Egypt. J. Bot. **Vol. 57,** No. 1 pp.119-137 (2017)

Fungi Associated with Maize and Sorghum Grains and their Potential for Amylase and Aflatoxins Production

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USING direct-plating technique, thirty-nine species belonging to 16 fungal genera were isolated from maize and sorghum grain samples (20 samples each) on Czapek's agar (Cz) and Czapek's supplemented with 40% sucrose agar (Cz40S) media at 28ºC. Widest spectrum of genera and species were recorded on sorghum (16 genera and 35 species) compared with maize grains (8 and 19). The highest total count (139 CFUs/ 100 grains) and the number of genera (14) and species (27) were identified from sorghum grains on Cz medium. The most common fungi on the grains tested were *Aspergillus flavus, A. niger, Eurotium amstelodami, E. rubrum, E. repens, Fusarium verticillioides* and *Rhizopus stolonifer*. Among 129 isolates screened for their abilities to produce amylase enzyme, 102 isolates could produce this enzyme, of which *A. terreus* exhibited the highest production (EI=1.73). HPLC analysis revealed that out of 6 strains of *A. flavus* tested, strain No. AUMC 11311 showed the highest production of aflatoxin B_1 and B_2 while the highest value of aflatoxin G_2 was produced by strain No. AUMC 11317. It could be concluded that fungi growing on grains and have the abilities of producing enzymes and/or aflatoxins might cause deterioration and spoilage to these grains.

Keywords: Fungi, Deterioration, Sorghum, Maize, Aflatoxins, Amylase.

Maize is the third most important food crop in the world surpassed only by wheat and rice. Yemen production of maize is estimated 221,078 tons according to the Statistical Agricultural Center in 2013. *Alternaria alternata, Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. parasiticus, Bipolaris maydis, Chaetomium globosum, Cladosporium cladosporioides, Cochlibolus lunata, Drechslera halodes, Epicoccum, Fusarium culmorum, F. graminearum, F. oxysporum, F. proliferatum, F. semitectum, Penicillium citrinum, P. funiculosum, P. oxalicum, Phoma herbarum, Rhizopus oryzae, R. stolonifer, Rhizoctonia solani* and *Trichoderma harzianum* were isolated from maize grains in Egypt (Ismail *et al.,* 2016), Pakistan, (Niaz and Dawar 2009), Ethiopia (Ofgea and Gure, 2015) and Colombo (Abe *et al.,* 2015).

Sorghum is located the fifth among cereal crops with 60 million tons

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annually produced in the world. It is the staple food grain for over 750 million people in Africa, Asia, and Latin America (Wilson *et al.,* 1995). Several studies on fungi associated with sorghum grains, were done and noticed that *Alternaria, Arthrinium, Aspergillus Botrytis*, *Cercosporella*, *Cladosporium*, *Colletotrichum*,, *Curvularia*, *Phoma*, *Rhizopus*, *Fusarium*, *Humicola* and *Trichoderma* were isolated (Abdel-Hafez *et al.,* 2014, Mohammed *et al.,* 2015 and Machio 2016).

One of the main ways of fungal attack of the grain is that production of cell wall- degrading enzymes. Several filamentous fungi have proven to be an important source of hydrolytic enzymes (Ogbonna *et al.,* 2015). *Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. oryzae* and *A. terreus*, *Emericella nidulans, Mucor racemosus,Mycosphaerella tassiana*, *Penicillium chrysogenum*, *Rhizopus oligosporus*, *R. stolonifer* and several others are used as sources of fungal α- amylases (Irfan *et al.,* 2012 and Singh *et al.,* 2014). In addition, amylases have immense applications on various fields in world market because of their wide applications in industries including food, brewing, distilling industry, textile, paper, pharmaceutical and bioconversion of solid wastes (Lall *et al.* 2015).

The other way of fungi to deteriorate the grains is by forming mycotoxins. Aflatoxins are natural secondary metabolites produced mainly by *Aspergillus flavus* group (section *Flavi*) that contaminant agricultural commodities in the field particularly in critical temperature and humidity conditions before or during harvest or in storage (Rustom, 1997 and Sweeney and Dobson 1998). Aflatoxins are classified to B_1 (AFB_1) and B_2 (AFB_2), produced by *A. flavus* and G_1 (AFG_1) and G_2 (AFG_2), produced by *A. flavus* as well as *A. parasiticus*. The WHO-International Agency for Research on Cancer (IARC) has classified all types of aflatoxins as carcinogenic agents to humans (IARC, 2002). Aflatoxins are largely associated with commodities produced in the tropics and subtropics crops, such as cotton, peanuts, pistachios, sorghum and maize (Yin *et al.,* 2008 and Kange *et al.,* 2015).

The objective of the current study was to assess the fungal diversity associated with maize and sorghum grains collected from Taiz Governorate, Yemen. Amylase production by isolated fungi and aflatoxigenic potential of some *Aspergillus flavus* strains were also evaluated.

Material and Methods

Collection of samples

Forty samples of maize (20 samples) and sorghum grains (20) were collected from different markets and local stores, in Taiz Governorate, Yemen (Table 1). The samples were put in clean polyethylene plastic bags, brought to the Mycological Laboratory and kept at 5ºC till fungal analysis.

Determination of moisture content (MC%)

The moisture contents of grain samples were estimated using the method described by Abdel-Hafez *et al.* (2014) and expressed as percentage of dry weight.

Germinability test

The viability of grains was assessed through their ability for germination. Filter papers were placed inside the Petri-dishes, moisten the paper until there was a tiny bit of standing water. Ten grains were put above the wet paper in each plate (Gummert, 2011). Put the plates in a warm place (30°C). Check the germination of grains every day, count germinated grain seedlings and the percentage of germination was calculated as following:

$$\% \text{ germination} = \frac{\text{No. of germination seeds}}{\text{No. of total seeds planted}} \times 100$$

Isolation and identification of fungi

The direct-plating technique was used to determine grain-borne fungi of maize and sorghum. Czapek's (Cz) and Czapek's supplemented with 40% sucrose (Cz40S) agar media incubated at 28ºC were used for isolation of fungi. Five grains of each sample were placed directly on the surface of each agar plate (Pitt *et al*., 1992). Four-replicate plates were used for each medium and the plates were incubated at 28°C for 7 days. The developing fungi were counted0, isolated, identified and calculated as colony forming units (CFUs) per 20 grains for each sample. Isolated fungi were identified mainly on the basis of their macro- and microscopic features following the keys of Raper and Fennell (1965), Booth (1971), Ellis (1971), Pitt (1979), Moubasher (1993), Leslie and Summerell (2006), Domsch *et al.* (2007), Pitt and Hocking (2009) and Ismail *et al.* (2015).

Screening for α- amylase production

One hundred and twenty-nine isolates collected from maize (41 isolates) and sorghum grains (88) were assayed for their abilities to produce α- amylase on modified Czapek's agar medium with the following composition (g/l): starch, 30; $NaNO_3$, 3; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.5; KCL, 0.5; $FeSO_4$, 0.01; agar, 15 and distilled water, 1000 ml (Bridge, 1985). The inoculated agar plates were incubated at 28°C for 5-7 days, then flooded with iodine solution. A clear zone around fungal growth indicates the production of α-amylase (Cowan, 1974). Enzyme index (EI) was calculated according to Ho and Foster (1972) as follows:

$$\text{Enzyme index (EI)} = = \frac{\text{Diameter of the outer limited of the clear zone}}{\text{Diameter of the fungal colony}} -$$

Screening for aflatoxins

Nineteen isolates of *Aspergillus flavus, A. flavus* var. *columnaris* and *A. tamarii*, collected during the current work, were screened for their aflatoxin potential using coconut agar medium (CAM). 100 g of shredded coconut were homogenized for 5 min with 300 ml hot distilled water, then filtered and the volume was completed into 1000 ml with distilled water (pH 7), after that 20 g agar were added. After sterilization, 15-20 ml was poured into sterile Petri-dishes. Fungal isolates were inoculated at the center of CAM agar plates and incubated at 28ºC in the dark for 7 days. Cultures were observed for fluorescence under UV light (365 nm). The positive results were detected as blue fluorescence and an uninoculated plate was observed as a reference (Davis *et al.*, 1987).

Extraction of aflatoxins from fungal isolates

To determine production of aflatoxin, six isolates of fungi were inoculated on potato dextrose broth. After 10 days incubation period, the content of each flask was homogenized with 100 ml chloroform, washed with equal volume of distilled water, dried over anhydrous sodium sulphate, filtered then concentrated under vaccum to near dryness, and diluted with 1 ml chloroform (Pons *et al*., 1972).

Assessment of aflatoxins by HPLC

For determination of aflatoxin from six fungal isolates, silica-based HPLC columns bonded with C8 or C18 groups are used with mobile phases consisting of binary or ternary mixtures of polar solvents. Commonly used solvent (deionized water, methanol and acetonitrile). In the reversed phase mode, the elution order of the common aflatoxins is B_1, B_2 and G_2 (Sekar *et al.* 2008).

Results

Moisture contents of the grains

Results in Table 1 showed that, moisture content of sorghum grains (10.6-20.2%) were much higher than that of maize grains (8.9-11.2%). The highest value of MC% for sorghum grains was recorded in sample number 37, while the lowest value was detected in sample No. 22. On the other hand, maize sample No. 11 showed the highest moisture content, while, Nos. 4 and 20 recorded the least MC% values.

Germinability of the grains

The percentage of germination for 20 grain samples ranging between, 40-100% of maize and 30–90% of sorghum grains. Maize grain Nos. 8, 10, 18, 20 gave 100% germination. While, the highest germinability of sorghum grains (90%) were detected in samples Nos. 28 and 35. On the other hand, low values of germinability were recorded in sorghum grain sample No. 30 and in maize grain sample No. 9 (Table 1).

TABLE 1. Localities, percentage moisture content (MC%) and percentage germinability (G%) of maize and sorghum grain samples.

Locality	Maize			Sorghum		
	No.	MC%	G%	No.	MC%	G%
Alshenany	1	10.1	70	21	14	50
Alshenany	2	10.8	60	22	10.6	70
Alshenany	3	9.1	60	23	11.6	60
Alshenany	4	8.9	80	24	14.2	80
Alshenany	5	9.7	90	25	11	80
Alsamsara	6	9.3	70	26	15.2	70
Alsamsara	7	9.2	90	27	12.5	80
Alsamsara	8	9.6	100	28	13.2	90
Alsamsara	9	10.1	40	29	13.2	50
Alsamsara	10	10.5	100	30	14.2	30
Alsamsara	11	11.2	70	31	14.8	80
Bearbasha	12	10.5	60	32	12.5	60
Bearbasha	13	11	70	33	12.4	70
Bearbasha	14	9.6	80	34	11.9	80
ALaamor village	15	10.8	80	35	12.7	90
ALaamor village	16	9.3	70	36	13.4	80
ALaamor village	17	9.7	90	37	20.2	70
26 September Street	18	10.5	100	38	12.7	50
26 September Street	19	10.1	50	39	14.3	80
26 September Street	20	8.9	100	40	14.6	70

Fungi recovered in the present investigation

All sorghum grain samples were contaminated with fungi, while 17 maize samples (out of 20) were contaminated by fungi. Thirty-nine species belonging to 16 fungal genera were isolated from maize and sorghum grain samples on Czapek's agar (Cz) and Czapek's supplemented with 40% sucrose agar (Cz40S) media. The total number of genera (16) and species (35) recorded on sorghum were highly significant than those obtained on maize (8 genera and 19 species) (Tables 1, 2, 3, 4 & Fig. 1,2).

TABLE 2. Mean ± standard error of total fungal counts and number of species isolated from maize and sorghum grains on Cz and Cz40S agar media.

Source & Media	*Aspergillus*	*Eurotium*	*Fusarium*	*Penicillium*	Total count	No of species
Maize Cz	102.75 ± 13.4^{b}	2 ± 0.4^{a}	19.5 ± 2.8^{c}	10.5 ± 3.1^{b}	121 ± 2.3^{a}	7.25 ± 0.6^{a}
Cz40	69.75 ± 3.1^{a}	45.25 ± 3.1^{b}	00 ± 00^{a}	10 ± 2.3^{b}	125.75 ± 7.2^{ab}	7.75 ± 0.3^{a}
Sorghum Cz	89.25 ± 2.9^{ab}	7.25 ± 3.0^{a}	5.75 ± 1.3^{b}	1.5 ± 0.9^{a}	138.25 ± 2.3^{b}	10.25 ± 0.5^{b}
Cz40	72 ± 2.0^{a}	58.25 ± 1.5^{c}	0.25 ± 0.3^{a}	0.75 ± 0.8^{a}	138 ± 1.2^{b}	10.25 ± 0.5^{b}
F	**4.8***	**145.9****	**34.4****	**7.02****	**4.8***	**11.2****

* = significant, ** = highly significant.

Aspergillus, *Eurotium*, *Fusarium* and sterile mycelia possessed more propagules on sorghum than on maize, while, *Penicillium* had highly significant propagules on maize than on sorghum. The total viable counts of fungi in sorghum on Cz (139 CFUs/ 100 grains) and on Cz40S (138) were significant than those in maize on Cz40S (126 CFUs/ 100 grains) and on Cz (121) (Tables 2, 3). The highest total fungal count was recorded in sorghum grains on Cz agar medium (139 CFUs/ 100 grains), followed by those isolated on Cz40S (138 CFUs per 100 sorghum grains) (Fig. 1, 2).

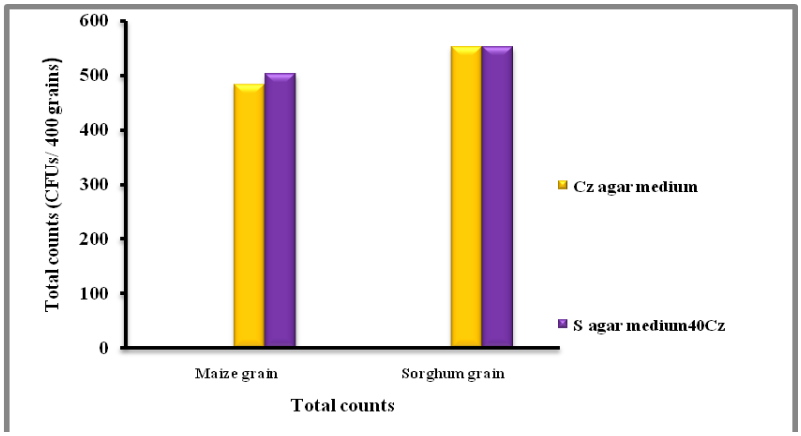

Fig. 1. Diagram illustrating the total counts of fungi isolated from maize and sorghum grains (per 100 grains) on Cz and Cz40S agar media.

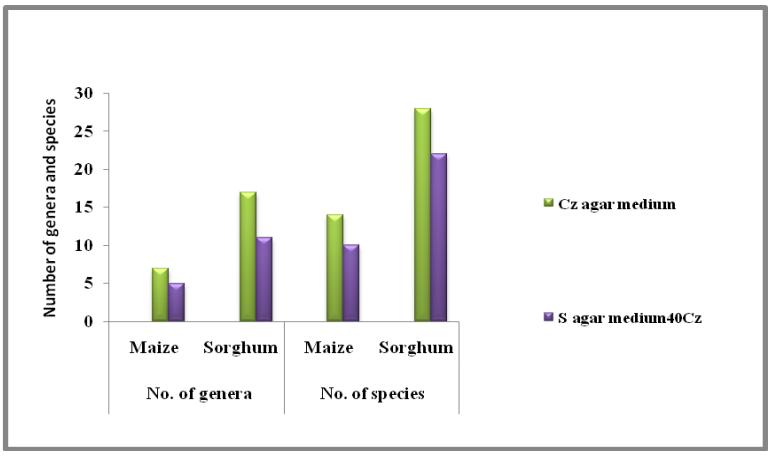

Fig. 2. Diagram illustrating the number of genera (NG) and species (NS) of fungi isolated from maize and sorghum grains (per 100 grains) on Cz and Cz40S agar media.

Fungi isolated from maize grains

Seventeen and 11 species related to 7 and 5 genera were recorded from maize grains on Cz and Cz40S agar media, respectively (Table 3). *Aspergillus* (7 and 6 species; isolated from 95% and 100% of total samples, comprising 65.6% and 55.6% of total fungi respectively) is the only genus isolated in high occurrence on both Cz and Cz40S. Whereas, *Rhizopus* (50% of total samples and 5.9% of total fungi), *Fusarium* (40% and 16.1%) and *Penicillium* (35% and 8.7%) were recorded in high frequency on Cz and *Eurotium* (85% of total samples and 35.9% of total fungi) was isolated highly on Cz40S. But *Penicillium* (30% and 7.9%) was isolated in moderate frequency on Cz40S. The most common species on both media were *A. niger* (60% and 85% of total samples), *A. flavus* (80% and 65%), on Cz were *Rhizopus stolonifer* (50%), *F. verticillioides* (40%), *Aspergillus tamarii* (25%) and *Penicillium pinophilum* (30%), on Cz40S were *Eurotium rubrum* (45%), *E. repens* (40%), *A. ochraceus* (30%), *A. tamarii* (30%) and *P. pinophilum* (30%) (Table 3).

Fungi isolated from sorghum grains

The results in Table 4 showed that 14 and 10 genera, represented by 27 + 1 variety and 22 species were isolated from sorghum grains samples on Cz and Cz40S agar media, respectively.

Aspergillus was recovered in high frequency (95% of the total samples on both Cz and Cz40S; black sterile mycelia and *Rhizopus* (50% each) on Cz and *Eurotium* (90% of total samples) on Cz40S. *A. flavus* (70% and 50% of the total samples), *A. niger* (55% and 30%) and *E. amstelodami* (30% and 55%) were the most common species on both Cz and Cz40S, respectively. *R. stolonifer* (50%) and *F. verticillioides* (30%) were the most common on Cz only (Table 4).

There are highly significant in total count of *Eurotium* on both media, while total counts of *Penicillium*, *Fusarium* and number of species recorded from maize and sorghum showed high significant difference (Table 2).

Amylase production

Among the 129 isolates tested, 102 (79.1% of the total isolates) were able to produce amylase enzyme. Of these isolates, only one isolate related to *A. terreus* and isolated from sorghum showed high capability (EI= 1.73). On the other hand, 8 isolates were moderate producers, they belonged to *Scopulariopsis brevicaulis*, *C. ovoidea*, *Fusarium solani*, *Cochliobolus spicifer* (1 isolate each), *Drechslera halodes* and *Penicillium griseofulvum* (2 isolates each). The remaining 93 positive isolates were weak producers. The negative producers (27 isolates) were all related to *Eurotium* group (Table 5).

TABLE 3. Total counts (TC, calculated per 100 grains in all samples), percentage total counts (TC%) and percentage frequency (F%) of fungi isolated from maize grain samples on Czapek's (Cz) and Czapek's supplemented with 40% sucrose (Cz40S) agar media at 28ºC.

Fungal Taxa	Czapek's agar			Czapek's 40% sucrose agar		
	TC	TC%	F%	TC	TC%	F%
Aspergillus P. Micheli ex Link	79.25	65.6	95	69.75	55.6	100
A. flavus Link	36.5	30.2	80	12	9.5	65
A. niger van Tieghem	27	22	60	42.5	33.8	85
A. ochraceus Wilhelm	0.25	0.2	5	3.5	2.8	30
A. sydowii (Bainier & Sartory) Thom & Church	2.75	2.3	10	2.5	1.98	5
A. tamarii Kita	5	4.1	25	7	5.6	30
A. terreus Thom	0.75	0.62	10			
A. vadensis Samson, de Vries, Frisvad & Visser	7	5.8	15	2.25	1.8	10
Cladosporium cladosporioides (Fresenius) de Vries	0.25	0.2	5			
Emericella nidulans (Eidam) Vuillemin	0.5	0.4	5			
Eurotium Link ex Gray				45.25	35.9	85
E. repens de Bary				27.5	21.8	40
E. rubrum Konig, Spieckermann & Bremer				17.75	14.1	45
Fusarium Link	19.5	16.1	40			
F. proliferatum (Matsush.) Nirenberg	0.75	0.6	5			
F. solani (Martius) Saccardo	4.5	3.7	5			
F. verticillioides (Saccardo) Nirenberg	14.25	11.8	40			
Mucor Fresenius	1.25	1	20	0.5	0.4	5
M. circinelloides Tieghem	0.75	0.6	15			
M. hiemalis Wehmer	0.5	0.4	5	0.5	0.4	5
Penicillium Link	10.5	8.7	35	10	7.9	30
P. duclauxii Delacroix	0.25	0.2	5			
P. pinophilum Hedgcock	10.25	8.5	30	10	7.9	30
Rhizopus stolonifer (Ehrenberg) Vuillemin	7.25	5.9	50	0.25	0.2	5
Sterile mycelia	2.5	2.1	15			
Total count	121			126		
No. of genera 8	7			5		
No. of species 19	17			11		

TABLE 4. Total counts (TC, calculated per 100 grains in all samples), percentage total counts (TC%) and percentage frequency (F%) of fungi isolated from sorghum grain samples on Czapek's (Cz) and Czapek's supplemented with 40% sucrose (Cz40S) agar media at 28ºC.

Fungal Taxa	Czapek's agar			Czapek's 40% sucrose agar		
	TC	TC%	F %	TC	TC%	F %
Alternaria. citri Ellis and Pierce emend., Bliss & Fawcett				0.25	0.18	5
Aspergillus	89.25	64.5	95	72	52.2	95
A. aculeatus Lizuka	9.25	6.7	25	20.75	15.03	30
A. brasiliensis Varga, Frisvad & Samson	6	4.3	10	3.5	2.54	10
A. clavatus Desmazieres	8.75	6.3	20			
A. flavus Link	24.5	17.7	70	12.75	9.24	50
A. flavus var. *columnaris* Raper & Fennell	2.75	1.98	10			
A. japonicas Saito				0.25	0.18	5
A. niger van Tieghem	33.25	24	55	17	12.3	30
A. parasiticus Speare				5	3.62	5
A. tamarii Kita	1.75	1.3	15	1.5	1.1	15
A. terreus Thom	0.5	0.3	10			
A. vadensis Samson, de Vries, Frisvad & Visser	2.5	1.8	5	11.25	8.15	25
Chaetomium globosum Kunze	0.5	0.3	5			
Cladosporium Link	0.25	0.2	5	1.25	0.9	15
C. cladosporioides (Fresenius) de Vries	0.25	0.2	5	0.5	0.36	10
C. sphaerospermum Penzig				0.75	0.5	5
Cochliobolus spicifer Nelson	0.5	0.3	10			
Curvularia Boedijn	1.5	1.1	20			
C. lunata (Wakker) Boedijn	1.25	0.9	15			
C. ovoidea (Hiroe &Watan.) Muntanole	0.25	0.2	5			
Drechslera halodes (Drechsler) Subram and Jain	2.5	1.8	25	0.25	0.18	5
Emericella nidulans (Eidam) Vuillemin				0.25	0.18	5
Eurotium Link ex Gray	7.25	5.3	40	58.25	42.2	90
E. amstelodami Mangin	3.75	2.7	30	20.5	14.9	55
E. intermedium Blaser				1.25	0.9	5
E. pseudoglaucum Blochwitz				6.25	4.53	15
E. repens de Bary				11.75	8.51	30
E. rubrum Konig, Spieckermann & Bremer	3.5	2.5	10	18.5	13.4	35
Fusarium Link	5.75	4.2	35	0.25	0.18	5
F. lateritium Nees	1	0.7	10			
F. verticillioides (Saccardo) Nirenberg	4.75	3.4	30	0.25	0.18	5
Mucor Fresenius	4.5	3.3	25	0.25	0.18	5
M. circinelloides Tieghem	4	2.9	25	0.25	0.18	5
M. racemosus Fresenius	0.5	0.3	10			
Penicillium Link	1.5	1.1	15	0.75	0.5	15
P. duclauxii Delacroix	0.25	0.2	5			
P. griseofulvum Dierckx	1.25	0.9	10	0.75	0.5	15
Phoma Saccardo	1.5	1.1	25			
P. glomerata (Corda) Wollenw & Hochapfel	0.25	0.2	5			
P. herbarum Westendorp	1.25	0.9	20			
Rhizopus stolonifer (Ehrenberg) Vuillemin	8.25	6	50	1.25	0.9	20
Scopulariopsis brevicaulis(Saccardo) Bainier	0.25	0.2	5			
Sterile mycelia (black)	14.25	10.2	50	3.25	2.4	20
Trichoderma longibrachiatum Rifai	0.25	0.2	5			
Yeast	0.25	0.2	5			
Total count	139			138		
No. of genera 16	14			10		
No. of species (35 + 1 variety)	27+1 variety			22		

TABLE 5. α- amylase enzyme production by the most commonly fungi recovered from maize and sorghum grains.

Species	Isolates tested	Source	Isolation medium	Enzyme index	OR
Aspergillus terreus	1	Sorghum	Cz	1.73	H
Scopulariopsis brevicaulis	1	Sorghum	Cz	1.5	M
Curvularia ovoidea	1	Sorghum	Cz	1.4	M
Fusarium solani	1	Maize	Cz	1.4	M
Cochliobolus spicifer	1	Sorghum	Cz	1.3	M
Drechslera halodes	2	Maize	Cz	1.3	M
		Sorghum	Cz	1.3	M
Penicillium griseofulvum	2	Sorghum	Cz	1.3	M
Alternaria citri	2	Sorghum	Cz40S	1	W
Aspergillus aculeatus	4	Sorghum	Cz	1.04-1.1	W
A. brasiliensis	5	Sorghum	Cz and Cz40S	1.03-1.1	W
A. clavatus	1	Sorghum	Cz	1.03	W
A. flavus	6	Maize	Cz and Cz40S	1-1.2	W
	5	Sorghum	Cz and Cz40S	1.02-1.2	W
A. flavus var. *columnaris*	2	Sorghum	Cz	1.1	W
A. japonicas	1	Sorghum	Cz40S	1.03	W
A. niger	7	Maize	Cz and Cz40S	1-1.1	W
	10	Sorghum	Cz and Cz40S	1.03-1.1	W
A. ochraceus	4	Maize	Cz40S	1.15-1.2	W
A. sydowii	2	Maize	Cz40S	1.15-1.2	W
A. tamarii	2	Maize	Cz and Cz40S	1.1	W
	1	Sorghum	Cz	1.2	W
A. vadensis	1	Sorghum	Cz40S	1.1	W
Chaetomium globosum	1	Sorghum	Cz40S	1	W
Cladosporium cladosporioides	2	Sorghum	Cz	1	W
C. sphaerospermum	1	Sorghum	Cz	1	W
Cochliobolus spicifer	2	Sorghum	Cz and Cz40S	1.05	W
Curvularia lunata	6	Sorghum	Cz and Cz40S	1-1.15	W
C. ovoidea	2	Sorghum	Cz and Cz40S	1-1.05	W
Drechslera halodes	1	Maize	Cz	1.2	W
	3	Sorghum	Cz	1-1.1	W
Fusarium lateritium	2	Sorghum	Cz and Cz40S	1.15-1.2	W
F. solani	2	Maize	Cz	1	W
F. verticillioides	3	Maize	Cz	1 – 1.2	W
	2	Sorghum	Cz	1	W
Mucor circinelloides	1	Sorghum	Cz	1.1	W
Penicillium duclauxii	1	Sorghum	Cz	1	W
P. griseofulvum	1	Maize	Cz	1.15	W
	3	Sorghum	Cz and Cz40S	1.04 – 1.2	W
P. pinophilum	3	Maize	Cz and Cz40S	1 – 1.1	W
Phoma glomerata	2	Sorghum	Cz	1	W
P. herbarum	1	Sorghum	Cz	1	W
Trichoderma longibrachiatum	1	Sorghum	Cz	1	W
Eurotium amstelodami	3	Maize	Cz40S	-Ve	-Ve
	6	Sorghum	Cz and Cz40S	-Ve	-Ve
E. intermedium	4	Sorghum	Cz and Cz40S	-Ve	-Ve
E. pseudoglaucum	1	Sorghum	Cz and Cz40S	-Ve	-Ve
E. repens	5	Maize	Cz40S	-Ve	-Ve
	4	Sorghum	Cz and Cz40S	-Ve	-Ve
E. rubrum	4	Sorghum	Cz and Cz40S	-Ve	-Ve

H= High production (EI= 1.6-1.7)
M= Moderate production (EI= 1.3-1.5)
W= Weak production (EI= 1-1.2).

Fluorescence (at 365 nm) of Aspergillus flavus group on Coconut agar medium (CAM)

Nineteen isolates of Section *Flavi*, including *A. flavus* (17 isolates), *A. flavus* var. *columnaris* and *A. tamarii* (one isolate each) collected from maize and sorghum grains were screened for their abilities to produce aflatoxin on CAM. The results revealed that 7 isolates (36.8% of total isolates) were able to produce fluorescence under U.V light at 365 nm, this indicates the production of aflatoxin. *A. flavus* strain No. AUMC 11314 gave very high fluorescence (++++), while strain Nos. AUMC 11311, AUMC 11312, AUMC 11313 and No. AUMC 11317 showed high aflatoxin production (+++) (Table 6).

TABLE 6. Fluorescence (at 365 nm) of *Aspergillus flavus* group recovered from maize and sorghum grains as revealed on coconut agar medium (CAM).

Species	Maize		Sorghum	
	Strain No.	Fluorescence on CAM	AUMC No.	Fluorescence on CAM
Aspergillus flavus	11325AUMC	-ve	AUMC 11321	-ve
A. flavus	11326AUMC	-ve	11311 AUMC	+++
A. flavus	11327AUMC	-ve	11322 AUMC	-ve
A. flavus	AUMC 11314	++++	AUMC 11323	-ve
A. flavus	AUMC 11315	++	AUMC 11312	+++
A. flavus	AUMC 11328	-ve	AUMC 11324	-ve
A. flavus	11316 AUMC	+	AUMC 11313	+++
A. flavus	AUMC 11329	-ve		
A. flavus	11330 AUMC	-ve		
A. flavus	AUMC 11317	+++		
A. flavus var. *columnaris*			11320 AUMC	-ve
A. tamarii	11331 AUMC	-ve		

fluorescence on CAM is expressed as –ve: negative result, +: weak intensity, ++: moderate intensity, +++: high intensity and ++++: very high intensity.

Assessment of mycotoxins by HPLC.

Using high performance liquid chromatography (HPLC), 6 strains of *A. flavus* collected from maize and sorghum grains (3 isolates each) showed production of aflatoxins B_1, B_2 and G_2 with various degrees. Strain No. AUMC 11311 produced the highest value of both aflatoxins B_1 and B_2 (0.733 mg/l and

0.034 mg/l), while, No. AUMC 11317 had the highest value of aflatoxin G_2 (0.871 mg/l) (Table 7).

TABLE 7. Mycotoxins screening potential of different isolates isolated from maize and sorghum by HPLC.

Species	No. Strain	Source	Aflatoxin		
			B_1	B_2	G_2
Aspergillus flavus	11314 AUMC	Maize	0.007 mg/l	0.016 mg/l	0.553 mg/l
A. flavus	11315 AUMC	Maize	0.003 mg/l	0.003 mg/l	0.613 mg/l
A. flavus	11317 AUMC	Maize	0.002 mg/l	0.002 mg/l	0.871 mg/l
A. flavus	11311 AUMC	Sorghum	0.733 mg/l	0.034 mg/l	0.442 mg/l
A. flavus	11312 AUMC	Sorghum	0.024 mg/l	0.013 mg/l	0.714 mg/l
A. flavus	11313 AUMC	Sorghum	0.006 mg/l	0.004 mg/l	0.585 mg/l

Discussion

Knowledge of the composition of mycobiota in cereal grains, during or post-harvest and in storage is an important step towards the prediction of possible mycotoxin contamination, accordingly, avoiding harmful effect on yield and grain quality.

Moisture content of the grain is a critical factor for fungal growth on the grain, leading to quality loss. Moisture contents of sorghum grain samples were much higher in sorghum than in maize. Moisture content is one of several factors known to influence fungal development and secondary metabolite production in agricultural products (Ezekiel *et al.,* 2014). Also, it has important role in enzyme production by fungi (Dar *et. al.,* 2014). It was reported that, storage fungi require moisture content ranging between 13-18% to invade cereal starchy grains (Moubasher *et al.,* 1972).

The germinability of grains was slightly higher in maize (40-100%) than in sorghum (30-90%), this may be due to high moisture contents in sorghum grains (up to 20.2%) which stimulate fungal growth. In agreement with the current results, Moubasher *et al.* (1980) stated that the germinability of peanut seeds declined with raising the moisture content.

Fungi isolated from maize grains

A total of 19 species belonging to 8 genera were collected from maize grains. *Aspergillus*, *Eurotium*, *Fusarium* and *Rhizopus* were the most common genera of which *A. flavus*, *A. niger*, *E. rubrum*, *E. repens*, *Rhizopus stolonifer* and *F. verticillioides* were the most encountered. El-Shanshoury *et al.* (2014) found that *A. flavus*, *A. niger*, *Penicillium* spp. and *Fusarium* spp. were the most common fungi in samples of cereal grains (maize and wheat) and peanut collected from central Delta province, Egypt. Also, Abe *et al.* (2015) recorded *Fusarium* and *Aspergillus* as the highest diversity fungi in maize grains of Brazil. Most fungi

isolated in the current study (*e.g. Aspergillus, Cladosporium, Curvularia, Fusarium, Mucor, Penicillium* and *Trichoderma*) were frequently found in maize from Pakistan , Saudi Arabia and Ethiopia (Niaz and Dawar, 2009, Mohamed *et al.*, 2013 and Ofgea and Gure, 2015).

Fungi isolated from sorghum grains

The current results showed that, number of genera (16) and species (35), as well as, total counts of fungi isolated from sorghum grains were relatively higher than those isolated from maize (8 genera, 19 species), this means that the higher moisture contents of sorghum (up to 20.2%) than those of maize grains (up to 11.2%) enhance fungal diversity and population on sorghum. *Aspergillus, Eurotium, Fusarium, Rhizopus* and black sterile mycelia were the most frequent genera. *A. flavus, A. niger, Rhizopus stolonifer, E. amstelodami* and *F. verticillioides* were the most common species.

A similar trend was reported for stored sorghum grains in Kenya (Kange *et al.*, 2015). In Nigeria, Abdulsalaam and Shenge (2011) recorded *Aspergillus, Fusarium, Rhizoctonia* and *Curvularia* species as the most common in washed sorghum grains. In a study of Ismail *et al.* (2012), *Aspergillus* and *Eurotium* were isolated in high incidences from cereal baby foods locally produced in Uganda.

amylase production -α

The highly production of α- amylase by to *A. terreus* isolated from sorghum. In the current work is supported by the studies of Khairnar (2014) and Ogbonna *et al.* (2015) who found that strains of *A. niger* and *A. terreus* were highly amylase producers. In accordance with our results, *A. niger, A. flavus, A. japonicus, A. terreus, Cladosporium cladosporioides* and *Chaetomium globosum* were also capable of producing amylase with the strains of *A. niger* and *C. cladosporioides* are the best producers (Reddy and Sreeramulu, 2016). Our finding on the lack of amylase production by the xerophilic species of *Eurotium*, disagree with those of Ulfig *et al.* (2009) and Shivani and Kumar (2015). The above mentioned fungi were previously recorded as α- amylase producers from various substrates (Dar *et al.*, 2014, Pathak *et al.*, 2014 and Lall *et al.*, 2015).

Aflatoxin production

The current results revealed that 36.8% of total isolates tested (19) were able to produce aflatoxins. In this respect, Ismail *et al.* (2016) stated after screening 47 isolates collected from peanut, corn and wheat on CAM that, two *A. flavus* strains (from corn) and 5 (from wheat) showed intense blue fluorescence indicating very high production of aflatoxin B. While 12 strains from peanut and 5 from wheat were high producers. Several studies reported the aflatoxigenic potential of different *A. flavus* strains collected from various seeds and grains all over the world (Riba *et al.*, 2010, Ezekiel *et al.*, 2012, Kana *et al.*, 2013 and Fakruddin *et al.*, 2015).

The results obtained by HPLC showed that, 6 strains of *A. flavus* could produce aflatoxins B_1, B_2 and G_2, but strains No. AUMC 11311 (for aflatoxins B_1 and B_2) and No. AUMC 11317 (for aflatoxin G_2) were the highest producers. Ezekiel *et al.* (2014) observed that, toxigenic strain of *A. flavus* obtained from fonio millet produced higher amounts of aflatoxin B than those from sesame. Moreover, the level of aflatoxin G was higher than that of aflatoxin B. In accordance with the current results, Aflatoxins B_1, B_2, G_1 and G_2 produced previously by several isolates of *A. flavus* collected from various substrates (El-Maraghy and Zohri, 1988, Feizy *et al.*, 2012, Abu-Taleb *et al*., 2012, Kange *et al*. 2015, Lai *et al.*, 2015 and Hamed *et al*., 2016).

Conclusion

The incidence of moulds and levels of mycotoxins in foods should be frequently and routinely determined. Also, there is an urgent need for further studies on fungi associated with stored cereal grains and their enzymes and mycotoxin production.

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(Received 31 /8 / 2016
accepted 26 /11 / 2016)

الفطريات المصاحبة لحبوب الذرة الشامية والذرة الرفيعة وقدرتها على أنتاج أنزيم الاميليز والافلاتوكسينات

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تمثل الذرة الشامية والذرة الرفيعة من أهم الحبوب الموجودة في اليمن والبلدان الأخرى ويعتبر نمو الفطريات وما تنتجه من الإنزيمات والسموم الفطرية من أهم العوامل التى تؤدي إلى فساد الحبوب وفي النهاية فقدان الجودة. تم فى هذا البحث عزل وتعريف الفطريات الملوثة لعدد 40 عينة من حبوب الذرة الشامية والذرة الرفيعة بإستخدام وسطى العزل شابكس آجار و40٪ سكروزآجار باستخدام تقنية الـزرع المباشر للبذورعلى سطح الوسط الغذائي، تم عـزل 39 نوعاً تنتمي الى 16 جنسا. وأظهرت النتائج أن أعداد الأجناس والأنواع المسجلة على الذرة الرفيعة (14 جنس و 27 نوع) كانت أعلى من تلك المسجلة على الذرة الشامية (7 جنس و 17 نوع (وأن أعلى تعداد فطري ظهر من الذرة الرفيعة (139 مستعمرة فطرية لكل 100 حبة) بينما أقلها كان على حبوب الذرة الشامية (121 مستعمرة فطرية لكل 100 حبة) ، كما تبين من النتائج أن أكثر الفطريات شيوعاً و انتشاراً هى اسبرجيلس فلافس ، نيجر، إيروتيم أمستيلُودامى، إيروتيم ربريم ، إيروتيم ريبنس ، فيوزايم فيرتسيلوديس و ريزوبس استولينيفر. وقد أُجريت إختبارات لمعرفة مقدرة 129 عزلة فطرية على إنتاج إنزيم الأميليز ، واتضح من النتائج أن 102 عزلـة لها قدرة على إنتاج أنزيم الأميليز حيث أظهرت عزلة الأسبرجلس تيريس أعلى إنتاجيـــــة.

تم إجراء تحليل HPLC لعدد 6 سلالات من فطرة الأسبرجلس فلافس لإختبار قدرتها على إفراز سموم الأفلاتوكسين (ب$_1$، ب$_2$، جـ$_2$) ، أظهرت السلالة رقم AUMC 11311 أعلى إنتاج للأفلاتوكسين ب$_1$ و ب$_2$ في حين أنتجت سلالة رقم AUMC 11317أعلى أنتاج للأفلاتوكسين جـ$_2$، وهذا يدل على أن هذه الفطريات قد تسبب فساد وتلف الحبوب المخزنة ، وعليه يوصى بإجراء إختبارات دورية على الحبوب المخزنة لمعرفة الفطريات الملوثة وقدرتها على إفراز الانزيمات والسموم وذلك لتلافى فسادها.