

*Afflation Contamination of Some Crop Seed Types and their Products  
in the Gezira State, Sudan*

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**ABSTRACT**

The present study investigated the aflatoxin contamination levels as well as infection with *A. flavus*, in samples of groundnut collected from different areas in Gezira State. Aflatoxin contamination levels were at their minimum in samples collected from the villages (producing areas) compared to very high levels in samples collected from the main central markets in Wad Medani. However, samples collected from local markets in the producing areas were less contaminated. Moreover, the infection with *A. flavus* followed the same pattern of aflatoxin contamination with higher infection in samples collected from Wad Medani markets. Aflatoxin contamination was high in groundnut seeds from insect damaged pods, while, the sound intact ones were almost free from aflatoxin. Regarding aflatoxin contamination of different groundnut seed products, the present study showed that grey and red roasted nuts were both highly contaminated. However, more aflatoxins accumulated when these nuts were sold in thin plastic bags. The legumes seeds (*Phaseolus*, Faba bean, and Chick pea) were highly contaminated with aflatoxins and also infected with *A. flavus* compared to the cereal seeds (wheat & sorghum). Seeds of crops other than the groundnut, collected from the two main markets

of Wad Medani town, included both bagged and non- bagged samples. Seeds of these crops sold in thin plastic bags had higher levels of moisture content and also higher incidence of *A. flavus* as well as, large amounts of aflatoxins.

**Key words:** Aflatoxins, *A. flavus*, groundnut

## INTRODUCTION

Aflatoxin is a name given for a group of toxins, which are produced by two fungi, *Aspergillus flavus* and *A. parasiticus*. Aflatoxins occur naturally in a wide range of commodities (Abdel-Rahim, 2005). From samples collected from various industrial sources in Taiwan, three out of eight peanut samples, 9 of 13 peanut butter, 4 of 12 peanut cakes and 8 of 17 peanut oils were found to contain aflatoxins. Campbell (1969) analyzed a wide variety of local foods in Philippines, and he found that all the 29 samples of the Philippines locally produced peanut butter contained more than 30 ppb, aflatoxins.. The toxins were also obtained in other peanut products, as well as in corn, bean, cassava, rice, and coconut products. In Cannery Islands, Frazier (1968) recorded a list of dehydrated products, prepared for human consumption, in which aflatoxins had been detected. These include wheat flour, dry pea meal, corn bread, peanut butter and others.

Peanut plays an important role in the diets of rural population in the Sudan, particularly children, this is because of the high protein (21-30%), Fat (41-53%) and carbohydrates (11-27%) contents. It is also rich in calcium, potassium, phosphorus, magnesium and vitamin E. The by- products of its oil extraction are widely used for feeding livestock, and greatly used in the form of cakes for the exporting poultry industry in the country (Salih, et al., 1995). Moreover, Sudan was the second exporting country of peanut after the USA during 1970s. It was exporting about 22% of the total world export, at that time. However, during the past few years there are many cases of Sudanese groundnut export lots which have been rejected by the importing countries, due to high levels of aflatoxin contamination (Salih, et al., 1995). However, despite the importance and the wide

use of peanut and its products in the Sudan e.g. (oil, peanut butter, groundnut paste, seeds and roasted nuts), very few studies were made to investigate the problem of aflatoxin contamination in these commodities (Habish and Abdalla, 1973; Hag Elamin, *et al.*, 1988, and Abdel Rahim, *et al.*, 1989).

The aim of the present study was to investigate the levels of aflatoxin contamination in some groundnut seeds and their products as well as the contamination of some other crop seeds. The effect of storage of these products in thin plastic containers and selling, was also studied.

## ***MATERIALS and METHODS***

### ***Collection of samples***

*Samples of seeds of different crops ( Groundnut, Phaseolus, Faba bean, and Chick pea, Wheat and sorghum) were collected from producing areas (Basatna, Kareaba and Sureeba), from some local markets (El-Hoosh, Medina Arab and Al-Fao) as well as from the two Wad Medani main markets (Al-Markazi and Al-Malaga). The samples were collected during the seasons of 2006 and 2007. These samples were collected randomly. Packed and non-packed seeds were collected separately. The size of each sample was around 0.5 kg.*

*Samples of three groundnut products (paste, red and grey roasted nuts), packed and non-packed were collected from the markets and investigated for their aflatoxin production.*

*Sound intact pods of groundnut and other crops as well as insect, or harvest damage ones, were collected and also investigated for their fungal infection and aflatoxin contamination.*

### ***Determination of fungal growth***

*In all fungal tests, plates containing potato dextrose agar (PDA) and malt extract agar (MA) media, supplemented with rose Bengal to discourage bacterial growth, were used. Sterile Petri-dish's containing moist filters were also used for fungal isolation. For determining fungal growth, in sound intact pods, before shelling, pods were soaked in 0.1% mercuric chloride solution for 5 minutes and washed twice in sterile distilled water, each, for 5 minutes. Shells were then removed aseptically and seeds were transferred into plates containing PDA, MA media or sterile moist filter papers. A total of 100 seeds from each sample, were used (10 seeds per plate). Plates of all treatments were incubated at 30° C for 5 days and fungal contamination was recorded (Abdalla, 2001).*

*For determining fungal growth in damaged pods of groundnuts, the pods were spray – disinfected by 0.1% mercuric chlorides solution rather than being soaked as was recommended for sound and intact pods (McDohald and Harkness, 1963). A sample of 100 seeds was transferred into Petri-dishes (10 seeds per plates) containing PDA, or MA. Plates were incubated as above and investigated for fungal growth as mentioned.*

*For the determination of fungal growth in groundnut paste, the paste was cut into small pieces (about the groundnut seed size) and was surface sterilized as in the case of damaged seeds. After sterilization 100 pieces were transferred into plates (10 pieces per plate) of solidified PDA or MA. Plates were incubated as mentioned above and percentage fungal contamination was made (Suleiman, et al., 2008).*

### ***Detection of Aflatoxins***

*For aflatoxin detection, seeds of each crop type were thoroughly mixed and about 100g were grounded in a coffee grinder. From the ground paste 20g were drawn and extracted with 100 ml chloroform. After concentration, the extract was analyzed chromatographically, using a developing solvent of chloroform and methanol (95:5 v/v) as was described by Jones (1972) and Abdalla (2001).*

## **RESULTS AND DISSCATION**

*Results (Table, 1) Show that seeds of groundnut collected from the villages of Basatna, Kareeba and Al Sureeba. (Producing sites) were almost free from aflatoxins. However, appreciable amounts of the aflatoxins were reported on seeds of pods collected from the local markets (Medina Arab, Al Fao and Al Hoosh). In contrast very high levels of aflatoxins were found on seeds collected from the two main markets (Malaga and Mercazi) in Wad Medani. According to McDonald (1969), seeds from producing areas are usually free from aflatoxins, because they are freshly produced and have not been stored for long periods. The high levels of contamination reported for seeds collected from the main two markets of Wad Medani, may be due to the bad handling procedures and some of them were sold in polythene bags, which increase the moisture content, the condition that is conducting to aflatoxin production (Sanders, 1968; Diener and Davis, 1967; Hag Elamin et al., 1988; and Abdel Rahim et al., 1997). The effect of groundnut pod condition on aflatoxin production of seeds was also studied in the present work. Pods were graded into three categories, sound intact, insect damage, and mechanical damage pods. Seeds from insect and mechanical damaged groundnut pods were found to be highly contaminated with A. flavus, and accumulated large amounts of aflatoxins (Table, 2). However, seeds from*

healthy intact pods were almost free from aflatoxins. Schrooder and Ashworth (1965), Ahmed (1981), and Hag Elamin et al. (1988) found that sound intact pods of groundnut gave seeds free from aflatoxin, although, a very low amount of *A. flavus* was shown to be present.

Table 1. Percent infection with *A. flavus* and the level of Aflatoxin of groundnut seeds collected from different location in the Gezira State.

Location	Infection with <i>A. flavus</i>	Aflatoxin level
Basatna	0.0	-
Kareeba	0.0	-
Sureeba	0.01	-
Al Hoosh	2.0	+
Medina Arab	7.0	++
Al Fao	5.0	++
Mercazi	25.0	+++
Malaga	30.0	+++

- No toxin, + Low level, ++ Moderate level, +++ High level of aflatoxin

Table 2. Effect of the groundnut pods condition on percent infection with *A. flavus* and the level of aflatoxin contamination.

Pod condition	% Infection with <i>A. flavus</i>	Aflatoxins ppm
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<i>Sound intact pods</i>	<i>0.01</i>	<i>0.0</i>
<i>Harvest damage pods</i>	<i>20.6</i>	<i>0.3</i>
<i>Insect infested pods</i>	<i>35.1</i>	<i>5.6</i>

*According to Hag Elamin et al. (1988) and Abdel Rahim et al., (1997), the higher aflatoxin contamination of seeds of the insect damaged pods, could be due to the fact that invasion by insect will provide sites of injury and serve as vectors for A. flavus transmission in the seeds.*

*On the other hand, the presence of higher levels of aflatoxin, in seeds of mechanically damaged pods was suggested by El Nour and Ibrahim (1970) and Griffin (1970) to be due substances secreted from the wounded pods, which may stimulate growth of the fungus and allow more aflatoxin to be produced.*

*Regarding aflatoxin contamination of the different groundnut products (past, grey and red roasted nuts), it was found that grey and red roasted nuts were both highly contaminated (Table, 3). Although, the groundnut paste was the least to be contaminated with aflatoxins, a large amount of the toxins was accumulated when it is sold in plastic bags. Roasted and salted peanuts are ones of the popular food items in Western Sudan. They are also used for the preparation of peanut butter, confectionaries, and in a variety of bakery products (Salunkhe et al. 1991). Therefore, their contamination with aflatoxins is a serious and dangerous matter. Hag Elamin et al. (1988) also found that the paste accumulated less aflatoxins compared to the grey and red roasted nuts. However Salunkhe et al. (1991) and Suliman et al. (2008) reported that roasting of groundnut seeds at 175° C or above for 20 min., could lead to high reduction*

in aflatoxin  $B_1$ , in the contaminated seeds. The reduction was attributed to the heat effect on aflatoxins. Heat could thermodynamically enhance reactions between aflatoxin  $B_1$  and the other components of the peanut seeds. It was also reported that aflatoxins are not easily extracted in the presence of thermally destructed constituents of the seeds (Abdel Rahim, 2005).

Table 3. Aflatoxin contamination of some groundnut products from the different local markets in the Gezira State (ppm).

Location	G/nut paste		Roasted G/nut		Madamas grey	
	Bagged	Non - bagged	Bagged	Non - bagged	Bagged	Non - Bagged
Al_Hoosh	6.3	0.1	7.0	0.2	9.2	0.01
Medina_Arab	8.0	2.0	9.1	0.5	10.1	0.20
Al_Fao	8.5	3.0	10.2	0.6	9.3	0.33
Merkazi	25.3	5.0	29.5	6.6	25.3	6.1
Malaga	28.5	3.0	29.8	7.0	20.0	8.0

The incidence of *A. flavus* in seeds of crop other than groundnut collected from the villages and the three local markets, under investigation, are shown on Tables 4 and 5, respectively. Phaseolus, faba bean and chick peas were the most infected all through, the three villages and the three markets, while, wheat and sorghum gave very low percentages of *A. flavus* infection. However, wheat and sorghum are cultivated there and usually a new crop is available. On the other hand phaseolus and faba bean are usually bought from the central and main markets, to be sold in the villages. Higher levels of infection in these seeds could be due to the bad handling procedures and the storage conditions. It was well established that the storage conditions can greatly affect the incidence of *A. flavus* in commodities (Sanders, 1968; Diener and Davis, 1967).

Table 4. Percent *A. flavus* infection in seeds of crops other than groundnut from the three villages

Crop	Kareeba	Sureeba	Basatana
Wheat	2.20	3.01	2.25
Faba bean	16.25	18.48	15.23
Phasolus	30.33	30.67	40.23
Chich pea	8.22	7.30	12.50
Sorghum	2.67	1.22	1.23

Table 5. Percent *A. flavus* infection in seeds of crops other than groundnut collected from the three local markets.

Crop	Local markets		
	Al Hoosh	Medina Arab	Al Fao
Wheat	2.33	2.67	1.28
Faba bean	15.00	20.33	22.33
Faseolus	27.33	38.67	55.67
Chick pea	16.15	18.33	19.11
Sorghum	3.67	3.23	5.67

The moisture content, *A. flavus* incidence and the levels of aflatoxin contamination of seeds of crops other than groundnut collected from the two main central markets in Wad Medani city, were also studied in this work. The seeds included; those which were sold in plastic bags and those which were non-bagged.. The moisture content in the bagged seeds was much greater than that of the non-bagged ones (Table, 6). It has been reported that the moisture content was the key factor in aflatoxin production by *A. flavus* (Sanders, 1968; and Hag Elamin *et al.* 1988). However, Davis and Diener (1966) came to

the conclusion that no detectable amounts of aflatoxin B1 could be produced at low relative humidity levels. They also added that the optimum moisture content for aflatoxin production is depending on the type of the crop seeds tested.

Table 6. Moisture content of seeds of crops other than groundnut collected from the two main markets and stored for 1 month

Crop	Al Malaga		Al merkazi	
	Bagged	Non-bagged	Bagged	Non-bagged
Wheat	17.50	9.22	18.22	8.00
Faba bean	12.67	8.33	15.67	7.63
Faseolus	18.40	6.40	17.22	6.40
Chick pea	16.00	8.35	15.33	8.55
Sorghum	13.60	9.70	10.60	8.90

Mean = 15.5

SD = 3.14

The incidence of the fungus *A. flavus* was higher in seeds sold in thin plastic bags in both markets as was reflected by percent infection (Table, 7). The moisture was reported by many authors, as a very important factor for growth of this fungus (McDonald and Harkness, 1963 and Al-Jali *et al.*, 1997).

Table 7. Percent infection of seeds of crops other than groundnut collected from the two main markets in Wad medani and stored for 1 month.

Crops	Al Malaga		Al merkazi	
	bagged	Non- bagged	bagged	Non- bagged
Wheat	58.13	28.67	50.33	29.50
Faba bean	95.50	59.20	90.20	26.33
Faseolus	95.53	71.00	96.00	65.00
Chick pea	88.20	55.06	90.00	51.67
sorghum	67.33	62.20	81.03	61.50

Mean = 15.5

SD = 78.05

The results on Table (8) indicated that all types of seeds sold in thin plastic bags accumulated large amounts of aflatoxins compared to non-bagged ones. As mentioned above, plastic bags increased the moisture content of the seeds and at the same time enhanced growth of the fungus, the conditions that no doubt lead to large production of aflatoxins. Surveys carried out over the past decade in many parts of the world showed that aflatoxins can contaminate some of the staple food materials consumed by human, particularly, plant foods that contribute proteins and calories, especially grains and oil seeds (Amla et al, 1974; Abdalla, 2001 and Abdel-Rahim, 2005).

Table8. Levels of aflatoxin contamination (ppm) of seeds of crops other than groundnut collected from the two markets of Wad Medani and stored for 1 month.

<i>Crops</i>	<i>Bagged</i>	<i>Non-bagged</i>	<i>Bagged</i>	<i>Non-bagged</i>
<i>Wheat</i>	3.26	0.21	2.91	0.91
<i>Faba bean</i>	5.16	1.16	3.67	1.68
<i>Faseolus</i>	9.60	3.25	3.67	2.50
<i>Chick pea</i>	6.33	2.33	5.33	2.10
<i>Sorghum</i>	3.50	0.91	1.90	0.80

## CONCLUSIONS

*Aflatoxin contamination levels were at their minimum in groundnut samples collected from the villages in the Gezira state (Basatna, Kureeba, Sureeba) and the local markets (Al-Hoosh, Al-Fao, and Al-Medina Arab), while, high levels were recorded on seeds of the two main markets in Wad Median (Al-Malaga and Al-Merkazi).*

*Aflatoxin contamination was found to be high in seeds from insect damaged pods, compared to the mechanical harvested damage pods. On the other hand healthy intact pods are free from aflatoxin contamination.*

*Regarding aflatoxin contamination of the different groundnut products (paste, grey and red roasted nuts), both grey and roasted nuts were highly contaminated when compared to the healthy groundnut seeds.*

*Pagged samples of phaseolus and faba bean seeds were the most infected with A. flavus, all through, the three villages and the three markets while, wheat and sorghum samples gave very low percentages of fungal growth. However, the same pattern was observed for aflatoxin of bagged and non- bagged seeds of these crops. The moisture content in the bagged seeds was much greater than that of the non- bagged seeds. Moisture content was the key factor in aflatoxin production by A. flavus.*

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## تلوث بذور بعض أنواع المحاصيل ومنتجاتها بالأفلاتكسينات

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### الخلاصة

تم في هذا البحث دراسة مستوى التلوث بالافلاتكسينات وكذلك الإصابة بالفطر (*A. flavus*) في عينات الفول السوداني التي تم جمعها من مناطق مختلفة بولاية الجزيرة . ووجد أن مستويات التلوث كانت أقل في العينات التي جمعت من القرى (مناطق الإنتاج) مقارنة بمستويات عالية جداً في العينات التي جمعت من الأسواق بمدينة ود مدني. ومع ذلك فإن العينات التي تم جمعها من الأسواق المحلية بمناطق الإنتاج كانت أقل تلوثاً. وبالإضافة لذلك فقد أتبعَت الإصابة بالفطر (*A. flavus*) نفس النسق. التلوث بالافلاتكسينات كان عالياً في بذور الفول السوداني التي تم جمعها من قرون الفول المصاب بالحشرات في حين كانت البذور السليمة خالية من الافلاتكسينات. وفيما يتعلق بتلوث منتجات بذور الفول السوداني بالافلاتكسينات فقد أثبتت الدراسة أن الفول المحمص (الرمادي والأحمر) كلاهما كان عالي التلوث خاصةً عند وضعهما في أكياس البلاستيك. بذور البقوليات (الفاصوليا، الفول المصري والبسلة) كانت أكثر تلوثاً بالافلاتكسينات ونسبة الإصابة بالفطر فيها كبيرة مقارنة مع بذور الحبوب (القمح، الذرة الرفيعة). وضع تلك البذور في أكياس البلاستيك أدى إلى ارتفاع الرطوبة ودرجة الإصابة بالفطر وكذلك إلى وجود كميات كبيرة من الافلاتكسينات.