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Diversity and Environmental Specificity of Fungal Isolates from Soils and Cereal Grains in Western Kenya

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ABSTRACT

Background and Objective: Mycotoxin contamination in maize and groundnuts has undermined Kenya's health care system for decades. This study aimed to determine the diversity of mycotoxin fungi in maize, groundnut and soils of Western Kenya and farmers' awareness of on-field mycotoxin mitigation measures. **Materials and Methods:** Infected maize, groundnut and soil samples were collected from Homa Bay, Migori, Siaya and Busia Counties. Semi-structured questionnaires were used to collect socio-economic data on mycotoxin awareness and mitigation measures used by farmers. Pure fungal isolates were obtained for diversity assessment on PDA and incubated at 25-27°C. A light microscope at ×400 magnification was used for the morphological identification of spores and mycelia, while species were identified using plant pathology reference books and journals. **Results:** Thirty-five diverse fungal isolates were obtained from all samples. The genus *Aspergillus* was the highest in terms of isolation frequency, with 14 diverse pathotypes, followed by *Penicillium* (8 isolates), *Fusarium* (4 isolates) and the rest (9 isolates). Busia County had the most diverse number of isolates, while Siaya had the least. Among sample categories, most fungi were obtained from soil samples (30 isolates) while maize and groundnuts recorded 19 and 9 isolates respectively. While 22 isolates were specific to counties, 13 were environmentally non-specific. Farmers in Western Kenya had partial knowledge of best pre- and post-harvest mycotoxin mitigation practices. **Conclusion:** Mycotoxin-producing fungi were the most dominant fungi in maize, groundnut and soils of Busia, Siaya, Homa Bay and Migori counties of Western Kenya. These fungal species were highly specific to the environments.

KEYWORDS

Mycotoxin, adulteration, specificity, *Zea mays*, *Arachis hypogea*

INTRODUCTION

Mycotoxins contaminate various food substances and agricultural products worldwide, posing serious health risks to humans and animals[1,2]. The presence of mycotoxin in significant quantities may induce acute to chronic health effects such as endocrine disruption in humans and animals[3]. Human exposure

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to mycotoxins may result from consumption of plant-derived foods with toxin adulteration. Additionally, exposure may be instigated by metabolites in animal products such as meat and eggs or exposure to air and dust-containing toxins[4]. Over 65% of the Kenyan population is exposed to chronic levels of mycotoxin poisoning[5-6]. Common mycotoxin-producing fungi, such as *Aspergillus flavus* species, thrive well in substrates of grains such as maize and groundnuts which are the most widely consumed crops[7-9]. Maize-groundnut intercrops are very popular in the Western region of Kenya[10]. This is because groundnut crop is known to fix large amounts of nitrogen and is considered an ideal option for rotation and intercropping with maize fields to boost yields[11-13]. The cereal-legume intercrop system is highly practiced by farmers as a mean of diversification to mitigate climate change and for managing striga weed menace which is a major constraint to crop productivity in this region.

However, a bulk of farmers, producers and consumers have little knowledge of best farming practices against mycotoxin accumulation in grain/field or the experience in dealing with mycotoxin contamination in foodstuff and grain[14-16]. Therefore, people in these regions are at a high risk of aflatoxin contamination. Inappropriate pre-and post-harvest procedures augment colonisation by mycotoxin-producing fungi of field crops and post-harvest produce[17-20]. Inadequate drying and improper storage coupled with high temperature and humidity levels are essential contributing factors to the accumulation of mycotoxins up to unacceptable levels in developing countries[21-23]. The risk is further aggravated as these farmers chiefly sell their surplus through informal markets which are highly unregulated[5,24]. Such farmers' practices are risk factors for mycotoxin contamination. Enhancing farmer, producer and consumer knowledge on mycotoxin mitigation measures right from the early stages of production to storage is highly desired as a means to mitigate the problem of mycotoxin contamination. However, sufficient farmers' knowledge on causes and sustainable management of these mycotoxin fungi on soils, maize and groundnuts in Western Kenya is still lacking several studies conducted in Western Kenya such as those of Were *et al*.[25] and Lewis *et al*.[26] in Homa bay and Bungoma counties detected the presence of aflatoxin in these regions. Others such as Lewis *et al*.[26] and Nelson *et al*.[27] determined aflatoxin levels in sample grains. Additionally, information on the occurrence, diversity and farmer knowledge of mycotoxin fungi in Busia County among others is still inadequate. This implies that the diversity of mycotoxin-producing fungi and non-toxigenic species in the region is a critical research gap and a vital step in identifying potential bio-control agents. Therefore, this study was established to assess the diversity of fungi in maize and groundnuts in Western Kenya and the levels of awareness of the causes and mitigations of aflatoxins in maize and groundnut grains among smallholders in Western Kenya.

MATERIALS AND METHODS

Study location and environmental characteristics: The study duration was from September 2019 to April 2022. Surveys were conducted in three sub-counties (sub-regions) of four counties of Western Kenya namely Busia, Siaya, Homa Bay and Migori. The four study regions were selected on the basis that maize and groundnuts are among the major food crops grown by the majority of farmers there. The four sampling sites have different agroecological conditions, ambient temperatures and annual rainfall. The climatic data of these four regions is unique and favours mycotoxin fungi establishment and growth (Table 1).

Sample collection, sample size determination and sampling technique: The study areas were grouped into clusters based on regional boundaries[32]. Sub-counties were selected randomly from each cluster to represent a sampling hub. Three significant towns serving sub-county headquarters within the four counties were selected as sampling hubs. The sampling sites include villages representing different AEZs within a 20 km radius of the sampling hub. The GPS-detecting applications and administrative boundaries were used for site location and mapping. Two hundred and fifty grams each of maize, groundnut grains (from the previous season) and soil samples were collected from selected farmers with a long history of

Table 1: Weather details of sampling sites of Western Kenya

Country	Annual average temperature (°C)	Annual rainfall (mm)	Highest humidity (%)	Source
Busia	21.8	2291	82.59-May	Atsiaya *et al.*[28]
Siaya	21.4	2154	82.74-April	Maina *et al.*[29]
Homa Bay	21.7	1331	71.5-May	Ogenga *et al.*[30]
Migori	21.0	1522	76.88-April	Olela *et al.*[31]

maize-groundnut intercropping systems. The process generated information on the incidence and prevalence of aflatoxin under different farming practices. During the sample collection, a total of 46 maize and groundnut farmers, 11 (Busia), 5 (Siaya), 15 (Homabay) and 15 (Migori), were selected and interviewed on grain storage methods and level of awareness on mycotoxin fungi with reference to commonly recommended mitigation measures using a semi-structured questionnaire[33]. Data on farmer level of awareness and commonly practiced mitigation measures in the region were subjected to analysis using descriptive statistics and presented in bar graphs with standard error bars using Microsoft Excel 365.

Isolation and culture purification of grain and soil fungi: Two hundred and fifty grams of grain (maize and groundnut) and soil samples which were aseptically collected from each farmer as described in section 2.2 above were used for fungal extraction. Isolation and purification of the mycotoxin fungi from diseased groundnut, maize seed and soil was done according to Salano *et al.*[34] and Owino *et al.*[35]. Five grains per sample were surface sterilised in 1% sodium hypochlorite (NaOCl) for 2 min, rinsed thrice in sterile distilled water, then plated on sterile PDA media and incubated at 27°C in the dark. After 5 days, a single conidium representing each collection was transferred to a freshly prepared PDA and set at similar conditions. Sub-cultures were made from the emerging colonies and pure cultures were obtained for subsequent studies[36]. Soil samples were passed through a 2 mm mesh sieve to remove crop residues and 1 gram of the sample was suspended in 9 mL sterile distilled water. The suspension was serially diluted to 10^{-3} and a 200 µL aliquot of the dilution was uniformly spread in duplicates on PDA[37]. The plates were incubated for five days, after which colonies of *Aspergillus* and other fungi were counted and the number of Colony Forming Units per gram (CFU/g) of soil was calculated according to Okayo *et al.*[38]:

$$\text{Soil (CFU / g)} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

Fungal diversity assessment and identification: Mycotoxin fungal isolates from all regions of the study were subjected to morphological descriptive assays and identification was done using various pathology reference journals such as[39-43]. Additionally, the assessment of diversity was based on observations using a biological light microscope (model MOMxianweijing2933 from Beijing, China) at ×400 magnification as well as in the petri-dishes to classify and identify isolates with respect to spore shape and mycelial growth characteristics such as mycelial colour, (front and reverse side colour) and texture. The Isolates were also grouped based on agro-ecological zones (counties) of isolation to document those specific to certain regions and those found across all the regions considered in the study.

RESULTS

Morphological diversity and identification of soil and grain fungi in Western Kenya: Diversity assessment and identification assay recorded a total of 35 diverse fungal isolates from groundnut grain, maize grain and soil samples across the four counties of Western Kenya. Out of the 35 diverse isolates, the genus *Aspergillus* was the highest in terms of frequency with 14 diverse pathotypes, followed by the genus *Penicillium* (8 isolates) and *Fusarium* (4 isolates), respectively (Table 2 and 3). Also, even within a fungal species, significant morphological differences were observed physically in plates and at the microscopy level in terms of mycelial colour, substrate colour and spore shape. While Busia County had the highest number of diverse isolates in all sample types, Siaya County had the lowest number of isolates.

Table 2: Occurrence and isolation frequency of environmental/region-specific fungal isolates

	Counties in Western Kenya												
	Busia			Siaya			Homa Bay			Migori			
Identified and unidentified fungus (region and sample specific)	M	G	S	M	G	S	M	G	S	M	G	S	Frequency of isolation
MMT3 (Unidentified)	+	-	-	-	-	-	-	-	-	-	-	-	1
Aspergillus terreus (MCBT6)	-	-	+	-	-	-	-	-	-	-	-	-	1
Aspergillus tubingensis	-	-	+	-	-	-	-	-	-	-	-	-	1
Aspergillus candidus	-	-	+	-	-	-	-	-	-	-	-	-	1
Monascus species	-	-	+	-	-	-	-	-	-	-	-	-	1
MCMT4a (Unidentified)	-	-	+	-	-	-	-	-	-	-	-	-	1
MCMT3 (Unidentified)	-	-	+	-	-	-	-	-	-	-	-	-	1
MCMBT3 (Unidentified)	-	-	+	-	-	-	-	-	-	-	-	-	1
Arthrinium sacchari	-	-	-	-	-	+	-	-	-	-	-	-	1
Coniothyrium olivaceum	-	-	-	-	-	+	-	-	-	-	-	-	1
Epichloe species	-	-	-	-	-	+	-	-	-	-	-	-	1
Aspergillus terreus (MGW1)	-	-	-	-	-	-	+	-	-	-	-	-	1
Aspergillus flavipes	-	-	-	-	-	-	+	-	-	-	-	-	1
Aspergillus tamarii	-	-	-	-	-	-	-	+	-	-	-	-	1
MCHB2 (Unidentified)	-	-	-	-	-	-	-	-	+	-	-	-	1
MCRN1 (Unidentified)	-	-	-	-	-	-	-	-	+	-	-	-	1
Aspergillus species (MSE2)	-	-	-	-	-	-	-	-	-	+	+	+	3
Aspergillus parasiticus	-	-	-	-	-	-	-	-	-	+	-	-	1
Aspergillus oryzae	-	-	-	-	-	-	-	-	-	-	-	+	1
Penicillium species (MCSW3)	-	-	-	-	-	-	-	-	-	-	-	+	1
Phialemoniopsis endophytica	-	-	-	-	-	-	-	-	-	-	-	+	1
Aspergillus species (MCSW1)	-	-	-	-	-	-	-	-	-	-	-	+	1
Number of fungi per sample	1	0	7	0	0	3	2	1	2	2	1	5	24
Number of fungi per region (Country)		8			3			5			8		

M: Maize grain, G: Groundnut grain and S: Soil sample

Table 3: Occurrence and isolation frequency of environmental non-specific fungal isolates

	Counties in Western Kenya												
	Busia			Siaya			Homa Bay			Migori			
Isolate identity	M	G	S	M	G	S	M	G	S	M	G	S	Frequency of isolation
Aspergillus flavus (MUG5)	+	-	-	+	-	+	+	-	-	+	-	-	5
Aspergillus niger	+	+	+	+	-	+	-	-	+	+	-	+	8
Penicillium aurantiogriseum	+	+	-	-	+	-	-	+	+	-	+	-	6
Penicillium species (MMT2)	+	+	+	-	-	-	-	-	-	-	+	-	4
Fusarium oxysporum (MGW5)	+	-	+	+	+	-	+	-	-	-	-	-	5
Fusarium proliferatum	+	-	-	-	-	+	-	+	-	-	-	-	3
Aspergillus nomius	-	-	+	-	-	-	-	-	+	-	-	-	2
Aspergillus flavus (GMT3)	-	+	-	-	+	-	-	+	-	-	-	-	3
Fusarium species (MCBT1)	-	+	+	-	-	+	-	-	+	-	-	+	5
Penicillium chrysogenum	-	-	+	-	-	-	-	+	-	-	-	+	3
MCMT4b (Unidentified)	-	-	-	-	-	+	-	-	+	-	-	-	2
Trichoderma harzianum	-	-	-	-	-	-	+	-	-	-	-	+	2
Biatriospora species	-	-	-	-	-	-	-	-	+	-	-	+	2
Number of fungi per sample	6	5	6	3	3	5	3	4	6	2	2	5	50
Number of fungi per region (Country)		17			11			13			9		

M: Maize grain, G: Groundnut grain and S: Soil sample

Aspergillus flavus (MUG5) was the most common isolate in maize samples, as it was detected in all sample regions. This isolate, however, was not detected in groundnut samples. *Fusarium oxysporum* (MGW5) and *Aspergillus niger* (MBT2) were detected in three out of four counties. *Aspergillus flavus* (GMT3) was the

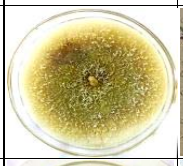
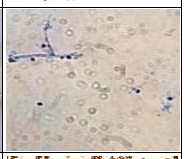
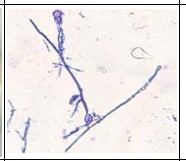
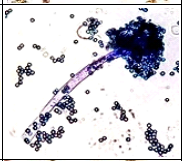
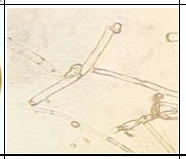
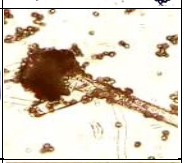
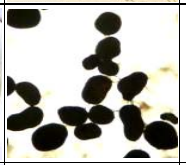
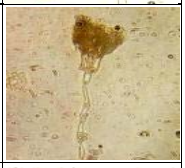
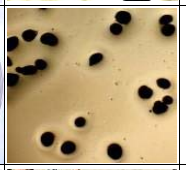
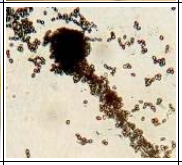
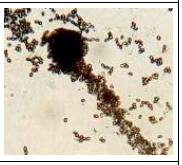
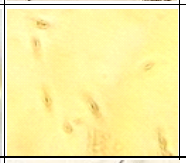
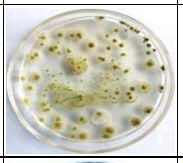
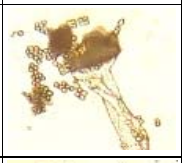
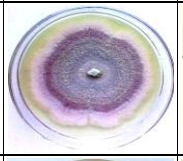
No.	Identification	Mycelial morphology and colour	Spore morphology (×400 magnification)	No.	Identification	Mycelial morphology and colour	Spore morphology (×400 magnification)
1	MMT3 **(**Unidentified**)**			12	*Coniothyrium olivaceum*[50]		
2	*Aspergillus terreus* **(**MCBT6**)**[44]			13	*Epichloe* species		
3	*Aspergillus Tubingensis*[45]			14	*Aspergillus terreus* (MGW1)[44]		
4	*Aspergillus Candidus*[46]			15	*Aspergillus flavipes*[51]		
5	*Monascus* species[47]			16	*Aspergillus Tamarii*[52]		
6	MCMT4a (Unidentified)			17	*Penicillium* species (MCSW3)[53]		
7	MCMT3 (Unidentified)			18	MCRN1 (Unidentified)		
8	*Aspergillus* species[48]			19	*Aspergillus parasiticus*[54]		
9	MCHB2 (Unidentified)			20	*Aspergillus oryzae*[55]		
10	MCMBT3 (Unidentified)			21	*Phialemoniopsis endophytica*[56]		
11	*Arthinium sacchari*[49]			22	*Aspergillus* species (MCSW1)[57]		

Fig. 1: Environmental specific diverse fungal isolates

most common isolate among groundnut samples and was detected in Busia, Siaya and Homa Bay counties. Alternately, *Aspergillus niger* (MBT2) was the most common isolate in soil samples, as was detected in all counties of the sample regions (Table 2). Thirty-one isolates were characterised to the generic level, while four isolates remained unidentified.

Environmental specificity and non-specificity of fungal isolates in Western Kenya: Generally, the majority of the fungal species isolated from maize, groundnut and soil samples were specific to the environments in which the samples were collected (Fig. 1). Twenty-two diverse fungal isolates were specific to one region/county (Fig. 1). Among the isolates, 10 were identified as *Aspergillus* species, with only one isolate from the *Penicillium* genus. Most of these isolates were isolated from Busia County samples (Table 2). However, out of the 9, Busia County-specific isolates, 8 were isolated from soil samples, one isolate obtained from maize samples and none detected in groundnuts. Siaya County recorded the least number of unique isolates (3) detected in soil samples only. Additionally, out of the 22 region-specific isolates, only four were specific to maize samples, while only one was specific to groundnut samples (Table 2).

On the other hand, thirteen diverse fungal isolates were non-specific to their environments (Fig. 2). Among these isolates, 12 were detected in Homa Bay County and 10 in Busia County, while Siaya and Migori

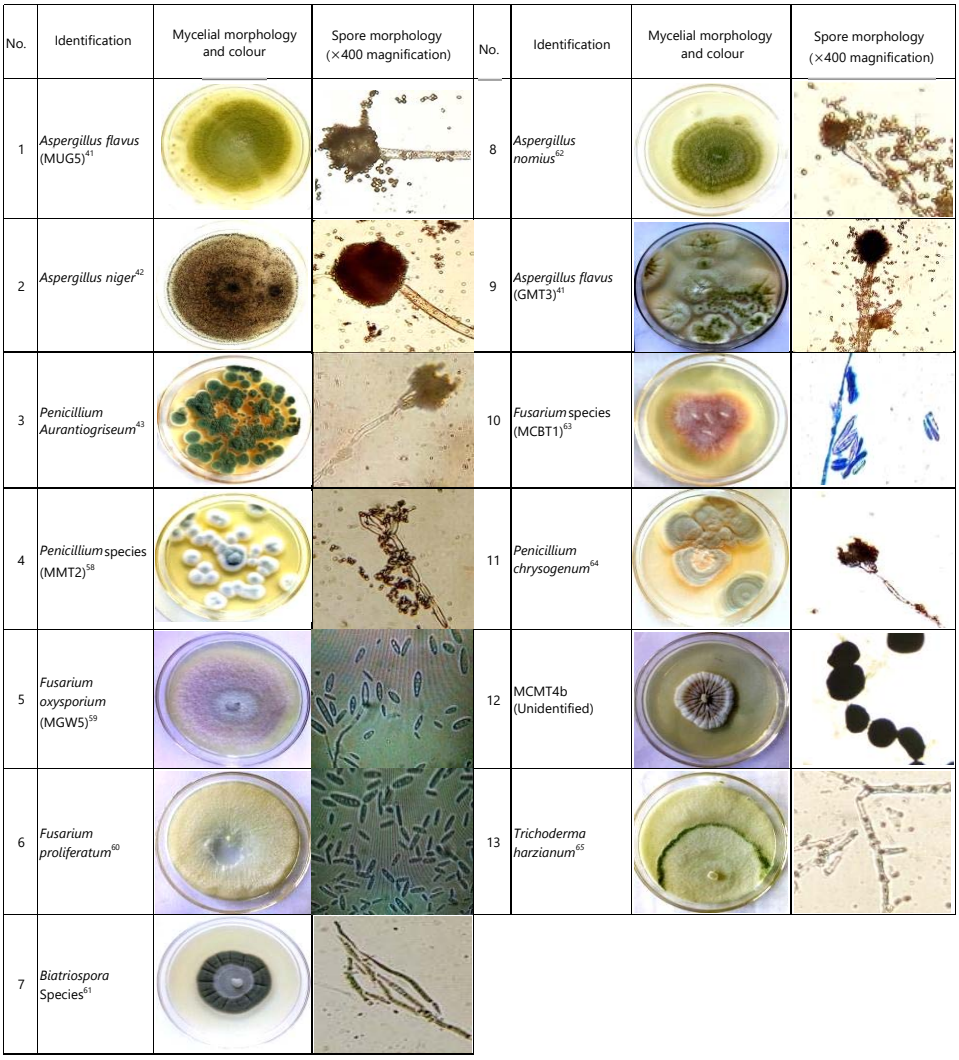


Fig. 2: Environmental non-specific diverse fungal isolates

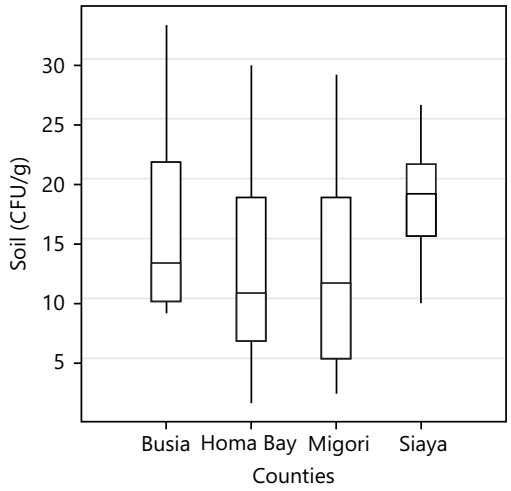


Fig. 3: Colony forming units per unit gram of fungal isolates in soil samples from Busia, Homa Bay, Migori and Siaya Counties

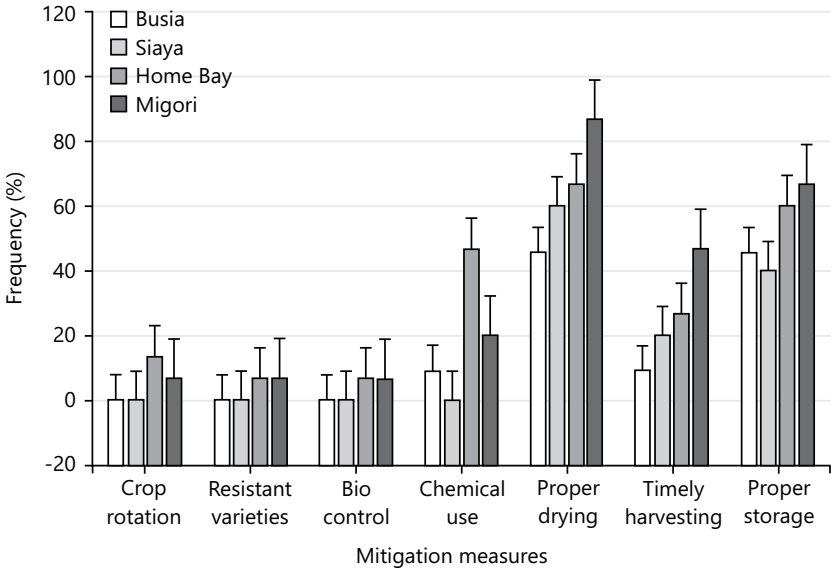


Fig. 4: Response frequencies of farmer awareness on mitigation measures against mycotoxin accumulation in groundnut and maize

counties recorded 8 diverse fungal isolates each (Table 3). However, in terms of isolation frequency, these isolates were most frequently detected in Busia County followed by Homa Bay County and lastly in Migori County.

Colony Forming Units (CFUs) of soil-inhabiting fungal species in Western Kenya: At the county level, there were no significant differences in CFU/g between Busia, Homa Bay, Migori and Siaya Counties. In terms of variation from one CFU to the other, Busia, Homa Bay and Migori were the highest. However, Siaya had the least variation in terms of CFU, though with the highest mean above 17 CFU/g. Additionally, with respect to sample distribution (whiskers), Busia County recorded the highest number of colony-forming units per unit gram (>30 CFU/g), while Homa Bay had the lowest (<5 CFU/g). Also, Siaya had the highest mean, while Homa Bay County had the lowest mean of CFU/g in soil samples (Fig. 3).

Farmer awareness levels on mitigation of mycotoxins: Farmer assessments on mycotoxin management revealed low awareness levels on mitigation measures. Proper drying and proper storage of grains were the most known mitigation measures against mycotoxin accumulation as they recorded at least 40%

awareness levels across the four counties. However, other mitigation measures such as the use of resistant varieties, use of chemicals and crop rotation scored less than 20% across the four counties (Fig. 4).

DISCUSSION

Fungal diversity and environmental specificity: The fungal diversity assessment study revealed that the soils of Western Kenya are enriched with a comprehensive genetic bank of soil and seed-inhabiting fungi. Therefore, mycotoxin accumulation remains a major threat to safe maize and groundnut products in western Kenya. This finding was mainly because the most predominant fungal isolates from the collected samples belonged to the three major mycotoxin-producing genera: *Aspergillus*, *Penicillium* and *Fusarium*[66]. The great diversity displayed in mycotoxin and infectious fungal species isolated portrays their abundance and possible widespread distribution through evolution[36,67]. For example, *Aspergillus flavus* was the most frequently detected fungus and occurred in two different pathotypes implying that the fungus has a great evolutionary potential. In addition to confirming its ubiquitous nature, the findings may also imply that mycotoxin accumulation will remain a challenge in the region for decades if not adequately alleviated[68-70]. High diversity in mycotoxin-producing fungi is an imminent management threat. However, such diversity also sets the stage towards the identification and development of biological mycotoxin control measures where non-toxigenic strains are identified and used.

In addition to diversity within a genus, inter-genera diversity was the highest. The presence of many fungal isolates from other genera insinuates the possibility of identifying other bio-control agents against mycotoxin accumulation in grains and soils. *Aspergillus flavus* (MUG5) was detected in both maize and soil samples. This finding implies that the fungus is both a soil and seed-borne pathogen. These findings compare well with those of Monda *et al.*[71] who isolated *Aspergillus flavus* from soil samples obtained from maize fields and suggested that the most effective way of managing this pathogen is by reducing its accumulation in the soil environment as well as on grain. Further analysis of fungal diversity disclosed that the majority of fungal isolates were obtained from the soil samples (Table 2 and 3). By comparing the counties, the magnitude of fungal diversity in soil samples positively correlated with diversity levels in maize and grain samples (Table 3). For example, Busia County also had the most diverse isolates of all the sample types. However, this scenario was more evident in mycotoxin fungi than in the rest of the isolates. This could be associated with the nature of their spores. For example, *Aspergillus* and *Penicillium* species produce many air-borne spores and hence are transmitted directly from the soil to seed in the field[72,73].

Besides soils, maize grains were more infected by fungal isolates obtained in the study than groundnuts. This occurrence could be due to a pathogen or race specificity. The division of *Forma specialis* of these mycotoxin fungi into different races explains why fungi with similar morphologies may have dissimilar infection rates on host plants[74]. However, this study cannot fully ascertain this phenomenon because the fungal isolates were not positively identified to the race level. Also, the grains assessed were only samples from the study region; hence the study is bound by the limitation of the sampling process.

For sample-specificity, 22 out of the total 35 fungal isolates were environmentally specific. This finding was expected due to the eminent environmental differences known to exist among the four counties. Further, the occurrence and prevalence of toxigenic and non-toxigenic fungi is highly dependent on the environmental conditions[75]. Busia County had the most diverse number of isolates, while Siaya County had the least diverse number of isolates (Table 2 and 3) probably due to variations in awareness levels on mitigation of fungal accumulation and variations in environmental conditions[76]. Respondents from Busia County were least aware of post-harvest mitigation measures against the accumulation of aflatoxin fungi. Such unawareness is a gateway to practices such as improper drying of grains providing sufficient moisture for fungal growth and proliferation and this may contribute to the diversity and abundance of fungi[77].

Farmer awareness levels and mycotoxin fungal diversity: Farmers in the sampled regions had little knowledge of mitigation measures against mycotoxin accumulation in grains (Fig. 4). These findings add tremendously to the scientific body of knowledge by revealing the current status of aflatoxin awareness levels in Western Kenya regions a subject that has been scarcely documented[78-81]. This knowledge has a ripple effect on fungal diversity because farmers are unlikely to deliberately use pre-and post-harvest practices that inhibit mycotoxin accumulation. For example, a common storage practice such as the use of gunny bags favors moisture build-up promoting fungal growth when placed on floors[21,82,83]. Low awareness levels on mycotoxin mitigation encourage practices that expose the grains to conditions that favors penetration and multiplication of mycotoxin-producing fungi[84-86]. Farmers are also unlikely to consistently practice programs such as crop rotation which reduce inoculum load and insect pest infestations[87]. Understanding these aspects by farmers is likely to reduce the inoculum load of most toxigenic fungi. Risk exposure indicators include knowledge of the causes of aflatoxin contamination and possible mitigations[5,6]. This knowledge is also mostly shared in farmer discussion forums. However, such initiatives are not practiced consistently in Western Kenya.

Implications, applications, recommendations and limitations of the study: The study findings are vital since they prompt further ground-truthing on the status of mycotoxin risk and vacuity of sustainable solutions in Western Kenya. The occurrence nature of identified fungal species in terms of diversity and environmental specificity is valuable information that can guide relevant geographical scope for identified bio-controls. Critical limitations of this study include the small geographical scope and number of plant species assessed. Additionally, molecular species identification was not conducted. Therefore, genetic chractersization of fungal species identified and *in-vitro* studies to identify species with the capacity to suppress mycotoxin fungi in Western Kenya is recommended.

CONCLUSION AND RECOMMENDATIONS

The study findings demonstrated that *Aspergillus* species are the most dominant fungi in maize, groundnut and soils of Busia, Siaya, Homa Bay and Migori Counties of Western Kenya. Although most fungal isolates in the region are sample and regional specific, mycotoxin fungi are non-specific to environments and samples. This study also revealed that farmers in Western Kenya have little awareness of appropriate mycotoxin mitigation measures. Therefore, there is a need for characterization and identification of mycotoxin-producing fungi in the region for sustainable management. Additionally, deliberate efforts need to be made to enhance information dissemination to farmers on on-farm mycotoxin management strategies.

SIGNIFICANCE STATEMENT

This study aimed to assess the diversity of mycotoxin fungi in maize, groundnut and soils in Western Kenya and farmers' awareness of mycotoxin mitigation measures. Thirty-five diverse pure cultures of fungal isolates were obtained from sampled cereal grains and soils of Western Kenya. The genus *Aspergillus* had the highest isolation frequency, with fourteen diverse pathotypes, followed by *Penicillium* (eight isolates), *Fusarium* (four isolates) and the rest (nine isolates). Busia County had the most diverse number of isolates, while Siaya had the least. While twenty two isolates were specific to counties, thirteen were environmentally non-specific. Farmers in Western Kenya had partial knowledge of best pre- and post-harvest mycotoxin mitigation practices.

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