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Aflatoxin contamination levels in farmer's preferred maize varieties and exotic lines in Tanzania

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Abstract

Aflatoxins are carcinogenic compounds produced by fungi like *Aspergillus*, which contaminate key crops such as maize, leading to food insecurity, poor health, and economic challenges, particularly in African countries where maize is a staple. High temperature, humidity, and insect damage increase aflatoxin contamination. Efforts to manage this issue are underway, with the most promising solution being the development of maize genotypes that accumulate lower levels of toxins and resist fungal infection. The present study was carried out to determine the reaction of maize genotypes to aflatoxin contamination in twenty selected exotic and local maize genotypes. The experiment was conducted at TARI-Ilonga in a screen house, and it was performed in accordance with a completely randomized design, with each germplasm replicated three times. The plants were inoculated with an *A. flavus* conidial suspension using the needle inoculation technique. An ultra-performance liquid chromatography fluorescence detector was used to quantify aflatoxin levels in the seeds of the maize genotypes. The results revealed varying degrees of aflatoxin contamination across the maize genotypes tested, ranging from 1.6 to 770.1 µg/kg. This study identified nine genotypes (G3, G10, G11, G12, G14, G15, G17, G18, and G20) that had very low aflatoxin concentrations (1.6–9.5 µg/kg). The identified genotypes can be tested in various environments and proposed for release as varieties or for use as parental materials in breeding programs to enhance food security, improve farmer incomes, and promote good health and sustainable agricultural practices in maize-growing regions.

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Introduction

Maize is one of the most important staple food crops in the world and is used for human consumption and animal feed and in many industries as a component of industrial goods (Okoth *et al*., 2017). More than 1.2 billion people in Sub-Saharan Africa rely on maize as their primary staple food crop, which accounts for almost 30% of their total caloric intake (Grace *et al*., 2015). Tanzania is ranked among the 25 highest maize-producing countries in the world (Suleiman and Kurt, 2015). With 5.9 million tons produced in 2022, Tanzania ranks fifth in Sub-Saharan Africa after South Africa, Nigeria, Ethiopia, and Egypt. Approximately 96.6 million tons of maize were produced on approximately 42.4 million hectares of land in Africa (FAOSTAT, 2022). Maize production in Sub-Saharan African countries is constrained by both biotic and abiotic factors (Logrieco *et al*., 2021). Tanzania, which is the largest producer of maize in East Africa, still faces many challenges in terms of maize production before it can realize its full business potential (Bjornlund and Van Rooyen, 2020). One of these challenges is the persistently high postharvest losses, which can reach up to 40 % in some rural areas (Suleiman and Kurt, 2015). *Aspergillus flavus* is a parasitic fungus that is saprotrophic and opportunistic and seriously damages crop in the field. High temperatures, drought condition, and insect damage are the primary causes of fungi infection and toxin production (Agag, 2004). Maize plants are susceptible to drought stress, which weakens the plant and increases its vulnerability to fungal infection (Kebede *et al*., 2012). Climate change is expected to worsen this situation by creating more favorable conditions for aflatoxigenic mold growth and toxin production (Benkerroum, 2019). Due to inadequate farming practices and poor storage management, mycotoxins and insect pests cause an average annual loss of 20-30 % in maize, accounting for 5-7 % of the crop (Shukla *et al*., 2019). Most of the maize varieties grown and consumed in Tanzania are susceptible to *A. flavus* infection and aflatoxin contamination (Kamala *et al*., 2015; Mtega *et al*., 2020). Aflatoxin, fumonisin, and ochratoxin are the three most significant mycotoxins in Tanzania (Suleiman and Kurt, 2015).

There are seven agro-ecological zones in Tanzania, which greatly differ in terms of the amount of rainfall, temperature, altitude and soil characteristics. The differences in soil characteristics and climatic conditions cause these agroecological zones to experience differences in potential and challenges for achieving sustainable agriculture. Maize is an important food and feed for livestock and is mostly grown in each of these seven agroecological zones. Most of the maize varieties grown and consumed in Tanzania are susceptible to *Aspergillus flavus* (*A. flavus*) infection and aflatoxin contamination (Kamala *et al*., 2015; Mtega *et al*., 2020). *Aspergillus flavus* and *Aspergillus parasiticus* are the main producers of aflatoxins, which are carcinogenic secondary metabolites found in agricultural foods and animal feeds (Wacoo *et al*., 2014).

Maize plants are susceptible to drought stress, which weakens the plant and increases its vulnerability to fungal infection (Kabede *et al*., 2012). The contamination of maize by aflatoxins when consumed by humans can cause both acute and chronic poisoning. In humans, aflatoxins can result in acute hepatic toxicity in chronic diseases such as liver cancer. Aflatoxins are associated with depression of the immune system, stunted growth, cancer and even death (Benkerroum, 2019; Strosnider *et al*., 2006). Aflatoxin exposure from maize consumption in infants and young children in Tanzania has been found to reach as high as 10,926 ng/kg body weight per day, which is a health concern for maize consumers (Shirima *et al*., 2015). According to a study developed by Boni *et al.,* (2021) in 10 districts of Tanzania, the average aflatoxin level in maize samples ranged between 12.47 and 162.40 µg/kg. The estimated average probable daily intake of aflatoxin B1 from maize in Tanzania ranges between 151.98 and 272.89 ng/kg body weight/day (Boni *et al*., 2021).

Furthermore, maize is an essential ingredient in feed formulations for livestock such as pigs and chickens. Feeding on aflatoxin-contaminated feeds has adverse effects on the health and productivity of livestock since it can lead to liver cancer, inborn defects and malformations, decreased body weight and growth, immunosuppression, and even death (Massomo, 2020; Ncube and Maphosa, 2020).

Preventing aflatoxins in the field and during storage is the best way to mitigate their effects (Bruns, 2003; Chulze, 2010). Aflatoxin contamination prior to harvest is a complicated process influenced by multiple biotic and abiotic factors. Consequently, to minimize aflatoxin contamination, a multifaceted strategy is required (Cary *et al.*, 2011). Preharvest methods for preventing aflatoxins can be roughly divided into three categories: 1) increase fungal resistance in the host (crop), 2) escape from fungal contamination, and 3) decrease in the number of toxic fungi. In particular, this includes weed control; appropriate disease and pest management, including the use of biocontrol agents; crop rotation; tillage; timely planting of agro-ecologically adapted or disease-resistant cultivars; appropriate crop densities; and management of irrigation and fertilization. According to Jaime and Cotty (2010), areas that were previously planted in maize had much higher infection percentages by *A. flavus* than fields that were previously planted in other crops, such as cotton or sorghum, with the latter having the lowest populations of *A. flavus*. These findings indicate that ongoing maize crop production greatly contributes to the development of toxic fungal populations in the soil, increasing the risk of fungal infection and aflatoxin contamination. Crop rotation is necessary because certain crops, such yams, millet, sorghum, cassava, and soy, are poor substrates for the growth of *A. flavus*. Regrettably, socioeconomic constraints such as the reduction in the size of smallholder farms due to the expansion of the rural population and the increasing shortage of land prevent most African countries from implementing efficient crop rotation (Jayne *et al.*, 2012; Thierfelder *et al.*, 2013) and over depend on maize (Martin *et al.*, 2000).

To achieve agricultural sustainability across the continent, better agricultural practices are essential for improving agricultural productivity and lowering postharvest losses. Cultural practices has been implemented, but its use has had a limited impact on the spread of infection and the accumulation of mycotoxins (Munkvold, 2003). The use of biocontrol techniques, enhanced post-harvest management, and best practices in agriculture have not been enough to lower contamination. Host resistance factor therapy has long been used to decrease aflatoxin accumulation in maize (Brown *et al.*, 2001; Cary *et al.*, 2011; Warburton *et al.*, 2011).

Discovering maize genotypes resistant to *A. flavus* infection and aflatoxin contamination is vital for improving maize production, particularly within agroecological farming systems in Tanzania. Integrating resistant maize genotypes into agroecological farming systems can improve maize production sustainability, enhance food safety, and boost resistance against pests and diseases in Tanzania. Since the middle of the 1970s, efforts have been made to create inbred lines of cereals that are resistant to aflatoxin contamination. Due to health problems and loss of income as a result of aflatoxin contamination, there is a need to reduce postharvest losses and produce safe food and feeds by identifying aflatoxin-resistant maize genotypes. This study aimed to screen maize genotypes for resistance to aflatoxin contamination. Thus, the information generated from this study will inform maize breeders on the genotypes and lines to use when developing maize varieties that are resistant to aflatoxin contamination.

Materials and methods

Study area description

The experiment was conducted at the Tanzania Research Institute (TARI) Ilonga in the coastal zone of the screen house. The Institute is located at latitude -6°15.344" S, longitude 37° 39' 32.38" E and stands at an elevation of 491 m above sea level. It receives an average annual precipitation of 274.93 millimeters (10.82 inches) and has 245.26

rainy days (67.19% of the time) annually, from March to May, with peaks in April. The mean annual temperature ranges between 16°C and 32.8°C, and the average relative humidity is 62%. The study was conducted during the 2023 main cropping season.

Maize genotypes used in the study

The experiment used twenty maize genotypes from Tanzania and the International Maize and Wheat Improvement Center (CIMMYT) Kenya. Table 1 presents the list of maize genotypes and lines used in the study.

Table 1. Maize genotypes used for the present experiment

Code	Genotype name	Source	Status
G1	Situka M1	TARI-Selian	Released variety (2001)
G2	Kilima	TARI-Selian	Released variety (1983)
G3	Vumilia K1	TARI-Selian	Released variety (2007)
G4	Lishe K1	TARI-Selian	Released variety (2001)
G5	CKL174188	CIMMYT	Inbred Line
G6	CML495	CIMMYT	Inbred Line
G7	CKL21181	CIMMYT	Inbred Line
G8	CKL173531	CIMMYT	Inbred Line
G9	CKL21274	CIMMYT	Inbred Line
G10	CKL174164	CIMMYT	Inbred Line
G11	CKL173302	CIMMYT	Inbred Line
G12	CKL21198	CIMMYT	Inbred Line
G13	CKL21199	CIMMYT	Inbred Line
G14	CKL174148	CIMMYT	Inbred Line
G15	CKL174184	CIMMYT	Inbred Line
G16	CKL174161	CIMMYT	Inbred Line
G17	CKL211166	CIMMYT	Inbred Line
G18	CKL174179	CIMMYT	Inbred Line
G19	Staha	TARI-Ilonga	Released variety (1983)
G20	T105	TARI-Tumbi	Released variety (2016)

Inbred line - Parental lines; Released variety - Commercial crop variety

Experimental design and planting

The 20 maize genotypes were planted in polyethylene bags following a completely randomized design (CRD) in the screenhouse at TARI-Ilonga. Each bag was filled with 20 kg of forest topsoil. Two seeds of each tested maize genotype were planted per bag, and the plants were replicated three times. The spacing between the rows was 75 cm, and that between plants was 30 cm. The trial was planted on 28th April, 2023, and the crops were given optimum agronomic management, such as irrigation, weeding and fertilization.

Fungal inoculation

S-type strain of toxigenic *A. flavus* obtained from the International Institute of Tropical Agriculture (IITA) headquarter Dar es Salaam, Tanzania, was cultured in Petri dishes containing potato dextrose agar (PDA). After seven days, the inoculum was removed from the culture using Tween 20 and distilled water, after which the concentration was adjusted to 3×10^7 conidia mL^{-1} by using a hemocytometer. At 20 days after mid-silk (at which 50% of the plants in the bag had silk emerged), each plant was inoculated with an *A. flavus* conidial suspension via the needle inoculation technique (Scott and Zummo, 1988). The inoculum was applied down the silk channel onto the ear with 3.5 ml of inoculum injected inside the husk onto the kernel from the tip of the ear. The inoculum was injected over the kernel without damaging the kernel using a hypodermic needle. To guarantee uniform dispersion of the conidia, the inoculum was shaken prior to each injection. The ears were allowed to mature and were harvested after each diseased ear was tagged.

Data collection

The maize cobs were harvested on 18th September, 2023. The plants of each genotype were harvested separately and air-dried to 14% moisture content before storage to prevent mold growth, insect infestation, and spoilage, which are common in grains with higher moisture levels. The maize genotype samples were subsequently sent to the International

Livestock Research Institute (ILRI) Nairobi for quantification of aflatoxin.

Sample preparation and analysis

Extraction of aflatoxin from maize samples

The AOAC Official Method was employed to extract aflatoxins from maize flour (AOAC Official Method 2006.08). Maize kernels were ground into a fine powder using a laboratory mill to achieve a particle size of less than 1 mm, preferably between 0.5 mm and 1 mm, ensuring uniformity for effective extraction. A sample of 5.0±0.1 g was precisely weighed into a 50 ml polypropylene centrifuge tube, using a sterilized weighing spatula cleaned with 70% ethanol and dried with a paper towel between samples. To this, 25 ml of 70% methanol was added. The mixture was shaken at 250 rpm for at least 20 minutes at room temperature using a mechanical orbital shaker. Following this, the extract was centrifuged at 3500 rpm for 10 minutes. A 2 ml aliquot of the extract was diluted in a 1:1 (v/v) ratio with 1% acetic acid and transferred to a 2 ml centrifuge tube. The final step involved filtering the sample through a 0.2 µm PTFE syringe filter into an HPLC vial for analysis.

Determination of aflatoxin concentration using UPLC-FLD

Chromatographic separation was carried out using a Nexera UHPLC system (Shimadzu Corporation, Kyoto, Japan), equipped with a SIL-30AC autosampler, LC-20AD Prominence pumps, and an RF-20AXS Prominence Fluorescence detector. The separation of aflatoxins B_1, B_2, G_1 and G_2 was achieved using a Synergi Hydro-RP analytical column (2.5 µm particle size, 100 mm × 3.00 mm) from Phenomenex (Torrance, CA, USA), operated at a flow rate of 0.4 mL/min. The process utilized an isocratic binary mobile phase comprising 40% methanol (mobile phase A) and 60% 1% acetic acid (mobile phase B). A 10 µL injection volume was used, with the column oven maintained at 50°C. Each liquid chromatography run lasted 9 minutes, and 30% methanol served as the column flushing solution. Fluorescence detection was performed at excitation and emission wavelengths of λex = 365 nm and λem = 435 nm, respectively. A standard calibration curve, created by plotting peak areas against the known concentrations of a series of injected standards, was established and used to determine sample concentrations using LabSolutions software version 5.89 (Shimadzu Corporation *et al.*, 2014). Aflatoxins B1, B2, G1, and G2 were identified by comparing the retention times (Table 2) of their chromatographic peaks in the test samples with those of their corresponding standard chromatographic peaks (Table 3).

Table 2. Retention time

Toxin	Retention time (minutes)
AFG2	3.857
AFG1	4.598
AFB2	5.699
AFB1	6.935

Table 3. Standard concentrations

Standard reference	Analyte concentration (ng/ml)				
	AFG2	AFG1	AFB2	AFB1	Total
Standard 1	103.350	102.950	102.950	103.200	412.450
Standard 2	51.675	51.475	51.475	51.600	206.225
Standard 3	25.838	25.738	25.738	25.800	103.113
Standard 4	12.919	12.869	12.869	12.900	51.556
Standard 5	2.584	2.574	2.574	2.580	10.311
Standard 6	0.517	0.515	0.515	0.516	2.062

Table 4. Limit of detection in aflatoxin testing

LOD specifics		
Analyte	LOD (µg/kg)	Matrix
Aflatoxin G2	0.072	Maize
Aflatoxin G1	0.223	
Aflatoxin B2	0.086	
Aflatoxin B1	0.36	

Table 4 indicates the lowest concentration of aflatoxin that can be reliably detected by a testing method. Achieving a low LOD is essential for ensuring the reliable identification of aflatoxin contamination in food and feed samples.

Results

Method performance

The use of ultra-performance liquid chromatography (UPLC) with a fluorescence detector (FLD) for aflatoxin quantification has important implications for maize crop breeding. The UPLC–FLD method

successfully separated four types of aflatoxins, aflatoxin B_1, B_2, G_1, and G_2, as shown in the UPLC chromatogram (Fig. 1).

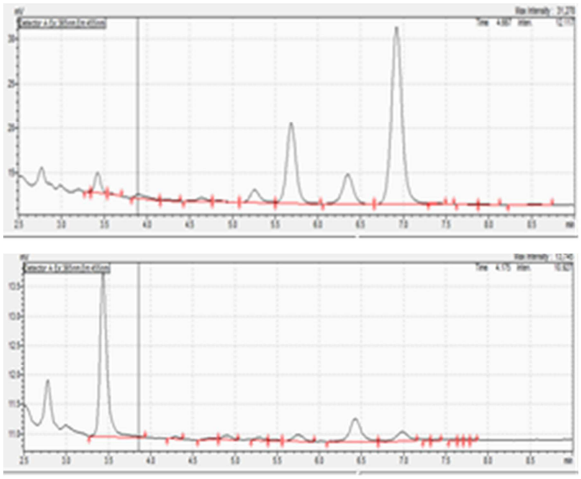

Fig. 1. Representative selected UPLC chromatograms for Samples 24_052 and 24_057

The calibration curve for each aflatoxin had a regression r^2 value of 0.999, indicating an almost perfect linear relationship between the aflatoxin concentration and detector response, and demonstrating the precision and accuracy of the method. An r^2 value near 1.0 reflects a nearly perfect correlation between aflatoxin concentrations and detector responses. This high linearity means that the method yields consistent and reproducible results, which are vital for reliable quantification. Each peak represents a different compound that was separated on the chromatography column. The height of each peak is proportional to the amount (concentration) of the compound. The concentration of compounds in each sample can vary, leading to differences in peak heights and areas.

Table 5. Aflatoxin concentration (µg/kg) in 20 maize genotype samples from the artificially inoculated maize genotypes

Maize genotype	Sample code	Toxin concentration (µg/kg)				Total Aflatoxin (µg/kg)
		Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	
G1	24_044	48.0	3.6	0.6	0.2	52.3
G2	24_045	683.1	76.0	4.8	6.2	770.1
G3	24_046	2.4	1.9	2.1	<LOD	6.3
G4	24_047	68.6	7.7	5.6	0.3	82.1
G5	24_048	340.6	62.9	7.4	4.9	415.7
G6	24_049	481.4	27.1	14.5	1.9	524.9
G7	24_050	401.2	69.8	2.7	3.7	477.4
G8	24_051	354.5	25.7	4.6	2.0	386.8
G9	24_052	197.3	23.5	10.6	1.8	233.1
G10	24_053	0.9	0.6	<LOD	<LOD	1.6
G11	24_054	1.9	4.0	2.4	<LOD	8.3
G12	24_055	3.7	2.7	3.1	<LOD	9.5
G13	24_056	5.1	2.9	3.4	0.1	11.6
G14	24_057	2.0	0.3	0.7	<LOD	2.9
G15	24_058	0.7	0.6	6.1	<LOD	7.4
G16	24_059	86.3	3.9	0.3	0.3	90.8
G17	24_060	<LOD	3.9	4.4	<LOD	8.7
G18	24_061	2.8	2.9	2.4	<LOD	8.1
G19	24_062	2.9	3.7	8.1	<LOD	14.7
G20	24_063	0.4	1.2	5.3	<LOD	6.9

LOD = limit of detection

Different levels of aflatoxins (B_1, B_2, G_1, G_2) were detected in the targeted maize germplasm (G1-G20) used in the present study (Table 5), ranging from not detected to 770.1 µg/kg. High concentration of more than all four aflatoxin types was observed in sample G2, for which the total aflatoxin concentration was 770.1 µg/kg. Samples with moderate concentrations of aflatoxins included samples, in µg/kg: G5 (415.7), G6 (524.9), G7 (477.4), and G8 (386.8).

Sample G9 had a medium level of aflatoxin accumulation (233.1 µg/kg). Some samples had relatively low accumulation of aflatoxins compared with others, such as samples G1 (52.3), G4 (82.1), G16

(90.8), G19 (14.7), and G13 (11.6). Never the less, less aflatoxin accumulations (6.3 – 9.5 µg/kg) were detected in some maize germplasms (G3, G10, G11, G12, G14, G15, G17, G18, and G20), this indicates that nine maize genotypes had very low aflatoxin accumulation. Some samples had accumulation of aflatoxin below the limit of detection (LOD) of the testing method. This is indicated as "<LOD" in the table. Even though these samples have accumulation below the LOD, this does not necessarily mean that they are completely free of aflatoxins.

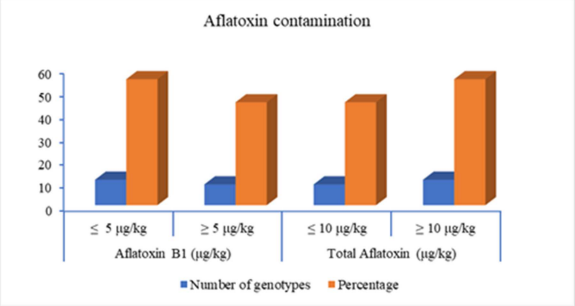

Fig. 2. Aflatoxin contamination distribution in tested maize samples

Aflatoxin contamination status of the maize samples tested

Fig. 2 shows the distribution of aflatoxin B_1 and total aflatoxin levels among the 20 tested maize genotypes. Aflatoxin B_1 levels were below or equal to 5 µg/kg in 11 genotypes (55%), while 9 genotypes (45%) exceeded this threshold. Similarly, total aflatoxin concentrations were ≤10 µg/kg in 9 genotypes (45%), with the remaining 11 genotypes (55%) showing levels above 10 µg/kg.

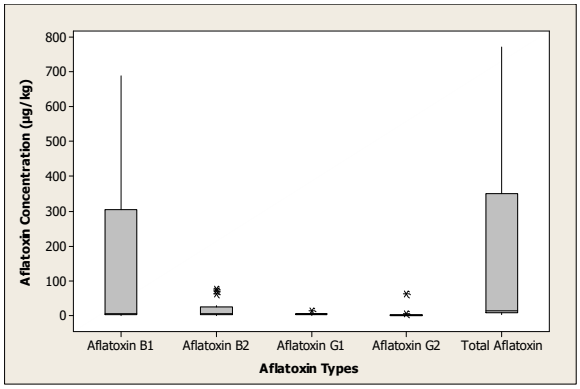

Fig. 3. Box plot for aflatoxin concentration across 20 maize genotypes

Table 6. Analysis of variance for aflatoxin quantification in 20 maize genotypes tested

Source of variation	df	SS	MS	F	P-value
Genotype	19	1.39E+07	732835	11.46	<0.001
Residual	1	63728.3	63728.3		

The boxplot shows distribution of aflatoxin levels across the maize genotypes. There was greater variability of total aflatoxin among tested maize genotypes. The highest aflatoxin accumulation and the largest variability among maize genotypes were recorded in aflatoxin B_1 followed by aflatoxin B_2, G_1 and G_2. Most of the maize genotypes had aflatoxin accumulation greater than their means (Fig. 3).

Table 6 indicated the p-value is less than 0.05, this conclude that there is a significant difference in total aflatoxin levels among the genotypes. This suggests that at least one genotype produces significantly different levels of total aflatoxin compared to the others.

Discussion

The ability to accurately and consistently measure aflatoxin levels allows breeders to evaluate and identify maize genotypes that are more resistant to aflatoxin-producing fungal infestations. Aflatoxins, particularly B_1, are highly toxic and carcinogenic, and contamination levels that surpass regulatory limits pose serious health concerns. According to the IARC Working Group (2012) (Lyon, 2014), aflatoxins are classified as Group-1 carcinogens, which indicates that there is enough proof of their ability to cause cancer. Since aflatoxin is a genotoxic carcinogen, exposure levels should be as low as possible because there is no safe amount of exposure. Decreased levels of aflatoxins in maize reduce the risk of exposure to these potent carcinogens, ensuring that maize-based food products are safer for consumption. This process could lead to the development of maize varieties that are resistant to toxigenic fungi, which is crucial for food safety and public health.

The data from the current study indicated varying degrees of aflatoxin contamination across the maize genotypes tested, with some of the genotypes showing significantly higher levels of aflatoxin accumulation than others. G2 displayed the highest aflatoxin contamination, with a total concentration reaching 770.1 µg/kg, significantly above global safety limits. This genotype contained all four types of aflatoxins (B1, B2, G1, and G2), making it unsafe. Such extreme contamination underscores G2's vulnerability to *Aspergillus* infection and aflatoxin buildup. Maize genotypes with elevated aflatoxin levels exhibit susceptibility, whereas those with lower aflatoxin concentrations may exhibit a certain degree of resistance. Nine maize genotypes (G3, G10, G11, G12, G14, G15, G17, G18, G20) exhibited very low aflatoxin levels, ranging from 6.3 to 9.5 µg/kg, within or near regulatory limits. These genotypes are promising candidates for breeding programs focused on developing maize varieties resistant to aflatoxin contamination. G17, in particular, if used in breeding for resistance, demonstrated contamination levels below the detection limit for Aflatoxin B_1 and a total aflatoxin content of 8.66 µg/kg, reinforcing its value as a resistance source. These low contamination levels suggest genetic resistance, which is crucial in controlling aflatoxin buildup in field conditions (Warburton *et al.*, 2011). The presence of "<LOD" denotes concentrations below the detection limit of the testing method, this does not guarantee they are entirely free from aflatoxins, as the detection method may not pick up minute quantities. Overall, the data underscore the variability in aflatoxin contamination among the tested maize genotypes. The results obtained from this study correspond to those of other studies on screening for aflatoxin resistance in maize genotypes conducted which reported different levels of aflatoxin contamination and accumulation in maize genotypes tested for resistance (Fountain *et al.*, 2019; Guo *et al.*, 2017; Willium *et al.*, 2015; Ortega *et al.*, 2014). The difference in the level of aflatoxin contamination may have been due to the resistance of the maize genotypes to the physical composition of the kernel, the cob husk covering, and the tight coating, which are connected to lower levels of aflatoxin (Edwards, 2006) or lipid content in seed embryos, which is associated with resistance to aflatoxins (Chen *et al.*, 1998). Maize genotypes with low total aflatoxin concentrations can be used as donor parents to improve widely cultivated and consumed maize genotypes.

The main requirements for developing cultivars resistant to aflatoxin contamination and preharvest *A. flavus* infection are the availability of genetic variability for resistance and access to dependable and efficient screening techniques. The uneven aflatoxin distribution among the tested germplasm, with 55% of genotypes having Aflatoxin B_1 levels below 5 µg/kg and 45% exceeding this level, points to varying resistance mechanisms. Likewise, 45% of genotypes had total aflatoxin concentrations below 10 µg/kg, while 55% had higher levels, highlighting the complexity of resistance against aflatoxins. This variation suggests that aflatoxin resistance in maize is likely governed by multiple genes, a hypothesis supported by QTL mapping studies (Warburton and Williams, 2014). The data from this study indicated that 55% of the genotypes have aflatoxin B_1 levels less than or equal to 5 µg/kg and that 45% of the genotypes have total aflatoxin levels either less than or equal to 10 µg/kg. These genotypes show resistance to aflatoxin infection and contamination and are within the maximum tolerable limits for aflatoxins for the East African region (Community, 2011) and the European Union (EC, 2006). Several genotypes exhibit promise for tolerating aflatoxin buildup and *A. flavus* (Grace *et al.*, 2015; Okoth *et al.*, 2017). These genotypes can be used in maize breeding programs to improve widely cultivated maize genotypes or tested in different agroecosystems to assess their adaptability and production potential; thereafter, new varieties can be suggested.

Conclusion

Aflatoxin contamination is a serious threat in maize production, particularly in the tropics where the environment is conducive to aflatoxin infection and contamination. Reliability in breeding for resistance remains one of the most effective methods for

reducing aflatoxin contamination in maize. The result shows a highly significant difference among maize genotypes concerning the accumulation of aflatoxins. In this experiment, with an alpha of 0.05 and 20 observations, the test is well-powered (98.7%) to detect an effect, meaning it is highly likely to correctly identify a true difference or effect. Whereas some genotypes were resistant, with low contamination levels, others, like G2, were very susceptible, with high levels of aflatoxin. From these results, the key interest should be in genotypes that contain low levels of aflatoxin for any breeding program related to the search for maize genotypes that are resistant to the contamination of aflatoxin. Nine maize genotypes, G3, G10, G11, G12, G14, G15, G17, G18, and G20, were found to be resistant to both aflatoxin B_1 and total aflatoxin. Thus, the identified maize genotypes that are resistant to aflatoxin contamination could be used in maize breeding programs to improve widely cultivated and consumed maize varieties. The genotype-by-environment interaction is a vital factor in breeding for aflatoxin resistance in maize. Understanding how different genotypes perform under varying environmental conditions allows breeders to develop more resilient and adaptable varieties that can thrive in diverse agroecological zones while minimizing aflatoxin contamination risks. The identified maize genotypes resistant to aflatoxin contamination that have not been released need to be tested in multilocation trials for aflatoxin contamination and other important agronomic traits to propose their release as new varieties.

Adopting aflatoxin-resistant maize genotypes in maize-growing agroecological zones will reduce poverty by improving farmer incomes, enhancing food security by increasing the availability and safety of maize, and promoting sustainable agricultural practices by reducing chemical inputs and food wastage. Addressing aflatoxin contamination is crucial for advancing sustainability in agriculture, the environment, and technology, ensuring public health protection, and maintaining economic stability for farmers and communities dependent on maize production.

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