



Fungal and Mycotoxin contamination of stored maize grains in Kebbi state, north-western Nigeria

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ABSTRACT

Studies were conducted to determine the fungal and mycotoxin contamination of stored maize grains in Kebbi state. A total of 93 samples of maize grains comprising 31 each from three (3) emirates councils (Argungu, Gwandu and Yawuri) were analysed for the presence, incidence and levels of Aflatoxins (B1, B2, G1, G2), Fumonisin B1 and phenotypic characterization of fungal contaminants, using standard mycological techniques and High Performance Liquid Chromatography (HPLC). Total Fungal Count (TFC) on contaminated products ranged from 1.8×10^3 to 2.3×10^4 CFU/g. *Fusarium graminearum* and *Aspergillus flavus* was the major source of mycotoxins contamination and the most frequently isolated followed by *A. parasiticus* and *A. niger*. The least in occurrence were *Penicillium notatum* and *Aspergillus fumigatus*. Aflatoxins account for 76%, 87% and 87% in Gwandu, Argungu, and Yawuri emirates samples at concentrations ranging from 0.1-211 µg/kg, 0.2-116 µg/kg and 0.1-252.5 µg/kg respectively. Fumonisin B₁ was detected (38.6%, 48.4% and 38.6%), at 1.2-18.1 µg/kg, 0.4-29.8 µg/kg and 0.3-17.3 µg/kg concentrations for Gwandu, Argungu, and Yawuri emirates respectively. The results show that there were significant ($P < 0.05$) differences between the samples analyzed in the State. Findings from this study affirm substantial contamination of maize with fungi and mycotoxins which are potentially hazardous to human health. Research needs to be done on management strategies for controls of mycotoxins and develop storage structures convenient for the storage of maize grains over a long period of time.

Keyword: Maize grains, Fungi, Mycotoxin, Contamination and Kebbi State

INTRODUCTION

Fungi are among the oldest and largest group of living organisms. They lack a vascular system and do not have good connection among segments and thus easily fragment into multiple bodies [1, 2]. Over 100,000 cases of plant fungal diseases account for 70% of major crop diseases, resulting in economic losses of billions of dollars each year [3].

Mycotoxin is a poisonous substance produced by toxigenic strains of fungi. Mycotoxin producing fungi are spread by wind, insects and decomposed soil wastes. These fungi are present in air, soil and water [4]. Mycotoxins are quite stable molecules which are extremely difficult to remove or eradicate and maintain their toxic properties along the tropic level in a food chain from market to consumption [5]. Approximately 400 secondary metabolites with toxigenic potential are produced by over 100 moulds. Such mycotoxins includes aflatoxins, fumonisins, ochratoxin, trichothecenes, zearalenone, penitrem and ergot alkaloids [6]. The Food and Agriculture Organization [7] estimated that 25% of the world's agricultural commodities are contaminated with mycotoxins, leading to significant economic losses [8]. The toxins are produced at different stages of harvest and processing of food or animal feed and can be detected in a wide variety of cereals, pulses and vegetable crop products, in foods and beverages (fruits, juice, beer and wine), feed and animals products (dairy, meat, milk and eggs) being involved in human and animal diseases. Consumption of food contaminated with mycotoxin can result in teratogenic, carcinogenic and oestrogenic or immune-suppressive effects [9]. Report by Shephard [10] indicates that between 25% and 50% of staple crop products, meat and milk products of animal origin are contaminated with mycotoxins due to infestation by pathogenic fungi. Farmers' practices of production and handling of plant at pre- and post-harvest stages may provide favourable conditions for colonization of fungi and mycotoxin contamination [11].

The studies are of paramount importance to serve as an effort to create awareness on toxigenic fungi and mycotoxin contamination on maize grains for human consumption in the state and to give valid recommendations for safe consumption and marketing of products in Kebbi State, Nigeria. Information on the prevalence of mycotoxins producing fungi is limited in Kebbi state, Nigeria which could be the basis for the reduction of mycotoxicoses in Nigerian. Little work has been done on mycotoxin contamination of plant products with mycotoxin producing fungi in Kebbi state.

This research is aimed to investigate the fungi and mycotoxin contamination on maize grains in Kebbi State, Nigeria.

MATERIALS AND METHODS

Sampling

The State has four emirates namely: Argungu, Gwandu, Yawuri and Zuru. Each Emirate has between 4 - 10 local governments' areas. For the purpose of this study, three emirates (Argungu, Gwandu and Yawuri) were randomly selected by balloting; a system random sampling technique was used for sampling of maize grains in the markets and stores of the selected local governments in the emirates.

Selection of markets/Stores

Markets and stores from selected local government areas were identified through local government departments' of agriculture and traditional authorities.

Mycological Analyses

The isolation of mycotoxigenic fungi was carried out according to agar dilution method as described by Pittet [12]. Ten (10) grams from each groundnut kernel and cake powder sample were homogenized with 90 ml of buffer peptone water and serial decimal dilutions (10^{-1} to 10^{-4}) were performed. Fungal species were isolated on Potato Dextrose Agar. The medium was poured

into sterile Petri dish and 0.1 ml of each sample suspension was spread-plated onto the PDA media. The plates were incubated for 5 to 7 days at 25°C. Fungal isolates were sub-cultured on Sabouraud Dextrose agar (Oxoid, UK) and incubated for 5 to 7 days at 25°C for purification. Fungi were identified by using taxonomic schemes based on microscopic observation and culture appearance including colonies colours, texture, reverse colour, hyphae arrangement, conidia shape and nature of spores [13]. The total fungal count for each plate was expressed as colony-forming units per gram of sample (CFU/g). Each genus or specie identified was then expressed as percentage (%) of the total isolated fungi. The total colonies of fungi were enumerated and results were reported in mean and average fungal counts according to Pitt and Hocking, [14] and Dachoupakan *et al.* [15].

Identification of mould

The identification of fungal strains and the determination of each species of fungi were done using the keys of Klich [16] for *Aspergillus* spp. and Pitt and Hocking [14] for *Penicillium* sp. and other genera. This was done by observing both microscopic characteristics and morphology of the colonies on PDA and SDA media.

Analysis of Aflatoxin

Samples were prepared to extraction, clean up and analysis for the presence of B1, G1, M1, B2, G2 and M2 according to the method described by Ehrlich and Lee [17] without modification. Methylene chloride and phosphoric acid were used for the simultaneous extraction of AFB1, AFB2, AFG1 and AFG2.

Extraction of aflatoxins

Approximately 50 grams of groundnut kernel and cake portion of pulverized samples were weighed into a 500 ml Erlenmeyer flask and 25 ml of 1M phosphoric acid and 250 ml of methylene chloride were added. The flask was placed on a mechanical shaker for 30 minutes and the content filtered under pressure on Buchner funnel fitted through an 18 cm circle rapid filter paper. Two hundred milliliter of the filtrate were collected and 50 ml aliquot was taken from the filtrate and placed in separate 100 ml Erlenmeyer flasks with glass stoppers, for aflatoxin assay.

High performance liquid chromatography (HPLC) analysis of aflatoxins

Aflatoxins were analyzed on a Cecil 1100 series HPLC system equipped with a UV detector set at a wavelength of 365 nm as described by Cora *et al.* [18]. The Altraspher ODS column, 4.6 mm x 250 mm was used at ambient temperature of 25°C. Acetonitrile/ water/acetic acid (10:50:40, v/v/v) were used as the mobile phase pumped at a flow rate of 0.8 ml/min. The injection volumes of both samples and standards were 20 µL.

Aflatoxin standard curve

The analysis was carried out with Aflatoxins standards (Sigma Chemical Company, St. Louis, MO, USA) of known concentrations. Aflatoxin B1, G1 and Aflatoxin B2, G2 eluted at distinct retention times of 1.673 min and 1.524 min, respectively. Calibration curves with correlation coefficient (R_2) of 0.91 and 0.99 were established for Aflatoxin B1, G1 and Aflatoxin B2, G2, using a series of dilutions containing (0.004, 0.008, 0.012 and 0.016 µg/ml) and (0.01, 0.02, 0.03 and 0.04 µg/ml) respectively for each standard. The limits of detection (LOD) were estimated as follows: known concentrations of Aflatoxin standards were prepared, successively diluted and subjected to HPLC until the minimum concentration at which the analyte could be detected was established. The LOD of the HPLC instrument with regards to both toxins was determined to be 0.21 and 0.18 µg/kg while the limits of quantification (LOQ) were

estimated based on the standard deviations of response and slope; this gave 0.42 and 0.33 µg/kg respectively.

Fumonisin Extraction in Maize grains

Samples were freeze dried and homogenized. The analysis was performed by fluorescent detection method [19]. Twenty grams (20 g) of animal tissues sample were mixed with 10 ml of 1M hydrochloric acid and mixed with 10 ml of methanol. The mixture was vortexed for 2 minutes and centrifuged at 1500 rpm for 10 minutes at 10°C. Then the organic phase was collected and applied to a C₁₈ cartridge at a flow rate of 1.0-1.5 ml/min previously preconditioned with 5 ml of methanol and 5 ml of methanol/water (3:1). The cartridge was then washed successfully with 5 ml of methanol/water (3:1) followed by 5 ml of methanol and lastly the fumonisins were eluted with 10 ml of 5% acetic acid in methanol. The eluted extract was immediately dried down under a stream of nitrogen gas at 60°C. The residue was refrigerated and only redissolved in 200 µl of methanol prior to derivatisation and HPLC analysis.

Quantification of the (actual) mycotoxins

Quantification of the actual mycotoxins was based on the formula adapted by legesse [20].

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

Where:

S = volume of standard with same colour intensity as sample (µl);

Y = concentration of Mycotoxins 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).

Data Analysis

The SPSS 21.0 (Windows version, IL, USA) was used for data analysis. Means for the distribution of concentrations of Mycotoxins, comparison of means of TFC across products and overall (%) for fungal species, were calculated and tested for significance at 95% confidence level by one-way ANOVA. The Duncan's multiple range tests was further used to separate the mean.

RESULT

Fungi Associated with Maize Grains Contamination

The total plate count of the visible colonies after serial dilutions and microscopic examination showed different morphological and cultural characteristics that formed the basis of identification of probable fungi isolates (Table 1). All maize samples analyzed in this study had fungal contamination at varying levels. A total of 155 fungal isolates belonging to 8 identified fungal species (*Aspergillus niger*, *A. flavus*, *A. fumigates*, *A. parasiticus*, *Fusarium Moniliforme*, *F. graminearum*, *F. verticilliodes* and *Penicillium*) were recovered from the analyzed samples. Maize samples from Yawuri emirates had the highest total fungal count of 2.3×10^4 cfu/g while those from Argungu emirate had the least fungal load, 1.8×10^3 cfu/g (Table 1). Although there was no significant ($P > 0.05$) difference in the fungal loads across all the emirates, on the overall, the incidence of *Aspergillus flavus* was the highest (28.17%) being significantly ($P < 0.05$) higher than the proportion of all other fungal species. *Penicillium species* occurred the least (3%) although its incidence was significantly ($P < 0.05$) lower than the incidence of *Aspergillus* and *Fusarium*. The details are presented in Table 1.

Table 1: Occurrence and Distribution of Fungi Contaminating Maize grains in three (3) Emirates of Kebbi State, Nigeria

Percentage (%) of fungal species occurring in Maize grains samples

Products	Emirates	*TFC (cfu/g)	Percentage (%) of fungal species occurring in Maize grains samples							
			<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus parasiticus</i>	<i>Fusarium moniliforme</i>	<i>Fusarium graminearum</i>	<i>Fusarium verticillioides</i>	<i>Penicillium notatum</i>
Maize grains	Gwandu	2.1 x 10 ⁴	11	7	1	6	8	8	8	1
	Argungu	1.8 x 10 ³	6	8	0	9	2	13	3	3
	Yawuri	2.3 x 10 ⁴	14	8	2	8	6	20	3	1
	Mean	2.1 x 10 ⁴								
Overall(%)			20 ^b	15 ^b	2 ^d	15 ^b	10 ^{cd}	26 ^a	9 ^d	3 ^d

*TFC (cfu/g): Total fungal count in colony forming units per gram

Overall (%). Mean with different letters in the same row are statistically different (p < 0.05) according to Duncan's test.

Table 2: Aflatoxin Concentration (µg/kg) of Maize Grains Samples Collected from three Emirate Council

Aflatoxin	Emirate Council	Frequency of Contamination (%)	Concentration range of positive samples (µg/kg)	Mean ± Std. dev.
AFB1	Gwandu Emirate	24/31 (76%)	1.0-211.0	38.36±60.42 ^{ab}
	Argungu Emirate	26/31 (87%)	0.3-116	29.76±38.63 ^b
	Yawuri Emirate	26/31 (87%)	0.7-252.5	45.51±96.31 ^a
AFB2	Gwandu Emirate	19/31 (62.0%)	0.1-56.0	12.74±17.65 ^{ab}
	Argungu Emirate	25/31 (81.0%)	0.2-68	14.06±20.16 ^b
	Yawuri Emirate	24/31 (76.1%)	0.1-91.0	15.090±17.00 ^{ab}
AFG1	Gwandu Emirate	16/31 (51.6%)	0.5-83.0	14.31±28.44 ^a
	Argungu Emirate	22/31 (71.4%)	0.3-41	7.50±13.71 ^b
	Yawuri Emirate	17/31 (57.8%)	0.3-76.8	14.081±15.51 ^{ab}
AFG2	Gwandu Emirate	13/31 (42.1%)	1.2-12.0	5.343±7.92 ^a
	Argungu Emirate	10/31 (32.3%)	0.3-10	1.08±2.67 ^b
	Yawuri Emirate	12/21(57.1%)	1.0-23.0	3.281±6.57 ^a
Total AF	Gwandu Emirate	24/31 (76%)	0.1-211	84.85±123.87 ^a
	Argungu Emirate	26/31 (87%)	0.2-116	53.11±80.11 ^b
	Yawuri Emirate	26/31 (87%)	0.1-252.5	81.67±141.74 ^a

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance.

Table 3: Fumonisin B1 Concentration (µg/kg) of Maize grains collected from three Emirates of Kebbi State

Fumonisin B1	Emirates Council	Frequency of Contamination (%)	Range of concentration of positive samples (µg/kg)	Mean ± Standard deviation
MAIZE	Gwandu Emirate	12/31(38.6%)	1.2-18.1	1.40 ±2.75 ^b
	Argungu Emirate	15/31(48.4%)	0.4-29.8	3.57 ±6.56 ^a
	Yawuri Emirate	12/31(38.6%)	0.3-17.3	1.13±2.36 ^b

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance

Aflatoxin Contamination in Maize grains

The presence of Aflatoxin in Maize grains obtained in Gwandu Emirate is presented in Table 2. Sixteen samples out of twenty one (76.2%) in Gwandu Emirate were contaminated with the Aflatoxin. The highest levels of Aflatoxin were Aflatoxin B₁ (1.0 – 221.0 µg/kg), followed by AFG1 and AFB2 (1.5 – 113.0 and 1.1 – 76.0 µg/kg) respectively. The lowest concentrations of Aflatoxin were recorded for AFG2 (1.8 - 22.0 µg/kg). In the Argungu Emirate, eighteen samples out of twenty one (85.7%) were found to be contaminated with Aflatoxin. The results indicate that the highest levels of Aflatoxin were Aflatoxin B₁ (1.3 – 156.0 µg/kg), followed by AFB2 and AFG1 (0.2 – 88.0 and 0.5 – 61.0 µg/kg) respectively, the lowest Aflatoxin concentrations were recorded for AFG2 (0.5 - 12.0 µg/kg). In

Yawuri Emirate, eighteen samples out of twenty one (85.7%) were contaminated with Aflatoxin. The results reveal that the highest levels of Aflatoxin were Aflatoxin B₁ (0.9 – 450.5 µg/kg). This levels represent the highest range of concentration found in all the remaining sampling emirates in the State, while the moderate levels were found on AFB2 and AFG1 (0.1 – 101.0 and 1.0 – 96.8 µg/kg) respectively. The lowest Aflatoxin concentrations were recorded for AFG2 (1.1 - 28.0 µg/kg).

Fumonisin B1 Contaminating Maize grains

The Fumonisin B1 investigated in Maize grains from Kebbi State was presented in Table 3. The frequencies of contamination of maize are 38.1%, 47.6% and 38.1% for Gwandu, Argungu and Yawuri emirates respectively. The concentrations ranged between 1.2 – 12.1, 0.4 – 27.0 and 0.3 – 11.1 µg/kg in the

respective emirates, with mean of 1.40, 3.57 and 1.03 for the respective emirates. There are significant differences in the levels between Argungu and other emirates.

DISCUSSION

Fungal species isolated in this study indicate the extent of genera contamination of maize grains in Kebbi State. Molds species reported in this study were found reported on same maize grains and other cereals in other parts of the country [21, 22]. The market fungi identified in this study have been showed in other studies [23, 24]. The species of fungi isolated in this study are said to be undesirable in foods. The presence of *Aspergillus* species in the cereal samples is an indication of possible health hazards as some species of *Aspergillus* are known to cause food intoxication and food poison [24]. It was however observed that market had incidence of fungi this could be due to the fact that, grains are mostly transported to markets after harvest for sales in locally made woven baskets and sacks under conditions that encourage the incubation of these contaminants and left exposed in open bowls in the market [25]. The observed variation in incidence of fungi could be climate dependent [26] and the fungi may differ from those of other studied area. *Fusarium* toxins which equally occurred in all the emirates are produced by over 50 species of *Fusarium* especially, *F. graminearium* and *F. culmorum*, and have a history of infecting the grain of developing cereals such as wheat and maize.

The occurrence of high aflatoxin content in Yawuri may be due to variation in environmental factors which are favourable for the growth of aflatoxigenic moulds which may be responsible for the detection of high aflatoxin level in this area which is more to the tropic when compared with Gwandu and Argungu emirates. This statement was supported by Ellis [27] who observed that temperature is an important factor for the growth of fungi especially *Aspergillus* spp. [26] supported this findings as he amplified that aflatoxin levels was significantly ($P < 0.05$) affected by temperature [28] reported that mycotoxigenic fungi are notable to inhabit a good number of food and grains to build up mycotoxins when conditions of the environment are complimentary for their development in the field and at storage locations. Furthermore, [29] in Turkey reported on the effects of Ochratoxin A and total aflatoxin levels in corn, and reported corn grain to be highly contaminated above permitted limit 10 µg/kg, this reported agreed with the present study in which corn was found to have higher level of contaminant. Similarly, Fumonisin B1 investigated in Maize grains contamination of maize are 38.1%, 47.6% and 38.1% for Gwandu, Argungu and Yawuru emirates respectively. The concentrations ranged between 1.2 – 12.1, 0.4 – 27.0 and 0.3 – 11.1 µg/kg in the respective Emirates, this was lower than other reports within and outside the country. [30] reported 80% occurrence of Total fumonisin within 2.7–10,904 µg/kg in unprocessed flour/grain in rural Northern Nigeria, this is consistent with our current findings, Abdus-Salaam et al. [31] reported FB1, FB2 and FB3 in rice with occurrences and mean values of 39.5%, 21.1%, 18.4% and 18.52 µg/kg, 8.75 µg/kg, 5.54 µg/kg, respectively Salau [4] also reported groundnut kernel contamination (85.7%) in a concentrations range between 0.90µg/kg to 646.00µg/kg from Sokoto state. From China, Xing et al. [32] reported Fumonisin B1 and deoxynivalenol as the primary mycotoxin of corn in 3 China provinces, they also reported 100% occurrence of fumonisin within 16.5–315.9 µg/kg. Also there are other reported cases of fumonisin B1, B2 and B3 in rice, corn, millet, sorghum and their derivatives in other hot temperate regions; Europe and Africa [33, 34]. These makes its presence in common staples of

great interest hence it poses a public health concern.

The demonstrated presence of mycotoxigenic fungi in cereal crops millet and maize in this study has public health implications as low grade, cheap, mouldy grains are consumed by animals and humans in the study area and other parts of the country resulting in high risk of human and animals mycotoxicoses with adverse effects on crop and livestock production, and therefore national economy and trade. This makes regulation of mycotoxins in our foods and feedstuffs, an imperative.

CONCLUSION

This study revealed that fungi such as *Aspergillus* spp, *Penicillium* spp. and *Fusarium* spp are the major fungi that infect stored maize grains in different emirates of Kebbi State. The presence of *Aspergillus* spp. (52%) and *Fusarium* spp. (45%) validated maize grain contamination by Aflatoxins and Fumonisin. Therefore this study shown that the consumers of maize in Kebbi State Nigeria are not excluded from the risk associated with the consumption of Aflatoxins and Fumonisin. The unsafe levels of contamination reported raises concerns with respect to the health of the individuals in this state. As a result, it should bring stakeholders together to review the food value chain from farm to folk and identify critical control points in managing the situation. Furthermore, there is a strong need to train maize producers, traders and marketers in Kebbi state, with respect to storage fungi and their effective management.

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