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Full Length Research Paper

Fungal and aflatoxin contamination of some human food commodities in Nigeria

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This paper provides data of investigation of fungal, aflatoxins M_1 (AFM_1) and B_1 (AFB_1) contamination of three hundred and forty three samples of five different food commodities from three States in Nigeria. Maize samples from Niger (43) and Kogi (50) States and dried yam chips (50) from Niger State were purchased and assessed for fungal contaminants. Seventy (30 each from the maize samples and 11 from yam chips) of the two hundred and forty five fungi isolated from the maize and dried yam chips samples (77 and 151 from maize from Niger and Kogi respectively, and 17 from yam chips) were screened for their mycotoxin producing potentials in albino mice. One hundred samples of imported powdered milk marketed in Lagos metropolis were also analyzed for AFM_1 using column chromatography for clean up and thin layer chromatography coupled with a densitometer equipped with winCATs soft ware for quantification. The Aflatoxin B_1 contents of fifty marketed samples each of beans and wheat from Minna were also determined using thin layer chromatography with visual estimation. *Fusarium* species, *Aspergillus fumigatus* and *Aspergillus flavus* were the major seed borne fungi in maize from the two States. While the predominant fungal contaminants of dried yam chips were *Fusarium, Aspergillus* and *Mucor* species. Toxicity screening results showed that 68.57% of the fungi tested were toxigenic and were mostly isolates of *Aspergillus, Fusarium, Penicillium, Rhizopus* and *Mucor*. AFM_1 was detected in 19 samples out of the 100 milk samples analyzed at levels ranging from 0.02 - 0.41 µg/kg. AFB_1 was found in 29 out of the 50 beans (63.5 - 106.2 µg/kg) samples analyzed while 54% of the 50 marketed wheat samples (102.9 - 198.4 µg/kg) were also contaminated with the toxin. While the AFM_1 found in the milk samples were below the 0.5 µg/kg permitted by European Union, Codex Alimentarius Commission and National Agency for Food and Drug Administration and Control, Nigeria, the AFB_l levels in beans and wheat were above the permissible limits of these regulatory agencies (4 - 5 µg/kg).

Key words: Fungi, aflatoxins, maize, yam, milk, beans, wheat.

INTRODUCTION

Fungi are ubiquitous plant pathogens and so are major spoilage agents of foods and feedstuffs. The infection of plants by various fungi not only results in reduction in crop yield and quality with significant economic losses but contamination of grains with fungal poisonous secondary metabolites called mycotoxins. The ingestion of such mycotoxin contaminated grains by animals and human beings has enormous public health significance because these toxins are nephrotoxic, immunotoxic, teratogenic and mutagenic which are capable of causing acute and chronic effects in man and animals ranging from death to disorder of central nervous, cardiovascular and pulmonary systems and intestinal tract (Bhat and Vasanthi, 2003). Of greatest concern is the relevance of these toxins in human hepatoma and oesophageal cancer, increased susceptibility to diseases especially in children and childhood pre-five mortality and reduced life expectancy (Beardall and Miller, 1994; Miller, 1996; Marasas, 2001).

The significant economic and health hazards caused by fungi and mycotoxin especially in developing countries that have poor food storages is of great concern so to ensure a healthy food supply thereby minimizing

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consequences to food security, international trade and animal and human health, there is need to monitor fungal and mycotoxin contamination periodically so as to meet international and national mycotoxin regulatory standards. The present study therefore investigates food quality with respect to fungal contamination in maize and dried yam chips collected from various areas in Kogi and Niger States of Nigeria. The mycotoxin producing potentials of the fungi isolated from the food products were also determined in albino mice. The work also sought to determine the concentrations of AFM_1 in differrent samples of various brands of powdered milk marketed in Lagos metropolis and AFB_1 contents of marketed beans and wheat in Niger State.

MATERIALS AND METHODS

Sampling

Sampling of commodities was conducted according to the method employed by Bainton et al. (1980). Statistically representative samples were randomly collected from field, market and storage facilities between December, 2000 and June, 2008. Field samples were randomly collected from farms just before harvest. Contents of traditional storage facilities and sacks in the markets were thoroughly mixed and samples taken from many points of the facilities. Between 0.5 - 1 kg of each sample of the various food commodities was collected. Sampling was done during the dry season. Except for powdered milk samples that were imported all food commodities studied were produced and processed in Nigeria. The samples were put in sealed polythene bags and transported to the laboratory where they were stored at -4°C until analysis.

A total of 343 dried samples of marketed powdered milk (100) from Lagos Metropolitan, maize (93) from Niger (43) and Kogi (50) States and fifty each of marketed dry yam chips, beans and wheat from Niger State intended for human consumption were sampled. The maize samples from Niger State were collected from field (22), market (9) and local silo, “rumbu” (6) from the twenty five local government area of Niger State while those from Kogi State were sampled from four local government areas (LGA’s) in the Eastern Senatorial District of Kogi State.

Isolation, identification and toxicity screening of fungi from maize and dry yam chips

Isolation and identification of fungi

Fungi were isolated and cultured according to the methods of Smith and Moss (1985) and Halfon-Meiri and Barkai-Gola (1990). The maize and yam chips samples were thoroughly mixed to obtain homogeneity and ten grains of maize and yam particles each were randomly selected, surfaced sterilized using 5.25% sodium hypochlorite solution (Reckitt and Colman, Nigeria Limited, Industrial Estate, Agbara, Ogun State) and washed aseptically with ten successive 100 ml volume of sterilized distilled water before plating on the potatoes dextrous agar (PDA). The plates were incubated at 28°C for 5 - 7 days. Each of the fungi that emerged was sub-cultured onto a fresh SDA slant in a 15 ml slant bottle to obtain a pure culture.

Each pure culture was characterized and identified on the basis of their morphological and microscopic characteristics using the keys of Pitt and Hockings (1997).

Determination of toxin producing potentials of fungi from maize and dry yam chips

The ability of the fungal isolates from these two food commodities to produce toxic metabolites was evaluated in albino mice in three steps mass culturing of fungi for toxin production, extraction of toxins and toxicity assay of extracts in mice. The fungal isolates were first cultured on autoclaved moisture equilibrated maize and incubated at 28°C for 14 days as static culture in order to allow for mycotoxins production and the toxins extracted into Methylene chloride (Gbodi, 1986). The extracts were filtered through fast fluted filter paper. The filtrates were evaporated on a water bath until the methylene chloride was distilled off. The residue was collected in a vial, labelled appropriately and stored in a deep-freezer at -20°C until used for toxicity testing.

The screening of maize and dried yam chips fungal culture extracts for toxicity in mice was conducted as described by Reddy and Hayes (2001). Thirty representative fungal isolates of the 77 from maize from Niger State were screened for toxicity in mice that is two isolates each of the fifteen different species isolated. Similarly, 30 non *Aspergillus* species of the 151 fungi isolated from maize from Kogi State were also screened for their mycotoxin producing potentials as well as eleven non *Aspergillus* species of the 17 fungi isolated from dried yam chips were screened for toxicity. Three mice each received 0.2 ml intraperitoneal injection of the fungal extract dissolved in dimethyl sulphoxide (DMSO). The mice were observed for signs of toxicity for 14 days. A group of 3 mice were injected with only DMSO to serve as controls. The toxicity of the extracts was arbitrarily categorized into four classes viz: 1) Very toxic (all three mice injected with extract died); 2) Moderately toxic (two of the three mice died); 3) Mildly toxic (one of the three mice died); 4) Non-toxic (no mice died).

Aflatoxin M1 analysis in powdered milk

Aflatoxin M_1 contents of the powdered milk samples were extracted, cleaned up and estimated by thin layer chromatography with quantification by densitometry as described in the Association of Official Analytical Chemist (AOAC) Method 908.16 (AOAC, 2000). The content of each tin was properly mixed and 20 g of the samples of powdered milk were taken separately and weighed into 250 ml separating funnel. 40 ml of distilled water was used to homogenize the milk samples. 50 ml of acetone, 5 g of sodium chloride and 1 ml of 0.1 N phosphoric acid were all added. This was shaken for 10 min. 100 ml of dichloromethane was later added and mixed gently. The mixture was filtered through filter paper into a dark vial bottle to avoid the decomposition of AFM_1.

Column chromatographic clean up was also carried out using a disposable column (15 ml) mounted on the plug syringe hole of mycosep clean-up apparatus. The column was filled with 2.0 g of silica gel plugged with filter paper and 2 g of sodium sulphate anhydrous. Sample extract was washed in the column with 10 ml toluene-acetic acid (9:1) to remove coloured compounds and later 10 ml of hexane-ether-acetonitrile (5:3:2) to remove fat from the extract solution. AFM_1 was later eluted with 15 ml chloroform-acetone (4:1). This was collected into evaporating tube and evaporated on a steam bath to dryness.

For the thin layer chromatographic estimation, dry extract in the glass vial was reconstituted with 400 µl of toluene-acetonitrile (9:1) and was vortex-mixed for about one minute. Eighty microlitre of the test solution was spotted on an imaginary line on the TLC plates (2 cm from the bottom of the plates) by using a micro syringe. 10, 20, 30 µl of AFM_1 standard (0.06 µg/ml) were also spotted along with the test solution. The AFM_1 standard was purchased from Sigma Chemical Company, St Louis, USA. Chloroform-acetone - isopropanol (87:10:33) was used for plate development. AFM_1 was quantified by a densitometer (Camag TLC scanner 3 equipped with

Table 1. Incidence of fungi in maize samples from field, Sack, “rumbu” and markets in Niger State.

Species	Incidence				
	Field	**Sack**	**Rumbu**	**Market**	**Total infected samples**
Aspergillus flavus	7/22*	4/6*	1/6*	5/9*	17/43*
Aspergillus fumigatus				1/9	1/43
Aspergillus glaucus	3/22	1/6		1/9	5/43
Aspergillus nidulan			1/6		1/43
Aspergillus niger	3/22	2/6	3/6	3/9	11/43
Aspergillus parasiticus				1/9	1/43
Aspergillus terreus			1/6		1/43
Aspergillus versicolor			2/6		2/43
Fusarium spp	8/22	3/6	4/6	1/9	16/43
Mucor spp	3/22	5/6		2/9	10/43
Rhizopus spp	5/22		2/6	3/9	10/43
Syncephalastrum spp			1/6		1/43
Penicillium spp				1/9	1/43
Total	29/22	15/6	15/6	18/9	77/43.

Note: Results are presented as fungal isolates per total number of samples analyzed. *Number of samples collected.

winCATs soft ware) at an extinction wavelength 350 nm and emission cut off of 400 nm (ModelG25 Muttenz, Switzerland).

Determination of aflatoxin B_1 in beans and wheat

The samples were screened and analyzed for aflatoxin B_1 using a multi-mycotoxin assay method (Ehrlich and Lee, 1984). In this method, methylene chloride and phosphoric acid are used for the simultaneous extraction of mycotoxins. A portion of the initial methylene chloride/phosphoric acid extract was subjected to a specific clean-up procedure for aflatoxin B_1 and the toxin quantified by thin layer chromatography with visual estimation

For the extraction, 50 g portion of pulverized samples was weighed into 500 ml Erlenmeyer flask and 25 ml 1M-phosphoric acid and 250 ml of methylene chloride were added. The flask was shaken for 30 min using a shaker and the content filtered under pressure on Buchner funnel fitted with 18 cm circle rapid filter paper. About 200 ml of the filtrate was collected and from this, 50 ml aliquot was placed in separate 100 ml Erlenmeyer flasks with glass stoppers, for AFB_1.

The filtrate was subjected to column chromatographic cleaned up by defatting with hexane, washing with diethyl ether and the toxin eluded in diethyl ether, methanol and distilled water (96:3:1) The elute was evaporated to dryness and the dry extract kept for AFB_1 analysis. The dried extract were reconstituted in benzene-acetonitrile (98:2) and spotted on thin layer chromatographic plates. The plates were developed in ether-methanol-water (96:3:1, v/v/v) and were estimated by visual comparison of fluorescence intensity of samples with that of standards. Aflatoxin was confirmed by spraying the thin layer chromatographic plates with aqueous sulphuric acid (50:50, v/v), dry and viewed under long wave, and the spots fluoresced yellow.

The quantification of the mycotoxins was done by visual comparison of the intensities of the standards and samples. This involved the comparison of the fluorescence intensities of the spots with same retention factor (R_F) of the mycotoxins in the samples with those of corresponding standard and determines which of the sample spot matches any of the standards. The corresponding aliquot volumes were then recorded and the concentrations of the mycotoxins in the samples in µg/kg were then calculated as follows:

$$\text{Mycotoxin content } (\mu g/kg) = \frac{S \times Y \times V}{W \times Z}$$

Where:

S = volume of standard with same colour intensity as sample (µl);
Y = concentration of mycotoxin standard used in µg/ml;
V = volume of solvent required to dilute sample contained in final extract;
W = effective weight (g) of original sample contained in final extract;
Z = volume of spotted sample equivalent to standard (µl).

Statistical analysis

Mean ± standard deviation and analysis of variance (students’t-test) of data generated were calculated using SPSS (version 10.0) software. The statistical level of significance was fixed at P<0.01 (99%).

RESULTS

Fungi isolated from maize in Niger State

Table 1 shows the types and occurrence of fungi found in field, stored and marketed maize samples collected during the harmattan season from all the twenty-five local governments’ areas of Niger State. The incidence data are presented as fungal isolates found per total number of samples. Seventy seven fungal isolates were isolated and identified in this crop. During this season, *A. flavus* (17/43) was the most common fungi in maize followed by *Fusarium species* (16/43), *A. niger* (11/43), *Rhizopus* spp 10/43) and *Mucor* spp (10/43). The *Aspergillus* spp had the highest incidence (39/43) in maize during the harmattan season. Other fungal isolates found in this cereal include *Syncephalastrum* spp and *Penicillium* spp.

Table 2. Toxicity of extracts of fungi Isolated from maize samples in Niger States in mice.

Very toxic (6)	Incidence	Moderately toxic (4)	Incidence	Mildly toxic (9)	Incidence	Non-toxic (11)	Incidence
Aspergillus flavus	1/6	*Aspergillus glaucus*	1/ 4	*Aspergillus flavus*	1/9	*Fumigatus*	2/11
Niger	1/6	*niger*	1/ 4	*A. glaucus*	1/9	*Nidulans*	1/11
Prasiticus	1/6	*Rhizopus* spp	1/ 4	*Nidulan*	1/9	*Versicolor*	2/11
Penicillium spp	2/6	*Fusarium* spp	1 /4	*A. parasiticus*	1/9	*Fusarium* spp	2/11
Fusarium spp	1/6			*Terreus*	2/9	*Mucor* spp	1/11
				Fusarium spp	2/9	*Rhizopus* spp	1/11
				Mucor spp	1/9	*Syncephalastrum* spp	2/11

Note: Number in brackets indicates the number of fungi screened at that level of toxicity and the incidence values is given by the number of toxigenic isolates found per total fungi tested. Very toxic = All three mice died (3/3), moderately toxic = two of three mice died (2/3), mildly toxic = one of three mice died (1/3), non-toxic = no mice died (0/3).

Table 3. Incidence of fungal species contaminating maize in Kogi State.

S/No.	Fungus	Incidence
1	*Aspergillus niger*	1/50
2	*Aspergillus fumigatus*	39/50
3	*Aspergillus flavus*	4/50
4	*Aspergillus* spp	15/50
5	*Fusarium oxysporum*	9/50
6	*Fusanum* spp	46/50
7	*Penicillium notatum*	15/50
8	*Penicillium* spp	17/50
9	*Rhizopus* spp	3/50
10	*Pithomyces chartanum*	2/50
	Total	151/50

Note: Results are presented as fungal isolates per total number of samples analyzed.

The fungi, *Syncephalastrum* was unique for maize from Niger State and was not isolated in same cereal in Kogi. The field fungi identified from maize include *Aspergillus flavus, A. glaucus, A. niger, Fusarium species, Mucor* spp, and *Rhizopus* spp (Table 1). The storage fungi isolated from these maize sam ples stored in sack and "rumbu" were *A. flavus, Aspergillus nidulans Aspergillus glaucus, A. niger, Aspergills terreus* and *Aspergus versicolor'*. Others include *Mucor* spp. *Rhizopus* spp, *Fusarium* spp, *Penicillium* species and *Syncephalastrum* spp.

In course of the toxicity assay, all mice that died did that between 9 - 48 h. Table 2 shows the toxicity screening results of extracts of some fungal isolates from the samples. The data are presented according to level of toxicity and under each level; the incidence of toxigenic fungi is given per fungal isolates tested. Of the thirty isolates screened, 19 (or 63.3%) produced toxic metabolites. Of these toxic nineteen isolates, 6 (31.5%) were very toxic, 4(21.1%) were moderately toxic while 9 (47.4%) were found to be mildly toxic. Of the nine isolates that produced mildly toxic metabolites six were *Aspergillus* species and the other three were *Fusarium* spp and *Mucor* spp. Two *Aspergillus* spp, one each of *Rhizopus* and *Fusarium* Species constituted the moderately toxic isolates. Of the six very toxic isolates, three were *Aspergillus* Species, two *Penicillium* spp and one *Fusarium* spp.

Fungi isolated from maize in Kogi State

Table 3 shows the incidence of fungi in maize from Kogi state while Table 4 shows the result of toxicity screening of the isolates in mice. The fungi isolated were mainly *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* species. *Fusarium* species (38.4%) recorded the highest incidence followed by *Aspergillus* (34.4%), *Penicililum* (23.3%) and *Rhizopus* (2.0%). *Pithomyces chartarum* (1.3%) was only isolated in maize from this State and not from Niger State. Of the thirty isolates used for toxicity screening, twenty isolates of the genera, *Fusarium*, *Penicillium* and *Rhizopus* were found to produce toxic metabolites while the non toxic fungi were *Pithomyces chartarum* and species of *Fusarium* (6) and *Rhizopus* (1).

Fungi isolated from dry yam chips

Table 3 shows the incidence of fungi in maize

Table 4. Toxicity screening of fungi extracts isolated from maize in Kogi State in mice.

Very toxic (8)	Incidence	Moderately toxic (6)	Incidence	Mildly toxic (7)	Incidence	Non toxic (9)	Incidence
Penicillium notatum	(2/8)	*Rhizopus* spp	(2/6)	*Fusarium* spp	(4/7)	*Pithomyces chartarum*	(2/9)
Fusarium spp	(3/8)	*Fusarium* spp	(3/6)	*Penicillium* spp	(2/7)	*Fusarium oxysporum*	(3/9)
Penicillium spp	(3/8)	*Penicillium* spp	(1/6)	*P. notatum*	(1/7)	*Fusarium* species	(3/9)
						Rhizopus species	(1/9)

Note: Number in brackets indicates the number of fungi screened at that level of toxicity and the incidence values is given by the number of toxigenic isolates found per total fungi tested. Very toxic = all three mice died (3/3), moderately toxic = two of three mice died (2/3), mildly toxic = one of three mice died (1/3), non-toxic = no mice died (0/3).

Table 5. Incidence of fungi isolated from dried yam chips in Niger State.

Fungus	Frequency of occurrence
Fusarium spp	6/50
Aspergillus spp	4/50
Aspergillus niger	1/50
Penicillium spp	2/50
Mucor spp	3/50
Geotrichum candidum	1/50
Total	17/50

Note: Results are presented as fungal isolates per total number of samples analyzed.

from Kogi state while Table 4 shows the result of toxicity screening of the isolates in mice. The fungi isolated were mainly *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* species. *Fusarium* species (38.4%) recorded the highest incidence followed by *Aspergillus* (34.4%), *Penicililum* (23.33%) and *Rhizopus* (2.0%). *Pithomyces chartarum* (1.3%) was only isolated in maize from this State and not from Niger State. Of the thirty isolates used for toxicity screening, twenty isolates of the genera, *Fusarium*, *Penicillium* and *Rhizopus* were found to produce toxic metabolites while the non toxic fungi were Pithomyce *chartarum* and species of *Fusarium* (6) and *Rhizopus* (1).

Fungi isolated from dry yam chips

Fungal growth was observed in only 17 out of the 50 samples of dried yam chips cultured. The fungi isolated were *Fusarium* spp, *Aspergillus* spp, *Aspergillus niger, Penicillium* spp, *Mucor* spp and *Geotrichum candidum*. Table 5 shows the frequency of occurrence of each isolates. Toxicity tests with fungal culture extracts of eleven (11) out of the twelve non-*Aspergillus* isolates in albino mice showed *Fusarium* spp, *Penicillium* spp, *Geotrichum candidum* and *Mucor* spp to be toxic. The Table 6 shows the toxicity levels of these extracts in albino mice.

Aflatoxin M_1 concentrations in powdered milk

The number of AFM_1 contaminated samples and the levels (mean values and standard deviation) found in them are presented in Table 7. Of the hundred (100) samples of powdered milk collected, 7 brands (19 samples) were found to be contaminated with AFM_1 at levels that did not exceed the regulatory limit of 0.5 µg/kg set by National Agency for Food and Drug Administration Control (NAFDAC) and the European Union and Codex Alimmentarius Commission.

Aflatoxin B_1 contents of beans and wheat

The concentrations of aflatoxin B_1 in beans and wheat are presented on Table 8. The results reveal high levels of AFB_1 (16.2 - 274 ug/kg) in the two grains examined, the lowest concentrations being observed in beans while wheat had the highest. Significantly ($P < 0.01$) higher mean values were recorded for wheat than beans which indicates a higher susceptibility to aflatoxin contamination of the former than the later. Twenty nine beans samples of the fifty analyzed were contaminated with the toxin with samples from Central market having the highest incidence and concentrations. Of the twenty seven contaminated wheat samples, those from Central and Bosso markets had the highest incidence and contents respectively. The results also show that the location of sample collection had significant ($P < 0.01$) effect on the AFB_1 contents as concen-trations varied significantly with location except

Table 6. Toxicity of extracts of fungi isolated from dried yam chips in mice.

Very toxic (6)	Incidence	Moderately toxic (2)	Incidence	Mildly toxic	Non-toxic (3)	Incidence
Fusarium spp	(2/6)	*Fusarium* spp	(2/2)	None	*Fusarium* spp	(1/3)
Penicillium spp	(1/6)				*Mucor* spp	(1/3)
Geotrichum candidum	(1/6)				*Penicillium* spp	(1/3)
Mucor spp	(2/6)					

Note: Number in brackets indicates the number of fungi screened at that level of toxicity and the incidence values is given by the number of toxigenic isolates found per total fungi tested. Very toxic = all three mice died (3/3), moderately toxic = two of three mice died (2/3), mildly toxic = one of three mice died (1/3), non-toxic= no mice died (0/3).

Table 7. Mean values of Aflatoxin M_1 in powdered milk samples marketed in Lagos metropolis.

Milk (Brand)	Number of contaminated samples	Mean values (µg/kg)	Range
A	2/2	0.220 ± 0.269	0.02 - 0.41
B	1/2	0.150 ± 0.212	0.00 - 0.30
C	4/5	0.325 ± 0.175	0.00 - 0.41
D	3/4	0.108 ± 0.525	0.00 - 0.11
E	1/2	0.070 ± 0.980	0.00 - 0.14
F	4/5	0.066 ± 0.126	0.00 - 0.29
G	4/5	0.016 ± 0.305	0.00 - 0.07
H	0/6	-	
I	0/2	-	
J	0/2	-	
K	0/2	-	
L	0/2	-	
M	0/4	-	
N	0/2	-	
O	0/2	-	
P	0/2	-	
Q	0/2	-	
R	0/2	-	
S	0/5	-	
T	0/2	-	
U	0/5	-	
V	0/5	-	
X	0/3	-	
Y	0/5	-	
Z	0/5	-	
A_1	0/5	-	
B_1	0/4	-	
C_1	0/5	-	
D_1	0/5	-	

except for wheat sampled from Central and Kpakungu markets. Samples of both beans and wheat from Central market had the highest incidence of the toxin.

DISCUSSION

Assessment of the incidence of fungal and mycotoxins contamination of some Nigerian cereals and powdered milk as conducted in this work gives the quality of the studied food products. All the grain samples contained more than one species of fungi with maize having more fungal infestation than dry yam chips. Similarly 56% of the beans and wheat samples analyzed contained aflatoxin B_1 at levels unacceptable by national and international mycotoxin regulatory bodies. This is an indication that these food commodities that are intended for human consumption in Nigeria are of low quality

Table 8. Aflatoxin B_1 levels (ug/kg) in beans and wheat from markets in Minna, Niger State.

Beans					Wheat				
Location (Market)	**No. of samples collected**	**No. of contaminated samples**	**Mean ± SD**	**Range**	**Location (Market)**	**No. of samples collected**	**No. of contaminated samples**	**Mean ± SD**	**Range**
Bosso	10	6	77.3 ± 37.7^{AEFG}	19.3 - 116.8	Bosso	10	3	198.4 ± 70.0^{TUWYZ}	137.6 - 275.0
Central	10	7	$106 \pm 39{,}5^{ABCD}$	52.1 - 137.6	Central	10	8	107.6 ± 68.2^{RST}	40.0 - 256.0
Chanchaga	10	6	63.5 ± 28.3^{CFHJ}	16.2 - 109	Gwari	10	4	134.5 ± 35.8^{RUVW}	87.0 - 175.0
Kpakungu	10	5	87.8 ± 23.6^{DGIJ}	58.4 - 125.1	Kpakungu	10	7	102.9 ± 53.0^{VXZ}	40.1 - 169.0
Tunga	10	5	93.5 ± 20.0^{BEHI}	68.8 - 130.0	Kwangila	10	5	133.2 ± 69.3^{SXY}	85.5 - 204.4
Total	50	29				50	27		
Mean ± SD			59.29 ± 14.85^{Q}	16.2 - 137.6				85.66 ± 16.19^{Q}	40.0 - 275. 0

Note: Results are expressed as Mean ± SD (Standard deviation) and range and values with similar superscripts are significantly different at $P < 0.01$.

and could constitute health hazard to the Nigerian populace. The ratio of fungi isolates per sample for maize was 2.4 while that of yam chips was 0.34 which implies more fungal infestation of maize than dried yam chips. Maize as a grain is an ideal and therefore a better substrate for fungal growth (FAO, 1983) than most crops including dried yam chips and though not determined in this work, is likely to have higher moisture content, 15 - 20% than the chips, 6 - 15% (Bankole and Mabekoje, 2004) hence the higher fungal contamination in maize than the yam chips. The high susceptibility of maize to fungal growth and mycotoxins production than most crops as observed in this survey has also been proven by Jelinek et al. (1989) and Okoye (1992) in Nigeria and at international level. Many of the fungi found in our study have also been found to cause spoilage to maize in Nigeria and other parts of the world. Gbodi (1992) identified the following fungi inmaize;*Fusarium*species,*Fusarium moniliforme*, *Helminthosporium* species, *Penicillium* species, *Phoma sorghina* species and *Rhizopus* species among others which have been isolated from maize in our work. The fungal profile of the studied dried yam chips is also in agreement with the findings of Bankole and Mabekoje (2004) as all the fungal species (*Aspergillus*, *Penicillium*, *Fusarium* and *Mucor*) isolated were also found by these workers in same food product from the South Western region of the country. However, the fungal contamination of the dried yam chips observed in the samples was lower than that reported in the South Western Nigeria samples and this could be because of the lower humidity of Niger State as compared to the wet rainforest climate of the later that is more conducive for fungal growth than in our study area.

In terms of mycofloral contamination marketed (2.5) maize had higher fungal isolates per samples than stored (2) and field (1.3) samples. Crops are infected in the field by fungi and these field fungi persist and proliferate during storage when favourable conditions prevails (Bainton et al., 1980). Therefore fungi incidence is likely to be higher in badly stored grains than those on the field as observed in this work. The exposure of marketed samples to the ubiquitous fungi and their spores in the air and the aerobic condition of the atmosphere would encourage more fungal growth than the relatively anaerobic conditions of storage systems such as sack and “rumbu”. This might explain the higher fungi occurrence in market samples than those from the stores.

The results of toxicity screening test showed that species of *Aspergillus*, *Fusarium, Penicillium, Rhizopus, Mucor* and *Geotrichum* from maize and dried yam chips produced toxic metabolites that were deleterious to mice. The presence of these for crops, implies that various mycotoxins including the major one namely aflatoxins, sterigmatocystin, ochratoxin A, citrinin and patulin elaborated by *Aspergillus* and *Penicillium* species and fumonisins, zearalenone and the trichothecenes produced by *Fusarium* species (Scott, 1994) are therefore likely to be found in these food commodities. Rhizonin A, a metabolite of *Rhizopus* and *Mucor* (Wilson et al., 1984) could also contaminate the studied grain and yam chips. Though no mycotoxin has been ascribed to *Geotrichum candidum*, it causes lesions of the skin, bronchi, mouth, lungs and intestine

(Beardall and Miller, 1994). This all points to potentially serious health hazards to human beings and animals in the Middle Belt State and indeed Nigeria as a whole as these toxins have acute, chronic and carcinogenic effects on human beings and animals (Gbodi and Nwude, 1988; Peraica et al., 1999). Many known toxigenic *Fusarium*, *Aspergillus* and *Penicillium* spp were found to be non toxic in this work. This could be as a result of mutation as repeated process of sub culturing may lead to loss of mycotoxin producing ability in the organism (Gbodi et al., 1986) and could also be that the strains of these toxigenic fungi do not have the genetic capacity to elaborate toxins (FAO, 1979).

Aflatoxins M_1 and B_1 in the milk and wheat and beans samples, respectively were analyzed using thin-layer chromatographic technique which is perceived by modern scientists as an insensitive technique but to the scientists in the developing world, it remains a simple, quick and inexpensive procedure for mycotoxin analysis. The detection limit of the technique as determined in the present study as well as by other workers (Bankole and Mabekoje, 2004) with regards to aflatoxins particularly B_1 is 2 µg/kg which is quite sensitive enough to meet the standards of both national and international regulatory agencies and therefore validates the results obtained.

Aflatoxin M_1 contamination of milk and milk products is a worldwide problem and Nigeria is not an exception as this study has shown 19% AFM_1 contamination of marketed powdered milk in the country. Garrido et al. (2003) detected the toxin in 79.9% of milk samples collected from Brazil, at concentrations between 0.05 and 0.2 µg/kg. Visconti et al. (1985) had also showed the presence of the toxin in Italian diary system. The AFM_1 contents found in marketed powdered milk in Lagos like those in the aforementioned works were all below the international regulatory limit and this might be because the milk samples were all imported from Europe and so compliance to the limit must have been achieved right at the point of manufacture. The hazards of AFM_l have been fully recognized; of great public health concern is the fact that the toxin is a potent carcinogen. Based on the result obtained, the occurrence of AFM_1 does not appear to be serious public health hazard in Nigeria with regards to imported milk products. Since AFM_1 is a dietary metabolic residue of AFB_1 which is recurrent at unsafe levels in Nigerian foodstuffs, it is likely that the former might be found at high levels in locally produced and processed milk products. A survey of AFM_1 in local milk and milk products will therefore give a better AFM_1 profile of the country and is therefore recommended.

The incidence and concentration of AFB_1 found in the beans and wheat samples were more elevated than those reported by other workers (Zinedine et al., 2006; Odoemelam and Osu, 2009) in Nigeria and elsewhere. Fungal growth and mycotoxin contamination are dependent on climate and storage conditions and therefore vary with locations, with hot and humid climate, poor storage conditions and poor agricultural practices exacerbating fungal and mycotoxin contents in foods and feedstuffs (Ominski et al., 1994). Grains in rural area of Northern Nigeria which is where our samples are from, are handpicked at harvest, left to dry for weeks at a threshing ground, threshed, packaged and transported in sacks to markets where they are sold in open containers. The complex effects of relative humidity, temperature, precipitation and insect and rodent infestation and their daily variation may interplay to provide conditions conducive for fungal growth and aflatoxin contamination on such exposed and poorly stored grains. These methods of harvesting, storage and transportation which differs from those in other parts of the country could probably explain the more elevated incidence and contents of AFB_1 in beans and wheat in this work as compared to results of the other workers.

The beans were all grown locally within Minna and its environs in Niger State so the significant differences in their AFB_1 levels might not be due to variation in climate but as a result of differences in harvesting, storage, transportation and handling conditions. The significant effect of locations on the incidence and AFB_1 levels of the wheat samples implies that the samples were from sources with different relative humidity, temperature, storage conditions and even agricultural practices. The marketed wheat samples purchased from Central, Kpakungu and Kwangila markets were actually imported and bought by retailers from Lagos-Bida axis while those sampled from Bosso and Gwari markets were grown in Jos and Nigeria. The imported grains had higher toxin incidence than the Nigerian grown wheat because they were transported and likely stored under poor conditions for a longer period than the later. It might also be because the transit towns (Lagos and Bida) of the imported wheat are warmer and more humid than the cold and drier climatic conditions in Jos where the home grown grains are from. The hot and humid conditions of Lagos and Bida are more favourable for fungal growth and mycotoxins production than the cold drier climate of Jos (Javis, 1976) hence the higher toxin contamination in the former that the later.

It stands to reason that the thick and hard coat of bean hinders penetration of fungi into the seed with resultant less fungal growth and mycotoxin contamination as compared to other grains. This might account for the significantly lower aflatoxin contamination observed in the beans than the wheat samples. Aflatoxin B_1 concentrations in the beans (59.29 ± 14.85 ug/kg) and wheat (85.66 ± 16.19 ug/kg) analyzed were above the National Agency for Food and Drug Administration and Control (NAFDAC) and European Union (5 ug/kg) tolerance level for aflatoxin in grains for human consumption. The chronic consumption of these crops with unsafe levels of $AFB_{1,}$ that is, immunosuppressive, nephrotoxic and hepatocarcinogenic (Gbodi and Nwude, 1988; Peraica et al., 1999), has grievous public health implications which calls for control and regulation of mycotoxins in the country. The desired control of mycotoxins can be achieved by reducing fungal infection of crops by rapid drying and correct storage of the harvested crops using effective anti-mould preservatives. Properly designed, mycoflora and mycotoxin surveys and monitoring programmes can reduce the fungal and mycotoxins in our foods. It is high time Nigeria enforced the legislation against mycotoxins.

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