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Distribution of indigenous strains of atoxigenic and toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* in maize and peanuts agro-ecological zones of Kenya

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Abstract

Background: Grains of important food and export crops in Africa are susceptible to contamination by toxin-producing moulds. Aflatoxins are mycotoxins associated with liver damage and cancer in humans and animals. These toxic substances are produced by fungi (such as *Aspergillus flavus* and *Aspergillus parasiticus*) in food and feed exposed to poor conditions during crop cultivation, storage or processing of harvest. The presence of aflatoxins in especially maize and peanuts in Kenya is of great concern. Recent developments in the application of atoxigenic strains of these fungi as biological control agents against toxigenic strains could be a solution to the problem. The objective of this study was to isolate, identify and characterize atoxigenic and toxigenic strains of *A. flavus* and *A. parasiticus* in Kenya, and investigate possible application of atoxigenic strains in control of aflatoxin levels in maize and peanuts. Fungal communities in soils of maize and peanut fields were examined to determine the distributions of aflatoxin-producing *Aspergillus* species and to identify endemic atoxigenic strains. 220 isolates belonging to *A. flavus* and *A. parasiticus* were collected randomly from soils of maize and peanuts fields in seven agro-ecological zones and characterized using morphological and physiological examination.

Results: *Aspergillus* section Flavi was detected in all the 57 soil samples collected in Kenya. Members of Aspergillus section Flavi L strain was the most common (54 %), followed by S-strains (35 %). Among *Aspergillus, A. flavus* was the most predominant (63.2 %), followed by *A. parasiticus* (27.7 %), *A. tamari* (5.5 %) and *A. nomius* (2.7 %). The mean CFU of the *Aspergillus* colonies per gram of soil was highly variable among the districts, ranging from 3.0×10^3 to 1.72×10^6 ($p < 0.05$). The mean pH across the collection sites also varied according to the respective agroecological zones (pH 5.5–6.8) which is within the optimal pH requirement for the members of section Flavi. There was no significant variation in temperature across the sampling sites ($p > 0.05$). The results also showed that *A. flavus* was detected in all the zones examined.

Conclusions: Each of the regions had atoxigenic strains of potential value which can be employed as biological control agents in the management of aflatoxicoses.

Keywords: Distribution, Atoxigenic, Toxigenic, *Aspergillus flavus*, *Aspergillus parasiticus*, Agroecological zones

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Background

The genus *Aspergillus*, a member of the phylum *Ascomycota*, includes over 185 known species. Members of *Aspergillus* section Flavi are characterized by their ability to produce flavine-derived secondary metabolites which include the aflatoxins. Aflatoxins are mycotoxins associated with hepatotoxicity, mutagenicity and carcinogenicity in humans and animals [1, 2]. Species capable of producing aflatoxins include *Aspergillus flavus*, *Aspergillus parasiticus*, and several less common taxa such as *Aspergillus nomius, Aspergillus tamarii*, *A. pseudotamarii*, *A. minisclerotigenes* and *A. bombycis* [3, 4]. Other *Aspergillus* outside of the section Flavi are also known to produce aflatoxins and species with this ability have been shown to be more diverse than previously thought [5].

Grains of important food and export crops in Africa, such as maize and peanuts, are susceptible to contamination with different toxin-producing moulds. Of particular importance are aflatoxins that pose serious health risks to humans and animals. In Kenya, the toll on human health from periodic outbreaks that record case fatality rates of 39 % [6] remain common. In addition, food grains contaminated with aflatoxin-producing fungi also lose their quality based on nutritional value, taste, smell, appearance, and are no longer suitable for consumption or sale. The major challenge is that the contaminated cereals such as maize may appear just like normal grains without any outward physical signs of fungal infection [7].

The earliest reported case of aflatoxicosis in Kenya was in 1977, where several dogs and poultry died in Nairobi, Coast and Rift Valley Provinces after feeding on poorly stored grains, while in 1978, samples of human and dog food (flour) were found to be contaminated with [8]. In 1984, twelve people died in Machakos district of Kenya after consuming aflatoxin contaminated maize, while in 1995, about 172 samples of maize analyzed were found to have aflatoxin. In 2004, there was an outbreak of acute aflatoxicosis in Kenya, one of the most severe episodes of human aflatoxin poisoning in history. A total of 317 cases were reported by July 2004, with a case fatality rate of 39 % [9, 10]. This epidemic resulted from ingestion of contaminated maize [11]. Other outbreaks occurred in 1981, 2001, 2004, 2005, 2006, 2007 and 2008 resulting in sickness, death and destruction of contaminated maize [8, 9, 11–13]. Maize from the affected areas contained as much as 4400 ng g^{-1} aflatoxin B1, which is 440 times greater than the 10 ng g^{-1} tolerance level set by the Kenya Bureau of Standards. Most of the aflatoxin poisoning outbreaks occurred in remote villages and, therefore, the number of people affected could have been higher than reported [11]. The aflatoxin contamination in maize has been associated with drought combined with high temperatures as well as insect injury [14].

Aspergillus flavus and the closely related subspecies *A. parasiticus* have a world-wide distribution and normally occur as saprophytes in soil and on many kinds of decaying organic matter. They readily infest several important crops such as corn, cottonseeds, peanuts and tree nuts. They are widely distributed with greater quantities of the fungus occurring in warm climates [4, 15].

Gene flow within *A. flavus* is primarily limited by a vegetative compatibility system [16, 17] that delineates the species into numerous genetic groups called Vegetative Compatibility Groups (VCGs). *A. flavus* VCGs clonal lineages [18] that exist in complex communities are composed of many VCGs. VCGs vary in many characteristics; including aflatoxin-producing ability. Populations of *A. flavus* in an individual agricultural field contain isolates of many VCGs [4, 19]. VCGs with aflatoxin-producing potential are known to vary less among isolates within a VCG than among isolates from different VCGs [18]. Communities resident in different fields, areas, and regions may vary widely in average aflatoxin-producing ability [20–22]. Isolates and VCGs that do not produce aflatoxin, called atoxigenic, are common within *A. flavus* communities [20–23]. Surveys of *A. flavus* isolates from various geographic regions have revealed differences in the proportions of isolates that produce low, medium, and high amounts of aflatoxins [5, 24, 25]. For instance, in Argentina [26] and Iran [27] less than 30 % of the *A. flavus* isolates were capable of producing aflatoxin whereas in Nigeria the number was much higher, exceeding 50 % [28]. In the southern USA most *A. flavus* isolates produce aflatoxin [24, 25]. In the USA, there are currently several atoxigenic strains of *A. flavus* used to reduce crop contamination.

Biological control strategies directed at utilizing non-aflatoxin (atoxigenic) strains to limit aflatoxin contamination on crops have been pursued for almost two decades [29]. These strategies seek to give atoxigenic strains a competitive edge and exclude their aflatoxin-producing relatives, therefore decreasing the potential for contamination in crops and the environment. Successful strategies have been accomplished by using native non-aflatoxin-producing strains such as *A. flavus* [30].

In this study, we sought to identify the natural occurence of different strains/species of *Aspergillus* and to identify the ecological prevalence of both aflatoxin-producing and non-producing strains. Identification of the proportion of non-aflatoxin-producing strains in natural environments is an important step towards estimating their applicability in the biological control of the more medically and economically important toxigenic strains in Kenya.

Methods

Field survey locations

Field surveys were done in 6 agro-ecological zones (AEZ) in Kenya where maize and peanut crops are widely cultivated. The study covered 5 districts Makueni (1.8°S, 37.62°E), Makindu (−1°17′31.437″, 36°49′19.005″N/E), Kwale (3°3′4°45′, 38°31′39°31′), Kibwezi (−2°25′00″S, 37°58′00″E) and Msambweni (4°29′29.8″S, 39°28′33.7″E) (currently counties) selected due to their historical association with frequent and serious outbreaks of aflatoxin contamination of farm produce. To assess fungal populations, soil samples were collected from 57 fields arbitrarily chosen within the different districts and agro-ecological zones on the condition that fields were radially separated by at least 5–20 km from each other. A field was equivalent to a small-holder subsistence farm.

Sample collection and preparation

Soil samples were collected and processed as described by Dorner et al. [31]. From each field, three to five scoops were randomly taken using a sterile trowel from the top 4–6 cm of soil. Samples were transported in sterile containers to the Kenyatta University laboratory where they were sorted, pooled per field, oven-dried at 40 °C for 5 days, and stored in sealed plastic bags at room temperature (22–26 °C) until use. Aliquots of soil for pH measurements were suspended in distilled H_2O (20 % soil, wt/vol).

Isolation and quantification of fungal species

For fungal isolation, soil samples were retrieved from storage, dried in a forced air oven (48–50 °C for 48 h), pulverized in plastic bags to remove clods and homogenized by hand-mixing.

Aspergillus section Flavi were isolated and quantified by the dilution plate technique on Modified Rose Bengal Agar (MRBA), [32]. In sterile 7 ml capacity polystyrene tubes, 1 g of soil was suspended in 3 ml of sterile water, mixed for 20 min on a rotary shaker, and plated on MRBA at appropriate dilutions. Dilution was to allow colony densities of less than 10 per plate. Plates were incubated in the dark for 3 days at 31 °C. Colonies of *Aspergillus* section Flavi were then identified by colony morphology. About 5–10 isolates per soil sample were transferred to 5/2 agar (5 % V-8 juice and 2 % agar, pH 5.2) and grown for 5 more days, unilluminated at 31 °C. Isolates were then classified on the basis of colony characteristics and conidial morphology at ×400 magnification. *Aspergillus* section Flavi colonies were identified by their characteristic growth pattern, retention of Rose Bengal within mycelia, and production of characteristic conidiophores after 3 days on MRBA. Isolates with abundant small sclerotia (average diameter <400 mm) were preliminarily classified as strain SBG [5] while isolates with smooth conidia and large sclerotia (average diameter over 400 mm) were classified as the L strains of *A. flavus* [33]. *A. tamarii, A. nomious* and *A. parasiticus* were initially identified by colony and spore morphology [3]. All preliminary identifications were confirmed by color reaction on AFPA (*A. flavus* and *A. parasiticus* agar) [34]. Quantities of *Aspergillus* section Flavi in soils were calculated as colony-forming units (CFU) per gram of soil. Several 3 mm plugs of sporulating culture were transferred to 4-dram vials containing 5 ml of sterile distilled H_2O. These conidial suspensions were maintained at 4 °C for further analysis.

Aflatoxin-production by the fungal isolates

To determine the relative frequency of toxigenic and atoxigenic strains of *Aspergillus* section Flavi strain distribution across the country, isolates were randomly selected from each of the collection sites in order to determine their aflatoxin-production capability and also to establish the strains that produce the greatest quantities of aflatoxins and the frequency of occurrence of non-aflatoxin producers in Kenya. A total of 57 isolates (both L and S strains) were fermented in Adye and Matales medium with 22.4 mM urea as the sole nitrogen source and adjusted to pH 4.7 prior to autoclaving [5]. Vials (15 ml containing 5 ml A&M) were seeded with approximately 2×10^3 conidia suspended in 100 ml water. After incubation at 32 °C in the dark for 5 days, the pH of the medium was measured, 3 ml acetone was added, and the contents mixed by inversion. Vials were allowed to set for 1 h to allow lysis of fungal cells and extraction of aflatoxins from mycelia and conidia. Subsequently, the mycelia was collected on Whatman No. 4 filter paper, dried in a forced air oven (48 °C, 3 days), and weighed to quantify fungal biomass. The filtrate was diluted appropriately, spotted alongside standards of aflatoxin B1, B2, G1 and G2 (BORATEC limited, Kenya), and separated on thin layer chromatography (TLC) plates (silica gel 60, 20 mm) with the development solvent being a mix of diethyl ether, methanol and water in a 96:3:1 ratio. Aflatoxin was quantified in situ on the TLC plates with a scanning densitometer (model cs-930, Shimadzu Scientific Instruments, Inc., Tokyo). Extracts that initially did not show detectable aflatoxins were diluted with an equal volume of water and extracted with 3 ml methylene chloride to concentrate any latent aflatoxin. Aflatoxins were partitioned into the methylene chloride fraction, from which they can be retrieved by drying and the residues dissolved in 100 ml of methylene chloride. The solution was then subjected to TLC as already described.

Data analysis

Analyses were done with SPSS version 15.0 for Windows (release 15.0.0, Vista Hotfix Applied) and Microsoft Excel 2007. Pearson correlation coefficients were generated to assess relationships of ecological and biological variables with $\alpha = 0.05$ and 0.01 for a 2-tailed t test. Analysis of variance was performed on all data with the general linear model (GLM), suitable for unbalanced data. The analyses for percentage values, CFU g^{-1}, and aflatoxin concentrations were preformed with data transformed, using the arcsine of the square roots, the natural logarithms (ln), and the logs (count +1), respectively. Sampling sites and the agroecological zones were treated as class variables.

Results

Distribution of *Aspergillus* section Flavi in Kenya

Aspergillus section Flavi was detected in all the soil samples from 57 fields situated within the AEZs studied. A total of 220 section Flavi colonies were successfully transferred from MRBA to 5/2 agar and subsequently identified by macroscopic, microscopic and growth characteristics in AFPA medium. Out of all the *Aspergillus* section Flavi isolates, *A. flavus* was predominant (63.8 % of section Flavi), followed by *A. parasiticus* (28 %), *A. tamarii* (5.6 %) and about 2.3 % of the isolates assigned to *A. nomius* (a recently discovered strain among the Section Flavi) as shown in Table 1.

The most common group (forming 85 % of the *Aspergillus* section Flavi isolated from the seven AEZs) were identified as L- strain with an average of 60.6 % in all the districts. The S- strains had a mean incidence of 39.4 %. Kwale district had the highest incidence of L strains (73 %) and the least of S-strain, while the reverse was true in Kibwezi district (L-strain being 48.5 % and S-strains being 51.5 %), (Table 1; Fig. 1).

The mean colony-forming units (CFU/g of soil) of the *Aspergillus* were extremely variable among the districts, ranging from 3.0×10^3 to 2.6×10^6. The CFU counts were significantly different between the districts. However, the CFU counts were not significantly different within the agroecological zones ($p > 0.05$), (Table 2).

There was a significant positive correlation (0.594, $p = 0.009$) between pH and the proportion of S strains in the sampled colonies. On the other hand, the correlation of pH and the percentage of L strains was negative (-0.594, $p = 0.005$). The proportions of L and S strains in the CFU yield was negatively correlated (Table 2).

The mean soil pH among the districts sampled ranged from 6.12 in Kwale (weakly acidic) to 7.19 (weakly alkaline) in Kibwezi district (Table 1).

Incidences of toxigenic and atoxigenic strains of aspergillus section Flavi in Kenyan districts

A total of 57 isolates, each representing the collection sites were randomly examined for their aflatoxin-production potential. Out of all the isolates, 45.9 % were positive for different aflatoxins and were categorized as toxigenic. On the other hand, 54.1 % showed no detectable aflatoxins in their TLC profiles (Fig. 2), hence they were classified as atoxigenic strains. Msambweni district had the lowest atoxigenic/toxigenic strain ratio (41.7:58.3 %) while Makindu district had the highest atoxigenic/toxigenic strain ratio (75:25 %) (Table 3; Fig. 1).

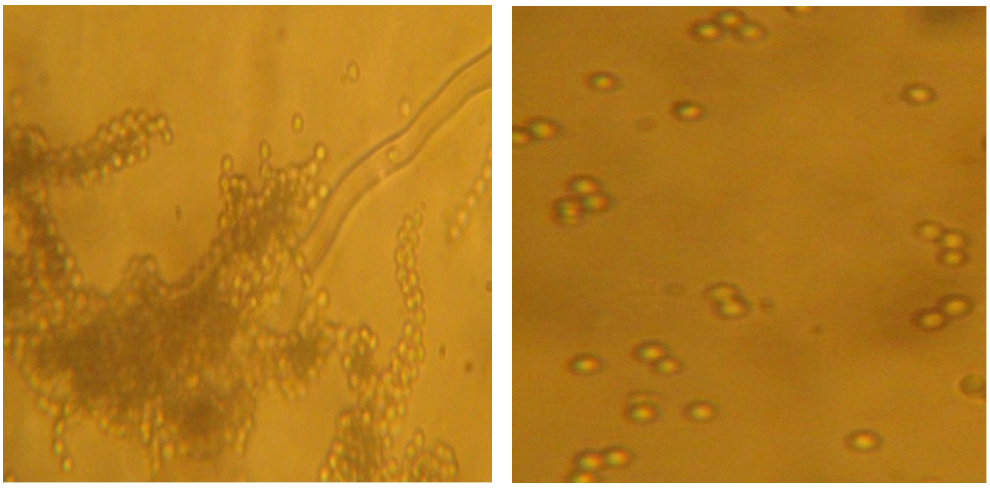
Table 1 Proportions of *Aspergillus* section Flavi of fields in five districts, soil pH, and colony-forming units per gram of soil in Kenya across the sampled agroecological zones

AEZ	District	Soil pH		CFU g^{-1} soil								
		Range	Mean	Range	Mean	No. of isolates[a]	% *A. flavus*[b]	% *A. parasiticu*[b]	% *A. tamarii*	% *A. nomius*	% L	% S
LM4/LM3/LM5	Kibwezi	6.5–7.41	7.19	5.0×10^4–1.41×10^6	3.54×10^5	26	75.7	21.5	2.8	0	48.5	51.5
CL3/L4	Kwale	5.6–6.6	6.12	4.6×10^4–2.6×10^6	6.692×10^5	38	60.6	31.3	8.1	0	73	27
LM3/LM4	Makindu	5.2–7.45	6.68	9.2×10^3–8.0×10^5	1.474167×10^5	44	49.5	34.2	9.1	5.6	59.6	40.4
LM4/LM5	Makueni	6.15–6.9	6.61	6.3×10^4–1.46×10^6	4.684×10^4	59	68.5	28.3	0	3.3	66.3	33.7
CL3/CL4/L3	Msambweni	6.2–7.6	6.59	3.0×10^3–2.6×10^6	7.915×10^4	53	64.6	24.6	8.03	2.8	55.4	44.6
						220	63.78	27.98	5.605	2.34	60.56	39.4

LM lower midland zones, *L* lowland zones, *CL* coastal lowlands (3-semi humid, 4-transitional, 5-semi arid)

[a] Summary of the isolates

[b] Percent data (% means of variables)



S- strain L- strain

Fig. 1 Microscopic features of *Aspergillus* spp Conidial features (×400)

Table 2 Pearson's correlation coefficients of relationships among the quantity of *Aspergillus* section Flavi population in soil (CFU g^{-1}), soil pH, AEZ and the isolates

	pH	CFU	% Af	% Ap	% At	% An	% L	% S
pH	1.000							
CFU	0.009	1.000						
% Af	0.061	−0.118	1.000					
% Ap	0.014	0.132	−0.893*	1.000				
% At	−0.294	0.038	−0.066	−0.319	1.000			
% An	0.280	0.029	−0.618*	0.470**	−0.193	1.000		
% L	−0.594*	−0.037	−0.338	0.236	0.105	0.228	1.000	
% S	0.594*	0.037	0.338	−0.236	−0.105	−0.228	−1.000*	1.000

CFU was log transformed (value + 1) prior to analysis

Percent data were arcsine square root transformed prior to analysis

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Aflatoxin production by toxigenic strains and quantification

Aflatoxin-producing aspergilli were isolated from all the districts surveyed (Table 3). As expected, *A. flavus* and *A. tamari* produced only B aflatoxins while *A. parasiticus* produced both B and G subtypes. Aflatoxin-producing potential varied widely among isolates from the same districts, and agroecological zones (Table 3). Only two isolates of *A. tamarii* produced detectable aflatoxin (B). *Aspergillus nomius* isolates did not show any toxigenic potential.

The majority of aflatoxin producers were the L type members of section Flavi. However, the highest

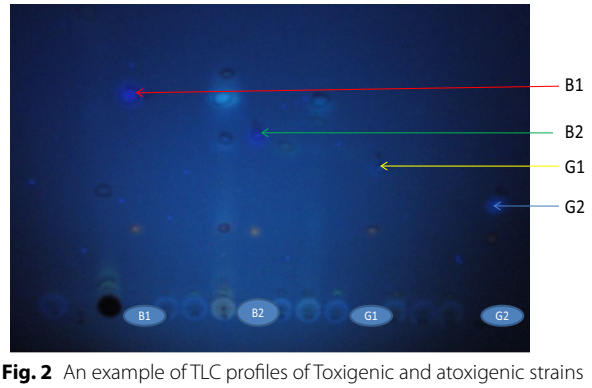


Fig. 2 An example of TLC profiles of Toxigenic and atoxigenic strains

Table 3 Incidence of atoxigenic and toxigenic strains surveyed in all the 57 sites within the districts in Kenya

District	Selected isolates surveyed	% toxigenic	% atoxigenic	Range of toxin yield per district (ng g^{-1})	Mean aflatoxin (ng g^{-1})	Toxigenic (N)	% B producers	% B & G producers
Kibwezi	9	44.4	55.6	4.0×10^3–4.23×10^6	1.53×10^6	4	50	50
Kwale	11	54.5	45.5	6.65×10^4–4.65×10^6	9.41×10^5	6	66.7	33.3
Makindu	8	25	75	1.742×10^4–9.157×10^5	4.67×10^5	2	50	50
Makueni	17	47.1	52.9	2.4×10^4–8.45×10^5	2.06×10^5	9	100	0
Msambweni	12	58.3	41.7	9.68×10^3–8.1×10^5	3.58×10^5	7	85.7	14.3
	57[a]	45.86[b]	54.1[b]				70.5[b]	29.5[b]

concentrations of aflatoxins were detected among the S type strains ranging from 2.25×10^5 to 4.65×10^6 ng g^{-1}. Mean aflatoxin production varied significantly among the districts with the least being Kibwezi (4.0×10^3 ng g^{-1}) and the highest being Kwale (4.65×10^6 ng g^{-1}) (Tables 3, 4). The B aflatoxins were the most common metabolite (70.5 % as compared to the BG aflatoxins, 29.5 %) (Table 3).

Discussion

Aspergillus section Flavi were detected in all 57 soil samples collected from various agroecological zones of maize and peanut growing areas in Kenya. This widespread distribution and prevalence conforms to previous findings of similar studies in Israel, Thailand, North America and West Africa [19, 28, 35]. Several members of the section *Flavi* including *A. niger, A. fumigatus, A. clavatus, A. ochraceus, A. terreus* and *A. versicolor* have been shown to be major mycotoxin-producing contaminants in various agricultural produce in Kenya. Our study supports a high incidence of *Aspergillus* section Flavi with *A. flavus* being the most predominant (63.2 %), *A. flavus* could indeed be linked to B1 aflatoxicosis contamination that has been shown to be prevalent as was determined by Probst et al. [9]. Members of *A. parasiticus* were less prevalent but nevertheless produced high concentrations of both B and G aflatoxins (Tables 3, 4). They, therefore, contributed significantly to the aflatoxicosis potential of resident fungal communities, a situation similar to that observed in West Africa by Donner et al. [28]. While all species produced B aflatoxin in different quantities, only *A. parasiticus* produced G aflatoxins among all groups studied in Kenya.

The negative correlation of pH and proportions of L strains could be an indication of the poorer adaptation of these strains to higher pH optima compared to the S strains. However, the adaptations of the two strains is not mutually exclusive as each of the two strains occur in different proportions within all the pH ranges observed. In addition, the range of soil pH variation across the examined regions was narrow, ranging from weak acidic to weak alkaline. The higher incidence of S strains in more alkaline soils has previously been observed in North American soils among isolates of *A. parasiticus, A. tamarii* and *A. flavus* [24]. However, in Nigeria [28], a negative correlation was observed. The effect of pH has been shown to play a lesser role in *A. flavus* population dynamics, with climate being cited as a more important determinant [35]. Therefore, other shared factors within the study areas such as temperature maxima of 40 °C, average humidity, semi aridity and transitional savannah ecosystems provide a range of biophysical conditions conducive for elevated levels of aflatoxin-producing fungi.

The aflatoxin-producing potential of *Aspergillus* communities is higher when S strain is present, as L strain isolates produce on average only 33 % of the toxin yield from S strain isolates [24]. In West Africa, S strain isolates produce greater quantities of aflatoxins than L strain isolates. This corroborates the finding of this study, in which the S strains produced the highest concentration of aflatoxins in Kenya. All the isolates of *A. flavus* (both L and S strains) did not produce the G aflatoxins but produced the B toxins. The percentage of *A. flavus* L-strain isolates that produced aflatoxins varied with geography and climate (Fig. 3).

Aflatoxin-producing fungi vary widely in many characteristics, including virulence for crops and aflatoxin-producing capacity [33]. *Aspergillus flavus* and *A. parasiticus* are most commonly implicated as causal agents of aflatoxin contamination. *Aspergillus flavus* has two morphotypes, the typical or L strain (sclerotia of >400 µm in diameter) and the S strain (sclerotia of <400 µm in diameter) [33, 36]. S-strain isolates produce more aflatoxins than L-strain isolates, on average. Many L-strain isolates produce no aflatoxins ("atoxigenic") [24].

All members of *A. flavus* lack the ability to synthesize G aflatoxins due to a 0.8–1.5 kb deletion in the 28-gene aflatoxin biosynthesis cluster [18]. In contrast to cases in the United States, studies conducted in West Africa found out that an unnamed taxon (sometimes called strain S_{BG})

Table 4 Aflatoxin production and quantification among isolates

District	Sample ID	Strain	Species	Aflatoxins	Total aflatoxin conc. (ng g^{-1})
Msambweni	MSAMRI1CRM	AT	*A. tamarii*	B	4.7×10^5
Msambweni	MSAVA3DG	AT	*A. tamarii*	B	7.8×10^4
kibwezi	KIKAT1DG	L	*A. parasiticus*	B & G	4.0×10^3
Kwale	KMAN2S2CRM	L	*A. parasiticus*	B & G	7.8×10^4
Kwale	KMWAL2S3W	L	*A. parasiticus*	B & G	6.65×10^4
Kwale	KMWAL1S2W	L	*A. flavus*	B	5.552×10^5
Kwale	KMWAL2S1CRM	L	*A. flavus*	B	8.41×10^4
Makindu	MAKAU2CRM	L	*A. parasiticus*	B & G	1.742×10^3
Makueni	MAUKA3W	L	*A. flavus*	B	1.2×10^5
Makueni	MMUK3DG	L	*A. flavus*	B	7.8×10^4
Makueni	MMUK2CRM	L	*A. flavus*	B	2.4×10^4
Makueni	MAUKA1CRM	L	*A. flavus*	B	1.25×10^5
Makueni	MANGE1DG	L	*A. flavus*	B	2.45×10^4
Makueni	MAU1DG	L	*A. flavus*	B	7.4×10^4
Msambweni	MSADZO1DG	L	*A. parasiticus*	B	7.582×10^5
Msambweni	MSAKI2CRM	L	*A. flavus*	B	1.27×10^4
Msambweni	MSAKI 3W	L	*A. flavus*	B	8.1×10^5
Msambweni	MSAKI2S3W	L	*A. parasiticus*	B & G	9.68×10^3
Kibwezi	KIKAL1CRM	S	*A. flavus*	B	2.25×10^5
Kibwezi	KIMIT 2W	S	*A. parasiticus*	B & G	1.67×10^6
Kibwezi	KIMIT3CRM	S	*A. flavus*	B	4.23×10^6
Kwale	KMAK2W	S	*A. flavus*	B	4.65×10^6
Kwale	KMWAL1S1W	S	*A. parasiticus*	B	2.15×10^5
Makindu	MAKAU3CRM	S	*A. flavus*	B	9.157×10^5
Makueni	MAUKA3CRM	S	*A. parasiticus*	B	3.64×10^5
Makueni	MAWA1CRM	S	*A. flavus*	B	8.45×10^5
Msambweni	MSAKI1S1W	S	*A. flavus*	B	2.5×10^5

Samples that produced no aflatoxins are not shown

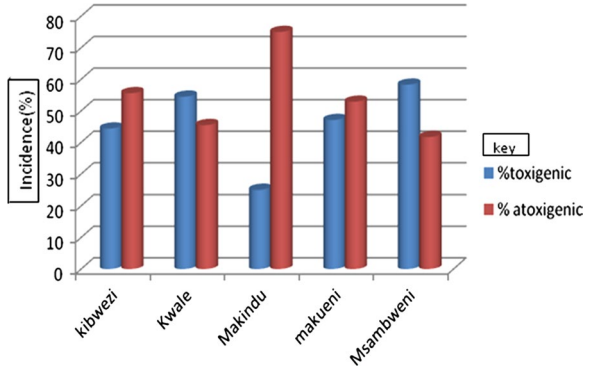


Fig. 3 Incidence of toxigenic and atoxigenic isolates of aspergillus section Flavi from soil samples of maize and pea nut growing areas in Kenya. S-strain L-strain

is commonly implicated in contamination events [5]. Strain S_{BG} is morphologically similar to the S strain of *A. flavus*, but DNA-based phylogenies reveal strain S_{BG} to be a distinct species ancestral to both *A. flavus* and *A. parasiticus* [37, 38].

Aflatoxin levels unacceptable for human consumption may occur even in areas with relatively low frequencies of aflatoxin producers. In the current study, 59.3 % of aflatoxin-producing L-strain isolates produced more than 10×10^5 ng g^{-1} (14.3 ppb) aflatoxin B1. This combined with high incidences allows the L-strain to be the largest contributor to the average aflatoxin-producing ability of fungal communities in the five districts (Fig. 1; Table 4) and a potentially important causal agent of contamination in Kenya. Similar results were observed in Nigeria [28]. This is in contrast, with earlier studies conducted in Kenya where the S-strain was observed to be the primary cause of maize aflatoxin contamination [9]. The current study shows that S-strain is present in low frequency or absent in certain sites.

Incidences of atoxigenic strains of section Flavi varied widely among districts and agroecological zones

(Table 1). The non-aflatoxin-producing strains are common in crop environments [21, 23, 24]. The atoxigenic strains of *A. flavus* and/or *A. parasiticus* have been employed as biopesticides directed at minimizing crop contamination with aflatoxins [4, 39]. Successful and effective biocontrol strategies necessitates that high ratios of atoxigenic to toxigenic strains be exhibited [40]. In the current study, high incidences of atoxigenic strains of section Flavi members were found in Kibwezi and Makueni districts both in lower midland zones (LM3, LM4 and LM5). These native populations provide potential biological control agents to manage the toxigenic strains.

Aflatoxin contamination of crops can be minimized by early harvest, prevention of insect damage and proper storage [41]. However, despite the careful management, unacceptable aflatoxin levels may occur from unpreventable insect damage to the developing crop or from exposure of the mature crop to moisture either prior to harvest or during storage in modules, handling, transportation or even use [33].

For many diseases, traditional chemical control methods are neither always economical nor are they effective, and fumigation as well as other chemical control methods may have unwanted health, safety and environmental risks in Kenya and many other developing countries. The antifungal abilities of some beneficial microbes have been known since the 1930s, and there have been extensive efforts to use them for plant disease control since then. However, they are only now being used commercially [33].

Aflatoxins cannot be readily removed from contaminated foods by detoxification. Therefore, there is interest in developing a biological control method that can increase crop safety by decreasing toxin content and this depends on the competitive displacement of toxigenic isolates using atoxigenic isolates of the same species. It has also been reported that aflatoxin production is inhibited by lactic acid bacteria, *Bacillus subtilis* and many moulds. This inhibition may result from many factors including competition for space and nutrients. In general, competition for nutrients required for aflatoxin production but not for growth and production of anti-aflatoxigenic metabolites by co-existing micro-organisms [42].

Currently, atoxigenic *A. flavus* L-strain isolates are used to competitively exclude aflatoxin producers during crop infection and thereby limit contamination in U.S. agriculture [4]. Such atoxigenic strains are highly effective against the S strain [4]. Adaptation and deployment of similar technologies in Africa could provide a promising strategy for prevention of future aflatoxicoses in East Africa while enhancing export possibilities for maize and peanuts [43].

Aspergillus section Flavi was resident in all sampled maize fields and quantities of section Flavi were higher on average in Kenya and compares to the ones in West Africa [35]. The incidences of the section Flavi in Kenyan soils demonstrates the fungal growth on crop-associated with organic matter. Corncobs, peanut pods and other crop debris harbor section Flavi for at least 3 years after harvest [44].

This study has reported high level of atoxigenic species of *Aspergillus* section Flavi. In vitro analysis showed that there is a growth competition between atoxigenic and toxigenic spp of *Aspergillus* section Flavi. The existing methods of biological control of aflatoxicosis by use of the atoxigenic strains can therefore be tested and adapted to Kenyan agriculture. The study has also established that the production of aflatoxins by these species depends on the prevailing physiological conditions such as temperature and moisture. Poor management practices such as poor storage, late harvesting, among others are predisposing conditions for the proliferation of the fungi, so there is need for education on the best practices so as to minimize on contamination. Furthermore, morphological analysis of *Aspergillus* section Flavi is limited with mechanisms of getting the true identity of the fungal species. There is need to adopt rapid and sensitive molecular techniques to support the morphological identifications. Finally, there is need to screen all the farm produce for possible aflatoxin contamination to reduce economic burdens related to the menace. We, therefore, hope this study will be taken into consideration by regulatory authorities in Kenya and beyond.

Conclusion

In conclusion, we report an ubiquitous presence of the members of the Aspergillus section Flavi within maize and peanut growing fields in Kenya. The diversity is characterized by a significant presence of atoxigenic *A. flavus* and *A. parasiticus*. Such atoxigenic variants have been previously applied in the biological suppression of natural populations of toxigenic strains and thereby minimizing aflatoxin contamination in peanuts [40], corn [45] and cotton [4]. The existing methods of biological control of aflatoxicosis by use of atoxigenic strains can therefore be tested and adapted to Kenyan agriculture. Control of natural populations of aflatoxin-producing fungi will lead to overall reduction of aflatoxicosis in farm produce as reported to be common in the regions studied [7, 9].

Abbreviations

CFU: colony-forming units; AEZ: agroecological zones; TLC: thin layer chromatography; AFPA: *Aspergillus flavus* and *Aspergillus parasiticus* agar; MRBA: Modified Rose Bengal Agar; VCGs: Vegetative Compatibility Groups.

Authors' contributions
OOD carried out TLC analysis and drafted the manuscript. FMK participated in statistical analysis and manuscript drafting. MMG participated in the design of the study. NNJ and participated in sample collection and processing. FO was involved in fungal culturing. YOM participated in sample processing and fungal culture analysis. KUE conceived the study, was involved in design and coordination. All authors read and approved the final manuscript.

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Compliance with ethical guidelines

Competing interests
The authors declare that they have no competing interests.

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References

1. Hesseltine CW. A millennium of fungi, food, and fermentation. Mycologia. 1965;57:149–97.
2. Ainsworth GC, Austwick PKC. Fungal diseases of animals. 2nd ed. Slough: Commonwealth Agricultural Bureau; 1973.
3. Klich MA, Pitt JI. Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Trans Br Mycol Soc. 1988;91:99–108.
4. Cotty PJ. Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the populations of *A. flavus* infecting cotton bolls and on the aflatoxin content of cottonseed. Phytopathology. 1994;84:1270–7.
5. Cotty PJ, Cardwell KF. Divergence of West African and North American Communities of Aspergillus Section Flavi. Appl Environ Microbiol. 1999;65:2264–6.
6. Centre for Disease Control. Outbreak of aflatoxin poisoning: Eastern and Central Provinces, Kenya. Weekly Reports. September 2004.
7. Muthomi JW, Njenga LN, Gathumbi JK, Chemining'wa GN, et al. The occurrence of aflatoxins in maize and distribution of mycotoxin-producing fungi in Eastern Kenya. Plant Pathol J. 2009;8:113–9.
8. Ministry of Agriculture (MOA) The role of post harvest in the control of aflatoxins in cereals and pulses. Ministry of Agriculture Headquarters, Nairobi 2008.
9. Probst C, Njapau H, Cotty PJ. Outbreak of an acute aflatoxicosis in Kenya in 2004: identification of the causal agent. Appl Environ Microbiol. 2007;73:2762–4.
10. Nyikal J, Misore A, Nzioka C, Njuguna C, Muchiri E, Njau J, Maingi S, Njoroge J, Mutiso J, Onteri J, Langat A, Kilei lK, Nyamongo J, Ogana G, Muture B, Tukei P, Onyango C, Ochieng W, Tetteh C, Likimani S, Nguku P, Galgalo T, Kibet S, Manya A, Dahiye A, Mwihia J, Mugoya I, Onsongo J, Ngindu A, DeCock KM, Lindblade K, Slutsker L, Amornkul P, Rosen D, Feiken D, Thomas T, Mensah P, Eseko N, Nejjar A, Onsongo M, Kesell F, Njapau H, Park DL, Lewis L, Luber G, Rogers H, Backer L, Rubin C, Gieseker KE, Azziz-Baumgartner E, Chege W, Bowen A. Outbreak of aflatoxin poisoning—Eastern and Central Provinces, Kenya, January–July 2004. Morb Mortal Wkly Rep. 2004;53:790–3.
11. Lewis L, Onsongo M, Njapau H, Schurz Rogers H, Luber G, Kieszak S. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environ Health Perspect. 2005;113:1763–7.
12. Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev. 2003;16:497–516.
13. Kenya Plant Health Inspectorate Service (KEPHIS). Mycotoxins and Food Safety. KEPHIS Headquarters, Nairobi, Kenya 2006.
14. Betran FJ, Isakeit T. Aflatoxin accumulation in maize hybrids of different maturities. Agron J. 2003;96:565–70.
15. Boyd ML, Cotty P. Characterization of Aspergillus section Flavi communities from natural habitats in the Sonoran Desert. Inoculum. 1998;49:11.
16. Papa KE. Heterokaryon incompatibility in *Aspergillus flavus*. Mycologia. 1986;78:98–101.
17. Bayman P, Cotty PJ. Vegetative compatibility and genetic diversity in the *Aspergillus flavus* population of a single field. Can J Bot. 1991;69:1707–11.
18. Ehrlich KC, Cotty PJ. An isolate of *Aspergillus flavus* used to reduce aflatoxin contamination in cottonseed has a defective polyketide synthase gene. Appl Microbiol Biotechnol. 2004;65:473–8.
19. Ehrlich KC, Kobbeman K, Montalbano BG, Cotty PJ. Aflatoxin-producing Aspergillus species from Thailand. Int J Food Microbiol. 2007;114:153–9.
20. Schroeder HW, Boller RA. Aflatoxin production of species and strains of the *Aspergillus flavus* group isolated from field crops. Appl Microbiol. 1973;25:885–9.
21. Lisker N, Michaeli R, Frank ZR. Mycotoxigenic potential of *Aspergillus flavus* strains isolated from groundnuts growing in Israel. Mycopathologia. 1993;122:177–83.
22. Cotty PJ, Howell DR, Bock C, Tellez A. Aflatoxin contamination of commercially grown transgenic Bt cottonseed. In Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council of America, Memphis, TN; 1997. p. 108–110.
23. Joffe AZ. Aflatoxin produced by 1,626 isolates of *Aspergillus flavus* from groundnut kernels and soils in Israel. Nature. 1969;221:492.
24. Cotty PJ. Aflatoxin-producing potential of communities of Aspergillus section Flavi from cotton producing areas in the United States. Mycol Res. 1997;101:698–704.
25. Horn BW, Dorner JW. Regional differences in production of aflatoxin B1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. Appl Environ Microbiol. 1999;65:1444–9.
26. Vaamonde G, Patriarca A, Pinto VF, Comerio R, Degrossi C. Variability of aflatoxin and cyclopiazonic acid production by Aspergillus section flavi from different substrates in Argentina. Int J Food Microbiol. 2003;88:79–84.
27. Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Allameh A, Kazeroon-Shiri A, Ranjbar-Bahadori S, Mirzahoseini H, Rezaee M-B. A survey on distribution of Aspergillus section Flavi in corn field soils in Iran: population patterns based on aflatoxins, cyclopiazonic acid and sclerotia production. Mycopathologia. 2006;161:183–92.
28. Donner M, Atehnkeng J, Sikora RA, Bandyopadhyay R, Cotty PJ. Distribution of Aspergillus section Flavi in soils of maize fields in three agroecological zones of Nigeria. Soil Biol Biochem. 2009;41:37–44.
29. Dorner JW. Biological control of aflatoxin contamination of crops. Toxin Rev. 2004;23:425–50.
30. Cotty PJ, Mellon JE. Ecology of aflatoxin producing fungi and biocontrol of aflatoxin contamination. Mycotoxin Res. 2006;22:110–7.
31. Dorner JW, Cole RJ, Connick WJ, Daigle DJ, McGuire MR, Shasha BS. Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts. Biol Control. 2003;26:318–24.
32. Cotty PJ. Comparison of four media for the isolation of *Aspergillus flavus* group fungi. Mycopathologia. 1994;125:157–62.
33. Cotty PJ, Lee LS. Aflatoxin contamination of cottonseed: comparison of pink bollworm damaged and undamaged bolls. Trop Sci. 1989;29:273–277.
34. Pitt JI, Hocking AD, Glenn DR. An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. J Appl Bacteriol. 1983;54:109–14.
35. Cardwell KF, Cotty PJ. Distribution of Aspergillus section Flavi among field soils from the four agroecological zones of the Republic of Benin, West Africa. Plant Dis. 2002;86:434–9.
36. Horn BW. Ecology and population biology of aflatoxigenic fungi in soil. Toxin Rev. 2003;22:351–79.
37. Egel DS, Cotty PJ, Elias KS. Relationships among isolates of Aspergillus sect. Flavi that vary in aflatoxin production. Phytopathology. 1994;84:906–12.
38. Ehrlich KC, Yu J, Cotty PJ. Aflatoxin biosynthesis gene clusters and flanking regions. J Appl Microbiol. 2005;99:518–27.
39. Dorner JW, Cole RJ, Blankenship PD. Effect of inoculum rate of biological control agents on preharvest aflatoxin contamination of peanuts. Biol Control. 1998;12:171–6.

40. Dorner JW, Cole RJ, Blankenship PD. Use of a biocompetitive agent to control preharvest aflatoxin in drought stressed peanuts. J Food Prot. 1992;55:888–92.
41. Cotty PJ. Effect of harvest date on aflatoxin contamination of cottonseed. Plant Dis. 1991;75:312–4.
42. Munimbazi C, Bullerman LB. Inhibition of aflatoxin production of *Aspergillus parasiticus* NRRL 2999 by *Bacillus pumilus*. Mycopathologia. 1998;140:163–9.
43. Bankole SA, Adebanjo A. Mycotoxins in food in West Africa: current situation and possibilities of controlling it. African J Biotechnol. 2004;2:254–63.
44. Jaime-Garcia R, Cotty PJ. *Aspergillus flavus* in soils and corncobs in south Texas: implications for management of aflatoxins in corn-cotton rotations. Plant Dis. 2004;88:1366–71.
45. Brown RL, Cotty PJ, Cleveland TE. Reduction in aflatoxin content of maize by atoxigenic strains of *Aspergillus flavus*. J Food Prot. 1991;54:623–6.

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