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CHEMICAL AND MYCOTOXICOLOGICAL STUDIES ON EGYPTIAN COTTON SEEDS AND THEIR MEALS *"Gossypium barbaduse"*

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ABSTRACT

Six cotton cultivars represent the two Egyptian cotton categories, i.e. extra long staple length (Giza 45, Giza 70 and Giza 86) and long staple (Giza 85, Giza 80 and Giza 83) were used in this study. It was found that, the whole seed of Giza 70 attained the highest content for each moisture (7.01%), protein (23.0%) gossypol (0.45%) and Aflatoxins (10.04) ug/kg. Meanwhile, Giza 45 had the highest content of oil (24.5%), and Giza 83 showed the highest content of ash (5.89%). Giza 86 recorded the lowest content of Aflatoxins (4.73 ug/kg) and total fungal count. On the other hand, cotton seed meal of Giza 70 gave the highest content for each moisture (5.9%), and ash (6%), while the meal of Giza 85 had the highest content of oil (5.24%).

INTRODUCTION

Cotton seeds are the second best potential source of plant proteins after soybean, and the fifth best oil producing plant after soybean, palm tree, colza, and sunflower (Texier, 1993). There is a mounting interest in cotton seed quality due to the worlds' demand for food, especially protein and oil. Egypt produces as much as 389.000 tons of cotton seeds annually. Improving the productivity of cultivars should increase such amount. Shortage of about a million tons of edible oil (Turner *et al.*1976).

Anthony (1998) mentioned that, for many materials, water activity is an important property for safety. He predicted food safety and stability with respect to microbial growth, chemical / biochemical reaction rates, and physical properties.

The major components in mechanical extracted cotton seed meal have been found to be 92.3% dry matter, 46.1% crude protein, 18.1% fiber, 32.3% neutral detergent fiber, 11.4% crude fiber and 7.2% ash (Mohamed, 2001).

Substantial economic losses of foods and feeds usually occur due to their deterioration by molds. In addition spoilage of stored crops is often accompanied by the formation of mycotoxins. Mycotoxins are a potential threat to both human and animal health, (Abdel-Mallek *et al.*, 1994).

Aflatoxin, are commonly found mycotoxin in agricultural commodities, is known to be the most potent toxin and a powerful carcinogen for man and animals (Linsel, 1982). He also added that Ochratoxin A, another important mycotoxin, is produced by *Aspergillus ochraceus* and some others, as *Aspergillus* and *Penicillium* species, Ochratoxin A is a nephrotoxin and hepatotoxic mycotoxin. Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus* strains, which are both widespread in nature. The occurrence of

aflatoxins were found to be more common in oil seeds and cereals than horticultural crops (Anonym, 1979).

In the present study, the meals and seeds of the major Egyptian cotton seeds Giza (45, 70, 86, 85, 80, 83), were investigated for their major chemical composition, fungi associated and natural mycotoxins contamination.

MATERIALS AND METHODS

Materials:

Pure aflatoxins (B_1, B_2, G_1 and G_2), ochratoxin A and zeralenone were purchased from Sigma, and Chemical Company P. 0 Box 4508, St. Louis, U.S.A.

Aspergillus parasiticus NRRL 2999, *A. Ochraceus* NRRL 3174 and *Furarium moniliforme* KJ 4353 were obtained from Standard Association of Australia 80 Arthur St., North Sydney, NSW.

The investigated six forementioned Egyptian cotton seeds were extracted by the Local Oil Mill in Khan-Elkhalily to get cotton seeds-oil and their meals.

Methods:

The chemical compositions of whole cotton seed or for cotton seed meal (oil, protein and moisture content (%)).The peroxide value was determined from different cotton seed oil samples were measured according to A.O.A.C. (2000), while gossypol content (%) was measured according to Smith (1958). Ash content (%) was determined according to (Mohamed 2001).

The water activity (a_w) was measured at 35°C with equilibrium moisture absorption of microcrystalline cellulose and standard curve (Vos and Lobuza, 1974 and A.O.A.C. 2000).

The myxotoxins (Aflatoxins, Ochratoxin A and zearalenone) were extracted and determined according to method A.O.A.C. (2000). Fifty grams of each grind whole seeds sample were extracted by acetone – H_2O (85 + 15) for determination of aflatoxins. Other fifty grams were extracted by 25 ml 0.1 M H_3PO_4 and 250 ml $CHCl_3$ for oxhratoxin A. Fifty grames plus 25g diat earth and 20 ml H_2O, plus 250 ml $CHCl_3$ were used for determination of zearalenone. Compare fluorescent intensities of mycotoxins in sample with the standard spots by destometric analysis on thin layer chromatography (TLC) plate (silica gel 60. without fluorescent indicator, Merck) and HPLC (high performance liquid chromatography) were carried out. A direct competitive enzyme-linked immunosorbent assay (ELISA) screening method for aflatoxins at 20 µg/g was studied (Trucksess *et al.*, 1989).

Determination of fungi:

100 seeds of each investigated sample were devided to two groups. First group was devided to five samples, each was placed on potato Dextrose Agar (APD) (Difco) with 30 mg/l of chlorotetracycline as antibacterial agent. Sample was added just before pouring the dishes, which were incubated at

25°C for 5-8 days. The other 50 seeds of each cotton seeds under observation were taken, superficially sterilized with a solution of 2% sodium hypochlorite for 2 min, and subsequently washed three times with sterile distilled water (Jimanez *et al.*, 1991). Species of *Aspergillus, Penicillium, Fusarium, Alternaria* and *Mucorales*, were identified according to Nelson *et al.*, (1983) and Pitt and Hocking, (1985).

The isolated aflatoxins producing strains of *A. parasiticus* as well as ochratoxin A producing strains of *A. ochraceus* and *Fusarium moniliform* strains which produce zeralenone toxin from the six Egyptian cotton seeds and their meals were cultured on Glucose Yeast Extract Sucrose (GYES) broth media at 28°C for 15 days according to Cutuli, *et al.*, (1991).

Aflatoxins (B1, B2, G1 and G2) were produced by growing a toxigenic *A. parasiticus*. Ochratoxin A was produced by growing *A. ochraceus* and zearalenone by *Fusarium moniliforme* on cotton seed and its meal for 15 days at 30°C and assayed for toxin according to Luca *et al.*, (1989); El-Naghy *et* al, (1991) and Cotty, (1994).

10, 15, 20 and 25 ml from each cotton seeds oil were put in 100 ml Czapek's-Dox Agar media for the production of aflatoxins, Ochratoxin A and zeralenone by fungi *Aspergillus flavus* , *Aspergillus ochracus* and *Fusarium moniliform*, respectively each at 28°C for 15 days, (Garber and Cotty 1995).

RESULTS AND DISCUSSION

(1) Chemical constituents of whole cotton seeds and their meals:

Data in Table (1) illustrate the average contents of main chemical constituents for cotton seeds and their meals. The moisture content of whole Egyptian cotton seed varieties ranged from 5.26 to 7.01 %, oil ranged from 20.0 to 24.5 %, protein from 20.8 to 23.0 %, gossypol from 0.32 to 0.45 % and ash from 4.32 to 5.89 %.

Table (1): Contents of moisture, oil , protein , gossypol and ash (%) for whole Egyptian cotton seed varieties and their meals.

Varieties	Water activity		Moisture		Oil		Protein		Ash		Gossypol
	Seed	Meal	Seed	Meal	Seed	Meal	Seed	Meal	Seed	Meal	Seed
Giza 45	0.76	0.67	5.47	5.15	24.5	4.6	21.8	38.0	4.45	5.70	0.37
Giza 70	0.74	0.70	7.01	5.90	20.8	4.8	23.0	40.0	4.32	6.00	0.45
Giza 86	0.68	0.64	6.52	5.69	21.7	4.7	22.08	41.0	4.89	5.99	0.32
Giza 85	0.69	0.71	6.97	5.74	24.2	5.2	21.3	36.0	5.22	5.24	0.39
Giza 80	0.74	0.71	6.81	5.73	21.1	5.0	20.8	34.9	4.35	5.59	0.44
Giza 83	0.70	0.68	5.26	5.10	20.0	4.7	20.9	37.9	5.89	5.00	0.38

It is worth to mention that the cotton varieties Giza 70 attained the highest content for each moisture, protein and gossypol . Giza 45 had the highest value of oil (24.5%) followed by Giza 85 , Giza 86, Giza 80 and Giza 83 (24.2, 21.7, 21.1 , 20.8 and 20.0%, respectively). While Giza 83 gave the highest value of ash (5.89%) followed by Giza 85, Giza 86 , Giza 45, Giza 80

and Giza 70 (5.22 , 4.89 , 4.45, 4.35 and 4.32% respectively). These results are in harmony with those obtained by Namich (1997) and Mohamed (2003).

The moisture content of Egyptian cotton seed meals ranged from 5.10 to 5.90 %, oil from 4.6 to 5.2 %, protein from 34.9 to 41.0 % and ash ranged from 5.00 to 6.00 %.

It is rather interesting to note that, the meal of Giza 70 gave the highest values for each moisture, protein and ash. While the meal of Giza 85 had the highest content of oil (5.2 %) followed by Giza 80, Giza 70, Giza 83, Giza 86 and Giza 45 (5.0, 4.8, 4.7, 4.7 and 4.6%, respectively). These results are in agreement with those reported by Mohamed (2001).

Variation in moisture and substrate in cotton seed was linked with the ability of *A. parasiticus* to grow on the seed and producing aflatoxins (Lillehoj *et al.*, 1987).

The data in table (1) showed that moisture content and water activity of cotton seed were 5.10-5.90% and 0.68 - 0.76 a_w, respectively, while those for cotton seed meals ranged between 3.26-4.81% and 0.64-0.71, respectively. It is clear that only slight differences could be observed in the six different types of Egyptian cotton seeds or cotton seed meals, concerning both moisture content and water activity.

(2) Mycotoxins and fungi found in cotton seeds and their meals:

Results of the mycotoxins analysis of the six investigated cotton seeds samples are shown in Table (2). Aflatoxins were detected in five samples. The concentrations of aflatoxins ranged between 4.73-10.40 ug/kg. Only two samples Giza 70 and Giza 83 contained the four types of aflatoxins (B_1, B_2, G_1 and G_2). Usually aflatoxins B_1 is the predominant aflatoxin found in agricultural commodities, while aflatoxins G_1 and G_2 are rarely found in cotton seed (Scott, 1978).

Table (2): Natural occurrence of mycotoxins in Egyptian cotton seeds (ug/kg).

Cotton seeds	Aflatoxins					Ochratoxin A	Zearalenone
	B_1	B_2	G_1	G_2	Total		
G45	-	3.02±0.4	2.08±0.7	-	5.10±0.5	-	-
G70	2.95±1.2	1.49±2.6	2.82±2.6	3.14±1.2	10.40±7.6	-	-
G86	2.97±3.0	1.76±0.6	-	-	4.73±3.6	-	-
G85	-	-	-	-	-	-	10.92±6.5
G80	6.66±0.5	1.67±0.7	-	-	8.3±1.2	-	6.90±5.4
G83	2.21±0.5	2.97±1.2	1.00±0.5	4.00±0.6	10.18±2.8	-	-

$\overline{X}$ ± SE

Cotton seed G70 was found to have the highest both protein (23%) and aflatoxins (10.4 ug/kg). In this respect, Mellon and Cotty (1997) suggested that protein composition and stored saccharides may be important factor influencing aflatoxin contamination in cotton seeds. These levels were under the allowed local limits of mycotoxins (E.O.S., 1990), hence the recommended maximum allowance is 10 ppb of aflatoxins (B_1, B_2, G_1 and G_2) in foods and feeds. In this concept, the obtained results are in agreement with

those reported by Wood, (1989) and Zeringue, *et al*, (1999). They found significant levels of contamination of cotton seed with aflatoxin in Arizona and Southern California. Many authors have stated or suggested that there weren't ochratoxin A or zearalenone on cotton seed, the natural occurrence of OA is generally associated with starch-rich foodstuffs. In this study OA was not found and zearalenone was measured only in two out of six cotton seed samples, namely G85 and G80 at concentration of 10.92 and 6.90 ug/kg, respectively (Table 2). Meanwhile, both cotton seed meal and cotton seed-oils were found to be free of any mycotoxins. The used heat in preparation process should reduce or destroy mycotoxins.

The significance of fungal contamination during the storage of cotton seeds were investigated and species belonging to fungal genera were isolated as shown in Tables (3 and 4), *Aspergillus* was the most frequent genus and it emerged in 45-80% in both cotton seeds and their meals.
The incidence of *Aspergillus niger* was the highest flowed by *A. favus*. These results are in agreement with those found by Mazen *et al.* (1990); Isakeit and Dunlap, (1995) and Verma *et al.* (1997).

(3) Mycotoxins amount produced by isolated moulds from cotton seeds:

Contamination by mycotoxins producing fungi were investigated in Table (3). The *A. flavus* was isolated and the produced aflatoxins were found to be 7.50, 6.25, 16.66 and 6.25% for seeds (G45 and G83) and meals (G80 and G83), respectively, while *A. ochraceus* and *F. monibform* produced 7.14% and 18.18%, respectively. Such strains were mycotoxigenic and the obtained data was in agreement with Jodral *et al.* (1992), who isolated *A. flavus* aflatoxigenic fungi from cotton seeds. Results in (Table 4) also appeared that *A. flavus* which isolated from sterilized cotton seeds produced only Aflatoxins (B_1 and G_2). Aflatoxigenic *A. flavus* also was isolated from both seeds and their meals for G83.

Results appeared in Table (5) show that the higher peroxide value the higher mycotoxins production as indicated in G86 followed by G45, which is in agreement with Passi *et al*, (1984) and Lillehoj *et al.*, (1987), who found that in vitro synthetic lipoperoxides greatly stimulated aflatoxin production by *Aepergillus parasiticus* and *A. flavus*. They also found that the high peroxide value in cotton seeds oil could be due to the higher of aflatoxin production and this might be due to the peroxidation of lipids of the endoplasmic reliculum of *A. parasiticus* by highly reactive C13 radicals formed by intraction with NADPH-Cytochrome P-450 system.

From the above mentioned data it is clear that cotton seeds containing 0.45 and 0.44% gossypol (G70 and G80, respectively) showed complete reduction for mycotoxins (Table 1), which is in agreement with those obtained by Garber and Cotty (1995).

Table (3): Fungi associated with Egyptian cotton seeds and their meals

Investigated samples	Contamination (%)	Fungi and their counts/100 grains																
		Total fungal count	*Aspergillus*										*Pencillium*		*Fusarium moniliform*		Other	%
			flavus	%	*niger*	%	*parasiticus*	%	*versicobr*	*ochrocus*	%	T.A.C.*	sp	%	sp	%		
Cotton seed																	-	-
G45	100	80	6	7.50	74	92.50	-		-	-		80	-	-	-		15	21.43
G70	100	70	-	-	50	71.43	-		-	5	7.14	55	-	-	-		-	-
G86	40	55	-	-	45	81.82	-		-	-		45	-	-	10	18.18	10	11.11
G85	100	90	-	-	70	77.78	-		-	-		70	10	11.11	-		-	-
G80	80	75	-	-	75	100.00	-		-	-		-	-		-		-	-
G83	60	80	5	6.25	25	31.25	25	31.25	-	-		55	25	31.25	-			-
Meal																		
G45	36	60	-	-	60	100.00	-		-	-		60	-	-	-		-	-
G70	80	80	-	-	75	93.75	-		-	-		75	5	6.25	-		-	-
G86	60	75	-	-	75	100.00	-		-	-		75	-	-	-		-	-
G85	50	70	-	-	70	100.00	-		-	-		-	-	-	-		-	-
G80	30	60	10	16.66	50	83.33	-		-	-		60	-	-	-		-	-
G83	40	80	5	6.25	75	93.75	-		-	-		80	-	-	-			-

* T.A.C. = Total Aspergillus count

Table (4): Mycotoxins production in liquid medium (ug/L) by strains isolated from untreated and treated cotton seeds*.

Cotton seed	Mycotoxins					
	Aflatoxins by *A. flavus*				Ochratoxin A *by A. ochracus*	Zearalenon by *F. moniliform*
	B_1	B_2	G_1	G_2		
Untreated seeds						
G45	11.70±4.5	9.60±2.7	5.87±2.7	4.99±5.4	-	-
G70	-	-	-	-	40.15±4.3	-
G86	-	-	-	-	-	78.20±5.6
G83	9.05±4.5	5.71±5.1	5.24±5.5	3.79±3.7	-	-
Treated seeds						
G80	25.28±10.6	-	13.30±12.3	-	-	-
G83	16.50±3.2	-	14.81±4.9	-	-	-

S.D. Standard Divination

*Cotton seed treated by 2% Sodium hypochlorite

Table (5): Mycotoxins production on untreated and treated cotton meal*.

Samples	Peroxide value	Mycotoxins					
		Aflatoxins by *A. Flavus*				Ochratoxin A by *A. Ochracaceus*	Zearalnone by *F. moniliform*
		B_1	B_2	G_1	G_2		
Untreated meal							
G45	9.16	61.12	-	50.74	-	57.87	-
G70	8.91	4.01	-	3.45	-	39.80	15.47
G86	9.33	35.12	20.88	38.16	37.50	38.30	13.50
G85	8.66	28.00	-	-	-	20.40	9.34
G80	9.00	-	-	-	-	-	-
G83	9.00	-	40.25	33.05	-	-	18.86
Treated meal							
G45	9.16	32.01	23.98	-	-	15.35	12.19
G70	8.91	2.12	1.24	7.57	7.47	-	-
G86	9.33	33.41	-	24.30	-	17.79	-
G85	8.66	6.88	1.12	4.14	3.00	-	10.72
G80	9.00	9.68	1.98	8.07	2.80	-	13.42
G83	9.00	9.83	4.61	6.70	2.45	16.54	3.59

*Cotton meal treated by 2% Sodium hypochlorite

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دراسات كيميائية وميكوتوكسينية على بذرة وكسب القطن المصرى

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تمت الدراسة على ست أصناف أساسية من القطن المصرى فائق طول التيلة (جيزة ٤٥ – جيزة ٧٠ – جيزة ٨٦) وطويل التيلة (جيزة ٨٥ – جيزة ٨٠ – جيزة ٨٣).

أهم النتائج المتحصل عليها:

سجلت بذور القطن المصرى صنف جيزة ٧٠ أعلى كمية من الرطوبة (٧,٠١%) والبروتين (٢٣%) والجيسبول (٠,٤٥%) والأفلاتوكسينات (١٠,٠٤% ميكروجرام/كيلو جرام). وبذور القطن المصرى صنف جيزة ٤٥ احتوت على أعلى نسبة من الزيت (٢٤,٥%) بينما الصنف جيزة (٨٣) احتوت على أعلى كمية من الرماد (٥,٨٩%) . وصنف جيزة (٨٦) أحتوى على أقل كمية لكلا من سموم الأفلاتوكسينات (٤,٧٣ ميكروجرام/كيلو جرام) ونسبة التلوث بالفطريات. فى حين كسب بذرة قطن صنف جيزة (٧٠) احتوى على أعلى كمية لكل من الرطوبة (٥,٩) والرماد (٦%) بينما كسب بذرة قطن صنف جيزة ٨٥ احتوى على نسبة زيت عالية وصلت إلى (٥,٢٤%).