REVIEW PAPER

Aflatoxin management in Sub-Saharan Africa: A call for intergrated aflatoxin management

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Abstract

Aflatoxin presents a major global public health and economic issue, particularly impacting maize producers, consumers, and markets. This toxic, mutagenic, and carcinogenic substance is produced by *Aspergillus* fungi and frequently contaminates maize, a key staple crop in Sub-Saharan Africa (SSA). The region's climate and inadequate food storage practices exacerbate maize contamination, posing significant health risks, including liver cancer, immune system suppression, and stunted growth in children. SSA is responsible for 40% (or 59,900) of the 155,000 annual cases of global aflatoxin-related liver cancer cases, driven by favorable conditions for fungal growth and poor food safety practices. Despite efforts to reduce contamination through agricultural best practices, biocontrol methods, and improved post-harvest management, these interventions have been insufficient. Challenges arise due to the complexity of contamination and inconsistent adoption by smallholder farmers. There is a need to combine different mitigation measures in order to achieve effect control of aflatoxin contamination in maize. This review explores the extent of aflatoxin contamination in maize, assesses the effectiveness of current mitigation methods, and underscores the need for combining different control measures including breeding resistant maize varieties as a long-term solution to improve food safety in SSA.

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Introduction

Aflatoxins are carcinogenic secondary metabolites found in agricultural foods and animal feeds biosynthesized by fungus from genera *Aspergillus* and *Fusarium* (Shephard, 2008).

They contaminate various agricultural products during crop development and after crop maturity and/or harvest (Cotty and Bayman, 1993). Both during preharvest and storage, maize kernels are contaminated with aflatoxin. Because of its important place in the food and feed supply chain and susceptibility to *Aspergillus flavus* contamination, maize contamination is a major global concern (Ostry *et al.*, 2021). The level of contamination is influenced by the host's vulnerability, the environment's propensity for infection, and the activity of the vector.

When the plant is exposed to drought stress as a result of cultural practices, leads to higher level of aflatoxins as it increases the exposure of susceptible silk to *A. flavus* airborne spores (Duncan *et al.*, 1981; Munkvold 2003). Aflatoxins produced by fungi are mycotoxins that contaminate a range of economically important crops such as maize, groundnut, sorghum, sugarcane, pearl millet, rice, cotton seed, and wheat (Andrade and Caldas, 2015). Both humans and animals are at risk for health problems when exposed to aflatoxin through food and feed products (Yu Zhang, 2016). The contaminations are natural toxicants that can't be eradicated easily from the diet and feeds. It results reduction in the economic value of the grain (Edwards 2006) and grain quality rendering it unfit for human consumption as well as animal feed. Aflatoxin infection on maize occurs from the field to storage (Sforza *et al.,* 2006). These fungi scatter from the soil, organic matter, and alternative hosts to developing crops (Cotty and Bayman, 1993). Post-harvest losses due to fungal infestations cause cob rots, discoloration of grain, and accumulation of mycotoxins hence resulting in huge losses (Chandrashekar *et al.*, 2000). The parasitic fungus *Aspergillus flavus*, which is saprotrophic and opportunistic, severely damages field crops. Due to faulty agricultural practices and poor storage management, mycotoxins and pests cause an average annual loss of maize of 20% to 30% (Shukla *et al.,* 2022).

Aflatoxin contamination is influenced by both biotic and abiotic factors that causes development of mold and toxins (Moreno and Kang, 1999; Edwards, 2006). The primary abiotic factors that cause plant stress and thereby enhance a plant's vulnerability to infection are moisture stress, a rise in temperature, and lower soil fertility (Moreno and Kang, 1999).

Naturally occurring fungi can produce aflatoxins if the combination of physical, chemical, and biological factors is appropriate. Chemical factors include, but are not limited to, such factors as air chemical composition and substrate type. Temperature and moisture are some of the physical ones, while the biological ones are those related to host species. In particular, high temperature, humidity stress, and insect damage act as dominant factors for mold infection and toxin production (Agag, 2004). Different studies reported the optimal temperature for the production of aflatoxin. (Lillehoj, 1983) reported the aflatoxin production temperature for *A. flavus* and *A. parasiticus* ranges from 12 to 41 °C, with optimal production between 25 and 32°C. (Weidenbörner, 2001), reported higher temperature stimulates the production of aflatoxin B relative to aflatoxin G. The optimal production of aflatoxin B_1 occurs between 24 and 28 °C whereas 30 °C is optimal for aflatoxin G_1 formation. An equal amount of aflatoxins B and G is induced by low temperatures between 8 and 10 °C. In feeds, the synthesis of aflatoxin increased at a temperature above 27 °C, humidity greater than 62%, and moisture level above 14% (Russo and Yanong, 2010). With climate change, this situation worsens due to increasingly suitable conditions for the production of aflatoxigenic mold growth and toxin production (Benkerroum, 2019).

The impact of eating contaminated food in SSA is underestimated because aflatoxin contamination is known to be frequent in low-income nations, for which there is little to no documentary evidence (Grace *et al.,* 2015). Furthermore, important players who could be crucial in controlling the situation, such farmers, have little knowledge in the causes, consequences, and countermeasures of aflatoxin contamination. Essentially, they are unwilling to bear the expenses of containing aflatoxin contamination, particularly as the majority of their transactions take place in unregulated black marketplaces (Sirma *et al.,* 2014). The health impact of exposure to aflatoxin contamination is significantly underreported due to lack of coordinated monitoring and medical surveillance. Moreover, mycotoxins are overlooked as important public health hazards and their control is inadequately supported and not prioritized by many African nations (Joint *et al.,* 2017). In cross-sectional and prospective studies conducted in sub-Saharan Africa, growth suppression in humans has been noted, with strong correlations found between aflatoxin exposure (Turner *et al.,* 2003; Turner *et al.,* 2007) The study conducted by (Liu and Wu, 2010) reported the Liver cancer development is the health impact for which chronic aflatoxin ingestion is most strongly associated. Worldwide, there are between 550,000 and 600,000 new cases of liver cancer reported annually; of these, 25,200 to 155,000 cases can be linked to aflatoxin exposure. To guarantee the safety and quality of food items, governments and international organizations enforce rules and guidelines regarding aflatoxin levels. A multifaceted strategy involving farmers, legislators, academics, and international organizations is needed to address aflatoxin contamination (Sundsmo *et al.,* 2015). The implementation of efficient regulatory measures, infrastructural improvement, and improved farming methods are the long-term answers that Sub-Saharan Africa needs to protect its economic stability and public health.

Since the problem of aflatoxin contamination starts in the field therefore, strategies for prevention need to start from the developing plant in the field (Munkvold, 2003). The most commonly used strategy for controlling aflatoxin contamination is through development of preharvest host resistance (Cary *et al.,* 2011). The availability of resistant genetic materials to aflatoxin contamination has been reported (Menkir *et al.,* 2008). To create disease-resistant cultivars, plant breeding and varietal selections have been employed (Menkir *et al.,* 2013). This review will discuss on the current status of aflatoxin in Sub-Saharan Africa, its effects, existing mitigation measures and their challenges, and the potential of utilizing integrated management practices for reducing the effects caused by aflatoxin contamination.

Importance of maize in Sub-Saharan Africa

Maize is one of the most significant crops in the world, mostly used for human and animal feed as well as an essential component of numerous industrial goods (FAOStat, 2021). More than 1.2 billion people in SSA depend on maize as their essential staple food crop. Maize makes up more than 30% of the calories consumed by people in sub-Saharan Africa (https://www.iita.org/cropsnew/maize/). The developing world's need for maize will nearly double from its current level by 2050 (Food and Nations, 2017; Rosegrant *et al.,* 2014; Rosegrant *et al.,* 2017). Tanzania is among the top 25 nations in the world for maize production (Suleiman and Kurt, 2015). In 2021 world maize production was 1.21 billion tones in 205 million hectares. In Africa maize production was 96.6 million tons produced on nearly 42.4 million hectares across all of Africa (FAOStat, 2022) (Fig. 1).
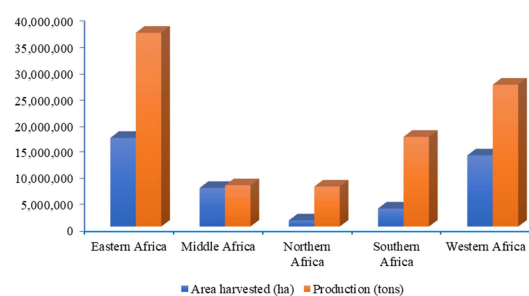

Fig. 1. Maize estimated area and yields by selected developing regions of Africa in 2021, data sourced from FAOSTAT 2022
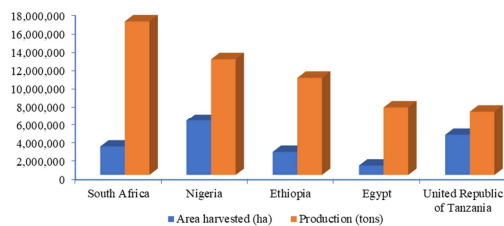
Fig. 2. Estimated area planted maize and production in year 2021 for the top five countries in Africa. Data source: FAOSTAT 2022

In the year 2021, Tanzania was the 5th largest producer of maize in Africa after South Africa, Nigeria, Ethiopia and Egypt respectively (FAOStat, 2022). Tanzania is the 2nd largest maize producer (19%) in eastern Africa after Ethiopia (29%) (Fig. 2). Maize is an important component for different industrial products and serves as a model organism for biological research (Shiferaw *et al.*, 2011). Maize-based dishes have deep cultural roots in many African communities, forming an integral part of their culinary heritage. Maize often features prominently in various rituals, ceremonies, and cultural celebrations across Sub-Saharan Africa (Ekpa, 2020). Maize is often used in food aid programs to combat hunger and malnutrition in vulnerable communities. Successful maize farming can alleviate poverty by providing a steady source of income for smallholder farmers. Maize's multifaceted role as a staple crop, economic driver, resilient crop, cultural symbol, and tool for agricultural development underscores its immense importance in Sub-Saharan Africa's socio-economic fabric.
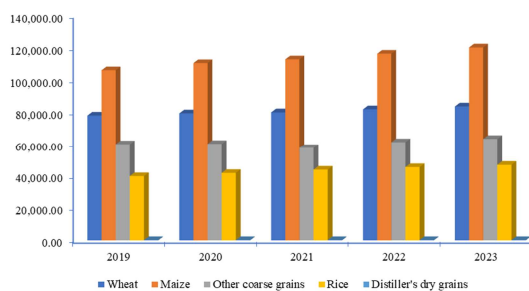

Fig. 3. Cereals consumption trend in Africa (unit tons, thousands), data sourced from OECD. Stat

Maize is mostly consumed as compared to other cereals in Africa (Fig. 3). More than 116 million tons of maize are thought to be consumed annually, with 30% and 21% of that consumption occurring in SSA and the rest of the world, respectively. Around 14 SSA nations rely mostly on white maize as a food source (85–95%) (https://www.iita.org/about/). There are several ways to eat maize: boiled or roasted green maize, steamed goods, porridges, drinks, bread, and snacks (Ranum *et al.*, 2014). Africa has a variety of cuisines made from maize, with each nation having its own food products, processing techniques, and ways of eating them (Mensah *et al.*, 2013). Within the same sociocultural group, variations in the ways that maize meal is processed and consumed can occasionally be used as a means of expressing social status and personal cultural identity (Ohna *et al.*, 2012). Maize cultivation supports the livelihoods of numerous farmers in Sub-Saharan Africa, providing income and sustenance for their families. It's a crucial commodity in regional and international trade, impacting the agricultural economy significantly (https://www.iita.org/cropsnew/maize/).

Contaminations, on the other hand, are a problem since they lower the market price for maize, which largely affects developing nations and slows down their economies. Several farmers still use local varieties of maize. Poor farming techniques used by them result in plant diseases and fungal spore contamination of maize (Jallow *et al.,* 2021). The fungus creates aflatoxins and stains the maize under unfavorable climatic conditions. Due to its economic importance and its use as food and feed for livestock, there is a need of developing resistant maize varieties to aflatoxin contamination.

Current status of aflatoxin contamination of maize in Sub-Saharan Africa
Aflatoxin contamination in Sub-Saharan Africa holds significant importance due to its widespread occurrence and detrimental effects on health, agriculture, and economies in the region (Table 1). Many African countries are widely exposed to aflatoxins contamination (Montesano *et al.* 1997) (Fig. 4).

Table 1. Drivers for aflatoxin contamination

| SL	Drivers	Challenge	Option for management
1.	Environmental Conditions	• Warm, humid conditions favour the growth of aflatoxin-producing fungi, such as *Aspergillus flavus*, making control difficult in tropical and subtropical areas. • Climate change worsens the situation by causing unpredictable weather, higher temperatures, and increased rainfall, all of which encourage fungal growth.	• Breeding resistant varieties through marker-assisted techniques can produce crops that are less susceptible to fungal infection. • Breeding for maize genotypes that can withstand the adverse weather conditions
2.	Pre-harvest Contamination	• Inadequate pest control can cause insect damage to crops, creating pathways for fungal infection. • Soil quality problems and water stress weaken plants, making them more vulnerable to contamination.	• Breeding resistant varieties through marker-assisted techniques can produce crops that are less susceptible to fungal infection. • Biological control using non-toxic strains of *Aspergillus* (like Aflasafe) can reduce the growth of toxic strains. • Pest control through integrated pest management (IPM) helps reduce insect damage, which can lower the risk of contamination. • Improved farming practices such as crop rotation, and better irrigation can strengthen plants and reduce fungal infections.
3.	Post-harvest Management:	• Improper drying and storage after harvest allow fungi to continue growing. • Traditional storage methods, such as using poorly ventilated spaces or unsuitable bags, fail to keep moisture levels low enough to prevent fungal contamination.	• Effective drying through solar or mechanical methods helps reduce moisture in harvested crops, limiting fungal growth. • Hermetic storage solutions, like airtight bags or silos, reduce the oxygen and moisture that fungi need to thrive during storage. • Timely harvesting of crops at appropriate moisture levels minimizes prolonged field exposure, which can lead to contamination.
4.	Lack of modern storage facilities	• Poor infrastructure, like inadequate drying equipment and storage facilities, limits efforts to prevent contamination.	• Community-based initiatives that promote shared storage and drying facilities can reduce post-harvest contamination risks.
5.	Lack of Awareness	• Many farmers and traders are unaware of the dangers of aflatoxin, as well as detection and prevention techniques.	• Farmer and trader education through training programs and awareness campaigns on aflatoxin prevention and safe handling practices is essential.
6.	Economic Factors	• Smallholder farmers often lack the funds to invest in better storage solutions, pesticides, or seeds that are resistant to aflatoxin. • Testing for aflatoxin and implementing prevention methods can be costly for small-scale producers.	• Financial support and subsidies for aflatoxin-reducing technologies, such as biological controls, resistant seeds, and drying equipment, can help smallholder farmers. • Stronger regulations and the introduction of affordable testing kits will improve food safety monitoring. • Market incentives for aflatoxin-free products can encourage farmers to adopt safer practices.
7.	Regulatory Challenges	• Many regions have weak enforcement of food safety standards and lack uniform regulations for aflatoxin. • There is often limited capacity for regular testing along the food supply chain.	• Advanced detection tools that are portable, affordable, and easy to use can help detect contamination early in the supply chain. • Digital traceability systems, like blockchain, can track contamination levels, improving transparency and ensuring a safer supply chain.
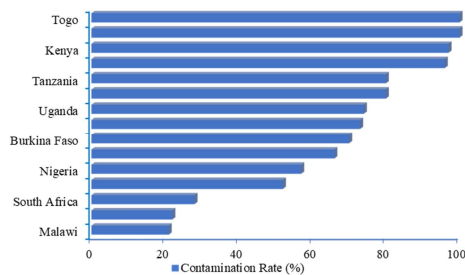
Fig. 4. Aflatoxin contamination rate in Sub-Saharan Africa countries, data sourced from Nji *et al.*, 2022

The region has conducive environmental, agricultural and storage condition for *Aspergillus* fungi infection and aflatoxin biosynthesis (Gnonlonfin *et al.*, 2013).

Aflatoxins can affect a wide range of food commodities, including oilseeds, cereals, milk, tree nuts, dried fruits and meat. Groundnut and maize are susceptible to aflatoxin contamination. According to (Gong *et al.*, 2016), maize and groundnuts are among the most significant food crops in sub-Saharan Africa due to their high daily consumption rates and use as key components in weaning meals for kids. Due to their large daily consumption, maize and groundnuts are crucial aflatoxin-prone crops to take into consideration (Gong *et al.*, 2016). Maize plants exposed to drought stress are more susceptible to infection by toxigenic fungi than are unstressed plants (Kebede *et al.,* 2012). When the plant is exposed to drought stress as a result of cultural practices, leads to higher level of aflatoxins as it increases the exposure of susceptible silk to *A. flavus* airborne spores (Jones *et al.*, 1981; Munkvold, 2003).
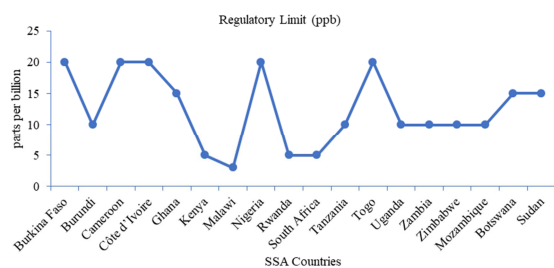

Fig. 5. Aflatoxin limit in SSA, data sourced from FAO corporate documents repository. Worldwide regulation for mycotoxins in food and feed 2003

Sub-Saharan Africa countries are in greater health risk as they rely on these commodities for sustenance

and income. Aflatoxin contamination often leads to significant food losses, impacting food security and causing economic hardships for farmers and communities. Since aflatoxin levels in maize are strictly controlled in many parts of the world, this is more of an economic than a health issue (Warburton and Williams, 2014). As a result, the majority of nations and areas have put in place safety laws to restrict exposure and lower public danger, both directly and indirectly (Chilaka *et al.* 2022). Trade barriers arise due to strict regulations on aflatoxin levels in exported crops (Fig. 5). This limits international market access for African produce, affecting economic growth and trade opportunities. The level of aflatoxin contamination in Africa is aggravated by low agronomic input practices, unsuitable grain storage, extreme weather conditions and inadequate knowledge and actions in management of mycotoxin (Shephard, 2003). Sub-Saharan Africa lack on food sufficiency and diversity which expose individuals to aflatoxin contamination (Strosnider *et al.,* 2006). There have been reports of excessive aflatoxin contamination in maize all over Africa (Table 2). Study in Kenya by Sirma *et al.* (2014), and Sirma *et al.* (2019) detected aflatoxin exposure from milk contributes relatively little to the incidence of liver cancer. Turner *et al.* 2003 in Gambia detected high dietary levels of aflatoxins exposure to sampled children (6-9 years) and established the maximum level for aflatoxin ingestion was between 2 and 10 μg/kg. Maize from Ghana have been reported to show occurrence of fumousins and aflatoxin (Kpodo *et al.*, 2000) and also from Benin (Fandohan *et al.*, 2005).

Consumption of aflatoxin contaminated food in Kenya lead to outbreak of 125 sudden deaths in 317 reported cases (Azziz-Baumgartner *et al.,* 2005; Lewis *et al.*, 2005), 15 and 16 deaths reported in 2005 and 2006 respectively (Anonymous, 2006). In Kenya About 20 different types of *Aspergillus* strain have been reported, and aflatoxin B_1, B_2, G_1, G_2 and M_1 are of demonstrated toxicological importance (Wu *et al.*, 2009). Maize, peanuts, and

their products are the most contaminated food crops in Kenya (Omara *et al.*, 2021). In the year 2016, two regions in the central part of Tanzania reported 68 cases and 20 deaths due to consumption of aflatoxin contaminated food. The contamination ranged: 10-51,100 µg/kg and 2.4-285 µg/kg for case and control households, respectively (Kamala *et al.*, 2018). In year 2017, the northern part of Tanzania 8 cases was reported with 4 deaths occurred (50%) due to consumption of contaminated maize (Outbreak News, 2017). The study conducted by Boni *et al.*, 2021 in 10 districts of Tanzania, found the mean aflatoxin level for maize samples was 12.47 µg/kg and the highly

contaminated sample had 162.40 µg/kg. Maize accounts for 41% of Tanzanian household weekly calorie intake (Abt., 2012). According to Boni *et al.* (2021), the calculated average probable daily consumption of aflatoxin B_1 from maize ranges between 151.98 and 272.89 µg/kg body weight/day, far above 10 µg/kg and 4 µg/kg maximum tolerable limits for aflatoxins for East African region and European Union respectively (EAC, 2011; EC, 2010). High dietary exposure to aflatoxins and/or fumonisins from foods based on maize has been linked to stunted growth in children in Tanzania, according to research done by (Kimanya *et al.*, 2010; Shirima *et al.*, 2015; Magoha *et al.*, 2016).

Table 2. Survey of maize food products contaminated with aflatoxin in some SSA countries

Class of aflatoxin	Incidence rate (Sample size)	Detection range	Country	Reference
AFB1	317	44.14 to 354.53 µg/kg	Kenya	(Azziz-Baumgartner *et al.*, 2005)
AF Total	317 (350)	20 to > 1,000 µg/kg	Kenya	(Lewis *et al.*, 2005)
AF Total	83 (49)	11 to 51,100 µg/kg	Tanzania	(Kamala *et al.*, 2018)
AFB1	99 (200)	12.47 to 162.40 µg/kg	Tanzania	(Boni *et al.*, 2021)
AF Total	37.7 (326)	<LOD to 341 µg/kg	Ghana	(Agbetiameh *et al.*, 2018)
AFB1, AFG1	26.6 (61)	0.02 to 0.19 µg/kg	Egypt	(Abdallah *et al.*, 2019)
AF Total	25.8 (256)	0 to 3760 µg/kg	Uganda	(Sserumanga *et al.*, 2020)
AF Total	100 (150)	20 to 91.04 µg/kg	Ethiopia	(Chauhan *et al.*, 2016)
AF Total	88.4 (112)	0 to 3,000 µg/kg	Niger and Benin	(Bakoye *et al.*, 2017)
AFB1	76 (70)	75.9 µg/kg	Togo	(Hanvi *et al.*, 2020)
AF Total	(70)	>10 µg/kg	Nigeria	(Adutenji *et al.*, 2014)
AF Total	(125)	LOD up to 115.6 µg /kg	Malawi	(Matumba *et al.*, 2014)

Effects of Aflatoxins on human, animal health and plants

Different types of aflatoxins have been identified and considered to be important and are designated as B_1, B_2, G_1, G_2, M_1, and M_2, respectively (Wacoo *et al.,* 2014). Aflatoxin B_1 is the most potent followed by G_1 and M_1 (JECFA, 2017). Naturally occurring Aflatoxins are B_1, B_2, G_1, and G_2. They are named according to the color of the light (blue or green) under UV light, and their relative movement in thin-layer chromatography (Bennett *et al.,* 2007). Among these, the most common and widespread in the world is aflatoxin B1 which accounts for 75% of all contamination of food and feeds (Ayub and Sachan, 1997). Aflatoxins M_1 and M_2 are interconnected with cow milk upon ingestion of aflatoxin B1 and B2 respectively, as their hydroxylated products (Stroka and Anklam, 2002). Aflatoxin has been categorized by

The International Agency for Research on Cancer (IARC) as a Group 1 carcinogen, it can suppress the body's immune system and interact with hepatitis B virus infection (Cancer and Organization, 2012; Sabran *et al.*, 2012).

Aflatoxins on human health

Aflatoxin-contaminated maize can kill humans and livestock both acutely and chronically when consumed (Benkerroum, 2019). Aflatoxin-induced acute hepatic damage can lead to chronic illnesses such as liver cancer in humans. It has been linked to immune system depression, growth retardation, cancer, and even mortality (Strosnider *et al.*, 2006). One in ten people in Sub-Saharan Africa frequently have hepatitis B and C. Aflatoxin consumption increases the incidence of liver cancer by almost ten times when compared to hepatitis exposure alone

(Turner *et al.*, 2003). According to the study by Jiang *et al.* (2005), individuals with high levels of aflatoxin B1 had a lower percentage of CD31 and CD191 cells than those with low levels of aflatoxin B_1, indicating that aflatoxin and HIV interact to suppress the immune system in humans.

Aflatoxins on animal health

Maize is a vital component of feed formulations for nongrazing animals like pigs and chickens. Numerous studies conducted in East Africa found high levels of aflatoxin contamination in animal feeds. As a result, consuming aflatoxin-contaminated feed negatively impacts an animal's health and productivity because it can cause liver cancer, birth deformities, malformations, decreased body weight and growth, immunosuppression, and even death (Massomo 2020; Ncube and Maphosa, 2020). Aflatoxin exposure and intake in less severe cases can cause vomiting, depression, bleeding, jaundice, general body weakness, appetite loss, stunted growth, malnutrition, and even unexpected fatalities in animals (Massomo, 2020). Compared to pigs, ducks, and turkeys, ruminants and chickens are more resistant to aflatoxin contamination (Benkerroum, 2020). Because of their potency and toxicity, aflatoxins are the main cause of health risks and are also responsible for losses resulting from contaminated processed foods and feeds (da Rocha *et al.*, 2014; Massomo, 2020).

Aflatoxins on plants

Aflatoxin exposure in plants slowed down root elongation, seed germination, and seedling growth. It also prevents the creation of certain enzymes, carotenoid, and chlorophyll (Jonathan *et al.*, 2016). Aflatoxin infection on maize occurs from the field to storage. Farmers in Africa may not be able to access prospective markets both inside and outside of their countries If the maximum tolerated limits for aflatoxin contamination in maize commodities exceeds. For example, in 2013 the World Food Program deemed about 60,000 bags of maize harvested under the Kenyan Hola Agricultural Irrigation Scheme unsuitable for

human consumption because the level of aflatoxin contamination above the maximum threshold limit (Omondi, 2019). In November 2019, aflatoxin contamination forced the recall of five kinds of maize flour from Kenyan retailers (Mutahi, 2019). Likewise, in The Kenya Bureau of Standards prohibited 17 brands in January 2020 because their maize flour did not reach the maximum threshold for aflatoxin (Asamba, 2020). The same is happening to Tanzania, when sends maize to its neighbors, any shipments that are found to have aflatoxins in excess of what is acceptable (EAC) partner states of 10 μg/Kg would either not sell for much or could be rejected and destroyed.

Mechanism of aflatoxin contamination in maize

Aspergillus flavus reproduce only asexually (Brown *et al.*, 1998). Colonization of the kernel surfaces by *A. flavus* is extremely important in the epidemiology of this disease development (Marsh and Payne, 1984; Abbas *et al.*, 2009). From the glume tissue surrounding the kernel surface, the fungal takes one of the two ways to enter the intact seed. From the surface of rachis and spikelet where they grow and enter at the junction between the bracts and rachillas (Marsh and Payne, 1984; Smart *et al.*, 1990). The fungus can also grow through a continuous air space from the rachis into the spikelet (Smart *et al.* 1990). *A. flavus* grows saprophytically as they colonize the living plant tissue and plant tissues in the soil. Plant residues save as the reservoir of the fungus where they can overwinter and resume growing under favorable conditions. The air and insects are the media for transporting the conidia on the host plant or remains in the field (Abbas *et al.*, 2009). Under suitable physical, chemical, and biological factors, naturally occurring fungi can develop aflatoxins. The chemical factors include the composition of the air and the nature of the substrate. The physical factors include temperature and moisture. Biological factors are those associated with the host species. The major determining factors for mold infestation and toxin production are high temperature, humidity stress and insect damage (Agag, 2004).

Aflatoxin mitigation methods in maize and their challenges

Different methods that have been used to mitigate aflatoxin contamination. i) Cultural practices like; timely planting, ideal plant densities, enough plant nutrition, preventing drought stress, managing other plant infections, weeds, and insect pests, as well as appropriate harvesting, are among the management techniques that lower the frequency of mycotoxin contamination in the field (Bruns, 2003). Cultural practices have been employed but with limited effects on infection and mycotoxin accumulation (Lillehoj and Wall, 1987; Moreno and Kang, 1999; Munkvold, 2003). ii) Several organisms have been examined for biological control against aflatoxin contamination, including bacteria, yeasts, and nontoxigenic (atoxigenic) strains of the organisms (Yin *et al.*, 2008), only atoxigenic strains of the causal species have made it to the commercial stage. According to studies conducted in the lab and in the field, these strains have been demonstrated to lower aflatoxin concentrations by 70–99%, hence lowering toxic contamination (Atehnkeng *et al.*, 2008; Atehnkeng *et al.*, 2014). The use of non-toxigenic fungi such as Aflasafe have been observed to minimize aflatoxin contamination. However, there are concerns about their accessibility in terms of distribution costs, effectiveness during droughts, allergy risk, and *Aspergillus* genotype dynamics. Furthermore, the use of Aflasafe need a lot of resources, including labor, cash, and time input (Abbas *et al.*, 2009). iii) Chemical control, Citric acid and lactic acid are more effective with inhibition rates as high as 86–92% and 67%, respectively, for the control of aflatoxins. Other organic and inorganic acids that have been tested for this purpose include tartaric acid, propionic acid, citric acid, and hydrochloric acid. Treatment with salts and acids, as well as alkaline compounds also reduced Aflatoxin contamination by up to 18–51%. Other chemicals include sodium bisulfite, Sodium hydrosulfite, and Ammonium persulfate has shown a reduction in aflatoxin contamination. Different adsorbents materials like activated charcoal, zeolites, complex carbohydrates like cellulose and polysaccharides, artificial polymers like polyvinyl pyrrolidone and cholestyramine, alumino, like clay, yeast, bentonite, and diatomaceous earth, and active carbon (Sipos *et al.,* 2021), The use of chemicals to most farmers are not easily accessible in terms of availability and affordability. iv) Aflatoxin development in crops can be stopped by adequately drying them before storage (Turner *et al.,* 2005). Temperature and moisture have an impact on the development of toxic fungus in products that are stored. Field-harvested maize stored at high moisture content can cause aflatoxin contamination to grow tenfold in three days (Hell *et al.*, 2008). It is generally advised to dry harvested goods as soon as possible, ideally to safe moisture levels of 10% to 13% for grains. It is challenging to do this by simple sun-drying in many African regions due to the high humidity levels. Mestres *et al.* (2004) noticed that even during the dry season, drying is not finished before loading grains into stores, which increases the risk of aflatoxins contaminating the products. Developed countries can prevent postharvest development of aflatoxins through modern infrastructure and grain storage practices but it's a challenge to developing countries. Aflatoxin contamination may still happen even when these managements practice are in place (Bandyopadhyay and Cotty, 2013; Bandyopadhyay *et al.,* 2016).

Due to the economic importance and significance of maize as a staple food crop, as well as the threat that aflatoxin contamination poses to food safety, several research initiatives have been launched in SSA and the globe at large to find maize genotypes that are resistant to aflatoxin contamination (Brown *et al.*, 2001; Menkir *et al.*, 2006; Grace *et al.*, 2015; Okoth *et al.*, 2017; Sserumaga, 2018). Host resistance strategy has gained eminence due to advances in identification of resistance traits (Cary *et al.*, 2011). The primary objective of host plant resistance is to prevent the fungus from spreading and colonizing developing plants (silk &cob) (Brown *et al.* 1999). Studies have revealed that, the traits for resistance to *Aspergillus flavus* infection are genetically controlled (Brown *et al.*, 1998; Li, 2004; Li and Kang, 2006).

Aspergillus flavus entry and infection are physically hindered by the physical composition of the kernel, cob husk covering, and tightness which are connected to lower levels of aflatoxin contamination (Edwards, 2006). Kernel proteins inhibit the biosynthesis of aflatoxin with a slight effect on fungal growth (Huang *et al.*, 1997). The protein inhibits the production and activity of fungal α-amylase which later restricts the fungal from using endosperm starch and fermentable sugars (Fakhoury and Woloshuk, 2001; Zhang *et al.,* 2010). The availability of genetic variability for resistance and access to reliable and effective screening methods are primary needs for creating cultivars resistant to pre-harvest aflatoxin contamination. There are a number of maize genotypes that show promise in terms of resistance to aflatoxin accumulation (Grace *et al.*, 2015; Okoth *et al.*, 2017), however no commercially cultivated resistant maize genotypes exist in SSA particularly in East African region (Mahuku *et al.*, 2019; Mutiga *et al.*, 2019), despite significant progress in discovering genes linked to pre-harvest aflatoxin contamination in maize and many other crops (Warburton *et al.*, 2013; Fountain *et al.*, 2015). Furthermore, even when various genotypes are cultivated under similar conditions, variations in aflatoxin contamination are frequently observed in the field (Kebede *et al.*, 2012). This review is written to describe aflatoxin contamination in maize in SSA and emphasize on potential of integrated approaches that are used to minimize aflatoxin contamination in maize including different methods for developing resistant maize genotypes.

Conventional breeding

Since the early 1970s, plant breeders and pathologists have been working to find genotypes of different crops that are resistant to aflatoxin accumulation in order to reduce aflatoxin contamination (Zuber *et al.*, 1978; Brown *et al.*, 1999; Moreno and Kang, 1999). Conventional breeding relies on combining traits from numerous populations within a species. The selected progeny is backcrossed with the recurrent parent for generations after the desired trait from the donor line is introduced into the elite recipient line to eliminate the non-targeted linked traits (Chen *et al.*, 2019). With conventional breeding, transfer of gene can only be done between plants that can sexually mate with each other. This restricts the number of new qualities that can be added to the ones that the species already possesses. When plants are crossed, a variety of qualities, including undesired traits are transferred along with the desired trait (Fig. 6). The resistance of maize to aflatoxin contamination is polygenic in nature and complex, identifying markers associated with resistance to aflatoxin contamination can help transfer resistance attributes to favored germplasm of maize (Cary *et al.*, 2011). Combining conventional breeding with marker-assisted selection to find quantitative trait loci linked to resistance can result in the transfer of resistance to superior germplasm (Williams *et al.*, 2005). Crops developed using conventional breeding including rice, wheat, maize, sorghum, lupine, common bean, potato, sweet potato, and tomato.
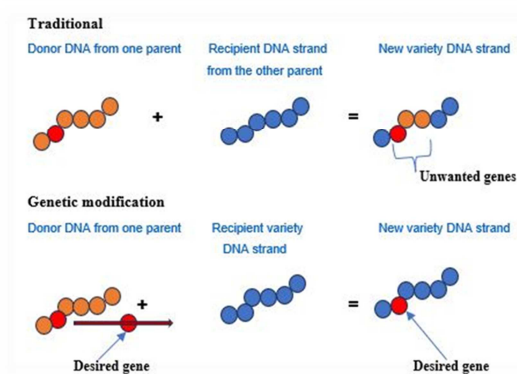

Fig. 6. Plants produced by traditional plant breeding methods receive a mixture of genes from both parents—genetic modification allows the selection of specific genes, rather than a mixture

Marker assisted breeding (MAB)

Marker-assisted breeding (MAB) is the use of molecular biotechnologies, more especially molecular markers, in conjunction with linkage maps and genomics to modify or enhance plant attributes based on genotypic testing (Jiang, 2013) (Fig. 7). Molecular markers (Table 3) are detectable DNA sequences located at certain locations of the genome and associated with the inheritance of a character or gene (FAO, 2004).

Table 3. SSR Markers associated with resistance to aflatoxin resistance

SL	Marker	Crop	Reference
1.	MpM1	Maize (Zea mays)	(Mylroie *et al.*, 2013)
2.	AGGS2289	peanut (Arachis hypogaea L.)	(Yu *et al.*, 2019)
3.	GM1916	peanut (Arachis hypogaea L.)	(Yu *et al.*, 2019)

Table 4. QTLs associated with resistance to aflatoxin resistance

SL	QTLs	Crop	Reference
1.	Chromosomes 1, 2, 3, 5, 8, and 10.	Maize (*Zea mays*)	(Womack *et al.*, 2020)
2.	Chromosomes 4,8, and 10	Maize (*Zea mays*)	(Dhakal *et al.*, 2016)
3.	Chromosome 2, 3, and 7	Maize (*Zea mays*)	(Busboom and White, 2007)
4.	Chromosome 8	Maize (*Zea mays*)	(Zhang *et al.*, 2016)
5.	Chromosome 1, 3, 4, 8, and 9.	Maize (*Zea mays*)	(Mayfield *et al.*, 2011)
6.	Chromosomes 1–8	Maize (*Zea mays*)	(Warburton and Williams, 2014)
7.	chromosomes except 4, 6, and 9	Maize (*Zea mays*)	(Brooks *et al.*, 2005)

According to Thottappilly *et al.* (2000) molecular markers are defined as naturally occurring polymorphism, comprising of proteins and nucleic acids that can be detected as being different. Markers can easily distinguish genotypes alleles that are susceptible or resistant to trait of interest (Mylroie *et al.*, 2013).

Thus, the use of markers can help identify progenies/genotypes with the trait of interest at an early stage of plant development (Francia *et al.,* 2005). The marker-assisted selection can help in nullifying the problems associated with phenotypic selection used in conventional breeding which is affected with genotype x environment interaction for some quantitatively inherited traits including aflatoxin resistance (Mohan *et al.*, 1997; Tabor *et al.*, 2002; Krishna *et al.*, 2023). Use of markers reduces breeding cycles as compared to conventional breeding since individuals are selected on the basis of genetic potential, not solely observable characteristics. A quantitative trait is a phenotype that can be measured and arises from both genetic and environmental influences that is not all or none but rather dispersed in magnitude among a population (Philibin and Crabbe, 2015). Quantitative trait loci (QTL) are regions on the chromosome containing the genes responsible for quantitative traits (Nadeem *et al.*, 2018). Several QTLs associated with resistance to aflatoxin contamination in different crop affected with aflatoxin contamination have been identified and introgressed into widely cultivated varieties (Williams, 2006). Crossing two parents in a biparental population can balance allele frequencies and increase the chance to detect rare QTLs. The narrow genetic diversity from only two parents limits the number of detected QTLs (Liu and Zeng, 2000; Pascual *et al.*, 2016). Multiparental populations (MPPs) have become popular for quantitative trait loci (QTL) detection (Li *et al.*, 2021). The segregating populations developed through crossing two parents with contrasting trait is used in construction of linkage map and identification of the QTLs (Pascual *et al.*, 2016). The creation of a linkage map using simple sequence repeat (SSR) markers and analysis of restriction fragment length polymorphisms (RFLP) helped in identifying chromosome regions responsible for aflatoxin production in the kernel and resistance to *A. flavus* (White *et al.,* 1995; Dhakal *et al.*, 2016).
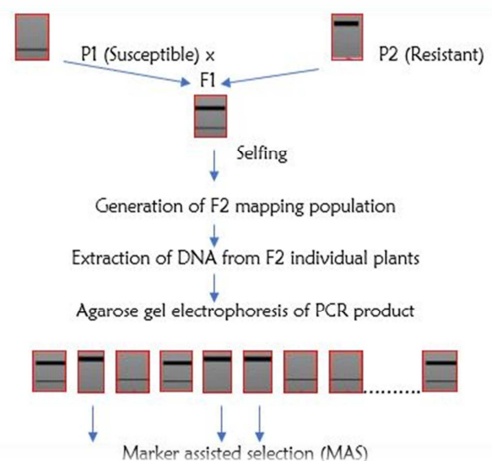
Fig. 7. Basic procedure for Marker assisted selection

On chromosomes 4, 8, and 10, the QTL linked to husk coverage and aflatoxin resistance was discovered

(Dhakal *et al.*, 2016). Single-nucleotide polymorphism (SNP) and simple sequence repeat (SSR) molecular markers were used to genotype the maize population. The results showed that two QTLs in bins 6.06 and 7.03 were the most promising for marker-assisted resistance introgression (Smith *et al.* 2019). From CML69, Caribbean Composite germplasm with stability expression across the environment, QTL in resistant lines with significant enough phenotypic effects to be employed in marker-assisted introgression have been found (Ogunola *et al.,* 2021). QTL linked to decreased aflatoxin accumulation in different regions of maize chromosome have been found from different studies (Table 4). Breeders can develop effective breeding strategies using marker-assisted selection to breed for resistance to *A. flavus* infection and aflatoxin accumulation in maize.

Genetic engineering
Genetic engineering is the direct application of biotechnology to modify an organism's genetic makeup (Christou, 2013). Genetic engineering works with a fragment of DNA in constructing an individual with new characteristics. Plant genetic engineering rely on traditional transgenic technique or the modern genome-editing methods. Genome editing permits modifications of the native plants DNA, including the insertion, replacement, and deletion of DNA segments at specific locations and lengths (Barrangou and Doudna, 2016).

On the other hand, transgenic technology introduces novel features into a model organism by randomly integrating foreign DNA into its genome. Conventional transgenic techniques involve transforming plants to introduce desired traits into their genome at random locations (Lorence and Verpoorte, 2004). Effective plant breeding is now possible without the introduction of transgenic plants due to technological advancements in genome editing (Araki and Ishii, 2015). Transgenic and cisgenic crops are the two kinds of genetically engineered crops that have been used in agricultural production. Transgenic crops are the result of transferring genetic material

between two different species. Within that species, the genes in cisgenic crops have undergone "edited" processes. Making better use of already-existing gene alleles and reducing linkage drag are the two advantages of cisgenics (Vasudevan *et al.*, 2023). The time it takes to breed plants is greatly reduced by this technology, which combines traditional techniques with state-of-the-art biotechnological instruments. As a result, it is possible to modify plant genomes without significantly altering the plant population as a whole, and the environmental impacts of cisgenic and transgenic plants differ (Vasudevan *et al.,* 2023). In genome editing methods, nucleotide substitutions are employed to create distinct variations (Scheben *et al.*, 2017). Using CRISPR/CRISPR-associated protein (CRISPR/Cas), breeding efforts can be accelerated and the genome sequence of any crop can be changed (Jinek *et al.*, 2012; Chen *et al.*, 2019). The CRISPR/Cas system was developed for genome editing based on RNA-guided DNA interference (Koonin *et al.*, 2017). CRISPR/Cas-mediated gene knockout, insertion, and replacement is being utilized to improve trait of interest such as yield, quality, disease resistance, and stress tolerance traits in crops by changing the target genes or rearranging chromosomes in elite cultivars. Plants resistant to biotic stress, including fungi, bacteria, viruses, and insects, have been created through the application of CRISPR/Cas-mediated gene deletion (Chen *et al.*, 2019).

Plant molecular bio-engineering have been conducted to determine the genetic makeup and mechanism of resistance to aflatoxin contamination in maize and cottonseed (Cary *et al.*, 2011; Gaikpa and Miedaner, 2019). *Aspergillus flavus* was genetically modified to generate the enzyme β-glucuronidase by genetic engineering; the activity of this enzyme can be examined to determine the extent of fungal infection in kernels (Brown *et al.*, 1999).

Additionally, a number of genes involved in the production of aflatoxin B_1 have been cloned, and the function of the gene product produced by these genes has been described (Chang *et al.*, 1992; Trail *et al.*, 1994).

A number of investigations have revealed proteins found in maize kernels that may prevent the growth of aflatoxin and *A. flavus*. Among these is chitinase that was separated from the Tex6 maize inbred line, which, at a concentration of 20 µg/mL, inhibits *A. flavus* growth by 50%. According to Chen *et al.* (2010) pathogenesis related protein 10 (PR-10) possesses antifungal and ribonucleolytic properties against *A. flavus*. *Aspergillus flavus* kernel resistance has been demonstrated to be conferred by a number of resistance related proteins (RAPs), such as Tripsin inhibitor (TI) (Chen *et al.,* 1998).

Inhibiting hyphal tip growth is the ability of proteins like Zeamatic (Guo *et al.*, 1997) and Mod-1/RIP-1 (Nielsen *et al.*, 2001; Weissinger *et al.*, 2007) that have been found in maize kernels. Aflatoxin association-mapping panel of 300 maize lines was made available for usage, with 30–40 of those lines exhibiting good resistance in various conditions (Mylroie *et al.*, 2013). When compared to traditional breeding, genetic engineering has a number of benefits. i) It makes it possible to modify, remove, add, or adjust particular genes of interest with the least number of unwanted alterations to the remaining crop genome. In comparison to conventional breeding, this means that crops displaying desired agronomic qualities can be achieved in less generation. ii) Genetic material from different species can be exchanged. As a result, the raw genetic material present inside the species is not restricted to the genes available within the species. iii) new genes can be introduced into crops that are vegetative propagated by plant transformation, which is made possible by genetic engineering (Dong and Ronald 2019). These characteristics make genetic engineering an effective method for increasing plant disease resistance.

Mutation breeding

Plant mutation breeding, also known as variant breeding, is a technique for creating novel crop varieties that involves using chemical or physical radiation to cause spontaneous genetic diversity in plants. Mutagenic agents such as radiation and certain chemicals can be used to induce mutations and produce genetic variants from which desired mutants can be selected (Van *et al.*, 2019). Natural selection uses variation, which is produced by mutation, as a driving force in evolution (Sciences, Earth *et al.*, 2016). Since a mutation in the DNA sequence affects all copies of the encoded protein, mutations can be particularly harmful to a cell or organism (Drummond and Wilke, 2008). Mutagenesis, or the process of introducing mutations into an organism's genome, has been used in plant breeding. Mutation breeding reduces time for breeding as compared to traditional breeding (Yali and Mitiku, 2022). Even when marker-assisted breeding is used to increase selection efficiency, producing and screening a large number of mutants is time-consuming and tedious (Scheben *et al.*, 2017). Mutation breeding in maize for aflatoxin contamination involves inducing mutations in maize plants to develop varieties that are more resistant to aflatoxin-producing fungi. No records of mutation breeding or commercial released varieties as a result of mutation breeding for aflatoxin resistance in maize so far. The greatest option is to induce mutations when genes necessary for resistance to a certain illness or stress are not present in the gene pool (Bado *et al.*, 2015). Plants such as wheat, barley, rice, potatoes, soybeans, and onions are examples of plants that have been created by mutation breeding (For FAOs' Mutant Variety Database, visit http://www-mvd.iaea.org/MVD/default.htm). Breeders can use mutation breeding and select from the mutated lines that show reduced susceptibility to aflatoxin contamination. The selected lines can be tested for desirable agronomic traits and thereafter released as new varieties.

Conclusion

An integrated approach to managing aflatoxin contamination in maize addresses the issue at every stage of the production process. By implementing pre-harvest measures such as breeding resistant crops, improving pest management, and adopting sustainable agricultural practices, along with post-harvest solutions like effective drying, hermetic storage, and timely

harvesting, the risk of aflatoxin contamination can be greatly reduced. Availability of different methods for developing aflatoxin-resistant maize is important as it enables maize breeders from different countries with different breeding capacities to develop resistant maize genotypes and adaptable to various growing conditions. Raising awareness among farmers and stakeholders, enhancing regulatory policies, and offering financial support also strengthen these efforts. The use of advanced technologies for early detection and monitoring further supports timely interventions. Overall, a holistic and integrated management strategy is critical for reducing aflatoxin contamination and promoting food safety, particularly in regions prone to contamination. Effectively managing aflatoxin contamination requires a coordinated approach, combining technological solutions, education, policy improvements, and economic incentives.

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