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REVIEW

Pre- and post-harvest aflatoxin contamination and management strategies of *Aspergillus* spoilage in East African Community maize: review of etiology and climatic susceptibility

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Abstract

Globally, maize (*Zea mays* L.) is deemed an important cereal that serves as a staple food and feed for humans and animals, respectively. Across the East African Community, maize is the staple food responsible for providing over one-third of calories in diets. Ideally, stored maize functions as man-made grain ecosystems, with nutritive quality changes influenced predominantly by chemical, biological, and physical factors. Food spoilage and fungal contamination are convergent reasons that contribute to the exacerbation of mycotoxins prevalence, particularly when storage conditions have deteriorated. In Kenya, aflatoxins are known to be endemic with the 2004 acute aflatoxicosis outbreak being described as one of the most ravaging epidemics in the history of human mycotoxin poisoning. In Tanzania, the worst aflatoxin outbreak occurred in 2016 with case fatalities reaching 50%. Similar cases of aflatoxicoses have also been reported in Uganda, scenarios that depict the severity of mycotoxin contamination across this region. Rwanda, Burundi, and South Sudan seemingly have minimal occurrences and fatalities of aflatoxicoses and aflatoxin contamination. Low diet diversity tends to aggravate human exposure to aflatoxins since maize, as a dietetic staple, is highly aflatoxin-prone. In light of this, it becomes imperative to formulate and develop workable control frameworks that can be embraced in minimizing aflatoxin contamination throughout the food chain. This review evaluates the scope and magnitude of aflatoxin contamination in post-harvest maize and climate susceptibility within an East African Community context. The paper also treats the potential green control strategies against *Aspergillus* spoilage including biocontrol-prophylactic handling for better and durable maize production.

Keywords *Aspergillus* section *Flavi* · Post-harvest practices · Maize · Aflatoxins · East African Community · Gene pathways · Aflatoxin biosynthesis

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Introduction

Maize or corn (*Zea mays* L.) is an important food/feed and industrial cereal crop in Sub-Saharan Africa, particularly in the East African region (Kornher 2018; Santpoort 2020). Primarily cultivated in tropical and warm temperate climates, the nutritional and dietary value of maize ranks highly compared to other agricultural and food crops (Kornher 2018). The cereal offers various benefits in numerous households including dietary provision, poverty reduction, animal feed supply, food security, and foreign exchange (Chukwudi et al. 2021), especially in the Sub-Saharan region, maize serves as an important cereal crop for nearly 1.2 billion people (Suleiman and Rosentrater 2015). According to Suleiman and Rosentrater (2015), maize alongside rice and wheat is ranked as the top three most cultivated cereals worldwide. Africa contributes approximately 7% of global corn production, with two-thirds of this quantity coming from Eastern and Southern Africa (Verheye 2010; Suleiman and Rosentrater 2015).

Across the East African Community, all parts of the maize plant have different uses—the stalk, cob, and tassels. After harvest, maize stalks are mostly used as fodder for domesticated dairy animals. Additionally, shredded corn stalks can serve as supplementary organic matter for composting. Divergent uses of the maize plant also apply to corn tassels that are known to contain vital compounds, including antioxidants, medicinal substrates, and phytochemicals (Kortei et al. 2021). Lastly, the kernels are attached to the cob and contain the most important part of the maize plant, the kernels, which are consumed in many different forms. Maize kernels can be consumed either as whole grains, ground into flour, or eaten fresh (roasted/boiled) from the cob. The importance of corn transcends beyond the food and feed industries to reach the nutraceutical sector. In the latter, maize has been shown to contain antioxidant properties due to the presence of anthocyanin and phenolic compounds in the cereal (Jacinto et al. 2018). Five types of corn including yellow, white, red, blue, and high carotenoid have all been shown to contain nutraceutical molecules, which provide both nutritive benefits and medicinal value to the human body (Jacinto et al. 2018).

Maize production has been continually threatened by a plethora of factors, with mycotoxins being one of the prevalent problems (Mutegi et al. 2018; Kortei et al. 2021; Meijer et al. 2021). The OECD-FAO Agricultural Outlook 2020–2029 (2020) deems that it is crucial to address the growing aflatoxin menace, especially considering that maize consumption is projected to increase by approximately 16% by 2027 in the Sub-Saharan region where livestock and human populations are expanding rapidly. Given the perennial challenges of aflatoxin contamination in maize, and the dietary importance of this staple within the East African Community, a review of the current situation within this region is therefore warranted. The present review assesses *Aspergillus* colonization in post-harvest maize and the resultant aflatoxin contamination problem in an East African Community context. The paper begins by providing an overview of aflatoxins, their primary producers, and etiological agents.

The article discusses aflatoxin contamination, occurrence, distribution, climate susceptibility, and feasible mitigation strategies that are discussed at length in an individualized context within all the seven member states of the East African Community in the subsequent sections. In this context, several peer-reviewed articles discussing the aflatoxin situation at post-harvest within an East African context were used to prepare the current review. Specific attention was given to papers highlighting aspects related to the following topics: post-harvest practices, *Aspergillus* section *Flavi*, aflatoxin contamination in stored maize, and aflatoxin biosynthesis. The paper concludes with a discussion of post-harvest practices that influence aflatoxin contamination of maize. Conclusively, the authors propose the establishment of complementary and comprehensive programs that are capable of addressing post-harvest losses resulting from aflatoxin contamination in maize, an initiative that would possibly streamline farmers' efforts to adopt good agricultural practices both at the smallholder and large-scale level for better on-farm productivity.

Overview of aflatoxins

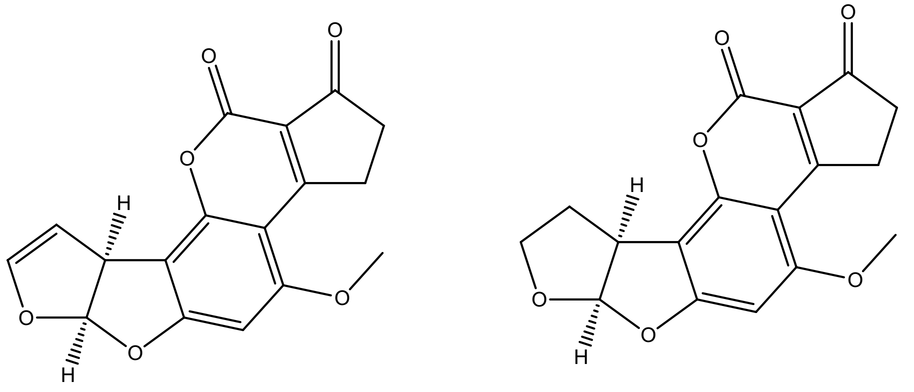
Aflatoxins are, by far, the most widely studied group of mycotoxins (Benkerroum 2020). The first report about their discovery and occurrence traces back to the 1960s in England during which the "Turkey X disease" caused more than 100,000 deaths of turkey (Blount 1961), 20,000 ducklings, and other partridge poults (Wogan et al. 2012). They are the most important mycotoxins with respect to their occurrence, effects on human health, toxicity, and trade (Hell and Mutegi 2011). Aflatoxins (*Aspergillus flavus* toxins) are naturally occurring, potent, and carcinogenic metabolites primarily produced by several species of *Aspergillus* fungi, especially the strains *A. flavus*, *A. parasiticus*, and *A. nomius*. Other *Aspergillus* species that produce aflatoxin albeit to a lesser extent include *A. bombycis*, *A. minisclerotigenes*, *A. parvisclerotigenus*, *A. ochraceoroseus*, and *A. pseudotamarii* (Probst et al. 2012; Okoth et al. 2018; Frisvad et al. 2019). Interestingly, *A. nomius* has a mycotoxin profile that is similar to that of *A. parasiticus* although it morphologically resembles *A. flavus* (Peterson 2016). *Aspergillus flavus* can be further classified according to either those that produce large sclerotia

(L-morphotype) or those that produce small sclerotia (S-morphotype) (Okoth et al. 2012; Mohale et al. 2013).

Both morphotypes of *A. flavus* produce only type-B aflatoxins (AFB_1 and AFB_2). Although the S-morphotype is not very commonly isolated, it is a potent producer of aflatoxin and produces numerous sclerotia (Mohale et al. 2013). Some other species belonging to S-morphotype produce both B and G toxins, e.g., minisclerotigenes and parvisclerotigenes. Unnamed strain SBG is another class of *Aspergillus* with an S-morphotype that produces both B and G toxins (Singh et al. 2020). Aflatoxins (AFs) are highly oxygenated, difuranocoumarin derivatives of which more than 20 different analogs or types are known to occur (Kew 2013; Sana 2019). Among the 20 known, four are considered the major types of aflatoxins; B_1, B_2, G_1, and G_2, produced primarily by *Aspergillus* section *Flavi* (Fig. 1) (Kuboka et al. 2019). The nomenclature of these four analogs stems from the color they fluorescence under long-wave ultraviolet (UV) illumination (B, blue; G, green) (Kuboka et al. 2019). The numerical subscripts relate to their relative chromatographic mobility. Of the four major AFs, AFB_1 is often the highest in toxicity followed by AFG_1 and AFG_2 (Hussain & Anwar 2008).

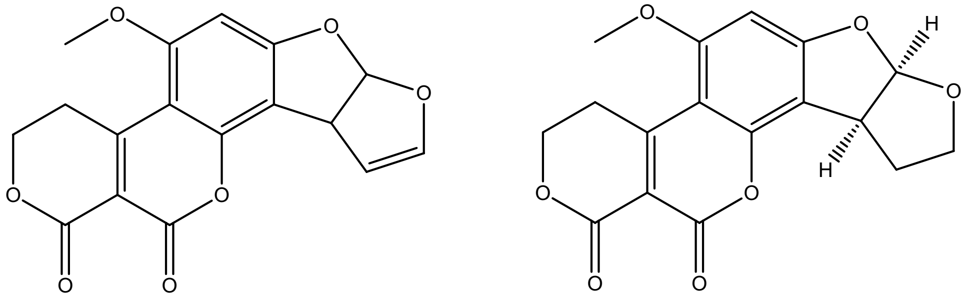
Aspergillus flavus produces only AFB_1 and AFB_2 while *A. parasiticus* produces the same metabolites along with AFG_1 and AFG_2. Notably, *A. flavus* produces other additional toxins including cyclopiazonic acid, aflatrem, and aflatoxicol (Omara et al. 2020; Omara et al. 2021), while *A. parasiticus* additionally produces parasiticol (Stubblefield et al. 1970) (Fig. 2), an aspect that further increases toxicity in contaminated crops (Duran et al. 2007; Abbas et al. 2011). However, these additional toxins are many times less toxic than aflatoxins and are not regulated.

Two additional metabolic products, aflatoxin M_1 and M_2 are found in either urine or milk of lactating animals fed on aflatoxin-contaminated rations, human urine as well as breast milk. The M-type aflatoxins are metabolic derivatives of the B-type AFs and are known to fluoresce blue-violet under UV radiation (Omara et al. 2021). Type-1 AFs refer to a specific classification of aflatoxins. Aflatoxins are a group of toxins produced by certain molds, particularly *Aspergillus* species. Type-1 aflatoxins typically include AFB1, AFB2, AFG1, and AFG2, which are some of the most potent and



Aflatoxin B_1

Aflatoxin B_2

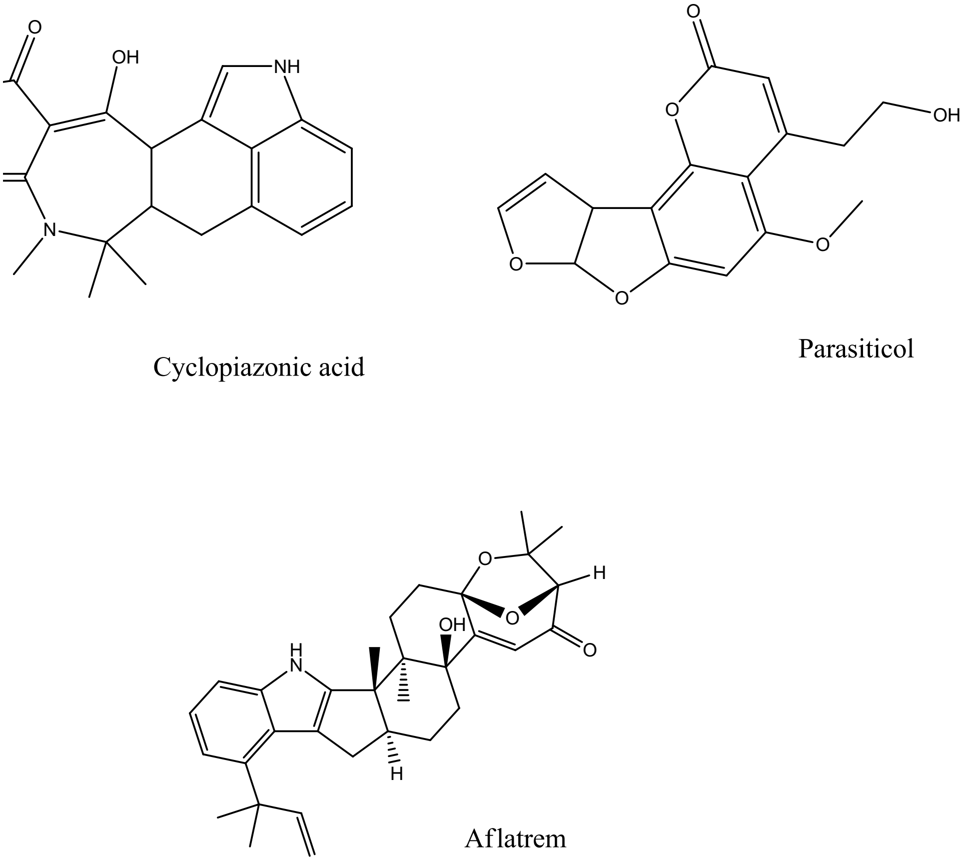


Aflatoxin G_1

Aflatoxin G_2

Fig. 1 Chemical structures of some of the major analogs and types of aflatoxins. Structures are modified from Omara et al. (2020)

Fig. 2 Additional toxins produced solely by *Aspergillus flavus* (Duran et al. 2007; Stubblefield et al. 1970)



Cyclopiazonic acid

Parasiticol

Aflatrem

well-studied aflatoxins (Popescu et al. 2022). In the context of aflatoxins, C_8–C_9 double bonds refer to a specific structural feature within the aflatoxin molecule. The double bond between the carbon atoms at positions 8 and 9 in the chemical structure is crucial for the toxin's biological activity. This double bond is particularly significant in aflatoxin B1 (AFB1), contributing to its high toxicity (Liu et al. 2019). In aflatoxins, the furan ring structure is part of what makes these molecules biologically active and capable of binding to DNA, leading to mutagenic effects and the cyclopentenone ring contributes to the molecule's overall toxicity and reactivity. The presence of this ring in B-type aflatoxins (AFB1 and AFB2) is part of what makes these compounds particularly dangerous (Liu et al. 2019).

Aflatoxins M_1 and M_2 are both hydroxylated metabolites of aflatoxin B_1 with the metabolic conversion being undertaken by enzymes within the animals' system (Hussain & Anwar 2008). Most often, animals that have been fed on maize heavily contaminated with AFB_1 end up having copious amounts of AFM_1 in the resulting milk (Kang'ethe et al. 2017; Kuboka et al. 2019); with the problem being more prevalent in Sub-Saharan Africa. On toxicological grounds, all the analogs illustrated in Fig. 2 are deemed multiplicatively mutagenic, teratogenic, and genotoxic in the order $AFB_1 > AFG_1 > AFB_2 > AFG_2$. This order is reflective of the unique potency of the C_8–C_9 double bond in the furan rings of the type 1 AFs (Omara et al. 2021). Moreover, the lethal nature of the cyclopentenone ring within the B-type aflatoxins is vividly demonstrated in the above order. The following section specifically discusses *Aspergillus* section *Flavi*, where the main producers of aflatoxins are highlighted in more detail.

Aspergillus section *Flavi*

Aspergillus section *Flavi* has for a long time attracted global attention due to its economic and public health impact (Norlia et al. 2019; Benkerroum 2020). More specifically, its toxigenic potential is distributed across divergent plant species like tree nuts, groundnuts, chili, sesame, and many others and subsequently infects a wide array of grains and cereals. As previously mentioned, members of this group constitute natural producers of aflatoxins, and they can exist in the soil either as conidia, sclerotia, or mycelia if present in plant tissues (Frisvad et al. 2019). The taxonomy of *Aspergillus* species has evolved over the past decades from simple morphological identification into polyphasic approaches that integrate biochemical, molecular, and genetic traits (Rasheed et al. 2019). The species consequently become added, repositioned, or re-classified within the *Aspergillus* genus (Arias et al. 2021). By way of adopting traditional schemes of identification, *Aspergillus* section *Flavi* contains a total of 33 species, which are then sub-divided into two clusters of closely related phylogenetic and morphologically similar species. The first cluster comprises *A. flavus*, *A. parasiticus*, *A. minisclerotigenes*, *A. aflatoxiformans*, and *A. nomius*, all of which are known to possess toxigenic potential (Taniwaki et al. 2018; Moral et al. 2020). The second category comprises *A. tamarii*, *A. sojae*, and A. *oryzae*, species that are atoxigenic and hence economically important in the production and manufacture of fermented foods (Atehnkeng et al. 2008; Benkerroum 2020; Hong et al. 2015).

Taxonomic schemes of identification for *Aspergillus* section *Flavi* were made in the first place using morphological diagnostic traits such as colony color production on various culture media and conidial wall ornamentation (Okayo et al. 2020). Secondary characteristics of identification include sclerotia production, conidial head aeration, and length of the stipe (Samson et al. 2007; Varga et al. 2007, Cho et al. 2022). In some cases, secondary metabolites have been used for species recognition in section *Flavi*, with the extrolites widely used in identification being aspergillic acid, kojic acid, cyclopiazonic acid, and aflatoxins (Samson et al. 2007; Rodrigues et al. 2011). Morphological identification of species within *Aspergillus* section *Flavi* is often difficult due to the high divergence present among isolates recovered from the same species (Norlia et al. 2019). Hence, molecular techniques are mostly relied on to study the taxonomy, morphology, and phylogeny of *Aspergillus* section *Flavi* (Samson et al. 2007) and to make rapid detection and prediction on the effects in humans and animals (Frisvad et al. 2019; Rasheed et al. 2019).

Factors affecting *Aspergillus* colonization and aflatoxin production in post-harvest maize during storage

Moisture content, temperature, and relative humidity are tripartite factors that significantly determine the rate of growth of *Aspergillus* fungi during storage. More specifically, *A. flavus* and *A. parasiticus*, the main producers of aflatoxin, experience either accelerated or slowed growth based on the availability of the aforementioned parameters. Relative humidity and moisture content are interrelated, with their levels being used to ascertain the ability of aflatoxigenic fungi to germinate. Maize is easily contaminated by aflatoxins if they are poorly dried before storage or fail to meet a safe level of moisture content (Klich 2007; Al-Hindi et al. 2018). In Kenya, the Ministry of Agriculture recommends that maize be dried to a moisture content of below 13.5% to reduce the possibility of aflatoxigenic fungi thriving in storage facilities (Omara 2019; Omara et al. 2021).

According to Dorner (2008), inadequate and poor drying of cereals prior to storage favors the growth of *Aspergillus* fungi at the post-harvest level. Owing to the fact that corn is naturally hygroscopic, it tends to absorb moisture from its immediate surroundings. Consequently, the degradation of maize grains through mold growth becomes rife if moisture and humidity levels are favorable (Waliyar et al. 2013, 2015). Additionally, secondary sources of moisture such as condensation, leaking roofs, or underground seepage can accelerate the growth of aflatoxin-producing fungi. Perennial poverty among most households is another contributing factor to the increased occurrence of aflatoxigenic contamination in Kenyan foods (Omara et al. 2021). Poor farmers are often pushed by hard economic times to sell their maize which is either premature or poorly dried in order to provide for their families. Adhering to recommended moisture content and temperature levels allows both shelled and unshelled maize to be stored for periods reaching approximately 1 year (Waliyar et al. 2015). During this time, the farmer can enjoy guaranteed food security for their family.

Stored maize can be used to prepare an assortment of meals, either in the form of grains, flour, or whole ear. Combined, humidity and temperature not only influence mold growth but also gene expression of aflatoxin biosynthesis (Abdel-Hadi et al. 2012; Bernáldez et al. 2017). The occurrence of *Aspergillus* fungi in a variety of cereals and foods apart from corn has been documented. Taniwaki et al. (2018) extensively studied the prevalence of aflatoxin in food and concluded that it occurs in other foods including coffee, rice, sorghum, peanuts, pigeon peas, cassava, cocoa, beans, and millet. Aflatoxin has also been reported to occur in fruits such as grapes and apple juice (Nan et al. 2022; Pushparaj et al. 2023). It should be noted that the presence of *Aspergillus* fungi in food does not always equate to the production of aflatoxin. Some fungal species might be simply present in food but do not produce any mycotoxins. Numerous strategies relating to aflatoxin mitigation have been studied, discussed, and documented (Ayalew et al. 2017). However, none of the proposed methods have been able to completely combat or eliminate aflatoxin production in food and edible commodities. Generally, aflatoxin contamination in maize begins at pre-harvest and worsens during post-harvest (Peles et al. 2019).

In Kenya, the contamination of maize-based products (*ugali*, *githeri*, *uji*, *muthokoi and mukimo*) with aflatoxin has been reported (Lusweti and Obade 2015; Nabwire et al. 2020; Omara et al. 2021), with the primary source of contamination being the raw maize (pre-harvest or post-harvest) sourced to prepare these meals. However, Kenyan research on aflatoxin is concentrated more on maize, milk, peanuts, and animal feed owing to the ranking of their importance

(Okoth 2016) as dietary staples and frequently consumed meals. Except for milk, the above meals are equally high aflatoxin-risk prone due to their ability to function as optimum substrates for aflatoxigenic fungi; however, regarding milk, it is not a substrate for aflatoxigenic fungi because it can be contaminated after milch animals consume contaminated feed (Okoth 2016).

General health impacts of aflatoxins

Aflatoxin exposure in biofluids and its impacts on human, particularly child, health in the East African Community (EAC) are well-documented in studies conducted in Kenya (Osoro et al. 2024), Tanzania, and Uganda (Gichohi-Wainaina et al. 2023). Numerous studies highlight the severe health risks posed by aflatoxins, including stunting, immunosuppression, and liver cancer. For example, Lauer et al. (2019) investigated maternal and infant aflatoxin exposure in Uganda and found significant levels of aflatoxin in both mothers and infants, potentially impacting growth and development (Lauer et al. 2019). Research in Tanzania has also linked aflatoxin exposure in children to growth impairment (Gichohi-Wainaina et al. 2023; Xu et al. 2018). Additionally, high levels of aflatoxin in Tanzania have been associated with stunted growth and compromised immune systems in affected individuals (Osoro et al. 2024). Continued ingestion of food or feed contaminated with aflatoxin leads to aflatoxicosis, a severe health condition that can degenerate into the chronic or acute stage (Peles et al. 2019). The severity of one's condition after contracting aflatoxicosis is dependent on multivariate factors including age, nutritional diet, duration of exposure, underlying medical conditions, and environmental parameters (Wagacha and Muthomi 2008).

The most common symptoms of aflatoxicosis include diarrhea, weight loss, fever, vomiting, lethargy or body weakness, anorexia, and stunted growth in children (Azziz-Baumgartner et al. 2005). In acute cases, aflatoxicosis is known to result in organ and tissue damage, with chronic toxicity being experienced in the liver, heart, and kidney tissues (Wang and Tang 2004; Williams et al. 2004; Xu et al. 2017; Benkerroum 2020). Similar symptoms are experienced in animals and livestock, with their susceptibility being dependent on species and age variation (Norlia et al. 2019). The International Agency for Research on Cancer (IARC) classifies one of the most potent AFs, aflatoxin B1 (AFB1) as a Group 1 carcinogen due to its ability to cause liver cancer in humans (Wu and Khlangwiset 2010) while also binding to both nucleic acids—DNA and RNA (Li and Liu 2019; Mutocheluh and Narkwa 2022). The subsequent parts of this review shed light on the East African Community, where each member state is discussed and analyzed in terms of its geographical positioning, climatic patterns, and farming cultivation practices; and how all of these factors cumulatively predispose the post-harvest maize to aflatoxin contamination and accumulation.

Aflatoxins in the East African Community

Maize is the most widely consumed cereal within the East African Community. The susceptibility of this staple food to mycotoxins, particularly aflatoxins, is excessively high, given that environmental factors are additionally favorable to the multiplication and proliferation of aflatoxigenic fungi. Cumulatively, factors such as high humidity, water stress, elevated temperatures coupled with insect invasion, cereal damage, and poor pre and post-harvest practices predispose maize and groundnuts to aflatoxin contamination. Maize-based meals provide optimum substrates for fungal growth and subsequent aflatoxin contamination (Daniel et al. 2011; Kimanya 2015; Mutiga et al. 2015a; Sasamalo and Mugula 2018; Omara et al. 2020), an aspect that further aggravates the mycotoxin menace, given that these foods are consumed almost daily across most households in the EAC. Aflatoxins are the most frequently encountered and studied mycotoxins within the East African Community (Kimanya 2015; Smith et al. 2016; Nabwire et al. 2020; Mugizi et al. 2021); followed closely by fumonisins, zearalenone and deoxynivalenol (Kimanya 2015; Omara et al. 2020; Mugizi et al. 2021; Omara et al. 2021). Aflatoxin B_1 accounts for the highest toxicity profile across EAC countries and is responsible for nearly 75% of all food contamination cases related to AFs. The *Aspergillus* genus is responsible for all aflatoxin poisoning cases with the species *A. flavus* and *A. parasiticus* being the most common across all seven countries. Overreliance on maize for EAC households predisposes them to aflatoxin poisoning, posing significant food safety and health concerns within the region. Maximum tolerable levels for AFB_1 in food range from at least 1 to 20 μg/kg in most countries. For the 29 listed EU member states, the tolerable limit for AFB_1 in food is set at 2 μg/kg (Dövényi-Nagy et al. 2020).

The economic impact of aflatoxin is substantial, with Africa losing an estimated $670 million annually in rejected export trade due to contamination (Meijer et al. 2021) For instance, Uganda and Tanzania lose approximately $16 million and $5.3 million, respectively, due to reduced agricultural exports caused by aflatoxin (Lukwago et al. 2019). Efforts to harmonize aflatoxin regulations and testing methods, such as the proposed adoption of AgraQuant Elisa Mycotoxin Test Kits, aim to reduce trade disputes by standardizing toxin detection at border points (Lukwago et al. 2019). However, the implementation of these methods faces challenges due to differences in infrastructure and capacity to manage contamination across member states (Meijer et al. 2021). Addressing aflatoxin contamination requires a multi-faceted approach, including harmonizing standards, improving detection and management infrastructure, educating stakeholders on prevention, and enhancing regional cooperation. These strategies can help improve food safety, enhance trade relations, and reduce the economic impact of aflatoxin contamination in the EAC region (Keyser and Sela 2020).

The EAC has developed comprehensive aflatoxin control policies focusing on several key areas (Ortega-Beltran and Bandyopadhyay 2021):

- Regulatory framework—establishes maximum allowable aflatoxin levels in food and feed, harmonizes standards across member states
- Surveillance and monitoring—implement regular monitoring programs, rapid testing methods, and laboratory analyses to detect aflatoxin contamination.
- Good agricultural practices (GAP)—promotes best practices for pre-harvest, harvest, and post-harvest handling, and trains farmers on crop management techniques.
- Post-harvest handling and storage—provides guidelines for proper drying, storage, and transportation of crops to prevent mold growth, and encourages innovative storage solutions.
- Public awareness and education—conducts campaigns to educate farmers, traders, and consumers about aflatoxin risks and prevention methods.
- Research and development—supports research on aflatoxin-resistant crops and biological control methods, and collaborates with research institutions.
- Risk assessment and management—conducts risk assessments to identify vulnerable areas and populations, and develops plans to manage aflatoxin outbreaks.
- Trade and market access—aligns control measures with international standards, implements certification programs, and ensures compliance to facilitate export opportunities.

Aflatoxins in Kenya

In Kenya, the causative fungi responsible for aflatoxin production are predominantly *A. flavus* and *A. parasiticus* (Wagacha & Muthomi 2008; Okoth et al. 2012; Okoth 2016; Mitema et al. 2018); with other secondary producers of AFs such as *A. tamarii*, *A. niger*, *A. tamarii*, *A. terreus*, and *A. versicolor* also having been isolated in mills and soils across Eastern and Western Kenya (Smith et al. 2016). According to Oloo et al. (2019), the *Aspergillus* species *A. minisclerotigenes* exhibited a higher potential for biosynthesis of aflatoxins compared to *A. flavus.* Aflatoxins in Kenya pose severe threats to human health owing to their dose-dependent nature, whereby if consumed at high doses, they result in severe toxicological effects in the body.

Aflatoxin contamination in Kenyan maize is known to occur either at pre-harvest, peri-harvest, or post-harvest (Mahuku et al. 2019). Pre-harvest factors that are dominantly known to result in aflatoxin contamination include soil type, maize cultivars, fungal species, climatic conditions, water activity, and agricultural practices. During harvest, aflatoxin contamination can be influenced by the optimum harvesting period and correct drying of maize. At post-harvest, contamination of maize by AFs is greatly affected by storage conditions, containers, structures, and durations (Gachara et al. 2018). Dövényi-Nagy et al. (2020) showed that from the aforementioned *Aspergillus* species, *A. flavus* and *A. parasiticus* are the two most important fungi in the contamination and colonization of agricultural crops, including maize. Of the two, *A. flavus* is the main producer of AFs in Kenya (and across the world). This ubiquitous fungus can be isolated from a broad range of climatic regions and is frequently found between latitudes of 16 and 35°, especially in warm temperate zones.

Recent studies have highlighted the critical role of the *Aspergillus* section *Flavi* community structure in the development of lethal levels of aflatoxins, particularly in Kenyan maize. Probst et al. (2012) investigated the community structure of *Aspergillus* section *Flavi* and its impact on aflatoxin levels. They found that specific strains within this community are more capable of producing high levels of aflatoxins, which can lead to lethal aflatoxicosis. This work underscores the importance of understanding fungal community dynamics in predicting and mitigating aflatoxin contamination (Probst et al. 2012).

In another significant study, Probst et al. (2012) identified deadly strains of *Aspergillus* in Kenya that are distinct from other aflatoxin producers. These strains exhibited unique genetic and phenotypic characteristics, contributing to their high toxicity. The findings of this study emphasize the need for targeted interventions and monitoring programs to control these potent aflatoxin-producing strains. Probst et al. (2014) further explored the diversity of aflatoxin-producing fungi in Sub-Saharan Africa and their impact on food safety. The study highlighted the extensive variation among *Aspergillus* species and strains in their ability to produce aflatoxins. This diversity complicates control efforts and necessitates comprehensive strategies to address aflatoxin contamination effectively (Probst et al. 2014).

In Kenya, the tropical climatic pattern is somewhat erratic with high temperatures, high humidity, and periodic droughts preceding most harvest seasons. This East African country has two distinct rainy seasons; the long rains that are witnessed from March to June and the intermittent rains that occur from October to December. Climatic zones are further partitioned into agro-ecological zones, contingent on suitable temperature and rainfall for food crops. In particular, the Rift Valley region of Kenya is endowed with fertile soils with temperatures averaging between 21 and 26 °C. Evidently, the Kenyan climatic pattern predisposes the country to experiencing frequent aflatoxin outbreaks. Owing to the health concerns associated with the ingestion of this mycotoxin, most countries have set legal limits for the amount of AFs allowed in both food and feed (Dövényi-Nagy et al. 2020).

In 21 countries spread across Asia, Africa, and Latin America, the set legal limit for AFB_1 in food is 5 µg/kg. The set legal limit for the same stands at 10 µg/kg, The East African Community (EAC) has approved specific regulatory limits for aflatoxins to ensure food safety within its member states. The EAC has set the maximum allowable level for AFB1 in food at 5 µg/kg, aligning with international standards to facilitate trade and protect public health (Dövényi-Nagy et al. 2020).

Nonetheless, the country has been a hotspot for fatal aflatoxicosis with the worst outbreak having occurred in 2004 in which out of the 317 cases reported, 125 of them were death fatalities (Lewis et al. 2005). During this outbreak, maize samples had aflatoxin B_1 levels of > 40,000 µg/kg, nearly four thousand higher than the allowed legislative limit (Muture and Ogana 2005). In the subsequent years (2005, 2006, 2007), comprehensive studies were conducted and it was revealed that most of the maize samples were laced with AFs at levels far greater than the 10 µg/kg restricted limit (Nsabiyumva et al. 2023). In a parallel study conducted in western Kenya to assess aflatoxin prevalence in maize flour, Mutiga et al. (2015a) revealed that nearly 50% of their samples tested positive for AFs, with 15% of them having toxin levels above 10 µg/kg.

Aflatoxin outbreaks in Kenya trace back to 1978 when the first poisoning case was reported. In the same year, aflatoxin contamination in dog meals exceeded 150 µg/kg with the highest being 3000 µg/kg. In the years 1984 and 1985, more fatal cases were reported where aside from humans, dogs, and poultry succumbed after consuming aflatoxin-laced meals (Mutegi et al. 2018). Most often, the fatalities reported do not reflect the actual magnitude of aflatoxicosis owing to the absence of streamlined monitoring and surveillance systems. Notably, aflatoxin research in Kenya has remained centralized in the Eastern region, which is synonymous with recurrent aflatoxicosis outbreaks. Few studies have been conducted in the western part, where the bulk of maize is cultivated. Studies by Komen et al. (2006), Mutiga et al. (2015a), Smith et al. (2016), Mitema et al. (2018), and Gachara et al. (2022) have demonstrated that western Kenya is aflatoxin-prone after carrying out research in Homa Bay, Uasin Gishu, Trans-Nzoia, Elgeyo Marakwet, Bungoma, and Nyanza counties. Findings from research conducted in the above counties demonstrated that aflatoxin contamination is equally serious and prevalent in western counties, just like it is in the eastern region, but often goes unreported due to the minimal attention accorded to the western part of the country. Evidently, the aflatoxin menace is densely distributed across the country and not only restricted to one geographical or agro-ecological zone. The heightened importance of safe maize cultivation and production with low aflatoxin levels underscores the need for greater vigilance in high maize-growing regions.

Aflatoxins in Uganda

As an agrarian, landlocked country, Uganda relies heavily on farming to feed and sustain its populace. Just like in Kenya, *A. flavus* and *A. parasiticus* are the main producers of aflatoxins in Uganda (Echodu et al. 2018; Wokorach et al. 2021b). The former fungus maintains its ubiquitous nature in the country, producing type-B AFs alongside other mycotoxins such as kojic, aspergillic, and cyclopiazonic acids (Omara et al. 2020). The production of AFs in Uganda is exacerbated by the prevailing climatic conditions—heavy rainfall, erratic dry spells, and high humidity. Further, biophysical factors including soil substrate composition, host-plant susceptibility, and fungal populations cumulatively contribute to the aggravation of aflatoxin contamination of foodstuff. Notwithstanding, literacy levels have been shown to play a pivotal role in the proliferation of aflatoxins in Ugandan foods (Sserumaga et al. 2020). Like in Kenya, aflatoxin contamination is equally rife in Uganda, with the most contaminated cereal crops, being peanuts (*Arachis hypogaea* L.), maize, millet (*Panicum miliaceum*), and their local products (Echodu et al. 2018; Wokorach et al. 2021a, b). However, other cereals such as rice (*Oryza sativa*) and sorghum (*Sorghum bicolor* L.) are equally affected by aflatoxigenic contaminants. Notably, maize is also the most important staple in the country. Its susceptibility to aflatoxin contamination remains high, a factor that aggravates the food insecurity crisis in the country.

Uganda, which is indeed a major maize exporter within the East African Community (EAC), has faced significant trade challenges due to aflatoxin contamination in its maize. Aflatoxins, which are toxic compounds produced by certain fungi, have been detected in Ugandan maize at levels exceeding the EAC standard of 10 parts per billion (ppb) (Akullo et al. 2023). This has led to notable trade disputes, particularly with Kenya and South Sudan. In 2021, Kenya imposed and subsequently lifted a ban on maize imports from Uganda, citing high aflatoxin levels. Despite lifting the ban, Kenya implemented strict import conditions requiring certificates of conformity to ensure compliance with safety standards (Akullo et al. 2023).

Similarly, South Sudan has periodically detained Ugandan maize trucks, demanding rigorous testing and certification to prevent contaminated maize from entering its market, These incidents highlight the regional impact of aflatoxin contamination and the ongoing efforts to maintain food safety and trade integrity within the EAC.

Comparable to Kenya, maize is the primary staple cereal in Uganda, with production estimated at more than two metric tons per hectare (Wokorach et al. 2021b). Apart from human consumption, maize functions as a major ingredient in animal feed. Hence, its contamination by aflatoxins places both animals and humans at heightened health risks. Interestingly, Uganda was among the first countries globally where aflatoxin poisoning cases were reported. More specifically, the year 1967 witnessed the death of a 15-year-old boy, whose autopsy revealed he had consumed cassava laced with 1700 μg/kg of AFs. Considering the regulated legal limit of aflatoxin in food stands at 10 μg/kg, the aforementioned mycotoxin content exceeded safety levels by a hundred and seventeen times. Flash-forward, numerous toxicological studies continue to be conducted on maize, more so pertaining to its aflatoxin content (Kitya et al. 2010; Lukwago et al. 2019).

In a pioneer survey conducted to determine the AF content of stored cereals in between harvests in Uganda, Alpert et al. (1970) reported that 29.6% of the 480 samples tested showed detectable aflatoxin levels. The prevalence of aflatoxins in maize was highest at 45%, followed by peanuts and cassava at 18% and 12%, respectively. High AF levels in maize correlate with those of Kenya, where evidently the cereal remains predisposed to aflatoxigenic contaminants. Despite the dangers posed by aflatoxin poisoning in cereals, very few (if none) studies have been performed on Ugandan brewed beer; one of the widely consumed foods that utilizes all major cereals. Ugandan beer is often brewed using maize, barley, and sorghum through a mixed-culture fermentation process. Consequently, the brewing route becomes highly ideal for the growth of aflatoxigenic fungi (Sserumaga et al. 2020), while effortlessly minimizing the chances of detection by consumers (Muzoora et al. 2017).

Aflatoxins in Tanzania

Maize in Tanzania is the primary staple food choice for the majority of the populace owing to its ability to enhance nutritive value, alleviate poverty, and provide consistent income (Pauw & Thurlow 2011; Homann et al. 2013). According to Mollay et al. (2020), nearly 75% of all homegrown maize is utilized for human consumption across Tanzanian households. Geographically, Tanzania's location predisposes it to frequent aflatoxin poisoning cases, as is the case with its neighboring countries Kenya and Uganda. Rainfall patterns are erratic in this East African country, with most parts relying on the long rains for food production. Consequently, maize is often stored for prolonged durations in order to cater to high demand during the off-season (Mollay et al. 2020). In light of this, the lengthy storage periods make maize vulnerable to aflatoxin contamination. Additionally, the aforementioned conditions make Tanzania a conducive environmental hub for AF growth and subsequent production.

In recent years, Tanzania has been grappling with aflatoxin contamination and exposure as evidenced by several studies. In a tripartite study conducted by Kamala et al. (2015) in Morogoro, Mbeya, and Manyara, 50% of all maize-based meals intended for human consumption were reported to be contaminated with at least one of the four AFs (AFB_1, AFB_2, AFG_1 or AFG_2). A further 28% of the samples had aflatoxin levels above the legal limit of 10 μg/kg. In a separate study in the aforementioned Manyara region, AFs were detected in at least 32% of maize kernels, at a mean of 3.4 ± 0.3 μg/kg and ranges of 2.1–16.2 μg/kg (Nyangi et al. 2016). In a different study that sought to determine aflatoxins and fumonisins (FB) co-occurrence in rural Tanzanian villages, Kimanya et al. (2008) collected 129 maize samples across four regions; Tabora, Iringa, Ruvuma, and Kilimanjaro. Findings revealed that 18% ($n = 23$) had AF levels up to 158 μg/kg. Alarmingly high AF levels are not only restricted to maize samples alone but also occur in maize-based products. Makori et al. (2019) reported total AF (AFB_1, AFB_2, AFG_1, or AFG_2) contamination in complementary maize flour brands with concentration levels ranging from 0.4 to 2129 μg/kg in 42.5% of the tested samples.

Biomarker concentrations, which have shown to be highly useful in elucidating toxicity exposure, have been detected in infants and young children (IYC) in some Tanzanian villages. According to Shirima et al. (2013), chronic exposure to IYC occurs primarily through contaminated maize-based diets. In a cross-sectional survey conducted on 148 young children (aged between 1 and 2 years) across Tabora, Iringa, and Kilimanjaro (different AEZs), AF biomarker concentrations were detectable in 84% of the tested children. Apparently, most of the latter were weaned exclusively on maize-based meals. Similar findings were reported in a study conducted in Haydom, where 72% of children showed detectable AFB_1-lysine (AFB_1-lys) biomarker concentrations in plasma samples averaging at 6.6 pg/mg albumin. A further 80% of urine samples tested had traceable amounts of urinary fumonisin B_1 (UFB_1). Notably, mycotoxin co-occurrence is a grave concern across Tanzania (and the East African Community) owing to the known genotoxicity effects. Specifically, AFB_1 and FB_1 mycotoxins induce regenerative proliferations in the target human tissues (Mollay et al. 2020) in addition to the known mycotoxigenic effects. Most often, co-exposure has been shown to occur among people who are highly dependent on maize and maize-based foods.

In Tanzania, the co-occurrence of AFB_1 and FB_1 is common in maize and maize-based complementary diets

(Kimanya et al. 2008; Suleiman and Rosentrater 2015; Kamala et al. 2018a, b; Mollay et al. 2020). These findings are further corroborated by Gong (2016) who revealed the co-occurrence of mycotoxins in IYC-maize-dependent diets. Myriad combinations of mycotoxins AFB_1, AFB_2, AFG_1, AFG_2, FB_1, and FB_2 were reportedly present in 82% of the maize-based porridge samples tested. Surprisingly, all fumonisins (FB_1 and FB_2) showed the highest percentile occurrence of 100% ($n = 101$). These findings demonstrate that mycotoxins (particularly AFs and FBs) are a primary health concern in Tanzania. Their ability to infiltrate deeply into matrices of maize and other cereal crops causes detrimental effects on human health.

Aflatoxins in Rwanda

The economic stability of Rwanda remains highly sustained by the agricultural sector, which contributes approximately 31% of the country's GDP. Statistically, at least an estimated 80% of Rwandese inhabitants practice either small or large-scale farming. The majority of the farmers have embraced the cultivation of crops categorized under the Crop Intensification Program (CIP). In the latter, the Rwandese government endeavored to triple the production of certain cereal and food crops in the span of 6 years. Among the crops targeted under the CIP umbrella body was maize, which doubles up as a staple crop in Rwanda. Primarily, maize farming is promoted owing to its nutritive value and cushioning abilities in the fight for food security. Like other countries in the EAC, the Rwandan climatic conditions are favorable for the growth and proliferation of mycotoxic fungi. Consequently, numerous cases of mycotoxin accumulation and contamination have been reported across the country (Matsiko et al. 2017; Nishimwe et al. 2019).

Generally, Rwanda enjoys warm climatic patterns, an aspect that promotes the germination of mycotoxigenic fungi. Given that the optimum growth temperature for aflatoxins and fumonisins ranges between 16 and 35 °C, the country's weather patterns are already favorable for the growth of the aforementioned mycotoxins. The risk of the latter is further exacerbated by inappropriate pre- and post-handling of foodstuffs coupled with poor storage practices, particularly in the case of maize. Nonetheless, Rwanda does not suffer from pronounced levels of aflatoxin or mycotoxin poisoning in comparison to other East African (and Sub-Saharan) countries. Owing to this, minimal studies have been conducted on the characterization of mycotoxigenic fungal species and their metabolites. It is therefore not surprising that scanty literature exists in the Rwandan context regarding the contamination of maize by aflatoxins and other associated food spoilage fungi. Nevertheless, several scholars have designed experimental studies to gauge the severity of the aflatoxin problem in Rwanda.

In their study, Nishimwe et al. (2019) designed a study for a determination of the level of aflatoxin B_1 in maize sold across markets in Kigali, the country's capital. Additionally, the authors investigated whether there was any correlation between AFB_1 in maize flour, gender, and education levels of the vendors. The implications of this study demonstrated the need for conducting expanded surveys so that the scope of aflatoxin contamination could be understood on a national level. In a separate study, Niyibituronsa et al. (2020) evaluated mycotoxin levels (aflatoxins and fumonisins) in maize across selected districts in Rwanda. Maize samples ($n = 227$) were analyzed for AFs and FBs using Reveal Q+, and the results were read by Accuscan Gold Reader. The results showed that the average aflatoxin level in the surveyed districts was 6.69 ± 13 μg/kg.

Generally, 90% of all grains scored below the legal limit of aflatoxin (10 μg/kg) regulated across the East African region. However, the highest AF level recorded reached 100.9 μg/kg. Fumonisin levels in the previously mentioned study were generally low with amounts ranging from 0 to 2.3 μg/kg. Evidently, this study revealed that aflatoxin and mycotoxin contamination is not as prevalent and rampant in Rwanda, as is the case in other East African countries. Supplementary research experiments on the aflatoxin situation in. Nonetheless, streamlined efforts are required by the Rwandan Standard Board and related authorities to strengthen the monitoring of mycotoxin contamination and shield consumers from aflatoxicoses and mycotoxicoses.

Aflatoxins in Burundi

By far, Burundi is the sole country in the East African Community that has very few occurrences or outbreaks related to aflatoxin poisoning. Despite the country having a relatively hot and humid climate coupled with agricultural practices that favor mold growth, data on molds and mycotoxins in foods is very limited and almost non-existent. Few scholars, however, have attempted to shed light on the AF situation in the Sub-Saharan country. In one of the earliest research on "Molds and Mycotoxins in Foods from Burundi", Munimbazi and Bullerman (1996) conducted a study to isolate and identify the main fungal species contaminating Burundian staples and further evaluate their mycotoxin-producing potential. In their findings, the researchers isolated *Aspergillus*, *Fusarium*, and *Penicillium* from maize kernels, rice, finger millet, sorghum, peanuts, mung beans, and haricot beans. Further, the authors dominantly found aflatoxins, fumonisins, and cyclopiazonic acid (CPA) from the previously mentioned cereals. Sixty-seven out of the 95 isolates of *Aspergillus* (*A. flavus*) all produced AFs and CPA. An additional 51 isolates out of the 56 isolates of *Fusarium* (*F. verticillioides*) were found to produce fumonisins.

Munimbazi and Bullerman (1996) concluded that despite them having isolated major mycotoxins in staple cereals, their prevalence in Burundi remains highly unreported or unsubstantiated. In a separate study by Udomkun et al. (2018a, b), the occurrence of AFs in agricultural produce in Burundian local markets was studied. Samples of maize, cassava, sorghum, soybeans, milk, and their processed products were collected randomly between May and July 2016. After subjecting the samples to aflatoxin analysis using Reveal Q+, all samples ($n = 244$) were found to contain AFs with levels ranging between 1.3 and 2410 µg/kg. Also, over half of the samples (51%) contained aflatoxin levels above the EU maximum tolerable limit. Udomkun et al. (2018a, b) concluded aflatoxin contamination in Burundi is a plausible threat to public health and food sufficiency in the nation. The authors moved to propose that mycotoxin surveillance programs at the pre and post-harvest levels be introduced to monitor the safety of foodstuffs in Burundi. The implementation of robust risk assessment programs could greatly aid in abating aflatoxin contamination by establishing stringent regulatory thresholds for mycotoxins in local crops and cereals.

Aflatoxins in South Sudan

Deemed the youngest country in the East African Community, South Sudan gained independence from Sudan in 2001. Very little information (if any) is available on mycotoxin poisoning and contamination in South Sudan. Having joined the EAC in 2016, the only available information on aflatoxin accumulation relates to the former Sudan before the split into Sudan and South Sudan. Hardly any literature or publications exist citing accurate information on the aflatoxin situation in the latter state. Hence, it would be inaccurate to report on the mycotoxin scenario in South Sudan since all available data converges toward the Republic of Sudan.

Aflatoxins in the Democratic Republic of Congo

As previously discussed, low- and middle-income countries tend to be predisposed to aflatoxin contamination due to the socio-economic and poor governance nexus. Belonging to the LMIC category, the Democratic Republic of Congo has been ranked as one of the poorest countries in Sub-Saharan Africa, with a GDP per inhabitant of 556.81 (Mutegi et al. 2018). The country's relative humidity and temperature create an optimal environment for the proliferation of aflatoxin-producing fungi (Kamika & Takoy 2011; Kamika et al. 2016). However, there is a dearth of information regarding the occurrence, distribution, and exposure of humans and animals to aflatoxins as well as mitigation and control strategies of the menace, especially in major cereals like maize (Kamika et al. 2016). In addition to climatic factors, socio-economic parameters play a pivotal role in the spread of aflatoxin contamination. Factors including low living standards, informal marketing systems, lack of streamlined transport services, and limited knowledge and information on pre and post-harvest cumulatively contribute to the mycotoxin menace. Additionally, the DRC is known to experience recurrent political and social conflicts that directly impact health, nutrition, and lifestyle (Udomkun et al. 2018a, b).

Resource-poor households experience the strain of conflict the most and the interplay between food availability, adequacy, and country stability becomes critical when addressing the aflatoxin challenge in this democratic state. Despite the scarcity of information on the subject, several cases have been reported of aflatoxin contamination in food and feed in the DRC. In studies conducted by Udomkun et al. (2018a, b), 300 samples of maize, groundnuts, and cassava collected from farmer households in eastern DRC were subject to aflatoxin testing. In 68% of samples, total AFs were above 4 µg/kg, with the highest detectable limit standing at 2270 µg/kg. Local farmers blamed poor soil composition, high humidity, and poor storage practices as the potential causes of crop contamination. In a parallel study, Kamika et al. (2016) assessed aflatoxin content in maize samples ($n = 50$) collected in different stages along the food supply chain. The results showed that 32% of the maize samples collected during pre-harvest were positive for AFs, with AFB_1 ranging from 1.5 to 51.23 µg/kg and 3.1 to 103.89 µg/kg for total AFs. AF levels further rose 500 times more reaching an alarming rate when the maize was transported to city stores and distribution vendors. In studies conducted by Kamika et al. (2014), the authors analyzed cereal samples ($n = 29$) collected from informal markets in Kinshasa for total aflatoxins AFB_1, AFB_2, AFG_1, and AFG_2, and mycoflora. A total of 95% of the samples were laced with aflatoxigenic fungi ranging between 20–49,000 and 40–21,000, with 75% of them exceeding the regulated WHO aflatoxin limit of 10 µg/kg. From their findings, the authors advised that future research on total AFs should be undertaken, especially given the fact that DRC is listed as one of the African countries with the highest prevalence rates of liver cancer.

Similar findings were reported by Manjula et al. (2009) after analyzing dried maize and cassava samples for aflatoxin content, and results were flagged off for having values above the 10 µg/kg limit. In an attempt to control aflatoxins in traditional cassava, Yalala et al. (2019) substituted traditional ferment with *Rhizopus oryzae* strains as starters (microferments) in a controlled fermentation environment. The results showed that aflatoxin production can be controlled during fermentation when aflatoxin-inhibiting microbial biomass is utilized to invade and colonize the metabolic activities of aflatoxigenic fungi (Yalala et al. 2019). Evidently, the

Democratic Republic of Congo suffers from frequent aflatoxin contamination cases, an aspect that appears to be standard across the East African Community.

Aflatoxin control strategies

Selection, efficacy, and advantages of utilizing non-aflatoxigenic *Aspergillus* strains for bio-control of aflatoxigenic strains

Bio-control methods have been proven to be pioneering, innovative, and effective in the fight against aflatoxin contamination in crops. The application of non-aflatoxigenic (AF^-) bio-control agents displace the growth of aflatoxigenic fungi (AF^+) in cereals and grains in the field, during storage and even transportation (Dorner 2008; Khan et al. 2021). Even when environmental conditions are optimal to support fungal growth, Non-aflatoxigenic strains do not impact germination but displace toxigenic strains in the soil before the toxigenic strains can establish in the field (Atehnkeng et al. 2008, 2022; Dorner 2008; Khan et al. 2021). Hence, topical applications of AF^- *Aspergillus* strains in the field substantially reduce aflatoxin contamination of crops. Notably, the non-aflatoxigenic strains are capable of dispersing spore communities of *Aspergillus*, thereby improving safety within the treated farm fields. According to Bandyopadhyay et al. (2016), a single dose of AF^- *Aspergillus* strains can benefit both treated crops and second-season crops, even if the latter missed the first treatment. The advantages of AF^- strains for bio-control obviously have ripple effects that trickle down several years even after one round of application (Atehnkeng et al. 2022; Khan et al. 2021).

Efficacy of non-aflatoxigenic *Aspergillus* strains as biocontrol agents

In an in vitro study conducted by Ehrlich et al. (1985), it was revealed that co-inoculation of AF^- *Aspergillus* strains with AF^+ ones significantly reduced the production of aflatoxins in maize. Under field conditions, the potential of AF bio-control using AF^- strains shows a marked reduction of aflatoxin production in other crops like peanuts (Dorner 2008) and cotton (Cotty 1994). A tenfold to a 100-fold increase in propagule density of the *Aspergillus* fungal community has been shown to occur when a mixture of non-aflatoxigenic *A. flavus* and *A. parasiticus* is applied against the AF^+ strains (Agbetiameh et al. 2020). Dorner (2008) reported a marked reduction of aflatoxin concentrations ranging between 74.3 and 99.8% when the AF^- *Aspergillus* strains were applied to peanut crops. Specifically, the findings were able to show that these AF^- strains were more dominant in the displacement of AF^+ species in the soil, thereby controlling soil-borne infections in crops. Notably, certain strains of *Aspergillus* possess high efficacy in AF reduction.

Ehrlich et al. (2004) demonstrated that the bio-control of aflatoxin production by inoculation using AF36, which is an AF^- strain, ultimately displaced AF^+ species in cottonseeds. Similar results were observed when Chang et al. (2007) co-inoculated TX9-8 (an AF^- strain) with aflatoxigenic strains in a 1:1 ratio. Competitive exclusion was able to occur whereby the vigorous growth of the AF^- TX9-8 strains displaced the aflatoxigenic strains. Despite the AF^- *Aspergillus* strains being highly effective in mitigating aflatoxin contamination of crops, As several papers have reported, the mechanism is competitive displacement (Bandyopadhyay et al. 2016), touch inhibition (Sweany and Damann 2020), aflatoxin degradation (Maxwell et al. 2021).

Aflatoxin bio-control

Aflasafe® bio-control approach

Aflasafe® is a multi-strain bio-control product developed using native AF^- strains of *Aspergillus* fungus. The AF^- strains do not produce aflatoxins and their mechanism of action functions by outcompeting the toxigenic *Aspergillus* strains. Selected *Aspergillus flavus* genotypes that do not produce aflatoxin have shown significant success as bio-control agents in mitigating aflatoxin contamination at pre and post-harvest. Popularly known as AF^- strains, these AF^- *A. flavus* genotypes competitively displace the AF^+ species from soil and crop debris (Cotty 1994). Such bio-control methods significantly reshape the fungal community that usually grows in association with the food crops whereby the AF^- strains are able to dominate the toxigenic fungal colonies (Cotty 1994). Resultantly, the aflatoxin potential of fungi in the field is greatly reduced (Adhikari et al. 2016). Aflasafe® mainly contains four distinct AF^- genotypes of the L-morphotype (Bandyopadhyay et al. 2016). According to Senghor et al. (2020), this multi-genotype strategy shows greater potential for effectively establishing *Aspergillus* colonies that hold low potential in aflatoxin production.

In field trials, this aflatoxin-reducing technology has been shown to reduce aflatoxin contamination by between 80 and 99% (Bandyopadhyay et al. 2016). Aflasafe® application has been shown to protect maize crops throughout the entire growing season and storage periods. Inoculation with Aflasafe® inoculum is optimally done when the soil is wet. Favorable moisture levels in the soil have been shown to accelerate the growth of AF^- strains on the carrier (dead sorghum grains) of the active ingredient in isolates. In America, products for aflatoxin bio-control have been in continued use for over 15 years. In Africa, the first country to legally adopt the use of Aflasafe® was Nigeria, after which many African countries followed suit (Bandyopadhyay et al. 2022). In Kenya, its adoption officially commenced in 2015 after the government approved it for controlling aflatoxin production in maize. Each African state has a country-specific suffix that is used to denote the origin of the Aflasafe® brand (e.g., KE01 for Kenya; TZ01 and TZ02 for Tanzania). Noteworthy mentions should also be made to the fact that each Aflasafe® product contains AF$^-$ strains that are endemic to a particular target country.

Aflasafe® KE01 denotes a Kenyan-specific Aflasafe® brand legally registered with the country's Pest Control Products Board (PCPB). More specifically, Aflasafe® KE01 is comprised of four indigenous AF$^-$ strains of *A. flavus* that are native to Kenya. The four AF$^-$ active ingredients of Aflasafe® KE01 are C6-E, C8-F, E63-I, and R7-H belong to the L-morphotype of *A. flavus* and were isolated from maize grains collected in Kenya (Bandyopadhyay et al. 2016; Moral et al. 2020). Upon application, the AF$^-$ strains within Aflasafe® KE01 produce numerous spores on dead sorghum grains that act as carriers, thereby acting as a food source and competitively displacing AF$^+$ strains. Similarly, the Aflasafe® registered brand in Tanzania, Aflasafe® TZ01 and TZ02 are both comprised of four AF$^-$ isolates as the main active ingredient of the biocontrol product. More specifically Aflasafe® TZ01 is derived from the isolates TMS 193–3, TMH 104–9, TGS 364–2, and TMH 30–8 (Mahuku et al. 2023); while Aflasafe® TZ02 is constituted from the isolates TMS 64–1, TGS 55–6, TMS 205–5 and TMS 137–3.

The use of Aflasafe® in aflatoxin control and mitigation is a timely move because, like most African countries, Kenya and Tanzania both lack an intensive national aflatoxicoses surveillance system. To this end, it becomes overly difficult to ascertain whether aflatoxin exposure and aflatoxicoses outbreaks are limited to certain regions termed "aflatoxin hotspots" (like the Eastern region in Kenya). What is more, scanty literature exists, conclusively affirming whether exposure to this mycotoxin varies demographically, ecologically, or socioeconomically. Aflasafe® provides a feasible, scientifically proven, and reliable bio-control approach to combat aflatoxins and minimize subsequent risk exposure to this lethal toxin (Ortega-Beltran and Bandyopadhyay 2021). In light of this, it is evident that the adoption and application of Aflasafe® are highly capable of dramatically reducing aflatoxin contamination, while simultaneously boosting income for millions of farmers through the reduction of crop losses. Additionally, the distribution of aflatoxin-free maize along the food value chain guarantees that consumers are protected from being exposed to lethal mycotoxins. Intensified training ought to be conducted with maize farmers in the East African Community in order for them to individually weigh the merits of adopting this technology, versus its drawbacks; from which they can be free to decide whether they can utilize it on their farms for aflatoxin biocontrol. Suffice to say, several downstream efforts for commercialization purposes must be addressed before an onset of widespread adoption of Aflasafe® bio-control by farmers is witnessed in the EAC and subsequently made a reality.

Inhibition of aflatoxin production using lactic acid bacteria and plant extracts as bio-control agents

Lactic acid bacteria

Lactic acid bacteria (LAB) are a non-sporulating, gram-positive, anaerobic, and fermentative group of bacteria that rely extensively on carbohydrates for metabolism (Perczak et al. 2018). The ability of LAB to ferment carbohydrates and produce lactic acid as an end-product is the primary reason why they are widely used in the food and hospitality industry, more so, for the bioconversion of fermented dairy products, meat, and vegetables (Perczak et al. 2018). LAB are equally essential for the production of wines, coffee, cocoa, sourdough, and many other indigenous fermented foods (Nuraida 2015; Zannini et al. 2016), while additionally improving their texture, flavor, and shelf life (Indira et al. 2011). The role of LAB in inhibiting the growth of food spoilage fungi has been demonstrated by various researchers (Schillinger & Villarreal 2010; Ndagano et al. 2011; Gerez et al. 2013; Zannini et al. 2016; Oranusi et al. 2022).

Principally, the antagonistic effect between the AF$^+$ fungi and LAB occurs due to the low molecular weight compounds produced by the lactic acid bacteria; including organic acids such as lactic acid and acetic acid, hydroxyl fatty acids, hydrogen peroxide, and other phenolic compounds (Gerez et al. 2013; Perczak et al. 2018). The ability to lower the pH by LAB is what in turn inhibits microbial growth and equally kills all susceptible micro-organisms altogether. During heterofermentation, LAB have been shown to produce high amounts of acetic acid and trace amounts of proprionic acid, both of which contain higher contents of lactic acid in an undissociated form (Perczak et al. 2018). What is more, low pH is additionally known to strengthen the antifungal properties of various salts contained in propionic acid.

One of the potentially interesting components involved in fungal growth inhibition is reuterin, a compound formed by LAB during anaerobic conditions (Langa et al. 2014). Reuterin suppresses all ribonuclease activity, an enzyme primarily involved in the synthesis of DNA (Perczak et al. 2018). In so doing, reuterin is able to inhibit the growth of *Aspergillus* and *Fusarium* species, the two main genera known for aflatoxin production. The efficacy of lactic acid bacteria in mycotoxin reduction an elimination has been demonstrated in numerous studies with the most effective species belonging to the *Lactobacillus* genus. Specifically, these include species such as *Lactobacillus acidophilus*, *Lactobacillus lactis*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum.* Out of these, *L. rhamnosus* has been shown to be the most effective in the elimination of mycotoxigenic fungi, removing up to several mycotoxins at once (Zinedine et al. 2005; Hatab and Yue 2012), with the efficacy being higher at pH levels of 4 (Zinedine et al. 2005).

The primary mechanism of action of LAB in mycotoxin removal is cell binding, where the bacteria binds to the toxins and metal ions. The cell walls of lactic acid bacteria contain peptidoglycan matrices, polysaccharides, lipoteichoic and teichoic acids—substances that are capable of adsorbing themselves onto mycotoxins, including aflatoxins, and permanently eliminating them. Additionally, LAB are able to biologically control aflatoxins through the adhesion mechanism, in which the level of efficacy is pegged solely on the bacterial concentration present. The ability to remove aflatoxins from agricultural commodities has been investigated widely. In a study by Motameny et al. (2012), *L. rhamnosus*, *L. acidophilus*, and *L. plantarum* were introduced into a gastrointestinal model in order to target the removal of AFB_1. The results showed that *L. plantarum* was the most successful in aflatoxin elimination (28%), followed closely by *L. acidophilus* (22%) and *L. rhamnosus* (18%).

In a parallel study, (Elsanhoty and Ramadan (2013) sought to compare the abilities of heat-treated and viable *L. rhamnosus*, *L. sanfranciscensis*, *L. acidophilus*, and *Bifidobacterium angulatum* to remove aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) from phosphate-buffered saline (PBS) liquid media. Among the four tested bacterial strains, viable *L. rhamnosus* showed the greatest efficacy in binding all four AFs and even confirmed superior efficiency after four washes. The latter results demonstrate that viable *L. rhamnosus* forms the most stable complexes with aflatoxins upon binding. Hernandez-Mendoza et al. (2009) echo these findings in their study that aimed to investigate the binding abilities of AFB_1 by *L. casei* and *L. reuteri* at different incubation times (0, 4, and 12 h) and pH (6, 7.2, and 8). Both *Lactobacillus* strains showed the highest affinity for AFB_1-binding action at pH 7.2 after both 4 and 12 h of incubation (80 and 80% for *L. reuteri* and 67.8 and 55.6% for *L. casei*, respectively).

The AFM_1 binding efficacy between *L. bulgaricus* and *S. thermophilus* was investigated by Khoury et al. (2011), in which it was shown that *L. bulgaricus* had the highest binding efficiency (87.6%). When the same bacterial strain was used to investigate its AFM_1 binding efficacy in yogurt processing, 58.5% efficiency was achieved compared to 37.7% for *S.thermophilus*, with binding efficiency being shown to increase with time. The effectiveness of lactic acid bacteria in removing AFB_1 from the Moroccan sourdough was investigated by (Zinedine et al. 2005), in which it was revealed that *L. rhamnosus* was more effective (44.89%) at 30 °C and pH 6.5. Evidently, lactic acid bacteria demonstrate massive potential in the elimination of aflatoxins (and other mycotoxins) from a wide variety of matrices. The mechanism of removal principally relies on the ability of mycotoxins to bind onto LAB cells, where they in turn become inactivated by various antifungal products, such as acetic acid. However, despite the promising research findings on aflatoxin inhibition by lactic acid bacteria, comprehensive studies need to be conducted to determine the exact mechanisms of enabling the mycotoxin binding process to be permanent. In practice, advanced technological schemes are required in order to commercialize this biocontrol approach and enable it to be used both at large-scale and the smallholder farm level globally.

Plant extracts

Traditionally, essential oils and plant extracts have demonstrated strong antibiotic, antifungal, and antimicrobial properties; an aspect that catalyzed their introduction to the food and beverage industry. In particular, plant extracts have been shown to be strong fungal growth inhibitors, making them safe bio-control alternatives in the elimination of aflatoxins in foods (El-Habib 2005; Iram et al. 2015; Yooussef et al. 2016). Plants with aqueous extracts contain chemically active compounds that inhibit aflatoxin biosynthesis (Reddy et al. 2009), thereby eliminating any mycotoxin print in foodstuffs. Given that aflatoxins are generally thermally and chemically stable, their management and elimination in agricultural commodities become essential in all steps of production (Ponzilacqua et al. 2018). Like plant extracts, essential oils (EOs) are capable of mycotoxin removal, in which they damage the enzymatic pathway of fungal cells by interfering with the synthesis of structural compounds and proteins. Their mechanisms of action function by denaturing the enzymes in-charge of sporulation and amino acids responsible for germination (Ponzilacqua et al. 2018). A good example of such plant compounds is limonene and monoterpenes, which inhibit the enzymatic action of pectin methylsterase, an important constituent of the main components of the fungal cell wall (Ponzilacqua et al. 2018).

Several in vitro studies on plant extracts containing essential oils and their inhibitory effects against aflatoxins and *Aspergillus* growth have been widely conducted (Table 1). *Ageratum conyzoides* leaf extracts showed the greatest inhibition abilities on AFs production by *A. flavus* at concentrations above 0.1 μg/mL according to a study done by Nogueira et al. (2010). Similarly, EOs extracted from *Lippia alba*, a plant species belonging to the Verbenaceae family, were able to completely inhibit in vitro production of AFB_1 at a tested concentration of 0.6 to 1.0 μg/mL (Shukla et al. 2009). *Curcuma longa* (turmeric) is one of the renowned spices worldwide and is commonly used in oriental and authentic food preparations. Originally from Southeast Asia, turmeric is widely used in food industries as both a coloring and seasoning agent. The synergistic action of its main ingredient, turmerone, promotes antifungal, antimicrobial, antioxidant, and anti-inflammatory activities (Khattak et al. 2005).

The complete inhibitory activity of *C. longa* plant extracts on the production of AFB_1 and AFB_2 was investigated by Ferreira et al. (2013), in which 0.5% (v/v) of the EOs was added to yeast extract sucrose agar (YESA) media. Results showed 96% and 98.6% inhibition against AFB_1 and AFB_2, respectively. It has been hypothesized that the inhibition mechanisms that occur are normally based on the peroxidation and cell lipid oxygenation caused by phenolic compounds. The effect of dried plant extracts on the inhibition of AF production has been demonstrated in different studies, most of which targeted common culinary and garnishing spices. Plant extracts of *Syzigium aromaticum* (clove), *Allium sativum* (garlic), *Ocimum sanctum* (basil), and *C. longa* effectively inhibited AF production by 100%, 75%, 85.7%, and 72.2%, respectively, when used at 5 g/kg concentration.

Cuminum cyminum (cumin) is a popular spice globally, with significant importance in the culinary industry. Studies by Kedia et al. (2014) showed that at 0.5 µg/mL concentration of essential oils extracted from cumin, aflatoxin production was inhibited significantly. In summary, the potential application of plant extracts in the mitigation of aflatoxins continues to attract greater attention within the scientific community because of their biologically safe, environmentally friendly, biodegradable, renewable, and potentially low-cost nature. (Vijayanandraj et al. 2014).

Post-harvest handling of maize, the management process and its impact on aflatoxin contamination, and subsequent buildup

Post-harvest grain management forms a critical component of food production in most developing countries, including those in the East African Community. However, the enormous challenges associated with this farming practice impact negatively on the quantity of remaining harvests that are relied upon to sustain communities after a given harvesting season. The losses at the post-harvest level have been documented to occur from farm to plate, and are often attributed to poor grain shelling and sorting, incomplete drying of cereals, poor storage practices as well as ineffective grain distribution. The latter aspect holds a particularly harmful ripple effect whereby it ends up negatively affecting future purchasing and consumption behaviors once the maize is transported to distribution centers.

Effective post-harvest practices are crucial in managing aflatoxin contamination in maize and other crops. The "dry chain" concept, which involves maintaining low moisture levels throughout the post-harvest period, is a significant method to mitigate aflatoxin contamination. This concept emphasizes the use of hermetic storage and proper drying techniques to reduce the moisture content of grains, thereby inhibiting fungal growth and aflatoxin production (Bradford et al. 2018). Post-harvest practices not only affect the quality of crops but also have direct implications on child health. Research has shown that poor post-harvest handling and storage practices can lead to increased aflatoxin levels in food, which in turn, pose serious health risks to children. Aflatoxin exposure is linked to stunted growth and impaired immune function in children, highlighting the need for improved post-harvest management strategies (Kamala et al. 2018a, b).

In Tanzania, various post-harvest management practices have been implemented to control aflatoxin contamination. These practices include the use of improved storage facilities, timely harvesting, and proper drying methods. Studies have demonstrated that these practices significantly reduce aflatoxin levels in maize, contributing to safer food supplies and improved public health outcomes (Kamala et al. 2018a, b). The socio-economic aspects of aflatoxin management are also critical, as evidenced by several studies by Vivin Hoffmann. Her research highlights the economic impact of aflatoxin contamination on households and the cost-effectiveness of various management strategies. In Kenya, Hoffmann's work underscores the importance of public awareness, regulatory frameworks, and market incentives in driving the adoption of effective aflatoxin control measures (Magnan et al. 2021; Pretari et al. 2019).

According to Xu et al. (2022), virtually all post-harvest strategies are essential in preventing aflatoxin accumulation and buildup during storage, especially given the fact that the growth ability of aflatoxigenic fungi is often accelerated during this period. Studies have shown that appropriate and strict adherence to the above-mentioned practices after harvest can significantly minimize AF contamination by up to 88% or more (Unnevehr & Grace 2013).

Drying of maize grains marks the onset of post-harvest activities, in which the overall aim is to eliminate unwanted moisture levels that may trigger fungal growth and subsequent aflatoxin production (Midega et al. 2016). Direct sunlight is undoubtedly the most feasible option for drying maize for both smallholder and large-scale farmers. As an old-age traditional practice, sun drying of maize and other cereals, if done effectively has been shown to minimize aflatoxin accumulation and contamination. Drying maize kernels does not completely eliminate (devoid) moisture from grains. Drying reduces moisture content to safe levels (e.g., 12–13% for corn). Agronomy extension officers and agricultural experts advocate for the use of a polyethylene sheet for drying the harvest, as opposed to pouring the maize cobs directly on the ground. The recommended moisture level of well-dried maize ought to be below 13% (Ng'ang'a et al. 2016), and it is important for farmers to ensure optimal moisture levels have been achieved prior to storage to discourage any fungal proliferation or toxin production. Typically, the measurement for moisture content in maize is often determined by a representative sample that is picked randomly from the entire harvested batch (Xu et al. 2022), whereby farmers either bite kernels with their teeth or puncture them with a thumbnail (Liu et al. 2016). Cracked kernels are interpreted to be well-dried while the reverse (spongy, soft, or tender) are deemed not ready for storage and subjected to further drying.

Grain shelling and sorting often marks one of the initial steps at post-harvest and is often followed by the drying phase. The simplicity of this activity is often overlooked by farmers, yet it holds massive potential in eliminating aflatoxin buildup in a significant fashion. Damaged, infected, or shriveled kernels should be removed prior to shelling, to ensure that the outcome of this process is done specifically on healthy and non-infected maize grains. However, caution should be taken if the shelling process is done manually, since this may be the only option available to most smallholder farmers (Xu et al. 2022). Most often, farmers will adhere properly to shelling and sorting practices but fail to utilize clean and well-dried containers for the same. Therefore, it is essential that containers utilized for this activity are dried well, to avoid any contact between the grains and underlying moisture that may catalyze the onset of *Aspergillus* growth in storage.

Moisture management and proper storage have been demonstrated to be powerful practices that are critical in the mitigation of aflatoxin contamination (Walker et al. 2018). Hermetic bags, clean sealed containers, and the triple-layered Purdue improved crop-storage (PICS) bag should be utilized for storage. All the latter three have been shown to hold a plethora of benefits including minimizing pest invasion, fungal growth, grain deterioration, and prolonged safe storage of maize. In studies conducted by Gachara et al. (2022) in the Rift Valley region of Kenya, it was shown that farmers who utilized either one of the above-mentioned forms of storage were able to preserve nearly 80% of their harvest until the next planting season. Therefore, adopting proper storage should be a principal practice that ought to be followed throughout the post-harvest period to effectively minimize aflatoxin accumulation. Grain storage holds multifaceted aspects, where apart from utilizing appropriate preservation containers, aeration, cleanliness and ventilation of the storage facilities are equally important. The storage granaries, silos, or barns should be properly constructed, giving room for optimum aeration levels. Further, cleanliness within the storage space should be strictly maintained to avoid any gradual buildup of pests, fungal colonies, or even unwanted moisture. While adherence to proper post-harvest practices has been shown to significantly minimize aflatoxin accumulation, the adoption of good agricultural practices can mitigate this challenge even more robustly.

FAO (2003) broadly defines good agricultural practices (GAP) as the knowledge and techniques employed in addressing agronomic, economic, and environmental sustainability for both on-farm production and post-production processes with the overall goal of ensuring safe and healthy agricultural produce. Effective utilization of GAP and its regular monitoring has been shown to improve the quality of most agricultural products, while also reducing most post-harvest losses. In this context, GAP then offers a double win for both smallholder and large-scale farmers who become largely incentivized to continue adhering to the practice in subsequent farming seasons. Several GAP measures have been identified as having a large impact on aflatoxin reduction, control, and mitigation. They include crop rotation, insect and pest management, fertilizer or compost application, timely harvesting, and proper harvesting techniques (Udomkun et al. 2018a, b; Wokorach et al. 2021b; Xu et al. 2022) as well as the earlier discussed post-harvest practices. The adoption of GAP is often problematic in developing countries, including those in the EAC, primarily because maize cultivation is widely done under the smallholder farming system (Massomo 2020), where most farmers grow, cultivate, and consume their maize. As such, it becomes extremely difficult to monitor these individualized agricultural practices and eventually quantify them as optimal in the mitigation of the aflatoxin menace. For instance, some farmers reiterated that crop rotation and irrigation are expensive GAP practices, given that the majority of the farming done in Sub-Saharan Africa is predominantly rain-fed (Mutiga et al. 2015a; Udomkun et al. 2018a, b).

Similar sentiments are echoed when it comes to the adoption of new technology, with most farmers being largely unwilling to embrace novel techniques in place of their conventional farming practices (Udomkun et al. 2018a, b; Xu et al. 2022). Evidently, the adoption of GAP would be more successful at the smallholder farm level if policies and regulations from local governments were introduced and implemented in a stringent fashion. Good agricultural practices provide an optimum avenue through which aflatoxin accumulation in maize grains can be mitigated at the farm level, especially for the aflatoxin-prone regions in an East African Community context.

Conclusion

Mycotoxins are among the most extensively researched yet potent toxins concerning food safety. The global attention they attract is due to their economic importance as contaminants with multifaceted effects on both animal and human health. In particular, the prevalence of aflatoxins continues to rise along the agricultural food chain, largely due to the absence of national surveillance systems that can flag potentially contaminated crops. This review has validated this concern, particularly within the context of the East African Community (EAC). It thoroughly discusses *Aspergillus* colonization and aflatoxin contamination of post-harvest maize, examining the etiology and primary causative agents, the climatic predisposition of the EAC region, and the specific gene pathways involved in aflatoxin biosynthesis.

Overall, aflatoxin accumulation and buildup in stored maize cereals remain a significant problem, despite numerous interventions aimed at mitigating this agronomic issue. Further research is needed in South Sudan and Burundi to conclusively determine if the magnitude of the problem is as severe as in other EAC countries and to enhance the understanding of this issue at a more advanced level. The adoption of local measures to minimize aflatoxin accumulation in post-harvest maize by farmers in the EAC has helped mitigate this perennial problem, albeit minimally. Therefore, it is paramount to develop aggressive, intensive, and rigorous agricultural and agronomic practices to significantly reduce the intensity and severity of aflatoxin contamination within the EAC.

The absence of robust control measures capable of suppressing the growth of aflatoxigenic *Aspergillus* species exacerbates the aflatoxin menace in this geographical region. Aflatoxin contamination in cereals, especially maize, cannot be overlooked within the EAC, as it forms the staple diet in most countries. There is an urgent need to diversify diets to reduce exposure to aflatoxin-contaminated foods in this region.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

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