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The Implication of Aflatoxin B_1 Contamination Through Dietary Intake of Farm Products Sold at Selected Markets in Damaturu, Yobe State, Nigeria

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Abstract
A survey was conducted on 100 farm products sold at selected markets in Damaturu to access the levels of AFB1 contamination using enzyme-linked immunosorbent assay (ELISA). The samples collected include; 20 g each of cereals (rice, millet, maize paste 1, maize 2 and wheat flour), legumes (red beans, groundnut 1, soya bean, white beans, groundnut 2), vegetables (spinach, red onions, garlic, egusi, baobab leaves (kuka) hot pepper, sweet pepper, clove 1, clove 2, moringa, locust bean, okra, dried ginger) and tuber (white cassava). A food sample questionnaire was used to determine the frequency of food consumed and consumption rates. Results of the study revealed that one of the Aflatoxigenic moulds (Aspergillus Niger) is associated with cereal/legume products from both open and stored markets except for protein, minerals, and tuber products. A. flavus (66.67%), A parasiticus (66.67%), Fusarium (50%), and A flavus (50) were associated with cereals, legumes, and beverages in stored markets respectively.

*The cereal products collected from open and stored markets were found to be contaminated with AFB1 at concentrations of 0.9ppb and 4.1ppb respectively. However, Protein and tuber products were found to be free of any contamination. AFB_1 was recorded in beverages(0.66ppb), cereals/legumes (0.37ppb), and minerals (0.5ppb) under the stored market, these results are lower than the limits established by NAFDAC of 10ppb on ready-to-eat foods and 20ppb on other food items. Based on the results obtained, cereal-based food recorded the highest consumption (*16.98kg – 88kg*) rate per day among the different individuals in each age group while beverages recorded the lowest consumption (1.5g-8.0g) rate per day. The estimated daily intake of AFB_1 from individual age groups based on the consumption of different types of food ranged from 0.03-1.16 ng kg^{-1} body weight day^{-1}. These results suggest that the dietary intake of AFB1, in Damaturu has no adverse health effects on the population.*

Keywords: Aflatoxin B1, cereal, legumes, minerals, beverages

INTRODUCTION
Mycotoxins occurring in food produce are secondary metabolites of filamentous fungi, which can contaminate many types of food crops throughout the food production and processing chain. Although numerous fungal toxins are known, a limited number of toxins are basically considered to cause nutritional losses and play significant roles in food quality and safety (Shephard, 2008). They occur majorly, worldwide in the cereal, grains from producers and traders as well as processors and are all associated with serious health problems for consumers according to Meyer *et al.,* 2019. Humans are exposed to mycotoxins directly

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through food crops of plant origin, by air (both indoors and outdoors) and indirectly through foods of animal origin.

Apart from the diseases caused by plant pathogenic fungi in several crops in the field and in storage, association of these mycobiota with agro-products during transit or storage could lead to their contamination with dangerous fungi derived toxins (mycotoxins). Mycotoxins are a group of structurally diverse group of low molecular weight compounds produced mainly by filamentous fungi (and sometimes yeasts) which are toxic to biological systems including plants, livestock and humans (Zain, 2011). Fungal infections leading to mycotoxin contamination of feed or foodstuff are predisposed by overcrowding crops in the field, insect infestation of crops in the field or store, and wounds on produce during threshing and processing. In addition, due to improper handling of mycotoxin contaminated hay by farm-hands in western Nigeria, toxic dermatitis and conjunctivitis have been observed (Amadi and Adeniyi, 2009). According to Enyiukwu *et al.,* 2014, 25% of Agricultural crops covering several cereals, legumes, nuts, and coffee are mycotoxin contaminated. In a survey in Nigeria up to 98-100% incidence of the nephrotoxic metabolite ochratoxin A (OTA) from *Aspergillus ochraceus* and *Penicillium verrucosum* were detected in cereals and oil crops such as maize, millet, sorghum, sesame and fonio all obtained from Niger and Oyo states respectively (Adejumo and Adejumo, 2014). A similar survey in the Niger Delta region also recorded 100% incidence of aflatoxin B_1 in bread fruit, wheat, millet, and guinea corn. Some fungi can produce more than one mycotoxin while some mycotoxins are produced by more than one fungal species (Zain, 2011). In Africa however, the mycotoxins considered of most Agricultural importance include aflatoxins, ochratoxins, fumonisins, beauvaricin and the trichothecenes among other. These toxins affect several biological systems in a variety of ways according to Coulombe, 1993. A plethora of harmful effects on plants, livestock and humans have been attributed to these toxins. In several plant families, toxic fungal metabolites have been reported to decrease growth, yield, and yield parameters of crops. Consumption of cereal and cereal-based products acts as the primary source of energy in developing nations, while contamination with aflatoxin, can result in specific nutritional deficiencies. Ingestion of food products with extremely high doses of aflatoxin can cause some disorders such as edema, hemorrhage, severe liver damage, and even death (Martins *et al.,* 2012). Cereals have been identified as a major route of dietary exposure to mycotoxins, especially when these mycotoxins are carried over to products produced from these cereals (Makun *et al.,* 2012; Chala *et al.,* 2014.). Sorghum, millet, and wheat are important cereal crops that are prevalent in Africa. However, these crops, like other cereal crops, are susceptible to fungal proliferation during pre and post-harvest periods of crop cultivation. This colonization of the crops by toxigenic fungi could lead to the production of secondary metabolites like mycotoxins (Chala *et al.,* 2014; Taye *et al.,* 2018).

Despite all the efforts to mitigate the presence of aflatoxin in food products, unprecedented climate change continues to provide the favorable conditions for fungal multiplication into colonies and production of high levels of mycotoxins (Paterson *et al.,* 2010). As such, the production environment and handling practices increase the risk of aflatoxin contamination especially in developing countries, thus presenting serious health problems to both humans and animals (Montes *et al.,* 2009). The danger to humans is even more pronounced because cereals like millets, maize, and sorghum are some of the staples and components of complementary foods in most Northern part of Nigeria and some part of sub-Saharan African countries, thus increasing the risk of exposing humans and animals to significantly high levels of aflatoxins in their diets at an early stage. Exposure to aflatoxin in children may cause stunted growth and in severe cases, leads to liver failure and even death. Unfortunately, many

rural and urban communities in developing countries may not be aware of this as indicated by Bhat *et al.,* 2003. Therefore, this research is aimed at determining the Implication of aflatoxin b_1 Contamination through dietary intake of farm products sold at selected markets [open market (*Sunday market*) and Store market (*Bayan Tasha*)] in Damaturu.

MATERIALS AND METHODS

Collection of samples

Purposive sampling technique which was a representative of the people selling farm produce in the selected markets was adopted. The sampling frame for the study consisted of 100 samples (20 g each of cereals, legumes, protein, tubers, beverages, and minerals) with sampling size of 50 each for the two selected markets in Damaturu Yobe State. Samples were collected from Sunday market and 'Bayan tasha'; the samples were labeled appropriately and stored at a room temperature in Desert Research laboratory, Yobe State University Damaturu until further analysis.

A simple questionnaire was administered to 100 respondents to determine the frequency of foods consumption and their consumption sizes. A portable weighing scale was used to weigh participant samples. A 120 kg graduated weighing scale was used to take the weights of several individuals within the two markets.

Isolation of fungal species

The samples were surface sterilized in accordance with Samson et al. (2010) procedure. The grain samples were thoroughly mixed after each (approximately 50g) seed were randomly selected and washed in 350 mL of 0.5% ethanol solution and rinsed with distilled water. 2-3 kernels were placed using sterile forceps onto potato dextrose agar (PDA) and incubated at room temperature (25^0C) for 7 days.

Morphological features of the fungal isolates

The fungal isolates were identified based on their macroscopic and microscopic characteristics. The macroscopic characteristics were used in identification of the fungal isolates based on the mycelia growth, pattern on PDA and the reverse (Samson *et al* 2010). The isolates were characterized microscopically using slide culture technique. An agar block of the desired dimensions was cut from PDA plate using sterilized scalpel blade. The agar block was placed on the surface of sterile microscope slide in a petri dish containing moistened cotton wools. The four quadrants of the agar block were inoculated with fungal isolate and a sterile cover slip was placed onto the surface. The lid of petri dish was replaced and the plate was incubated at 30 for 5 days. After the incubation period, the cover slip was removed, placed on a microscope slide containing a drop of lactophenol cotton and observed microscopically for the characteristic shape and arrangement of spores. The identification was done both macroscopically and microscopically with reference to Larone Atlas of mycology considering the colour of the mycelia and shape of the conidiophore, vesicle and conidia (Daphne and Joel, 2013). The isolates were maintained on PDA slants at 25^0C for preservation.

Extraction of Aflatoxin B1 and ELISA Quantification

The method of Hell *et al*. (2009) was applied for the extraction of aflatoxin from the food and vegetable samples. A 70% methanol solution was prepared by making up 700 ml methanol to 1 litre. Twenty grams (20g) of each food sample were weighed into a conical flask, into which 100 ml of 70% methanol was added to realize a 1:5 (w/v) proportion. The conical flask was capped and the mixture was shaken for 5 minutes. The extract was filtered through a Whatman No. 1 filter paper. The extract was used for Aflatoxin B1 quantification. Conjugate

solution (200µl) was pipetted into dilution wells, 100µl of each sample extract was added in to the dilution wells, mixed well and 100µl from dilution wells was transferred into antibody coated wells and incubated at room temperature for 15 minutes, after incubation it was washed 5 times with distilled water and kept to dry up. Substrate solution (100µl) was pipetted into the antibody coated wells and incubated for 5 minutes, after which 100µl stop solution was pipetted into the antibody coated wells. The strips were read with a STAT FAX ELISA reader using 450 nm filter and 630 nm differential filters.

Range of Quantification: The colour intensity (OD) is inversely proportional to the amount of aflatoxin present in the sample. Levels of aflatoxin in the samples were obtained from a graph curve that was plotted using the OD of the calibrator wells and the concentration of the calibrator (see Fig 2)

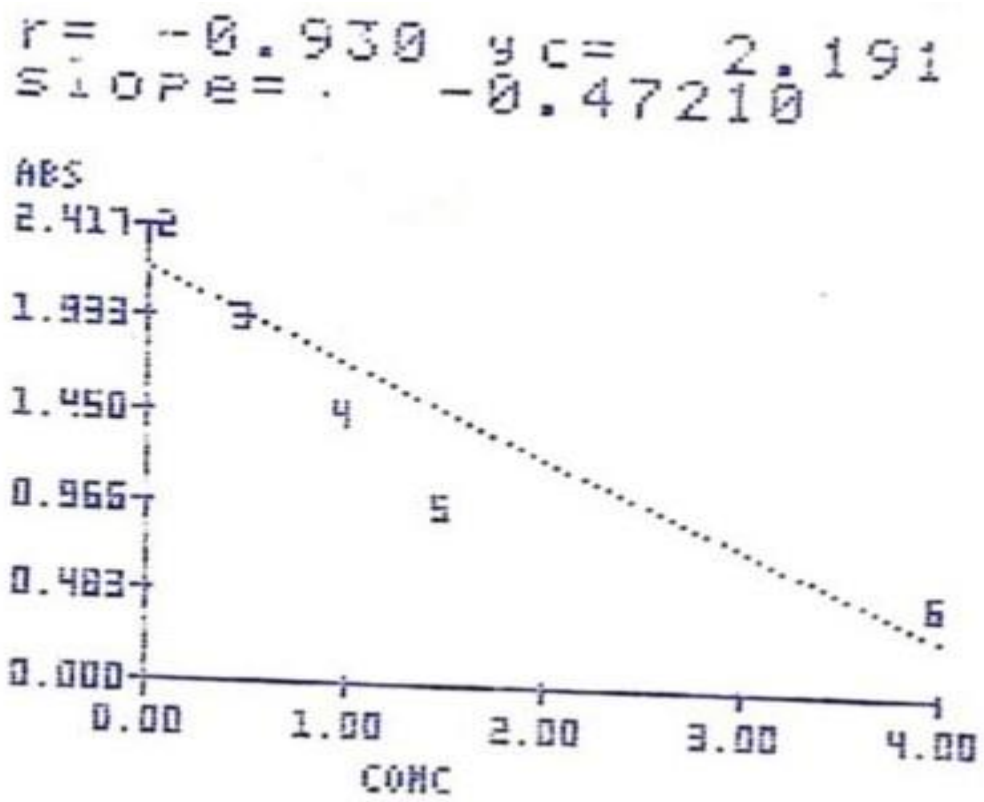

Fig 2. Gives calibration curve of OD (absorbance) of the calibrator wells and the concentration of the calibrator

Estimation of Dietary Exposure: The method used by Nugraha *et al.* (2018) and approved by Joint Committee on Food additives (JECFA) was adopted in this study. This was achieved for Aflatoxin (AFT) based on the contamination level and the estimated consumption.

$$\text{Estimated daily intake (EDI)} = \frac{\text{contamination level X consumption rate}}{\text{Body weight per person (kg)}}$$

Estimated daily intake = Estimate of the amount of toxin which can be ingested daily (ng/kg bw/day) Contamination level = Mean toxin content in a certain foodstuff (ng/g)
Consumption rate = the amount of the foodstuffs ingested on daily basis (gram/day)

Statistical Analyses

The data generated in the course of this research were arranged in Microsoft Excel Package while Descriptive statistics was employed for data analysis. These includes calculations of

mean, standard deviation, standard error of mean.

RESULTS

Table 1 shows the distribution of the Aflatoxigenic fungi of different food category stored cereals were contaminated with *A. paraciticus, A. flavus* (66.67%) with *A. niger* at open market cereals. The highest percentage of aflatoxigenic mould (100%) was detected in cereal/legumes product collected from both open market and store. Protein product collected from two markets was free of moulds. *A flavus* were detected on stored beverages (50%) waste minerals in both open and store markets were found to be contaminated with *A. niger. A. fusarium* was detected in legumes collected from store, while no mould was found in open market legumes.

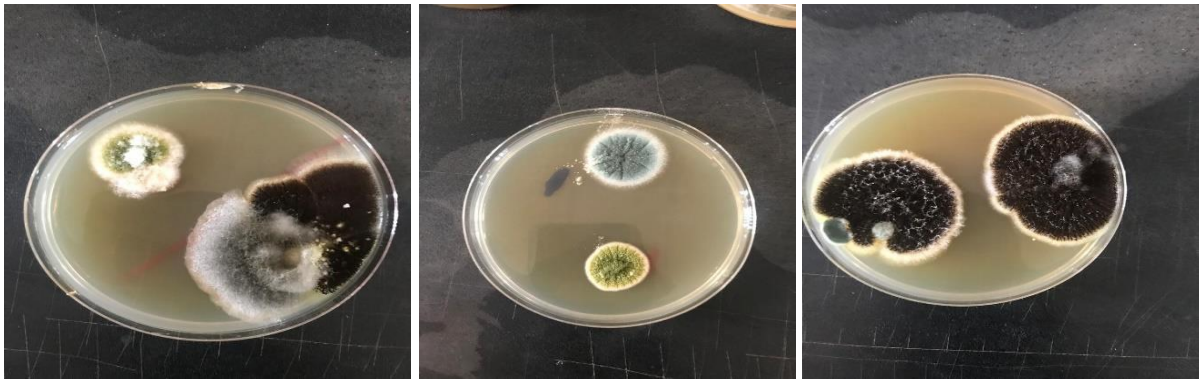

Fig. 1 Macroscopic characteristics of fungal species isolated from grains

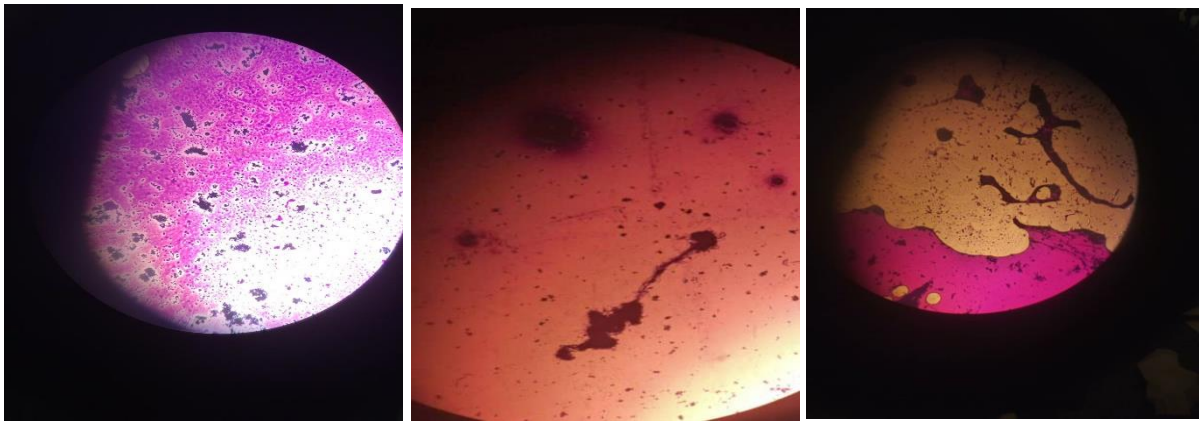

Fig 2: Microscopic characteristics of Fungi species isolated from grains

Table 1: Distribution of moulds isolated from different foods categories

Food category	No. toxigenic species	Species associated with grains	
	%	Open market	Stored grains
Cereals	66.67	*A.niger*	*A. paraciticus, A. flavus, A. niger*
Legumes	50	*ND*	*Fusarium spp*
Cereals/legumes	100	*A. flavus, A. niger*	*A. flavus, A. niger*
Protein	Nil	*ND*	*ND*
Tubers	0	*A. niger*	*ND*
Beverages	50	*A. niger*	*A. flavus*
Minerals	0	*A. niger*	*A. niger*

ND = not detected,

Table 2 shows the level of Aflatoxigenic B_1 in store and open markets products. Aflatoxin B_1 was detected in cereals at mean concentration of 0.9ppb at open market whereas 4.1ppb was recorded in store market. Proteins and tubers recorded zero (0) levels of Aflatoxin in both open and store markets. Legumes products in open market has the least concentration of 0.37ppb while higher level of 5.5ppb was recorded in store market. Beverages in store have greater level of Aflatoxin concentration of 4.26ppb whereas least record of 0.66ppb was recorded in open market. More so, other food types such as cereals/legumes product in the store and open markets recorded 4.81ppb and 0.64ppb of Aflatoxin concentration respectively. The levels of the toxin in minerals was 4.77ppb and 0.5ppb in both store and open markets respectively.

Table 2: Level of aflatoxin B_1 contamination (ppb) in different food produced in selected markets

Types of food	The mean levels of Aflatoxin					
	Open market			Stored market		
	Mean (M)	S.D	S.E	Mean (M)	S.D	S.E
Cereal	0.9	1.6	0.75	4.1	2.89	1.29
Legumes	0.37	0.49	0.2	5.52	2.73	1.11
Cereal/Legumes	0.64	0	0.00203	4.81	0.01	0.00577
Protein	ND	ND	ND	ND	ND	ND
Tubers	ND	ND	ND	ND	ND	ND
Beverages	0.66	0.69	0.23	4.26	3.25	1.08
Minerals	0.5	0.62	0.36	4.77	1.33	0.77

ND = Not detected, SD = standard deviation, S.E = standard error

Table 3 shows the data collected on the types and quantity of food items consumed by the sampled population. The result shows the consumption of different types of food per day between the age groups and mean intake of AFB_1 (ng kg^{-1} body weight day^{-1}). The result indicates cereal based food had the highest consumption rate per day between different groups which ranged from 16.98kg – 88kg. The mean intake of cereals/legumes food by the population ranges between 22.5kg to 73kg per day, while legumes intake was 20.05kg to 66kg. tubers minerals and beverages recorded the lowest consumption rate per day between the age groups as indicated in the Table.

The estimated daily intake of AFB_1 in several food commodities consumed by the different age groups indicated that, cereal based foods at age 1-21years and above has mean intake of 0.89 gkg^{-1} body weight day^{-1}, 1.10 gkg^{-1} body weight day^{-1} and 1.35gkg^{-1} body weight day^{-1} respectively. Cereal/legumes dietary intake of AFB_1 was between the ranged of 0.85 gkg^{-1} body weight day^{-1} to 1.14 gkg^{-1} body weight day^{-1} based on the one-day recall. The EDI of protein and Tubers is not detected as indicated in the Table 3.

Table 3: Simulation Data of food intake in different age groups across the two markets

Age group (year)	Average body weight (kg)	Food category	Mean/food consumed per day/kg^{-1}	Mean of food intake gkg^{-1} bw dy^{-1}
1-5		Cereals	16.98	0.89
		Legumes	20.05	0.44
	17	Protein	10.06	N.D
		Tubers	4.8	N.D
		Minerals	1.0	0.02
		Beverages	1.6	0.06
6-10		Cereals	30.90	1.11
		Legumes	26.05	0.38
	25	Protein	18.05	N.D
		Tubers	9.0	N.D
		Minerals	1.6	0.03
		Beverages	1.5	0.04
11-15		Cereals	72.0	1.75
		Legumes	52.0	0.52
	37	Protein	48.09	N.D
		Tubers	30.0	N.D
		Minerals	5.0	0.06
		Beverages	7.0	0.12
16-20		Cereals	72.0	1.35
		Legumes	52.0	0.40
	48	Protein	48.09	N.D
		Tubers	30.0	N.D
		Minerals	5.0	0.05
		Tubers	7.0	0.09
21 and above		Cereals	88	1.16
		Legumes	66	0.35
	68	Protein	52	N.D
		Tubers	30	N.D
		Minerals	7.0	0.05
		Beverages	8.0	0.07

DISCUSSION

Aflatoxins are frequently detected in cereals and other food products. These food samples are liable to aflatoxigenic fungi under excessive moisture/humidity and during storage. The contamination of food products by Aflatoxin B_1 is commonly regarded as one of the most critical risks to humans due to its timely assessment of the levels in ensuring food security, safety, and the health of all individuals concern as reported to be a group 1 carcinogenic with hepatotoxic, mutagenic, immunosuppressive, teratogenic properties according to the assertion of IARC, 2012.

The findings of this study as seen in the Table 1 showed that cereal based products collected from stores are contaminated with one or more Aflatoxigenic fungi than in the open market. *Aspergillus paraciticus and A. flavus* isolated in this study are known naturally occurring contaminants of cereals and other products, in the field and during storage (Reddy *et al.,* 2010). *Aspergillus and Penicillium spp* are also known to be important fungi that are generally associated with stored products. The non-aflatoxigenic fungi recorded in the open market may be that food processing has induced changes in the optimal conditions favouring fungal proliferation. As food products in the stores are more favoured by cold and damp conditions with humid temperatures that enhanced fungal growth (Thomson and Henke, 2010). *Aspergillus flavus* isolated in cereal/legumes-based food in the open markets may be likely due to exposure to the food products from the farms at pre-harvest. Most of these products are directly brought from the farm at the time of harvest to the market. *Aspergillus flavus* is the

most favorable susceptible source of contamination of crops at the time of harvest (Scheidegger and Payne, 2003). The fungus appears to form many sclerotia in the insect-damaged kernel before harvest and once this happens, the *A. flavus* continue to grow but in the field and open market products.

The *Fusarium spp* appears to be more dominant in store, the occurrence of *fusarium* isolated from the store as in table 1 suggested that, it has greater adaptability under different environmental conditions. Covarelli *et al* (2014) reported that *Fusarium spp* is predominance fungi isolated from stored foods.

Among the *Aspergillus niger isolated* (50%) from almost all the food categories, none of them was a mycotoxin producer, but their presence could be important as biodeterioration which indicates poor food hygiene quality.

Aflatoxin B1 was detected in stored cereals at a concentration of 4.1ppb and open market cereals at 0.9ppb levels. These results were not surprising, given that 66.67% of aflatoxigenic fungi are isolated from the stored samples. *A parasiticus* and *A. flavus* are known natural fungi-producing aflatoxin B1. The findings regarding aflatoxin in cereals are similar to those in other studies by Blankson *et al.* (2018) who reported that 96% of processed cereal based food from Ghana contained AFB1 levels higher than the EU permissible limits of 0.1ug kg^{-1} but lower than the maximum acceptable limits of 10ppb recommended by NAFDAC. Similar results from previous studies in Nigeria indicated that cereal-based products have higher contamination level (Makun *et al* (2010), Manjula *et al* (2010), and Oyedele *et al* (2017).
Aflatoxin contamination in cereals and their derivatives was reported by Cotty *et al* (2007) who observed that excessive moisture/humidity during storage affects the level of aflatoxin contamination.

Samples collected from the store in this study indicated that most of the cereals have higher moisture content (10.76%) than the legumes which recorded lower moisture contents. These might be explained according to the study of Gallo *et al* (2016) reported that fungal biomass and AFB1 production depends on temperature and moisture contents, water activities of 0.96aw, and temp between 25-28^{0}C support fungal proliferations. The low occurrence of aflatoxin B1 in legumes may be due to the lower moisture content (3.8%) as revealed by Al-Shikli *et al.,* (2017).

The mean level of aflatoxin B1 found in this study in all food categories ranging from 0.37ppb-5.2ppb is lower than the level established by NAFDAC and EC (10ppb of total aflatoxin on not ready to eat foods).(EC 2006)

The estimated daily intake of the aflatoxin B1 from individual age groups based on consumption of different types of food categories ranged from 0.03-1.16 ug kg/body weight. The main contributor to the dietary intake of the toxin was cereal-based food. Thuvander *et al* (2011) reported that dietary intakes of total aflatoxin for swedes cereal-based food contained 0.80ug kg^{-1} body weight day^{-1}.

According to the European Commission, the maximum allowed level for cereals is 2mg/kg per day for aflatoxin and products containing maize-based cereal at 100mg/kg, thus the results obtained in this study ranged from 0.03-1.16 35gkg^{-1} body weight day^{-1} indicates a low degree of human exposure to aflatoxin B1 in Damaturu through the ingestion of different types of food from the two markets, however sufficient drying and good practices for the harvesting of crops should be applied to reduce the risk of crop spoilage by aflatoxin, and regulations should be proposed to control rodent or insect activity during storage.

CONCLUSION

This study has shown that grains stored inside shops of the market are susceptible to invasion and colonization by diverse moulds including aflatoxigenic isolates of *Aspergillus flavus* and *Aspergillus paraciticus*. However, the presence of *Aspergillus niger* and other non-aflatoxigenic-fungi in the grains is also an indication of poor post harvest handling practices and at the time of storage. Storage of food grains under clean, dry conditions with low kernel moisture content (about 8-10%) and at low temperature will go a long way of protecting fungal infestation thereby reducing aflatoxin contamination. In addition, packaging, removal of spoiled or mouldy grains, and mechanical drying before storage can greatly limit mould growth and aflatoxin formation.

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