

## Original Research Article

# FUNGAL AND MYCOTOXIN CONTAMINATION OF STORED MAIZE IN KOGI, NORTHCENTRAL NIGERIA: AN IMPLICATION FOR PUBLIC HEALTH

### ABSTRACT

The maize value chain in the Kogi State and most parts of the country from where maize is purchased into the State lacks mechanisms that ensure grain quality and safety. This study was designed to evaluate toxigenic fungi and associated mycotoxins in maize produced within different agro-zones of Kogi State. Harvested and stored maize seeds under different storage conditions were collected from three different zones (Zone B Bassa, Zone C Lokoja, Zone D Idah), ~~cultured~~. Different fungal species were isolated by culturing using the spread plate technique on potato dextrose agar (PDA) and identified microscopically. Mycotoxin production by isolated fungi was subsequently evaluated for Deoxynivalenol (DON) contamination using the High-Performance Liquid Chromatography technique (HPLC). The outcome of the study was statistically analysed using simple frequencies and percentages. *Aspergillus* spp. and *Penicillium* spp. were the fungi found to be associated with the stored seeds in Kogi, while *Fusarium* spp. *Mucor* spp. and *Rhizopus* spp. were the field fungi identified. Of the thirteen samples collected, the most common genera were *Aspergillus* (isolated from 41.67% of the evaluated samples), *Fusarium* (27%) and in a lesser extent *Rhizopus* spp. (8.33%). The result also shows DON was detected in 92.3% of the stored maize samples, making it one of the widespread mycotoxin contaminants of maize grain. Implications of this study for human and animal health and economic development were discussed and appropriate recommendations made especially for adoption of proper storage technology among small-scale farmers for improved maize quality and safety.

### Keywords

Mycotoxin, toxigenic fungi, contamination, deoxynivalenol (DON), health.

### 1 Introduction

Maize is the third most important cereal crop in the world [1], and one of the most staple foods in the Northern Nigeria. Nigeria is one of the largest maize producing countries in Africa [2]. The nutritional components which include carbohydrates, potassium, vitamins, minerals and fibers

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can be compared to those of sorghum, rice, cassava, yam, potato etc. This crop serves a vital link in the human food chain in Nigeria and most parts of the world. Maize grains are presently used in the food industries as an important component in weaning foods for infants and it is equally valued by adults [3].

Since the crop became known, its utilization has driven high production all over Nigeria especially in Kaduna, Taraba, Adamawa, Niger, Nasarrawa and Benue States [2]. Maize is used in many important starchy foods for human and animal consumption, particularly in northern and western Nigeria as 'ogi' or 'eko tutu', 'kunu', and 'koko', as tradition fermented porridge [4].

These names are local names used in Nigeria so use of brackets will be appropriate.

Maize grain is not consumed soon after harvest but often stored for many months to be sold or consumed later. It has been reported by several researchers that fungal infestation in maize results in color change, decreases in nutritional values, and reduction of overall quality and quantity of the maize. Fungi are agents of food contamination and many species are saprobes, found in a variety of habitats and are ubiquitous agents of decay. Several of these fungal species have been found associated with production of mycotoxins, which are of public health importance [5].

~~Major fungi associated with grain storage, including maize, are *Aspergillus flavus*, *Fusarium* sp. and others. Fungal load in maize presents a major risk for humans and animals, through production of mycotoxins (especially Aflatoxins).~~ While in storage, grains are mostly susceptible to infection by species of fungi. Infected grains by fungi result in reduced germination, visible mould discoloration; chemical and nutritional changes, increased its mustiness, production of carcinogenic toxins and finally leading to spoilage of grains in many ways [6]. Major fungi associated with grain storage, including maize, are *Aspergillus flavus*, *Fusarium* sp. and others.

Fungal growth in maize is facilitated by hot and humid conditions [7]. In tropical and subtropical countries, a large proportion of the grain (such as maize) is harvested and stored under hot and humid conditions, and most farmers lack proper knowledge, equipment and methods of drying grains [8]. Subsequently, the maize is stored while still relatively moist and warm; both warmth and high moisture contents can result in rapid deterioration of the grains and promote the growth of microorganisms (e.g. fungi and bacteria) and insects in the grains [9]. Maize, like other stored products is hygroscopic in nature and tends to absorb or release moisture. Even if properly dried

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after harvest, exposure to moist and humid conditions during storage will cause the (kernel) to absorb water from the surroundings, leading to increased maize moisture contents, which result in enhanced deterioration. To maintain high quality maize during storage, maize should be protected from weather (including relative humidity and temperature), growth of microorganisms, and insects [10]. Also poor harvesting practices, unsuitable storage conditions, improper transportation, marketing, and processing also contribute to fungal growth. These environmental conditions as well as the food production chains are characteristic in most parts of Kogi where ~~this staple maize food~~this staple maize food are-is susceptible to toxigenic fungi and obviously their mycotoxin ~~contaminants~~contaminants. Fungal presence and growth in these grains therefore present a major risk for humans and animals, through production of mycotoxins.

As occurrence of fungi contaminant of maize increases; this poses a threat to the health of both humans and livestock. In order to effectively reduce its presence on grains and adverse health impact, especially the toxigenic types, it is important to detect them as quickly as possible and identify and control all the environmental factors which promote their growth and development [11] possibly through reengineering, sensitization, awareness, and other effective interventions.

### 1.1 Fungi that invade stored seeds

Storage fungi are those that grow on products in storage; one characteristic that they share in common is the ability to grow without free water. they comprise several species of *Aspergillus* spp. and a few of *Penicillium* spp. as stated by [12]. All these have the ability to grow in grain and seeds whose moisture contents are in equilibrium with relative humidity of 70% - 90% [12]. Most of these fungi are common on a great variety of organic and inorganic materials especially decaying vegetation, food products, fabrics and insulating materials made of plant fibres, paints, coatings, leather goods and glues. They occur almost everywhere and contaminate all grains and seeds.

-Fungi are known to cause pathological problems in maize seeds, therefore, imparting injuries on them. The field fungi often colonize seed primordial and maturing seeds and reduce seed yield, qualitatively and quantitatively.

Fungi belonging to facultative saprophytes and facultative parasites may lower the quality of seeds by causing discoloration, others are; reduction or elimination of germination capacity and

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several other physiological alterations in seeds [13]. These disorders have their sources both from field and stores. Shetty [14] reported that seed borne fungi are commonly found within or outside the seed; the inoculum may be carried on seed surface, usually as propagules such as spore, sclerotium or fragment of mycelia or they may be as dormant mycelia or sclerotium within the various tissues of the seed. Some have been found in the endosperm, ovules and on the pericarp and seed coat.

### 1.2 Mycotoxigenic Fungi

Several genera and species of filamentous fungi produce polypeptide or polyketide-derived mycotoxins that have significant agricultural, epidemiological and economic impact. *Aspergillus*, *Fusarium*, and *Penicillium* species are responsible for the majority of agricultural mycotoxin contamination. These fungi are common components of the microbial flora associated with many agronomic crops, including maize, peanuts, tree nuts, grapes, coffee, cotton, wheat, barley, and other cereal grains [15]. Thus those species of fungi that have toxic effect on humans and animals are referred to as mycotoxigenic fungi.

### 1.3 Mycotoxin

Mycotoxin is a toxic secondary metabolite produced by organisms of the fungus kingdom and is capable of causing disease and death in both humans and animals. The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops. One mold species may produce many different mycotoxins, and several species may produce the same mycotoxin [16].

Most fungi are aerobic (use oxygen) and are found almost everywhere in extremely small quantities due to the minute size of their spores. They consume organic matter wherever humidity and temperature are sufficient. Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high. The reason for the production of mycotoxins is not yet known; they are not necessary for the growth or the development of the fungi [17]. Because mycotoxins weaken the receiving host, the fungus may use them as a strategy to better the environment for further fungal proliferation. The production of toxins depends on the surrounding intrinsic and extrinsic environments and these substances vary greatly in their

toxicity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms [18].

### 1.3.1 Types of mycotoxins

Aflatoxins (AFLs), OchratoxinsA (OTA), trichothecenes as Vomitoxin (DON), Zearelenone (ZEA), Fumonisin B1 and B2 (FUMO B1, FUMO B2), tremorgenic toxins, sterigmatocystin, citrinin, patulin and ergot alkaloids are types of mycotoxins. Of particular interest in this study is the vomitoxin or deoxynivalenol (DON) of the trichothecene family.

#### 1.3.1.1 Deoxynivalenol (DON)

Deoxynivalenol is a mycotoxin produced by fungi of the *Fusarium* genus, i.e. *Fusarium culmorum* and *Fusarium graminearum*. Due to the high toxicity of *Fusarium* toxins and high occurrence of the fungi species producing them, these mycotoxins belong to the most animal and human health endangering ones, which are abundant in various cereal crops (wheat, maize, barley, oats, and rye) and processed grains (malt, beer and bread). Chemically, it belongs to trichothecenes. In contaminated cereals, 3- and 15-acetyl DON can in significant amounts (10 – 20%) occur concomitantly with DON. The fungi producing trichothecenes are soil fungi and are important plant pathogens which grow on the crop in the field [19].

Studies have shown that short-term and sub-chronic exposure to DON decreased body weight, weight ~~gain and gain~~, and feed consumption in rats and mice. Haematological effects were also observed. Conflicting results ~~are observed~~ are observed for the effect of DON on organ weights ~~reported that reported that~~ spleen and liver weights and the liver-body ~~and kidney and kidney~~-body weight ratios increased in Sprague-Dawley rats gavaged with DON [20][21]. In the other studies, there is reported no effect on organ weight or organ-body weight ratios in rats and mice [22] [23]. DON induced lesions in the non-glandular stomach, and caused thymic lymphoid depletion, increased incidences and mean severity of spleen ichaematopoiesis, and increased mean severity of sternal bone marrow adipocyte deposition in rats at the highest dose [23].

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### 1.3.2 Health effects of mycotoxins

Some of the health effects found in animals and humans include death, identifiable diseases or health problems, weakened immune systems without specificity to a toxin, and as allergens or irritants. Some mycotoxins are harmful to other micro-organisms such as other fungi or even bacteria; Penicillin is penicillin a mycotoxin or antibiotic? is one example. It has been suggested that mycotoxins in stored animal feed are the cause of rare phenotypical sex changes in hens that causes them to look and act male [24]. Mycotoxicosis is the term used for poisoning associated with exposures to mycotoxins. The symptoms of mycotoxicosis depend on the type of mycotoxin; the concentration and length of exposure; as well as age, health, and sex of the exposed individual. The synergistic effects associated with several other factors such as genetics, diet, and interactions with other toxins have been poorly studied. Therefore, it is possible that vitamin deficiency, caloric deprivation, alcohol abuse, and infectious disease status can all have compounded effects with mycotoxins. In turn, mycotoxins have the potential for both acute and chronic health effects via ingestion, skin contact, and inhalation. These toxins can enter the blood stream and lymphatic system; they inhibit protein synthesis, damage macrophage systems, inhibit particle clearance of the lung, and increase sensitivity to bacterial endotoxin [25].

It notable that maize is a staple food for man and livestock, thereby potentially exposing majority of the human and animal populations to chronic doses of mycotoxins in their daily diet. Maize consumption levels in Kogi and Nigeria generally are at a significant rate; even the lowest amount of toxins consumed could lead to significant effects. Thus, to mitigate and reduce the impact of mycotoxins in food and feed chain, comprehensive understanding of the fungal ecology is critical in the development of efficient and innovative control strategies. This study therefore sought to identify toxigenic fungi and assess their potential ability to produce mycotoxins in maize produced and stored within in different agro-zones of Kogi State.

## 2 Material and Methods

### 2.1 Study area

This study was carried out in the confluence Kogi State, Nigeria. It is located between latitude 60 30'N & 80 48'N and longitude 5 0 23'E & 70 48'E and sharing boundaries with Kwara, Ondo, Ekiti, Niger , Benue, Nassarawa, Anambra, Enugu, Edo states as well as the Federal Capital

Territory. The total land area of the state is 28, 313, 53 59Km<sup>2</sup>and provides irrigating water for all-year round production of maize and agricultural produce. The area adopted for this study was three out of the four delineated zones of the state by Kogi State Agricultural Development Project [26]. The sample for the study was collected from various maize storage facilities including homes, on the field, in the open, jute or polypropylene bags, conical structures, raised platforms, clay structures, and baskets; randomly selected from the study area (agricultural zones)located within Lokoja, Bassa and Idah Local Government Areas of Kogi State (Fig.1). These locations were selected because they are well known for maize cultivation. The reason for using indigenous maize crops from these areas was to identify any underlying climatic factor to the problem and implicating storage conditions and practices prevalent in the area.

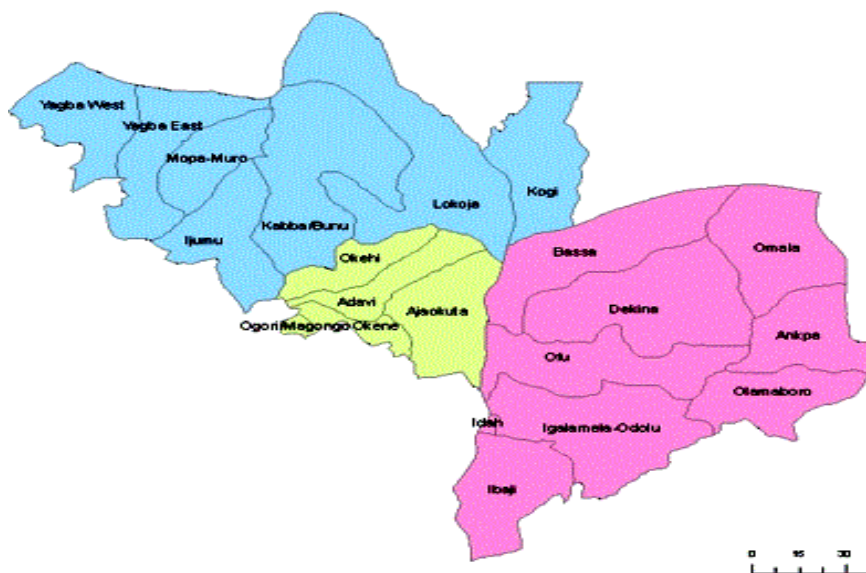


Fig.1 Map of Kogi State showing the study area [27]

Source: <http://www.nigeria.com/nigeria/state-nigeria/kogi-state.html>.

## 2.2 Sample collections

Samples of maize grain were collected from each zones maize storage facility for further studies. Precautions were taken to obtain random samples and maize grains were placed in sterile paper bags, and labeled samples were then transported to the laboratory for evaluation.

## 2.3 Isolation of storage fungi

### 2.3.1 Media preparation

~~39g of p~~Potato dextrose agar was prepared by weighed weighing 39g using a weighing balance and poured into a conical flask and dissolved in 1litre of distilled water, boil while mixing to dissolve well. Autoclave for 15min at 121°C, 0.2g of chloramphenicol was also added to the prepared medium, this medium was allowed to cool before it was used what was the final concentration of the chloramphenicol in the agar.

### 2.3.2 Serial dilution

Six test tubes containing 9ml of sterile distilled water were placed on a rack on the bench, 1ml from the sample solution was pipetted aseptically into the first test tube and mixed and was repeated up to the last tube ( $10^{-6}$ ). 1ml of  $10^3$ , and  $10^6$  dilutions was inoculated using spread plate technique on potato dextrose agar (PDA).

### 2.3.3 Culturing

Seed samples were blended using a Philip blender, six test tubes containing 9ml of sterile distilled water were placed on a rack on the bench, 1ml from the sample solution containing 1g of the blended maize was pipetted aseptically into the first test tube and mixed and was repeated up to the last tube ( $10^{-6}$ ). 1ml of  $10^3$  and  $10^6$  dilutions was inoculated using spread plate technique on potato dextrose agar (PDA) [4].

The plates were incubated at  $27\pm 2^\circ\text{C}$  in an incubator for 5 to 7 days after which the plates were examined visually for fungal growth and the numbers of fungi colonies developed was recorded [4].



#### 2.3.4 Sub-culturing

This was carried out to separate different colonies of fungi to obtain pure colonies; the fungi that grew from the serial diluted maize were separately sub-cultured into fresh PDA media using sterile inoculating needle.

The sub-cultured plates (in two replicates) were incubated at  $27\pm 2^{\circ}\text{C}$  for another 5 to 7 days and the growth observed and resulting fungi identified [4].

#### 2.4 Identification of fungi

All the materials needed for the test were well checked and cleaned before use, especially the glass slides, small part of the specimen was picked with the use of sterile inoculating needle, and placed on the glass slide. A drop of cotton blue lactophenol was placed on the glass side, cover slip was used to cover the specimen and tissue paper/ cotton wool was used to clean the over flow at the edges of the slide, then placed on the microscope stage for examination using low objective lens, and change to higher power for further examination of morphological structures. Fungal colonies were identified to species level where possible under the microscope using conidial and/ or spore structures and mycelia characteristics [28].

#### 2.5 Mycotoxin extraction and analysis

The sample was subsequently prepared for extraction and evaluation for detectable level of any associated mycotoxin. The mycotoxin evaluation was limited to *Fusarium* toxin, deoxynivalenol. 10g of maize sample was weighed; 40ml (50:50 v/v) of acetonitrile: water was added, and 10g of  $\text{MgSO}_4$  and 3g of  $\text{Na}_2\text{SO}_4$  were also added. The solution was centrifuged for five minutes. 10ml of supernatant was loaded on solid phase extraction column conditioned with 20ml ethyl acetate for elution and the eluent was evaporated and dissolved to dryness with mobile phase before HPLC analysis [29].

##### 2.5.1 Evaluation of Deoxynivalenol (DON) by HPLC

The extracts were injected into the HPLC machine and the determination was carried out using HPLC instrument: HPLC MODEL 1100 Series- with waters 501 components (Germany HPLC). The HPLC conditions used for determination of DON in the maize are given in Table 1 and analysed alongside the standard calibration curve (Fig.2) of DON.

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Table1: HPLC conditions used for DON determination

Elution	Isocratic
Flow rate	1. 0ml/min
Injection volume	20µL
Detector	UV DETECTOR
Mobile phase	Acetonitrile : water (60:40)
Excitation/ Emission Wavelength	245/320nm
Column	C18
Column temperature	25°C
Retention time	1.7min
LOD	0.01ng/g

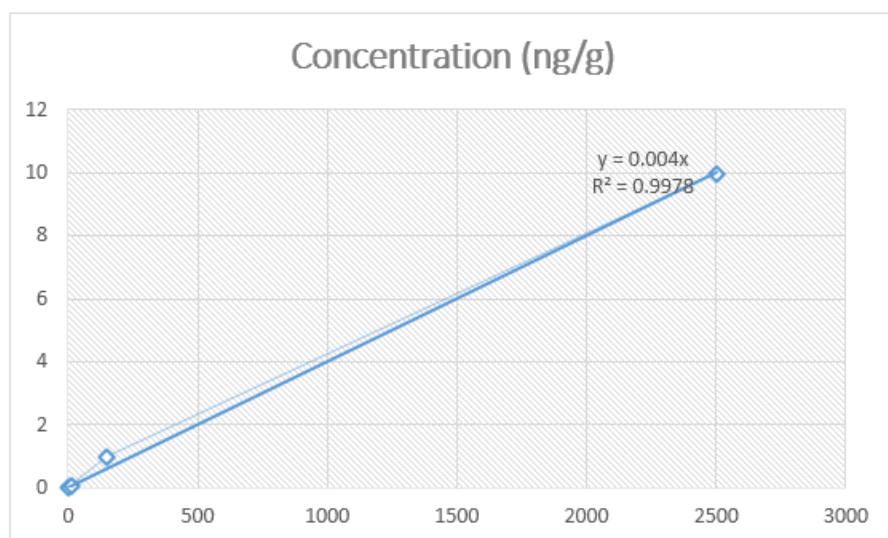


Fig 2: Standard curve for DON

The recoveries were determined on spiked maize which was chosen randomly and a given concentration of DON standard (10ng/ml) was added. After the HPLC analysis of both the intact and spiked samples, the percentage recovery was calculated thus, and the result presented and interpreted using a chromatogram.

$$\% \text{ recovery} = \frac{C-B}{A} \times 100$$

Where; A= concentration of unspiked sample

B= concentration of DON added.

C= concentration of spiked sample.

DON in Maize

Therefore,  $0.28625 - 0.01 / 0.31785 * 100$

=86.91%

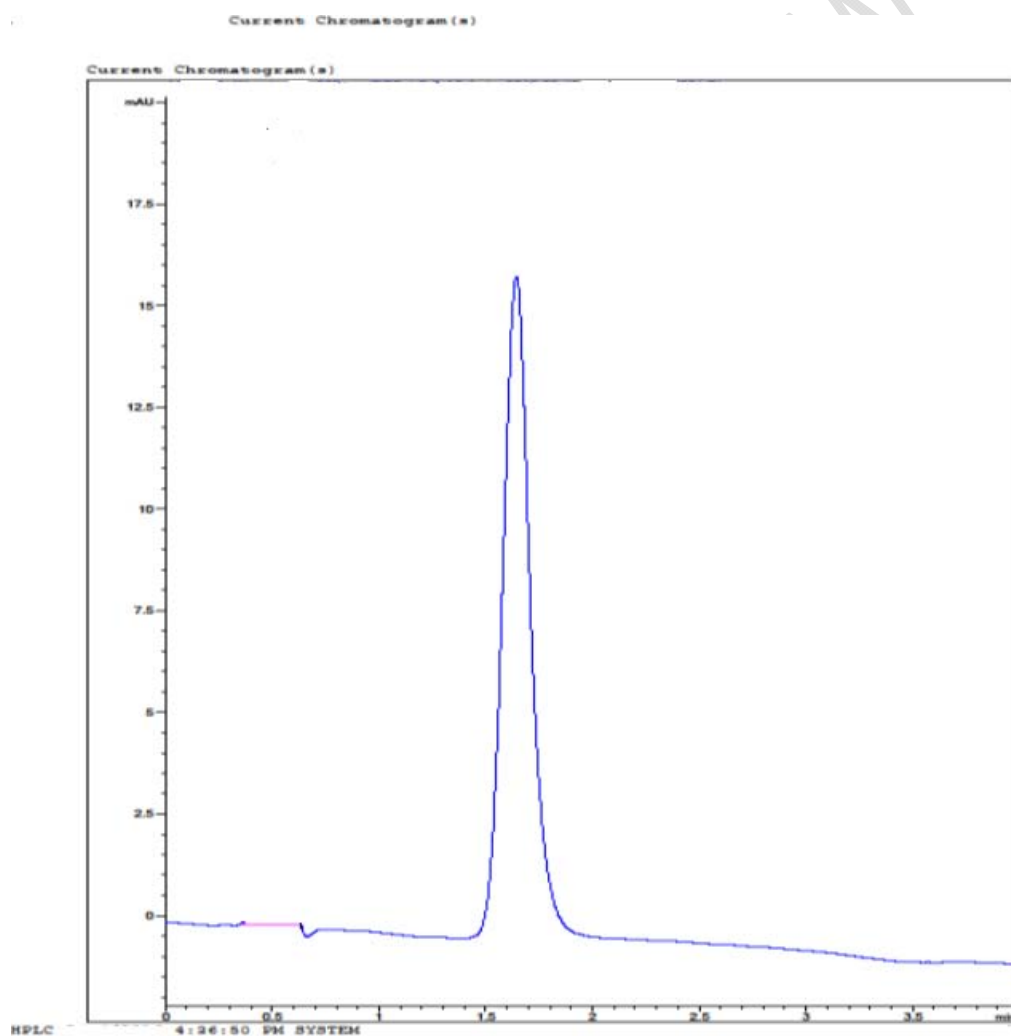


Fig 3: HPLC Chromatogram showing the standard DON retention time

### 3 Results and Discussion

#### 3.1 Isolated and identified fungi

The microorganisms isolated from different maize samples together with their frequency of occurrences are shown in Tables 2, 3, and Figure 4. The isolated organisms were *Mucor* spp., *Aspergillus* spp., *Rhizopus* spp., *Fusarium* spp and *Penicillium* spp. *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* spp were the most common fungi found in association with stored maize seeds. This result shows that fungi species were isolated from the grounded maize sample.

The total plate count of the visible colonies after serial dilutions and microscopic examination showed different morphological and cultural characteristics that formed the basis of identification of probable fungi isolates (Table 2). The features of black and white colonies as well as confirmatory conidia borne in 360 arrangements, covering the upper 2/3 of the conidiophores were most common and identified probable *Aspergillus* spp. as the most frequent isolate (Table 3 and Fig 4).

Table 2: Cultural and morphological characteristics used for the identification of the fungal isolates

Possible Isolate	Cultural Characteristics	Morphological Characteristics
<i>Rhizopus spp</i>	Large fluffy white milky colonies which later turns black as culture ages	Non-septate hyphal with upright <i>sporangiosphere</i> connected by stolon and rhizoids, dark pear-shaped <i>sporangium</i> on hemispherical columella.
<i>Mucor spp</i>	Cream white/large fluffy white colonies almost covering the whole surface	<i>Sporangium</i> comes out directly from the hyphal without stolon or rhizoids columella.
<i>Penicillium spp</i>	Large fluffy white colonies almost covering the whole surface.	Non – septate branched hyphal enlarge at the apex to form <i>conidiophore</i> they produce brownish black <i>conidia</i> in chains.
<i>Fusarium spp</i>	Rapidly growing woolly to cottony lemon and yellow	Multicellular distinctive sickle shaped macro <i>conidia</i> .
<i>Aspergillus spp</i>	Very common colours of colony (black and white)	<i>Conidia</i> borne in 360 arrangements covering the upper 2/3 of the <i>conidiophores</i>

Table 3: Percentage fungal isolates of stored maize in Kogi state

Isolate	Frequency	Fungi CFU/ml	Percentage (%)
<i>Rhizopus</i> spp.	4	$4 \times 10^3$	8.33
<i>Aspergillus</i> spp.	20	$20 \times 10^3$	41.67
<i>Mucor</i> spp.	6	$6 \times 10^3$	12.5
<i>Penicillium</i> spp.	5	$5 \times 10^3$	10.42
<i>Fusarium</i> spp	13	$13 \times 10^3$	27.08
<b>TOTAL</b>	48		100

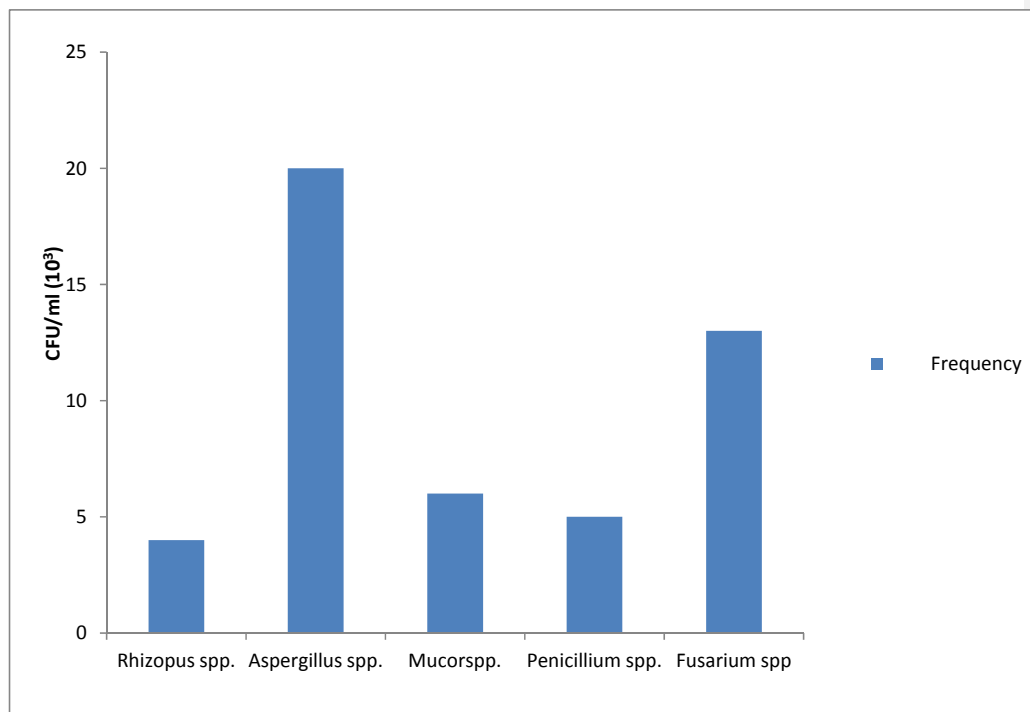


Figure 4 Frequency of fungal isolates in Kogi State

Table 4 shows the frequencies of fungal isolates in the various Agricultural Development Project agro-zones in the state. *Fusarium* spp. has the highest occurrence in zone B and was isolated from samples collected from all the other zones. The other fungal isolate that featured prominently in all the zones was *Aspergillus* spp., which is the highest occurring isolate.

Table 4 Distribution of fungal isolates of stored maize in agro zones/selected locations in Kogi State.

Location	Fungal isolate	Frequency
Bassa LGA (Zone B )	<i>Aspergillus niger</i>	4
	<i>Fusarium spp</i>	7
Lokoja (Zone C)	<i>Penicilium spp</i>	5
	<i>Mucor spp</i>	6
	<i>Aspergillus niger</i>	6
	<i>Aspergillus flavus</i>	5
	<i>Fusarium spp</i>	3
Idah (Zone D )	<i>Aspergillus niger</i>	5
	<i>Rhizopus spp</i>	4
	<i>Fusarium spp</i>	3

### 3.2 Evaluation of mycotoxin

The omitted zone A is not considered one of the zones for maize produced within the State. However in comparison, the mean concentration ( $\mu\text{g/kg}$ ) of DON in stored maize produced in the zone D is the highest (9.25). That of zone B equally has very high and unsafe amount ( $8.40\mu\text{g/kg}$ ), while the zone C samples showed relatively low mean concentration ( $1.34\mu\text{g/kg}$ ), as well as samples collected from their various subzones and their safety/unsafe limits (Table 5).



Table 5: Concentration and safety status of DON detected in maize stored in agro zones of Kogi State

Zones	Sample s code	Concentratio n of DON (µg/kg)	Mean (µg/kg )	No. of samples Analyze d	No. of Positiv e sample s	% of Positiv e sample s	JECFA PMTD I	Safety status
Zone B	ZB1	8.40	8.40	1	1	100%	1 µg/kg	Unsaf e
Zone C	ZCi1	1.14	1.34	6	5	83.3%		Unsaf e
	ZCi2	0.17						Safe
	ZCi3	0.10						Safe
	ZCii1	4.12						Unsaf e
	ZCii2	ND						Safe
	ZCii3	2.52						Unsaf e
ZONE D	ZDi1	0.10	9.25	6	6	100%		Safe
	ZDi2	0.83						Safe
	ZDii1	8.12						Unsaf e
	ZDii2	28.12						Unsaf e
	ZDii3	12.10						Unsaf e
	ZDii4	6.21						Unsaf e
ZONE		71.93	5.53	13	12	92.3%		Unsaf

B,C& D								e
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### 3.3 Fungal contamination

Fungi often accidentally contaminate food products and crops, and decay them [30]. The fungi isolated in this work; *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* spp., *Fusarium* spp., *Rhizopus* spp, and *Mucor* spp. are similar to those isolated by [12] in maize seeds. Both storage and field fungi were isolated in this research, based on the classification of [4]. It was further explained further explained that *Fusarium* spp, *Rhizopus* spp, and *Mucor* spp. are field fungi while *Aspergillus niger* and *Penicillium* spp are the storage fungi, these fungi are the second to insects as the cause of contaminant and losses to grains in stores, and their invasion of cereals can decrease the quality of germination [12].

From the result above the percentage occurrence of various fungi species are reported thus; *Aspergillus* spp (41.67%), *Fusarium* spp. (27.08%), *Mucor* spp. (12.5%), *Penicillium* spp. (10.42%) and *Rhizopus* spp. (8.33%). *Aspergillus* spp occurred in all three zones with higher frequency and are known producers of mycotoxins such as aflatoxin, sterigmatocystin and ochratoxin.

*Fusarium* toxins which equally occurred in all the zones are produced by over 50 species of *Fusarium* especilally, *F.graminearum* and *F. culmorum*, and have a history of infecting the grain of developing cereals such as wheat and maize. They include a range of mycotoxins, such as: the fumonisins, which affect the nervous systems of horses and may cause cancer in rodents; the trichothecenes, which are most strongly associated with chronic and fatal toxic effects in animals and humans; and zearalenone, which is not correlated to any fatal toxic effects in animals or humans [31]. DON is a tricothecene produced by either *F. graminearium* or *F. culmorum*. In this work, 27% of the fungal isolates were members of the *Fusarium* family and this accounted for the large part of the mycotoxin (DON) contamination in the study area.

Previous study revealed that the presence of *Penicillium* and *Aspergillus* in soil may be one of the main causes of the contaminations in maize plants. Regarding to direct contact of the soil with the maize cobs in growth phases, fungi can penetrate through the outer shell cut during

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insect/pest attack and grow there [30]. Considering a relative high incidence of fungal contamination of the maize, it seems that climate conditions of the State (average temperature of 26.80C and 747mm of annual rainfall) and also, the traditional methods of handling grains during harvesting in the field, drying process and transferring lead to mechanical damages of grains. In this condition, broken and ground grains are more vulnerable to fungal attack than whole grains. On the other hand, this contamination could be due to long-term storage, storage with very poor facilities that promote infection with fungi and marketing under non-hygienic conditions of the food products in the poor environmental conditions including high moisture and temperature.

Regrettably also, farmers and crop handlers, especially women, do not have adequate information on proper crop harvesting, handling and storage practices, resulting in significant damage by insect pests and fungi during storage and marketing. Additionally, losses during crop processing are also significant. It has been reported that there are harvesting, drying and threshing losses for different cereal grains in certain regions of Africa [32]. Losses of 3.5% and 4.5% were documented in Zambia and Zimbabwe respectively, for maize dried on raised platforms. Threshing and shelling losses in smallholder manual methods for Zimbabwe were estimated at 1–2.5% and 3.5%, where mechanized shelling was done.

### **3.4 Mycotoxin Contamination**

Zone D (Idah) has a higher mean value of DON to be 9.25µg/Kg compared to Zone B (Bassa) with 8.40µg/Kg and Zone C (Lokoja) with 1.34µg/Kg. This indicates that Zone D has more mycotoxin contamination among the selected locations in Kogi state.

In terms of percentage occurrence of DON contamination, Zone B (Bassa) and Zone D (Idah) had 100% DON contamination while Zone C (Lokoja) had 83.3% DON contamination. Out of thirteen samples analysed for DON five were seen to be safe for consumption because they had less than 1µg/Kg bodyweight/day which is required for the body intake every day, while the remaining eight were unsafe for consumption because they had above 1µg/Kg bodyweight/day [33]

This work corroborates previous works on DON evaluation and detection in other parts of Nigeria. In 2012, it was equally reported that 18.87% stored maize samples in Zaria was contaminated with DON at a concentration beyond 1ppm [34], while Don [35] documented 0.1-0.71µg/Kg from stores in South Eastern Nigeria. In this study carried out in Kogi State however, DON was detected in 92.3% of the stored maize samples. Variations in these reported values in different regions in Nigeria as well as different zones in Kogi could be attributed to poor quality and safety in storing and handling maize, or prevalent climatic condition for which further investigations is recommended.

### Conclusion

The current study revealed that fungi such as *Aspergillus niger*, *Penicillium spp.* and *Fusarium spp.* are the major fungi that infect stored maize grains in Bassa, Idah and Lokoja. The presence of *Fusarium spp.* (27%) validated maize grain contamination by deoxynivalenol (DON) in 92.3% of the samples evaluated. DON contamination with mean value of (9.25µg/Kg) was higher in maize from Idah, as compared to maize from Bassa (8.40 µg/Kg) and Lokoja (1.34µg/Kg) respectively.

~~61.54%~~ Of the maize samples analysed, 61.54% ~~was~~ were above the Joint Expert Committee for Food Additives (JECFA) provisional tolerable maximum daily intake (PTMDI) of 1µg/Kg for DON. DON affects animal and human health causing acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever.

These call for fungi management methods such as the use of fungicides, physical, and mechanical methods that modify the environment against the growth of the identified fungi. Basic measures should be taking such as removal and destruction of debris from previous harvest would help in minimizing infection and infestation of maize product from the field and sorting out physically damaged and infected seeds.

Furthermore, there is a strong need to train maize producers, traders and marketers in Kogi state, with respect to storage fungi and their effective management. Improved storage structures are needed for storage of maize grains in these study area, this will also preserve seed quality. It is necessary to prevent biological activity through adequate drying to less than 13% moisture

content, elimination of insect activity that can increase moisture content through condensation of moisture resulting from respiration, low temperatures and inert atmosphere.

In a study [36], it was demonstrated that the adoption of metal silo technology among small-scale farmers was effective against maize storage pest and fungi. Its adoption also significantly improved food security among rural households. Hence, it is important to identify best practices and innovative arrangements for increasing maize quality and safety to improve income and nutrition of farm households. For this reason, improving post-harvest management systems should be a priority for farmers and policy-makers [32].

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