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Determination of Aflatoxin Levels in Cereals and Leguminous Grains Selected Cereals and Leguminous Grains Retailed in Wukari, Taraba State

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Abstract

Aflatoxins are group of secondary fungal metabolites produced by *Aspergillus* species, such as *Aspergillus flavus* and *Aspergillus parasiticus*. The aflatoxin producing moulds can grow on cereals and legumes in the field, poorly dried harvested crops in storage, processed food, and feed products. The study was carried out with the aim to determine the level of aflatoxin contamination of cereals grain and legumes retailed in Wukari, Taraba State Nigeria. A total Sixty-three (63) samples were procured from different vendors from the markets namely, new and old markets in Wukari and Dorowa Market, which comprises of 3 samples each of millet, maize, rice, groundnut, sesame seeds and soy beans. The samples were grounded and extracted with 80% (v/v) methanol. The enzyme-linked Immunosorbent Assay (ELISA) technique was used in quantifying the total aflatoxin content of the samples. The results revealed that there were no significant differences in the aflatoxin levels of shelled melon seeds while there was a significant difference in shelled groundnut, soybeans and millet samples purchased from all the markets. Aflatoxin levels in cereals and legumes retailed in Wukari, Taraba State ranged from 0.57-1.17µg/kg in shelled groundnut samples, 0.47-2.27µg/kg in shelled melon samples, 1.53-3.17µg/kg in sesame seed samples, 0.10-0.20µg/kg in soybean samples, 3.11-13.10µg/kg in maize samples, 6.13-15.4µg/kg in millet

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samples, and 0.471.0µg/kg in parboiled rice samples. the levels of aflatoxin in the samples which was observed to be highest in millet sample procured from Dorowa market with a value of 15.43±0.15% and lowest in soy bean bean sample procured from Dorowa and New market Wukari with a value of 0.10±0.00%. These aflatoxin levels were within the permissible limits for total aflatoxins recommended by FDA and NAFDAC for all products intended for food, hence these cereals and legumes analysed in this study are safe for human consumption.

Keywords: Aflatoxin, *A. flavus, A. parasiticus*, Cereals, Enzyme-linked Immunosorbent Assay, Legumes

INTRODUCTION

Cereals are crop plants from the grass family Poaceae that are grown for their edible starchy seeds or grains and are botanically known as caryopsis (Ukonmah and Eruotor, 2012). The term 'cereal' refers to both the plant and the grain, and it is also loosely applied to the grain product. The cereal grain is a single-seeded indehiscent fruit, or caryopsis, with the pericarp entirely bonded to the seed coat (Ukonmah and Eruotor, 2012). The family *Fabaceae* (Leguminosae) includes legumes. Legumes are second only to cereals as a source of human nourishment and provide essential proteins. Legumes offer comparatively high levels of important amino acids such as lysine and tryptophan, which fully supplement the protein provided by cereals. Their proteins have comparatively low levels of the sulfur-containing amino acids methionine and cystine, which are rather abundant in grain protein (Ukonmah and Eruotor, 2012).

Fungi are those that grow on stored products; one feature they all have in common is the ability to develop in the absence of free water; they include numerous Aspergillus spp. and a few Penicillium spp. (Olusegun and Hussaini, 2013). All of these have the potential to grow in grains and legumes. They exist practically everywhere and contaminate all grains and legumes (Olusegun & Hussaini, 2013). Aflatoxins are a group of secondary fungal metabolites generated by *Aspergillus* species, including *A. flavus* and *A. parasiticus*. *A. bombycis*, *A. ochraceoroseus*, *A. nomius*, and *A. pseudotamari* are also aflatoxin-producing species, but they are considerably less common (Bennett and Klich, 2003). Aflatoxins are highly toxic, carcinogenic, mutagenic, immunosuppressive, and teratogenic substances generated as secondary metabolites by *A. flavus* and *A. parasiticus* (Krishnamurthy and Shashikala, 2006). During a drought, aflatoxins become more frequent, and thus more of a food safety

problem, because little rainfall and high temperatures promote the growth and survival of the moulds that make them.

Crops stressed by drought and high temperatures, as well as those weakened by insect or other damage, are more vulnerable to mould growth and aflatoxin contamination. Aflatoxin-producing moulds can grow on field crops, poorly dried harvested crops in storage, processed foods, and feed items (Abbas, 2005). Environmental and storage factors both contribute to aflatoxin formation. Other aspects include water activity, moisture content in feeds and substrates, as well as insect damage (Arrus *et al.,* 2005).

Aflatoxin's principal kinds include B_1, B_2, G_1, G_2, M_1, and M_2 (Wrather, 2008). Aflatoxin B_1 is produced in abundance and is the most poisonous, followed by G_1, B_2, and G_2. Aflatoxin B_1, B_2, G1, and G2 are classed as Group I human carcinogens, while M_1 is a Group 2B probable human carcinogen (Krishnamurthy and Shashikala, 2006). Aflatoxin B_1 causes carcinomas in animals and has a high association with cancer incidence in humans (Jolly et al., 2009; Meggs, 2009).

Aflatoxin B_1 is found in the highest concentrations in contaminated commodities, while total aflatoxins (AFT) are the sum of all aflatoxins' related chemicals. The Food and Agriculture Organisation (FAO) reports that the world's maximum tolerated levels of aflatoxin B_1 are 1-20 μg/kg in foods and 5-50 μg/kg in cattle feed (FAO, 2004). Aflatoxin B_1 is mostly found in contaminated food, and humans are exposed to it virtually exclusively through their diet.

Aflatoxin B_1 contamination has also been documented in swine and poultry production (Viegas et al., 2013). This study aims to determine aflatoxin contamination in selected cereals and leguminous grains retailed in Wukari, Taraba State, with the goal of suggesting control methods.

MATERIALS AND METHODS

A total of 63 samples of various agricultural commodities, including maize, rice, shelled groundnut, soy beans, sesame seed, shelled melon, and millet, were procured from grain vendors operating in the markets of Wukari metropolis. These markets are namely Wukari New Market, Wukari Old Market and Dorowa Market which has

distance of above 40km from Wukari city. The collection of samples took place during the period spanning from April to May of the year 2023.

Three sellers were picked in a random manner at distinct points within each market, and a total of seven samples were procured from each of these vendors. The study involved the collection of three samples each of maize, rice, unshelled dried groundnut, soya beans, Beni seed, unshelled melon, and millet. Samples weighing approximately 100 grams were procured from each vendor and thereafter placed in individual clean containers. These samples were promptly brought to the Laboratory for analysis.

Quantification of aflatoxins by ELISA method, sample preparation and extraction

The sorghum, maize, millet, beans, and groundnuts were selected as representative samples for analysis. These samples were pulverized to a particle size where 75% of the material passed through a mesh sieve. A total of 50 grams of the pulverized samples was gathered and placed into a conical flask, followed by the addition of 5.0 grams of NaCl. The samples were subsequently combined with 100 mL of methanol solution containing 80% methanol and subjected to high-speed blending for a duration of 3 minutes. The samples were let to undergo sedimentation and afterwards passed through filter paper to collect the filtrate. A total of 5ml of the filtrate was thoroughly added to 20ml of distilled water and then filtered using a glass fibre filter (www.beaconkits.com). A total of twenty-one wells were allocated within a micro well strip holder, with each well designated for a certain sample or standard. Next, a volume of 50 microliters of enzyme conjugate was accurately measured from the bottle with a green cap and dispensed into each of the designated test wells. Using a micro pipette, 50 microliters of each sample and standard were extracted and added to the respective test wells containing the enzyme conjugate. Subsequently, 50 microliters of antibody were dispensed into each test well. The plate was gently shaken to ensure thorough mixing of the contents, and then incubated at room temperature for 10 minutes. After incubation, the contents of the wells were discarded, and the wells were cleansed by repeatedly filling them with distilled water and carefully draining and discarding it five times. This washing procedure was conducted with the intention of avoiding any disruption to the wells from their holder. After the completion of the previous washing step, the absorbent paper towel was positioned on the level surface of the test wells and gently tapped in order to eliminate any remaining remnants of the wash solution. A volume of 100 μL of the substrate from the blue-capped vial was measured and dispensed into each

of the test wells. The plate was then gently shaken and incubated at room temperature (37°C) for duration of 10 minutes. A volume of one hundred microlitre of stop solution was measured and dispensed from a bottle with a red cap into each individual test well. The plate rack was then gently shaken to ensure proper mixing. The observed phenomenon involves a transition in coloration from blue to yellow. Subsequently, the test wells are subjected to analysis using a micro well ELISA reader, namely at a wavelength of 450 nm and with the implementation of a differential filter set at 630 nm. The optical density (OD) measurements were collected from each micro well, and the corresponding concentrations were determined using a graph curve derived from the OD values and the known concentrations of the standards (www.beaconkits.com).

Data Analysis

Result of different determinants were reported as mean ± standard deviation. Significance were determined using analysis of variance (ANOVA). Differences between means was analysed using (specific posthoc test). Data analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 23. A significant level of $P< 0.05$ was employed to determine statistical significance.

RESULTS

Aflatoxin levels of Food Samples purchased from three Markets in Wukari

The Aflatoxin levels of food samples purchased from the three markets studied had the following ranges: Shelled Groundnut samples: 0.5-1.17 μg/kg, Shelled melon samples: 0.47-3.27μg/kg, Sesame seeds: 1.53-3.17μg/kg, Soy beans: 0.10-0.2μg/kg, Maize: 3.11-13.10μg/kg, Millet: 6.13-15.43 μg/kg, Parboiled rice: 0.43-1.00μg/kg,

From the results shown below in Table 4.22, it can be seen that Sesame seeds and Millet samples had their highest Aflatoxin levels in M_3 with values: 3.17±0.25 and 15.43±0.15 respectively. Maize samples purchased from M_2 (Old Market) showed a significance decrease in their Aflatoxin level with value: 3.11±2.59 compared the ones purchased from M_1 (New market) and M_3 (Old Market) with values: 13.10±0.30 and 10.97±0.25 respectively. However, samples purchased from M_1 and M_3 have the same Aflatoxin levels with values 0.1±0.00 and 0.1±0.00 respectively, While Soybean sample purchased from M_2 showed a slightly higher value of 0.2±0.00.

Table 1: Aflatoxin Levels of Food Samples Purchased from Three Markets in Wukari

FOOD SAMPLES	M_1 (µg/kg)	M_2 (µg/kg)	M_3 (µg/kg)
Shelled groundnut	0.57 ± 0.06^a	1.17 ± 0.12^c	0.83 ± 0.06^b
Shelled melon seeds	3.27 ± 4.10^a	1.07 ± 0.06^a	0.47 ± 0.06^a
Sesame seeds	2.07 ± 0.15^b	1.53 ± 0.02^a	3.17 ± 0.25^c
Soy beans	0.10 ± 0.00^a	0.20 ± 0.00^a	0.10 ± 0.00^a
Maize	13.10 ± 0.30^c	3.11 ± 2.59^a	10.97 ± 0.25^b
Millet	6.13 ± 0.25^a	12.07 ± 0.15^b	15.43 ± 0.15^c
Parboiled rice	1.00 ± 0.12^b	0.47 ± 0.12^a	0.63 ± 0.12^a

Results presented as mean ± standard deviation. Results within the same row with the same superscript indicate no significant differences (p.0.05), while results with the different superscripts within a row indicate significant differences ($p<0.05$).

DISCUSSION

Aflatoxin is an important naturally occurring mycotoxin in agricultural products. They are produced by several species of *Aspergilli*. Not all strain of *Aspergillus species* produce aflatoxin (Frisvad *et al.,* 2007*)*. In table 1 above, the following ranges were obtained for levels of aflatoxin in food samples analysed in the three markets under study: shelled groundnut had 0.5-1.17µg/kg, shelled melon had 0.47-3.27µg/kg, sesame seed had 1.53-3.17µg/kg, soybeans had 0.10-0.20µg/kg, rice had 0.47-1.0µg/kg, maize had 3.11-13.10µg/kg and millet had 6.13-15.43µg/kg. Total aflatoxin levels found in other grain samples analysed in this study were relatively lower than that detected in maize and millet samples. The total aflatoxin level found in millet sample from Dorowa Market Wukari had the highest aflatoxin contamination levels; this could be due to differences in storage and postharvest handling between the markets. This finding was consistent with that of Shitu *et al.* (2021) who reported a high aflatoxin contamination level in millet seed samples in their research. This finding is not in agreement with that of Batagarawa *et al.* (2015) who reported a contamination level of 0.62µg/kg in millet from Katsina state, and Ezekiel, (2014), who reported a range of 0.08-1.40 µg/kg for millet. The differences in contamination level

might be due to the difference in environmental factors (temperature and relative humidity) that favour the growth of aflatoxigenic moulds and agricultural practices between the study areas. Soybean samples recorded the lowest aflatoxin levels in the present study. Soybean procured from New Market and Dorowa Market Wukari had the lowest aflatoxin contamination level of 0.10µg/kg, this is indicative of the quality of soybean sample analysed in this study. The findings were also consistent with Niyibituronsa *et al.* (2018) who reported a low aflatoxin levels in soybean samples grown in Rwanda. All cereals and leguminous grains analysed in this study had aflatoxin values which did not exceed the maximum acceptable standard of total Aflatoxin limit of European Union (EU) standard adopted by Nigeria in safe to eat food and feeds (Imade *et al.,* 2021).

CONCLUSION

The study has revealed variations in aflatoxin contamination from various selected grains retailed in three markets from Wukari. Aflatoxin contamination wwas observed to be highest in millet sample procured from Dorowa market. However, this highest aflatoxin content has not exceeded the maximum acceptable standard of total aflatoxin limit of European Union (EU) adopted by Nigeria in safe to eat food and feeds, hence the grains analysed are safe for human consumption.

Recommendation

i. Stringent policies and legislation should be implemented to regulate aflatoxin contamination levels in food commodities. These regulations should align with the sacceptable limits set by organizations such as the National Agency for Food and Drug Administration and Control (NAFDAC).
ii. Food vendors and farmers should be educated about the importance of proper drying of grains before storage. Proper drying techniques can help minimize the risk of fungal growth and aflatoxin contamination.
iii. Consideration should be given to using non-toxic antifungal chemicals as part of storage strategies to prevent fungal growth in stored grains and reduce toxin production. These strategies can help mitigate the risk of aflatoxin contamination in food commodities.

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