The incidence of mycological and aflatoxin contamination of groundnuts collected from Khartoum state in Sudan

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Abstract

This study assessed the groundnuts collected from Khartoum state. Forty groundnut samples were collected randomly at informal six markets in the three cities (Khartoum, Omdurman, Bakri) and analyzed for mycoflora and aflatoxins (B1, B2, G1, and G2) using standard methods. The procedure of Vicam (Vicam, 2016) was followed in this test. . The results indicated the incidence of contamination of different groundnut seeds types by *Aspergillus* spp. collected from different markets in Khartoum State varied from 96.5 % in crushed seeds collected from Souq-klaklah in Khartoum city to 7.6% in roasted seeds from Souq-Bahri illustrate that the highest average of *Aspergillus* contamination incidence was reported in crushed seeds (92.95 %) followed by raw seeds (63.40%), whereas roasted seeds showed the lowest incidence (11.70%).

Keywords: Aspergillus; aflatoxigenic fungi; mycotoxin; aflatoxin; groundnut; Khartoum; Sudan

1. Introduction

Developing countries, especially in Africa, face many socio-economic challenges of which poor food security and food safety are paramount (Schmidhuber and Tublello, 2007)**.** Aflatoxins are more prevalent in tropical and sub-tropical areas where environmental conditions such as high temperature and humidity prevail, which favor the growth of fungi and the production of mycotoxins on the crops (Klich, 2007). More often discreet and only a few perceptible, molds form a part of many microorganisms which contaminate foods in storage or before when the conditions are favorable (D'mello, 2003). Concerning the above, many countries, as well as multilateral agencies have established regulations to protect human beings from consuming highly contaminated food (Kamika and Takoy, 2011

Poor food safety in Africa remains a major concern, especially with regard to the consumption of food contaminated with aflatoxins (Sephard, 2003; Wagacha and Muthomi, 2008).

Considered as the most important mycotoxin, aflatoxin is a natural potent carcinogen known to affect both humans and animals. The evidence on a synergistic interaction between aflatoxin and hepatitis B virus contamination in the incidence of liver cancer with hepatocellular carcinoma as the most prevalent type has been reported (Moss, 1996). Groundnuts are the third most important crop in Sudan. It is cultivated mainly to give rain Western Sudan region (Darfur and Kordofan) Irrigation system in the state) and Gezira. Peanuts and their products are the most important for that National export and local production for local consumption. Traditional Peanut butter "Dakuwa" is sold homemade by street vendors as one of the most popular meals Nationwide, especially among the poor Municipalities (Omer 2001)

Unfortunately, exposure to aflatoxin is often sporadic and hidden, so this problem is largely ignored in many developing countries. Since food consumption is one of the major health factors in humans, continuous monitoring of aflatoxin levels in peanut samples should be performed to prevent consumption of food contaminated with aflatoxin. However, there is evidence to suggest that aflatoxin contamination is a major food-safety concern in Sudan where the environmental conditions and socio-economic problems are conducive to poor storage management and subsequent food spoilage and aflatoxin contamination. This study investigated the presence aflatoxins in groundnuts samples collected from local markets in Khartoum- Sudan.

2. Materials and methods

2.1. Collection of Samples

Groundnuts samples (40 in number) were collected from retailers in six sites which were namely central and local whole markets in Khartoum proper; Kalakla and Shabi markets in Khartoum city; Bahri and Soug 6 markets in Khartoum North, and Omdurman and Shabi in Omdurman city. A ground weight of 25 g was taken from each sample using a digital sensitive balance and kept in a small plastic bag for the test. The procedure of Vicam (Vicam, 2016) was followed in this test. The steps of this test are as follows:
Determination of aflatoxins:

2.2. Sample extraction

1. Five grams of the test sample weighed and added to a 40 extraction tube.
2. 10 ml of 70% methanol were taken with a 10 ml graduated cylinder and poured into a 40 ml extraction tube.
3. The 40 ml extraction tube was then covered and the mixture was shaken well by hand for 1 min.
4. The mixture then was left to stand for 3 min.

2.2.3. Aflacheck procedure

1. Strip test dilution tube was placed in the paper rack found in the kit box.
2. Distilled water, 250 µL, was added to the strip test dilution tube using a 250 µL strip test pipettor.
3. Sample extract, 250 µL, was transferred to the strip test dilution tube by a new strip test pipettor. 4. The solution was mixed by capping the strip test dilution tube and agitating by hand. 5. Aflacheck® strip test (arrows pointing down) was inserted into the solution tube and left to develop. 6. The result was taken negative when two lines (of the test and the control) were noticed and positive when only one line (of the test) was observed in the test strip. The time needed is estimated as 3 min. 7. The time of development was up to 5 min for a better result. If after 5 min no test line appears then the results can be interpreted as positive. 8. When no line was observed the test was repeated using another test strip. The Afalcheck® test strip reflected one line in three samples from each of the two groups of samples from the two sites in Khartoum proper whereas all the other test samples showed the same result. However, SPSS statistics program was used for the significance at 5% level.

2.2.4. Fungal isolation

Forty groundnuts seeds per sample were surface sterilized with 10% Chlorox solution for 1 min, followed by immersion in sterile distilled water for 1 min. Surface sterilized seeds were then placed on freshly prepared potato

dextrose agar (PDA) plates (five seeds per plate) and incubated for four days at 25°C. The incidence was calculated as percentage of infected seeds compared to total number of seeds tested. Pure cultures of different out growing fungi were obtained by transferring fungal colonies to new PDA plates using sterile toothpicks, and incubating the plates for 5-7 days at 25°C.

2.2.5. Species identification

Isolates were identified to a species level based on morphological (phenotypic) features as described by Raper and Fennell (1965), Cotty (1994), and Okuda et al. (2000). For this purpose, isolates representing each pure culture were grown on and PDA at 25°C for 5-7 days. Fungal colonies that grew rapidly and produced colors of white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores were broadly classified as Aspergillus spp. The major distinction currently separating *A. niger* from the other species of Aspergillus is the production of carbon black or very dark brown spores from *Biseriate phialides* (Raper and Fennell, 1965). Those, which produce conidia with smooth surface and colonies typical of *A. flavus* were recorded as *A. flavus.*

3. Results and Discussion

3.1. Incidence of Aspergillus contamination

The incidence of contamination of different groundnut seeds types by *Aspergillus* spp. collected from different markets in Khartoum State varied from 96.5 % in crushed seeds collected from Souq-klaklah to 7.6% in roasted seeds from Souq-Bahri (Table 1). Data in Fig. 1 illustrate that the highest average of *Apergillus* contamination incidence was reported in crushed seeds (92.95 %) followed by raw seeds (63.40%), whereas roasted seeds showed the lowest incidence (11.70%).

Aspergillus flavus populations are genetically diverse and phenotypic variations have been well documented. Isolates vary considerably in their ability to produce aflatoxins and colonize plants. They generally can be grouped into two sclerotial morphotypes, L strains and S strains also named *A. flavus* var. *parvisclerotigenus* (Saito and Tsuruta, 1993). L strain isolates produce abundant conidiospores and sclerotia that are usually larger than 400 µm in diameter, whereas S strain isolates produce fewer conidiospores and numerous sclerotia that are usually smaller. The S strain isolates typically produce higher amounts of aflatoxins than the L strain isolates on the same media. The aflatoxigenic trait of the S strain isolates seems very stable. In contrast, a significant portion of A. flavus L strain field isolates do not produce aflatoxins (Chang et al., 2006).

3.2. Identification of Aspergillus species associated with groundnut seeds

Two *Aspergillus* spp. were found to be associated with groundnut samples collected from different markets in Khartoum State. The first species isolated from the collected samples was *A. niger*. The major distinction separating *A. niger* from the other species of *Aspergillus* is the production of carbon black or dark brown spores. *A. flavus* was the second species identified in this study. Colonies of this fungus were characterized by yellowish-green pigments, consisting of a dense felt of conidiophores or mature vesicles bearing phialides over their entire surface (Fig 2)

Aflatoxin contamination is the most important quality problem in groundnuts worldwide with serious commercial implications. To minimize the health and economic implications associated with the presence of aflatoxins in food and feedstuff, several guidelines have been developed and legislation has been adopted, both at international and national levels. According to the Act as well as the WHO, all foodstuffs containing more than 10 µg/kg aflatoxin, of which AFB1 should not be more than 5 µg/kg, are deemed contaminated and not fit for human consumption. In addition, the Codex Alimentarius Commission on Food Additives and Contaminants set the limit for total aflatoxin at15 µg/Kg, half of this limit being for AFB1(Henry et al., 1999). Astringent regulation has been reported by the European Commission which set the maximum limit for aflatoxins at 4 µg/Kg and for AFB1 at 2 µg/Kg (Wu, 2004).

Table (1): Proportion of groundnut seeds infected by *Aspergillus* spp. collected from different markets in Khartoum State

Location	Market	Seed infection (%)		
		Row	Crushed	Roasted
Khartoum	Souq-Klaklah	90.5	96.5	8.5
	Souq-Elshabi	80.4	94.7	9.3
Khartoum-North	Souq-Bahri	75.3	98	7.6
	Souq-6	54	87	11.4
Omdurman	Souq-Omderman	35.7	92.2	16
	Souq-Elshabi	44.5	89.3	17.2
Average		**63.40**	**92.95**	**11.70**

Table 2: Aflacheck® test results of Groundnuts samples

Sample	Raw	Crushed	Roasted	SE	CV
Souq-Klaklah	3.3533^{a}	93.00^{a}	0.5600^{ab}	7.3820	57.59
SouqOmdurmann	1.1111^{b}	34.00^{bc}	1.1700^{a}	0.5969	123.09
Souq-Elshabi	1.0578^{b}	50.30^{b}	0.8933^{ab}	0.0558	72.52
Souq-6	0.2667^{b}	120.00^{a}	0.6067^{ab}	0.3257	97.39
K. shabi	0.2600^{b}	7.27^{c}	0.5433^{ab}	0.5265	19.644
Souq-Bahri	0.1233^{b}	5.67^{c}	0.4833^{b}	0.2245	17.676

The study shows that groundnuts samples from Khartoum are more susceptible to fungi spoilage. This might be due to environmental factors, the socio-economic situation as well as lack of enforcement of food regulation. The study suggests that the government should learn from Sudan in terms of policy design and implementation of food safety and security measures such as:

- To initiate an awareness program on mycotoxin contamination among subsistence, emerging-commercial and commercial farmers.
- To design and build good warehousing which meets all safety requirements for storage of foods such as peanut.
- To also establish a good groundnut, transfer system from the storage facility to the shelling plants to selling points.
- To develop adequate sanitary facilities as well as an effective pest control program.

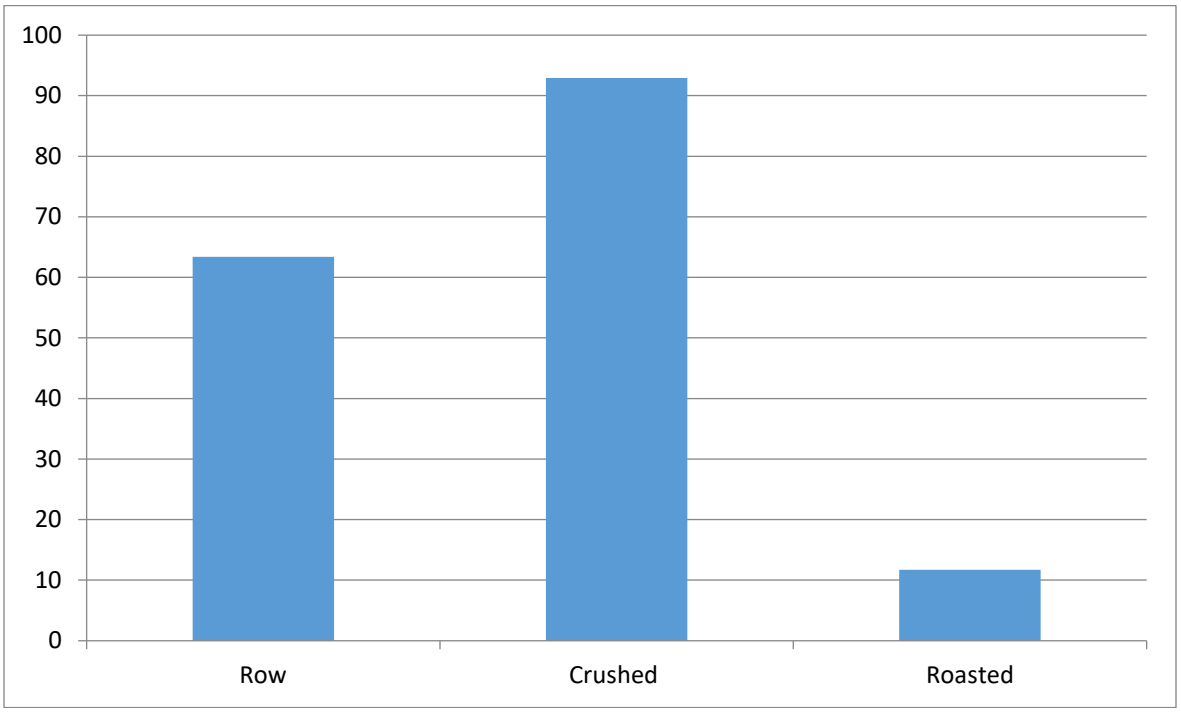

Fig. 1. Incidence of *Apergillus* infection in groundnut seeds (row, crushed and roasted) collected from different markets in Khartoum State.

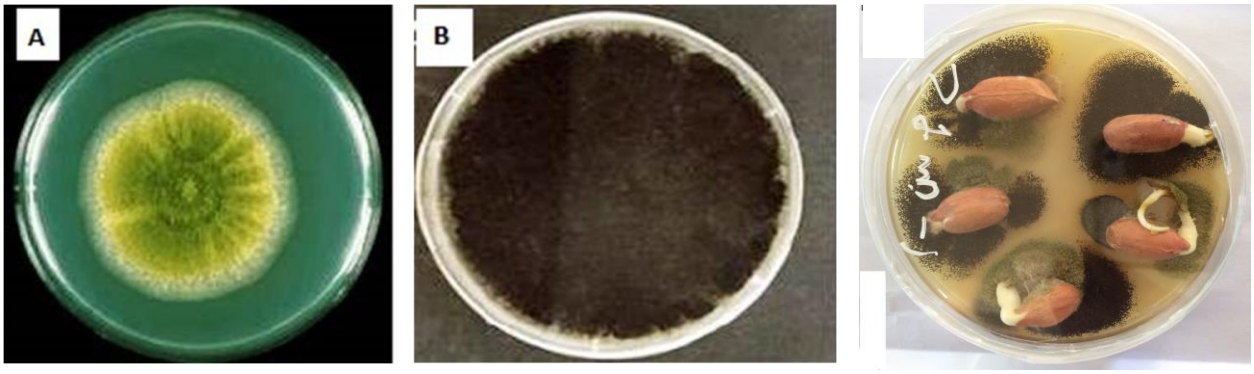

Fig. 2. *Aspergillus* spp. isolated from groundnut samples. (A): *A. flavus*, (B): *A. niger*, C: groundnut seeds contaminated by *Aspergillus* spp.

4. Conclusion

The results obtained in this study reflect high contamination of groundnuts with aflatoxins which are very alerting and alarming for the importance of controlling these deadly carcinogens. However, this study also satisfied the training needs about the use of the Aflacheck® test for the store managers of the Agricultural Bank of Sudan who will, by their turn, transfer this technology to other beneficiaries and will elevate the safety level of food, using available and easy to use method, at least for a very important and susceptible sector of the Sudanese community to these carcinogens, the children

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Conflict of Interest

There are no conflicts of interest.

References

Schmidhuber J. and Tublello, F.N. 2007. Global security under climate change. PNAS 104(50): 19703-19708.

Klich, M.A. 2007. Aspergillus flavus: the major producer of aflatoxin. Molecular Plant Pathology 8(6): 713-722

D'mello J. P. F. 2003. Mycotoxins in cereal grains, nuts and other plant products. In: D'Mello J.P.F. (ed.) Foods safety: Contaminants & toxins. Wallingford: Cab International Publishing. pp. 65-88.

Kamika I. and Takoy, L.L. 2011. Natural occurrence of aflatoxin B1 in peanut collected from Kinshasa, Democratic Republic of Congo. Food Control 22(11): 1760-1764

Ciegler A. and Bennett J. W. 1980. Mycotoxins and mycotoxicoses. Bioscicence 30(8): 512-515.

Viljoen J. H. 2003. Mycotoxins in grain and grain products in South Africa and proposals for their regulation. Ph.D. Thesis. Pretoria: University of Pretoria

Brera C., De Santis B., Debegnach F. and Miraglia M. 2008. Mycotoxins. In: Pico, Y. (ed.), Food contaminants and residue analysis. Oxford: Elsevier, pp. 363- 419.

Capriotti A L., Caruso G., Cavaleire C. Foglia P., Samperi R. and Lagana A. 2011. Multiclass mycotoxin analysis in food, environmental and biological 85 matrices with chromatography/mass spectrometry. Mass Spectrometry Reviews, In press. DOI: 10.1002/mars.20351

Shephard, G.S. 2009. Aflatoxin analysis at the beginning of the twenty-first century. Analytical and Bioanalytical Chemistry 395: 1215-1224.

Wagacha J. M. and Muthomi J. W. 2008. Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. International Journal of Food Microbiology 124: 1-12.

Moss, M.O. 1996. Mycotoxic fungi. In: Eley, A.E. (ed.) Microbial Food Poisoning, 2 nd edition. New York: Chapman & Hall. pp. 75-93**.**

Omer, R.E., Verhoef, L., Veer, P.V., Idris, M.O., Kadaru, A.M.Y., Kampman, E., Bunschoten A. and Kok F. J. 2001. Peanut butter intake, GSTM1 genotype and hepatocellular carcinoma: a case-control study in Sudan. Cancer Causes and 99 Control 12: 23-32.

Raper K B, Fennell DI (1965). The genus *Aspergillus*. Williams and Wilkins. Baltimore, Maryland. pp.686.

Cotty P J (1994). Comparison of four groups for the isolation of A. flavus group fungi. Mycopathologia. 125: 157-162.

Okuda T, Klich MA, Seifert KA, Ando K (2000). Integration of modern taxonomic methods for *Penicillium* and *Aspergillus* Classification. In: Samson RA, Pitt JI Editors. Hardwood Academic Publishers: Reading, UK. pp 83-100.

Scott P.M. Natural poisons. In Official methods of analysis of the Association of Official Analytical Chemists, 15th Edition; Helrich, K., Eds.; AOAC: Arlington, USA, 1990; pp. 1184- 1213.

Omer R. E. Studies on the effect of field storage environment on aflatoxin contamination of the Rahad Agricultural Scheme. M.Sc Thesis, Khartoum University, Sudan, 1992. 21.

Omer R. E, Bakker M.J, Van'tveer P. Hooganboom ,R.L.A.P, Polman, T.H.G, Alink G.M, Idris M.O, Kadaru A.M.Y, Kok F. J. Aflatoxin and liver cancer in Sudan. Nutr Cancer. 2001;32:174–80.

Henry S H., Bosch F.X., Troxell T.C. and Bolger P.M. 1999. Reducing liver cancer-global control of aflatoxin. Science 286(5449): 2453-2454.