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Aflatoxin Contamination Levels in Sesame Seeds Sold in Benue State, North Central Nigeria

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Abstract

Storage of sesame is increasingly becoming important among the growers and users of the crop in Benue state, north central Nigeria. The aim is to sell the produce and maximize benefits/ or for evacuation for export markets. These envisaged advantages often fail due to fungi and its attendant aflatoxin contamination. This study aimed to determine aflatoxin production in Sesame storage in Benue State, Nigeria. Four samples were collected each from four different markets within each of the six surveyed LGA resulting in a total of 96 samples. Markets from selected local government areas were identified through local government departments' of agriculture and traditional authorities. Using high performance liquid chromatography (HPLC) analyses; the fraction for the total aflatoxin was subjected to a specific column chromatographic clean up. HPLC analysis revealed four types of aflatoxin viz: AFB_1, AFB_2, AFG_1, and AFG_2 respectively. AFB_1 had the highest frequency of occurrence and the levels were: AFB_1 =3.95-11.75µg/kg, AFB_2= 0.00-2.35µg/kg; AFG_1= 0.00-2.06 µg/kg; AFG_2=0.00-1.47µg/kg respectively. These values were above the European Union Maximum Residue Limits (MRL) of 2 µg/kg for aflatoxin B_1 and 4 µg/kg for total aflatoxin. In conclusion, considering the benefits of sesame, it is recommended that they should source for other type of crops that are not highly susceptible to aflatoxin and also several treatments are be applied to reduce the levels of contamination in sesame before its utilization for safety purposes.

Keywords

Sesame *Sesamum indicum*, Aflatoxin Level, Contamination, Safety

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1. Introduction

Seed storage is of great significance factor affecting seed longevity. Many rsearchers reported that the speed of decline in seed quality is dependent on storage, length of storage, type of seeds and seed quality [1]. Sesame (*Sesamum indicum* L.) is rich in different unsaturated fatty acids and minerals [2] and probably one of the oldest and most traditional oil seed crops known to mankind [3]. It is mainly cultivated in the Savannah zones of Nigeria and many other parts of the world [4], whose oil is often preferred to ground nut oil because of its better flavour-enhancing property, 100% free of cholesterol, while the seeds are used as spices and livestock feed. It is a major cash crop export of Nigeria

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to countries like: Japan, China and Turkey [5]. Sesame contains ~ 50-52% oil, 17-19% protein and 16-18% carbohydrate [6]. The fat content of sesame seeds is about (~) 50% [7]. Fatty acids such as oleic acid ~ (43%) linoleic acid ~ (35%), Palmitic acid ~ (11%), and stearic acid ~ (7%) contributing to 96% of total fatty acids [8]. Food stuffs rich in oil, such as nuts and seeds (peanuts, pistachio etc) [9-12] are sensitive to AF- producing fungal invasion and may therefore, be contaminated with AFs, particularly AFB_1.

Sesame is one of the oil seeds cultivated in Nigeria, being the fifth on the global list of highest producing Nations and first in Africa [13]. Nigeria contributed 450,000t to the global production of 5,631, 443t of sesame seeds in 2016 [13] with export quantities of 172,839t [14]. One major limiting factor to the export of sesame to the European Union (the major market for several African countries) is contamination with mycotoxins (especially aflatoxins) beyond the regulatory limits of 2 and 4 µg/kg for aflatoxin B_1 and total aflatoxins respectively set by the European commission for oil seeds, (peanuts, nuts, dried fruits and cereals) intended for human consumption (European Commission [15] whilst worldwide limits for AFB_1 range between 1 and 20 µk/kg and 0 and 35 for total aflatoxin µg/kg [16]. Aflatoxins (AFs) are highly toxic secondary metabolites produced by certain species of *Aspergillus* mainly *Aspergillus flavus* and *A. parasiticus* [17, 18]. Among these, aflatoxin B_1 (AFB_1) represents the most common contaminant and the most potent liver carcinogen, and classified by the International Agency for Research on Cancer (IARC) as a group 1 carcinogen [19]. Besides, AFs have negative economic impact on several crops and food chain [20, 21].

Sesame is attacked by many fungi including *Fusarium oxysporum*, *F. sesame* and *Macrophomina phaseolina* [22, 23]; *Alternaria brassicola*, *A. redicina*, *Aspergillus alba, A. flavus, A. niger, A. viridus, Cephalosporium* spp., *Curvularia* spp., *Drechslera* spp., *Fusarium* spp and *Penicillium* spp from nine sesame cultivars in Pakistan [24]. In a similar report, Sabry et al [25] isolated *Alternaria* spp. *Aspergillus parasiticus*, *A. flavus*, *A. niger*, *Penicillium* spp., *Cladosporium* spp from sesame seeds in Egypt., whereas *Alternaria*, *Aspergillus*, *Cercospora*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* were associated with sesame seeds in Plateau state, Nigeria [26]. Diverse fungi, including notable storage moulds were found as contaminants of sesame seeds in Benue and Nasarawa state Nigeria [27]. The findings of these research workers indicate that mycoflora of sesame seeds may not be the same for different places and conditions. At present, aflatoxins are yet to be regulated at the Local market in Nigeria. It therefore, becomes highly necessary for continuous monitoring and tracking compliant and violates agricultural commodity shipments at the local market before they get to the international market [27]. For bountiful yield, healthy seeds should be given priorities among the most important things in agronomic practices. Global losses in food production due to seed borne diseases are important negative factors in world agriculture; hence, seeds should be examined on a regular basis. However, information is still scanty on (1) mycoflora of stored sesame seeds and (2) aflatoxin contamination level in stored sesame seeds in the study area.

2. Materials and Methods

Benue state is located in the Southern Guinea Savanna which is a transition belt between the grassland savanna in the North and the rainforest in the South. Benue State, Nigeria is designated with (latitude 6°21′ - 8°10′ N and longitude 7°44′ E - 9°55′ E).

2.1. Sampling

The State has three agricultural zones namely: North which comprises (Buruku, Guma, Gboko, Tarka, Makurdi, Gwer-West and Gwer) Local government Areas, East (Konshisha, Vandeikya, Kwande, Ushongo, Katsina-Ala, Ukum and Logo), and South (Apa, Ado, Agatu, Otukpo, Ohimimi, Okpokwu, Ogbadibo, Obi and Oju). In this study, two zones that are known as the belt of sesame production in the State were chosen (BNARDA, 2011). Of these zones, a total of six LGAs including Guma, Gboko and Makurdi (Benue North) and Otukpo, Obi and Ogbadibo (Benue South) were included in the survey. Four samples were collected each from four different markets within each of the six surveyed LGA resulting in a total of 96 samples. Markets from sampled areas were identified through department of Agriculture in their local government areas.

2.2. Representative Sample Size Determined by (DRSS)

Sample size was determined by using the formula:

$$S = \frac{x^2 PQ}{l^2}$$

S = Total number of markets/locations

x = Score of confidence interval

P = Prevalence

Q = 1-p

l = error of estimation

The confidence interval was 95% while error of estimation was 0.05 with an estimated prevalence of 47% (26)

2.3. Extraction of Aflatoxin and Clean up

Samples were analysed for Aflatoxin B_1, G_1, B_2 and G_2 after undergoing proper extraction and cleaning using a standard method [28]

2.4. Extraction of Aflatoxin

Sesame sample impoverished form was weighed into Erlenmeyer flask (500 ml). Adding to the flask were phosphoric acid 25ml (250 ml) and methylene chloride 250 were added. After placing the flask, on the mechanical shaker for 30 minutes, the content was filtered using a Buchner funnel and a rapid filter paper of 18cm in diameter. Exactly 50 ml aliquot of the filtrate was transferred into another flask for aflatoxin quantification.

2.5. Aflatoxin Clean-up

Column chromatographic method was used for the analysis of Afs fractions. Exactly 150ml of Dichloromethane (DCM) was dispersed into the chromatographic column that was initially set up with a glass wool. The content was poured half way to the column but Anhydrous sodium sulphate (Na_2SO_4) was added. Also added to the column was Silica gel. Filtrate (50ml) was obtained and defatted with N-hexane (130ml) and Ether (130ml) step wisely. Extraction of aflatoxin was done in the mixture of Ether, methanol and water in the ratio 96; 3:1 respectively. After collecting the extract into a new beaker it was evaporated and placed in glass vials. Storage was done at 0°C for analysis.

2.6. High Performance Liquid Chromatography (HPLC)

HPLC system on a Cecil 1100 series connected to a UV detector set up for aflatoxin analysis at 365 nm [29]. The ambient temperature was set at 25°C. While the chromatographic column had a dimension of 4.6mm by 250ml. the mobile phase was a mixture of Acetonitrile/ water/acetic acid in the mixture of (10:50:40,) respectively at a pumping rate of 0.8 ml/min while the injection volume was 20 µL in sample and standard.

2.7. Preparation for Aflatoxin Analysis

Aanalysis standards of known concentrations were used. AFB_1, AFG_1 were eluted at retention times of 1.673 min while AFB_2 and AFG_2 were eluted 1.524 min retention time. Coefficient of determination (R^2) for these two groups of aflatoxin is 0.91 and 0.99 respectively in the construction of calibration curve in a series of dilution for the standard preparation. Preparation was done in the HPLC coloumn until minimum concentration was established for the analyte. The LOD's scores were set at 0.21 and 0.18 µg/kg respectively. The LOQ (Limit of quantification) as determined through the Calibration curve were given as 0.42 and 0.33 µg/kg respectively. The actual aflatoxin was quantified in µg/kg using the formula:

Actual aflatoxin (µg/kg) is equal to

$$\frac{S \times Y \times V}{W \times Z}$$

S = volume of standard having similar color intensity as sample (µl);

Y = Mycotoxin standard concentration

V = volume of solvent diluted the final extract required to dilute sample contained in final extract;

W = weight (g) of original sample in final extract;

Z = volume of sample equivalent to standard (µL)

2.8. Statistical Analysis

Statistical Analysis was done using GENSTAT version 17 for the analysis of variance. Mean separation was done by DMRT 0.05 level of significance**.**

3. Results

The study revealed the types, frequency and the levels of aflatoxin concentration in six local government areas of Benue state Figures 1-6.

Table 1 shows the mean concentration levels of AFB_1 and AFB_2 recovered from six local government areas (LGAs) in Benue State. The LGAs are: Guma, Otukpo, Ogbadibo, Gboko, Makurdi and Obi. Mean concentration levels of AFB_1 were found to be higher than those determined from AFB_2. The mean concentrations of AFB_1 in the six LGAs were in the range of 5.60 – 6.65 µg/kg and had low variability with samples from Makurdi having the least average AFB_1 concentration (5.6 µg/kg) while samples from Gboko had the highest concentration on the average (6.65 µg/kg). The highest maximum AFB_1 concentration was found in samples obtained from Guma LGA (11.75 µg/kg) followed by those obtained from Ogbadibo LGA (8.40 µg/kg) and Otukpo LGA had the least maximum AFB_1 concentration (7.48 µg/kg). Mean AFB_2 concentration levels unlike AFB_1 varied greatly across the LGAs involved in survey. The highest average concentration of AFB_2 was recorded in samples obtained from Otukpo LGA (1.76 µg/kg) followed by those obtained from Obi LGA (1.74 µg/kg). The highest maximum AFB_2 concentration was found in Otukpo (2.35 µg/kg) followed by Ogbadibo and Makurdi which had concentration levels of 2.31 µg/kg, on the average respectively. Samples obtained from Gboko LGA had the lowest maximum AFB_2 (2.15µg/kg). In Ogbadibo and Makurdi LGAs however, the minimum AFB_2 concentrations

were both 0.00 µg/kg (Table 1)

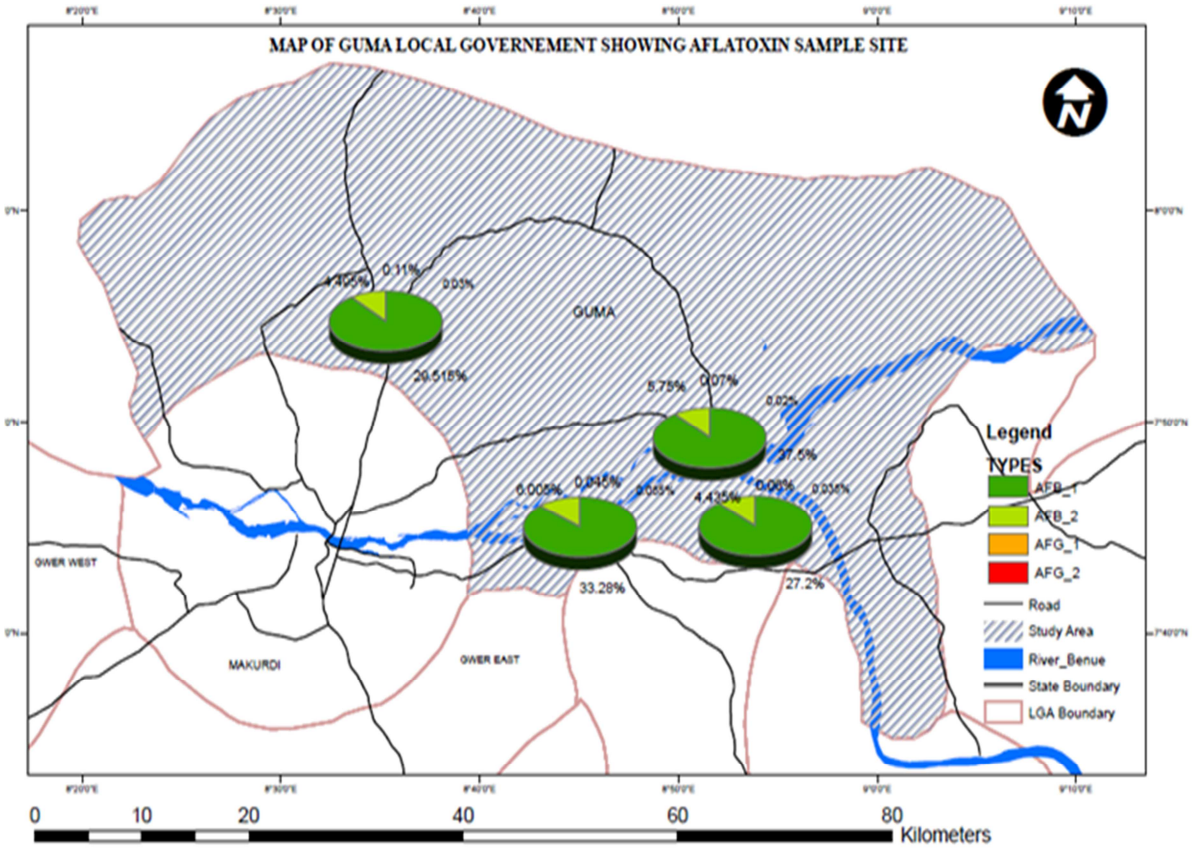

Figure 1. Map of Guma LGA showing aflatoxin types.

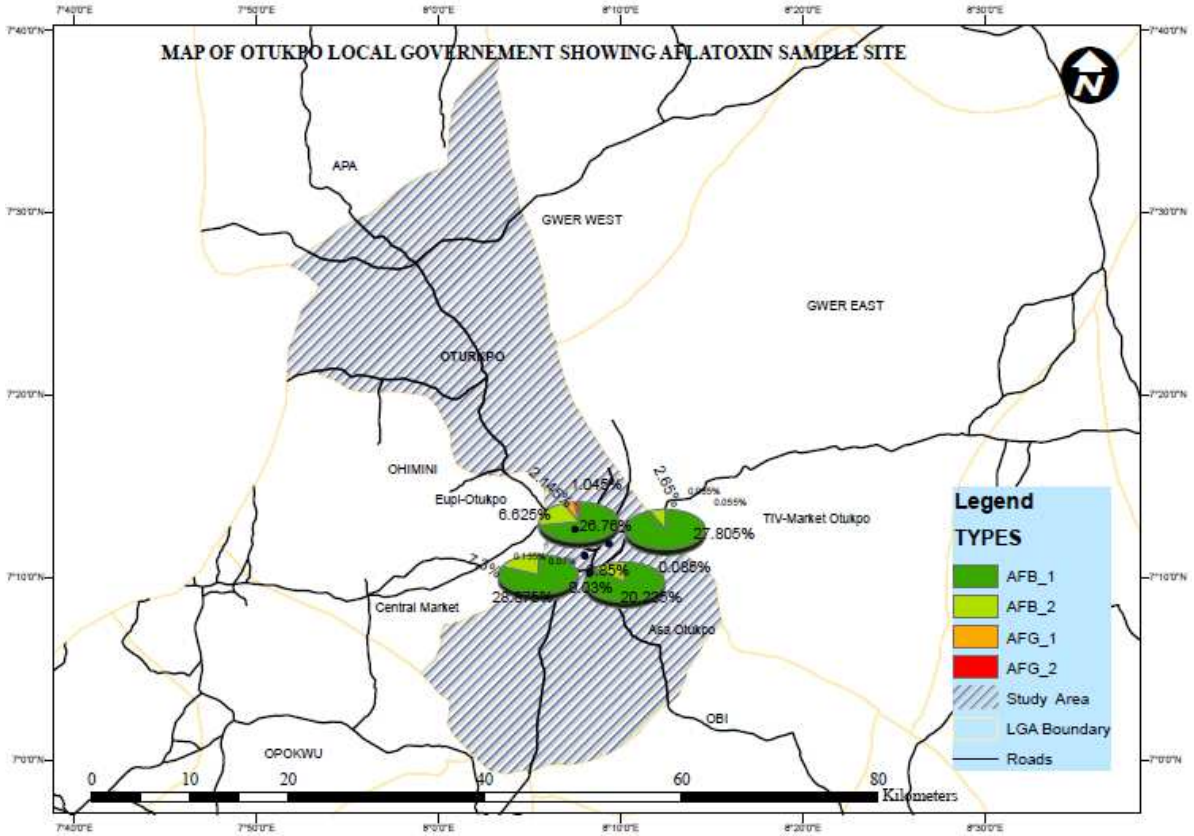

Figure 2. Map of Otukpo LGA showing aflatoxin types.

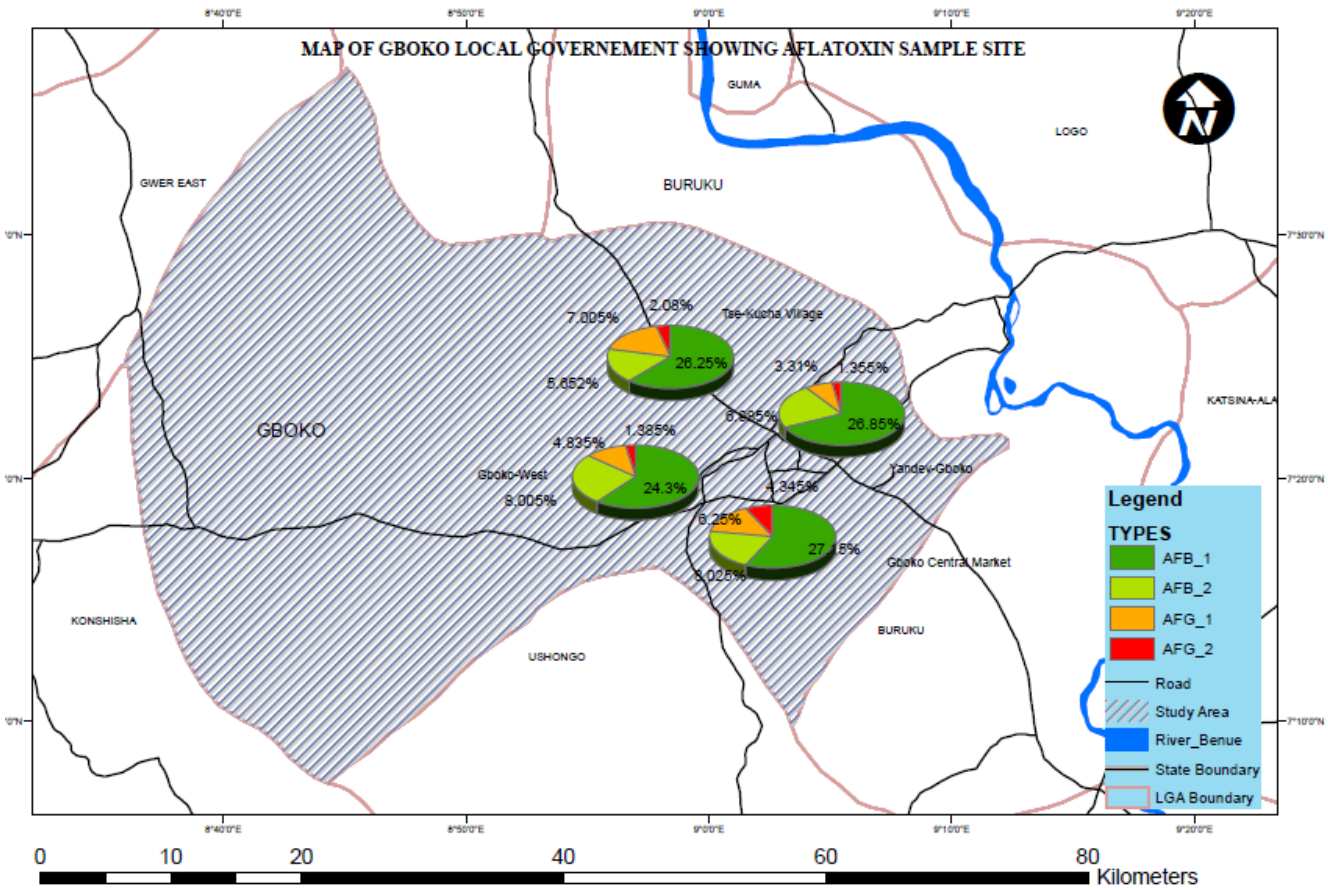

Figure 3. Map of Gboko LGA showing aflatoxin types.

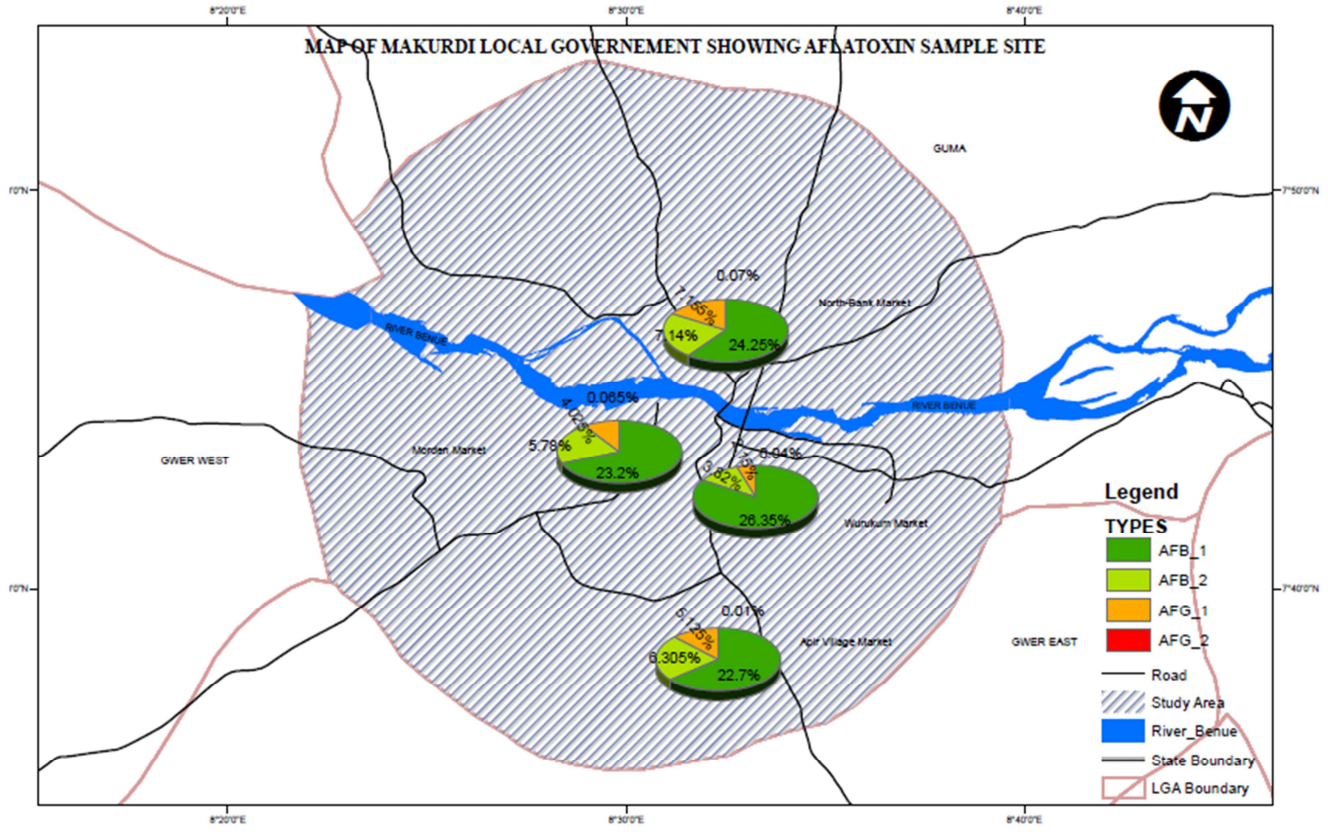

Figure 4. Map of Makurdi LGA showing aflatoxin types.

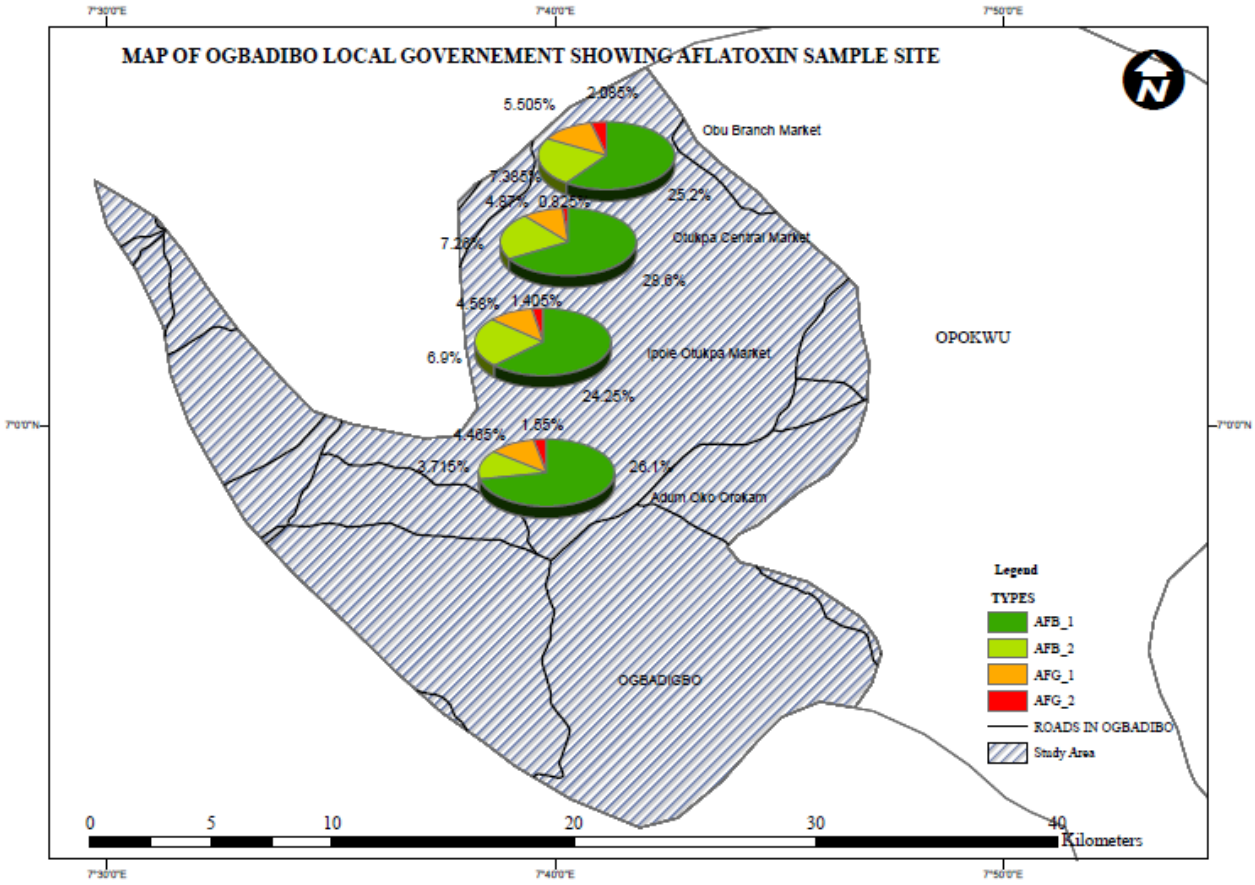

Figure 5. Map of Ogbadibo LGA showing aflatoxin types.

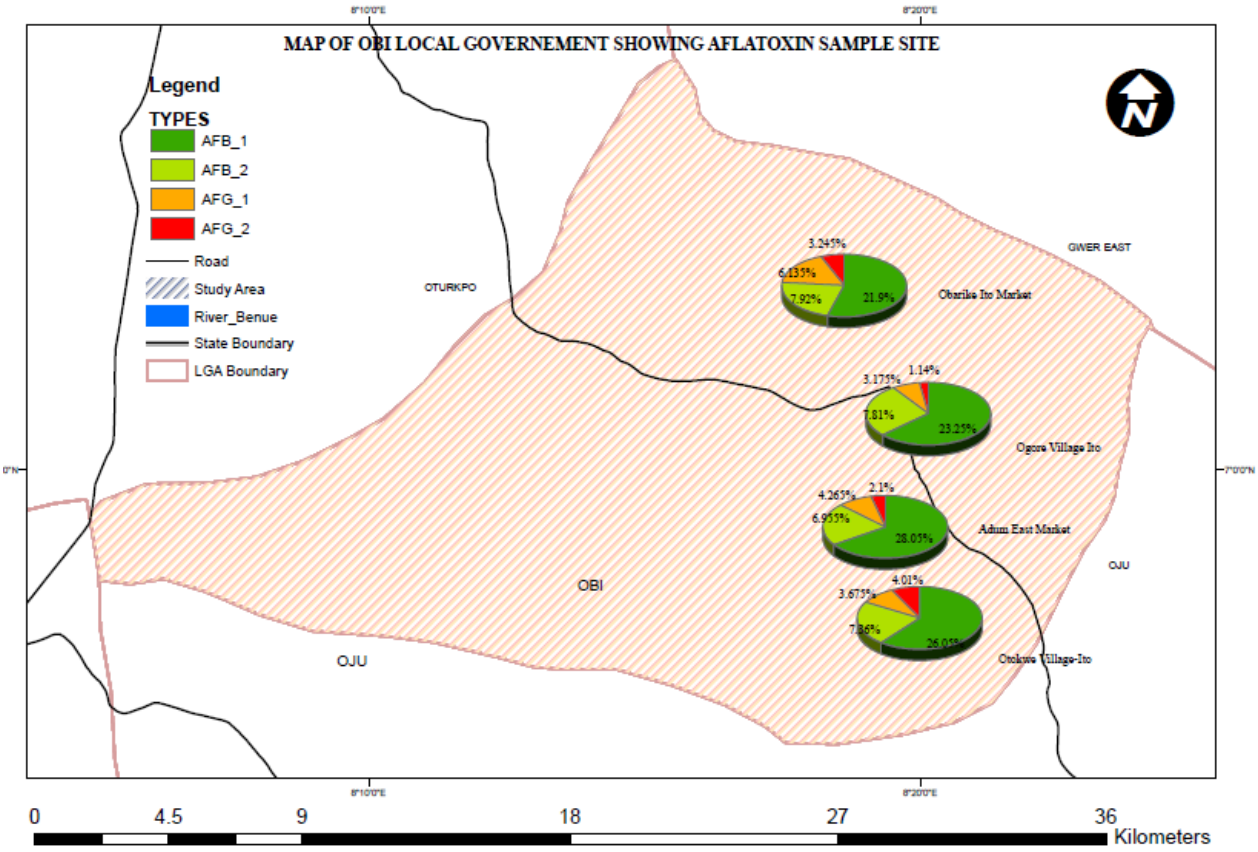

Figure 6. Map of Obi LGA showing aflatoxin types.

Table 1. Mean concentration levels of AFB_1 and AFB_2 in Benue State.

LGA	AfB1 (µg/kg)			AfB2 (µg/kg		
	Mean±SE (µg/kg)	Min.AfB_1	Max.AfB_1	Mean±SE (µg/kg)	Min.AfB_2	Max.AfB2
Guma	6.54±1.01	6.01	11.75	0.92±0.02	0.44	2.21
Otukpo	6.39±0.07	3.97	7.48	1.76±0.11	0.20	2.35
Ogbadibo	6.53±1.22	5.15	8.40	0.93±0.01	0.00	2.31
Gboko	6.65±1.05	4.70	8.05	1.44±0.42	1.06	2.15
Makurdi	5.6±1.00	3.95	9.70	1.35±0.02	0.00	2.31
Obi	6.45±0.06	4.85	7.65	1.74±0.66	1.08	2.20

*SE= Standard Error; *Min. = Minimum value; *Max. = Maximum value

Table 2 shows the mean concentration levels of AFG_1 and AFG_2 recovered from six local government areas (LGAs) in Benue State. The LGAs included Guma, Otukpo, Ogbadibo, Gboko, Makurdi and Obi. A mean concentration level of AFG_1 was found to be higher than those determined from AFG_2. The mean concentrations of AFG_1 in the six LGAs were in the range of 0.01 – 1.87 µg/kg and had high variability with samples from Gboko having the highest average AFG_1 concentration (1.87 µg/kg) while samples from Guma had the least concentration on the average of 0.01 µg/kg. The highest maximum AFB_1 concentration was found in samples obtained from Ogbadibo LGA (2.06 µg/kg), Gboko LGA (2.06 µg/kg), Makurdi LGA (2.06 µg/kg) and Obi LGA (2.06 µg/kg). Otukpo LGA had the least maximum AFG_1 concentration (0.03 µg/kg). Mean AFG_2 concentration levels like AFG_1 varied greatly across the LGAs involved in the survey. The highest average concentration of AFG_2 was recorded in samples obtained from Obi LGA (0.63 µg/kg) followed by those obtained from Ogbadibo LGA (0.39 µg/kg). The highest maximum AFG_2 concentration was found in Obi (1.47µg/kg) followed by Gboko which had concentration level of 1.17 µg/kg. Samples obtained from Guma LGA had the lowest maximum AFG_2 (0.02 µg/kg).

Table 2. Mean concentration levels of AFG_1 and AFG_2 in Benue State.

LGA	AfG1 (µg/kg)			AfG2 (µg/kg)		
	Mean±SE (µg/kg)	Min.AfG_1	Max.AfG_1	Mean±SE (µg/kg)	Min.AfG_2	Max.AfG_2
Guma	0.01±0.00	0.00	0.04	0.01±0.00	0.00	0.02
Otukpo	0.75±0.02	0.00	0.03	0.21±0.00	0.00	0.88
Ogbadibo	1.12±0.41	0.00	2.06	0.39±0.00	0.00	0.86
Gboko	1.87±0.03	0.00	2.06	0.31±0.00	0.00	1.17
Makurdi	0.76±0.01	0.00	2.06	0.01±0.00	0.00	0.04
Obi	0.57±0.00	0.00	2.06	0.63±0.00	0.00	1.47

*SE= Standard Error; *Min. = Minimum value; *Max. = Maximum value

Table 3: shows percentage frequency distribution of the aflatoxin types spread across six concentration ranges. AFB_1 was the most recovered, occurring in four out of the six categories. The result shows that all samples were positive for AFB_1 having residue levels which were greater than 3.0 µg/kg and distributed in categories 3, 4, 5 and 6. Of the total number of positive samples analyzed for AFB_1, 53.13% had contamination levels in the range of 5.1 – 7.0 µg/kg (category 4). Aflatoxin B_2, AFG_1 and AFG_2 recovered from the samples occurred only in category 1 and 2. Aflatoxin B_2, occurred the most in category 2 (86.46% of positive samples) while AFG_1 and AFG_2 occurred in category 1 (52.08% and 89.58% of positive samples, respectively).

Table 3. Frequency distribution of aflatoxin types in sesame collected in Benue State.

Aflatoxin type	N	N^+	Percentage frequency of samples contaminated at different ranges (%)					
			Category1	Category2	Category3	Category4	Category5	Category6
			≤1.0 µg/kg	1.1 – 3.0 µg/kg	3.1 – 5.0 µg/kg	5.1 – 7.0 µg/kg	7.1 – 9.0 µg/kg	≥9.1 µg/kg
AFB_1	96	96	0	0	10.42	53.13	32.29	4.17
AFB_2	96	90	13.54	86.46	0.00	0.00	0.00	0.00
AFG_1	96	84	52.08	47.92	0.00	0.00	0.00	0.00
AFG_2	96	71	89.58	10.42	0.00	0.00	0.00	0.00
AF_{total}	96	96	0.00	0.00	0.00	6.25	38.54	55.21

*N = total number of samples collected
*N^+ = samples in which aflatoxin was detected
*AF_{total} = Total aflatoxin

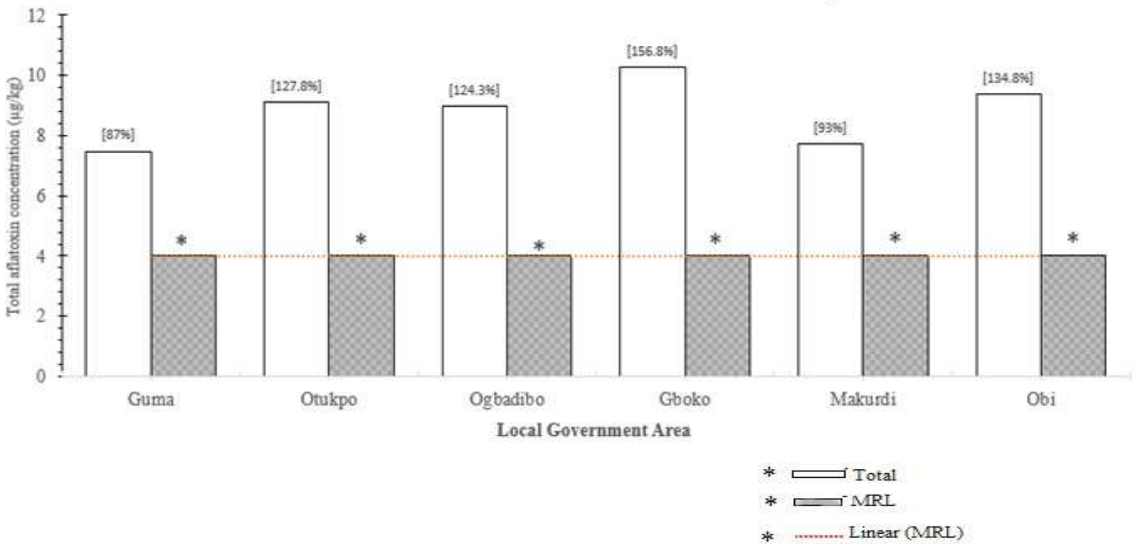

Figure 7. Total aflatoxin concentration levels recovered from sesame sourced in Benue state.

Figure 7 shows the total aflatoxin concentration levels recovered from sesame samples obtained from six local government areas in Benue State and the European Union maximum residue limit (EU-MRL) for sesame. All samples collected were above the EU-MRL for sesame consumption as presented. Guma, Otukpo, Ogbadibo, Gboko, Makurdi and Obi had mean total aflatoxin of 7.48 µg/kg, 9.11 µg/kg, 8.97 µg/kg, 10.27 µg/kg, 7.72 µg/kg and 9.39 µg/kg, respectively. The difference between concentration levels determined in samples and EU-MRL were statistically higher ($p<0.05$) in all the LGAs involved in the survey. Total aflatoxin concentration in samples obtained from Gboko had a percentage increase of 156.8% when compared to the EU-MRL of 4 µg/kg. The least concentration even though above the EU-MRL was found in samples obtained from Guma with a percentage increase over the EU-MRL of 87%.

4. Discussion

This study analyzed 96 samples for the presence of aflatoxins in sesame obtained from different markets in Guma, Otukpo, Ogbadibo, Gboko, Makurdi and Obi Local Government areas in Benue State. Four types of aflatoxins (AFB_1, AFB_2 AFG_1 and AFG_2) were detected. Of the aflatoxins detected, AFB_1 had the highest frequency of occurrence. This finding corroborates previous findings of Hosseininia *et al.* [2] who reported that AFB_1, AFB_2 AFG_1 and AFG_2 are the most encountered aflatoxin types on sesame with AFB_1 been the most frequent. Similarly, Yentur *et al.* [30] reported AFB_1 and AFG_1 as the most frequently occurring types of Aflatoxin in stored oil-based crop samples. All contaminated sesame samples were at levels above the EU maximum residue limits (MRL) of 2 µg/kg for aflatoxin B_1 and 4 µg/kg for total aflatoxin. The EU MRL has been adopted and is currently used as the standard in Nigeria [32].

The study has for the first time, revealed aflatoxin spread in sesame in the major sesame-producing areas in Benue State. Among the Local government surveyed, samples of sesame obtained from Gboko LGA had the highest aflatoxin load, 156.8% above the EU-MRL for total aflatoxin. This is contrast to the findings of Ezekiel and Sombie [26], Mba and Akuesi [32] who reported that sesame is less succeptible to aflatoxin. These differences could be ascribed to differences in environmental factors of handling, transportation storage facilities among others. In support of the current study however, Luttfullah and Hussain [10] showed that crops or foodstuff with high oil content as is the case of sesame are good substrates for the growth of toxigenic strains of *A. flavus* which engenders aflatoxin contamination.

Variations in findings of aflatoxin contamination levels are not uncommon as survey findings may be influenced by a number of factors. Although the levels of AFB_1 (3.95- 11.75µg/kg), AFB_2 (0.00 – 2.35), AFG_1 (0.00 – 2.06), AFG_2 (0.00 – 1.47) now being reported for sesame are within the range reported elsewhere, differences between these results and those obtained from other researchers may be due to variation in geographical origin, seasonal variation of the samples tested and weather conditions. Makun *et al.* [31] opined that difference in harvest and storage conditions as well as the agricultural practices (in each location) also influenced the level of aflatoxin contamination of food commodities. Three environmental factors (temperature, relative humidity and amount of rainfall) influence the production of aflatoxin in the field and during storage. Studies done on the effect of environmental conditions on aflatoxin contamination showed that, when the conditions were favourable, the occurrence of aflatoxin was highly related to these factors [33]. Nonetheless, the current research has further demonstrated the high

aflatoxin load associated with sesame marketed in the Benue states. This precarious situation presents a serious concern and the need for urgent deployment of mitigation strategies in the survey area and sesame supply chain.

5. Conclusion

In this study, four types of aflatoxin were identified in sesame seeds: AFB_1, AFB_2, AFG_1, and AFG_2 respectively. AFB_1 had the highest frequency of occurrence. All values were above the European Union Maximum Residue Limits (MRL) of 2 µg/kg for aflatoxin B_1 and 4 µg/kg for total aflatoxin. In conclusion, considering the benefits of sesame, it is recommended that they should be diversification of diet such that over dependence on crops that are highly susceptible to aflatoxin is reduced and also several treatments should be applied to reduce the levels of contamination in sesame before its utilization. Sanitation measures should be considered before storage. Also, several treatments should be applied to decrease the level of contamination before use.

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