ORIGINAL ARTICLE

Incidence, Distribution and Phenotypic Characterisation of Aflatoxigenic Fungi Contaminating Commonly Consumed Food Grains in Katsina State, Nigeria

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ABSTRACT

Introduction: The use of traditional storage facilities by most of the grain farmers and traders in Nigeria promotes fungal contamination of stored grains and subsequently mycotoxins which are potent carcinogens, neurotoxic, hepatotoxic and immunotoxic when consumed. This study was conducted to determine the incidence and phenotypes of mycotoxigenic fungi associated with commonly consumed food grains in Katsina state, Nigeria. **Method:** Fungal population in 21 composite samples each of maize, wheat, rice and peanuts from three open markets were determined using standard mycological techniques. *Aspergillus* spp obtained from the samples were screened for aflatoxigenicity and subsequently characterised by Attenuated Total Reflectance Fourier Transformed Infrared (ATR-FTIR) spectroscopy. **Results:** A total of 136 filamentous fungi belonging to 19 species were isolated, of which *Aspergillus flavus* (18.4%), *Mucor racemosus* (13.2%) and *Aspergillus niger* (10.3%), were predominant. The highest level of contamination was found in the peanuts ($1.8 \times 10^5 \pm 2.5 \times 10^5$ CFU/g). All the 12 *Aspergillus parasiticus* and 18 (72%) of *Aspergillus flavus* isolates obtained from the samples produced aflatoxin B1 on solid media as observed under ultraviolet light and confirmed by Thin Layer Chromatography. The ATR-FTIR spectra of both toxigenic and atoxigenic *Aspergillus* spp showed similar pattern. **Conclusion:** The levels of the mycotoxigenic fungi in the food grains, except for rice, were above the permissible limit of 100 to 10,000 CFU/g set by ICMSF, this signifies that they are unsafe for use as food or feed ingredients and hence, the need for more stringent control measures.

Keywords: Aflatoxigenicity, ATR-FTIR, Food grains, Incidence, Mycotoxigenic fungi

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INTRODUCTION

Mycotoxins are heterogeneous group of low molecular weight toxic organic compounds that are produced as secondary metabolites by some filamentous fungi as they grow on various substrates. Fungi, owing to their ubiquitous nature and ability to produce and germinate from spores of various types as well as possession of several virulence factors, have been identified as major group of biological agents associated with many health hazards especially those related to agricultural products (1–3). Many fungal species, particularly *Fusarium* spp, *Aspergillus* spp and *Penicillium* spp can contaminate various crops in the field and/or during storage and consequently produce wide array of toxic metabolites, mycotoxins (3–7) which are toxic to humans, animals and plants (8,9).

Fungal food spoilage and foodborne toxicities have resulted in illnesses and deaths of humans, animal and plants, as well as spoilage of large per cent of the worlds' food products leading to a significant decrease in plants and animal productivities and overall economic loss in many countries (10,11). Thus, the need for rapid and reliable techniques for the detection of mycotoxigenic fungi and their mycotoxins in foods is of paramount importance.

Nigeria is one of the major grain-producing African countries. It is the largest producer of rice in west Africa (12). In 2019, it produced over 30 million metric tons of

maize, rice, sorghum and wheat grains (12). However, poor storage facilities and favourable environmental conditions such as warm temperature has led to the contamination of various agricultural products in Nigeria by mycotoxigenic fungi and mycotoxins. This has contributed to the rejection of over 120 Nigerian food products by the European Union from 2013 to 2016 alone due to their poor quality (13).

Katsina state is one of the major grain producing and consumer state in Nigeria where traditional storage facilities are commonly used by both farmers and grain sellers (14). However, although, there were various reports of fungal contamination of agricultural products in the state, most of those published reports focused on vegetables (4,15–20). Therefore, the aim of the present study is to determine the incidence and distribution of mycotoxigenic fungi contaminating food grains in Katsina state, evaluate the toxigenicity of the most dreadful species isolated (*Aspergillus flavus* and *Aspergillus parasiticus*) and determine the Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy (ATR-FTIR) spectra of the toxigenic and atoxigenic isolates among them. Being the first study of this nature from Katsina state, the data generated (incidence, distribution and ATR-FTIR spectral profiles) will serve as reference data for the incidence/prevalence of mycotoxigenic fungi in cereals and peanuts and will help in evaluating the safety of Katsina state foods. It could be used in generating and predicting the degree of fungal and aflatoxin contamination of grains in Katsina for effective aflatoxin control. It is hypothesised that rapid yet simple cost-effective techniques such as preparative Thin Layer Chromatography and FTIR-ATR can be harness for rapid determination of mycotoxigenic fungi in foods or feeds.

MATERIALS AND METHODS

Study area, sample collection and preparation

The study area is Katsina state which is one of the major agricultural states in Nigeria with a total population of 5,801,584 million peoples of which more than 90% are involved in one or more farming practice (21). It is divided into three major senatorial zones, namely Katsina North (KN), Katsina South (KS) and Katsina Central (KC) consisting of 12, 11 and 11 Local Government Areas (LGA) respectively. Each LGA has a central open market, and for this study, one open market was randomly selected from each zone making three open markets namely, Daura (KN), Dutsinma (KC) and Funtua (KS), with an average temperature at the time of the 3-day sampling were 33.1°C, 29.6°C and 28.2°C respectively. A total of 84 food grain samples comprising 21 composite samples each of maize, wheat, rice and peanuts were purchased from the three selected open markets. These food products were selected for this study because of their high consumption rate in the state (21). Each composite sample was made from three different samples of same variety and brand purchased from different vendors. The total sample size for this study was estimated at 95% confidence level based on the previously reported prevalence rate (80%) of fungi and mycotoxin contamination of food grains in other part of Nigeria (22). Each of the food samples was collected in a sterile polythene bag (23), labelled and transported to laboratory for analysis. Each of the samples was ground into crystalline powder, stored in a sterile air-tight container in dark cold room at 4°C until use.

Determination of fungal load in each sample

Determination of incidence of fungi in each sample was carried out as described elsewhere (7) with some modifications. Ten grams from each sample was homogenised with 90 ml of Brain Heart Infusion broth (BHIB) (MERK 1.10493 Milliphore) medium and serial decimal dilutions (10^1 to 10^4) was performed using test tubes containing 9 ml each of sterilised BHIB. The test tubes were incubated overnight at 30°C (24). Subsequently, an inoculum of 1 ml from each test tube was pipetted and spread-plated onto sterilised Potato Dextrose Agar (PDA) (HiMedia, M096) media plates in triplicate each and incubated for five days at 28±2°C. Plates were observed at day three and day five and various mould fungal colonies that developed from each PDA plate were counted according to their types, and the overall total colony counts in each plate were determined and recorded as total colony-forming units (CFU) per gram of the sample.

Identification of the fungal isolates

Various fungal colonies from the PDA plates were sub-cultured on to Sabouraud Dextrose agar (SDA) (HiMedia, M063) plates and incubated for 3 to 5 days at 28±2°C to obtain pure isolates in each sample. Additionally, isolates (*Aspergillus* species only) were also sub-cultured on AFPA (*Aspergillus flavus* and *Aspergillus parasiticus* Differential Medium, Oxoid UK CM0731) plates for further phenotypic characterisation. Subsequently, each isolate was subjected to slide culture, stained with lactophenol cotton blue (LPCB) stain, observed under microscope using x10 and then x40 objective lenses. Isolates were then identified based on their microscopic characteristics (types and arrangement of hyphae, conidiophore vesicle, conidia heads, ornamentation, phialides and fruiting body) and macroscopic/cultural appearance on SDA and AFPA (colonies colours, texture, reverse colour, hyphae arrangement, conidia shape and nature of spores) with the aid of the references and keys (7,25–28).

Aflatoxigenicity screening

All the *Aspergillus flavus* and *Aspergillus parasiticus* isolates obtained from the peanuts and cereal samples were subjected to aflatoxigenicity test. Each of the isolates was sub-cultured on SDA and AFPA media and incubated at 30°C for five days. All the plates were viewed under UV light at 365 nm. Isolates that

produced blue fluorescence around their colonies were identified as aflatoxigenic (toxigenic species) while those that do not fluoresce were categorised as non-aflatoxigenic (atoxigenic species) (29). As a modification of the method to enhance the fluorescence, few drops of methanol:water, 3:1 (v/v) were added on the surface of the colonies to dissolve and bring the aflatoxin (if any) in the medium to the surface for better fluorescence.

Confirmatory test for the aflatoxigenicity of the isolates by thin layer chromatography (TLC)

To confirm the production of aflatoxin in the solid media by the toxigenic isolates, this method was adopted and modified from previously described method of extracting and quantifying mycotoxins from fungal growth (30). Twenty millilitre (20 ml) of methanol:water, 3:1 (v/v) solvent was added to the surface of the Aspergillus colonies in each plate, allowed to stay for 30 minutes, poured the suspension into separate beakers, filtered with Whattman number one filter paper, dried in drying oven at 45˚C and the resulting extracts were spotted on glass coated silica gel plates (20 x 20 cm, MERK 60 F254) alongside the AFB1 standard solution (Pribolab China, STD#1041U) as positive control and blank (methanol-water solvent) as negative control. The plates were developed in TLC tank (Kontes, Vineland USA) using Dichloromethane-methanol, 5:1 (v/v) as mobile phase. The mobile phase was predetermined and validated by means of preparative thin layer chromatography (Prep. TLC) prior to the aflatoxigenicity screening. The plates were viewed under UV at 365 nm (31). The isolates whose extracts produce TLC bands with blue fluorescence under UV that corresponds to that of the AFB1 standard were confirmed as aflatoxigenic isolates.

Fourier transformed infrared spectroscopy (FTIR) of the toxigenic and atoxigenic isolates

The toxigenic and atoxigenic *Aspergillus* species section *flavi* were analysed by Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy (ATR-FTIR) as described elsewhere (32) with little modification. A small portion of fungal growth from the fungal culture previously soaked with the methanol:water, 3:1 (v/v) solvent was placed on the ATR diamond crystal using sterile forceps and scanned from 650 to 4000 cm^{-1} for 16 times to increase the signal to noise ratio and averaged as a single spectrum. FTIR spectra were recorded in the absorption range between 650 and 4000 wavenumber per centimetre at a resolution of 4 cm^{-1}. Prior to each sample scan, a background scan was made and later subtracted from the sample spectrum. A baseline correction, smoothening, normalisation and automated pick picking for each spectrum was done using FTIR Opus software version 6.5 (Bruker, Germany). The types of chemical bonds/functional groups present in the samples were identified by interpreting the infrared absorption spectrum using IR spectra Table. Spectrum comparison was carried out to detect the differences between the toxigenic and atoxigenic *Aspergillus* spp.

Validation of the ATR-FTIR Method

Prior to adoption, the FTIR method was assessed for specificity, repeatability, and reproducibility. The specificity was assessed by comparing the FTIR spectra of the blank (solvent), toxigenic *Aspergillus* spp and atoxigenic *Aspergillus* spp for the presence of interference peaks. The repeatability (intra-day precision) was determined and expressed as percentage relative standard deviation (% RSD) of wavenumbers specific to fungal mycelium (32) obtained by analysing three replicates of 5-day old *Aspergillus* cultures. On the other hand, the reproducibility (inter day precision) was assessed through repeated measurements of different triplicate sub-cultures of the *Aspergillus* spp once in a week for three weeks. The variation between the inter day wavenumbers were expressed as % RSD.

RESULTS

Incidence of mould fungi in the samples

All the samples analysed in this study were contaminated by at least one or more mould fungi except for eight samples (three wheat samples and five rice samples) which were only contaminated by yeast fungi. A total of 136 mould fungal isolates were obtained from the 84 food samples analysed. Of these 136 isolates, 46 (33.8%) were obtained from the peanut samples; 45 (33.1%) were obtained from the maize samples, 23 (16.9%) were isolated from wheat samples and 21 (16.2%) isolates from the rice samples (Table I). The average contamination level in all the samples ranges from 1.0×10^3 to 8.4×10^5 CFU/g. The highest average fungal count was found in the peanuts ($1.8 \times 10^5 \pm 2.5 \times 10^5$ CFU/g) followed by wheats ($1.5 \times 10^5 \pm 1.6 \times 10^5$ CFU/g), maize ($1.1 \times 10^5 \pm 1.9 \times 10^5$ CFU/g), and rice ($8.9 \times 10^4 \pm 1.6 \times 10^5$ CFU/g).

Table I : Summary of incidence of mould fungi in the peanuts and food grains from Katsina state, Nigeria

Statistics	Samples			
	Peanut Samples	Maize Samples	Wheat Samples	Rice Samples
Sample Size (N)	21	21	21	21
No of fungal isolates obtained (n = 136)	45	44	22	21
Mean (CFU/g)	1.8×10^5	1.1×10^5	1.5×10^5	8.9×10^4
Standard Error of Mean	5.5×10^4	4.1×10^4	3.6×10^4	3.4×10^4
Median	1.1×10^5	4.1×10^5	1.0×10^5	5.0×10^3
Standard Deviation	2.5×10^5	1.9×10^5	1.6×10^5	1.6×10^5
Minimum	3.0×10^3	3.0×10^3	1.0×10^2	1.0×10^2
Maximum	1.1×10^6	8.4×10^5	6.0×10^5	5.0×10^5

Morphological characterisation of the fungal isolates

The macroscopic and microscopic characterisation of the 136 fungal isolates obtained from the 84 food samples analysed revealed that these isolates belonged to 19 different fungal species with variable frequencies of isolation. Of the 136 isolates, 25 (18.4%) were identified as *Aspergillus flavus*, 18 (13.2%) *Mucor racemosus*, 13 (10.3%) *Aspergillus niger*, 12 *Aspergillus parasiticus* (8.8%), 9 *Fusarium graminearum* (7.6%), 7 *Rhizopus stolonifer* (5.2%), 6 (4.4%) *Aspergillus fumigatus*, 6 (4.4%) *Penicillium chrysogenum*,6 (4.4%) *Penicillium notatum*, 5 (3.7%) *Fusarium solani*, 5 (3.7%) *Fonsecaea pedrosoi*, 4 (2.9%) *Penicillium cheresanum*, 3 (2.3%) *Acrimonium kliense*, 3 (2.2%) *Fusarium verticillioides*, 2 (1.5%) *Aspergillus nidulans*, 2 (1.5%) *Fusarium proliferatum*, 2 (1.5%) *Paecilomyces lilacinus*, and 2 (1.5%) *Penicillium verrucosum* (Table II).

Similarly, the distribution and frequency of occurrence of the isolates in each food category are shown in Fig. 1. Of the 19 species of mould fungi identified, peanuts samples were contaminated by 16 species, maize samples were contaminated by 15 species, while the rice and wheat samples were contaminated by 9 species each (Fig. 1).

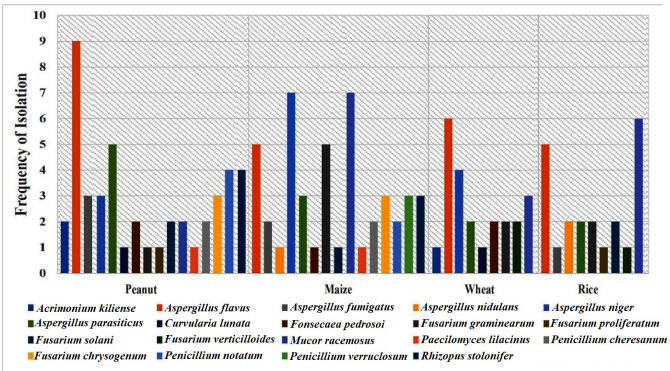

Fig. 1 : Distribution of the fungal isolates in the cereals and peanut samples

Aflatoxigenicity screening

To determine the health significance of the *Aspergillus flavus* and *Aspergillus parasiticus* species isolated from the food samples analysed in this study, the isolates were screened for their ability to produce aflatoxin B1 (AFB1) on solid media. Of the 25 *Aspergillus flavus* and 12 *Aspergillus parasiticus* isolates screened for their ability to produce aflatoxin on solid media (SDA and AFPA), 18 (72%) of the *Aspergillus flavus* isolates were toxigenic while all the 12 *Aspergillus parasiticus* isolates were toxigenic as evidenced by the blue fluorescence around their colonies under UV light at 365 nm. The intensity of the blue fluorescence was higher when few drops of methanol:water, 3:1 (v/v) solvent was added to the surface of the *Aspergillus* colonies (Fig. 2). Similarly, the result of the TLC confirmatory test for the aflatoxigenicity of the *Aspergillus* isolates gave same number of toxigenic and atoxigenic species. In fact, some of the AFB1 TLC bands produced by the isolates showed more intense blue fluorescence under the UV light compared to the standard AFB1, indicating that some of the atoxigenic isolates produced AFB1 at concentration higher than that of the standard AFB1 (0.5 µg/ml) used (Fig. 3).

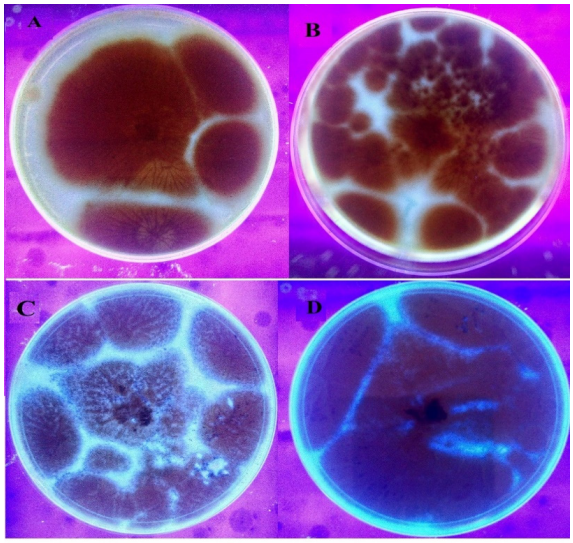

Fig. 2 : Appearance of the toxigenic and atoxigenic *Aspergillus* spp under UV. The intensity of the blue flourescence was enhanced by adding few drops of methanol:water (3:1). A and B are toxigenic showing blue fluorecence arround their colonies under UV light at 365 nm. C and D are the atoxigenic species.

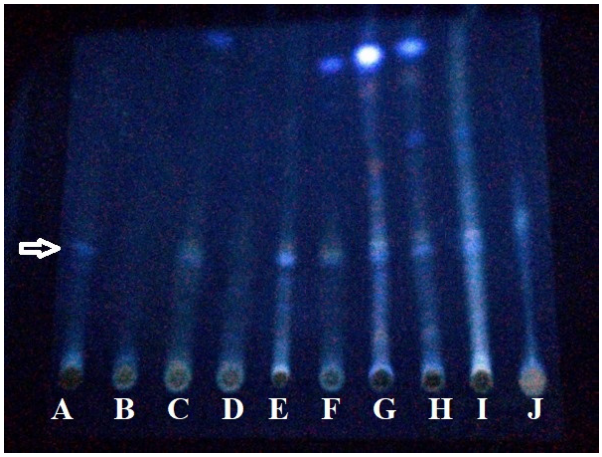

Fig. 3 : TLC plate showing the AFB1 bands detected from the plate extract of the atoxigenic and toxigenic *Aspergillus* isolates. The arrow showed the position of the AFB1 band. A is the AFB1 standard (0.5 µg), B is the blank (negative control), C to J are the samples. C, E, F, G, H, I and J are for toxigenic isolates as evidenced by their blue band which corresponds to that of the AFB1 standard used. D is for the atoxigenic isolate as it has no AFB1 band.

Fourier Transformed Infrared Spectroscopy (FTIR) of the toxigenic and atoxigenic isolates

The result of ATR-FTIR spectroscopy showing the Mid-Infrared (MIR) spectral fingerprints of the atoxigenic and toxigenic *Aspergillus* spp isolated in this study is shown in Fig. 4. Being phylogenically related species, the spectra of both *Aspergillus flavus* and *Aspergillus parasiticus* were very much similar. They have many peaks denoting some common functional groups such as lipids, proteins, carbohydrates, phospholipids, nucleic acids, polysaccharides as well as primary and

Table II : Morphological characterisation of the fungal isolates and their frequency of isolation in the food samples

Isolate Code	Characteristics		Name of Isolate	Frequency of Isolation	
	Macroscopy	Microscopy		n	%
001	Globous, velvety and pale grey colonies with a pale reverse side	Hyaline, septate, unbranched hyphae with short erect conidia at the hyphal tips and the apices of the phialides bear clusters of hyaline conidia	*Aspergillus kiliense*	3	2.2
002	Bright, yellow-green, umbonate colonies with whitish areal hyphae and white border, and a pale yellow to milk reverse side	Septate hyaline hyphae with long rough conidiophores bearing globose vesicles containing uniseriate or biseriate phialides covering the entire vesicle	*Aspergillus flavus*	25	18.4
003	Bluish Green umbonate colonies with whitish areal hyphae and white-grey border, and a pale yellow to milk reverse side	Septate hyaline hyphae with long conidiophores bearing flask-shaped vesicles containing uniseriate or biseriate phialides covering upper third of the vesicle	*Aspergillus fumigatus*	6	4.4
004	Velvety colonies with dark green to olive colouration and a yellow reverse side	Septate hyaline hyphae with short conidiophores bearing small globose vesicles with biseriate phialides covering the upper half of the vesicles. Numerous Hulle cells were also seen	*Aspergillus nidulans*	3	2.2
005	Downy white to yellow umbonate colonies with dark brown to black pinpoints and white border, with white to a pale yellow reverse side	Branched septate hyaline hyphae with long smooth conidiophores bearing large globose vesicles surrounded by radiate biseriate phialides	*Aspergillus niger*	14	10.3
006	Dark green umbonate colonies with whitish aerial spores at the centre, yellowish border and white to a pale yellow reverse sides	Septate hyphae with short rough conidiophores bearing thick-walled spherical conidia, small globose vesicles with biseriate phialides covering upper third of the vesicles	*Aspergillus parasiticus*	12	8.8
007	Black downy colonies with somewhat dusty surface and a pale reverse side	Unbranched septate hyphae with erect conidiophores bearing smooth-walled, olivaceous pale brown swollen conidia, septated horizontally only, arrange in triple or pentagonal arrangements	*Culvularia lunata*	2	1.5
008	Cottony colonies with brown-black colour at both front and reverse sides	Dark brown septate hyphae with suberect a pale brown septate and highly branched conidiophores bearing brown and barrel-shaped conidia	*Fonsecaea pedrosoi*	5	3.7
009	Cottony, velvety colonies with pink in centre and white border with a pale brown reverse side	Branched septate hyphae bearing multicellular spindle-like septate conidia	*Fusarium graminearum*	10	7.6
010	White cottony colonies tinged with purple, and light purple reverse sides	Septate hyaline hyphae with medium length branched medium length conidiophores bearing phialides with abundant single-celled and clavate microconidia. Few macroconidia also were seen, and chlamydospores were absent	*Fusarium proliferatum*	2	1.8
011	Cream velvety lavender colonies with cottony surface and milk reverse side	Branched hyphae bearing short phialides with clusters of two-celled kidney-shaped microconidia. A moderate number of thick-walled curved macroconidia and some single chlamydospores were also seen	*Fusarium solani*	5	3.7
012	Purple colonies with a cream surface, slightly raised centre and a purple reverse side	Medium phialides with many pink septated microconidia and fewer number of straight macroconidia. Chlamydospores were absent	*Fusarium verticillioides*	3	2.2
013	Cottony white growth spotted with black and greenish colour, globose elevation and a pale yellow to greyish reverse side	Aseptate hyphae bearing large globose sporangia with short branched phialides and no rhizoids	*Mucor racemosus*	18	13.2
014	Pinkish velvety colonies with a whitish cottony periphery and a yellowish reverse side	Hyaline septate hyphae bearing branched conidiophores that support brush-like clusters of slender phialides with extended tapering tips	*Paecilomyces lilacinus*	2	1.5
015	Milk umbonate colonies with a cottony periphery and a pale yellow reverse side	Hyaline septate hyphae bearing simple conidiophores that support multiple chains of single-celled conidia	*Penicillium cheresanum*	4	2.9
016	Blue-green umbonate colonies with yellowish pigment and milk border with a pale yellow reverse side	Hyaline septate branched hyphae with numerous conidiophores bearing brush-like clusters of phialides that bear chains of conidia	*Penicillium chrysogenum*	6	4.4
017	Blueish green and umbonate colonies with milk border and a pale yellow reverse side	Hyaline septate branched hyphae with numerous conidiophores bearing brush-like clusters of phialides that bear chains of conidia	*Penicillium notatum*	6	4.4
018	Blueish green and umbonate colonies with wide milk border and a pale yellow reverse side	Hyaline septate branched hyphae with numerous conidiophores bearing brush-like clusters of two-stage phialides that bear chains of conidia	*Penicillium verruculosum*	3	2.2
019	Cottony white, lid-lifting growth spotted with a black and greenish colour and a pale yellow reverse sides	Rhizoid - borne, long, unbranched sporangiophores bearing round black sporangia with round columellae.	*Penicillium stolonifer*	7	5.6

secondary amides (Amide I and Amide II) (32,33). These molecules produce same vibrational pattern making the spectra to look similar. Differences between the two species were only observed in some wavenumbers (2927 to 4000 cm^{-1} and 600 to 800 cm^{-1}) along the spectral regions (Fig. 4).

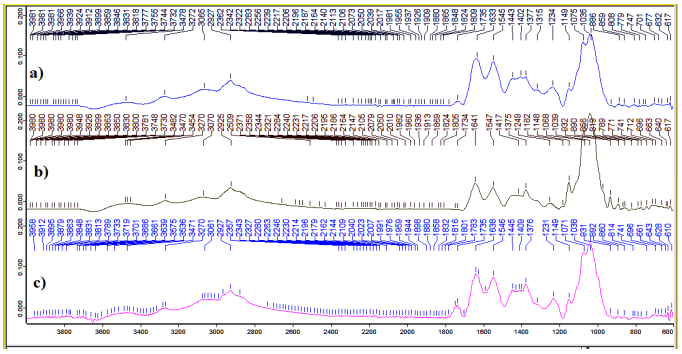

Fig. 4 : Average ATR-FTIR Spectra of the fungal isolates after subtracting the spectrum of the culture media: a) Atoxigenic *Aspergillus flavus*, b) Toxigenic *Aspergillus flavus* and c) Toxigenic *Aspergillus parasiticus*

Validation of the ATR-FTIR method

The specificity test showed that the method is specific as there was no interference peaks between the spectra of the blank (solvent) and that of the *Aspergillus* species (Fig. 5). Also, consistent results were obtained in all the intra-day precision (repeatability) measurements with a % RSD of 2.16 – 3.05%. Similar results were obtained for the inter day precision (reproducibility) measurements with a % RSD of 1.73 – 2.34% (Fig. 5), indicating high precision and reliability of the method.

DISCUSSION

Apart from insects, fungi are the most common organisms associated with grain infestation and spoilage (34), and they are second to bacteria in terms of food borne infection and poisoning. The higher incidence of both mycotoxigenic fungi and saprophytic fungi obtained from the peanuts and food grain samples in this study, therefore, signifies great public health threats in terms of risks of foodborne infection, intoxication, deterioration and consequently diseases and severe economic losses.

The average contamination level in all the foods, except for rice, was above the permissible limit of 10 to the power of 2 and 10 to the power of 5. These figures can also be revised to: "100 to 10000" CFU/g set by International Commission for Microbiological Specification for Foods (35). This high level of fungal contamination may be due to the nature of the sampling environment (open markets) as opined by a previous researcher (36) who obtained high fungal counts in dry meat products sold in open markets in Nigeria. Also, a similar trend was observed in which peanut products from some major open markets in Sokoto state, Nigeria showed a high level of fungal contamination (7). In a study conducted across the five agroecological zones of Nigeria (37), a high incidence of *Aspergillus flavus* was observed in groundnut cakes samples collected from all the five zones. It was concluded that *Aspergillus* spores in the market environment settled and contaminate the peanut cakes as a result of poor handling and packaging noticed from the vendors. Our result is similar to many published research works that also report fungal contamination in peanuts and grains above the permissible limit set by ICMSF (29,38).

Several factors such as warm temperature, high moisture/ relative humidity and moderate pH were identified as important abiotic factors that favours the growth of foodborne fungi. Daura market which is situated in the hottest region of our study area has a significantly higher level of contamination ($p < 0.01$) compared to Dutsinma and Funtua markets. Of the 136 fungal isolates in this study, 51 were isolated in Daura, while Dutsinma and Funtua had 43 and 42 isolates respectively. There was no significant difference ($p > 0.01$) between the level of contamination of Dutsinma samples and those of Funtua. In terms of the distribution of the isolates, all the species of *Acrimonium kiliense*, *Curvularia lunata* and *Fusarium proliferatum* were only isolated in Daura; *Aspergillus nidulans* was only isolated in Funtua market; *Fusarium verticilloides* was only isolated in Dutsinma market; *Fonsecaea pedrosoi* was only isolated in Daura and Funtua samples; while all the other species were distributed across the three markets. However, most of the fungal species obtained in this study were also reported as contaminants of other agricultural products in many open markets in Katsina state, Nigeria. Species of *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus niger*, *Mucor hiemalis*, *Rhizopus stolonifer*, *Phoma glomerata*, *Penicillium citrinum* and *Nigrospora sphaerica* have been reported as the main fungi contaminating condiments and spices in Katsina central market (4), Kafur central market, Katsina state (16), and Funtua central market, Katsina (20). Similarly, it has been reported that *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus niger*, *Fusarium oxysporum*, *Lasiodiplodia theobromae*, *Penicillium corylophilum*, *Rhizopus stolonifer* and *Verticillium* spp are the main fungi contaminating the three varieties of pepper (*Capsicum chinense*, *C. frutescens L.* and *C. annum L*) sold in Dutsinma central market, Katsina (15). In another study (19), *Aspergillus flavus*, *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus parasiticus*, *Penicillium digitatum.*, *Penicillium citrinum* and *Mucor* spp were reported as the main fungi contaminating common vegetables in Kankara LGA of Katsina state.

Peanuts are among the major crops that are vulnerable to fungal invasion both before and after harvesting. They have very high moisture content and many nutrients including all the twenty essential amino acids, minerals, vitamins, fibres, polyphenols, antioxidants, and several proteins (39) which serve to provide a

good carbon source for the proliferation of microbial contaminants especially fungi. This alongside some extrinsic, implicit and processing factors makes peanuts a suitable ecosystem for various fields and storage fungi (7). Of the four food categories (peanuts, rice, maize and wheat) analysed in this study, peanuts samples were the most contaminated. All the samples (peanuts) were contaminated by at least one or more mould fungi belonging to the genera *Acrimonium*, *Aspergillus*, *Curvularia*, *Fonsecaea*, *Fusarium*, *Mucor*, *Paecilomyces*, *Penicillium* or Rhizopus. Several literatures from Nigeria (7,37) reported high contamination of peanuts and peanut products by both toxigenic and atoxigenic fungi.

Similarly, all the fungal genera that contaminate the peanut samples in this study were also found in the maize samples and is in agreement with other published data that report mycofloral contamination of maize in Nigeria (17,40). This further suggests a common source of contamination of both peanuts and the maize. The same observation goes to the rice and wheat samples and was also like other published works reporting fungal contamination of these cereals in Nigeria (41–44). Our result however is not in agreement with that of El Raheem and colleagues (45). They analysed peanuts, rice, maize and wheat in Egypt for mycoflora and observed that rice samples had the highest contamination level, followed by maize, wheat and then peanuts. This disparity may be due to the nature of the food brands and difference in the environmental conditions between the two countries as changes in environmental factors such as temperature, relative humidity, pH and other are known to affect the growth and distribution of fungi in the environment.

Furthermore, the predominance of the mycotoxigenic fungal species (*Aspergillus* spp, *Fusarium* spp and *Penicillium* spp) contaminating the peanuts and food grains in Katsina state Nigeria as identified in this study were also reported in nuts and grain products by other researchers in Nigeria (7,38,42). The presence of these species signifies potential risk to the people in the study area since all of them are well known toxigenic fungal candidates. *Aspergillus flavus* produce aflatoxin B1 and B2, *Aspergillus parasiticus* produce aflatoxin B1, B2, G1 and G2, *Fusarium verticillioides* and *Fusarium proliferatum* produce fumonisin B1 and B2 (7,41). In order to determine the health significance of the *Aspergillus flavus* and *Aspergillus parasiticus* species isolated from the food samples analysed in this study, the isolates were screened for their ability to produce aflatoxin B1 (AFB1) on solid media. We observed a significantly ($p < 0.05$) high incidence of toxigenic species compared to the atoxigenic ones. This is in agreement with the finding that reported a high incidence of toxigenic fungi in rice samples obtained from fields, stores and markets in Niger state of Nigeria (42). All the tested isolates of *Aspergillus flavus* and *Aspergillus parasiticus* were found to be excellent producers of aflatoxins. Similarly, high prevalence of toxigenic *Aspergillus flavus* was also reported in stored maize grains across the five agro-ecological zones of Nigeria (38). A similar trend of the high incidence of toxigenic fungi was also reported in Argentina peanut growing fields (46), in peanut seeds in Kenya (47) and nuts from Saudi (48). However, a study conducted to investigate the incidence and consumer awareness of toxigenic *Aspergillus* section flavi and aflatoxin B1 in peanut cake from Nigeria, revealed significantly ($p < 0.05$) lower incidence of toxigenic *Aspergillus flavus* compared to the atoxigenic strains (37). This signifies possible fluctuation in the natural occurrence of atoxigenic and toxigenic species of *Aspergillus flavus* in different regions and seasons in Nigeria.

FTIR has been described as a simple, reliable, sensitive yet rapid, non-destructive cost-effective spectroscopic technique that could be employed for rapid identification/characterisation of microorganisms and their metabolites on the basis of their unique chemical composition (49) that differ from one microorganism to another. In particular, ATR-FTIR is considered the easiest and accurate infrared spectroscopic technique because it does not require samples preparation compared to some other FTIR methods. This technique has been applied for the identification of fungi in environmental samples (33,49). The ATR-FTIR spectral fingerprints of the atoxigenic and toxigenic *Aspergillus* spp in this study were very similar, possibly, due to the common molecular features they share being members of the same species. These molecules produce the same vibrational pattern making the spectra to look similar. Observed in this study and as reported earlier (50), members of *Aspergillus* spp have unique characteristic wavenumbers in the regions 1765 – 1590 cm^{-1}, 1470 – 1275 cm^{-1}, 1170 – 1000 cm^{-1} and 930 – 715 cm^{-1} along their FTIR spectra corresponding to Amide I, Amide II, polysaccharides and fungal finger prints respectively. The main differences between the toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* as observed in the present study lies in 2927 to 4000 cm^{-1} and 600 to 1630 cm^{-1}. The *Aspergillus parasiticus* isolates had wavenumbers 3958.49 cm^{-1}, 3878.99 cm^{-1}, 3719.38 – 3517.20 cm^{-1}, 3436.1 – 315.59 cm^{-1} and 3075.17 – 3061.14 cm^{-1} at the fingerprint region which are absent in both the toxigenic and atoxigenic *Aspergillus flavus* isolates. In addition, it also has the wavenumbers 1626.65 cm^{-1}, 1589.13 cm^{-1}, 1429.17, 1398.67 cm^{-1}, 1376.14 cm^{-1}, 930.78 cm^{-1}, and 891.609 cm^{-1}, all of which were absent in the *Aspergillus flavus* isolates. On the other hand, the *Aspergillus flavus* isolates have wavenumbers of 3965.62 – 891.61 cm^{-1}, 3887.83 – 3889.38 cm^{-1}, 3850.35 – 3859.07 cm^{-1}, 3800.29 – 3804.10 cm^{-1}, 3743.95 – 3755.31 cm^{-1}, and 856.48 – 859.01 cm^{-1}, which were absent in the *Aspergillus parasiticus* isolates.

When the spectra of the toxigenic and atoxigenic species were compared, both toxigenic isolates (*Aspergillus*

flavus and *Aspergillus parasiticus*) have wavenumbers of 3939.46 – 3940.61 cm^{-1}, 3775.69 – 3776.63 cm^{-1}, 1633.13 – 1637.53 cm^{-1}, 1443.39 – 1445.47 cm^{-1}, 1401.79 – 1409.2 cm^{-1}, 1315 cm^{-1}, 1230.77 – 1233.8 cm^{-1} and 1071.32 – 1074.63 cm^{-1}, all of which were absent in the spectra of the atoxigenic *Aspergillus flavus* isolates. Previous researcher (49), noted a similar pattern in which the *Aspergillus flavus* had a clear peak at 1375.31 cm^{-1} which was absent in the *Aspergillus parasiticus,* while the *Aspergillus parasiticus* had smaller peaks between 500 cm^{-1} to 800 cm^{-1} which were absent in the toxigenic *Aspergillus flavus* isolates.

Similarly, when the spectra of the toxigenic and atoxigenic *Aspergillus flavus* isolates were compared, the toxigenic isolates have wavenumbers of 3955.18 cm^{-1}, 3791.12 cm^{-1}, 928.92 cm^{-1}, and 886.23 cm^{-1} which were absent in the atoxigenic isolates. On the other hand, the atoxigenic isolates have wavenumbers of 3454.31 cm^{-1}, 1640.89 cm^{-1}, 1416.85 – 1068.22 cm^{-1}, 972.016 cm^{-1}, and 890.33 cm^{-1}, which were absent in the toxigenic isolates.

CONCLUSION

This study determined the incidence, distribution, phenotypic characterisation and toxigenicity of mycotoxigenic fungi contaminating commonly consumed food grains in Katsina state, Nigeria. The average level of fungal contamination in the four categories of foods analysed (peanuts, maize, rice and wheat), except for the rice samples, were above the minimum permissible limit of 10^2 to 10^5 CFU/g set by International Commission for Microbiological Specification for Foods (ICMSF), this signifies that they are unsafe for use as food or feed ingredients and hence, the need for more stringent control measures. The distinct ATR-FTIR spectral profile of the toxigenic and atoxigenic *Aspergillus* spp obtained in this study could be used as a reference spectrum for rapid identification of these dreadful fungal species in foods and feeds in Katsina state, Nigeria. Intervention studies that will assess the knowledge and `practice of the grain farmers, traders and public in Katsina towards fungal and aflatoxin contamination of grain products and develop effective awareness campaign on the dangers associated with ingestion of the contaminated products are recommended.

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