A REVIEW OF SELECTED PREHARVEST MANAGEMENT OPTIONS OF ASPERGILLUS FLAVUS AND AFLATOXIN CONTAMINATION OF MAIZE IN TANZANIA

Goodluck P. Tito*, Jovin K. Mugula[2] and Richard Raphael Madege[1]
[1]Department of Crop Science and Horticulture,
[2]School of Engineering and Technology
Sokoine University of Agriculture, P. O. Box 3005, Chuo Kikuu, Morogoro, Tanzania

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ABSTRACT
Maize (Zea mays L.) is a staple food in Tanzania, but it is often susceptible to aflatoxin contamination caused by the Aspergillus flavus fungi. Aflatoxin contamination in crops is influenced by insufficient knowledge of pre-harvest management practices. Due to the toxic nature of aflatoxins, their proportions and concentrations in various food ingredients are subject to strict regulations in developed countries. The contamination resulting from aflatoxins remains one of the critical mycotoxin challenges in Tanzania because it affects food safety, security, trade, and human health. Either, an integrated combination of intervention measures such as biocontrol is the perfect strategy for sustainable reduction of A. flavus and aflatoxin production in maize. This paper explores several agricultural approaches that potentially reduce aflatoxins production in maize. Selected bio-controls such as Trichoderma spp and Atoxigenic A.flavus are among these strategies. The anticipation of this appraisal is to stimulate improvement of the existing aflatoxin management methods and inventions to exploit their effectiveness in managing toxigenic A.flavus and Aflatoxin production at harvest.

Keywords: Aflatoxin contamination, Aspergillus flavus and Trichoderma asperellum.

1. INTRODUCTION
Mycotoxins are groups of naturally occurring secondary metabolites of fungi that are toxic and contaminate maize before and after harvesting (Smith et al., 2012). Mboya and Bogale (2012) stated that mycotoxin contamination in maize risks human and livestock health. The critical mycotoxins in maize are aflatoxin, fumonisin, deoxynivalenol, and ochratoxin (Kimanya et al., 2012). Aflatoxins are secondary metabolites of Aspergillus fungi (Wild and Gong, 2010) from two main species; *Aspergillus flavus*, which has aflatoxin B1, and B2 *Aspergillus parasiticus*, with aflatoxin B1, B2, G1 and G2 (Omar, 2013). Aflatoxin B1 is acute and linked to liver cancer, immunosuppression, growth destruction, death upon high dosage, and prolonged exposure to aflatoxin, which significantly adversely affect agriculture, food security, and trade (Shephard, 2008). Aflatoxin is widespread in the food chain and causes significant adverse health consequences since it is common in maize and peanuts, which are essential in developing countries' diets (Smith et al., 2012).
Aflatoxins cause severe problems and are rich in maize because their contamination occurs under favourable climates in the field (Krnjaja et al., 2013). Conditions favourable for fungal growth are high temperature and humidity, low soil fertility, drought and insect infestation, monsoon, and unexpected rain during harvest (Kamala et al., 2015). Countries with latitudes between 40ºN and 40ºS, like Tanzania, favour growing conditions suitable for the fungi populations and their

exposure to infect the crops (Hussaini et al., 2012). IITA reported that about $1.2 billion in world trade is lost to aflatoxin contamination, with African economies losing $450 million annually (IITA, 2013). Schmalle III and Munkvold (2015) report that more than four billion people in developing nations are at aflatoxins risk. In Tanzania, it is reported that newborns and children experience high exposure to aflatoxin through maize intake (Shirima et al., 2014) and breast milk from mothers whose maize is their primary diet (Magoha et al., 2014). Thus, a holistic, integrated management approach to aflatoxin in maize is undeniably mandatory to reduce the occurrence and rise of aflatoxin contamination in Tanzania and to minimise the health risk of aflatoxins to consumers (Massomo, 2020).

1.1 Maize Production

More than 70 million tons of maize in Africa have produced annually (Macauley, 2015). Either, Maize is susceptible to contamination by a variety of mycotoxins, including aflatoxin; The risk of exposure in Africa is higher than in other parts of the world because it is one of the major cereal crops consumed throughout the continent, and its favourable environmental condition for fungal growth (Kimanya et al., 2014; Wilson and Lewis, 2015). Maize (Zea mays) is one of the most important food crops grown in all regions of Tanzania (Ismail et al., 2015; Kimanya et al., 2010). More than half of the arable land in Tanzania is devoted to cereal crops, but maize is the leading and most preferred food crop among all food crops and produced crops (Suleiman and Rosentrater, 2015). National consumption is estimated at over 3 million tons per year.

In contrast, rural residents' daily per capita maize consumption is 450 g (Smith and Subandoro, 2012). Maize accounts for 31% of total food production and more than 75% of cereal consumption in Tanzania (Suleiman and Rosentrater, 2015). Suleiman and Rosentrater also show that Tanzania's estimated annual per capita maize consumption is about 128 kg. The potential seen in maize has been overshadowed by reports that maize is a more suitable substrate for mould infections and the production of mycotoxins harmful to humans and animals than other grains such as sorghum (Kpodo et al., 2000; Bandyopadhyay et al., 2007). In 2020, Boni et al. reported the prevalence of aflatoxin contamination in 200 maize samples collected from 10 districts of Tanzania. Quantification detected aflatoxin in samples gathered from all districts, and the prevalence of aflatoxin contamination ranged from 10 to 80%; this shows that maize contaminated with aflatoxin is common in Tanzania; therefore, people are more exposed due to food preferences.

1.2 Pre-harvest contamination of maize

Maize is highly susceptible to aflatoxin contamination when cultivated in soils with *Aspergillus flavus*. At the beginning of the growing season, sclerotia of Aspergillus fungi are exposed to the soil surface and then rapidly germinate spores to produce large quantities of conidia for the next season (Cotty, 2001). Fungal conidia in maize fields germinate earlier before silking; throughout the production season, infected plant tissues become a secondary conidial inoculum, mainly when environmental conditions are favourable for their spread (Scheidegger and Payne, 2003). Aflatoxin contamination is also influenced by aerial spores, whereby fungal dispersal by air leads to contamination of maize in the field during grain filling (Saori and Nancy 2011). Besides, moisture at the soil surface that promotes conidia germination is retained from the shade provided by the maize canopy; therefore, infection is severe in maize when caught by rain earlier or at harvest (Jaime-Garcia and Cotty, 2003). Therefore, management of plant canopy through plant spacing and moisture management by adhering to a good irrigation scheme is crucial.

2. ASPERGILLUS FLAVUS AND AFLATOXINS

2.1 Aspergillus flavus

A. flavus is a saprophytic soilborne fungus that infects, and its metabolites contaminate food crops in the field. After harvest, it is also responsible for producing Aflatoxin B1, a carcinogenic and the most potent toxin (Guru Prasad, 2014). According to Benkerroum (2020) aflatoxin producing *Aspergillus* species are described by three genera; *Flavi* (producing aflatoxins B and G), *Ochraceorosei* (producing aflatoxins B 1 and B 2), and *Nidulantes* (producing aflatoxin B 1). Remarkable variation has been noticed in the genus *Flavi* concerning the strain's capability to colonise and produce aflatoxins (Frisvad et al., 2019; Probst et al., 2014). On the contrary, Atoxigenic strains of *A. flavus* cannot produce aflatoxins. In contrast, some are more toxigenic and can produce large amounts of aflatoxins, exceeding 10,000 μg/kg (Benkerroum, 2019). The *A. flavus* strains are grouped into the S and L strains depending on morphologic characteristics and gene variations for aflatoxin production (Cotty,1989). The S strain has relatively small-sized sclerotia with a diameter of < 400 μm, producing abundant small sclerotia, fewer conidia, and larger quantities of aflatoxin B than the L strain, with less but more significant sclerotia with a diameter of > 400 μm, produces numerous conidia. In contrast, the amounts of the B aflatoxins vary (Cotty,1989). However, the S strain usually produces more aflatoxin than the L strains, irrespective of the aflatoxins produced (Benkerroum 2019: Probst et al., 2014).

2.2 Aflatoxins

Aflatoxins are secondary metabolites of aspergillus fungi responsible for contamination of agricultural produces, and after high exposure, it can result in sickness or death of consumer (Guchi, 2015). In 1961 aflatoxin was reported in England for the first time as a "Turkey X" disease that infects turkey and chicken from feeds with peanut meal blend; later, they were discovered as aflatoxins affecting livestock and humans (Richard, 2012). According to Benkerroum (2020), eighteen different types of aflatoxins have been documented. However, based on the higher levels of toxicities and leading incidence, they are; aflatoxin B 1, B 2, G 1, and G 2. These types of aflatoxin are identified depending on the colour of the light they emit when exposed to UV light, whether blue or green and their relative measure in thin-layer chromatography (N. Benkerroum, 2019;2020). Since aflatoxins are flavourless, colourless and odourless substances, it is challenging for farmers or consumers to notice and understand their existence in contaminated food products (Ncube and Maphosa, 2020). According to Massomo (2020), studies conducted in Tanzania, including a countrywide survey in 2013 regarding aflatoxin at harvest whereby 19%of 100 samples were found to have aflatoxin levels exceeding the maximum tolerable limit, with areas like Kilindi, Mvomero, and Kilosa identified with the highest risk. Boni et al., 2020 from the survey show that the mean aflatoxin level for maize collected in 10 regions of Tanzania had average contamination of 12.47 μg/kg, while the highly contaminated sample was 162.40 μg/kg.

2.2.1 Effects of Aflatoxins on Humans

Aflatoxins are natural poisonous substances practically tricky to eliminate from the food and feeds, later contributing to extensive contamination of maize production and its products.

Aflatoxin is a severe fungal contaminant leading to acute and permanent poisoning of man and livestock (Benkerroum, 2020). Exposure to aflatoxins in contaminated food is among the causes of hepatocellular carcinoma and the fifth most commonly reported cancer in humans globally (Williams and Windham, 2015). Besides, the International Agency for Research on Cancer (IARC) considers aflatoxin as a group 1a carcinogen capable of suppressing the immune system and may act together with hepatitis B virus infection (Benkerroum,2019).

Either, Periodic incidences of acute poisoning due to aflatoxin in maize were firstly reported in Tanzania in June 2016, whereby 68 cases and 20 deaths were reported from two regions of central Tanzania (Kamala et al., 2018). Eight (8) cases and four (4) deaths from the Manyara region (Outbreak News Today., 2017). As a result, most countries have established standard levels tolerable for human consumption (Shephard, 2008). However, these tolerance levels have little applicability to underprivileged, small-scale African farmers who depend on maize for daily sustenance and income. Thus, Kumar et al., (2000) listed the universal levels tolerable and the concerns of feeding products with aflatoxin (AFB1) for humans. The study clarifies that aflatoxins levels from 20 ppb (parts per billion) are the highest level acceptable for humans; when they exceed 100 ppb. It may slow the growth of children, but as it increases from 200 ppb to 400ppb will have the same implication on adult growth too, and beyond 400 ppb may lead to liver damage and finally cause cancer (Kumar et al., 2000).

3.FACTORS AFFECTING AFLATOXIN PRODUCTION IN MAIZE

The growth and multiplication of most fungi, including Aspergillus, depends on conducive environmental conditions such as temperature ranging from 25°C to 35 °C, water activity above 0.7 *a*w and atmospheric humidity at 77% or greater (Shuaib et al., 2010). Among the significant determining aspects in fungal occurrence and toxin production causing aflatoxins are water, high temperature and insect injury to the host plant. Similarly, crop growth stages, poor fertility management, crop densities, and weed competition raise fungal populations and toxin production (Dhanasekaran et al., 2011).

3.1 Fungal population

The soilborne fungus associated with the crop in the field, such as *Aspergillus,* severely impacts the frequency and severity of aflatoxin production in maize (Grubisha and Cotty,2010). The Toxigenicity of *Aspergillus* species differs depending on the strains' inherent features, their interaction with other microorganisms in the environment, and their association with the host crop (Benkerroum, 2020). On the contrary, the proportion of non-aflatoxigenic strains increased in the field reduces the ability of occupier fungal populations to produce their metabolites and finally decreases aflatoxin contamination in crops (Cotty et al., 2008). Additionally, Cotty et al., 2008 reported that in Arizona and South Texas, the relatively high toxicity of the S strains infested a moderately low quantity of cottonseed but was associated with a bulk of the aflatoxin pollution. Therefore, an increase in toxigenic strains would result in the rise of aflatoxin contamination. However, increasing the population of non-aflatoxigenic strains and other similar biocontrol agents has proven effective reduces the ability of aflatoxin production and their vast contamination.

3.2 Changes in climate condition

Climate change is a global driver of growing food security problems; its projected influence on food mycotoxins is of great concern (Battilani et al., 2016). Tropics and Sub-Sahara tropics countries, such as Tanzania, are more prone to aflatoxin contamination influenced by temperature and rainfall appropriate for the growth of *A. flavus* (Pratiwi et al., 2015). Also, a higher temperature is related to a rise in the production of *A. flavus* conidia, higher kernel infection rate, and conidia dispersal; in so doing, these condition contributes to high aflatoxins accumulation (Reddy et al., 2014). For that case, Mahuku et al., (2019) reported that maize collected from two Kenya regions during harvesting has a difference in levels of Aflatoxin B1 subjective to fungal population and variability in environmental conditions.

3.3 Pest invasion

In Tanzania, the crucial limitations to maize cultivation involve insect pests, rodents, weeds and pathogens (Akowuah et al., 2015). Broken grains are considerably susceptible to fungal attack and, therefore, to aflatoxin and other related mycotoxin contamination (Ostry et al., 2014). Besides, weed competition and insects feeding on developing kernels are tenser than insects feeding on cob and silk to facilitate the fungus' infection and production of aflatoxins (Kebede et al., 2012).

4. PRE-HARVEST MANAGEMENT OF *A. FLAVUS* AND AFLATOXIN CONTAMINATION

Since aflatoxin contaminates maize during cultivation, the significance of managing crop fungal infection and buildup of aflatoxins prior to harvest is emphasized by Bandyopadhyay et al., (2016) and Moral et al., (2020); this is reasonable since it is the field inoculum that sparks enormous aflatoxin contamination after harvesting and during storage. It mainly occurs when crop production is exposed to conditions enhancing fungal spread and aflatoxin production (Mahuku et al., 2019). Other pre-harvest management involves Good agricultural practices (GAP) tactics to minimize crop stress, improve crop vigour, and later decrease the succeeding exposure of maize to aflatoxigenic fungi, such as applying suitable soil, water and pest management techniques (Mutiga et al., 2019). Certain strains of fungi, bacteria, and yeast can manage pre-harvest and post-harvest plant pathogens and pests by testing a collection of selected biocontrol agents against *A. flavus* (Sivparsad et al., 2016).

4.1 Biocontrol controls and their combinations

Biological control methods to plant diseases management covers a slight decrease in the number or the influence of pathogens attained by the introduction of unlike biocontrol agent mechanisms, the activity of native or introduced biocontrol agents rise by changing the environment to enhance the activity of antagonist's microbes (Thambugala et al., 2020). Integrating biocontrol might offer a consistent pre-harvest control method to minimize aflatoxin contamination of maize. Biocontrol association between *A.flavus* and other biocontrols at pre-harvest minimises the buildup of aflatoxin in maize (Sivparsad et al., 2016). Particular mechanisms like antagonism, antibiosis, mycoparasitism or competition are common for some biocontrol microbes against plant pathogens. However, other biocontrol agents can operate in more than one mechanism in some cases (Elad and Freeman, 2002). The success of most biocontrols has been realized in a controlled environment such as greenhouses or laboratories, where necessary ecological parameters are maintained compared to open fields (Xu et al., 2011).

Moreover, to improve the efficacy of biocontrol made by a single microbe, the interest in combining several biocontrol agents is increasing in recent studies assessing various functional mechanisms in mixed biocontrol agent populations (Guetsky et al., 2011). It is essential to consider direct and indirect interactions between mixtures of biocontrol agent populations since the combined use of biocontrol agents results in improvement, reduction or similar biocontrol efficiency (Xu et al., 2011).

4.1.1 Atoxigenic *Aspergillus flavus*

This method consists of field application of non-toxigenic *A.flavus* biocontrol for its competitive exclusion mechanism against toxigenic A.*flavus*; reducing crop infection of *A. flavus* and later minimizing the level of aflatoxin contamination (Sivparsad et al., 2016). However, In 2019 Pitt et al., revised the benefits of atoxigenic *A. flavus* strain as a biocontrol agent. Findings show that field application of non-toxigenic *A. flavus* reduces the frequencies and magnitudes of aflatoxins in maize due to its effectiveness in disrupting and minimising the capability of native toxigenic *A. flavus* to produce the aflatoxins (Cotty,2008). Such studies inspired the International Institute of Tropical Agriculture (IITA) to manufacture Aflasafe TM, an African-registered biocontrol product. In Tanzania, two products, Aflasafe TZ01 and TZ02, were registered in 2018; these products have consistently reduced aflatoxin contamination in the field (Shah, 2019).

***4.1.2 Trichoderma* species**

Other biocontrol agents than atoxigenic *A. flavus*, antagonist microbes like fungi, demonstrate a great result in managing toxigenic *A. flavus* growth and reducing aflatoxin production on maize at harvest (Woo et al., 2014). Trichoderma is filamentous fungi, including rhizosphere strains related to the root ecosystem that naturally resides in our environment, primarily found in the soil (López-Bucio et al., 2015). Moreover, Trichoderma is among the antagonist fungi commonly used as bio fungicides in managing plant diseases worldwide (Woo et al., 2014). Besides, Trichoderma can control various foliar and soilborne plant pathogens. Other benefits are enhancement of plant resistance to abiotic stresses, plant mineral nutrition use, plant growth stimulation, and crop productivity (López-Bucio et al., 2015). The exceptional performance of Trichoderma as a biocontrol agent is due to its ability to display multiple antagonistic mechanisms against plant pathogens (Hermosa, 2014). Also, they are metabolically flexible and fast-growing fungi capable of feeding on numerous organic matters, making them an outstanding competitor in the soil ecosystem. In addition, their secondary metabolites act as a chemical weapon to inhibit plant pathogens by their interference antagonism (Hermosa, 2014).

5.CONCLUSION AND RECOMMENDATION

This review reveals that available control practices are ineffective enough to prevent excessive levels of aflatoxin contamination in food. Therefore, more research involving biocontrol agents is required to discover a practical, economical and safe method to prevent aspergillus infection on maize grain. Success in this will be realised by developing the most effective biocontrol agent with multiple effects against plant and soilborne pathogens under field conditions, either by combining several biocontrol agents with compatible modes of action or singly but with multiple modes of action against several pathogens. Finally, more effort is needed to raise awareness among farmers and consumers regarding the pre-harvest management of aflatoxin and its consequences on human health. The awareness should not only focus on areas with severe

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aflatoxin cases but countrywide since all maize consumers are victims of the maize products that originated from the infected areas.

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