Assessment of Aflatoxin B_1 Content of some Local Rice Cultivars in Kaduna State-Nigeria

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Abstract
Thirty nine samples of rice were collected from thirteen Local Government Areas in Kaduna State and analysed for Aflatoxin B_1 using the Enzyme-linked Immunosorbent Assay (ELISA) method. Concentrations of aflatoxin B_1 was highest (177.2 µg/kg), in sample obtained at Sanga Local Government Area. These values were above the permissible limits described by some regulatory bodies like National Agency for Food and Drug Administration and Control (NAFDAC), European Union (EU) and European African Community (EAC). The concentrations (0.3µg/kg) of samples obtained from Kauru Local Government Area were observed to be the lowest. Generally the rice samples investigated were found to contain varying concentrations of aflatoxin B1.This implies that direct consumption of the rice without pretreatment to reduce the aflatoxin content could be detrimental to health.

Keywords: Aflatoxin, Kaduna, Mycotoxicosis, Mycotoxicosis, Rice

Introduction

Cereal grains (rice, corn, wheat etc) are staple foods that provide more food energy worldwide than any other type of crop (Okpiaifo *et al*., 2020). Rice is the second largest staple member of the cereals family internationally. It is known as *Oryza glaberrima* (African rice). It belongs to the family *Poacea;* In Nigeria, the paddy rice species is popularly referred to as "*Shinkafa*" by the Hausas. It is called *Iresi* and *Osikapa*, by the Yoruba, and Igbo respectively. It is called *Chinkafa* by the Tiv people. Rice is the most widely consumed staple by a large part of the world's population (20 %). It is the second largest consumed cereal after wheat. It is primarily a profit crop, and in Nigeria, it is produced mainly as a cash crop. The crop is cultivated in virtually all agro-ecological regions of Nigeria (Amin *et* al., 2015; Ben-Chendo *et al*., 2017). Kamai *et al*., 2020, listed major rice producing States in Northern Nigeria as: Kebbi, Borno, Kano, Kaduna Benue etc. Studies have shown that Nigeria is the largest producer of Rice (paddy) in Africa with an average production rate of 8 million metric tons and is ranked 14^{th} in the world with China being the first according to 2019 ranking.

Food spoilage is a worldwide concern, arising mainly from chemicals, environmental factors, cross-contamination during processing, from food packaging materials and through natural toxins (especially those produced by fungi). It usually poses a health concern, leading to strict regulations in food products by national and international governments (Di Stefano *et al*., 2014). Favourable climatic and environmental factors usually promote fungi prevalence. Indeed, fungi are found growing on crops and foodstuffs (cereals, nuts, spices, dried fruits, apples and coffee beans). The health effect of fungal infection due to mycotoxin contamination depends on the degree of contamination (Freire, 2016).

Exposure to mycotoxins may lead to carcinogenic, immunosuppressant and estrogenic effects due to their toxicity (Pradeep *et al.*, 2017). Aflatoxins which are also mycotoxins are known to infect corn, corn silage, all cereal grains, sorghum, peanuts, and other oilseeds etc. According to Najeeb and Farag (2019), aflatoxin B1 is commonly found in rice. Reddy and Muralidharan (2009), observed that 67.8% of rice cultivated in India are contaminated with aflatoxin B1 (AFB1) with concentrations ranging from 0.1 to 308.0 µg/kg. Similarly, Amin *et al*., reported that hepatocellular carcinoma (HCC) incidence being reported in some countries is linked to consumption of AFB1 in food staples.

Owing to the significance most families attached to rice as a priority meal, the need to define the toxic status of locally produce rice cannot be underplayed. It is in this light that this study considered the assessment of aflatoxin B1 in indigenous rice produced by farmers across Kaduna State.

Materials and Methods

Sample Collection

Choice of sampling points was based on farming practices and the degree of involvement. Each identified local government was divided into 3 zones (in a Y shape); four samples of 250 g of whole grain were collected from each zone and mixed up to give a composite of 1 kg. A total of 39 samples were collected from thirteen local government areas (Kubau, Soba, Giwa, Makarfi, Kudan, Ikara, Kauru, Kajuru, Chikun, Sanga, Lere, Kachia, and Sabon Gari) of Kaduna state for this study. Samples were collected directly from farmers so as to ensure the originality.

Study area

Kaduna State, located in north-western Nigeria, has a population of 7,474,369 going by 2013 projection. It has 23 local government areas (LGAs). The state is located on Latitude is 10.609319 and longitude is 7.429504. It has a GPS Coordinates of 10^0 36' 3

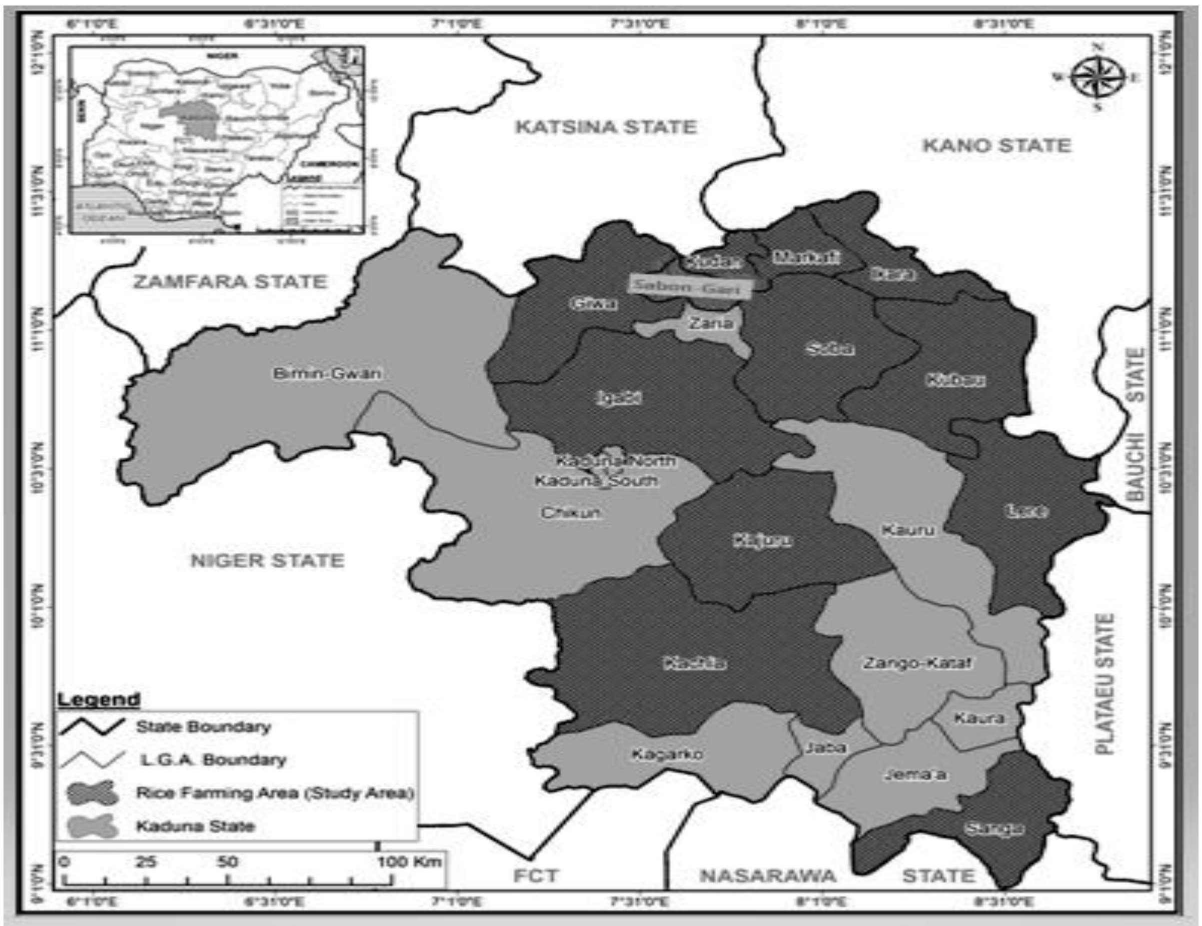

Figure 1: Map of Kaduna State Showing the Sample Sites

Sample Preparation and Extraction

Sample extraction was performed following manufacturer's instruction (Aqra Quant Total Aflatoxin Assay) test kit. A 25 g of powdered rice sample was weighed into a 250 mL conical flask and to this 5 g of sodium chloride was added in 100 mL of aflatoxin extracting solution (containing 70 % methanol and 30 % water). The conical flask was capped and sealed with paraffin, then shaken at about 140 rpm on a horizontal shaker for 30 minutes. The content was allowed to settle and filtered through a fluted filter into a 150 mL beaker. From the beaker, 15 mL of the filtrate was taken and introduced into a 50 mL graduated cylinder, and then 30 mL of distilled water was added. The content of the graduated cylinder was filtered through a glass fiber filter into a 150 mL beaker; this filtrate was used in the immune affinity column or Alfa test column.

Determination of Aflatoxin B_1 by ELISA method

The method described by Ramon *et al*., 2002 was adopted, with slight modification. A 96 well ELISA plates were used as the solid phase. The wells of the plates were coated with 100 µL of 5 µgmL^{-1} BSA- AFB_1 solution in carbonate/bicarbonate buffer (100 nM at pH 9.8). The plate was incubated at 37 °C for 1 hr in an incubator. Aflatoxin B_1 working standards was prepared by dilution method (using 1:10 diluted extract) at concentrations ranging from 25 ng to 10 picogram/mL in 100 µl volume; following method of Ramon *et al*., (2002).

AFB_1 was calculated using the relation:

AFB_1 (µg/kg) :

$$\left(\frac{A\times D\times E}{G}\right) \; or \; \frac{A\times E}{C\times G}$$

Where:

A = AFB_1 concentration in diluted or concentrated sample extract (ng/mL)

D = Times dilution with buffer

C = Times concentration after cleanup
E = Extraction solvent volume used (mL)
G = Sample weight (g)

Statistical Analysis

GraphPad Prism 8.4.3 (686) was employed in statistical treatment of data obtained for this study using one-way ANOVA (within local governments) and the Tukey's multiple comparisons test (between local governments) at 95% confidence interval.

Results and Discussion

Result of aflatoxin B_1 (AFB_1) concentrations in the 13 Local Government Areas of Kaduna State are as presented in Table 1 and Figures 1-14. Samples from Kubau Local Government have AFB_1 concentration values of 27.21 µg/kg, 10.11 µg/kg and 13.31 µg/kg for kb_1, kb_2 and kb_3 respectively. Soba Local Government (So_1, So_2 and So_3) recorded AFB_1 concentrations of 44.70 µg/kg, 0.80 µg/kg and 11.30 µg/kg respectively. AFB_1 in samples from Giwa Local Government showed varying concentration values of 6.91 µg/kg and 2.70 µg/kg for Gw_1 and Gw_2, while Gw_3 was 2.04 µg/kg respectively. Samples from Mk_1, Mk_2 and Mk_3 in Makarfi Local Government revealed concentrations of 15.71 µg/kg, 20.71 µg/kg and 28.80 µg/kg respectively.

In Kauru Local Government, the following values were recorded Ka_1 (1.71 µg/kg), Ka_2 (23.17 µg/kg) and Ka_3 (0.30 µg/kg) in the the three sampled sites. Kajuru Local Government, Kj_1, Kj_2 and Kj_3 recorded AFB_1 concentrations of 14.11 µg/kg, 23.11 µg/kg and 21.08 µg/kg. Results obtained from Sanga local Government (Sa_1, Sa_2 and Sa_3) gave the following concentrations in the order as: 0.51 µg/kg, 177.20 µg/kg and 101 µg/kg respectively. Concentration of Aflatoxin B_1 from Lere Local Government was found to be: Le_1 (2.40 µg/kg), Le_2 (35.90 µg/kg) and Le_3 (3.20 µg/kg). Result from Chikum Local Government listed concentrations for Ch_1 (14.80 µg/kg), Ch_2 (4.71 µg/kg) and Ch_3 (17.50 µg/kg). AFB_1 sampled in Kachia Local Government at points Kc_1, Kc_2 and Kc_3, yielded the following concentrations; 1.81 µg/kg, 15.70 µg/kg. Kc_1 and 12.10 µg/kg respectively. Samples from Sabon Gari local government was collected at points Sb_1, Sb_2 and Sb_3. Sb_2 recorded the highest concentration (11.91 µg/kg), Sb_3 (7.01 µg/kg) was next, then Sb_1 (1.61µg/kg).

The four major Aflatoxins are known as aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2), several researches have implicated AFB_1 as the most toxic and classified as a group I carcinogenous aflatoxin by the International Agency for Research on Cancer (IARC). It has been found to be associated with liver cancer and acute hepatitis based on epidemiological studies (Reddy *et al*., 2011; Ruadrew *et al*., 2013; El tawila *et al*., 2013; Súarez-Bonnet *et al*., 2013).

Table 1: Concentration (µg/kg) of Aflatoxin B_1 in the Rice sample

Local Government Area	Sample Identity	AFB_1 Mean Concentration (µg/kg)	International standards (µg/kg) EU	EAC	NAFDAC
Kubau	kb_1	27.21±0.01	2.00	5.00	10.0
	kb_2	10.11±0.01			
	kb_3	13.31±0.01			
Soba	So_1	44.70±0.01			
	So_2	0.80±0.01			
	So_3	11.30±0.02			
Giwa	Gw_1	6.91±0.01			
	Gw_2	2.70±0.01			
	Gw_3	2.04±0.01			
Makarfi	Mk_1	15.71±0.01			
	Mk_2	20.71±0.01			
	Mk_3	28.80±0.01			

Kauru	Ka_1	1.71±0.01
	Ka_2	23.17±0.06
	Ka_3	0.30±0.01
Kudan	Kd_1	19.61±0.01
	Kd_2	14.40±0.01
	Kd_3	19.71±0.01
Ikara	Ik_1	14.81±0.01
	Ik_2	14.81±0.01
	Ik_3	0.41±0.01
Kajuru	Kj_1	14.11±0.01
	Kj_2	23.11±0.01
	Kj_3	21.08±0.01
Sanga	Sa_1	0.51±0.01
	Sa_2	177.20±0.01
	Sa_3	101.±0.01
Lere	Le_1	2.40±0.01
	Le_2	35.90±0.01
	Le_3	3.20±0.01
Chikum	Ch_1	14.80±0.01
	Ch_2	4.71±0.01
	Ch_3	17.50±0.01
Kachia	Kc_1	1.81±0.01
	Kc_2	15.70±0.01
	Kc_3	12.10±0.01
Sabon Gari	Sb_1	1.61±0.01
	Sb_2	11.91±0.01
	Sb_3	7.01±0.01

Key: *kb_1 = Pambegua, kb_2 = Anchau, kb_3 = Zuntu, So_1 = Damari, So_2 = Maigana, So_3 = Tashan Ice, Gw_1 = Yakawada, Gw_2 = Kaya, Gw_3 = Gangara, Mk_1 = Meyere, Mk_2 = Makarfi Town, Mk_3 = Gubuci, Ka_1 = Chawai Kafin-Fadama, Ka_2 = Anguwan-juri, Ka_3 = Fadamam Badarin kasa, Kd_1 = Hunkuyi, Kd_2 = Zabi, Kd_3 = Kudan Town, Ik_1 = Sabon Gari, Ik_2 = Saya Saya, Ik_3 = Jamfalan, Kj_1 = Kasuwan Magani, Kj_2 = Maraba, Kj_3 = Kufana, Sa_1 = Gwantu, Sa_2 = Fadan Karshi, Sa_3 = Aboro, Le_1 = YarKasuwa, Le_2 = Lahadi, Le_3= Mariri, Ch_1 = Kujama, Ch_2 = Mahuta, Ch_3 = Maraban-Rido, Kc_1 = Gummel, Kc_2 = Katari, Kc_3= Kurmin Musa, Sb_1 = Likoro, Sb_2 = Saka-Dadi, Sb_3 = Jama'a*

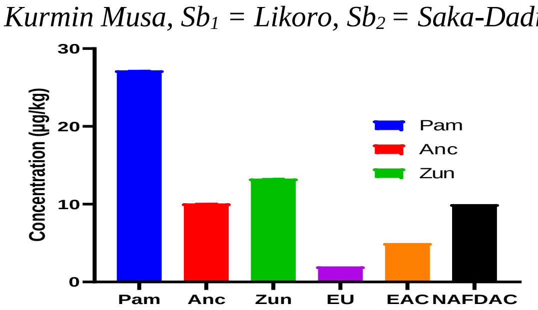

Fig. 1 Kubau LG

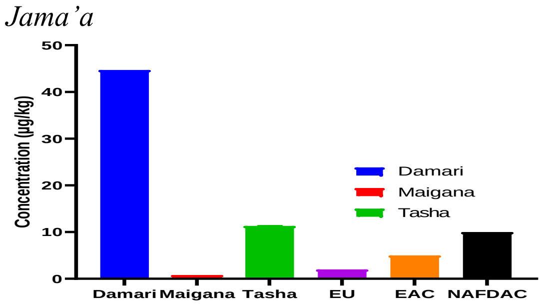

Fig. 2 Soba LG

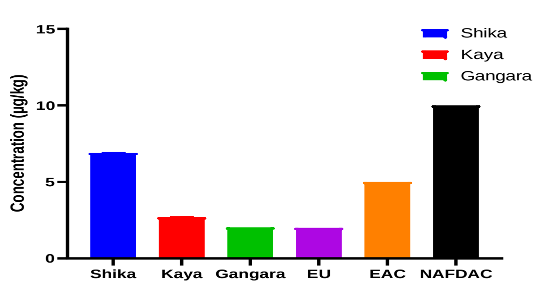

Fig. 3 Giwa LG

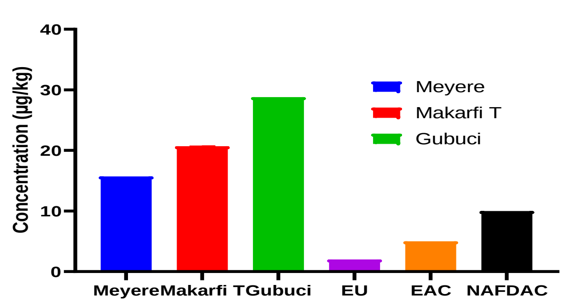

Fig. 4 Makarfi LG

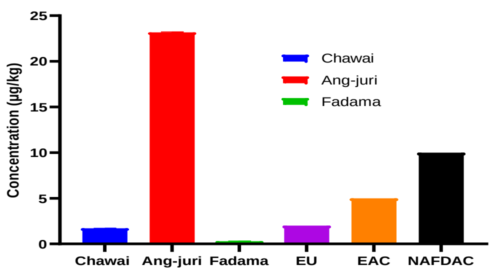

Fig. 5 Kauru LG

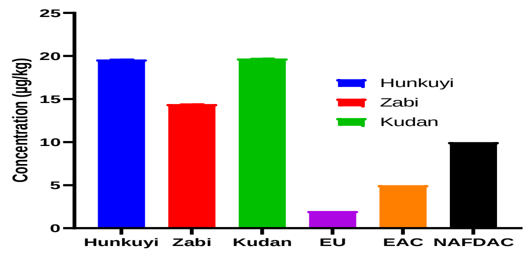

Fig. 6 Kudan LG

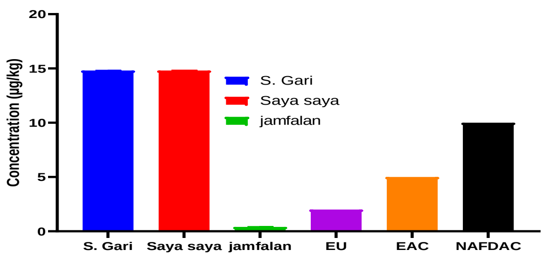

Fig. 7 Ikara LG

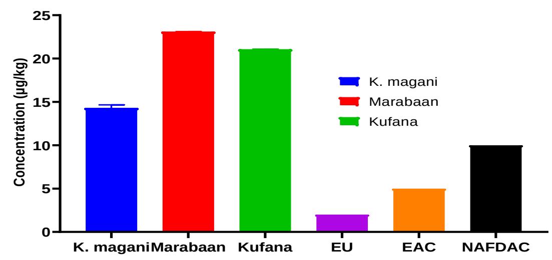

Fig. 8 Kajuru LG

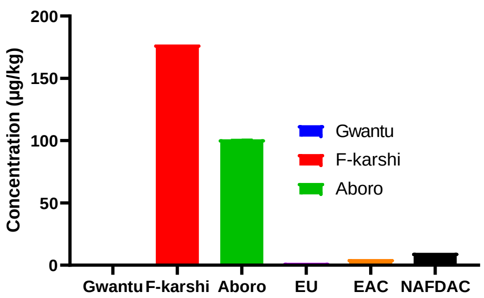

Fig. 9 Sanga LG

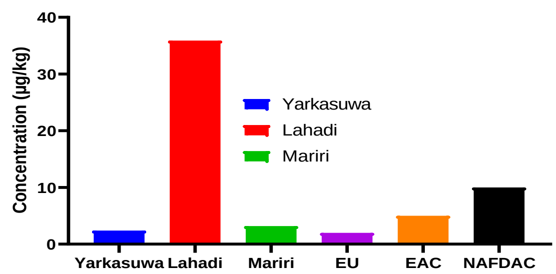

Fig. 10 Lere LG

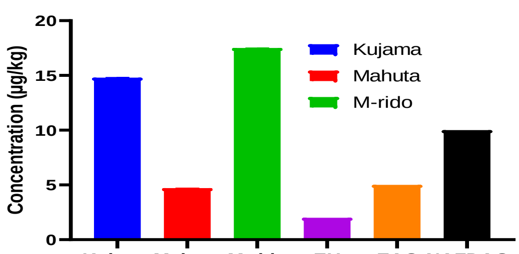

Fig. 11 Chikun LG

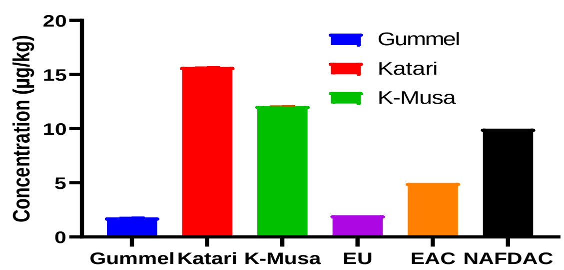

Fig. 12 Kachia LG

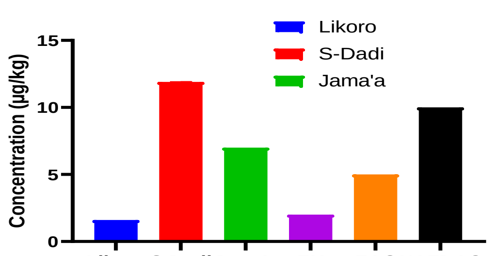

Fig. 13 Sabon Gari LG

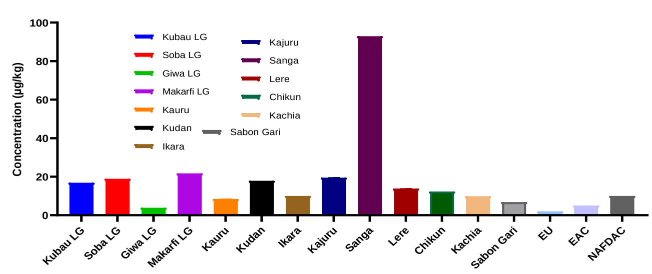

Fig. 14 Average Aflatoxin B_1 Concentration Between Local Governments

Findings from this work showed concentration of aflatoxin at So_1 and So_3 were above the EC and EAC permissible limits of 2.0 and 5.0 µg/kg , while that recorded at So_2 was below the set limit. Gw_1 gave values slightly above the EAC permissible limit but well above the EC permissible limit, while Gw_2 and Gw_3 reported values below the permissible limit. At Kauru, only sample Ka_2 were above the set limits, while Ka_3 recorded the lowest value. All concentrations obtained for Kudan local Government were above recommended limits. Two samples from Ikara Local Government (Ik_1 and Ik_2) were found to be above the permissible limits.

Results from Kajuru Local Government were also above the permissible limits. Sa_1 revealed a very low contamination value, while Sa_2 recorded a very contrasting concentration, it recorded the highest value for any of the samples analysed and is very much above the permissible limits. Samples Le_1, Le_2 and Le_3 (Lere LGA) were all above the permissible limits. Result for Chikum Local Government implicated Ch_3 as the most contaminated, followed by Ch_1 while Ch_2 was the lowest, all concentrations documented are above the set permissible limits. For Kachia, sample Kc_1 gave concentration below the recommended limits while Kc_2 and Kc_3 values were above the recommended limits. In Sabon Gari Local Government, point Sb_2 recorded the highest concentration, which was above the recommended limits, followed by Sb_3, while Sb_1 recorded concentration was below the recommended limits.

Guided by the European Union standard, the maximum concentration of Aflatoxin in food stuffs as established by the European Commission Regulation No 1881/2006, stipulates the lowest permitted value of AFB_1 in food to be 0.002 µg/kg. The value of total Aflatoxins in food for direct consumption is pegged at 4.0 µAg /kg, and for other food products to 15.0 µg /kg. In 2009, the European Agency for Food Safety (EFSA) raised from the concentration value from 4 to 10 µg /kg, but maintaining the European regulations (Georgievski *et al*., 2016).

Result for this research as compared with the NAFDAC limit (10 µg/kg) for raw food items were found to be well above it, except for Giwa (GW_1, GW_2 and GW_3), Soba (So_2), Kauru (Ka_1 and Ka_3), Ikara (Ik_3), Sanga (Sa_1), Lere (Le_1 and Le_3), Chikun (Ch_2) and Sabon Gari (Sb_1 and Sb_3). Similar high concentrations have been recorded in beans (59.29) and wheat (85.66) (Makun *et al*., 2009). All local governments studied recorded at least a sample above the NAFDAC tolerance level. Tukey's post hoc test revealed that there exist no significant difference between Ikara (10.01 µg/kg) and NAFDAC limit (10.0 µg/kg)

Similar research conducted in Pakistan, showed rice (*Basmati*) contained higher levels of AFB_1, these levels were 4.9–8.8, and 8.9–12.5 µg/kg, respectively (Iqbal *et al*., 2014). Rice (white, *Basmati* and parboiled) from Spain, Mexico, Pakistan, USA and other sources also exceeded levels of AFB_1 tolerated in cereals in the European Community (Súarez-Bonnet *et al*., 2013). In Austria and West Scotland, AFB_1 levels were ≤10 µg/kg in rice varieties originating from India, Pakistan, Italy, Egypt and other places (Ruadrew *et al*., 2013, Reiter *et al*., 2010).

Batagarawa *et al.*, (2015), reported aflatoxins to be responsible for impacting negatively on health, especially on humans, there is little awareness about aflatoxins among farmers, rural traders, and consumers in Nigeria, leading to global trade losses estimated at $1.2bn. Odoemelam and Osu (2009), assessed AFB_1 contamination of Some edible foods (Wheat, Millet, Guinea Corn, Breadfruit and Groundnut) from major markets in the Niger Delta region of Nigeria, recording concentrations in the range of 17.01-20.53 µg/kg for wheat, 34.00– 40.30 µg/kg for millet, 27.22-36.13 µg/kg for guinea corn, 40.06-48.59 µg/kg for breadfruit and 74.03-82.12 µg/kg for groundnut, these values compare favourably with current research. Olorunmowaju *et al*., (2014) also studied the Simultaneous Occurrence of Aflatoxin and Ochratoxin A in Rice from Kaduna State, Nigeria. AFB_1 was determined at concentrations between 4-292µg/kg; Similarly, Onyedum *et al*., (2020), discovered aflatoxins had 100% occurrence in all analysed samples at concentrations within 2.1 – 248.2 µg/kg.

Separate studies undertaken by Apeh *et al.,* (2016) and Pradeep *et al.,* (2017) on concentration of aflatoxin B_1 in some cereal drinks showed the following concentrations: sorghum (0.96-21.74 µg/Kg), *burukutu* (1.27-8.82 µg/Kg), *Pito* (0.69-2.00 µg/Kg), millet grain (1.05-14.96 µg/Kg), millet dough (0.81-

3.78 µg/Kg) and sesame (0.79-60.05 µg/Kg). Samples studied were found to be unsafe for consumption and designated as containing the most potent genotoxic and carcinogenic aflatoxin.

A one-way ANOVA conducted compared AFB_1 within the local government areas and found there was significant difference at ($P < 0.05$) and P value < 0.0001; a post hoc (Tukey's) multiple comparisons test at 95 % CI of difference, test similarly indicate significant difference in AFB_1 content within and between local governments at $P < 0.05$ and P value <0.0001 for all samples.

Endemic low level exposure to AFB1 could constitute a risk factor for humans; applying the contamination criteria as updated by the EFSA as reported by Georgieyski et al. (2016), which modified the concentration of aflatoxin that could affect human health from its earlier level of 4 to 10 µg/kg; results obtained for this research showed results recorded for Kubau, Makarfi, Kudan and Kajuru local government areas were above 10 µg/kg. Soba, Ikara, Sanga, Chikun and Kachia local governments recorded two samples each above the safety limits, while one sample each was recorded to be above the permissible limit in Kauru, Lere and Sabon Gari local governments.

Conclusion

There is significant difference in concentration of aflatoxin AFB_1 in all 39 samples investigated. High percentages (81.82 % and 69.7%) AFB_1 contaminations were recorded in this research. The implication of this is that the consumption of this rice could be detrimental to health. Therefore, recommended that urgent steps should be taken in reducing the contamination of rice by AFB1.

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