Surveillance of Aflatoxin Levels in Maize (*Zea mays* L.) Grains Sold in Some Major Markets of Kaduna State, Nigeria

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Received: 11/01/2024 **Revised: 27/01/2024** **Accepted: 20/02/2024**

Aflatoxin contamination in maize is a significant food safety challenge in Nigeria, causing post-harvest losses and posing a threat to public health. This study evaluated the levels of aflatoxin contamination in maize grains sold in five major markets (Samaru, Sabon Gari, Railway, Central Market, and Tudun Wada markets) in Kaduna State. The grains were stored in sacks for 9–11 months before being marketed. Maize grains (one kilogram) were randomly collected from two cluster points in the selected markets in March 2023. Grains were obtained from five locations (vendors) in each market cluster. Aflatoxin was extracted from ground samples using the enzyme-linked immunosorbent assay (ELISA) protocol. The mean aflatoxin levels in maize grains sold in the markets ranged from 6.53±0.7 µg/kg to 60.87±1.87 µg/kg. Analysis of variance (ANOVA) of the aflatoxin levels showed a significant difference ($p \leq 0.05$) across the sampled locations. Mean aflatoxin levels in samples from Samaru 1, Samaru 2, and Kaduna Central Market 1 were 9.27±0.37, 6.53±0.7, and 9.67±0.55 µg/kg aflatoxin levels, respectively. These levels were within the permissible aflatoxin limit of the Standard Organization of Nigeria (10.00 µg/kg). Aflatoxin levels in all the samples were higher than the permissible limit of the European Union/World Health Organization limit (4.00µg/kg). Thus, limiting exports of our commodities. Even though the moisture contents recorded were within the recommended level for storage, the grains were still contaminated with aflatoxin. The incidence of aflatoxin contamination in the grains could therefore be linked to improper farmers' awareness, poor storage practices, and weather conditions. In conclusion, this study showed a high level of aflatoxin contamination (highest mean = 60.87±1.87) in maize grains sold in some major markets in Kaduna. Only maize grains sampled at Samaru and Kaduna Central (Cluster 1) markets are within the permissible limits and acceptable for consumption in Nigeria.

Keywords: Aflatoxin, contamination, maize, post-harvest, mycotoxin

https://dx.doi.org/10.4314/etsj.v15i1.2

INTRODUCTION

Aflatoxins which are natural toxicants are the most lethal fungal toxins that can cause both acute and chronic poisoning in humans and livestock. They pose a significant risk to consumer welfare and agricultural economies, resulting in a number of health hazards and financial losses (Mutiga *et al.*, 2015). Maize (*Zea mays* L.), a major dietary staple, is susceptible to contamination by aflatoxins, primarily caused by *Aspergillus* species. *Aspergillus flavus* Link (Ascomycota, Eurotiales), is a pervasive contaminant of agricultural produce (Bennet & Kale, 2007). Aflatoxin-producing fungi are prevalent in sub-Saharan Africa, where warm and humid weather provides ideal conditions for fungal growth (Massomo, 2020). Environmental stress in the field, such as dryness, can promote fungal development on crops and the production of toxins. Additionally, warm and humid conditions during storage further enhance aflatoxin development. Therefore, poor drying of crops contributes to increased aflatoxin accumulation during storage. Seasonal and annual variations in contamination levels can occur due to factors like regional farming and storage practices, soil characteristics, and climate (Xu *et al.*, 2018).

In Nigeria, maize is largely cultivated by smallholder farmers on over 6.5 million hectares of land across diverse agro-ecological zones of the country (IARC, 2015; PWC, 2021). Cultivated maize is widely used for human consumption (80%), animal feed (20%), pharmaceutical industries, food manufacturers, breweries, flour mills and other industries. Maize consumption per capita is estimated to be around 35 kg per year, contributing approximately 10% of the country's daily calorie intake (Wossen *et al*., 2023).

The World Resources Institute estimates that a number of environmental effects along the food production and supply value chains result in the loss or waste of about 23% of the food that is available in Sub-Saharan Africa. One of the main causes of food loss that contributes to food insecurity in Africa is the post-harvest loss of grains (Chomba, 2022). It has been reported by the Food and Agricultural Organization of the United Nations, FAO (2019) that reducing post-harvest losses could

benefit around 2 million African smallholder farmers by increasing their income and food security. Nigeria, a major maize producer in Africa, faces challenges in ensuring the safety of its maize products for its population. Kaduna State in northern Nigeria is a large agricultural hub with a sizable maize market. Despite the importance of maize, the seeds are often compromised by the presence of aflatoxin (Onyedum *et al*., 2020), which can be attributed to poor farming and storage practices that lead to frequent contamination by aflatoxigenic fungi (Bankole *et al*., 2006; Johnson *et al*., 2018), climatic changes (Battilani *et al*., 2016; Medina *et al*., 2017), oxidative stress (Reverberi *et al*., 2010) and light exposure (Kovac *et al*., 2018). As a result, maize seeds prices in the market are significantly lowered, posing a threat to farmers and dealers who may incur substantial financial losses (Keta *et al*., 2019). Maize is susceptible to various diseases and pests such as rust, rot, aphids, etc., both in the field and during storage. However, aflatoxin contamination can be identified by the presence of olive green or gray-green colors on corn kernels, whether they are in the field or in storage. On-site tests quickly detect the potential presence of aflatoxin, but they cannot provide precise quantitative results. The actual levels of aflatoxin are determined through specific analytical tests, as the toxins are produced internally within the kernels (Gary *et al*., 2012). Methods that are used for aflatoxin determination include thin-layer chromatography, ELISA, high performance liquid chromatography, etc. (Uzeh & Adebowale, 2021).

Microbial contamination (including aflatoxin) has been extensively documented, with several outbreaks directly linked to the consumption of commodities purchased from open markets (Ezekiel *et al*., 2012). However, it receives less attention in developing countries. Aflatoxin contamination is common in peanuts, maize, tree nuts, dried fruits, animal feeds, as well as several other staple foods (Kolawole *et al*., 2020; Meijer *et al*., 2021). Several studies on aflatoxin contamination have been conducted in different parts of Nigeria. Information on aflatoxin contamination in Kaduna is available for selected grains and feeds (Batagarawa *et al*., 2015), groundnut kernels (Wartu *et al*., 2015), and dried vegetables (Nafisa *et al*., 2017). However, there is little information available on the level of aflatoxin contamination in maize grains, despite maize being a major staple sold in Kaduna State, Nigeria. Therefore, it is crucial to assess the presence of aflatoxin contamination in the available grains. Consequently, this study was conducted to determine the levels of aflatoxin contamination in maize grains sold at major markets in Kaduna State, Nigeria.

LITERATURE REVIEW

Aflatoxin, its Toxicity, Occurrence, and Different Types

Aflatoxins are a group of natural mycotoxins produced as toxic secondary metabolites by toxigenic strains within the fungi *Aspergillus* section (de Almeida *et al*., 2019; Ayeni *et al*., 2020; Chang *et al*., 2023). There are currently more than 20 distinct aflatoxins. Three categories of aflatoxins are Aflatoxin B (AFB), Aflatoxin G (AFG), and Aflatoxin M (AFM) types (Negash, 2018). The most prevalent are aflatoxin B_1 and B_2 (AFB_1 and AFB_2), aflatoxin G_1 and G_2 (AFG_1 and AFG_2), and aflatoxin M_1 (AFM_1) (Benkerroum, 2020; Ajmal *et al*., 2022). The nomenclature for the aflatoxin series is based on their fluorescence emission pattern under ultraviolet (UV) light, occurrence and the nature of the compounds present (Chang *et al*., 2023). B and G aflatoxins emit blue and green fluorescence at 450 nm and 425 nm, respectively as distinguished by thin layer chromatography. Class M afltoxins are named from the letter M in milk due to the widespread occurrence in contaminated livestock milk and milk products. The subscript 1 and 2 numbers in aflatoxins denote major and minor compounds, respectively (Ting *et al*., 2020; Chang *et al*., 2023).

Aflatoxin is a family of toxic chemicals that are immunotoxic, hepatoxic, carcinogenic, and genotoxic (Tirmenstein & Mangipudy, 2014; Chang *et al*., 2023). These aflatoxins affect food and feed, leading to harmful effects on humans and animals. The toxicity levels of different aflatoxin classes vary; B_1, G_1, B_2, and G_2 aflatoxins are arranged in decreasing order of toxicity (Kumar *et al*., 2017). AFB_1 is the most ubiquitous food-contaminating aflatoxin and is of particular concern due to its immunotoxic and cancer-causative potentials (Chauhan *et al*., 2016; Ajmal *et al*., 2022). Hence, the International Agency for Research on Cancer classified AFB_1 as a group 1 or class 1 human carcinogen (IARC, 2012). Some diseases linked to aflatoxin toxicity include hepatocellular injury and necrosis, cholestasis, hepatomas, acute hepatitis, periportal fibrosis, bleeding, jaundice, fatty liver alterations, cirrhosis in malnourished infants, and Kwashiorkor (Tirmenstein & Mangipudy, 2014).

The universal aflatoxin-producing molds thrive in soil and spread through agricultural commodities such as dairy, cereals, nuts, tea, spices, chocolate, and feed for fish and animals (Negash, 2018). These commodities are prone to aflatoxin contamination worldwide, particularly in tropical countries, and may occur during the harvesting, processing, transportation, and storage of the commodities (Meijer *et al*., 2021; Ajmal *et al*., 2022; Chang *et al*., 2023). Researchers from all around the world have evaluated the aflatoxin contamination levels in various grains, staple foods, and by-products (Chang *et al*., 2023). These studies were conducted in Pakistan,

Malaysia, Kenya, Malawi, Nigeria, and several other countries around the world (Mwalwayoa & Thole, 2016; Ayeni *et al*., 2020; Ajmal *et al*., 2022; Kamano *et al*., 2022; Chang *et al*., 2023). In many cases, especially in African countries, the aflatoxin levels are above the acceptable limits, and this poses a serious health risk to consumers. For example, a study suggests that aflatoxin contamination of maize and groundnuts in Africa, which are crucial parts of the diet, is exceedingly high (Negash, 2018).

Mycotoxigenic and Aflatoxigenic Fungi, and Food Contamination

Fungi are a kingdom comprising beneficial, parasitic, and pathogenic groups classified differently from plants, bacteria, and some protists due to their chitinous cell walls (Ayofemi & Adeyeye, 2019). Mycotoxins are byproducts of fungi secreted on metabolizing organic medium. Mycotoxins, which are secreted by aflatoxin-producing fungi, are known as aflatoxin (Ayofemi & Adeyeye, 2019; Salisu *et al*., 2022). There are several toxigenic molds currently identified. However, mycotoxin-producing molds significant to human health include those contaminating cereals such as maize, wheat, barley, oats, rice, and groundnuts (Ayofemi & Adeyeye, 2019). Mold fungi of the species *Aspergillus* section *Flavi* (*Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nominus*) are the most economically important toxigenic fungi (Salisu *et al*., 2022). Other toxigenic molds, including *Aspergillus niger*, *Penicillium* spp., and *Fusarium* spp., are involved in postharvest decay and contamination of foods, feeds, and produce (Ayofemi & Adeyeye, 2019; Salisu *et al*., 2022).

Occurrence and Research Overview of Aflatoxin Levels on Commodities in Nigeria

Aflatoxin contamination in foods is a worldwide concern, especially in countries with tropical and subtropical climates (Norlia *et al*., 2019). Aflatoxin contamination occurs during pre-harvest and post-harvest periods and deteriorates during storage. The primary determinants of aflatoxigenic fungal growth during storage are temperature, relative humidity, and moisture content (Norlia *et al*., 2019). Maize, peanuts, and animal feeds were the most investigated African commodities for aflatoxin contamination. For almost a decade, 99% of studies indicated that AFB_1 contamination exceeded the European Union legal limit in several African countries (Meijer *et al*., 2021). Nigeria is the country with the most African research on aflatoxin contamination levels (Meijer *et al*., 2021). Aflatoxin B_1 levels in maize sold in several markets in Benue State, Nigeria, was screened by Ubwa *et al*. (2012) using thin-layer chromatography. In their investigation, Ubwa *et al*. (2012) did not detect the aflatoxin level in both stored and oven-dried fresh maize collected from the markets. They concluded that the aflatoxin levels could be either negligible or below the detection limit. Furthermore, the detection technique might be insufficient for the study. However, Ayeni *et al*. (2020) found that 99% of the maize was contaminated with total aflatoxins (range: 0.65–265 µg/kg) in their investigation of maize samples sold in open marketplaces in Ondo State, Nigeria. They employed the ELISA method for total aflatoxin screening. This level exceeds permissible limits and poses a high level of health risk for maize consumers. Studies on aflatoxin contamination levels were conducted by researchers in Kaduna State on harvested and stored groundnut kernels (Wartu *et al*., 2015) and dried vegetables (Nafisa *et al*., 2017). Other aflatoxin levels reported from foods and processed commodities in Nigeria include maize samples (Onilude *et al*., 2012), street-vended snacks (Ezekiel *et al*., 2012), locally processed peanut butter (Uzeh & Adebowale, 2021), and dairy cattle feeds (Omeiza *et al*., 2018).

Aflatoxin Regulation

Numerous international and local agencies are involved in implementing regulatory systems aimed at limiting the exposure of populations to aflatoxin. These regulations include permissible aflatoxin AFB_1, AFM_1, and/or total aflatoxins (AFB_1, AFB_2, AFM_1, and AFM_2) levels in specific foods or for all foods (Chang *et al*., 2023). Notable among the international organizations is the United Nations Office for Disaster Risk Reduction (UNDRR), which set a limit for maximum levels of aflatoxin in foods, including various nuts, grains, dried figs, and milk, in the range of 0.5–15 µg/kg. The maximum limit of total aflatoxins set by the United States Food and Drug Administration (FDA) stands at 20 µg/ kg for all foods (Chang *et al*., 2023). The European Union (EU) has different maximum acceptable limits for different foods. For instance, the EU set 2 µg/ kg and 4 µg/ kg for afatoxin B_1 and total aflatoxins, respectively, for most cereals and products derived from cereals (EU, 2023). The maximum allowable limits set by the EU for aflatoxins in rice and maize are 10 µg/ kg for total aflatoxins and 5 µg/ kg for aflatoxin B_1 (EU, 2023).

The international permissible limits are highly stringent. However, there exist differences in the risks, food consumption patterns, and levels of aflatoxin contamination in agro-ecological regions around the globe; these permit countries to set their own maximum acceptable limits within the ambit of science-based evidence (Gong *et al*., 2015). Presently, aflatoxin regulations are in full practice in over 50 different countries (Batagarawa *et al*., 2015). In Nigeria, aflatoxin limits are set by the Standards Organization of Nigeria (SON) and the National Agency for Food and Drug

Administration and Control (NAFDAC) to ensure food safety control systems (PACA, 2018). SON has set maximum limits for aflatoxins in maize and sorghum (10 µg/kg), groundnut (20 µg/kg), and groundnut cake and sesame seed (4 µg/kg) (PACA, 2018). Similarly, NAFDAC regulation on packaged goods and export-bound products sets aflatoxin levels at 4 µg/kg for ready-to-eat foods and 10 µg/kg for raw food items (Batagarawa *et al*., 2015).

MATERIALS AND METHODS

Sample Collection

Maize samples were collected from three markets in Zaria (Sabon Gari, Samaru and Tudun Wada) and two markets in the central metropolis of Kaduna (Railway and Central markets). These locations were selected based on a purposive sampling. The locations were selected based on their large population, status as major economic hubs in Kaduna State, and the presence of major markets. Maize grains were purchased randomly from two cluster points in the selected markets in March 2023. This was just before the rainy season when stored maize from the previous season is sold. In each market cluster, maize was purchased randomly from five vendors. Each purchase (1kg) was collected in aseptic polyethylene bags and transported to the laboratory at the Department of Crop Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria for processing.

The moisture content was measured using a moisture content meter. All samples were pulverized into a fine powder using an electric blender (MX- AC400, Panasonic) not later than 3 days after collection. Representative samples weighing 50g each were obtained through repetitive quartering and stored at -20°C until aflatoxin analysis was conducted (samples were processed promptly and not retained for longer than five days).

Sample Preparation

Twenty grams (20g) of each ground sample were weighed and placed in a beaker. The samples were then transferred into a reagent bottle and mixed with 25ml of aceto-nitrite. To ensure a homogeneous mixture, the samples were shaken at 250rpm for 3 minutes using an orbital shaker. Next, the samples were filtered into a centrifuge tube using filter paper. The resulting filtrate was collected in a test tube and used as aflatoxin extract for quantification purposes (Batagarawa *et al*., 2015; Nafisa *et al*., 2017).

Enzyme-linked Immunosorbent Assay (ELISA) for Total Aflatoxin

The quantification of total aflatoxin was conducted using the indirect ELISA technique as described by the manufacturer's instructions (the AqraQuant Total Aflatoxin Assay test kit was used). Three replicates were tested for each sample. First, 150µl of diluted toxin-Bovine Serum Albumin (BSA) conjugate was dispensed into each well of the ELISA plate, and the plate was incubated in a shaking oven at 37°C for 1 hour. After that, the content was discarded into a disposal chamber. Next, the plate was washed three times with phosphate-buffered saline (PBS)-Tween, with each wash taking 3 minutes. Following the washes, approximately 150µl of 0.2% BSA was added to each well, and the plate incubated again in a shaking oven at 37°C for 30 minutes. After the incubation period, the plate was removed, and its contents were discarded in a waste sink. The plate was then washed again using the same method as described above. To continue, 150µl of antiserum was added to each well and the plate was incubated for an additional 30 minutes at 37°C. After the incubation, the plate was removed, its contents were discarded, and the plate was washed three times with PBS-Tween at 3-minute intervals after each wash (Wartu *et al*., 2015; Felagha *et al*., 2016).

A standard solution of aflatoxin was prepared by diluting 1.5µl of the standard into a mixture of methanol and 1X phosphate-buffered saline with Tween® detergent (PBST) at a ratio of 1:1 with a total volume of 0.6ml. This prepared standard solution was then used to prepare a solution in a serial dilution using a 1:1 mixture of methanol and PBST as the diluent. For each sample, 20µl was diluted in 180µl of a 0.2% BSA solution and the mixture vortexed vigorously. The absorbance of the standard and sample was measured at a wavelength 405nm using an ELISA plate reader (LABTRON LMPR-A30, United Kingdom). The corresponding aflatoxin concentration in each well was estimated from the standard curve plotted using the percentage binding against the total aflatoxin standards (Felagha *et al*., 2016).

Data Analysis

The collected quantitative data for total aflatoxins in the maize samples was coded, entered in Microsoft Excel spreadsheets, and analysed with R software (version 4.0.3). Analysis of variance (ANOVA) was conducted to determine the significant difference in aflatoxin levels of maize grains collected from the different markets. The correlation coefficient between aflatoxin levels and moisture content of stored maize seeds was also assessed.

RESULTS AND DISCUSSION

Aflatoxin levels in the sampled maize seeds showed significant ($p \leq 0.05$) variation across all locations. The results of the ELISA analysis for the maize samples revealed that all maize samples were contaminated with aflatoxin, with levels ranging from 6.53µg/kg to 60.87µg/kg. This observed incidence of aflatoxin

contamination is consistent with previous reports of 100% incidence in maize from Nigeria (Onyedum *et al*., 2020; Williams *et al*., 2015). The percentage moisture content and aflatoxin levels in maize samples are given in Table 1 and figure 1, respectively. The mean values of the aflatoxin levels are presented in Table 2. The moisture contents of the sampled seeds, which had been in storage for 9- 11 months, ranged from 2.1% to 11.4%. These moisture contents are thus satisfactory for maize seed storage. However, the samples had high aflatoxin levels. It was further observed that maize samples from three locations (Kaduna Central Market 1, Samaru 1, and Samaru 2) were within the permissible limit of Standard Organization of Nigeria (10.0ug/kg). Similar ranges in aflatoxin levels have been reported by Kolawale *et al*. (2020). All the values were higher than the European Union (EU)/World Health Organization (WHO) permissible limit (4.0ug/kg). Thus, limiting the exports of commodities to other countries and consequently affecting revenue generation.

Aflatoxin contamination in this study suggests that poor storage conditions during prolonged storage may have played a role, even at low moisture levels. According to Villers *et al*. (2014), if the moisture is between 12 and 13%, the growth of the fungus is halted. Therefore, it is imperative to suggest that the high aflatoxin levels observed in this study could be due to infestation by *Apergillus flavus* from the field or farm. This infestation could have proliferated under poor storage facilities and low temperatures resulting from incomplete drying of maize seeds immediately after harvest. This highlights the importance of drying immediately after harvest to prevent the proliferation of *Apergillus* spp. which produces aflatoxin during storage. It is evident that low moisture content alone in dry maize seeds does not guarantee aflatoxin-free seeds under prolonged seed storage as samples within the recommended storage moisture content in this study were contaminated with aflatoxin, exhibiting negative correlation (-0.3) between maize moisture and aflatoxin levels (Figure2). Our study also aligns with the evidence provided by Oliveira *et al*. (2009) for high aflatoxin levels. They suggest that aflatoxin growth is influenced by weather conditions before or after harvest. Pre-harvest aflatoxin contamination of maize is associated with drought and temperatures during grain fill. When soil moisture is below normal and temperatures are high, the number of *Aspergillus* spores in the air increases, leading to crop infection through areas of damage caused by insects. Once infected, plants experience stress, favouring aflatoxin production. Post-harvest aflatoxin contamination can develop if grain is improperly managed during the drying and storage processes, especially if it is during unfavourable humidity and temperature (Xu *et al*., 2018; Massomo, 2020).

Table 1: Sampling locations, GPS and moisture contents of maize seeds used

MARKET	SC	GPS	MC (%)
Sabon Gari	SG1	11°6'27"N, 7°43'46"E	2.90
	SG2	11°6'28"N, 7°43'45"E	2.10
Kaduna Central	KC1	10°31'2"N, 7°25'43"E	5.40
	KC2	10°31'2"N, 7°25' 46"E	3.70
Samaru	SM1	11°9'42"N, 7°38'49"E	4.30
	SM2	11°9'42"N, 7°38'51"E	10.10
Railway	RW1	10°31'2"N, 7°25'43"E	2.50
	RW2	10°31'2"N, 7°25'41"E	4.80
Tudun Wada	TW1	11°6'9"N, 7°40'38"E	11.40
	TW2	11°6'7"N, 7°40'44"E	8.10

Key: SC- Sample Code, GPS- Global Positioning System, MC- Moisture Content

Table 2: Aflatoxin levels in maize grains collected from some markets

SN	Location	Sample Code	Aflatoxin level (µg/kg)
1	Railway 1	RW1	60.87±1.87[a]
2	Railway 2	RW2	13.14±0.92[e]
3	Samaru 1	SM1	9.27±0.37[fg]
4	Samaru 2	SM2	6.53±0.78[gh]
5	Sabon Gari 1	SG1	32.53±1.13[b]
6	Sabon Gari 2	SG2	24.47±2.32[d]
7	Kaduna Central 1	KC1	9.67±0.55[fg]
8	Kaduna Central 2	KC2	11.50±0.51[ef]
9	Tudun Wada 1	TW1	28.70±1.00[c]
10	Tudun Wada 2	TW2	29.67±1.43[bc]
11	Standards Organization of Nigeria	SON	10.00±0.00[ef]
12	EU/WHO	EU/WHO	4.00±0.00[h]

Values are mean ± standard error. Means with the same superscripts along column are not significantly different ($p \leq 0.05$)

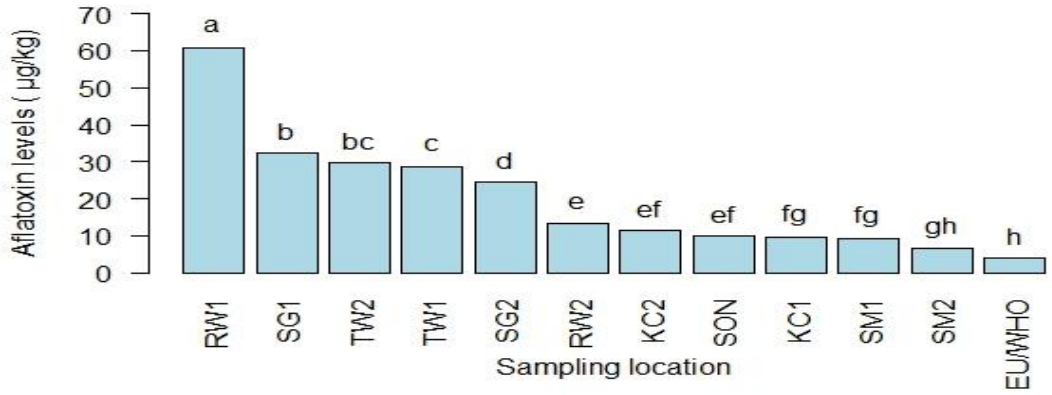

Figure 1: Bar plot of the aflatoxin levels for the sampled maize grains

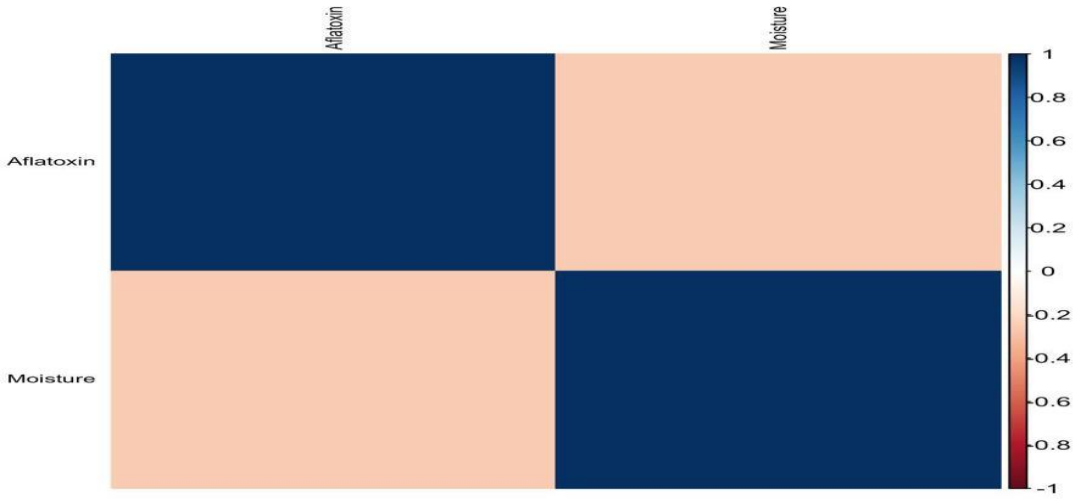

Figure 2: Correlation coefficient of aflatoxin levels and moisture content of stored maize seeds

CONCLUSION

This study showed a high level of aflatoxin contamination (highest mean = 60.87±1.87) in maize grains sold in some major markets in Kaduna State, Nigeria. The aflatoxin levels in seven out of the ten locations sampled were above the maximum tolerable limit (15.0 µg/kg) set by the SON for maize consumption in Nigeria. This is an indication that majority of the grains on the market (70%) are contaminated with aflatoxin. The aflatoxin levels were all higher than EU/WHO limit (4.0 µg/kg). Thus, limiting the exports of commodities to other countries. Therefore, appropriate pre- and post-harvest measures should be adopted to reduce aflatoxin contamination of maize in the state. Farmers must have an understanding of good post-harvest practices to guarantee acceptable levels of aflatoxin in stored maize. Improving post-harvest management practices could also help mitigate losses throughout the maize value chain and thereby contribute to poverty reduction and food insecurity in the country.

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