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Isolation and characterisation of aflatoxigenic *Aspergillus* species from maize and soil samples from selected counties of Kenya

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Aflatoxin contamination of maize put the health and well-being of Kenyan people at risk, primarily children. Aflatoxigenic fungi can infect grains from pre-harvest stages in the field to post-harvest stages in the stores. The aim of this study was to isolate and characterize aflatoxigenic *Aspergillus* spp. from maize and soil samples from selected counties of Kenya: Makueni, Nyeri, Bungoma, Uasin Gishu and Siaya. Isolation was done using the direct plating technique of surface-sterilized grains on Czapek Dox Agar medium and plating of serially diluted soil samples. *Aspergillus* colonies were purified and identified using colony growth characteristics, colony colour on PDA media and microscopic characterisation. In total, 174 *Aspergillus* isolates were obtained, where 82.3% came from maize samples while 17.7% were from soil samples. Makueni County had the highest number of *Aspergillus* isolates at 58.1%, Nyeri 12.6%, Uasin Gishu 10.3%, Siaya 10.3% and Bungoma 8.6%. The characterization process identified 10 different *Aspergillus* spp.; 78.5% were *Aspergillus flavus,* 8.0% *A. versicolor,* 3.4% *A. parasiticus,* 2.3% *A. clavatus*, 2.3% *A. sydowii*, 2.3% *A. fumigatus,* 1.1% *A. glaucus*, 1.1% *A. nidulans*, 0.6% *A. candidus* and 0.6% *A. wentii*. The results evidence that maize grains and fields in the various counties are highly contaminated with aflatoxigenic *Aspergillus* species.

Key words: Aflatoxin, *Aspergillus,* maize.

INTRODUCTION

Aflatoxin is one of the most famous mycotoxins produced by several species of *Aspergillus* including *A. flavus* and *A. parasiticus* in a wide variety of agricultural commodities including grains (maize), legumes and nuts (Patten, 1981). The main aflatoxin producing fungi *A. flavus, A. parasiticus* and *A. nomius* can infect maize from pre-harvest stages in the field to post-harvest stages in the stores. Species of *Aspergillus* are almost ubiquitously present in soils of tropical areas (Ranajit et al., 2005). Although, most species of *Aspergillus* are not of much consequence in agriculture, some species are found in plant products, particularly oil-rich seeds. Contamination of seeds with highly poisonous aflatoxins results from the presence of toxigenic strains of four species of *Aspergillus: A. flavus, A. parasiticus, A. nomius* and *A. bombycis,* each producing a combination of different types of aflatoxins (Ranajit et al., 2005).

In Africa, aflatoxins have an impact on human and

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Abbreviations: PDA, Potato dextrose agar; **CZ,** Czapek Dox Agar; **ANOVA,** analysis of variance.

animal health and on trade. Aflatoxin has been reported to be associated with the exacerbation of the energy malnutrition syndrome in children and vitamin A malnutrition in animals. In various animal models, in addition to being hepatotoxic, aflatoxin causes significant growth faltering and is strongly immune-suppressive at weaning (Wild et al., 1992). Similar effects have been reported in human population in a few African countries such as Ghana and it has been recently shown that 99% of all children weaned from mother's milk to maize-based diets in Benin and Togo had aflatoxin in their blood, indicating ingestion of aflatoxin-contaminated food (Ranajit et al., 2005).

In developing countries, the contamination of crops with aflatoxin leads not only to economic losses, but also has a severe impact on human health. In Africa, a continent that relies on vulnerable crops such as groundnuts and maize as dietary staples, aflatoxin contamination causes major health problems (Shephard, 2003). People in rural areas may have no option but to consume contaminated crops on a daily basis. This moderate, chronic intake of aflatoxin via food can lead to severe pathological conditions, including liver cancer, immune system deficiency and impaired development of children (Williams et al., 2004). Malnutrition, a common condition in rural Africa, increases disease prevalence and further reduces the ability of the human body to cope with aflatoxin exposure. Chronic aflatoxin poisoning reduces life expectancy.

Acute aflatoxin poisoning is caused by ingestion of high levels of the toxin. Immediate consequences are severe liver damage, acute jaundice and hepatitis, which may subsequently result in death (Bennett and Klich, 2003). Although on a global basis, deaths from acute aflatoxin poisoning are rare, Kenya has experienced dramatic outbreaks of mycotoxin poisoning resulting in loss of lives. In 2004, an acute aflatoxicosis outbreak occurred in Machakos, Kenya resulting in 317 cases and 125 deaths, while cases of liver cancer have been linked to high levels of aflatoxins in the Lake Victoria Basin (LVB) (Anonymous, 2004).

MATERIALS AND METHODS

Source of maize and soil samples

A total of 113 maize and 113 soil samples obtained randomly from maize farms and rural households in Makueni, Nyeri, Bungoma south, Moiben and Ugunja districts were used in this study. These districts were selected to represent the following counties respectively: Makueni, Nyeri, Bungoma, Uasin Gishu and Siaya (Figure 1). Makueni, Nyeri and Siaya counties were selected due to the fact that aflatoxin poisoning cases have been previously reported in those counties, while Bungoma County lies on a transit route where exchange of maize from other countries such as Uganda is possible. Uasin Gishu County was selected due to the fact that there is high maize production in the county every year. In each district, four villages were selected, that is, towards the east, west, north and south of the district. In each village, five homes (200 to 300 m apart) were randomly selected based on information gathered from local residents as to which homes had renowned maize farmers and sampling was done in those homes. Most of these areas are in mid-altitude agro ecological zones with warm and humid conditions such as Ugunja while Nyeri and Moiben districts have high relative humidity thus mould invasion is primarily due to inadequate drying and improper storage (Pitt, 2000). These factors favour development of moulds and production of mycotoxins (Kaaya et al., 2006). These areas have unpredictable rainfall patterns making it difficult for small scale farmers to efficiently dry their produce. In Nyeri, Bungoma south, Moiben and Ugunja districts 20 maize samples (500 g each) and 20 soil samples (100 g each) were collected. However, in Makueni district 33 maize samples (500 g each) and 33 soil samples (100 g each) were collected. Soil samples were collected from maize farms. This higher number of samples collected from Makueni district was attributed to the fact that the district had several reported cases of aflatoxin poisoning. Soil samples were collected from floors of maize stores, maize farms and the bare ground in the homestead where maize is usually dried. The samples were collected in properly labeled khaki paper bags to minimize saprophytic fungal contamination and transported in a cool box to the laboratory for analysis. The samples were stored at 4°C until further analysis.

Isolation from maize and soil samples

The maize grains were surface sterilized in 2% sodium hypochlorite and rinsed three times with sterile distilled water. A total of 20 grains were randomly picked per sample and plated (five per plate) on Czapek Dox Agar medium (CZ) amended with 50 mg of streptomycin and 50 mg of penicillin. The soil samples were first serially diluted before plating. One gram of the soil sample was dissolved in 9 ml sterile distilled water and serially diluted to 10^{-4}. One millilitre of the 10^{-3} and 10^{-4} dilutions were plated in CZ amended with 50 mg of streptomycin and 50 mg penicillin. The plates were then incubated at 28°C for 7 days and the number of kernels showing growth of *Aspergillus* species in each Petri dish was counted (Plate 1A) while for the dilution plates the number of *Aspergillus* colonies per plate was counted (Plate 1B). *Aspergillus* colonies were sub-cultured on potato dextrose agar (PDA) and incubated at 28°C for 7 days. Treatments were replicated four times and the experiment was done in a complete randomized design.

Identification and characterisation of *Aspergillus* species

The resulting cultures were identified to species level based on cultural and morphological characteristics like colony diameter, colony colour on agar and reverse, colony texture and zonation (Klich, 2002). Morphological features were studied under the microscope and the major and remarkable microscopic features that were considered were conidiophores, conidial shape, phialides and metulae, presence and shape of vesicles (Larone, 1995). Contemporary diagnosis of the *Aspergillus* species was based on the descriptions and keys of Klich (2002). For microscopic characterisation, slide cultures of the isolates were prepared and incubated in moist chambers at 28°C for 5 days before observation under a light microscope.

Data analysis

SAS version 9.0 was used in the data analysis. One way analysis of variance (ANOVA) was used to test whether the *Aspergillus* isolates obtained from maize and soil in the five districts were significantly different from each other based on their frequency of occurrence.

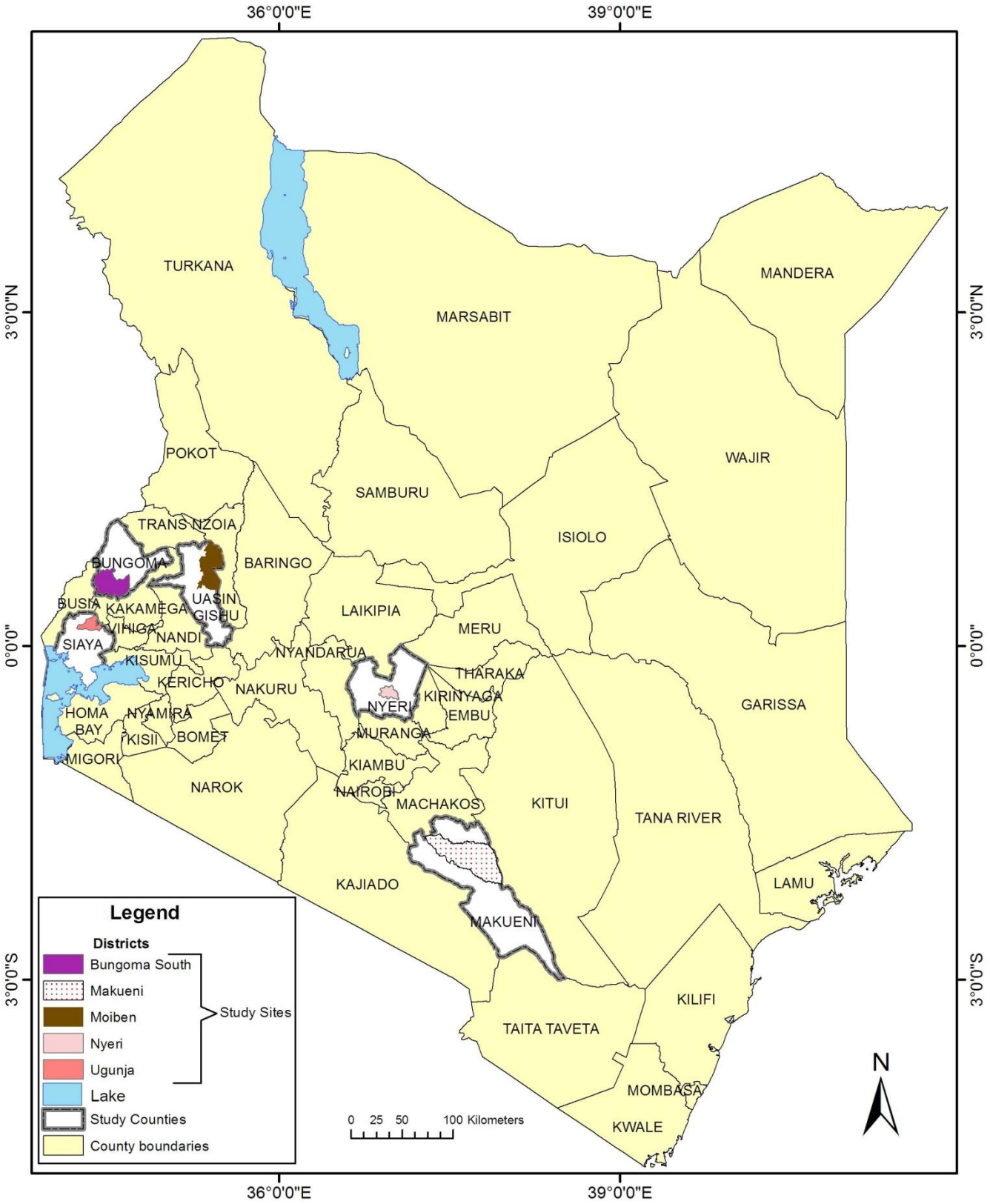


Figure 1. Map of the study area. Key: Bungoma (Mean temperature: 20°C, RH: 68 to 76%), Makueni (Mean temperature: 22.5°C, RH: 48 to 57%) Uasin Gishu (Moiben) (Mean Temp: 17.5°C, RH: 75 to 80%), Nyeri (Mean temperature: 16°C, RH: 64-83%) and Siaya County (Mean temperature: 23.5°C, RH: 53 to 60%). Source: Cartographer, Department of Environmental Science, Egerton University (2013).

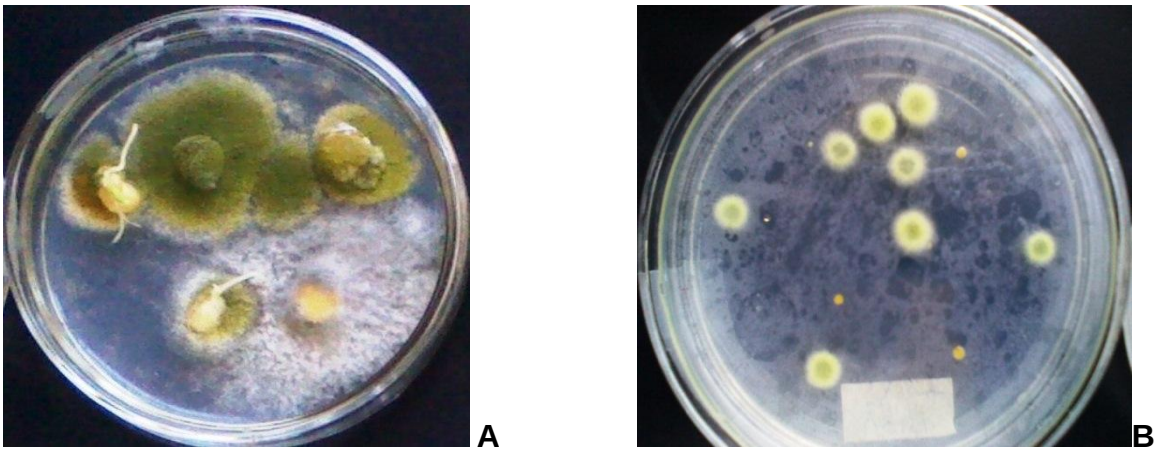


Plate 1. (a) *Aspergillus* spp. growth on maize kernels cultured on CZ media. (b) *Aspergillus* spp. colonies growth from soil serial dilutions cultured on CZ media.

Table 1. Frequency of *Aspergillus* isolates from maize and soil samples and distribution of various *Aspergillus* species across all the five districts.

Source	District						
	Makueni	**Nyeri**	**Moiben**	**Ugunja**	**Bungoma**	**Total**	**% Total**
Maize isolates	99	17	7	14	7	144	82.3
Soil isolates	2	5	11	4	8	30	17.7
***Aspergillus* species**							
A. flavus	96	15	4	10	11	136	78.5
A. versicolor	0	2	8	2	2	14	8.0
A. parasiticus	1	0	1	4	0	6	3.4
A. clavatus	0	0	4	0	0	4	2.3
A. sydowii	1	2	0	1	0	4	2.3
A. fumigatus	1	2	0	0	1	4	2.3
A. glaucus	1	0	1	0	0	2	1.1
A. nidulans	1	1	0	0	0	2	1.1
A. candidus	0	0	0	1	0	1	0.6
A. wentii	0	0	0	0	1	1	0.6
Total No.	101	22	18	18	15	174	100
Total (%)	58.0	12.6	10.4	10.4	8.6		100

One way ANOVA was also used to test whether the distribution of the various *Aspergillus* spp. were significantly different in all the five districts.

RESULTS

Aspergillus isolates from maize and soil and their incidences across all the districts

The incidence of the maize and soil isolates from all the districts was recorded as shown in Table 1. The percentage ratio of maize to soil *Aspergillus* isolates in each district was as follows: Makueni 98%: 2%, Nyeri 77.3%: 22.7%, Moiben 38.9%: 61.1%, Ugunja 77.8%: 22.2% and Bungoma South 46.7%: 53.3%. In Makueni, Nyeri and Ugunja districts, there was a higher number of maize isolates than soil isolates, while in Moiben and Bungoma South districts there was slightly more soil isolates than maize isolates.

The high number of isolates in maize than in soil especially in Makueni may be due to on-farm maize grain processing as this has been reported to be a common practice among the farmers, especially of eastern Kenya (Makueni) (Strosnider et al., 2006). Results obtained by Muthomi et al. (2012) showed that maize and maize products sampled at farm level had a higher risk of contamination by *Aspergillus* spp. and aflatoxins. These results show that maize and soil across all the five districts are highly contaminated with fungi of the genus *Aspergillus*. The frequency of the *Aspergillus* isolates

across all the five districts analyzed through ANOVA, was not significantly different (P = 0.5489). This was expected because the ubiquitous nature of these *Aspergillus* spp. enables them to grow on dead organic matter everywhere in nature, their presence is only visible to the unaided eye when mould colonies form and they derive energy from the organic matter in which they live (Ryan and Ray, 2004).

Morphological, cultural and microscopic characterisation of the *Aspergillus* species

Morphological, cultural and microscopic features of the isolates were studied and recorded (Table 2). A total of 10 *Aspergillus* species were identified and characterized. Most isolates of the same species, despite originating from different districts, showed similarities in their morphological and cultural characteristics in PDA media. *A. flavus* strains EM324, EM244, EM182 and EM1112 from Makueni; NM091, NM083 and NM084 from Nyeri; BM071 and BS116 from Bungoma UM127 from Ugunja and RS013, RM023-2 from Moiben, all had similar surface colour of olive green with whitish margins and reverse colour of creamish to yellow on PDA. Similarly, there were only slight variations in their colony diameters which ranged between 37 to 42 mm. For *A. glaucus,* strains RS024 from Moiben and EM211 from Makueni also had less variable colony colour of green with yellow areas and reverse colour of creamish yellow, and the colony diameter ranged between 26 and 29 mm. *A. parasiticus* strain UM082 (Plate 2b) showed characteristics similar to *A. flavus* strains (Plate 2b) apart from the colony colour which was conifer green. *A. versicolor* strains BS203 from Bungoma had a colony diameter of 17.0 mm which differed with other *A. versicolor* strains RS016 and RS162 from Moiben which had colony diameters ranging from 32 to 34 mm. *Aspergillus clavatus* strains were from Moiben district and were all similar in their morphological characteristics. *Aspergillus sydowii* strains from Makueni, Nyeri and Ugunja were not different from each other in their morphological characteristics. *Aspergillus fumigatus* from Makueni, Nyeri and Bungoma districts also did not show major differences in their morphological characteristics. *Aspergillus nidulans* strains from Makueni and Nyeri were not different from each other morphologically. *Aspergillus wentii* and *A. candidus* occurred singly from Bungoma and Ugunja districts, respectively, and had no strains to be compared with. The cultural, morphological and microscopic characteristics of the various *Aspergillus* spp. were recorded as shown in Table 2. The 10 different *Aspergillus* spp. identified and characterized are shown in Plate 2a - j. Plate 3a and b show microscopic characteristics of *A. flavus* and *A. parasiticus*. Identification of *A. flavus* is not an easy task due to its similarities with *A. parasiticus* and *A*. *nomius*. However, the other *Aspergillus* spp. are distinctly different from each other, and with the help of the descriptions and keys by Klich (2002), it was possible to achieve a reliable identification and discrimination of the various isolates of *Aspergillus* species.

Distribution of the *Aspergillus* species and their incidences in the five districts

The *Aspergillus* species, having been identified and characterized to species level as shown in Table 2, were grouped according to their species and district of origin and data recorded as shown in Table 1. *Aspergillus flavus* species was the most frequently occurring species in almost all the districts apart from Moiben district which had more *A. versicolor* than *A. flavus*. These results (Table 1) are evidence that *A. flavus* is the major contaminant of maize and soil across all the five districts under this study. Statistically, these results further showed that there was no significant difference (P = 0.0699) in the frequency of occurrence among the various *Aspergillus* species found in all the five districts at a 95% confidence limit. SAS version 9.0 was used in the data analysis.

DISCUSSION

The high occurrence of *Aspergillus* spp. moulds in the maize and soil samples in the various districts can be attributed to factors such as warmth and humidity in the LVB region (Ugunja), high relative humidity with low temperatures in Nyeri, Bungoma and Moiben districts leading to improper drying of the maize and high temperatures with drier conditions in Makueni district which predisposes maize to the moulds at pre-harvest stage in the field and post-harvest stage in storage (Okoth et al., 2012). The fungus forms sclerotia that allows for saprophytic survival for extended periods in the soil, maize residue and maize-cobs (Wagacha and Muthomi, 2008). The propagules in the soil and crop debris act as the primary source of contamination, infecting maturing maize crops (Atehnkeng et al., 2008b).

These results were also anticipated in the Lake Basin Region (Ugunja), because of the relatively high temperature and relative humidity which provided optimum growth conditions for the *Aspergillus* spp. (Anonymous, 2004). Higher number of *Aspergillus* spp. was recorded in grain samples from the semi-arid Makueni district than those from the humid regions in Moiben, Nyeri, Bungoma and Ugunja. These results are in agreement with the findings of Muthomi et al. (2012) where higher *Aspergillus* spp. isolation frequencies were recorded in grain samples from the semi-arid eastern region than those from the humid North Rift regions.

Table 2. Cultural, morphological and microscopic characterization of *Aspergillus* isolates.

Group no.	Isolate	Colony colour on PDA		Colony size (mm)	Conidiophore	Conidial head	Shape of Vesicles	Seriation	Conidial shape	Isolate name
		Conidia	Reverse							
G3	EM324	Olive green with whitish margin	Yellowish with grey margin	41.3±3.1	Short, finely roughened wall and colourless	Radiate	Subclavate	Uniseriate	Spherical	*A. flavus*
G7	NM091	Olive green with dirty white margin	Cream centre with alternating grey and cream periphery	35.3±5.1	Short, smooth walled and colourless	Radiate	Subclavate	Uniseriate	Spherical	*A. flavus*
G8	NM083	Olive green with white margin	Creamish	34.7±0.6	Slightly long, rough walled and colourless	Radiate	Subclavate	Biseriate	Spherical	*A. flavus*
G9	EM244	Olive green with white margin	Cream centre with alternating grey and cream rings	34.0±4.0	Short, finely roughed walled and colourless	Radiate	Subclavate	Uniseriate	Spherical	*A. flavus*
G13	NM084	Olive green with cream margin	Cream center with alternative grey and cream concentric rings	37.7±2.5	Short, smooth walled and colourless	Radiate	Subclavate	Uniseriate	Spherical	*A. flavus*
G16	EM182	Olive green with whitish margin	Creamish	32.3±3.2	Short, smooth walled and colourless	Columnar	Subclavate	Uniseriate	Spherical	*A. flavus*
G17	BM071	Olive green with cream margin	Cream center with alternative grey and cream concentric rings	36.7±2.3	Short, rough walled and colourless	Columnar	Subclavate	Uniseriate	Spherical	*A. flavus*
G18	EM1112	Olive green with whitish margin	Cream with grey margin	35.0±1.0	Short, smooth walled and colourless	Radiate	Globose	Uniseriate	Spherical	*A. flavus*
G19	UM127	Olive green with whitish margin	Cream with grey margin	28.3±1.5	Slightly long, smooth walled and colourless	Radiate	Subclavate	Uniseriate	Spherical	*A. flavus*
G21	BS203	Grey with a yellow at the middle	Greenish centre with creamish margin	17.0±2.7	Short, finely roughened wall and colourless	Columnar	Subclavate	Biseriate	Spherical	*A. versicolor*
G22	RS016	Creamish centre with green to sulphur yellow margins	Sulphur yellow	34.0±1.0	Short, finely roughened wall and colourless	Radiate	Globose	Biseriate	Spherical	*A. versicolor*
G31	RS024	Green with sulphur margin	Yellow	26.7±2.1	Long, smooth walled and colourless	Radiate	Subclavate	Uniseriate	Spherical	*A. glaucus*
G32	BS023	Sulphur yellow centre with alternating grey and sulphur yellow concentric rings	Sulphur yellow	26.0±2.0	Short, smooth walled and colourless	Columnar	Subclavate	Biseriate	Spherical	*A. wentii*

Table 2. Contd

G34	RS162	Grey centre with yellowish margin	Yellow	32.7±1.2	Short, smooth walled and colourless	Radiate	Globose	Biseriate	Spherical	*A. versicolor*
G38	US098	Greyish blue with white margin	Yellow	29.0±1.0	Slightly long, smooth walled and colourless	Radiate	Globose	Biseriate	Spherical	*A. sydowii*
G41	RS013	Olive green with cream margin	Yellow centre with cream margin	36.0±2.0	Long, smooth walled and colourless	Radiate	Globose	Uniseriate	Spherical	*A. flavus*
G49	UM082	Conifer green with cream margin	Yellow centre with cream margin	26.7±0.6	Slightly long, smooth walled and colourless	Radiate	clavate	Uniseriate	Spherical	*A. parasiticus*
G50	BM092	Grey centre with green margin	Cream centre with grey margin	41.3±3.1	Slightly long, smooth walled and colourless	Radiate	Globose	Uniseriate	Spherical	*A. fumigatus*
G58	US184	White	Deep yellow	15.7±1.5	Short, finely roughened wall and colourless	Radiate	Globose	Uniseriate	Spherical	*A. candidus*
G64	RM143	Bluish green with white margin	Brown center with alternating cream and brown concentric rings	31.7±1.5	Short, finely roughened wall and brownish	Radiate	Subclavate	Uniseriate	Spherical	*A. clavatus*
G68	ES042	Green	Deep red	23.7±2.1	Short, smooth walled and colourless	Columnar	Subclavate	Biseriate	Spherical	*A. nidulans*
G71	BS116 RM023-2	Olive green	Creamish yellow	36.0±2.0	Short, smooth walled and colourless	Columnar	Subclavate	Uniseriate	Spherical	*A. flavus*
G72	EM211	Green centre with alternating yellow and green concentric rings	Cream	29.0±1.7	Slightly long, smooth walled and colourless	Columnar	Subclavate	Uniseriate	Spherical	*A. glaucus*

E, Eastern (Makueni); R, Rift valley (Moiben); B, Bungoma; N, Nyeri; U, Ugunja; M, maize; S, soil; (01-32), sample number in each district; (1 -12), isolate number from each sample; (-2), purification number.

Mechanical damage during and after harvest, facilitates entry of the fungal spores either in maize cobs or grains (Pitt, 2000). This could explain why very high quantities of the *Aspergillus* spp. were isolated from the maize samples because some of them had damaged grains that might have predisposed the grains to the fungus infection.

These results resonates with the study of Muthomi et al. (2012) in which the specific *Aspergillus* spp. isolated from whole and unprocessed maize grain and soil from North Rift and Eastern regions were: *A. flavus*, *A. niger*, *A. fumigatus*, *A. versicolor*, *A. terreus*, *A. clavatus* and *A. ochraceus*. The most frequently isolated were *A. flavus* and *A. niger*, while A*. clavatus* was the least frequently isolated *Aspergillus* species and was mainly isolated in samples from the humid North Rift region. Similarly, in this study, *A. flavus* had the highest incidences in all the districts apart from Moiben. Moiben (North Rift) had the highest incidence of *A. clavatus* and this support the findings of Muthomi et al. (2012) where *A. clavatus* was predominantly isolated from the humid North rift

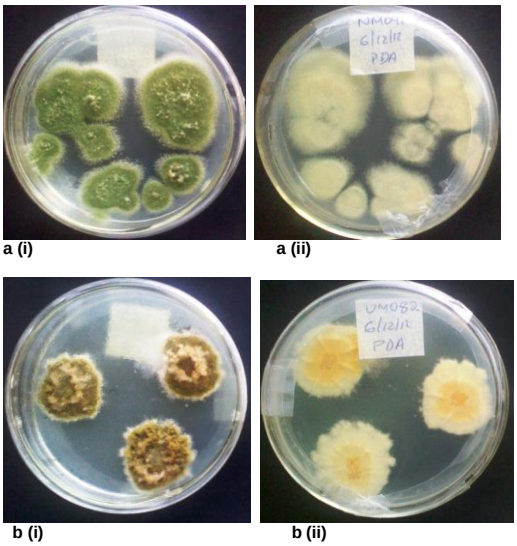


Plate 2. Morphological and cultural characteristics of the 10 *Aspergillus* species growing on PDA media after seven days of growth at 28°C. a (i) *A. flavus* NM091 (surface); a (ii) *A. flavus* NM091 (reverse); b (i) *A. parasiticus* UM082 (surface); b (ii) *A. parasiticus* UM082 (reverse).

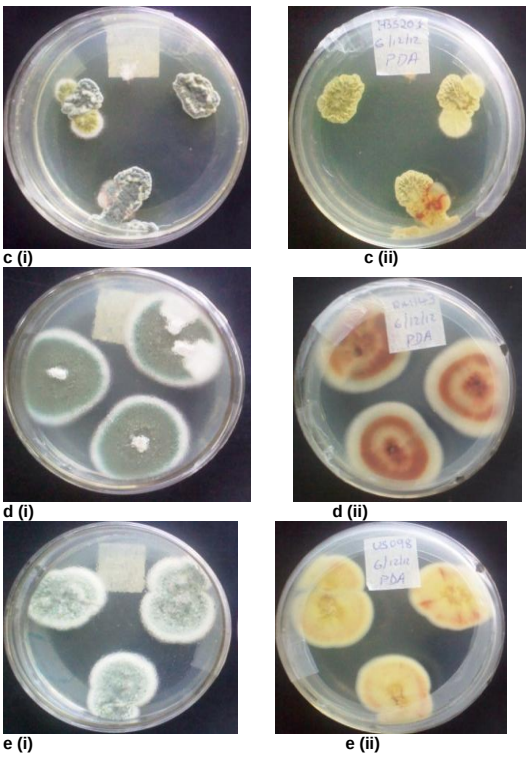


Plate 2 Contd. Morphological and cultural characteristics of the 10 *Aspergillus* species growing on PDA media after seven days of growth at 28°C. c (i) *A.versicolor* BS203 (surface) ; c (ii) *A. versicolor* BS203 (reverse); d (i) *A. clavatus* RM143 (surface) d (ii) *A. clavatus* RM143 (reverse); e (i) *A. sydowii* US098 (surface) 2e (ii) *A. sydowii* US098 (reverse).

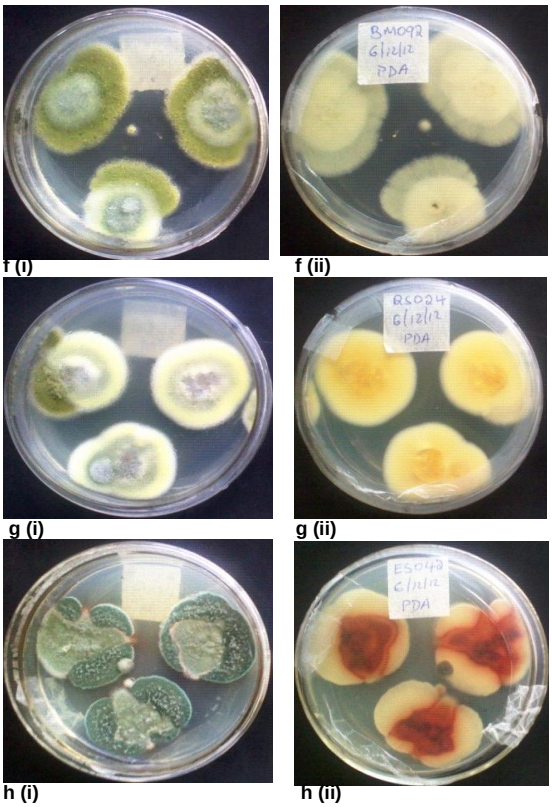


Plate 2 Contd. Morphological and cultural characteristics of the 10 *A.* species growing on PDA media after seven days of growth at 28°C. f (i) *A. fumigatus* BM092 (surface) ; f (ii) *A. fumigatus* BM092 (reverse); g (i) *A. glaucus* RS024 (surface); g (ii) *A. glaucus* RS024 (reverse); h (i) *A. nidulans* ES042 (surface); h (ii) *A. nidulans* ES042 (reverse).

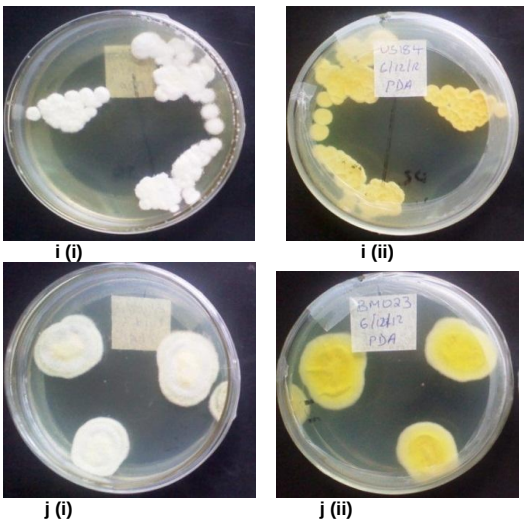
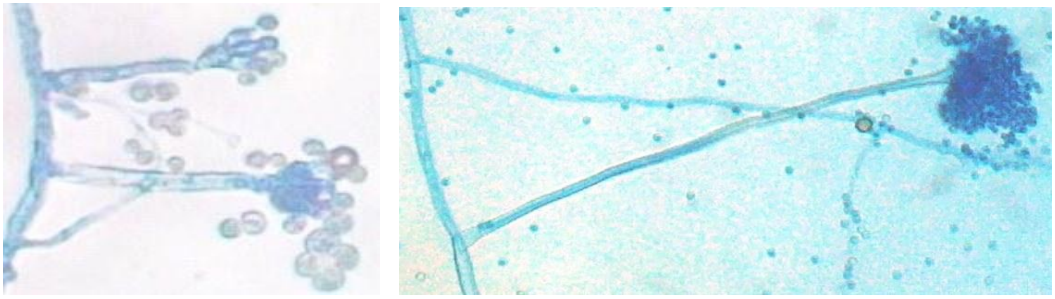


Plate 2 Contd. Morphological and cultural characteristics of the 10 *Aspergillus* species growing on PDA media after seven days of growth at 28°C. i (i) *A. candindus* US184 (surface) ; i (ii) *A. candindus* US184 (reverse); j (i) *A. wentii* BM023 (surface); j (ii) *A. wentii* BM023 (reverse).



(a) (b)

Plate 3. Microscopic characteristics of *A. flavus* (EM244) and *A. parasiticus* (UM082). a: Uniseriate conidial heads with subclavate vesicle of *A. flavus* strain EM244. b: Radiate conidial head shape attached to a long stipe of *A. parasiticus* strain UM082.

region.

The pervasive nature of *Aspergillus* spp., their high ability to colonize diverse substrates and lack of effective control measures (Souza et al., 2005) could have contributed to their high occurrences in maize and soil from the five districts. *Aspergillus* spp. are more commonly associated with cereals during drying and storage. *A. flavus* and *A. parasiticus* have a particular affinity for cereals and can be recognized by yellow-green or grey green colour on maize kernels in the field and in storage (Varga et al., 2011). This study found out that *A. flavus* was the most prevalent *Aspergillus* spp. in all the districts except for Moiben district where *A. versicolor* was the predominant species.

These results are in line with the findings of Okoth et al. (2012) and Muthomi et al. (2012) who reported that *A. flavus* was the most dominant *Aspergillus* spp. in Makueni and Nandi counties and also in Eastern region and North Rift region, respectively. Grain samples collected from farmers in the semi-arid eastern region (Makueni) during the short rainy seasons had higher incidences of *A. flavus,* of up to 14% as compared to grain harvested during the long rainy seasons (Muthomi et al., 2012). This statement supports the high levels of *A. flavus* in Makueni district since sampling in this study was done immediately after the short rainy season maize harvest in all the districts. Additionally, variations in fields' cropping history, cultivation practices, sowing dates, seed varieties planted and/or soil types can differ greatly in aflatoxigenic fungi and aflatoxin contamination (Munkvold et al., 2009). These dynamics may clarify the variances in the incidences and type of the *Aspergillus* spp. isolated from the five districts. Similarly, damaged maize kernels also favours the growth of *A. flavus* as compared to any other *Aspergillus* species (Pitt, 2000). This could be the reason why the most frequently isolated *Aspergillus* species in the five districts was *A. flavus.*

Identification of *A. flavus* is not an easy task due to its similarities with *A. parasiticus* and A. *nomius*. However, the other *Aspergillus* spp. are distinctly different from each other, and with the help of the descriptions and keys by Klich (2002), it was possible to achieve a reliable identification and discrimination of the various *Aspergillus* spp. isolates. The results of this study showed that even "good" maize perceived to be safe for human consumption is highly contaminated with moulds of the genus *Aspergillus* with *A. flavus* being the major contaminant. All the "good" maize samples from Makueni, Nyeri, Moiben, Ugunja and Bungoma South districts were found to have high levels of *A. flavus* contamination as compared to their respective soil samples. It is evident that residents of these districts consume the "good" maize oblivious of the health risks that they and their animals are exposed to.

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