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DETECTION OF AFLATOXINS ASSOCIATED WITH *Aspergillus spp* IN SOME PEANUT GROWING AREAS IN EGYPT

Emara, M.F.*;A. A. Abo Hagar*; A. A. El Deeb and Akila S. Hamza***
*** Central Laboratory for Food & Feed. Agric. Res. Cent. Giza.**
**** Plant Pathology Res. Inst. Agric. Res. Cent. Giza.**

ABSTRACT

Peanut seed samples collected from different localities in Ismailia and El-Sharkia governorate in the two seasons, 1999 and 2000 showed that the percentages of contamination with aflatoxin were 17% and 11.4% in the Ismailia governorate, while the percentages in El-Sharkia governorate were 18.4% and 4% in 1999&2000 seasons.

The highest percentages of seed contaminated with the yellow mold were obtained from peanut samples presenting Ismailia (west Kantara locality) followed by El-Sharkia governorate (Belbies locality), 68.3%&48,5%in 1999 season. Seed presenting the 2000 season which Abou- Hamaad (El- Sharkia) and Faied (Ismailia) localities recorded the mold at 43.8% and 37.3% respectively. The common isolated pathogenic fungi from seeds in the governorate was *Aspergillus flavus* followed by *A. parasiticus*, while *A. ochraceus* was the lowest in this respect. The highest rate of *A. flavus* frequency was showed in peanut seeds collected from Ismailia followed by El- Sharkia governorate in the first season, while the opposite results were obtained in the second season. Also, the frequency in *A. flavus* presence was higher in the seeds than obtained in the peanut shells of samples collected from the governorate in the two seasons.

The highest percentage and frequencies of *A. flavus* and *A. parasiticus* were recorded in peanut samples contaminated with high level of aflatoxins. Also, all contaminant samples with aflatoxins were found infected with *A. flavus* and/or *A. parasiticus*. Data obtained here indicated that East Kantara and El-korain in Ismailia and El-Sharkia governorate, respectively, were free from contamination with aflatoxins in the two successive seasons of the investigation

Keywords: Aflatoxins, Peanut, *Aspergillus flavus* , *A. parasiticus, A. ochraceus*

INTRODUCTION

Aflatoxins are carcinogenic metabolites produced by several members of the *Aspergillus flavus* group in grains and foods (*Shapira et al., 1996*). *A. parasiticus* Speare, generally produces Aflatoxins, B_1,B_2,$G_1$and G_2 (*Delucca et al., 1987*). *Ellis et al. (1994)* revealed that aflatoxin B_1 production by *A. parasiticus* was detected at levels greater than the regulatory limit which is 20 ng g^{-1} in peanuts at 20 °C and 25 °C (52.95ng g^{-1} Max.). Aflatoxins the serious problem of groundnut in many parts of the world are secondary metabolites formed by toxigenic strains of *Aspergillus flavus* and *A. parasiticus* . These compounds are only a few of over 120 mycotoxins produced by fungi (*Nichols, 1983*). The term aflatoxins include four compounds of bis-furancoumarin metabolites produced by certain strains of *A. flavus* and *A. parasiticus*, named B_1,B_2,$G_1$and G_2, which occur naturally in plant products. The contamination of peanut with aflatoxins was high in

samples showed highest percentage of infectious diseases and pathogenic fungi frequencies (*Hassan et al., 2002).*

This paper reports the occurrence of yellow Mold of peanut, and evaluate the presence of aflatoxin contamination in peanut samples collected from Ismailia and El-Sharkia governorates.

MATERIALS AND METHODS

Analysis of peanut samples for detection of aflatoxin:-

The extraction of aflatoxins was conducted according to *A.O.A.C (1998)*. The samples were blended with 250ml methanol –water (55:45,v/v) and 100ml hexane for 1min. at high speed. The mixture was transferred to the centrifuge tube and centrifuged for 5 min. At 2000 rpm and an aliquot from the aqueous methanol phase (25 ml) was taken into a separator contained chloroform. The separator was shaken (30–60 sec.), the bottom layer (chloroform) was separated and concentrated using rotary evaporator. The residue was quantitatively transferred using small volumes of chloroform. The solvent was completely removed under nitrogen.

Determination of aflatoxin:-

Aflatoxins were determined using thin layer chromatographic technique as follows:-

The dried film representing the aflatoxins in the samples was dissolved in a known amount of chloroform. The aflatoxin standards were spotted along with the samples. The plates were developed using a mixture of acetone – chloroform (1:9,v/v). the chromatoplates were detected under UV lamp at 365nm, the concentration of aflatoxin were calculated using the formula:-

$\mu g/Kg = (S.Y.V.) / (X.W)$

Where:

S= μL of standard to unknown.

Y= concentration of aflatoxin standard in μg/ml.

V= μL of final dilution of sample extract.

X=μL sample extract giving a spot intensity equal to S.

W= mass of the sample, represented by the final extract in g.

Determination of yellow rot diseases of peanut percentage:-

Peanut samples were collected from Ismailia and El-Sharkia governorates during 1999 and 2000 seasons. These samples were examined and divided into diseased pods and apparently healthy pods. Diseased pods showed different degrees of discoloration ranging from superficial russiting to complete yellowing or yellow-greening of the hulls as well as various stages of hull and seed decay. Randomized samples of 100 pods were replicated four times in each, and investigated to determine percentage of yellow pod rot disease of peanut.

Isolation and determination the frequencies of the isolation pathogenic fungi:-

Fungi associated with the samples of peanut pods were isolated according to Garren and Porter (1970). Ten-seeded fruits were shelled then 1 cm^2 pieces of shell and seed with intact tests were surfaced. Disinfected for 3 minutes in 0.5%Nacl and planted on potato dextrose agar (PDA) and plain agar medium (5/plate in 5 replicates). Plates were examined after 7 days incubation at 30 °C for fungal structures. Fungi associated with peanut samples were recorded on both seeds and shells. Reading were expressed as isolation frequencies (i.e., No. of infected seeds with particular fungus whole sample of 100 seeds) according to *Marei (2000)*.

RESULTS AND DISCUSSION

Peanut samples were collected from different localities of Ismailia and El-Sharkia governorates in two seasons, 1999 and 2000, respectively, as shown in Table (1) and(2). The percentage of contamination of aflatoxin were 17.0% and 11.42% in the two seasons for Ismailia governorate. While the percentages in El-Sharkia governorate were 18.42% and 4.0% in 1999 and 2000 seasons. Also, the collected samples from East Kantra, Abou-Sowier, Ismailia governorate and El-Korain, Sharkia governorate were free from aflatoxin in the two seasons. On the other hand, the highest percentage of aflatoxin were found in samples collected from West Kantara and Faied, Ismailia governorate, and Abou-Hammad and Belbies, Sharkia governorate in 1999 and 2000 seasons. *Kannaiyan et al., (1989)* analyzed 28410 samples of groundnut for aflatoxin contamination and found that 6.3% of samples. Seed health testing of these samples contain 75 µg/kg aflatoxin. These samples were rejected for export revealed that all were contamination with *A. flavus*.

Results in Tables (3) and (4) indicate that the percentage of yellow Mold of peanut was higher in the samples having high level of aflatoxin. Similar results were obtained by *Wilson and Jay (1976)*, *Quitco et al. (1989)*. *Youssef et al. (1999)*, *Marei (2000)* and *Hassan et al. (2002)*, who found that the percentage of yellow Mold of peanut (*A. flavus* and *A. parasiticus*) were higher in peanut samples having higher aflatoxin contamination. This result is in-agreement with those obtained by *Faar et al. (1989)* who mentioned that *A. flavus*, *A. parasiticus* are preharvest pathogens of peanuts and several tree nut crops. Aflatoxin metabolites of *A. flavus* were investigated in groundnut meal for the presence of aflatoxin B_1 in 2192 samples, B_2 in 1158 samples, and G_1 and G_2 in 603 samples each. The mean of the contamination by aflatoxin B_1 was 266 µg/kg, by B_2 it was 22, G_1 3.2 and G_2 0.7 µg/kg. The maximum yield for aflatoxin B_1 was 10100 µg/kg, for B_2 it was 450, G_1 130 and G_2 30 µg/kg. Aflatoxins B_2, G_1 and G_2 accounted for 9.3% of the total aflatoxin and their toxicity was equal to 2% of aflatoxin B_1 (*Strszelecki et al., 1900)*.

Table (1): Incidence of aflatoxin in peanut samples and its contamination in some localities of Ismailia and Sharkia governorates in 1999 season.

Governorate	localities	No. of Samples	Total samples contaminated with aflatoxin	%	Main toxin found	Level of aflatoxin (ppb)		
						Shell	Seed	
							B_1	B_2
Ismailia	Ismailia	45	4.0	8.88	B_1-B_2	0.0	3.6-138-141-304	0.0-10.1-46-47
	Faied	14	3.0	21.43	B_1-B_2	0.0	21-105-3960	7-35-1320
	West Kantara	15	7.0	46.66	B_1-B_2	0.0	3-3.2-300-396-504-570-770	0.0-0.0-56-100-132-190-260
	East Kantara	6	-ve	0.0		0.0	0.0	0.0
	Etal- El- Kabier	11	3.0	27.27	B_1-B_2	0.0	48-65-570	16-0.0-190
	Abou-Sowier	9	-ve	0.0		0.0	0.0	0.0
	Total	100	17.0	17.0	B_1-B_2			
Sharkia	Belbies	10	1.0	10.0	B_1-B_2	0.0	204	68
	Abo-Hammad	13	4.0	30.76	B_1-B_2	0.0	24-171-780-1200	57- 80- 260- 400
	El-Korain	6	-ve	0.0		0.0	0.0	0.0
	Fakous	7	1.0	14.28	B_1-B_2	0.0	15	5
	El-Hossinea	2	1.0	50.0	B_1-B_2	0.0	108	36
	Total	38	7.0	18.42				
Total No. from the two Governorate		138	24.0	17.39				

Table (2): Incidence of aflatoxin in peanut samples and its contamination in some localities of Ismailia and Sharkia governorates in 2000 season.

Governorate	localities	No. of Samples	Total samples contaminated with aflatoxin	%	Main toxin found	Level of aflatoxin (ppb)		
						Shell	Seed	
							B_1	B_2
Ismailia	Ismailia	30	-ve	0.0	B_1-B_2	0.0	0.0	0.0
	Faied	40	7.0	17.0	B_1-B_2	0.0	75-96-100-110-217-280-3150	0.0-0.0-41-0.0-0.0- 89-1050
	West Kantara	17	5.0	29.41	B_1-B_2	0.0	1.2-107-185-225-1078	0.0-69-0.0-48-294
	East Kantara	18	-ve	0.0		0.0	0.0	0.0
	Etal- El- Kabier	16	4.0	25.0	B_1-B_2	0.0	65-290-1020-1070	0.0-303-0.0-160
	Abou-Sowier	19	-ve	0.0		0.0	0.0	0.0
	Total	140	16.0	11.42				
Sharkia	Belbies	13	1.0	7.69	B_1	138	174	0.0
	Abou-Hammad	22	2.0	9.09	B_1-B_2	0.0	51-56	0.0-64
	El-Korain	15	-ve	0.0		0.0	0.0	0.0
	Fakous	7	-ve	0.0		0.0	0.0	0.0
	El-Hossinea	18	-ve	0.0		0.0	0.0	0.0
	Total	75	3.0	4.0				
Total No. from the two Governorate		215	19.0	8.83				

Table(3): Average of yellow Mold disease of peanut and frequencies of the isolated pathogenic fungi (*Aspergillus spp.*) from shells and seeds of peanut samples collected from Ismailia and Sharkia governorates in 1 999 season.

Governorate	localities	No. of Samples	% of yellow pod rod	Frequencies percentage of isolated pathogenic fungi									
				A. flavus			*A. parasiticus*			*A. ochraceus*			
				On shells	On seeds	Mean	On shells	On seeds	Mean	On shells	On seeds	Mean	
Ismailia	Ismailia	45	3.9	13.8	20.8	17.3	5.8	10.6	8.2	0.0	0.0	0.0	[illegible]8.5
	Faied	14	45.8	30.8	58.7	44.75	30.2	37.8	34.0	0.0	12.8	6.4	2[illegible]3.38
	West Kantara	15	68.3	32.7	83.9	58.3	29.8	43.6	36.7	0.0	10.5	5.25	3[illegible]3.42
	East Kantara	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	[illegible].0
	Etal- El- Kabier	11	10.5	19.2	32.8	26.0	10.7	22.3	16.5	0.0	4.6	2.3	1[illegible]1.93
	Abou-Sowier	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	[illegible].0
	Total	100	21.53	16.08	32.7	24.65	12.75	19.05	15.9	0.0	4.65	2.33	1[illegible].29
Sharkia	Belbies	10	18.5	17.7	26.8	22.25	12.8	17.8	15.3	0.0	10.7	5.35	17[illegible].47
	Abou-Hammad	13	48.5	22.9	40.6	31.75	10.0	18.2	14.1	0.0	12.3	6.15	15[illegible].16
	El-Korain	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0[illegible].0
	Fakous	7	16.3	10.8	25.0	17.90	0.0	10.8	5.4	0.0	0.0	0.0	7. 77
	El-Hossinea	2	20.7	0.0	46.7	23.35	10.0	20.6	15.3	0.0	10.2	5.1	14 .58
	Total	38	20.20	10.28	27.82	19.05	6.65	13.48	10.02	0.0	6.64	3.32	10 .99
Total No. of all samples		138	20.87	13.18	27.94	--	9.66	16.27	--	0.0	5.65	--	—

Table(4): Average of yellow Mold disease of peanut and frequencies of the isolated pathogenic fungi (*Aspergillus spp.*) from shells and seeds of peanut samples collected from Ismailia and Sharkia governorates in 2000 season.

Governorate	localities	No. of Samples	% of yellow pod rod	Frequencies percentage of isolated pathogenic fungi									
				A. flavus			*A. parasiticus*			*A. ochraceus*			
				On shells	On seeds	Mean	On shells	On seeds	Mean	On shells	On seeds	Mean	
Ismailia	Ismailia	30	6.7	10.0	30.0	20.0	0.0	22.0	11.0	0.0	16.8	8.4	13.13
	Faied	40	37.8	15.0	25.0	20.0	12.6	18.4	15.5	0.0	10.0	5.0	13.5
	West Kantara	17	18.3	0.0	22.8	11.4	0.0	30.8	15.4	0.0	5.0	2.5	9.77
	East Kantara	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Etal- El- Kabier	16	12.7	10.5	26.3	18.4	13.7	17.3	15.5	0.0	12.8	6.4	13.43
	Abou-Sowier	19	0.0	0.0	10.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	1.67
	Total	140	12.58	5.92	19.02	12.47	4.38	14.75	9.57	0.0	7.43	3.72	8.58
Sharkia	Belbies	13	37.6	18.8	40.6	29.7	0.0	32.4	16.2	0.0	0.0	0.0	15.30
	Abou-Hammad	22	43.8	20.0	46.80	33.4	14.8	38.6	26.7	0.0	10.0	5.0	21.70
	El-Korain	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Fakous	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	5.0	1.7
	El-Hossinea	18	32.8	0.0	30.8	15.4	0.0	12.8	6.4	0.0	0.0	0.0	7.27
	Total	75	19.03	6.47	23.64	15.7	2.96	16.67	9.82	0.0	4.80	2.47	8.97
Total No. of all samples		190	15.81	6.84	21.33	--	3.67	15.71	--	0.0	6.12	--	--

Data presented in Tables (3) and (4) indicated that the percentages of yellow Mold of peanut, the frequencies of aflatoxigenic fungi *A. flavus* and *A. parasiticus* and the contamination with aflatoxin were higher in seeds than shells. Similar results were obtained by a number of investigators, *Jackson (1965), Dienner et al. (1966),* and *Porter and Garren(1968)* who reported that 7% of sound seeds of freshly dug peanut fruit were contaminated with toxigenic strains of *A. flavus* while 3% contaminated with other species of Aspergillus. They added also, that *A. flavus* was found at 6% in young immature peanut fruits and thought that the fungus invade and establish in growing geocarp. *A. flavus* was found on about 1% of shells and on 5% of seeds. *Ito et al. (1992)* found that 27.5% of forty isolates of *Aspergillus spp.* Obtained from seed produced aflatoxins.

Results in Tables 1,2,3 and 4 showed that the percentage of yellow Mold disease of peanut, and the frequencies of contaminated peanut with aflatoxin were higher in the samples collected from localities of Ismailia than the collected samples from El-Sharkia governorate in 1999 and 2000 seasons. These results are in-agreement with those obtained by *Wilson and Bell (1984), Chen et al. (1993), Youssef (1999)* and *Marei (2000)* who reported that infection with the pathogenic fungi appeared in the field before harvest and increased in Ismailia than South Tahrir. This may be due to that the peanut are cultivated in Ismailia for several years while the inoculum density increased year after year causing higher infection with the yellow Mold. *Wyk et al. (1998)* indicated that *A. flavus* was isolated from groundnut seeds collected from 15 depots in the groundnut-producing areas of South Africa. All samples were contaminated with *A. flavus* and the contamination was ranging from 2.0 to 53,8% of seeds.

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الكشف عن الأفلاتوكسينات المصاحبة لفطر الأسبرجلس فـــي الفـــول الســـوداني المنزرع في بعض مناطق نموه بمصر

محمد فهمي عماره *، أمل عبد العزيز أبوحجر*، عبد الرحمن عبد اللطيف الديـــب ** و عقلية حمزة *

*** المعمل المركزي الأغذية والأعلاف**

**** معهد بحوث أمراض النباتات – مركز البحوث الزراعية – جيزة**

جمعت عينات من بذور فول سوداني لتمثل مناطق زراعته في محافظتي الإســماعيلية و الشرقية وذلك على مدار موسمي ١٩٩٩&٢٠٠٠ و قد وجد أن نسبة الإصابة في البذور الممثلـــة لمحافظة الإسماعيلية هي ١٧% ، ١١,٤% و في محافظة الشرقية ١٨,٤% , ٤% على التوالي. وجد أن أعلى نسبة أصابه بمرض عفن ثمار الفول السوداني الأصفر خلال موسم ١٩٩٩ كانت في العينات التي تم تجميعها من محافظة الإسماعيلية (منطقة قنطرة شرق) يليـــها محافظـــة الشـــرقية (منطقة بلبيس) حيث كانت النسبة ٦٨,٣% , ٤٨,٥%على التوالي . أما في موسم ٢٠٠٠ فكـــانت نسبه الإصابة في البذور المجمعة من مناطق أبو حماد (محافظـــة الشـــرقية) و فـــايد (محافظـــة الإسماعيلية) ٤٣,٨% , ٣٧,٣% على التوالي.

ظهرت أعلى اصابه بالفطرين *Aspergillus flavus, A. parasiticus* على بذور الفول السوداني الناتجة من المحافظتين أما الفطر *A. ochraceus* فكان أقلهم تواجـــدا. كما لوحظ أن أعلى معدل تكرار للإصابة بفطر *A. flavus* على بذور الفول السوداني التي تـــم تجميعها من محافظة الإسماعيلية يليها محافظة الشرقية و ذلك خلال الموسم الأول بينمـــا كـــانت النتائج عكسية خلال الموسم الثاني. أيضا أوضحت النتائج أن أعلى تكرار لظـــهور الفطـــر *A. flavus* كان على البذور عنه على قشرة ثمار الفول السوداني.

و قد سجلت الدراسة وجود علاقة طرديه بين تكرار ظهور الفطريـــن *Aspergillus flavus, A. parasiticus* و نسبة وجود الأفلاتوكسينات في عينات الفول السوداني وقد تأكد من النتائج أيضا أصابه كل العينات الملوثة بالأفلاتوكسينات بالفطرين .كمـــا أظـــهرت النتـــائج أن مناطق شرق القنطرة و القورين في محافظتي الإسماعيلية و الشرقية كانت خالية مـــن الإصابـــة بالأفلاتوكسين و ذلك خلال موسمي الدراسة.