



# Fungi Isolation, Identification and Detection of Aflatoxin in Cereals Sold In Major Markets of Katsina Metropolis

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## Research Article

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## Abstract

Aflatoxins are highly toxic and carcinogenic secondary metabolites produced by fungi. The study was aimed at isolating and identifying fungi associated with cereals sold in major markets in Katsina metropolis and detect aflatoxin in the cereals.. Sixteen (16) samples (four each) were collected from four different market namely; Chake, Central, Tsohuwar Kasuwa and Yarkutungu markets. Fungi were isolated and identified using standard microbiological procedures and aflatoxin was detected using thin layer chromatography (TLC). Fungal species isolated and identified were *Aspergillus niger*, *A. flavus*, *A. parasiticus* and *A. fumigatus* with the occurrence of 6(35.3%), 5(29.4%), 5(29.4%) and 1(5.9%) respectively. Blue florescence which indicated aflatoxin B was detected in the samples with retention factors of 0.52-0.88. Aflatoxin was not detected in the samples having the retention factor above 0.88. This study therefore concluded that cereal samples collected from major markets of Katsina metropolis were contaminated with one or more *Aspergillus* species and detection of aflatoxin B in some samples is a health hazard. Thus, fungal and aflatoxin contaminations of cereals at the field, during harvest or storage should be controlled through good agricultural, processing and storage practices as well as ensuring hygienic practices at the point of sales.

**Keywords:** Aflatoxin; *Aspergillus*; Cereals; Katsina Markets

## Introduction

Mycotoxins, secondary metabolites made by filamentous fungi, are known to have a wide range of negative health effects on both humans and animals, including death [1]. The Mycotoxins with the biggest impact on agriculture

are aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremor genic toxins, and ergot alkaloids [2]. These toxins come from several species of mycotoxigenic fungi belonging to various genera [3]. Some molds have the capacity to produce many Mycotoxins, and different fungi species can produce different mycotoxins. In contaminated

cereals and grains, Mycotoxins contamination is frequently multi-mycotoxin [4]. There are three genera of mycotoxigenic fungi that are a part of the human food chain: *Aspergillus*, *Fusarium*, and *Penicillium* [5,6]. Aflatoxin is the name for mycotoxins that arise from *Aspergillus* species, common post-harvest fungi found in grains. These poisons are harmful to both humans and animals, and they play a role in the global food industry's financial losses.

According to Sule, et al. [7], enormous amounts of food are wasted every year as a result of fungal contamination and aflatoxigenic fungi invasion. This type of spoilage is particularly common in tropical nations like Nigeria where storage issues are present [7]. About 25% of the world agricultural produce, according to the Food and Agriculture Organization is contaminated by mycotoxins, which result in significant losses for farmers [8]. The existence of mycotoxins in food has also been confirmed by the World Health Organization [9]. They are capable of produce more than 14 mycotoxins, of which aflatoxins are the most significant categories. *Aspergillus flavus* and *Aspergillus parasiticus* are the most significant aflatoxin-producing fungi, with rising importance in food and feed poisoning [10]. Because they produce blue or green fluorescence when exposed to UV light, respectively, the naturally occurring aflatoxins were given the designations B or G and produced aflatoxicosis when consumed in contaminated food or feed [11,12].

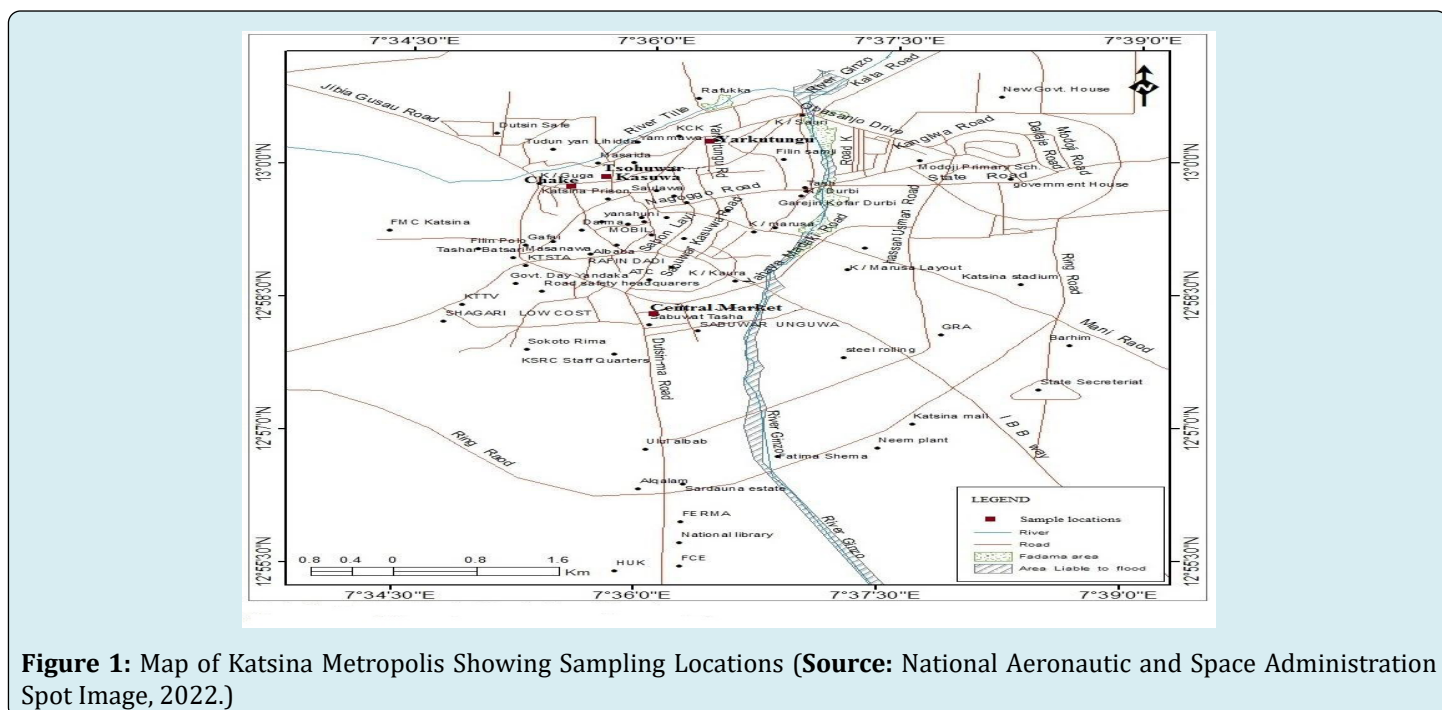
Lack of information and consumption of tainted food and feed around the world make aflatoxins a major cause of disease outbreaks [13]. Significant concern exists

regarding the presence of excessive quantities of aflatoxins in non-industrialized nations' diet. Consuming aflatoxins increases the risk of mutagenic, carcinogenic, teratogenic, and immunosuppressive health effects in both people and animals. Acute liver damage from aflatoxins can progress to chronic conditions such liver cancer, hemorrhages, oedema, and even instantaneous death in humans. Numerous cases of both human and animal aflatoxicosis have frequently gone unreported in every region of this country due to the sneaky nature of aflatoxin production and the health issues that resulted, which made the diagnosis of aflatoxicosis much more challenging. This shows that there has not been much research on aflatoxicosis in Nigeria and that there isn't much knowledge about the toxin. In view of the negative public health and economic impacts of aflatoxins, this study aimed to isolate and identify fungi and detect aflatoxin in cereals using thin layer chromatographic techniques.

## Materials and Methods

### Study Area

Sampling was carried out in Central, Yar Kutungu, Chake and Tsohuwar Kasuwa markets of Katsina metropolis, Katsina State. Katsina is a state in the northwestern geopolitical zone of Nigeria. According to National population commission (NPC, 2006), Katsina's estimated population was 5, 792, 578 people. The state is bounded by Niger republic to the north, Jigawa and Kano states to the east, Kaduna state to the south and Zamfara state to the west Figure 1.



**Figure 1:** Map of Katsina Metropolis Showing Sampling Locations (Source: National Aeronautic and Space Administration Spot Image, 2022.)

### Sample Collection

A total of Sixteen (16) samples (Four each) of rice, wheat, maize and millet were collected from four selected major markets (Figure 1) and were transported to the laboratory of the Department of Biological Sciences, Al-Qalam University Katsina for subsequent analyses.

### Sample Processing

About 80% of the collected samples were grounded to powdered form using sterilized mortar and pestle, while the remaining 20% were kept for direct plating during fungal isolation. The samples were stored at 4oC pending further analyses [14].

### Isolation and Identification of Fungi

Fungal isolation was carried out by direct plating method described by Gautam AK, et al. [15] and adopted by Shamsudeen, et al. [16]. Potato Dextrose Agar (PDA) with chloramphenicol (500mg/l) was prepared following the manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes, and allowed to cool. After cooling, prepared the medium was dispensed into petri-dishes and samples were inoculated onto the petridishes containing the prepared medium and incubated at room temperature for 5-7 days. After incubation, fungal colonies were purified by sub culturing onto freshly prepared PDA plates and incubated at room temperature for 5-7 days. Identification of pure fungal isolates was carried out by observing cultural characteristics such as pattern of growth and pigment production as well as microscopic characteristics such as hyphal morphology and conidia as described by the El-Hassan, et al. [17].

### Microscopic Slide Preparation and Observation

A drop of mounting fluid, lacto phenol cotton blue solution was placed on a grease free slide. Scrapings of the pure isolates were taken from the Potato Dextrose Agar (PDA) and transferred on the fluid using a sterilized, cooled wire needle. It was then pressed gently to enable it mix properly with the stain. A sterile forcep was used to place a cover slip over the slides and blotting paper was also used to wipe excess stain and then examined under low magnification (x10) and high magnification (x40) objectives [17,18].

### Extraction of the Samples

Thirty (30g) of the samples each was grounded using pestle and mortar. Twenty (20g) of each of the grounded samples was measured into a clean conical flask with seal. A 100ml of 70:30(v/v) methanol-water solutions was added and the flasks were sealed. The mixture was vigorously shaken for three (3) minutes, allowed to settled and then filtered through a filter paper and the filtrate was obtained, the residue was discarded and the filtrate was concentrated using a rotary evaporating machine. The concentrated extracts were poured in clean bijou bottles, labeled and kept in a refrigerator for further analysis [19].

### Detection of Aflatoxin in the Samples' Extracts

The chromate graphic plates were coated with silica gel. Thirty grams (30g) of silica gel was mixed with 75ml of distilled water; this was then used to coat the chromatographic plates and allowed to dry for 30minutes. The coated plates were activated by heating in an oven at 100oC for30minutes, then, the extracts from extraction were spotted on the plates using capillary tubes. Chloroform: methanol mixture of 97:3ml was used as the chromatographic solvent; it was run into the chromatogram or chromatographic tank to serve as the mobile phase of the chromatography. The spotted plates were 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 was marked immediately. the retention factor was determined using the relation;  $RF = \text{Distance Moved by Substance (DMS)} / \text{Distance Moved by Solvent (SF)}$  Aflatoxins was then detected by illuminating the plates with ultra violet light (UV) where blue or green fluorescence indicate aflatoxin B and G respectively [20].

## Results

### Identification of Fungal Isolates

The macroscopic and microscopic features of the identified fungal species were presented in (Table 1). The species identified were *A. niger*, *A. flavus*, *A. fumigatus*, and *A. parasiticus*.

Isolates Code	Macroscopic Features	Microscopic Features	Species Identified
W	Black mycelia growth and fully extended in the growth medium	Conidiophores that are not branched with bulb like end bearing conidia	<i>A. Niger</i>
X	Dark green colony that are umbonate	Spherical conidia beared on rough conidiophore	<i>A. parasiticus</i>

Y	Bluish green colony, that are hairy and flat	conidial heads were typically columnar. Conidiphore stipes were short, smooth walled conidia globose to subglobose,	<i>A. fumigates</i>
Z	Green colony with flat edges	conidial heads were typically radiate, conidia were globose to subglobose,	<i>A. flavus</i>

**Table 1:** Macroscopic and Microscopic Features of Fungi Species Identified

Sampling Location	Samples	<i>A. niger</i>	<i>A. flavus</i>	<i>A. parasiticus</i>	<i>A. fumigatus</i>
Chake Market	MZ	-	-	+	-
	RC	+	+	-	-
	ML	-	-	+	-
	WH	+	-	+	-
Central Market	MZ	-	+	-	+
	RC	+	-	-	-
	ML	+	-	-	-
	WH	+	-	-	-
T/Kasuwa Market	MZ	-	-	+	-
	RC	-	+	-	-
	ML	-	-	+	-
	WH	-	-	+	-
Yarkutungu Market	MZ	+	-	-	-
	RC	-	+	-	-
	ML	-	+	-	-
	WH	+	-	-	-

Key: MZ= Maize, RC = Rice, ML = Millet, WH = Wheat, + = Present, - = Absent

**Table 2:** Fungal Species Isolated From the Samples

### Occurrence of Fungal Species in the Samples

The occurrence of fungal species was presented in table 3. *A. parasiticus* had the highest occurrence of 6(35.3%)

followed by *A. flavus* and *A. niger* with the occurrence of 5(29.4%) each. the lowest of occurrence of 1(5.9%) was recorded with *A. fumigatus*.

Species	Occurrence	Percentage Occurrence (%)
<i>A. niger</i>	5	29.4
<i>A. flavus</i>	5	29.4
<i>A. parasiticus</i>	6	35.3
<i>A. fumigatus</i>	1	5.9
Total	17	100

**Table 3:** Occurrence of Fungal Species in the Samples

### Colour of the Samples' Extracts

The colour of the samples extracts collected from various markets was presented in Table 4. The colours are

dark yellow, yellow, pale yellow, turbid white, white and dark brown.

Sampling Location	Samples	Colour of the Extracts
Chake Market	MZ	Dark Yellow
	RC	White
	ML	Pale Yellow
	WH	White
Central Market	MZ	Yellow
	RC	Yellow
	ML	White
	WH	Turbid White
Tsohuwar Kasuwa Market	MZ	Pale Yellow
	RC	White
	ML	Pale Yellow
	WH	Pale Yellow
Yarkutungu Market	MZ	Yellow
	RC	White
	ML	Yellow
	WH	Dark Brown

Key: MZ= Maize, RC = Rice, ML = Millet, WH = Wheat

**Table 4:** Colour of the Samples Extracts

### Retention factor, Florescence and Aflatoxin Detected in the Samples

Table 5 shows the retention factor and aflatoxin detected in samples. The distance moved by the solvent (DMS) ranges

from 2.4-2.6cm and solvent front (SF) ranges from 1.3 to 2.3 cm. while the RF ranges from 0.52 to 1.9. Blue florescence (i.e aflatoxin B) was detected in samples collected from Chake, Yarkutungu and Tsohuwar Kasuwa markets but was not detected in samples collected from Central Market (Table 5).

Sampling Location	Samples	DMS(cm)	SF (cm)	RF	Florescence	Aflatoxin Detected
Chake Market	MZ	2.4	1.9	0.76	Blue	AFB
	RC	2.4	1.4	0.56	Blue	AFB
	ML	2.4	1.8	0.75	Blue	AFB
	WH	2.4	1.9	0.76	Blue	AFB
Central Market	MZ	2.6	1.8	1.9	ND	Absent
	RC	2.6	2.3	1.4	ND	Absent
	ML	2.6	1.9	1.8	ND	Absent
	WH	2.6	2	1.9	ND	Absent
Tsohuwar Kasuwa Market	MZ	2.5	2.1	0.88	Blue	AFB
	RC	2.5	1.9	0.79	Blue	AFB
	ML	2.5	1.9	0.79	Blue	AFB
	WH	2.5	1.3	0.54	Blue	AFB
Yarkutungu Market	MZ	2.5	1.8	0.72	Blue	AFB
	RC	2.5	1.8	0.72	Blue	AFB
	ML	2.5	1.3	0.52	Blue	AFB
	WH	2.5	1.7	0.62	Blue	AFB

Key: DMS = Distance Moved by the Solvent, SF = Solvent Front, RF = Retention Factor, AFB = Aflatoxin B, ND = Not Detected.

**Table 5:** Retention Factor, Florescence and Aflatoxin Detected

## Discussion

The present study revealed that samples collected from major markets of Katsina Metropolis were contaminated by *A. niger*, *A. flavus*, *A. parasiticus*, and *A. fumigatus*. The presence of these fungal species is an indication of possible health hazard as some species of the genus *Aspergillus* were reported to cause food intoxication and poisoning through production of toxic secondary metabolites termed aflatoxins [21]. Fungal contamination of food is a major concern in food safety as huge quantities of foods are wasted annually, because this contamination renders the food unfit for human and animals consumption as a result of physical damage and reduction of its nutritional value [7]. Also, fungal food spoilage and toxicities have resulted in illnesses and deaths of humans, animals and plants as well as the spoilage of large percentage of food products leading to significant decline in plants and animals productivities and overall economic loss in many regions of the world [22-24].

The occurrence of these species in cereal grains was reported by many researchers [17,20,25]. The presence of these species in the samples could be due to careless and improper handling, poor storage and unhygienic conditions as well as favorable environmental factors that favor the growth of food spoilage fungi [25].

In this study, analysis of samples' extracts using thin layer chromatography revealed the retention factor (RF) values that ranged from 0.52-1.9. Florescence was not detected in samples with retention factor value above 0.88. Blue florescent which indicated aflatoxin B was detected in samples with retention factor of 0.52-0.88. These retention factors were within the ranges to which aflatoxin B was detected by El-Hassan, et al. [17,20,26,27] reported that when similar compounds are extracted using the same method, and developed on thin layer chromatography using the same stationary and mobile phases, they are likely to have the same retention factor values. Occurrence of aflatoxin in any sample due to contamination by aflatoxigenic fungi either in field or during storage, makes the sample unfit for human and animal consumption as aflatoxins are group of toxic and carcinogenic secondary metabolites of fungal origin. *A. flavus*, *A. parasiticus*, and rarely other species and strains of *Aspergillus* were reported as potent aflatoxin producers [28]. Aflatoxins are very powerful hepatocarcinogens and their naturally occurring mixtures have been classified as a class I human carcinogen [29]. They play role in causing liver damage, liver cirrhosis and induction of tumor in both man and animals [16]. Health problems such as infertility in men [30] and reduction in salivary secretory immunoglobulin A levels [31] were reported.

## Conclusion

The results of this study showed that cereal samples analysed were contaminated with one or more fungal species that are known to cause food poisoning and foodborne intoxication. Detection of aflatoxin B in some samples is a potential health hazard as aflatoxins were reported to induce cancers, tumors and other health problems. Therefore, fungal and aflatoxin contaminations of cereals at the field, during harvest or storage should be controlled through good agricultural processing and storage practices as well as ensuring hygienic practices at the point of sales.

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