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Research Article

Mycoflora of Maize in Niger State, Nigeria

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Abstract
Fungi have been associated with contamination and spoilage of food. These organisms are known to thrive in different weather and environmental conditions. Maize kernel obtained from field, store and market from 25 Local Government Areas of Niger State within the month of August to September were analyzed for fungi occurrence using standard methods. Ten different fungus; *Aspergillus niger, A. ochraceus, A. flavus, A. fumigatus, A. parasiticus, Mucor spp, Fusarium spp, Penicillium spp, Yeast* and *Rhizopus spp*, were identified and isolated from the maize kernel. From the agro-ecological zones of the state, the most predominant fungi were *Aspergillus* species (241/345), *Rhizopus spp* (41/345) and *Mucor spp* (37/345) while *Penicilium spp* and *yeast* were the least identified. Within the zones, *Aspergillus species* have the highest occurrence; wet (65/95), driest (63/95), wettest (56/95) and dry zone (54/95) zones. The highest occurrence of fungi was found in the market sample (129) followed by store (112) and field (104). High percentage occurrence of 11.6% was recorded in Suleja, Wushishi, and Borgu Local Government Area, followed by 10.6, 10.1, 8.7, 9.7, 9.2, 8.7 and 8.2% in Bosso, Tafa, Madalla, Minna, Paiko, Bida and Sabon wuse respectively. Mold species known to be mycotoxin producers were reported in this work. This poses as a risk factor to food safety.

Key words: Fungi, Maize kernel, Niger State.

Introduction

Fungi such as those belonging to the *Aspergillus, Fusarium* and *Penicillium* genera are food safety compromisers due to their ability to produce secondary metabolites known as mycotoxins [1], these toxins contaminate agricultural commodities and when ingested by animals or humans may pose detrimental diseases [2]. It is estimated that annual economic losses in Asia and Africa as a result of grain mould are in excess of US$ 130 million [3]. Mycotoxins are generally associated with cancer, reduced growth, infertility, liver and kidney dysfunction, and death. These effects result from short term or long term exposure. Short time exposure to high level of aflatoxin in Kenya led to 125 deaths in 2004, while consumption of aflatoxin contaminated maize killed 106 natives of Western India in 1974 [4]. It is estimated by FAO that 25% crops grown all over the globe are contaminated by fungi toxins every year, annual losses from this is about 1 billion tons of foods and products. Fungi infestation of food and subsequent production of mycotoxin can occur at different points in the food value chain, such as during cultivation in the field, during processing, during transportation or storage [5,6]. Mycotoxin contamination of cereals or forages on field usually result from infection with a mycotoxigenic fungi or a symbiotic endophyte [7]. During processing, storage and transportation of harvested products, environmental conditions that favour fungi are the cause of contamination [8].
Maize (*Zea mays L.*) is one of the commonly cultivated food worldwide, which is a major staple in developing countries, mostly in the Sub-Saharan Africa. Maize being a common staple in

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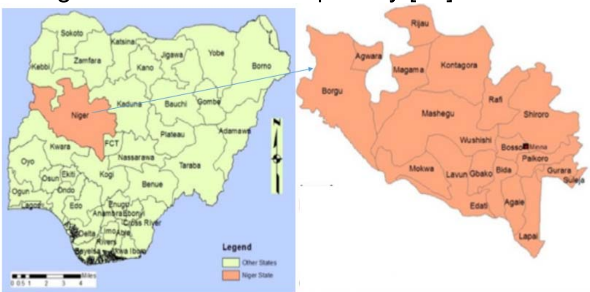
West Africa [9], has been a very important starchy food for both human and animal consumption particularly in northern Nigeria. Maize is used to produce 'koko' or 'ogi' a traditional fermented paste, it is also used to make 'tuwo' a stiff porridge this food is very common amongst the northerners and middle belts, maize is also eaten when roasted or boiled as a snack during its harvest season [10]. It is reported that, United States of America is the largest producer of maize in the world, while China and Brazil follow [3]. In Africa, Nigeria is the second largest producer of maize following South Africa, Nigeria produced 10,790,600 Mt of maize in 2014 [3]. Maize, which possesses large ears with many seeds / kernels, has been reported to be more susceptible to fungi and mycotoxin contamination than other cereals in Nigeria and in the United States [11,12]. Adesuyi [13] reported that of the grains produced in Nigeria in 1970 -1971, between 2.5 and 4.0% were lost to insects annually while between 1.25 and 3% were damaged by fungi. Okereke and Nwosu [14] reported an estimated loss of 58% of 1000 tonnes of maize due to insect and fungal attack.

From the time when the research on mycotoxins began in Nigeria in 1963, a lot of work have estimated mycotoxin levels in maize from different part of the country [11,15,9,16,17,18,19,20]. However little work has been done to ascertain the mycoflora in maize from Niger state, though Atehnkeng *et al*. [21] estimated the level of aflatoxin from three geo- agricultural zones of the state and Makun *et al*. [22] reported the occurrence of mycoflora contaminating maize from the state capital Minna. Since maize is one of the most cultivated food in Niger state which is used for several food and animal feed preparations, both locally and all over the country, it is therefore of paramount importance to know the occurrence of mycoflora in maize from the state. This work would therefore be relevant to food safety.

Material and Methods

2.1 Sample location and sample collection

Samples of maize (95) were collected during the rainy season (August-September), from different local government areas of Niger State according to the four microclimatic zones of the state (Table 1). Niger State is the largest Nigerian state by area (76,363 km²); it lies between 10°00′N 6°00′E of the coordinate. It is hot and humid for most part of the year especially between May and October (29.5°C and 73.1%) which is conducive for fungal growth. The state grows a large amount of maize amongst major crops like rice, sorghum, cowpea, millet, groundnut, yam and cassava, which are also grown in commercial quantity [23].



In each of the zones, samples were collected from field, store and market. Field samples were collected during the harvest period while those from the store were collected from locally built mud barns called "Rumbu" in Hausa language, the market samples were bought from different markets within the study area. About 300 g of each sample was collected, labeled and packaged in small containers and transported to the laboratory.

Table 1.

Niger State Local Government Areas (LGAs) according to microclimatic zones

S/No	Zones	Annual Rainfall Range (mm)	Local Government Area
I.	Wettest	>1400	Suleja and Tafa
II.	Wet	1200-1400	Borgu and Magama
III.	Dry	1000-1200	Agaie, Agwara, Bida, Bosso, Edati, Gbako, Gurara, Katcha, Kontagora, Lapai, Lavun, Mashegu, Minna, Mokwa, Munya, Paiko, Rijau, and Shiroro
IV.	Driest	<1000	Mariga, Rafi and Wushishi

2.2 Preparation of sample for analysis

Two grams (2g) each of the samples were blended into fine flour using a milling machine (Greiffenberger Antriebstenchnic, Marktredwitz, Germany). The flour was preserved in a clean dry container prior to usage.

2.3 Fungi Isolation and Identification

The method described by Cotty [37] was used. The method used a Modified Rose Bengal Media (Sigma-Aldrich Chemie GmbH) commonly called Clean up media (CU) for fungi isolation. The blended sample (1 g) was weighed into 10 mL of sterile-distilled water and mixed on a vortex mixer. The mixture was spread plated in three dilutions (50 µl, 100 µl and 200 µl) on a CU media. This is to enhance the collection of isolates of fewer than 15 colonies from plates. The plates were incubated for 72 hours at 31°C. After 72 hours, resulting fungi colonies were counted with a colony counter (Scienceware® colony counter system) under a magnifying lens. Aspergillus colonies that counted between 1-15 were transferred into a 5/2 media (containing 50 mL/L 5% V-8 juice, 2% Bacto agar (Difco) set at pH 5.0-5.2), all other fungi were transferred into a full strength PDA Media (Sigma-Aldrich Chemie GmbH) containing lactic acid (Sigma-Aldrich Chemie GmbH) and further incubated for clearer growth.

Fungi identification: Isolates were classified on the basis of conidial morphology and colony characteristics. Microscopic examination coupled with atlas guide was used to determine other fungi genera and species. References used include; [38,39,40].

Quantities of fungi were calculated as colony-forming units (CFU) per gram.

$$cfu/g = \frac{\text{Number of colonies X Reciprocal of the dilution factor}}{\text{Plating volume (1ml)}}$$

Results and Discussions

Ten distinct fungus *Aspergillus niger, A. ochraceus, A. flavus, A. fumigatus, A. parasiticus, Mucor spp, Fusarium spp, Penicillium spp, Yeast* and *Rhizopus spp*, were isolated from maize kernel samples collected from market, field and store during this survey in Niger State. Across the agro-ecological zones *Aspergillus species* were the most predominant fungi identified, followed by *Rhizopus spp* and *Mucor spp* while *Penicillium spp* and *yeast* were the least identified (Table 2). This is in tandem with various reports from within and outside Nigeria, which shows similar fungi, isolates [24,25,21,22]. *Aspergillus spp* being the dominant amongst other species isolated is in agreement with Adebajo *et al*. [9], and Bankole and Mabekoje [19]. *Aspergillus spp*. were recovered from 61/91 maize samples from Ghana and Nigeria [26]. According to the report of Gonza´ slez, *et al*. [25] the fungi that had higher occurrence in Northwestern Provinces of Argentina were *Aspergillus niger, A. flavus, A. parasiticus, Mucor spp,* and *Rhizopus spp* in maize. In maize kernels from Spain, Alborch *et al*. [27] reported occurrences of *Aspergillus spp*. (93.3%), *Penicillium spp*. (83.3%), *Mucorales* (53.3%), *Cladosporium spp*. (43.3%), *Fusarium spp*. (33.3%) and yeasts (23.3%). According to the authors, the remaining species (*Beauveria spp., Trichoderma spp., Alternaria spp., Geotrichum spp., Paecilomyces spp., Scopulariopsis spp*. and *Acremonium spp.*) were found in less than 20% of the samples. These reports from various continents clearly shows the ubiquity of fungi species. And explains why mycotoxin is a global challenge.

Among the most occurring fungi in our work and that of others reported, *A. flavus* is a popular name when it comes to production of the carcinogenous aflatoxin in maize worldwide [28,29], this fungi is further classified as L strain and S strain based on genetic, morphological and physiological features. Those with fewer but larger sclerotia are the L-strain types while those with numerous small sclerotia are the S-strains. The S-strains are more dedicated in production of aflatoxins than L-strains. *A. flavus* do not produce aflatoxin G series due to a 1.5-2.2kb deletion in the aflatoxin biosynthetic gene cluster, resulting in the loss of cypA gene. This gene is required for the production of G series aflatoxins. Since the gene is present in the *A. parasiticus* they are able to produce G series aflatoxins [28].

Another very important fungi mycotoxigenic fungi isolated in maize kernel is the *Fusarium species* which was just 2% of the total isolates. They are important for their role in causing a wide range of diseases to crops [30] and capable of producing several mycotoxins, including trichothecenes, zearalenone (ZEA), fumonisins, which are among the economically significant mycotoxins found in maize, rice and wheat (FAO, 2013). Xing *et al*. [31] isolated *F. verticillioides* (24.77%), *F. graminearum* (15.08%), *Fusarium spp*. (5.54%), *A. flavus* (4.93%), *A. niger* (7.51%), *Penicillium spp.* (3.86%), *Eupenicilliium sp*. (1.82%), *Alternaria sp*. (3.57%), *Trichoderma sp*. (2.20%) from maize kernel, establishing that *Fusarium, Aspergillus, Alternaria* and *Penicillium* occurred in pre-nature drying maize kernels as earlier claimed by Streit *et al*. [32]. In Qatar, *F. verticillioides* showed highest frequency distribution (34%), followed by *F. graminearum* (16%), *F. oxysporum* (15%), *F. proliferatum* (13%), *F. culmorum* (8%), *F. solani* (7%), *F. subglutinans* (4%) and *F. avenaceum* (3%) in the feed cereals [33].

Based on geographical peculiarities, we categorized our sampling points into zones. The occurrence of *Aspergillus species* was highest in wet zone (65/95) and driest zone (63/95), and lowest in wettest zone (56/95) and dry zone (54/95) how ever in all cases occurrence was over 56%. Out of the total 345 fungi identified, most fungi were identified from the wet zone (92) and driest zone (92), followed by the wettest zone (84) while the dry zone (77) has the least occurrence. The occurrence of fungi in maize from different local government of Niger State is presented in table 3, it shows that moulds occurred mostly in Suleja, Wushishi and Borgu LGAs, followed by Bosso, Tafa, Madalla, Minna, Paiko, Bida and Sabon wuse respectively.

Table 2.

Occurrence of fungi in maize from Niger State

Fungi species	Zone 1 n = 22	Zone 2 n = 25	Zone 3 n = 24	Zone 4 n = 24	Total Occurrence n = 95	Overall Occurrence %
A. niger	19	21	14	21	75	21.7
A. ochraceus	05	05	05	07	22	6.4
A. flavus	15	21	15	17	68	19.7
A. fumigatus	03	04	03	03	16	3.8
A. parasiticus	14	14	17	15	60	17.4
Mucor spp	08	06	14	09	37	10.7
Fusarium spp	03	02	03	01	09	2.6
Penicillium spp	02	02	01	03	08	2.00
Yeast	03	03	02	04	12	3.5
Rhizopus spp	12	14	03	12	41	11.9
Total fungi count	84	92	77	92	345	100

Fungi from cereals are commonly studied as field, store, and market source [21,10]. The fungi isolated from samples obtained from field, market and store in Niger State shows that *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. parasiticus* and *Rhizopus spp* predominated more in samples from market while *Mucor spp, Fusarium spp Rhizopus spp* and *Penicillium spp* predominated more in the stored sample. Generally, Table 2 indicated that market sample (129) has the highest occurrence of fungi followed by stored samples (112) while samples from the field has the least (104). A studies has shown a high presence of both field fungi (e.g. *Alternaria, Cladosporium,* and *Fusarium*) and storage fungi (e.g. *Aspergillus* and *Penicillium*) in maize from Guatemala [34], such outcome may be related with elevated moisture during harvesting and throughout storage, as well as possible pest-associated wounds [35]. Water activity (a_w) plays a significant role on the occurrence of fungi [31]. Widespread poor storage facilities and inadequate preservation methods, including inadequate drying may have also enhanced the growth of these fungi.

Table 3.

Occurrence of fungi in maize sample collected from different local government area of Niger state

Fungi Species	Tafa n= 6	Madalla n= 5	Suleja n= 6	Sabon Wuse n = 5	Borgu n =6	Minna n = 6	Bosso n = 6	Bida n = 6	Paiko n = 6	Wushishi n = 6	Frequency n = 58
A. niger	4	4	6	4	5	5	6	3	3	5	45
A. flavus	4	1	6	3	5	5	6	3	4	4	41
A. ochraceus	1	2	2	1	1	1	2	1	1	2	13
A. parasiticus	4	5	3	2	4	4	5	5	3	4	39
A. fumigatus		1	1		1				1	1	05
Mucor spp	3	2	2	3	1	3	1	6	3	2	26
Fusarium spp	1	1			1				2	1	06
Penicillium spp	1				1		1		1	1	05
Yeast	1	1		1	1					1	05
Rhizopus spp	2	1	6	2	4	2	1		1	3	22
Total occurrence	21	18	24	17	24	20	22	18	19	24	207
% Occurrence	10.1	8.7	11.6	8.2	11.6	9.7	10.6	8.7	9.2	11.6	100

Fungi are the major causes of spoilage in stored grains and ranked second only to insect infestation as a cause of deterioration and loss of food [10], the significant economic and health hazards caused by fungi and mycotoxin especially in developing countries cannot be over emphasized. The consequences of plant contamination by various fungi is not relegated to reduction in crop yield and quality with significant economic losses, but adulteration of grains with mycotoxins leads to serious diseases and even death [36]. Fungi are known to thrive in numerous conditions, but most importantly they grow in high temperate and moist environments, these conditions are more prevalent in wet zones, this may account for the high occurrence of fungi in wet zone (II). Other factors like insect infestation, agricultural practices, prolonged or bad storage system and microbial load could encourage fungal growth in grain in other conditions such as in a dry harsh environment as found in this study. Therefore, the continuous consumption of maize in Niger State without proper precautions may pose a threat to the health of human and animals alike.

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Conflict of Interest
There is no conflict of interest on the work nor this report

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