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Level of Aflatoxins in Selected Cereals in Ekiti State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aflatoxins are family of toxins produced by certain fungi found on agricultural crops such as maize (corn), peanuts, cottonseed, and nuts. The ingestion of such mycotoxin contaminated grains by animals and human beings has enormous public health significance. This has necessitated the evaluation of effects of *Asparagus africanus* (Lam - whole plant) extracts on aflatoxins production by fungi isolated from selected foods in Ekiti State, Nigeria. A total of 240 food samples were collected across the 16 Local Government areas in rural open markets. The food samples analyzed were: Igbemo rice *Oryza sativa* igbemo curtivar), maize (*Zea mays*) and groundnut (*Arachis hypogaea*). Aflatoxin-producing fungi and non aflatoxin producing fungi were isolated and identified using morphological method of identification on PDA and microscopic with lactophenol cotton blue stain. The molecular identification of the isolates was done using internal transcribe spacer (ITS) region. A total of 70 samples that contain *Aspergillus* spp were subjected to ELISA test to quantify and identify types of aflatoxins that were present in them. It was revealed that four types of aflatoxins were present in the food samples (AFB1, AFB2, AFG1, and AFG2). Also, different species of isolates were obtained from the samples viz: *Aspergillus flavus, Aspergillus*

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niger, Rhizopus stolonifer, Mucor mucedo, A. parasiticus, *Neurospora crassa, Botrytis cinerea, Fusarium oxyporum* and *Penicillium oxalicum. Aspergillus flavus* was prevalent among the isolates. The molecular identification showed three of the isolates were *Aspergillus flavus* with different percentage of ITS4 rDNA identity. The level of aflatoxins in some of the food samples analyzed is of great concern because of its ability to cause disease (in man and animal) and these toxins are introduced mostly during storage, that is, post-harvest.

Keywords: Aflatoxins; cereal foods; food safety; mycotoxins; Rhizopus stolonifera.

1. INTRODUCTION

The priority of the world is on Food security [1]. "Due to the population of the world, there is high demand for food, yet the global food supply is being challenged by several factors such as changes in the patterns of spatial and temporal distribution of water available for agriculture, temperature patterns and frequency of adverse weather conditions and geographical distribution of pests and diseases" [2]. "Food production requires continued innovation and resilience in farming systems, including better preparedness and improved risk management. In addition, food production must be achieved sustainably, i.e. without affecting agricultural land and natural ecosystems for future generations" [3].

"Cereals, including wheat, maize (corn) and rice, are the major staple foods required by the world's population, for both human and animal consumption. It is imperative that cereal production meets the demands of our growing world population, which is estimated to reach 9 billion by 2050" [1]. **"**Worldwide, cereal production has failed to meet consumption needs due to loss contamination of crops during storage and effects of pests attack is estimated at around 10% to 30% annually" [4] as well low crop productivity and low investments into the sector. In many developing countries losses of 10-15% in stored products including cereals and legumes are caused by insect and microbial activities [5]. Twenty-five million tons of cereals are lost during different postharvest stages including storage and post-production in Nigeria, Australia, USA, and West Asia [6]. The main biotic factors influencing cereals loss during storage are insects, moulds, birds and rats [6].

"Climate change has indirectly impacted land values in areas of cereals cropping due to increasing temperature and declining rainfall from 1990 to 2011; temperatures are projected to increase considerably by 2100. Extreme weather events in Nigeria such as droughts, floods and severe storms also have negative effects on cereals production, particularly due to erratic rainfall or high temperatures. These factors influence infection of crops by fungi that grow and produce mycotoxins (e.g. aflatoxins). Changes in CO_2 concentrations as a result of climate change also impact on wheat production" [7].

"Fungi are ubiquitous plant pathogens and are the major spoilage agents of foods and feedstuffs" [8,9]. "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" [10]. Aflatoxins are family of toxins produced by certain fungi found on agricultural crops such as maize (corn), peanuts, cottonseed, and nuts etc. Cassel *et al.,* [11] Alshannaq and Yu, [12]. Aflatoxin-producing fungi can contaminate crops in the field, at harvest, and during storage [13]. 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 [14]. They 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 [15].

Whilst food production is an important part of food security, less consideration has been given to postharvest losses of food supplies, especially losses related to a reduction in food quality due to increased activity of biological organisms such as pests and diseases, yet spoilage accounts for significant losses of edible grain. Indeed, the postharvest loss of cereals is highest during storage after harvest. Magan *et al*. [16] demonstrated that postharvest losses are caused by a wide variety of biotic and abiotic factors. These include mould, insects, mites and the key environmental factors of water and temperature respectively. The interactions between these factors affect the dominance of fungi, especially

mycotoxigenic species such as *Fusarium culmorum, Aspergillus ochraceus* and *Penicillium verruscosum*. Therefore, minimizing postharvest losses caused by these pathogens is an effective way to improve agricultural income '[17]. This study aims at isolating and identifying the aflatoxin-producing fungi from the selected food items such as Igbemo rice ("*Igbemo curtiva"*), maize (*Zea mays*) and groundnut (*Arachis hypogaea*); and also to determine the level of aflatoxin in these food samples.

2. MATERIALS AND METHODS

2.1 Collection of Food Samples

A total of 240 dried samples of Local Rice: 'Igbemo rice' (Igbemo curtiva), Maize (*Zea mays*) and Groundnut (*Arachis hypogaea* L) weighing 250g each were randomly collected from local markets across the 16 Local Governments Areas of Ekiti State in a sterile ziplock bag and were kept in the refrigerator until use.

2.2 Isolation of Fungi Associated with Food Samples

Ten (10) grains of Local Rice (Igbemo curtiva), Maize (*Zea mays*) and Groundnut (*Arachis hypogaea* L) were randomly selected from the samples already in ziplock bag and subjected to surface sterilization with 0.1% sodium hypochlorite solution for 2 minutes. The selected samples were rinsed with distilled water (the aim is to remove contaminant from the environment). The samples were picked with sterile forcep onto already prepared plates (direct plating method) aseptically and incubated at room temperature (25^{o}C) for 3 days (72 hours). The plates were observed for growth thereafter. Pure culture of fungal colonies obtained were sub-cultured on freshly prepared PDA plates and incubated aerobically at 25 ^{o}C for 3 days (72 hours) for subsequent characterization and taxonomic identification. As a control, a plate with only Potato Dextrose Agar (PDA) was incubated at 25°C for 3 days [18].

2.3 Cultural and Morphological Examination of Isolates on PDA

The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation [19]. All the colonies were observed. The morphological characteristics and appearance of the fungal isolates seen were identified.

2.4 Microscopic Examination of Isolates

From pure culture, fungal colony was picked with an inoculating needle and placed on a sterilized and grease-free glass slide containing two drops of lactophenol cotton blue stain. The fungal colony was covered with a cover slip and the slides were examined under the microscope with ×10 and × 40 objective lens. The fungi isolates were identified on the basis of their hyphae, spores, conidial, presence of rhizome and etc. Borman and Johnson, [20].

2.5 Molecular Characterization of Fungal Isolates

Molecular identification was done by ITS4rDNA gene as described by Al-Hindi *et al.* [21]. It involves four stages viz:

Fungal DNA extraction and preparation: A ZR Bashing Lysis Tube was filled with 50 mg (Net Weight) of resuspended fungus cells in up to 200 L of water or up to 200 mg of tissue. A750 mL of the lysis solution was put into the tube for at least five minutes. It was processed at maximum speed in the presence of a 2 mL tube holder assembly on the bead. The Lysis tube containing the solution was centrifuged for one min at a speed of over 10,000 rpm in a micro centrifuge. Fuor hundred (400 mL) of supernatant were centrifuged at 7000 rpm for a minute after being taken to a Zymo-Spin IV Spin Filter (orange top). The base of the Zymo-Spin IV Spin Filter was snapped off prior usage. One thousand two hundred (1200 L) of fungal DNA binding buffer was mixed with the filtrate in the collecting tube. The mixture was divided into 800ul portions, placed into a column of Zymo-Spin IIC in a collecting tube and centrifuged at 10000 rpm 1 minute. A total of 800ul can be stored in the Zygo-Spin IIC column. After being returned to the Zymo-Spin II C column in a collecting tube, the mixture underwent a 10-minute centrifugation at 10000 g. The Zymo-Spin II C column in the new collection tube was filled with 200 L DNA pre-wash buffer before being centrifuged at 10000 rpm for one minute. The Zymo-Spin IIC column received 500 L of fungal DNA wash buffer, and it was centrifuged at 10,000 rpm for one minute. DNA elution buffer was added directly to the column matrix after transferring the Zygo-Spin II C column to a clean 1.5 mL micro centrifuge tube. Then the column matrix was centrifuged at

10000 rpm for 30 seconds to elite the DNA [21].

Polymerase chain reaction (PCR): PCR cocktail mix consists of 2.5 µL of 10x PCR buffer, 1 µL of 25 mM $MgCl_2$, 1 µL of DMSO, 2 µL of 2.5 mMDNTPs, 0.1 µL of 5µ/µL Taq DNA polymeease and 3 µL of 10ng/µL DNA. Initial denaturation at 94 °C for 5 minutes, followed by 36 cycles of denaturation at 94 °C for 30 seconds, annealing at 54 °C for 30 seconds and elongation at 72 °C for 45 seconds followed by a final elongation step at 72 °C for 7 minutes and hold temperature at 10 °C forever. PCR amplification was carried out in GeneAmp PCR 9700. Amplified fragments were visualized on safe view-stained 1.5% Agarose electrophoresis gels. The size of the amplicon was about 600pb. DNA ladder used was Hyper Ladder from Bioline [21].

PCR fragments purification: The Centricon-100 column was assembled according to the manufacturer's recommendation. 2 mL of deionized water was added to the column. The entire sample was added to the column. The column was spun at 3000 x g in a fixed-angle centrifuge for 10 minutes. The waste receptacle was removed and the collection vial was attached. The column was inverted and spun at 270 x g for 2 minutes to collect the sample. This should yield approximately 40 – 60 µL of sample. Deionized water was added to bring the purified PCR fragments to the original volume [21].

Fungal DNA sequencing: Sanger sequencing was performed using 3130 XL Genetic Analyzer from Applied Biosystems. The sequences of primer used are ITS4: TCCTCCGCTTATTGATATGC and ITS5: GGAAGTAAAAGTCGTAAGAAGG. The obtained DNA sequence was blasted on the NCBI Gene library for finding similar sequences. Only those sequences were included that showed highest sequence similarities with the query sequence [21].

2.6 Quantitative Aflatoxins Content of Samples

The food samples from which aflatoxins producing organisms has been isolated were subjected to screening for presence of aflatoxins and quantified as well using ELISA kit a method described by Harish *et al.* [22].

Sample preparation and immunoaffinity: Five (5) grams of finely ground sample was extracted with 25 mL of 70% aqueous methanol using a laboratory homogenizer and filtered through a filter paper (Whatman no.1). 100 µL of each filtrate were diluted with 600 µL of dilution buffer and 50 µL of diluted sample employed to immunoaffinity column (RBiopharm Ag, Darmstadt, Germany) for cleaning up of the samples. The basis of washing of samples involved antigen-antibody reaction. The column containing gel suspension to which monoclonal antibodies were attached covalently. The antibodies are specific for the Aflatoxin B1, B2, G1, G2 and M. Total aflatoxins content finally eluted with 0.5 mL of HPLC grade methanol and analyzed through ELISA microplate Reader Model 680 (Bio-Rad India).

Quantification of total aflatoxin content: Quantitative analysis of total aflatoxin was performed by competitive ELISA using RIDASCREEN total aflatoxin kit, Darmstadt, Germany. Fifty (50 µL) of standard solution of aflatoxin and cleaned eluted sample were added to the wells of microtiter plate in replicates. Fifty (50 µL) of peroxidase enzyme conjugate and 50 µL of mouse monoclonal anti-aflatoxin antibodies were added to each well of microtiter plate and incubated at room temperature in the dark for 30 minutes. After washing thoroughly with 250 µL distilled water three times, 50 µL of urea peroxidase (substrate) and 50 µL of tetramethylbenzidine (chromogen) were added to each well to mix thoroughly and was further incubated for 30 minute at room temperature in the dark. Reaction was stopped by adding 100 µL 1M sulphuric acid (stop reagent) and the absorbance was measured at 450 nm using ELISA microplate reader Model 680 (Bio-Rad India). A calibration curve was drawn using a wide range of total aflatoxin standards with concentration of 0 ppt to 4050 ppt.

2.7 Aflatoxins Content of Food Inoculated with Isolates

One kilogram (1.0 kg) of food samples viz: Rice (*Igbemo curtiva*), Maize (*Zea mays*) and Groundnut (*Arachis hypogaea*) was weighed into 5L capacity bucket separately. The samples were inoculated with inoculum ($1.6x10^3$ CFU/mL) of the confirmed isolates from 3.4 above for 14 days and incubated at room temperature (25 ^oC ± 0.2) [23]. The samples were analyzed for aflatoxin content using Harish *et al*. [22] protocol.

3. RESULTS AND DISCUSSION

3.1 Results

Various Local Governments and number of food samples collected: The number of samples taken from each of Ekiti State's 16 Local Government Areas (LGA) is displayed in Table 1. In each Local Government, 15 samples of Maize, Rice (Igbemo cultivar), and Groundnut were gathered from various places. Ado, Ikere, Efon, and Ikole are only a few LGAs that are comprised of a single town. Some of these LGA's streets were used in place of towns.

Cultural and morphological characteristics of fungal isolates from Rice 'Igbemo cultivar', Maize (*Zea mays*), and Groundnut (*Arachis hypogaea*) in Ekiti: The morphological characteristics of isolates from rice (Igbemo cultivar). *Aspergillus* spp., *Rhizopus stolonifer*, *Mucor mucedo*, and *Penicillium citrinum* were predominant in all the food samples. *Neurospora crassa* and *Botrytis cinerea* were predominant on rice and maize samples. However, *Aspergillus parasiticus* and *Fusarium oxysporum* were predominant only on the rice samples as shown on Table 2.

Frequency of occurrence of fungal colonies: Ten (10) general of isolates were obtained from the samples. These isolates were found in all the samples. *Aspergillus flavus* has the highest number of occurrences which is 31 among the aflatoxins producing fungi while *Fusarium* has the least value of 2. On the other hand, among the non-aflatoxins producing fungi, *Rhizopus* spp. has the highest value of 48 while *Neurospora* spp has the least value as seen in Table 3.

Molecular identification of isolates: Three of the four isolates were identified molecularly as *Aspergillus flavus*, while one was *Trichoderma vridie*, according to Table 4 molecular identification of four isolates with various accession numbers.

Aflatoxins content of food sample from which fungi producing aflatoxins were isolated: The aflatoxins content of rice, groundnut and maize revealed the levels of AFB1, AFB2, AFG1, and AFG2. These were present in varying levels ranging from 0.00 to 85.58±0.03 ppm (AFB1), 0.00 to 88.22±0.30 ppm (AFB2), 0.00 to 78.34±0.028 ppm (AFG1), and 0.00 to 70.55±0.01 ppm (AFG2) (Table 5).

Aflatoxins content of food sample collected from each Local Government: The percentage distribution of aflatoxins (%) in the 16 LGA is shown in Table 6. Gbonyin LGA had the highest percentage (15.79%) followed by Ikole LGA (13.16%). The value of 10.53% is the same for Ado, Oye, and Ikere. Values in Irepodun and Ekiti West were undetectable (0 ppb).

Aflatoxins content of inoculated food samples: The AFB1, AFB2, AFG1, and AFG2 levels in healthy food samples of Rice, Maize, and Groundnut after inoculation with a verified isolate of *Aspergillus flavus* are shown in Table 7 with values of 40.00±0.02 ppm, 32.00±0.03 ppm, 44.00±0.03 ppm, and 38.00±0.04 ppm for maize respectively. Two kinds of aflatoxins (AFB2 and AFG1 — 44.56±0.02 ppm, 38.22±0.02 ppm) are present in healthy groundnut. Only AFB2 – 48.21±0.01 ppm was detected in the Rice sample; all others were undetectable.

3.2 Discussion

A total of 240 food samples (Rice '*igbemo cultivar*', Maize and Groundnut) were analyzed for aflatoxins content and isolation of aflatoxins producing fungi from the 16 Local Government area of Ekiti State. Fungal species isolated from rice were *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger, Botrytis cinerea*. The result obtained is in agreement with the result of Taligoola *et al.* [24] who reported that *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger* were isolated from rice form Libya. Similarly, this conforms to those of Amanloo *et al. [25]* who reported that *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger, Aspergillus fumigatus,* and *Rhizopus* sp were isolated from rice from Iran. Also, these are consistent with those of the study conducted by Asrani *et al*. [26] who found that *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus niger* are the major fungi in cereals. Chen *et al.* [27] stated that *Aspergillus flavus* and *Aspergillus parasiticus* are seldomly found in rice which does not conform to the result of this research work which may be as a result of the difference in environment, cultivation system and handling [28].

Fungal genera isolated from groundnut were *Penicillium* spp., *Rhizoous* spp., *Mucor* spp and *Aspergillus* spp. This conforms to the findings of Hamed *et al.,* [29] that the most common fungi in nuts are *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., and *Aspergillus flavus*. Also,

Madilo *et al.* [30] identified *Aspergillus flavus, Aspergillus niger, Aspergillus tamanii, Penicillium citrinum, Rhizopus stolonifer* in roasted groundnut sold in Bauchi State of Nigeria. Similarly, this is in agreement with the study of Aleiro and Ibrahim [31] who observed *Mucor* spp., *Aspergillus* spp., *Penicillium* spp., *Curvularia* spp., *Fusarium* spp. and *Rhizopus* spp. in dried vegetable sold in Central Market in Sokoto, Sokoto State. This conforms to those of Pitt *et al*. (2013) who reported that *Penicillium* spp. and *Rhizopus* spp. were found in peanuts. Also, this is consistent with the study of Akinnibosun and Osawaru [32] who found that *Neurospora* spp., *Trichoderma* spp., *Aspergillus niger, Aspergillus flavus, Mucor* spp., *Rhizopus* spp., *Penicillium* spp. and *Fusarium* spp. in peeled and unpeeled groundnut sold in Benin.

Fungal genera isolated from maize were *Mucor* spp., *Aspergillus* spp., *Botrytis* spp., *Rhizopus stolonifer* spp.*, Penicillium* spp., and *Aspergillus flavus.* This is in agreement with the result of Muthomi *et al.* [33] and Mtega *et al.* [34] who reported that maize samples had a higher contamination by *Aspergillus* spp. The reason for the high contamination by *Aspergillus* spp. may be their high ability to colonize substrates that is rich in carbohydrate. This also conforms to those of Abubakar and Sule [35] who reported that *Penicillium* spp. and *Rhizopus* spp. were isolated in maize from Cameroon. Similarly, this is in line with the result of Rosemary *et al.* [36] who reported that the following fungal species: *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp. and *Mucor* spp. were isolated in maize from Enugu State of Nigeria.

Table 1. Various Local Governments and number of food samples collected

No	Local Government	Town/Street	Maize sample	Rice sample	Groundnut sample	Total
1	Ado	Odo Ado	2	2	2	15
		Irona	1	2	2	
		Adebayo	2	1	1	
2	Ikere	Okekere street	1	1	1	15
		Odooja/Atiba street	2	2	2	
		Oke Osun street	1	1	1	
		Anaye street	1	1	1	
3	Gbonyin	Ode Ekiti	1	1	1	15
		Ijan Ekiti	1	1	1	
		Agbado Ekiti	2	1	1	
		Imesi Ekiti	1	2	2	
4	Ijero		5	5	5	15
5	Ise /Orun	Emure Ekiti	2	2	2	15
		Ise Ekiti	2	2	2	
		Orun Ekiti	1	1	1	
6	Ido/Osi	Ido Ekiti	2	2	2	15
		Osi Ekiti	1	1	1	
		Ifaki Ekiti	2	2	2	
7	Ekiti East	Omuo Ekiti	3	3	2	15
		Kota Ekiti	2	2	3	
8	Ekiti south	Ilawe Ekiti	1	2	2	15
		Ogotun Ekiti	1	1	1	
		Aramoko Ekiti	2	2	2	
9	Irepodun/ Ifelodun	Iyin Ekiti	2	2	2	15
		Igede Ekiti	2	2	2	
		Are Ekiti	1	1	1	
10	Efon		5	5	5	15
11	Moba	Otun	5	5	5	15
		Usi				
12	Ilejemeje	Ekanmaje	5	5	5	15
13	Emure		5	5	5	15
14	Oye	Oye	2	2	2	15
		Ilupeju	2	1	1	
		Ayegbaju	1	2	2	
15	Ikole	Asin	5	5	5	15

No	Local Government	Town/Street	Maize sample	Rice sample	Groundnut sample	Total
16	Ekiti West		5	5	5	15
	Total					240

Table 2. Cultural and morphological characteristics of fungal isolates from Rice '*igbemo cultivar*', Groundnut, and Maize in Ekiti State, Nigeria

Morphological Characteristics	Suspected Fungi Identified	Food samples
Yellow green colour on the surface, cream colour on the reverse, woolly in texture, septate hyphae.	*Aspergillus flavus*	Rice, groundnut, maize
Dark green colour on the surface, bright orange yellow colour on the reverse, suede like surface, velvety in texture, filamentous, septate hyphae.	*Aspergillus parasiticus*	Rice
White to grey colour on the surface, pale white colour on the reverse, filamentous, cottony in texture, non septate hyphae.	*Rhizopus stolonifer*	Rice, groundnut, maize
Colonies are typically colour white to grey, older colony become grey to brownish colour due to development of spores	*Mucor mucedo*	Rice, groundnut, maize
Orange, powdery conidia	*Neurospora crassa*	Rice, maize
Fluffy creamy growth that later turned pinkish with a yellowish reverse side	*Fusarium oxysporum*	Rice
White to brown colour with dark spots on the surface, dark coloured reverse, filamentous, woolly in texture, septate hyphae	*Botrytis cinerea*	Rice, maize
Green with green colour on the surface, orange coloured wrinkled reverse, filamentous, powdery in texture, septate hyphae	*Penicillium citrinum*	Rice, groundnut, maize

Table 3. Frequency of occurrence of fungal isolates from food samples collected

Isolates	(X) Occurrence	% Frequency
Aspergillus flavus	31	16.75
Aspergillus niger	12	6.49
Penicillium citrinum	15	8.12
Rhizoous stolonifer	48	25.41
Mucor mucedo	30	16.22
Botrytis cinerea.	19	10.27
Aspergillus parasiticus	17	9.19
Aspergillus fumigatus	10	5.41
Fusarium oxysporium	2	1.08
Neurospora crassa	1	0.54
Total	185	100.00

Table 4. Molecular identification of isolates

Isolate	Strain	Accession Number	E-value	ITS4 rDNA identity (%)	Isolates' Identity
B2	*Aspergillus flavusT13*	MN179300.1	0.0	90.73	*Aspergillus flavus*
B3	*Aspergillus flavus* Egy3	LC368455.1	0.0	89.56	*Aspergillus flavus*
B4	*Trichoderma viridie*	MN497567.1	0.0	71.19	*Trichoderma viridie*
B6	*Aspergillus flavus*	MK493826.1	0.0	75.91	*Aspergillus flavus*

Table 5. Aflatoxin content of food sample from which fungi producing aflatoxin were isolated

SN	Sample code	AFB1(ppb)	AFB2 (ppb)	AFG1(ppb)	AFG2(ppb)
1	GBG2	80.00±0.70	31.75±0.30	45.5±0.70	38.00±0.00
2	ISR1	0.00	32.00 ±0.20	0.00	0.00
3	EFG2	34.00±0.70	51.00±0.71	44.25±0.06	30.85±0.49
4	ADM2	0.00	44.56±0.00	38.27±0.07	0.00
5	IKOR3	0.00	57.22±0.30	35.10±0.01	30.00±0.71
6	IKM3	0.00	0.00	33.22±0.02	0.00
7	IKM2	32.45±0.01	51.00±0.71	34.75±0.35	30.35±0.00
8	GBR3	40.20±0.01	0.00	0.00	0.00
9	ADM3	38.55±0.07	44.56±0.00	33.22±0.01	30.58±0.03
10	IDM5	0.00	43.22±0.01	0.00	0.00
11	IJG3	0.00	57.22±0.30	0.00	0.00
12	IKR3	0.00	44.39±0.20	0.00	0.00
13	IDR3	0.00	44.28±0.02	0.00	0.00
14	GBG2	0.00	44.00±0.71	35.32±0.01	30.00±0.00
15	IKG3	44.28±0.03	38.25±0.01	76.33±0.014	70.55±0.01
16	EEG1	75.22±0.028	68.85±0.02	78.34±0.028	70.55±0.07
17	GBM3	85.58±0.03	66.00±0.71	55.64±0.028	39.67±0.01
18	OYG2	0.00	41.10±0.01	0.00	0.00
19	ADR3	0.00	33.22±0.01	0.00	0.00
20	IKM1	36.72±0.01	80.00±0.20	0.00	0.00
21	OYM2	62.00±0.01	88.22±0.30	33.21±0.014	30.58±0.01
22	GBG1	0.00	32.22±0.01	0.00	0.00
23	EEG2	43.22±0.01	63.12±0.02	0.00	0.00
24	IKG2	33.02±0.20	34.00±0.022	43.00±0.00	0.00
25	IKOR1	0.00	63.22±0.01	53.22±0.01	0.00
26	EEM1	52.00±0.01	0.00	0.00	0.00
27	ADR1	0.00	53.22±0.01	38.00±0.01	0.00
28	IKOG2	50.00±0.02	55.13±0.01	43.22±0.00	48.00±0.00
29	GBG2	0.00	33.22±0.04	55.00±0.02	0.00
30	IKR1	43.45±0.01	0.00	0.00	0.00
31	IKOM1	75.22±0.01	53.22±0.01	65.00±0.01	57.08 ±0.01
32	IKOR1	0.00	50.00±0.02	0.00	0.00
33	IKOM2	62.22±0.01	35.00±0.01	0.00	0.00
34	OYR3	53.00±0.01	0.00	0.00	0.00
35	OYM1	60.00±0.01	47.30±0.01	0.00	0.00
36	IMG2	0.00	53.40±0.02	0.00	0.00
37	MOR1	0.00	50.20±0.01	0.00	0.00
38	IMM1	83.00±0.01	35.22±0.01	51.00±0.01	45.21±0.00

Gbonyin Groundnut GBG, EFG Efon Groudnut, GBR: Gbonyin Rice, IKOR; Ikole Rice, ADM: Ado Maize, OYM: Oye Maize, EE: Emure/Ise. Values are means of duplicates ± standard deviation. The safe limit: 1-30ppb

This study showed the presence of 185 isolates belonging to eleven (11) different genera identified from stored foods obtained from Ekiti State, Nigeria. Among the genera, *Aspergillus* had the highest number of occurrence (70) followed by *Rhizopus* (48), *Botrytis* (19) and *Penicillium* (15). In all analyzed samples, the prevalent fungal genus was *Aspergillus* spp. which could have been as a result of their ubiquity character. This finding was in conformity with that of Nyirahakizimana *et al.* [37] who reported that the genera *Aspergillus* was the major genera that affects nuts and seeds. Similarly, this is in agreement with the study of Kumari and Ghosh, [38] who reported that *Aspergillus flavus and Aspergillus niger* were the most visible and frequent fungi in Almonds from Japan. Mukhtar *et al.* [39] reported that various fungi were isolated from fruits in Utako market, Abuja, Nigeria. *Aspergillus niger, Aspergillus parasiticus, Aspergillus fumigatus, Rhizoous stolonifer, Mucor mucedo* and *Alternaria* spp. in decreasing order. Similarly, Al-Hindi *et al.* [21] showed that the fungal genera *Aspergillus, Penicillium, Fusarium and Rhizopus* were isolated from agar wood where *Aspergillus flavus, Aspergillus parasiticus and Aspergillus niger* are the dominant fungi. His results also indicated that the most commonly isolated fungal genera were in the following

descending order: *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus*. Similarly, *Aspergillus* spp., *Penicillium* spp., and *Mucor* spp. were shown to be connected with dried cocoa beans during storage, according to research by Fagbohun [40]. Additionally, According to research by Kumari and Ghosh [38] *Aspergillus flavus*, *Rhizopus oryzea*, and *Rhodosporidium toruloides* are the most common fungi found in West Bengal connected with stored food grains which correlates with this research.

The identity of the isolates which was determined by molecular characterization shows that they hire similarities to strains of isolates in the *Aspergillus* and *Trichoderma* genus except for two that did not show any result. The species determination was confirmed by PCR with the primers specific for the ITS-rDNA region sequencing. Out of the 6 isolates (B2, B3, B4, B6, B5, B1), B2, B3, and B6 were *Aspergillus flavus,* B4 was *Trichoderma viridie,* B5 and B1 did not show any visible result. The prevalence of *Aspergillus flavus* in the stored foods was above 50%, which is very high, and indicates the possible high level production of aflatoxin in food samples under study [41]. Also, Al-Hindi *et al.* [21] detected among *Aspergillus* genera, *A. flavus* and *A. ochraceus* based on their morphology and confirmed by PCR using specific primers in his research on isolation and characterization of mycotoxigenic fungi in agarwood which is in line with this research.

Aflatoxin contamination of food samples collected showed the presence of four different types; Aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2). In this research, the concentration of species of aflatoxins decreases as follows AFB2, AFB1, AFG1 and AFG2. Among the detectable values of AFB1 concentration in food samples, eighteen (18) samples were below detection level (0 %) while twenty (20) samples were detected. AFB2 has the highest frequency of occurrence in all the samples with thirty three (33) samples being detected, while five (5) samples were below detection level. AFG1 was found in nineteen samples (19), while the remaining samples (19) were below detection level. Only thirteen samples were positive to AFG2, while others were 0 %.

Table 6. Percentage of aflatoxin content of food samples in each local government

SN	Local Government Areas	Distribution of Aflatoxin (%)
1	Gbonyin	15.79
2	Ikole	13.16
3	Oye	10.53
4	Ikere	10.53
5	Ado Ekiti	10.53
6	Ekiti East	7.90
7	Emure	7.90
8	Southwest	5.30
9	Ilejemeje	5.30
10	Ido/Osi	2.63
11	Efon	2.60
12	Moba	2.60
13	Ijero	2.60
14	Ido	2.60
15	Irepodun	0.00
16	Ekiti West	0.00

Table 7. Aflatoxins content of inoculated food samples

Samples	Isolate inoculated	AFB1 (ppb)	AFB2 (ppb)	AFG1 (ppb)	AFG2 (ppb)
HM	B2	40.00±0.02	32.00±0.03	44.00±0.03	38.00±0.04
HG	B2	0.00	44.56±0.02	38.22±0.02	0.00
HR	B2	0.00	48.21±0.01	0.00	0.00

Values are means of duplicates ± standard deviation

HG: Healthy groundnut, HM: Healthy Maize, HR: Healthy Rice. B2: confirmed Aspergillus flavus

It has been estimated that 25% of the world's crops are affected by mould or fungal growth that may result in contamination of toxic fungal secondary metabolites known as mycotoxins. Aflatoxigenic fungi are common soil habitants all over the world and they frequently contaminate agricultural crops with AFB2 and AFG1 which are present in the soil [26]. Aflatoxins, the toxic metabolites produced by different species of toxigenic fungi, can contaminate human food at various stages in the food chain. With the development of world markets for agricultural products and more attention paid to food safety, aflatoxins have become a problem in countries that previously did not have to worry about aflatoxins contamination [28]. As a result, numerous countries have established or proposed regulations for controlling aflatoxins in food and feeds. The tolerance levels for total aflatoxins (sum of aflatoxins B_1, B_2, G_1 and G_2) in different countries may range from 1 to 35 µg/kg for foods, with an average of 10 µg/kg and from zero to 50 µg/kg for animal feed, with an average of 20 µg/kg. In this present study, the level of aflatoxins in some food samples was relatively high with 85 ppb in maize. This may be due to the high level of substrate, that is, carbohydrate which promotes the production of aflatoxins [28]. Aflatoxins production is also widely affected by the substrate and various nutritional factors such as carbon, amino acids, nitrogen, lipids, and few trace elements. Substrate rich in carbohydrates supports more production as compared to oil as carbohydrate easily provides carbon which is needed for good fungal growth [42].

The distribution of aflatoxins in the local government area based on this work showed that Gbonyin has the highest percentage (15.79 ppb), Ikole (13.16 ppb), Oye, Ikere and Ado had the same value (10.53 ppb). Two of the local government had 0 ppb. Aflatoxins content of inoculated food sample was high in maize and all the four types of aflatoxins were present while groundnut had AFB2 and AFG1 while rice has AFB2. The aim is to be sure that the confirmed isolate can produce aflatoxins.

4. CONCLUSION

The level of aflatoxins in some of the food samples analyzed is of great concern because of its ability to cause disease (in man and animal) and these toxins are introduced mostly during storage, that is, post-harvest. Presence of aflatoxins in staple food like rice, maize and groundnut is of a great concern. Hence, farmers at the grass-root should be sensitized with the effects of consuming aflatoxin contaminated food.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Porter JR, Xie L, Challinor AJ, Cochrane K, Howden SM, Iqbal MM, Travasso MI. Food security and food production. Cambridge University Press, Cambridge, United Kingdom and New York. 2014;485-533.
2. Millar J, Roots J. Changes in Australian agriculture and land use: implications for future food security. International Journal of Agricultural Sustainability. 2012;10 (1): 25-39.
3. Hoffmann U. Assuring food security in developing countries under the challenges of climate change: key trade and development issues of a fundamental t transformation of agriculture. In Discussion Papers-United Nations Conference on Trade and Development (UNCTAD) (No. 201). United Nations Conference on Trade and Development; 2011.
4. Chelladurai V, Jayas DS, White NDG. Thermal imaging for detecting fungal infection in stored wheat. Journal of Stored Products Research. 2010;46:174–179.
5. Neethirajan S, Tuteja SK, Huang ST, Kelton D. Recent advancement in biosensors technology for animal and livestock health management. Biosensors and Bioelectronics. 2017;98:398-407.
6. Baloch MA, Thapa GB. The effect of agricultural extension services: Date farmers' case in Balochistan, Pakistan. Journal of the Saudi Society of Agricultural sciences. 2018;17(3):282-289.
7. Yang C, Ko B, Hensley CT, Jiang L, Wasti AT, Kim J, DeBerardinis RJ. Glutamine

oxidation maintains the TCA cycle and cell survival during impaired mitochondrial pyruvate transport. Molecular cell. 2014;56 (3):414-424.

8. Makun HA, Anjorin ST, Moronfoye B, Adejo FO, Afolabi OA, Fagbayibo G, Surajudeen AA. Fungal and aliatoxin contamination of some human food commodities in Nigeria. African Journal of Food Science. 2010;4(4):127-135.
9. Esan AO, Fapohunda SO, Ezekiel CN, Sulyok M, Krska R. Distribution of fungi and their toxic metabolites in melon and sesame seeds marketed in two major producing states in Nigeria. Mycotoxin Research. 2020;36:361-369.
10. Fan K, Qian S, Zhang Z, Huang Q, Hu Z, Nie D, Han Z. Recent advances in the combinations of plant-sourced natural products for the prevention of mycotoxin contamination in food. Critical Reviews in Food Science and Nutrition. 2023;1-17.
    Available:https://doi.org/10.1080/10408398.2023.2227260
11. Cassel EK, Campbell B, Draper M, Epperson B. Aflatoxins hazards in grain/Aflatoxicosis and livestock. South Dakota State University (SDSU). 2012; 444-445.
12. Alshannaq A, Yu JH. Occurrence, toxicity, and analysis of major mycotoxins in food. International Journal of Environmental Research and Public Health. 2017;14(6):632.
13. Garcia-Cela E, Kiaitsi E, Sulyok M, Krska R, Medina A, Petit Damico I, Magan N. Influence of storage environment on maize grain: CO2 production, dry matter losses and aflatoxins contamination. Food Additives and Contaminants: Part A. 2019; 36(1):175-185.
14. Benkerroum N. Chronic and acute toxicities of aflatoxins: Mechanisms of action. International Journal of Environmental Research and Public Health. 2020;17(2):423.
15. Awuchi CG, Ondari EN, Nwozo S, Odongo GA, Eseoghene IJ, Twinomuhwezi H, Okpala CO. R. Mycotoxins' toxicological mechanisms involving humans, livestock and their associated health concerns: A review. Toxins. 2022;14(3):167.
16. Magan N, Medina A, Aldred D. Possible climate-change effects on mycotoxin contamination of food crops pre- and postharvest. Plant Pathology. 2011;60: 150-163.
17. Tefera TM, Mugo SN, Tende R, Likhayo P. Methods of screening maize for resistance to stem borers and post-harvest insect pests. Crop Protection. 2011;30(3):240-245.
18. Toma FM, Abdulla NQF. Isolation and identification of fungi from spices and medicinal plants. Research Journal of Environmental and Earth Sciences. 2013; 5(3):131-138.
19. Tafinta IY, Shehu K, Abdulganiyyu H, Rabe AM, Usman A. Isolation and Identification of Fungi Associated with the Spoilage of Sweet Orange (Citrus sinensis) Fruits in Sokoto State. Nigerian Journal of Basic and Applied Science. 2013;21(3): 193-196.
20. Borman AM, Johnson EM. Interpretation of fungal culture results. Current Fungal Infection Reports. 2014;8:312-321.
21. Al-Hindi RR, Aly SE, Hathout AS, Alharbi MG, Al-Masaudi S, Al-Jaouni SK, Harakeh SM. Isolation and molecular characterization of mycotoxigenic fungi in agarwood. Saudi Journal of Biological Sciences. 2018;25(8):1781-1787.
22. Harish Chandra, Jyotsana Bahugun and Ajay Singh. Detection of Aflatoxin in Zea mays L. from Indian Markets by Competitive ELISA. Octa Journal of Biosciences International peer-reviewed journal Octa. Journal of Bioscience. 2013; 1(1):62-68
23. Wanjiku EK, Waceke JW, Wanjala BW. Mbaka JN. Identification and Pathogenicity of Fungal Pathogens Associated with Stem End Rots of Avocado Fruits in Kenya, International Journal of Microbiology; 2020.
24. Taligoola HK, Ismail MA, Chebon SK. Toxigenic fungi and aflatoxins associated with marketed rice grain in Uganda. Journal of Basic Appllied Mycology. 2010; 1:45-52.
25. Amanloo S, Kahhka R, Ramezani AA. The Mycotoxins contamination of the imported consumer rice and its producing fungi in Zabol. Jahrom Medical Science University. 2014;12:19-27.
26. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. Journal of Hepatology. 2019;70 (1): 151-171.

27. Chen Y, Lu Z, Zhao J, Zou Z, Gong Y, Qu F, Han L. Epidemiology and molecular characterizations of azole resistance in clinical and environmental Aspergillus fumigatus isolates from China. Antimicrobial Agents and Chemotherapy. 2016;60(10):5878-5884.
28. Kebede S, Afework M, Debella A, Ergete W, Makonnen E. Toxicological study of the butanol fractionated root extract of Asparagus africanus Lam., on some blood parameter and histopathology of liver and kidney in mice. BioMedical Cental Research Notes. 2016;9:1-9.
29. Hamed AM, Abdel-Hamid M, Gámiz-Gracia L, García-Campaña AM, Arroyo-Manzanares N. Determination of aflatoxins in plant-based milk and dairy products by dispersive liquid–liquid microextraction and high-performance liquid chromatography with fluorescence detection. Analytical Letters. 2019;52(2): 363-372.
30. Madilo FK, Glover RL, Islam MN, Roy, N. and Letsyo E. Microbiological Assessment of Groundnut (*Arachis hypogaea* L.) Sold for Consumption in Ghana. Journal of Food Quality. 2023;1(1):12.
31. Aliero AA, Ibrahim AD. Aflatogenic and Aflatoxin Contents of Dried Vegetables Sold in Sokoto Metropolis, Nigeria. Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS). 2014;5(7): 116-120.
32. Akinnibosun FI, Osawaru EE. Quality assessment of peeled and unpeeled roasted groundnut (*Arachis hypogaea* L.) Sold in Benin City, Nigeria. International Research Journal of Natural and Applied Sciences. 2015;2(3):18-32.
33. Muthomi JW, Mureithi BK, Chemining'wa GN, Gathumbi JK, Mutit EW. Aspergillus species and Aflatoxin b1 in soil, maize grain and flour samples from semi-arid and humid regions of Kenya. International Journal of Agri Science. 2012;2(1):22-34.
34. Mtega M, Mgina CA, Kaale E, Sempombe J, Kilulya KF. Occurrence of aflatoxins in maize and maize products from selected locations of Tanzania and the effects of cooking preparation processes on toxin levels. Tanzania Journal of Science. 2020; 46(2):407-418.
35. Abubakar S, Sule A. Technical efficiency of maize production in Rijau local government area of Niger state, Nigeria. Journal of Agriculture and Veterinary Science. 2019; 12(2):63-71.
36. Rosemary OC, Udeh Sylvester MC, Benedict AO, Ugwu Okechukwu PC. Isolation and characterization of fungi associated with the spoilage of corn (*Zea mays*). International Journal of Pharmaceutical Medicine and Biological Sciences. 2013;2(3):86-91.
37. Nyirahakizimana H, Mwamburi L, Wakhisi J, Mutegi CK, Christie ME, Wagacha JM. Occurrence of Aspergillus species and aflatoxin contamination in raw and roasted peanuts from formal and informal markets in Eldoret and Kericho towns, Kenya. Advances in Microbiology. 2013; 3(4):333-342.
38. Kumari R, Ghosh AK. Development of sensitive polyclonal antibodies against dominant stored wheat grain fungus for its immunological detection. Julius-Kühn-Archiv. 2018;(463).
39. Mukhtar MF, Wusa NR, Danladi UI, Salisu N. Isolation and characterization of fungal species from spoilt fruits in Utako Market, Abuja, Nigeria. Journal of Applied Sciences. 2019;19:15–19.
40. Fagbohun BJ. Combining dominant spectral features in airborne SWIR and TIR imagery for mineralogical mapping (Master's thesis, University of Twente); 2015.
41. Afzal N, Hassan SM, Mughal SS, Pando A, Rafiq A. Control of Aflatoxins in Poultry Feed by Using Yeast. American Journal of Chemical and Biochemical Engineering. 2022;6(1):21-26.
42. Ma X, Wang W, Chen X, Xia Y, Wu S, Duan N, Wang Z. Selection, identification, and application of Aflatoxin B1 aptamer. European Food Research and Technology. 2014;238(6):919-925.

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