



Analysis and Health Risk Evaluation of Aflatoxin B1 Levels in Groundnut (*Arachis hypogea*) and Maize (*Zea mays*) Samples from Wukari, Nigeria

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Abstract:

Aflatoxin B1 (AFB1) is a secondary fungal metabolite which is considered a public health concern worldwide due to its genotoxicity and carcinogenicity. In this study, we evaluated the levels and potential health effects of AFB1 in Groundnut and Maize samples from Wukari, Nigeria. Ten samples (each) of maize and groundnuts were collected randomly from local markets and analysed for AFB1. Our findings revealed the presence of AFB1 in all the samples investigated with concentrations ranging between 7.79 – 14.08 $\mu\text{g}/\text{kg}$ in groundnuts and 1.48 -15.50 $\mu\text{g}/\text{kg}$ in maize samples. Overall, 90% of the analysed samples contained aflatoxin B1 above the allowed

limit of 5 $\mu\text{g}/\text{kg}$. The assessment of chronic exposure and probabilistic health risk from consumption of AFB1 in the investigated food samples was done via chronic daily intake (CDI) and margin of exposure (MOE) evaluations. Obtained CDI values for groundnut ranged between 14.96 – 28.74 $\text{ng}\cdot\text{kg}^{-1}$ BW day^{-1} for children and 3.20 – 6.15 $\text{ng}\cdot\text{kg}^{-1}$ BW day^{-1} for adults while for maize the values were in the range of 5.38 – 56.42 $\text{ng}\cdot\text{kg}^{-1}$ BW day^{-1} for children and 1.15 – 12.09 $\text{ng}\cdot\text{kg}^{-1}$ BW day^{-1} for adults. MOE values ranged between 3.01 – 31.60 for children and 14.06 – 147.83 for adults. The obtained MOE values are by far lower than the recommended $\geq 10,000$ value thus indicating high carcinogenic risks to consumers of the food items. There is need to create more awareness and interventions on aflatoxin contamination of food sources in Nigeria.

Keywords: Aflatoxins, Maize, Groundnuts, Risk assessment, Food safety.

Introduction

Aflatoxins are a group of highly toxic secondary metabolites, produced by fungi of the genus *Aspergillus* spp which colonize many foodstuffs during agricultural production, harvesting, transportation, storage, and food processing. The two fungal species principally involved in aflatoxins production are *Aspergillus flavus* and *Aspergillus parasiticus*. There are about

20 well identified aflatoxins, but the four major ones are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) (Inan, *et al.*, 2007). AFM₁ and AFM₂ are derived from aflatoxin B types through different metabolic processes and are expressed in animals and animal products (Weidenborner, 2001, Wolf-Hall, 2010). Chemically, aflatoxins are difuranocoumarin derivatives in which a bifuran group is attached at one side of the



coumarin nucleus, while a pentanone ring is attached to the other side or a six-membered lactone ring is attached in the aflatoxin G series (Nakai, *et al.*, 2008, Bennett and Klich, 2003).

Aflatoxins are generally classified as carcinogenic by the International Agency for Research on Cancer (IARC, 2002). They are known to suppress the immune systems of humans and animals by interfering with the cells which are responsible for boosting immunity. Large doses of aflatoxins lead to direct death and damage, while small long-standing doses lead to immunologic or nutritional effects; both cases can lead to liver cancer (Marroquín-Cardona *et al.*, 2014). Children are more prone to the toxicity of aflatoxin as it increases the risk of early infections by reducing immunity. The carcinogenic nature of aflatoxin is due to its ability to damage DNA either by lipid peroxidation or by oxidation (Zhang *et al.*, 2015). Among the known aflatoxins, AFB₁ constitutes the most harmful type; it is a potent hepatocarcinogenic, mutagenic, teratogenic agent. The cytochrome p-450 present in the liver activates AFB₁ which is then converted into AFB₁ 8,9-epoxide. The compound AFB₁ 8,9-epoxide is responsible for various carcinogenic effects (Denissenko *et al.*, 1999). Apart from being carcinogenic, aflatoxin also has negative effects on the kidney, heart, liver, testis, and brain (Yilmaz *et al.*, 2018).

Aflatoxin contamination in crops is a global threat that compromises the safety of food, feed, and also influences the agricultural economy of a country. Crops can be contaminated by the *Aspergillus* fungi during the process of harvesting, storing, and transporting hence leading to the productions of several Afltoxins. AFB₁ which is produced by *Aspergillus flavus* and *Aspergillus parasiticus* has been shown to be the major Aflatoxin contaminant in foods.

Globally, there have many reports on the dietary exposure of aflatoxins by the consumption of cereal products. Risk characterization studies have been carried out using two different approaches; the risk of cancer, which considers the incidence of HBV (individuals with HBsAg+) and the carcinogenic potency of

aflatoxins (FAO/WHO, 2017); and the margin of exposure (MOE), which is obtained from the relationship between the reference dose level that causes a 10% increase in the incidence of cancer in rodents (BMDL₁₀) and estimated daily intake (EDI) (EFSA, 2007).

In Nigeria, Several crops have been associated with aflatoxin B₁ contamination. Among them, corns and groundnuts have been shown to be highly susceptible to the deleterious fungus-aflatoxins, hence resulting in harmful diseases in humans and animals when consumed. Given the severe adverse effects of aflatoxins on human health and the difficulty of eliminating them from food, there is need for regular evaluation and control of the toxin in food sources. The current study is aimed at investigating aflatoxin contamination in maize and groundnut (peanut) samples from Wukari in Taraba state Nigeria. The study would also evaluate the carcinogenic risk which the consuming population may be exposed to from consumption of the investigated food sources. Dietary risk assessments for aflatoxins are essential information that would guide in taking measures to reduce associated risks and guarantee food safety

Materials and Methods

Sample Collection and Preparation

Ten (10) samples each of maize (*Zea mays*) and Groundnut (*Arachis Hypogea*) were arbitrarily purchased from local grain markets in Wukari, Nigeria. Each sample was packed in a sterile plastic bag and transported to the laboratory at for analysis. The samples collected were finely ground and the resulting powdered samples were stored in air-tight containers.

Determination of Moisture Content

The moisture content of samples was determined using the standard oven method (AOAC, 1990). Three grams (3 g) of a sample was placed in a dry empty dish and dried to constant weight in an oven at 105 °C Thereafter, the moisture content was calculated based on percentage weight loss.

Extraction of Aflatoxins

Fifty grams (50 g) of finely ground sample was wrapped in a cellulose paper and placed in the thimble chamber of a soxhlet extraction apparatus. Extraction was carried out for five hours using methanol as solvent. The extract was collected and filtered through whatman No.1 filter paper. The filtrate was transferred into a separating funnel and 40 mL of chloroform was added followed by 5 g of anhydrous sodium sulphate (Na_2SO_4) and then washed with chloroform. The extract was evaporated to near dryness on a steam bath.

Determination of Aflatoxin B1

Thin-layer chromatographic plates were prepared using Silica Gel; the suspension of silica gel was applied to the plates at a thickness of 0.25 mm and left to gel. The plates were then dried at 80 °C for 2 hr after which they were stored in a drying cabinet. For the TLC analysis, the previously evaporated extracts were re-dissolved in 250 µl of chloroform. The samples extracts and aflatoxin B1 standards were spotted on the start line of the TLC using a micro pipette. The plates were developed in a development tank pre-saturated with methanol-water in the ratio 9:1). The spotted plate was placed vertically in the development tank and left for 35 to 45 mins until the solvent reached the stop line (10 cm) from the base line. The plate was then removed and allowed to dry. This process was repeated for all the samples and the developed plates were viewed under a long wavelength UV lamp. The AFB1 bands were carefully scraped and washed with a mixture of acetone and chloroform in the ratio 6:4 and then transferred to a cuvet. The absorbance was taken at 363 nm on a UV-Visible spectrophotometer. Quantification of AFB1 was done via a standard calibration curve.

AFB1 Exposure and Probabilistic Health Risk Assessment

In this study, the carcinogenic risk of AFB1 in the investigated food samples was evaluated via margin of exposure (MOE) estimation using the Monte Carlo simulation (MCS) model. Firstly, the chronic daily intake (CDI) of AFB1 was

calculated based on the equation below (IARC, 2002, Adel *et al.*, 2016, Dadar *et al.*, 2017).

$$\text{CDI (ng/kg)} = \frac{\text{Ci} \times \text{IRi} \times \text{EDi} \times \text{EFi}}{\text{BW} \times \text{AT}} \quad (1)$$

Ci = Content of AFB1 in studied foodstuffs (ng/g); IRi = daily intake of studied foodstuffs (g/day); EDi = Exposure duration (70 years for adults and 6 years for children); EFi = frequency of exposure (350 days/years); BW = mean body weight (15 kg for children and 70 kg for adults); AT ($365 \text{ days/y} \times \text{EDi}$) is the average exposure time (2190 and 25,550 days for children and adults, respectively).

Consumption data for Nigeria was taken from FAO database (Ranum *et al.*, 2014) which were as follows: maize: 60 g/person/day, peanuts: 30 g/person/day.

MOE was calculated using the following equation.

$$\text{MOE} = \frac{\text{BMDL10}}{\text{CDi}} \quad (2)$$

In this equation, BMDL10 is taken as $170 \text{ ng} \cdot \text{kg}^{-1} \text{ BW} \cdot \text{day}^{-1}$ for AFs (EFSA, 2007) and CDI is the chronic daily intake (ng/kg BW). According to EPA exposed population is at a safe range when the MOE value is above 10,000 (US EPA, 2011)

Results and Discussion

Tables 1 and 2 present the results obtained for moisture analysis and AFB1 determination. The values obtained for moisture content ranged from 2.7 – 4.4% for groundnut samples and 3.9 – 7.6 % for the maize samples. Moisture content of stored nuts and grains is known to greatly influence the growth of toxigenic fungi which could lead to aflatoxin contamination. Research has shown that aflatoxin-producing pathogens infect stored seeds and produce toxins when pod moisture level is above 8% and ambient

temperature exceeds 25°C (Flet, 2023). Also, the recommended moisture limit for stored maize has been given as 13.5 % (Ubwa *et al.*, 2012). The moisture content values recorded in this study are all below 8% therefore we infer that the aflatoxin contents recorded are not likely due to the moisture levels in the samples.

Our findings on AFB1 determinations showed that all the investigated samples were contaminated with the toxin at different levels. For the groundnut samples (Table 1), the highest concentration obtained was 14.97 µg/kg (Sample J) while the lowest concentration (7.79 µg/kg) was recorded for sample F. For the maize samples (Table 2), the most contaminated was sample Q with a concentration of 15.50 µg/kg while the least contaminated was sample P with a concentration of 1.48 µg/kg.

The reason for the high proportion of contaminated samples obtained in this study could be attributed to factors such as cultivation conditions, late harvesting, storage of insufficiently dried produce and damage to seeds during dehulling. Also, Nigeria has a tropical climate characterized by rainfalls, high humidity and high pre-harvest temperatures. These conditions are known to support the growth of *Aspergillus* fungi and hence could have led to the production of aflatoxins in the investigated crops.

Table 2. Moisture Content and Aflatoxin B1 Concentration in Groundnut Samples from Wukari, Nigeria

Sample	Moisture content (%)	AFB1 (µg/kg)
A	3.5 ± 0.06	9.42 ± 0.15
B	3.1 ± 0.15	12.6 ± 0.17
C	3.0 ± 0.12	12.05 ± 0.01
D	2.7 ± 0.08	11.38 ± 0.01
E	3.7 ± 0.02	13.08 ± 0.15
F	3.9 ± 0.10	7.79 ± 0.31
G	4.4 ± 0.04	8.51 ± 0.05
H	3.5 ± 0.12	7.87 ± 0.06
I	3.2 ± 0.01	14.08 ± 0.08
J	3.8 ± 0.14	14.97 ± 0.09
Overall Mean	3.48 ± 0.51	11.18 ± 2.62

Note: Values are expressed as mean ± SD for three determinations

Table 2. Moisture Content and Aflatoxin B1 Concentration in Maize Samples From Wukari, Nigeria

Sample	Moisture content (%)	Concentration (µg/kg)
K	4.2 ± 0.17	7.55 ± 0.12
L	4.8 ± 0.21	10.35 ± 0.07
M	7.6 ± 0.34	13.42 ± 0.42
N	3.9 ± 0.24	8.06 ± 0.15
O	3.5 ± 0.06	6.20 ± 0.23
P	4.0 ± 0.12	1.48 ± 0.05
Q	5.2 ± 0.04	15.50 ± 0.08
R	5.3 ± 0.08	11.45 ± 0.14
S	5.4 ± 0.14	5.70 ± 0.02
T	6.5 ± 0.22	2.22 ± 0.04
Overall Mean	5.04 ± 1.27	11.06 ± 0.03

Note: Values are expressed as mean ± SD for three determinations

The problem of AFB1 contamination in cereals and oilseeds is prevalent in many African countries. Studies in most countries in the continent show that almost half of the cereal production have aflatoxin content above permissible limits. A study in Senegal showed levels of up to 852.2 µg/kg in whole grain maize samples, while in Tanzania, the levels reached 1081 µg/kg in the same matrix (AfricaAIMS, 2016). According to the same source, in Uganda the amount of aflatoxin in maize ranged from 86 µg/kg to 3300 µg/kg with 20% – 65% of samples exceeding the maximum permissible limit. Related studies in Nigeria also show high contamination by aflatoxins in many locations within the country; aflatoxin evaluation in groundnut samples from Sokoto, Nigeria showed the presence of the toxin in 82.5% of analysed samples at concentrations ranging between 0.9 - 646.0 µg/kg (Salau *et al.*, 2016). A study of aflatoxin contamination in Selected Grains from Katsina and Zaria metropolis in northern Nigeria revealed that 79.3% of the entire samples were positive with aflatoxins level in the range of 0.1- >20 µg/kg (Batagarawa *et al.*, 2015). Samples of dry roasted groundnuts from southwestern Nigeria analysed for aflatoxin contamination revealed the presence of aflatoxin B1 in 64.2% of samples with a mean of 25.5 ppb (Bankole and Mabekoje, 2004).

Similarly groundnut samples collected from four agro-ecological zones of Nigeria showed the presence of aflatoxins in 39% of the samples with mean concentration of 216 $\mu\text{g}/\text{kg}$ (Oyedele *et al.*, 2017). In general, aflatoxin contamination appears to be highly common in Nigeria and also widespread in Africa.

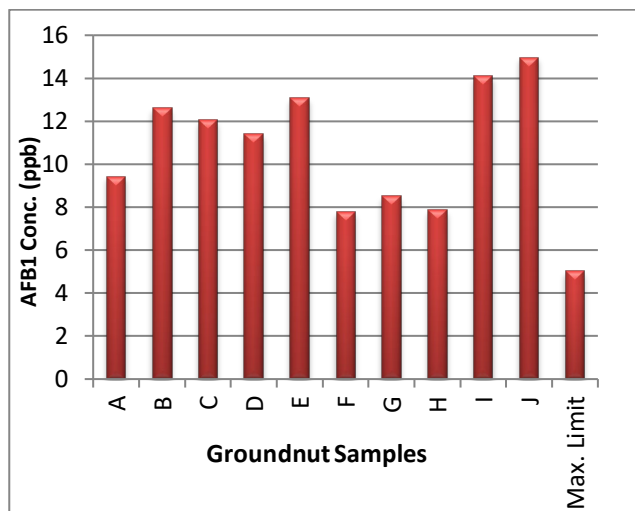


Figure 1. Comparison of AFB1 Levels in Groundnut Samples from Wukari, Nigeria with Maximum Permissible Limit

In view of the toxic and carcinogenic effects of aflatoxin contaminated foods, regulatory bodies in different countries have set tolerance limits for the toxin in different food categories. The US department of Agriculture (USDA) and Food and Drug Administration (FDA) set the tolerance limit of 20 ppb on foods while EU countries allow much lower ppb concentration of aflatoxins. Accepted levels for aflatoxins are variable for various foods in different countries. Currently, most nations have a maximum tolerable level of total aflatoxins in maize and peanuts ranging from 4 to 20 ppb however, aflatoxin B1 has a higher individual standard. The most recent USDA Foreign Agricultural Service (FAS) FAIRS Country Report for Nigeria indicates that the country applies Codex, European Union, and U.S. FDA standards in regards to mycotoxin maximum (www.fda.gov). The set limits for aflatoxin B1 in maize and groundnuts given by these regulatory bodies ranges from 2 – 5 ppb. In this study, the set

limit of 5 ppb was used to assess the level of AFB1 contamination in the investigated samples. For the groundnut samples (Figure 1), our findings show that all 10 investigated samples contained AFB1 above the permissible limits while for the maize samples (Figure 2), 8 out of the 10 samples (80%) showed higher than permitted AFB1 levels. Overall, 90% of the investigated samples were contaminated above tolerable limits

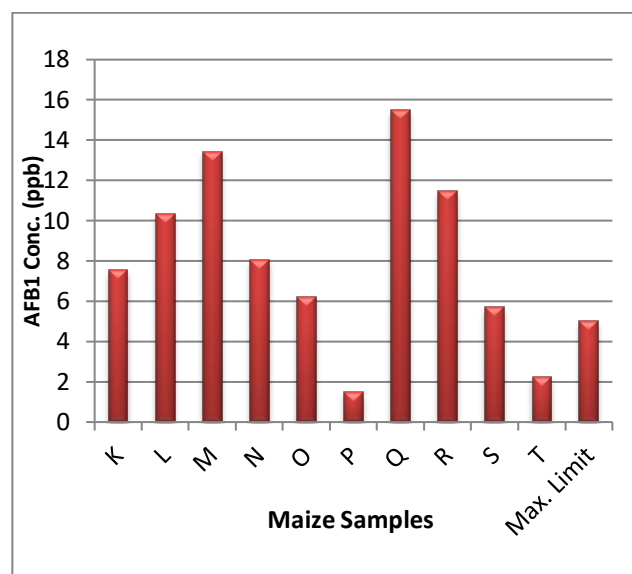


Figure 2. Comparison of AFB1 Levels in Maize Samples from Wukari, Nigeria with Maximum Permissible Limit

An Assessment of chronic exposure and probabilistic health risk from consumption of AFB1 in the investigated food samples was done through calculation of chronic daily intake (CDI) and margin of exposure (MOE). The results obtained are presented in Table 3. MOE is a harmonized approach to compare the margin between a dose and an exposure that causes cancer to humans or animals. This approach uses animal dose and dietary exposure in humans to derive a value which indicates carcinogenic risk. In this study, the risk evaluation was done for two weight groups; Adults and with 70 kg average weight and children with 15 kg average weight. The CDI values obtained for groundnut samples ranged from 3.20 – 6.15 $\text{ng}/\text{kg bw day}^{-1}$ for adults and 14.96 – 28.74 $\text{ng}/\text{kg bw day}^{-1}$ for

children while the MOE values were in the range of 27.62 – 53.125 and 5.92 - 11.36 for the adult and children categories respectively. The risk assessment for maize consumption revealed CDI range of 1.15 – 12.09 ng/kg bw day⁻¹ for adults and 5.38 – 56.42 ng/kg bw day⁻¹ for children while MOE values were 14.06 – 147.83 and 3.01 – 31.60 for the two groups respectively. The range of the MOE gives an indication of the level of concern with respect to carcinogenic risk; the larger the MOE, the smaller the potential risk and vice versa. A value of 10,000 is recommended as the limit of safe MOE for AFB1 (USEPA, 2011). Our findings in this study revealed that all the MOE values are lower than 10,000 thus indicating that consumption of the investigated foods poses high risk to public health. The exposure to the children category is much higher than the adult category. This is due to the lower body weight of children which leads to higher intake per unit body weight.

Table 3. Exposure Assessment and Risk Characterization of Aflatoxin B1 Intake through Consumption of groundnut and Maize from Wukari

Food type	Adults (70 kg BW)		Children (15 kg BW)	
	CDI (ng/kg)	MOE	CDI (ng/kg)	MOE
Groundnut	3.20–6.15	27.64 – 53.125	14.96 – 28.74	5.92 – 11.36
Maize	1.15 – 12.09	14.06 – 147.83	5.38 – 56.42	3.01 – 31.60

The MOE values found in this study are comparable to those reported in some African countries; Studies from Kenya, Botswana, Gambia and Tanzania reported MOE values of 6.5, 37.8, 621.7 and 202 respectively (Shephard *et al.*, 2008). Similarly AFB1 Margin of Exposure (MOE) values of 6.80, and 6.75 for children and adults were recorded for a study on maize consumption in Ghana (Kortei *et al.*, 2022). Exposure assessment and estimated cancer risk due to consumption of maize grains from related studies in Nigeria showed much higher risk than the current study; MOE values in the range of 0.01- 0.54 and 0.02 – 1.30 were reported for

children and adult groups respectively in a study on risk assessment of mycotoxins in stored maize grains consumed by infants and young children in Nigeria (Adetunji *et al.*, 2017). Similarly dietary risk assessment of aflatoxins among consumers of cereals, nuts and legumes in north-central Nigeria revealed mean MOE values of 0.035 for children and 0.21 for adults for AFBI consumption from peanuts. The values for maize consumption were 0.26 and 1.58 for children and adults respectively (Ezekiel *et al.*, 2021). Overall, the MOE values we report in this study were within the range of values that have been reported for related studies within the country.

Conclusion

This study has shown that the level of aflatoxin contamination in maize and groundnut samples from wukari, Nigeria is of great concern. AFB1 was found in all the investigated samples and 90% of the concentrations were above tolerable limits. The probabilistic Monte Carlo method which was used to assess the carcinogenic risk of the analysed foodstuff revealed margin of exposure (MOE) values that are by far lower than the 10,000 safe exposure value. This indicates high carcinogenic risks among the populations that consume the investigated food samples.

We recommend that regulatory bodies in the country take serious action against agricultural food products contamination by aflatoxins in order to safeguard public health. Actions such as regular monitoring for compliance across the chain of production to consumption should be implemented. There is also need to create awareness about aflatoxins among farmers and farm produce traders to implement practices that would help reduce aflatoxin contamination in farm produce.

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