

## IMPACT OF SOME AFLATOXIN-CONTAMINATED FOOD-GRAINS ON PUBLIC HEALTH

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## ABSTRACT

Samples of three food-grains stored for 3-7 months, in three different types of stores were evaluated for fungal inocula and aflatoxins contamination. Blood tests of certain human populations use to eat from such food-grain stocks, including serum aflatoxin  $B_1$ , some trace elements, some heavy metals and schistosomal immunodiagnostic tests were carried out. Recorded values were related to liver disease markers, *i.e.* hepatitis B surface antigen (HBs-Ag) and hepatitis C antibodies (Anti-HCV-Ab). Main concern of aflatoxins-grains-contamination was given.

Aspergillus flavus, A. niger and A. parasiticus recorded between 0 and 94%. Other seven non-aflatoxins producing fungi were recorded in different levels, *i.e.* Alternaria alternata, Cephalosporium sp., Cladosporium sp., Fusarium moniliforme, Penicillium spp., Stemphylium botryosum and Rhizopus nigricans. Aflatoxin B<sub>1</sub> was recorded as traces ( $<5\mu g/kg$ ) in maize sample stored for 4 months containing 17% moisture content (MC) and recorded 143.8  $\mu g/kg$  in wheat sample stored for 7 months containing 19% MC, while, B<sub>2</sub> toxin was separated from other three samples at levels of 67.2  $\mu g/kg$  (5 months + 15% MC), 77.6  $\mu g/kg$  (6 months + 15% MC) and 134.8  $\mu g/kg$  (5 month + 17% MC) from maize, wheat and maize grain samples, respectively.

Aflatoxin  $B_1$  medians in hepatitis patients were highly elevated than the upper limit of the range in all trace elements and heavy metals except mercury. Those having elevated aflatoxin  $B_1$  had higher percentages (95.2%) within hepatitis B surface antigen (HBsAg) positive cases than negative one (76.5%).

### INTRODUCTION

Public health is the first priority of physicians, agriculturists and ecologists. Obtaining high quality and healthy food commodities is a major issue for consumers allover the world. Therefore, extensive efforts are being made to enrich knowledge improving the agricultural production in terms of quantity as well as quality. Wheat, maize and faba bean are major components in the daily Egyptian meals, in addition to their importance for animal nutrition. These crops are attacked in field and during storage by some fungi that have the capability to produce hazardous mycotoxins to man and animal, such as *Aspergillus flavus* that can produce aflatoxins. It seems that local storage facilities owned by the small Egyptian farmers are very poor if compared with the internationally accepted storage standards, besides the warm weather in Egypt which usually results in excessive fungal molding of stored commodities (El-Abbasi, 1998 and Bekheet *et al.*, 2001) and most likely subsequent mycotoxin contamination.

Aflatoxins play a suspicious role, alone or combined with some other factors such as viral infection in the development of hepatocellular carcinoma (HCC). The hepatotoxic effect of aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub>) is well recognized in the time being as reported by Ross *et al.* (1992). Also, Abdel Tawab *et al.* (1999) proved that hepatic failure may results from a combined effect of the schistosomiasis (bilharzia disease caused by *Schistosoma mansoni*) and aflatoxin B<sub>1</sub>. Sun *et al.* (2002) suggested that there is a relation between aflatoxin B<sub>1</sub>, genetic and environmental factors to incite hepatitis diseases. There were relative contributions of environmental exposure and host susceptibility factors on production of aflatoxins in human blood which may result in the formation of  $B_1$  albumin adduct that increase the risk of liver cancer.

The main objective of the current collaborative study was to determine aflatoxins' contamination in some food crops (wheat, maize and faba bean) obtained from traditional store types (farmers or local market) after certain periods of storage under Egyptian conditions in relation to human liver cancer susceptibility.

### **MATERIALS AND METHOD**

#### **Collection of food-grains samples**

Grain samples subjected to different storage periods and types were collected from El-Shoabak El-Sharky village, Giza governorate during the year 2005. Storage period ranged from 3-7 months, while store types were in small room (locally called Ghorn), on rural house roof or in clay silo.

Details of collected samples are shown in Table (1). Moisture content in two replicates of the each sample was determined using the method of A.O.A.C. (1990). Samples were kept under deep freezing conditions at  $-20^{\circ}$ C for further tests.

# Detection and identification of fungi associated with grains samples of wheat, maize and faba bean

The standard blotter method described by ISTA (1996) was followed for detection and identification of fungi associated with grains under study. Two hundred grains of each sample were placed in Petri dishes on three layers of well-moistened blotters at the rate of 10 seeds/dish (for maize and faba bean) and 25 seeds/dish (for wheat). Dishes were then placed in incubator at  $25 \pm 2^{\circ}$ C under alternating cycle of 12 h day light and 12 h darkness for 7 days. At the end of the incubation period, dishes were examined using stereobinocular microscope (6-50X) for the presence of seed borne fungi. The compound microscope was also used for confirmation. Identification of the detected fungi was carried out following the description of Barnett and Hunter (1986), Booth (1985) and Klich and Pitt (1992).

Grain sample	Source	Storage period (month)	Storage type	Moisture content %
1. Maize-1	Farmer	3	Ghorn	14
2. Maize-2	Farmer	3	Clay silo	14
3. Maize-3	Farmer	5	House roof	15
4. Maize-4	Farmer	5	Ghorn	17
5. Maize-5	Retailer	4	Local market	17
6. Wheat-1	Farmer	7	Ghorn	19
7. Wheat-2	Retailer	6	Local market	15
8. Faba bean	Retailer	6	Local market	14

Table (1): Grain sample, source, storage period, storage type and moisture content of each sample.

#### **Determination of aflatoxins**

Aflatoxins  $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$  were extracted and determined in grain samples of food crops under study following the method adopted by Williams (1984).

Aflatoxins were quantified by measuring fluorescence intensity using ACD 60 spectrodenistometer was used in assaying a refrectance mode at excitation wave length 365 nm and emission wave length of 430 nm following the technique adopted by Dickens *et al.* (1980).

#### Patient population survey

Medical survey of the village was done to screen liver and other organs of human population, those used to consume mainly a part or more of the previous food-grains in their daily meals. Population work mainly as agricultural labors and some of them work in nearby factories of iron and steel, metal melting including lead, electricity station, cement industry and petroleum pipes companies. Eighty four individuals, regardless of sex and with at least fifteen years of age, representing 15 randomly selected houses from all locations of the village were studied.

#### **Blood analyses**

Because of human behaviour complexity, including nutrition, it was difficult to study the contribution of aflatoxins-grain-contamination as a single factor on human liver diseases. So, several human blood tests were carried out through the current investigation, including serum  $B_1$  toxin (Reichert *et al.*, 1988). Other blood tests, such as the determination of trace elements (copper, zinc, selenium and iron), heavy metals (aluminum, cadmium, arsenic, mercury, chromium, lead, manganese and nickel), schistosomal immunodiagnostic tests and their relations to liver disease markers (hepatitis B surface antigen (HBs-Ag) and hepatitis C antibodies (Anti-HCV-Ab)) were also done.

### **RESULTS AND DISCUSSION**

#### Fungal inocula associated with grains samples

Several fungal species were recovered from grains of food crop samples as shown in Table (2). *Aspergillus* spp. comprised the major proportion of the fungal isolates recovered from grains regardless of the source or type of grain collections. It was found that isolates belonging to *A. flavus* ranked the highest frequency followed by *A. niger* and *A. parasiticus* (Table 2). *Aspergillus* spp., the cosmopolitan candidates, are known as the most contaminant of food crops and their products allover the world (WHO, 1979). Some of *Aspergillus* spp. such as *A. flavus* and *A. parasiticus* were reported by several investigators as aflatoxin producers (Ominski *et al.*, 1994). It is clear also from Table (2) that isolates of *A. flavus* were isolated from maize grain samples almost in higher frequencies comparable with those of wheat or faba bean. High frequency percent of A. flavus was recorded in maize grains with low moisture content (MC, 14 %) when stored for three months. Whereas, storing maize grains containing higher moisture contents and stored for longer periods caused reduction in frequencies of this fungal species. Alternaria alternata was recovered only from wheat grain samples containing 19 % MC when stored for 7 months. Rhizopus nigricans showed the following higher frequency followed by Penicillium spp. Fungal isolates belonging to these two species are the most candidates which could be associated with food crops as reported by many investigators (Neergaard, 1983; El-Abbasi, 1990 and 1998). These two fungi were reported as endophytes in many important agricultural commodities that occur inside plant tissues without causing any apparent symptoms except for maize having high MC (Wilson 1995). However, members of genus Penicillium spp. can produce a variety of secondary metabolites (Samson and Frisvad, 2004). Rhizopus spp. is most likely to occur among food handlers during storage, transportation and marketing of plenty of food stuffs such as maize and peanuts (Al-Doory and Domson, 1984). Both fungal species, *Rhizopus* and *Penicillium* were frequently isolated from the majority of grains samples regardless MC in grains and period of grains storing. Fusarium moniliforme was also isolated from grain samples of crops under study in moderate frequencies up to 12%. This cosmopolitan fungus can infect all cereal crops causing stalk and grain rots (Shurtleff, 1984 and Frederiksen, 1986). Besides, it can be recovered from symptomless plant tissues and produce a variety of mycotoxins. This fungus could be recovered from both samples of maize and wheat regardless of the MC or storing period. *Cladosporium* spp. were isolated only from one of each of maize and wheat grain samples. Whereas, Alternaria alternata and Stemphylium botryosum were isolated just from one sample of wheat grains but could not be recovered from the other samples.

As isolates belonging to *Aspergillus* spp. were the most frequent contaminants to grains of sampled food crops under study, further study was carried out to detect and identify aflatoxins in food-grain samples.

# Aflatoxins concentration (µg/kg) in grain samples under various levels of MC and different storage periods

Since types of stores involved in the current investigation (4 different types, Table 1) did not show remarkable variations affecting neither fungal populations nor aflatoxins production in tested grain samples. Data presented herein most probably are very much related to moisture content of grain samples and/or storage period. In similar subject, Thompson and Henke (2000) stated that aflatoxin production ranged from 0 to  $151\mu g/kg$  in maize grain samples regardless of type of storage container, time of storage and climatic conditions, with 8% only of the tested samples produced aflatoxin levels that exceeded 50  $\mu g/kg$ .

Aflatoxins  $B_1$  and  $B_2$  were found to be the only mycotoxins detected in some grain samples under study (Table 3). These two types of aflatoxins were found in samples of maize or wheat containing 15 % MC or more and stored for 5 months or more. No aflatoxins were detected in faba bean grain samples in current study.

Aflatoxin  $B_1$  was recorded as traces (less than 5  $\mu$ g/kg) and 143.8 µg/kg in two samples; maize-5 stored for 4 months containing 17% MC and wheat stored for 7 months containing 19% MC. However, aflatoxin B<sub>2</sub> was more detected in grain samples than aflatoxin B<sub>1</sub>, but in other 3 samples, *i.e.* maize-3, wheat-2 and maize-4, since it recorded 67.2, 77.6 and 134.8 µg/kg, respectively. Fifteen percent of MC and above seemed to be a critical factor in all samples of stored food-grains. Moreover, no aflatoxin contamination recorded when moisture content was below 15%, regardless the storage period or type of stored food-grains.

Table (2): Frequency percentage of fungi associated with non-sterilized seeds examined using the blotter method after 7 days of incubation at  $25^{\circ}C \pm 2$  and alternative cycles of cool white/darkness.

	ą		Fungi associated with food grains									
Grain food samples (source)	Stora ge period (month)	MC %	Aspergillus flavus	A. niger	A. parasiticus	Alternaria alternata	Cephalosporium sp.	Cladosporium sp.	Fusarium moniliforme	<i>Penicillium</i> spp.	Stemphylium botryeosum	Rhitopus nigricans
1. Maize-1 (farmer)	3	14	86	- s	-		-	-		-	-	
2. Maize-2 (farmer)	3	14	87	94			-	-	12	30	-	30
3. Maize-3 (farmer)	5	15	7	-	5	-	-	-	-	9	-	27
4. Maize-4 (farmer)	5	17	15	19			-	23	б	17	-	22
5. Maize-5 (retailer)	4	17	7	10			2	-	б	37	-	50
б. Wheat-1 (farmer)	7	19	22	25		19	-	-	3	16	8	23
7. Wheat-2 (retailer)	б	15	17	4	-		-	9	-	-	-	37
8. Faba bean (retailer)	б	14	2	-	3		-		-	4	-	37

- <sup>a</sup> = not detected.

M.C. = moisture content of grains.

Table (3): Aflatoxin(s) incidence in stocks of eight food grains contained various levels of moisture content and stored for different storage periods.

Grain samples	Storage period	MC	Aflatoxins production (µg/kg)			
	(month)	%	<b>B</b> <sub>1</sub>	<b>B</b> <sub>2</sub>	G <sub>1</sub>	G <sub>2</sub>
1. Maize-1	3	14	0	0	0	0
2. Maize-2	3	14	0	0	0	0
3. Maize-3	5	15	0	67.2	0	0
4. Maize-4	5	17	0	134.8	0	0
5. Maize-5	4	17	Traces*	0	0	0
6. Wheat-1	7	19	143.8	0	0	0
7. Wheat-2	6	15	0	77.6	0	0
8. Faba bean	6	14	0	0	0	0

\* <5  $\mu$ g/kg.

Fortunately, samples of faba bean (the most common daily meal in Egypt) stored for 6 months with no aflatoxin contamination, which may be referred to unsuitable nutritive components such as certain kinds of lipids and/or carbohydrates required for fungal nutrition. No contamination with aflatoxins G<sub>1</sub> and/or G<sub>2</sub> was recorded. Regarding the storage aspect, indeed there are multi factors may affect stored grain quality and subsequent toxin contamination could be detected. Those factors are described by Ominski et al. (1994) who arranged them according to their importance in the following order; water activity (1), temperature (2), time (3), damage to the seeds (4), oxygen and carbon dioxide levels (5), composition of the substrate (6), fungal abundance (7), prevalence of toxigenic strains (8), spore load (9), microbial interaction (10) and invertebrate vectors (11). In this investigation, the attention was concentrated on 5 factors only, *i.e.* water activity expressed as moisture content, time (as storage period), composition of the substrates (as three different crops), fungal abundance (as the recorded fungi) and the prevalence of toxigenic fungi (mainly as Aspergillus spp.).

Generally, it could be concluded that aflatoxin(s) may contaminate maize and/or wheat grains containing 15% MC or more when stored in room conditions and loaded with high spore numbers of aflatoxigenic isolate of *Aspergillus flavus* is threatening food-grain quality during storage and that could be considered as a remarkable risk factor for human health.

# Determination of aflatoxin $B_1$ in human blood analysis (ng/kg) and its relations to other blood tests' values

Table (4) shows median of all studied numerical variables; trace elements, heavy metals and aflatoxin  $B_1$  in patients with hepatitis serologic findings. Selenium, cadmium and lead were higher than the reference values. Mercury in all subjects was within the reference range. All other variables showed that some cases had abnormal high levels that differed according to conditions of hepatitis cases.

Aflatoxin  $B_1$  median level was highly elevated than the upper limit of the reference values in all groups.

Table (5) shows that cadmium, lead, chromium and nickel had high percentages in all cases either positive or negative in the same mentioned order. But those having elevated lead and chromium had higher percentages within HBsAg positive cases than negative ones.

Those having elevated aflatoxin  $B_1$  had higher percentages (95.2%) within HBsAg positive cases than negative one (76.5%). Percentages of those having *Schistosoma* antigen were higher in HBsAg positive cases (83.3%) than negative ones (75.9%). Also, those having *Schistosoma* antibodies were much higher within HBsAg positive cases than negative ones (64.7% vs. 52.0%).

Variables	Negative HBsAg and Anti-HCVAb	HBsAg positive	Anti- HCVAb positive	Combined infection	Reference value
Trace elemen	<u>ts:</u>				
Copper	93.0	96.0	99.0	93.0	80-110 μg/L
Zinc	91.0	92.0	92.5	89.5	80-120 μg/L
Selenium	16.6	14.9	15.9	17.3	5.7 <b>-</b> 7.4 μg/L
Iron	111.5	108.0	108.5	108.5	65-120 μg/L
Heavy metals	<u>):</u>				
Aluminum	47.0	44.0	40.	32.5	14 <b>-</b> 62 μg/L
Cadmium	2.5	2.4	2.5	2.5	0.9 <b>-</b> 2 μg/L
Arsenic	50.0	51.0	44.0	48.5	$> 80 \ \mu g/L$
Mercury	99.0	111.0	127.0	120.0	100-300 µg/L
Chromium	28.0	30.0	32.0	31.0	20-30 µg/L
Lead	38.5	39.0	38.5	43.5	10-38 μg/L
Manganese	7.9	7.8	7.6	8.3	<b>7-12 μg/</b> L
Nickel	4.6	4.1	3.7	8.0	4.8 μg/L
<u>Aflatoxin B<sub>1</sub></u>	45.0	65.0	37.5	52.5	16.3-36.2 ng/kg

Table (4): Median values of trace elements, heavy metals and aflatoxin  $B_1$  in patients with hepatitis serologic findings.

Table (5): Percentage\* distribution of studied trace elements, heavy metals and aflatoxin  $B_1$ , *Schistosoma* antibodies and *Schistosoma* antigen among patients with hepatitis serologic findings.

Variables	Negative	HBs Ag positive	Anti-HCV Ab positive	Combined infection
<b>Trace elements:</b>				
Copper	32.0	13.3	30.0	0.0
Zinc	16.0	13.3	10.0	0.0
Iron	11.1	7.7	0.0	0.0
Heavy metals:				
Aluminum	8.0	0.0	0.0	0.0
Cadmium	88.5	76.5	88.9	100.0
Arsenic	0.0	5.9	0.0	0.0
Chromium	40.7	52.6	62.5	50.0
Lead	57.1	68.4	50.0	100.0
Manganese	10.5	0.0	0.0	25.0
Nickel	42.1	18.8	12.5	50.0
Aflatoxin B <sub>1</sub>	76.5	95.2	88.9	100.0
Schit. Antibod.	52.0	64.7	57.1	50.0
Schit. Antigen	75.9	83.3	57.1	25.0

\* Percentages refer to subjects having abnormal high levels of studied variables within normal, HBs Ag, anti-HCV antibodies or combined sero-positive subjects.

Results of human blood analyses mentioned in Tables (4 and 5) indicated that there are some factors could be associated with some liver diseases as markers or causes for hepatitis B and C in an Egyptian rural community. These factors were some heavy metals, some trace elements, aflatoxin B<sub>1</sub> grain contamination and *Schistosoma mansoni* infection. These results were close to those stated by Creppy (2002) and Sayed *et al.* (2005) who reported that aflatoxins exposure usually resulted from dietary sources. Creppy (2002) stated that prevention of aflatoxin B<sub>1</sub> adverse effect included reduction in mycotoxin level in food stuffs and further increasing the intake of diet components such as antioxidants and substances known to prevent carcinogenesis, mainly liver cell primary carcinoma. In a recent study in China, Zhi *et al.* (2008) confirmed the relationship between hepatitis B virus (HBV) infection and each of oxidative stress factors and aflatoxin B<sub>1</sub> (AFB1)

exposure which might substantially increase the risk for hepatocellular carcinoma (HHC).

So, adopting all measures that can reduce mycotoxin contamination in food-grains including harvesting process, postharvest practice, handling and storing of food stuffs, could be considered the major approaches to avoid public health disaster incited by mycotoxins, particularly aflatoxins.

Accordingly, sooner or later, the health care system would be confronted by a rush of cases of hepatocellular carcinoma (HCC). It is the time to educate the public about decreasing risk of aflatoxins exposure.

#### ACKNOWLEDGMENT

Deep appreciations are expressed to Theodor Bilhariz Research Institute (Project No. 70-M), Ministry of Health and Population, Egypt for facilitating this study.

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تأثير تلوث بعض حبوب الغذاء المخزونة بالأفلاتوكسينات على الصحة العامة

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تم تقييم عينات من ثلاث أنواع حبوب غذاء مخزونة فى ثلاث أنواع من المخازن لمدة تراوحت بين 3-7 شهور من حيث تلوثها باللقحات الفطرية والأفلاتوكسينات. كما تم إجراء بعض تحاليل الدم لبعض الأدميين الذين يستخدمون تلك الحبوب فى غذائهم ، وشملت هذه التحاليل مستوى التوكسين الفطرى بى-1 فى الدم ، بعض العناصر الصغرى ، بعض المعادن الثقيلة ودلالات أعراض البلهارسيا. تم ربط القيم المتحصل عليها بدلالات أمراض الكبد الوبائية بى ، سى. كما أعطيت أهمية رئيسية لتلوث الحبوب بالأفلاتوكسينات.

سجلت الفطريات أسبر جيلاس فلافس ، أسبر جيلاس نيجر وأسبر جيلاس بار اسيتيكس قيم تراوحت بين صفر -96%. كما سجلت سبع فطريات أخرى غير منتجة للأفلاتوكسينات نسب مئوية مختلفة و هي الفطريات ألترناريا ألترناتا ، نوع سيفالوسبوريوم ، نوع كلادوسبوريوم ، فيوز اريوم مونيليفورم، أنواع من البنيسيليوم، سنيميفيليوم بوتريوزم وريزوبس نيجريكانز. وقد تم تسجيل الأفلاتوكسين بي-1 في عينتين بمستوى آثار في عينة الذرة المخزنة لمدة 4 شهور بمحتوى رطوبي 17%، وبمستوى 143.8 ميكروجرام/جم في عينة قمح مخزنة لمدة 7 شهور بمحتوى رطوبي 19%. بينما تم تسجيل الأفلاتوكسين بي-2 في ثلاثة عينات أخرى بمستويات 67.2 ميكروجرام/كم (5 شهور بمحتوى رطوبي 15%) ، 77.6 ميكروجرام/كجم (6 شهور بمحتوى رطوبي ميكروجرام/كجم (5 شهور بمحتوى رطوبي 10%) من عينات ذرة ، قمح وذرة على ميكروجرام/كم (5 شهور بمحتوى رطوبي 10%) من عينات ذرة ، قمح وذرة على التوالي.

كانت قراءات متوسطات الأفلاتوكسين بى-1 فى عينات الدم لمرض الإلتهاب الكبدى بنوعيه التى تم اختبار ها عالية وكانت جميعها مرتبطة بمستويات مرتفعة من المعادن الثقيلة فيما عدا معدن الزئبق. كما سجل تواجد الأفلاتوكسين بى-1 نسبة عالية (2.95%) عند المرضى الذين كانت إختبارات الإلتهاب الكبدى بى موجبة مقارنة بمن كانت نتائج إختبارات عينات الدم لهم سالبة (76.5%).