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BIODIVERSITY OF MYCOBIOTA IN PEANUT SEEDS, CORN AND WHEAT GRAINS WITH SPECIAL REFERENCE TO THEIR AFLATOXIGENIC ABILITY

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ABSTRACT

The fungal biodiversity in 30 samples, 10 of each of peanut seeds, corn and wheat grains were estimated on two isolation media (AFPA and DRBC). The aflatoxigenic ability of isolates related to *Aspergillus* section *Flavi* using CAM agar plates was also assessed. The results indicated that the mean moisture content was relatively low in peanut (8.05%) while it was relatively high in corn samples (10.45%). A limited number of peanut seeds but a large number of corn grains were fungi-free on both media. On AFPA, the aflatoxigenic species contaminated 9, 5 and 7 of peanut, corn and wheat samples respectively. On AFPA and DRBC, the total number of genera and species recorded on wheat were higher than those on corn and peanut and only three genera, *Aspergillus*, *Penicillium* and *Fusarium* were isolated from the three substrates. *Aspergillus* possessed more propagules on peanut than on corn and wheat, whereas *Fusarium* and *Penicillium* had more propagules on corn than on peanut and wheat. The aflatoxigenic species *A. flavus*, and *A. niger* were isolated from the three substrates. These two species showed higher propagules on peanut and corn than on wheat. *P. chrysogenum*, *P. duclauxii* and *F. verticillioides* were recorded from corn and wheat grains, *P. aurantiogriseum* and *F. chalamydosporum* from corn and *P. brevicompactum*, *P. funiculosum* from wheat and *P. pinophilum* and *F. oxysporum* from peanut only. Flourescence at 365 nm of 43 *A. flavus* and other 4 fungal strains recovered from the analyzed substrates and grown on CAM agar plates revealed that all *A. flavus* strains showed blue color with different intensities indicating aflatoxin B production, while the other 4 non-*A. flavus* strains showed negative results.

Keywords: Peanut, corn, wheat, aflatoxigenic fungi, aflatoxins

INTRODUCTION

Most agricultural commodities are susceptible to fungal invasion before, during or after harvesting, owing to improper handling, drying, transportation and/or storage. Food contamination by fungi and their toxic metabolites (mycotoxins) still remains a serious global problem. It is estimated that as much as 25 % of the worlds' cereals are contaminated with fungi and known mycotoxins, while a higher percentage could be contaminated with toxins are yet unidentified (**Mannon and Johnson, 1985**). Peanuts and cereals are foods of intermediate moisture content, thus fungal and mycotoxin development may occur at the farm or at the site of storage, affects the yield, quality and nutritive value of the products. The degree of mould contamination in stored grains can be used as a measure of their quality (**Karunaratne and Bullerman, 1990**). Moreover, some grains may contain mycotoxins as a result of fungal growth (**CAST, 1989; FAO, 1990**). Mycotoxins which occur naturally in foods are of significance in terms of food safety. These are produced mainly by species of *Aspergillus*, *Penicillium* and *Fusarium* (**Bullerman, 1979**).
In countries like Egypt, where a hot and humid climate prevails, agricultural practices favour mould growth (**Rustom, 1997**), data on moulds, their mycotoxins as well as other deteriorating factors in foods are urgently needed. Peanuts, corn and wheat grains, which contribute protein and calories, form the basis for the staple diet of the majority of the people in Egypt and other developing countries. In general, such seeds and grains are high risk crops (**Mirocha, 1983**). Historically only *A. flavus*, *A. parasiticus* and *A. nomius* have been known as producers of aflatoxins (**Ehrlich *et al.*, 2007**). However, **Coppock and Christian (2007)** have since reported that *A. bombycis*, *A. ochraceoroseus* and *A. pseudotamarii* are also capable of producing aflatoxins.
The objective of this study was to investigate the mycobiota and possibly the aflatoxigenic species associated with peanut seeds, corn and wheat grains grown in Egypt.

MATERIAL AND METHODS

Collection of peanut, corn and wheat samples

Thirty samples, 10 of each of peanut seeds, corn and wheat grains were collected from Assiut and Sohag Governorates after harvesting during the period from January to May 2013. These samples were from those marketed for human consumption and were apparently in good conditions. Each sample (1 Kg each) was placed in additional sterile polyethylene bag. The samples were transferred into the laboratory and kept at 4ºC pending moisture content determination and mycological analyses.

Determination of moisture content

The moisture content was estimated by drying triplicates of known weight of the samples (peanut, corn and wheat) at 110º C for 24 h and then reweighed (**Magan and Lacey, 1985; Pitt and Hocking, 2009**). The moisture content is expressed as the average percentage of the weight loss of the three replicates.

Isolation, enumeration and identification of fungi

Two different media were used to assess the mycological quality of the samples: one for general isolation of fungi (dichloran rose Bengal chloramphenicol agar, DRBC of **King *et al.*, 1979,** modified by **Pitt and Hocking, 1997**), and the other for selective isolation of *Aspergillus flavus* and *Aspergillus parasiticus* fungi (*Aspergillus flavus/parasiticus* agar, AFPA of **Pitt *et al.*, 1983**).
Direct plating technique adopted by the second international workshop (**Pitt *et al.*, 1992**) was used, as it was recommended for isolating fungi from peanut seeds. The seeds and grains were briefly surface disinfected by 50 ml house-hold chlorine bleach (nominally 4-5 % active chlorine) added to 450 ml distilled water

for 2 minutes before culture. Peanut seeds, corn and wheat grains were cultured on the surface of agar plates (5 plates/sample, 5 particles/plate, with a total of 25 seeds or grains on each medium type for each sample).
DRBC agar plates were incubated at 28ºC for 7–10 days after which the growing colonies were counted, identified whenever possible from the original plates, otherwise sub-cultured on potato dextrose agar (PDA) slants, allowed to grow, then stored at 4ºC for later identification. On the other hand, the inoculated plates of AFPA medium were incubated for 42-48 hours at 30°C. After incubation the reverse of the Petri-dishes was examined for a bright yellow/orange coloration (**Pitt *et al.*, 1983; Pitt and Hocking, 2009**). The identification of the aflatoxigenic aspergilli and other moulds was confirmed on the basis of their macroscopic and microscopic features.

Identification of fungi

The identification of fungi was carried out using the methods, media and plating techniques described by **Raper and Fennell (1965); Ellis (1971); Pitt (1979); Moubasher (1993); Leslie and Summerell (2006); Domsch *et al*. (2007); and Pitt and Hocking (2009).**

Screening for aflatoxins

All *Aspergillus flavus* strains (43) in addition to some other fungal strains (4) collected during this study were screened for aflatoxin-producing ability using coconut agar medium (CAM). The medium was prepared according to **Davis *et al.* (1987)** as follows: 100 g of shredded coconut was homogenized for 5 min with 300 ml hot distilled water, then the homogenate was filtered through four layers of cheese cloth, completed to 1000 ml by distilled water, the pH of the clear filtrate was adjusted to pH 7 and 20 g agar were added. The medium was autoclaved for 15 min at 121ºC, cooled to about 40º – 45ºC, and poured while being stirred into sterile Petri-dishes. Fungal isolates were inoculated at the center of CAM agar plates and incubated at 25ºC in the dark for 7 days. Cultures were observed for flourescence under long-wave UV light (365 nm) after 3, 5 and 7 days. The positive results were shown as blue fluorescence and an uninoculated plate was observed as a reference.

RESULTS AND DISCUSSION

Moisture content of the samples

The moisture content in peanut samples ranged from 5.94 to 10.27% with a mean of 8.05±1.25 while those in corn grain samples fluctuated from 9.29 to 13.40% with a mean of 10.45±1.18. In wheat grain samples they varied from 7.51% to 11.58% with a mean of 9.56±1.24 (Table 1). In this respect, the moisture content of peanut seed samples collected from Uganda and Kenya ranged from 5.07% to 7.97% with a mean of 6.63% (**Ismail, 2000**), while for those collected from Kenya ranged from 3.3% to 6.9% (**Wagacha *et al.*, 2013**), and of maize collected from Egypt ranged from 8.75-16.76% (**Abdel-Hafez *et al.*, 2014**).

Overview on the mycobiota of peanut seeds and corn and wheat grains

From the summarized data presented in table (1) it could be noted that all samples from peanut seeds and wheat grains collected from Assiut and Sohag Governorates were infested with fungi as revealed on AFPA and DRBC media. However only 9 out of 10 samples of corn were contaminated as revealed on both media.
On AFPA, the aflatoxigenic species, that showed orange brown pigmentation after 48 hours of incubation at 30ºC (Fig 1), contaminated a total of 9, 5 and 7 of peanut, corn and wheat samples respectively. In this respect all 144 *A. flavus* isolates isolated from Algerian wheat were of bright orange reverse on AFPA plates (**Riba *et al.*, 2010**).
On DRBC, 10, 8 and 5 samples yielded aflatoxigenic species of the analyzed substrates. From peanut seeds analyzed from both governorates, 94.8% and 99.6% were infested with fungi on AFPA and DRBC, respectively, while only 42.8% and 47.2% of corn grains were contaminated with fungi on the two isolation media, respectively. Regarding wheat samples only 55.2% and 64% of the grains were contaminated with fungi on both media respectively (Table 1).
Limited numbers of peanut seeds (13 out of 250 on AFPA and only 1 on DRBC), while large numbers of corn grains (143 out of 250 and 132 out of 250) were fungi-free on AFPA and DRBC, respectively. On the other hand, 112 out of 250 and 90 out of 250 wheat grains were fungi-free on both media respectively. Regarding fungal isolates from the three substrates (peanut, corn and wheat), the largest number was reported from peanut (403 and 467/250 seeds analysed) while the least numbers were reported from corn (119 and 126/250 grains) on AFPA and DRBC, respectively (Table 1).

Fungal diversity in peanut seeds and corn and wheat grains on *Aspergillus flavus/parasiticus* agar medium (AFPA)

All peanut and wheat samples but nine corn samples were contaminated with fungi. However more propagules were obtained from peanut. The total number of genera and species recorded on wheat (13 genera and 20 species) were higher than those obtained on corn (8 and 17) and peanut (4 and 5) (Table 2). Only two genera, *Aspergillus* and *Penicillium* were isolated from the three substrates investigated (peanut, corn and wheat) on AFPA at 30ºC. *Aspergillus* possessed more propagules on peanut than on corn and wheat, but *Penicillium* had more propagules on corn than on peanut and wheat. From *Aspergillus*, the aflatoxigenic species (*Aspergillus flavus*), and *A. niger* were recovered from the three substrates. Both species showed high propagules on peanut and corn than on wheat (Table 2). These two species were also the most dominant in maize from Egypt (**Abdel-Hafez *et al.*, 2014**). In addition, two more *Aspergillus* species were recovered from corn (*A. fumigatus* and *A. tamarii*) but not from peanut or wheat, and two species were recovered from wheat (*A. candidus* and *A. clavatonanica*) but not from peanut or corn (Table 2). In this respect, **Riba *et al.* (2010)** found that out of the 150 strains of *Aspergillus* section *Flavi* isolated from Algerian wheat, 144 were identified as *Aspergillus flavus* and 6 as *Aspergillus tamarii*. Other *Aspergillus* species isolated belonged to the section *Nigri*, *Circumdati* and *Terrei*. **Abriba *et al.* (2013)** registered *Aspergillus flavus, A. parasiticus, A. terreus, A. niger, A. versicolor, A. ochraceus* and *A. nidulans* from peanut, maize and/or wheat in Nigeria.
None of *Penicillium* species was recorded from the three substrates investigated. *P. chrysogenum* was isolated from both corn and wheat grain samples only, *P. aurantiogriseum* and *P. duclauxii* from only corn and *P. brevicompactum* and *P. funiculosum* from only wheat. Unidentified *Penicillium* species was recorded from peanut and corn. Unidentified species of *Penicillium* were recorded from peanut, maize and/or wheat in Nigeria (**Abriba *et al.*, 2013**) and from maize in Egypt (**Abdel-Hafez *et al.*, 2014**).
Rhizopus stolonifer was recorded in high frequency from peanut and in rare frequency from wheat but was missing from corn. In agreement with our findings, species of *Rhizopus* were reported as dominant from peanut in Uganda (**Ismail, 2000**), but missing in maize from Egypt (**Nooh *et al.*, 2014**), however they were reported from maize and wheat but not from peanut in Nigeria (**Abriba *et al.*, 2013**).
Acremonium (2 species), *Chaetomium globosum*, *Nigrospora oryzae*, *Rhizoctonia solani* and species of *Fusarium* were recorded from corn and wheat but not from peanut. Also, *Acremonium potronii, Fusarium nygamai, F. oxysporum, F. verticillioides* were recorded only from corn but *Acremonium strictum* from only wheat. Some more species were registeded only from wheat grains (*Alternaria* represented by *A. alternata, A. chlamydospora* and *Alternaria* sp., *Epicoccum nigrum, Phaeoacremonium* sp., *Scytalidium lignicola* and *Stemphylium botryosum*, all of them are dematiaceous hyphomycetes), but *Mucor* and sterile mycelia from only peanut and *Trichothecium roseum* from only corn. Species of *Fusarium*, *Alternaria*, *Botryotrichum*, *Cladosporium*, *Setosphaeria* and *Tichothecium* were also reported from Egyptian maize (**Abdel-Hafez *et al.*, 2014**).

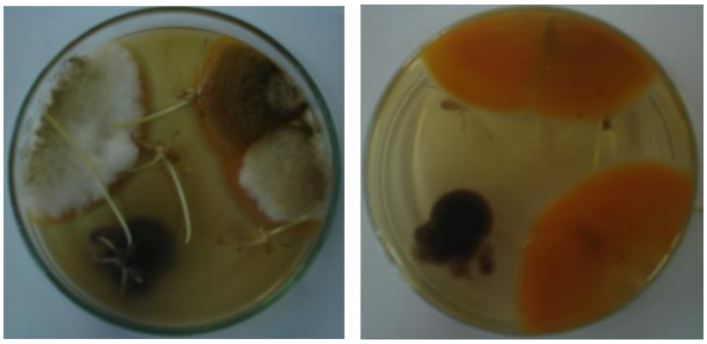

Figure 1 Bright yellow/orange coloration on AFPA indicting aflatoxigenic *Aspergillus* species

Table 1 Summarized data for the mycobiota analysis of peanut, corn and wheat samples collected from Assiut (A) and Sohag (S) Governorates on AFPA and DRBC media

Substrate	Peanut						Corn						Wheat					
Medium	AFPA			DRBC			AFPA			DRBC			AFPA			DRBC		
Governorate	A	S	Total	A	S	Total	A	S	Total	A	S	Total	A	S	Total	A	S	Total
Samples analyzed	5	5	10	5	5	10	5	5	10	5	5	10	5	5	10	5	5	10
Samples infested with fungi	5	5	10	5	5	10	4	5	9	4	5	9	5	5	10	5	5	10
Samples infested with aflatoxigenic species	4	5	9	5	5	10	3	2	5	4	4	8	4	3	7	2	3	5
Seeds (grains) analyzed	125	125	250	125	125	250	125	125	250	125	125	250	125	125	250	125	125	250
Seeds (grains) infested with fungi	125	112	237	125	124	249	40	67	107	38	80	118	60	78	138	57	103	160
% seeds (grains) infested with fungi	100	89.6	94.8	100	99.2	99.6	32	53.6	42.8	30.4	64	47.2	48	62.4	55.2	45.6	82.4	64
Fungi-free seeds (grains)	0	13	13	0	1	1	85	58	143	87	45	132	65	47	112	68	22	90
% fungi-free seeds (grains)	0	10.4	5.2	0	0.8	0.4	68	46.4	57.2	69.6	36	52.8	52	37.6	44.8	54.4	17.6	36
Total isolates obtained	250	153	403	241	226	467	49	70	119	43	83	126	62	78	140	64	112	176
Total aflatoxigenic isolates obtained	97	29	126	71	34	105	17	5	22	12	7	19	10	6	16	3	4	7
Genera	2	4	4	5	7	8	4	8	8	4	7	7	8	9	13	11	10	13
Species	3	5	5	9	9	13	6	16	17	6	11	14	14	10	20	16	11	22
% moisture contents: mean ± SD [min-max]	8.1±1.3 [5.94-10.27]						10.5±1.2 [9.29-13.40]						9.56±1.2 [7.51-11.58]					

Table 2 Colony forming units (CFUs, calculated per 250 seeds or grains in all samples), percentage CFUs and frequency of fungi isolated from peanut seeds, corn and wheat grains recovered on *Aspergillus flavus/parasiticus* agar at 30ºC.

Fungi	Peanut			Corn			Wheat		
	CFUs	%CFUs	%F	CFUs	%CFUs	%F	CFUs	%CFUs	%F
Acremonium				2	1.68	10	1	0.71	10
A. potronii Vuillemin				2	1.68	10			
A. strictum W. Gams							1	0.71	10
Alternaria							59	42.14	100
A. alternata (Fries) Keissler							52	37.14	90
A. chlamydospora Mouchacca							2	1.43	10
Alternaria sp.							5	3.57	30
Aspergillus	228	56.58	100	59	49.58	70	25	17.86	80
A. candidus Link							1	0.71	10
A. clavatonanica Bat. *et al.*							2	1.43	10
A. flavus Link	126	31.27	90	22	18.49	50	16	11.43	70
A. fumigatus Fresenius				1	0.84	10			
A. niger van Tieghem	102	25.31	90	35	29.41	70	6	4.29	40
A. tamarii Kita				1	0.84	10			
Chaetomium globosum Kunze				1	0.84	10	5	3.57	30
Epicoccum nigrum Link							3	2.14	30
Fusarium				12	10.08	60	4	2.86	20
F. nygamai Burgess & Trimboli				2	1.68	20			
F. oxysporum Schlechtendal				2	1.68	20			
F. verticillioides (Saccardo) Nirenberg				6	5.04	30			
Fusarium sp.				2	1.68	20	4	2.86	20
Mucor sp.	1	0.25	10						
Nigrospora oryzae (Berkeley & Broome) Petch				4	3.36	20	13	9.29	40
Penicillium	7	1.74	40	35	29.41	70	6	4.29	40
P. aurantiogriseum Dierckx				6	5.04	40			
P. brevicompactum Dierckx							1	0.71	10
P. chrysogenum Thom				11	9.24	30	4	2.86	20
P. duclauxii Delacroix				14	11.76	70			
P. funiculosum Thom							1	0.71	10
Penicillium sp.	7	1.74	40	4	3.36	10			
Phaeoacremonium sp.							1	0.71	10
Rhizoctonia solani Kühn				5	4.20	30	1	0.71	10
Rhizopus stolonifer (Ehrenberg) Vuillemin	151	37.47	100				14	10	10
Scytalidium lignicola Pesante							1	0.72	10
Stemphylium botryosum Wallr.							7	5	20
Sterile mycelia (dark & white)	16	3.97	60						
Trichothecium roseum (Persoon: Fries) Link				1	0.84	10			
Total number of propagules	403	100	100	119	100	90	140	100	100
Number of genera		4			8			13	
Number of species		5			17			20	

Fungal diversity in peanut seeds and corn and wheat grains on dichloran rose Bengal chloramphenicol agar medium (DRBC)

As the case on AFPA, more propagules were obtained from peanut than from wheat or corn on DRBC medium. On the other hand, broader spectrum of species was recorded on wheat (22 species) than on corn (14) or peanut (13) (Table 3).

Three genera, *Aspergillus*, *Fusarium* and *Penicillium* were isolated from the three substrates (Table 3).

Aspergillus possessed more propagules on peanut than on corn and wheat, while *Fusarium* and *Penicillium* had more propagules on corn than on peanut and wheat. From *Aspergillus*, the aflatoxigenic species (*Aspergillus flavus*) and *A. niger* were recovered from the three substrates. Both species showed higher propagules on peanut and corn than on wheat (Table 3). Several studies reported the predominance of *A. flavus* in peanut samples originating from Egypt (**El-Shanshoury *et al.*, 2014**), Nigeria (**Abriba *et al.*, 2013**), Uganda (**Ismail, 2000**), Kenya (**Wagacha *et al.*, 2013**), Iran (**Hedayati *et al.*, 2010**), Libya (**Attiatalla *et al.*, 2010**) and Saudi Arabia (**Deabes and Al-Habib, 2011**), in maize samples originating from Egypt (**Nooh *et al.*, 2014; Abdel-Hafez *et al.*, 2014**), Kenya (**Muthomi *et al.*, 2012**), Malaysia (**Reddy and Salleh, 2011**), Nigeria (**Ezekiel *et al.*, 2012**), Hungary (**Toth *et al.*, 2012**), and in wheat grain samples originating from Egypt (**Mazen *et al.*, 1984; Abdel-Hafez *et al.*, 1990; El-Shanshoury *et al.*, 2014**), Australia (**Berghofer *et al.*, 2003**), Argentina (**Vaamonde *et al.*, 2003**), Iran (**Ghiasian et al., 2004**), Nigeria (**Abriba *et al.*, 2013**), Libya (**Attitalla *et al.*, 2010**) and Turkey (**Bayder *et al.*, 2005**). It was also found that

the incidence of aflatoxigenic species *A. flavus* was higher in peanuts (69 %, 49. 38 %) than in wheat (13 %, 7.67 %) in Argentina (**Vaamonde *et al.*, 2003**) and Libya (**Attitalla *et al.*, 2010**), respectively.

In addition only one more *Aspergillus* species was recovered on peanut and wheat (*A. candidus*) but not from corn, while *A. terreus* was recovered on peanut and corn but not from wheat, and *A. fumigatus* was recovered on wheat but not from peanut or corn (Table 3). These aspergilli and others were previously reported from peanut, maize and/or wheat (*A. niger*; **Reddy and Salleh, 2011; El-Shanshoury *et al.*, 2014**), maize in Egypt (*A. terreus*; **El-Shanshoury *et al.*, 2014**), maize grains in Kenya (*A. flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, A. versicolor* and *A. clavatus*; **Muthomi *et al.*, 2012**).

None of *Fusarium* species was recorded from the three investigated substrates. Only *F. verticillioides* and an unidentified *Fusarium* species were recovered from both corn and wheat, *F. chlamydosporum* from corn, and *F. oxysporum* from peanut. Also, none of *Penicillium* species was represented in the three substrates. Only *P. chrysogenum* and *P. duclauxii* were recorded from both corn and wheat, *P. funiculosum* and *P. pinophilum* from peanut, *P. aurantiogriseum* from corn, and an unidentified *Penicillium* species was recorded from peanut and corn (Table 3). *Fusarium verticillioides, F. graminearum, F. proliferatum, F. equiseti* and *Penicillium* sp. were prevalent in corn samples in Malaysia (**Reddy and Salleh, 2011**), while the most dominant *Fusarium* species in maize grains in Egypt were in the following order: *F. verticillioides, F. oxysporum, F. solani, F. proliferatum, F. udum* and *F. nisikadoi* (**Abdel-Hafez *et al.*, 2014**).

Some other species were isolated from two substrates but not from the third e.g. *Nigrospora oryzae* from corn and wheat but not from peanut, *Rhizopus stolonifer* in high frequency from peanut and in low frequency from wheat but not from corn, and *Rhizoctonia solani* from peanut and corn but not from wheat. On the other hand, some other species were recorded from one substrate but not from the others e.g. *Macrophomina phaseolina*, unidentified species of *Acremonium* and *Trichoderma* and yellow sterile mycelia were recorded from peanut; *Alternaria alternata, Alternaria* sp., *Botroytrichum piluliferum, Chaetomium elatum, C. globosum, Chaetomium* sp., *Cladosporium cladosporioides, C. oxysporum, Epicoccum nigrum, Eurotium amstelodami, Stemphylium botryosum* and *Thermoascus aurantiacus* were recorded from only wheat; and *Chaetomium spirale* and *Setosphaeria rostrata* were recorded from only corn (Table 3). In this respect, six fungal genera, namely *Aspergillus, Penicillium,* and *Fusarium* from peanut, maize and wheat, and *Mucor* (maize), *Rhizopus, Alternaria* and *Cladosporium* (wheat) (**El-Shanshoury *et al.*, 2014**) and from Egyptian maize samples collected from 10 governorates including Assiut and Sohag prior to storage, however *Alternaria* and *Rhizopus* were not recorded from samples collected from Assiut and Sohag ((**Nooh *et al.*, 2014**). Also species of *Mucor* and *Rhizopus* dominated some corn samples collected from Malaysia (**Reddy and salleh, 2011**). **Misra *et al.* (2010)** isolated 16 co-existing fungal species with *A. flavus* as frequent on wheat grains in India and these were *A. fumigatus, A. japonicus, A. niger, A. tamarii, Emericella nidulans, Candida albicans, Chaetomium globosum, C. spirale, Cladosporium cladosporioides, Fusarium oxysporum, Memnoniella echinata, Mucor hiemalis, Penicillium citrinum, P. funiculosum, Torula convoluta* and *Trichoderma viride*. **Ismail (2000)** found also that the most common fungal species in peanuts collected from Kenya and Uganda were *Rhizopus stolonifer, Aspergillus parasiticus, Fusarium solani, Lasiodiplodia theobromae* and *Penicillium chrysogenum* on DRBC. **Abriba *et al.* (2013)** recorded species of *Aspergillus* (*A. flavus, A. parasiticus, A. terreus, A. niger, A. versicolor, A. ochraceus, A. nidulans*), *Penicillium* (2 species) and *Rhizopus* (1 species) from peanut, maize and/or wheat in Nigeria.

Flourescence of aflatoxigenic species and other fungal strains recovered from peanut seeds, corn and wheat grains on coconut agar medium (CAM)

The fluorescence at 365 nm of 47 fungal strains grown on CAM was observed. Twelve out of the 15 *Aspergillus flavus* strains recovered from peanut seeds showed intense blue color indicating aflatoxin B production and only 3 weak aflatoxigenic strains (showing faint colour), one of them (AUMC 9769) fluoresced greenish yellow color indicating aflatoxin G production (Fig. 2). The other 4 strains originating from peanut seeds (related to *Aspergillus candidus, A. niger, Macrophomina phaseolina* and *Penicillium funiculosum*) showed negative results (Table 4). Out of 11 *Aspergillus flavus* strains recovered from corn grain samples, only two showed strong aflatoxigenic ability (intense blue color) while 9 strains showed faint blue color indicating low aflatoxin-producing ability (Table 4). The flourescence at 365 nm of 17 *A. flavus* strains recovered from wheat grains revealed 5 strains as very highly aflatoxigenic and 5 strains were also highly aflatoxigenic (intense blue color), but only 7 strains showed faint blue color indicating lower production of aflatoxin B (Table 4). In this respect, **Riba *et al.* (2012)** screened 150 isolates that belong to *A. flavus* (144 isolates) and *A. tamarii* (6) isolated from Algerian wheat for aflatoxin production on coconut agar medium (CAM), and found only 45 isolates (30 %) were aflatoxigenic. Also, in the screening of **Ezekiel *et al.* (2012)** of 90 isolates of *Aspergillus* section *Flavi* on neutral red desiccated coconut agar medium (NRDCA) it was found that only 35.6 % of the isolates produced the characteristic fluorescence of aflatoxins.

Table 3 Colony forming units (CFUs, calculated per 250 seeds or grains in all samples), percentage CFUs and frequency of fungi isolated from peanut seeds, corn and wheat gra recovered on dichloran rose Bengal chloramphenicol agar at 28ºC.

Fungi	Peanut			Corn			Wheat		
	CFUs	CFUs%	F%	CFUs	CFUs%	%F	CFUs	CFUs%	%F
Acremonium sp.	1	0.21	10						
Alternaria							70	39.77	100
A. alternata							69	39.20	100
Alternaria sp.							1	0.57	10
Aspergillus	284	60.81	100	38	30.16	80	13	7.38	70
A. candidus	1	0.21	10				4	2.27	10
A. flavus	105	22.48	100	19	15.08	80	7	3.98	50
A. fumigatus							1	0.57	10
A. niger	177	37.90	100	18	14.29	70	1	0.57	10
A. terreus Thom	1	0.21	10	1	0.79	10			
Botrytrichum piluliferum Saccardo & Marchal							1	0.57	10
Chaetomium				4	3.17	30	13	7.38	30
C. elatum Kunze							2	1.14	10
C. globosum							7	3.98	30
C. spirale Zopf				4	3.17	30			
Chaetomium sp.							4	2.27	10
Cladosporium							12	6.82	60
C. cladosporioides (Fres.) de Vries							10	5.68	40
C. oxysporum Berk. & M.A. Curtis							2	1.14	20
Epicoccum nigrum							5	2.84	50
Eurotium amstelodami Mangin							1	0.57	10
Fusarium	6	1.28	30	14	11.11	70	5	2.84	20
F. chalamydosporum Wollenweber & Reinking				8	6.35	30			
F. oxysporum	6	1.28	30						
F. verticillioides				3	2.38	20	4	2.27	10
Fusarium sp.				3	2.38	20	1	0.57	10
Macrophomina phaseolina (Tassi) Goidanch	8	1.71	40						
Nigrospora oryzae				11	8.73	20	7	3.98	50
Penicillium	40	8.57	40	52	41.27	90	3	1.70	20
P. aurantiogriseum				1	0.79	10			
P. chrysogenum				13	10.32	30	1	0.57	10
P. duclauxii				28	22.22	40	2	1.14	10
P. funiculosum	1	0.21	10						

P. pinophilum Hedgcock	12	2.57	20						
Penicillium sp.	27	5.78	40	10	7.93	40			
Rhizoctonia solani	3	0.64	30	2	1.59	10			
Rhizopus stolonifer	122	26.12	90				19	10.80	30
Setosphaeria rostrata Leonard				5	3.97	10			
Stemphylium botryosum							26	14.77	50
Sterile mycelia (yellow)	1	0.21	10						
Thermoascus aurantiacus Miehe							1	0.57	10
Trichoderma sp.	2	0.43	20						
Total number of propagules	467	100	100	126	100	90	176	100	100
Number of genera		8			7			13	
Number of species		13			14			22	

Table 4 Flourescence (at 365 nm) of *Aspergillus flavus* and other fungal strains recovered from peanut seeds, corn and wheat grains as revealed on coconut agar medium (CAM).

Substrate	**Peanut**		**Corn**		**Wheat**	
Species	AUMC No.	Flourescence on CAM	AUMC No.	Flourescence on CAM	AUMC No.	Flourescence on CAM
A. flavus	9768	++	9783	++	9806	+++
A. flavus	9769	+ GY*	9784	+	9808	+
A. flavus	9770	++	9785	+	9810	+++
A. flavus	9771	+	9786	+	9813	++
A. flavus	10135	++	9787	+	10133	+
A. flavus	9801	++	9788	+	9816	++
A. flavus	9772	++	9790	++	9817	+
A. flavus	9773	++	9793	+	9843	++
A. flavus	9778	++	9794	+	9848	+
A. flavus	9779	++	9796	+	9849	+++
A. flavus	9780	++	9797	+	9850	+
A. flavus	10134	+	--		9851	++
A. flavus	9781	++	--		9852	+++
A. flavus	9782	++	--		9853	+++
A. flavus	9803	++	--		9854	+
A. flavus	--		--		9855	+
A. flavus	--		--		9856	++
A.candidus	9777	-ve	--		--	
A. niger	9802	-ve	--		--	
Macrophomina phaseolina	10137	-ve	--		--	
Penicillium funiculosum	9776	-ve	--		--	
Total *flavus*-strains tested	15		11		17	
Total positive strains	15		11		17	
Total negative strains	4 (non-*flavus*)		0		0	

*GY = Greenish yellow fluorescence, fluorescence on CAM is expressed as –ve: negative result, +: weak intensity, ++: high intensity, and +++: very high intensity.

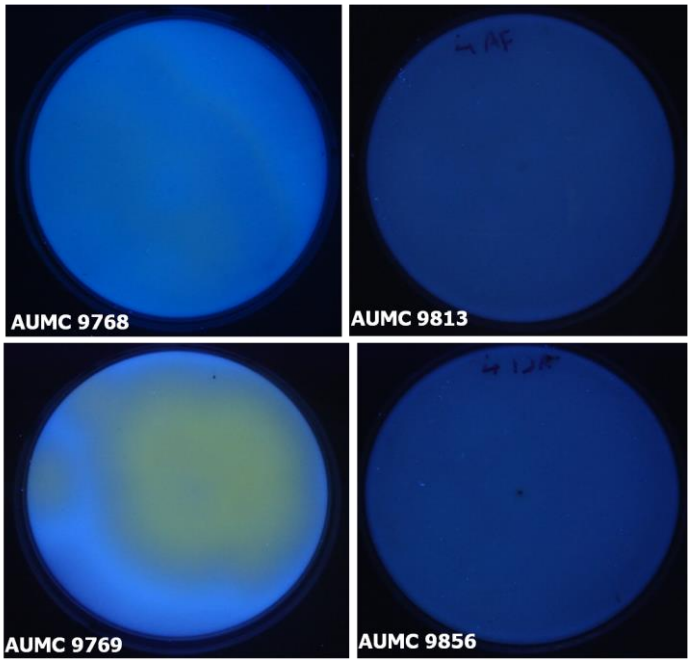

Figure 2 Blue fluorescence visible at 365 nm on coconut agar medium (CAM) for *Aspergillus flavus* strains Nos. AUMC 9768, AUMC 9813 & AUMC 9856 and greenish yellow fluorescence for AUMC 9769.

CONCLUSION

The number of fungal species recorded on wheat grains were higher than those recorded on corn and peanut. Only three genera (*Aspergillus*, *Penicillium* and *Fusarium*) were isolated from the three substrates with more propagules of *Aspergillus* being heavily contaminating peanut samples and more *Fusarium* and *Penicillium* propagules heavily contaminating corn samples. The aflatoxigenic species (*A. flavus*) was common on the three substrates on both isolation media. The results of flourescence at 365 nm of the 43 *A. flavus* and other 4 fungal strains recovered from peanut seeds, corn and wheat grains and grown on CAM agar plates, revealed that all *A. flavus* strains showed blue color with different intensities indicating aflatoxin B production, except one strain (AUMC 9796 originating from peanut seed) gave greenish yellow colour indicating aflatoxin G production, while the other 4 non-*A. flavus* strains showed negative results. Because of their deleterious effects, the incidence of moulds and levels of mycotoxins in foods should be frequently and routinely determined.

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