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Detection of Fungi and Aflatoxins Contaminated Peanut Samples (*Arachis Hypogaea* L.)

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Abstract Mycoflora associated with some peanut seed samples (*Arachis hypogaea* L.) which collected from five different Governorates, in Egypt yielded 1000 fungal isolates belonging to four fungal genera i.e. *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus*. Agar plate (PDA) medium was enhanced for seed health testing than blotter test method and gave higher numbers of fungal colony. The Behera (Nobaria location) samples were higher contaminated of fungi whereas the Aswan samples were less contaminated than other governorates. *Aspergillus* spp was the most frequently present, which recorded 40.71% of *A. niger*, 34.29% of *A. parasiticus*, 5.58% of *A. flavus* and 0.43% of *A. terreus. Rhizopus* was moderately encountered (6.85%), followed by *Penicillium* (8.14%), whereas *Fusarium oxysporum* was less frequent occurred. Disinfected seeds contained lessed fungi than non-disinfected ones. The Aswan samples were less fungal contaminated than the others (2.14%) and Sharkia samples were highly contaminated, followed by Behera (Nobaria) samples (12.0 and 10.71% of fungal frequency respectively). On the other hand, contaminated peanut pods yielded 1400 fungal isolates belonging to five fungal genera i.e. *Aspergillus, Epicoccum, Fusarium, Penicillium* and *Rhizopus.* Aspergillus genus was the most frequent and gave 13.5, 12.61, 5.9 and 3.4% with *A. parasiticus, A. niger, A. terreus* and *A. flavus*, respectively. Penicillium was moderately frequent with 26.9%, followed by *Fusarium* which gave 9.2%, 6.7% and 2.5% with *Fusarium oxysporum, F. solani* and *Fusarium* spp., respectively. *Rhizopus* genus was 16%, whereas *Epicoccum* sp. was lessed which gave only 3.4% in all disinfected pods using agar plate (PDA) medium. The Aswan samples was less fungal contaminated than others and gave a frequency of 14.3% in disinfected pod samples with agar plate test. The Monofya samples gave higher fungal frequency contamination of peanut pods (31.1%), followed by Sharkia and Giza pod samples which gave 21.0 and 17.6%, respectively. The Behera (Nobaria) samples were moderately contaminated and record 16.0%. *Epiccocum* sp. was less frequently encountered. Tested of aflatoxigenic fungi using either TLC and HPLC resulted that three isolates of *A. flavus* gave positive reaction with AFB_1 and AFB_2, while only one isolate of *A. parasiticus* produced AFB_1, AFB_2, AFG_1 and AFG_2 which record 172, 418.38, 1358.7 and 364.6 µg/kg, respectively.

Keywords: Peanut (*Arachis hypogaea* L.); Fungi; Mycotoxin; Aflatoxins.

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Introduction

Peanuts (*Arachis hypogaea* L., Family: Fabaceae) are a rich source of fat, proteins and vitamins. They are grown on a large scale in almost all the tropical and subtropical countries, especially in India, China, USA and in West Africa (Pitt *et al.*, 1993). Also, Peanut or groundnut (*Arachis hypogea* L.), a member of the legume family, is an important food and oil crop. It is currently grown on approximately 42 million acres worldwide. It is the third major oilseed of the world after soybean and cotton (FAO, 2011). In Egypt, peanut is one of the most important leguminous crops as well as in many parts of the world. It is used for human consumption, oil production, food industries and animal feeding. The total production of peanut in Egypt was 26255 metric tons harvested from 29338 feddan, with an average yield of 895 kg/ feddan (CAPMAS., 2006). Egypt is a major peanut exporting country and the European markets accounts for 68 percent of its peanut exports (FAO. 2011). Many fungi are serious parasites of pod and grains in the field (soil borne fungi), storage and transporting (storage fungi) causing pod and grains rot and their invasion can resulted various damage including yield loses of pod and/or grains in both qualitatively and quantitatively, discoloration, total decay and mycotoxin production.

In Egypt, the major fungal species associated with rotting of peanut pods were *Aspergillus* spp., *Fusarium* spp., *Macrophomina phaseolina, Rhizoctonia solani* and *Sclerotium rolfsii* (Emara *et al*., 2003). Seven fungi, *Aspergillus flavus, A. parasiticus, Fusarium moniliforme, F. solani, Macrophomina phaseolina, Rhizocotina solani* and *Sclerotium rolfsii*, have been frequently isolated with different frequencies from either pod shells or seeds of peanut (Mahmoud, 2004). In addition, several species of pathogenic fungi associated with peanut pods and caused pod rot disease have been reported worldwide, for example, *Fusarium* spp. (Adiver and Anahosur, 2002) and *Aspergillus* spp. (Xue *et al.*, 2003). The mycoflora in stored peanut samples (hulls and kernels) from Tupa state of Barzil resulted *Fusarium* spp. (67.7% in hulls and 25.8% in kernels) and *Aspergillus* spp.(10.3% in hulls and 21.8% in kernels) and the presence of five other genera (Vivian *et al.*, 2008). The aflatoxin-producing fungus *Aspergillus flavus* is a causal agent of preharvest contamination of food commodities such as oil seed crops worldwide. Peanut, corn and cottonseed are among the oil seeds that are susceptible to aflatoxin contamination during invasion of these crops by *A. flavus.* Contamination of agricultural commodities with aflatoxins can result in serious economic hardships to producers and adverse health impacts in both humans and domestic animals (Duran *et al.*, 2009). *Aspergillus* was consistently the most frequent genus in seeds and

inshell peanuts and was the dominant mycotoxigenic component of the mycobiota. The most common species were from *Aspergillus* section *Flavi* (4.7-78.3%), *Aspergillus* section *Nigri* (9.4-52.6%) and *Aspergillus* section *Circumdati* (5.1-30.9%) (Yousef Sultan and Naresh Magan, 2010). Peanuts are important substrates for the growth and subsequent aflatoxin production by different members of *Aspergillus*. The results indicated that all the shell and seed samples were infected with fungi. *Aspergillus flavus* was isolated from all seed samples but did not isolated from peanut shells. All seed samples were contaminated with aflatoxin (Abdel-Wahhab, et. al., 2011).

A. parasiticus produces aflatoxin B_1, B_2, G_1 and G_2, and *A. flavus* produces B_1 and B_2. The main contamination by B_1 was 266, B_2 was 22, G_1 was 3.2 and G_2 was 0.7 mg/kg. The maximum yield for aflatoxin B_1 was 10 mg/kg, B_2 was 450, G_1 was 130 and G_2 was 30 mg/kg. B_2, G_1 and G_2 totaled 9.3% of the four aflatoxins, and their toxicity were equal to 2% of that of aflatoxin B_1. The term aflatoxin usually refers to four compounds, of the group bis-furanocoumarin metabolites produced by four species of Aspergillus i.e., *A. flavus, A. parasiticus, A. tamorii* and *A. nominus* (Novas and Cabral, 2002). Aflatoxin produced when toxigenic strains of the fungi *Aspergillus flavus* Link. ex Fries and *A. parasiticus* Speare grow on peanuts and many other agricultural commodities. Aflatoxin concentration is the most important quality problem in peanuts worldwide with serious health implications for humans as well as livestock (Gong *et al.,* 2003). Peanut seeds are good substrate for growth and subsequent aflatoxin production by aflatoxigenic fungi (Xue *et al.,* 2003). Analysis of hulls showed that, 6.7% of the samples were contaminated with AFB1 (mean levels = 15–23.9 µg /kg) and AFB2 (mean levels = 3.3–5.6 µg/kg); in kernels, 33.3% of the samples were contaminated with AFB1 (mean levels = 7.0–116 µg/kg) and 28.3% were contaminated with AFB2 (mean levels = 3.3–45.5 µg/kg) Saleemullah, *et al.* 2006. Several types of aflatoxins exist, but the four main types are Aflatoxin B1, B2, G1 and G2, with Aflatoxin B1 being the most toxic Olaru, *et al*., (2008). Analysis of the toxigenic potential revealed that, 93.8% of the *A. flavus* strains isolated were producers of AFB1 and AFB2 (Viviane *et al.,* 2008). Samples of peanut products were analyzed for aflatoxins (AF) B1, B2, G1 and G2 by high performance liquid chromatography. The results showed 44.2% samples positive for at levels of 0.5 to 103.8 µg/kg (Carlose *et al.,* 2009). The aflatoxin-producing fungus *A. flavus* is a causal agent of pre-harvest contamination of food commodities such as oil seed crops worldwide. Peanut, corn and cottonseed are among the oil seeds that are susceptible to aflatoxin contamination during invasion of these crops by *A. flavus* (Duran *et al.,* 2009).

The aim of the present work was: collected peanut samples (seed and pods) fropm different Governorates in Egypt, isolation and identification of fungi associated with peanut seed and pods. The extraction and detection of mycotoxin production was also studied.

Materials and methods

Peanut samples

Peanut pod and seed (Kernels) samples were collected from five different governorates in Egypt, namely Aswan, Giza, Nobaria, Monofya and Sharkia. The samples were collected into plastic bags, a sub-sample from each bag was examined by naked eye in the laboratory and classified as follows: samples with breakdown, with dry brown lesions, with pink discoloration on the surface and healthy pods and seeds (no symptoms).

Isolation and identification of fungi

The seeds and pods were classified into two groups. The first group was disinfected (surface sterilized) with 1% sodium hypochlorite for 2 min then washed with sterilized distilled water (SDW) and dried between two layers of sterilized filter paper (SFP). The second group wsa untreated (non-disinfected). All groups of pod and seeds were placed onto potato dextrose agar plats (PDA medium) with streptomycin sulfate (100 mg/ml), as well as plated on two layers of moisted autoclaved filter paper (damp chumper). All Petri dishes were incubated at 27 °c for 4-7 days. All the developing fungal colonies were transferred and purified onto PDA medium by using single spores or hyphal tips techniques. The total fungal count and percentage of natural infection with fungi were calculated, and the frequency occurrence of different fungi associated with peanut pod and seeds were recorded. The growing fungal cultures on PDA slants were stored in a refrigerator until used. All fungal isolates were identified on the basis of their morphological characteristics by using specific media, in the Plant Pathology Department of the National Research Centre (NRC).

Aflatoxin production

Preparation of aflatoxins

In the Food Toxicology and Contamination Department of the National Research Centre (NRC), aflatoxins were produced by the inoculation of YES

medium in Erlenmeyer flasks containing 500 ml of liquid media with *Aspergillus flavus* and *A. parasiticus*. The infected medium was incubated at 25 ^{O}C for 15 days for aflatoxin determination. The criteria of purity of the standards were checked by determining the chromatographic purity and molar absorption. The absorbance at 350 nm was determined and concentration was calculated.

Extraction

The aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) were extracted according to the method of AOAC.49.49.12.18 (AOAC, 2007). Fifty ml of the liquid media representative of the sample were taken into 500 ml conical flasks. Twenty five ml water, 25 gm diatomaceous earth (celite) and 250 ml chloroform were added. The flask was securely closed with masking tape and shaken on a wrist action shaker for 30 min to extract the toxin. The content of the flask was filtered through fluted filter paper. The contents were transfer to a Buchner funnel pre-coated with about 5 mm layer of diatomaceous earth and the first 50 ml of filtrate were collected.

Clean-up procedures

The preparation of column chromatography was carried out according to Ogido *et al.* (2004).

Determination of aflatoxins

The determination of aflatoxins was carried out by using HPLC according to (AOAC, 2007). The HPLC instrument used was waters (474) system, equipped with quaternary pump. The fluorescence detector system was set at 360 nm excitation and 440 nm emission wavelengths. The chromatography column was phenomenex c18 (250x 4.6 mm), 5 µm. The mobile phase system (H_2O: MeOH: CH3CN, 30:60:10 v/v/v) was isocratically at flow rate of 1 ml /min (Han et al., 2004). The data were collected and integrated using Totalchrom Navigator Chromatography Manager Software.

Results

Fungal frequency associated with peanut seed samples

The results given in Table 1 and Table 2 showed that the total fungal frequency contaminated peanut seed samples yielded 1000 isolates, 700 of

them (70%) by using agar plate test (PDA medium) and 300 isolates (30%) with blotter test. Table 1 showed that, four fungal genera were identified and namely, *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* (Fig 1.a,b,c &d). *Aspergillus* was the most fungal frequently recorded with 40.71% of *A. niger*, 34.29% of *A. parasiticus*, 5.58% of *A. flavus* and 0.43% of *A. terreus. Rhizopus* was moderately recorded (6.85%), followed by *Penicillium* (8.14%), wherease, *Fusarium oxysporum* was less frequent (4%). The disinfected seeds were less fungal contaminated than non-disinfected seeds. Aswan sample was less fungal contaminated than other Governorates which gave 2.14%, whereas the Sharkia sample was highly contaminated, followed by Behera (Nobaria location) sample with disinfected seeds respectively and gave 12.0 and 10.71% of fungal frequency. Moderate fungal frequency occurred with disinfected Monifya and Giza samples which recorded 6.57and 6.43% respectively using (PDA) agar plate test. The Behera sample was higher frequency occurred which record 23.58% with non-disinfected peanut seeds using agar plate (PDA) test. On the other hand data (in Table 2) showed that the Behera sample gave higher fungal frequency in both disinfected and non-disinfected peanut seeds, with 14.0 and 21.0%, respectively using the blotter test method. The Aswan sample was less and gave zero%, while Sharkia and Monifya samples were moderate frequency with 8.0 and 9.0% for disinfected seeds respectively. The Sharkia sample gave moderate fungal frequency 14.0%, while Gharbia was less 5.0% for non-disinfected blotter test. From the previous results, it can be concluded that, agar plate (PDA) test was an enhanced test method for fungal isolates of seed health testing than blotter test method.

Table 1. Fungal frequency occurred with some peanut seed samples using Agar plate (PDA) which collected from different Governorates

Test Fungal isolate(s)		Agar plate (PDA)										Total
		Disinfected					Non disinfected					
		A	G	M	SH	N	A	G	M	SH	N	
Aspergillus	*T.C*	9	18	30	30	30	45	30	45	3	45	285
niger	*%*	1.3	2.8	4.3	4.3	4.3	6.4	4.3	6.4	0.4	6.4	40.71
Aspergillus	*T.C*	0	27	3	42	42	18	6	12	45	45	240
parasiticus	*%*	0.0	3.9	0.4	6.0	6.0	2.8	0.9	1.7	6.4	6.4	34.29
Aspergillus	*T.C*	0	0	3	9	0	0	3	9	0	15	39
flavus	*%*	0.0	0.0	0.4	1.3	0.0	0.0	0.4	1.3	0.0	2.1	5.58
Aspergillus	*T.C*	0	0	0	0	0	3	0	0	0	0	3
terreus	*%*	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.43
Fusarium	*T.C*	0	0	10	0	0	6	0	3	0	9	28
oxyspoium	*%*	0.0	0.0	1.4	0.0	0.0	0.9	0.0	0.4	0.0	1.3	4.0
Penicillum	*T.C*	6	0	0	0	3	15	3	6	6	18	57
sp.	*%*	0.9	0.0	0.0	0.0	0.4	2.1	0.4	0.9	0.9	2.8	8.14
Rhizopus sp.	*T.C*	0	0	0	3	0	0	6	0	6	33	48
	%	0.0	0.0	0.0	0.4	0.0	0.0	0.9	0.0	0.9	4.7	6.85
Total	*T.C*	15	45	46	84	75	87	48	75	60	165	700
	%	2.14	6.43	6.57	12.0	10.71	12.43	6.86	10.71	8.57	23.58	100.0

A: Aswan G: Giza M: Monofya SH: Sharkia N: Nobaria (Behera) T.C: Total count of fungi % : Fungal frequency occurred PDA: Potato dextrose agar

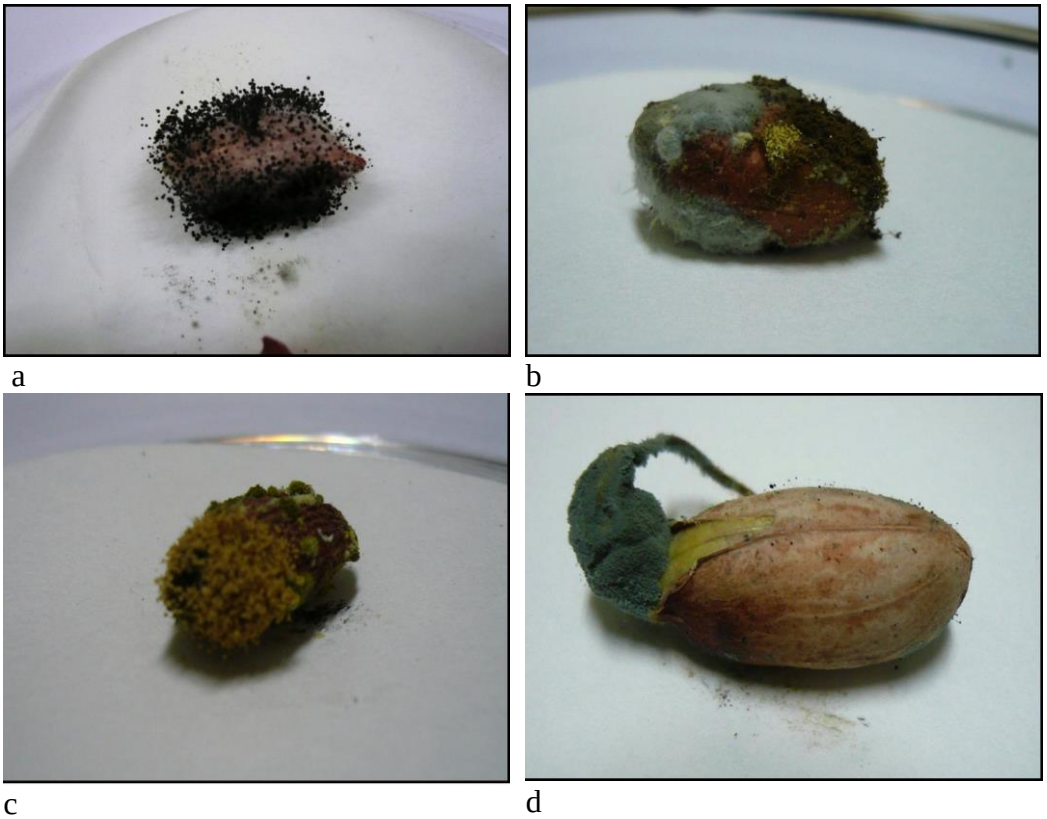

Fig. 1. A. *Aspergillus niger* associated with Non-disinfected peanut seed, Aswan sample, b-*Aspergillus flavus*, *A. terreus* and *Penicillum* sp. associated with Non- disinfected peanut seed, Aswan sample, c-*Aspergillus flavus and Aspergillus parasiticus* associated with Non- disifected peanut seed, Sharkia sample, d-*Penicillum* sp. associated with disinfected peanut seed, Aswan sample

Table 2. Fungal frequency occurred with some peanut seed samples using blotter test which collected from different Governorates

Test Fungal isolate(s)		Blotter test										Total
		Disinfected					Non disinfected					
		A	G	M	SH	N	A	G	M	SH	N	
Aspergillus niger	*T. C*	0	3	21	6	3	18	12	45	3	12	123
	%	0.0	1.0	7.0	2.0	1.0	6.0	4.0	15.0	1.0	4.0	41.0
Aspergillus parasiticus	*T. C*	0	0	3	18	33	3	3	6	36	39	141
	%	0.0	0.0	1.0	6.0	11.0	1.0	1.0	2.0	12.0	13.0	47.0
Aspergillus flavus	*T. C*	0	0	3	0	0	0	0	6	0	3	12
	%	0.0	0.0	1.0	0.0	0.0	0.0	0.0	2.0	0.0	1.0	4.0
Aspergillus terreus	*T. C*	0	0	0	0	0	6	0	0	0	0	6
	%	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	2.0
Fusarium oxyspoium	*T. C*	0	0	0	0	0	0	0	0	0	3	3
	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0
Penicillum sp.	*T. C*	0	0	0	0	6	0.0	0.0	0.0	3	6	15
	%	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	1.0	2.0	5.0
Rhizopus sp.	*T. C*	0	3	27	24	42	27	15	57	42	63	300
	%	0.0	1.0	9.0	8.0	14.0	9.0	5.0	19.0	14.0	21.0	100.0
Total	*T. C*	0	3	21	6	3	3	3	45	3	12	123
	%	0.0	1.0	7.0	2.0	1.0	1.0	1.0	15.0	1.0	4.0	41.0

A : Aswan G: Giza M: Monofya SH: Sharkia N: Nobaria (Behera) T.C : Total count of fungi % : Fungal frequency occurred PDA : Potato dextrose agar

Fungal frequency contaminated peanut pod samples

Isolation from peanut pod samples which collected from the five Governorates i.e. Aswan, Giza, Shrkia, Monifya and Behera (Nobaria location) resulted 1400 fungal isolates, 1190 of these isolates (85%) using agar plate medium (PDA) in Table 3 and 210 isolates (15%) using the blotter test method in Table (4). Table (3) indicates that, five fungal genera were identified, namely Aspergillus, Epicoccum, Fusarium, Penicillium and Rhizopus (Fig 2.a, b&c). Aspergillus genus was the most fungal frequent and gave 13.5, 12.61, 5.9 and 3.4% with *A. parasiticus, A. niger, A. terrus* and *A. flavus*, respectively. Penicillium was moderately frequent, with 26.9%, followed by Fusarium which gave 9.2%, 6.7% and 2.5% with *Fusarium oxysporum, F. solani* and *Fusarium* spp., respectively. *Rhizopus* genus frequency was 16%, while *Epicoccum* sp. was less and gave 3.4% in all disinfected pods using agar plate (PDA) medium. On the other hand, the Aswan sample was less fungal contaminated than others and gave 14.3% of fungal frequency in disinfected pod sample with agar plate test. The Monofya sample gave higher fungal frequency contamination of

peanut pods and record 31.1%, followed by Sharkia and Giza pod samples, which gave 21.0 and 17.6%, respectively. The Behera (Nobaria location) sample was moderate, with a record of 16.0 %.

Table 3. Fungal frequency occurred with some peanut pod samples using Agar plate (PDA) which collected from different Governorates

Test Fungal isolate(s)		Agar plate (PDA) Disinfected					Total
		A	G	M	SH	N	
Aspergillus	*T.C*	20	30	70	30	0	150
niger	*%*	1.7	2.5	5.9	25	0.0	12.61
Aspergillus	*T.C*	20	20	40	40	40	160
parasiticus	*%*	1.7	1.7	3.4	3.4	3.4	13.5
Aspergillus	*T.C*	0	0	40	0	0	40
flavus	*%*	0.0	0.0	3.4	0.0	0.0	3.4
Aspergillus	*T.C*	10	0	20	40	0	70
terreus	*%*	0.8	0.0	137	3.4	0.0	5.9
Fusarium	*T.C*	0	0	60	0	50	110
oxyspoium	*%*	0.0	0.0	5.0	0.0	4.2	9.2
Fusarium	*T.C*	0	0	60	20	0	80
solani .	*%*	0.0	0.0	5.0	1.7	0.0	6.7
Fusarium sp.	*T.C*	0	0	30	0	0	30
	%	0.0	0.0	2.5	0.0	0.0	2.5
Penicillum sp.	*T.C*	70	60	30	100	60	320
	%	5.9	5.0	2.5	8.4	5.0	26.9
Epicoccum sp.	*T.C*	10	10	0	0	20	40
	%	0.8	0.8	0.0	0.0	1.7	3.4
Rhizopus sp.	*T.C*	40	90	20	20	20	190
	%	3.4	7.6	1.7	1.7	1.7	16.0
Total		170	210	370	250	190	1190
		14.3	17.6	31.1	21.0	16.0	100.0

A : Aswan G: Giza M: Monofya SH: Sharkia N: Nobaria (Behera) T.C : Total count of fungi % : Fungal frequency occurred PDA : Potato dextrose agar

Table 4 showed that isolation from disinfected peanut pod samples, by using the blotter test method resulted, 210 fungal isolates belonging to four fungal genera i.e. *Aspergillus, Fusarium, Penicillium* and *Rhizopus*. *Penicillium* sp was the most frequent and gave 33.33%, followed by *Fusariun solani* with 28.58% and *A. Flavus* which record 14.29%, whereas *A. parasiticus* was less frequent 4.76%. On the other hand, the Monifya disinfected peanut pod sample gave higher fungal frequency 71.43% followed by Giza with 19.05% while, Behera (Nobaria location) was not contaminated (zero%). Aswan and Sharkia pod samples were moderately contaminated with fungal frequency of 4.76%.

Table 4. Fungal frequency occurred with some peanut pod samples using Blotter test which collected from different Governorates

Test Fungal isolate(s)		Blotter test Disinfected					Total
		A	G	M	SH	N	
Aspergillus parasiticus	*T.C*	10	0	0	0	0	10
	%	4.8	0.0	0.0	0.0	0.0	4.76
Aspergillus flavus	*T.C*	0	0	30	0	0	30
	%	0.0	0.0	14.3	0.0	0.0	14.29
Fusarium oxysporum	*T.C*	0	0	20	0	0	20
	%	0.0	0.0	9.5	0.0	0.0	9.52
Fusarium solani .	*T.C*	0	0	50	10	0	60
	%	0.0	0.0	23.8	4.8	0.0	28.58
Penicillum sp.	*T.C*	0	20	50	0	0	70
	%	0.0	9.5	23.8	0.0	0.0	33.33
Rhizopus sp.	*T.C*	0	20	0	0	0	20
	%	0.0	9.5	0.0	0.0	0.0	9.52
Total	*T.C*	10	40	150	10	0	210
	%	4.76	19.05	71.43	4.76	0.0	100.00

A : Aswan G: Giza M: Monofya SH: Sharkia N: Nobaria (Behera) T.C : Total count of fungi % : Fungal frequency occurred PDA : Potato dextrose agar

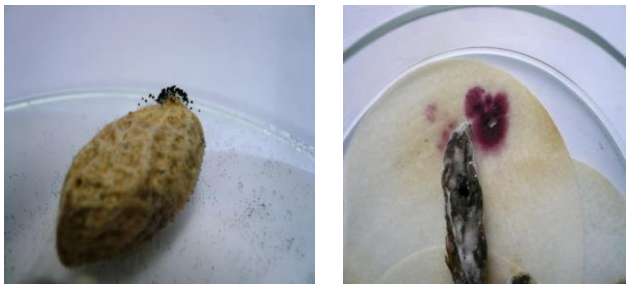

Fig. 2. a-*Aspergillus flavus and A. parasiticus* associated with disinfected peanut pod, Monofya sample, b-*Aspergillus niger* associated with disinfected peanut pod, Giza sample, c-Fusarium associated with disinfected peanut pod, Monofya sample.

Aflatoxin production

Aspergillus group i.e. *A. niger, A. flavus, A. parasiticus* and *A. terrus* was tested using thin layer chromatography (TLC). The results given in Table 5 indicated that, three isolates of *Aspergillus flavus* were positive producers of aflatoxins B_1 and B_2. The results showed that only one isolate of *A. parasiticus* which was isolated from Behera (Nobaria location) peanut seed samples gave positive reaction and produced B_1, B_2, G_1, and G_2 aflatoxins.

Table 5. Aflatoxin production

Aflatoxigenic fungi	AFB_1	AFB_2	AFG_1	AFG_2
Aspergillus flavus	+	+	-	-
A. niger	-	-	-	-
A. parasiticus	+	+	+	+
A. terrus	-	-	-	-

AF: Aflatoxin

The identification of aflatoxions could easily be deduced from the constant retention time compared with the standard spiked in the HPLC chromatogram (Fig 3 a & b). The HPLC chromatogram for standard aflatoxins (ST) showing that AFG1 was elucated at 7.88 min., AFB1 at 10.33 min., AFG2 at 13.66 min. and AFB2 evaluated at 19.91 min. (Fig 1a). But, the HPLC chromatogram for aflatoxins extracted from peanut sample show that, AFG1 was elucated at 7.92 min., AFB1 at 10. 28min., AFG2 at 13.68 min. and AFB2 evaluated at 20.33 min. as shown as (Fig 3.b).

The results given in Table 6 showed the determination of aflatoxins B_1, B_2, G_1 and G_2 produced by *A. parasiticus* which was isolated from Behera (Nobaria location) peanut seeds after being inoculated artificially and incubated for two weeks at 25 ± 2 ℃. Aflatoxin B1 was found in concentration of 01.7 µg/g (172.0 µg/kg), aflatoxin B2 was found in concentration of 0.418 µg/g (418.38 µg /kg) , aflatoxin G1 was found in concentration of 1.35 µg/g (1358.7 µg/kg), aflatoxin G2 was found in concentration of 0.364 µg/g (364.6 µg/kg). The type of aflatoxin and quantity of aflatoxin µg/g and µg/kg are show in Fig 4.a/g & b/kg.

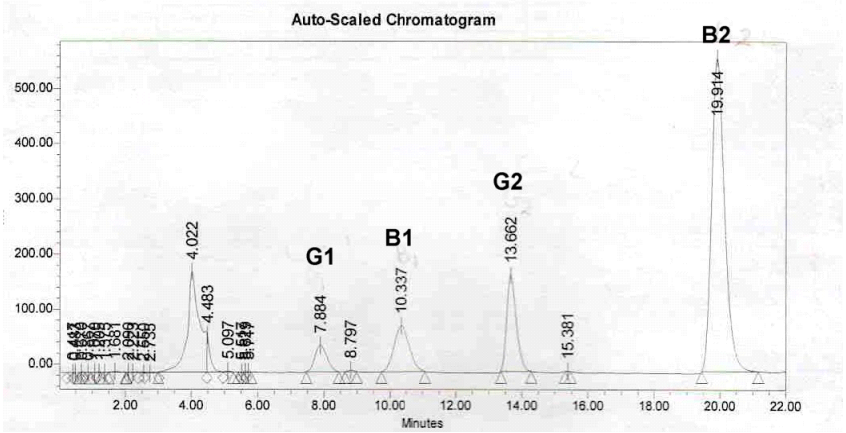

Fig. 3. a) HPLC Chromatogram for aflatoxins ST. Showing that AFG1 elucated at 7.88 min., AFB1 elucated at 10.33 min., AFG2 elucated at 13.66 min. and AFB2 evaluate at 19.91 min.

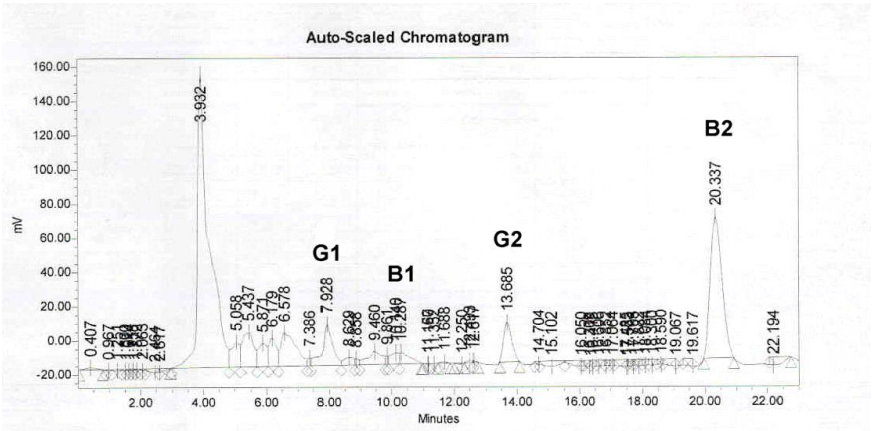

Fig. 3. b) HPLC Chromatogram for aflatoxins extracted from peanut samples showing that AFG1 elucated at 7.92 min., AFB1 elucated at 10.28 min., AFG2 elucated at 13.68 min. and AFB2 evaluate at 20.33 min.

Table 6. Quantitively of aflatoxins µg/kg

Quantity of aflatoxin	Type of aflatoxin				Total
	AFG1	AFG2	AFB1	AFB2	
µg/g	1.35	0.364	0.17	0.418	2.302
µg/kg	358.7	364.6	172	418.38	2313.68

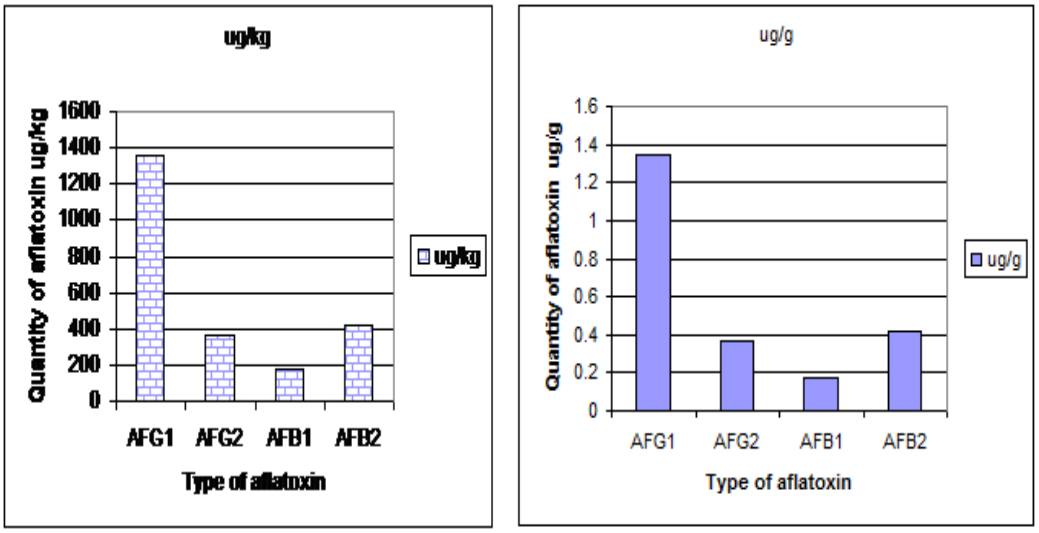

Fig. 4. a/g & b/kg) Type of aflatoxin.

Discussion

Mycoflora associated with peanut seed samples (*Arachis hypogaea* L.) collected from five different Governorates in Egypt namely Aswan, Giza, Behera (Nobaria location), Monofya and Sharkia resulted 1000 fungal isolates belonging to four fungal genera i.e. *Aspergillus, Fusarium, Penicillium* and *Rhizopus.* Agar plate (PDA) medium was an enhanced method for seed health testing than, the blotter test method, and gave higher numbers of fungal colony Embaby and Mona 2006 and Embaby, et.al. 2008. The Behera (Nobaria location) sample was highly contaminated with fungi, whereas the Aswan sample was less contaminated than others. Aspergillus genus was the most frequent and recorded 40.71% of *A. niger*, 34.29% of *A. parasiticus*, 5.58% of *A. flavus* and 0.43% of *A. terreus. Rhizopus* was moderate and gave 6.85% followed by *Penicillium* 8.14%, while, *Fusarium oxysporum* was less frequent (4%). Disinfected seeds were less fungal contaminated than the non-disinfected ones. Aswan sample was less fungal contaminated than others, and gave 2.14% while Sharkia sample was highly contaminated, followed by Behera (Nobaria) sample and gave 12.0 and 10.71% of fungal frequency respectively. On the other hand, contaminated peanut pods yielded 1400 fungal isolates belonging to five fungal genera i.e. *Aspergillus, Fusarium, Penicillium* and *Rhizopus. Aspergillus* was the most fungal frequency occurred and gave 13.5, 12.61, 5.9 and 3.4% with *A. parasiticus, A. niger, A. terrus* and *A. flavus* respectively. *Penicillium* was moderate fungal frequency with 26.9% followed by Fusarium which gave 9.2%, 6.7% and 2.5% with *Fusarium oxysporum, F. solani* and *Fusarium* spp. respectively followed by *Rhizopus genus* 16%, while *Epicoccum* sp. was less fungal frequency occurred which gave 3.4% in all disinfected pods using agar plate (PDA) medium. On the other hand, Aswan sample was less fungal contaminated than others and gave 14.3% of fungal frequency in disinfected pod sample with agar plate test. Monofya governorate sample gave higher fungal frequency contaminated of peanut pods which record 31.1% followed by Sharkia and Giza pod samples which gave 21.0 and 17.6% respectively while, Behera (Nobaria location) was moderate which record 16.0. *Epiccocum* sp. was less fungal frequency occurred. Similar results were obtained by Mohamed 2004 and Vivian *et al* (2008).

Three isolates of *A. flavus* gave positive reaction with AFB_1 and AFB_2, while only one isolate of *A. parasiticus* produced AFB_1, AFB_2, AFG_1 and AFG_2 which record 172, 418.38, 1358.7 and 364.6 µg/kg respectively. Similar data were also obtained by Gong *et al* (2003), Saleemullah, *et. al.* (2006), Carlos, *et. al.* (2009) and Duran, *et. al*. (2009).

References

Abdel-Wahhab, M. A., Sehab, A. F., Hassanien, F. R., El-Nemr, Sh. E., Amra, H. A. and Abdel-Alim, H. (2011). Efficacy of ozone to reduce fungal spoilage and aflatoxin contamination in peanuts. International Journal of Nuts and Related Sciences 2:01-14.

Adiver, S. S. and Anahosur, K. H. (2002). Pod rot of groundnut Caused by Fusarium. Indian Phytopathology 55:315-318.

AOAC, (2007). Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International 17th edition. Nature Toxins. AOAC International, Arlington, Virginia, USA. pp. 49.

CAPMAS, (2006). Central Agency for Public Mobilization and Statistic .Cairo, Egypt. Retrieved form www.msrintranet.capmas.gov.eg/pls/fdl.

Carlos, A. F., Natalia, B., Roice, E. and Andrezza, M. (2009). Determination of aflatoxins in peanut products in the northeast region of Sao, Brazil. International Journal of Molecular Sciences 10:174-183.

Duran, R. M., Cary, J. W. and Calvo, A. M. (2009). The role of vea in *Aspergillus flavus* infection of peanut, corn and cotton. The Open Mucology Journal 3:27-36.

Emara, M. F., Hagar, A. A. A., El-Deeb, A. A. and Hamza, A. S. (2003). Detection of aflatoxin associated with Aspergillus spp. In some peanut growing areas in Egypt. Journal of Agricultural Science Mansoura University 28:8063-8072.

Embaby, E. M. and Abdel–Galil, M. M. (2006). Seed borne fungi and mycotoxins associated with some legume seeds in Egypt. Journal of Applied Sciences Research 2:1064-1071.

Embaby, E. M., Swelium, M. A., Hazaa, M. M., Abdel–whhab, M. A. and El-Shahat, E. A. (2008). Effect of *Aquilegia vulgaris* L. (Columbine) extract on *Aspergillus flavus* contaminated corn grains (*Zea maize* L.) in Egypt. Egyptian Journal of Applied Sciences 23:62-73.

FAO. (2011). FAO Achievements in Egypt. Retrieved form http://neareast.fao.org/-FCKupload/File/acheg.pdf.

Gong, Y., Egal, S., Hounsa, A., Turner, P., Hall, A., Cardwell, K. and Wild, C. (2003). Determinants of aflatoxin exposure in young children from Benin and TogoWest Africa: the critical role of weaning. International Journal of Epidemiology 32:556–562.

Han, D., Macdonald, S. J., Boughtflower, V. and Brereton, P. (2004). Simultaneous determination of aflatoxins and ochratoxin A in food using a fuully automated immunoaffinity column clean up and liquid chromatography-fluorecence detection. Journal of chromatography A 1059: 13-16.

Mahmoud, E. E. Y. (2004). Integrated controls of pod rot diseases of peanut. (Doctor of Philosophy's Thesis). Ain Shams University.

Novas, V. and Cabral, D. (2002). Association of mycotoxin and sclerotia production with compatibility groups in Aspergillus Flavus from Argentina. Plant Disease 86:215-219.

Ogido, R., Oliveira, C. A. F., Ledoux, D. R., Rottinghaus, G. E., Correa, B., Butkeraitis, P, Reis, T. A., Gonçales, E. and Albuquerque, R. (2004). Effects of prolonged administration of aflatoxin B1 and Fuminsin B1 in Laying Japanese quail. Poultry science 83:1953-1958.

Olaru, R., Vasile, A. and Ivanescu, M. (2008). Determination of total aflatoxins in non-animal food stuffs. Revista Romana de Medicina Veterinara 18:144-153.

Pitt, J. I., Hocking, A. D., Bhudhasama, K., Miscamble, B. F., Wheeler, K. A. and Tanboon-Ek, P., (1993). The normal mycoflora of commodities from Thailand. 1. Nuts and oilseeds. International Journal of Food Microbiology 20:211-226.

Shah, H., Iqbal, A. and Khalil, I. A. (2006). Aflatoxin contents of stored and artificially inoculated cereals and nuts. Food Chemistry 98:699–703.

Viviane, K. N., de Oliveira Rocha, L., Gonçalez, E., Fonseca, H., Ortega, E. M. M. and Corrêa, B. (*2008*). Distribution of fungi and aflatoxins in a stored peanut variety. Food Chemistry 106:285-290.

Xue, H. Q., Isleib, T. G., Payne, G. A., Wilson, R. F. and Novitzky, W. P. (2003). Comparison of aflatoxin production in normal and lugh-oleic backcross-derived peanut lines. Plant Disease 87:1360-1365.

Yousef, S. and Magan, N. (2010). Mycotoxigenic fungi in peanuts from different geographic regions of Egypt. Mycotoxin Research 26:133-140.

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