Ass. Univ. Bull. Environ. Res. Vol. 15 No. 1 March 2012

AUCES

FUNGAL POPULATIONS AND MYCOTOXINS OF WHEAT GRAINS IMPORTED TO EGYPT

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ABSTRACT:

The fungal populations and mycotoxins were evaluated on 38 wheat grain samples imported to Egypt from different countries. The incidences, isolation frequencies and relative densities of both storage and field fungi were determined. 61 species and one unidentified species in addition to 3 species varieties appertaining to 21 genera were isolated on glucose-Czapek's agar medium (CZ), glucose-Czapek's agar containing 6% NaCl (CZ-NaCl) and malt agar medium (MA). The most predominant mycobiota were, *Aspergillus* species (43.4%) followed by *Eurotium* species (13.7%), *Rhizopus* (13.2%), and *Alternaria* species (7.7)% of the total isolates. The most common species which contaminated samples were *A. flavus* (18.2%) followed by *A. flavus* var. *columnaris* (12.7%), *Alternaria alternata* (9.3%), and *Eurotium amstelodami* (3.8%) of the total isolates. Statistical analysis revealed a high significant correlation between, fungal total count, number of genera, and number of species on different media. Meanwhile, the mean number of genera and species on MA and CZ were significantly increased than CZ-NaCl. Evaluation of naturally occurance of mycotoxins in wheat grain, showed 10.5% of samples containing sterigmatocystin. The most predominant mycotoxigenic species were *A. flavus* var. *columnaris* and *A. flavus*. Nevertheless, the ability of *Aspergillus* of section *Flavi* to produce mycotoxins (aflatoxins B_1, B_2, G_1 and G_2 and sterigmatocystin) was 35.4% of isolates. These hazardous compounds are known to decrease the food quality and can cause acute or chronic intoxication to humans and animals.

INTRODUCTION:

Wheat is one of the most important cereal crops in the world and the main staple food in Egyptian homes. Egypt depends upon imported wheat grains to compensate the deficiencies in local production. Indeed, the government imports about 45% of our yearly needed wheat. A large proportion of this imported wheat grains are from USA, Canada, Europe and Australia. In North African countries, the foods most susceptible to aflatoxin contamination are locally produced or imported cereals such as wheat. This crop is a staple in dry Mediterranean regions of North Africa, where its consumption in the form of couscous, pasta, macaroni, spaghetti, bread, and frik is a cultural tradition. Mould contamination of grains can occur during the harvest and the

post-harvest periods under unsuitable conditions of temperature and humidity (Doohan *et al.*, 2003).

The mycobiota of wheat and wheat products was found to be dominated by *Aspergillus* species from sections *Nigri* and *Flavi* (Riba *et al.*, 2008). A number of fungal species have been associated with wheat, belonging mainly to the genera *Fusarium* and *Alternaria*, known as field fungi and the so-called storage fungi, such as *Penicillium, Aspergillus* and *Rhizopus* (Bottalico and Perrone, 2002; Gohari *et al.*, 2007 and Juan *et al.*, 2008). Among these genera, some species are of major concern because of their toxigenic properties for producing a range of toxic secondary metabolites known as mycotoxins (Betina, 1984 and Saberi *et al.*, 2004).

In respect to natural occurance of mycotoxins, sterigmatocystin was detected in one of the 29 samples of heated wheat grain (Scott *et al.*, 1972). Also, it has been found naturally in feedstuffs and other cereal grains (Jeswal, 1990; Scott, 1990; Sarah *et al.*, 1996 and Scudamore *et al.*, 1998). The ingestion of mycotoxin contaminated grains may lead to a wide array of biological effects being genotoxic, carcinogenic, embryotoxic and teratogenic (IARC, 1993 and Bennett & Klich, 2003). Such contamination can lead to reducing and downgrading of the grain quality, making it unsafe for human and livestock consumption (Goswami and Kistler, 2004; Osborne and Stein, 2007).

Four aflatoxins are commonly produced in foods, aflatoxins B_1, B_2, G_1, and G_2. These mycotoxins are produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and other *Aspergillus* in section *Flavi* (Samson *et al.*, 2006; Pildain *et al.*, 2008). Aflatoxins (AFs) are the most potent natural carcinogens known (JECFA, 1997), affecting animal species, including humans. These hazardous compounds that occur simultaneously in food or unprocessed materials can cause acute or chronic intoxication to humans and animals (Wild and Hall and 1996; Placinta *et al.*, 1999), including Aflatoxins and Ochratoxins from *Aspergillus* spp. and *Penicillium* spp. (Payne, 1998; Frisvad and Samson, 2004), Sterigmatocystin from *Emericella nidulans* (Youssef *et al.*, 2008), and many other toxic compounds. Mycotoxins are often unavoidable and of a worldwide preoccupation (Bennett and Klich, 2003), and their contamination of foods and feeds is a significant problem (Zaina, 2011).

The present investigation was designed for isolation and identification of both storage and field fungi, evaluation of natural occurance of mycotoxins of the grains, and for studying the ability of isolates of *Aspergillus* from section *Flavi* to produce mycotoxins. These inspections were done to control grain contamination and represent the final category of food quality control.

MATERIALS AND METHODS:

Sample collection:

Thirty-eight wheat grain samples were collected from ships, which arrived to Damietta port during October 2003-December 2005. The samples collected at the time of discharge ships, and putt in sterile polyethylene bag under

refrigeration (5°C) until the time of examination. In addition, the data of samples were collected such as source and kind of grains in addition to exported to imported time.

Moisture content determination:

Soon after grain collection, the samples were subjected to moisture content analysis by the high constant temperature oven method as in ISTA (1985).

Media used for isolation of fungi:

-Glucose-Czapek's Rose Bengal agar medium (CZ) containing (g/l): glucose, 10; $NaNO_3$, 3; K_2HPO_4, 1; KCl, 0.5; $MgSO_4$, 0.5; Rose Bengal, 0.05; chloramphenicol, 0.5; Agar, 16; per liter distilled water.

-Glucose-Czapek's Rose Bengal agar medium containing 6% NaCl (CZ-NaCl): as mentioned above but with adding 60 g NaCl (Mislivec and Bruce, 1977 and Mislivec *et al.*, 1979 and Weidenbörner and Hindrof, 1989). The cultures were incubated at 28±2°C for 5-15 days.

-Malt agar medium (MA) containing (g/l): malt extract, 20; glucose, 20; peptone, 1; Agar, 20.

Detection of fungal flora:

- Isolation of storage fungi:

The direct plating technique was employed as described by Pitt and Hocking (1985). Three hundred grains were randomly selected for fungal isolation from each sample and directly plated on poured plates. Sixty plates each containing 5 grains (20 plates of CZ, CZ-NaCl, and MA).

-Isolation of field fungi:

The previous process was carried out but seeds were exposed to surface disinfection by a commercial 5% aqueous solution of sodium hypochlorite for 1 minute, then rinsed twice in sterile distilled water and dried in a laminar flow cabinet (Wareing, 1997).

-Counting of fungi:

The fungal colonies recovered were counted and expressed as:

1-Colonies forming units (CFU). The developing colonies were counted as (CFU per 100 seeds) and isolated on the slants of the previous media for identification process.

2-The isolation frequency (F%).

3-Relative density (RD%) of genera and species were calculated according to Marasas *et al.* (1988).

4-Number of cases of isolation (NCI) and E-Occurrence remarks (OR) which was categonized as follows:

H=High occurrence (NCI more than 50% of the samples).

M=Moderate occurrence (NCI ranged between 30-50% of the samples).

L=Low occurrence (NCI ranged between 13-29% of the samples).

R=Rare occurrence (NCI less than 13% of the samples).

-Identification of fungi:

The fungal isolates were identified according to the following authorities: *Aspergillus* species according to Raper and Fennell (1965) and Moubasher (1993).

Penicillium species according to Raper and Thom (1949), and Moubasher (1993). *Fusarium* species according to Domsch *et al.* (1980). Dematiaceous Hyphomycetes according to Ellis (1971 and 1976) and others fungi were identified according to Domsch *et al.* (1980) and Moubasher (1993).

Statistical analysis:

The results were analyzed using SAS system included:

1-Correlation coefficients between fungal total counts, number of genera and species of storage and field fungi on different media.

2-Correlation between types of media for variable mean fungal total counts, number of genera and species.

3-Correlation between storage and field fungi for variable mean fungal total counts, number of genera and species.

4-Correlation between storage and field fungi for variable mean fungal total counts, mean number of genera and mean number of species of wheat grain samples.

5-Correlation coefficient between moisture content and total count (storage and field fungi) of wheat grain samples on different media.

Cultivation of *Aspergillus* of section *Flavi* for toxin production:

The most potent fungal isolates (*A. flavus*) were grown in 250 ml flasks each containing 50 ml Czapek's peptone yeast extract liquid medium (CZPY) containing (g/l): sucrose, 30; peptone, 10; $NaNO_3$, 2; K_2HPO_4, 1; yeast extract, 1; KCl, 0.5; $MgSO_4$, 0.5. The media were sterilized at 1.5 atm. for 20 min. and incubated at 28°C for 10 days under static conditions.

Toxin extraction:

-Extraction of *Aspergillus* toxins:

After the above incubation the content of each flask (medium+mycelium) was homogenized for 5 min in a high-speed blender (1600 rpm.) with 100 ml chloroform. Extraction procedures were repeated three times and the chloroform extracts were combined, washed with equal volume of distilled water, dried over anhydrous sodium sulphate, filtered then concentrated under vacuum or a stream of nitrogen to near dryness (Eman *et al.*, 2005).

-Extraction of grains toxins:

Fifty grams of each sample were soaked with 100 ml chloroform in 250 ml flask for 24 h in shaker. The defatted residue was re-extracted for another 24 h in shaker with 100 ml chloroform, then chloroform extracts were combined, washed with an equal volume of distilled water, dried over anhydrous sodium sulfate, filtered then concentrated and left to dry. The dried materials were transferred to vials with small amount of chloroform, which was evaporated to near dryness and cleaned up (Zohri and Sabah, 1992).

Detection of mycotoxins:

-Thin Layer Chromatography (TLC):

The analysis of extract on TLC for the presence of different mycotoxins was performed

according to the standard procedures of Roberts and patterson (1975) and Samson *et al.* (1995).

Identification of mycotoxins:

The developed plates were detected before and after spraying with the different reagents under short wave (254 nm) and long wave (356 nm) ultra violet irradiation. Mycotoxins were identified by comparison with appropriate reference standards after each of the following treatments:

Aflatoxins: Aflatoxin B_1 and B_2 fluorescent bright blue and Aflatoxin G_1 and G_2 fluorescent green under long wave UV light (Chelkowski *et al.*, 1974). The TLC was developed in a saturated chamber with chloroform/ acetone (9:1, v/v). Then Aflatoxin spots were observed under long wave ultraviolet light (λ=356 nm).

Citrinin: Citrinin fluoresces lemon yellow under long wave UV light (Saito *et al.*, 1971).

Sterigmatocystin: The compound exhibits a dull brick red fluoresce under short wave UV light. Fluorescence change to yellow on spraying with aluminum chloride solution (20 g $AlCl_3$, $6H_2O$ in 100 ml ethanol) and the plates heated at 100°C for 5 minutes (Josefsson and Möller, 1977).

Ochratoxin A: It fluoresces greenish- blue under long wave UV light and changes to deep blue on exposure to ammonia fumes (Nesheim, 1976).

Patulin: The toxin is observed on TLC plates as dark spot on a light background. It can be visualized as yellow fluorescent spot after spraying with P-anisaldehyde reagent, fluoresces pale blue under long wave UV light after exposure to ammonia fumes (Scott *et al.*, 1970).

Zearalenone: Zearalenone fluoresces blue-green under long wave UV light and more greenish under short wave UV light and gives a green spot with 50% sulphuric acid in methanol that quickly turns to yellow (Eppley, 1968 and Roberts and Patterson, 1975).

RESULTS AND DISCUSSION:

Wheat is one of the world's most important food crops. Foods made from wheat and its derivatives are a major part of a diet for over a third of the world's people. The storage life of grains depends mainly on two physical factors; temperature and moisture content. Thus, survival and reproduction of biological agents in grain depend on the temperature and moisture levels (White, 1995). Moreover, mould contamination of grains can occur during the harvest and the post-harvest periods under suitable conditions of temperature and humidity (Doohan *et al.*, 2003), which play an important role in the growth and geographical distribution of fungi (Kosiak *et al.*, 2004). Hence, the identification of wheat mycoflora is becoming essential in order to control food contamination by fungi. In this respect, the moisture content of wheat grains under study showed variation

from 7-12% moisture content and the majority of samples have 9%. The wheat grain samples were imported from different countries viz: USA (18 samples), France (10), Australia (4), Russian (4) and 2 samples from Panama (Table 1). Imported grains may be shipped from temperate to tropical regions, subsequently transported, and stored within the recipient country (Wareing, 1997). One of the problems associated with this is the uptake or migration of water from one part of a grain bulk to another (Sellam and Christansen, 1976), another problem is the increase in water activity (*a*w) with increasing temperature for a given moisture content (Pixton, 1982, and Boxall and Gough, 1993). Both of these factors can led to an increase in the growth of fungi and spoilage of the grain. This can illustrate the high counts of CFU obtained during the mycological analysis of the imported grain samples.

The data presented in this study gave detailed information on the mycobiota which represented in both storage and field fungi of wheat grain samples imported at Damietta port. The results indicated that the grain samples were heavily contaminated with different fungi as reported in Tables 2 & 3. Indeed, mycological analysis of wheat samples using three media revealed that 61 species and one unidentified species in addition to 3 species varieties belonging to 21 genera were identified in the present work. *Aspergillus* (15 species and 2 varietes) and *Penicillium* (11 species) exhibited the broadest spectra of species (Raper and Fennell, 1965).

In addition to the previous genera and species, our results indicated other species as follows: *Absidia corymbifera, Alternaria* (4 species), *Cladosporium* (4 and one unidentified species), *Chaetomium globosum, Cochliobolus* (2), *Emericella* (one species), *Eurotium* (4 species), *Doratomyces stemonitis, Epicoccum nigrum, Mucor* (3), *Nigrospora sphaerica, Paecilomyces variotii, Rhizopus stolonifer, Scopulariopsis brevicaulis, Setosphaeria* (2), *Stemphylium botryosum,* and *Ulocladium* (3 species) and sterile mycelia, in addition to *Fusarium* (one species and one species variety).

The most recovered genera, in terms of frequency from wheat samples were *Aspergillus, Eurotium, Penicillium, Rhizopus stolonifer, Alternaria, Cladosporium* and *Fusarium.* These results agree with other published literatures dealing with fungi of cereal grains. In this respect, Mislivec *et al.* (1975) reported that mold flora of beans, before and after disinfection, were dominated by species of the *Aspergillus glaucus* group, the toxicogenic species were *A. ochraceus, Penicillium cyclopium, P. viridicatum,* and some species of *Alternaria, Cladosporium* and *Fusarium.* In addition, Bresler *et al.* (1995) determined the extent of mycofloral grains in Argentina before and after surface disinfection, and confirmed that *Aspergillus, Penicillium, Fusarium* and *Alternaria* were the most predominant genera.

Table 1 : Wheat grain sample numbers, their sources, storage periods, moisture contents (%) and their natural occurance of mycotoxins detected.

Samle No.	Source	Storage period*	Moisture content (%)	Natural occureance of mycotoxins detected
1	USA	22	10	-
2	USA	23	12	-
3	USA	21	11	-
4	Panama	28	12	-
5	USA	24	8	-
6	Australia	25	8	-
7	Panama	28	8	-
8	Australia	25	9	-
9	USA	18	8	-
10	Australia	22	8	-
11	USA	19	7	-
12	USA	23	9	-
13	USA	23	7	-
14	USA	17	8	-
15	USA	22	9	-
16	France	27	11	-
17	France	33	10	-
18	France	20	9	-
19	France	22	12	-
20	France	20	10	-
21	Australia	18	9	-
22	France	24	12	-
23	USA	22	9	-
24	USA	24	8.5	Sterigmatocystin
25	USA	18	9	Sterigmatocystin
26	USA	20	9	Sterigmatocystin
27	Russia	15	9	-
28	Russia	17	9	Sterigmatocystin
29	France	26	9	-
30	France	22	9	-
31	USA	23	9	-
32	USA	25	9	-
33	Russia	14	9	-
34	Russia	16	9	-
35	France	19	9	-
36	France	20	9	-
37	USA	25	9	-
38	USA	23	9	-

(*) Day of shipping.

Table 2 : Colonies forming units (CFU), relative density (RD %), number of cases of isolation (NCI), occurrence remarks (OR) and frequency (F %) of wheat storage fungi on glucose-Czapek's agar medium (CZ), glucose-Czapek's agar containing 6 % NaCl (CZ-NaCl) and malt agar medium (MA)

Genera and species	CZ					CZ- NaCl					MA				
	CFU	RD%	NCI	OR	F %	CFU	RD%	NCI	OR	F %	CFU	RD%	NCI	OR	F %
Absidia corymbifera	31	1.13	11	L	28.94	-	-	-	-	-	18	0.81	10	L	26.31
Alternaria	232	8.46	18	M	47.36	106	4.38	12	M	31.57	252	11.35	25	H	65.78
A. alternate	207	7.55	18	M	47.36	101	4.18	12	M	31.57	217	9.77	25	H	65.78
A. tenuissima	25	0.91	8	L	21.05	-	-	-	-	-	17	0.77	5	L	13.15
A. pharagmospora	-	-	-	-	-	5	0.21	3	R	7.89	9	0.14	4	R	10.52
Aspergillus	1350	49.23	38	H	100	847	35.03	36	H	94.73	866	39.01	37	H	97.36
A. awamori	85	3.10	17	M	44.73	53	2.19	12	M	31.57	62	2.79	20	M	52.63
A. candidus	46	1.68	11	L	28.94	38	1.57	7	L	18.42	17	0.77	9	L	23.68
A. clavatus	6	0.22	4	R	10.52	-					9	0.41	7	L	18.42
A. flavus	718	26.19	34	H	89.47	464	19.19	30	H	78.94	369	16.62	33	H	86.84
A. flavus var. *columnaris*	259	9.45	18	M	47.36	260	10.75	19	M	50	206	9.28	22	H	57.89
A. fumigatus	135	4.92	25	H	65.78	4	0.17	1	R	2.63	99	4.46	23	H	60.52
A. niger	11	0.40	8	L	21.05	2	0.08	2	R	5.26	17	0.77	12	M	31.57
A. ochraceus	7	0.26	4	R	10.52	8	0.33	2	R	5.26	5	0.23	5	L	13.15
A. oryzae	3	0.11	2	R	5.26	2	0.08	2	R	5.26	5	0.23	4	R	10.52
A. tubingensis	-	-	-	-	-	14	0.58	2	R	5.26	-	-	-	-	-
A. parasiticus	48	1.75	9	L	23.68	-	-	-	-	-	34	1.53	10	L	26.31
A. sulphureus	3	0.11	1	R	2.63	-	-	-	-	-	1	0.05	1	R	2.63
A. terreus	7	0.26	3	R	7.89	-	-	-	-	-	9	0.41	5	L	13.15
A. terreus var. *africanus*	4	0.15	4	R	10.52	-	-	-	-	-	10	0.45	7	L	18.42
A. versicolor	10	0.36	6	L	15.78	2	0.08	2	R	5.26	15	0.68	9	L	23.68
A. wentii	8	0.29	5	L	13.15	-	-	-	-	-	8	0.36	5	L	13.15
Chaetomium globosum	-	-	-	-	-	-	-	-	-	-	4	0.18	7	L	18.42
Cladosporium	26	0.95	4	R	10.52	34	1.41	7	L	18.42	15	0.68	5	L	13.15
C. cladosporioides	6	0.22	3	R	7.89	12	0.50	4	R	10.52	10	0.45	7	L	18.42
C. oxysporum	-	-	-	-		16	0.66	6	L	15.78	-	-	-	-	-
C. sphaerospermum	2	0.07	1	R	2.63	-	-	-	-	-	5	0.23	4	R	10.52
Cladosporium sp.	18	0.66	2	R	5.26	6	0.25	1	R	2.63	-	-	-	-	-
Cochliobolus	5	0.18	3	R	7.89	-	-	-	-	-	6	0.27	7	L	18.42
C. lunatus	2	0.07	2	R	5.26	-	-	-	-	-	1	0.05	2	R	5.26
C. spicifer	3	0.11	1	R	2.63	-	-	-	-	-	5	0.23	4	R	10.52

Cont. Table 2 :

Genera and species	CZ					CZ- NaCl					MA				
	CFU	RD%	NCI	OR	F %	CFU	RD%	NCI	OR	F %	CFU	RD%	NCI	OR	F %
Emericella nidulans	2	0.07	2	R	5.26	-	-	-	-	-	4	0.18	4	R	10.52
Epicoccum nigrum	1	0.04	1	R	2.63	-	-	-	-	-	-	-	-	-	-
Eurotium	93	3.39	16	M	42.10	969	40.07	35	H	92.10	52	2.34	16	M	42.10
E. amstelodami	43	1.57	13	M	34.21	538	22.25	34	H	89.47	28	1.26	11	L	28.94
E. chevalieri	38	1.39	12	M	31.57	131	5.42	23	H	60.52	13	0.59	8	L	21.05
E. proliferens	2	0.07	2	R	5.26	-	-	-	-	-	4	0.18	3	R	7.89
E. repens	10	0.36	4	R	10.52	300	12.41	27	H	71.05	7	0.32	5	L	13.15
Fusarium	16	0.58	4	R	10.52	-	-	-	-	-	15	0.68	5	L	13.15
F. moniliforme var *anthophilum*	15	0.55	4	R	10.52	-	-	-	-	-	11	0.50	4	R	10.52
F. oxysporum	1	0.04	1	R	2.63	-	-	-	-	-	4	0.18	2	R	5.26
Mucor	93	3.39	10	L	26.31	130	5.38	14	M	36.84	46	2.07	11	L	28.94
M. circinelloides	60	2.19	10	L	26.31	71	2.94	11	L	28.94	29	1.31	14	M	36.84
M. fuscus	7	0.26	2	R	5.26	-	-	-	-	-	5	0.23	2	R	5.26
M. racemosus	26	0.95	6	L	15.78	59	2.44	11	L	28.94	12	0.54	5	L	13.15
Paecilomyces variotii	2	0.07	1	R	2.63	-	-	-	-	-	-	-	-	-	-
Penicillium	85	3.10	22	H	57.89	109	4.51	8	L	21.05	64	2.88	14	M	36.84
P. aurantiogriseum	12	0.44	6	L	15.78	8	0.33	2	R	5.26	5	0.23	3	R	7.89
P. chrysogenum	22	0.80	11	L	28.94	37	1.53	6	L	15.78	18	0.81	10	L	26.31
P. citrinum	15	0.55	9	L	23.68	16	0.66	5	L	13.15	13	0.59	9	L	23.68
P. corylophilum	4	0.15	2	R	5.26	-	-	-	-	-	5	0.23	3	R	7.89
P. funiculosum	10	0.36	2	R	5.26	15	0.62	3	R	7.89	-	-	-	-	-
P. jenseni	1	0.04	1	R	2.63	-	-	-	-	-	3	0.14	3	R	7.89
P. puberulum	1	0.04	1	R	2.63	-	-	-	-	-	5	0.23	5	L	13.15
P. purpurogenum	6	0.22	2	R	5.26	26	1.08	2	R	5.26	3	0.14	2	R	5.26
P. variabile	1	0.04	1	R	2.63	-	-	-	-	-	2	0.09	2	R	5.2
P. verrucosum	13	0.47	6	L	15.78	7	0.29	3	R	7.89	10	0.45	8	L	21.05
Phoma herbarum	-	-	-	-	-	-	-	-	-	-	2	0.09	2	R	5.26
Rhizopus stolonifer	679	24.76	30	H	78.94	129	5.33	7	L	18.42	804	36.22	36	H	94.73
Scopulariopsis brevicaulis	8	0.29	5	L	13.15	-	-	-	-	-	8	0.36	6	L	15.78
Setosphaeria holmii	-	-	-	-	-	1	0.04	1	R	2.63	3	0.14	2	R	5.26
Stemphylium botryosum	13	0.47	6	L	15.78						12	0.54	8	L	21.05
Ulocladium atrum	4	0.15	1	R	2.63	-	-	-	-	-	8	0.36	3	R	7.89
Sterile mycelia	102	3.72	10	L	26.3	93	3.85	8	L	21.05	50	2.25	8	L	21.05
Total count	4642					4613					3536				
Number of genera	16					8					17				
Number of species and varieties	46+3					27+1					46+3				

Table 3 : Colonies forming units (CFU), relative density (RD %), number of cases of isolation (NCI), occurrence remarks (OR) and frequency (F %) of wheat field fungi on glucose-Czapek's agar medium (CZ), glucose-Czapek's agar containing 6 % NaCl (CZ-NaCl) and malt agar medium (MA)

Genera and species	CZ					CZ- NaCl					MA				
	CFU	RD%	NCI	OR	F %	CFU	RD%	NCI	OR	F %	CFU	RD%	NCI	OR	F %
Alternaria	295	17.98	32	H	84.21	143	10.08	17	M	44.73	298	34.94	32	H	84.21
A. alternate	222	13.53	31	H	81.57	123	8.67	17	M	44.73	289	33.88	32	H	84.21
A. chlamydospora	22	1.34	5	L	13.15	-	-	-	-	-	-	-	-	-	-
A. phragmospora	39	2.38	10	L	26.31	20	1.41	7	L	18.42	-	-	-	-	-
A. tenuissima	12	0.73	5	L	13.15	-	-	-	-	-	9	1.06	6	L	15.78
Aspergillus	706	43.02	36	H	94.73	422	29.74	31	H	81.57	369	43.26	28	H	73.68
A. awamori	40	2.44	11	L	28.94	15	1.06	11	L	28.94	15	1.76	9	L	23.68
A. candidus	28	1.71	6	L	15.78	28	1.97	5	L	13.15	4	0.47	3	R	7.89
A. flavus	310	18.89	31	H	81.57	188	13.25	24	H	63.15	127	14.89	28	H	73.68
A. flavus var. *columnaris*	94	5.73	12	M	31.57	66	4.65	10	L	26.31	152	17.82	16	M	42.10
A. fumigatus	109	6.64	16	M	42.10	12	0.85	3	R	7.89	36	4.22	11	L	28.94
A. niger	20	1.22	11	L	28.94	2	0.14	2	R	5.26	10	1.17	6	L	15.78
A. ustus	2	0.12	2	R	5.26	-	-	-	-	-	3	0.35	3	R	7.89
A. parasiticus	8	0.49	5	L	13.15	4	0.28	1	R	2.63	15	1.76	5	L	13.15
A. sulphureus	3	0.18	1	R	2.63	-	-	-	-	-	1	0.12	1	R	2.63
A. terreus	-	-	-	-	-	5	0.35	2	R	5.26	-	-			
A. tubingensis	88	5.36	5	L	13.15	92	6.48	4	R	10.52	-	-	-	-	-
A. versicolor	-	-	-	-	-	8	0.56	6	L	15.78	-	-			
A. wentii	4	0.24	2	R	5.26	1	0.07	1	R	2.63	6	0.70	3	R	7.89
Chaetomium globosum	2	0.12	1	R	2.63	-	-	-	-	-	3	0.35	2	R	5.26
Cladosporium	19	1.16	11	L	28.94	21	1.48	7	L	18.42	40	4.69	17	M	44.73
C. cladosporioides	7	0.43	5	L	13.15	6	0.42	3	R	7.89	17	1.99	11	M	28.94
C. macrocarpum	-	-	-	-	-	3	0.21	1	R	2.63	-	-	-	-	-
C. oxysporum	3	0.18	3	R	7.89	2	0.14	3	R	7.89	12	1.41	7	L	18.42
C. sphaerospermum	3	0.18	2	R	5.26	-	-	-	-	-	11	1.29	5	L	13.15
Cladosporium sp.	6	0.37	2	R	5.26	10	0.70	2	R	5.26	-	-	-	-	-
Doratomyces stemonitis	1	0.06	1	R	2.63	-	-	-	-	-	-	-	-	-	-
Eurotium	17	1.04	7	L	18.42	374	26.36	22	H	57.89	19	2.23	10	L	26.31
E. amstelodami	4	0.24	3	R	7.89	194	13.67	20	H	52.63	11	1.29	7	L	18.42
E. chevalieri	6	0.37	4	R	10.52	27	1.90	13	M	34.21	6	0.70	4	R	10.52
E. proliferens	2	0.12	2	R	5.26	-	-	-	-	-	2	0.23	2	R	5.26
E. repens	5	0.30	2	R	5.26	153	10.78	14	M	36.84	-	-	-	-	-

Cont. Table 3 :

Genera and species	CZ					CZ- NaCl					MA				
	CFU	RD%	NCI	OR	F %	CFU	RD%	NCI	OR	F %	CFU	RD%	NCI	OR	F %
Fusarium	7	0.43	2	R	5.26	-	-	-	-	-	-	-	-	-	-
F. anthophilum	4	0.24	2	R	5.26	-	-	-	-	-	-	-	-	-	-
F. oxysporum	3	0.18	1	R	2.63	-	-	-	-	-	-	-	-	-	-
Mucor racemosus	26	1.58	3	R	7.89	64	4.51	4	R	10.52	4	0.47	2	R	5.26
Nigrospora sphaerica	3	0.18	1	R	2.63	4	0.28	1	R	2.63	4	0.47	3	R	7.89
Penicillium	70	4.27	20	H	52.63	42	2.96	12	M	31.57	48	5.63	21	H	55.26
P. aurantiogriseum	7	0.43	4	R	10.52	7	0.49	4	R	10.52	9	1.06	5	L	13.15
P. brevicompactum	4	0.24	2	R	5.26	-	-	-	-	-	-	-	-	-	-
P. chrysogenum	17	1.04	8	L	21.05	22	1.55	8	L	21.05	20	2.34	11	L	28.94
P. citrinum	11	0.67	5	L	13.15	5	0.35	4	R	10.52	10	1.17	6	L	15.78
P. funiculosum	9	0.55	5	L	13.15	-	-	-	-	-	-	-	-	-	-
P. purpurogenum	8	0.49	2	R	5.26	-	-	-	-	-	-	-	-	-	-
P. variabile	4	0.24	1	R	2.63	-	-	-	-	-	-	-	-	-	-
P. verrucosum	10	0.61	6	L	15.78	8	0.56	3	R	7.89	9	1.06	5	L	13.15
Rhizopus stolonifer	67	4.08	12	M	31.57	10	0.70	2	R	5.26	-	-	-	-	-
Scopulariopsis brevicaulis	4	0.24	1	R	2.63	-	-	-	-	-	-	-	-	-	-
Setosphaeria rostrata	1	0.06	1	R	2.63	-	-	-	-	-	4	0.47	3	R	7.89
Stemphylium botryosum	13	0.79	4	R	10.52	1	0.07	1	R	2.63	7	0.82	5	L	13.15
Ulocladium	20	1.22	3	R	7.89	-	-	-	-	-	11	1.29	4	R	10.52
U. atrum	9	0.55	3	R	7.89	6	0.42	2	R	5.26	5	0.59	3	R	7.89
U. botrytis	5	0.30	1	R	2.63	-	-	-	-	-	4	0.47	1	R	2.63
U. chartarum	6	0.37	1	R	2.63	-	-	-	-	-	2	0.23	2	R	5.26
Sterile mycelia	384	23.40	20	M	52.63	332	23.40	15	M	39.47	46	5.39	8	L	21.05
Total count	2769					2420					1638				
Number of genera	15					10					11				
Number of species and varieties	42+ 2					29+1					29+1				

Table 4 : Statistical analysis of performances on wheat grain

Correlation No.	Statistical analysis results					
	Inter-correlation item	Total count	No. of Genera	No. of Species		
1	Total count	-	0.39602**	0.48993**		
	No. of genera	-	-	0.86798**		
	No. of species	-	-	-		
2	Media	Cz	Cz+ NaCl	Malt	LSD	Comparison wise
	Mean	57.671 A	50.816 A	40.434 B	8.5897	Total count
	Mean	4.4342 A	3.1842 B	4.5789 A	0.5593	No. of Genera
	Mean	8.0000 A	5.6842 B	8.0263 A	1.1721	No. of Species
3	Kind of fungi	Media	Mean TC	Mean No. of genera	Mean No. of species	
	Storage	Cz	102.92	4.46	9.46	
		Cz + NaCl	103.23	2.07	6.07	
		Malt	92.92	5.15	10.84	
	Field	Cz	31.69	3.61	6.30	
		Cz + NaCl	22.15	3.53	6.00	
		Malt	21.84	4.53	8.15	
4	Media	Storage fungi	Field Fungi	LSD	Comparison wise	
	Mean	64.737 A	34.544 B	7.0135	Total count	
	Mean	4.5877 A	3.5439 B	0.4567	No. of Genera	
	Mean	8.6754 A	5.7982 B	0.957	No. of Species	
5	Total count			Moisture content		
	Storage fungi on Czapek's			0.15491 Ns		
	Storage fungi on Czapek's with NaCl			0.10766 Ns		
	Storage fungi on malt			0.16587 Ns		
	Field fungi on Czapek's			0.03644 Ns		
	Field fungi on Czapek's with NaCl			-0.10581 Ns		
	Field fungi on malt			0.35055 *		

(❀) Correlation number:

1-Correlation coefficient between fungal total counts, number of genera and number of species of storage and field fungi of wheat grain samples on different media (*,**: Significant at 0.05, and 0.01 levels, respectively).

2-Correlation between types of media (Cz, Cz with NaCl and malt) for variable mean fungal total counts, mean number of genera and mean number of species of wheat grain samples (Different letters means significant, Same letters means non significant).

3-Values mean of storage and field fungi for total counts, number of genera and number of species of wheat grain samples.

4-Correlation between storage and field fungi for variable mean fungal total counts, mean number of genera and mean number of species of wheat grain samples.

5-Correlation coefficient between moisture content and total count of (storage and field fungi) of wheat grain samples on different media.

Table 5: Screening test of mycotoxins produced by *Aspergillus* section Flavi

Mycotoxins	*A. flavus* (16)	*A. flavus* var. *columnaris* (16)	*A. parasiticus* (10)	*A. oryzae* (16)	Total isolates (48)
Aflatoxin B_1, B_2, G_1 and G_2	1	5	-	-	6
Aflatoxin B_1	3	-	-	-	3
Aflatoxin B_1 and B_2	-	2	-	-	2
Sterigmatocystin	1	1	-	-	2
Sterigmatocystin and aflatoxin B_1, B_2, G_1 and G_2	-	-	1	-	1
Sterigmatocystin and aflatoxin G_1 and G_2	-	1	-	-	1
Sterigmatocystin and aflatoxin B_1	-	-	1	-	1
Aflatoxin G_1	-	1	-	-	1

Many other moulds were also isolated in moderate frequency, including mainly *Cochliobolus* and *Setosphaeria*, while others in a low frequency as *Emericella, Epicoccum, Peacilomyces, Phoma, Scopulariopsis, Stemphylium, Doratomyces, Nigrospora, Absidia, Ulocladium, Mucor,* and *Chaetomium* (Tables 2 and 3). As represented in the same tables, the most frequent fungus was *Aspergillus,* dominant in all cases, and present in 43.4% af all isolates compared with an isolation frequency (%), 100, 94.7, and 97.3 followed by 94.7, 81.5 and 73.6 of storage and field fungi on Cz, Cz-NaCl, and MA, respectively. Consequently, the order of frequency of isolated species varied in storage and field fungi was, *Penicillium,* dominated with 57.89% in storage fungi of wheat grains on Cz compared with 52.63 and 55.26% of field fungi on Cz and MA, respectively. Also, the dominace of *Eurotium*, on Cz-NaCl was 92.1 and 57.9% of storage and field fungi of wheat grain samples, respectively. Additionally, *Rhizopus stolonifer* dominated in storage fungi and isolated from 78.9 and 94.7% of wheat grains on Cz and MA, respectively (Tables 2 and 3). These data were supported by those obtained by El-kady and El-Maraghy (1990) and Baliukoniene *et al.* (2003).

The predominance of *Penicillium* species in this study was in agreement with Baliukoniene *et al.* (2003) who isolate 8 fungal genera from 55 samples of wheat and barley grains were isolated and the most prevalent genera were *Penicillium*, *Aspergillus* and *Fusarium.* The current result was similar to other results reported on grains mycoflora (Orsi *et al.*, 2000; Ono *et al.*, 2002 and Berghofer *et al.*, 2003). Howevere, Riba *et al.* (2008) found that the occurrence and the levels of the genus *Penicillium, Fusarium, Alternaria* and *Mucor* were substantially lower than those of *Aspergillus* in wheat grain samples.

The predominant of *Aspergillus* species in this study was similar to the finding of Berghofer *et al.* (2003) that the most common fungi in wheat grains were *Aspergillus, Penicillium, Cladosporium* and *Eurotium* spp. In Egypt, El- Kady and El- Maraghy (1990) inspect the mycoflora of 50 wheat grain samples and found that *Aspergillus, Penicillium, Fusarium* and *Rhizopus* were the most prevalent genera. Nearly similar observation has been previously reported by EL- Maghraby (1989). Also, similar observations have been previously reported by many investigators working with other grains and seeds (Mislivec *et al.*, 1975, 1979; Lee *et al.*, 1986; Mishra and Daradhiyar, 1991; Adebajo *et al.*, 1994; Adisa, 1994; Bresler *et al.*, 1995; Uddin and Chakraverty, 1996; González *et al.*, 1997; Orsi *et al.*, 2000; Ono *et al.*, 2002, Baliukoniene *et al.*, 2003 and Al-Hazmi, 2011). The predominance of *Eurotium* species found in this study came in agreement with other reports on grain mycoflora (Mislivec *et al.*, 1975, 1979; Rabie *et al.*, 1997 and Berghofer *et al.*, 2003).

Interestingly, *Alternaria* species was dominated in storage fungi of wheat grains on MA with a frequecy of 65.7% which was remarkably lower than that reported as field fungi (84.2%) on both Cz and MA. These results

support the previous reports given by Barbara *et al.* (2004), that *Alternaria* spp. dominated in the acceptable samples with *Alternaria infectoria*, of grain samples of wheat, barley and oats collected from Norway in 1997 and 1998. Also, our results were in line to that reported by many other investigations (Mislivec *et al.*, 1975; 1979; Lee *et al.*, 1986; Bresler *et al.*, 1995; Uddin and Chakraverty, 1996; González *et al.* (1997); Gohari *et al.*, 2007; Bensassi *et al.*, 2009 and 2011).

Statistical analysis of the results showed a high significant correlation between fungal total count, number of genera and number of species on different media (Table 4, correlation 1). The data also showed a significant decrease in the mean fungal total counts obtained on MA than on Cz and Cz-NaCl. While, the mean number of genera and species on MA and Cz were significantly increased than Cz-NaCl (Table 4, correlation 2).

The results of storage and field fungi of wheat grains cultured on different media showed a significant decrease in the counts of field fungi compared with storage fungi of wheat grain samples for mean total counts, mean number of genera and species (Table 4 correlations 3&4). Mislivec *et al.* (1975) reported that surface disinfections substantially reduced the mold incidence. Generally, the correlation between moisture content and fungal total counts was not significant except the moisture content with total counts of field fungi isolated on MA was significantly correlated (Table 4, correlation 5).

The evaluation of natural contaminantes of mycotoxins in 38 wheat grain samples by thin layer chromatographic technique revealed that 10.5% of the total samples containing sterigmatocystin which represented in four wheat samples (Table 1), while the remaining samples were not contaminated by any other mycotoxin. The samples, which were positive for sterigmatocystin in the study of Anonymous (1980) were highly molded. Also, sterigmatocystin was detected in one of the 29 samples of heated wheat grain (Scott *et al.*, 1972) and in feedstuffs and other cereal grains (Jeswal, 1990; Scott, 1990; Sarah *et al.*, 1996 and Scudamore *et al.*, 1998). Perenzin *et al.* (2001) found that 62% of wheat samples collected from experimental field plots in northern Italy (Lombardy) were contaminated by aflatoxins, while a total 43.6% of the maize samples were contaminated with aflatoxins B1 (Karami-Osboo *et al.*, 2012), however, no aflatoxins were detected in the current work, that agrees with the finding of Behfar *et al.* (2008) that none of 32 wheat flour samples was contaminated by aflatoxins.

Cereals represent a main food for the Egyptian population, therefore it has a high social, economic and nutritional relevance. Furthermore, they are usually stored in conditions which favour mould growth and mycotoxin production. On the other hand, there were many investigations reported the presence of mycotoxins on grains. Fandohan *et al.* (2005) declared that aflatoxins and fumonisins naturally contaminated maize through the

traditional processing. Also, Olsson *et al.* (2002) reported the presence of ochratoxin A (OA) and deoxynivalenol (DON) in ten barley samples with normal odor, and 30 with some kind of off-odor were selected from Swedish granaries. Zinedine *et al.* (2007) confirmed that the incidence of AFB1 in wheat flour commercialized in Morocco was about 17.6%, and that levels of contamination ranged from 0.03 to 0.15 µg/kg. Some food processing methods have been shown to result in reduction or elimination of aflatoxins (Murphy *et al.* 2006). AFB1was detected by HPLC in 56.6% of the wheat samples and derived products (flour, semolina and bran) with contamination levels ranging from 0.13 to 37.42 µg/kg (Riba *et al.*, 2010).

The evaluation of mycotoxins production by *Aspergillus* species of section *Flavi,* recovered from wheat grain samples, resulted in 35.4% of the isolates have the ability to produce mycotoxins. Hence, 48 different fungal isolates (16 isolates were belonged to *A. flavus* var. *columnaris,* 16 isolates to *A. flavus,* 10 isolates to *A. parasiticus,* and 6 isolates to *A. oryzae*) were tested. Ten isolates of *A. flavus* var. *columnaris* produced toxins; 5 of them produced aflatoxins B_1, B_2, G_1 and G_2; two produced aflatoxin B_1, B_2; one produced sterigmatocystin, one produced sterigmatocystin and aflatoxin G_1 and G_2, and the other produced aflatoxin G_1 (Table 5). The isolates of *A. flavus* var. *columnaris* has the highest ability to produce Aflatoxin (62.5%) of its strains.

The ability to produce aflatoxins from the isolated *A. flavus* may vary according to the type of commodity from which they were isolated (El-Kady *et al.*, 1979; Mishra and Daradhiyer, 1991).

Interestingly, 5 isolates of *A. flavus* produced toxins, 3 of them produced aflatoxin B_1, one produced aflatoxin B_1, B_2, G_1, G_2, and the other produced sterigmatocystin. These results are in agreement with the finding of Eman *et al.* (2005), she noticed that of 45 isolates of *A. flavus*, ten have the ability to produce aflatoxins B_1, B_2, G_1, G_2 and sterigmatocystin. Also, this result was similar to that of Pitt and Hocking (1997).

Finally, 2 isolates of *A. parasiticus* produced toxins, one of them produced sterigmatocystin, aflatoxins B_1, B_2, G_1 and G_2 and the other produced sterigmatocystin and aflatoxin B_1. Moreover, all *A. oryza* strains did not produced any toxins (Table 5). In agreement to this, *A. parasiticus* produced aflatoxins B_1, G_1, NIV, DON and T-2 toxins at high levels (Attala *et al.*, 2003). The reported mycotoxins were previously produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and others *Aspergilli* in section *Flavi* (Samson *et al.*, 2006 and Pildain *et al.*, 2008).

AFB1 had produced on CYA medium by *Aspergillus* of section *Flavi* strains (Riba *et al.*, 2010). A number of fungal species associated with maize, mainly belonging to the genera of *Fusarium* and *Aspergillus* have been reported to produce mycotoxins that cause mycotoxicoses in domestic animals and human (Karami-Osboo *et al.*, 2012).

CONCLUSION:

The samples of wheat grains which imported to Egypt from different countries were contaminated with several fungi (storage and field). Moreover, 10.5% of these grains were naturally contaminated with sterigmatocystin comparable to, 35.4% of *Aspergillus* of section *Flavi* isolates produced mycotoxins (aflatoxins B_1, B_2, G_1 and G_2 in addition to sterigmatocystin). Hence, the imported grains must be evaluated for their containmaination with mycoflora and mycotoxins. In addition, precautions must be taken during processing, transport, packaging, and storage to avoid grain contamination by mycoflora and mycotoxins.

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Ass. Univ. Bull. Environ. Res. Vol. 15 No. 1 March 2012

دراسة الحياة الفطرية وسمومها في حبوب القمح المستوردة لمصر

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لأن القمح من الحبوب الغذائية الرئيسية لسكان مصر، ولما له من أهمية اقتصادية وغذائية عالية فقد ركزت هذه الدراسة على تواجد الفطريات فى عينات القمح، وتقييم الوجود الطبيعي للسموم على هذه العينات، وكذلك مقدرة عدد من العزلات الفطرية التي تم عزلها خلال الدراسة على إفراز السموم الفطرية معملياً.

تم جمع ثمانية وثلاثين عينة من حبوب القمح المستوردة من الولايات المتحدة الأمريكية وفرنسا وأستراليا وروسيا وبنما، والتي تصل إلى ميناء دمياط عن طريق السفن خلال الفترة من أكتوبر ٢٠٠٣ إلى ديسمبر ٢٠٠٥. أظهرت نتائج المحتوى الرطوبى للعينات أنه يتراوح من ٧-١٢٪، وكانت معظم العينات تحتوى على ٩٪ من المحتوى الرطوبى.

أمكن خلال هذه الدراسة عزل وتعريف ٦١ نوعاً فطرياً بالإضافة إلى ٣ أصناف تنتمي إلى ٢١ جنساً فطرياً بالإضافة إلى نوع غير معرف، وذلك باستخدام ثلاثة أوساط غذائية، وهى : الجلوكوز شابكس أجار، الجلوكوز شابكس أجار والمضاف إليه ٦% كلوريد الصوديوم ووسط مستخلص الشعير.

سادت أجناس فطرة الأسبيرجيلس بنسبة ٤٣,٤٪ تلتها فطرة الأوروشيم بنسبة ١٣,٧٪، وفطرة الريزوبس بنسبة ١٣,٢٪، ثم فطرة الألترناريا (٧,٧٪) من مجموع العزلات الفطرية. كانت أكثر الأنواع الفطرية شيوعاً والملوثة للعينات هى فطرة الأسبيرجيلس فلافس (١٨,٢٪)، وأسبيرجيلس فلافس صنف كولومناريس (١٢,٧٪)، وتلتها فطرة ألترناريا ألترناتا (٩,٣٪)، ثم فطرة الأوروشيم امستيلودامى (٣,٨٪) من مجموع العزلات الفطرية.

أظهرت التحاليل الإحصائية لفطريات حبوب القمح وجود معامل ارتباط معنوي كبير بين المجموع الكلى وعدد الأجناس وعدد الأنواع لعينات القمح على الأوساط الغذائية المستخدمة.

في محاولة لإختبار السموم الفطرية التي قد توجد طبيعيا على عينات القمح المستخدمة وجد ان العينات ملوثة طبيعيا بنسبة ١٠,٥٪ بالاستريجماتوسستين.

فى دراسة تقييم مدى سمية بعض عزلات مجموعة الأسبيرجيلس فلافس والتي أجريت على ٤٨ عزلة مختلفة وجد أن ٣٥,٤٪ من العزلات لها القدرة على إنتاج السموم الفطرية المسماه بالأفلاتوكسين B_1 وB_2 و G_1 وG_2 بالإضافة إلى الأستريجماتوسستين.

بما لهذه المركبات من خطورة معروفة فى إنخفاض كفأة الغذاء ولآثارها الضارة على صحة الإنسان والحيوان فلا بد من فحص الحبوب المستوردة بكافة أنواعها للوجود الطبيعي للسموم والفلورا الفطرية والسموم الناتجة عنها. وكذلك باستخدام أساليب معالجة المواد الغذائية التى تؤدي إلى انخفاض أو القضاء على الفلورا الفطرية وسمومها (ذلك عن طريق أخذ الاحتياطات أثناء عملية التجهيز والنقل والتعبئة والتغليف والتخزين والتوزيع).