

SHORT COMMUNICATION

**ASPERGILLUS FLAVUS INFECTION AND AFLATOXIN B<sub>1</sub> CONTAMINATION OF GROUNDNUT AND ITS PRODUCTS IN A MARKET IN SAMARU, ZARIA, NIGERIA**

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**SUMMARY**

The concentration of aflatoxin B<sub>1</sub> produced by *Aspergillus flavus* has long been referred in human and animal toxicity, declining production and trade in groundnuts. The health implication of aflatoxin B<sub>1</sub> has made it one of the most regulated mycotoxins worldwide. This study was aimed at assessing the infection of groundnuts by *A. flavus* and the subsequent aflatoxin B<sub>1</sub> contamination of groundnut products. The percentage incidence of *A. flavus* was higher in shelled roasted groundnut than in unshelled roasted groundnut. However, the percentage incidence in raw groundnut was lower in both shelled and unshelled, though unshelled raw groundnuts had the lowest incidence. The results demonstrate the natural presence of AFB<sub>1</sub> in groundnut and its by-products. The highest aflatoxin levels were observed in roasted (shelled) groundnuts (58.04µg/kg) which was significantly different (P<0.05) from groundnut oil, groundnut cake (kuli kuli) and roasted (unshelled) groundnut. The level of AFB<sub>1</sub> in groundnut oil was significantly higher (P<0.05) than groundnut cake (kuli kuli) and roasted (unshelled) groundnut. The results showed no significant difference (P>0.05) between groundnut cake and roasted (unshelled) groundnut samples. This fact warrants concern when considering that consumers of those products are large. The relatively high incidence of AFB<sub>1</sub> urges the need for regular monitoring and a more stringent food safety system in order to control these toxins at the lowest possible levels in food products.

**Key words:** *Aspergillus flavus*, Aflatoxin B<sub>1</sub>, groundnut and groundnut products

**GROUNDNUT** (*Arachis hypogaeae*) also known as peanut, goober, or monkey nut (14), is a legume crop grown in the tropics and subtropics, being important to both small and large commercial producers that is used as both a grain legume (14) and an oil crop (due to its high oil content). Groundnut is mainly grown in the northern part of Nigeria, in large quantities as compared to other regions of the country. Kano, Kaduna, Taraba, Bauchi, Borno, and Adamawa states account for 83-88% of national production with Kano state as the major producing area. Nigeria is a major groundnut exporting country and is ranked the 4th in groundnut production globally (14). Groundnuts in Nigeria are grown in commercial quantities mostly for the extraction of their oil which is used in cooking, for biodiesel fuel, laxatives, dye, shampoo, insecticide, explosives and glue. The cake which is the by-product of the oil extracted nut is used as a material in animal feed and also in the production of peanut flour. Apart from extracting the oil content of the nuts, the nut is also commonly used as a snack and can be boiled, roasted, fried or crushed into candies, cake, (kuli-kuli) (14). Groundnut is one of the crops prone to *Aspergillus flavus* infection and subsequent accumulation of aflatoxins.

Aflatoxin is produced by certain fungi called *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus flavus* is a saprotrophic and pathogenic fungus with a cosmopolitan distribution (1). Ramírez-Camejo *et al.* (2) reported that it is best known for its colonization of cereal grains, legumes, and tree nuts. Postharvest rot typically develops during harvest, storage, and/or in transit. *A. flavus* infections can occur while hosts are still in the field (pre-harvest), but often show no symptoms until postharvest storage and/or in transport. In addition to causing pre-harvest and postharvest infections, many strains produce significant quantities of toxic compounds known as mycotoxins, which, when consumed, are toxic to mammals. The presence of those molds does not always indicate that harmful levels of aflatoxin are present, but does indicate a significant risk. *A. flavus* is an opportunistic human and animal pathogen (3). Under unfavorable conditions of harvesting and storage, groundnut and other agricultural products may be invaded by toxigenic strains of the common mold *Aspergillus flavus* and this could result in the elaboration of a highly toxic class of mycotoxins commonly referred to as aflatoxins. Presently, more than 50 countries have established regulations for controlling aflatoxins in foods and

feeds. The Standards Organization of Nigeria (SON) has set a 4 µg/kg (4), 20 µg/kg (5) and 4 µg/kg (6) standards for maximum total aflatoxin concentrations for maize, raw groundnuts and groundnut cake (*kulikuli*) respectively. Despite aflatoxin regulatory standards, unpackaged food for domestic consumption are not regulated. This means that aflatoxin-contaminated grain can easily enter the Nigerian consumption stream due to low awareness about aflatoxins and their health impact among consumers and sellers.

## MATERIALS AND METHODS

### Sample collection

A total of forty samples comprising of 10 roasted groundnut, 10 unshelled roasted groundnut, 10 groundnut cakes and 10 groundnut oil were randomly sampled (100g each) in clean labeled polyethene bags from Samaru markets at different points and from different hawkers. The collected samples were sealed and kept at room temperature for 2 days. Roasted groundnut samples were labeled G1, unshelled roasted groundnut as G2, groundnut cake as G3 and groundnut oil as G4.

### Isolation and identification of *Aspergillus flavus* from infested groundnut seeds

One hundred and eighty seeds each from scarified and non-scarified pod

samples were surface sterilized in 1% sodium hypochlorite for three minutes and rinsed with distilled water three times. The seeds were then placed in fifteen Petri dishes (9 cm) layered with moistened filter paper (10 seeds per plate). The plates were arranged on a laboratory table and observed daily and recorded for seven days for the growth of *Aspergillus flavus*. Moisture was supplemented intermittently as the filter paper dried out. Care was taken not to overly wet the filter paper or the seeds to minimize germination and sprouting.

Percentage incidence was calculated using the formula;

$$\text{Incidence (\%)} = \frac{Nafl}{N} \times 100$$

Where;

Nafl – number of seeds with *A. flavus*  
N – Total number of seeds

Sub-cultures of *A. flavus* were made by transferring mycelia mass growing on infected seeds using a heat sterilized metal pick to Potato Dextrose Agar amended with streptomycin (PDAs) plates. The process was repeated from the first sub-culture until pure cultures were established. The pure cultures were then preserved in PDAs slants for further experiments. The cultures were then identified on the basis of colony characteristics and conidial morphology. Pure cultures were further sent to Centre for Agriculture

and Bioscience International (CABI) for confirmation.

### **Sample preparation (extraction of aflatoxins)**

Fifty grams of groundnut seeds were blended. Twenty grams of the powder was homogenized and extracted (filtration) in 100 ml 70% methanol containing 0.5% KCl after agitating for 30 min at 300 rpm.

### **ELISA analysis**

The filtrate was then loaded alongside standards of aflatoxin B<sub>1</sub> and incubated with specific antibodies and conjugates/substrate and read off an Enzyme Linked Immunosorbent Assay (ELISA) reader at 405 nm. Values obtained for aflatoxin B<sub>1</sub> standards were used to draw a curve on a spread sheet, taking aflatoxin concentrations on the "X" axis and optical density values on the "Y" axis. The formula below was used to obtain the result of the unknown

$$\text{AFB}_1 (\mu\text{g/kg}) = \frac{A \times D \times E}{G}$$

A = AFB<sub>1</sub> concentration in diluted or concentrated sample extract (ng/ml)

D = Number of times diluted with buffer

E = Extraction solvent volume used (ml)

G = Sample weight (g)

### **Data analysis**

Analyses were performed with SAS (version 9.1.3, SAS Institute Inc., Cary, NC). Analysis of variance was performed on all data with the general linear model (GLM). Least significant difference (LSD) test was performed to compare treatment means at the 5% level.

## **RESULTS**

### **Isolation, identification and incidence of *Aspergillus flavus* from infested groundnut seeds**

Fungi isolated from the various groundnut products was *Aspergillus flavus*. The cultural and morphological characteristics of the isolates were studied. Culture of *Aspergillus flavus* was green in colour and covered 90 mm petri dish in five days at ambient temperature. Macroscopic examination shows the conidia whose shape is globose, ellipsoid and slightly spherical.

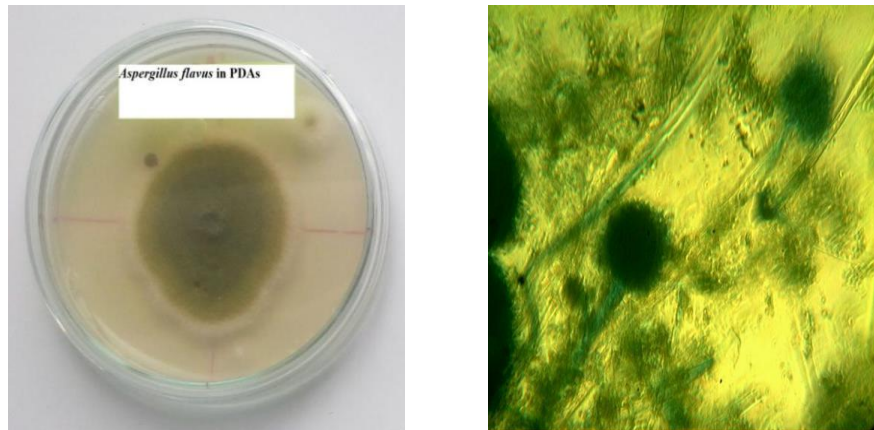


Plate I: A= Culture of *Aspergillus flavus* on plates B= *Aspergillus flavus* conidia (x40)

Table 1 shows the incidence of *A. flavus* on groundnut seeds. The percentage incidence of *A. flavus* was higher in shelled roasted groundnut (81.7%) than in unshelled roasted groundnut (42.2%). However, the

percentage incidence in raw groundnut was lower in both shelled (9.4%) and unshelled (1.7%), though unshelled raw groundnuts had the lowest incidence (1.7%).

**Table 1:** Incidence of *Aspergillus flavus* on groundnut seeds collected from Samaru market, Zaria, Nigeria

Type of groundnut	Raw	Roasted
Number of seeds	180	180
No. of seeds infected with <i>A. flavus</i> (Shelled)	17	147
% incidence of <i>A. flavus</i> (Shelled)	9.4	81.7
No. of seeds infected with <i>A. flavus</i> (Unshelled)	3	76
% incidence of <i>A. flavus</i> (Unshelled)	1.7	42.2

Table 2 shows the variation in AFB<sub>1</sub> levels within groundnuts samples from Samaru market. Generally, the obtained result shows that shelled roasted groundnut had significantly higher levels of AFB<sub>1</sub> (58.04µg/kg) compared to the rest. This was followed by groundnut oil

(29.74µg/kg) which was also significant but lower than shelled roasted groundnuts. The difference in mean of groundnut cake (11.81µg/kg) and unshelled roasted groundnut (09.13µg/kg) was not significant when compared to unshelled roasted groundnut (09.13µg/kg).

**Table 2:** Aflatoxin B<sub>1</sub> concentration in groundnut and groundnut products samples from Samaru market.

Processed forms	Aflatoxin B <sub>1</sub> (µg/kg)
Roasted Groundnut (shelled)	58.04±28.29 <sup>a</sup>
Groundnut Oil	29.74±13.71 <sup>b</sup>
Groundnut Cake	11.81±4.94 <sup>c</sup>
Roasted Groundnut (unshelled)	09.13±2.63 <sup>c</sup>

± = Standard Error of the Mean, Values with different superscript across the column are significantly different (P<0.05).

## DISCUSSION

*Aspergillus flavus* was isolated and identified. The confirmatory features conform to those described by Diba *et al.*, (7) on *A. flavus* isolates from groundnut seeds. Pure cultures of *A. flavus* identified from macroscopic characterization showed, upon microscopic examination, the characteristic globose, ellipsoid and slightly spherical conidia. The microscopic features observed are common features for the specie and have been widely described (7). *Aspergillus flavus* commonly occur on agricultural commodities such as groundnuts, maize, and cottonseed (8). The soil serves as a reservoir and primary source of inoculum of *A. flavus* (9) where it survives mainly as a saprophyte and grows on dead plant and animal tissue which is important for the recycling of nutrients (10). However, this fungus is also a parasite and can use living organisms such as maize,

groundnuts, cotton, and even humans as hosts (11). *Aspergillus flavus* is the predominant species responsible for aflatoxin contamination in groundnuts pre- and postharvest (12).

The incidence of aflatoxin in high concentrations in groundnut seeds (and products thereof) has been reported by various researchers (13, 14). Roasted groundnut (shelled) had the highest AFB<sub>1</sub> concentration. This may not be unconnected with the hygienic status of handlers of these food products. Abalaka and Elegbede (15) reported high AFB<sub>1</sub> concentrations in roasted groundnuts from markets in Port Harcourt. In two different studies conducted in Nigeria, (16) reported that Groundnuts cultivated in the northern parts of Nigeria were contaminated with Aflatoxin levels up to 2000µg/kg, while (17) reported Aflatoxin B<sub>1</sub> levels of between 37 –

455µg/kg in Groundnut purchased from markets in Ibadan, Oyo State Nigeria. From the study groundnut oil had significant AFB<sub>1</sub> concentrations. Akano and Atanda (18) reported AFB<sub>1</sub> concentrations ranging between 0.11-45.0µg/kg. Groundnut cake (kuli kuli) and unshelled roasted groundnuts had AFB<sub>1</sub> concentrations lower than the Nigerian regulatory limit of 20 µg/kg for groundnuts. AFB<sub>1</sub> concentrations in kuli kuli, a groundnut product widely consumed in different forms by a vast majority of Nigerians, range between 4.10-268.00 µg/kg, which is above the 2 µg/kg limit for Nigeria and 20 µg/kg limit for groundnut (19). The range of AFB<sub>1</sub> contamination in roasted ground nut in present study was comparable as reported by (20) who documented that about 64.2% roasted ground nuts samples were found contaminated with AFB<sub>1</sub> with mean concentration 25.5 µg/kg. In another study *A. flavus* was found in 58.3% of the groundnut samples (21).

The high occurrence of AFB<sub>1</sub> in roasted peanut may be attributed to the fact that mostly in markets the roasted peanut with shell or without shell stored in jute bags from which these kernels can absorb moisture from environment. Water activity of the substrate plays an important role in fungal growth (22). The present study urges the need to implement the regulations of AFB<sub>1</sub> in food

commodities because the more economically developed parts of the world have already implemented limits on mycotoxins (23, 24). In developing countries like Nigeria, such protection is absent because of high poverty levels. It has been documented that factors inducing fungal spoilage and AFB<sub>1</sub> production are more present in developing countries than elsewhere (25). Several authors have affirmed that AFB<sub>1</sub> problems are more serious in developing countries where the climatic conditions (water activity, moisture and temperature), transportation, marketing, and storage practices are inadequate and considered conducive for fungal growth and mycotoxin productions.

## **CONCLUSION**

The results demonstrate the natural presence of AFB<sub>1</sub> in groundnut and its by products. The highest aflatoxin levels were observed in roasted (shelled) groundnuts, followed by groundnut oil, groundnut cake (kuli kuli) and roasted (unshelled) groundnut. This fact warrants concern when considering that consumers of those products are large. The relatively high incidence of AFB<sub>1</sub> urges the need for regular monitoring and a more stringent food safety system in order to control these toxins at the lowest possible levels in food products.

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