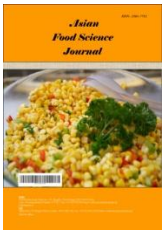

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Mycoflora and Aflatoxin Producing Fungi from Some Storage Cereal Grains Sold in Darki Market, Wudil Local Government Area, Kano State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author Authors FIEH, EBA and SFU designed the present study. FIEH conducted the survey wrote the manuscript and discussed the results. EBA and SFU supervised the overall implementation of investigation. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study was aimed at determining the mycoflora and aflatoxin producing fungi in some storage cereals sold at Darki market, Wudil Local Government Area, Kano State. Nigeria.
Study Design: A total of thirty samples (ten samples containing 250g each of maize, millet and wheat) were obtained from storage facilities of traders in different location of Darki, market.
Place and Duration of Study: Storage facilities of Darki market, Wudil, Kano State, between May 2021 and July 2021.
Methodology: Samples were subjected to serial dilution, enumeration of fungal load using pour plate method, inoculation and isolation of fungi using direct plating method, extraction and detection of aflatoxin using thin layer chromatography and molecular analysis using PCR.
Result: Total fungal (mold) count on Potato Dextrose Agar recorded the highest count of 6.5×10^3 in wheat sample 4 (WH_4) while the least count of 2.2×10^2 was recorded in wheat sample 6 (WH_6). The fungal isolates obtained from this study were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* spp, *Mucor* spp, and *Rhizopus* spp. The percentage of occurrence of fungi isolated from the cereal

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samples was highest in *Aspergillus niger* (34.43%) and the least (11.48%) was recorded in *Mucor spp.* Extraction and detection methods revealed a blue fluorescence on the TLC plates indicating the presence of aflatoxin B in the samples. The standard calculated range of Retention factor value was between 0.58 and 0.75. After DNA extraction, the fragment size obtained by PCR reactions with primer pairs ITS1/ITS4 showed 600bp for most of the isolates. All the Aspergillus spp, *Fusarium* spp. and *Rhizopus* spp showed positivity for the molecular markers ITS1/ITS4 by having positive bands. Only *Mucor* spp showed negative results for ITS1/ITS4 primers and showed no band.

Conclusion: This study shows that most of the cereals were contaminated with either one or more molds but within the satisfactory and marginal limits. However, aflatoxin contamination is a threatening issue Hence aflatoxins contamination of crops at harvest conditions should be controlled to some extent by the implementation of good agricultural practices and good storage practices.

Keywords: Aflatoxins; darki; maize; millet; molecular analysis; thin layer chromatography; wheat; wudil.

1. INTRODUCTION

Cereals and cereal products constitute large portion of food resources and consumed by a large number of people worldwide [1]. The cereals are annual common grass members of the grass family (a monocot family Poaceae, also known as Gramineae) which usually have long, thin stalks, such as wheat, rice, maize, sorghum, millet, barley and rye, whose starchy grains are used as food [2]. Food security on the African continent has been worsening and deteriorating in recent decades. The proportion of the malnourished population has remained predominant in most Sub-Saharan African countries [3]. Mycotoxins are toxic secondary metabolites produced by filamentous fungi in a wide range of agricultural commodities worldwide, including cereals, nuts, legumes, spices, fruits, and their products [4]. Among various types of mycotoxins, aflatoxins (AFs) are highly toxic and are known to contaminate a wide variety of foods such as maize, groundnuts, dried fruits, meat and milk-based products [5,6,7]. Aflatoxins are naturally occurring mycotoxin that is largely produced by *Aspergillus flavus* and *Aspergillus parasiticus* species of fungi. This is a highly toxic secondary metabolite that contaminates a number of crops, causing a great economic loss [8]. The major cereal crops in Nigeria are rice, maize, sorghum, wheat, millet, sugar cane and fonio millet with rice ranking as the sixth major crop in terms of the land area while sorghum account for 50% of the total cereal production and occupies about 45% of the total land area devoted to cereal production in Nigeria [9]. Contamination of agricultural produce mainly cereals, oily seeds and nuts such as peanuts with mycotoxins producing fungal species compromises the safety of food and poses a serious health risk to consumers [10,11].

Majority of the inhabitants of rural areas especially in the northern part of Nigeria consume cereals as their main staple foods and may stand a chance of being infected by aflatoxin. In Nigeria, cereals are sold in the open market with less or no regulation of quality. Contamination of cereals such as rice, maize and millet is an important issue for grain quality and from consumer's health point of view. In cereals however, aflatoxin contamination is a threatening issue and its negative effects on human health most especially infants and young children, are very alarming. The significant economic and health hazards posed by aflatoxin especially in developing countries that have poor food storages is of great concern, hence the need for the study.

Microbial and aflatoxin contamination is a major concern in food safety as they affect humans, animal and economic growth of any nation [12]. In Kano State, there is paucity of information on mycoflora and aflatoxin producing fungi from stored cereal grains. Hence, the present study was designed to document the mycoflora and aflatoxin producing fungi in some stored cereals (maize, millet, wheat) grain sold at Darki market, Wudil Local Area, Kano State, Nigeria in order to educate the public about good agricultural practices, storage, harvest and handling of the grains (cereals) to reduce the level of mycotoxin contamination.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of thirty (30) samples, ten (10) samples containing 250g each of maize, millet and wheat were obtained from storage facilities of traders in different location of Darki market respectively. The samples were collected twice at an interval of 2 weeks. The collected samples were labeled, packaged in sterile polyethylene bags, and immediately transported to the microbiology laboratory, Kano University of Science and Technology, Wudil for analysis.

2.2 Sample Preparation

About 80% of the collected samples were subjected to powdered form by grinding with a sterilized mortar and pestle, while the remaining 20% was kept to be used for direct plating during fungal isolation. The samples were stored at 4^oC pending further analysis [13]. Ten (10) grams of each grounded cereal sample was weighed into 90mls of peptone water; it was properly shaken and then used as stock. Several dilutions was be made to be achieve up to 4 fold (10^{-4}) for each prepared samples using 1ml from the stock homogenate and 9mls of peptone water for serial dilution. Then a serial dilution of 1ml was transferred into 9mls of peptone water in a test tube to give 10^{-2} dilutions. This process was continued until 10^{-4} dilutions [14].

2.3 Enumeration of Fungal Loads

For inoculation and enumeration of fungal loads, method described by Jallow *et al*., 2018 [14] was adopted. One (1) ml of 10^{-2} and 10^{-3} dilutions of each sample was inoculated in duplicate by spread plate method on a correspondingly labeled agar plate containing Potato Dextrose Agar (PDA) containing 500mg/l chloramphenicol (the antibiotics is aimed at suppressing the growth of bacteria) which was prepared using the manufacturers instruction manual. The plate was incubated at room temperature for 5-7days. Fungal growths were observed within the 5-7days in plate colonies and after the incubation period, fungal loads was then calculated in colony forming units per gram of sample (CFU/g) as:

$$\text{CFU/g} = \frac{\text{number of colonies} \times \text{reciprocal of the dilution factor}}{\text{Plating volume (1ml)}}$$

2.4 Isolation and Identification of Fungi

Fungal isolation was carried out by using direct plating method described by Gautam *et al*., 2012 [15] and adopted by Shamsudeen *et al*., 2017 [16]. Potato Dextrose Agar (PDA) with chloramphenicol (500mg/l) was prepared according to manufacturer's instructions and sterilized by autoclaving at 121^oC pressure for 15 minutes, and allowed to cool to about 45^oC. After cooling the media was poured into petri-dishes. Five seeds each of maize grain, millet and wheat each was randomly chosen using sterile forceps and was placed on the prepared petri-dishes. The cultures was covered, labeled and incubated at room temperature for 5-7 days.

2.4.1 Sub-Culturing

To obtain a pure culture, each distinct fungal colony was sub-cultured on freshly prepared petri-dishes of PDA medium which was prepared using the manufacturer's preparation manual, and then incubated at room temperature for 5-7 days for subsequent taxonomic identification [17,18]. After the period of incubation the individual isolates were identified based on the features such as color, shapes and colonial appearance for all the samples [19].

2.4.2 Microscopy

A drop of mounting fluid, lactophenol cotton blue solution was placed on a grease free slide. Scrapings of the pure isolates was taken from the Potato Dextrose Agar (PDA) and transferred on the fluid using a sterilized, cooled wire needle. It was pressed gently to enable it mix properly with the stain [18]. A sterile forceps was then used to place a cover slip over the slides and blotting paper was used to wipe excess stain and then examined under low magnification (x10) and high magnification (x40) objective lens of the microscope.

2.5 Extraction of Aflatoxin

Methods adopted by Shamsudeen *et al*. 2017 [16] were used for the extraction of aflatoxin. Twenty (20g) of each of the grounded samples was measured into a clean jar with seal. A 100ml of 70:30(v/v) methanol-distilled water solutions was added to the jar. The mixture was vigorously shaken for three (3) minutes, allowed to settled, then filtered through a Whatman No. 1 filter paper, the filtrate was obtained and the residue was discarded. The filtrate was concentrated

using a rotary evaporating machine, then the concentrated extracts was poured in sterilized bijou bottles labelled and kept in a refrigerator for further analysis [20].

2.5.1 Detection of aflatoxin using thin layer chromatography (TLC)

The chromatographic plates were coated with silica gel. Thirty grams (30g) of silica gel was mixed with 75ml of distilled water, this was used to coat the chromatographic plates and allowed to dry for 30minutes. The coated plates were activated by heating in an oven at 100^{o}C for 30minutes, and then the extract from extraction was spotted on the plates using capillary tubes. Hexane: ethyl acetate mixture of 70:30ml was used as the chromatographic solvent; it was run into the chromatographic tank to serve as the mobile phase of the chromatography. The spotted plates were then dipped into the tank containing the solvent without allowing the solvent to touch the areas spotted with the extract. The solvent was allowed to move the substance (extracts) until the solvent stop moving. The distance moved by the solvent and that moved by the substance were marked immediately after removing from the solvent and measured for calculating the retention factor using the relation;

$$RF = \frac{\text{Distance Moved by Substance (DMS)}}{\text{Distance Moved by Solvent (SF)}}$$

The presence of aflatoxins will be detected by illuminating the plates with ultra-violet light (UV) were blue fluorescence indicate aflatoxin B and green fluorescence will indicate aflatoxin G respectively [16] (Sham.

2.6 Molecular Analysis

2.6.1 DNA extraction

Method adopted by Umaima *et al*. 2018 [21] was used for the extraction of DNA. Fungal mycelium was produced in 20ml Potato Dextrose Broth. Mycelium was harvested by filtration through mesh sieves, washed with sterile water and deposited on whatman filter paper to remove excess water. Mycelium was ground to a fine powder in liquid nitrogen using nitrogen and DNA was extracted using the DSBIO quick genomic DNA extraction kit (Dongsheng Biotech) by following the manufacturer's instruction manual.

2.6.2 PCR amplification

Method adopted by Omaima *et al*. 2018 [21] was used for PCR amplifications. The PCR amplifications were carried in a total volume of 25µl, containing 20mg genomic DNA, 1 X PCR buffer (20mM Tris-HCL, 10mM $(NH_4)_2SO_4$, 10mM $MgSO_4$, 0.1% Triton X-100), 0.2 unit of Taq DNA polymerase and ITS1/ITS4 (ITS1 and ITS4 are universal fungal primers: where ITS1 is the forward primer while ITS4 is the reverse primer). Restriction Fragment Length Polymorphism (RFLP) was used. The base pair for forward primer ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and reverse primer ITS4 (5' TCCTCCGCTTATTGATATGC 3'). ITS is a standard marker for fungal DNA barcoding. The PCR amplification was carried out according to the following temperature: Initial step of 2min at 94^{o}C, 90 sec at 52^{o}C, 2min at 72^{o}C and a final step of 7min at 72^{o}C. Electrophoresis of PCR-amplified products was performed in 1.5% agarose gel. The PCR products were stained with ethidium bromide and visualized with 305nm ultraviolet light.

3. RESULTS

A total of 30 (thirty) cereal samples were analyzed in this study which comprise of ten samples each of maize (MA), millet (MI), and wheat (WH) purchased from Darki market, Wudil Local Government Area, Kano. Results of fungal count in this study shows that for maize samples, sample MA_2 had the highest fungal count with 4.2×10^3, while the least count were recorded in sample MA_{10} with 3.85×10^2. In millet samples, the highest fungal count of 4.1×10^3 in sample MI_8 while the least count were recorded in sample MI_9 with a count of 4.15×10^2. In wheat samples, sample WH_4 had the highest fungal count with 6.5×10^3, while the least count were recorded in sample WH_6 with count of 2.2×10^2. In the overall thirty cereal samples wheat samples recorded both the highest and lowest fungal count of 6.5×10^3 and 2.2×10^2 as shown in (Table 1).

Morphological and microscopic descriptions of the fungal isolates show that five fungal isolates namely: *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* spp, *Mucor* spp, and *Rhizopus* spp were isolated from the samples analyzed as shown in (Table 2).

Distribution and occurrence of fungal isolates in the cereal samples (maize, millet and wheat) shows that *Aspergillus niger* had the highest

count of occurrence of 21, *Rhizopus* spp had occurrence count of 14, *Fusarium* spp had an occurrence count of 11, *Aspergillus flavus* had an occurrence count of 8, while the least occurrence count was recorded in *Mucor* spp with an occurrence count of 7 as shown in (Table 3).

Thin layer chromatography, retention factor and type of aflatoxin detected shows that the retention factor after separation of individual components using thin layer chromatography has a range of 0.58 to 0.75, while illumination under UV-light showed blue fluorescent spot indicating aflatoxin B in nine (9) samples out of the thirty samples analyzed as shown in (Table 4).

Molecular analysis using polymerase chain reaction (PCR) showed that out of the six isolates, five isolates had a single band at 600bp on lanes 1, 2, 4 and 5, lane 6 had a single band at 700bp while lane 3 had a negative band as shown in (Fig 1).The SDA reactions were setup using the DNA polymerase and random hexamers on genomic DNA extracted from single spores of fungal genera (*Aspergillus*, *Rhizopus*, *Fusarium* and *Mucor*). The genomic DNA for the amplification was used directly for the SDA reaction without any additional purification step. An aliquot (1.5 µL) of the reaction was run on 1.5% agarose gel and the amplification products visualized. The amplified product was found to be ca. 10 kb in size (Fig. 1) for all the fungal templates used for the reaction (Fig 2).

Table 1. Total fungal counts for maize, millet and wheat cereal samples

S/N	Samples	TFC (CFU/g)
1	MA_1	2.29×10^3
2	MA_2	4.2×10^3
3	MA_3	2.15×10^3
4	MA_4	2.5×10^3
5	MA_5	3.85×10^3
6	MA_6	1.65×10^3
7	MA_7	1.76×10^3
8	MA_8	3.4×10^3
9	MA_9	2.4×10^3
10	MA_{10}	3.85×10^2
11	MI_1	4.0×10^3
12	MI_2	3.85×10^3
13	MI_3	1.8×10^3
14	MI_4	3.9×10^3
15	MI_5	2.8×10^3
16	MI_6	2.15×10^3
17	MI_7	2.3×10^3
18	MI_8	4.1×10^3
19	MI_9	4.15×10^2
20	MI_{10}	2.45×10^3
21	WH_1	1.55×10^3
22	WH_2	2.65×10^3
23	WH_3	3.5×10^3
24	WH_4	6.5×10^3
25	WH_5	4.5×10^3
26	WH_6	2.2×10^2
27	WH_7	3.55×10^3
28	WH_8	5.75×10^3
29	WH_9	2.15×10^3
30	WH_{10}	5.05×10^3

Key: S/N= Serial Number, MA= Maize samples, MI= Millet samples, WH= Wheat samples

Table 2. Morphological and microscopic description of fungal isolates

Isolates	Colonial Morphology	Microscopy	Organism
A	Pin like black powdery colony, reverse side is white	Non branched conidiophore with bulb end carrying conidia which appears dark brown	*Aspergillus niger*
B	Green growth, colony is plain with flat edges and has a white fruiting body	Radiating conidial heads with rough conidiophores.	*Aspergillus flavus*
C	Colony is pinkish white fluffy dense growth. Reverse side is tan or dark violet in color	Short crescent shaped conidiophores with abundant micro conidia	*Fusarium* spp
D	Rapid growing wooly colony which appears white when new but turns greyish brown with aging. The reverse side remains pale white	It has a broad hyphae which are non-septated. Sporangium has a thin wall. It has no rhizoids	*Mucor* spp
E	Deeply cottony sponge like growth grey-brown	It has well developed rhizoids. Sporangiospores are attached to stolons	*Rhizopus* spp

Table 3. Distribution of fungal isolates in maize, millet wheat samples obtained from Darki town

S/N	Samples	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium* spp	*Mucor* spp	*Rhizophus* spp
1	MA_1	+	-	-	-	-
2	MA_2	-	-	-	-	-
3	MA_3	+	-	-	-	-
4	MA_4	+	-	-	-	+
5	MA_5	+	-	-	-	-
6	MA_6	+	-	+	-	-
7	MA_7	+	-	+	-	-
8	MA_8	+	-	-	-	+
9	MA_9	+	-	-	+	-
10	MA_{10}	+	-	-	-	-
11	MI_1	+	+	-	+	-
12	MI_2	-	+	+	+	-
13	MI_3	+	+	+	-	-
14	MI_4	-	+	+	-	+
15	MI_5	+	+	+	-	+
16	MI_6	-	-	+	-	-
17	MI_7	+	+	+	-	+
18	MI_8	+	+	-	-	-
19	MI_9	+	+	-	-	+
20	MI_{10}	+	-	+	-	-
21	WH_1	+	-	-	+	+
22	WH_2	-	-	-	-	+
23	WH_3	-	-	-	-	+
24	WH_4	+	-	+	+	+
25	WH_5	+	-	+	-	+
26	WH_6	+	-	-	+	-
27	WH_7	+	-	-	-	+
28	WH_8	-	-	-	+	-
29	WH_9	-	-	-	-	+
30	WH_{10}	-	-	-	-	+
	Total	21	8	11	7	14
	Percentage (%)	34.43%	13.11%	18.03%	11.48%	22.95%

KEY: + = present, - = absent

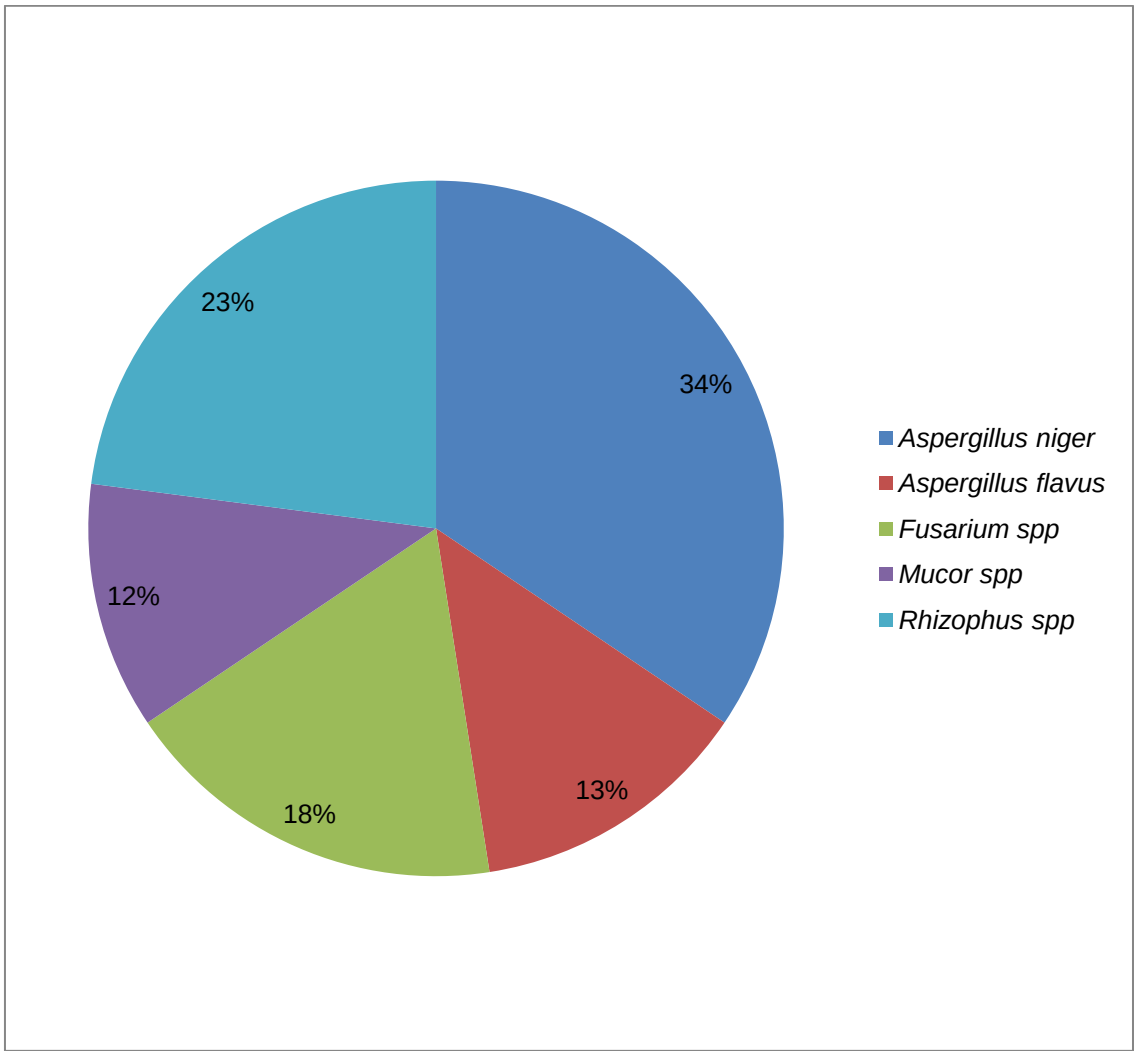


Fig. 1. Percentage distribution of fungal isolates in maize, millet wheat samples obtained from Darki town

Table 4. Retention Factor and Fluorescence under UV light for Maize, Millet and Wheat Samples

S/N	Samples	DMS(cm)	SF(cm)	RF	Fluorescence
1	MA_1	4.3	6.5	0.66	Absent
2	MA_2	4.3	6.5	0.66	Absent
3	MA_3	4.2	6.5	0.65	Absent
4	MA_4	4.0	6.5	0.62	Absent
5	MA_5	4.1	6.5	0.63	Absent
6	MA_6	4.6	6.5	0.71	Absent
7	MA_7	4.9	6.5	0.75	Blue
8	MA_8	4.7	6.5	0.72	Absent
9	MA_9	4.7	6.5	0.72	Absent
10	MA_{10}	4.6	6.5	0.71	Absent
11	MI_1	4.6	6.5	0.71	Blue
12	MI_2	4.5	6.5	0.69	Blue
13	MI_3	4.5	6.5	0.69	Blue
14	MI_4	4.5	6.5	0.69	Blue
15	MI_5	4.7	6.5	0.72	Blue

S/N	Samples	DMS(cm)	SF(cm)	RF	Fluorescence
16	MI_6	4.7	6.7	0.70	Absent
17	MI_7	4.8	6.7	0.72	Blue
18	MI_8	4.8	6.7	0.72	Blue
19	MI_9	4.7	6.7	0.70	Blue
20	MI_{10}	4.8	6.7	0.72	Absent
21	WH_1	4.1	6.7	0.61	Absent
22	WH_2	3.9	6.7	0.58	Absent
23	WH_3	4.3	6.7	0.64	Absent
24	WH_4	4.2	6.7	0.63	Absent
25	WH_5	4.2	6.7	0.63	Absent
26	WH_6	4.0	6.5	0.62	Absent
27	WH_7	4.0	6.5	0.62	Absent
28	WH_8	4.0	6.5	0.62	Absent
29	WH_9	4.1	6.5	0.63	Absent
30	WH_{10}	4.2	6.5	0.65	Absent

KEY: MA = Maize sample, MI = Millet sample, WH = Wheat sample

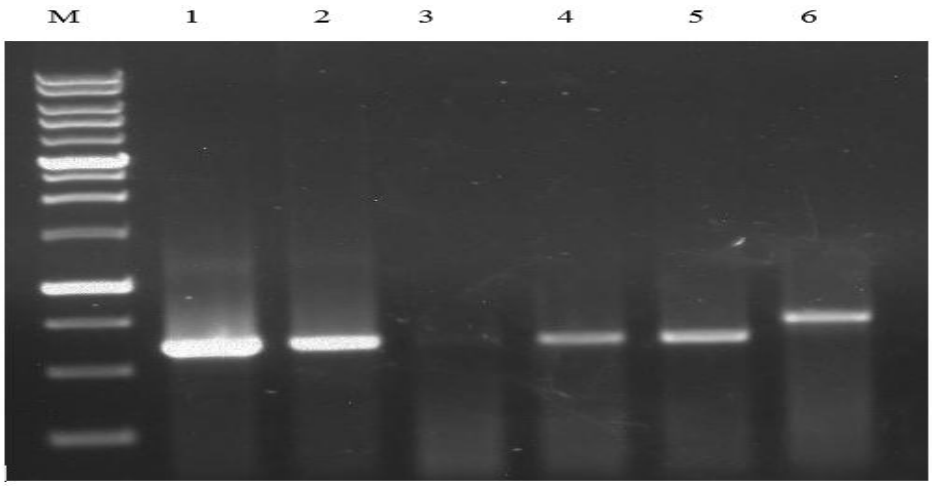
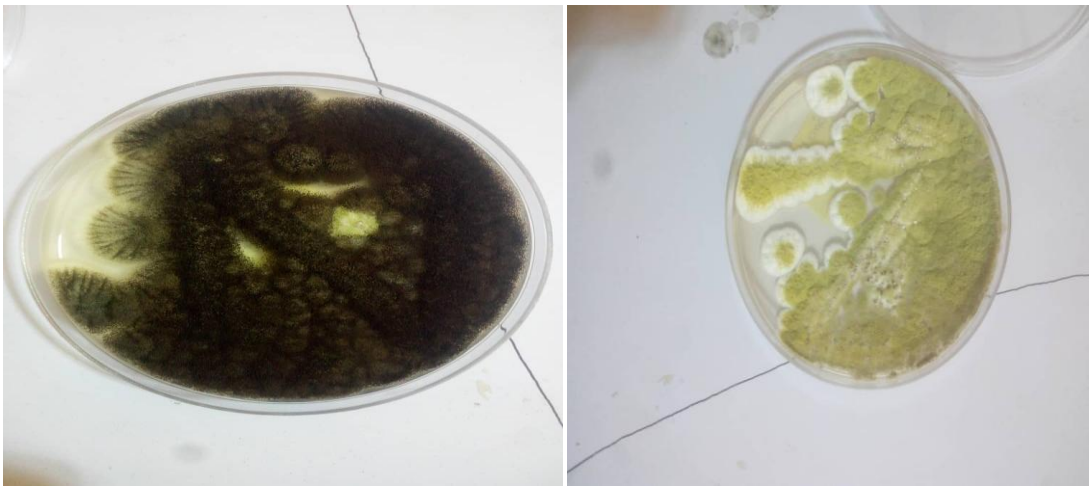
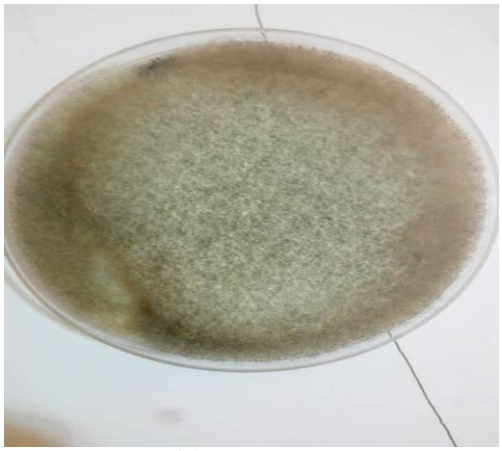


Fig. 2. Agarose gel electrophoresis (1.5%) for amplified genome by PCR protocol in UV transilluminator (M; marker, 250bp DNA ladder: Lane 1 & 2 – *Aspergillus* spp., 3 – *Mucor* spp., 4 – *Aspergillus* spp., 5 – *Fusarium* spp., and 6 – *Rhizopus* spp. respectively)



Aspergillus niger *Aspergillus flavus*

Fusarium spp



Rhizopus spp

Mixed growth of *Aspergillus niger* and Fusarium spp

Mixed growth *Aspergillus flavus* and *Aspergillus niger*

4. DISCUSSION

This study shows that the cereal (maize, millet, wheat) samples analyzed contained a high number of fungi; this could be due to possible source of contamination. Various factors influencing aflatoxin contamination are soil type, fungal species in the soil, climate, and improper agricultural practices, as well as the weather condition during harvesting and post-harvest conditions. The variations in fungal counts obtained may be due to introduction during exposure to and direct contact with agricultural products in the market [22]. The fungal counts ranged from 2.2×10^2 to 6.5×10^3 CFU/g in the cereal samples and the ranges were within the acceptable limits of 10^2 to 10^5 as stated by [23] Food and Agricultural Organization (1992).

In this study, five different fungi were isolated and identified as: *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp *Fusarium*s pp, *Mucor* spp and similar findings of some of these fungi have been reported earlier [24,17,25]. Fungi are the major enemies to stored grains [17]. Fungi are part of the normal flora of food products; however, some produce toxic metabolites such as mycotoxins. Mycotoxins are harmful to human health also degrades food taste and flavor, with consequence economic losses.

In this study the fungi with highest frequency of occurrence was *Aspergillus* spp and this report is

similar to the findings of [26] who reported *Aspergillus* spp as the common isolated fungi. *Aspergillus niger* had a percentage rate of 34.43% which was the highest. However, *Aspergillus niger* is not known to be pathogenic, however, in very rare cases it could be an opportunistic microorganism capable of colonizing and causing ear, nose and lungs infection on people with acute illness and immuno-compromised individuals [27].

The presence of these fungi is an indication of possible health hazards as some species of *Aspergillus* are known to cause food intoxication and food poison [28]. Among the three different cereal samples analyzed (Maize, Millet and Wheat), Millet samples had the highest occurrence of *Aspergillus flavus* and this agrees with the work of [29]: which reported that millet samples recorded highest occurrence of *Aspergillus flavus*. The high frequency of *A. flavus* observed in millet sample may be as a result of the contact of these substrates with soil. Also previous studies had shown that *Aspergillus flavus* frequently occurred in the field and this is attributed to agro-ecological condition of the field [29].

The thin layer chromatography, retention factor and type of toxin detected revealed that aflatoxin was present in nine (9) samples and absent in twenty-one (21) samples. Eight of the aflatoxins were detected in millet samples (MI_1 to MI_5 and MI_7 to MI_9) while only one was detected in maize sample (MA_7). The retention factor after separation of individual components using thin layer chromatography (TLC) had a range of 0.58-0.75. Samples detected with aflatoxin had retention factors of 0.69, 0.70, 0.71, 0.72 and 0.75. This is in accordance with the work of [30] who reported having RF values of 0.59-0.80. It has been reported by [31], that when similar compounds are extracted using the same extraction method and developed on thin layer chromatography using the same stationary and mobile phase, they are likely to have the same RF values. For molecular analysis after DNA extraction, PCR reactions were conducted with primer pairs ITS1/ITS4. The products of the PCR reactions were amplified, run on 1.5% agarose gel and the amplification products visualized. The amplified product was 100kb as the size of amplified DNA fragments for all the fungal templates used for the reaction. The fragment size obtained by PCR reaction with primers ITS1/ITS4 was 600bp for most of the isolates. All the *Aspergillus* spp, *Fusarium* spp. And *Rhizopus* spp showed positivity for the molecular markers ITS1/ITS4 by having positive bands. Only Mucor spp showed negative results for ITS1/ITS4 primers and showed no band. Identifying fungi based on morphology alone is challenging, since there are a limited number of morphological characteristics that can be used for identification. However PCR can serve as a confirmatory procedure for identification of fungi to the species level. The implication of PCR in this study is to know the *Aspergillus* species in the grains in order to create awareness to the general public to stay away from mycotoxins.

5. CONCLUSION

This study shows that most of the cereals was contaminated with either one or more molds but within the satisfactory and marginal limits. The presence of these fungi in cereals is an indication of possible health hazards as some species of *Aspergillus* are known to cause food intoxication and food poisoning. However, aflatoxin contamination is a threatening issue and its negative effects on human health most especially infants and young children, are very alarming. Hence, aflatoxins contamination of crops at pre-harvest and post-harvest conditions should be controlled to some extent by the implementation of good agricultural practices (GAPs). It is recommended that mass awareness and enlightenment campaign should be carried out in order to educate the farmers, about good agricultural practices, storage, harvest and handling of the grains (cereals) to reduce the level of mycotoxin contamination. Additionally, further study should be done and PCR should serve as an adoptable standardized procedure for identification of microorganisms rather than using morphology alone.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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