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Prevalence and distribution of aflatoxin (AfB1) in groundnut and groundnut-based products in Northwestern Nigeria

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The kernel of groundnut and groundnut-based products are easily contaminated by aflatoxin: a mycotoxin produced by the fungus *Aspergillus flavus and A. parasiticus*. A total of 526 samples of groundnut and groundnut-based products were collected from six states in Nigeria namely Kano, Jigawa, Katsina, Kebbi, Sokoto and Benue States and analyzed for Aflatoxin B1 (AfB1) contamination using the Enzyme-linked Immunosorbent Assay (ELISA) technique. Results of the analysis revealed that both groundnut kernel and processed products had varying levels of AfB1 contamination. While AfB1 contamination levels varied between 7.82 and 12.33 µg/kg in kernels of local groundnut varieties, they ranged between 3.79 and 6.79 µg/kg in those of improved groundnut varieties. Mean AfB1 levels in groundnut-based products ranged from 12.30 to 99.37 µg/kg, with the highest recorded in *kuli-kuli* - a by-product of groundnut oil processing. Variability between mean AfB1 contamination levels in groundnut kernels of improved and local varieties were significant while no statistical difference was found between mean AfB1 contamination levels in groundnut kernels between/amongst the states. Outcomes of the study suggest that an integrated approach including the use of improved groundnut varieties, appropriate crop management practices and awareness creation on food safety, and notably on aflatoxin, could mitigate contamination in the groundnut value chain.

Key words: Aflatoxin B1, prevalence, groundnut, distribution, Nigeria.

INTRODUCTION

Groundnut, (also called Peanut) is the edible kernel of the legume *Arachis hypogea*. It plays an important role in the diets of many households in Africa and Asia due to its high nutritive value, high oil, fiber and protein contents.

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Apart from oil, they are consumed, used or sold in many forms - roasted, boiled, processed and sprinkled onto foods or included in different local dishes in Nigeria (Vabi et al*.,* 2018). Groundnut kernels are often pressed to obtain groundnut oil which is widely used in many rural and urban households of countries in West and Central Africa (WCA). Also, groundnut and groundnut products are important snacks for travelers especially those on religious and tourist expeditions. The crop was useful in the elimination of malnutrition in some African countries (Guimon and Guimon, 2012).

Despite the wide-ranging uses and popular cultivation of groundnut alongside cereal crops by resource-limited farmers in the Semi-Arid region of Africa and Asia, the crop is prone to pre-and post-harvest contamination by aflatoxin producing fungi notably *Aspergillus flavus* (Williams et al., 2004; Bediako et al*.,* 2019). When subjected to end of season drought stress, groundnut becomes more susceptible to aflatoxin contamination (Pitt et al., 2013; Kanyi, 2018). The habitually cited health risks of aflatoxins include immuno-suppression, impaired growth, liver cancer and death depending on the quantity and frequency of ingestion (Williams et al*.,* 2004; Wagacha and Muthomi, 2008). Due to the potential health risks of aflatoxins, many countries have established maximum limits (MLs) or levels for key agricultural commodities including groundnut. These regulations single out aflatoxin as the most regulated mycotoxins underlining public and private sector concerns for food safety in both developing and developed economies. For example, the MLs set by the EU vary between 2-12 µg/kg for AfB1 and 4-15 µg/kg for total aflatoxins (European Union Commission Regulation, 2006 as amended; FAO, 2015). The MLs of the US Food and Drug Administration is 20 ppb (= 20µg/kg) for total aflatoxins in all foods except milk (Food and Drug Administration, FDA, 2000; Bediako et al*.,* 2019). The MLs set by the Standard Organization of Nigeria (SON, 2006) for groundnut kernel is 20 and 4 µg/kg for K*uli-kuli* - a by-product of groundnut.

However, in Africa, large proportions of groundnut and groundnut-based products contain aflatoxins exceeding MLs. Between 22 to 54% of groundnut samples collected in Mali during the 2009 and 2010 cropping seasons, contained AfB1 contamination levels above 20 µg/kg (Waliyar et al*.,* 2015). In Malawi, 21 and 8% of samples of groundnut kernel were contaminated with AfB1 above 20 µg/kg during 2008 and 2009 cropping seasons, respectively (Gong et al*.,* 2015). In Botswana, nearly 78% of kernels of groundnut samples had total aflatoxin (AF_T) ranging between 12 and 329 µg/kg (Mphande et al*.,* 2004; Monyo et al., 2012) while in Benin, all of the kernel of groundnut produced and placed on domestic markets were contaminated with AfB1 (Hell et al*.,* 2003). In Ghana, between 5 and 15% of kernels of groundnut samples were discarded during sorting due to potential for aflatoxin contamination (Awuah et al., 2006).

In Nigeria, between 30 to 90% of marketed and/or stored kernels of groundnuts were contaminated by aflatoxin of which between 25 to 83% exceeded the Nigerian and EU MLs of 20 and 4 µg/kg, respectively (Ezekiel et al*.,* 2012a). Average aflatoxin concentrations were reported between 43 and 118 µg/kg and 39 and 198 µg/kg for AfB1 and AFT, respectively in Southwestern Nigeria (Ezekiel et al., 2012a, b). Similar reports from the Kaduna and Port Harcourt cities in North and South Nigeria respectively, showed that between 14 and 25% of groundnut kernel and groundnut-based products exceeded the US and Nigeria MLs of 20 µg/kg.

This scenario demonstrates that aflatoxin contamination levels well above permissible limits by national and international regulatory agencies are rampant in countries of West and Central Africa (Akano and Atanda, 1990; Arowora and Ikeorah, 2010; Atanda et al*.*, 2013; Ifeji et al*.,* 2014; Salau et al., 2017). In order to enhance knowledge and consciousness on aflatoxins in the groundnut value chain, this paper presents the prevalence and distribution of AfB1 in groundnut and groundnut-based products in Northwestern Nigeria.

MATERIALS AND METHODS

Sampling and collection of samples

A combination of purposive and random sampling procedures was used to collect groundnut and groundnut-based products from different sites in five States of Northwestern Nigeria (Kano, Jigawa, Katsina, Kebbi and Sokoto) and Benue State in North central Nigeria (Figure 1). At least 100 g of shelled groundnut of improved and local varieties were collected from farmers who hosted varietal demonstrations. Similarly, groundnut-based products were collected from vendors of these products in markets of local government areas (LGAs) of these states. Samples of improved groundnut kernel and local groundnut varieties were packaged and labeled by location. The total number of samples by type is summarized in Table 1.

Detection and quantification of AfB1

Except, groundnut oil, all samples were blended separately using a dry mill kitchen grinder (Kanchan Multipurpose Kitchen Machine, Kanchan International Limited, Mumbai, India). After crushing each sample, the blender was washed with 3.5% Sodium hypochlorite (NaOCl) and carefully rinsed with water to avoid cross contamination. 20 g of each blended sample was titrated with 100 ml of 70% methanol (v/v 70 ml absolute methanol in 30 ml distilled water) containing 5 g potassium chloride and further blended in Waring Commercial blender until homogeneity was reached. The mixture was then transferred into a 250 ml of labelled conical flasks and shaken on a Benchmark orbital (Model ORBI-Shaker) shaker for 30 min. Filtration was done using Whatman filter paper number 4. The extract was then diluted with Phosphate buffer, 1:10 phosphate buffer saline in Tween-20 (1 ml of extract and 9ml of buffer) and analyzed for aflatoxin contamination using the Indirect

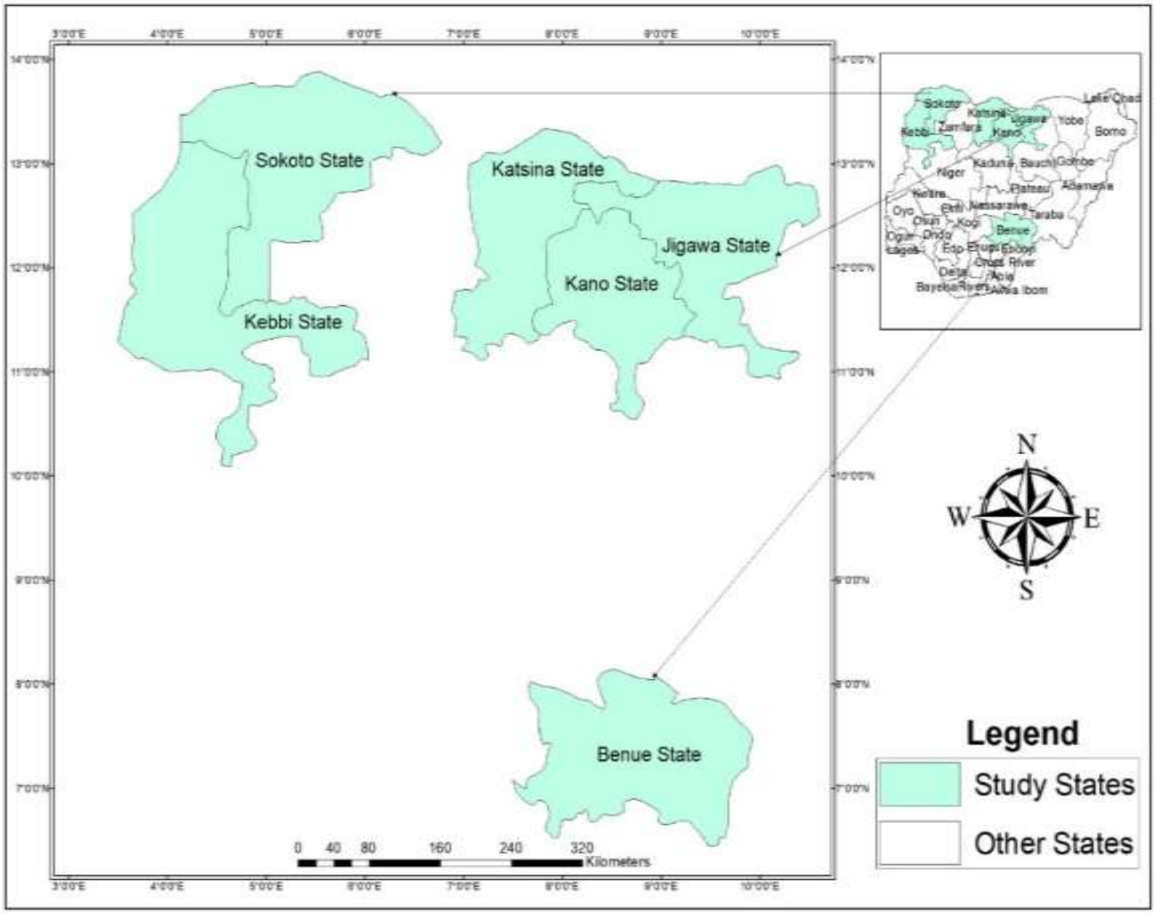

Figure 1. States (Administrative units) included in the study.

Table 1. Summary of samples collected for aflatoxin detection and quantification.

State	Improved groundnut varieties				Bulked local varieties	Groundnut-based products			
	SAMNUT 23	SAMNUT 24	SAMNUT 25	SAMNUT 26		Kulikuli	G/N oil	Fried G/N	Totals
Jigawa	6	13	2	0	39	12	3	1	76
Kano	31	71	29	6	37	31	4	7	216
Katsina	0	25	9	9	15	18	4	5	85
Kebbi	2	22	6	1	15	9	4	1	60
Sokoto	2	45	0	1	21	19	0	1	89
Total	41	176	46	17	127	89	15	15	526

Competitive Enzyme-Linked Immunosorbent Assay (ELISA) as described by Waliyar et al. (2015) and the ELISA protocol provided by ICRISAT - International Crops Research Institute for the Semi-Arid Tropics.

AfB1 BSA antigen was coated unto an ELISA plate (Nunc, Maxisorp). The plates were incubated at 37°C for one hour before the toxin solution was collected and stored in a large glass bottle for disposal. The plates were washed in three changes of PBS–Tween, allowing a holding time of three minutes per wash. The plates were blocked with a 150 µl per well solution of 0.2% bovine serum albumin (BSA) in PBS–Tween and incubated at 37°C for one hour. The blocked plates were then washed in three changes of PBS–Tween allowing three minutes for each wash. To the washed plates, 100 µl of groundnut kernel extract was added followed by 50 µl of antiserum. Instead of the groundnut kernel extract, 100µl aliquots of different concentrations of between 25 and 100 ng were added into the first 20 wells (two rows of 10 wells each) to serve as a standard. The plates were then incubated for one hour at 37°C to facilitate reaction between the toxins and the antibody (ICRISAT, 2010).

The plates were subsequently washed in three changes of PBS–Tween allowing 3 min for each wash. A dilution of 1:1000 goat anti-rabbit IgG labeled with alkaline phosphatase was prepared in PBS-Tween containing 0.2% BSA. A 150µl aliquot was added to each well, and incubated for one hour at 37°C. The plates were washed in three changes of PBS–Tween. A 150µl aliquot per well of substrate solution (p-nitro phenyl phosphate prepared in 10% diethanolamine buffer, pH 9.8) was added and incubated for about one hour at 37°C. Absorbance was measured at 405 nm in an ELISA plate reader (Multiskan Plus, Labsystems Company, Helsinki, Finland).

AfB1 levels were detected and quantified using a spectrophotometer by giving optical density values at a 405 nm wavelength. A linear regression curve was plotted for optical density values and a standard curve used to extrapolate with known correlation coefficients thereby giving concentrations in microgram per kg (µg/kg). All samples were analyzed in duplicate replication. The Statistical Package for the Social Sciences (SPSS) version 16 was used to summarize and analyze the values. The results are presented using means, percentages and standard errors.

RESULTS

Table 2 presents the pattern of AfB1 contamination in the samples subjected to the analyses. On average, only 9% of the samples met the European Union (EU) limit for AfB1 with another 39% revealing AfB1 levels between the 2.1 - 8 µg/kg ML. This gives rise to a total of 48% which met the EU limit. About 91% of the samples had AfB1 contamination levels up to 20 µg/kg, which are within the US and Nigeria limits for total aflatoxins in groundnut. The mean AfB1 contamination levels in all the states ranged between 1.06 and 162.8 µg/kg, though there were variations between and amongst states.

Katsina State had the highest percentage of contaminated samples for all classes (0-2, 2.1-8, 8.1-20 and above 20 µg/kg). This was followed by Kano State with the highest percentage of samples within the 2.1-8.0 µg/kg class. None of the samples from Sokoto met the 0-2 µg/kg limit. Across the states, minimum mean AfB1 concentration ranged from 0.1 µg/kg in Jigawa, Kano and Katsina states to 4.31 µg/kg in Sokoto State. Maximum mean AfB1 levels ranged from 26.91 µg/kg in Sokoto to 397.4 µg/kg in Kebbi State. Similar comparisons of the MLs of AfB1 contamination were reported by Hoeltzl et al*.* (2012).

AfB1 contamination in kernels of local and improved groundnut varieties

Mean AfB1 contamination levels in groundnut kernel are presented in Table 3 and Figure 2. Although mean AfB1 contamination levels varied between local and improved groundnut varieties within each State, the ranges were broadest in Sokoto (6.04 - 13.12 µg/kg) and Kano States (6.19 - 13.19 µg/kg) (Figure 2). Individually, all improved varieties, SAMNUTs 23, 24, 25 and 26 were in the lower range of contamination from ≤1 µg/kg in SAMNUT 25 in Sokoto and Benue and SAMNUT 26 in Jigawa, Kebbi and Benue; to 10.49 µg/kg in SAMNUT 25 in Jigawa State. Local groundnut varieties had AfB1 concentrations levels in a higher range between 10.15 µg/kg in Benue State to 13.74 µg/kg in Sokoto State. Mean AfB1 contamination levels in groundnut kernel of improved varieties were almost half those of local varieties. SAMNUT 26, AfB1 contamination levels ranged from ≤ 1 to 6.87 µg/kg; with minimum and maximum AfB1 contamination levels lowest of all the varieties. The variation between mean AfB1 levels in improved and local varieties was statistically significant ($P \leq 0.05$).

All the improved groundnut varieties taken together showed AfB1 contamination levels ranging from 3.79 µg/kg in Katsina State to 6.97 µg/kg in Kebbi State, while AfB1 contamination levels of bulked local varieties ranged from 7.82 µg/kg in Kano State to 12.33 µg/kg in Kebbi State.

AfB1 contamination in groundnut-based products

In the case of processed products (Figure 3), powder *kuli-kuli* collected from Sokoto State had the highest mean AfB1 contamination of 135.44 µg/kg, followed by those collected from Kano (8.03 µg/kg). Jigawa and Kebbi States had similar AfB1 contamination levels of 0.10 µg/kg. Groundnut cake samples from Kebbi State had the highest AfB1 contamination (122.26 µg/kg), followed by samples from Sokoto State (75.61 µg/kg), Jigawa State (48.47 µg/kg) and Katsina State (18.32 µg/kg). The lowest levels of samples contaminated with AfB1 were found in groundnut cake from Kano State (13.53 µg/kg), in groundnut oil from Kebbi State (71.70 µg/kg), Jigawa State (48.65 µg/kg), Kano State (10.70 µg/kg) and Sokoto (8.23 µg/kg). Roasted groundnut from Kebbi State also had the highest level of AfB1 contamination (72.70 µg/kg), followed by Katsina (8.40 µg/kg) Kano (8.24 µg/kg) and Jigawa (0.60 µg/kg). Of all the groundnut-based products collected from the five states, the highest AfB1 contamination levels were found in *Kuli-kuli* powder.

AfB1 contamination in both groundnut kernel and groundnut-based products according to LGAs

Only three LGAs (Safana in Katsina State, Bebeji and Tofa in Kano State) had the least number of samples with AfB1 contamination ranging between 6.57 and 7.67 µg/kg for groundnut kernels as shown in Figure 4. This was followed by one LGA in Jigawa State (Dutse), one LGA in Kebbi State, Shagari in Sokoto State, Musawa in Katsina

Table 2. AfB1 contamination levels in groundnut kernel and groundnut-based products.

State	No. of samples	Percentage range of (µg/kg)				Mean ± SE	Minimum (µg/kg)	Maximum (µg/kg)
		0-2	2.1-8	8.1-20	Above 20			
Jigawa	76	8	8	70	14	18.65±3.44	0.1	193.4
Kano	216	14	61	20	5	8.13±0.57	0.1	89.4
Katsina	85	19	42	34	5	9.17±1.36	0.1	104.3
Kebbi	60	3	25	55	17	29.87±9.19	0.7	397.4
Sokoto	69	--	59	36	5	9.20±0.58	4.31	26.91
Means	101	9	39	43	9	15.0±3.01	1.06	162.8

Table 3. AFB1 contamination levels in groundnut kernels and groundnut-based products.

State	Improved varieties			Local varieties			Groundnut based products		
	Mean ±SE	Min. (µg/kg)	Max. (µg/kg)	Mean ±SE	Min. (µg/kg)	Max. (µg/kg)	Mean ±SE	Min (µg/kg)	Max (µg/kg)
Jigawa	6.03±0.78	0.1	8.95	11.91±0.34	8.98	16.88	12.3±1.43	16.99	193.4
Kano	4.89±0.20	0.1	7.12	7.82±0.09	7.12	9.07	18.98±2.15	9.33	89.4
Katsina	3.79±0.41	0.1	7.2	8.01±0.09	7.32	8.81	18.36±3.63	8.82	104.3
Kebbi	6.97±0.42	0.7	9.12	12.33±0.49	9.76	16.00	99.37±33.98	16.01	397
Sokoto	6.37±0.18	0.6	7.61	12.02±0.58	8.23	16.32	91.47±20.13	17.2	277.2

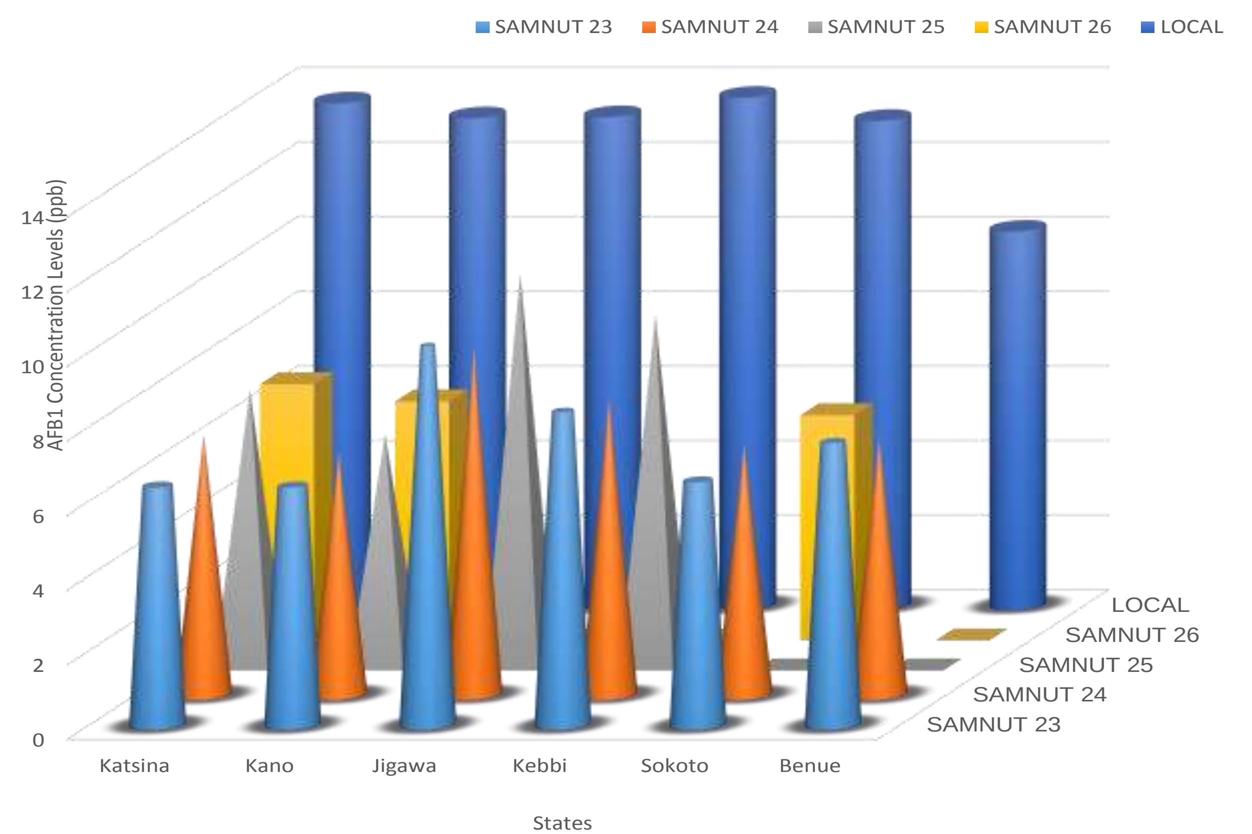

Figure 2. Concentrations in groundnut kernels.

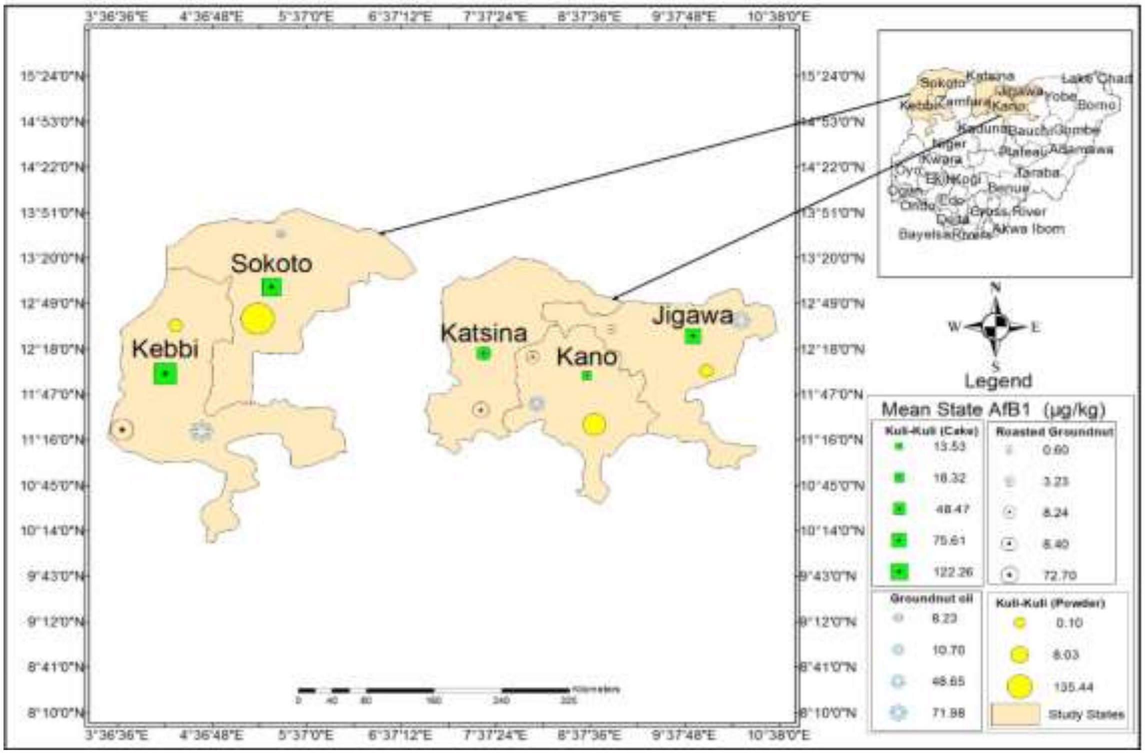

Figure 3. AfB1contamination in groundnut-based products in Northwestern Nigeria.

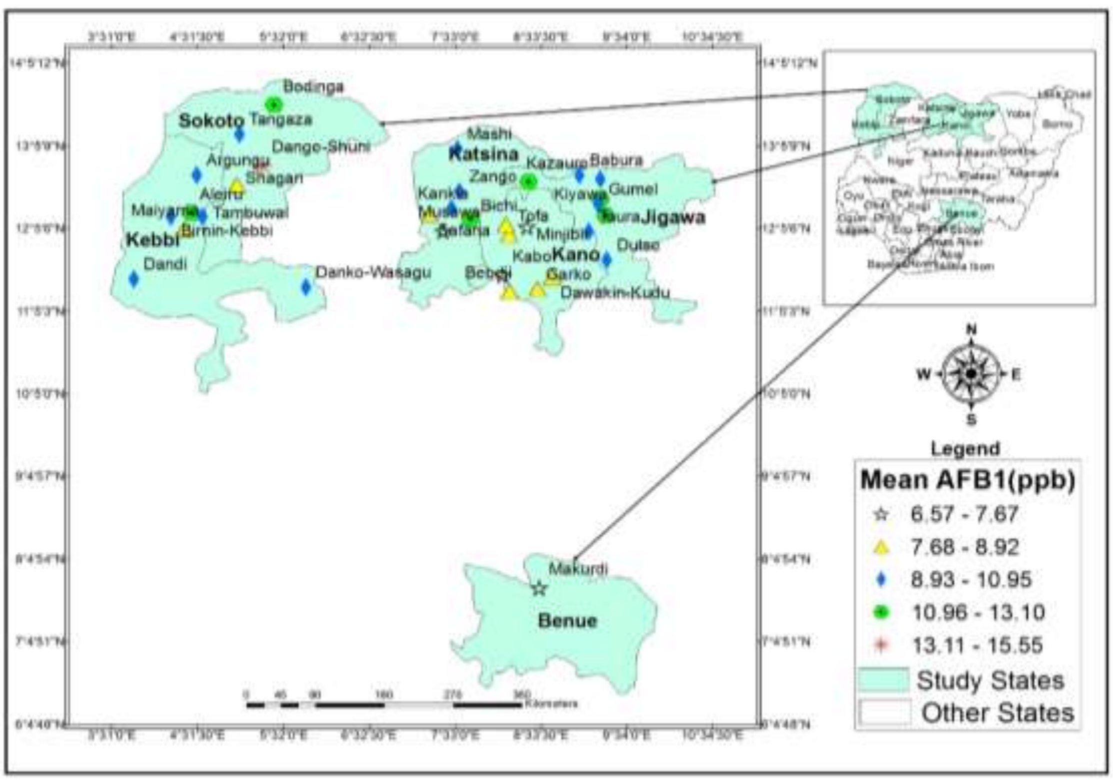

Figure 4. LGAs with groundnut samples of AfB1 contamination in Northern Nigeria.

State and four LGAs in Kano State (Minjibir, Garko, Bebeji and Dawakin-Kudu) between 7.68 and 8.92 µg/kg. About 40% of the LGAs (12 out of 31) had samples with AfB1 contamination levels ranging between 8.93 and 10.95 µg/kg. Three of these LGAs had samples contaminated with AfB1 levels above 10.96 µg/kg with Tangaza (Sokoto State), Maiyama (Kebbi State) and Zango (Katsina State) having samples with AfB1 contamination levels between 10.96 and 13.10 µg/kg and two LGAs namely Argungu and Birnin-Kebbi (both in Kebbi State) having samples with the highest mean AfB1 contaminations ranging from 13.11 to 15.55 µg/kg.

DISCUSSION

Aflatoxin and pesticide contamination in agricultural commodities has become public health concerns (Wagacha and Muthomi, 2008; PACA, 2017). Previous studies reported results similar to those of this study especially for *kuli-kuli* with AfB1 contamination level ranging between 53 and 2,820 µg/kg (Ezekiel et al., 2013; Kayode et al., 2013; Ogara et al., 2017; Oluwabamiwo et al., 2017). These results also confirm those of Chen et al. (2013) with high levels of AfB1 contamination in groundnut-based products. This plethora of high levels of AfB1 notably in groundnut-based products bring to the open sufficient actions required for the effective management of aflatoxins in Nigeria.

AfB1 contamination levels were lower in kernels of improved groundnut varieties than in those of local varieties. Higher AfB1 contamination levels in local groundnut varieties may be associated with inability for them to escape from end of season droughts as they take longer days to mature (120 -150 days) compared to improved groundnut varieties with maturity periods of between 75 - 85 days (Jalloh et al., 2013; Ajeigbe et al., 2015; Vabi et al., 2019). The maturity periods of these local groundnut varieties coincide with periods of moisture stress, a condition that favors rapid growth and multiplication of aflatoxin producing fungi. Jalloh et al. (2013) suggested that promoting extra short duration groundnut varieties that escape temporal and spatial rainfall regimes are highly encouraged. The short duration and high yielding groundnut varieties being promoted by development partners of the Nigerian Federal Ministry of Agriculture and Rural Development (FMARD) are able to escape drought stress since they are harvested earlier than local groundnut varieties.

In terms of distribution, AfB1 contamination levels were higher in both kernel and groundnut-based products from Kebbi and Sokoto States than in other States. The cropping season in these two states occurs between June and October with mean annual rainfall ranging between 500 and 1,300 mm. Also, annual average temperature in these States is 28.3°C though maximum daytime temperatures in Sokoto can be as high as 40°C most of the year. Therefore, the hot humid conditions in these States favor the growth of aflatoxin-producing fungi as reported by Mclean and Berjak (1987) and Atanda et al. (2013). Stress due to drought enhances the vulnerability of crops to *Aspergillus* infection and increase of AfB1 production. While climatic and storage conditions play vital roles in the growth of aflatoxin producing fungi (Mutegi et al., 2013), inappropriate agricultural management practices including the choice of groundnut varieties to be grown are options for the effective management of aflatoxin as demonstrated by several authors notably Salau et al. (2017).

In a similar connection, promoting improved groundnut varieties alongside recommended crop management practices have consistently been reported to be alternatives for effectively managing aflatoxin in agricultural sector value chains (Kumar and Popat, 2010; Hell et al., 2003; Arowora and Ikeorah, 2010; Waliyar et al., 2015; Vabi et al., 2016). Recently, strategies have been embarked upon to develop groundnut germplasms that exhibit improved genetic resistance to *A. flavus* infection, and therefore aflatoxin contamination (Sharma et al., 2017).

Conclusion

Results of this study validate the presence of AfB1 in the kernels of both improved and local groundnut varieties in Kano, Jigawa, Katsina, Kebbi, Sokoto and Benue States of Nigeria. AfB1 contamination levels were lower in groundnut kernels than in groundnut-based products with high levels in groundnut cake *(kuli-kuli)* demonstrating urgent public health concerns. Though AfB1 contamination levels vary between groundnut kernels and groundnut-based products and between the states included in the study, they underline the imperative of proactive measures to mitigate aflatoxin contamination at all levels beginning from the farm. This notwithstanding, results of this study reveal that the promotion of improved groundnut varieties may constitute a leeway for the effective mitigation of AfB1 contamination. In addition to improved groundnut varieties, appropriate farm-level and post-harvest management practices should greatly reduce chances of aflatoxin contamination in the groundnut value chain.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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