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An overview of challenges and elimination of aflatoxin contamination in maize (*Zea mays*)

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Abstract
Maize is an important cereal crop for domestic markets as well as for foreign trade in several developing and developed countries. It is also one of the most valuable cash crops in India. However, its production is constrained by *Aspergillus* species, which cause quantitative losses and produce highly toxic and carcinogenic chemical substances known as aflatoxins. This article critically reviews Aflatoxin contamination in maize (*Zea mays* L.) in India and its elimination. Although maize has a huge potential as a cash crop to improve livelihoods of farmers and traders in various parts of India, its market is declining and export of the crop has come to a standstill. This is due to aflatoxin contamination of the crop and the difficulty of meeting tolerance limits by importers and food processors, leading to rejection of the crop and reduction in market demand. Aflatoxin contamination is both a pre-harvest and post-harvest problem. Many developed countries have laid down specific regulations for import and export of those items in terms of economic implications. In the light of present status different measures (prevention, elimination, and decontamination/inactivation) have been discussed in this review to minimize the risk of aflatoxin contamination in pre-harvest, harvest, post-harvest, processing stage and storage conditions.

Keywords: Aflatoxin, *Zea mays*, *Aspergillus flavus*, maize, toxin

1. Introduction
Maize plays an important role in the livelihoods of poor peoples and in the rural economy of many developing countries. *Aspergillus flavus* is a fungus of economic and toxicological importance due to the production of aflatoxins and other chemicals with deleterious properties (e.g., aspergillic acid, cyclopiazonic acid, kojic acid, helvulic acid, etc.). This fungus is ubiquitous in the environment, being readily isolated from plants, air, soil, and insects (Wicklow *et al.*, 2003, Matthew *et al.*, 2017) [113, 78]. Aflatoxins are cancerous secondary metabolites produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus* in agricultural foodstuff such as maize, peanuts grains, cereals, and animal feeds. Aflatoxins are difuranocoumarin molecules synthesized through the polyketide pathway (Bennett and Klich, 2003) [16]. Six out of 18 different types of aflatoxins that have been identified are considered important and are designated as B_1, B_2, G_1, G_2, M_1, and M_2, respectively (Dors *et al.*, 2011) [40]. These aflatoxin groups exhibit molecular differences. For example, the B-group aflatoxins (B_1 and B_2) have a cyclopentane ring while the G-group (G_1 and G_2) contains the lactone ring (Gourama and Bullerman, 1995) [52]. Whereas the B-group aflatoxins exhibit blue fluorescence, the G-group exhibits yellow-green fluorescence under ultraviolet (UV) light, thus making the use of fluorescence important in identifying and differentiating between the B and G groups. Aflatoxin B_1 is the most common (Hussein and Brasel, 2001) [65] and the most widespread (Cullen *et al.*, 1993; Kok, 1994) [32, 72] in the world and accounts for 75% of all aflatoxins contamination of food and feeds (Ayub and Sachan, 1997) [7]. Aflatoxins M_1 and M_2 are hydroxylated products of aflatoxins B_1 and B_2, respectively, and are associated with cow milk upon ingestion of B_1 and B_2 aflatoxins contaminated feed. Moreover, once formed from B_1 and B_2 forms, aflatoxins M_1 and M_2 remain stable during milk processing (Stroka and Anklam, 2002) [104].

Aflatoxins are a group of chemicals produced by certain mould fungi. These fungi, *Aspergillus flavus* and *Aspergillus parasiticus* can be recognized by olive green or graygreen, respectively, on maize kernels, in the field or in storage (Fig. 1). Although aflatoxins are not automatically produced whenever grain becomes mouldy, the risk of aflatoxin contamination is greater in damaged, mouldy maize than in maize with little mould. Aflatoxins are harmful or fatal to livestock and are considered carcinogenic (cancer causing) to animals and humans. In the midwest, aflatoxin levels are highest during hot, dry summers. The prime conditions for the fungus to produce toxin are warm (>70°F) nights during the later stages of grain fill (August/

September) in a period of drought. In high risk years, aflatoxin screening may be done at the elevator or where the maize is marketed. Rapid, on site tests can determine the possible presence of aflatoxin, but they do not provide specific quantitative results. The toxins are produced inside the maize kernels and their presence can be determined only by specific analytical tests. Because aflatoxin levels can vary greatly from kernel to kernel, sampling the load, bin, or unit of grain is the most critical step in determining actual levels of aflatoxin (Isaac *et al.*, 2019) [67].

Fig 1: Growth of *Aspergillus flavus* in artificial culture (left) and *Aspergillus* ear rot symptoms on maize ear (right)

Maize is one of the cereals most susceptible to aflatoxin contamination (Wilson *et al.*, 2006) [120]. High consumption of maize coupled with frequent and elevated aflatoxin levels, leads to a high aflatoxin risk. The development and dissemination of aflatoxin management practices are essential to reduce exposure to aflatoxins by consumers and producers dependent on maize for food and income generation. In this review article, we briefly describe an overview of challenges and elimination of aflatoxin contamination in India and different elimination approaches that can be used to reduce aflatoxin contamination in maize.

2. Importance of maize production in India

Globally, maize is known as "Queen of cereals" because it has the highest genetic yield potential among the cereals. It is cultivated on nearly 150 m ha in about 160 countries having wider diversity of soil, climate, biodiversity and management practices that contributes 36% (782 MT) of the global grain production. The United States of America (USA) is the largest producer of maize contributes nearly 35% of the total production in the world and other important growing countries are China, Brazil, India, Argentina, Ukraine, and Mexico.

Maize is grown throughout the year in India. It is predominantly a *kharif* crop with 85 per cent of the area under cultivation in the season. Maize is the third most important cereal crop in India after rice and wheat. It accounts for 9 per cent of total food grain production in the country. Maize plays an important role in the livelihoods of the poor farmers and in the rural economy of many developing countries as well as different part of our country. The major maize producing states in India are Karnataka, Andhra Pradesh, Tamil Nadu, Rajasthan, Maharashtra, Bihar, Uttar Pradesh, Madhya Pradesh and Gujarat account for 85 per cent of India's maize production and 80 per cent of area under cultivation which are also identified as potential production areas. Production of maize in India is dominated by Andhra Pradesh and Karnataka which contributes to 38 per cent of the total production.

The dependency of a significant population in terms of employment generation and diversity in the usage of maize. Indian maize is performing comparatively low in terms of yield as compared to world average. The difference in yield between India and world yield for maize is huge and works out to roughly 130%. The difference in yield with the leaders in maize production is even much larger, for example, with US it is more than 400% and with China it is nearly 225%. Interestingly, Argentina and Ukraine with less than half of area under maize than India are able to produce significantly more than India. The large gap in yield of maize production in India. It is clearly evident that the huge amount of effort required in improving the yield and total production of maize in India. It is imperative for all the stakeholders involved in the maize sector to join hands to leapfrog it to a stage where the production is at least able to meet the demand of the domestic consumption.

Maize accounts for the major share of the coarse grain production; showing a steady upward trend over the last decade on growing domestic demand (poultry feed and industrial use) and increasing productivity (better hybrid seeds). Study suggests that growth of the poultry, starch industries and consequent demand from these sectors is likely to outstrip domestic production of maize in near future. India is likely to continue to import small quantities of food grade maize (e.g., sweet corn etc.) and popcorn for the food processing industry due to growing consumer demand and low domestic supplies. This poses a huge challenge in front of maize production scenario in India of meeting the ever growing domestic demand from diverse segments catering to food and non-food usage of maize. Although, India has received maize production level of 26 MT, it would require 45 MT of Maize by the year 2022. The existing productivity level of Maize should double from 2.5 tonnes/ha presently to 5 tonnes/ha with subsequent increase in farmer's income by 2022 (FICCI and NCDEX, 2014) [48].

3. Overview of aflatoxins

The genus *Aspergillus* belongs to the Deuteromycetes (Fungi Imperfecti; Hyphomycetes); their teleomorphs can be found in the Ascomycetes. The fungi are found in many commodities as good substrate for growth because of the large number of enzymes which they can use for their

development (Hell, 1997) [57]. Pelczar *et al.* (1993) [91] indicated that the Ascomycete produce sexual spores (ascospores) endogenously in a well differentiated ascocarp but the Asccomycetes and the Deuteromycetes reproduce vegetatively by conidia. Hell (1997) [57] stated that aflatoxins are only produced by two related species: *Aspergillus flavus* and *Aspergillus parasiticus*, with the latter species producing specifically the G type of aflatoxin.

Aflatoxin is a very powerful hepatocarcinogen, and naturally occurring mixtures of aflatoxins have been classified as a class 1 human carcinogen (IARC, 1993) [66]. The IARC (1993) [66] also concluded that there was inadequate evidence for the carcinogenicity of aflatoxin M_1. EHSO (2005) [43] explained that aflatoxins are toxic carcinogenic byproducts of the moulds *Aspergillus flavus* and *Aspergillus parasiticus*. The name aflatoxin was derived from a toxin producing fungus which caused a disease referred to as "Turkey X disease" in England in 1960 which resulted in the death of 100,000 young turkeys. The fungus was identified as *Aspergillus flavus* in 1961 and the toxin was named aflatoxin due to its origin (A.flavis-Afla). EHSO indicated further that *Aspergillus flavus* is 7 common and widespread in nature. The mould is found in the soil, decaying vegetation and grains undergoing microbial deterioration. Keller *et al.* (1994) [71] reported that scientists made the attempt to isolate genes associated with aflatoxin biosynthesis through cloning of genes in order to understand the enzymes regulating the biosynthesis. Furthermore, the information gained on the regulation of the genes in the pathway could help to develop control strategies through inhibition of these controlling genes.

The chemical structures of some aflatoxins are shown in Fig. 2 (Cole and Cox, 1981) [27].

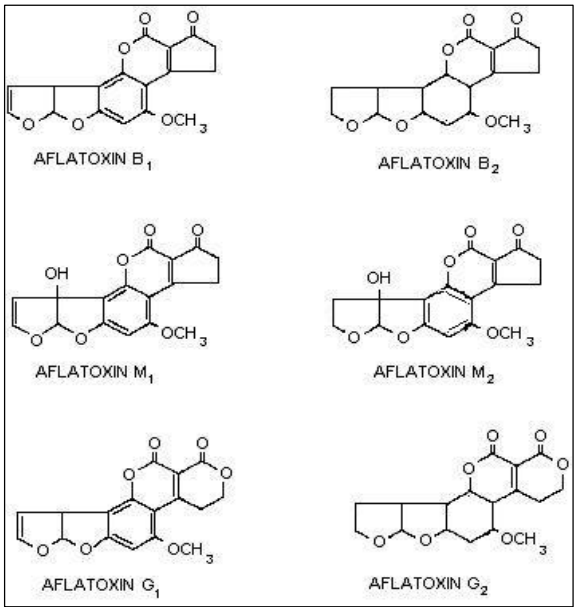

Fig 2: Chemical structure of aflatoxins

4. Maize contamination by aflatoxins

Maize, like other cereals growing above ground, gets infected with airborne fungal spores and aflatoxin contamination during plant growth (preharvest), during harvesting and after harvest during handling (postharvest). Improper practices during cultivation will carry the fungus to the postharvest processing and storage stage. Improper storage also leads to fungus attack and aflatoxin contamination. Like any other crop, maize can get contaminated with not only aflatoxin but also fumonisin and other mycotoxins which are harmful for humans.

The agricultural commodities that are prone to aflatoxins toxicity are maize and maize products, peanuts, cottonseed, milo, animal feed and majority of tree nuts (Beatriz *et al.*, 2005; Binder *et al.*, 2007) [14, 18]. Aflatoxins toxicity has always remained a topic of debate in terms of international market as well as economic development of country which are part of trade market. To overcome these challenges many countries have set maximum acceptable levels of aflatoxins in food and food products and animal feed (Diener *et al.*, 1987; European Commission 2006) [35, 44]. Previous studies proposed that the occurrence of aflatoxins in food products mainly influenced by favorable conditions such as high moisture content and temperature (Wu *et al.*, 2011) [121]. The extent of contamination by aflatoxins also varies with different geographic location, agricultural and agronomic practices, storage condition of crops and more importantly processing of food materials under favorable temperature and humidity conditions (Chauhan *et al.*, 2008) [24]. In many developing countries of Africa continent, aflatoxins toxicity of food have been companion with increased risk of hepatocellular carcinoma in the presence of hepatitis B virus infection (Henry *et al.*, 1999) [61] and esophageal cancer respectively (Wild and Turner 2002) [116]. Intensive exposures of B_1 at a concentration in excess of 2 ppm are reported to cause non-specific liver problems and death within few days. Whereas, chronic effect of B_1 leads to immunosuppression and nutritional deficiency (Peraica *et al.*, 1999) [92].

Maize as an agricultural commodity is considered as one of the best substrate for the fungi to grow and produce toxicogenesis. Many surveys across the globe showed that this crop can be highly contaminated with aflatoxins (Munkvold 2003) [85]. Indian maize is very complicated, involving substantial alteration in crops cultivated around the different parts of country (Choudhary and Kumari, 2010) [25]. Maximum quantities of maize produced are stored under poor and unsatisfactory storage conditions for considerable period of time. Traditional storage of maize in India is like drum made up of mud, bamboo strips, hangs of cob with roof and pits. In comparison of these storage conditions, the technology involves storage of maize in polyethylene bags and gunny bags (Choudhary and Kumari, 2010) [25]. Previous reports proposed that extended storage of maize under unacceptable storage conditions enhances fungal growth which promotes the production of respective mycotoxins (Chauhan *et al.*, 2008) [24]. Despite the fact that maize is a crucial food to Indian population and is vulnerable to aflatoxins risk due to different geographical and climatic conditions and poor handling of crop and storage, limited surveys have been reported on the relation of fungal mycotoxins in the crop and ways to protect the food from contamination in India.

5. Ecology and life cycle of *Aspergillus flavus*

Aspergillus flavus is a saprophytic fungus that survives on dead plant tissue and sometimes behaves as a weak and opportunistic pathogen (Cleveland *et al.*, 2005) [122]. The sources of inocula for *A. flavus* and *A. parasiticus* are sporogenic sclerotia, conidia and mycelia that over-winter in plant debris (Scheidegger and Payne, 2003) [98]. In fields repeatedly cropped to maize or rotated between maize, groundnut and cotton, conidia from sporogenic sclerotia are the primary source of *A. flavus* inocula. Conidia adjacent to the developing maize cobs germinate in the soil following the

release of carbon and nitrogen substrates by injured maize cobs and result in colonization of the cobs. Hot humid conditions favour the release of spores on plant residues, and these spores are dispersed by wind through the field (Dawit and Berehanu, 1985) [33]. Conidia that adhere to insect bodies are physically moved to plant parts and tassels in maize. Smaller, generally immature kernels are more easily infected in a shorter period of time than kernels in more mature cobs. Infections of maize kernels at other maturity stages are relative to the survival of the fungus and necessarily to a new infection at a later stage of maturity (Fig. 3).

Soil populations of *A. flavus* in soils under maize cultivation can range from 200 to >300,000 colony forming units (cfu) g^{-1} soil (Abbas *et al.*, 2004a, Zablotowicz *et al.*, 2007) [2, 123] and can constitute from ≤0.2% to ≤8% of the culturable soil fungi population. The major soil property associated with maintaining soil populations of *A. flavus* is soil organic matter. From the study it was also evident that higher populations of *A. flavus* are maintained in the soil surface of no-till compared to conventional till soils (Zablotowicz *et al.*, 2007) [123].

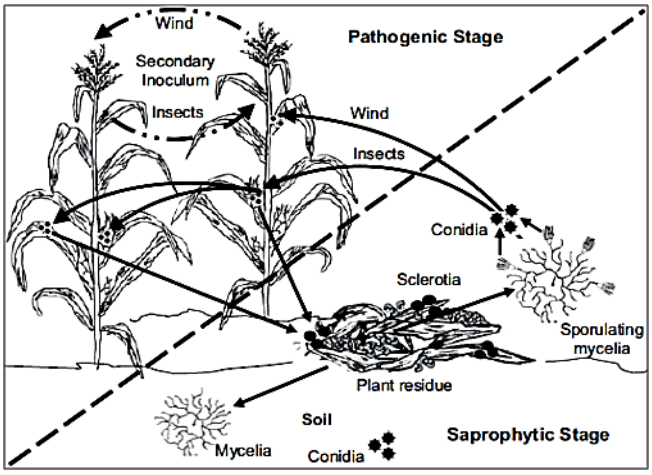

Fig 3: Life cycle of *A. flavus* in a maize crop during saprophytic and pathogenic stages (Courtesy: Abbas *et al.*, 2009) [1]

6. Economic importance of aflatoxins

Aflatoxins are naturally occurring mycotoxins that are predominatly produced by two species of fungi: *Aspergillus flavus* and *A. parasiticus*. Aflatoxins are some of the most potent toxic substances found in foods and feeds. Since the 1960s, when they were first discovered as responsible for the death of 100,000 turkeys in England, aflatoxins have been a subject of concern of many studies. Aflatoxins are highly toxic and can cause serious harm to human and animal health. Numerous studies have linked aflatoxins to various diseases, such as cancer of liver and hepatitis B and C. High levels of aflatoxin were detected in children with *kwashiorkor* (childhood malnutrition from protein insufficiency) in the Sudan, Durban, South Africa and Nigeria. In Gambia, 93% of sampled children (6-9 years old) were tested and found to be positive for aflatoxin albumin adducts. Aflatoxins are ubiquitous but are commonly found in warm and humid climates (Dohlman, 2003) [38]. Hence, most commodities from tropical countries, especially maize and groundnut, are easily contaminated with aflatoxins.

Aflatoxin contamination of human and animal feeds poses serious health and economic risks worldwide. FAO estimates that 25% of the world food crops are affected by mycotoxins each year and constitute a loss at post-harvest (Goto *et al.*, 1996) [51]. According to Cardwell *et al.* (2004) [23] aflatoxin contamination of agricultural crops causes annual losses of more than $750 million in Africa. In the US, it was reported that income losses due to aflatoxin contamination cost an average of more than US$100 million per year to US producers (Coulibaly *et al.*, 2008) [31]. Aflatoxin due to the invasion by *Aspergillus flavus* of the maize cobs is a serious problem in the international maize market and has seriously hampered the export business of the developing countries (FAO, 2002) [46].

To restrict exposure of maize to aflatoxin contamination, many countries and governmental agencies have set safety regulations, limiting the average concentration of aflatoxin on maize and maize products. For instance, in 1974 the Food and Drug Administration (FDA) proposed a tolerance level at 15 parts per billion (ppb) for aflatoxin in maize products. These regulations on food crops due to aflatoxin toxicity have a considerable economic impact on crop production and consequently on farm organization. According to FAO, many developing countries are unable to sell large quantities of maize on the international market because of aflatoxin contamination (FAO, 2002) [46].

7. Detection of aflatoxin

Aflatoxin not only has adverse effects on human health but also cause serious economic losses when tons of foods have to be discarded or destroyed as a result of aflatoxin contamination. To ensure food safety, maximum levels for aflatoxins in food and feed have been set by national and international organizations and various approaches have been developed for the determination of aflatoxin concentrations in food and feed commodities. Due to aforesaid consideration effect of aflatoxin in food and feed products, the detection methodology are mentioned below.

A. Chromatography

Chromatography is one of the most common methods for quantifying aflatoxin. In the beginning of aflatoxin analysis and research, Gas Chromatography (GC) was frequently used for detection and quantification of compounds. Later on, new chromatography based techniques were developed for aflatoxins. Examples of these improvements are Liquid Chromatography (LC), Thin Layer Chromatography (TLC) (Stroka *et al.*, 2000) [105], and High-Performance Liquid Chromatography (HPLC) (Bacaloni *et al.*, 2008) [8] which now a days is the most commonly used chromatographic technique for detection of a wide diversity of mycotoxins, especially for aflatoxin derivatives (De Rijk *et al.*, 2011) [34]. Frisvad and Thrane (1987) [49] described an HPLC method to identify 182 mycotoxins and other fungal metabolites based on their alkylphenone retention indices and diode array spectra. Coupling of HPLC with mass spectroscopy or tandem mass spectroscopy allows for highly accurate determination of toxin concentrations and compound identification in one analysis (Sobolev, 2007) [103]. Alternatively, fluorescence detection of the unmodified aflatoxins is widely used in HPLC applications as well as in Thin Layer Chromatography. Furthermore, there are combinations of the methods above with pre-process techniques, which can detect the concentration of aflatoxin in a solution in a better way. For example, immunoaffinity column sample clean-up followed by a normal or reverse phase of HPLC separation with fluorometric detection is mostly used for quantitative determination of M_1 due to the characteristics of specificity, high sensitivity and simplicity of operation (Muscarell *et al.*, 2007) [87].

B. Immunoassay

Immunochemical detection for aflatoxins is based on antibody-antigen reactions (Ab-Ag) (Lee *et al.,* 2004) [75]. Since different kinds of aflatoxin molecules can be considered as antigens, it is possible to detect them by developing antibodies against the compounds. Most of the immunological methods are based on enzyme-linked immunosorbent assays (ELISA), which have good sensitivity, speed and simplicity. In addition, some lateral flow immunoassays (LFIAs) also are applied for the qualitative and semi-quantitative detection of aflatoxin in food, feed and milk (Ho and Wauchope, 2002; Anfossi *et al.,* 2011; Salter *et al.,* 2006) [64, 4, 96]. Even though several reports have been published on the immunochemical determination of aflatoxin in food.

C. Biosensors and other methods

Biosensors, an alternative to improve the disadvantages of the previous methods, are multidisciplinary tools with an enormous potential in detection and quantification of aflatoxin. There are all kinds of biosensors that base their performance on different physical or biochemical principles, such as optical, optoelectronic, electrochemical, piezoelectric, DNA and combined. Thus, such devices have a huge impact in healthcare, food management, agronomical economy and bio-defense (Nayak *et al.,* 2009) [88]. Many kinds of biosensors are applied to detect aflatoxin. However, they mainly work in conjunction with immunochemical methods. Such junctions are based on the high affinity of antigen-antibody interaction and have the aim of increasing the sensitivity and shortening the detection time of the toxic element (Dinckaya *et al.,* 2011) [37].

Further methods exist which are less common than the previously described methods but have a wide utility as well. The most important are those ones that base their principle on electrochemistry, spectroscopy and fluorescence. Compared with traditional methods for aflatoxin determination, electrochemical techniques offer some advantages such as reliability, low cost, *in-situ* measurements, fast processes, and easier methodology than common chromatography techniques through a similar performance. Spectroscopy techniques have been popularized due to the characteristics that fast, low cost and non-destructive analytical methods suitable to work with solid and liquid samples. Among them, near infrared spectroscopy (NIRS) is an excellent method for a rapid and low cost detection of aflatoxin in cereals (Fernandez-Ibanez *et al.,* 2009) [47]. When incorporated with a bundle reflectance fiber-optic probe, NIRS was successfully applied to quantify aflatoxin B_1, ocharatoxin A and total aflatoxins in paprika (Hernandez-Hierro *et al.,* 2008) [62]. Aflatoxins have a native fluorescence due to their oxygenated pentahetherocyclic structure, which is the basis of most analytical and microbiological methods for detection and quantification of aflatoxins (Rojas-Duran *et al.,* 2007; Rasch *et al.,* 2010) [95, 93].

8. Elimination of aflatoxin contamination in maize

Elimination of aflatoxin contamination in maize is complex. Both preventative and curative procedures may be necessary. Aflatoxin occurrence and severity in field crops is largely a matter of uncontrollable natural events. The complete elimination of aflatoxin from human food, while desirable, is almost impossible to achieve, as aflatoxin is distributed in a wide range of agricultural products where it is an unavoidable natural contaminant. However, Pre-harvest, harvest, post-harvest, processing stage and storage strategies employed to reduce aflatoxin in food for better quality. This is confirmed by Hameeda *et al*. who state that commodities contaminated with aflatoxin have a lower market value and cannot be exported (Hameeda *et al.,* 2006) [56].

I. Pre-harvest management

Significant levels of mycotoxins can occur in the food crops in the fields. Some of the strategies for prevention of mycotoxins in the field are reduction in plant stress through irrigation, mineral nutrition and protection from insect damage. Avoidance of environmental conditions that favour infection in the field e.g. drought, insect infestation, primary inoculum, delayed harvesting etc. Good cultural practices viz. crop rotation, cropping pattern, irrigation, timely planting, harvesting and use of biopesticides have protective actions that reduce mycotoxin contamination of field crops. Breeding of cultivars resistant to fungal infection, use of crop protection chemicals that are antifungal agent, identification of plant constituents that disrupt aflatoxin biosynthesis or fungal growth and their use in new biochemical marker-based breeding strategies to enhance resistance and protection in crops. Development of transgenic plants resistant to fungal infection, development of transgenic cultivars capable of catabolism/interference with toxin production, development of crops genetically engineered to resist insect damage, development of crop seeds containing endophytic bacteria that exclude toxigenic fungi, exclusion of toxigenic fungi by pre infection of plants with biocompetitive non-toxigenic fungal strains are includes in pre-harvest managements. The fungal genome of *A. flavus* has been sequenced to understand the regulation of aflatoxin formation by environmental factors. This information can be used in development of host resistance against aflatoxin contamination by studying the effects of various physiological parameters e.g. drought stress on gene expression in toxigenic fungi. Mechanical damage of seeds may incur during harvesting. When damage is kept to a minimum during this phase, subsequent contamination is significantly reduced. Field crops should be harvested in timely manner to reduce moisture or water activity level to a point where mycotoxin formation will not occur.

Field contamination is considered a significant source of inoculum in maize, thus efforts have focused on preharvest management through integrated approaches for controlling critical factors known to increase fungal infection and aflatoxin production (Cole *et al.,* 1989; Mehan *et al.,* 1991a) [28, 81]. These practices includes growing resistant cultivars, avoiding end-of-season drought stress, reducing inoculum sources and avoiding cob damage through timely harvest and postharvest handling. This research has been facilitated by sick plots containing highly aggressive, toxigenic strains of *A. flavus* that can be used to screen large amounts of germplasm (Waliyar *et al.,* 1994) [110] and an inexpensive ELISA for quantitative estimation of aflatoxin concentration in maize kernels. Aflatoxin contamination of crops has been shown to take its roots from both pre and post-harvest conditions. This contamination could occur in the field during storage and in transit (Hell *et al.,* 2008) [58]. Three methods of aflatoxin management has identified: "pre and post-harvest management and detoxification". Pre-harvest management of aflatoxin is the best and most widely explored strategy.

A. Host resistance

Aflatoxin contamination in maize seeds caused by *Aspergillus flavus* hampers international trade and adversely affects health of consumers of maize and its products. It can occur in the field when the crop is growing, during harvesting, curing, in

storage and transportation. Aflatoxin research on maize at India focuses on identification and utilization of genetic resistance to preharvest seed infection and aflatoxin production by *A. flavus* and pre and post-harvest management practices to minimize contamination. Breeding for aflatoxin resistance has been a major issue in maize for nearly four decades but it should be require more work on this aspect in our country. Despite global efforts, progress in aflatoxin resistance breeding has been limited due to the low level of resistance to different components of resistance (preharvest seed infection and aflatoxin production and *in-vitro* seed colonization by *A. flavus*) their variable performance due to high genotype and environment interaction, lack of reliable screening protocols and limited understanding of genetics of resistance.

Recently germplasm has identified either genetic resistance to infection and growth of *A. flavus* in the grain or the ability to suppress fungal production of aflatoxin following infection (Brown *et al.*, 1999; Moreno and Kang, 1999) [20, 83]. Early resistant germplasm characterization studies used the percentage of infected kernels in an ear or the grain aflatoxin levels to identify resistant lines (Zuber *et al.*, 1983; Widstrom *et al.*, 1984; Widstrom *et al.*, 1986; Scott and Zummo, 1988-18) [124, 114, 115, 99]. These early sources of resistance include Mp313E, SC54, Mp420, and Tex6 (Scott and Zummo, 1988; Hamblin and White; 2000) [99, 55]. Newer breeding lines and populations with high and repeatable resistance under varying environments have been released; these includes Mp715, Mp717, GT-MAS:gk, CML176, CML269; CML322 and Tx114 (Betran *et al.*, 2002; McMillian *et al.*, 1993; Williams and Windham, 2001 & 2006; Guo *et al.*, 2001) [17, 80, 117, 54]. All resistant lines is identified to date from tropical germplasm in their backgrounds. Thus, they tend to be tall, late and prone to lodging, in addition to lower yielding than commercial hybrid checks. It has been very difficult to transfer the resistance from these older breeding lines into a more agronomically acceptable idiotype using only phenotypic selection because of the highly quantitative nature of host plant resistance to *A. flavus* infection and aflatoxin accumulation in maize. However, some of the newest breeding lines including Mp718, Mp719, Tx736, Tx739, and Tx740 (Williams and Windham, 2012; Mayfield *et al.*, 2012, Marilyn *et al.*, 2014) [119, 79] that have recently been released show a much better plant type and high resistance.

B. Biological control

Aflatoxin contamination events are more prevalent during times of high heat and drought conditions which may stress the host plant possibly weakening plant defence mechanisms, thereby, facilitating *A. flavus* infection (Cotty and Jaime-Garcia, 2007; Hill *et al.*, 1983; Sanders *et al.*, 1984; Ehrlich *et al.*, 2015) [30, 63, 97, 42]. Biological control appears to be the most promising approach for control of aflatoxin in both pre and post harvested crops. Several bacterial species such as *Bacillus subtilis, Bacillus pumilus, Streptomyces aureofaciens* and *Pseudomonas putida* have shown the ability to inhibit fungal growth and production of aflatoxins by *Aspergillus* spp. in laboratory experiments. Several strains of *B. subtilis* isolated from the non-rhizophere of maize soil were also able to inhibit aflatoxin accumulation (Nesci *et al.*, 2005) [89]. Another potential means for aflatoxin control is by fungal biocontrol agent in the field. Biological control of aflatoxin production in crops in the US has been approved by the Environmental Protection Agency and two commercial products based on atoxigenic *Aspergillus flavus* strains are being used (afla-guard® and AF36®) for the prevention of aflatoxin in peanuts, corn and cotton seed (Dorner, 2009) [39]. In Africa, atoxigenic strains of *A. flavus* have been identified to competitively exclude toxigenic fungi in the maize and peanut fields. These strains have been shown to reduce aflatoxin concentrations in both laboratory and field trials by 70 to 99% (Atehnkeng *et al.*, 2014) [6]. A mixture of four atoxigenic strains of *A. flavus* of Nigerian origin has gained provisional registration as AflaSafe® to determine efficacy in on-farm tests. This inhibitory result from many factors including competition for space and nutrients in general, competition for nutrients required for aflatoxin production but not for growth and production of antiaflatoxigenic metabolites by co-existing microorganisms. Yeast species can develop quickly in leaf, fruit and flower surfaces excluding the other microorganism growth by means of competition for space and nutrient. The use of yeasts in postharvest biocontrol formulations apparently presents advantages over other organisms. Yeasts are easy to cultivate, fast growing and are present in a variety of environmental niches (Walker 2011; Sejakhosi *et al.*, 2013; Bandyopadhyay *et al.*, 2016; Antonio *et al.*, 2018) [111, 100, 9, 5].

C. Cultural control

Management practices that reduce the incidence of mycotoxin contamination in the field include timely planting, optimal plant densities, proper plant nutrition, avoiding drought stress, controlling other plant pathogens, weeds and insect pests and proper harvesting (Bruns, 2003) [21]. During the growing period several factors influence fungal colonization and aflatoxin production including the soil type and condition, rate of evapotranspiration, availability of viable spores, end-season drought stress, damage to maize cobs by insects and mechanical damage during harvesting (Borgemeister *et al.*, 1998; Kaaya *et al.*, 2006) [19, 68]. It is impossible to control all of these factors but some cultural practices can greatly reduce the amount of fungal infection. Some of these practices include: summer ploughing, selecting planting dates to take advantage of periods of higher rainfall and avoiding end of the season drought effects, seed dressing with systemic fungicides or biocontrol agents, maintaining good plant density in the fields, soil amendment with gypsum and farmyard manure, removing prematurely dead plants, managing pests and diseases, timely harvesting, excluding damaged and immature cobs, drying cobs quickly, controlling storage pests and only storing cobs/seeds with < 10% moisture content (Diener *et al.*, 1987; Hell *et al.*, 2008) [35, 58]. The use of mechanical threshers and seed storage bins also can reduce aflatoxins in maize. Although most of these practices are cost-effective and practical under subsistence farming conditions, they remain largely unadopted by subsistence farmers due to various socio-economic constraints including farmers' attention to other revenue generating activities and a lack of appropriate structures for drying and storage (Mestre *et al.*, 2004) [82].

II. Post-harvest management

Even if the contamination occurs or persists after this phase, the hazards associated with toxin must be managed through post-harvest procedures, if the product is to be used for food and feed purposes. Storage and processing are the major areas where contamination can be prevented. Removal of damaged grain and drying of grain to the minimal moisture level. Control of insect and rodent activity and maintenance of appropriate moisture levels and temperature. Appropriate

packaging is often successful way of excluding insects and moulds. Frequent cleaning of food/feed delivery systems and short term storage. Use of antifungal agents such as propionic acid and acetic acid (Hell *et al.,* 2008; Kaaya and Kyamuhangire, 2006) [58, 68]. Thermal inactivation is also one of the alternatives for products that are usually heat processed. Fumonisins and ochratoxin levels have been shown to be lower in thermally processed maize and maize products (Giorni *et al.,* 2007; Battilani *et al.,* 2008) [50, 13].

Aflatoxins are compounded through excessive heat, high humidity, lack of aeration in the stores, and insect and rodent damage resulting in the proliferation and spread of fungal spores. Thus strategies to minimize quantitative and qualitative post-harvest losses have been developed (Hell *et al.,* 2008) [58]. The stores are constructed to prevent insect and rodent infestation and to prevent moisture from getting into the grains. It is difficult to promote new storage technologies, such as the use of metal or cement bins to small-scale farmers due to their high cost. Many farmers nowadays store their grains in bags, especially polypropylene which are not airtight, but there is evidence that this method facilitates fungal contamination and aflatoxin development (Hell *et al.,* 2000; Udoh *et al.,* 2000) [58, 108]. Presently, there are efforts to market improved hermetic storage bags in African countries, based on triple bagging developed for cowpea (Murdock *et al.,* 1997) [86] which has been or is being tested for other commodities (Ben *et al.,* 2009) [15].

The compounds used for seed fumigation like ethylene oxide and methyl bromide were found to significantly reduce the incidence of fungi including toxigenic species on stored groundnuts and melon seeds (Bankole *et al.,* 1996) [12]. Among the chemical compounds tested in feeds propionic acid, sodium propionate, benzoic acid and ammonia were the best anti-fungal compounds followed by urea and citric acid (Gowda *et al.,* 2004) [53].

Sorting out of physically damaged and infected grains (known from colorations, odd shapes and size) from the intact commodity can result in 40-80% reduction in aflatoxins levels (Park, 2002; Fandohan *et al.,* 2005; Afolabi *et al.,* 2006) [90, 45, 3]. The advantage of this method is that it reduces toxin concentrations to safe levels without the production of toxin degradation products or any reduction in the nutritional value of the food. This could be done manually or by using electronic sorters. Clearing the remains of previous harvests and destroying infested crop residues are basic sanitary measures that are also effective against storage deterioration. Cleaning of stores before loading in the new harvests was correlated to reduced aflatoxin levels (Hell *et al.,* 2008) [58]. Separating heavily damaged ears i.e. those having greater than 10% ear damage also reduces aflatoxin levels in maize (Sétamou *et al.,* 1998) [101]. Wild hosts which constitute a major source of infestation for storage pests should be removed from the vicinity of stores (Hell *et al.,* 2008) [58].

A. Processing stage

Maize is source of human food, forage, flour, and other processed products for industry in many countries. Increase in aflatoxin content can occur if the phases of grain drying, storage and processing are poorly managed (Smith, 1997; Chulze, 2010) [102, 26].

(i) Grain handling

Do not hold high moisture grain in wagons or trucks longer than 6 hours. Place high moisture maize being held for drying in a holding bin using forced air to keep it as cool as possible. Use sound sanitation practices in handling grain. Clean augur wells and pits, and clean around dump stations before and after each use. Minimize physical damage by conveyors or from dropping the grain into tall bins.

(ii) Dry properly

Drying temperature and drying time may have an effect on the development of aflatoxin in stored grain. Slow drying with low heat over long periods could promote aflatoxin development.

(iii) Layer-in-bin drying

Drying maize in deep layers can produce conditions highly favourable for mould development. The drying temperature for this method is increased only 10 to 20 °F above outside conditions. The relative humidity in the top layer of grain remains very high for an extended period of time.

(iv) Batch-in-bin drying

A lot of grain is batch dried in bins. Wet grain is usually placed about 3 to 4 feet deep in a drying bin and hot air (about 140 °F) is forced through the grain, drying the batch normally in less than 24 hours. The grain is then cooled for storage in the same bin or moved to a storage bin and cooled by aeration fans.This method can be used when storage is available to unload the dryer before putting additional wet grain into the dryer. If the heater is adequate to raise air temperature sufficiently, this method can increase drying rate by a factor of 3 over the layer-in-bin drying method. Chances of aflatoxin being produced with this system are decreased since higher temperature and faster drying are used. To prevent further fungi and mould growth in storage, dry to an average moisture content of 12 percent.

(v) Column dryers

Aflatoxin production in column dryers operated at high temperatures (180-200 °F) and short drying time (1-2 hours) is very unlikely. This is true for batch or continuous flow column type dryers.

(vi) Maintain proper storage conditions

Keep moisture in stored grain below 12-13 percent to stop the development of aflatoxin. Also keep insect activity to a minimum. Cooling the grain below 60°F with outside air as soon as temperatures permit is beneficial in arresting the development of insect populations. Scalping grain before storage will help remove trash, cracks and shrivelled kernels, which are usually high in aflatoxin content. Consider using a grain cleaner at the bin to remove trash before placing the grain into storage.

(vii) Industrial processing

Industrial detoxification processes include using "inorganic salts and organic acids, and ammoniation which can eliminate the aflatoxin producing fungus with ammonia vapour as well as natural acids, salts and plant extracts. Ammoniation appears to have the most practical application for the decontamination of agricultural commodities (USAID, 2012) [109].

B. Storage

Aerate grain in storage when it is at least 10°F warmer than outside air temperature and humidity is below 65 percent. An air flow rate of 1/10 cubic feet per bushel per minute is adequate for cooling grain. Drying fans can be used to cool

grain in a few hours when outside air conditions are favourable (Turner *et al.,* 2005) [107]. Air should be pulled from the top of the bin and exhausted through the bottom to prevent moisture condensation at the top of the grain during aeration. Never add heat during aeration. Grain held in storage should be inspected and probed every 3 to 4 weeks. Check for insect activity, high temperatures, mould growth or sprouting at the top of the grain. Mould fungi and aflatoxin levels in maize are normally higher in the fine material commonly. Removal of the fines can reduce aflatoxin levels up to 50 percent. Storage is an important activity in agriculture because it reduces losses at post-harvest stage and also gives opportunity to sellers and producers to increase their net revenue as a result of price increase. It is therefore believe that the length of storage affects the quality and enhances contamination of aflatoxin (Kaaya *et al.,* 2006) [68].

C. Chemical control

Ammonia at 0.5-7% coupled with long exposure time, ambient temperature and pressure has been successfully used to inactivate aflatoxin in contaminated commodities, such as maize, cotton seed and groundnut. This process has been approved by safety and regulatory agencies, such as Food and Agriculture Organization (FAO), Food and Drug Administration (FDA), and United States Department of Agriculture (USDA) (Moustafa *et al.,* 2001) [84]. *In vitro* studies indicated that the fungicide chlobenthiazone is highly effective in inhibiting aflatoxin biosynthesis by cultures of *A. flavus*; however, aflatoxin synthesis by *A. parasiticus* was, in fact, stimulated by this fungicide (Wheeler, 1991) [112]. Various surfactants, including some used in pesticide formulations, reduced aflatoxin biosynthesis by >96% (Rodriguez and Mahoney, 1994) [94]. Use of natural oils from thyme (Kumar *et al.,* 2008) [73], lemongrass (Bankole and Joda, 2004), and other herbs has also been studied and shown to supress aflatoxin in certain crops in Asia. A three year field study has indicated that none of five fungicides (azoxystrobin, pyraclostrobin, propiconazole, tetraconazole, dithiocarbamate) or fungicide mixtures of trifloxystrobin + propiconazole and azoxystrobin + propiconazole, applied to maize at mid silking, were effective insignificantly reducing aflatoxin contamination (Bruns and Abbas, 2006; Doukas *et al.,* 2012; Mateo *et al.,* 2017; Markoglou *et al.,* 2011) [22, 41, 77, 76]. The herbicide glufosinate has been reported as having antifungal activity against certain phytopathogenic fungi *in vitro* (Uchimiya *et al.,* 1993) and has shown activity in reducing infection of maize kernels *in vitro* (Tubajika and Damann, 2002; Lagogianni and Tsitsigiannis, 2018) [106, 74].

9. Conclusion

Producing aflatoxin free maize and other grains ensures food safety. The health and economic wellbeing of a farmer is an important investment in the agricultural sector. Since it is difficult to identify maize that are contaminated. Aflatoxin is a type of mycotoxin produced by *Aspergillus* mould. Aflatoxin is the most well-known and researched mycotoxin. India is most favourable for aflatoxicogenic fungi and aflatoxin contamination, especially B_1. Reports show that maize and groundnut are the most contaminated commodities in the country. These two commodities are most important in day to day dietary sources of the people in different part of country. The level of contamination for most commodities in the country is also very much greater than the international standard. There are basically six groups of aflatoxins, B_1, B_2, G_1, G_2, M_1 and M_2; from which B_1 are the most potent aflatoxins to cause health damage to human and animal. B_1 is the most the most common contaminant of most Indian commodities. Aflatoxins are toxic to human and animal and cause different diseases. There are two main ways people are usually exposed to aflatoxin. The first is when someone takes in a high amount of aflatoxins in a very short time. This can cause liver damage, liver cancer, mental impairment, abdominal pain, vomiting, death and others. The other way people suffer aflatoxin poisoning is by taking in small amounts of aflatoxins at a time, but over a long period. This might happen if a person diet has a small amount aflatoxin. This can cause growth and development impairment, liver cancer, DNA and RNA mutation and others. It is important to avoid consuming maize that are shrivelled, broken, discoloured, or show symptoms of fungal infection. As much as farmers grade maize for the market, they also need to grade for consumption. Use of good crop production practices and postharvest handling techniques can effectively reduce fungal contamination and maintain the quality of the produce. Such efforts would then open up opportunities for farmers to access rewarding markets that currently have stringent quality standards.

10. References

1. Abbas HK, Wilkinson JR, Zablotowicz RM, Accinelli C, Abel CA, Bruns HA *et al*. Ecology of *Aspergillus flavus*, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. Toxin Reviews. 2009; 28(2-3):142-153.
2. Abbas HK, Zablotowicz RM, Locke MA. Spatial variability of *Aspergillus flavus* soil populations under different crops and corn grain colonization and aflatoxins. Can. J Bot. 2004a; 82:1768-1775.
3. Afolabi CG, Bandyopadhyay R, Leslie JF, Ekpo EJA. Effect of sorting on incidence and occurrence of fumonisin and fumonisin-producing *Fusarium* species on maize (*Zea mays* L.) in Nigeria. Journal of Food Protection. 2006; 69:2019-2023.
4. Anfossi L, D'Arco G, Calderara M, Baggiani C, Giovannoli C, Giraudi G. Development of a quantitative lateral flow immunoassay for the detection of aflatoxins in maize. Food Additives and Contaminants: Part A: Chemistry, Analysis, Control, Exposure and Risk Assessment. 2011; 28:226-234.
5. Antonio M, Esther GC, Amedeo P, Peter JC, Paola B. Biological Control Products for Aflatoxin Prevention in Italy: Commercial Field Evaluation of Atoxigenic *Aspergillus flavus* Active Ingredients. Toxins. 2018; 10(30):2-14.
6. Atehnkeng J, Ojiambo PS, Cotty PJ, Bandyopadhyay R. Field efficacy of a mixture of atoxigenic *Aspergillus flavus* Link: Fr vegetative compatibility groups in preventing aflatoxin contamination in maize (*Zea mays* L.). Biol. Control. 2014; 72:62-70.
7. Ayub M, Sachan D. Dietary factors affecting aflatoxin bi carcinogenicity. Malaysian Journal of Nutrition. 1997; 3:161-197.
8. Bacaloni A, Cavaliere C, Cucci F, Patrizia F. Determination of aflatoxins in hazelnuts by various sample preparation methods and liquid chromatography-tandem mass spectrometry. Journal of Chromatography A. 2008; 1179:182-189.
9. Bandyopadhyay R, Ortega-Beltran A, Akande A, Mutegi C, Atehnkeng J, Kaptoge L *et al*. Biological control of aflatoxins in Africa: current status and potential

challenges in the face of climate change. World Mycotoxin Journal: Special Issue: Mycotoxins in a changing world. 2016; 9(5):771-789.
10. Bankole SA, Joda AO. Effect of lemon grass (*Cymbopogon citratus* Stapf) powder and essential oil on mould deterioration and aflatoxin contamination of meion seeds (*Colocynthis citrullus* L.). Afr. J Biotechnol. 2004; 3:52-59.
11. Bankole SA, Joda AO. Effect of lemon grass (*Cymbopogon citratus* Stapf) powder and essential oil on mould deterioration and aflatoxin contamination of meion seeds (*Colocynthis citrullus* L.). Afr. J Biotechnol. 2004; 3:52-59.
12. Bankole SA, Eseigbe DA, Enikuomehin OA. Mycoflora and aflatoxin production in pigeon pea stored in jute sacks and iron bins. Mycopathologia. 1996; 132:155-160.
13. Battilani P, Barbano C, Piva G. Aflatoxin B_1 contamination in maize related to the aridity index in North Italy. World Mycotoxin Journal. 2008; 1:449-456.
14. Beatriz LS, Alessandra BR, Paulo AM, Miguel MJ. *Aflatoxins, ochratoxin* A and zearalenone in maize-based food products. Braz. J Microbiol. 2005; 36:289-294.
15. Ben D, Liem P, Dao N, Gummert M, Rickman J. Effect of hermetic storage in the super bag on seed quality and milled rice quality of different varieties in Bac Lieu, Vietnam. International Rice Research Notes. 2009; 31(2):12-20.
16. Bennett JW, Klich M. "Mycotoxins". Clinical Microbiology Reviews. 2003; 16(3):497-516.
17. Betran FJ, Isakeit T, Odvody G. Aflatoxin accumulation of white and yellow maize inbreds in diallel crosses. Crop Science. 2002; 42(6):1894-1901.
18. Binder EM, Tan LM, Chin LJ, Hadle J, Richard J. Worldwide occurrence of mycotoxins in commodities feeds and feed ingredients. Anim. Feed Sci. Technol. 2007; 137:265-282.
19. Borgemeister C, Adda C, Sétamou M, Hell K, Djamamou B, Markham RH *et al*. Timing of harvest in maize: Effects on post-harvest losses due to insects and fungi in central Benin, with particular references to *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). Agriculture, Ecosystem and Environment. 1998; 69:233-242.
20. Brown RL, Chen Z, Cleveland TE, Russin JS. Advances in the development of host resistance in corn to aflatoxin contamination by *Aspergillus flavus*. Phytopathology. 1999; 89(2):113-117.
21. Bruns HA. Controlling aflatoxin and fumonisin in maize by crop management. Journal of Toxicology, Toxin Reviews. 2003; 22:153-173.
22. Bruns HA, Abbas HK. Effects of glufosinate Ammonium and urea on aflatoxin and fumonisin levels in corn. Plant Health Progress, 2006. doi:10.1094/PHP-2006-0324-01-RS.
23. Cardwell KF, Desjardins D, Henry SH. The cost of achieving food security and food quality, 2004. (http://www.apsnet.org/online/ festure/mycotoxin/ top.html) (Accessed on July 1, 2012).
24. Chauhan Y, Wright G, Rachaputi N Modelling climatic risks of aflatoxin contamination in maize. Aust. J Exp. Agric. 2008; 48:358-366.
25. Choudhary AK, Kumari P. Management of mycotoxin contamination in preharvest and postharvest crops: Present status and future prospects. Journal of Phytology. 2010; 2(7):37-52.
26. Chulze SN. Strategies to reduce mycotoxin levels in maize during storage: a review. Food Additives and Contaminants. 2010; 27:651-657.
27. Cole RJ, Cox RH. Handbook of Toxic fungi metabolites. Academic Press New York, London, 1981, 937.
28. Cole RJ, Sanders TH, Dorner JW, Blankenship PD. Environmental conditions required to induce preharvest aflatoxin contamination of groundnuts: Summary of six years research, 1989.
29. McDonald D, Mehan VK. (Eds.). Aflatoxin Contamination of Groundnuts: Proceedings of the International Workshop (6-9 October, ICRISAT, Patancheru, India). ICRISAT, Patancheru, India, 1987, 279.
30. Cotty PJ, Jaime-Garcia R. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. International Journal of Food Microbiology. 2007; 119:109-115.
31. Coulibaly O, Hell K, Bandyopadhyay R, Hounkponou S, Leslie JF. Mycotoxins: detection methods, management, public health and agricultural trade, Published by CAB International, 2008. ISBN 9781845930820.
32. Cullen JM, Newberne PM, Eaton D, Groopman J. Acute hepatotoxicity of aflatoxins. The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance, 1993, 3-26.
33. Dawit A, Berehanu A. Prevalence of *Aspergillus flavus* in Ethiopian cereal grains a preliminary survey. Ethiopian Medical Journal. 1985; 23:143-148.
34. De Rijk TC, Arzandeh S, Kleijnen HCH, Zomer P, Vander Weg G, Mol JGJ. Aflatoxins determination using in-line immunoaffinity chromatography in foods. Food Control. 2011; 26:42-48.
35. Diener U, Cole R, Sanders T, Payne G, Lee L, Klich M. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Ann. Rev. Phytopathol. 1987; 25:249-270.
36. Diener UL, Cole RJ, Sanders TH, Payne GA, Lee LS, Klich MA. Epidemiology of Aflatoxin, in formation by *Aspergillus flavus*. Annual Review of Phytopathology. 1987; 25:240-270.
37. Dinckaya E, Kınık O, Sezginturk MK, Altug C, Akkoca A. Development of an impedimetric aflatoxin M1 biosensor based on a DNA probe and gold nanoparticles. Biosensors and Bioelectronics. 2011; 26:3806-3811.
38. Dohlman E. Mycotoxin hazards and regulations: Impacts on food and animal feed crop trade, International trade and food safety: Economic theory and case studies, Jean Buzby (ed.), Agricultural Economic Report 828. USDA, ERS, 2003.
39. Dorner JW. Development of biocontrol technology to manage aflatoxin contamination in peanuts. Peanut Sci. 2009; 36:60-67.
40. Dors GC, Caldas S, Feddern V. Aflatoxins: contamination, analysis and control in Aflatoxins. Biochemistry and Molecular Biology, In Tech, Shanghai, China, 2011, 415-438.
41. Doukas EG, Markoglou AN, Vontas JG, Ziogas BN. Effect of DMI-resistance mechanisms on cross-resistance patterns, fitness parameters and aflatoxin production in *Aspergillus parasiticus* Speare. Fungal Genetics and Biology. 2012; 49:792-801.
42. Ehrlich KC, Moore GG, Mellon JE, Bhatnagar D. Challenges facing the biological control strategy for eliminating aflatoxin contamination. World Mycotoxin

Journal: Special Issue: Aflatoxins in maize and other crops. 2015; 8(2):225-233.
43. EHSO. (Environmental Health and Safety on Line). *Aspergillus flavus* conidial head; http: //Nww.ehso.com, Accessed on February 5, 2005.
44. European Commission (EC). Commission Regulation (EC) No. 1881/2006 of 19 December setting maximum levels for certain contaminants in foodstuffs. Off. J Eur. Union. L. 2006; 364:5-24.
45. Fandohan P, Zoumenou D, Hounhouigan DJ, Marasas WF, Wingfield MJ, Hell K. Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. International Journal of Food Microbiology. 2005; 98:249-259.
46. FAO (Food and Agriculture Organization). Food and Agricultural Statistics. Rome, Italy, 2002, 27-44.
47. Fernandez-Ibanez V, Soldado A, Martinez-Fernandez A, De la Roza-Delgado B. Application of near infrared spectroscopy for rapid detection of aflatoxin B_1 in maize and barley as analytical quality assessment. Food Chemistry. 2009; 113:629-634.
48. FICCI & NCDEX. Maize in India. Policy paper, 2014, 32.
49. Frisvad JC, Thrane U. Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (diode array detection). Journal of Chromatography A. 1987; 404:195-214.
50. Giorni P, Magan N, Bertuzzi PA, Battilani P. Studies on *Aspergillus* section *Flavi* isolated from maize in northern Italy. International Journal of Food Microbiology. 2007; 113:30-333.
51. Goto T, Wicklow DT, Ito Y. Aflatoxin and cyclopiazonic acid production by a sclerotium-producing *Aspergillus tamarii* strain. Applied Environmental Microbiology. 1996; 62:4036-4038.
52. Gourama H, Bullerman LB. Detection of molds in foods and feeds: potential rapid and selective methods. Journal of Food Protection. 1995; 58(12):1389-1394.
53. Gowda N, Malathi V, Suganthi R. Effect of some chemical and herbal compounds on growth of *Aspergillus parasiticus* and aflatoxin production. Animal Feed Science and Technology. 2004; 116:281-291.
54. Guo BZ, Li RG, Widstrom NW, Lynch RE, Cleveland TE. Genetic variation within maize population GT-MAS: gk and the relationship with resistance to *Aspergiilus flavus* and aflatoxin production. Theoretical and Applied Genetics. 2001; 103(4):533-539.
55. Hamblin AM, White DG. Inheritance of resistance to aspergillus ear rot and aflatoxin production of corn from Tex6. Phytopathology. 2000; 90(3):292-296.
56. Hameeda B, Rupela OP, Reddy G. Antagonistic activity of bacteria inhabiting composts against soil-borne plant pathogenic fungi. Indian Journal of Microbiology. 2006; 46(4):389-396.
57. Hell K. Factors contributing to the distribution and incidence of aflatoxin producing fungi in stored maize in Benin. An unpublished Ph.D. Dissertation, University of Hannover, 1997.
58. Hell K, Cardwell KF, Setamou M, Poehling HM. The influence of storage practices on aflatoxin contamination in maize in four agro-ecological zones of Benin, West Africa. Journal of Stored Products Research. 2000; 36:365-382.
59. Hell K, Fandohan P, Bandyopadhyay R, Cardwell K, Kiewnick S, Sikora R *et al*. Pre- and postharvest management of aflatoxin in maize. In Leslie JF, Bandyopadhyay R, Visconti A. (Eds.) Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CABI Publishing, Wallingford, UK, 2008, 210-219.
60. Hell K, Fandohan P, Bandyopadhyay R, Cardwell K, Kiewnick S, Sikora R *et al*. Pre and postharvest management of aflatoxin in maize. In Leslie JF, Bandyopadhyay R, Visconti A. (Eds.) Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CABI Publishing, Wallingford, UK, 2008, 210-219.
61. Henry SH, Bosch FX, Troxell TC, Bolger PM. Reducing liver cancer-global control of aflatoxin. Science. 1999; 286:2453-2454.
62. Hernandez-Hierro JM, Garcia-Villanova RJ, Gonzalez-Martin I. Potential of near infrared spectroscopy for the analysis of mycotoxins applied to naturally Aflatoxins-Recent Advances and Future Prospects 312 contaminated red paprika founding the Spanish market. Analytica chimica acta. 2008; 622:189-194.
63. Hill RA, Blankenship PD, Cole RJ, Sanders TH. Effects of soil moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. Applied and Environmental Microbiology. 1983; 45:628-633.
64. Ho JA, Wauchope RD. A Strip Liposome Immunoassay for Aflatoxin B_1. Analytical Chemistry. 2002; 74:1493-1496.
65. Hussein HS, Brasel JM. Toxicity, metabolism and impact of mycotoxins on humans and animals. Toxicology. 2001; 167(2):101-134.
66. IARC. Some naturally occurring substances: Food items and constituents, heterocyclic amines and mycotoxins. IARC monographs on evaluation of carcinogenic risk to humans, Lyon, France, International Agency for Research on Cancer, 1993, 56.
67. Isaac WO, Gloria M, Ankar-Brewoo Herman EL, Luke PMT, Edmund OB, William OE. Aflatoxin Exposures and Risks in Maize-Based Foods Consumed by University Students. Acta Scientific Nutritional Health. 2019; 3(2):110-119.
68. Kaaya AN, Kyamuhangire W. The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda. International Journal of Food Microbiology. 2006; 110:217-223.
69. Kaaya AN, Kyamuhangire W. The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda. International Journal of Food Microbiology. 2006; 110:217-223.
70. Kaaya AN, Kyamuhangire W, Kyamanywa S. Factors affecting aflatoxin contamination of harvested maize in the three agro-ecological zones of Uganda. Journal of Applied Sciences. 2006; 6:2401-2407.
71. Keller NP, Butchko RAE, Sarr B, Phillips TD. A visual pattern of mycotoxin production in maize kernels by *Aspergillus* spp. Phytopathology. 1994; 845:483-488.
72. Kok WT. Derivatization reactions for the determination of aflatoxins by liquid chromatography with fluorescence detection. Journal of Chromatography B: Biomedical Sciences and Applications. 1994; 659(1-2):127-137.

73. Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK. Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post-harvest fungal infestation of food commodities. Innovative Food Science and Emerging Technologies. 2008; 9:575-580.
74. Lagogianni CS, Tsitsigiannis DI. Effective chemical management for prevention of aflatoxins in maize. Phytopathologia Mediterranea. 2018; 57(1):186-197.
75. Lee NA, Wang S, Allan RD, Kennedy IR. A rapid aflatoxin B_1 ELISA: development and validation with reduced matrix effects for peanuts, corn, pistachio and soyabeans. Journal of Agricultural Food Chemistry. 2004; 52:2746-2755.
76. Markoglou AN, Doukas EG, Ziogas BN. Effect of anilinopyrimidine resistance on aflatoxin production and fitness parameters in *Aspergillus parasiticus* Speare. International Journal of Food Microbiology. 2011; 146:130-136.
77. Mateo EM, Gómeza JV, Gimeno-Adelantado JV, Romera D, Mateo-Castro R, Jiménez M. Assessment of azole fungicides as a tool to control growth of *Aspergillus flavus* and aflatoxin B_1 and B_2 production in maize. Food Additives & Contaminants. 2017; 34:1039-1051.
78. Matthew AA, Nelson O, Francis KA. Aflatoxin contamination in cereals and legumes to reconsider usage as complementary food ingredients for Ghanaian infants: A review. Journal of Nutrition & Intermediary Metabolism. 2017; 10:1-7.
79. Mayfield K, Betran FJ, Isakeit T. Registration of maize germplasm lines Tx736, Tx739, and Tx740 for reducing preharvest aflatoxin accumulation. Journal of Plant Registrations. 2012; 6(1):88-94.
80. McMillian WW, Widstrom NW, Wilson DM. Registration of GT-MAS: gk maize germplasm. Crop Science. 1993; 33:882.
81. Mehan VK, McDonald D, Haravu LJ, Jayanthi S. The Groundnut Aflatoxin Problem, Review and Literature Database. ICRISAT, Patancheru, India, 1991a.
82. Mestres C, Bassa S, Fagbohoun E, Nago M, Hell K, Vernier P *et al*. Yam chip food sub-sector: hazardous practices and presence of aflatoxins in Benin. Journal of Stored Products Research. 2004; 40:575-585.
83. Moreno OJ, Kang MS. Aflatoxins in maize: the problem and genetic solutions. Plant Breeding. 1999; 118(1):1-16.
84. Moustafa AM, Losso JN, King JM, Park DL. Ammoniation of aflatoxin contaminated raw peanuts at ambient temperature and pressure. In: Proceedings of the Institute of Food Technologists Annual Meeting. June 23-27, New Orleans, Louisiana, 2001, 30-55.
85. Munkvold GP. Cultural and genetic approaches to managing mycotoxins in maize. Ann. Rev. Phytopathol. 2003; 41:99-116.
86. Murdock LL, Shade RE, Kitch LW, Ntoukam G, Lowenberg-Deboer J, Huesing JE *et al*. Postharvest storage of cowpea in sub-Saharan Africa. In Singh BB *et al*. Eds., Advances in Cowpea Research. Co-publication IITA and JIRCAS. IITA, Ibadan, Nigeria, 1997, 302-312.
87. Muscarella M, Lo Magro S, Palermo C, Centonze D. Validation according to European Commission Decision '02/657/EC of a confirmatory method for aflatoxin in milk based on immunoaffinity columns and high performance liquid chromatography with fluorescence detection. Analytica chimica acta. 2007; 594:257-264.
88. Nayak M, Kotian A, Marathe S, Chakravortty D. Detection of microorganisms using biosensors-A smarter way towards detection techniques. Biosensors and Bioelectronics. 2009; 25:661-667.
89. Nesci AV, Bluma RV, Etcheverry MG. *In-vitro* selection of maize rhizobacteria to study potential biological control of *Aspergillus* section Flavi and aflatox in production. Eur. J Plant Pathol. 2005; 113:159-171.
90. Park DL. Effect of processing on aflatoxin. Advances in Experimental Medicine and Biology. 2002; 504:173-179.
91. Pelczar JMJ, Chan ECS, Krieg NR, Pelczar MF, Edwards DD. Microbiology: Concepts and Applications, McGraw-Hill, Inc., 1993, 279-286.
92. Peraica M, Radic B, Lucic A, Pavlovic M. Toxic effects of mycotoxins in human health. Bull WHO. 1999; 77:754-766.
93. Rasch C, Bottcher M, Kumke M. Determination of aflatoxin B_1 in alcoholic beverages: comparison of one- and two-photon-induced fluorescence. Analytical and Bioanalytical Chemistry. 2010; 397:87-92.
94. Rodriguez SB, Mahoney NE. Inhibition of aflatoxin production by surfactants. Appl. Environ. Microbiol. 1994; 60:106-110.
95. Rojas-Duran TR, Fente Vazquez BI, Franco CM, Sanz-Medel A, Cepeda A. Study of a room temperature phosporescence phenomenon to allow the detection of aflatoxigenic strains in culture media. International Journal of food microbiology. 2007; 115:149-158.
96. Salter R, Douglas D, Tess M, Markovsky B, Saul SJ. Inter laboratory study of the Charm ROSA Safe Level Aflatoxin M1 Quantitative lateral flow test for raw bovine milk. Journal of AOAC International. 2006; 89:1327-1334.
97. Sanders TH, Blankenship PD, Cole RJ, Hill RA. Effect of soil temperature and drought on peanut pod and stem temperatures relative to *Aspergillus flavus* invasion and aflatoxin contamination. Mycopathologia. 1984; 86:51-54.
98. Scheidegger KA, Payne GA. Unlocking the secrets behind secondary metabolism: A review of *Aspergillus flavus* from pathogenicity to functional genomics. Journal of Toxicology. 2003; 22:423-459.
99. Scott GE, Zummo N. Sources of resistance in maize to kernel infection by *Aspergillus flavus* in the field. Crop Science. 1988; 28:504-507.
100. Sejakhosi M, Angel M, Magan N. Effect of environmental factors on *in vitro* and *in situ* interactions between atoxigenic and toxigenic *A. flavus* strains and control of aflatoxin contamination of maize. Biocontrol Science and Technology. 2013; 23(7):776-793.
101. Sétamou M, Cardwell KF, Schulthess F, Hell K. Effect of insect damage to maize ears, with special reference to *Mussidia nigrivenella* (Lepidoptera: Pyralidae), on *Aspergillus flavus* (Deuteromycetes: Monoliales) infection and aflatoxin production in maize before harvest in the Republic of Benin. Journal of Economic Entomology. 1998; 91:433-438.
102. Smith JE. Aflatoxins. In: Handbook of Plant and Fungal Toxicants (J.F.P. D'Mello ed.) : CRC Press, Boca Raton, NY, 1997, 269-285.
103. Sobolev VS. Simple, rapid, and inexpensive clean up method for quantitation of aflatoxins in important agricultural products by HPLC. Journal of agricultural and food chemistry. 2007; 55:2136-2141.
104. Stroka J, Anklam E. New strategies for the screening and determination of aflatoxins and the detection of aflatoxin

producing moulds in food and feed. TrAC-Trends in Analytical Chemistry. 2002; 21(2):90-95.
105. Stroka J, Van Otterdijk R, Anklam E. Immunoaffinity column clean-up prior to thin-layer chromatography for the determination of aflatoxins in various food matrices. Journal of Chromatography A. 2000; 904:251-256.
106. Tubajika KM, Damann KE Jr. Glufosinate-ammonium reduces growth and aflatoxin B_1 production by *Aspergillus flavus*. J Food Prot. 2002; 65:1483-1487.
107. Turner PC, Sylla A, Gong YY, Diallo MS, Sutcliffe AE, Hall AJ *et al*. Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in West Africa: a community-based intervention study. The Lancet. 2005; 365:1950-56.
108. Udoh JM, Cardwell KF, Ikotun T. Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. Journal of Stored Products Research. 2000; 36:187-201.
109. USAID and Danya International. Aflatoxin: A Synthesis of the Research on Health, Agriculture and Trade, 2012, 36.
110. Waliyar F, Ba A, Hassan H, Bonkoungou S, Bosc JP. Sources of resistance to *Aspergillus flavus* and aflatoxin contamination in groundnut genotypes in West Africa. Plant Disease. 1994; 78:704-708.
111. Walker GM. *Pichia anomala*: cell physiology and biotechnology relative to other yeasts. Antonie van Leeuwenhoek. 2011; 99:25-34.
112. Wheeler MH. Effects of chlobenthiazone on aflatoxin biosynthesis in *Aspergillus parasiticus* and *A. flavus*. Pesticide Biochemistry and Physiology. 1991; 41:190-197.
113. Wicklow DT, Bobell JR, Palmquist DE. Effect of intraspecific competition by *Aspergillus flavus* on aflatoxin formation in suspended disc culture. Mycol Res. 2003; 107:617-623.
114. Widstrom NW, Wilson DM, McMillian WW. Ear resistance of maize inbreds to field aflatoxin contamination. Crop Science. 1984; 24:1155-1157.
115. Widstrom NW, Wilson DM, McMillian WW. Differentiation of maize genotypes for aflatoxin concentration in developing kernels. Crop Science. 1986; 26:935-937.
116. Wild CP, Turner PC. The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis. 2002; 17:471-481.
117. Williams WP, Windham GL. Registration of maize germplasm line Mp715. Crop Science. 2001; 41:1375.
118. Williams WP, Windham GL. Registration of maize germplasm line Mp717. Crop Science. 2006; 46:1407-1408.
119. Williams WP, Windham GL. Registration of Mp718 and Mp719 germplasm lines of maize. Journal of Plant Registrations. 2012; 6(2):200-202.
120. Wilson JP, Jurjevic Z, Hanna WW, Wilson DM, Potter TL, Coy AE. Host specific variation in infection by toxigenic fungi and contamination by mycotoxins in pearl millet and corn. Mycopathologia. 2006; 161:101-107.
121. Wu F, Bhatnagar D, Bui-Klimke T, Carbone I, Hellmich R, Munkvold G *et al*. Climate change impacts on mycotoxin risks in US maize. World Mycol. J. 2011; 4:79-93.
122. Yu J, Cleveland TE, Nierman WC, Bennett JW. *Aspergillus flavus* genomics: Gateway to human and animal health, food safety, and crop resistance to diseases. Revista Iberoamericana de Micología. 2005; 22:194-202.
123. Zablotowicz RM, Abbas HK, Locke MA. Population ecology of *Aspergillus flavus* associated with Mississippi Delta soils. Food Addit Contam. 2007; 24:1102-1108.
124. Zuber MS, Darrah LL, Lillehoj EB. Comparison of open pollinated maize varieties and hybrids for preharvest aflatoxin contamination in the southern United States. Plant Disease. 1983; 67:85-187.