International Scholars Journals

International Journal of Bacteriology and Mycology ISSN 2756-3669 Vol. 12 (1), pp. 001-011, January, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Fungal ear rot pathogens in maize cropping systems: A comparative analysis of push-pull and monocropping systems in Western Kenya

Maxwell J. Owuor[1,2*], Charles A. O. Midega[1], Meshack Obonyo[2] and Zeyaur R. Khan[1]

[1]International Centre of Insect Physiology and Ecology, P. O. Box 30- 40305, Mbita, Kenya.

[2]Department of Biochemistry, Faculty of Science, Egerton University, P. O. Box 536-20115, Egerton, Kenya.

Accepted 14 September, 2022

It is imperative to establish the distribution and density of soil fungal communities as a requisite for formulating strategies for management of ear rot infections and mycotoxin contamination. In a two seasons study, short (SR) and long rainy (LR) seasons, we investigated the distribution of *Aspergillus* and *Fusarium* fungi causing ear rots and producing mycotoxins from 120 soil samples collected from maize fields under push-pull (PP) and maize monocrop (MM) systems in Western Kenya. Cultural methods were used for identification of *Aspergillus* and *Fusarium* species, while molecular techniques were used for confirmation of *Fusarium* section *Liseola*. Detection of total aflatoxins in cultures of section *Flavi* isolates was carried out by enzyme-linked immunosorbent assay (ELISA). A total of 338 fungi were isolated; 80% were identified as *Aspergillus* and 4.4% *Fusarium*. The distribution of fungi was significant with season but not cropping systems. The frequency of occurrence was higher during the LR (68.4%) than the SR (31.6%). In cropping systems, the frequency of occurrence of *Aspergillus flavus* was higher in MM (60.2%) than PP (39.8%). However, *Aspergillus parasiticus* was more frequent in PP (71.4%) than MM (28.6%); and during the SR (78.6%) than the LR (21.4%). Majority (81.3%) of *A. flavus* and *A. parasiticus* were toxigenic. There was low recovery of *Fusarium* species in soil samples. These findings show that soils from both cropping systems are potential for *Aspergillus* infection and aflatoxins contamination; however, low *Fusarium* distribution in soil suggest external inoculum source for *Fusarium* ear rot infections common in most maize fields in Western Kenya.

Key words: *Aspergillus*, *Fusarium* section *Liseola*, push-pull, soil.

INTRODUCTION

Fungi are part of diverse living components of soil, with several of them living as saprophytes and symbionts contributing to various soil services including structure formation, organic decomposition, recycling of major elements (for example carbon, nitrogen and phosphorus) and toxic removal (Aislabie and Deslippe, 2013). Pathogenic fungi also exist as major causal agents of soil borne diseases affecting roots, stalks, leaves and ears of

*Corresponding author. E-mail: maxwelljuti@gmail.com.

various crops including maize (Shurtleff, 1980). Nevertheless, the presence of certain non-pathogenic (mainly saprophytes) or pathogenic fungi on grains, soils and other reservoirs are potential for ear rot infection and mycotoxin production, especially species in the *Aspergillus* and *Fusarium* genera (Horn et al., 1995; Pereira et al., 2011).

The *Aspergillus* genus is divided into sections (or subgenus groups) of which *Flavi* is most important in agriculture as cause of ear rot diseases and producer of aflatoxins (Gnonlonfin et al., 2011). Several species are classified under *Flavi*, but *Aspergillus flavus* Link*, Aspergillus parasiticus* Speare and *Aspergillus nomius* Kurtzman (Rodrigues et al., 2007) are prominent isolates in maize and soil samples. Amongst these species, *A. flavus* and *A. parasiticus* are prolific producers of aflatoxins with the former being the most abundant in both air and soil (Hedayati et al., 2007), hence affecting more of aerial crops like maize. On the other hand, *A. parasiticus* is mostly reserved in soils with high isolation frequency of peanuts fields (Garber and Cotty, 2014).

The filamentous fungus with equal importance in maize production is *Fusarium*. Most of its members are producers of three important agricultural mycotoxins which include: fumonisins, deoxynivalenol and zearalenone (D'Mello et al., 1999). They are also causative agents of root and ear rots in maize resulting in yield losses (Sutton, 1982). Three *Fusarium* species with high frequency of isolation in maize include: *Fusarium graminearum* Schwabe, *Fusarium verticillioides* (Sacc.) Nirenberg*, Fusarium proliferatum* (T. Matsushima) Nirenberg and *Fusarium subglutinans* (Wollenweb and Reinkings) P. E Nelson, T.A. Toussoun and Marasas (Leslie and Summerrel, 2006).

Soil is the primary habitat for *Fusarium* and *Aspergillus* species. The population of *Aspergillus* and *Fusarium* propagules in the soil (field) increases the risk for maize infections and mycotoxin contamination (Horn, 2003). In order to safeguard against losses, fungal distribution in food and soil ecology is imperative for effective formulation of prevention and control measures. In soil fungal ecology, cultural practices greatly encourage or discourage fungal distribution. For instance, rotation of susceptible crops like wheat with maize together (Schaafsma et al., 2005) increase fumonisin incidence. Addition of organic matter either by cultural practice through minimum tillage, or application of organic amendments increases *Aspergilli* propagules (Zablotowicz et al., 2007) while decreasing those of *Fusarium* in soil (Alakonya et al., 2008).

Among several strategies used for soil management in western Kenya, push-pull technology (PPT), a companion cropping system where maize or sorghum is intercropped with moth repellent (push) forage legumes in the genus *Desmodium,* edged with moth attractive (pull) grasses such as Napier grass (*Pennisetum purpureum* Schumach) or *Brachiaria*, is mostly adopted by smallholder farmers (Khan et al., 2011). Although, PPT is known for insect pest management, it contributes to soil health improvement which is potentially impactful on soil fungal community. The technology improves organic matter content of the soil, nitrogen fixation, overall improvement in soil macro- and micro arthropods and conservation of soil moisture (Khan et al., 2011). However, distribution of *Aspergillus* and *Fusarium* ear rot fungi in soil under push-pull remains unknown. In this context, the aim of this study was to investigate the level of soil-borne *Aspergillus* and *Fusarium* species in push-pull and maize monocrop plots in western Kenya.

MATERIALS AND METHODS

Field survey

The study sites included Kisumu, Siaya and Vihiga sub-counties (districts) of Western Kenya (Figure 1). Soils in these sites are generally vertisols, ferralsol and nitosols showing a natural decline in soil fertility predominantly manifested by occurrence of purple witch weed, *Striga hermonthica* (Del.) Benth. (Orobanchaceae), soil erosion and deficient nitrogen and phosphorus. However, heterogeneity in soil fertility exists amongst smallholder farms in the region where there is less investment in external inputs to restore soil fertility. Push-pull technology has been disseminated for pest control and soil fertility improvement for over 10 years in the region (Khan et al., 2011).

Sample collection

The sampling method of Horn and Dorner (1998) was adopted for soil sampling with slight modifications. Briefly, transect which runs 5 km from one push-pull cluster to the next was made. A total of 60 fields were sampled at maize silking period during the short and long rainy seasons of 2014 and 2015, respectively. In a cluster, four push-pull and maize monocrop fields were sampled by removal of 4 subsamples of soil with a sterile trowel from the top 4 to 6 cm of soil at intervals of 2 to 4 m. The soil subsamples collected from each field were mixed and placed in a paper bag and air dried at 25°C for 1 week. The soil was then carefully mixed and sieved through a no.10 USA standard sieve (2.00 mm opening) (Dual Manufacturing Company, Franklin Park, IL 60131, USA) and stored at 4°C.

Isolation of fungi

The dilution plate technique by Cotty (1994) and Leslie and Summerell (2006) were used for *Aspergillus* and *Fusarium* recovery, respectively. One gram of thoroughly mixed soil samples was suspended in 9 ml of distilled water. These resultant solutions were serially diluted to 10^{-3}. One milliliter of 10^{-2} and 10^{-3} were plated in quadruplicate in Petri dish (90 x 15 mm) containing a quarter strength potato dextrose agar (PDA) (HiMedia Laboratories Pvt. Ltd) amended with 30 mg chloramphenicol. The plates were then incubated at 31°C for 6 days in the dark for *Aspergillus* recovery, and at 25°C for 14 days for *Fusarium*. Colonies of *Aspergillus* and *Fusarium* that grew on each plate were counted and their population determined as colony forming unit (CFU) per gram and calculated as follows:

Total fungal colonies = Number of colonies x dilution factor/weight of soil (1 g)

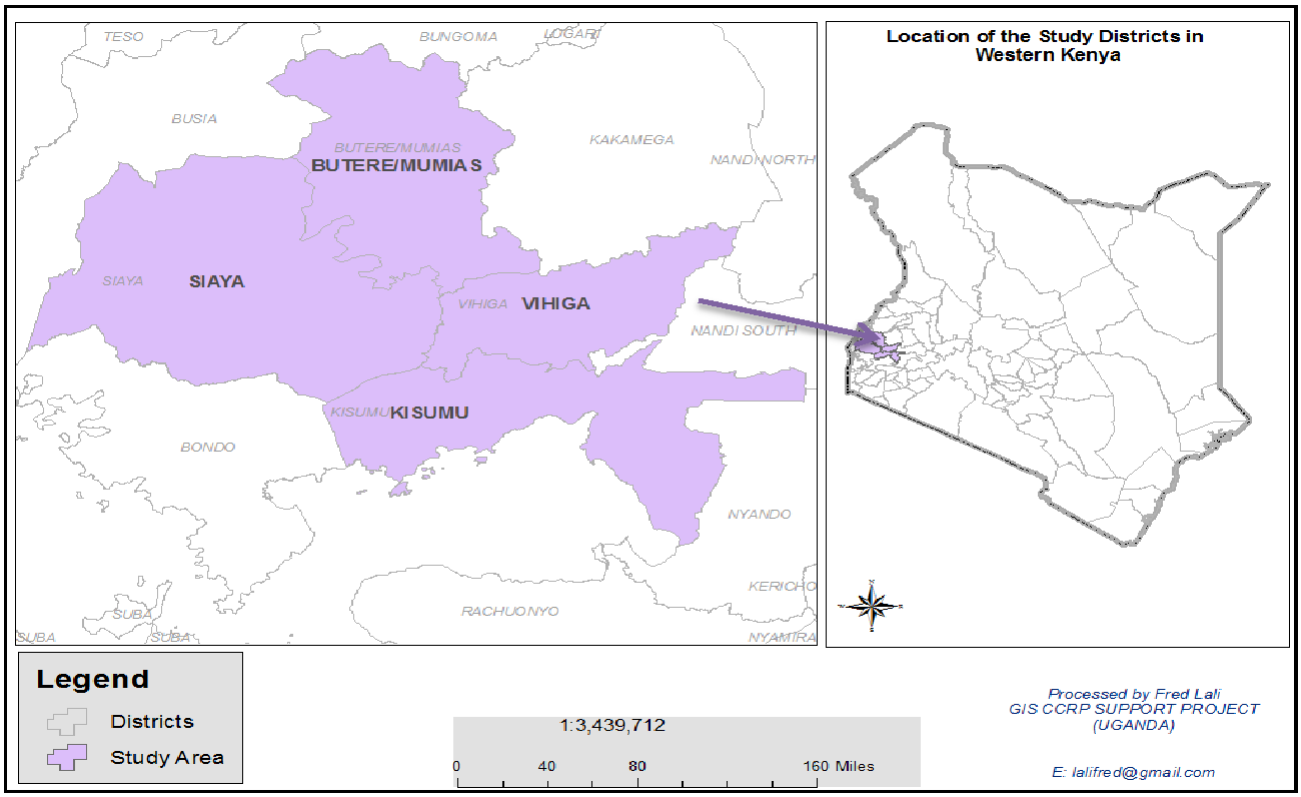


Figure 1. Map showing study districts in Western Kenya.

Colonies of *Aspergillus* and *Fusarium* were then sub-cultured on full strength PDA amended with 30 mg chloramphenicol.

Morphological identification of *Aspergillus* and *Fusarium*

The colonies on PDA identified as *Aspergillus* were transferred aseptically onto Czapek Dox Agar (CZ) (Oxoid Ltd, Basingstoke, Hampshire, England) plates and incubated at 31°C for five days. Their colony characteristics (colour and reverse) were observed. Those characterized to belong to *Aspergillus* section *Flavi* were confirmed on Aspergillus Flavus Parasiticus Agar (AFPA) base (HiMedia Laboratories Pvt. Ltd) plates incubated at 25°C for five days for positive orange reverse. Microscopic features such as: head serration, vesicle and conidia were observed in a compound light microscope (Carl Zeiss MicroImaging GmbH 37081, Gottingen, Germany) using keys by Klich (2002).

Fusarium colonies recovered were grown on PDA plates and observed for pigmentation on both top and reverse, and on Spezieller Nahrstoffarmer Agar (SNA) for macroconidial features. Further identification using species-specific primers was used for identification of *F. verticillioides, F. proliferatum* and *F. subglutinans*.

Molecular identification of *Fusarium* section *Liseola*

DNA extraction

Fusarium isolates, 13 in total, culturally identified to belong to *Fusarium* section *Liseola* were grown as monosporing cultures on PDA plates for seven days at room temperature. For each isolate, mycelium was harvested for total DNA extraction according to Gherbawy et al. (2001). One gram of freshly harvested mycelium was ground in liquid nitrogen with a mortar and pestle into a very fine powder. Fifty milligrams of the ground mycelium was transferred into 1.5 ml Eppendorf tube and mixed with 700 µL 2 x CTAB buffer. The contents of Eppendorf tube was incubated at 65°C for 30 min before addition of 700 µL of chloroform : isoamyl alcohol (24:1 v/v), and a brief mixing. The mixtures were then centrifuged at 10,000 *g* for 30 min and supernatant was transferred into another tube. Isopropanol, 700 µL in volume was added and mixed with the supernatant and left to chill overnight at -20°C. This content was centrifuged again at 10,000 *g* for 5 min, after which the supernatant was discarded and pellets washed twice in 1 mL of 70% ethanol and left to dry under a vacuum. The pellets were afterwards resuspended in 700 µL distilled water. The quality of DNA was evaluated in 1% agrose gel electrophoresis.

Detection of Fusarium DNA using species-specific primers

The following primer pairs, VER 1/2, PRO 1/2 and SUB 1/2 were used for identification of *F. verticillioides*, *F. proliferatum* and *F. subglutinans*, respectively in PCR assay according to Rahjoo et al. (2008): *F. verticillioides*, VER 1/2 (F: 5′-CTT CCT GCG ATC TTT CTC C-3′, R: 5′-AAT TGG CCA TTG GTA TTA TAT ATC TA-3′); *F. proliferatum*, PRO 1/2 (F: 5′-CTT TCC GCC AAG TTT CTT C-3′, R: 5′-TGT CAG TAA CTC GAC GTG TTG-3′); *F. subglutinans,* SUB ½ (F: 5′-CTG TCG CTA ACC TCT TTA TCC A-3′, R: 5′-CAG TAT GGA CGT TGG TAT TAT TAT ATC TAA-3′).

The PCR assay was done in a total volume of 25 µL of master mix comprising 5X buffer, 25 mM of each dNTP, 25 mM $MgCl_2$, 0.2 µL of Ampli Taq polymerase (Applied Biosystems, USA), 2.0 µl of

Table 1. Incidence of ear rot fungi recovered from maize fields in western Kenya.

Variable	Fungal genera				
	Aspergillus	***Fusarium***	***Trichoderma***	***Penicillium***	**Total fungi**
	n (%)				
District					
Kisumu (N=40)	106 (82.8)	2 (1.6)	4 (3.1)	16 (12.5)	128 (37.9)
Siaya (N=40)	88 (77.2)	7 (6.1)	13 (11.4)	6 (5.3)	114 (33.7)
Vihiga (N=40)	80 (83.3)	6 (6.3)	2 (2.1)	8 (8.3)	96 (28.4)
Season					
SR (N=60)	85 (84.2)	2 (2.0)	6 (5.9)	8 (7.9)	101 (29.9)
LR (N=60)	189 (79.8)	13 (5.5)	13 (5.5)	22 (9.3)	237 (70.1)
Cropping system					
PP (N=60)	130 (78.3)	8 (4.8)	7 (4.2)	21 (12.7)	166 (49.1)
MM(N=60)	144 (83.7)	7 (4.1)	12 (7.0)	9 (5.2)	172 (50.9)
Total (N=120**)**	274 (81.1)	15(4.4)	19 (5.6)	30 (8.9)	

n, Number of isolates; N, number of samples; SR, short rain; LR, long rain; PP, push-pull; MM, maize monocropping. The percentage (%) incidence was calculated based on the total counts across the table while incidence of fungi between variables was compared within a column in the table. The incidence of total fungi was significant with season (t (118) =5.513, p=0.001) and *A. flavus* (t (118) =2.1683, p<0.001).

each primer and 5 µL of fungal template DNA. Reactions were performed in Proflex PCR system thermocycler (Applied Biosystems, USA) under the following conditions: denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 50 s, annealing at 56°C for 50 s, extension at 72°C for 1 min, final extension at 72°C for 7 min with cooling at 4°C for final recovery of the samples. The amplified products were then visualized in 1.2% agarose gels stained with ethidium bromide.

Test for aflatoxigenicity

Twenty seven species identified belonging to *Aspergillus* section *Flavi* were grown on PDA at 31°C for a period of 7 days and total aflatoxin were extracted from their cultures according to method described by Rao et al. (1997). A whole sample comprising agar, mycelia and spores was ground in a blender for 5 min. Two grams of the blended mixture was used to extract total aflatoxins in 10 mL of 60% methanol. The mixture was then filtered (Whatman #1) and the filtrate analysed by using ELISA Kits for total aflatoxin (Helica Biosystem Inc.).

Data analysis

All analyses were done using R version 3.3.1. The incidences of fungi were presented in counts and percentages as score of total counts. The data for total fungi, *Aspergillus* and *A. flavus* were normalized by log *x*+1 transformation before mean comparison. However, means of *A. parasiticus, A. fumigatus, Aspergillus niger, Aspergillus terreus, Aspergillus tamarii* and *F. verticillioides* were compared with Mann-Whitney U test or Kruskal-Wallis test.

RESULTS

Distribution of fungi in soil

A total of 338 fungi in four genera were isolated from soil samples (Table 1). From these isolates, 80% were *Aspergillus*, 8.9% *Penicillium*, 5.6% *Trichoderma* and 4.4% *Fusarium*. The incidence of fungi with district was not statistically significant. However, the incidence of *Fusarium* was low in Kisumu (1.6%) than Siaya (6.1%) and Vihiga (6.3%). This was converse to *Penicillium* which had high (12.5%) incidence in Kisumu than Siaya (5.3%) and Vihiga (8.3).

There was significant difference (t (118) = 4.6018, p<0.001) in distribution of total fungi in short and long rainy season. SR had lower (29.9%) incidence than LR (70.1%). Similarly, the incidence of *Aspergillus* was significant (t (118) = 2.1683, p<0.001) in SR and LR season. Incidence of total fungi and in both PP and MM was not significant. However, *Penicillium* had high incidence in PP (12.7%) than MM (5.2%).

Identification of *Aspergillus* and *Fusarium* species

Three species belonging to *Aspergillus* section *Flavi* were identified by colony reverse on AFPA agar (Plate 1). The three species further identified on CZ based on their conidial colour and head serration were *A. Flavus;* yellow green surface and numerously biseriated [Plate 2 a(i) and a(ii)]; *A. parasiticus,* conifer green surface and mainly uniseriated [Plate 2 b(i) and b(ii)]; and *A. tamarii,* dark green surface and abundantly uniserate [Plate 2 c(i) and c(ii)]. Other *Aspergillus* species equally identified on PDA by other features were *A. terrei;* sand brown surface with columnar conidial ornamentation [Plate 2 d(i) and d(ii)]; *A. fumigatus,* blue grey surface and subglobose vesicle [Plate 2 e(i) and e(ii)]*;* and *A. nigri,* black surface and brownish, relatively long and smooth conidiophore [Plate 2 f(i) and f(ii)].

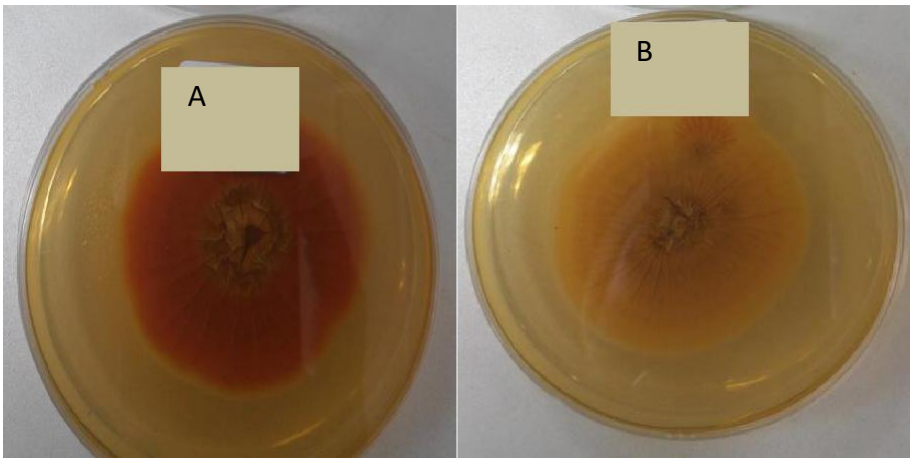


Plate 1. Colony reverses of two isolates in *Aspergillus* section *Flavi* showing bright orange (A) and yellow (B) colour after incubation on AFPA for 5 days at 25°C. Orange colour is positive for *Flavi* section.

There was low recovery of *Fusarium* species causing ear rots; however, 13 isolates recovered morphologically belong to *F. verticillioides* [Plate 2 g(i) and g(ii)]. Out of these isolates (13), 9 were positive (Figure 2) for *F. verticillioides* after molecular characterization with TEF-gene.

Incidence of *Aspergillus* and *Fusarium* species

The respective distribution of six *Aspergillus* and *Fusarium* species in push-pull and monocrop systems were as follows: *A. flavus,* 39.8 and 60.2%; *A. fumigatus,* 55.2 and 44.8%; *A. niger,* 35.6 and 64.4%; *A. parasiticus,* 71.4 and 28.6%; *A. terreus,* 53.3 and 46.7%; *A. tamari,* 20 and 80%; *F. verticillioides,* 50 and 50%; and *F. graminearum,* 66.7 and 33.3%, respectively (Table 2). A high incidence of total and individual fungi was observed during long than short rainy seasons respectively, except *A. parasiticus* which was abundant in short (78.6%) than long rainy season (21.4%). Majority of aflatoxigenic fungi were positive for aflatoxins (81.5%) with only 8.3 and 23.7% of *A. parasiticus* and *A. flavus,* respectively being atoxigenic (Table 3).

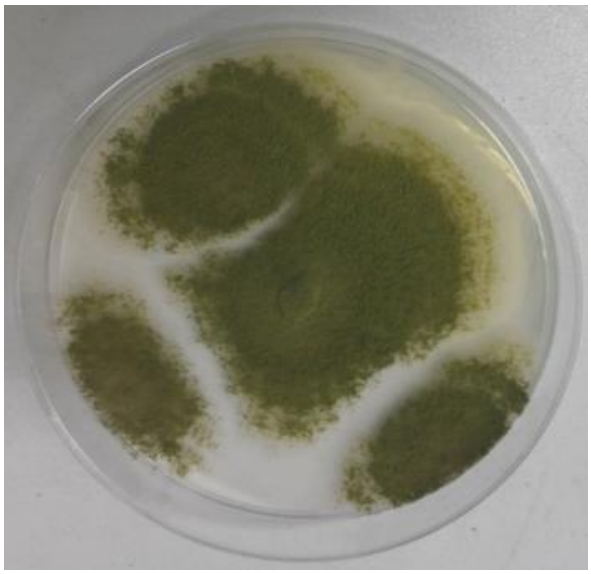
The population of *Aspergillus* and *Fusarium* species in soil

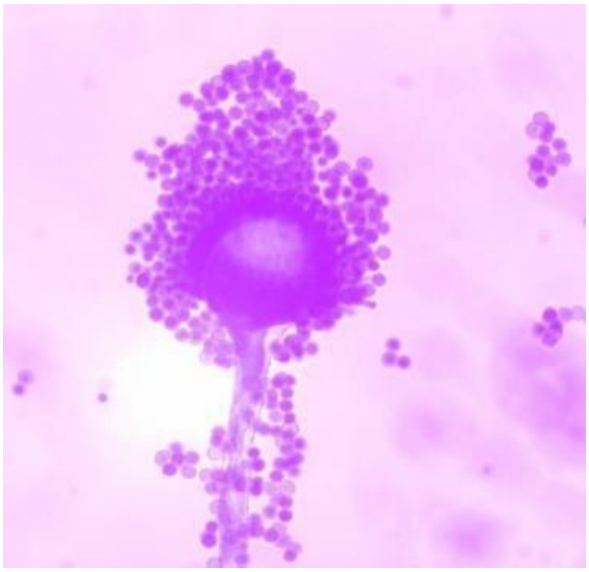
In general, there was no significant difference in population of total and individual fungal species between the two cropping systems (Table 4). However, low population of fungi was observed in push-pull (2,266.1 $CFUg^{-1}$) than in monocrop plots (2,499.9 $CFUg^{-1}$). *A. parasiticus* was the only species which had high population in push-pull (333.3 $CFUg^{-1}$) than in the monocrop system (133.3 $CFUg^{-1}$), with relatively small insignificant difference ($p<0.067$). During long rainy season, a significantly high population of *A. flavus*, *A. fumigatus*, *A. terreus* ($p<0.001$) and *A. parasiticus* ($p<0.05$) were also observed.

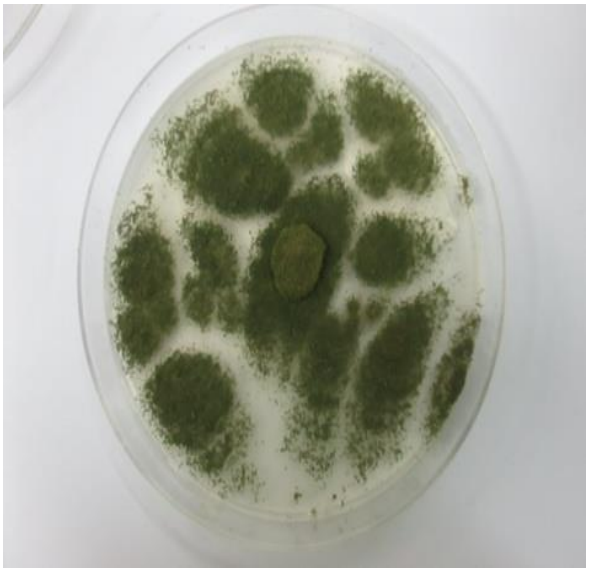
DISCUSSION

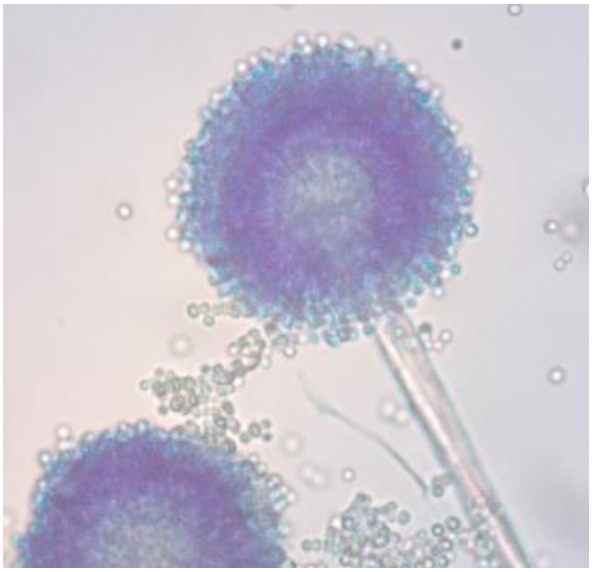
There was higher (averagely 80%) incidence of *Aspergillus* than other fungi in all the districts, cropping systems and seasons observed in this study. This corroborates findings of other studies in different agro-ecological areas in Kenya that reported relatively higher incidence of *Aspergillus* relative to other fungi (Okoth et al., 2012; Karanja, 2013). However, insignificant difference in incidence of *Aspergillus* between push-pull and maize monocrop systems contradicted the finding which showed significant increases in *Aspergillus* population with minimum tillage and organic matter amendments (Zablotowicz et al., 2007). Thus, more *Aspergillus* expected on a conserved system like push-push which improves organic matter content in the soil and reduces the amount of tillage was not observed. This observation could be explained on the basis that historically, and depending on the cropping season and amounts of rainfall, most farms in western Kenya more often have maize intercropped with food legumes such as common bean (*Phaseolus vulgaris* L.) and peanuts (*Arachis hypogaea* L.) (Mudavadi et al., 2001). Such edible legumes provide beneficial ecological services of soil improvement through addition of organic matter and nitrogen fixation that could increase *Aspergillus* incidence in the soil (Mudavadi et al., 2001).

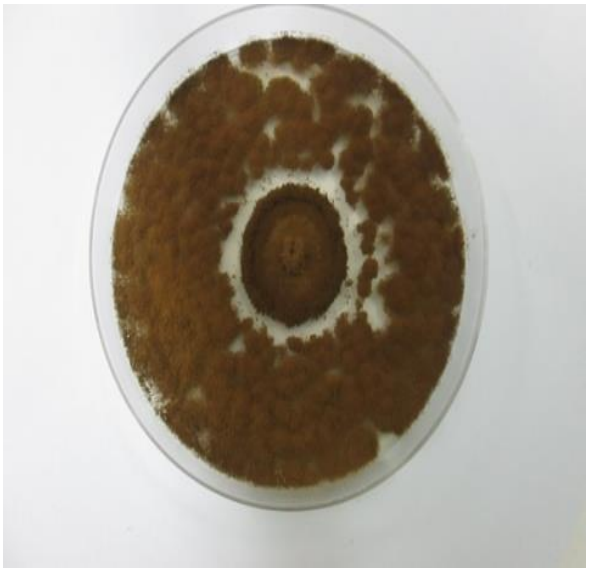
Soil as the main reservoir for both *A. flavus* and *A. parasiticus* has relatively higher frequency of the former

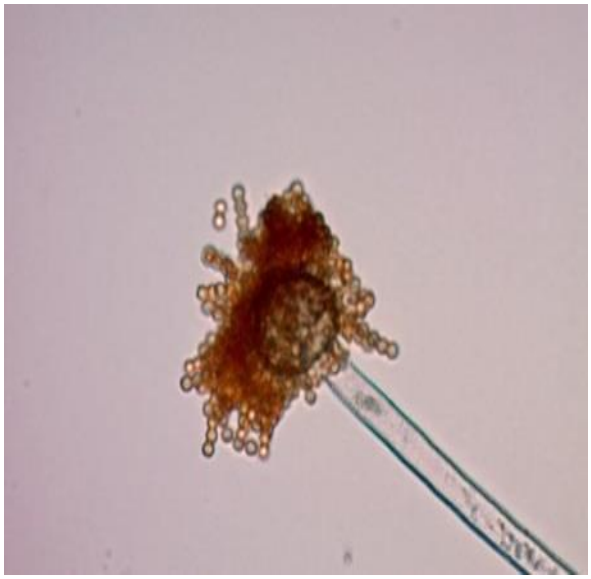
a(i) 

a(ii) 

b(i) 

b(ii) 

c(i) 

c(ii) 

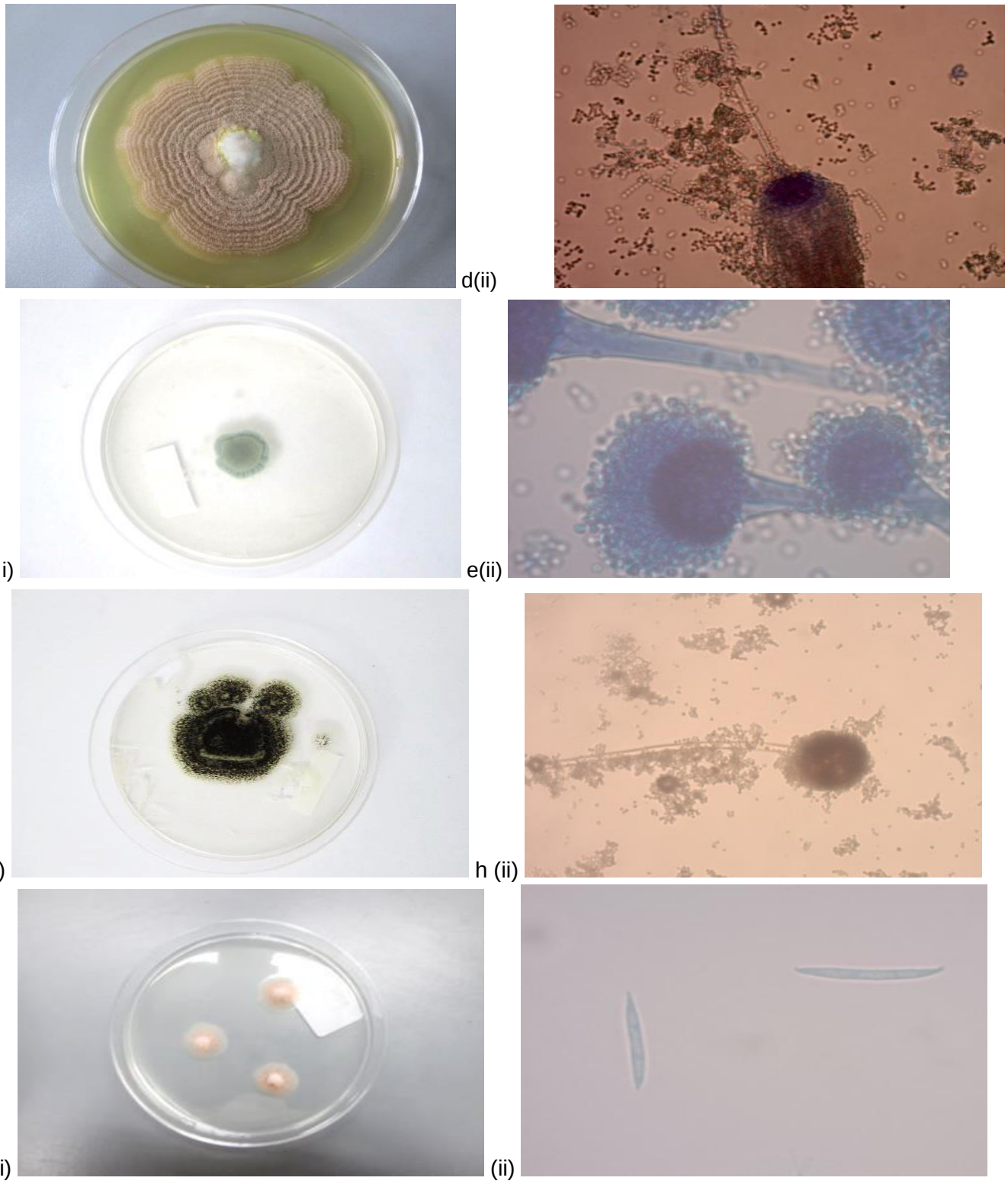


Plate 2. The cultural and morphological traits of the 6 *Aspergillus* species *and Fusarium verticillioides* growing in PDA and CZ after 7 days of incubation. a(i) *A. flavus* greenish yellow surface on CZ; a(ii) a biseriate conidial head with a globose vesicle of *A. flavus* (Mg=1000×); b(i) *A. parasiticus* ivy green surface on CZ, b(ii) *A. parasiticus* with uniserate, globose and conidia in chains (Mg=1000x); c(i) *A. tamarii* dark brown surface on PDA, c(ii) globose vesicle, as observed under the microscope (Mg=1000×); d(i) *A. terreus* sand brown surface on PDA; d(ii) columnar conidial ornamentation in *A. terreus* (Mg=500×); e(i) *A. fumigatus* blue grey surface on CZ; e(ii) *A. fumigatus* subglobose vesicle (1000x); f(i) *A. niger* black surface; f(ii) brownish, relatively long and smooth conidiophore of *A. niger* (Mg=400×); g(i) *Fusarium verticillioides* surface on PDA; g(ii) *Fusarium verticillioides* macroconidia (Mg=1000x).

Figure 2. Gel electrophoresis of PCR amplified translation elongation factor-1 alpha gene (611 bp) on 13 isolates of *Fusarium* section *Liseola*. Isolates denoted as V, *F. verticillioides*; S, *F. subglutinans*; and P, *F. proliferatum*. Lane L, 1 kb base pair ladder; +, positive control for *F. verticillioides*. Electrophoresis was performed on 1.2% agarose gel.

Table 2. Population of *Aspergillus* and *Fusarium* spp. in different cropping systems and seasons.

Fungi species	Cropping system		Season	
	Push-pull maize monocrop		Short rain	Long rain
A. flavus	39 (39.8)	59 (60.2)	31 (31.6)	67 (68.4)
A. fumigatus	37 (55.2)	30 (44.8)	12 (17.9)	55 (82.1)
A. niger	16 (35.6)	29 (64.4)	9 (24.3)	28 (75.7)
A. parasiticus	20 (71.4)	8 (28.6)	22 (78.6)	6 (21.4)
A. terreus	16 (53.3)	14 (46.7)	2 (6.7)	28 (93.3)
A. tamarii	1 (20.0)	4 (80.0)	1 (20.0)	4 (80.0)
F. verticillioides	9 (50.0)	9 (50.0)	2 (11.1)	16 (88.9)

n, number of isolates (%); raw percentages calculated based on counts within district, cropping system and season.

Table 3. Percentage of selected section *Flavi* isolates tested for aflatoxigenicity.

Species	Number of isolate	Toxigenic (%)	Atoxigenic (%)
A. flavus	15	73.3	23.7
A. parasiticus	12	91.7	8.3
Total	27	81.5	18.5

Aspergillus species than the latter (Klich, 2007). However, the frequency of *A. parasiticus* is comparatively higher and more endemic in soils where peanut or sugarcane is grown relative to that under maize (Garber

Table 4. Population (CFU g^{-1}) of *Aspergillus* and *Fusarium* species in different cropping systems and seasons.

Fungi	Cropping system			Season		
	PP (mean CFUg^{-1})	MM (mean CFUg^{-1})	P-value	LR (mean CFUg^{-1})	SR (mean CFUg^{-1})	P-value
A. flavus	650.0	983.3	0.405	1,116.6	516.7	0.0012
A. fumigatus	616.7	500.0	0.330	916.7	200.0	0.001
A .niger	266.7	483.3	0.090	433.3	316.7	0.550
A. parasiticus	333.3	133.3	0.067	100.0	366.7	0.054
A. terreus	266.7	233.3	0.464	466.7	33.3	0.0001
A. tamarii	16.7	66.7	0.311	66.7	16.7	0.311
F. verticillioides	116.0	100.0	0.761	200.0	16.7	0.0045
Total	2,266.1	2,499.9	0.856	3300.0	1466.9	0.001

PP, Push-pull; MM, maize monocrop; CFUg^{-1}, colony forming unit per gram of soil; LR, long rainy season; SR, short rainy season; significance level (p=0.05).

and Cotty, 2014). Although, not measured in the current study, soil temperature has been reported to influence incidence of these fungi, with lower temperatures favoring *A. parasiticus* relative to *A. flavus* (Horn, 2005). Optimally, *A. parasiticus* grow at temperature of 22°C, while *A. flavus*, at 30 to 37°C (Horn, 2005). This cool soil temperature is encouraged by cultural practices such as cover cropping, reduced tillage (Sławiński et al., 2012), and wet season (Horn et al., 1995). In the push-pull system, *Desmodium* provides soil cover for a longer period due to its perennial nature as compared to annual edible intercrop legumes common (bean and peanuts) in western Kenya. The push-pull system also manifest limited tillage practices during land preparation and weeding for conservation, and from cover cropping of *Desmodium,* respectively. This explain probable low soil temperature in PP thus higher population of 71.4% was observed on *A. parasiticus* in soil samples from push-pull as compared to 28.6% in maize monocrop systems. The long dry spells which increases soil temperature in long rainy season than the short rainy season (Mugalavai et al., 2008) also account for low (21.4%) population of *A. parasiticus* during the long rainy season relative to 78.6% during the long rainy season in this study.

Several studies on aflatoxin production have reported fewer incidences of non-aflatoxin (atoxigenic) producers amongst *A. parasiticus* isolates (Tran-Dinh et al., 2009; Barros et al., 2006), except in few cases (Okoth et al., 2012; Salano et al., 2016). The current study supports these findings as 8.3% of *A. parasiticus* isolates as compared to 23.7% of *A. flavus* were positive for aflatoxin production. With more aflatoxigenic fungi, the merit of conserved systems in increasing soil agricultural sustainability might also expose crops to aflatoxin contamination by increasing their *A. flavus* propagules in soils (Zablotowicz et al., 2007). However, contamination of maize is not entirely dependent on the population of *A. flavus* in the soil since maize intercropping which encourages more *A. flavus* has shown low aflatoxin contamination as compared to sole cropping system (Mutiga et al., 2015). Therefore, as revealed in these studies, intercrops are able to reduce *Aspergillus* infections and contamination through other factors such as increased soil nitrogen and limiting insect damage (Brun, 2003).

The frequency of *A. parasiticus* or ratios of *A. flavus/A. parasiticus* (4:1) in this study suggest the potential levels of contamination in maize. Studies show that *A. parasiticus* is comparatively a poor colonizer of aerial plants like maize (Horn, 2003) and have low spore density in air (Horn et al., 1995) than *A. flavus* (Hedayati et al., 2007). Indeed, study by Angle et al. (1982) observed almost complete infection of maize ears with *A. flavus* despite high incidence of both *A. parasiticus* and *A. flavus* in soil. Therefore, increased frequency of occurrence of *A. parasiticus* in push-pull relative to maize monocrop warrants further investigation.

The observations of this study presented *A. terreus*, *A. niger* and *A. fumigatus* as equally abundant in soil, with respective 30, 45 and 69 isolate counts as compared to 98 of *A. flavus*. This observation corroborates reports of most studies on distribution of microflora in the soil (Horn et al., 1995; Horn, 2005). However, they contradicted study by Salano et al. (2016) which reported higher (55) count of *A. niger* than *A. flavus* (26) in eastern province of Kenya. The high presence of these species portends less impact on grain quality as they are not chief producers of agriculturally important mycotoxins (D'Mello et al., 1999; Gnonlonfin et al., 2011). However, recent studies have reported production of fumonisin and ochratoxins A by *A. niger* (Mogensen et al., 2010; Palencia et al., 2010) and territrem by *A. terreus* (El-Sayed Abdalla et al., 1998), while *A. fumigatus* is known causal agent of invasive aspergilosis (Hedayati et al., 2007)

The observations in this study illustrated low (18) isolate count of *Fusarium* section *Liseola* and no *F. graminearum* isolates in soils. This was similar to the study by Okoth and Siameto (2010) on soils in maize fields. The most plausible explanation for this occurrence could be their inherent scarcity (Okoth and Siameto,

2010) or effects of organic matter in the soil (Alakonya et al., 2008) from intercropping systems common in western Kenya. But importantly, low soil *Fusarium* incidence indicated more *Fusarium* infection from aerial spores and external sources.

The cultural identification in *Fusarium* section *Liseola* is demanding and limiting (Summerell et al., 2003), thus molecular methods are used for confirmation. In molecular identification of *F. verticillioides* using translation elongation factor 1-alpha (TEF) gene, 140 isolates culturally identified as *F. verticillioides*, 133 and 4 isolates were confirmed as *F. verticillioides* and *F. proliferatum,* respectively (Rahjoo et al., 2008). Therefore, further identification of species in *Fusarium* section *Liseola* using TEF genes is more accurate and reliable. Evidentially, in this study, 13 isolates were initially identified by cultural characteristics as *F. verticillioide;* 9 isolates were positive for *F. verticillioides* using TEF gene. However, *F. proliferatum* and *F. subgluinans* were not present amongst the isolates.

In conclusion, seasons had significant influence on distribution of *Aspergillus* and *Fusarium* fungi in soil, while cropping system did not. The high *Aspergillus* fungi in the soil in this study show that soil fungal community within the field is a potential risk for aspergillus ear rot infection and aflatoxin contamination, while the low frequency of *F. verticillioides* and *F. graminearum* in the soil samples suggest external inoculum as important for both gibberella and fusarium ear rot infection in the field.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank McKnight foundation and International Center for Insect Physiology and Ecology for all the support provided during this study. Much appreciation also goes to the technical staff and push-pull farmers for their co-operation during this study. They are also indebted to Dr Ulrike Fillinger for assisting with the microscope.

REFERENCES

Aislabie J, Deslippe JR (2013). Soil microbes and their contribution to soil services. In. Dymond JR ed. Ecosystem services in New Zealand – conditions and trends. Lincoln, New Zealand, Manaaki Whenua Press. pp. 112-161.

Alakonya AE, Monda EO, Ajanga S (2008). Management of *Fusarium verticillioides* Root Infection Court in Maize Using Organic Soil Amendments. World Appl. Sci. J. 5(2):161-170.

Angle JS, Dunn KA, Wagner GH (1982). Effect of cultural practices on the soil population of *Aspergillus flavus* and *Aspergillus parasiticus*. Soil Sci. Soc. Am. J. 46:301-304.

Barros G, Chiotta ML, Torres A, Chulze S (2006). Genetic diversity in *Aspergillus parasiticus* population from the peanut agroecosystem in Argentina. Lett. Appl. Microbiol. 42(6):560-566.

Brun AH (2003). Controlling aflatoxin and fumonisin in maize by crop management. J. Toxicol. 22:153-173.

Cotty PJ (1994). Comparison of four media for the isolation of *Aspergillus flavus* group fungi. Mycopathologia 125:157-162.

D'Mello JPF, Placinta CM, Macdonald AMC (1999). *Fusarium* mycotoxins: A review of global implications for animal health, welfare and productivity. Anim. Feed Sci. Technol. 80:183-205.

El-Sayed Abdalla A, Zeinab Kheiralla M, Sahab A, Hathout A (1998). *Aspergillus terreus* and its toxic metabolites as a food contaminant in some Egyptian bakery products and grains. Mycotoxin Res. 14(2):83-91.

Garber NP, Cotty PJ (2014). *Aspergillus parasiticus* communities associated with sugarcane in the Rio Grande Valley of Texas: Implications of global transport and host association within *Aspergillus* section *Flavi*. Phytopathology 104(5):462-471.

Gherbawy YAMH, Adler A, Prillinger H (2001). Genotypic identification of *Fusarium subglutinans*, *F. proliferatum* and *F. verticillioides* strains isolated from maize in Austria. Proc. Egypt. J. Biol. 3:37-46.

Gnonlonfin GJB, Adjovi Y, Gbaguidi F, Gbenou J, Katerere D, Brimer L, Sanni A (2011). Scopoletin in cassava products as an inhibitor of aflatoxin production. J. Food Saf. 31(4):553-558.

Hedayati M, Pasqualotto A, Wam P, Bowyer P, Denning D (2007). *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiology 153(6):1677-1692.

Horn BH (2003). Ecology and population biology of aflatoxigenic fungi in soil. J. Toxicol. Toxin Rev. 22:315-379.

Horn BW (2005). Colonization of wounded peanut seeds by soil fungi: selectivity for species from Aspergillus section Flavi. Mycologia 97:202-217.

Horn BW, Dorner JW (1998). Soil populations of Aspergillus species from section Flavi along a transect through peanut-growing regions of the United States. Mycologia 1:767-776.

Horn BW, Greene RL, Dorner JW (1995). Effect of Corn and Peanut Cultivation on Soil Populations of *Aspergillus flavus* and *A. parasiticus* in Southwestern Georgia. Appl. Environ. Microbiol. 62:2472-2475.

Karanja LW (2013). The occurrence of *Aspergillus* section *Flavi* in soil and maize from Makueni, County, Kenya. M.Sc.Thesis, University of Nairobi Kenya.

Khan ZR, Midega C, Pittchar J, Pickett J, Bruce T (2011). Push–pull technology: a conservation agriculture approach for integrated management of insect pests, weeds and soil health in Africa. Int. J. Agric. Sustain. 9(1):162-170.

Klich M A (2002). Biogeography of Aspergillus species in soil and litter. Mycologia 94:21-27.

Klich MA (2007). *Aspergillus flavus*: the major producer of aflatoxin. Mol. Plant Pathol. 8:713-722.

Leslie JF, Summerell BA (2006). The *Fusarium* laboratory manual. 1st ed. Ames, Iowa: Blackwell Publishing.

Mogensen JM, Frisvad JC, Thrane U, Nielsen KF (2010). Production of fumonisin B2 and B4 by *Aspergillus niger* on grapes and raisins. J. Agric. Food Chem. 58:954-958.

Mudavadi PO, Otieno KJ, Wanambacha W, Odenya JO, Odendo M, Njaro OK (2001). Smallholder dairy production and marketing in western Kenya: a review of literature. Smallholder Dairy (Research and Development) Project Research Report. ILRI (International Livestock Research Institute), Nairobi, Kenya.

Mugalavai EM, Kipkorir EC, Raes D, Rao MS (2008). Analysis of rainfall onset, cessation and length of growing season for western Kenya. Agric. For. Meteorol. 148:1123-1135.

Mutiga SK, Hoffmann V, Harvey JW, Milgroom MG, Nelson RJ (2015). Assessment of aflatoxin and fumonisin contamination of maize in Western Kenya. Phytopathology 105(9):1250-1261.

Okoth S, Nyongesa B, Ayugi V, Kang'ethe E, Korhonen H, Joutsjoki V (2012). Toxigenic potential of Aspergillus species occurring on maize kernels from two agro-ecological zones in Kenya. Toxins (Basel). 4:991-1007.

Okoth S, Siameto E (2010). Suppression of Fusarium spp. in a Maize and Beans Intercrop by Soil Fertility Management. J. Yeast Fungal Res. 1(2):35-43.

Palencia ER, Hinton DM, Bacon CW (2010). The black *Aspergillus*

species of maize and peanuts and their potential for mycotoxin production. Toxins 20:399-416.
Pereira P, Nesci A, Castillo C, Etcheverry M (2011). Field studies on the relationship between *fusarium verticillioides* and maize (Zea mays l.): effect of biocontrol agents on fungal infection and toxin content of grains at harvest. Int. J. Agron. Volume 2011 (2011), Article ID 486914, 7 pages. doi:10.1155/2011/486914
Rahjoo V, Zad J, Javan-Nikkhah M, Gohari AM, Okhovvat SM, Bihamta MR, Razzaghian J, Klemsdal SS (2008). Morphological and molecular identification of Fusarium isolated from maize ears in Iran. J. Plant Pathol. 90:463-468.
Rao CY, Fink RC, Wolfe LB, Liberman DF, Burge HA (1997). A study of aflatoxin production by Aspergillus flavus growing on wallboard. J. Am. Biol. Saf. Assoc. 2(4):36-42.
Rodrigues P, Soares C, Kozakiewicz Z, Paterson RRM, Lima N, Venâcio A (2007). Identification and characterization of *A. flavus* and aflatoxins. In: Méndez-Villas, A. (Editor) – "Communicating Current Research and Educational Topics and Trends in Applied Microbiology. pp. 527-534.
Salano EN, Obonyo MA, Toroitich FJ, Odhiambo B O, Aman BO (2016). Diversity of putatively toxigenic *Aspergillus* species in maize and soil samples in an aflatoxicosis hotspot in Eastern Kenya. Afr. J. Microbiol. Res. 10(6):172-184.
Schaafsma AW, Tamburic-Ilincic L, Hooker DC (2005). Effect of previous crop, tillage, field size, adjacent crop, and sampling direction on airborne propagules of Gibberella zeae/Fusarium graminearum, Fusarium head blight severity, and deoxynivalenol accumulation in winter wheat. Can. J. Plant Pathol. 27:217-224.
Shurtleff MC (1980). Compendium of Corn Diseases. 2nd Edition, American Phytopathology Society. P 105.
Sławiński C, Cymerman J, Witkowska-Walczak B, Lamorski K (2012). Impact of diverse tillage on soil moisture dynamics. Int. Agrophys. 26:301-309.
Tran-Dinh N, Kennedy I, Bui T, Carter D (2009). Survey of Vietnamese peanuts, corn and soil for the presence of *Aspergillus flavus* and *Aspergillus parasiticus*. Myopathologia 168:257-268.
Zablotowicz RM, Abbas HK, Locke M A (2007). Population ecology of *Aspergillus flavus* associated with Mississippi Delta soils. Food Addit. Contam. 24:1102-1108.