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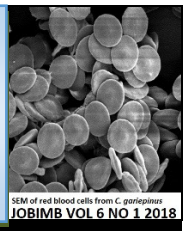

The Occurrence of *Aspergillus* Species and Aflatoxin in Groundnut Sold in Gombe Metropolis

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ABSTRACT

The production of groundnut is constrained by several factors, among which is the contamination with *Aspergillus* sp. In addition to causing quantitative losses, some of the *Aspergillus* sp. produce highly toxic and carcinogenic chemical substances known as aflatoxins. Aflatoxin contamination of groundnut has gained global significance due to deleterious effects the contaminant has on human and animal health. This study aimed at investigating the occurrence of *Aspergillus* sp. in different groundnut samples as well as analyzing the samples chemically to detect the presence of aflatoxin in the groundnut samples. Thirty (30) samples of groundnuts were collected from five local markets in Gombe metropolis, namely: Gombe main market, Gombe old market, Riyal, Tashan Dukku, and Arawa market. The kernels were examined for the occurrence of *Aspergillus* sp. and contamination with aflatoxins. All samples were subjected to microbiological analysis by culturing them on suitable growth medium followed by chemical analysis by Thin-Layer Chromatography (TLC) technique. Twenty-four samples (80%) gave positive readings with TLC technique, and in culture, *Aspergillus* flavus was isolated from nineteen samples (63%). The concentrations of aflatoxin in these samples were ranged from low to very high, in range of (23.67 – 134.66 µg/Kg kernel).

INTRODUCTION

Groundnut (*Arachis hypogaea L.*), which is also known as peanut, earth-nut, monkey nut and goobers, is a member of the Papilionaceae, largest and most leguminous crop [1] . It serves as component of crop rotation in many countries [2,3]. Groundnuts are also significant source of cash in developing countries that contribute significantly to food security and alleviate poverty [4]. Developing countries account for 97% of the world's groundnut area and 94% of the total production [5]. It is mainly native to the warmer regions. It frequently provides food for humans, livestocks, and in the absence of meat, it forms a valuable protein component in diet. It is one of the world`s most important oil seed crops, ranking as the 13th most important food crop and 4th most important oil seed crop of the world and cultivated in more than 100 countries in six continents [6]. Groundnut kernels contain 40-50% fat, 20-50% protein and 10-20% carbohydrate and are rich in vitamins and minerals. Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used for culinary purpose. Oil pressings, seeds and haulms of groundnut are used as animal feed, while the oil cake is used as an industrial raw material and fertilizer [7]. Groundnut crops are grown for their seeds, the oil, the meal, and the vegetative residues [8].

Two-third of the world groundnut crops are crushed for oil, which is used mainly for cooking. It may also be used for margarines and vegetable glee, for shortenings in pastries and bread, in soaps, pharmaceutical and cosmetic products, lubricant and emulsion for insecticides and as fuel for diesel engines [9]. These multiple uses of groundnut plants make it an excellent cash crop for domestic as well as foreign trade in several developing and developed countries. However,

groundnut yield in some part of the world and particularly in Africa is lower than the world average due to prevailing abiotic, biotic and socio-economic factors [2,3,10].

Mature seeds of groundnut are liable to infection by the mould *Aspergillus species* if they become damp in the soil or store and a resulting by-product of the metabolism of those fungi can produce a series of toxic substances, the aflatoxins [11]. Feed produced from meals containing aflatoxin induces obvious and hidden effects in livestock varying from a general loss of condition to death, or long-term degeneration of internal organs from cancer should contaminated kernels be sold as edible nut, human poisoning can occur [12]. Aflatoxin contamination of groundnut prevents groundnut producers from accessing bigger southern markets, increases dependency on foreign food aid, stifles economic opportunities and adversely affects consumer health.

Aspergillus spp. are very widely distributed fungi like *Penicillium* and their spores are abundant in the air and in the soil. Eighteen groups of *Aspergilli* were listed and recognized 132 species. *Aspergillus* spp. are facultative parasites. They invade host plant tissues directly or attack tissues that have been predisposed by environmental stresses such as dry weather or damages caused by insects, nematodes, natural cracking, and harvest equipment [13].

Aflatoxin is the name for a group of toxins known as B1, B2, G1, G2, M1 and M2 (carcinogenic compounds) that are produced mainly by two fungi called *Aspergillus flavus* and *Aspergillus parasiticus*. These toxins occur naturally and have been found in a wide range of commodities (including peanuts) used for animal and human consumption. Depending on their levels, toxins can severely affect the liver and induce a human carcinogen, i.e., causes cancer. In many developing countries, Aflatoxin is a major health risk to both humans and animals due to the high levels of the contaminated products consumed [14].

Aflatoxins are a group of structurally related toxic polyketide-derived secondary metabolites produced by certain stains of *A. flavus* and *A. parasiticus* [15]. Aflatoxins are the major mycotoxins that are most commonly associated with groundnut [16]. In view of the extreme toxicity and the widespread distribution of the aflatoxins, much effort has been expended towards achieving effective control of the contribution of foods and feeding stuffs of groundnut origin likely to contain aflatoxin [17]. This research was aimed at detecting the occurrence of *Aspergillus sp.* in different samples of groundnut as well as analyzing the samples chemically to detect the presence of aflatoxin in the samples.

MATERIALS AND METHODS

A total of 30 samples were collected from five local markets in Gombe metropolis namely: Gombe main market, Gombe old market, Riyal, Tashan Dukku, and Arawa market i.e six samples each from market. The samples were labeled as sample A, B, C, D and E representing cross their collection site and each subgroup were labelled from 1-6 making the total number of thirty (30) samples from all over the metropolis.

Isolation of *Aspergillus* spp. from the Samples

A method described by Hayate, and Idris [18] were adopted in isolating the fungi. This was performed by weighing 10 g of the groundnut sample and blending with 90mls of distilled water [18]. 1ml was taken from each mixture and serial dilution of in to 1-5 folds were time. 1ml was taken from 10^{-1}, 10^{-3}, and 10^{-5} dilutions, inoculated on to the surface of freshly prepared Sabouraud dextrose agar (SDA) plates and incubated at 25^0C for 7 days. Pure cultures of different out growing fungi were obtained by transferring fungal colonies to new PDA plates using sterile wireloop and incubating the plates at 25^0C for seven days. The pure cultures were examined microscopically using Lactophenol Cotton Blue (LPCB) stain and were classified to the species level by comparing the culture characteristics with the one given in the atlas [19].

Chemical Analysis of Samples for Aflatoxins

The general procedures used in the chemical analysis of the samples for the presence of aflatoxins as previously described by Jones [20] as follows:-

Defatting of Samples

About 50 g from each of the sample types were weighed and grinded. 20 g of the grinded samples were weighed and then transferred into different beakers (50 mL). The samples were extracted with aromatic - free petroleum spirit (100 ml) for four hours as described by [21].

Extraction of aflatoxin from samples

The TPI standard procedure described by Jones and Lee was used in extracting aflatoxin from the samples [20,22]. This was done by weighing 10 g defatted sample into a 250 ml wide - mouthed bottle, 10 ml of water was then added and mixed thoroughly using a gloss rod. 100 ml of chloroform was then added as described by Carling [23] and the bottle was stopped with a rubber bung coated with aluminum foil to protect the rubber from attack by the chloroform. The content was placed on a wrist shaker for 30 min to extract the toxin. The extract was then filtered through a Whatmann No.1 filter paper as described previously [23]. The filtrate obtained was further concentrated by evaporating excess chloroform on a water bath [23].

Detection of aflatoxin by thin-layer chromatography (TLC)

The method of Iong, *et al.* was used in detecting the aflatoxin content of the extracts [24]. In this procedure, ten 12 cm x 12 cm silica gel chromatographic plates were used in all the sample extracts. The silica gel chromatoplates were then spotted with the extract at a position 2 cm from the bottom of the plates. A development solvent (chloroform-methanol) (95:5 v/v) as described by Dicken was used as the mobile solvent system [25].

The chromatoplates were developed by standing each plate in a chromatography tank containing the solvent to a depth of 1cm. The Chromatography tank was placed in a dark cupboard and careful observation was made in assessing the level of the solvent front on the plates in order not to exceed 10cm of the required limit to cover the solvent system. The plates were then removed from the chromatography tank and left to dry as described by Peterson, *et al.* [26]. The plates were irradiated with an ultraviolet light of 365 mu as [25] to observe the presence of florescent spots of extracts [25]. The retention factor (R_f) was calculated as in **Equation 1**, recorded to determine the concentration of aflatoxins using **Equation 2**.

$$R_f = \frac{\text{Distance movement by compound}}{\text{Distance movement by solvent}} \quad \textbf{(Eqn 1)}$$

Concentration of aflatoxin B1 in µ/Kg = $\frac{(S \times Y \times V)}{(X \times W)}$ (**Eqn. 2**)

Where:

S ≡ µl aflatoxin B1 standard equal to unknown.
Y ≡ concentration of aflatoxin standard µg/ml.
V ≡ µl of final dilution of sample extract.
X ≡ µl of sample extract spotted to giving fluorescent intensity equal to S.
W ≡ weight of sample in gram of original sample contain in fin extract.

RESULTS AND DISCUSSION

The results of analysis of groundnut samples for the presence of *Aspergillus flavus* and aflatoxin were presented as follows:

Table 1 shows the result of the total fungal plate count across various selected markets in Gombe metropolis namely: Gombe main market, Gombe old market, Riyal, Tashan Dukku, and Arawa market. Highest number of fungi was observed in Gombe main market with (7.05×10^4 cfu/ml), followed by Gombe old market with (5.33×10^4 cfu/ml), while Tashan Dukku showed the least with (2.05×10^4 cfu/ml).

Table 1. Total fungi Plate Count across the various selected market in Gombe.

S/N	Market	Fungal plate counts (cfu/ml)
1	Gombe Main market	7.05×10^4
2	Gombe old market	5.33×10^4
3	Arawa market	4.37×10^4
4	Riyal market	4.35×10^4
5	Tashan Dukku market	2.05×10^4

Table 2 shows the results of the occurrence of different fungi isolated from the various selected markets in Gombe metropolis. The result shows that *Aspergillus flavus* has the highest frequency of occurrence with 19 (63%), followed by *Asprgillus niger* with 8 (27%), while, *Aspergillus parasiticus* showed the least frequency with 3 (10%).

Table 2. Frequency distribution and percentage frequency for the occurrence of fungi isolated from the samples.

Species	Frequency of occurrence	% Frequency
Apergillus flavus	19	63
Aspergillus niger	8	27
Aspergillus parasiticus	3	10
Total	30	100

Table 3 shows the result for the occurrence of aflatoxins extracted from the groundnut samples in Gombe metropolis. The result revealed that aflatoxin B_1 has the highest frequency of occurrence with 11 (37%), followed by aflatoxin B_2 with 9 (30%), while, aflatoxin G_1 showed the least with 4 (13%).

Table 3. Frequency distribution and percentage frequency for the occurrence of aflatoxins extracted from the samples

Aflatoxin	Frequency of occurrences	% Frequency
Aflatoxin B_1 (AFB_1)	11	37
Aflatoxin B_2 (AFB_2)	9	30
Aflatoxin G_1 (AFG_1)	4	13
Negative	6	20
Total	30	100

Table 4 shows the result of the mean concentration of aflatoxins extracted from the groundnut samples in selected markets in Gombe metropolis, namely: Gombe main market, Gombe old market, Riyal, Tashan Dukku, and Arawa market. The result revealed that Gombe main market has the highest contamination of aflatoxins with (74.35 µg/Kg), followed by Gombe old market with (37.72 µg/Kg), while Tashan Dukku market with (15.67 µg/Kg) has the least contamination of aflatoxins

Table 4. Concentration of aflatoxins detected in the groundnut samples.

S/N	Location	Cocentration of aflatoxin (µg/ Kg)
1	Gombe main market	74.35
2.	Gombe old market	37.32
3.	Arawa market	26.90
4.	Riyal market	18.67
5.	Tashan Dukku	15.67

DISCUSSIONS

Aflatoxin has been the subject of many studies because of its deadly toxicity to certain domesticated animals, including turkeys, ducks and trout. In addition increased incidence of human hepato-carcinoma is associated with ingestion of sublethal doses of aflatoxin [27].

The result of the total fungal plate count across various selected markets in Gombe metropolis, namely: Gombe main market, Gombe old market, Riyal, Tashan Dukku, and Arawa market, revealed highest number of fungi in Gombe main market (7.05×10^4 cfu/ml), followed by with Gombe old market (5.33×10^4 cfu/ml), while Tashan Dukku showed the least with (2.05×10^4 cfu/ml). The highest fungal count observed in Gombe main market might be attributed to long-term storage in the store by the marketers. Interestingly, this study is in agreement to that of Weiss *et al*. who reported that mature seeds of groundnut are liable to infection by the mould if they become damp in the soil or store [11].

So also, the results of the occurrence of different fungi isolated from the various selected markets in Gombe metropolis revealed highest frequency of occurrence by *Aspergillus flavus* with 19 (63%), followed by *Aspergillus niger* with 8 (27%), while, *Aspergillus parasiticus* showed the least frequency with 3 (10%). The highest frequency of occurrence by *Aspergillus flavus* might be attributed to a particular affinity of the *Aspergillus flavus* to the use of groundnut as a good substrate for growth. This correlates with the findings of Lanfont and Lanfont [28] that *A. flavus* have a particular affinity for groundnut as a substrate for growth. The result was also consistent with Chala, *et al*. who detected 5-11,900 µg/kg total aflatoxin from same samples suggesting heavy groundnut contamination by *Aspergillus flavus* and associated fungus in the region [28, 29].

Furthermore, the result of the occurrence of aflatoxins extracted from the groundnut samples in Gombe metropolis. The result revealed that aflatoxin B_1 has the highest frequency of occurrence with 11 (37%), followed by aflatoxin B_2 with 9 (30%), while, aflatoxin G_1 showed the least with 4 (13%). The highest frequency of occurrence by aflatoxin B_1 might be attributed to the high prevalence of aflatoxin B_1 in contaminated groundnut. This result is consistent to the findings of Park *et al.* [30] who analyzed 40 groundnut and 30 peanut butter samples, AFB_1 was found in 5 peanut butter samples with mean AFB_1 concentration of 12 mg/kg, and 10 peanut samples had AFB_1

concentration ranging from 19-32 mg/kg [30]. It also tallies with the survey done in Philippines on groundnut-based products which revealed that 60% of the samples were positive for aflatoxin B1 in range of 1.00 - 244 µg/Kg [31].

Finally, the result of the mean concentration of aflatoxins extracted from the groundnut samples in selected markets in Gombe metropolis, namely: Gombe main market, Gombe old market, Riyal, Tashan Dukku, and Arawa market revealed that Gombe main market has the highest contamination of aflatoxins with (74.35 µg/Kg), followed by Gombe old market with (37.72 µg/Kg), while Tashan Dukku market with (15.67 µg/Kg) has the least contamination of aflatoxins. This may be due to long time storage of the groundnut in the store by the marketers. The result is in agreement with the work of Bakhiet *et al.* on the survey and determination of aflatoxin levels in stored groundnut in Sudan who reported 58.33% of 60 stored groundnut tested were positive to aflatoxin B_1 with the concentration range of 17.57 – 404.00 µg/Kg [32]. In addition, there are several surveys that show a relatively lower level of contamination of aflatoxin B1 in groundnuts and their products. Siame *et al*. who did a study in Botswana, reported that the levels of aflatoxin B1 in a range of (0.8 – 16.00 µg/Kg) for the raw shelled shelled samples and for peanut butter were (3.2 – 16.00 µg/Kg). In Tokyo, Japan, Tabata *et al*. found that several groundnut products were contaminated by aflatoxin B_1 in a range of 0.4 – 21.7 µg/Kg [33].

According to Ali *et al*., when the initial content of aflatoxin was high in the raw shelled groundnut, a high level of aflatoxin contamination can be expected in its final products such as peanut candy and peanut butter [31]. On the other hand, the low level of aflatoxin contamination in the peanut products has always been associated with the use of high quality raw materials (raw shelled groundnut) that contain an acceptable low initial level of aflatoxin. Besides, various groundnut processing techniques, such as shelling, drying under sunlight, boiling with salty water, and roasting, were also found to be useful in reducing the aflatoxin content in the products [34]. The percentage for the occurrence of aflatoxins in the samples analyzed in this research indicates a higher level of contamination of the groundnut in the local markets. Groundnut is one of the major sources of protein especially among the peasants, thus the presence of aflatoxins in the samples presents a higher health risk to consumers, considering the fact that local processing techniques employed to groundnut food contributes partially to the destruction of aflatoxins in such foods [34].

Higher levels of contamination of the samples may be attributed to the suitable environmental conditions that favor the growth and formation of aflatoxins by the fungi. These conditions (temperature and humidity) are at a favorable level for growth of the fungi in tropical countries such as Nigeria.
Though groundnut has been shown to be one of the commodities mostly infected by the fungi, high percentage of occurrence by the fungi may be attributed to the harvesting, processing, and condition for storage of this commodity in markets and stores.

Conclusively, the percentages for the occurrence of *Aspergillus* spp.in the samples analyzed indicate the ubiquitous nature of *Aspergillus* spp. and potential for contamination of food stuffs and animal feeds of groundnut origin. The presence of a golden yellow extract after evaporating the solvent is an inference to the toxicity of the samples analyzed. The toxicity level is attributed to the presence of metabolites (aflatoxin) of the fungi.

The comparison of R_f values of the extract with that of the control concluded that the sample extract comprises the aflatoxins.

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