Identification and Toxigenicity of *Aspergillus* spp. from Soils Planted to Peanuts in Eastern Zambia

S. M. C. Njoroge*, K. Kanenga, M. Siambi, F. Waliyar and E. S. Monyo[1]

ABSTRACT

It is not known which aflatoxigenic species are present in Zambia. Therefore, soil samples were collected during May to June 2012, at the end of the growing season in Eastern Province, from 399 farmers' fields that had been planted to groundnut (*Arachis hypogaea* L.) in Nyimba, Petauke, Mambwe, and Chipata Districts. Population densities of *Aspergillus* spp. were estimated by plating 10^{-3} soil dilutions on modified dichloran rose Bengal (MDRB) media. To test for toxigenicity, colonies were randomly selected from MDRB dilution plates, single-spored, transferred to vials with yeast extract sucrose (YES) liquid media, and grown for a week at room temperature. Agra Strip ® lateral flow cards were then used to test the filtered extracts, from the YES cultures, for total aflatoxin at 4 and 20 parts per billion (ppb). We identified *Aspergillus flavus* (small and large sclerotia strains), *A. parasiticus*, *A. niger*, *A. nomius*, *A. oryzae*, *A. tamarii*, and *A. terreus*. 100% of the S-strain *A. flavus* isolates produced aflatoxin at 4 and 20 ppb, whereas 86% and 56% of the L-strain *A. flavus* isolates produced aflatoxins at 4 and 20 ppb, respectively. All the *A. nomius* isolates produced aflatoxins at 4 and 20 ppb. 79% and 64% the *A. parasiticus* isolates produced aflatoxins at 4 and 20 ppb. To our knowledge, this is the first peer reviewed report from Zambia documenting the population densities of *A. flavus* across different agroecologies. In addition, it is also the first report on the identification of different *Aspergillus* spp., such as *A. nomius*, *A. terreus*, *A. oryzae*, and *A. tamarii* from Zambia. This information, taken together with cropping practices, soil characteristics, agroecological and climatic data, can form a basis for developing holistic pre-harvest aflatoxin mitigation strategies.

Key words: Aflatoxigenic, Aflatoxin, Population densities.

Peanut (*Arachis hypogaea* L.) is the second most cultivated crop in Zambia. In 2013, groundnut was cultivated on 207,249 Ha, mostly by subsistence farmers, and the average yield was 515 Kg/Ha (FAOSTAT, 2013). Within Zambia, peanut production is highest in the Eastern Province where the crop is grown on 80,000 Ha in different agroecological zones, from the cooler and wetter plateau, i.e., Chipata District, to the hotter and drier Luangwa Valley, i.e., Mambwe District. Like in most countries in southern Africa, the cropping season starts at the onset of the rains in December, and peanut is grown in rotation with other staples and cash crops such as maize, cotton, sunflower, tobacco, etc. Farmers, however, prioritize sowing corn, cotton, tobacco, and sunflower over peanut, which are planted up to 3 weeks after the rains commence.

Aflatoxins are chemical compounds produced by the opportunistic pathogens *Aspergillus flavus* Link ex Fries, (Teleomorph *Petromyces flavus*) *A. parasiticus* Speare (Teleomorph *P. parasiticus*), and *A. nomius* (Teleomorph *P. nomius*) (Abbas *et al.*, 2004; Amaike and Keller, 2011; Horn *et al.*, 2009a; Horn *et al.*, 2009b; Horn *et al.*, 2011; Paterson *et al.*, 1997), however, only *A. flavus* and *A. parasiticus* have an impact on agriculture (Horn, 2003; Klich, 2007). Oil seed crops such as corn, peanut, cotton, and sunflower are susceptible to aflatoxin contamination. Aflatoxins are a common contaminant of foods, especially the staple diets of many in developing countries (Williams *et al.*, 2004) and the US Food and Drug Administration considers aflatoxins to be unavoidable contaminant of foods (Williams *et al.*, 2004). Dietary exposure to aflatoxin can lead to aflatoxicosis, defined by Williams *et al.* (2004), as poisoning from ingesting aflatoxins. Aflatoxicosis, can either be acute, which is severe intoxication that leads to direct liver damage followed by sickness or death (Azziz-Baumgartner *et al.*, 2005), or chronic, which is subsymptomatic exposure that may lead to nutritional and immunological consequences (Williams *et al.*, 2004). However, all doses i.e., chronic and acute, have an accumulative effect on the risk for cancer (Williams *et al.*, 2004). In developing countries, exposure to aflatoxins may begin early, when infants are exposed tog weaning and adult foods (Gong *et al.*, 2002).

[1]First author: International Crops Research institute for the Semi-Arid Tropics (ICRISAT), P.O. Box 1096, Lilongwe, Malawi; Second author: Zambia Agriculture Research Institute, Chipata, Zambia; Third and fifth authors: ICRISAT, P. O. Box 39063, Nairobi, Kenya; Fourth author: ICRISAT, Patancheru 502 324, Telagana, India.
*Corresponding author's E-mail: s.njoroge@cgiar.org

Aflatoxin contamination is a serious problem in Africa (Atehnkeng *et al*., 2008; Kaaya and Warren, 2005; Matumba *et al*., 2015; Monyo *et al*., 2012; Mutegi *et al*., 2009; Setamou *et al*., 1997; Wagacha and Muthomi, 2008) and also worldwide (Amaike and Keller, 2011; Munkvold, 2003). Despite the magnitude of the aflatoxin problem, only a few studies have attempted large scale comparisons of soil populations of aflatoxigenic fungi over large geographic areas which represent differences in climatic, soil type, and cropping systems (Horn, 2003). Reports on characterization of aflatoxigenic fungi have been published mostly from the southeastern US (Abbas *et al*., 2004; Boyd and Cotty, 2001; Horn, 2003; Horn, 2005; Horn *et al*., 1995; Sweany *et al*., 2011; Jamie-Garcia and Cotty, 2006). In Africa, there have been reports from Kenya (Okoth *et al*., 2012) where the authors tested isolates from two different agroecologies – the hotter and drier Makueni, where the acute outbreak of aflatoxicosis occurred (Lewis *et al*., 2005), and the cooler and wetter Nandi, a corn growing region in the Rift Valley. Okoth *et al*. (2012) reported that there were no differences in the occurrence of *A. flavus* between the two tested regions, however, S-strain *A. flavus* was more prevalent in Makueni compared to Nandi, where L-stains were more prevalent. In Nigeria, Atehnkeng *et al*. (2008) collected maize samples from three agroecological zones and determined the identity of isolated species and also the incidence of toxigenic versus atoxigenic strains. However, most reports from Africa document the prevalence of aflatoxin contamination in different commodities and researchers often do not differentiate or characterize *Aspergillus* species (Monyo *et al*., 2012; Matumba *et al*., 2015).

It is important to study and document factors that influence aflatoxin contamination, such as understanding the activities and population structure of aflatoxigenic fungi in the soil, because this is a pre-requisite for developing effective measures to control aflatoxin contamination (Horn, 2003). Other important factors also should be considered when developing preharvest aflatoxin mitigation measures, i.e., the crops grown and their resistances to aflatoxin contamination, soil characteristics, agronomic and cultural practices, weather, and the occurrence of drought. Taken together, these would be used to develop a holistic approach to aflatoxin mitigation.

To our knowledge, there are no published reports from Zambia on the population density of *A. flavus*, and characterization of aflatoxigenic fungi. Therefore, the objective of this study was to identify aflatoxigenic fungi from soils cropped to peanut, estimate the population densities of *A. flavus*, and to characterize isolates for aflatoxin production.

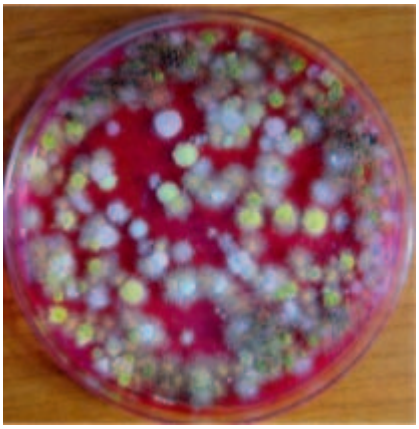

Fig. 1. Colonies of soilborne fungi growing on modified dichloran Rose Bengal medium (MDRB) after dilution plating and ready for enumeration.

Materials and Methods

Population density of *A. flavus*.

Geo-referenced soil samples were collected from fields cropped to groundnuts in Nyimba, Petauke, Mambwe, and Chipata Districts during May to June 2012. All these districts are important in groundnut production, with Nyimba and Mambwe being located in the hotter Luangwa Valley, and Petauke and Chipata being in the cooler Plateau. From each field, soils were sampled from at least 5 distant spots, 0 to 10 cm deep along the ridge, and aggregated into a single composite sample. From Nyimba, Petauke, Mambwe, and Chipata, a total of 100, 100, 95, and 104, soil samples were collected, respectively. For processing, samples were taken to ICRISAT laboratories in Lilongwe, Malawi. Estimation of the population densities of *A. flavus* was done using methods from Horn *et al*. (1995). Briefly, soil samples were air dried for 1 week and then a subsample of 3.3 g was measured into test-tubes containing 10 ml of 0.2% water agar, and vortexed. 2 ml of the mixture was then added into another test-tube containing 8 ml of 0.2% water agar and vortexed. Four replicates, 0.2 ml each, of the mixture were then plated on Modified Dichloran Rose Bengal (MDRB) media. Petri dishes were then incubated for 3 days at 37 °C and colonies (Fig. 1) were counted under the dissecting microscope (Jenco International Inc, Portland, USA).

Identification of *Aspergillus* spp.

The following reference isolates were obtained from the Centraalbureau voor Schimmelcultures

(CBS) Fungal Biodiversity Centre: i) *A. parasiticus* CBS 100939 98.0708 EX-B12-1045; ii) *A. terreus* var *terreus* CBS 601.65 10.1474 EX-B12-1045; iii) *A. nomius* CBS 260.88 07.1480 EX-B12-1045; iv) *A. caelatus* CBS 763.97 05.0604 EX-B12-1045; v) *A. fumigatus* CBS 127.803 10.1913 EX-B12-1045; and vi) *A. flavus* var *flavus* CBS 573.65 07.1168 EX-B12-1045. Working cultures of these isolates were grown on Czapek's agar (CzA), Potato Dextrose Agar (PDA), and Malt Extract Agar (MEA). Test tubes with slants of CzA were used for long-term storage at both room temperature and at 4 °C. A total of 91 isolates were randomly selected from cultures growing on MDRB soil dilution plates and transferred onto plates with CzA. Two weeks later, hyphal-tip transfers were taken from the edge of these cultures and transferred onto plates with CzA, PDA, and MEA. Plates were then held at room temperature and the morphological characteristics of the colonies were recorded two weeks later. To observe microscopic characteristics, slides from each of the cultures were mounted and observed at ×200, ×400, and ×1000, using a Jenco brightfield compound microscope (Jenco International Inc, Portland, USA). *Aspergillus* spp. were then identified by comparing with the isolates from CBS and also by using the *Aspergillus* spp. taxonomic key from Domsch *et al*. (2007).

Toxigenicity of *Aspergillus* spp.

Hyphal-tip isolates were grown on CzA for two weeks. Single spore isolate transfers were aseptically taken from the edge of the colony using a sterile needle, into Yeast Extract Sucrose (YES) media in 10-ml vials. The vials were then sealed and incubated at room temperature for 1 week. The negative and positive controls were, non-inoculated and inoculated YES media with a toxigenic strain from CBS collection, respectively. 1 week later, 10-g of the YES media was weighed into sterile polypropylene Falcon® tubes. 20 ml of 70% (v/v) methanol was then added into the tubes and vortexed for 1 minute. The mixture was then filtered through a Whatman # 1 filter paper into a conical flask. 50 µl of the filtrate was then pipetted into micro-wells and total aflatoxin (B_1+B_2+G_1+G_2) production was determined using strip tests Agra Strip ® (Romer Labs, Austria) at 4 and 20 ppb.

Results and Discussion

In this study, we documented the population densities of *A. flavus* across two agroecologies in eastern Zambia. Population densities of *A. flavus* varied amoung districts. The mean population density of *A. flavus* was 2.6, 1.8, 2.0, and 2.4 log CFU/g of dry soil in Chipata, Mambwe, Nyimba, and Petauke Districts, respectively. Mambwe and Nyimba Districts, which fall in the comparatively warmer Luangwa Valley, had lower populations of *A. flavus* than Chipata and Petauke Districts, located in the cooler and wetter plateau.

Several studies have documented population densities at different scales—from single fields to larger geographical zones, at different sampling frequencies, and also from fields with different cropping histories. Horn (2005) sampled two cultivated fields in Georgia at five different times and showed that for populations of *A. flavus* L strain, field A had lower mean densities at 2.7 log CFU/g compared to field B at 3.1 log CFU/g. A similar observation was also reported on population densities of *A. flavus* S strains from these fields (Horn, 2005). From an earlier study, also conducted in Georgia, Horn (1995) found that populations of *A. flavus* and *A. parasiticus* did not differ significantly at the beginning of the season and remained stable during the season in fields cropped to corn and peanuts. However, populations of *A. flavus* relative to *A. parasiticus* increased significantly in one drought stressed corn field after harvest and debris dispersal. In Virginia, which has relatively cooler weather, populations of *A. flavus* did not exceed 2.0 log CFU/g soil in peanut cultivated soil (Griffin and Garren, 1974).

Variations in population densities of *A. flavus*, both between and within districts were therefore expected. However, we expected higher populations in the hotter and drier Luangwa Valley compared to the cooler and wetter plateau. Several factors, such as flooding, could explain for the documented differences in population densities of *A. flavus*. Parts of the Luangwa Valley are prone to floods. Farmers interviewed during sample collection noted that some fields cropped to groundnuts had been exposed to recurrent flooding events, with water standing in these fields for a few days. For example, in the sampled Chikowa region of Mambwe District, the Kasengwa, Lwuo, and Kabilubilu rivers flooded during the 2011/2012 growing season, whereas in the Masumba region of Mambwe District, the Msandile river flooded affecting fields cropped to groundnuts and other crops. In such areas, it is common practice to plant along the flood plains of the rivers, because farmers try to take advantage of the fertile alluvial soils deposited whenever these rivers break their banks.

Population densities of *A. flavus*, an aerobic fungus found in the upper profiles of the soil (Horn, 2003), could therefore have been reduced because of these flooding events. Colony forming

Table 1. Characterization of *Aspergillus* spp. isolates on Czapek's Agar, Potato Dextrose Agar, and Malt Extract Agar and the determination of afltoxigenicity of the isolates.

Species	No. of isolates	Colony colour on			% Toxigenic[d]	
		CzA[a]	PDA[b]	MEA[c]	4 ppb	20 ppb
A. tamarii	6	Brown/cinnamon	Green/brown	Green/brown	–	–
A. flavus (S-strain)[e]	32	White/yellow	White/green	Light green	100	100
A. flavus (L-strain)[f]	11	White/yellow	White/green	Light green	86	58
A. parasiticus	19	Dirty green	Dark green	Light green	100	100
A. oryzae	8	Green/white/yellow	White	Green/white	–	–
A. terreus	6	Cinnamon	Light green	Light green	–	–
A. nomius	6	Yellow/white	White	Light green	100	100
A. niger	3	Black	Black	Black	–	–

[a]Czapek's Agar.
[b]Potato Dextrose Agar.
[c]Malt Extract Agar.
[d]Aflatoxigenicity was determined by testing filtrates of fungal isolates, growing on Yeast Extract Sucrose media, with Agra Strip® (Romer Labs, Austria) for total aflatoxin (AFB_1+AFB_2+AFG_1+AFG_2).
[e]Isolates with mean sclerotia size less than 400 μm in diameter.
[f]Isolates with mean sclerotia greater than 400 μm in diameter.

units estimated through soil dilution plating mostly arise from conidia and hyphae (Horn, 2003). Conidia are asexual spores that are hydrophobic and are mostly found in the upper 6 cm of the soil (Horn, 2003). However, in agricultural soils, *Aspergillus* spp. can be found up to 30 cm deep, possibly due to ploughing activities that result in mixing of soil at such depths (Horn, 2003). Flooding events could reduce the densities of both conidia and hyphae in the upper soil profiles by either depositing soils from the flooding river, or by washing down these propagules further down the soil profile.

Many papers on characterizing A*spergillus* in both agricultural and non-agricultural fields have been published (Boyd and Cotty, 2001; Karthikeyan *et al*., 2009; Okoth *et al*., 2012; Solozarno *et al*., 2014). Boyd and Cotty (2001) reported that *A. flavus* and *A. tamarii* were the only species isolated from plant debris and pod samples collected from the dessert in Arizona. In contrast, Okoth *et al*. (2014), reported that *A. flavus* was the most abundant species from section *Flavi* isolated from corn in two districts in different agroecologies in Kenya. *A. tamarii* and *A. parasiticus* were also isolated. Interestingly, Okoth *et al*. (2014) reported that *A. parasiticus* and *A. tamarii* were isolated more frequently in the cooler district of Nandi compared to Makueni which is also receives significantly lower rainfall. Of the 91 *Aspergillus* spp. isolates characterized in our study, the most abundant species was *A. flavus* (47%), followed by *A. parasiticus* (21%). Other species identified were *A. tamarii* (7%), *A. oryzae* (9%), *A. terreus* (7%), *A. nomius* (7%) and *A. niger* (3%) (Table 1).

A. flavus populations are genetically diverse (Chang *et al*., 2005; Karthikeyan *et al*., 2009) and is evident in the different capabilities of isolates producing toxin (Karthikeyan *et al*., 2009; Okoth *et al*., 2014). There are many methods, both cultural and analytical that can be used to determine toxigenicity. Abbas *et al*. (2004) reported that cultural methods were equally as effective in determining toxigenicity and were cheaper than testing for toxin using HPLC or ELISA.

In our study 100% of the S-strain *A. flavus* isolates produced AFB_1 at 4 and 20 ppb, whereas 86% and 56% of the L-strain isolates produced AFB_1 at 4 and 20 ppb, respectively. Non-toxigenic isolates have sequence breakpoints in the aflatoxin gene cluster, and Chang *et al*. (2005) showed that such deletions are not a rare occurrence in natural populations. Recently, Solozarno *et al*. (2014) reported that 60% of *A. flavus* isolates from a corn field in Mississippi were toxigenic however they did not differentiate isolates based on S- and L-morphotypes. Interestingly, S morphotypes have been identified in Asia, United States, Africa, South America, but not in Europe (Boyd and Cotty, 2001; Okoth *et al*., 2014; Perrone *et al*., 2014). Karthikeyan *et al*. (2009) also tested isolates of *A. flavus* recovered from corn for toxin production and reported that 63% (32 out of 52 isolates) produced AFB_1 and the rest did not.

Our results also showed that 79% and 64% of tested *A. parasiticus* isolates produced aflatoxins at 4 and 20 ppb, respectively. *A. nomius* isolates were also tested for toxin production and all isolates produced aflatoxins at 4 and 20 ppb (Table 1). However, by adopting for this study, a cut-off

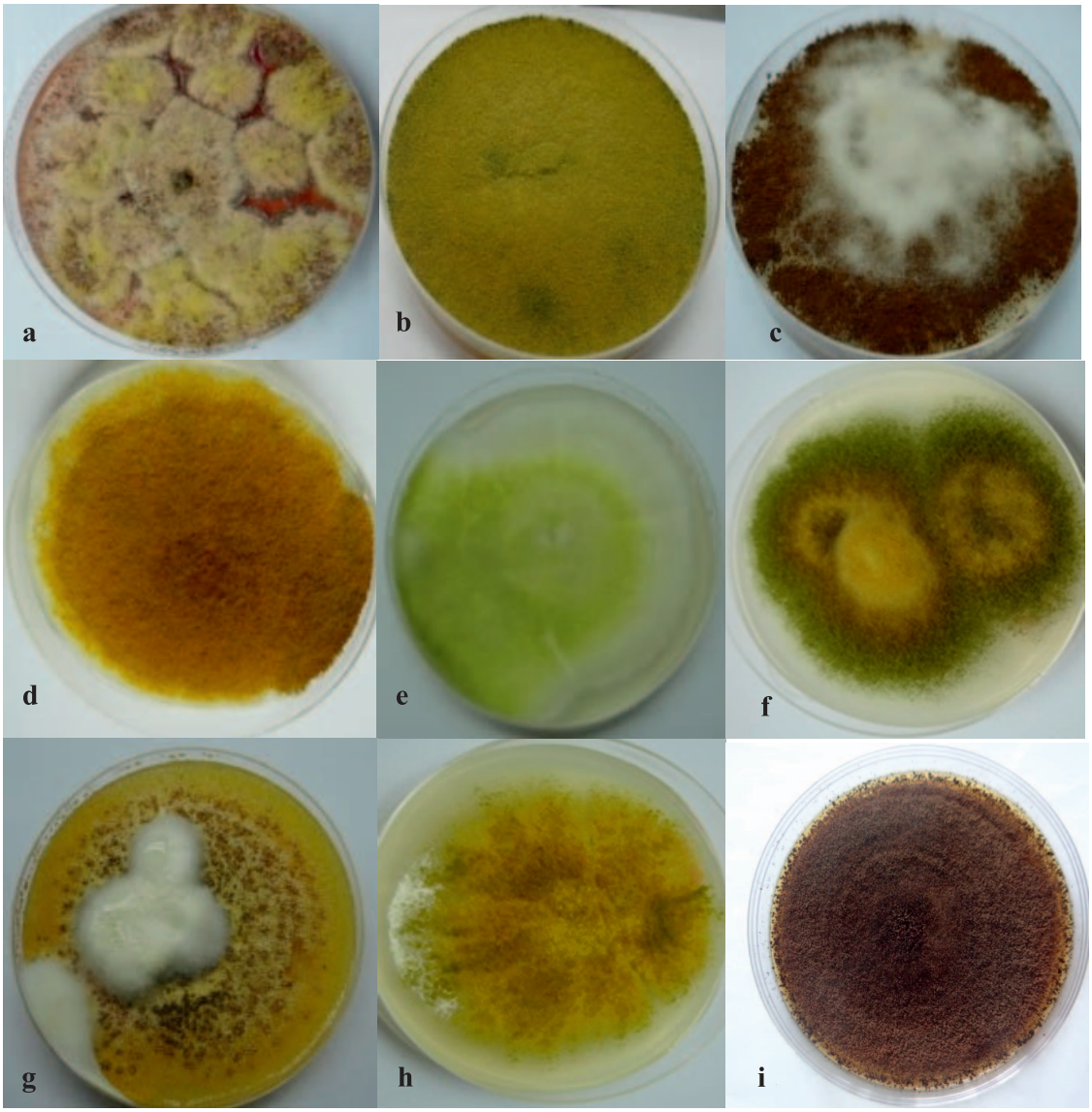

Fig. 2. Colonies of *A. flavus* (a), *A. parasiticus* (b), *A. tamarii*, (c and d), *A. oryzae* (e and f), *A. nomius* (g), *A. terreus* (h), and *A. niger* (i), respectively, all growing on Czapek's agar (CzA).

lower detection limit of 4 ppb, we could be under-reporting on the percentage of toxigenic isolates present. Interestingly, *A. flavus* isolates may lose the ability to produce aflatoxin in-vitro following successive culture transfers (Sweany *et al.*, 2011) and this too, could have affected our results. Therefore, to better determine toxigenicity, a combination of assays may be used. Abbas *et al.* (2004), reported that out of 517 isolates tested, combining cultural assays reduced false positives for aflatoxigencity to 0% and false negatives to 7%.

In this study, we also sought to identify and characterize for the first time in eastern Zambia, fungi from *Aspergillus* spp. isolated from soils cropped to groundnut. 91 *Aspergillus* spp. isolates, randomly selected from soil dilution plates (Fig. 1), were identified and characterized for aflatoxin production. We identified *A. flavus* (small (S) and large (L) sclerotial strains), *A. parasiticus*, *A. niger, A. nomius*, *A. oryzae*, *A. tamarii,* and *A. terreus*. *A. flavus* colonies were identified as greenish-yellow to yellow-white colonies on CzA (Fig. 2A), but were mostly green on MEA (Fig. 3A), and white-green colonies on PDA (Fig. 4A). Some *A. flavus* colonies were sclerotial with sparse hyphal growth on nutrient agar. *A. flavus* S- and L-strain isolates had sclerotia $\leq$400 µm or >400 µm in diameter, respectively. Under microscopic examination, conidia of *A. flavus* colonies were mostly smooth-walled and were borne on phialades, which were attached to metulae on globose vesicles (Fig. 5A). *A. flavus* is a common fungus in the soil and has been isolated in arid, temperate, and humid agro-

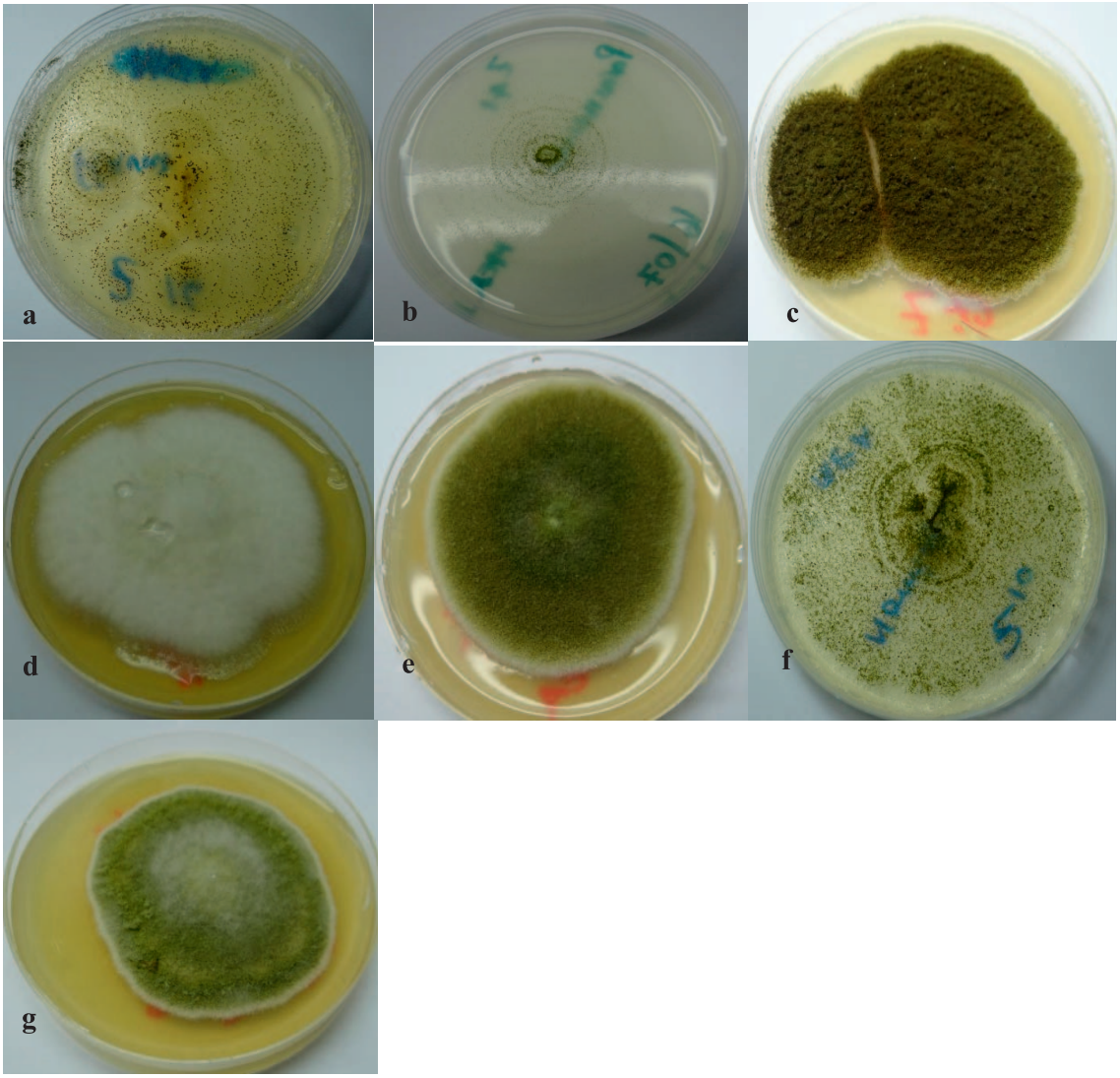

Fig. 3. Colonies of *A. flavus* (a), *A. parasiticus* (b), *A. tamarii*, (c), *A. oryzae* (d and e), *A. nomius* (f), and *A. terreus* (g), respectively, all growing on Malt Extract Agar (MEA).

ecologies worldwide (Abbas *et al*., 2004; Boyd and Cotty, 2001; Griffin and Garren, 1974; Horn, 2005; Horn 2003; Okoth 2014; Sweany *et al*., 2011)

In contrast, *A. parasiticus* colonies were pale green on both CzA (Fig. 2B) and MEA (Fig. 3B), and dark green on PDA (Fig. 4B). Under microscopic examination, conidial walls of *A. parasiticus* were echinulate and the stalks of conidiophores were rough-walled. Conidia were borne on phialades which lack metulae (Fig. 5B). *A. parasiticus* is also a common soil inhabitant which is mostly isolated in cooler temperate areas but is distributed worldwide (Domsch *et al*. 2007).

A. tamarii were comparatively easier to identify. Colonies of *A. tamarii* were chocolate-brown to cinnamon-brown on CzA (Fig. 2C and D), whereas on MEA, colonies were greenish with tints of brown (Fig. 3C), and green with more pronounced brown on PDA (Fig. 4C). Under microscopic observation, conidia of *A. tamarii* were echinulate and characteristically attached in chains, radiating from phialades lacking metulae (Fig. 5C). *A. tamarii* does not produce aflatoxin but produces another neurotoxin—cyclopiazonic acid (CPA) (Boyd and Cotty, 2001). The impact of CPA contamination in food is however an area not well researched into, especially in Africa. In the US, *A. tamarii* is mostly isolated from soil but occasionally isolated from plants (Horn, 2007). However, Horn (2007) discusses that the distribution of *A. tamarii* is not well defined in the US, but in contrast, Okoth *et al*. (2011) reported that *A. tamarii* was mostly found in the cooler areas of Nandi in Kenya, compared to the hotter and drier regions of Makueni.

For *A. oryzae*, colonies on CzA varied from yellow-white (Fig. 2E) to green-brown (Fig. 2F),

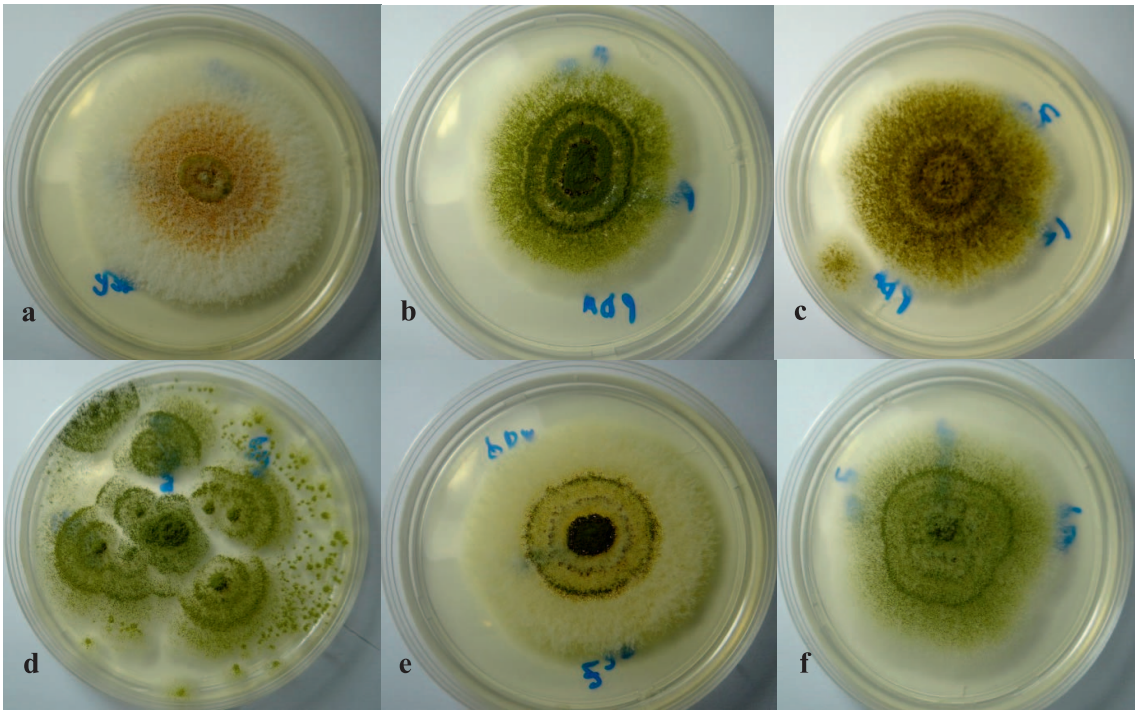

Fig. 4. Colonies of *A. flavus* (a), *A. parasiticus* (b), *A. tamarii*, (c), *A. oryzae* (d), *A. nomius* (e), and *A. terreus* (f), respectively, all growing on Potato Dextrose Agar (PDA).

whereas on MEA colonies were white (Fig. 3D) or green (Fig. 3E). Compared to *A. flavus*, conidia of *A. oryzae* were echinulate, and were borne on conidiophores with characteristically long stalks (Fig. 6A). In addition, unlike for *A. flavus*, *A. oryzae* colonies did not form sclerotia. *A. oryzae* is important in food processing in Asia where it is used to ferment products and in the making of soy sauce, sake, etc (Domsch *et al.*, 2007). The fungus has also been isolated worldwide in soils from cultivated fields and also in agricultural products.

For *A. nomius,* colonies were green to yellow with white sectors on CzA (Fig. 2G), light green on MEA (Fig. 3F) and white fluffy colonies on PDA (Fig. 4E). *A. nomius* colonies formed sclerotia (Figs. 2G and 3F), mostly larger than 400 μm in diameter. Under the microscope, conidia were slightly echinulate with globose vesicles and had roughened conidiophores (Fig. 6B). There are few reports on the isolation and identification of *A. nomius*, a comparatively newer aflatoxin producing species. In the US, *A. nomius* is most reliably found in agricultural soils of the Mississippi Delta (Horn, 2007).

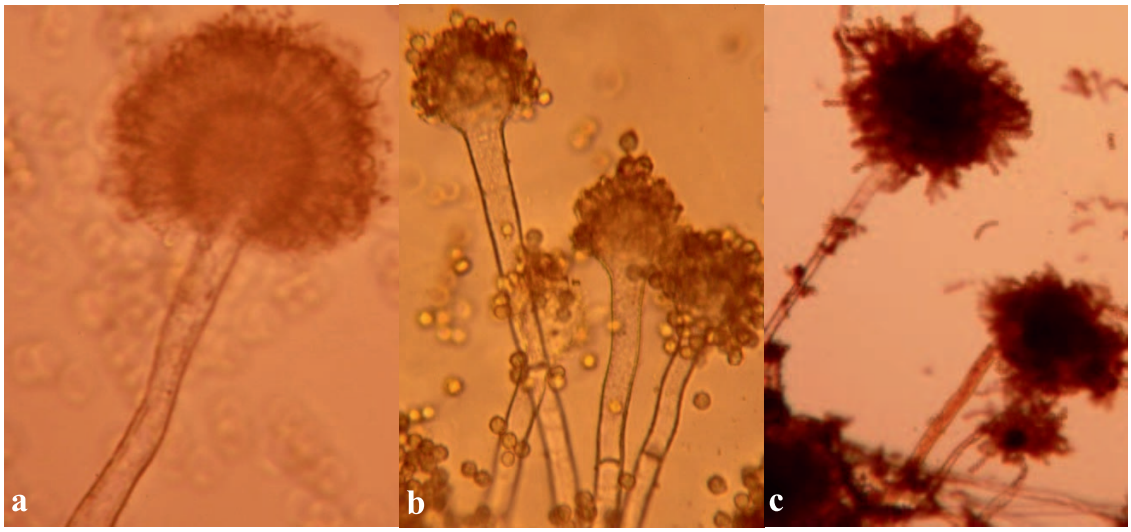

Fig. 5. *A. flavus* (a), *A. parasiticus* (b), and *A. tamarii* (c).

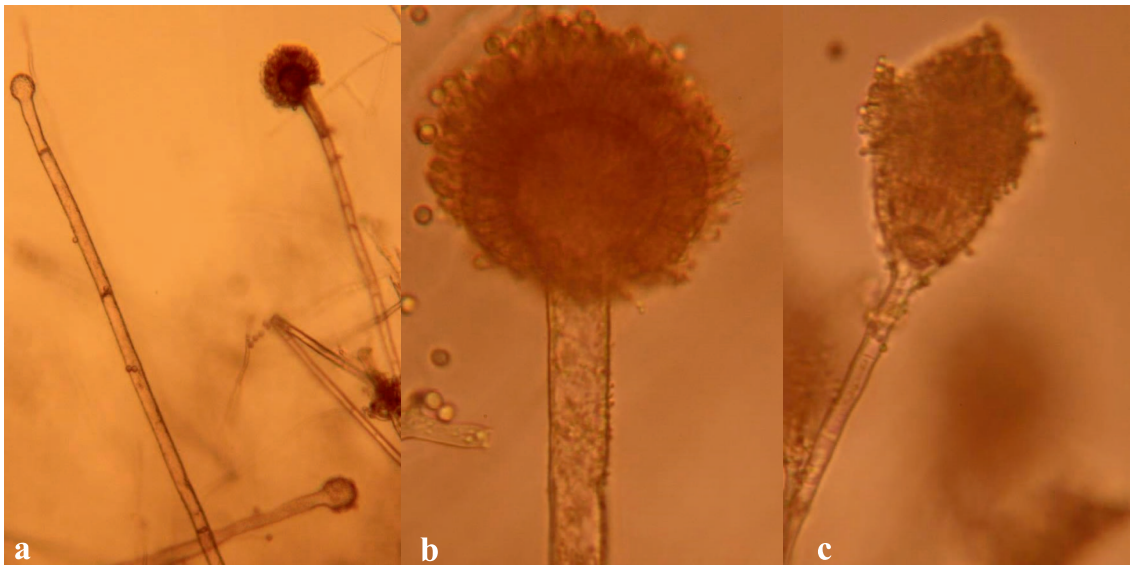

Fig. 6. ***A. oryzae*** **(a),** ***A. nomius*** **(b), and** ***A. terreus*** **(c).**

For *A. terreus*, colonies were cinnamon to light brown on CzA (Fig. 2H) and light green on both MEA (Fig. 3H) and on PDA (Fig. 4F). Under microscopic observation, vesicles of *A. terreus* were semi-spherical and had conidia arising from phialades attached to metulae (Fig. 6C). Conidia heads of *A. terreus* were strongly columnar arising from the top sides of the vesicles (Fig. 6C). Lastly, colonies of *A. niger* were characteristically black, both on CzA (Fig 2I) and on PDA. *A. niger* (Fig. 7) is an important spoilage fungus that can also attack young peanut seedling in the field causing crown rot (Melouk and Damicone, 1997), and produces ochratoxins (Perrone *et al*., 2007). Its importance in causing crown rot and reducing plant stand has not been documented in Zambia. *A niger* is ubiquitous in soil (Melouk and Damicone, 1997).

Fig. 7. ***A. niger***

Summary and Conclusions

Despite the importance of aflatoxin contamination and increased focus on mitigation efforts, information on the identification of aflatoxigenic fungi in Africa is still limited. To our knowledge, this is the first peer reviewed report from Zambia documenting the population densities of *A. flavus* across different agroecologies. In addition, it is also the first report on the identification of different *Aspergillus* spp., such as *A. nomius*, *A. terreus*, *A. oryzae*, and *A. tamarii* from Zambia. However, our findings on the presence of these different *Aspergillus* spp. is not surprising since these fungi have been isolated in many different ecologies worldwide (Abbas *et al*., 2004; Boyd and Cotty, 2001; Domsch *et al*., 2007; Horn, 2003; Horn, 2005; Horn *et al*., 1995; Jamie-Garcia and Cotty, 2006; Monyo *et al*., 2012; Okoth *et al*., 2012). The significance of our research is the documentation of the presence of different toxigenic species, such as the S-strain *A. flavus* and also *A. nomius*, but also L- strain *A. flavus* and *A. parasiticus*.

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