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ORIGINAL ARTICLE

Assessment of Aflatoxin B1 (AFB1) in Stored Groundnuts in Nasarawa State

Pedro Akharenegbe[*1,2], Aleruchi Chuku[1], John Mawak[3], Bashir Sani[1]

[1]*Department of Microbiology, Faculty of Science, Federal University of Lafia, PMB 146, Lafia, Nasarawa State, Nigeria*

[2]*Department of Science and Laboratory Technology, Faculty of Science, Federal University of Lafia, PMB 146, Lafia, Nasarawa State, Nigeria*

[3]*Department of Microbiology, Faculty of Science, University of Jos, PMB 2084, Jos, Plateau State, Nigeria*

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ABSTRACT: Aflatoxin B1 is the most common, toxic, and carcinogenic aflatoxin produced by some species of *Aspergillus*. This study assessed the distribution, prevalence and concentration levels of AFB1, and the moisture content of groundnut stored in Nasarawa State. A total of 300 groundnut samples were collected from *rubum* and analyzed quantitatively and qualitatively for AFB1 using standard thin layer chromatography procedures. Results indicated a 90% prevalence rate of AFB1 in the samples, with concentration levels ranging from 11.49±9.15µg kg^{-1} to 76.29±14.39µg kg^{-1}. The groundnut samples had a moisture content of 3.66±3.21% to 6.26±2.48%, which was observed to be positively and significantly correlated to AFB1 levels. About 51% and 60% of the samples had AFB1 levels above the limits of the Standard Organisation of Nigeria and United States Food and Drug Administration (>20µg kg^{-1}) and the European Union (4µg/kg) respectively. All samples (100%) from Nasarawa Eggon, Wamba, Karu, and Nasarawa LGAs had detectable limits of AFB1, while Doma and Obi had the lowest rate of positive samples (73.91%). Toto (78.26%) and Nasarawa Eggon (73.91%) LGAs had AFB1 levels above EU and SON/USFDA recommended limits. Samples from Nasarawa North (38.51±26.01µg kg^{-1}) agricultural zone had the highest AFB1 concentration levels and moisture contents, followed by Nasarawa South (34.03±9.72µg kg^{-1}) while Nasarawa West (28.15±21.28µg kg^{-1}) had the least values. The concentration levels of AFB1 and moisture content varied significantly ($p<0.05$) across both LGAs and agricultural zones. The findings of this study raise considerable health concerns. There is a need for improved consumer awareness, routine assessment of groundnut and groundnut products, and improvement in storage practices to curtail outbreaks of AFB1 ingestion and intoxication.

INTRODUCTION

Some toxigenic mold species produce secondary metabolites pre- and post-harvest called mycotoxins. These mycotoxins are known to contaminate diverse groups of food and food crops including spices, nuts, fruits, and oilseeds, with the Food and Agriculture Organisation and the World Health Organisation estimating that 25% of global cereals produced are contaminated with mycotoxins [1–3]. In addition, about 100 countries have feed/food laws and regulations because of the hepatotoxic and carcinogenic effects of mycotoxins [4, 5].

Aflatoxins, one group out of over 400 mycotoxins, are

*Corresponding author: peddyimo@yahoo.com (P. Akharenegbe)
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produced by members of the *Aspergillus* genus (*A. flavus, A. parasiticus, A. niger,* and *A. nomicus*) [6, 7]. Aflatoxins are the most common and most toxic mycotoxin. They have been designated as group 1 carcinogens since 1993 by the World Health Organisation (WHO) and International Agency for Research on Cancer (IARC) [1, 8, and 9]. Aflatoxins are heterocyclic aromatic hydrocarbons first discovered and isolated in 1960 from an outbreak of Turkey-X disease [10–12].

Aflatoxin B1 (AFB1) is one of six groups of secondary metabolites – AfB2, AfG1, AfG2, AfM1, and AfM2 – consisting of difuranocoumarin compounds [13]. Out of the six aflatoxins, AFB1 is the most prevalent, most potent carcinogen, has the highest toxicity level with a 0.36 mg kg^{-1} average lethal dose (LD_{50}), and has been tagged a class 1 carcinogen by IARC [8, 14–17]. AFB1 has also been reported to be highly teratogenic and mutagenic [11]. The maximum allowable level of AFB1 ranges from 2-30 µg kg^{-1}, with most countries setting a limit of 20 µg kg^{-1} [1, 11]. Early detection and prevention are the recommended control method, as AFB1 is highly stable, toxic, and is distributed widely in foodstuffs, especially in warmer climates [18]. AFB1 has been found in a wide variety of food crops such as cereals, spices, fruits, soy products, and of particular interest, peanuts [19].

Peanut (*Arachis hypogea*), also referred to as groundnut, monkey nut, or pindar, is a leguminous crop and a cheap source of nutrients grown globally. It is consumed in several varieties, such as flakes (popularly known as *kuli-kuli* in Nigeria), roasted, boiled, soup, oil, and as animal feeds [20, 21]. Currently, Nigeria is the 3rd largest producer of groundnut globally, sitting ahead of Sudan and the United States of America and just behind China – which ranks first – and India. In Nigeria, Nasarawa State is amongst the top groundnut-producing states that collectively produce over 80% of Nigeria's groundnut. Nasarawa State is also amongst the highest consumers of groundnut and groundnut products in Nigeria [22, 23].

Given the national and internal groundnut production position of Nasarawa State and Nigeria, the lack of data on AFB1 in Nasarawa State is worrisome. Therefore, this pioneering study in Nasarawa state aims to assess the prevalence of AFB1 in stored groundnuts in Nasarawa State.

MATERIALS AND METHODS

Study and sample area

This study was conducted in Lafia, the capital city of Nasarawa State, located in the North Central Region of Nigeria. Nasarawa State is one of the highest groundnut-producing States in Nigeria, and most of its citizens are predominantly farmers. Laboratory analysis and detection of AFB1 was carried out at the laboratory of the Microbiology Department, Federal University of Lafia.

With 13 Local Government Areas and about 3 million population size, it is one of the smallest states in terms of population in Nigeria. The LGAs are grouped into three (3) ecological agricultural zones, which are: Nasarawa North zone (Akwanga, Nasarawa Eggon and Wamba), Nasarawa South zone (Awe, Doma, Keana, Lafia and Obi), and Nasarawa West zone (Karu, Keffi, Kokona, Nasarawa and Toto).

Sample collection

A cross-sectional randomized study design approach was adopted in the collection of samples. About 4-5kg of groundnuts were collected from 300 storage facilities – called *rubum* in Hausa – from each Local Government Area in the agricultural zones. In Lafia, a total of 24 samples were collected, while a total of 23 samples each were collected from other LGA.

Detection of Aflatoxin B1 (AFB1)

Sample preparation, detection, and quantification of AFB1 were carried out as described herein [24, 25]. About 20g of each groundnut sample were macerated and homogenized in 100ml of 80% methanol in a rotary shaker at 400rpm for 30 minutes and subsequently filtered. An 8:8:5 mixture consisting of 40ml of filtrate, 40 ml of 10% sodium chloride, and 25ml of hexane was prepared, vortexed, and allowed to stand for a minute. After standing for a minute, 25ml of dichloromethane was then added, mixed, and allowed to stand for another minute at room temperature resulting in the stratification of the mixture. Finally, draining of the bottom layer of

the mixture was done through anhydrous sodium sulfate (25g) bed into a beaker and evaporated to dryness in a fume hood.

The extracts were reconcentrated with 1ml of dichloromethane, transferred into an Eppendorf tube, and subjected to scanning densitometry. On thin-layer chromatography (TLC) aluminium (20 × 10 cm) silica gel 60 F254 plates (Merck, Darmstadt, Germany), 4µl of each extract was carefully spotted together with aflatoxin B1 standards (Supelco, Bellefonte, PA). The TLC plates were observed under ultraviolet light at 365 nm for the presence or absence of aflatoxins B1. Quantification and analysis of total AFB1 present in each sample were determined using the CAMAG TLC Scanner 3 scanning densitometer and winCATS 1.4.2 quantification software (Camag, AG, Muttenz, Switzerland), with a 1µg kg^{-1} limit of detection.

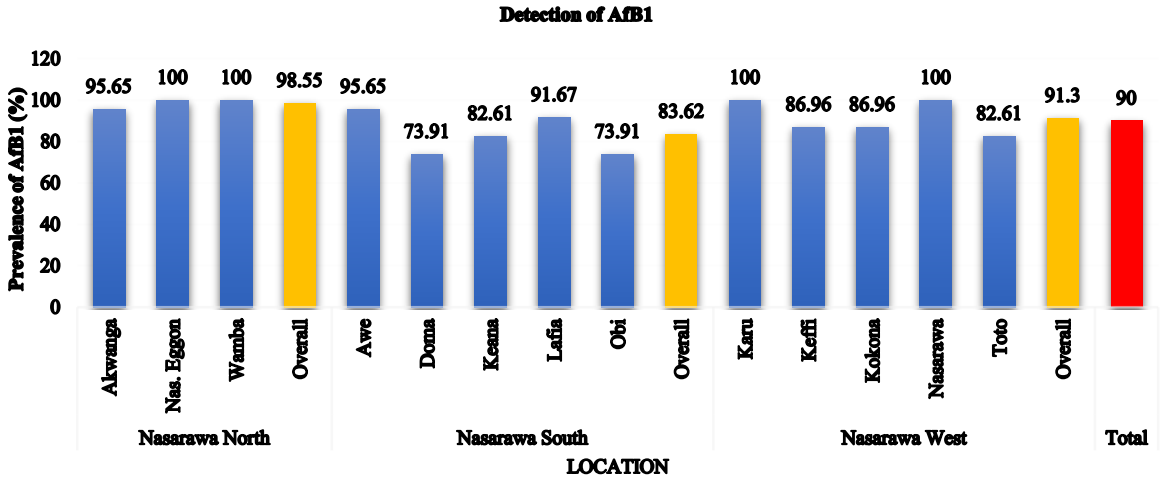

Figure 1. Detection rates of AFB1 in groundnut stored in Nasarawa State.

Determination of moisture content

The moisture content of the groundnut samples was determined using MCA110-T rapid moisture analyzer (Bioevopeak Company Ltd. China). 50g of each groundnut sample was inserted into the moisture analyzer in triplicates, and the percentage moisture content of the samples was observed from the digital reader. The average of the triplicate sample was recorded and taken as the percentage moisture

Statistical analysis

Data obtained was analyzed using R Console software (Version 4.0.2). Shapiro-Wilk normality test was carried out, and it revealed that the data was not normally distributed. Hence, the data were log-transformed, and one-way analysis of variance (ANOVA) was carried out. The Chi-square test was used to determine the statistical significance of the mean difference of aflatoxins as well mean of the moisture content. The different means were separated using Tukey's HSD test. The level of significance was set at $P < 0.05$. Linear regression analysis was used to establish the predictor effect of moisture content on the aflatoxin content of groundnut.

RESULTS

Detection and prevalence of aflatoxin B1 (AFB1) from stored groundnuts in Nasarawa State

Laboratory detection of AFB1 from the stored groundnut samples showed an overall prevalence rate of 90% (270/300). A 100% (23/23) prevalence of AFB1 was observed in samples from Nasarawa Eggon, Wamba, Karu and Nasarawa LGAs, while Doma and Obi LGAs had the least AFB1 prevalence of 73.91% (17/23). Based on agricultural zones, Nasarawa North (98.55%; 68/69) had the highest prevalence rate, closely followed by Nasarawa West (91.30%; 105/115) and Nasarawa South (83.62%; 73/116) had the least prevalence of AFB1 as

shown in Figure 1. However, there was no significant difference (p.0.05) in the prevalence rate of AFB1 in the study location.

About 60% and 51% of positive samples had AFB1 limits above EU standard (4µg kg^{-1}) and SON/USFDA standards (20µg kg^{-1}), respectively, as shown in Figure 2. Based on EU standards, Toto LGA had the highest (78.26%; 18/23) samples with unsafe levels of AFB1, while Obi and Keffi LGAs had the lowest (43.48%; 10/23) samples with unsafe levels. Quite similarly, SON/USFDA standards showed that Keana, Obi and Keffi LGAs had the lowest (39.13%; 9/23) samples with unsafe levels, and Nasarawa Eggon had the highest (73.91%; 17/23) samples with unsafe levels.

Concentration levels of AFB1 and moisture content from stored groundnuts in Nasarawa State.

The mean levels of AFB1 detected in the groundnut samples analyzed ranged from 11.49±9.15µg kg^{-1} (Karu) to 76.29±14.39µg kg^{-1} (Nasarawa Eggon), as shown in Table 1. Nasarawa West agricultural zone had the lowest average detected level of 28.15±21.28µg kg^{-1}, while Nasarawa North zone had the highest average AFB1 level of 39.51±26.01µg/kg. The concentration levels of AFB1 varied significantly ($p<0.05$) in both the local government areas and agricultural zones.

Groundnut samples from Nasarawa North agricultural zone had the highest mean moisture content of 5.34±2.85%, followed by Nasarawa South (4.74±5.41%), and Nasarawa West had the least mean moisture content of 4.08±3.08%. Moisture content levels were significantly different ($p<0.05$) among the agricultural zones and local government areas. Within LGAs studied, Nasarawa LGA had the least (3.66±3.21%) mean moisture content, while Nasarawa Eggon LGA had the highest (6.26±2.48%) mean moisture content.

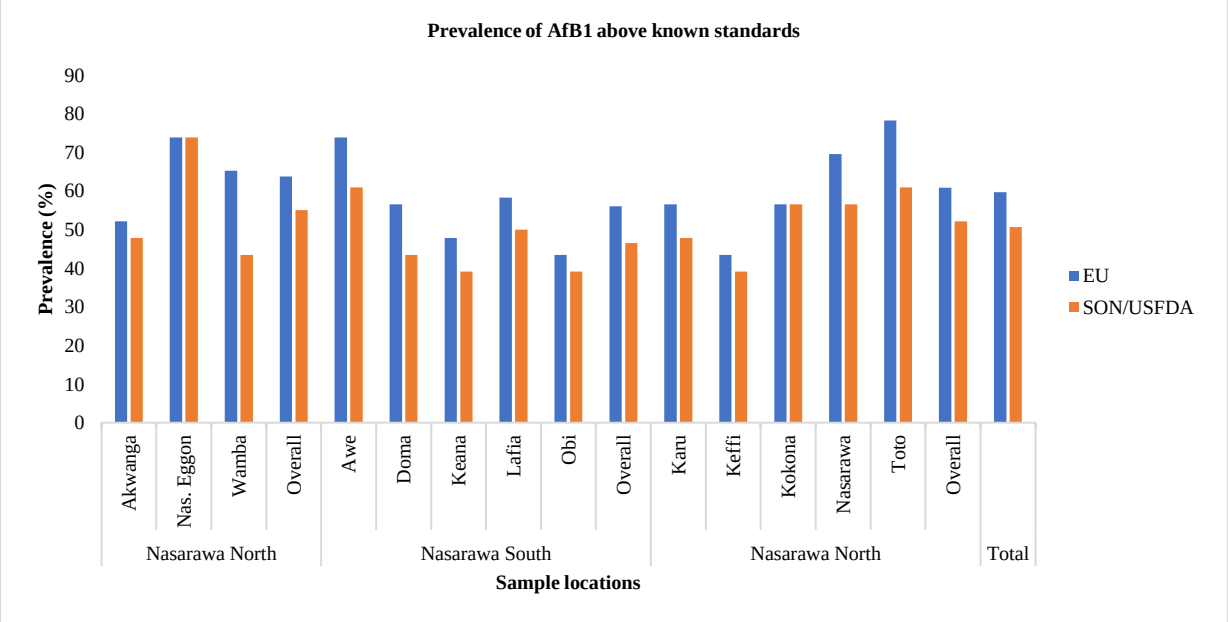

Figure 2. Prevalence of AFB1 in stored groundnut samples above EU (European Union) and SON/USFDA (Standard Organisation of Nigeria and United States Food and Drug Administration)

Table 1. Mean incidence levels of AFB1 (µg kg^{-1}) and moisture content (%) of stored groundnuts in Nasarawa State.

Agricultural Zone	LGAs	Mean ± SD of AFB1 levels (µg kg^{-1})		Mean ± SD of moisture content (%)	
		LGA	Zone	LGA	Zone
Nasarawa North	Akwanga	21.01±18.73	39.51±26.01	4.94±2.90	5.34±2.85
	Nasarawa Eggon	76.29±14.39		6.26±2.48	
	Wamba	21.23±10.25		4.81±3.02	
Nasarawa South	Awe	30.15±9.49	34.03±9.72	4.37±3.16	4.74±5.41
	Doma	37.29±8.87		5.83±8.95	
	Keana	22.72±13.84		4.38±0.91	
	Lafia	51.12±15.36		5.26±4.86	
	Obi	28.85±16.39		3.83±4.07	
Nasarawa West	Karu	11.49±9.15	28.15±21.28	4.23±2.31	4.08±3.08
	Keffi	13.73±12.22		4.03±3.27	
	Kokona	26.08±12.78		4.34±4.05	
	Nasarawa	19.99±9.21		3.66±3.21	
	Toto	69.47±4.97		4.13±2.51	

Prediction of aflatoxin B1 contents in groundnuts as a function of Moisture content

There was a significant positive relationship between moisture content and aflatoxin B1 in the samples from Nasarawa State. The regression model predicted a linear relationship between aflatoxin B1 and moisture content, as shown in Figure 3. The result showed that moisture content had a significant effect ($P<0.001$) on the production of aflatoxin B1 (AFB1) in stored groundnuts in Nasarawa State

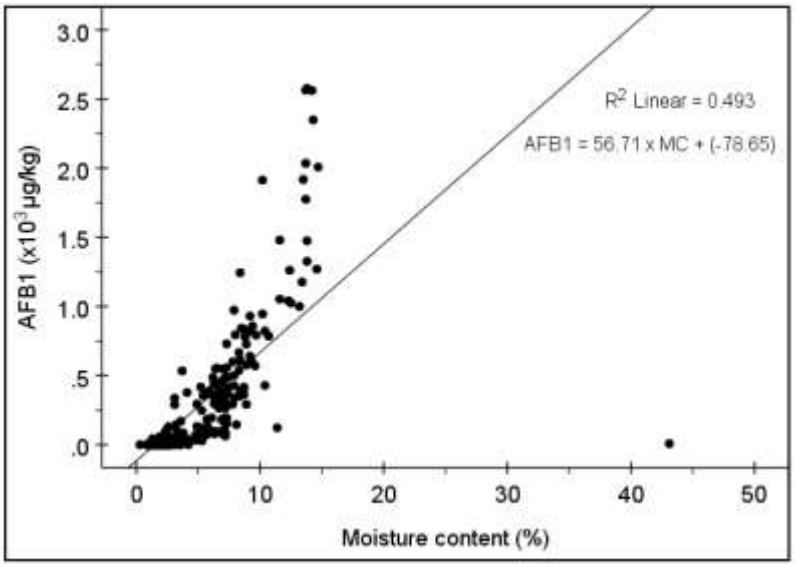

Figure 3. Aflatoxins B1 production as a function of moisture content in stored groundnuts from Nasarawa State

DISCUSSION

The health implications associated with AFB1 cannot be overemphasized, with reports showing more carcinogenicity evidence in AFB1 than in other aflatoxins [26]. In addition, there has been an increase in groundnut production due to the introduction of new and improved variants, despite the poor availability of data on aflatoxin B1 levels in stored groundnut and storage facilities. This led to investigating the distribution and

prevalence of AFB1 in stored groundnut in Nasarawa State. This study showed that 90% of the stored groundnut samples had detectable limits of AFB1, with AFB1 detected in all samples from Nasarawa Eggon, Wamba, Karu, and Nasarawa local government areas. The prevalence rate in this study is far higher than the 14% [27] and 24% [28] previously reported but concurs with other reports where over 90% prevalence was observed in peanut and peanut products from Taiwan, Benin and Thailand [6, 18, 29, 30].

Concentration levels of AFB1 in this study ranged from 11.49±9.15µg kg^{-1} to 76.29±14.39µg kg^{-1}. About 60% of samples had AFB1 levels above the European Union (EU) standard of ≤4µg kg^{-1}, while about 51% of the samples had AFB1 concentration levels above the 20µg kg^{-1} maximum limit of the Standard Organization of Nigeria (SON) and the United States Food and Drug Administration (USFDA). These findings are in tandem with previous reports where levels of AFB1 were above EU, SON and USFDA standards [6, 18, 31–33]. Nasarawa North had more prevalence and high concentration of AFB1 levels compared to other agricultural zones. This could be attributed to the region being colder than other regions of the State [34]. The high AFB1 levels and prevalence rate observed in this study are alarming, considering the toxicity level and public health burden of ingesting AFB1 and the consuming population. Ingestion of AFB1-contaminated foods is reported to lead to hepatoxicity and carcinogenicity, causing diseases such as carcinoma, cirrhosis in children, kwashiorkor, and chronic gastritis [6]. AFB1 has also been reported to cause a significant level of toxicity in laboratory and farm animals, resulting in multiple interferences with protein metabolism, micronutrient synthesis, and immune and neural responses [35], with reports suggesting that similar effects could occur in humans exposed to high levels of AFB1 [7, 36, 37].

The moisture content of the groundnut samples ranged from 3.66±3.21% to 6.26±2.48%. A significant positive correlation (r^2 = 0.493; $p<0.05$) between moisture content and AFB1 levels was observed. This signifies that the higher the moisture content, the higher the probability of the presence and high concentration of AFB1. This concurs with several reports where samples from more humid areas had a higher prevalence and concentration of AFB1 than less humid or drier regions [38–41].

The high AFB1 prevalence and levels observed in this study could be attributed to the geography of Nasarawa State, post-harvest practices, and storage conditions. Nasarawa State is classified as having a rainy tropical climate with an annual rainfall of about 2,000mm, characterized by a seven-month rainy period accompanied by a dry winter season. Humidity ranges from 55% in the dry season and up to 95% in the rainy season. This hot humid climatic condition has been reported to favour and enhance optimum growth condition for the growth of fungi that produces aflatoxins [42, 43]. Storing periods and conditions [43] could also be responsible for the traits observed in this study, with literature stating that groundnuts are highly susceptible to mycotoxin contamination because of their nutritional contents [44, 45]. Therefore, longer storing periods, especially under poor conditions, could enhance fungal growths, mycotoxins synthesis, and contamination.

CONCLUSIONS

Analysis of samples of groundnuts stored in *'rubum'* in Nasarawa State showed high prevalence (90%) and high concentration levels (11.49±9.15 – 76.29±14.39µg kg^{-1}) of aflatoxin B1. Although prevalence and concentration levels of AFB1 varied among the local government areas and agricultural, the difference were not statistically significant. Results of this is alarming and ring the bells for urgent regulatory authorities (local and national) to intervene and check levels of AFB1, mycotoxins, and other microbial toxins in food storage facilities in the State and beyond due to the deleterious effects these toxins could have on humans and animals, the public health burden it could cause as well as the economic consequences.

CONFLICT OF INTERESTS

No conflict.

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