

Effect of Groundnut Pod Condition on the Microbial Content and Aflatoxin Contamination in the Groundnut Seeds

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ABSTRACT

The present study was investigated the effect of groundnut pod condition on the microbial content and aflatoxin contamination in the groundnut seeds in Sudan, which collected from irrigated area (Gezira) and rain-fed area (Al-fao and Kordofan). The samples were investigated for their fungal growth using potato dextrose agar (PDA) media and for their aflatoxin contamination using thin layer chromatography (TLC) technique. High percentage of the groundnut seeds were found unshelled sound intact (53.33-63.00%), while the low percentage was unshelled shrink/damaged (10.33-19.34%). The infection by *A. flavus* and aflatoxins contamination were found to be high in the split samples either shelled or unshelled which collected from Gezira area (56.67%), whereas, the low percentage was (10.00%) in the unshelled shrink/damaged samples which collected from Kordofan area. Microbial content showed that the sound intact seeds either shelled or unshelled were free from *A. flavus* and aflatoxins, while the split and shrink/damaged samples either shelled or unshelled were infected by *A. flavus* and contaminated by aflatoxins. Moreover, High percentage of fungus infection other than *A. flavus* were obtained (40.00-43.33%) in split and shrink/damaged shelled samples, however, low percentage were obtained (10.00%) in intact samples either shelled or unshelled which collected from Gezira area.

Keywords: Groundnut, aflatoxins, *A. flavus*, shelled, unshelled

INTRODUCTION

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Groundnut (peanut, earthnut), (*Arachis hypogaea* L.) is one of the most important food and oil crops cultivated and utilised in most parts of the world as annual legume native to south America (Murphy, 1993; Gibbons *et al.* 2002). It is known by many other local names such as goober peas, monkey nuts, pygmy nuts and pig nuts (Wikipedia, 2016), which belong to the genus *Arachis* of the family *Fabaceae* (Erickson, 1990; Salunkhe *et al.*, 1991; Abd elazem, 2006). It is a major cash crop and is widely grown practically in all the tropical and subtropical regions of the world (Willume and Siha, 1999; Mariod 2005).

In the seventies, groundnut was one of the most exported crops in Sudan. It was the second exporting country after the United States of America; it was exporting about 22% of the total world export, and the revenue exceeded one hundred million dollars annually. Since the beginning of the eighties, the export of groundnut started to decline to less than one million dollars. Many factors were considered as the reasons for deterioration and instability of the groundnut export; including reduction groundnut production, increasing of local consumption, and existence of new competitors in the international market as well as reduction in the quality levels of which the contamination with aflatoxin is one of its main reasons (Osman and Khalid, 2006). Contamination of groundnut and their products with aflatoxins was reported in several studies carried out in Turkey, China, Iran, Senegal and Sudan (Idris *et al.* 2013; Li *et al.* 2009; Atanda *et al.* 2013).

Mycotoxins are a large group of secondary metabolic products from fungi or molds, which pose serious risks in both humans and livestock. Fungal growth and mycotoxin production may occur in the field and/or during storage, under suitable temperature and humidity conditions (Bryden, 2012). Aflatoxins are mycotoxins produced by two species of *Aspergillus* (*A. flavus* and *A. parasiticus*) *A. flavus* is a fungus which is especially found in areas with hot and humid climates (Abdel-rahim, 2005). The four major types of aflatoxins are B1, B2, G1 and G2. While M1 and M2 are metabolites of B1 and B2 are found in the milk of mammals fed with aflatoxin contaminated diets (Egal *et al.* 2005). Groundnut can become particularly susceptible to *Aspergillus*, resulting in aflatoxin contaminated groundnuts, which could be used for human consumption and animal feed (Okello *et al.* 2013). As per the International Agency for Research on Cancer (IARC), aflatoxins are considered group 1 carcinogen (Zinedine and Mañes, 2009; IRAC, 2015). Majority of the aflatoxins reported till date are potentially carcinogenic, teratogenic, tremorogenic, nephrotoxic, immunotoxic or hemorrhagic (Bhat *et al.* 2010). The liver is the primary target organ for aflatoxins long-term intake of feeds contaminated with aflatoxins results in negative effects on the liver, such as hepatic cell and tissue injury, as well as gross and microscopic abnormalities (Williams *et al.*, 2011; Gholami-Ahangaran *et al.*, 2016). Good agricultural practices during both pre-harvest and post-harvest conditions would minimize the problem of contamination by mycotoxins (Stephen-Blezinger, 2002). The aim of this study was to investigate the presence of *A. flavus*, microbial content and aflatoxin contamination in groundnut seeds obtained from different areas in Sudan.

MATERIALS AND METHODS

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A total of 54 samples of groundnut seeds decorticated (shelled) and unshelled were obtained from Gezira (18 samples), Al-fao region (18 samples) and Western Sudan (18 samples), which were collected from different local and central markets.

The samples were investigated for their fungal growth using potato dextrose agar (PDA) media. The groundnuts samples were washed several time with sterile distilled water, and then dried on a filter paper to remove any residual water. Five pieces of these dried groundnut seeds were distributed on the surface of a solidified PDA medium in a Petri-dish. Inoculated plates were incubated in an incubator at 28 - 30°C. The plates were investigated daily for fungal growth (McDonald and Harkness, 1963). Colonies of the fungus, *A. flavus* green colour were detected and calculated as percent from the 100 seeds of each treatment. Contamination with fungi other than *A. flavus* was also calculated.

Aflatoxins were determined; as follow, pods of each sample were thoroughly mixed, spread on a clean surface and quartered. From each quarter about 100 g were weighed and the seeds were ground in a coffee grinder (Type Mcoc). 20 g from each of the ground material were extracted with hexane (boiling rang 60 - 80°C) in 100 ml soxhelt extraction for 4 hours (Coomes *et al.*, 1965). The residual solvent was dried from the defatting material by heating in a forced drought oven at 105°C for 30 minutes. From the defatted sample 10 g were taken and placed into 250 ml flask, 10 ml of distilled water were added, and were then thoroughly mixed. An amount of 100 ml chloroform were then added and the flasks were Stoppard with a rubber plug coated with aluminium foil to protect the rubber from being attacked by the chloroform. The flasks were shaken on a griffin shaker for 30 minutes to ensure good extraction. The content of each flasks were filtered after shaking, through a filter paper (24 cm). The chloroform was then evaporated to dryness in a water bath 70°C (AOAC, 1999). Quantitation of the toxin was accomplished by the TLC technique, standard aflatoxins B1, B2, G1 and G2 were used throughout. The pre-coated chromatographic papers were used and heated in an oven at 105°C For 1 hour. The papers were then cooled in a dust free atmosphere for 30 minutes before being placed into a plate cabinet. The dried extracted samples were washed in a known volume of chloroform. An amount of 5 to 25 µl of the solution was spotted on the prepared TLC paper by a micro syringe. The papers were then dried before being developed in a chromatic tank. The loaded chromatographic papers were developed in a diethyl ether solution in a chromatographic tank, and then were allowed to dry before they were redeveloped in a solution of a mixture of chloroform-methanol (97:3 v/v, respectively). The solution was allowed to move for 10 cm above the base line of the paper. The papers were dried and examined in a dark room under ultra violet light lamp (peat emission 366 mm, philips Hp w 125 watts type) at a distance of about 30 cm from the lamp (Diener and Davis, 1968; Jones, 1972).

The data were collected and subjected to analysis of variance (ANOVA). Means were tested and separated by using the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ as reported by Steel *et al.* (1997).

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RESULTS AND DISCUSSION

Table (1) presented grading of groundnut seed samples into decorticated (shelled) and unshelled, pods were graded into three categories: sound intact, split and shrink/damaged harvest. The ANOVA analysis proved that there are no significant difference ($P \leq 0.05$) between the samples. High percentage of intact unshelled was obtained ranged (53.33-63.00%), while low percentage of shrink/damaged unshelled was obtained ranged (10.33-19.34%).

Table 1: Percentage of sound intact, split and shrink/damaged groundnut seeds collected from Gezira, Al-fao and Kordofan area.

Treatments	Pod condition	Percentage		
		Gezira	Al-fao	Kordofan
Decorticated (Shelled)	Intact	25.67 c	46.33 b	37.67 b
	Split	47.33 b	34.67 c	34.67 b
	Shrink / Damaged	27.00 c	19.00 e	27.66 c
Unshelled	Intact	53.33 a	63.00 a	61.67 a
	Split	27.33 c	24.67 d	28.00 c
	Shrink / Damaged	19.34 d	12.33 f	10.33 d
SE±		0.98	0.85	1.04
CV%		14.93	11.18	17.00

* Means in the same column followed by the same letter (s) are not significantly different according to the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

* Where as SE Standard Error.

* Where as CV Coefficient of Variation.

Table (2) presented microbial content and aflatoxin contamination of the groundnut seeds (different categories) which collected from Gezira area. The ANOVA analysis proved that the sound intact samples either shelled or unshelled were free from *A. flavus* and aflatoxins. The percentage of incidence of *A. flavus* in split shelled and unshelled samples were found average 56.67% , whereas, shrink/damaged shelled and unshelled samples were found average 40.00%. The split and shrink/damaged either shelled or unshelled samples were contaminated by aflatoxins. High percentage of *A. niger* infection was found average 36.67% in shrink/damaged shelled samples, while low percentage was average 16.67% obtained in intact shelled samples. High percentage of *Rhizopus* and other fungi infection were obtained ranged (40.00-43.33%) in split and shrink/damaged shelled samples, however, low percentage were obtained ranged (10.00-23.33%) in intact samples either shelled or unshelled.

Table 2: Microbial content and aflatoxin contamination of the groundnut seeds (different categories) collected from Gezira area.

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Treatments	Pod condition	Percentage				Aflatoxin contamination
		<i>A. flavus</i>	<i>A. niger</i>	<i>Rhizopus</i>	Other fungi	
Decorticated (Shelled)	Intact	0.00 c	16.67 d	23.33 c	10.00 c	-
	Split	56.67 a	30.00 b	40.00 a	40.00 a	+
	Shrink / Damaged	40.00 b	36.67 a	40.00 a	43.33 a	+
Unshelled	Intact	0.00 c	23.33 c	20.00 c	10.00 c	-
	Split	56.67 a	26.67 bc	30.00 b	26.67 b	+
	Shrink / Damaged	40.00 b	26.67 bc	30.00 b	26.67 b	+
SE±		0.91	1.48	1.62	1.54	
CV%		35.6	42.8	47.2	50.5	

* Means in the same column followed by the same letter (s) are not significantly different according to the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

* Where (-) = Non detected and (+) = Detected

* Where as SE Standard Error.

* Where as CV Coefficient of Variation.

Table (3) presented microbial content and aflatoxin contamination of the groundnut seeds (different categories) which collected from Al-fao area. The ANOVA analysis proved that the sound intact samples either shelled or unshelled were free from *A. flavus* and aflatoxins. The incidence of *A. flavus* in split shelled was found average 33.33%, while split unshelled was found average 26.67%. Shrink/damaged shelled was found average 20.00%, whereas, shrink/damaged unshelled was found average 16.67%. The split and shrink/damaged either shelled or unshelled samples were contaminated by aflatoxins.

High percentage of *A. niger* infection was found average 23.33% in shrink/damaged samples either shelled or unshelled, however, low percentage was 10.00% obtained in intact samples either shelled or unshelled. High percentage of *Rhizopus* infection was found average 33.33% in split unshelled samples, while low percentage was 13.33% obtained in intact shelled samples. High percentage of other fungi infection were found average 26.67% in shrink/damaged unshelled samples, whilst low percentage was found average 13.33 % in intact samples either shelled or unshelled.

Table 3: Microbial content and aflatoxin contamination of the groundnut seeds (different categories) collected from Al-fao area.

Treatments		Percentage	Aflatoxin contamination
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	Pod condition	<i>A. flavus</i>	<i>A. niger</i>	<i>Rhizopus</i>	Other fungi	
Decorticated (Shelled)	Intact	0.00 e	10.67 c	13.33 e	13.33 c	-
	Split	33.33 a	16.67 b	23.33 cd	20.00 b	+
	Shrink / Damaged	20.00 c	23.33 a	26.67 c	23.33 ab	+
Unshelled	Intact	0.00 e	10.67 c	20.00 d	13.33 c	-
	Split	26.67 b	23.33 a	33.33 a	23.33 ab	+
	Shrink / Damaged	16.67 d	23.33 a	30.00 b	26.67 a	+
SE±		0.82	1.05	1.41	1.27	
CV%		22.5	29.2	42.0	41.8	

* Means in the same column followed by the same letter (s) are not significantly different according to the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$

* Where (-) = Non detected and (+) = Detected.

* Where as SE Standard Error.

* Where as CV Coefficient of Variation.

Table (4) presented microbial content and aflatoxin contamination of the groundnut seeds (different categories) which collected from Kordofan area. The ANOVA analysis proved that the sound intact samples either shelled or unshelled were free from *A. flavus* and aflatoxins. The incidence of *A. flavus* in split shelled was found average 30.00%, whilst split unshelled was found average 20.33%. Shrink/damaged shelled was found average 23.33%, while shrink/damaged unshelled was found average 10.00%. The split and shrink/damaged either shelled or unshelled samples were contaminated by aflatoxins.

High percentage of *A. niger* infection was found average 30.00% in split shelled samples, whereas, low percentage was 16.67% obtained in intact shelled samples. High percentage of *Rhizopus* infection was found average 33.33% in split unshelled samples, however, low percentage was 16.67% obtained in intact unshelled samples. High percentage of other fungi infection were found average 33.33% in shrink/damaged unshelled samples, while low percentage was 23.33% of intact unshelled samples.

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Table 4: Microbial content and aflatoxin contamination of the groundnut seeds (different categories) collected from Kordofan area.

Treatments	Pod condition	Percentage				Aflatoxin contamination
		<i>A. flavus</i>	<i>A. niger</i>	<i>Rhizopus</i>	Other fungi	
Decorticated (Shelled)	Intact	0.00 d	16.67 d	26.67 b	26.67 bc	-
	Split	30.00 a	30.00 a	26.67 b	30.00 ab	+
	Shrink / Damaged	23.33 b	26.67 b	30.00 ab	30.00 ab	+
Unshelled	Intact	0.00 d	20.00 c	16.67 d	23.33 c	-
	Split	20.33 b	26.67 b	33.33 a	30.00 ab	+
	Shrink / Damaged	10.00 c	23.33 bc	20.00 c	33.33 a	+
SE±		1.05	1.05	1.14	1.41	
CV%		36.5	23.8	26.4	35.6	

* Means in the same column followed by the same letter (s) are not significantly different according to the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$

* Where (-) = Non detected and (+) = Detected.

* Where as SE Standard Error.

* Where as CV Coefficient of Variation.

Overall the sound intact samples either shelled or unshelled were free from *A. flavus* and aflatoxins contamination, this result is similar to results reported by Ahmed (1981), Hag Elamin *et al.* (1988) and Abdel-Rhim *et al.* (2010). The split and shrink/damaged samples either shelled or unshelled were infected by *A. flavus* and contaminated by aflatoxins. There are many factors affect the aflatoxins contamination; bad handling, storage conditions which increase the moisture content and substances secreted from the wounded pods which may stimulate growth of *A. flavus*, thus, allowed more aflatoxin production (El Nour and Ibrahim, 1970; Griffin, 1970). Moreover, the fact that invasion by insect provide sites of injury and serve as vectors for *A. flavus* transmission in the seeds. Orum *et al.* (2007) postulated that temperature, soil condition, day length, crop sequence history, insect levels, rainfall frequency and management practice may influence aflatoxin producing *Aspergillus* communities. Many authors reported an increased of aflatoxin contamination in post-harvest groundnut samples; which were produced and stored during the production period, normally from October to January, then again stored till April or even May, waiting for prices to increase and then transferred to the biggest market, also equipped with many factories and mills. The seeds can again be stored in a couple of months in mills until oil extraction. This long storage period under hot and relatively humid conditions might also be

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responsible for aflatoxin contamination (Idris *et al.* 2010; Mutegi *et al.* 2013; Mariod and Idris, 2015).

Peanut pods are easily infected by aflatoxin-producing *Aspergillus* species from field soil. To assess the aflatoxin-producing *Aspergillus sp.* in different peanut field soils, soil is the main source of inoculum for aflatoxigenic *Aspergillus* species, and since peanut pods grow underground, they are in direct contact with the soil fungal population. The soil type, landform and rainfall had a greater influence on the growth of aflatoxin-producing *Aspergillus* in different agroecological zones. (Zhang *et al.* 2017). However, the results indicated that the aflatoxins contamination in the irrigated area is relatively high than the rain-fed area, which contrasted with previous reports indicate that groundnuts grown under rain-fed conditions are subjected to drought stress and accumulate more aflatoxins before digging than those grown under irrigation (McDonald and Harkness, 1963). Ding *et al.* (2014) researched the distribution of aflatoxins contamination in post-harvest groundnut in China, the highest was observed in the Yangtze River (YR) ecological region and the lowest in Northeast zone (NE).

Almost, the samples of the groundnut seeds are contaminated with aflatoxin. The infection by *A. flavus* and aflatoxin contamination were found to be high on the split samples either shelled or unshelled which collected from Gezira area. Adopting internationally recommended harvest procedures at farm levels by implementing hazard analysis and critical control point (HACCP) procedures as well as adopting good agriculture and good manufacturing practices (GAP and GMP) might significantly reduce the aflatoxins contamination in fresh produce. Some of the important criteria to be practiced at the farm level include: time of harvesting (early harvesting is recommended), handling of produce without injury, drying to acceptable moisture and water activity levels, proper transportation and premarketing storage to prevent damp storage abuse and minimizing insect infestation.

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