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Natural Occurrence of Fungi and Aflatoxins Contamination in Maize, Rice and Sorghum from Gashaka Taraba State, Nigeria

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Abstract

This work aimed to determine fungi profile and Aflatoxin levels in maize, rice, and sorghum consumed in Gashaka Local Government, Taraba State, Nigeria. Eighteen samples of maize (6), rice (6), and sorghum (6) from markets and stores were analyzed for fungi profiling and Aflatoxins using the conventional method of identification and Ultra High-Performance Liquid Chromatography (UHPLC) method respectively. Forty-one fungi species were isolated in maize, 36 in rice, and 26 in sorghum. Rice samples collected from the markets had the highest mean fungal load of (12.47 ± 10.01) x 10^4 CFU/g while sorghum samples from the market had the lowest fungal load of (2.03 ± 1.27) x 10^4 CFU/g. The predominant genera isolated were *Aspergillus* sp, *Fusarium,* and *Penicillium* sp. *Aspergillus flavus* (14.63%) and *Aspergillus tamarii* (14.63%) were the most prevalent species in maize. *Aspergillus niger* (13.88 %) and *Aspergillus flavus* (11.11 %) were predominant in rice. *Fusarium solani* (19.23%) and *Fusarium oxysporum* (13.79 %) were more prevalent in sorghum. Aflatoxins analysis revealed that 16.66% and 27.77 % of the samples exceeded the 2 µg/Kg and 4 µg/Kg EU maximum regulatory limit for AFB_1 and total aflatoxins, respectively, in all cereals intended for direct human consumption. Rice from the market had the highest Aflatoxin B_1 contamination (15.52±0.0 µg/kg), followed by maize from the market (4.15±2.28 µg/kg). Aflatoxins G_2 (2.09±0.00 µg/kg) and Aflatoxin B_1 (1.87±0.18 µg/kg) were most prevalent in stored maize. This study confirms low levels of Aflatoxins contamination in cereals from Taraba State but levels in rice exceed the European Union regulatory levels, raising public health concerns.

Keywords: aflatoxins, fungi, maize, rice, sorghum

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1. Introduction

In Nigeria, maize is a significant crop produced by smallholder farmers throughout several agroecological zones, covering approximately 6.5 million hectares of land, consistent with other nations in Sub-Saharan Africa [1]. It is the biggest cereal crop in area and production volume and is Nigeria's most-eaten staple food [1]. Approximately 80 percent of the maize grain produced is used for human consumption and used as animal feed while the remaining 20 percent is utilized for industrial processing of varied goods [1]. With a per capita consumption of roughly 35 kilograms per person yearly, maize contributes to an estimated 10% of the daily calorie intakes in the nation making it a principal source of mycotoxin. Maize is a significant source of income

for farmers and contributes greatly to agro-industrial development, particularly in the animal feed business [2]. Nigeria is Africa's top rice producer, with roughly 8.5 million tons produced in 2023. In 2023, nation consumed 7.8 million tons of rice [3], making it one of most popular basic foods. With its consumption throughout country, it is essential to disseminating mycotoxins.

Sorghum is a crop cultivated across globe for its versatile uses as a source of income, grain for foods and feeds, sweet stems, pasturage, fodder, fiber, broomcorn, fuel, bioethanol, and alcoholic beverages [4]. Sorghum is an important food source because of its nutritional quality and resilience to harsh environmental conditions [5]. It is the fifth most cultivated cereal in the world and second in Africa with estimated production of 58.7 million tons and 27.5 million tons in 2020 respectively [6]. Along with maize and rice, it is one of the staple cereals in Nigeria. The estimated annual economic losses encountered with sorghum and sorghum products are more than $130 million [7]. However, many fungal species are considered common contaminants of food and feed because they are ubiquitous and can thrive in various environmental conditions. Filamentous toxigenic moulds produce mycotoxins. The presence of moulds and mycotoxins in foods and feed reduces the quality and harms the supply chains including crop producers, animal producers, grain handlers and distributors, processors, consumers, and society as a whole [8].

It is estimated that 25 to 50% of the foodstuffs are contaminated with mycotoxins. Generally, cereals and nuts are the primary sources of mycotoxins. Mycotoxicosis is the disease caused by consuming food or feed contaminated with mycotoxins and it is non-transmissible, unresponsive to antibiotics, and associated with seasonal outbreaks. Aflatoxin, (*Aspergillus flavus* toxin), a major mycotoxin is produced primarily by the *Aspergillus flavus* and *A. parasiticus*. Aflatoxins (AF) are furanocoumarins and occur in various chemical forms such as AFB_1, AFB_2, AFG_1, AFG_2, and AFM_1 [9]. It is known to cause gross liver damage, resulting in liver cancer (hepatocarcinogen), and colon and lung cancer. Aflatoxin B_1 is classified as a carcinogen by the International Agency for Research on Cancer. The AFB_1 suppresses the immune system, promotes inflammation, and inhibits the growth of humans and animals [9]. This work aims to evaluate the natural occurrence of fungi and the aflatoxins contamination in maize, rice, and the sorghum samples from the Gashaka local government of the Taraba State, Nigeria.

2. Material and Methods

2.1. Study Area

Gashaka Local Government Area (LGA) is situated in the Southeast of Taraba State and extends to approximately between 11°00′–12°00′E and 07°30′–08°00′N. This LGA is bordered to the Southeast by the Republic of Cameroon, to the north and east by Adamawa State, and the west by Kurmi and Bali LGAs while to the south by Sardauna LGA (Fig.1). Situated within the Derived Savannah, this region boasts a mountainous terrain and unpredictable atmospheric conditions that range from tropical moist-humid to dry-humid in the lowlands and sub-temperate in the highlands. The rainy season spans from April to November, while the dry season prevails from November to April [10].

2.2. Sample Collection

A total of 18 samples of maize, rice, and sorghum were collected from both stores and markets in February 2024. Samples were collected following the European Commission Regulation [11]: 1kg of each sample was collected, labeled, packaged in polythene bags, and taken to the laboratory. A total of six samples for each crop were collected from the markets (3) and Stores (3).

2.3. Sample Preparation

Before analysis, each sample was milled using a sterile mechanical blender (Labinco, Breda, and The Netherlands). Ten (10) g of each were separated to be used for fungal isolation. All the samples were stored at 4 °C in a freezer to prevent further post-harvest accumulation of molds before analysis.

2.4. Fungal Isolation, Macroscopic and Microscopic Identification

The mycological analytical procedure involving four steps was used including fungal isolation on potato dextrose agar (PDA), sub culturing on PDA, malt extract agar (MEA), and yeast extract agar (YEA), macro- and microscopic identification [12]. Primarily, each milled sample was subjected to a six-level serial dilution technique in which 1 g was diluted in a 9-mL of sterile distilled water, vortexed, and subsequently, 1 mL of the suspension was transferred to a 9 mL of sterile distilled water, and vortexed. 100 µL of each suspension was inoculated on solid PDA containing 1 % Chloramphenicol in 90-mm Petri dishes and incubated at room temperature for 3-5 days. Between the 3rd and 5th day of incubation, all colonies were counted and results were presented as the number of fungal colonies per gram of sample calculated and expressed in colony-forming units per gram (CFU/g) as shown in Equation 1.

$$\mathrm{CFU/g} = \frac{\text{Number of colonies} \times \text{Reciprocal of the dilution factor}}{\text{plating volumes (ml)}}$$

The determination of each species of fungi was done using the keys of Klich and Pitt [13], Klich [14], and Nyongesa [15], for *Aspergillus* spp*,* Pitt and Hocking [16] for *Penicillium* and other genera. The fungal morphology was studied macroscopically by observing colony features (color, shape, size, and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue-stained slide mounted with a small portion of mycelium.

2.5. Determination of Aflatoxins Using the UHPLC Method

Aflatoxin standard containing AFB_1, AFB_2, AFG_1, and AFG_2 in the ratio 4/1/4/1 was purchased from Trilogy Analytical Laboratory, USA. De-ionized was gotten from Elga Water Purification System by Veolia Water Solutions and Technologies, UK. Methanol and Acetonitrile (HPLC – Gradient Grade) were obtained from VWR International FontenaySous-Bois France. The Aflaclean™ immunoaffinity columns (IAC) for Aflatoxins B_1, B_2, G_1, and G_2 were purchased from LCTech GmbH Germany.Sodium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, and potassium chloride, utilized in the preparation of phosphate buffer saline solution (PBS), were sourced from Sigma Aldrich Darmstadt, Germany.

- ***Aflatoxin Standard Preparation***

The total aflatoxin standard as 5 µg/mL solution was made to an intermediate solution of 1 µg/mL. From it, a four-point calibration was prepared as shown below
Standard 4: 80 µL of 1 µg/mL was made up to 2 mL with 50 % methanol (this is equivalent to 40 ng/mL).
Standard 3: 1 mL of standard 4 was added to 1 mL of 50 % methanol (equivalent to 20 ng/mL).
Standard 2: 1 mL of standard 3 was added to 1 mL of 50 % methanol (equivalent to 10 ng/mL).
Standard 1: 400 µL of 10 ng/mL was made up to 2 mL with 50 % methanol (equivalent to 2 ng/mL).

2.6. Sample Preparation

The samples were ground using a sterile mechanical blender (Labinco, Breda, The Netherlands and stored in the freezer at a temperature of - 18 ºC until it was needed for analysis. A 12.5 g of the finely ground maize samples and 1.25 g of sodium chloride were weighed into a 50 mL centrifuge tube. A 25 mL of 80 % methanol was added and subsequently agitated on an orbital shaker at 400 rpm for 10 minutes. It was then centrifuged at 4000 rpm for 10 minutes. 2 mL of the supernatant was diluted with 14 mL of phosphate-buffered saline (PBS). The mixture was passed through the Aflaclean^{TM} immunoaffinity columns by gravity. The column was washed with 20 mL of PBS and air was passed through the column to remove residual liquid. The trapped aflatoxins were eluted (by back flushing) with 1 mL of 100 % methanol and it was collected in an amber glass vial. After the elution, 1 mL of water was passed through the column and it was collected in the same vial. Thereafter, 10 µL was injected into the HPLC system.

2.7. Instrumentation and Chromatographic Condition

The analytical process was carried out with a reverse phase HPLC – Dionex Ultimate 3000 UHPLC (Thermo Fisher Scientific, Waltham, MA, United States) equipped with a quaternary pump, autosampler, and a fluorescence detector (FLD). Chromatographic separation was performed on Thermo Scientific ODS Hpersil C18 column (250 mm x 4.6 mm and 5 µm particle size) from Massachusetts, United States. The mobile phase was a mixture of methanol and water (40: 60). Each of the mobile was on a different line (proportioning valve) of the pump system and it was isocratically delivered at a flow rate of 1 mL/min for a run time of 30 minutes. The analytical column oven was maintained at 40 ºC. The fluorescence detector was set at an excitation and emission wavelength of 362 nm and 425 nm respectively. Recording and evaluation of the chromatogram was carried out with chromeleon version 7.2 software. The standard showed sufficient linearity with a correlation coefficient of 0.9995, 0.9999, 0.9999, and 0.9998 for AFG_2, AFG_1, AFB_2, and AFB_1 respectively.

2.8. Statistical Analysis

All the analytical data generated were subjected to statistical analysis using SPSS (version 10.0) software. The statistical level of significance was fixed at $P < 0.05$ (95%).

3. Results and Discussion

Fig. 2 shows the fungal load in the selected grain collected from stores and markets in Gashaka, Taraba State Nigeria. Rice samples collected from the markets had the highest mean fungal load of (12.47 ± 10.01) x 10^4 CFU/g while sorghum samples from the market had the lowest fungal load of (2.03 ± 1.27) x 10^4 CFU/g. Although there was no consistent trend, i.e., decrease or increase in fungi load from store to market ($P<0.05$). All the samples had fungal load above the permissible limit of (1 x 10^4) in ready-to-eat foods based on the microbiological criteria [17]. A total of 103 molds isolated from 18 maize, rice, and sorghum samples collected in markets (9) and stores (9). Based on macroscopic and microscopic observations, the mould strains grouped (Table 1) into 27 species belonging mainly to 7 fungal genera, namely *Aspergillus* sp (42.87%), *Fusarium* sp (33.50%), *Penicillium* sp (18.32%), *Rhizopus* sp (3.46%), *Absidia* sp (0.5%), *Curvularia* sp (0.3%) and *Cladosporium* sp (0.1%). *Aspergillus* sp was the most present genus among all isolated genera. 12 species were identified, *Aspergillus candidus, Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceus, Aspergillus oryzae, Aspergillus tamarii, Aspergillus terreus, Aspergillus parasiticus. Aspergillus versicolor*.

This is consistent with previous studies indicating *Aspergillus* exhibits dominance in Nigerian foods [18-20]. The dominance of *Aspergillus* in all samples may also be attributed to its ability to utilize a wide variety of organic substrates and adapt well to a broad range of environmental conditions as outlined [21]. *Aspergillus flavus* was the most dominant species (12.62%) followed by *Aspergillus niger* (10.67%). *A. flavus* and *A. niger* are frequently isolated from maize, with *A. flavus* being a major producer of aflatoxins [22]. The high prevalence of *Aspergillus flavus* implies that frequent consumption of these grains is a contributory factor to the high exposure of Aflatoxin in Nigeria [23-24]. The second most present genus was the *Fusrium* sp with 7 species. These were: *Fusarium cerealis, Fusarium equiseti, Fusarium graminearum, Fusarium oxysporum, Fusarium proliferatum, Fusarium solani, Fusarium verticillioides*. The most present species were: *Fusarium solani* (9.87%), followed by *Fusarium oxysporum* (9.06 %). This finding is consistent with previous studies that showed the *Fusarium* in grains like maize and rice in the Nigeria [19], in Brazil [25], in the Ethiopia [26].

F. verticillioides and *F. proliferatum* are prevalent in grains, and are reported to be producers of fumonisins, a mycotoxin associated with serious health risks, like esophageal and liver cancers [27]. The occurrence of *Penicillium* in the samples is consistent with previous studies where *Penicillium* was reported to be the predominant fungal genera [23-28]. *Penicillium* species detected were *Penicillium citrinum, Penicillium pinophilum, and, Penicillium verrucosum* in order of their prevalence. Other fungal species identified were *Rhizopus spp, Absidia corymbifera, Cladosporum oxysporum, Culvaris affinis, Curvularia clavata,* and *Curvularia spp.* Citrinin is a mycotoxin produced by *P. citrinum*, *P. expansum*, *P. radicicola*, and *P. verrucosum* which has various health effects like nephrotoxicity, effects on embryos, and intestinal cell apoptosis [29]. Aflatoxins contamination in maize, rice, and sorghum samples from stores, and markets from Gashaka, Taraba State is presented in Table 2 below. Rice samples from the market had the highest mean of 10.98 ± 10.3 µg/Kg. Sorghum samples from the stores had the lowest mean concentration of 0.19 ± 0 µg/Kg. Sorghum samples were less contaminated by aflatoxins than maize and rice.

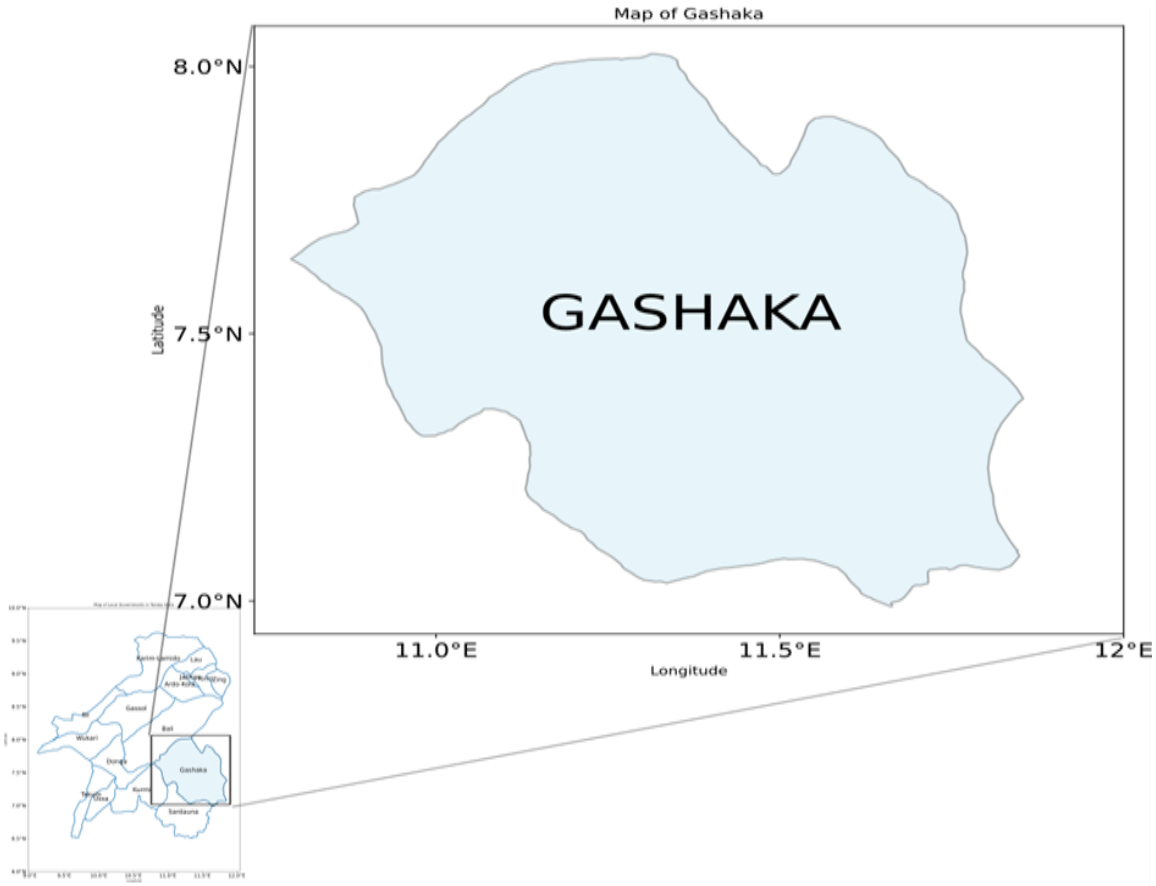

Fig. 1: Figure of Gashaka local government of Taraba State, Nigeria

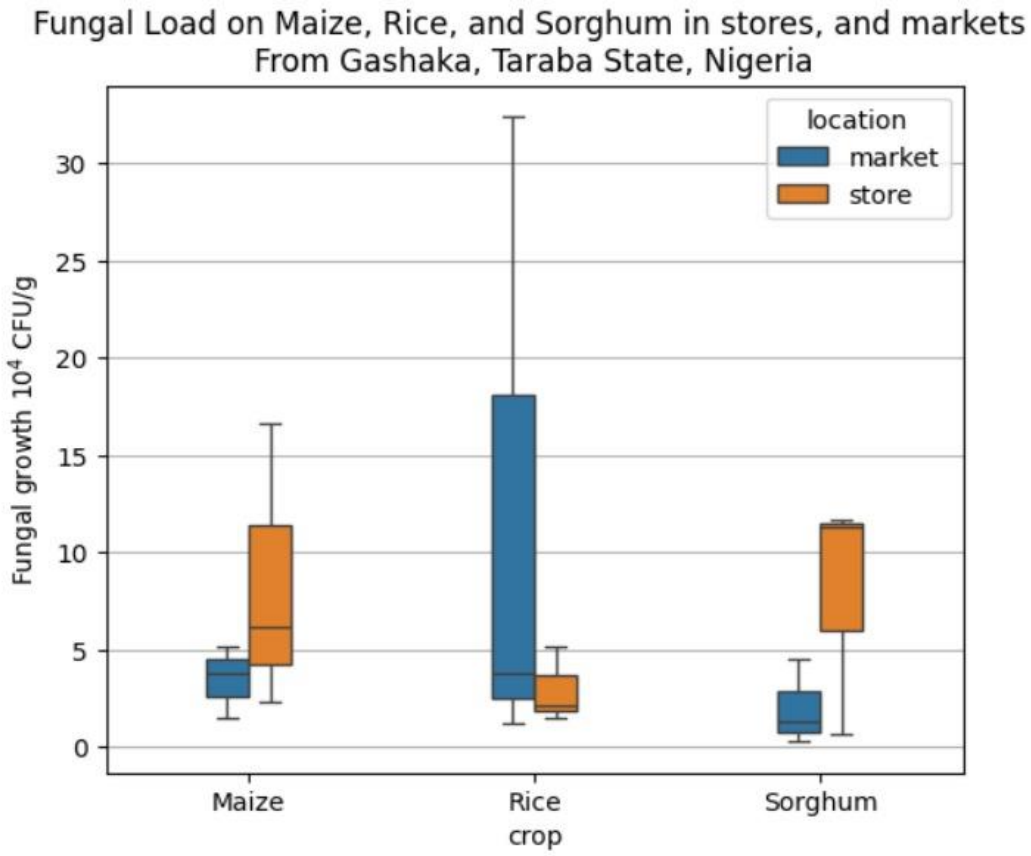

Fig. 2: Fungal Load in Maize, Rice, and Sorghum (store, and market) From Gashaka, Taraba State, Nigeria

Table 1: Fungi profile in Maize, Rice, and Sorghum from Gashaka Local government of Taraba State, Nigeria

Fungal species	Maize n=6			Rice n=6			Sorghum n=6			Total n=18
	Store n=3	Market n=3	Total n=6	Store n=3	Market n=3	Total n=6	Store n=3	Market n=3	Total n=6	
Aspergillus candidus	1(33.33)	1(33.33)	**2**	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	2
Aspergillus carbonarius	1(33.33)	2(66.66)	**3**	1(33.33)	2(66.66)	**3**	0(0.00)	0(0.00)	**0**	6
Aspergillus clavatus	1(33.33)	1(33.33)	**2**	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	2
Aspergillus flavus	3(100)	3(100)	**6**	2(66.66)	2(66.66)	**4**	2(66.66)	1(33.33)	**3**	13
Aspergillus fumigatus	0(0.00)	0(0.00)	**0**	1(33.33)	0(0.00)	**1**	0(0.00)	0(0.00)	**0**	1
Aspergillus niger	1(33.33)	3(100)	**4**	3(100)	2(66.66)	**5**	1(33.33)	1(33.33)	**2**	11
Aspergillus tamarii	3(100)	3(100)	**6**	1(33.33)	1(33.33)	**2**	0(0.00)	0(0.00)	**0**	8
Aspergillus terreus	0(0.00)	1(33.33)	**1**	0(0.00)	1(33.33)	**1**	0(0.00)	0(0.00)	**0**	2
Aspergillus ochraceus	0(0.00)	0(0.00)	**0**	1(33.33)	0(0.00)	**1**	0(0.00)	0(0.00)	**0**	1
Aspergillus oryzae	0(0.00)	0(0.00)	**0**	3(100)	1(33.33)	**4**	0(0.00)	0(0.00)	**0**	4
Aspergillus parasiticus	1(33.33)	1(33.33)	**2**	1(33.33)	1(33.33)	**2**	0(0.00)	0(0.00)	**0**	4
Penicillium citrinum	1(33.33)	0(0.00)	**1**	2(66.66)	1(33.33)	**3**	0(0.00)	0(0.00)	**0**	4
Penicillium pinophilum	0(0.00)	1(33.33)	**1**	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	1
Penicillium verrucosum	1(33.33)	0(0.00)	**1**	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	1
Fusarium cerealis	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	0(0.00)	1(33.33)	**1**	1
Fusarium equiseti	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	1(33.33)	0(0.00)	**1**	1
Fusarium graminearum	1(33.33)	1(33.33)	**2**	1(33.33)	0(0.00)	**1**	1(33.33)	0(0.00)	**1**	4
Fusarium oxysporum	2(66.66)	0(0.00)	**2**	0(0.00)	1(33.33)	**1**	3(100)	1(33.33)	**4**	7
Fusarium proliferatum	1(33.33)	1(33.33)	**2**	1(33.33)	0(0.00)	**1**	1(33.33)	0(0.00)	**1**	4
Fusarium solani	1(33.33)	2(66.66)	**3**	1(33.33)	2(66.66)	**3**	2(66.66)	3(100)	**5**	11
Fusarium verticilloides	1(33.33)	1(33.33)	**2**	1(33.33)	2(66.66)	**3**	2(66.66)	0(0.00)	**2**	7
Absidia corymbifera	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	0(0.00)	1(33.33)	**1**	1
Cladosporum oxysporum	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	1(33.33)	0(0.00)	**1**	1
Culvularia affinis	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	1(33.33)	0(0.00)	**1**	1
Curvularia clavata	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	0(0.00)	1(33.33)	**1**	1
Curvularia spp	0(0.00)	0(0.00)	**0**	0(0.00)	0(0.00)	**0**	1(33.33)	0(0.00)	**1**	1
Rhizopus spp	0(0.00)	1(33.33)	**1**	1(33.33)	0(0.00)	**1**	0(0.00)	1(33.33)	**1**	3
Total	19	22	**41**	20	16	**36**	16	10	**26**	103

n: number of analyzed samples

Table 2: Occurrence of Total Aflatoxin in Maize, Rice, and Sorghum (µg/Kg)

	Maize n=6		Rice n=6		Sorghum n=6	
Mycotoxins	Store n=3	Market n=3	Store n=3	Market n=3	Store n=3	Market n=3
AFB_1	1.87 ± 0.18	4.15 ± 2.28	0.21 ± 0.01	15.52 ± 0	0.19 ± 0	ND
AFB_2	0.32 ± 0.3	0.46 ± 0.39	ND	ND	ND	ND
AFG_1	2.09 ± 0	1.55 ± 0	ND	ND	ND	ND
AFG_2	0.56 ± 0	0.15 ± 0.0034	ND	ND	ND	ND
Total Aflatoxin	2.01 ± 0.87	2.77 ± 2.05	0.27 ± 0.01	10.98 ± 10.3	0.19 ± 0	ND

AFB_1: Aflatoxin B_1, AFB_2: Aflatoxin B_2, AFG_1: Aflatoxin G_1, AFG_2: Aflatoxin G_2, ND: Non-Detectable, n: number of samples

16.66% and 27.77 % of the samples exceeded the 2 µg/Kg and 4 µg/Kg EU maximum regulatory limit for AFB_1 and total aflatoxins in all cereals intended for direct human consumption [11]. Aflatoxin contamination ranged from 0–15.52 µg/Kg in market samples and from 0–2.09 µg/Kg in store samples. Rice had the highest level of fungal load and aflatoxin contamination due to its high starch content [30]. Fungal and aflatoxin contamination in sorghum is relatively lower as indicated in this study because certain sorghum species exhibit resistance to fungal and aflatoxin contamination [31]. These results aligned with other studies in Nigeria. Mycotoxin contamination of stored maize grains was assessed in Kebbi State Nigeria [32]. Of the 21 samples from the Gwandu Emirate, 16 (76.2%) were contaminated with aflatoxin. The highest aflatoxin level was AFB_1 (1.0–221.0 µg/kg). AFB_1, AFB_2, AFG_1, and AFG_2 together accounted for 67% of all aflatoxins (total aflatoxins) detected in maize with a maximum of 3863 µg/kg and a mean value of 128 µg/kg. Comparing maize to other foods, aflatoxin was more common overall. In another study, twenty-one rice samples from the field (ten), store (six), and market (five) from the traditional rice-growing areas of Niger State, Nigeria analyzed for aflatoxins, aflatoxins detected in all samples, at total aflatoxin concentrations of 28–372 µg/kg [33].

4. Conclusions

Gashaka is located in the Derived Savanna in Nigeria and is characterized by unpredictable atmospheric conditions ranging from tropical moist-humid to dry-humid in the lowlands and sub-temperate in the highlands supporting the proliferation of fungi and the production of Aflatoxins in grains. Seasonal variations, particularly during the wet and dry seasons, create favourable conditions for various fungal species to thrive. Poor storage conditions in these humid environments contribute to the rapid growth of fungi in grains. The common fungal genres in Maize, Rice, and sorghum found in this study were *Aspergillus*, *Penicillium*, and *Fusarium*. Although the number of samples analysed in this study was limited, some samples' high levels of aflatoxin clearly show the necessity for increased surveillance. Many more samples from different processing points around the country should be analysed. Our results in this pilot work indicated that because of high aflatoxins in some samples, a considerable percentage of the rice and maize from the Nigerian market could be considered unsafe for human consumption.

Conflict of interest

Authors declare no conflict of interest in this study.

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