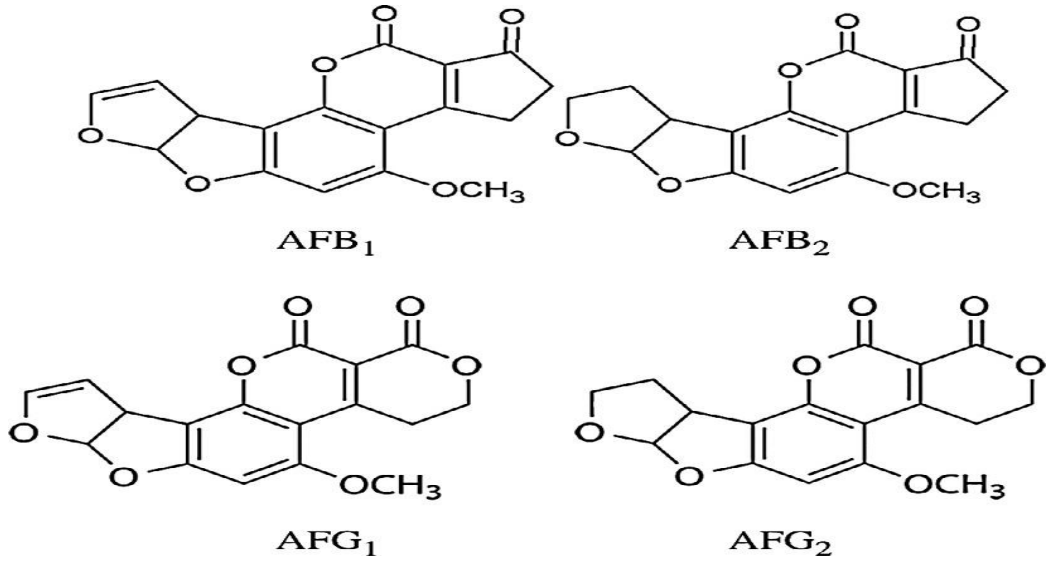

Figure 1. Molecular structure of aflatoxins B1, B2, G1, and G2.
Source: Marin et al. (2013).

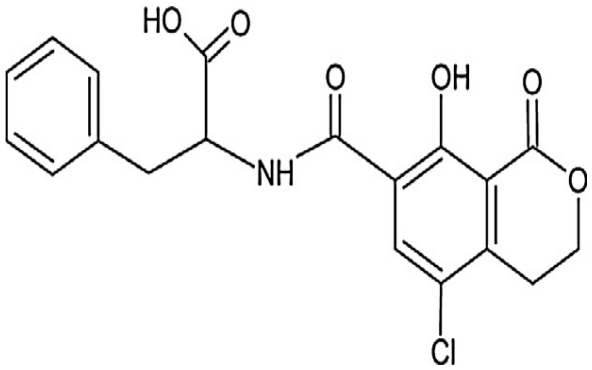

Figure 2. Molecular structure of ochratoxin A.
Source: Marin et al. (2013).

al*.,* 1965). It is a phenylalanyl derivative of a substituted iso-coumarin (R)-N-[5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-y1)-carbonyl]-L phenylalanine (Figure 2).

Ochratoxin A is produced by two main genera of fungi, *Aspergillus* and *Penicillium* with main producing species of *Aspergillus* Section *Circumdati*, *Aspergillus* Section *Nigri, Penicillium verrucosum,* and *Penicillium nordicum* (EFSA, 2006a).

Ochratoxin A is the most toxic member of the ochratoxin which is structurally similar to the amino acid phenylalanine. Thus, it has an inhibitory effect on a number of enzymes that use phenylalanine as a substrate, particularly Phe-tRNA synthetase, resulted in the inhibition of protein synthesis. Ochratoxin A is a mitochondrial poison, which causes cellular damage, oxidative burst, lipid peroxidation, and oxidative phosphorylation. Furthermore, it increases cell apoptosis and it is a stable and heat resistant which is not damaged by common food preparation temperature (above 250^{o}C for several minutes reduce its concentration (Marin et al*.*, 2013).

Fumonisins

Fumonisins are fusarium toxins first discovered in 1988 [Gelderblom et al. (1988) cited in Marasas (2001)] and constitute the large family of compounds (Antonio et al*.,* 2018) which are produced by a number of fungi most dominantly *Fusarium verticillioides* and *Fusarium proliferatum.* Other fungal species, including *F. dlamini, F. nygamai* and *F. napiforme* also produce fumonisins (EFSA, 2005a). Fumonisins have strong structural similarity to sphinganine which are the precursor of sphingolipids (Figure 3).

There are about 12 types of known fumonisin types and the most important ones are FB1, FB2, and FB3 of which FB1 is most toxic. They are the mostly found in maize grown in warmer areas. Since *F. verticillioides* and *F. proliferatum* grow in a wide range of temperatures only at relatively high water activities (aw > 0.9), FBs are formed prior to harvest or during the early stage of storage and its concentration does not increase during storage except under extreme conditions. They are fairly heat-stable, and toxicity can be minimized only during processes where temperature is beyond 150°C (Marin et al*.,* 2013). The chemical name of this mycotoxin is 1,2,3-propanetricarboxylicacid,1,10-[1-(12-amino-4,9,11-trihydroxy-2 methyl tridecyl)-2-(1-methylpentyl)-1,2-ethanediyl]ester.

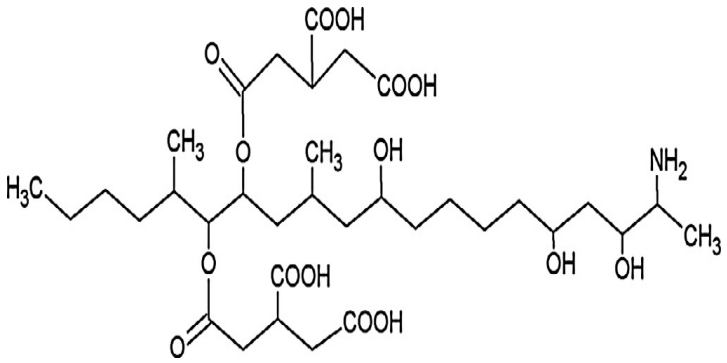

Figure 3. Molecular structure of FB1.
Source: Marin et al. (2013).

Factors favoring fungal proliferation and mycotoxin production

The favoring conditions for mycotoxin production relate mainly to poor hygienic practices during transportation, improper storage, processing, high temperature and moisture content and heavy rains (Bhat and Vasanthi, 2003). These conditions are typically observed in different African countries including Ethiopia. The demand for the storage of food substances has been increased due to the increasing population.

Researchers have found a variety of factors which favor the production of mycotoxins. Those are grouped as physical, chemical, and biological factors. Physical factors include environmental conditions viz temperature, relative humidity, and insect infestation while chemical factors include the use of fungicides or fertilizers as well as biological factors depend on the interactions between the colonizing toxigenic fungi and the substrate, in fact some plant species are more susceptible to colonization while environmental conditions may increase the vulnerability of others are more resistant (Margherita et al*.,* 2012). In other ways thus factors can be either intrinsic, extrinsic, processing or implicit each of which including moisture content, water activity, substrate type, plant type and nutrient composition; climate, temperature, oxygen level; drying, blending, addition of preservatives, handling of grains; insect interactions, fungal strain and microbiological ecosystem respectively (Gabriel and Puleng, 2013).

Mycotoxin analysis techniques

Determination of mycotoxin level in food sample is usually accomplished by certain steps: sampling, preparation, extraction followed by a cleanup and detection which is performed by many instrumental and non-instrumental techniques (Ahmad and Jae-Hyuk, 2017) (Figure 4).

Chromatographic techniques

This technique is the most commonly used method for mycotoxin analysis. Thin layer chromatography (TLC) is one of earliest quantitative method for mycotoxin screening based on visual assessment or instrumental densitometry. However, recent advances in mycotoxin analysis have introduced fast and convenient chromatographic technologies for both detection and quantification such as high performance liquid chromatography (HPLC) coupled with ultraviolet, diode array, fluorescence or mass spectrometry detectors and ultra HPLC with reduced column packing material. Highly advanced coupling liquid chromatography techniques, mass-spectrometry and HPLC coupled mass spectrometric or fluorescence detectors are frequently used in mycotoxins analysis while other chromatographic techniques are rarely used because of limited sensitivity and specificity. HPLC-FLD (HPLC coupled with fluorescence) is used for single mycotoxin analysis and HPLC-MS/MS (HPLC coupled with mass spectrometry) is the best choice for simultaneous determination of multiple mycotoxins (Ahmad and Jae-Hyuk, 2017).

These are the ultimate methods used for the identification/confirmation of the identity of mycotoxins, including those which are masked and do not fluoresce or do not absorb visible UV light. Such methods allow the identification and sometimes the quantization of many mycotoxins in a single sample. As mycotoxins are real problems for health, there will always attract attention and, certainly, methods for their analysis will continue to improve. Because of the potential co-occurrence of such contaminants, the challenge is to develop screening methods for their rapid simultaneous detection of multiple families of mycotoxins from the same sample. But the differences in their chemical and physical properties and of concentration range of interest have made simultaneous detection very difficult. In this regard HPLC technique coupled with mass spectrometry or multiple detectors have good prospects. Luminex's xMAP technology is another technology comprising existing

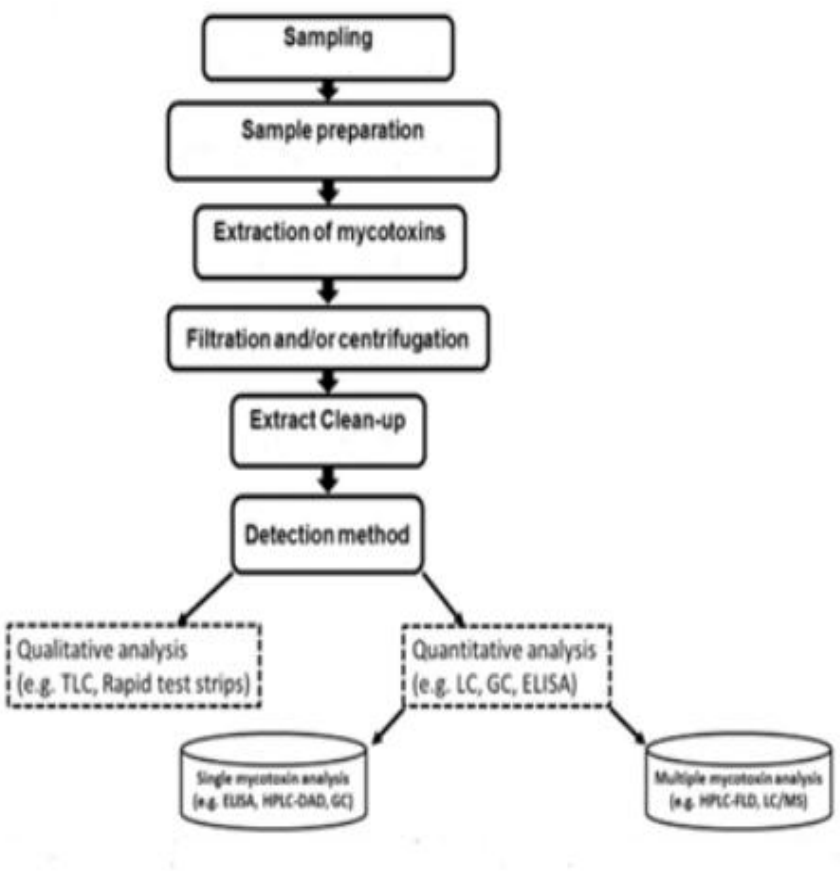

Figure 4. Common steps in mycotoxin analysis (Source: Ahmad and Jae-Hyuk, 2017).

technologies-flow cytometry, microspheres, lasers, digital signal processing and traditional chemistry. The range of applications is considerable throughout the drug-discovery and diagnostics fields, as well as in basic research. Microspheres are dyed to create 100 distinct colors. Each microsphere has a 'spectral address' based on red/infrared content. The suspendable microspheres are coated with capture reagents such as antibody or oligonucleotides. Sample is then added to microspheres and the analyte is captured by the microspheres. A fluorescent reporter tag is then added and results are read using a compact microsphere analyzer. The advantages of the technology are: high speed, high throughput, multi-analyte detection, versatility and reproducibility.

Immunochemical techniques

Enzyme linked immunosorbent assay

Immunochemical techniques are a widely established technology employed mainly for rapid and sensitive screening of mycotoxins in unprocessed commodities/ raw materials. Enzyme Linked Immunosorbent Assay (ELISA) provides rapid screening, with many kits commercially available for detection and quantification of all major mycotoxins (Ahmad and Jae-Hyuk, 2017). This assay enables the qualitative, semi-quantitative and quantitative determination of mycotoxins in food and feed. The principle is based on the use of antibodies and specific color changes. ELISA tests are found commercially in different forms such as single disposable membrane-based test, micro titer plate and tube assay methods (Kristine and Florian, 2018).

Fluorescence polarization immunoassay

Fluorescence polarization immunoassay is a newly developed immunoassay technique based on the indirect measurement of the changes of molecule rotation in a solution. A fluorochrome labeled mycotoxin with a low molecular weight acts as the antigen. The aggregation with the anti-mycotoxin antibody results in the formation of an immune complex, gaining in weight and therefore slowing the rotation rate of the molecule. That causes an increase in polarization of emitted light which can be

detected by fluorescence polarization reading instruments. The deficiency of such assays is the problem of cross reactivity which is not completely deleted and hence further research is needed to evaluate this influence (Kristine and Florian, 2018).

Biosensor technology

Biosensors enable the detection of the analyte in a sample because of the interaction between the analyte and biological sensitive elements such as enzymes, tissues, nucleic acids or antibodies. The interaction results in a signal which can be detected by a transducer (optical or physicochemical detection) and is transformed in an utilizable measured variable (Kristine and Florian, 2018).

Molecular techniques

DNA and aptamer based biosensors

It is reported that DNA biosensor based method is used to analyze AFM1 in milk samples. In this technique, thiol-modified single stranded DNA probe is immobilized on a monolayer of cysteamine and gold nanoparticles. The DNA biosensor particularly bound the AFM1 and detection of the process is carried out with electrochemical impedance spectroscopy and cyclic voltammetric techniques (Dinckaya et al*.,* 2011). Another form to use DNA in biosensors is aptamer based technique. Aptamers are peptide molecules (DNA or RNA duplex structures) which can bind with specific analyte. Chen et al*.* (2012) reported a DNA duplex structure with an anti-OTA-aptamer such as fluorophore and quencher and binding ochratoxin A to this structure leads to an increase of the fluorescence (Kristine and Florian, 2018).

Molecular imprinting polymers

It is a synthetic technique designed to imitate natural recognition entities viz antibodies and biological receptors. This highly selective molecular technique uses cross-linked polymers which are electrochemically prepared by the reaction of monomer and cross linker in the presence of mycotoxins (Ahmad and Jae-Hyuk, 2017).

Impacts of mycotoxins

Some countries have set permitted levels of mycotoxins in food to control and reduce the effects of fungal toxins. These levels are variable and depend up on economic status of the countries. In US, Food and Drug Administration (FDA) has permitted a total amount of 20 ng/g aflatoxin in livestock feed and 0.5 g/kg or 50 ng/l in milk and In European countries, permitted levels of aflatoxin M1 in milk, milk products and baby food are 0.005 mg/kg. Also, different countries have set different regulations for permitted levels of aflatoxin in livestock feed. For instance, European Union (EU) has set permitted levels of aflatoxin from 0.05 to 0.5 µg/kg. Factors such as weather conditions are also effective in determining permitted levels of aflatoxin. Permitted levels of this toxin in tropical countries are higher compared to mild and cold countries. In Africa, only some countries such as Ivory Coast, Egypt, Kenya, Malawi, Nigeria, South Africa, Senegal etc. have permitted level of mycotoxins in food and feed ingredients and the rest including Ethiopia have no result. This contributes more effect of mycotoxins to human health and world economy at large (Demissie, 2018).

Health impact

Mycotoxins cause diseases in human and animals called mycotoxicosis and its severity depends on the toxicity rate (Peraica et al., 1999). Mycotoxins are all heat-stable and not destroyed by cooking and normal industrial processing (Margherita et al*.,* 2012) and are endangering human health, animal production and countries economy (WHO, 2006). Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. Aflatoxin B1 is a potent liver carcinogen in humans and is acutely toxic at high levels of exposure. Its exposure is also associated with childhood stunting (Geremew, 2015). In countries with chronic aflatoxin contamination, animal production is severely reduced thereby minimizing dietary protein and milk quality. Poor awareness about aflatoxins, appropriate control measures to control contamination in the field and in storage and the negative health effects of aflatoxin consumption are reported in most African countries including in Ethiopia (Antonio et al., 2018). Reasons for this are the wide spread occurrences of mycotoxins at frequently high levels and food consumption patterns that can result in large intake of a single cereal such as corn. Additional factors on health impact are also prevalent poverty and malnutrition (Kristine and Florian, 2018).

Generally, mycotoxins are carcinogenic, mutagenic, immunotoxic, hepatotoxic, teratogenic, neurotoxic, foetotoxic, hemorrhagic, nefrotoxic, estrogenic and dermotoxic and specifically aflatoxins cause diseases such as aflatoxicosis, hepatocarcinogenicity, encephalopathy and Reye's syndrome; whereas ochratoxin causes balcan endemic nephropathy (BEN) and kidney tumors as well as fumonisins cause esophageal cancer, hepatocarcinogenicity, pulmonary

edema, leukoencephalomalacia, hepatotoxicity and nephrotoxicity (Margherita et al*.,* 2012). Moreover, mycotoxins have been linked to birth defects in many animals, nervous system problems (tremors, limb weakness, staggering, and seizures), and tumors of the liver, kidneys, urinary tract, digestive tract, and the lungs (USDA, 2006).

Economic impact

The economic effects attributed to mycotoxin infection are widely felt in all sectors of the production and consumption of grain products. It is directly derived from crop, livestock losses, and indirectly, from the cost regulatory programs designed to reduce risks to animal and human health. Contamination can result in direct economic impact through limited yields, price discounts, restricted end markets and export rejections from importers. Mycotoxin contamination has an adverse economic effect in reducing the yield for food and fiber crops and food contamination with mycotoxin results in the huge and universal economic crisis (USDA, 2006; Geremew, 2015). The livestock industry is also mostly affected by mycotoxins. It makes animals more prone to disease by weakening their immune system and decrease vaccination response. In other ways, it may cause loss in productivity in the dairy cow industry, specifically in the case of aflatoxins, additional losses involve the clearance times farmers have to wait in order to allow animals to excrete all AFM1 from their systems (Marroquín et al*.,* 2014).

Occurrence of mycotoxin in Ethiopia

From the African perspective, aflatoxins, ochratoxins, and fumonisins are considered to be widespread in major dietary and export oriented crops (Vismer et al*.,* 2015). Even if the proportion is different, research by Ayalew (2002) revealed that ochratoxins, fumonisins and aflatoxins dominantly occur in Ethiopia. As Dereje et al*.* (2012) reported the total collected groundnut samples were found to be 100% positive for *Aspergillus* species and shows that the groundnut production in the study region is at high risk of contamination. According to Ezekiel et al*.* (2018), mycotoxins have been present in Ethiopian alcoholic and non-alcoholic beverage input crops such as barley, maize, millet, sorghum, teff and wheat. Chauhan et al*.* (2016) analyzed that all maize samples intended for human consumption have shown aflatoxin toxicity higher than those recommended by Food and Drug Administration and European Union regulatory levels as determined by chromatographic techniques. Moreover, Tameru et al. (2008) found that *Fusarium* and *Aspergillus* toxins were higher in storage than pre-harvest samples exceeding the safe limits of European countries. Since the storage practice of cereals like sorghum in underground pits increase the moisture content in the grain, sorghum samples were reported containing fumonisins with higher concentration of 2.2 µg/g and zearalenone, deoxynivalenol and nivalenol with lower frequency (Ayalew et al*.,* 2006) and microbiological analysis of the samples revealed that fifteen species of fungi were identified from the maize samples. Aspergilli were the most frequent fungi, occurring in 94% of the samples followed by *Fusarium* species (76.5%) and *Penicillium* species (64%) (Ayalew, 2010). And in southern Ethiopia, 100 maize samples were analyzed and resulted in mean fumonisin concentration of 1.68 µg/g (Tameru et al*.,* 2009). Abebe et al*.* (2017) also reported the occurrence of urinary aflatoxin in children causing aflatoxicosis. Additionally, according to the semi-annual report of feed the future innovation lab for the reduction of post-harvest loss (2016), pests and mycotoxins have both been identified as critical issues and especially maize, wheat, and chickpea and were found to be highly infested commodities.

Mycotoxin prevention and control strategies

Prevention

It has been accepted that prevention of mycotoxin contamination of crops is the primary measure and alternative over the other control methods. In the field, it involves good agronomic practices that increase plant healthy growth and prevent infection by toxigenic fungi. These practices include prevention of drought stress and using resistant varieties, crop rotation aimed to reduce of mycotoxigenic fungal biota, optimum maturity harvest with rapid drying and good storage conditions as well as overall field management (Gabriel and Puleng, 2013).

Since fungi cannot grow in properly dried foods, efficient drying of commodities and maintenance of the dry state is an effective control measure against mycotoxin production. To prevent mycotoxin production, drying should be done soon after harvest and as rapidly as feasible. The water content for safe storage corresponds to water activity of about 0.7 (is an effective technique used throughout the world for controlling fungal spoilage and mycotoxin production in foods). While it is possible to control fungal growth in stored commodities by controlled atmospheres or use of preservatives or natural inhibitors, such techniques are almost always more expensive than effective drying, and are thus rarely feasible in developing countries.

After harvest, the most important factor to prevent fungal growth within the grain is reducing the moisture content down to create unfavorable condition for fungal growth. Also antifungal preservatives may be used as a control strategy and the application of different substances such as organic acids, antibiotics, herbs,

spices, essential oils, pesticides, fumigants, antioxidants and chlorine has been reported to be effective (Ayalew, 2002).

Damaged grain is more prone to mycotoxin contamination and thus it is important to avoid damage before and during drying, and in storage. Insects are a major cause of damage. Field insect pests and some storage species damage grain on the head and promote fungal growth in the moist environment of the ripening grain. In storage, many insect species attack grain, and the moisture that can accumulate from their activities provides ideal conditions for the fungi. To avoid moisture and mould problems, it is essential that numbers of insects in stored grain be kept to a minimum. Such problems are compounded if the grain lacks adequate ventilation, particularly if metal containers are used.

Appropriately timed ventilation, fan-forced if necessary, will greatly assist the maintenance of the commodity at below 0.7 aw. Ideally, all large-scale storage areas should be equipped with instruments for measuring humidity, so that air appropriate for ventilation can be selected. Sealed storage under modified atmospheres for insect control is also very effective for controlling fungal growth, provided the grain is adequately dried before storage, and provided diurnal temperature fluctuations within the storage are minimized. If commodities must be stored before adequate drying this should be for only short periods of no more than, say, three days. Use of sealed storage or modified atmospheres will prolong this safe period, but such procedures are relatively expensive and gaslight conditions are essential. A proven system of storage management is needed, with mycotoxin considerations an integral part of it. A range of decision-support systems is becoming available covering the varying levels of sophistication and scale involved (Olusegun et al*.,* 2013).

Control

Aflatoxin control strategies have been developed since the 1960s. Generally, these strategies can be divided into three groups: pre-harvest control (field management and use of biological and chemical agents), harvest management and postharvest detoxification (use of natural and chemical agents and irradiation) (Gabriel and Puleng, 2013). Moreover, based on the technique or the method applied, mycotoxin control strategies can be physical, chemical or biological.

Physical control

Physical approaches include hand sorting, washing and crushing combined with de-hulling (Marta and Bedaso, 2016). Research found that gamma irradiation at doses from 15- 30 kilo gray resulted in mycotoxin reduction in groundnut kernels. Also, cooking and steaming for a long time under pressure reduces mycotoxin load (Ephrem, 2015).

Chemical control (mycotoxin detoxification)

Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market. However, in order to protect food quality and the environment, low persistent synthetic fungicides are still relevant at present to prevent diseases of food crops (Pal and Gardener, 2006). In recent years, the need to develop fungal disease control measures using phyto-chemicals as alternative to synthetic chemicals has become a priority of scientists worldwide. Phytochemicals are naturally occurring, non-nutritive biologically active chemical compounds of plant origin, have some protective or disease-preventive properties. Some phytochemicals are injurious to fungi and could be used to protect crops, animals, humans, food and feeds against toxigenic fungi and mycotoxin, Therefore, it is important to find a practical, cost effective and non-toxic method to prevent fungal contamination and mycotoxins load in stored farm produce. Use of natural plant extracts and bio control agents provides an opportunity to avoid chemical preservatives. A multitude of fungi toxic plant compounds (often of unreliable purity) is readily available in the fields (Toba et al*.,* 2013).

Biological control

Biological control has been an emergent alternative to efficiently manage mycotoxins production and hence, reducing the use of chemical compounds. Toxigenic fungi are either true pathogens as *fusarium* species or secondary pathogens or saprophytes and effective secondary colonizers as *Aspergillus* and *Penicillium* species. The use of biocontrol agents for toxigenic fungi control has focused on the efficacy in terms of control of germination/growth/colonization by the fungi to raw or processed food commodities and reduction in the production of the associated mycotoxin by often targeting the biosynthetic genes involved in toxin bio-synthesis (Medina et al*.,* 2017).

Microbial strategies

Nowadays, research proved and more focus has been given to microbial control of mycotoxins. As it have been reported by Guan et al*.* (2011), it is possible to control aflatoxin B1 by AFB1 binding with probiotics/dairy strains of lactic acid bacteria such as *Lactobacillus, Lactococcus*, *Bifzdobacterium sp*. and *Propionibacterium* and yeast

strains (*Saccharomyces cerevisiae*) (Tsitsigiannis et al*.,* 2012).

Moreover, microbial transformation such as bacterial biotransformation for instance bacterial strain *Nocardia corynebacterioides* (formerly *Flavobacterium aurantiacum);* fungal biotransformation by non-aflatoxin-producing filamentous fungi, edible fungal strains and biotransformation by its producing fungi which is dependent on mycelial lysis and high-aeration and microbial enzyme transformation such as peroxidase enzyme such as laccase enzymes from various sources play an important role in controlling AFB1 contamination (Guan et al*.,* 2011).

Fumonisins particularly fumonisin B1 (FB1) have gained international concern by its agro economic and food safety effect. Various reports have been forwarded with the microbial control of FB1 by interaction between *Fusarium* and potent bacterial antagonists. *L. rhamnosus* is reported to have an effect on production of mycotoxin production by *F. proliferatum, F. verticillioides* and *F. graminearum.* Furthermore; different bacterial strains such as *Azotobacter armeniacus, B. subtilis, Bacillus* spp*., Burkholderia cepacia* and others act against *F. verticillioides, Clonostachys rosea i*mposes *F. verticillioides, F. proliferatum* and fungal strains such as *F. verticillioides, F. proliferatum* act against *F. graminearum* (Tsitsigiannis et al*.,* 2012).

Additionally, many bacterial strains belonging to *Streptococcus, Bifidobacterium, Lactobacillus, Butyribrio, Phenylobacterium, Pleurotus, Saccharomyces, Bacillus* and *Acinetobacter* genera and certain fungi belonging to the genera *Aspergillus* (*A. fumigatus, A. niger, A. carbonarius, A. japonicus, A. versicolor, A. wentii* and *A. ochraceus*), *Alternaria, Botrytis, Cladosporium, Phaffia, Penicillum* and *Rhizopus* (*R. stolonifer* and *R. oryzae*) have more than 95% OTA degradation and some have shown detoxifying properties. Similar to aflatoxins and fumonisins, *Saccharomyces* yeasts can be used for the decontamination of OTA (Reddy et al*.,* 2010).

Biotechnology for mycotoxin elimination

Traditional approaches to study host plant resistance to mycotoxins especially *A. flavus* was not efficient in identifying the specific metabolites or components which have direct effect on aflatoxin biosynthesis. The absence of durable sources of resistance in the germplasm of various crops led to concerns in updating knowledge on biological mechanisms to control aflatoxin biosynthesis and the efficiency of host-plant resistance factors to aflatoxin deposition with in crops. Knowledge on biotechnological strategies is considered with the following three basic requirements; knowledge about the fungus; environmental factors (drought stress); and host-plant resistance. Genetic studies have done to monitor the molecular characteristics of the toxin in the fungus. Environmental factors such as drought have a direct effect on the suppression of bio-competitive phytoalexins and antifungal proteins or protective compounds usually phenols which influence aflatoxin synthesis and retard seed maturation. As drought increases aflatoxin contamination, drought tolerance trait does not seem to be sufficient by itself to reduce aflatoxin production. Therefore, identification of useful variations among genotypes provides molecular tools for selection of resistant lines of which genetics, genomics and proteomics have to be further analyzed.

Advances in genomics, marker development and genetic engineering technology have the potential to improve food safety from aflatoxin contamination. Research advances in microarrays, fungal expressed sequence tags (EST), and whole genome sequencing have led to discovery of many genes responsible for host plant interactions and aflatoxin contamination. It starts with candidate gene identification and going to mutagenesis (Targeting induced local lesions in genomes), molecular breeding approaches and genetic engineering. Finally, Host-induced gene silencing (HIGS) is done by which the pathogen is directed by the host plant to down-regulate the expression of its own genes (Bhatnagar-Mathur et al*.,* 2015). HIGS is a promising technology in which the pathogenic fungi is directed by the host plant to down-regulate the expression of its own genes, without requiring the host plant to express a foreign protein (Nakayashiki, 2005).

Gene manipulation studies are extensively undergoing to control the molecular regulation of aflatoxins; success has been achieved in identification of genes involved in aflatoxin biosynthesis and their subsequent cloning for use as “molecular tools” for identifying agents and compounds which act as inhibitor in the aflatoxin biosynthesis pathway. This is controlled by specific Cys6Zn2 DNA binding proteins, AflR, along with a number of co activators such as AflJ, LaeA, VeA, VelB and VosA that adjust the timing of AflR’s activity by forming a complex in the nucleus (Figure 5). This knowledge has opened the possibility of identifying resistance mechanisms which inhibit aflatoxin biosynthesis and fungal growth, apart from providing a robust and economical way of indirect measurements of fungal toxin control (Ehrlich, 2014).

Molecular breeding approaches

Marker identification to speed up resistance traits transferred into agronomically viable genetic backgrounds is necessary due to the polygenic and complex resistance to mycotoxin contamination (Bhatnagar-Mathur et al*.,* 2015). Quantitative trait loci (QTL) studies are also used to map resistance-associated protein (RAP) genes associated with maize aflatoxin resistance such as an embryo-specific protein, heat shock and

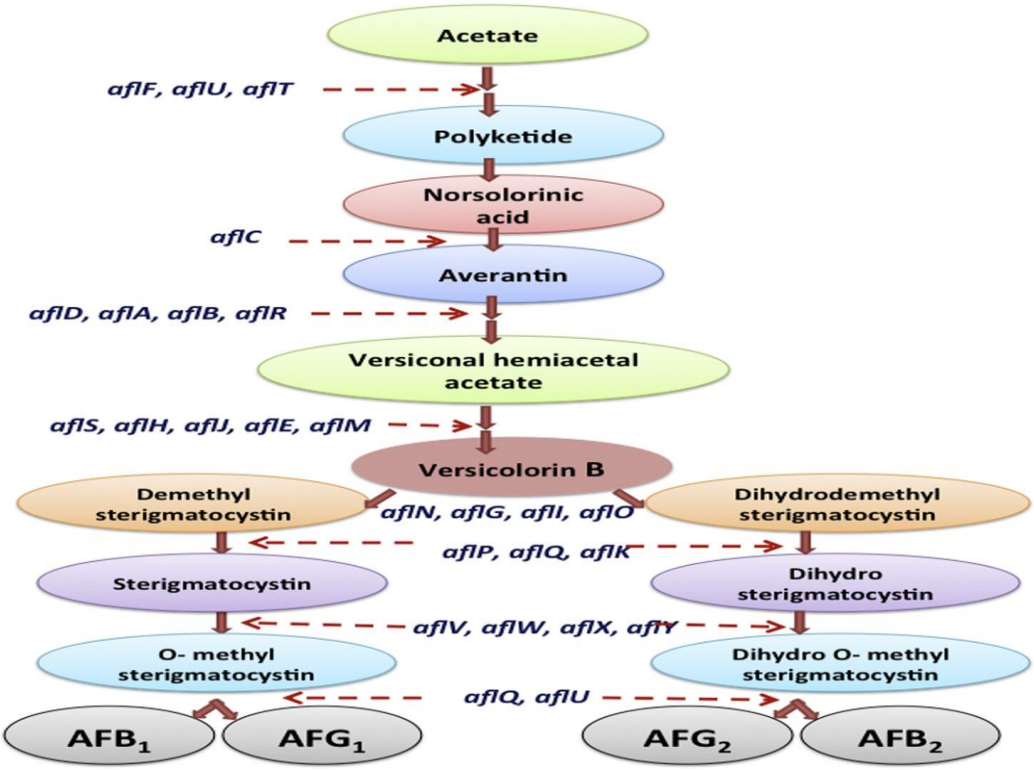

Figure 5. Schematic representation of intermediates and key genes involved in aflatoxin biosynthetic pathway. Source: (Bhatnagar-Mathur et al., 2015).

glucanase genes as well as a glucose dehydrogenase. MpM1, the first gene-based marker specifically developed for resistance to aflatoxin in maize, has now been integrated into existing marker-assisted selection programs for incorporating resistance into elite maize breeding lines. Converting the underlying genetic and molecular information in to normal language continues to be a major challenge due to large genomic regions containing these QTL. To address these problems/ difficulties, molecular markers closely linked to the QTL are needed to facilitate the breeding process by reducing breeding cycle (Brown et al., 2013).

Proteomics are also being used as a novel tool in mycotoxin research to identify RAPs and the candidate resistance genes associated with the resistance mechanisms among the resistant lines, in comparison with susceptible lines. The discovery of storage and stress-related proteins as biomarkers for aflatoxin is potentially useful for breeders to find appropriate strategies to improve plant resistance and stress tolerance of host plants against contamination (Wang et al., 2010).

RECOMMENDATIONS

Since mycotoxins are ubiquitous (Gizachew et al., 2016) and they can appear everywhere in every commodity thereby inducing numerous economic and health crisis, appropriate and environmentally friendly prevention and control strategies shall be given priority. Moreover the government shall consider the use of (biotechnological) molecular approaches to control mycotoxins and researches have put forward an insight into molecular based techniques. Because mycotoxins have worldwide distribution and effect, researchers in Ethiopia and the WHO in collaboration with FDA should set out appropriate consumption limit standard and sound measures.

CONFLICT OF INTEREST

The author has not declared any conflict of interests.

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