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Assessment of Aflatoxin Contamination in Maize and Groundnuts during Storage in Giwa Community, Kaduna State Nigeria

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Abstract

Contamination of food and feed by aflatoxins has become a worldwide cause of public health concern due to its significant impact on human health and crop market value. The present study aimed to assess the aflatoxin contents of maize and groundnut from stores and warehouses in the Giwa community, Kaduna State, Nigeria. A total of ninety (90) grain samples of maize and groundnut were collected between October to December 2020 and analyzed for total aflatoxins using Enzyme-Linked Immuno Sorbent Assay (ELISA). Eighty-four 84(93.3%) of the samples had detectable aflatoxin levels (0.2-9.8ppb), while six 6(6.7%) had none. There was no significant difference (P>0.05) in the mean total aflatoxin content of the grains from stores, warehouses and household foodstuff samples. This could be attributed to agricultural practices and low temperature and humidity storage conditions, which were the same for all the stored grains. Overall, aflatoxin concentrations of the grain samples were within the acceptable limit (10ppb for maize and 15ppb for groundnut) for food safety set by NAFDAC. However, there could be further contamination during storage, particularly when temperature rises and humidity increases. Hence, improved storage conditions and monitoring of grain before sale are recommended to avoid contamination during storage and to ensure a healthy and safe food supply along the trade chain and to the consumers.

Keywords: Aflatoxin. Maize. Groundnut. Grain stores. Warehouses. ELISA.

INTRODUCTION

For billions of people around the world, maize and groundnut are stapled crops (Eskola *et al.*, 2020). However, in warm agricultural areas, both crops are regularly infected by aflatoxin-producing fungi (Agbetiameh *et al.*, 2017) with subsequent contamination with aflatoxins before, during, and after harvest (Bandyopadhyay and Cotty, 2013). The main causes of mould growth and spread on food crops in many developing nations are a combination of inadequate agricultural techniques, insufficient crop drying, and damp meteorological conditions. Due to these, harvested maize, groundnuts, and other staple agricultural goods have unsafe amounts of mycotoxins, particularly aflatoxins (Agbetiameh *et al.*, 2017; Agriopoulou *et al.*, 2020).

Aflatoxin-containing substances have a detrimental effect on both human and animal health. They are extremely poisonous and carcinogenic (Bryden, 2012). Consuming products tainted with aflatoxin may have long-term or short-term repercussions, including fatalities (Agbetiameh *et al.*, 2017). The two species of aspergillus that produce aflatoxin most frequently are *Aspergillus flavus* and *Aspergillus parasiticus* (Benkerroum, 2020). When the soil moisture is below average and the temperature is high during grain loading, the amount of *Aspergillus* spores in the air increases, causing pre-harvest aflatoxin contamination of maize. These spores spread to crops through insect-damaged areas (Ubwa *et al.*, 2012). When grains are incorrectly handled during the drying and storage processes, i.e. under favourable humidity and temperature circumstances, postharvest aflatoxin contamination can develop (Ubwa *et al.*, 2012). According to ICRISAT (2018), local farmers typically cultivate maize and groundnuts under rain-fed circumstances with insufficient preventive controls to reduce aflatoxin contamination. As a result, eating groundnuts and maize could expose many people in Nigeria to aflatoxin (Atanda *et al.*, 2013; Keta *et al.*, 2019). Numerous studies across Africa, especially Nigeria, have shown levels of toxins in foods and other agricultural products that are significantly higher than those permitted limits established by national and international regulatory agencies (Makun *et al.*, 2010; Ubwa *et al.*, 2012; Jimoh and Kolapo, 2014; Sule *et al.*, 2015; Chauhan *et al.*, 2016; Kachapulul *et al.*, 2017; Keta *et al.*, 2019). As a result, the problems with food safety brought on by

mycotoxin contamination in Nigerian consumer agricultural goods must be addressed.

Assessing mycotoxin contamination of grains from whole suppliers would give insight into the foodstuff contamination level at the start of the food chain supply. This study aimed to assess the aflatoxin contents of maize and groundnut from stores and warehouses in the Giwa community, Kaduna State, Nigeria, using Enzyme-Linked Immuno Sorbent Assay (ELISA).

MATERIALS AND METHODS

Study Area

Giwa local Government area lies between longitude 11.25 °N, latitude 7.47°E and about 640m above sea level. Giwa is a large food crop growing and trading community with most of its residents being agriprenuers. Maize, beans, soya bean, groundnut and sorghum (especially with maize) on a large scale are the common grains cultivated by the resident of the community. Grain storage facilities include small stores and moderate and big warehouses in which they keep their foodstuffs, most of which are located in the market. They also keep some of the foodstuffs at home in a dedicated room. Large numbers of storage facilities are similar, made up of mud with no windows. The map of Giwa L.G.A is presented in Figure 1.

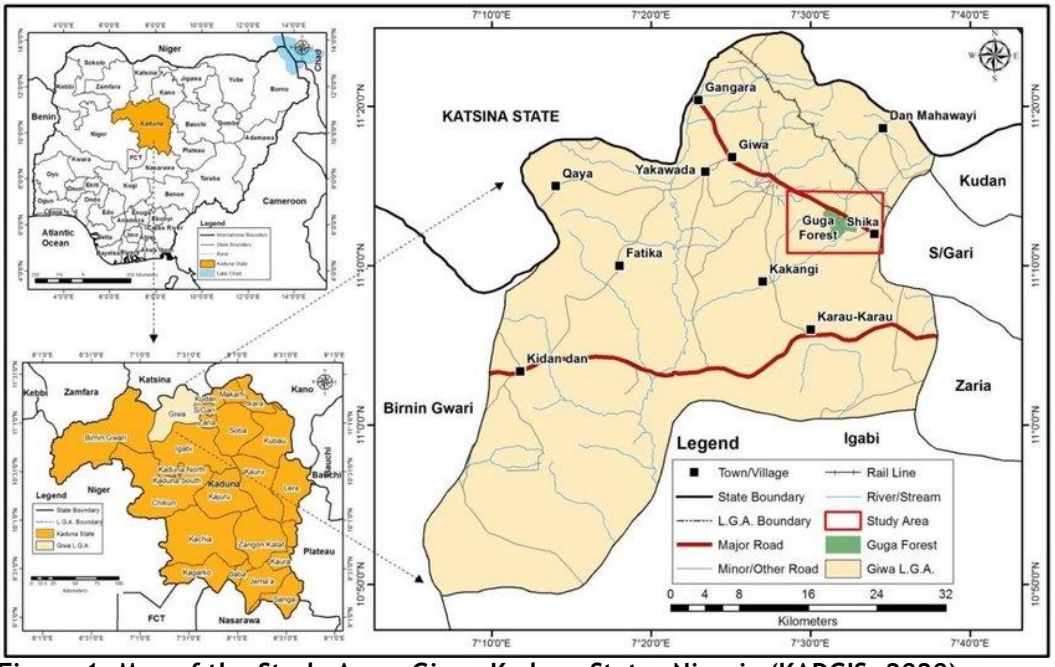

Figure 1: Map of the Study Area: Giwa, Kaduna State, Nigeria (KADGIS, 2020)

Sample Collection

Samples were collected from warehouses in the market and from stores at home. A total of ninety (90) grain samples (45 maize and 45 groundnuts) were collected for the study. Sampling units were; maize store in a residential building, groundnut store in a residential building, maize warehouse, groundnut warehouse, and maize and groundnut for household consumption (stored in the kitchen). Six (6) grain samples of maize and groundnut were collected from stores and warehouses, and six (three maize and three groundnuts) samples were from household foodstuff. The sampling was done three times at an interval of one month on 22nd October, 19th November and 21st December 2020.

Meteorological data collection

Meteorological data of the studied area over the studied period (October to December) was collected from Meteorological Unit, Department of Soil Science, Institute of Agricultural Research, Ahmadu Bello University Zaria. The data include temperature (T), relative humidity (RH), and rainfall.

Total Aflatoxin Extraction

The analysis was carried out by processing 30 samples at a time. Ten grams (10g) of grain samples were ground to powder using a miller (model-HGB2WTG4). Two grams (2g) were weighed and poured into an extraction bottle. Ten millilitres (10ml) of 70% methanol was added and mixed on a rotary shaker at 2000rpm for 10minute. The sample mixture was filtered, and 100µl of the filtrate was diluted using 600µl of distilled water and set for ELISA.

Total Aflatoxin Quantification using ELISA

Wells were placed on a micro well plate, 50µl of enzyme-antibody conjugate was measured using micro pipette and dispensed in each well.

Another aliquot of 50µl of each of the sample (diluted filtrate) and the standard were added into appropriate test wells, then 50µl of antibody was dispensed into each test well, the plate was shaken gently to mix the content and allowed to incubate at room temperature for 30 minutes. The contents of the wells were discarded and washed by filling with distilled water and discarding it five times. Following the last wash, the absorbent paper towel was placed on the flat surface of the test wells and tapped to remove the last of the wash solution. Hundred microlitre (100µl) of the substrate was measured and dispensed into test well, the plate was shaken gently and incubated at room temperature for 10 minute. Aliquot of 100µl of stop solution was measured and dispensed into each test well and shaken gently. The absorbance was read using an ELISA reader (spectrophotometer, STAT FAX 303/PLUS) at 450 nm, and the aflatoxin concentration automatically generated (RIDASCREEN* Aflatoxin Total ELISA kits user guide, 2019).

Statistical Analysis

One-way ANOVA was used to compare the mean (six concentrations for samples from stores and warehouses and three concentrations for samples from household foodstuff) total aflatoxin concentrations in stored grains among the sampling sites, using Science Analytical Software (SAS-JP Pro 14). The mean total aflatoxin contents of the grains were presented in the form of multiple bar chat, with error bars representing the standard deviation from the mean. The p-value for each month was presented on the graph.

RESULTS

Description of Sampling Sites

The warehouses shown in Plate I are typical of size 4m x 5m x 3m. They have small vents near the roof, which serve as a source of ventilation. The grains are customarily arranged to lean against the walls from all sides, closing on towards the door. A typical warehouse can be filled to more than 80% of the floor surface but is not stacked to the roof to allow for ventilation. The warehouses used were all built and put to use together. They have been in use for up to thirty years. The stores at home are typical of size 3m x 3m x 2.5m with similar ventilating holes as the market warehouses. They have been in use for a long time, usually the house's age. The duration of use is typically thirty years. The stored grains were harvested product of the year, and were within three and five weeks of storage for maize and groundnut respectively.

Plate I: Stored grains in warehouses in the study area (Right: groundnut warehouse; left: maize warehouse)

Mean Total Aflatoxin Concentration of Grains Stored in Warehouses and Homes of Farmers

Figures 2 and 3 represent the mean total aflatoxin concentration of maize and groundnut grain samples. Out of the ninety (90) grain samples analyzed, 84 (93.33%) had detectable aflatoxin levels (0.2-9.8ppb), while 6 (6.67%) had none.

One-way Analysis of Variance (ANOVA) showed that there was no significant difference ($p>0.05$) in the means of total aflatoxin level among the samples collected from different units at each sampling period for both maize and groundnut. Wider error bars were observed, signifying that data sets were scattered around the mean.

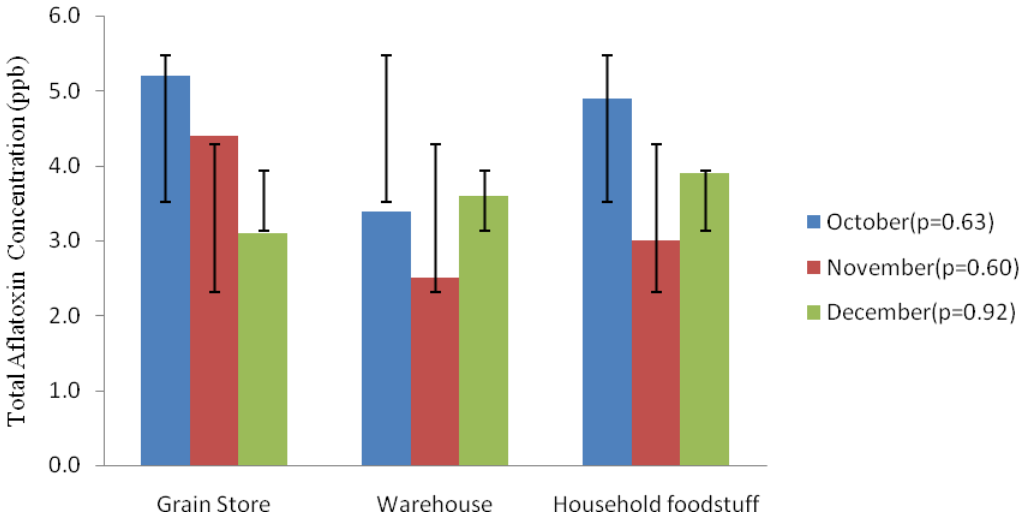

Figure 2: Mean Aflatoxin Concentration (ppb) of Maize Samples Collected from Store and Warehouse in Giwa

Climatic conditions at the months of sampling:
October: T°C (18±8 - 32±2), RH% (44±16 - 92±11), Rainfall mm (3±8)
November: T°C (17±1 - 32±1), RH% (24±4 - 65±1), Rainfall mm (0±0)
December: T°C (16±1 - 33±2), RH% (18±4 - 51±6), Rainfall mm (0±0)
*Values are presented in mean±standard deviation.

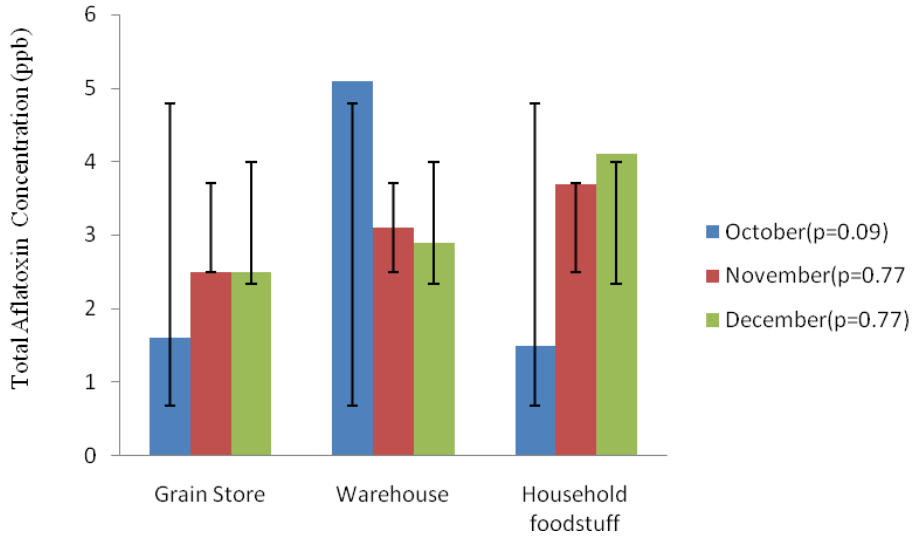

Figure 3: Mean Aflatoxin Concentration (ppb) of Groundnut Samples Collected from Store and Warehouse in Giwa

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DISCUSSION

It was observed that the levels of total aflatoxin in the stored grains were within the permissible limits (10ppb for maize and 15ppb for groundnut) specified by the NAFDAC (Anhwange and Adie, 2020). Non-occurrence of drought in the year during crop cultivation, deficient rainfall during harvesting, very low-temperature range and reduction in relative humidity through the sampling time could be the reason for the low aflatoxin content of the stored foodstuffs (Strelec *et al.*, 2010; Benkerroum, 2020).

The freshness of the grains, i.e., grains were freshly stored within three and five weeks of storage for maize and groundnut, respectively, could also be the reason for low aflatoxin contamination of the stored foodstuffs. Moreover, there was no significant difference (P>0.05) in the mean total aflatoxin content of the grains in store, warehouse and household foodstuffs. These could be attributed to the community's farmers' cultivation, harvest and storage procedures, which might be similar (Strelec *et al.*, 2010).

Mould contamination would undoubtedly increase when there is high humidity and temperature, leading to the total aflatoxin raise above the permissible limit. Hocking (2007) reported that if both temperature (20-38°C) and moisture (16-24%) are favourable for *Aspergillus flavus,* aflatoxin can be produced within 48 hours.

The inconsistent increase and decrease in the aflatoxin content of the grains through the three months sampling period and the wider error bars observed might be due to the nature of the aflatoxin contamination of the grains. Aflatoxin contamination is not uniform on grain kernels, so the whole aflatoxin content from a sample could be from one or two contaminated kernel(s) (Udomkun *et al.*, 2017). As such, the differences observed in this study do not signify further or reduced contamination of the grains. This could be the reason why broader ranges of permissible limits were given by regulatory bodies around the globe (Eskola *et al.*, 2020; CAC42, 2021).

Similar to this study is that of Williams *et al.* (2015), who reported mean total aflatoxin content of 3.20 ± 0.12 µg/kg in maize samples with non-detectable levels in groundnut samples; they attributed the low level of contamination to the freshness status of the grain. It could also be attributed to climatic conditions that prevailed in the region where the grain was cultivated and improved agricultural practices by the farmer(s) who grow the grains (Benkerroum, 2020). In addition, Agbetiameh *et al.* (2017) reported that over 15% of maize and 11% of groundnut samples in Ghana exceeded the aflatoxin limit sets by the Ghana standard authority. This is similar to this study's finding, where a low aflatoxin level was contained in maize and groundnut. The reason for this could be as mentioned above.

However, in Kaduna State of Nigeria, Sule *et al.* (2015) study revealed higher aflatoxin contamination levels in maize and maize products (mean aflatoxin level of 177ppb and 102ppb in old and new maize grains) with over 80% having aflatoxin contamination above the permissible limit of 10ppb. This is far above the level observed in this study, which could be attributed to climatic conditions during the cultivation period (Benkerroum, 2020). In Ethiopia, Chauhan *et al.*, (2016) reported aflatoxin contamination of >50ppb in 53% of maize/maize products, with the remaining samples being contaminated beyond the permissible limit. This is also far above the amount found in this study, the reason for this could also be as mentioned above.

CONCLUSION

Eighty-four 84(93.33%) of the analyzed grain samples had detectable aflatoxin levels (0.2-9.8ppb), while six 6(6.67%) had none. According to NAFDAC, the aflatoxin content of the grains was within the permissible limit of 10ppb (for maize) and 15ppb (for groundnut). One-way Analysis of Variance (ANOVA) reveals no significant difference (p>0.05) in the mean total aflatoxin contamination among the grain samples in grain stores, warehouses and household foodstuffs through the sampling period.

Recommendations

Educating farmers and grain tradesmen about the possible presence of mycotoxin especially aflatoxin and its associated health risk, as well as, preventive measures to mycotoxins contamination of grains will reduce its occurrences, this can be achieved by enlightening the community by relevant stakeholders in Public Health and Agriculture.

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