

## CHEMICAL COMPOSITION OF THE SEEDS AND MEALS OF SOME EGYPTIAN COTTON VARIETIES AND POSSIBLE CONTAMINATION WITH MYCOTOXINS

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### Abstract

The chemical composition and natural occurrence of mold and mycotoxins contamination in whole cottonseeds and their meals were carried out for six Egyptian cotton cultivars (Giza 45, Giza 70, Giza 86, Giza 85, Giza 80 and Giza 89). The results showed that there were differences between the varieties. Since both the cottonseed meal and cottonseed-oil were found to be free of any mycotoxins for all the varieties, so they are safe for the health of both human and animal. The relationship between moisture % and natural occurrence of zeralenone showed significant positive correlation, also between protein % and aflatoxin contamination. While there was a high significant negative correlation between total fungal count and protein % and between oil % and natural occurrence of aflatoxins. Giza 70 variety exhibited special behavior, it attained the highest content for moisture and gossypol among all cottonseeds and their meals, and the highest protein content for cottonseed meal. It also had the lowest total fungal count.

### INTRODUCTION

Cottonseeds are the fifth best oil producing plant after soybean, palm tree and sunflower and the second best potential source of plant proteins after soybean. Hassan et al., (2005) evaluated the performance of five cultivars of Egyptian cotton, Giza 80, Giza 89, Giza 85, Giza 89 and Giza 90 for oil and protein %. The results showed that Giza 85 exceeded the other varieties regarding oil % content (22.28 %). On the other hand, Giza 89 gave the lowest oil % content (20.56 %) and the differences between them were significant. Regarding protein % character, the highest value (24.28 %) was obtained from Giza 90 among the examined cultivars, but Giza 85 and Giza 89 gave the lowest protein % (21.21% and 21.25%, respectively) and the differences between Giza 90 and both Giza 85 and Giza 89 genotypes were significant. Amal (2003) reported that, oil and protein % of brown cottonseed are 16.4 % and 18.7 % respectively, which are obviously lower than those components of either Giza 80 (21.8 % oil and 20.8 % protein) or for Giza 89 (22.6 % oil and 19.9% protein). Gossypol content mg /g for brown cottonseed is

higher (5.33 %) compared with both Giza 80 and Giza 89 (4.04 and 4.14 % respectively).

Cottonseed meal produced by expeller process contains: 94% dry matter, 41.0 % crude protein, 12.5 % crude fiber and 4.5 % oil, while meal produced by solvent process contains 92% dry matter, 41.5 % crude protein, 12.5 % crude fiber and 1.5 % oil. The major components in mechanical extracted cottonseed 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 (Barraza et al. 1991).

Seeds commonly may carry a heavy spore load from the field into storage. Eleven genera and 31 species in addition to 2 species varieties were isolated from stored cottonseeds collected from different localities in Egypt. The most common species on 2% glucose-Czapek's agar at 28°C using the dilution and the seed plate methods were: *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. tamarii*, *Mucor racemosus* and *Rhizopus stolonifer*. On 2% cellulose-Czapek's agar, *A. niger*, *A. flavus*, *A. terreus*, *A. fumigatus*, *A. zonatus*, *Mucor racemosus* and *Rhizopus stolonifer* were more frequently grown. Screening of fungal species and strains isolated for aflatoxin production by the fluorescence technique revealed that, only two strains of *A. flavus*, two strains of *A. oryzae* and one strain of *A. fumigatus* were positive for aflatoxin production. Only aflatoxin B2 was produced by *A. flavus* and the optimal conditions for toxin production on synthetic agar medium is described (El-Naghy et al., 1991).

The present investigation aims to study the following points

1. The chemical composition, fungal flora and mycotoxins contamination of whole cottonseed and their meal for six Egyptian cotton cultivars (Giza 45, Giza 70, Giza 86, Giza 85, Giza 80 and Giza 89).
2. The differentiation between the varieties in chemical composition and natural occurrence of fungi and mycotoxins.
3. The relationship between this chemical composition and occurrence of mold and mycotoxin contamination.

## MATERIALS AND METHODS

This study was carried out on six commercial Egyptian cottonseed varieties Giza 45, Giza 70, Giza 86, Giza 85, Giza 80, and Giza 89. These varieties were obtained from Cotton Maintenance section, Cotton Research Institute, Agriculture Research Center. The Local Oil Mill in Khan-Elkhalily was used to obtain cottonseeds-oil and their meals. The chemical composition of whole Egyptian cottonseeds and their meals: oil (%), protein (%), gossypol (%), ash (%) and moisture content were determined

according to A.O.A.C. (2000) and water activity ( $a_w$ ) was measured at 35 °C with equilibrium moisture absorption of microcrystalline cellulose and standard curve (Vos and Lobuza, 1974). Fungal flora was determined according to Jimenez et al. (1991).

The mycotoxins (Aflatoxins, Ochratoxin A and Zearalenone) were extracted from the cottonseeds and their meals and determined according to method A.O.A.C. (2000). Pure aflatoxins (B1, B2, G1 and G2), ochratoxin A and zearalenone were purchased from Sigma, Company, St. Louis, U.S.A.

The statistical procedures outlined by Little and Hills (1978) were applied to the data obtained in this study.

## RESULTS AND DISCUSSION

### Chemical composition of whole cottonseeds and their meals

Tables 1 and 2, illustrate the average content of main chemical Composition for Egyptian cottonseeds and their meals. The moisture content of cottonseed ranged from 5.26 % for variety Giza 89 to 7.01 % for Giza 70, while the water activity ( $a_w$ ) ranged from 0.68 % for Giza 86 to 0.76 % for Giza 45 respectively. On the other hand regarding the cottonseed meal the moisture content were ranged from 5.1% for variety Giza 45 to 5.9 % for Giza 70, while the water activity ranged from 0.6 % for Giza 86 to 0.71 % for Giza 85 and Giza 80 respectively. It is clear that slight differences could be observed between the six different varieties of Egyptian cottonseeds and their meals, concerning both moisture content and water activity.

Oil content % for cottonseed ranged from 20.8 % for variety Giza 70 to 24.5 % for Giza 45, and ranged from 4.6 % for Giza 45 to 5.2 % for Giza 85 for the cottonseed meal. Giza 45 had the highest value of oil 24.5% followed by Giza 85 , Giza 86, Giza 80 and Giza 89 respectively for the cottonseed but Giza 85 had the highest oil % content for the cottonseed meal 5.2 % followed by Giza 80, Giza 70, Giza 86, Giza 89 and Giza 45 respectively.

Protein % content ranged from 19.92 % for Giza 89 to 23.08 % for Giza 86 for cottonseed, while it ranged from 34.9 % for Giza 80 and 41.0 % for Giza 86, for cottonseed meal. For the cottonseed Giza 86 attained the highest protein % content 23.08 % followed by Giza 70, Giza 45, Giza 85, Giza 80 and Giza 89, while Giza 86 had the highest protein % content followed by Giza 70, Giza 45, Giza 89, Giza 85 and Giza 80 respectively for cottonseed meal.

Gossypol content (%) for cottonseed ranged from 3.14 % for Giza 86 to 4.32 % for Giza 70, and it ranged from 4.6 % for Giza 45 to 5.0 % for Giza 80, for cottonseed meal.

Giza 70 had the highest gossypol content (4.32 %) followed by Giza 80, Giza 85, Giza 45, Giza 83 and Giza 86 for cottonseed, while Giza 70 attained the highest gossypol content followed by Giza 89, Giza 86, Giza 85, Giza 45 and Giza 80 respectively for cottonseed meal.

For cottonseed, ash % content ranged from 4.32 % for Giza 70 to 5.89 % for Giza 89, and ranged from 5.0 % for Giza 89 to 6.0 % for Giza 70 for the cottonseed meal. Giza 89 gave the highest value of ash 5.89% followed by Giza 85, Giza 86, Giza 45, Giza 80 and Giza 70 respectively for the cottonseed, while Giza 70 attained the highest ash % value 6.0 % followed by Giza 86, Giza 45, Giza 80, Giza 85 and Giza 89 respectively for the cottonseed meal.

These results are in agreement with Namich (1997) and Amal (2003). It is worthwhile to mention that the cotton variety Giza 70 attained the highest content for moisture and gossypol of both cottonseed and its meal, and the highest protein content for cottonseed meal.

Table 1. Chemical composition: water activity, moisture %, oil %, protein %, gossypol % and ash % for whole Egyptian cottonseed Varieties.

Varieties	Moisture %	Water activity $a_w$	Oil %	Protein %	Gossypol (%)	Ash %
Giza 45	5.47	0.76	24.5	21.80	3.90	4.45
Giza 70	7.01	0.74	20.8	22.80	4.32	4.32
Giza 89	5.26	0.70	21.0	19.92	3.62	5.89
Giza 86	6.52	0.68	21.7	23.08	3.14	4.89
Giza 85	6.97	0.69	24.2	21.30	4.00	5.22
Giza 80	6.81	0.74	21.1	20.80	4.20	4.35
LSD 0.05	0.017	0.018	0.178	0.178	0.148	0.170

Table 2. Chemical composition: water activity moisture %, oil %, protein %, Gossypol % and ash % for Egyptian cottonseed meals.

Varieties	Moisture %	Water activity	Oil %	Protein %	Gossypol (%)	Ash %
		$a_w$				
Giza 45	5.15	0.67	4.6	38.0	4.6	5.70
Giza 70	5.90	0.70	4.8	40.0	4.8	6.00
Giza 89	5.10	0.68	4.7	37.9	4.7	5.00
Giza 86	5.69	0.64	4.7	41.0	4.7	5.99
Giza 85	5.74	0.71	5.2	36.0	5.2	5.24
Giza 80	5.73	0.71	5.0	34.9	5.0	5.59
LSD 0.05	0.017	0.0178	0.102	0.178	0.178	0.199

#### Natural occurrence of fungi in Egyptian cottonseeds and their meals

The presence of fungal contamination in cottonseed was investigated in this study and species belonging to fungal genera were isolated as shown in Table 3. The total fungal count isolated from the seeds of Giza 45, Giza 86 and Giza 89 varieties were the highest value compared with the other varieties which followed by Giza 80, Giza 70 and Giza 86 respectively. It is worthwhile to mention that seeds of Giza 70 variety had the lowest total fungal count which may be attributed to the existence of the highest gossypol content in it.

Three species (*Aspergillus*, *Penicillium* and *Fusarium*) were isolated from cottonseeds and their meals. The most common species was *Aspergillus*, it was the most frequent genus and it emerged in 45-80%. The incidence of *Aspergillus niger* was the highest followed by *Aspergillus flavus*. Seeds of Giza 45 variety had the highest total *Aspergillus sp.* count followed by Giza 85, Giza 70, Giza 89 and Giza 86 respectively. *Penicillium sp.* occurs only in the seeds of two varieties only Giza 85 and Giza 89. While *Fusarium sp.* occurs in the seeds of Giza 86 variety only. These results are in agreement with those found by Mazen et al. (1990).

Table 3. Natural occurrence of fungi in Egyptian cottonseeds

Cottonseed Varieties	Total fungal content (clony count/100 seeds)	<i>Aspergillus sp.</i>				<i>Penicillium sp.</i>	<i>Fusarium sp.</i>
		<i>flavus</i>	<i>niger</i>	<i>parasiticus</i>	<i>ochrocos</i>		
Giza 45	80	6	74	-	-	-	-
Giza 70	55	-	50	-	5	-	-
Giza 89	80	5	25	25	-	25	-
Giza 86	55	-	45	-	-	-	10
Giza 85	80	-	70	-	-	10	-
Giza 80	75	-	75	-	-	-	-

Regarding the cottonseed meal the total fungal count was similar to the whole cottonseeds as shown in Table 4, ranging from 60-80 colony / 100 grains. Also the most common species were *Aspergillus*, it was the most frequent genus and it emerged in 60-80 %. The incidence of *Aspergillus niger* was the highest followed by *Aspergillus flavus*. Seeds of Giza 89 variety had the highest total *Aspergillus sp.* count followed by Giza 70, Giza 86 and Giza 80, Giza 85, Giza 80 and Giza 45 respectively Giza 45 the lowest variety in total fungal count. *Penicillium sp.* occurs only in the meal of Giza 70 variety with small occurrence (5 colony / 100 grains). While *Fusarium sp.* did not occurs in the meals of all the varieties. It is worthwhile to mention that variation in moisture and substrate in cottonseed was linked with the ability of *Aspergillus parasiticus* to grow on the seed and produce aflatoxins.

Table 4. Natural occurrence of fungi in the Egyptian cottonseed meals.

Meal	Total fungal content (colony count/100 seeds)	<i>Aspergillus sp.</i>				<i>Penicillium sp.</i>	<i>Fusarium sp.</i> <i>moniliform</i>
		<i>flavus</i>	<i>niger</i>	<i>parasiticus</i>	<i>ochrocos</i>		
Giza 45	60	-	60	-	-	-	-
Giza 70	80	-	75	-	-	5	-
Giza 89	80	5	75	-	-	-	-
Giza 86	75	-	75	-	-	-	-
Giza 85	70	-	70	-	-	-	-
Giza 80	60	10	50	-	-	-	-

#### Mycotoxins detected in different cottonseeds and their meals

The results of the mycotoxins analysis of the six investigated cottonseed samples are shown in Table 5, both cottonseed meal and cottonseed-oil were found to be free from any mycotoxins. This may be due to the preparation processes which may reduce mycotoxins levels or destroy them.

Aflatoxins were detected in five out of six cottonseed samples. The concentrations of aflatoxins ranged between 4.73 and 10.40 ug/kg. Only two samples Giza 70 and Giza 89 contained the four types of aflatoxins ( $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$ ). Usually aflatoxin  $B_1$  is the predominant aflatoxin found in agricultural commodities, while aflatoxins  $G_1$  and  $G_2$  are rarely found in cottonseed. It is worthwhile to mention that these levels were under the allowed local limits of mycotoxins E.O.S.,(1990), hence the recommended maximum allowance is 10 ppb (ug/kg) of aflatoxins ( $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$ ) in foods and feeds. Also product standards for aflatoxins according to (United States, European

Union and Codex Alimentarius) showed that for animal feeds and other than cottonseed meal or corn standard for aflatoxins was 20 ppb, usda (GIPSA) (1998)

Table 5. Natural occurrence of mycotoxins in Egyptian cottonseed, cottonseed meal and oil of seed (ug/kg).

Sample	Natural occurrence of Aflatoxins					Natural occurrence of Ochratoxin A	Natural occurrence of Zearalenone
	B <sub>1</sub>	B <sub>2</sub>	G <sub>1</sub>	G <sub>2</sub>	Total		
Cottonseed Giza 45	-	3.02±0.4	2.08±0.7	-	5.10±0.5	-	-
Giza 70	2.95±0.2	1.49±0.6	2.82±0.6	3.14±0.2	10.40±0.6	-	-
Giza 89	2.21±0.5	2.97±0.2	1.00±0.5	4.00±0.6	10.18±0.8	-	-
Giza 86	2.97±0.3	1.76±0.6	-	-	4.73±0.3	-	-
Giza 85	-	-	-	-	-	-	10.92±0.5
Giza 80	6.66±0.5	1.67±0.7	-	-	8.3±0.2	-	6.90±0.4
cottonseed meal for all six varieties	zero	Zero	Zero	zero	Zero	zero	zero
Oil for all six varieties	zero	Zero	Zero	zero	Zero	zero	zero

On the other hand, cottonseed for Giza 70 was found to have the highest of 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 cottonseed.

In this study ochratoxin A was not found, while zearalenone was found only in two varieties out of six cottonseed samples, Giza 85 and Giza 80 at concentration of 10.92 and 6.90 ug/kg respectively.

Park et al. (1999) showed the associated hazards related to mycotoxin formation and possible corrective actions. It is clear that many factors related to cultivation,

harvesting and processing influence the level of mycotoxin contamination in seeds. Thus by controlling these factors we can minimize the mycotoxins.

**The relationship between the chemical composition and natural total fungal count and mycotoxin contamination**

The data listed in Table 6 revealed that, there was a significant positive relationship correlation between moisture % and natural occurrences of zearalenone and also between protein % and aflatoxin contamination. There was a highly significant negative correlation between total fungal count and protein % and between oil % and natural occurrence of aflatoxins. Mellon and Cotty (1997) suggested that protein composition and stored saccharides may be important factors influencing aflatoxin contamination in cottonseed.

Table 6. Simple correlation coefficients between the levels of total fungal count, Aflatoxin and zearalenone toxins and the chemical composition of cottonseed.

	Total fungal count	Aflatoxins	Zearalenone toxin
Water activity	0.147	0.435	-0.241
Moisture %	0.469	-0.217	0.518*
Oil %	0.385	-0.755**	0.401
Protein %	-0.950**	0.33*	-0.352
Ash %	0.389	-0.089	0.074
Gossypol %	0.174	0.188	0.339

**CONCLUSION**

The Giza 70 variety exhibited special behavior, Giza 70 attained the highest content for moisture and gossypol in both its cottonseed and meal, and the highest protein content for cottonseed meal. It also had the lowest total fungal count which may be due to having the highest gossypol content. Since both the cottonseed meal and cottonseed-oil were found to be free of any mycotoxins for all the varieties, they are safe for the health of both human and animal.

The relationship between moisture % and natural occurrences of zearalenone showed a significant positive correlation, also between protein % and aflatoxin contamination. While there was a high significant negative correlation between total fungal count and protein % and between oil % and natural occurrence of aflatoxins.

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## التركيب الكيميائي لبذور وكسب بعض أصناف القطن المصري ودراسة التلوث المحتمل للميكوتوكسينات بها

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٢. سموم ملوثات الغذاء - المركز القومى للبحوث - القاهرة

أجرى هذا البحث لدراسة التركيب الكيميائي وتحديد مستوى التواجد الطبيعي للفطريات ومدى التلوث بالميكوتوكسينات التي تفرزها هذه الفطريات في كل من بذرة وكسب ستة أصناف من القطن المصري (جيزة ٤٥ - جيزة ٧٠ - جيزة 89 - جيزة ٨٥ - جيزة ٨٠ - جيزة ٨٦). وتم تقدير كل من النسبة المئوية للزيت والبروتين والجوسيبول والمحتوى من الرطوبة لبذرة وكسب هذه الاصناف، وكما تم تحديد مستوى التواجد الطبيعي للفطريات ومدى التلوث بالميكوتوكسينات التي تفرزها هذه الفطريات (الافلاتوكسينات - الاوكراتوكسين - الزيرالينون)، وقد تم دراسة العلاقة بين هذا التركيب الكيميائي وتواجد الفلورا الطبيعية والميكوتوكسينات التي تفرزها اذا نمت على البذور أو الكسب.

و قد اظهرت النتائج ما يلي :

١. وجود اختلافات صنفية بالنسبة للنقاط موضع الدراسة (التركيب الكيميائي - مستوى التواجد الطبيعي للفطريات - مدى التلوث بالميكوتوكسينات التي تفرزها هذه الفطريات).
٢. خلو زيت وكسب جميع أصناف القطن المصري موضوع الدراسة تماما من السموم الفطرية بعد انتاجها من البذور بطريقة العصر الميكانيكي مما يعنى أنها آمنة تماما على صحة الانسان والحيوان.
٣. أظهر الصنف جيزة ٧٠ سلوكا خاصا بالمقارنة بباقي الاصناف موضع الدراسة . فقد وجد أنه أعلى الاصناف في محتوى بذرته و كسبه من الرطوبة و الجوسيبول، بينما الكسب الناتج منه كان اغنى كسب فى البروتين بالمقارنة بكسب باقى الاصناف. ووجد أنه أقل الاصناف تلوثا بالفطريات الطبيعية (الفلورا الطبيعية).
٤. بدراسة العلاقة بين التركيب الكيميائي للبذرة أو الكسب الناتج منها وتواجد الفطريات و الميكوتوكسينات التي تفرزها وجد عموما أن العلاقة بين محتوى الرطوبة وتواجد توكسين الزيرالينون وكذلك بين محتوى البروتين وتواجد الافلاتوكسين علاقة معنوية موجبة. بينما وجد أن العلاقة بين العد الكلى للفطريات ومحتوى البروتين وكذلك بين تواجده الافلاتوكسين ومحتوى الزيت علاقة عالية المعنوية سالبة.
٥. وبعد اجراء هذه الدراسة عموما نوصى بمراعاة بعض الاحتياطات لتقليل الاخطار التي يمكن أن تحدث نتيجة تواجده الفطريات التي يودى نموها الى هدم و تلف البذور نتيجة استهلاك مكوناتها الغذائية أو نتيجة افراز الميكوتوكسينات الضاره بصحة الانسان والحيوان نتيجة نمو هذه الفطريات على البذور أو الكسب: فقبل الزراعة يجب اختيار

أصناف مقاومة - اختيار دورة زراعية جيدة - استخدام برنامج فعال للسيطرة على الحشرات و الحشائش- تنظيم برامج الري، أما أثناء الجنى يجب المحافظة على المحصول من الرطوبة و الحشرات و العوامل البيئية الأخرى كما يجب تخزين المحصول فى مكان جاف نظيف لمنع نمو الفطريات. و بعد الجنى و أثناء عمليات التصنيع يجب أن تجرى جميع العمليات التصنيعية بدقة عالية لضمان الوصول لمنتج على الجودة من زيت و كسب.