NATURAL OCCURRENCE OF SOME TOXIGENIC FUNGI IN SOME AGRICULTURAL COMMODITIES USED IN ANIMAL FEEDS

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ABSTRACT

The possible occurrence of mycotoxigenic fungi in feed and foods, and rational decisions on the status of foods suspected to contain mycotoxins, are ever present problems in the food industry around the world During the present study, One hundred and thirteen feed ingredient samples of maize (n=64) and sorghum (n=49) were collected from Assuit Governorate during the period from September 2015 to October 2016. Freshly harvested samples were collected directly from farms during harvesting season (40 maize and 25 sorghum samples). Stored samples were collected from storage sites owned to the farmers; 24 aggregate samples from each of maize and sorghum during the period from January to October 2016. Two different isolation media were used to assess the mycological status of the samples. Dichloran rose Bengal chloramphenicol agar (DRBC) for general isolation of fungi, and Aspergillus flavus and parasiticus agar (AFPA). The moisture contents of samples and aflatoxigenic ability of the isolates were investigated. A total of 65 species assigned to 26 genera were isolated from all samples investigated (40 freshly harvested maize samples, 25 freshly harvested sorghum samples and the stored maize and the stored sorghum (24 samples each). The total count of fungi isolated from freshly harvested maize samples, freshly harvested sorghum samples, stored maize and stored sorghum samples ranged from 1-34, 12-34, 8-32 and 9-41 CFU respectively on DRBC agar and ranged from 2-32, 8-34, 11-36 and 14-34 CFU on AFPA agar. The mean count of fungi in the stored maize samples decreased gradually after 3 months of storage till the 9th month of storage then increased in the last period of storage, while in the stored sorghum samples the mean count increased gradually after 3 months of storage till the 9th month of storage then decreased in the last period of storage. Finally care should be taken in the preparation of feeds in order to prevent the adverse effects of the mycotoxins associated with the raw materials.

Key words: Toxigenic fungi, agricultural commodities, animal feeds

INTRODUCTION

There are wide year to year fluctuations in the levels of fungal contamination in feeds depending on many factors, such as adverse conditions favoring fungal invasion and growth (Magan and Olsen, 2004). Tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest and flash floods led to fungal proliferation and production of mycotoxins (Bhat and Vasanthi, 2003).

Fungi could cause about 50-80% of damage on farmers' maize during the storage period if conditions are favorable for development (Kossou and Aho, 1993). Maize is considered as one of the most important cereal crops in Egypt (FAO, 2004). It is mostly used as food and also contributed in livestock feed as concentrates in poultry feeds or silage maize in large animals (Nooh *et al.,* 2014). Maize may be contaminated both pre- and post-harvest with mycotoxigenic fungi, which include Aspergillus, Fusarium and Penicillium spp., which not only reduces its quality but also are capable of producing mycotoxins that have toxic and/or carcinogenic effects in humans and animals (Van Asselt *et al.,* 2012). Sorghum is the fourth most important cereal in Egypt (after maize, wheat and rice) (Abdel-Hafez *et al.,* 2014).

Growth of aflatoxigenic fungi occur in damaged high moisture seeds. The critical moisture content for growth of A. flavus in starchy cereal grains, is 17-18%, soybeans 17-17.5% and for peanuts is 9-10.5%. The upper limit of moisture for growth of A. flavus and aflatoxins production is about 30%. Aspergillus flavus will grow slowly below 13°C, and most rapidly at 37°C, but does not produce AFs at temperatures below 13°C or above 42°C with optimum growth at 25-37°C (Klich *et al.,* 1992). The high fungal contamination in the field may be masked because of the presence of high amounts of asymptomatic kernels. Therefore, even if kernels appear healthy, care should be taken after harvesting in order to avoid subsequent growth and development of the fungal

species infecting the kernels and production of mycotoxins during storage (Bacon and Hinton, 1996).

The present work was designed to study the prevalence of some toxigenic fungi in freshly harvested and stored maize and sorghum at Assiut Governorate through assessment of the aflatoxigenic ability of the Aspergillus section Flavi isolates.

MATERIALS AND METHODS

[1] Chemicals: All chemicals used were obtained from El Nasr Chemical Company (Egypt) and Merck Company (Germany). Chemicals that were used for preparation of the four media (Aspergillus flavus and Aspergillus parasiticus agar, Dichloran Rose-Bengal Chloramphenicol Agar, Czapek Yeast Extract Agar (CYA) and Coconut Agar medium were Agar agar, Chloramphenicol, Copper sulfate, Dichloran, Dipotassium hydrogen phosphate, Ferric ammonium citrate, Glucose, Magnesium sulfate heptahydrate, Magnesium sulfate, Peptone, Potassium chloride, Potassium dihydrogen phosphate, Rose Bengal, Shredded coconut, Sodium nitrate, Sucrose, Yeast extract and Zinc sulfate.

[2] Media composition: Dichloran rose bengal chloramphenicol agar (DRBC), Aspergillus flavus and parasiticus agar (AFPA) and Czapek yeast extract agar (CYA) were prepared accordind to Pitt and Hocking (2009) where Coconut agar medium (CAM) was prepared according to Davis *et al.* (1987)

[3] Samples and sampling: One hundred and thirteen feed ingredient samples of maize (n=64) and sorghum (n=49) were collected from Assuit Governorate during the period from September 2015 to October, 2016.

(a) Freshly harvested samples: Freshly harvested samples were collected directly from farms during harvesting season (40 maize and 25 sorghum samples). Each sample was collected from 3 different fields at the same city to form a representative sample. In case of maize 10 cobs were collected from each field and in case of sorghum, 1 kg sample were taken from each field.

(b) Stored samples: Stored samples were collected from storage sites Manfalout, Manqabad, Abnub, Al-Fath, Abu-Tij and Sidfa. Twenty-four aggregate samples from each of maize and sorghum at January, April, July and October 2016. The samples were placed in a double sterile polyethylene bags to minimize the loss of water content and provide sufficient aeration, sealed and transferred immediately to the laboratory for mycological and aflatoxins analysis as soon as possible. After homogenization of the sample; one hundred grams were stored at 4 ℃ for mycological examination as soon as possible and three hundred grams were stored at freezer for aflatoxins analysis as soon as possible. In addition to three replicates 50 gm each were used immediately for estimation of moisture content.

[4] Determination of moisture content was done according to Pitt and Hocking (2009).

[5] Isolation, enumeration and identification of fungi: Isolation and enumeration were done according to the method of King *et al.* (1979), which modified by Pitt and Hocking (2009) for general isolation of fungi, and Aspergillus flavus and parasiticus agar AFPA for selective isolation of Aspergillus flavus and Aspergillus parasiticus. Direct plating technique adopted by the second international workshop was used (Pitt *et al.*, 1992) while the identification of the aflatoxigenic aspergilla and other moulds was carried out according to Moubasher (1993), Leslie and Summerell (2006), Domsch *et al.* (2007), Pitt and Hocking (2009) and Ismail *et al.* (2016).

[6] Screening for aflatoxins: Production of aflatoxins was readily detectable by direct visualization under UV light of a beige ring surrounding colonies after an incubation period; when present, the ring exhibited blue fluorescence (Almoammar *et al.*, 2013). All Aspergillus Strains (A. flavus, A. parasiticus and A. flavus var. columnaris) and some other fungal strains were screened for aflatoxins-producing ability on coconut agar medium (CAM). The medium was prepared according to Davis *et al.* (1987). Cultures observed for fluorescence under long-wave UV light (365 nm) after 3, 5 and 7 days. The positive results were shown as blue fluorescence and an un-inoculated plate was observed as a reference.

RESULTS AND DISCUSSION

The results were summarized in tables 1-3, and figures 1-5.

Table 1: Frequency percentage (F%) and percentage total counts (TC%), of Alternaria spp., Aspergillus spp., A. flavus, A. niger, A. parasiticus, Fuserium spp. and Penicillium spp. isolated from freshly harvested maize and sorghum on DRBC and AFPA media.

Taxa / Media	On DRBC agar				On AFPA agar			
	Fresh maize		Fresh sorghum		Fresh maize		Fresh sorghum	
	F%	TC%	F%	TC%	F%	TC%	F%	TC%
***Alternaria* spp.**	42.5	9.13	100	41.19	40	10.51	100	41.69
***Aspergillus* spp.**	85	35.74	88	26.68	90	40.49	84	22.15
A. flavus	47.5	7.37	80	8.74	52.5	15.46	80	10.62
A. niger	57.5	16.19	76	17.16	57.5	13.82	72	10.92
A. parasiticus	7.5	7.21	ND-	ND	7.5	6.96	ND	ND
***Fusarium* spp.**	35	11.69	76	4.99	25	12.36	68	12
***Penicillium* spp.**	32.5	4.32	36	2.49	27.5	4.18	44	2.31

ND=not detected

Table 2: Frequency percentage (F%) and percentage total counts (TC%), of Alternaria spp., Aspergillus spp., A. flavus, A. niger, A. parasiticus, Fuserium spp. and Penicillium spp. isolated from stored maize and sorghum on DRBC agar.

Grains type	Maize								Sorghum							
Storage period	After 3 months		After 6 months		After 9 months		After 12 months		After 3 months		After 6 months		After 9 months		After 12 months	
Taxa	F	TC	F	TC	F	TC	F	TC	F	TC	F	TC	F	TC	F	TC
Alternaria spp.	16.6	0.6	16.6	0.8	16.6	1.2	50	2.3	100	45.5	100	45.5	100	28.1	83.3	24.1
Aspergillus spp.	100	71.1	100	60.5	100	75	83.3	68.2	100	30.7	100	23.9	83.3	48.9	83.3	46.6
A. flavus	100	40.4	66.6	30.7	50	25	83.3	31.7	50	5.2	83.3	4.7	16.6	2.1	50	12.5
A. niger	100	30.0	83.3	20.1	100	33.7	83.3	43.1	100	22.8	83.3	13.1	83.3	34.5	50	23.3
A. parasiticus	ND	ND	16.6	0.8	33.3	8.7	ND	ND	33.3	2.6	ND	ND	50	12.2	16.6	5.8
Fusarium spp.	33.3	1.8	16.6	1.7	33.3	3.7	33.3	1.5	100	7.8	83.3	6.5	66.6	6.3	33.3	5.8
Penicillium spp.	16.6	0.6	33.3	3.5	33.3	2.5	50	10.8	16.6	1.9	50	2.3	33.3	4.2	16.6	0.8

ND=not detected

Table 3: Frequency percentage (F%) and percentage total counts (TC%), of Alternaria spp., Aspergillus spp., A. flavus, A. niger, A. parasiticus, Fuserium spp. and Penicillium spp. isolated from stored maize and sorghum samples on AFPA agar.

Grains type	Stored maize								Stored sorghum							
Storage period	3 months		6 months		9 months		12 months		3 months		6 months		9months		12months	
Taxa	F	TC	F	TC	F	TC	F	TC	F	TC	F	TC	F	TC	F	TC
Alternaria spp.	33.3	2.4	16.6	4.2	33.3	1.8	33.3	1.4	100	50	100	50.4	100	34.9	100	30.7
Aspergillus spp.	100	77.9	83.3	70.3	100	75.7	83.3	70.8	100	27.3	83.3	10.7	100	44.9	66.6	47.1
A. flavus	100	51.5	66.6	41.5	66.6	26.2	83.3	33.3	66.6	15.3	50	4.0	50	4.7	33.3	10.4
A. niger	83.3	25.7	66.6	16.9	83.3	28.9	83.3	31.2	83.3	9.3	66.6	5.3	83.8	27.8	66.6	36.6
A. parasiticus	ND	ND	16.6	2.5	33.3	16.8	ND	ND	16.6	1.3	ND	ND	50	11.2	ND	ND
Fusarium spp.	50	1.8	16.6	1.6	33.3	1.8	ND	ND	66.6	10	100	16.1	50	5.3	33.3	1.9
Penicillium spp.	16.6	1.2	66.6	5.1	33.3	5.6	33.3	7.6	16.6	0.6	33.3	2.0	16.6	1.2	50	1.9

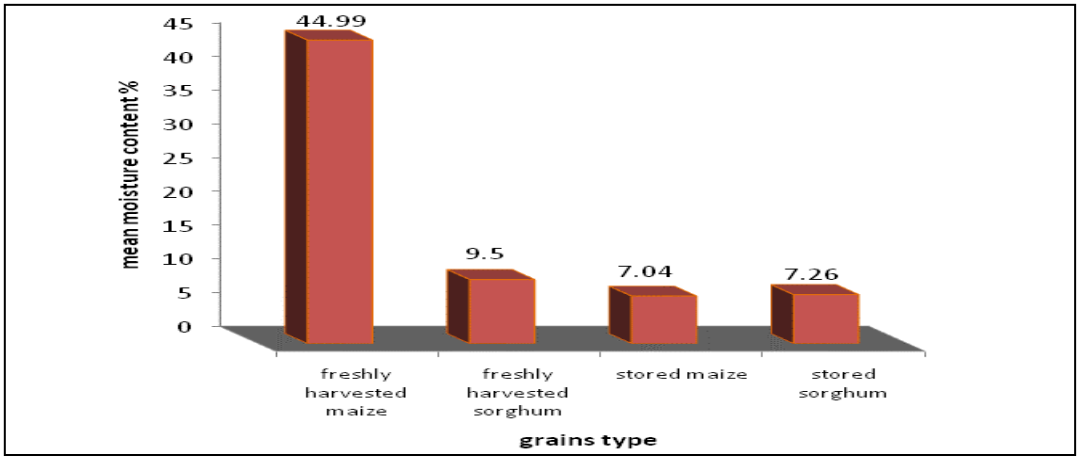

Fig. 1: Shows the moisture content of the examined samples**.**

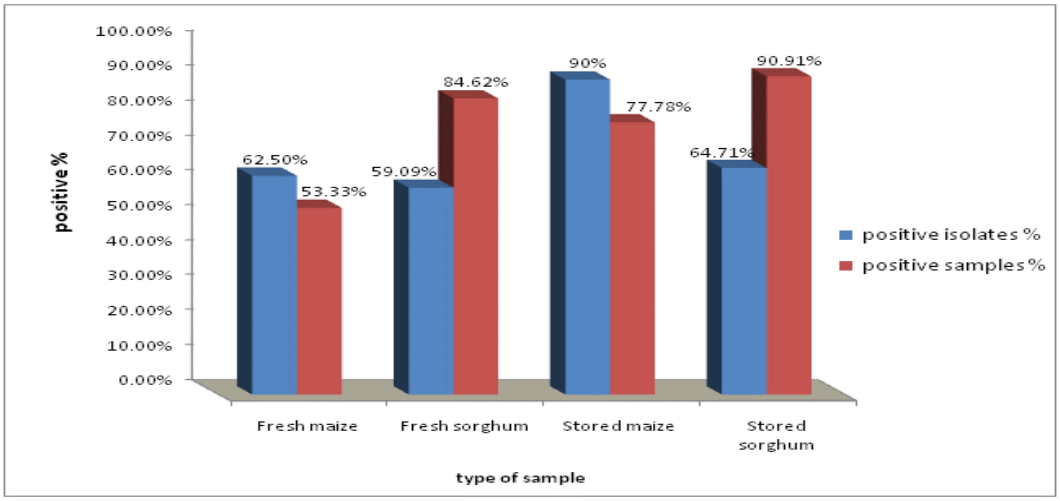

Fig. 2: Positive % of A. flavus isolates on CAM and the % of samples from which these isolates were originated that contaminated with aflatoxins using UPLC.

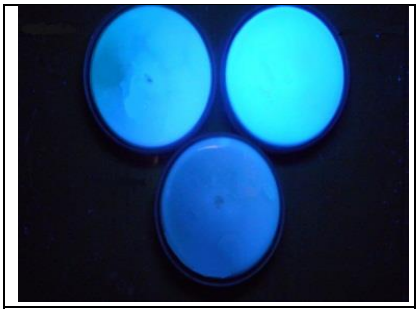

Fig. 3: *Aspergillus flavus* isolates give blue fluorescensse on CAM platespropably indicating aflatoxins production with different intensity.

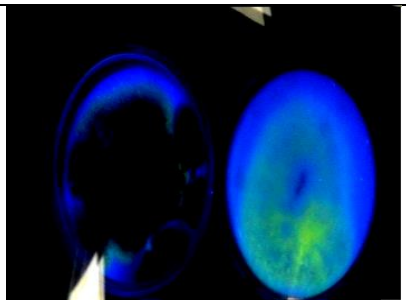

Fig. 4: *Aspergillus flavus* isolate: A) with black sclerochia give high blue fluorescensse, B) give yellowish blue fluorescensse on CAM plates.

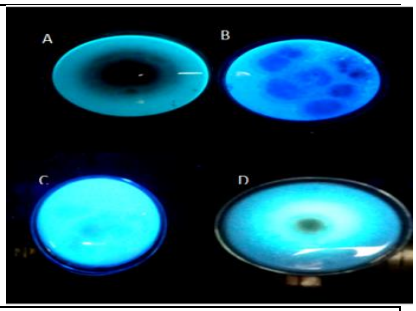

Fig. 5: Showing A) *Aspergillus chevallieri* isolate gave blue fluorescensse, B) *A. melleus* isolate gave blue fluorescensse, C) *A. stellatus* isolate gave green fluorescensse and D) *A. repens* isolate gave green fluorescensse on CAM plates.

Moisture content: In freshly harvested maize samples; the percentage moisture content ranged from 19.37% to 65.38% with a mean of 44.99±11.38%. In contrast, the percentage moisture content of the freshly harvested sorghum samples was low ranging from 8.54% to 11.12% with mean of 9.50±1.03%. On the other hand, the stored maize samples had moisture content ranging from 2.53% to 9.37% with a mean of 7.04±1.59% while; in the stored sorghum samples it ranged from 2.45% to 11.74% with a mean of 7.26±2.27%. Within the stored maize samples the total mean moisture content decreased gradually from 8.71%, 7.23%, 7.21% to 5.02% after 3, 6, 9 and 12 months respectively. Also in the stored sorghum samples, the total mean moisture content decreased from 8.64%, 8.04%, 6.74% to 5.40% after 3, 6, 9 and 12 months respectively. Maize and the other stored products are hygroscopic in nature and tend to absorb or release moisture (Suleiman *et al.,* 2013).

Bispo dos Santos *et al.* (2012) and Di Domenico *et al.* (2015) concluded that moisture was significantly influenced by the interaction of storage type with time. Mendoza *et al.* (2017) concluded that maize is naturally high in moisture after harvest. They collected freshly harvested maize from three different altitudes and found that the mean moisture content was 31 ± 2%, 25 ± 6% and 28 ± 6% respectively. In our investigation, the high moisture content in the freshly harvested maize samples may be due to the early harvesting in order to be used as human food. Another explanation concluded by Vasconcelos *et al.* (2002) where they found that, farmers prefer the early harvest, to reduce the exposure to adverse environmental conditions, insect and fungal attacks, even if the harvesting of immature seeds occur. Sauer *et al.* (1984) found that maize samples with less than 11% moisture averaged 14% kernel invasion by storage fungi (all Aspergillus and Penicillium plus the Mucorales) and those with higher than 13% had 44% invasion and similar results were also reported by Reed *et al.* (2007) and Suleiman *et al.* (2013).

Fungal biodiversity and total counts: During the present investigation a total of 65 species assigned to 26 genera were isolated from 40 freshly harvested maize samples, 25 freshly harvested sorghum samples, 24 each of stored maize and stored sorghum samples that collected from different localities in Assiut governorate. The total number of species and genera was 42 species and 19 genera on the freshly harvested maize, 33 and 13 on the freshly harvested sorghum, 34 and 17 on the stored maize and 39 and 17 on the stored sorghum samples.

On DRBC agar, the highest total count of fungi was registered in the freshly harvested and the stored sorghum samples and were in the range of 12-34 and 9-41 CFUs, respectively while the lowest was from the freshly harvested and the stored maize samples (1-34 and 8-32 CFUs respectively). These results were in accordance with El-Kady *et al.* (1982), Lahouar *et al.* (2015) and Abdel-Sater *et al.* (2017) who found that the fungal infection rates and biodiversity in sorghum were higher than that of maize.

On AFPA agar, the highest total count of fungi was registered in the stored sorghum and maize samples and was in the range of 14-34 and 11-36 CFUs, respectively while the lowest was from the freshly harvested sorghum and maize samples (8-34 and 2-32 CFUs, respectively). Noteworthy that AFPA medium is a selective medium for Aspergillus flavus and A. parasiticus which are well-known to be storage fungi that may explain the reason behinds the higher count range in the stored samples than the fresh samples on the AFPA agar.

The mean count of fungi in the stored maize samples decreased gradually after 3 months of storage till the 9th month of storage then increased in the last period of storage and these may be due to the beginning of substrate deterioration. While in the stored sorghum samples the mean count increased gradually after 3 months of storage till the 9th month of storage then decreased in the last period of storage.

Fungi isolated from the freshly harvested and stored maize

Aspergillus was the most common genus being isolated from both the freshly harvested (90% of samples) and stored maize samples (83.33% to 100% of samples). This was in accordance with the findings of Abdel- Sater *et al.* (2017) While, Lazzaro *et al.* (2015) and Xing *et al.* (2017) found that Fusarium or Pencillium were the predominant species this difference could be explained by the difference in geographical zones, methods of cultivation and storage or the difference of hybrids types.

In freshly harvested maize, A. niger, A. flavus and A. parasiticus contaminated 57.5%, 47.5%, and 7.5% of samples constituting 16.19%, 7.53% and 7.05% of the total count on DRBC agar. A. niger, A. flavus and A. parasiticus contaminated 57.5%, 52.5%, and 7.5% of the samples constituting 15.91%, 13.82% and 6.49% of the total count on AFPA agar. Where the A. niger had the highest frequency and total count percentage.

In stored maize, A. niger and A. flavus were the most common species contaminating 66.67% to 100% and 50% to 100% of samples over the 12 months while A. parasiticus was isolated only from 16.67% and 33.33% of samples after 6 and 9 months of storage (on both DRBC and AFPA). The lowest total count percentage of the A. flavus was during summer (after 9 months of storage) while, the highest percentage count of A. niger and A. parasiticus at the same period of storage.

Aspergillus flavus is usually considered a storage fungus but it can invade grains in the field (Christensen, 1982). A. flavus is related to warm or tropical areas and is less frequently encountered in cold areas Jedidi *et al.* (2017) and Mendoza *et al.* (2017) isolated A. flavus from 66.67% and 60% of the freshly harvested maize samples in Tunisia and Mexico, respectively, while Lazzaro *et al.* (2015) and Xing *et al.* (2017) isolated A. flavus in range doesn't exceed 14% of the freshly harvested maize from Brazil, Italy and china. In Egypt, Ismail *et al.* (2016) and Abdel-Sater *et al.* (2017) isolated A. flavus from 50- 94% of the stored maize samples.

Logrieco *et al.* (2003) referred the dominance of the A. niger to its black spores that made it highly resistant to sunlight and sun-drying. Ismail *et al.* (2016), Xing *et al.* (2017) and Abdel Sater *et al.* (2017) found that A. niger was dominant species. Pitt and Hocking (2009) stated that the relatively minor distribution of A. parasiticus is related to the fact that it is a common fungus of soil and nuts rather than cereals, also Hassan *et al.* (2017) and Jedidi *et al.* (2017) found that A. parasiticus was isolated in low frequency.

Alternaria, Fusarium and Pencillium were isolated in moderate frequency from the fresh maize samples (40-42.5%, 25-35% and 27.5-32.5% of the samples, on AFPA agar DRBC and agar respectively). Also Cladosporium, Rhizopus and yeasts were of moderate frequency; contaminating 42.5%, 30% and 37.5% of the fresh maize samples on DRBC agar and 32.5%, 32.5% and 27.5% on AFPA agar. Fusarium had the highest count followed by Alternaria and Penicillium.

In stored maize, Pencillium (a storage fungus) was revealed from 16.67% to 66.67% of samples and its' total percentage count increased from 0.61% after 3 months to 10.85% after 12 months on DRBC agar and from 1.23 to 7.94 on AFPA agar. Alternaria (a field fungus) was recovered in rare frequency (16.67% to 33.33% of samples) and count (0.61% to 4.24%of total propagules) during storage. Fusarium (a field fungus) contaminated the samples in range from 16.67 to 50% and constituted 1.55% to 3.75% of the total count percentage where the highest frequency occurred after 3 months of storage (winter season). Rhizopus stolonifer and yeasts were isolated in low frequency while, Cladosporium cladosporioides was isolated in moderate frequency from the stored samples.

Mendoza *et al.* (2017) reported that high presence of both field fungi (e.g. Alternaria, Cladosporium, and Fusarium) and storage fungi (e.g. Aspergillus and Penicillium) in the fresh maize. Jedidi *et al.* (2017) concluded also that the frequencies of contamination with Aspergillus, Fusarium and Alternaria were higher in freshly harvested samples, whereas Penicillium species were more frequent in stored samples. Cladosporium and Alternaria were isolated from 20% and 14.19% of the freshly harvested samples. Jurjevic *et al.* (1999) reported a high occurrence (up to 93.6%) in fresh maize samples collected from 14 regions of Croatia. Lazzaro *et al.* (2015) and Xing *et al.* (2017) isolated the Penicillium in low frequency ranged from 0.87% to 9.2% of the fresh maize samples.

In Egypt, Aziz *et al.* (2006) and Ismail *et al.* (2016) isolated Penicillium from the stored maize with high frequency (70-100% of samples) while, Nooh *et al.* (2014) isolated it in moderate frequency (17.6% to 27.1% of samples from Assiut). Hassan *et al.* (2017) and Abdel-Sater *et al.* (2017) isolated Penicillium from 38.8%, 28%, 40% and 35% of the stored maize

samples collected from Romania, Pakistan, Qatar and Yemen, respectively.

Species of Fusarium and Alternaria are considered as field fungi invading more than 50% of maize grains before harvest (Robledo-Robledo, 1991). There was a reduction in their occurrence during storage and lose pathogenicity during storage Susan *et al.* (2005) and Madbouly *et al.* (2012) reported that the surface disinfection of seeds with sodium hypochlorite removed most saprophytic microorganisms found on the seed surface and decreased the overall incidence of Fusarium species to 2% in maize.

Covarelli *et al.* (2011) found that the most prevalent genus being isolated from the freshly harvested maize was Fusarium that contaminated 66.4% to 76.8% of samples while, Lazzaro *et al.* (2015) and Xing et al. (2017) isolated it in range from 7.5% to 47.3% and 15.08% to 24.77% of the fresh maize samples, respectively. In case of the stored maize, Soliman (2003) and Ismail *et al.* (2016) isolated Fusarium with frequency ranged from 10-90% and 60-70% of samples that were collected from Egypt while, Aziz *et al.* (2006) and Nooh *et al.* (2014) isolated it from only 20% and 12.9% of samples, respectively.

During our investigation, Talaromyces duclauxii (=Penicillium duclauxii) was isolated frequently in range of 33.33-83.33% of the stored maize samples with the highest frequency after 6 months (autumn) constituting 6.75-18.42% of the total propagules on the DRBC agar and 1.23-9.72% on AFPA agar, this was in accordance with Ismail *et al.* (2016).

Tabuc *et al.* (2009) and Abdel-Sater *et al.* (2017) found that 45%, 43.2% and 50% of the stored maize samples were contaminated with Rhizopus stolonifer while, Nooh *et al.* (2014) and Ismail *et al.* (2016) isolated it from 1.08% to 5.5% and 0% of samples collected from Assiut, respectively. Tabuc *et al.* (2009) found that Cladosporium spp. contaminated 15% of samples from Romania but it was missing in maize from Egypt (Aziz *et al.*, 2006).

Fungi isolated from the freshly harvested and stored sorghum

In freshly harvested sorghum samples, Alternaria (field fungus) and Aspergillus were the most common genera being isolated from 100% and 88% of samples on DRBC agar and from 100% and 84% of samples on AFPA agar. Alternaria and Aspergillus constituted 41% and 26.98% of total propagules on DRBC agar, respectively and nearly the same percentages on AFPA agar (41.54% and 22.15%).

In stored sorghum samples, Alternaria and Aspergillus were the most common fungus recovered from 100% of the samples over 12 months of storage. On DRBC agar, Alternaria (a field fungus) decreased from 45.75% of the total propagules after 3 months of storage to 24.17% after 12 months while Aspergillus (a storage fungus) increased from 30.72% of the total propagules after 3 months of storage to 46.67% after 12 months of storage. Also on AFPA agar, Alternaria decreased from 50% of the stored samples total count after 3 months to 30.72% after 12 months and Aspergillus increased from 27.33% of the stored samples total count after 3 months to 47.06% after 12 months. The decrease in the percentage count of Alternaria spp. and the increase in Aspergillus spp. percentage count were in accordance with the mean moisture content of samples which decreased gradually from 8.64% after 3 to 5.40% after 12 months.

Gonzalez *et al.* (1997) isolated Aspergillus from 40 to 60% of the freshly harvested sorghum samples in Argentina and isolated Alternaria from 90 to 100% of the samples during three successive years. Sreenivasa *et al.* (2010) isolated Aspergillus and Alternaria from 88.6% and 84.1% of the freshly harvested sorghum samples collected in India. El-Kady *et al.* (1982), Soliman (2003) and Lahouar *et al.* (2015) isolated Aspergillus from 100%, 27.7% and 87.2% of the stored sorghum samples collected from Egypt and isolated Alternaria from 12%, 73.1% and 81.2% of the samples, respectively. Abdel-Sater *et al.* (2017) isolated Aspergillus from 95% of samples collected from Yemen while, there was no Alternaria. Also, Yassin et al (2010) and Mahmoud *et al.* (2013) found that Aspergillus was dominant in the stored sorghum samples collected from Saudi Arabia while, Alternaria isolated with very low frequencies.

In this study, on DRBC agar, A. flavus and A. niger contaminated 80% and 76% of the freshly harvested sorghum samples while A. niger possessed the highest percentage count (17.16% of the total count compared to 8.74% for A. flavus). Also on AFPA agar, A. flavus and A. niger contaminated 80% and 72% of the samples and A. niger possessed the highest percentage count (10.92% of the total count compared to 10.62% for A. flavus). A. parasiticus was not detected.

In stored sorghum samples, the most common Aspergillus species were A. niger and A. flavus with frequency ranged from 50% to 100% and 50% to 83.33% of samples. A. flavus percentage count decreased gradually during the first 9 months of storage while increased after 12 months (from 5.23% to 4.79%, 2.13% then 12.5% on DRBC and from 15.33% to 4.03, 4.73% then 10.46% on AFPA). Noteworthy that, the highest A. niger percentage count was after 9 months of storage (34.57% on DRBC and 27.81% on AFPA) where the percentage count of the A. flavus was the lowest (2.13% on DRBC and 4.73% on AFPA). A.

parasiticus was isolated from 33.33% to 50% of samples with the highest frequency (50%) and percentage count (12.23% on DRBC and 11.22% on AFPA) was after 9 months of storage.

Gonzalez *et al.* (1997), da Silva *et al.* (2000), and Sreenivasa *et al.* (2010) found that A. flavus predominated over A. niger in the freshly harvested sorghum with frequency ranged from 42.1% to 86% for A. flavus and from 0.7% to 59.1% for A. niger. Hussaini *et al.* (2009) and Yassin *et al.* (2010) isolated A. niger with frequencies higher than that of the A. flavus in the stored sorghum samples. In contrast, Lahouar *et al.* (2015) and Abdel-Sater *et al.* (2017) isolated A. flavus with frequencies higher than A. niger in the stored sorghum. A. parasiticus was isolated in low frequencies from stored sorghum samples collected from Egypt (El-Kady *et al.*, 1982), Nigeria (Hussaini *et al.*, 2009) and Egypt and Tunisia (Lahouar *et al.*, 2015), (8%, 14.19% and 9.9% of samples, respectively).

In this study, Fusarium, Cochliobolus and Cladosporium were also of high frequency; contaminating 68-76%, 68-72% and 68% of the freshly harvested sorghum samples (on DRBC and AFPA agar, respectively) while, Penicillium and Nigrospora oryzae appeared moderately and contaminated 36-44% and 40% of the freshly harvested samples. Also, the total percentage count of the Fusarium (4.99-12% of total count), Cladosporium (8.31-12.01%) and Cochliobolus (6.08-6.31%) were higher than that of the Pencillium (2.31-2.49%) and Nigrospora oryzae (1.87-2%).

Fusarium decreased gradually in frequency from 100% in stored sorghum samples after 3 months to 33.33% after 12 months of storage. Its percentage count decreased from 7.84% to 5.83% of total count and from 10% to 1.96% on DRBC and AFPA agar, respectively. The decrease in frequency of isolation and percentage count of the Fusarium spp. could be explained by the low moisture content of the grains and being a field fungus (da Silva *et al.*, 2000). Penicillium contaminated 16.67%, 50%, 33.33%, 16.67% of the stored samples and constituted 1.96%, 2.39%, 4.26% and 0.83% of the total count on DRBC agar while it contaminated 16.67%, 33.33%, 16.67% and 50% of the samples and constituted 0.67%, 2.01%, 1.18% and 1.96% of the total count on AFPA agar after 3, 6, 9 and 12 months, respectively. Cladosporium and Nigrospora oryzae decreased in frequency and count during storage while Cochliobolus increased during storage.

Gonzalez *et al.* (1997) and Sreenivasa *et al.* (2010) found that Fusarium contaminated the freshly harvested sorghum with high frequencies (100% and 93.2%, respectively), however da Silva *et al.* (2000) and Hussaini *et al.* (2009) isolated it with lower frequency (25% and 18.75% of samples, respectively). On the other hand, and in contrast to our finding Chala *et al.* (2014) and Lahouar *et al.* (2015) isolated the Fusarium with high frequency from the stored samples (100% and 95.3%, respectively) while El-Kady *et al.* (1982) and Mahmoud *et al.* (2013) isolated it from 16% and 31.24% of samples. Soliman (2003) found that Pencillium and Fusarium were rarely isolated from stored sorghum samples.

Gonzalez *et al.* (1997) found that Cladosporium contaminated 67% and 40-90% of the freshly harvested samples while Sreenivasa et al. (2010) isolated it from only 6.4% and 22.7% of samples. El-Kady *et al.* (1982) isolated Cladosporium from 16% of stored samples collected from Egypt. da silva *et al.* (2000) and Hussaini *et al.* (2009) found that Cladosporium contaminated the freshly harvested sorghum with frequencies higher than that of the stored one (6.4% and 25% in the fresh samples and decreased to 0.7% and 14.19% in the stored samples, respectively). Cochliobolus was isolated in a range from 14.3% to 60% of the freshly harvested sorghum by da Silva *et al.* (2000) and Sreenivasa *et al.* (2010) while isolated in low frequency from the stored samples analyzed by El-Kady *et al.* (1982) and Yassin *et al.* (2010). Nigrospora oryzae isolated with low frequency (11% and 8.6% of samples) by da Silva *et al.* (2000).

Our results were in accordance with Hussaini *et al.* (2009) who isolated the Penicillium in moderate frequency from the freshly harvested samples while, Gonzalez *et al.* (1997) and Sreenivasa *et al.* (2010) isolated it in high frequency. Penicillium was isolated in low frequency and count from the stored sorghum samples analyzed by Chala *et al.* (2014) and Lahouar *et al.* (2015). Abdel-Sater *et al.* (2017) concluded that Penicillium had highly significant propagules on maize than on sorghum.

Sreenivasa *et al.* (2010) and Lahouar *et al.* (2015) concluded that there is a lack of ac¬curate data on the frequency and relative percentage of fungi in sorghum grains so, further studies should be performed.

Aflatoxigenic ability of Aspergillus flavus on coconut agar medium (CAM):

15 isolates out of the 24 A. flavus isolates (62.5%) from the freshly harvested maize samples were aflatoxin producers on CAM while only 53.33% of the positive isolates had the favorable conditions to produce aflatoxins in samples under the field conditions (with levels ranging from 1.73-111.76 ppb). In freshly harvested sorghum, 13 (59.09%) out of the 22 A. flavus isolates tested were positive to aflatoxin production on CAM while 84.62% of the positive isolates had the favorable conditions to produce aflatoxins in samples under the field condition (with levels ranging from 1.84-122.72 ppb). The percentages of aflatoxigenic isolates were

nearly similar in both the freshly harvested maize and sorghum while the percentage of samples, from which these isolates originated, that contaminated with aflatoxins were higher in sorghum than in maize.

For the stored maize, 18 (90%) isolates out of the 20 A. flavus isolates were aflatoxin producers on CAM while 77.78% of the positive A. flavus isolates had the favorable conditions to produce aflatoxins in samples during storage (with levels ranging from 1.97-88.5 ppb). For the stored sorghum, 11 (64.71%) out of 17 the A. flavus isolates gave blue florescence on the CAM while 90.91% of the positive A. flavus isolates had the favorable conditions to produce aflatoxins in samples during storage (with levels ranging from 9.53-241.70 ppb). The percentages of aflatoxigenic isolates were higher in the stored maize and sorghum samples than the freshly harvested samples and also the percentage of samples, from which these isolates originated, that contaminated with aflatoxins were higher in the stored samples than in the freshly harvested samples.

Higher percentage of the aflatoxigenic isolates were found in the stored maize in comparison with the stored sorghum samples while the higher percentage and levels of aflatoxins contamination were found in the stored sorghum samples.

Four out of the five A. parasiticus isolates from freshly harvested maize samples were positive on CAM with different levels of which one was giving green fluorescence, while three out of the four A. parasiticus isolates from stored maize were positive for the aflatoxin production on CAM. Six out of the seven A. parasiticus isolates from stored sorghum were recorded to produce blue fluorescence on CAM and one produced green fluorescence. Only one isolate from the A. flavus var. columnaris strains, that isolated from the four types of grains, showed high intensity fluorescence and the remaining were negative.

In stored maize samples, two A. tamarii, one A. stellatus, one A. chevallieri and one A. repens isolates were tested. The two A. tamarii isolates were negative. A. stellatus isolate produced high intense green fluorescence on CAM while A. chevallieri and A. repens gave high intense blue fluorescence and very high green fluorescence, respectively on CAM and these are probable indication for ochratoxins production. A. montevidensis was negative for the fluorescence on the CAM. In stored sorghum samples one A. candidus and one A. melleus isolates were tested. A. candidus isolate were recorded as negative producers while A. melleus isolate gave intense blue fluorescence (probable indication for ochratoxins production).

In Serbia, Jakić-Dimić *et al.* (2009) isolated A. flavus from 18.7% of the stored maize samples analyzed and detected aflatoxins in 18.3% of the samples while higher percentage was obtained from stored sorghum samples where 40% of samples were contaminated with A. flavus and aflatoxins (up to 50 ppb) at the same time. Ismail *et al.* (2016) found that 100% of A. flavus isolated from maize samples gave blue fluorescence on CAM indicating aflatoxins production while only two stains (out of 11) showed strong aflatoxigenic ability (intense blue color) and 9 strains showed faint blue color indicating low aflatoxins-producing ability.

Fifty-nine Aspergillus flavus strains isolated from 10 freshly harvested and 130 stored Brazilian sorghum samples and were tested for their ability to produce aflatoxins by da Silva *et al.* (2000) of which thirty-eight (64.4%) of them produced detectable levels of aflatoxins (AFB1+AFB2) at concentrations ranging from 12.00 to 3282.50 ppb. A. flavus isolates have been identified in 63.5% of the maize samples from the fields in 2009 and 2010, and 18.8% of these isolates were able to produce aflatoxins on maize kernels with mean levels ranged from 63.3±0.6 to 4721±43 ppb in 2009 and 2010 (Dobolyi *et al.*, 2013). Abdel-Sater *et al.* (2017) screened 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 collected from Yemen for their abilities to produce aflatoxins on CAM and found that 7 isolates (36.8% of total isolates) were able to produce fluorescence under U.V light, indicating the production of aflatoxin. Four isolates (40%) of the ten A. flavus isolates collected from maize and three (42.85%) of the seven A. flavus isolates collected from sorghum were positive. Both of A. tamarii and A. flavus var. columnaris isolates were negative.

IN CONCLUSION

High incidence of fungi and presence of aflatoxins in maize and sorghum samples indicate bad handling of these commodities during pre- or post-harvest and reflect improper storage conditions. There is an urgent need for strict regulations, regular inspection and control for the contamination of grains with fungi and aflatoxins.

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مدي تواجد بعض الفطريات المفرزة للسموم في بعض المحاصيل الزراعية المستخدمة في غذاء الحيوان

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تهدف الدراسة الحالية الي تقييم مدي تلوث حبوب الذرة الشامية والذرة العويجة بالفطريات المفرزة للسموم في محافظة اسيوط. وقد تم في اطار ذلك تجميع عدد ١١٣ عينة خلال الفترة من سيبتمبر ٢٠١٥ الي اكتوبر ٢٠١٦ وتشمل عدد ٤٠ عينة من الذرة الشامية المحصودة حديثا و ٢٥ عينة من الذرة العويجة المحصودة حديثا و ٢٤ عينة من كل من الذرة الشامية والذرة العويجة المخزنة. وقد تم عزل الفطريات الملوثة لهذه العينات باستخدام نوعان من الوسائط الغذائية وهي؛ دي كلوران روز بنجال كلورامفينيكول اجار (DRBC agar)و الاسيراجيلس فلافس وباراسيتيكس اجلر .(AFPA agar) كما تم قياس نسبة الرطوبة وقدرة عزلات الاسبراجيلس فلافس والاسبرجلس باراسيتيكس وغيرها علي انتاج سم الافلاتوكسين باستخدام الوسط الغذائي coconut agar) .(mediaوتم الفحص باستخدام مصدر للاشعة الفوق بنفسجية (UV-LIGHT) للكشف عن الخاصية الفلورنسية للسموم الفطرية باستخدام تقنية Thin Layer Chromatography وتاكيد النتائج للعينات الموجبة باستخدام Ultra Performance Liquid Chromatography.هذا وقد أوضحت النتائج عزل ٦٦ نوعا من الفطريات تنتمي الي ٢٦ جنس من اجمالي ١١٣ عينة تم فحصها. وقد تراوح العدد الكلي لانواع الفطريات المعزولة علي الوسط الغذائي دي كلوران روز بنجال كلورامفينيكول اجار مابين ١ الي ٣٤ مستعمرة في حالة الذرة الشامية المحصودة حديثا و ١٢- ٣٤ مستعمرة في الذرة العويجة المحصودة حديثا و ٨-٣٢ مستعمرة من الذرة الشامية المخزنة ومن ٩-٤١ مستعمرة من الذرة العويجة المخزنة. اما في حالة الوسط الغذائي الاسيراجيلس فلافس و باراسيتيكس اجار فكانت ٢-٣٢ و ٨-٣٤ و ١١- ٣٦ و ١٤-٣٤ مستعمرة علي التوالي.وبقياس نسبة الرطوبة للعينات وجد ان متوسط نسبة الرطوبة للذرة الشامية المحصودة حديثا والذرة العويجة المحصودة حديثا والذرة الشامية المخزنة والذرة العويجة المخزنة تساوي ٤٤.٩٩% ± ١١.٣٨ و٩.٥٠% ± ١.٠٣ و٧.٠٤% ± ١.٥٩ و ٧.٢٦% ± ٢.٢٧ علي التوالي. وكان الاسبراجلس هو اكثر الاجناس الفطرية شيوعا حيث تم عزله من ٨٥% من العينات علي الوسط الغذائي الاول (DRBC agar) و ٩٠% من العينات علي الوسط الثاني (AFPA agar) لعينات الذرة الشامية المحصودة حديثا وكان الالترناريا و الاسبراجلس اكثر الاجناس الفطرية شيوعا وتم عزلها من ١٠٠% و ٨٨% من العينات علي الوسط الاول و ١٠٠% و ٨٤% من العينات علي الوسط الثاني. في الذرة العويجة المحصودة حديثا لعينات الذرة الشامية المخزنة.وكان ايضا الاسبراجيلس هو الاكثر شيوعا طوال فترة التخزين وتم عزله من ١٠٠% من العينات بعد ٣ و ٦ و ٩ اشهر من الحصاد بينما عزل من ٨٣% من العينات بعد ١٢ شهر. أما عينات الذرة العويجة المخزنة ؛وكانت الفطريات الاكثر شيوعا هي الاسبراحلس والالتارناريا و الفيوساريم وعزلت من ١٠٠% من العينات بعد ٣ شهور من الحصاد. بينما عزل البنسيليوم من ١٦.٦٧% و بعد ٦ اشهر ؛ عزل كل من الاسبراجيلس والالترناريا من ١٠٠% من العينات وعزل الفيوساريم والبنسيليوم من ٥٠% و ٨٣.٣٣% من العينات. وبفحص قدرة عزلات الاسيراجلس فلافس علي انتاج سم الافلاتوكسين وجد ان ٦٢.٥% و ٥٩.٠٩% و٩٠% . و٦٤.٧١%منها انتجت الافلاتوكسين في الذرة الشامية المحصودة حديثا و الذرة العويجة المحصودة حديثا والذرة الشامية المخزنة والذرة العويجة المخزنة على التوالي .