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Determination of Aflatoxin Concentrations in Cereals and Legumes Marketed in Zaria Metropolis, Kaduna State, Nigeria

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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 in Zaria metropolis, Kaduna State, Nigeria. Ninety (90) samples were collected, which comprises of 18 samples each of millet, sorghum, maize, beans, and groundnuts respectively. The samples were subjected to proximate analysis. The grains were further subjected to cultural isolation and microscopic identification. The isolates were then screened for aflatoxin production ability with neutral red desiccated coconut agar and viewed under UV light (365nm). The remaining portions of the samples was 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 all the cereals and legumes analysed contain organic and inorganic nutrients that can support the growth of aflatoxigenic moulds and production of aflatoxins. Some major parameters such as carbohydrate content, crude protein, crude lipid, and ash contents were statistically significant ($p < 0.05$). Thirty-one (31) isolates from the 90 samples were confirmed to be *A. flavus* and seventeen (17) were *A. Parasiticus*, with percentage occurrence of 34.4% and 18.9% respectively. All the isolates were screened and demonstrated ability for aflatoxin production under Ultra-Violent light (390nm). The results also revealed a high concentration of aflatoxin (11.04 µg/kg) in millet and a low concentration in sorghum (1.07 µg/kg). The contamination levels within the grains were found to be statistically significant ($p < 0.05$). Aflatoxin contaminations also occurred in 48 samples out of the 90 samples analysed. The grains samples analysed were found to be contaminated with varying amounts of aflatoxins, which is harmful to humans and animals. Therefore, steps should be taken to ensure that grains are properly dried prior to storage.
Keywords: aflatoxin, *A. flavus*, *A. parasiticus*, cereals, Enzyme-linked Immunosorbent Assay, legumes

INTRODUCTION

Cereals are crop plants belonging to the grass family *Poaceae* that are cultivated for their edible starchy seeds or grains and botanically known as caryopsis (Ukonmah and Eruotor, 2012). The term 'cereal' applies to the entire plant as well as the grain and it is also loosely applied to the grain product. The cereal grain is a one seeded indehiscent fruit, or caryopsis, in which the pericarp is completely fused with the seed coat (Ukonmah and Eruotor, 2012).

Legumes belong to the family *Fabaceae* (Leguminosae). Legumes are second only to cereals as source of human food and provide much needed proteins. Legumes contain relatively high amounts of essential amino acids; lysine and tryptophan and thus fully compliment the protein supplied by cereals. Their Proteins contain relatively low amounts of the Sulphur containing amino- acids methionine and cystine, which are present in relatively high amounts in the protein of cereals (Ukonmah and Eruotor, 2012).

Fungi are those that grow on products in storage; one characteristic that they share in common is the ability to grow without free water they comprise several species of *Aspergillus* spp. and a few of *Penicillium* spp. (Olusegun and Hussaini, 2013). All these have the ability to grow in grain and legumes. They occur almost everywhere and contaminate all grains and legumes (Olusegun and Hussaini, 2013).

Fungi belonging to facultative saprophytes and facultative parasites may lower the quality of seeds by causing discoloration, others are; reduction or elimination of germination capacity and several other physiological alterations in grains (Neegaard, 1997).

Aflatoxins are group of secondary fungal metabolites produced by *Aspergillus* species, such as *A. flavus* and *A. parasiticus, A. bombycis*, *A. ochraceoroseus*, *A. nomius*, and *A. pseudotamari*are also aflatoxin-producing species, but they are encountered much less frequently (Bennett and Klich, 2003).

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive and teratogenic agents produced as secondary metabolites by *A. flavus* and *A. parasiticus* (Krishnamurthy and Shashikala, 2006).

Aflatoxins become more prevalent, and therefore more of a food safety concern, during drought because low rainfall and high temperatures encourage the growth and survival of the moulds that produce the toxins. Crops stressed by drought and high temperatures and/or weakened by insect or other damage, are more susceptible to mould growth and subsequent aflatoxin contamination. The aflatoxin producing moulds can grow on crops in the field, poorly dried harvested crops in storage, processed food, and feed products (Abbas, 2005).

The production of aflatoxin is enhanced by environmental conditions (temperature and relative humidity) and storage conditions. Other factors include water activity, moisture content in foods and substrates, in addition to the damage caused by insects (Arrus*et al.*, 2005).

The major types of aflatoxin are B_1, B_2, G_1, G_2, M_1 and M_2 (Wrather, 2008). Aflatoxin B_1 is produced most abundantly and the most toxic followed by G_1, B_2 and G_2 respectively. Aflatoxin B_1, B_2, G_1 and G_2 are classified as Group I human carcinogens whereas M_1 is classified as Group 2B probable human carcinogen (Krishnamurthy and Shashikala, 2006). Aflatoxin B_1 is responsible for carcinomas in animals, showing a strong relationship with the incidence of cancer in humans (Jolly *et al.*, 2009; Meggs, 2009).

Aflatoxin B_1 occurs in the highest amounts in contaminated commodities; and total aflatoxins (AFT) refers to the sum of the related compounds of aflatoxins. According to Food and Agriculture Organization (FAO), the world wide maximum tolerant levels of aflatoxin B_1 was reported to be in the range of 1–20 µg/kg in foods, and 5–50 µg/kg in dietary cattle feed in (FAO, 2004). Aflatoxin B_1 is mostly found in contaminated food and humans are exposed to aflatoxin B_1 almost entirely through their diet. Occupational exposure to aflatoxin B_1 has also been reported in swine and poultry production (Viegas*et al.*, 2013).

This research aimed at determining aflatoxin contamination in cereals and legumes marketed in Zaria Metropolis with the view to suggest control measures and to avoid eating food contaminated with aflatoxins.

MATERIALS AND METHODS

Study Area

Zaria metropolis is located at latitude 11° 07' N and longitude 07°42' E, and is one of the most important cities in Northern Nigeria (Uba*et al.*, 2008). It has a total area of 300 km^2 and consisting of six major settlements; Zaria City, Tudun Wada, Sabon Gari, Danmagaji, Kwangila and Samaru. Zaria metropolis, Kaduna State, is reported to have had a population of 1,018,827 in 2007 (TWG, 2007).

It has a tropical continental climate with a pronounced dry season, lasting up to six months (May - October). During the dry season, a cool period is usually experienced between November and February. This emanates from the influence of the Northeastern winds (harmattan) which control the tropical continental air mass coming from the Sahara. Hazy to dusty conditions and low temperatures characterize the North-East (NE) winds, as low as 10°C at night. A temperature of 40°C is sometimes recorded in the afternoon. The humidity also drops to less than 15% in December/January. Zaria experiences a brief period of hot but dry weather in March and April, followed by a progressive incursion of tropical maritime air mass from the Atlantic Ocean, which displaces the NE (harmattan) winds. During this short period, the mean daily maximum temperatures are stable, and they ranged from 38 - 42°C. After this, the South Westerly Monsoon winds laden with moisture bring the rain in thunderstorms and squalls with heavy fall of high intensities. The rainy season lasts from May to September/October with long-term annual rainfall of 1,040 mm in about 90 rain days. The relatively deep tropical ferruginous soils and climate conditions of Zaria are suitable and sustain a good cover of savanna woodland (Northern Guinea Savanna), with a variety of grasses woody shrubs and short trees. Six big markets were selected from the metropolis for analysis.

Collection of Samples

Ninety samples of millet, sorghum, maize, beans and groundnuts were collected from major grains sellers from markets in Zaria metropolis namely Zaria city, Tudun Wada, Sabon Gari, Kwangila, Danmagaji and Samaru market in Zaria, from July - August, 2019.

Three vendors were selected at different point in each market and five samples were purchased from each vendor. Ninety samples were collected; 18 samples of millet, sorghum, maize, beans and groundnuts respectively.

Approximately 100g samples were purchased from each vendor and the samples were placed separately in clean, sterile containers labelled appropriately and transported immediately to the Laboratory for analyses. Each sample was divided into two; one-half was used for proximate analysis and other half for mycological investigation and quantification of aflatoxins.

Proximate Compositions of Legumes and cereal grains

The analyses of proximate composition of the samples was carried out according to AOAC, (2010) method.

Determination of Moisture Content

A clean crucible was dried to constant weight in an air oven at 105°C, cooled in a desiccator and weighed (W1). Two grams of the sample was accurately weighed into the previously labelled crucible and reweighed (W2).

The crucible and sample were dried in oven to a constant weight (W3). The percentage moisture content was calculated thus:

$$\% \text{ Moisture content} = \frac{W3 - W1}{W2 - W1} \times 100$$

Determination of Ash Content

The porcelain crucible was dried in an oven at 100°C for 10 min, cooled in a desiccator and weighed (W1). Two grams of the sample was placed into the previously weighed porcelain crucible and weighed (W2). The sample was ignited and transferred into a furnace, which was then set at 550°C. The sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed cooled in the desiccator and weighed W3. The percentage ash content was calculated as:

$$\% \text{ Ash content} = \frac{W3 - W1}{W2 - W1} \times 100$$

Determination of Crude Lipid

A clean, dry 500ml round bottom flask, containing few anti-bumping granules was weighed (W1) and 300ml of petroleum ether (40-60°C) for extraction was poured into the flask fitted with soxhlet extraction unit. The extractor thimble containing twenty grams of the sample was fixed into the soxhlet extraction unit. The round bottom flask and a condenser were connected to the soxhlet extractor and cold-water circulation was put on. The heating mantle was switched on and the heating rate was adjusted until the solvent was refluxed at a steady rate. Extraction was carried out for six hours. The solvent was recovered and the oil dried in the oven at 70°Cfor one hour. The round bottom flask containing the oil was cooled in the desiccator and then weighed (W2). The lipid content was calculated thus:

$$\% \text{ Crude Lipid} = \frac{W2 - W1}{\text{Weight of sample}} \times 100$$

Determination of Crude Fibre

Two grams of sample was weighed into a round bottom flask. Hundred millilitres of 0.25M sulphuric acid solution was added and the mixture boiled under reflux for 30min. The hot solution was quickly filtered under sunction. The insoluble matter was washed several times with hot water until it was acid free. It was quantitatively transferred into the flask and 100ml of hot 0.31M sodium hydroxide solution added and the mixture boiled again under reflux for 30 minutes and quickly filtered under sunction. The insoluble residue was washed with boiling water until it was based free. It was dried to constant weight in the oven at 100°C, cooled in a dessicator and weighed (C_1). It was then incinerated in a muffle furnace at 550°C for 2 hours, cooled in the dessicator and reweighed (C_2).

The crude fibre was calculated thus:

$$\text{The loss of weight on incineration} = \frac{C_1 - C2}{\text{Weight of original sample}} \times 100$$

Determination of Nitrogen Content and Crude Protein

About 1.5g of the defatted sample in an ash less filter paper was dropped into 300ml Kjeldahl flask in order to digest the protein. Twenty-five millilitres of H_2SO_4 and 3g of mixed catalyst (weighed separately into an ash less filter paper) were dropped into the Kjeldahl flask. The flask was then transferred to the Kjeldahl digestion apparatus. The sample was digested until a clear green colour was obtained.

The digest was cooled and diluted to 100ml with distilled water.

Twenty millilitres of the diluted digest were measured into a 500ml Kjeldahl flask containing anti-bumping chips and 40ml of 40% NaOH, was slowly added by the side of the flask. The 250ml conical flask containing a mixture of 50ml of 2% Boric acid and 4 drops of mixed indicator was used to trap the ammonia liberated. The conical flask and the Kjeldahl flask were then placed on the Kjedahl distillation apparatus, with the tubes inserted into the conical flask and the Kjedahl flask. The flask was heated to distil out NH_3 evolved. The distillate was collected into the boric acid solution. After the boric acid turned green, 10 minutes were allowed for complete distillation of the ammonia present in the digest. The distillate was titrated with 0.1M HCl.

The nitrogen content and crude protein were thus calculated as:

Percentage Nitrogen = 14 x M x Vt x Tv x 100/ Weight of Sample (mg) x Va

% Crude protein = % Nitrogen (N2) x 6.25

Where M = Actual molarity of acid

Tv = Titre volume of HCl used

Vt = Total volume of diluted digest

Va = Aliquot volume distilled

Determination of Carbohydrate Content (by difference)

The sum of moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100% as illustrated below:

% Total Carbohydrate= 100 – (% Moisture + % Ash + % Fat + % Protein + % Fibre).

Isolation of Fungal Species

Media Preparation

Sweet potato yeast extract (SPYE) Agar was compounded as follows; 250g of fresh sweet potato was cut into smaller pieces and boiled in 1000mL of distilled water for 30 minutes. The extract was collected in a clean Erlenmeyer flask by passing the suspension through a clean muslin cloth. To the total volume of the extract in the Erlenmeyer flask, 1.3g of yeast extract and 20g of agar-agar was added and the pH adjusted to 5.5 and boiled until the agar dissolved. The contents of the flask were sterilized in an autoclave at 121°C for 15minutes. After cooling, streptomycin added to inhibit bacteria growth, poured in sterile Petri-dishes, and allowed to set and the plates were dried in an incubator.

Slants of the SPYE agar was also prepared as described above but in test tubes.

Neutral Red Desiccated Coconut Agar (NRDCA) was prepared by modification of the method of Davis *et al.*,(1987) as reported by Atanda *et al.*, (2011) as follows; two hundred grams of desiccated coconut was soaked in hot distilled water for 30 min., blended aseptically in a Warring blender for 5 min and filtered through two layers of cheese cloth. Two percent agar (Oxoid) was added to the filtrate, heated to boiling, cooled to about 50°C. To the filtrate, 0.1% neutral red stain was added and the pH was adjusted to 4.5. The media was then sterilized at 121°C for 15 min, cooled and poured uniformly into sterile Petri dishes.

Inoculation of Samples

Ten gram (10g) of each ground sample of millet, sorghum, maize, beans and groundnuts was separately added to 90ml of sterile distilled water and homogenized for 2 minutes to form a stock suspension. An aliquot of 0.5ml was spread on already prepared sweet potato yeast extract agar plate and the inoculated plates were incubated at room temperature for 3- 5 days (Bankole and Mabejoke, 2004). Observed colonies were sub cultured on SPYE agar in order to obtain pure isolates and the observed colonies were identified based on macroscopic and microscopic features (Pitt and Hocking 2009) and the isolates was sub-cultured into fresh sweet potato yeast extract agar slant and kept in the refrigeratoruntilrequired.

Screening of Isolates for Aflatoxigenic potential

The pure isolates of the samples were screened for aflatoxigenicity using desiccated neutral red coconut agar as described by Ezekiel *et al.*, (2013). Each isolate was inoculated on freshly prepared neutral red desiccated coconut agar, incubated at room temperature for 3-5 days. Isolates that absorbed and emitted very bright, moderate and weak UV light (fluorescence) at 365nm were confirmed to be capable of producing aflatoxins.

Quantification of Aflatoxins by ELISA Method

Sample Preparation and Extraction

Representative samples of millet, sorghum, maize, beans and groundnuts were pulverized to about 75% of the sample will pass through a mesh sieve. Fifty grams of the grounded samples was collected into a conical flask and 5.0 g of NaCl added. The samples were further mixed with 100ml of 80% methanol and blended at high speed for 3 minutes. The samples were allowed to settle and filtered through filter paper filtrate was collected and 5ml of the filtrate was mixed thoroughly with 20ml of distilled water and filtered through a glass fibre filter (Beacon, 2015).

Assay Procedure

Ninety-Five wells were placed in a micro well strip holder one for each sample and the standards, then 50 micro litres of enzyme conjugate were measured from the green capped bottle and dispensed in each test wells.

The micro pipette was used to take 50 micro litres of each sample and the standard added into appropriate test wells containing 50 micro litres of the enzymes conjugate, and then 50 micro litres of antibody was dispensed into each test well, the plate was shaken gently to mix the content and incubated at room temperature for 10 minutes. The contents of the wells were dumped, the wells were washed by filling with distilled water by pouring and dumping it five times carefully in order not disrupt the wells from the holder during washing procedure. 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 micro litres of the substrate from blue-capped bottle was measured and dispensed into each test wells, the plate was shaken gently and incubated at room temperature (37^{o}C) for 10 minutes. Hundred micro litres of stop solution from red capped bottle was measured and dispensed into each test well and shaken the plate rack gently. The colour changes from blue to yellow and then the test wells on micro well ELISA reader at 450 nm and a differential filter of 630nm. The optical density (OD) was taken from each micro well and the concentrations were obtained from a graph curve that was obtained from OD and the concentration of the standards (Beacon, 2015).

Data Analysis

The mean aflatoxin concentration and proximate compositions of the food commodities were analysed by Analysis of Variance (ANOVA) using SPSS Version 20. P ≤0.05 was considered significant.

RESULTS

Proximate Composition of the Grains and Legumes

Sorghum had the highest concentration of carbohydrates (77.73%) and moisture (9.73%) and the least in crude fibre (1.92%), crude protein (5.11%) and crude lipids (4.53%). In addition, groundnuts samples recorded the highest concentration of crude lipids (32.36%), crude protein (16.71%) and crude fibre (4.04%) and the least concentration of carbohydrates (36.05%). There were significant differences ($P<$ 0.05) in the Ash, crude lipid, crude protein and carbohydrate content of the samples (Table 1).

Distribution of Aflatoxigenic Moulds in the Grain Samples

Forty-eight 48 (53.3%) of the 90 samples were contaminated with fungal species. The occurrence of *Aspergillus flavus* was 31(34.4 %) while *Aspergillus parasiticus* was 17(18.9 %). Millet however had the highest occurrence of *A. flavus* 9(50%) and *A. Parasiticus* 5(28%) while sorghum has the least occurrence of *A.flavus* 3(16%) and *A.parasiticus* 2(11%). Similarly, maize and groundnut samples had the same percentage occurrences of 39% and 22% respectively for *A. flavus* and *A.parasiticus* (Table 2).

Aflatoxin Producing Ability of *A. flavus* and *A. parasiticus*

The two aflatoxigenic moulds (Table 2) were isolated namely *A. flavus* (31) and *A. parasiticus* (17). Of the *A. flavus* and *A. parasiticus* isolates tested for aflatoxin production, 07 *A. flavus* isolates had very bright fluorescence intensity, while *A. parasiticus* had 5 withvery bright fluorescence intensity. Nine isolates of *A.flavus* had weak fluorescence intensity followed by *A. parasiticus* with only 3. Thus, indicating the ability of the isolates to produce aflatoxin in large or small quantities (Figures 1 and 2).

Table1: Proximate Composition ofCereal Grains and Legumes (%) Sold in Zaria Metropolis

Food Commodity	Moisture	Ash	Crude lipid	Crude protein	Crude fibre	Carbohydrate
Millet	8.16^{a}	1.43^{b}	13.40^{b}	5.28^{b}	3.49^{a}	68.30^{a}
Sorghum	9.73^{a}	0.98^{b}	4.53^{c}	5.11^{b}	1.92^{a}	77.73^{a}
Maize	8.89^{a}	0.91^{b}	6.03^{c}	5.42^{b}	3.41^{a}	75.23^{a}
Beans	8.38^{a}	3.57^{a}	17.13^{b}	7.18^{b}	2.89^{a}	60.96^{a}
Groundnut	8.68^{a}	2.16^{a}	32.36^{a}	16.71^{a}	4.04^{a}	36.05^{b}

Means with different superscript along the columns are significant (P < 0.05)

Table 2: Distribution of Moulds in Cereal Grains and Legumes Sold in Zaria Metropolis

Food Commodity	No. of Sample Analysed	No. of fungal species & % occurrence of *A. flavus*	*A. Parasiticus*	Total No. of sample contaminated
Millet	18	9(50)	5(28)	14
Sorghum	18	3(16)	2(11)	5
Maize	18	7(39)	4(22)	11
Beans	18	5(28)	2(11)	7
Groundnut	18	7(39)	4(22)	11
Total	90	31(34.4)	17(18.9)	48

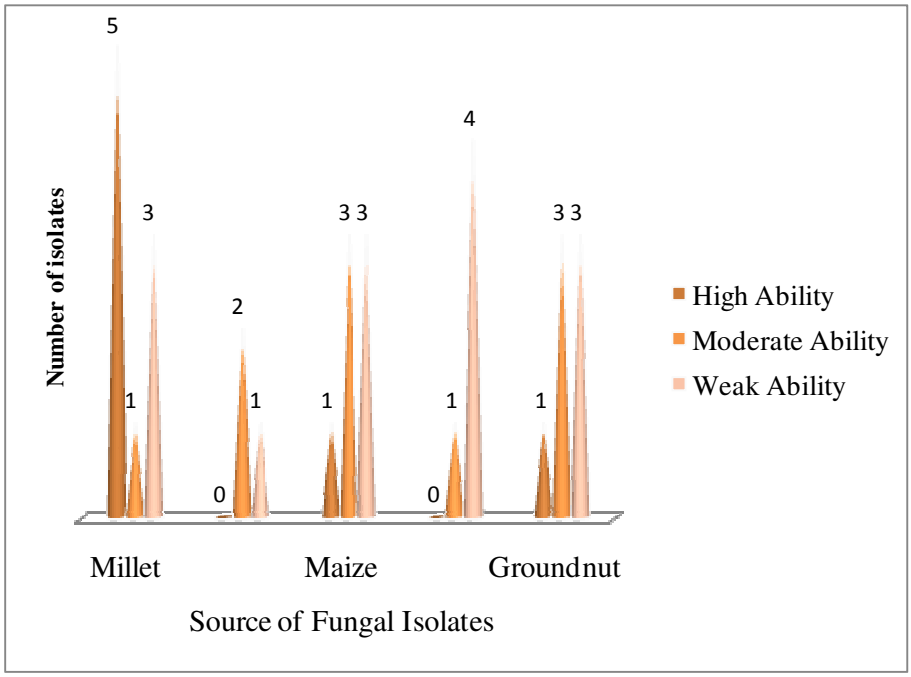

Figure 1: Aflatoxins Production Ability of *A. flavus* on Neutral Red Desiccated Coconut Agar under UV Light (365nm)

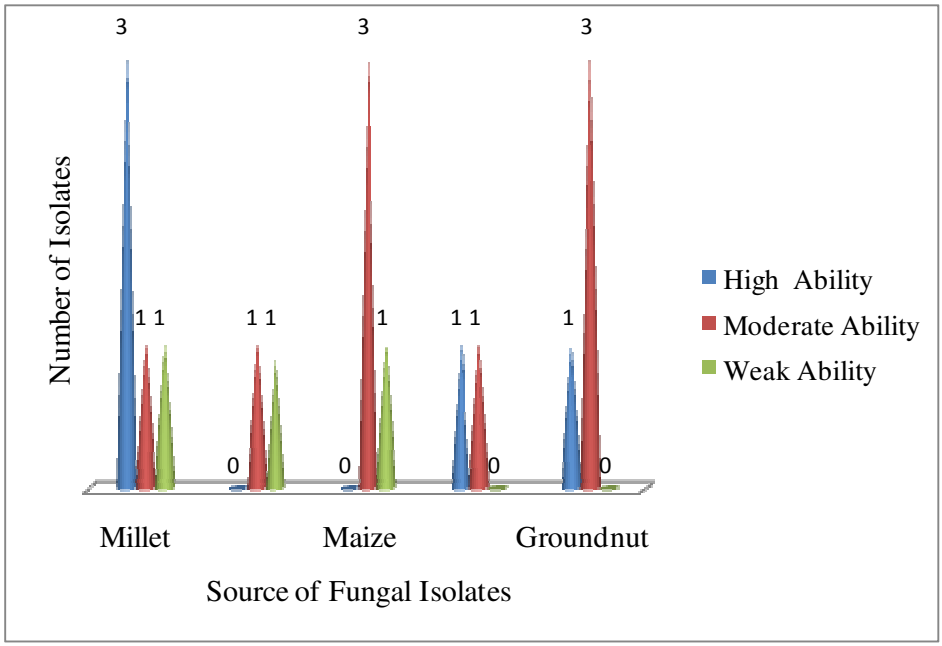

Figure 2: Aflatoxigenic Production Ability of *A. parasiticus* Isolates on Neutral Red Desiccated Coconut Agar under UV light (365 nm)

Aflatoxin Concentration of Grains and Legumes

The aflatoxin concentration of the grains and legumes marketed in Zaria metropolis, showed that the concentration ranged between 1.07 µg/kg (sorghum) to11.04 µg/kg for millet (Table 3). There were significant differences ($P \leq 0.05$) in the level of aflatoxin concentration of the samples.

The level of aflatoxins in the samples from Dan-Magaji Market was found to be the least with a concentration of 1.32µg/kg, the highest concentration of the aflatoxin was found in grain samples from Sabon Gari Market with a mean concentration of 5.17µg/kg as shown in Table 4 and the samples were not statistically significant.

Table 3: Mean Aflatoxin Concentrations of Cereals and Grain Legumes Sold in Zaria Metropolis

Food Commodity	Number of sample		Concentration of Aflatoxin	
	Analysed	Positive	Range (µg/kg)	Mean* (µg/kg)
Millet	18	9	0.4 – 52.0	11.04[a]
Sorghum	18	3	3.6 – 11.4	1.07[b]
Maize	18	7	0.4 – 18.1	2.17[b]
Beans	18	5	0.5 – 14.7	1.99[b]
Groundnut	18	7	0.6 – 16.9	2.44[b]

Means with different superscript along the column are statistically significantly different ($p \leq 0.05$).

Table 4: Aflatoxin Concentrations of Cereal Grains and Legumes Marketed in Zaria Metropolis According to Markets

Market	Number of sample		Concentration of Aflatoxin	
	Analysed	Positive	Range (µg/kg)	Mean* (µg/kg)
Zaria City	15	4	2.6 – 52.0	4.45[a]
Tudun Wada	15	5	2.3 – 9.2	1.89[b]
Sabon Gari	15	8	0.5 – 29.7	5.17[a]
Kwangila	15	5	3.0 – 40.0	4.77[a]
Samaru	15	4	0.6 -39.6	4.87[a]
Dan-Magaji	15	5	0.4 – 8.0	1.32[b]

Means with different superscript along the column are statistically significantly different ($p \leq 0.05$).

DISCUSSION

The moisture contents observed in the samples were low. However, long-term storage of the grains may increase the fungal growth. The moisture contents observed in this study was similar to that of Shitu*et al.*, 2018that reported moisture content of between 10.4 –12.7% in maize, sorghum and millet.Moisture and temperature are the main factors that influence post-harvest contamination of stored commodities by aflatoxigenic moulds (Hell and Mutegi, 2011).

The percentage ash content is an indication of minerals in the samples. The sorghum and maize samples had lesser mineral content based on their ash contents, whereas the millets, groundnuts and beans were rich in minerals required for growth of the moulds. This observed variation in the ash contents in the different grains might be due to genetic factors and environmental factors like irrigation frequency, soil composition and fertilizers (Ikram *et al.*, 2010). This is similar with the 1.4-3.3% ash content of maize as reported by Shitu*et al.*, (2018). The ash content observed is

however lower than the 5.1% in maize reported by Mlay*et al*. (2005). This might be due to The percentage crude protein content of the grains indicated that they were good sources of nitrogen, which is required for growth of moulds and aflatoxin production. The crude protein content observed in this study was lower than the 20.8 – 23.7% reported by Habibullah and Hamid, (2007) in beans. The possible reasons for the variation might be due to differences in storage conditions in the study area and genetic factor.

The carbohydrate content of grains indicates fermentable sugars required for the growth of moulds and subsequent aflatoxin production. The range carbohydrate content of the grains is similar to the 65 – 75%, and 71.7% reported by Subramanium and Metta (2000) for maize in India. Isma'il, (2016) however reported a concentration of 61.37% for sorghum and Adebolu (2005) reported a concentration of 65.63% for maize. The concentration of beans was as low as 36.05%. The reasons for the observed differences could bedue to lowcarbohydrate constituent of the two major components of starch (amylose and amylopectin) during germination or possible degradation along processing line.

The distribution of moulds isolate in this study showed that *Aspergillus flavus* was the most dominant than the *A. parasiticus* in all the five different grains samples analyzed. Millet samples recorded highest occurrence of *A. flavus* and *A. parasiticus* and produced the highest aflatoxin concentration than other samples analyzed, while sorghum samples had the least occurrence, hence recorded the least aflatoxin concentration. The observed variation might be due to differences in storage practices. The presence of *A. flavus and A. parasiticus* however, call for concern, as these moulds are known to produce aflatoxins and have been implicated in mycoses.

Between the two species, *A. flavus* was found to be dominant mould, which is in agreement with Klich*et al*. (2009) who reported a high incidence of *A. flavus*. Previous studies had shown that *A. flavus* frequently occurred in the field. The presence of both *Aspergillus flavus and A. parasiticus* in the grains and legumes is very common. According to Jelene*et al*., (2013) droughtcondition caused higher incidence and favours *Aspergillus flavus*. Similarly, Rossetto*et al., (*2005) also attributed the high frequency of the two moulds to the adaptation of these fungi to the substrates, especially during storage. The high frequency of *A. flavus* observed in millet samples may be because of the contact of the substrate with the soil. The incidence of the aflatoxigenic moulds in the agricultural products as observed in this study may be due agronomic practices like field drying and other environmental factors. to inadequate storage and handling practices, the ubiquitous nature of these moulds and environmental factors (Ibeh*et al.*, 1991).

The production of yellow pigmentation on the reverse side of plates of neutral red desiccated coconut agar is an indication of the presence of aflatoxin producing species thus obviating the need for UV light in the screening process (Atanda*et al*. 2006). Furthermore, Abbas *et al*. (2005) suggested the use of fluorescence production as an effective cultural method for the detection of aflatoxin producing ability. The isolates of *A. flavus* and *A. parasiticus* isolated from the samples were found to be capable of producing aflatoxins using Neutral Red Desiccated Coconut Agar (NRDCA) under UV light (365nm). Dyer and McCammon (1994) reported that the colour fluorescence under UV light is a useful tool in differentiation of toxigenic isolates, as *A. flavus* fluoresced pastel blue in a ring around each colony, while *A. parasiticus* fluoresced bluish white. Thus, fluorescence colouration could be used in differentiating *A. flavus* and *A. parasiticus*. This is an indication that neutral red desiccated coconut agar (NRDCA) enhance the aflatoxin detection ability of the medium. This finding is in agreement with the findings of Cotty (1997) and Yu *et al*. (2004) who reported that *Aspergillus* species produce aflatoxin in appreciable amounts on DCA by the isolates as reported by Olsen *et al*. (2008).

Aflatoxin is an important naturally occurring mycotoxin in agricultural products. They are produced by several species of *Aspergillus*. Not all strains of *Aspergillus* species produce aflatoxins (Frisvad*et al*., 2007).

Millet is a major staple food in Northern Nigeria. In this study, the mean level of aflatoxin found in millet from Sabon Gari market had the highest aflatoxin contamination level and concentration range far beyond the acceptable limit of 4µg/kg set by SON. This could be due to differences in storage conditions between the markets. This finding is not in agreement with that of Batagarawa*et al*. (2005) who reported a concentration 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 favours the growth of aflatoxigenic moulds and agricultural practices between the two study areas.

Sorghum is another staple food and an important starchy food for human and animal consumption in Nigeria. The mean level aflatoxins found in the sorghum samples are

within the acceptable limit. However, the range of aflatoxin concentration of sorghum samples from Kwangila market exceeded the limit set standard by NAFDAC, thus others are safe for human consumption. The low level of aflatoxin observed in sorghum might be due to the high phenol and tannin contents present in sorghum, which are known to inhibit fungal infestation (U.S. Grain Council, 2008). Though the aflatoxin concentration levels in samples were not disquieting, consistent consumption might result in long-term accumulation of the toxins, causing disease and metabolic disorder resulting in poor human and animal health.

The differences observed in aflatoxins concentration between millet and sorghum could be due to environmental factor (temperature and humidity), storage condition, handling processing and sensitivity of the method of quantifying the aflatoxins.

Maize is one of the most widely distributed food plants in the world (Bradburn, 1993). Maize samples analyzed in this study revealed that the mean aflatoxin concentration and the range are within the acceptable limit, except maize sample from Samaru market that had the highest level of contamination, which exceeded the limits sets by SON. The observed differences within the markets might be due to prolonged storage and method of handling. Atehnkeng*et al*. (2008) had earlier reported a concentration range of between 30.9 µg/kg-507.9µg/kg for maize from 11 districts across three agro-ecological zones of Nigeria. Manjula (2009) also reported mean aflatoxin levels of 0.55µg/kg and 0.46µg/kg in maize samples.

The contamination levels of groundnuts were within the acceptable limit except for samples obtained from Sabon Gari market, which exceeded the acceptable limit and is call for concern. This could also be due to handling practices, storage conditions and other environmental factors. This finding differed from that of Batagarawa*et al*. (2015) where 6.68µg/kg was for reported in groundnut and 14.7µg/kg for groundnut products in Zaria metropolis. In addition, Bankole and Adebanjo (2003) reported the aflatoxin contamination of Nigerian groundnuts at levels that ranged between 20-455µg/kg.

The bean samples recorded a low level of aflatoxin contamination. However, one sample exceeded the acceptable limit of 0.5 – 14.7 µg/kg and this call for concern as the level was higher than acceptable limits. The finding is however in contrast with the findings of Oranusi and Olarewaju (2013) who reported a contamination level of 21µg/kg for bean in Sango-Ota markets, Ogun State, Nigeria.

CONCLUSION AND RECOMMENDATIONS

Samples of cereals and legumes marketed in Zaria metropolis, Kaduna State, Nigeria had gross fungal and aflatoxin contamination. *Aspergillus flavus* and *A. parasiticus* were the major fungal contaminants of the grains and legumes, with *A. flavus*accounting the highest occurrence in the samples.In addition, the isolates of *Aspergillus flavus* and *A. parasiticus* demonstrated the ability to produce aflatoxins.The grains and legumes analyzed were found to contain aflatoxins at varying concentrations.With highest concentration(11.04 µg/kg) in milletand lowest concentration in sorghum samples (1.07 µg/kg). Thus, posing threat to lives and safety of humans and animals.

Therefore, there is need for stringent policy that will ensure and regulateaflatoxin contamination level in food commodity to suit with the acceptable limit set by National Agency for Food, Drug Administration and Control (NAFDAC). Food vendors and farmers should be educated to ensure that grains are properly dried prior to storage. Possible means of curbing the toxins by appropriate use of non-toxic antifungal chemical as part of storage strategies to minimize fungal growth in stored grains and subsequent toxin production should be practiced.

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