J. Mycopathol. Res. **61(2)** : 177-184, 2023; ISSN : 0971-3719 (Print), 2583-6315 (Online) doi. 10.57023/JMycR.61.2.2023.177
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Morphology and aflatoxigenicity determination using dichlorvos ammonia (DV-AM) method of soil-borne *Aspergillus* spp. isolated from Eastern Kenya

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Received : 24.02.2023 Accepted : 11.04.2023 Published : 26.06.2023

Soil fungi are an important part of the terrestrial ecosystem with attention focusing on their role in nutrient cycle as well as food safety. The present work aimed at studying *Aspergillus* spp. using morphological descriptors as well as their potential to produce aflatoxins putatively. One hundred soil samples were collected during the harvest prior to introduction of Aflasafe as a pre-harvest biological control strategy. Over 400 isolates were cultured, identified and clustered into groups. The report identified sixteen species of *Aspergillus* characterized on PDA and YES agar media. *Aspergillus flavus* was predominant at 41.5% and the least at 1.5% being *Aspergillus oryzae*. Others included: *A. parasiticus, A. tamarii, A. terreus, A. candidus, A. fumigatus, A. wentii, A. Penicillioides*and *A. ochraceus.*Fifty two percent of these were aflatoxin producers as determined by the Dichlorvos-Ammonia (DV-AM) method; an indication that there is a positive correlation between the frequencies of aflatoxigenic fungi isolation from the Eastern region of Kenya and the reported cases of aflatoxicosis. This supports the view that the Eastern region of Kenya is rich in diversity of aflatoxigenic *Aspergillus* species. The findings justify the need for integrated approaches starting at pre harvest strategies through to post harvest to develop viable solutions for mitigating and managing aflatoxin contamination.

Keywords: Aflatoxins,*Aspergillus*, Dichlorvos-Ammonia (DV-AM),morphology

INTRODUCTION

Fungal species belonging to the genus *Aspergillus* are of great economic importance due to their impact on human life. They are producers of metabolites that are useful as enzymes in industry, agriculture, medicine, natural cycling, bio-fertilizers (Dick, 2009; Karthikeyan *et al*. 2014; Al-Enazi*et al.* 2018).

However, certain species of *Aspergillus* can produce secondary metabolites, called mycotoxins, which are hazardous to human and animal health. Several mycotoxins have been isolated from food and feed material harboring fungi species. Of the over 52 naturally occurring toxic compounds produced by fungi, aflatoxins are the most important (Alshannaqand Yu, 2017; ObonyoandSalano, 2018). It is widely believed that two species of Aspergillus are major producers of aflatoxins namely: *Aspergillus flavus* and *Aspergillus parasiticus* although other less common species such as: *A. nomius, A. tamarii, A. pseudotamarii, A. minisclerotigenes*and *A. bombycis*are also able to produce aflatoxins(Roy *et.al.* 2013; Bhat and Reddy, 2017).Aflatoxigenic fungi commonly inhabit the soils and frequently contaminate peanuts,

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cottonseed, maize and tree nuts (Jamali *et al*. 2012).It is definitely essential to assess the diversity of aflatoxin-producing *Aspergillus* species (Zhang *et* al. 2017).

Over the last four decades, the incidences of acute aflatoxicosis from contaminated food have been documented in Kenya, leading to the belief that that the country in leading in severity and frequency of outbreak as well as the Eastern region being the hot-spot for aflatoxicosis. The most severe aflatoxicosis outbreak ever reported in Kenya occurred in Eastern Province in 2004. This outbreak resulted in 317 cases and claimed 125 lives, a case fatality rate (CFR) of 39 percent. CFR was significantly higher in Makueni district than in Kitui district (Lewis *et al*. 2005; Unnevehrand Grace, 2013). Since 2004, outbreaks among subsistence farmers have recurred annually in Eastern Province. Other outbreaks occurred in 2005, 2006, 2007 and 2008 resulting in sickness, death and destruction of contaminated maize (Lewis *et al*. 2005; Probst *et al*. 2007; Muthomi*et al.* 2009).

The diversity of fungi varies from place to place, depending on the physical, chemical and biological factors of their habitat (Madavasamy and Panneerselvam, 2012; Okun *et al.* 2015).The current study reports the occurrence of the aflatoxigenic soil-borne *Aspergillus* species in the four counties of Eastern Kenya using the DV-AM method; a method considered relatively cheap and fast yielding reliable results(Kushiro *et al.* 2018).Prior to this the only other study that has been conducted to ascertain aflatoxin production capacity is a combination of molecular and metabolic methods (ZarrinandErfaninejad, 2016; Okoth *et al.* 2018), which are considered resource intensive especially for countries in the developing world. The study of aflatoxigenicity among *Aspergillus* communities in different agro ecological zones is of great importance as it will promote the better understanding of their population dynamics especially in view of likely introduction of biological control program using non-toxigenic *Aspergillus* species.

MATERIALS AND METHODS

Sample collection

The samples used in this study were previously recovered from soil collected from farmers in the Eastern regions of Kenya in 2013, from a larger study carried out by Salano (Salano*et al*., 2016). The study site (Eastern Kenya) is semi-arid with annual conditions as follows: rainfall 250–500 mm, RH 60–70% and temperature 23–34°C (Freeman and Coe, 2002). Samples were collected in May and December 2013 approximately 2 months after harvest following the long and short rain season of the year. An altitudinal transect Machakos 1° 312 0.01203 S37° 162 0.01203 E [1000–1600 masl] to Kitui 1° 222 1.05603 S38° 02 37.98003 E [400–1163 masl]) was selected from which sampling points were set every 5 km in the 100 Km road. At each sampling, farmers on both sides of the main road were randomly selected (ObonyoandSalano, 2018). From each farm, a soil sample of between 50 - 60 g was taken at random using a sterile trowel from the top 4-6 cm of soil, collected in sterile 1kg brown paper bags, sealed and stored at room temperature (22?C - 26?C) before analysis.The trowel was cleaned with sodium hypochlorite in between the sampling households and wiped dry before the next use.

Fungal isolation

One gram of the soil was suspended in 9 mL of sterile distilled water and serially diluted to 10^{-4}. One mL of the 10^{-3} and 10^{-4} dilutions were plated in triplicate in Potato Dextrose Agar (PDA) media (HiMedia Laboratories Pvt. Ltd). The plates (Petri dishes (90 × 15mm; Aptaca™, Italy) were then placed in a growth chamber at a temperature of 28±2^{o}C for 7 days. Plates with growth were sub-cultured on PDA and Yeast Extract agar. The PDA media was amended with 50mg/L streptomycin sulfate and penicillin (Zhonghuo Pharmaceuticals, China) to inhibit bacterial growth and subsequently incubated at 30oC for 7 days.

Morphological identification of Aspergillus isolates

Pure cultures of *Aspergillus* isolates were identified to species level based on cultural and morphological characteristics such as: colony diameter, colony color on agar and reverse, colony texture and zonation. Morphological features were studied under the microscope (Motic BA210 microscope (Speed Fair Co., Ltd, Hong Kong)while taking into consideration the key outstanding microscopic features such as conidiophores, conidial shape, phialides and metulae, presence and shape of vesicles (Diba*et al.* 2007).

Contemporary diagnosis of the *Aspergillus* species was based on the descriptions and keys by Klich (Klich, 2002).

Aflatoxigenicity of the Aspergillus species

The Dichlorvos-Ammonia (DV-AM) method was adopted as described by Kushiro *et al*. (2018), to test for aflatoxigenicity. The fungi were separately cultured on PDA amended with 50mg/L streptomycin sulfate, penicillin (Zhonghuo Pharmaceuticals, China) and dichlorvos reagent.20 µL of 250-fold diluted dichlorvos (DV; Divipan, Amiran) methanol solution was spread onto the plate and left for more than 60 min to allow the solution to make PDA-DV agar medium for inoculation. Inoculation of the isolates was done at three points of the Petri dishes (90 × 15mm AptacaTM, Italy) and then incubated at 28ºC for seven days prior to testing for aflatoxigenicity.200 µL of ammonium hydroxide (AM) solution (reagent grade, 25%) was poured onto the inside of the lid of the petri plate and kept for 20 min to allow the AM vapor to disperse. The underside of aflatoxigenic (positive) colonies exclusively showed a brilliant purple-red colour while atoxigenic (negative) colonies rarely changed their colour. The results were then used to discriminate isolates as toxigenic or atoxigenic *Aspergillus* species.

RESULTS AND DISCUSSION

Identification of the Isolates

Based on the morphological characteristics on the two media sets, the isolated fungi were identified to the genus level. On the basis of macro and micro morphological characteristics, the species of interest in the genus *Aspergillus* was further characterized at the species level. Aspergillus were the most occurring fungi with 229 isolates (52%); *Penicillium* had 141 (32%); *Alternaria* 18 (4%); *Fusarium* 13 (4%);*Paecilomyces with* 11 isolates*; Verticillum*7 and*Trichoderma* 9 isolates had 2% each; *Acremonium* had 5 isolates, *Mucor* 4 and *Rhizopus* 5 that contributed 1% each as presented in the chart (Fig1).

Macroscopic and Microscopic characteristics of Aspergillus species on PDA and YES media

The morphological characteristics such as colony diameter, colony colour on agar and reverse, colony texture and zonation identified sixteen *Aspergillus* species as described in Table1. Microscopically outstanding features such as conidiophores, conidial shape, phialides and metulae, presence and shape of vesicles were used to distinguish the species of the isolate as shown in (Table2). The percentage occurrence of different species of *Aspergillu*s ranged from 1.5–42.2%. *Aspergillus flavus* had the highest occurrence (42.2%) while *A. oryzae, A. ustus and A. niger* (1.5%) had the lowest occurrences, followed by *A. tamarii* (15.3%) and *A. parasiticus* (9.4%) respectively as presented in Fig.2. Other identified species included A. terreus, A. *candidus*, *A. wentii, A. fumigatus, A. nidulans* and *A. syndowii* at 2.4% each. *Aspergillus versicolor* and *A. ochraceus* were at 3.5%. *Aspergillus penicillioides* was at 5.9% while *A. nomius* represented 4.7% of the isolates identified during the study. The relative abundance and diversity of the *Aspergillus spp*. is presented in Fig.2 as a percentage of their occurrence.

Among these species both toxigenic and atoxigenic species isolates have been identified using the DV-AM method as described. The test distinctively identified *A. flavus, A. parasiticus and A. nomius* as aflatoxin producers in the study. On this basis the detailed description of these species is given. However, it is good to note that not all the isolates within these three were positive for aflatoxin production.

Aspergillus flavus was identified by a yellowish green colony after seven days on PDA. Some isolates of *A. flavus* displayed dark olive-green colonies with white margins and turned olive yellow with age (e.g. 2ES-07-02, 2ES-54-04) as depicted inFig. 3c. They were predominantly uniseriate (e.g. 2ES-54-04, 2ES-42-01, 2ES-12-04, 2ES-02-01) as depicted in Fig.3f but some were biseriate (e.g. 2ES-07-02, 2ES-92-06) as in Fig. 3g with philiades radiating in all sides from metulae that are borne on subglobose or globose vesicles of variable size. The metulae obscured the entire surface of the vesicles. Conidia had a globose shape ranging with thin walls and rough texture. The conidiophores had a rough texture and thick walls were non-pigmented and unbranched (Fig.3f &g). On YES media, some isolates (e.g. 2ES-07-02) showed creamy white with wooly concentric rings while others had orange wooly surfaces (e.g. 2ES-02-01, 2ES-92-06) as depicted (Fig.3 d &e).

Aspergillus parasiticus colonies on PDA had a yellow to dark green front with a cream to pale yellow colour on reverse. On the reverse they had about 20-25 wrinkles having a length 10-15 mm as depicted in Fig. 3d. On YES media colonies had cottony beige concentric rings as in Fig.4c&d. The conidiophores vesicles were pyriform to globose with yellow/green radiate conidia. The philiades were predominantly uniseriate with the radiate conidia having rough conidial walls. The characteristic feature of this isolate was the presence of spherical and rough conidia with thick walls as well as rough conidiophores as seen in Fig. 4 e & f.

Aspergillus nomius surface were floccose consisting of white or very light yellow green long vegetative mycelium and sparse conidial colonies on PDA The reverse of the colonies were dull green. On YES the colony was velvety to floccose, consisting of white and green vegetative mycelium. Vesicles as well as the conidia were globose to subglobose as shown (Fig.5). The hyphae were septate with a globose to subglobose vesicles. The phialides were biseriate with globose to subglobose intermediately rough conidia. The distinguishing characteristic feature was its pyriform-ornamented conidiation.

Aflatoxigenicity of the Aspergillus species

From the findings of the current study, 40% of the *Aspergillus* species isolated from the soil were aflatoxin producers. *Aspergillus flavus* (e.g. 2ES-12-04, 2ES-52-01, 2ES-35-07) isolates were the most toxigenic species (52%) compared to *A. parasiticus* isolates (e.g. 2ES-92-06, 2ES-42-01, 2ES-86-01) that made 33% while *A. nomius* made the remaining 15 % (e.g. 2ES-22-02). A positive control (Fig.6a) presents a brilliant plum-red pigmentation on exposure to Dichlorvos-Ammonia (DV-AM) solution. This colour change made it easy to distinctively identify aflatoxigenic fungi from of non-aflatoxigenic ones (2ES-92-05, 2ES-87-01) as shown in Fig. 6. The colour change was monitored to change from the original colours of the respective reverse to purple-red through the pH change by ammonia vapour indicates different colour intensities an indication of different concentrations of aflatoxin production.

Since the discovery of aflatoxins in the early 1960s, many studies have been conducted to assess the occurrence and to describe the ecology of aflatoxin-producing fungi in natural and agricultural environments. Understanding the geographical distribution of the aflatoxin producing Aspergillus species will be a prerequisite to developing viable solutions for mitigating and managing aflatoxin contamination at different points of food production chain. Morphological characterization of macroscopic and microscopic characters of the isolates from the soil samples enabled identification of fungi of the genus *Aspergillus, Penicillium, Fusarium, Alternaria, Rhizopus, Mucor, Trichoderma, Verticillum, Acremonium* and *Paecilomyces. Fungi* belonging to *Aspergillus, Penicillium, Fusarium and Alternaria* species have been implicated in mycotoxin production in food and feed. This could be as a result of their supposed dominance in agricultural soils which serves as reservoir for primary inoculums for the infection of susceptible crops for both humans and animals (Moss, 2002; Akinola *et al*. 2019). In addition mycotoxin contamination is often an additive process, beginning in the field and increasing during harvest, drying, and storage.

As obtained in this study, a large portion of Aspergillus identified from soil samples were *A. flavus* accounting for 42.2% of total cultures isolated and 52% of these isolates being aflatoxigenic. This supports the report on A. flavus as the most common and prevalent *Aspergillus* species in nature and has been described as the main fungal contaminants in African foods and feeds (Muthomi *et al.* 2009; Anthony *et al*., 2012). The population size of A. flavus has been correlated with soil organic matter and nutritional status, with the most fertile soils containing the greatest concentration of *Aspergillus* (Zablotowicz*et al*. 2007). A. parasiticus in this study was quite low at 14.5% which can be attributed to the fact that A. parasiticus is more prevalent in peanuts and peanut soils than any other crop which was the case for the Eastern region that is mainly a maize growing zone. However, it is typically outcompeted by A. flavus when the two fungi are both present. In addition, *Aspergillus nomius* at 4.7% gave a positivity rate of 11% of the isolates of this species.

Aspergillus community in any agricultural ecosystem has an important influence on the extent to which food and feed becomes contaminated with mycotoxins. Previous studies on occurrences of aflatoxins and aflatoxicosis have shown that there

is a positive correlation between the frequency of aflatoxin producing fungi and levels of aflatoxins (Mutegi *et al*. 2009; Muthomi *et al*. 2009; Wagacha *et al*., 2013). Therefore strategies deployed in mitigation and control, should focus more on pre-harvest measures to ensure reduction in risks of mycotoxins in food and feed. Information especially on soil distribution and ecology of *A. flavus* is consequently considered a prerequisite for developing effective measures to prevent and to control aflatoxin contamination of crops notwithstanding the soil and crop management practices and a number of environmental factors that can influence the population size and spatial distribution of A. flavus in cultivated soils (Zablotowicz *et al.* 2007).

Notably, *A. flavus* and *A. parasiticus* have been implicated in aflatoxin contamination in Kenya but other Aspergillus species like *A. nomius, A. bombycis, A. ochraceoroseus, A. pseudotamarii, A. parvisclerotigenus, A. rambellii and A. tamarii*

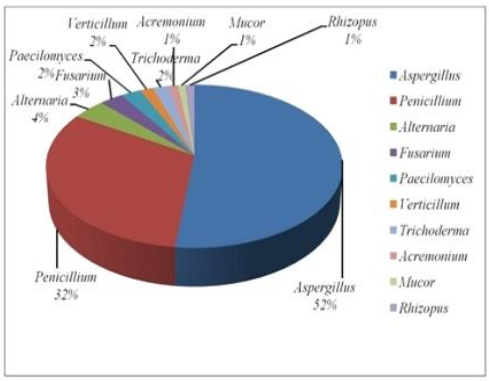

Fig. 1 : Occurrence of soil fungal species

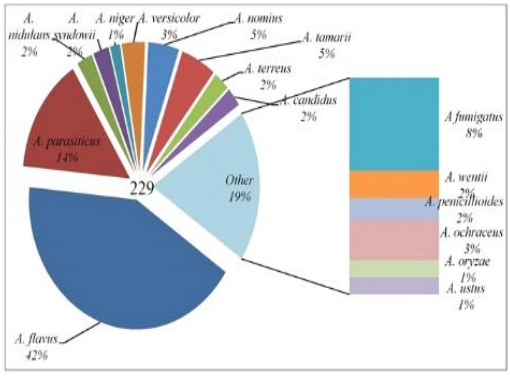

Figure 2: Percent diversity of the *Aspergillus* spp. Isolated from soil of Eastern Kenya

Fig. 2: Per cent diversity of the Aspergillus spp. Isolated soil of Eastern Kenya

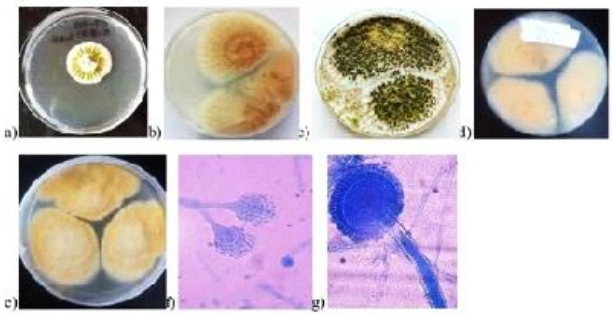

Fig. 3: *Aspergillus flavus* surface and reverse colonies on PDA (a, b & c); sclerotia dominating the surface of the colony (c) ; surface and reverse colonies on PDA (d & e); biseriate conidial head with a globose vesicle of A.flavus (f); uniseriate conidial head in (g). (x400)

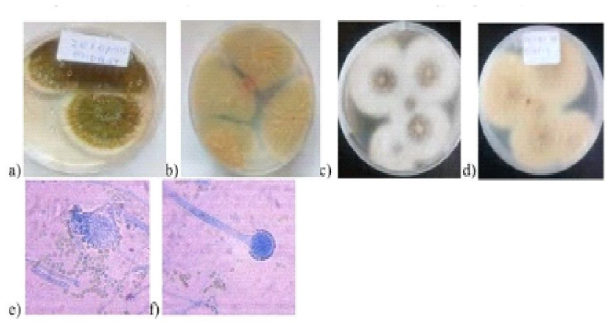

Fig. 4: *Aspergillus parasiticus* surface colonies on PDA (a & b); *A.parasiticus* surface colonies on YES (c & d); uniseriate conidial head with a globose vesicle with rough conidia (e) (x 400)

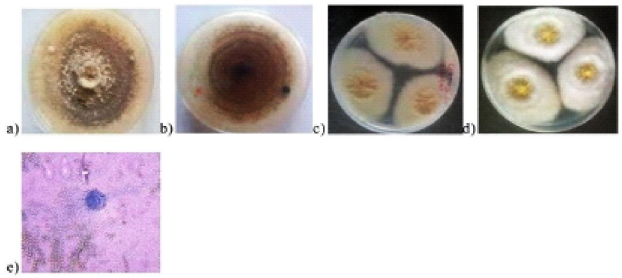

Fig. 5: *Aspergillus nominus* surface colonies on PDA (a& b); *A.nominus* surface colonies on YES (c & d); biseriate conidial head with a globose vesicle of conidia (e) (x 400)

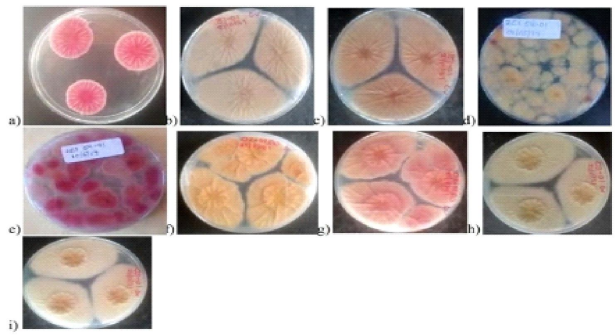

Fig. 6: Aflatoxeginicity test; (a) positive control; (b, c, d, f and h)shows cultures before exposure to DV-AM solution; (c, e and g) positive results after exposure to DV-AM solution; (i) negative result of Atoxigenic isolate before and after exposure to DV-AM

Table 1: Macroscopic features of *Aspergillus* species isolated

	Colony colour			Colony	Colony	Zonation	Growth	Soluble	Elevation	Size
	Surface		Reverse	Edge	Texture			Pigments		(mm)
A. flavus	PDA	Green	Cream to yellow to red brown	Entire	Granular	Radial wrinkles	Fast	None	Umbonate	65-75
	YES	White	Cream to yellow	Entire	Cottony	Irregular	Fast	None	Umbonate	55-60
A. parasiticus	PDA	Green with white mycelia	Brown	Entire	Rough	Wrinkled	Moderate	Brown	Umbonate	20-30
	YES	Light orange	Slightly orange	Entire	Velvety	Wrinkled	Slow	None	Umbonate	15 - 20
A. nidulans	PDA	Grayish green with white	Brown	Entire	Finely rough	Furrowed	Slow	Brown	Raised	35-40
	YES	White	White to cinnamon brown	Entire	Floccose	Smooth	Slow	None	Umbonate	20-25
A. syndowii	PDA	Cinnamon brown with orange tinge	Golden yellow	Undulate	Floccose	Contoured wrinkles	Moderate	Orange	Umbonate	35-40
	YES	Blue green	Cream white	Undulate	Velvety	Smooth	Moderate	Orange	Umbonate	30-35
A. niger	PDA	Dark brown to black	Colourless to yellow	Entire	Velvety	Radially furrowed	Fast	None	Umbonate	45-50
	YES	White	Cream	Entire	Cottony	Wrinkled	Moderate	None	Umbonate	30-35
A. versicolor	PDA	White to orange-cream with green ring at the centre	Bright orange	Entire	Floccose	Radially furrowed	Moderate	Red/ orange	Flat	25-30
	YES	White and orange gray with white margin	Yellow with a white margin	Entire	Velvety	Smooth	Slow	None	Umbonate	15-25
A. nomius	PDA	White or very light yellow	Dull orangish to light yellow	Filamentous	Fluffy	Dull	Fast	None	Flat	40-50
	YES	Parrot green	Pale brown	Serrated	Floccose	Punctiform	Fast	None	Flat	40-45
A. tamarii	PDA	Grayish green with white to dull white centres	cream yellow to red tinge	Entire	rough and powdery	Irregular	Moderate	Brown	Umbonate	35-40
	YES	Cream yellow to orange	Brown with cream margin	Entire	Rough	Irregular	Moderate	Red	Umbonate	30-35
A. terreus	PDA	beige to cinnamon-buff to brown	Yellow to gold	Undulate	Granular and powdery	Star shaped striations/ concentric ring	Moderate	Amber	Umbonate	35-45
	YES	White	Yellow	Entire	Velvety	Smooth	Moderate	None	Umbonate	30-35
A. candidus	PDA	Creamish white	Cream	Entire	Granular to floccose	Smooth	Slow	None	Umbonate	20-35
	YES	Creamy	Cream	Entire	Smooth	Smooth spiral free pattern	Slow	None	Raised	25-30
A fumigatus	PDA	Gray-green	Dark red-brown	Entire	Floccose	No wrinkles	Rapid	Red	Flat	50-60
	YES	Grayish white	light orange to red	Entire	Floccose	No wrinkles	Rapid	None	Umbonate	45-55
A. wentii	PDA	White	Dull yellow	Entire	Fluffy to cottony	Radial wrinkles	Rapid	None	Crateriform	45-55
	YES	White	Cream yellow to red tinge	Entire	Cottony	Granular	Rapid	None	Umbonate	35-40

also have the ability to produce aflatoxins (Peterson *et al.* 2001). However, in this study this does not rule out mycotoxin production owing to the presence of the species like *Aspergillus ochraceus, Penicillium spp*. as well as *A. niger* known to produce Ochratoxin A, the most potent ochratoxin whose effects on human as well as animal health have already been documented (Abarca *et al.* 2001; Visagie *et al*. 2014). It has been shown that *A. niger* also produced fuminosins though earlier it was thought to be produced by *Fusarium* species only. This points to an alarming scenario as the regulatory agencies in Kenya only test for aflatoxins. This could indicate that the toxin burden in the country is grossly underestimated.

Aflatoxin producers in the study accounted for 40 % of the isolates. Presentation of brilliant plum red pigmentation by these species can be attributed to the presence of anthraquinone intermediates in aflatoxin biosynthetic pathway. The colour change in the colony is due to colour changes of aflatoxin precursors i.e.versiconal hemiacetal acetate (VHA) and versiconol acetate (VOAc), which accumulate in the mycelia by the inhibition of an AF biosynthetic enzyme, VHA esterase by Dichlorvos. Since these precursors contain anthraquinone moieties in their structures, the mycelial colors are markedly changed from the original yellowish colours to purple-red through the pH change by ammonia vapor. Monitoring this colour change makes it easy to identify aflatoxigenic fungi. Non aflatoxin producers will not

Table 2: Microscopic features of *Aspergillus* species isolated

	Hyphae	Vesicle	Conidia Colour	Phialides/ serration	Conidiation	Shape of conidia	Conidial wall	Conidial Heads	Fruiting body
A. flavus	Branched septate	Globose to subglobose	Yellow/ greyish green	Biseriate covering nearly entire vesicle	Columnar	Spherical to ellipsoidal	Smooth to rough wall	Columnar	Cleistothecia present
A. parasiticus	Septate	Pyriform to globose	yellow/green	Predominantly uniseriate	Radiate	Radiate	Rough	pyriform to radiate	Cleistothecia absent
A. nidulans	Branched septate	hemispherical	Green	Biseriate, borne on the upper half of the vesicle	Columnar	globose to slightly elliptical	Smooth	Long and columnar	Cleistothecia present Hulle cells present
A. syndowii	Septate	Subglobose to pyriform	Brown	Biseriate	Pyriform	Spherical	Subglobose	Radiate	Hülle cells present
A. niger	Branched septate	Globose	Brown	Biseriate covering the entire surface of the vesicle	Radiate	spherical or sub spherical	Has warts and ridges	Radiate	Cleistothecia present
A. versicolor	Septate	sub spherical or ellipsoidal	Brown	Biseriate, covering the entire surface of the vesicle	Radiate	spherical	Smooth to rough wall	Radiate	Hülle cells present in some isolates
A. nomius	Septate	Globose to subglobose	yellowish green	Biseriate	pyriform-ornamented	globose to subglobose	Rough	Radiate	Cleistothecia absent
A. tamarii	Septate	globose	Olive green	uniseriate and biseriate	Radiate	Spherical	Spherical to sub spheroidal	compact loosely radiate	Cleistothecia absent
A. terreus	Septate	dome-shaped	Green	biseriate on upper two third of the vesicle	Radiate	spherical to broadly elliptical	Smooth	Densely columnar appearing fan-shaped	Cleistothecia absent
A. candidus	Septate	Sub spherical to spherical	Black	Biseriate, covering the entire surface of the vesicle	Radiate	Spherical to sub spherical	Smooth to rough wall	Radiate, appearing loosely columnar	Cleistothecia absent
A fumigatus	Branched septate	Dome shape	greenish grey	Uniseriate covering only upper portion of vesicle	Columnar	globose to subglobose	Finely rough	Columnar and compact	Cleistothecia present
A. wentii	septate	Globose	dark yellow to brown	Biseriate	Radiate	Globose	Smooth glassy	Radiate	Cleistothecia absent
A. penicillioides	Septate	oval or flask-shape	Green	uniseriate with several long	pyriform	spherical	Smooth to slightly	Columnar	Cleistothecia absent

undergo the colour change, as the colours of non-aflatoxigenic fungi do not change(Kushiro e*t al*. 2017).

CONCLUSION

On the basis of the results reported in this study, the prevalence of *Aspergillus* species and putatively aflatoxigenic strains in the agricultural soils of Eastern Kenya is high. Crop infection and subsequent aflatoxin contamination starts under field conditions and this should be the critical step to focus on aflatoxin prevention strategies. Although some species were easily identifiable due to the presence of dissimilitude in morphology in soil borne *Aspergillus,* misidentification is a possible impediment because they are highly complex, ever evolving and only a single characteristic can be used to separate them, there is no single method (morphological, physiological or molecular) that works flawlessly in identifying and recognizing all the species described so far. Furthermore, there may be disadvantages of classical identification methods as they are highly time consuming and require considerable mycological knowledge and experience to be accurately performed. Therefore, a combination of different methods i.e. the polyphasic approach should be used to achieve reliable identification and discrimination of putative aflatoxin producers. Affordable, practical, rapid tests that could be used in the field would be of great potential value.

ACKNOWLEDGEMENTS

The authors express their heartfelt gratitude to the farmers, county government in the greater Eastern Kenya for their co-operation. We appreciate Micah Lagat and Amos Mutai for their tremendous laboratory support. The experiments were carried out at the Mycotoxin Research Laboratory, Biochemistry Department Egerton University.This work was supported by the National Research Fund-Kenya; grant awarded to MAO and FJT on mitigating risks of mycotoxins in the food and feed chain.

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