INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 25, No. 4, p. 193-207, 2024

RESEARCH PAPER OPEN ACCESS

Effect of soil physio-chemical parameters on the prevalence of aflatoxin-producing fungal species in maize agro-ecosystems of Tanzania

Owekisha H. Kwigizile[*1,2], Ernest R. Mbega[1], Marco E. Mng'ong'o[3], Arnold A. Mushongi[4], Mashamba Philipo[1]

[1]*School of Life Sciences and Bioengineering (LiSBE), The Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania*

[2]*Tanzania Agricultural Research Institute, Mbeya, Tanzania*

[3]*Department of Crop Science and Horticulture, Mbeya University of Science and Technology, Mbeya, Tanzania*

[4]*Tanzania Agricultural Research Institute, Kilosa, Morogoro, Tanzania*

Key words: Maize, Agroecosystems, Soil, Aflatoxin-producers, Aflatoxins, Prevalence, Tanzania

http://dx.doi.org/10.12692/ijb/25.4.193-207 Article published on October 08, 2024

Abstract

Despite the significance of maize (Zea *mays* L.) in Tanzania, Aflatoxin contamination poses significant risks to food and nutrition security, human health, and economic losses. Aflatoxin emanates from farms and farming systems which are managed by small-scale resource poor farmers. Virtually such production conditions favor predominance of Aflatoxins in the food and feed systems. However, relatively little is known about contaminants relationship on the soil ecosystems. The current study explored correlation of soil physio-chemical characteristics and aflatoxin-producing fungal species, particularly *A. flavus* and *A. parasiticus,* in maize-growing regions of Tanzania. Soil samples were collected from seven districts of Babati and Kiteto (Manyara region), Chemba, Kondoa and Bahi (Dodoma region) and Nzega and Urambo (Tabora region) previously reported high level of contamination and analyzed for physio-chemical parameters. The macro-morphological identification method was used for fungal identification from soil samples. Results exposed sandy loam soil texture was dominant across districts, low proportions of clay particles and silt. Soil chemical properties differed significantly at ($P \leq 0.001$) for pH, organic matter, Total N, S, B, and EC, implicating that soil fertility status were diverse among studied districts. The correlations between soil characteristics and fungal prevalence revealed a significant correlation between certain soil physio-chemical parameters and aflatoxin-producing fungal abundance in maize agro-ecosystem. Two species namely *A. flavus (38.1%)* and *A. parasiticus* (22.2%) were dominant genera from soil samples compared to maize samples hence source of inoculums being from soil. These findings underscore the importance of soil management practices in mitigating aflatoxin contamination in maize.

* **Corresponding Author:** Owekisha H. Kwigizile ✉ owekisha.kwigizile@tari.go.tz

Introduction

Maize (*Zea mays*) is a staple and high-value crop used worldwide for food, feed, cash and agro-allied industries in sub-Saharan Africa (Nji *et al.*, 2022). In Tanzania, maize is the first most important cereal followed by rice supporting millions of livelihoods and contributing significantly to the country's food security. However, over 80% of maize is produced by small- scale resource poor farmers for subsistence and as a cash crop under low inputs and rain-fed agro-ecology conditions (Frederick *et al.*, 2020; Wilson and Lewis, 2017). Despite its importance, maize is one of the most susceptible crops to pre- and post-harvest contamination with myco-toxigenic fungi such as *Aspergillus flavus* and *A. parasiticus* mainly those in section Flavi. Fungi are indigenous to a mixture of environmental fields including soil, decaying vegetation, and food storage systems which predispose food and feed to aflatoxin accumulation (Abbas *et al.*, 2009; Cotty and Mellon, 2006; Fouché *et al.*, 2020; Horn, 2003; Pitt and Hocking, 2013), Generally these habitats vary widely with respect to biotic and abiotic factors but the soil remains the primary reservoir and source of inoculum for aflatoxigenic fungal biota.

Aflatoxigenic *Aspergillus* species produce toxic metabolites that are highly carcinogenic, and *A. flavus* and *A. parasiticus* are the most frequently implicated species in the contamination of food crops, including maize (Academic, 2021; Agape *et al.*, 2021; Boni *et al.*, 2021; Kibwana *et al.*, 2023). Aflatoxin contamination is an issue of food security, food safety, nutrition security, human health and trade. Consequently, the contamination has resulted to poor quality, quantity and low export earnings of the country from the maize sub-sector. Aflatoxigenic fungi reside in soils as conidia, sclerotia and hyphae, which act as primary inoculum, directly infecting maize crop through wind and insect dispersal (Garber and Cotty, 1997). The maize production and postharvest handling systems in SSA favor aflatoxigenic entry points along the value chain. Nonetheless, effective Aflatoxin management should prioritize the primary entry avenue in the micro-ecosystem for the aflatoxigenic agents particularly at pre harvest crop stages.

Aflatoxigenic *Aspergillus* species produce various mycotoxins such as aflatoxins, ochratoxins, fumonisin and others (Elias, 2016; Falade, 2019; Frisvad *et al.*, 2019; Gbashi *et al.*, 2019), which mostly affects crops including maize. Aflatoxins production is dominant among mycotoxins in the soil since it is the natural habitat of *Aspergillus* species that produce toxins. Aflatoxins are poisonous and dangerous naturally occurring secondary metabolites (Benkerroum, 2020; Elias, 2016; Mtega *et al.*, 2020; Negash, 2018; Shabeer *et al.*, 2022), and usually exist in four types namely AFB1, AFB2, AFG1, and AFG2 based on the florescence colour (B for blue and G for green under ultraviolet light), (Frisvad *et al.*, 2019; Pitt and Hocking, 2013). Aflatoxins pose significantly serious health risks in both human and animals consuming contaminated food and feed respectively (Gong *et al.*, 2016; Kimanya *et al.*, 2021; Kinyenje *et al.*, 2023; Liu *et al.*, 2012), that including mutagenic and carcinogenic effects, immune suppression, malnutrition, liver cancer, and death from acute exposure (Githang'a *et al.*, 2019; Kamei and Watanabe, 2005; Tola and Kebede, 2016), they also limit trade and value addition in crops (Kumar *et al.*, 2021; Mahuku *et al.*, 2023; Makhuvele *et al.*, 2020; Tai *et al.*, 2020). Aflatoxins contamination can materialize both in the maize fields (Fig. 1) during transportation and after the crop has been harvested and stored (Jaime-Garcia and Cotty, 2004; Mahuku *et al.*, 2019; Massomo, 2020).

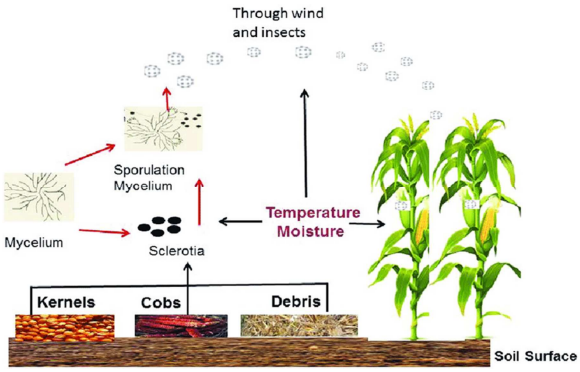


Fig. 1. Source of aflatoxigenic fungal inoculum and entry mechanism to maize from the soil

The presence and abundance of aflatoxin-producing agents in soils are influenced by various biophysical and chemical factors in the soil including microclimate, substrate availability, water activity (influenced by temperature), complex ecological relationships and soil nutrients and after harvest (Abbas *et al.*, 2009; Buckner *et al.*, 2016; Fouché *et al.*, 2020; Gugnani, 2003). Nevertheless, occurrence may vary by host crop, cropping systems and geographical location (Geiser *et al.*, 2007; Klich, 2007). Consequently, high adaptability of aflatoxigenic species to different environments allows for survival of such populations (Nji and Babalola, 2023).

Studies have reported soil properties such as texture, moisture content, pH, and organic matter to significantly influence the habitat suitability for aflatoxin-producing fungi (Fouché *et al.*, 2020; Wang *et al.*, 2023). Variability in soil nutrient levels especially N and P have been reported to play a critical role in the distribution of the fungal pathogen (Manoza *et al.*, 2017). Moisture stress particularly drought during crop growth stages results in increasing susceptibility to fungal colonization in crops (Fouché *et al.*, 2020). Knowledge on the interactions and association of aflatoxin-producing species with bio physio-chemical components versus the ecology of soil microbiota may determine the kind and role of secondary metabolites to be synthesized as a defensive response by host plant.

The development and wide-spread of aflatoxigenic fungal species, as well as host resistance and pathogenic interaction, are significantly influenced by soil microbial activity, which is greatly influenced by soil reaction status (Horn, 2003; Monda *et al.*, 2020; Wang *et al.*, 2023).

Particular mycotoxins bind strongly to soil organic carbon and clay minerals, thus, soil organic content and texture are essential (Kenngott *et al.*, 2022; Ncube and Maphosa, 2020; Schenzel *et al.*, 2012). Soil pH, soil cation exchange, and calcium concentration influence fungal diversity, hence management and maintenance of soil fertility would be among the sustainable Aflatoxin management options in the agricultural food systems. Thus, understanding the association between the aflatoxigenic fungal species including prevalence and composition of soils in maize growing agro-ecosystems is important for devising Aflatoxins contamination mitigation strategies which is currently less understood among researchers and other stakeholders in the maize agro-ecosystems. Furthermore, soil parameters have a more or less systematic pattern to warrant identify confidently population status targeted by the present study. Therefore, the main objective of this study was to investigate the correlation between soil physio-chemical characteristics and the prevalence of Aflatoxin-producing fungal species and growth in maize agroecosystems; eventually to understand relationships between soil health and Aflatoxin contamination in maize cropping systems in Tanzania. Understanding this correlation is critical because aflatoxin contamination poses significant risks to food security, human health, and economic stability in Tanzania. The findings of this study could inform soil management practices that reduce aflatoxin contamination, thereby improving crop yields and food safety.

Materials and methods

Description of the study area

The study was conducted in three regions of Tanzania with varying climatic conditions namely Manyara (4°18′54″S 36°57′14.76″E), Dodoma (6° 9' 40.2624" S and 35° 44' 43.5336" E), and Tabora (5° 1' 49.6596" S and 32° 49' 9.9516" E) (Fig. 2) which are main maize producers and reported to have high risk of Aflatoxin contaminations. The three regions form a semi-arid northern and central zone of Tanzania due to low and erratic rainfall. The climate of Dodoma is characterized by subtropical climate with warmer period and a cooler period due to altitude and season variations, annual rainfall amounts to 605 mm and temperature. The study area had a temperature ranging 17.7 to 29.3°C (https://www.climatestotravel.com/climate/tanzania/dodoma). Manyara has temperate highland tropical

climate with dry winter's climate with annual rainfall amounting to 88.19 mm and temperature of 25 to 32°C (https://weatherandclimate.com/tanzania/manyara), while Tabora is characterized by tropical savanna climate with average temperature of 24.20C and annual rainfall amounts 115.4 mm (https://weatherandclimate.com/tanzania/tabora), thus the three regions in the study area represented a wide range of climate variation in maize producing areas.

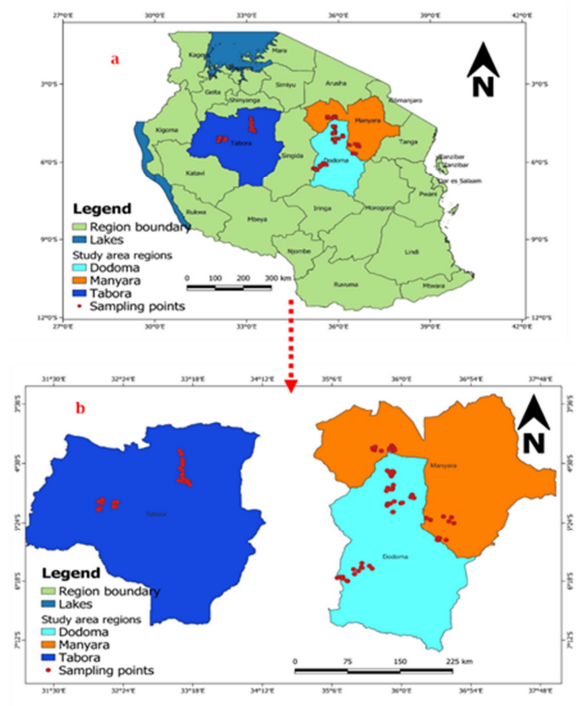


Fig. 2. The study area with sampling points where maize and soil samples were collected for the present study during 2022 to 2023

Study design and sampling methods

A purposive sampling method was adopted based on the history of aflatoxicosis, and a cross-sectional study design was used to select districts for field soil and maize sample collection, which were defined as maize fields that had been planted with maize in the previous season. Fields that had not planted maize in the previous season were not included. Various cropping regimes (intercropping and monoculture) were considered during sample collections.

Soil collection and sample preparation

Soil samples were collected from 7 maize-growing districts councils namely, Nzega DC and Urambo DC (Tabora), Bahi DC, Chemba DC, and Kondoa DC (Dodoma), Babati DC and Kiteto DC (Manyara) (Fig. 2) during 2022 cropping season. A total of 126 soil samples were collected from 21 villages, 18 soil samples per district and the distance between one samples field to another was at least 5 km apart. During soil sampling, each sample was taken from nine (9) spots in the field from the topsoil surface (0-30 cm depth) using hand held Auger in an X style (The diagonal sampling techniques) (Kumar *et al.*, 2008). Five points from the center of each sampling point in the perimeter of 5m were sampled and composited to make one soil sample (quartering method). Approximately 300 grams of soil were collected, packed in a brown paper plastic zipper bags and transported to the Soil Science Laboratory of Tanzania Agricultural Research Institute (TARI) – Uyole Center for air drying and further analysis.

Soil fertility biochemical and physical analysis

Soil samples were air dried, ground, and sieved (2 mm sieve) to determine the content of macronutrients (Nitrogen (N), Phosphorus (P), and Potassium, (K)) using a spectrophotometer, and micronutrients (Calcium (Ca), Sulfur (S), Magnesium (Mg), zinc (Zn), Boron (B), and iron, (Fe)) using a method described by Mehlich (1984). This involved diluting the soil by two dilution factors for testing phosphorus and nitrogen, and three dilution factors for testing potassium. The soil mixtures were filtered using Whatman filter paper to obtain clear filtrates to be further analyzed for Total nitrogen (N) using the Kjedal digestion-distillation method. The Bray and Kurtz method was used to determine the P concentration and K using flame photometer method. Soil texture and particle size distribution were determined by hydrometer method. Organic Carbon (OC) was determined by oxidation method as described by Walkley (1980). Soil pH was determined by suspension method.

Isolation of Aflatoxigenic fungal species

Aflatoxin-producing fungal species were isolated from the sampled soils. The serial dilution and direct plating method was used to dilute the soil sample

(Cetinbas *et al.*, 2018), aimed at minimizing the fungi load in the soil in each dilution. The soil mixture was diluted 7 times and labelled as 10-1 until 10-7. In a sterile dilution 50 mL Falcon tube, 1 g of soil was suspended in 10 mL of sterile distilled water and mixed for 10 minutes on a Shaker. Then 10 mL of the solution was transferred to the first vial by using a pipette. Afterward, other 9 mL of the solution from the first vials was transferred to the second vial and the steps continued until the last vial. About 0.1 mL of the solution in each vial was pipetted into the prepared plates with Potato Dextrose Agar growth media (PDA) mL supplemented with a detergent (triton-X) of at 10−4 and 10−5 dilutions. The solution was spread on the plate using a hockey stick and incubated in the dark for 5-7 days at room temperature (28˚C) (Odhiambo and Wagara, 2013). Each colony of fungi grown on the plate after incubation periods was then sub-cultured in a new plate and incubated again at room temperature for 7 days to produce pure fungal culture. Number of Colony Forming Unit (CFU) was determined by equations 1 and 2. For maize kernels, five kernels were selected randomly for each maize sample, and plated on growth media, incubated for 5 days, then single culture were isolated and sub-cultured to obtain pure culture before identification.

CFU/g=(Number of Colonies × Total dillution factor)/(Volume of culture plated in mL) (1)

Whereby:

Dillution Factor=(Final volume (diluent volume+Stock volume)Vf)/(Volume of the stock transfered (Vi)) (2)

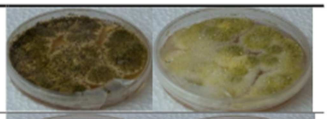
CFU = Colony Forming Unit.

The repairs of pure fungal culture was done by sub-culturing each of the different colonies onto another new PDA plate for macro-morphology identification

Identification of aflatoxin-producing fungi species

The morphological identification of aflatoxin-producing fungal species was conducted after 10 days of inoculation and the colonies were further observed phenotypically by observing macro-morphological characteristics of the fungal colonies that included colony development, color, texture, size, conidia appearance, and reverse color on petri-dishes (Okayo *et al.*, 2020). *Aspergillus flavus* was identified by greenish to yellowish colony color, black color for *A. niger*, Pale brown to dark brown for *A. terreus*, Yellowish to brownish as *A. parasticus* and White to cream color as *A. nomius* (Table 1).

Table 1. Phenotypic macro-morphology of different *Aspergillus* section Flavi isolated from soil samples in the solid potato dextrose agar (PDA) growing Media in three regions of Tanzania during cropping season 2022/ 2023

Aspergillus species	Phenotypic colour descriptions for colonies	Macro-morphology
Aspergillus flavus	Greenish to yellowish	
Aspergillus parasticus	Yellowish to brownish	
Aspergillus niger	Black	
Aspergillus terreus	Pale brown to dark brown	
Aspergillus nomius	White to cream	

Statistical data analysis

The collected data were analyzed by various statistical packages based on soil parameters and fungal population in the study districts. Collected data were analyzed using Jamovi 2.3.2.1, Gen Start 2014, 15th Edition and DATAtab Statistics Calculator Team (2024) (https://datatab.netsoftware). Descriptive statistics were performed to analyze data generated from the study site to determine mean values and compared to critical and recommended values to assess the status of soil fertility and occurrence of aflatoxin-producing species. To determine the difference of studied parameters across maize growing districts and cropping patterns, one-way ANOVA and Fisher's Unprotected least significance tests were conducted at a 5% level of significance ($P<0.05$).

Results and discussion

Soil texture and soil particle size distribution

Based on the present study, there is a significant difference ($P<0.05$) in soil particle distribution (Clay, Silt, and Sand) across studied districts. The results exposed that sandy loam soil texture was dominant across districts with sand proportion of 71.3 % (Nzega), 68.7 % (Chemba), 67.2 % (Urambo), 65.5 % (Bahi), and 64.6 % (Kondoa) (Fig. 3). These results indicated the low proportions of clay particles and silt as well. The observed particle size distribution might be due to interaction of parent materials, weathering, and climate variability. The particle size distribution affects soil properties such as porosity, water retention, and aeration (Kalonga *et al.*, 2024). Soil particle size distribution affects aflatoxin-producing fungi by influencing soil properties, water availability, and nutrient dynamics (Fouché, 2020). Aflatoxin-producing fungi, especially *Aspergillus flavus* and *A. parasiticus*, thrive in specific soil conditions (Cetinbas *et al.*, 2018; Gugnani, 2003; Horn, 2003; V. Kumar *et al.*, 2008). Thus, sandy soils drain quickly, potentially leading to drought stress and favoring Aflatoxin production by the fungal pathogen as a defensive mechanism against drought stress. Consequently, soil texture affects nutrient retention and availability for fungal growth and development. Aflatoxins can undergo transformation and degradation in the soil due to microbial activity, which are influenced also by soil particles distribution by playing a role in binding and releasing of these secondary metabolites (toxins).

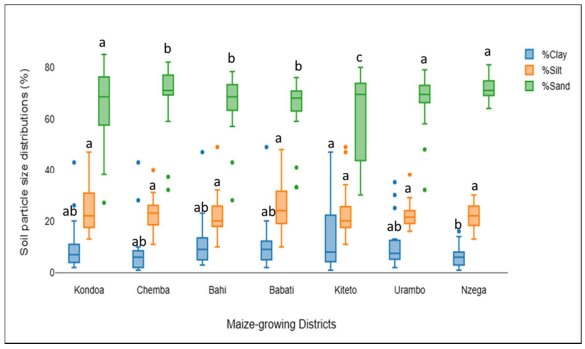


Fig. 3. Box plots showing soil particles distribution among studied maize growing districts in Tanzania during cropping season 2022/2023

Soil chemical properties

The soil pH regulates the biological, chemical, and physical characteristics of the soil, hence may play an important role in influencing the growth and activity of aflatoxin-producing fungal species. This affects the nutrients availability for growth and output, Aflatoxins contamination particularly *Aspergillus flavus* and *A. parasticus* from Section Flavi thrive across a wide pH range, but their optimal pH range lies between 3 and 7 (Horn, 2003; Kumar *et al.*, 2021; Ncube and Maphosa, 2020). Slightly higher pH around neutral (6-7) promote both fungal growth and development and subsequently Aflatoxins production. Acidic soils (low pH around 3) would limit the growth of aflatoxin-producing fungal species, however these conditions reduce the availability of essential nutrients like Phosphorus (P) within the soils as well, such conditions would create unfavorable environment for a wide range of crops growth and development including fungal species. Therefore a balanced pH would support both fungal colonization and subsequent Aflatoxins production. In the current study there was highly significant difference at ($P\leq0.001$) (Fig. 4a), where pH ranged from 5.8 to 6.9. Therefore monitoring pH levels in the maize cropping systems without compromising the nutrient availability would be paramount sustainable option to

mitigate Aflatoxin contaminations risks at pre harvest stages.

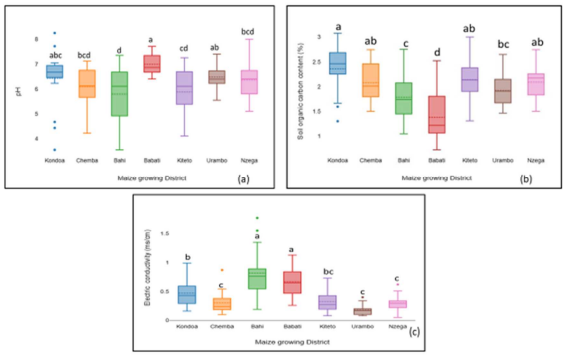


Fig. 4. Box plots showing different soil chemical fertility components for the studied districts (a) soil pH, (b) soil organic carbon (%), (c) Electric conductivity (ms/cm)

Soil organic carbon (SOC)

Plays an important role in the physical, chemical, and biological components of the soils. In the present study, SOC was significantly different (P≤0.001) among studied districts (Fig. 4b). The SOC influences the occurrence and behavior of aflatoxin-producing fungal species and subsequent Aflatoxin production (Pitt and Hocking, 2013; Schenzel *et al.*, 2012). Carbon sources are essential for fungal growth and metabolism. The optimum level of SOC for fungal growth ranges 3 – 7 % (Bulta, 2017). However the studied districts had SOC ranging 1.38 – 2.36 % (Fig. 4b) which is low. The SOC varied among studied districts such as Kondoa (2.36%), Kiteto (2.14%), Nzega (2.09%), Chemba (2.08 %), Urambo (1.92%), Bahi (1.78 %), and Babati (1.38 %). The low SOC levels influence on poor soil moisture retention, hence drought stress and limited nutrient availability which enhance aflatoxin production as stress outflow mechanism; while high SOC levels would mitigate drought effects and avail nutrients, that will encourage non toxigenic fungal species to thrive and increase, the condition that will outcompete the aflatoxigenic fungal species available in such soil, thus reducing Aflatoxin risk (Khan *et al.*, 2021; Kinyungu *et al.*, 2019; Nji and Babalola, 2023; Peles *et al.*, 2021). The increasing SOC in agricultural fields would enhance fungal growth but could also promote microbial competition against Aflatoxin producers, while decreasing SOC would reduce fungal activity but could inhabit toxigenic fungal strains. Therefore balancing SOC is essential for managing Aflatoxin risks in soils in the maize production ecosystems (Wang *et al.*, 2023).

Soil electrical conductivity (EC)

Is important in shaping the interactions between aflatoxin-producing fungal species and the maize ecosystems since it measures the salt content in the soil (Salinity). Soil ion concentration, an index that reflects the water-soluble salt content in the soil, which directly impacts nutrient availability hence growth and occurrence of fungal species. The EC influences the activity of soil microbes involved in essential processes, including the release of greenhouse gases like nitrogen oxides, methane, and carbon dioxide (Wang *et al.*, 2022). Some microbes within the soil produce the greenhouse gases while decomposing organic matter in soil. The optimal EC value for plant growth and development, usually falls between 0.8 and 1.8 µs/cm, not exceed 2.5 (Fouché, 2020). Based on the present study (Fig. 4c), the soil EC was highly significant different (P≤0.001) across all studied districts. However, the EC recorded within range of (0.18 to 0.82). Low soil EC would hinder Aflatoxin production by affecting fungal growth and development, however, further research is needed to fully understand its impact on Aflatoxin contaminations in the soils. The EC values among studied districts were lower in Urambo (0.18) and higher in Nzega district (0.3) which also indicated low fungal species (Fig. 4c).

Total Nitrogen, Phosphorus, Sulphur and Boron

The present study showed highly significant difference (P≤0.001) in Total Nitrogen (TN), Sulphur (S) and Boron (B) across the studied districts, except phosphorus (P) was not significantly different among studied districts. The TN concentration among districts ranged between 0.06 mg/kg – 0.1 mg/k which were very low compared to the recommended values (15 – 30 mg/kg) (Bulta, 2017). The low TN indicates poor soil fertility (Fig. 5a). The N availability

in the soil affects fungal growth and metabolism. Studies reports that N in nitrite and nitrate forms increase Aflatoxin production levels especially for *A. flavus* and *A. parasticus* (Kumar *et al.*, 2021), thus monitoring N level s in the agricultural soils would sustainably reduce pathogen prevalence and subsequently aflatoxins production.

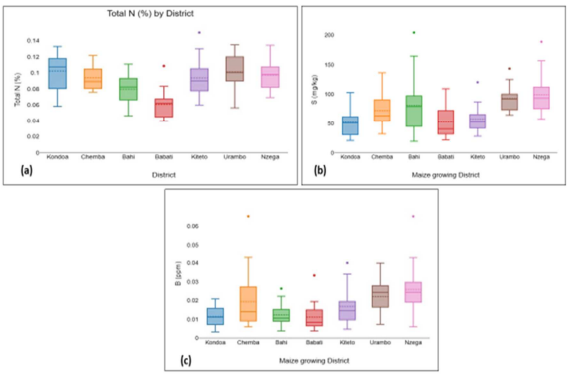


Fig. 5. Box plots showing a concentration of soil nutrients across studied maize growing districts in Tanzania during season ….(a) Total Nitrogen (N) %, (b) Sulphur(mg/kg) (S) concentration (ppm), and Boron (B) (ppm)

The concentration of TN and P were observed to have negative correlation with number of fungal isolates obtained from studied soil samples. However, the TN and P concentration had positive correlation with number of maize kernels vs fungal growth (Table 2). The results suggest that soils with unbalanced TN and P are likely to influence more fungal infestations in maize field compared to soils with balanced levels of TN and P.

Sulphur (S) and Boron (B) play essential roles in soil health and could also indirectly influence the occurrence of aflatoxin-producing fungal species. Sulphur (S) availability in the soil, would affects soil pH, microbial activity, and nutrient cycling (Kalonga *et al.*, 2024). However, low Sulphur (S) levels could lead to soil alkalinity, the condition that favor the growth of aflatoxin-producing fungi. Equally, adequate Sulphur (S) promotes a balanced soil ecosystem, potentially reducing the prevalence of *A. flavus* and *A. parasticus* species. Based on the current study, the amount of Sulphur (S) in the studied districts ranged from 51.8 – 99.2 ppm whereby Nzega district had highest content (99.2 ppm), followed by Urambo (92.9 ppm) which was high compared to optimum range of around 10 to 20 parts per million (ppm) in the soil for most crops (Bulta, 2017). Therefore, maintaining optimal Sulphur (S) and Boron (B) levels in the soil would promote plant health, maize in particular, but also would indirectly influence the occurrence of aflatoxin-producing fungi.

Table 1. Simple Person linier correlation of TN and P on the number of kernels showing fungal growth and number of isolates determined from soil samples in seven districts of Tanzania during cropping season 2022/2023

	Total N (%)	P(mg/kg)	No. of Kernels showed fungal growth	No. of isolates grown
Total N (%)	1	0.04	0.07	-0.05
P(mg/kg)	0.04	1	0.05	-0.1
No. of Kernels showed fungal growth	0.07	0.05	1	0.24
No. of isolates grown	-0.05	-0.1	0.24	1

Correlation between soil parameters and aflatoxin-producing fungal abundance

The present study determined soil nutrients to have significant influence on the number of fungal isolates determined from soil samples (Table 3). Moreover, the soil nutrients had influence on the number of kernels which depicted positive growth of fungal producing aflatoxins. The study findings indicate that soil nutrients may play a significant role in influencing both the number of fungal isolates detected in soil samples (Table 3) and the occurrence in kernels with positive fungal growth, specifically aflatoxin-producing fungal isolated from the soil. The correlation coefficients and P-

values as in Table 3 show the relation of soil nutrients and fungal species. For instance, the negative correlation with K (Cmol/kg) suggests that higher potassium levels may be associated with fewer fungal isolates.

Correlation between aflatoxin-producing fungi and maize kernels

Additionally, the present study reveals that soil nutrients may impact the occurrence of kernels with positive fungal growth, specifically those producing aflatoxin. Total N (%) had correlation coefficient of 0.07, which is a non-significant weak positive correlation (P=0.427), while K (Cmol/kg) portrayed a stronger negative correlation with a coefficient of -0.35. The p-value is <0.001, indicating statistically significant correlation. Soil health (with balanced physical and biochemical parameters) and nutrient availability are complex interactions, consequently multiple factors may contribute to fungal growth and eventually Aflatoxin production. However, further research and detailed analyses are needed to understand the precise mechanisms that might be involved.

Table 3. Pearson simple linier correlations between soil nutrients and aflatoxin-producing fungal isolates abundance from seven districts in Tanzania during cropping season 2022/2023

		Kernels with fungal growth	No. of isolates grown
Total N (%)	Correlation	0.07	-0.05
	p	0.427ns	0.554ns
P(mg/kg)	Correlation	0.05	-0.1
	p	0.605ns	0.279ns
S (mg/kg)	Correlation	-0.02	0.13
	p	0.862ns	0.136ns
K (Cmol/kg)	Correlation	-0.35	-0.17
	p	<.001***	0.054ns
Mg (Cmol/kg)	Correlation	-0.18	0.16
	p	0.046**	0.067ns
Ca (Cmol/kg)	Correlation	-0.06	0
	p	0.511ns	0.959ns
Fe (ppm)	Correlation	-0.11	0.09
	p	0.237ns	0.319ns
Mn (ppm)	Correlation	0.03	0.02
	p	0.707ns	0.826ns
Zn (ppm)	Correlation	-0.01	-0.02
	p	0.934ns	0.807ns
B (ppm)	Correlation	0.01	0.05
	p	0.919ns	0.604ns

Table 4. Mean Number of isolates obtained from soil samples in six districts of Tanzania during cropping season 2022/2023

Region	District	No. of soil samples	No. of colonies counted	Mean ± Sd
Dodoma	Kondoa	18	178	9.89 ± 9.07
	Chemba	18	252	14 ± 12.93
	Bahi	18	380	21.11 ± 14.6
Manyara	Babati	18	726	40.33 ± 42.8
	Kiteto	18	379	21.06 ±20.62
Tabora	Urambo	18	486	27 ± 25.65
	Nzega	18	177	9.83 ± 6.65

Occurrence of Aspergillus species isolated from soil samples

The present study (Table 4) showed that Babati district in Manyara region had a high number of colonies 40.3, followed by Urambo district in Tabora region. Chemba and Kondoa districts in Dodoma and Nzega district in Tabora displayed lower mean number of colonies 14, 9.9, and 9.8 respectively.

Results in Table 5 showed that *Aspergillus flavus* had high number of isolates from the soils among studied districts. These isolates were assigned

description colour greenish to yellowish and with a prevalence of 38.1%. Next was *A. parasticus* 28(22.2%), whereas *A. terreus* had lowest occurrences from soil (Table 5). These results are in hand with findings obtained by (Boni *et al.*, 2021; Kibwana *et al.*, 2023; Mfaume, 2019). The dominance of *A. flavus* might be due to wide range of adaptability compared to other species, thus more investigation and verification on its abundance in soil would generate enough information and eventually recommendations in management and mitigation strategies.

Table 5. Prevalence of *Aspergillus* spp isolated from soil and maize kernels samples (n=126) collected from six districts in Tanzania during cropping season 2022/2023.

Aspergillus spp	No. of isolates	Isolates colour	Prevalence (%)
Aspergillus flavus	48	Greenish to yellowish	38.1
Aspergillus parasticus	28	Yellowish to brownish	22.2
Aspergillus niger	21	Black	16.7
Aspergillus nomius	18	White to yellowish	14.3
Aspergillus terreus	11	Pale yellow to dark brown	8.7
Total	126		100

Table 6. Prevalence (%) of *Aspergillus* spp isolated from maize kernels and soil samples from six districts of Tanzania during cropping season 2022/2023

Aspergillus species	Soil	Maize kernels
Aspergillus flavus	36	22
Aspergillus parasticus	19	13
Aspergillus niger	13	7
Aspergillus nomius	11	7
Aspergillus terreus	7	3

Furthermore, it was determined that the number of *Aspergillus* species from section Flavi isolated from soil samples among studied regions and districts varied significantly ($P<0.05$) (Table 6). The study found that soil samples had high number of *Aspergillus* colonies, where the *A. flavus* had the leading proportion compared to other species of the Genera isolated from soil and maize samples. These findings are in support of results obtained from various studies as reported by (Cardwell and Cotty, 2002; Musleh and Al-ouqaili, 2018; Dhlamini, 2014; Odhiambo and Wagara, 2013; Okun *et al.*, 2015). From the present study this shows that source of inoculums were more from soil than maize crop, thus soil management would be an option to control the prevalence of the fungal pathogens and subsequent aflatoxin production within maize agro-ecosystems.

Conclusion

This study has highlighted that *Aspergillus species* are abundant in the soil. There was significance positive and negative correlation among studied soil parameters with number of *Aspergillus* isolates obtained, total N, S, K, OC, P, pH, and EC respectively. Soil fertility content was diverse among studied districts; therefore influenced the occurrence of aflatoxigenic fungal species. These findings suggest the need for the community of practice and policy makers to emphasize the use of fertilizers (inorganic and organic) for soil amendments. More fertilizer subsidies especially for N, P and S based fertilizers are recommended. Nevertheless, the study offered significant knowledge on the correlation between the wide range of aflatoxin-producing pathogens and soil nutrients concentration thereby contribute to understanding of the complex interactions between soil properties, fungal ecology, and aflatoxin contamination at pre harvest cropping stages.

Acknowledgments

The author wishes to thank Maize farmers, and Agricultural Extension officers in the study districts for their field support during soil and mazie sample collection. All collaborators at the Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania, Department of Crop Science and Horticulture, College of Agricultural Sciences and Technology, Mbeya University of Science and Technology, Mbeya, Tanzania and Tanzania Agricultural Research Institute, Kilosa, Morogoro, Tanzania for their technical and guidance support during the development of this research article.

References

Abbas HK, Wilkinson JR, Zablotowicz RM, Accinelli C, Abel CA, Bruns HA, Weaver MA. 2009. Ecology of *Aspergillus flavus*, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. Toxin Reviews **28**(2–3), 142–153. https://doi.org/10.1080/15569540903081590

Academic W. 2021. Aflatoxin contamination in Tanzania: quantifying the problem in maize and groundnuts from rural households. World Mycotoxin Journal **14**(4), 553–564. https://doi.org/10.3920/WMJ2020.2646

Agape K, Ndesendo VMK, Begum S. 2021. Screening of aflatoxin-producing fungi in maize and groundnuts from three regions in Tanzania. **47**(2), 609–615.

Benkerroum N. 2020. Aflatoxins: Producing-molds, structure, health issues, and incidence in Southeast Asian and Sub-Saharan African countries. International Journal of Environmental Research and Public Health **17**(4). https://doi.org/10.3390/ijerph17041215

Boni SB, Beed F, Kimanya ME, Koyano E, Mponda O, Mamiro D, Kaoneka B, Bandyopadhyay R, Korie S, Mahuku G. 2021. Aflatoxin contamination in Tanzania: quantifying the problem in maize and groundnuts from rural households. World Mycotoxin Journal **14**(4), 553–564. https://doi.org/10.3920/WMJ2020.2646

Buckner CA, Lafrenie RM, Dénommée JA, Caswell JM, Want DA, Gan GG, Leong YC, Bee PC, Chin E, Teh AKH, Picco S, Villegas L, Tonelli F, Merlo M, Rigau J, Diaz D, Masuelli M, Korrapati S, Kurra P, Mathijssen RHJ. 2016. Advanced biometric technologies and liveness detection in biometrics. IntechOpen. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics

Bulta AL. 2017. Assessment and mapping of status and spatial distribution of soil macronutrients in Kambata Tembaro. September 2016. https://doi.org/10.15406/apar.2016.04.00144

Cardwell KF, Cotty PJ. 2002. Distribution of *Aspergillus* section *Flavi* among field soils from the four agroecological zones of the Republic of Bénin, West Africa. Plant Disease **86**(4), 434–439. https://doi.org/10.1094/PDIS.2002.86.4.434

Cetinbas FC, Ahluwalia RK, Polymer P, Membrane E, Kulikovsky A, Smiley KT. 2018. Isolation and identification of soil fungi isolates from forest soil for flooded soil recovery. https://doi.org/10.1088/1757-899X/342/1/012028

Cotty PJ, Mellon JE. 2006. Ecology of aflatoxin-producing fungi and biocontrol of aflatoxin contamination. Mycotoxin Research **22**(2), 110–117. https://doi.org/10.1007/BF02956774

Dangwa N, Dhlamini Z, S A. 2014. Molecular characterization of aflatoxigenic *Aspergillus* species in dried traditional foods in Zimbabwe. Advances in Bioresearch **5**(1), 29–36. https://doi.org/10.15515/abr.0976-4585.5.29-36

Elias NKS. 2016. Aflatoxins: A silent threat in developing countries. African Journal of Biotechnology **15**(35), 1864–1870. https://doi.org/10.5897/ajb2016.15305

Falade T. 2019. Aflatoxin management strategies in Sub-Saharan Africa. Mycotoxins - Impact and Management Strategies. https://doi.org/10.5772/intechopen.78784

Fouché T, Claassens S, Maboeta M. 2020. Aflatoxins in the soil ecosystem: an overview of its occurrence, fate, effects, and future perspectives. Mycotoxin Research **36**(3), 303–309. https://doi.org/10.1007/s12550-020-00393-w

Fouché T. 2020. Aflatoxins in the soil ecosystem: an overview of its occurrence, fate, effects, and future perspectives.

Frederick B, Sabula L, Mruma S, Mzee F, Mtoka E, Masigo J, Ndunguru A, Swai E. 2020. Maize production manual for smallholder farmers in Tanzania. International Institute of Tropical Agriculture **32**.

Frisvad JC, Hubka V, Ezekiel CN, Hong SB, Nováková A, Chen AJ, Arzanlou M, Larsen TO, Sklenář F, Mahakarnchanakul W, Samson RA, Houbraken J. 2019. Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins, and other mycotoxins. Studies in Mycology **93**, 1–63. https://doi.org/10.1016/j.simyco.2018.06.001

Garber RK, Cotty PJ. 1997. Formation of sclerotia and aflatoxins in developing cotton bolls infected by the S strain of *Aspergillus flavus* and potential for biocontrol with an atoxigenic strain. Phytopathology. **87**(9), 940–945. https://doi.org/10.1094/PHYTO.1997.87.9.940

Gbashi S, Madala NE, De Saeger S, De Boevre M, Adekoya I, Adebo OA, Njobeh PB. 2019. The socio-economic impact of mycotoxin contamination in Africa. Mycotoxins - Impact and Management Strategies 3–22. https://doi.org/10.5772/intechopen.79328

Geiser DM, Klich MA, Frisvad JC, Peterson SW, Varga J, Samson RA. 2007. The current status of species recognition and identification in *Aspergillus*. Studies in Mycology **59**, 1–10. https://doi.org/10.3114/sim.2007.59.01

Githang'a D, Anzala O, Mutegi C, Agweyu A. 2019. The effects of exposures to mycotoxins on immunity in children: A systematic review. Current Problems in Pediatric and Adolescent Health Care **49**(5), 109–116. https://doi.org/10.1016/j.cppeds.2019.04.004

Gong YY, Watson S, Routledge MN. 2016. Aflatoxin exposure and associated human health effects, a review of epidemiological studies. Food Safety **4**(1), 14–27. https://doi.org/10.14252/foodsafetyfscj.2015026

Gugnani HC. 2003. Ecology and taxonomy of pathogenic aspergilli. Frontiers in Bioscience. **8**(SUPPL.). https://doi.org/10.2741/1002

Horn BW. 2003. Ecology and population biology of aflatoxigenic fungi in soil. Journal of Toxicology - Toxin Reviews **22**(2–3), 351–379. https://doi.org/10.1081/TXR-120024098

Jaime-Garcia R, Cotty PJ. 2004. *Aspergillus flavus* in soils and corncobs in South Texas: Implications for management of aflatoxins in corn-cotton rotations. Plant Disease **88**(12), 1366–1371. https://doi.org/10.1094/PDIS.2004.88.12.1366

Kalonga J, Mtei K, Massawe B, Kimaro A, Winowiecki A. 2024. Characterization of soil health and nutrient content status across the Environmental Challenges **14**(January), 100847. https://doi.org/10.1016/j.envc.2024.100847

Kamei K, Watanabe A. 2005. *Aspergillus* mycotoxins and their effect on the host. Medical Mycology **43**(SUPPL.1), 95–99. https://doi.org/10.1080/13693780500051547

Kenngott KGJ, Albert J, Meyer-wolfarth F, Schaumann GE. 2022. *Fusarium* mycotoxins in maize field soils: Method validation and implications for sampling strategy. 1–21.

Khan R, Ghazali FM, Mahyudin NA, Samsudin NIP. 2021. Biocontrol of aflatoxins using non-aflatoxigenic *Aspergillus flavus*: A literature review. Journal of Fungi **7**(5). https://doi.org/10.3390/jof7050381

Kibwana M, Kimbokota F, Christopher R, Mmongoyo JA. 2023. Aflatoxins in stored maize, maize flours, and stiff porridge consumed in schools: A case study of Dodoma region, Tanzania. Food Control **146**, 109519. https://doi.org/10.1016/J.FOODCONT.2022.109519

Kimanya ME, Routledge MN, Mpolya E, Ezekiel CN, Shirima CP, Gong YY. 2021. Estimating the risk of aflatoxin-induced liver cancer in Tanzania based on biomarker data. PLoS ONE. **16**(3 March), 1–11. https://doi.org/10.1371/journal.pone.0247281

Kinyenje E, Kishimba R, Mohamed M, Mwafulango A, Eliakimu E, Kwesigabo G. 2023. Aflatoxicosis outbreak and its associated factors in Kiteto, Chemba and Kondoa Districts, Tanzania. PLOS Global Public Health **3**(8), e0002191. https://doi.org/10.1371/journal.pgph.0002191

Kinyungu S, Isakeit T, Ojiambo PS, Woloshuk CP. 2019. Spread of *Aspergillus flavus* and aflatoxin accumulation in postharvested maize treated with biocontrol products. Journal of Stored Products Research **84**, 101519. https://doi.org/10.1016/j.jspr.2019.101519

Klich MA. 2007. *Aspergillus flavus*: the major producer of aflatoxin. **8**, 713–722. https://doi.org/10.1111/J.1364-3703.2007.00436.X

Kumar A, Pathak H, Bhadauria S. 2021. Aflatoxin contamination in food crops: causes, detection, and management: a review.

Kumar V, Vyas U, Singh D. 2008. Dynamics of soil population of *Aspergillus flavus* and aflatoxin contamination in groundnut-based production system in Gujarat. January.

Liu Y, Chang CCH, Marsh GM, Wu F. 2012. Population attributable risk of aflatoxin-related liver cancer: Systematic review and meta-analysis. European Journal of Cancer **48**(14), 2125–2136. https://doi.org/10.1016/j.ejca.2012.02.009

Mahuku G, Mauro A, Pallangyo B, Nsami E, Boni SB, Koyano E, Mponda O, Ortega-Beltran A, Atehnkeng J, Aquiline F, Samuel R, Njela J, Cotty PJ, Bandyopadhyay R. 2023. Atoxigenic-based technology for biocontrol of aflatoxin in maize and groundnuts for Tanzania. World Mycotoxin Journal. **16**(1), 59–73. https://doi.org/10.3920/wmj2021.2758

Mahuku G, Nzioki HS, Mutegi C, Kanampiu F, Narrod C, Makumbi D. 2019. Pre-harvest management is a critical practice for minimizing aflatoxin contamination of maize. Food Control **96**(June 2018), 219–226. https://doi.org/10.1016/j.foodcont.2018.08.032

Makhuvele R, Naidu K, Gbashi S, Thipe VC, Adebo OA, Njobeh PB. 2020. The use of plant extracts and their phytochemicals for control of toxigenic fungi and mycotoxins. Heliyon **6**(10), e05291. https://doi.org/10.1016/j.heliyon.2020.e05291

Manoza FS, Mushongi AA, Harvey J, Wainaina J, Wanjuki I, Ngeno R, Darnell R, Gnonlonfin BGJ, Massomo SMS. 2017. Potential of using host plant resistance, nitrogen, and phosphorus fertilizers for reduction of *Aspergillus flavus* colonization and aflatoxin accumulation in maize in Tanzania. Crop Protection **93**, 98–105. https://doi.org/10.1016/j.cropro.2016.11.021

Massomo SMS. 2020. *Aspergillus flavus* and aflatoxin contamination in the maize value chain and what needs to be done in Tanzania. Scientific African **10**, e00606. https://doi.org/10.1016/j.sciaf.2020.e00606

Mfaume J. 2019. Managing aflatoxin–producing fungi using indigenous atoxigenic strains of *Aspergillus* species in groundnut in Mtwara region, Tanzania.

Monda E, Masanga J, Alakonya A. 2020. Variation in occurrence and aflatoxigenicity of *Aspergillus flavus* from two climatically varied regions in Kenya. Toxins **12**(1). https://doi.org/10.3390/toxins12010034

Mtega M, Mgina CA, Kaale E, Sempombe J, Kilulya KF. 2020. Occurrence of aflatoxins in maize and maize products from selected locations of Tanzania and the effects of cooking preparation processes on toxin levels. Tanzania Journal of Science **2**(46), 407–418.

Musleh MH, Al-ouqaili MTS. 2018. Isolation of *Aspergillus flavus* from some clinical and environmental sources by HPLC and PCR techniques. May.

Ncube J, Maphosa M. 2020. Current state of knowledge on groundnut aflatoxins and their management from a plant breeding perspective: Lessons for Africa. Scientific African **7**, e00264. https://doi.org/10.1016/j.sciaf.2020.e00264

Negash D. 2018. A review of aflatoxin: occurrence, prevention, and gaps in both food and feed safety. Journal of Nutritional Health and Food Engineering **8**(2), 190–197. https://doi.org/10.15406/jnhfe.2018.08.00268

Nji QN, Babalola OO, Mwanza M. 2022. Aflatoxins in maize: Can their occurrence be effectively managed in Africa in the face of climate change and food insecurity? Toxins **14**(8). https://doi.org/10.3390/toxins14080574

Nji QN, Babalola OO. 2023. Soil *Aspergillus* species, pathogenicity and control perspectives. 1–16.

Odhiambo BO, Wagara IN. 2013. Isolation and characterization of aflatoxigenic *Aspergillus* species from maize and soil samples from selected counties of Kenya. African Journal of Microbiology Research **7**(34), 4379–4388. https://doi.org/10.5897/AJMR2013.5846

Okayo RO, Andika DO, Dida MM, K'otuto GO, Gichimu BM. 2020. Morphological and molecular characterization of toxigenic *Aspergillus flavus* from groundnut kernels in Kenya. International Journal of Microbiology. https://doi.org/10.1155/2020/8854718

Okun DO, Khamis FM, Muluvi GM, Ngeranwa JJ, Ombura FO, Yongo MO, Kenya EU. 2015. Distribution of indigenous strains of atoxigenic and toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* in maize and peanuts agro-ecological zones of Kenya. Agriculture and Food Security **4**(1), 1–10. https://doi.org/10.1186/s40066-015-0033-5

Peles F, Sipos P, Kovács S, Győri Z, Pócsi I, Pusztahelyi T. 2021. Biological control and mitigation of aflatoxin contamination in commodities. Toxins **13**(2), 1–19. https://doi.org/10.3390/toxins13020104

Pitt JI, Hocking AD. 2013. Fungi and Food Spoilage. Springer **53**(9).

Schenzel J, Forrer H, Vogelgsang S, Hungerbu K, Bucheli TD. 2012. Mycotoxins in the environment: I. Production and emission from an agricultural test field.

Shabeer S, Asad S, Jamal A, Ali A. 2022. Aflatoxin contamination, its impact, and management strategies: An updated review. Toxins **14**(5), 1–24. https://doi.org/10.3390/toxins14050307

Tai B, Chang J, Liu Y, Xing F. 2020. Recent progress of the effect of environmental factors on *Aspergillus flavus* growth and aflatoxins production on foods. Food Quality and Safety **4**(1), 21–28. https://doi.org/10.1093/fqsafe/fyz040

Tola M, Kebede B. 2016. Occurrence, importance, and control of mycotoxins: A review. Cogent Food and Agriculture **2**(1). https://doi.org/10.1080/23311932.2016.1191103

Wang H, Zhao R, Zhao D, Liu S, Fu J, Zhang Y, Dai N, Song D, Ding H. 2022. Microbial-mediated emissions of greenhouse gas from farmland soils: A review. Processes **10**(11), 1–14. https://doi.org/10.3390/pr10112361

Wang X, Wang D, Zhang S, Zhu M, Yang Q, Dong J, Zhang Q, Feng P. 2023. Research progress related to aflatoxin contamination and prevention and control of soils. Toxins **15**(8). https://doi.org/10.3390/toxins15080475

Wilson RT, Lewis J. 2017. The maize value chain in Tanzania. FAO 14–36.