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**Research Article**

# Aflatoxins in Groundnut Paste in Khartoum State, Sudan

**Esameldin Bashir Mohamed Kabbashi[1] and Salah Eldeen Abbas Ali[2]**

1. Food Research Center, Ministry of Science and Communications, Khartoum.
2. National Chemical laboratories, Federal Ministry of Health, Khartoum.

Corresponding Author: esameldinkabbashi@gmail.com, Tel.: +249 (912236967/ 922226554 and 122034853), Fax: +249 -185 – 311049



## Abstract

Aflatoxins are metabolites of the genus *Aspergillus*two species of which are capable of producing these chemicals *viz. A. falvus*and *A. parasitcus*. However, the former produces aflatoxin B1 and B2 and latter is responsible for producing both B types, G1 and G2. These fungi infect a lot of crops but the groundnut more commonly. These toxins have a lot of impacts on human health which include retarded growth in children, carcinogenicity, teratogenicity and they increase TB incidence. This study focused on the determination of the amount and type of aflatoxin in 12 samples of groundnut paste (dakwa) from different locations in Khartoum State, Sudan. The method used in this experiment is the AflaTest® HPLC. The obtained results showed that only two samples were contaminated with aflatoxin G with a magnitude of 6.17, and 6.76 µg/ kg G1 and G2 in sample one and 4.95 µg/ kg G2 in the other contaminated sample both collected from Geraif East location 1 and Geraif East location 2, respectively. The figures of the first sample (total aflatoxins) surpass the recommended upper limit by the EU and the Codex Alimentarius but the latter has a score lower than both EU and the Codex Alimentarius upper maximum limits (5 & 10 µg/ kg, respectively). These findings revealed a number of implications that include the dominance of the *A. parasiticus*in the test samples, the AFLG may be the common aflatoxin that contaminate dakwa in Khartoum State and the Geraif area peanut is the mostly infected compared

to other test areas that included two locations in Buri, two locations in Kalakla, two locations in Khartoum proper, two location in Kuku and two from Omdurman. The results of this study shed some light on the qualitative and quantitative aflatoxin contamination of dakwa in greater Khartoum besides an alert about the contamination intensity area wise. That is, the total aflatoxins (G1 & G2) in the contaminated sample one scored about 13 ppb which is higher than the maximum limit set by the Codex Alimentarius.

**Keywords:** Aflatoxins, *Aspergillus***,** AflaTest®, groundnut**,** Codex, and Khartoum.

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## Introduction

Aflatoxins are naturally occurring carcinogenic byproduct of common fungi on grains and other crops, particularly maize and groundnuts. They pose a significant public health risk in many tropical developing countries and are also a barrier to the growth of domestic and international commercial markets for food and feed [1]. It is also reported that domestic commodities most susceptible to aflatoxin are peanuts, corn, cotton – seed, and tree nuts (almonds, pecans, walnuts) and the most susceptible imported commodities to USA are Brazilian nuts and Pistachio nuts [2]. Moreover, aflatoxin is a toxic class 1 carcinogenic byproduct of fungi that colonize maize and groundnuts among other crops. However, more than 4.5 billion people (64% of the world inhabitants) in developing countries may be chronically exposed to aflatoxin in their diets [3]. Aflatoxin is also reported as a class 1 carcinogen that contributes to 28% of all new liver cancers. It also increases TB. The stunted children were found to have 30 – 40% more aflatoxin in their blood than those whose normal body weight. The percentage of stunted children was reported to be 46% for those less than five years of age in Malawi[4]. As climate shifts, so do the complex communities of aflatoxin – producing fungi. This includes changes in the quantity of aflatoxin – producers in the environment and alterations to fungal community structure. Fluctuations in climate also influence predisposition of hosts to contamination by altering crop development and by affecting insects that create wounds on which aflatoxin – producers proliferate. Aflatoxin contamination is prevalent both in warm humid climates and in irrigated hot deserts [5]. However, rain and temperature influence the crop phases differently with dry, hot conditions favoring aflatoxin contamination during the crop development and warm, wet conditions favoring it after maturation[5]. Postharvest contamination may take place due to a number of factors that include damage of kernels [3] crop stresses such as drought and insect infestation and inadequate drying and storing facilities [3] and soaking of shells to simplify hand shelling of groundnuts which induces ideal conditions for *Aspergillus* infection in Malawi[4]. The Association of Official Analytical Chemists (AOAC) members used a variety of methods in determining aflatoxins in peanuts which include the enzyme linked immunoassay (ELISA)[6], solid – phase radio immunoassay (RIA) [6], high pressure liquid chromatography [7] , differnt thin layer chromatography and minicolumn detection methods (Holaday – Velascoandmodified Holaday – Velasco methods)[8]**.** Difficulties in obtaining a representative sample of peanuts for aflatoxin analysis have been established in studies carried out by Whitaker and his coworkers [9]**.** However, random sampling of peanut butter jarsfrom a pallet is more representative than the analysis of any single case from that pallet. That is fewer jars randomly drawn will provide less error in estimation of the true level than will a larger number of samples drawn from a more limited area [10].Nevertheless, the discovery in 1963 that

aflatoxin, a potent hepatocarcinogento rats, was present in peanut meal and peanuts produced in the United States led to search for this toxin in peanut butter, a popular staple food for children [10]. Positive findings presented the FDA with a novel situation to control. Fortunately, the problem was resolved through the cooperative efforts of the FDA, USDA and the affected industries. This cooperation set the pattern for future control efforts [10]. International mycotoxin check sample program has had three aflatoxin – contaminated samples, raw peanut meal, finished peanut butter, and white corn meal, were analyzed in 139 laboratories in 34 countries using the BF, CB, Pons methods and HPLC for quantification. Significant and insignificant differences were reported, separately in different samples [11]. This study focused on the quantitative and qualitative contamination, using AfalTest®, of peanut butter in samples randomly collected from 6 areas and 12 locations in Khartoum State to shed illumination onto this silent human health enemy.

## Materials and Methods

Samples of peanut butter (1 kg each) were collected randomly from 6 areas (Buri, Khartoum proper, Kalakla, Kuku, Geraif East, and Omdurman), and 12 locations (2 samples from each area) within these areas in Khartoum State, Sudan. Each sample was homogenized gently before taking part of it for analysis. Analysis of these samples took place in the toxicology section, the National Health Laboratories of the Federal Ministry of Health, Khartoum. The procedure of AflaTest®[12] was followed in these analyses as follows

1.0. HPLC Set up.

2.0.*SampleExtraction*:
2.1. Weigh 25g of groundnut sample with 5g salt (NaCl) and place in blender jar.
2.2. Add to jar 100 ml methanol: water (80:20).
2.3. Cover blender jar and blend at high speed for 1 minute.
2.4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3.0.*ExtractDilution*
3.1. Pipet or pour 10 ml filtered extract into a clean vessel.
3.2. Dilute extract with 40 ml of purified water. Mix well.
3.3. Filter dilute extract through glass microfibre filter into a clean vessel.
4.0. Column Chromatography
4.1. Pass 4 ml of filtered diluted extract (4 ml = 0.2g sample equivalent) completely through AflaTest affinity column at a rate of about 1-2 drops/ second until air comes through column.
4.2. Pass 10 ml of methanol: water (20:80) through the column at a rate of about 2 drops/ second.
4.3. Repeat step 4.2 once more until air comes through column.
4.4. Place glass cuvette (VICAM part # 34000) under AflaTest column and add 1.0 mL HPLC grade methanol into glass syringe barrel. In this test clean glass flask WAS used instead.
4.5. E lute AflaTes t® column at a rate of 1 drop/second by passing the methanol

through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette. In this test clean glass flask used instead.

4.6. Add 1.0 ml of purified water to eluate. Inject 20-100 µl onto HPLC (Shimadzu®).

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0. Recovery: 76% at 20 ppb ($7B_1$:$1B_2$:$3G_1$:$1G_2$ aflatoxin mix).

6.0. Results of the aflatoxin were found by calculating the injected concentration of aflatoxin divided by the area of the standard in the chromatogram and multiplied the area of the sample. The results reflect that only two samples were contaminated with aflatoxin $G_1$ and $G_2$. The former has a score of 6.17 and 6.76 µg/ Kg (ppb) (G1 and G2, respectively and a total of 12.91 ppb) and the later 4.95 µg/ Kg (ppb) both were from Geraif East area locations 1 and 2, respectively.

## Results and Discussion

## Results

Ten test samples were found negative for aflatoxin. However, two samples were found positive for aflatoxin $G_1$ and $G_2$ from two locations in Geraif East. The recorded scores for both samples were 6.17 and 6.76 ($G_1$ and $G_2$ for sample 1) and 4.95 ($G_2$ for sample 2) µg/ Kg (ppb), respectively (Table, 1). However, the spikes of the aflatoxins $G_1$ and $G_2$ are displayed in Figures 1 (1.1. and 1.2) and 2 (2.1. and 2.2.), respectively. However, the contamination percentage of the test samples was 16.67%. Whereas, the difference in the retention time of the spikes between the standard(s) and the samples (Figures 1 and 2) are 0.139 and minute for $AFLG_1$ in sample one and 0.075 and 0.011 minutes for $AFLG_2$ in samples one and two, respectively. The corresponding data in another study in Finland recorded a 0.006 and 0.006 minute for $AFLG_1$ for aflatoxin in sample one and two and 0.096 and 0.003 minute for $AFLG_2$ for samples one and two, respectively [13]. The difference is more or less 0.1 minute when approximated to one decimal in both studies.

## Discussion

Table 1 displays the results of the sample analyses for aflatoxin in groundnut butter in Khartoum State. The contamination was confined to Geraif East area where two test samples taken from it recorded aflatoxin contamination of 12.93 ppb [$AFLG_1$, 6.17 and 6.76 $AFLG_2$ µg/ kg (ppb)] for sample 1 and 4.95 µg/ kg (ppb) $AFLG_2$ for the other sample (Table 1 & Figures 1 and 2). These results confirmed that the causal organism was *A. parasiticus* which is capable of producing both B and G aflatoxins [14]. This contamination affected 16.67% of the test samples. The aflatoxin contamination in groundnut samples checked in Khartoum State earlier was 10% in a range of < 0.5 – 135 µg/ kg (ppb) [15]. Nonetheless, groundnut samples, collected soon after harvest, from different districts in the irrigated region (Central Sudan) were free from aflatoxins. Samples collected from the rainfed region (Western Sudan) showed variable levels of aflatoxin ranging from 100% sample contamination in El Hamdi to only 10% in Casgeal[16]. It is also reported that the groundnut paste exhibited lower aflatoxin contamination than boththe gray and the red roasted groundnut and the contamination of groundnut products

collected from Behri reflected a higher contamination than those from Khartoum and Omdurman either [16]. These findings go with these of the present study that is the reported percentages of contamination are close and the contamination was higher in Behri than the other areas which agrees with what reported here. That is, Geraif East belongs geographically to Behri (East Blue Nile River). Also aflatoxin contamination was reported in peanut paste [16]. A lot of procedures are used in USA in the check points for aflatoxin which include a visual examination for the conidial heads of *A. flavus* and their presence will disqualify the suspect lots from allowance into the commerce and human consumption [17]. The other commonly used screening technique is the application of one of several minicolumn procedures to detect aflatoxin contamination above a predetermined level [8] & [18]. A preliminary survey in 1982 of aflatoxin levels in peanut butters indicated that 31 out of 32 samples of major national brand– named products examined contained <10 µg/ kg aflatoxin $B_1$and that 59% of these were below the limit of detection (2 µg/ kg). In contrast, of 25 peanut butters from specialist 'Health Food' outlets, 64% contained <10 µg/ kg aflatoxin $B_1$, the remainder ranging from 16 to 318 µg/ kg, with one sample having a total aflatoxin concentration of 345µg/ kg. Subsequent surveys in 1983 and 1984 of 'Health Food' products confirmed that these manufacturers were still experiencing some difficulty in complying with the 30 µg/ kg total aflatoxin voluntary guideline limit [19].A survey in 1984 was carried out of 228 retail samples of nuts and nut confectionery products comprising peanuts (shelled, unshelled, roasted and salted), mixed nuts, almonds (both unblanched and ground), brazils (in shell), hazelnuts (in shell), chocolate– coated peanuts, peanut brittle and coconut ice. The highest total levels of aflatoxins observed were in unshelled peanuts containing 4920µg/ kg and in a composite sample of visibly mouldedbrazils containing 17 926 µg/ kg [19]. The findings of this Study together with the cited literature may draw the attention to the importance of the checkup of groundnuts and its products for aflatoxin contamination. However, the dominance of *A. parasiticus* in the test sample may need a further study in the future.

**Table 1: Afaltoxins in groundnut butter in Khartoum State**

| Sampling Area | Sample Location | Aflatoxin Type | Aflatoxin Score (ppb) | Max. Allowed | |
|---|---|---|---|---|---|
| | | | | Codex | EU |
| Buri | 1 | None | 0.00 | 10 | 5 |
| | 2 | Do | 0.00 | Do | Do |
| Geraif East | 1 | $G_1$& $G_2$ | 6.17 & 6.76 | Do | Do |
| | 2 | $G_2$ | 4.95 | Do | Do |
| Kalakla | 1 | None | 0.00 | Do | Do |
| | 2 | Do | 0.00 | Do | Do |
| Khart. Prop. | 1 | Do | 0.00 | Do | Do |
| | 2 | Do | 0.00 | Do | Do |
| Kuku | 1 | Do | 0.00 | Do | Do |
| | 2 | Do | 0.00 | Do | Do |
| Omdurman | 1 | Do | 0.00 | Do | Do |
| | 2 | Do | 0.00 | Do | Do |

**Figure 1.1.: The Chromatogram of Sample 1 Geraif East**

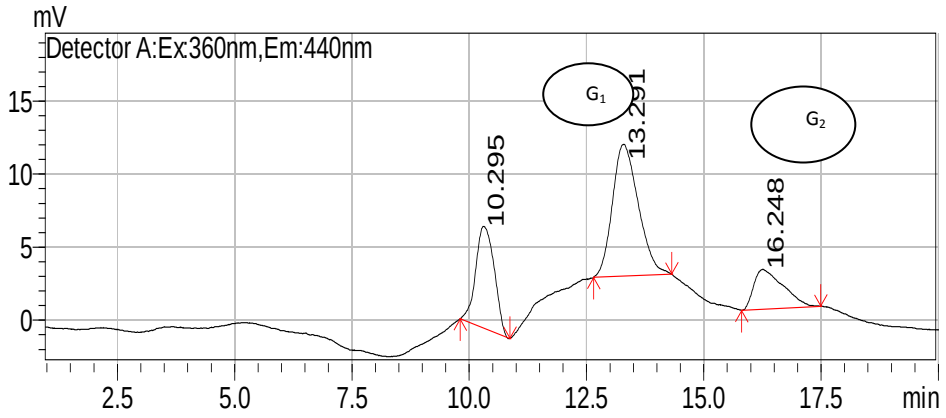



**Figure 1.2.: Chromatogram of the Standard spikes for Sample 1 Geraif East**

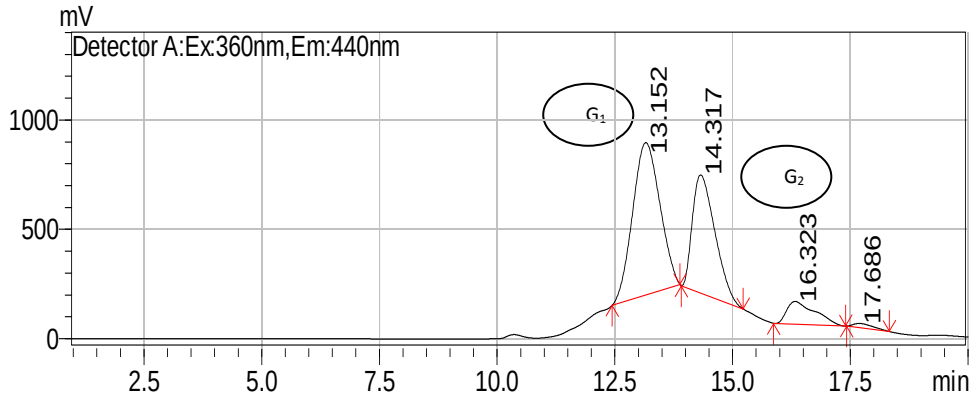



**Figure 2.1.: Chromatogram of the spikes for Sample 2 Geraif East**

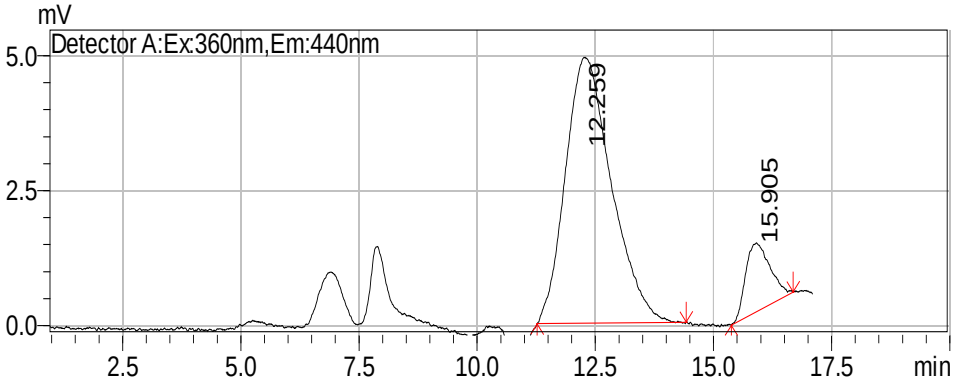
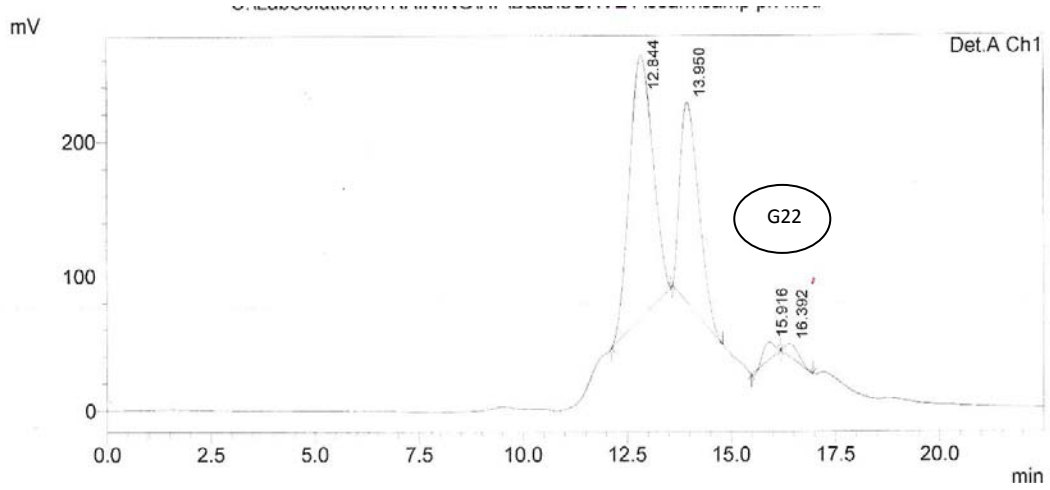


**Figure 2.2: Chromatogram of the spikes of the Standard for Sample 2 Geraif East**

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