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Studies on Fungal Spoilage of Stored *Zea mays* L. (Maize) Grains in Two Markets in Lagos State, Nigeria

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Authors' contributions

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

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ABSTRACT

Maize is one of the most essential staple foods consumed and its spoilage by fungi has been a serious concern to humans since the dawn of agriculture and food storage. Therefore, this research was aimed at isolating, identifying and determining the toxicity level of fungi causing spoilage of stored maize grains. Grain samples of maize were collected from Igando and Alaba markets in (Lagos State) from the top and bottom of maize bags from both market stores. Samples were cultured on Potato Dextrose Agar for fungal isolation. All isolates were identified using morphological and microscopic features. Also, the affordable qualitative method (ammonia vapour test) was carried out in order to identify some selected isolates that are aflatoxigenic. Petri- plates containing these isolates were flipped upside down and 2ml of concentrated ammonia solution (Extra Pure AR grade) was poured into the lid of inverted culture plates and observed for 10-15 minutes inside a desiccator for proper release of ammonia vapour. The genera of fungi isolated were; *Aspergillus* (57.15%), *Fusarium* (21.43%), *Penicillium* (7.14%) and *Rhizopus* (14.29%). The dominant genus was *Aspergillus.* The exposure of selected isolates to ammonia vapour led to varying degrees of colour changes which included pink, red and plum red. Similarly, isolates that were moderately poisonous were pink in colour, least poisonous showed red colour and very poisonous isolates indicated plum red colour. The findings from this study indicated that *Aspergillus* spp. are mostly responsible for spoilage of maize grains in storage and contamination with *A. flavus* can lead to poison production.

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1. INTRODUCTION

Zea mays (maize) is a grassy plant that belongs to the family (Poaceae). It is grown all over the world and is one of the most significant cereal crops. Maize is not only an important human food crop but also a key component of animal feed and a raw ingredient for a variety of industrial products. Maize is a surprising plant because one seed sown can yield over 400 kernels in return. Maize has a high yield, simple to prepare, easy to digest, and less expensive than other grains. Most part of the corn plant has profitable value: the seeds, leaves, stems, cotton, and corn can be used to make a wide range of culinary and non-food items [1]. *Z. mays* is grown in a variety of environments and is a staple crop in Africa consumed by people of diverse food preferences and socio-economic backgrounds.

The plant has shown to be a vital component of the agricultural system and influences the agricultural structure of many smallholder farmers, particularly in northern states of Nigeria [2]. According to Ayeni [3], maize is employed as a local cash crop with 30 percent of land allocated to maize cultivation. Several studies on maize production have found that the grain has increased across the country's agro-ecological zones. Report shows that Nigeria is the largest maize producer in Africa, followed by South Africa [4].

From the time when primitive man began to practice agriculture, fungi causing spoilage have been a threat. Grain storage practices have a direct impact on grain quality. This is because it can result in a constant rise in the humidity and warmth of the maize grains, favouring fungal growth [5]. If conditions are favourable for fungi to grow, they could cause 50 to 80 percent harm to farmers' corn during storage [6].Field fungi that infest maize cobs can do a lot of damage, especially in humid environments, lowering yield and quality, as well as feed and seed value. In addition, several pathogens can cause diseases in maize such as Cladosporium rot, Diplodia ear rot, Gibberella ear rot, Fusarium ear rot, Penicillium ear rot, Downy mildew of maize, Aspergillus ear rot and many others [7].

The spoilage of maize grains could limit availability to consumers and can result in economic losses to farmers. Contamination of corn causes spoilage, which affects human and animal health. The development of toxins (aflatoxins) by various fungal species that contaminate food and feeds is one of the most distressing elements of the invasion of grain by field and storage fungi. Incidence of aflatoxins in maize was also assessed and recorded in some parts in the Southwestern zone of Nigeria by [8] and [9]. In this regard, corn continues to attract attention, since it is one of the most important dietary products. Therefore, this research was aimed at isolating, identifying and determining the toxicity level of fungi causing spoilage of stored maize grains.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Samples

Maize grains samples were collected in maize stores at Alaba and Igando markets in Lagos State. These maize grains samples were brought to the laboratory for isolation. A total of sixty (60) grains samples [10] were collected and labelled A and B which represent Up and Down respectively because the maize grains were collected from bags stored up and down at their respective stores. The study was carried out in Lagos state, Nigeria from January to July 2021.

2.1.1 Preparation and sterilization of media

Potato Dextrose Agar (PDA) used for isolation of fungi was prepared according to standard procedure [11].

2.2 Isolation of fungi

The method of isolation used was the Agar plate method. Maize grains collected from different stores at Alaba and Igando markets were surface–sterilized by immersion in 2% sodium hypochlorite solution in a 250mL conical flask for one minute, followed by two successive rinses with sterile distilled water. The grains were dried with sterilized filter paper before placing them on the PDA medium containing chloramphenicol (0.25mg/ml). For grains collected at the Alaba market, different Petri-plates were used for grains at the top bags (3 grains per plate) and the bottom bags (3 grains per plate). This same process was also repeated for grains collected at the Igando market. All Petri plates were well labelled respectively. The Petri plates containing

grains were incubated at room temperature (28 ± 3°C) and growth of the fungi was observed.

2.2.1 Identification of isolated fungi

The incubated Petri-plates were observed after ninety-six hours (4 days) and sub-culturing was done until pure cultures were obtained. Then, slides were properly prepared, labelled, viewed, and identified by comparing their cultural and morphological characteristics as described in the Mycological Atlas of Robert and Ellen [12].

2.3 Qualitative Method for Determining Toxicity Level

The fungus screened for aflatoxin production was *Aspergillus flavus*. Therefore, pure samples of *A. flavus* isolates obtained previously were replicated and incubated for 4 days. After incubation, all pure samples were screened qualitatively using the Ammonia vapour test. Petri-plates containing these isolates were flipped upside down and 2mL of concentrated ammonia solution (Extra Pure AR grade) was poured into the lid of inverted culture plates and kept for 10-15 minutes inside a desiccator for proper release of ammonia vapour [13]. The colour changes on these plates were recorded after they were exposed to ammonia vapour.

3. RESULTS

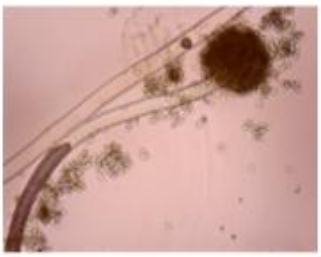
3.1 Fungi Isolated from Stored Maize Grains

Different fungi were isolated from maize samples collected from both markets (Alaba and Igando). Four genera were isolated and the frequency of occurrence was; *Fusarium oxysporum* (21.43%), *Aspergillus niger* (42.86%), *Aspergillus flavus* (14.29%)**,** *Penicillium* sp. (7.14%)**,** *Rhizopus stolonifer* (14.29%). This result shows that *A. niger* was the most predominant among other fungi isolated and *Penicillium* sp. occurred the least.

(i) F. oxysporum on PDA

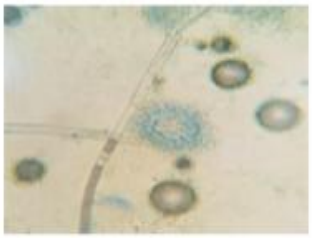
(ii) Photomicrograph of F. oxysporumx100

(iii) A. niger on PDA



(iv) Photomicrograph of A. nigerx100

(v) A. flavus on PDA

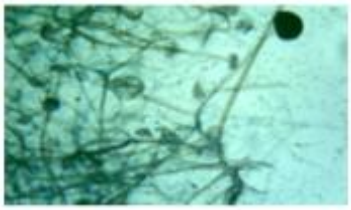


(vi) Photomicrograph of A. flavusx100

(vii) *Penicillium sp.* on PDA

(viii) Photomicrograph of *Penicillium sp*. x100

(ix) R. stolonifer on PDA



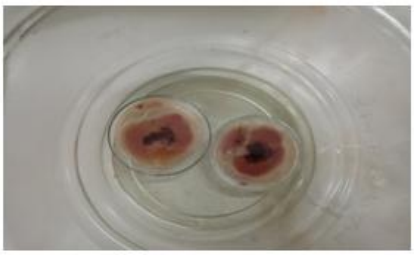
(x) Photomicrograph of R. stoloniferx100

Plate 1(i-x). Cultural and microscopic morphology of isolated fungi

3.2 Identification of Poisonous Isolates using Ammonia Vapour Test

After identification of poisonous *A. flavus* isolates using the qualitative method, it was found that these isolates showed varying degrees of colour changes which included plum red, red and pink. *A. flavus* isolates that were very poisonous were plum red in colour, moderately poisonous showed pink colour and least poisonous isolates indicated red colour.

(A)



(B)

Plate 2(a-b). *A. flavus* isolates inside a desiccator during the test for aflatoxin toxicity level

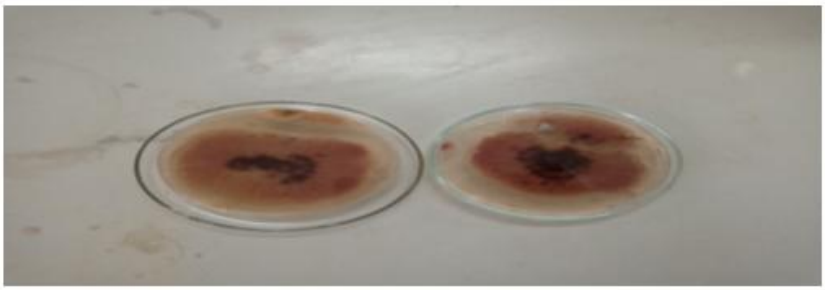


Plate 3. Change of colour developed on *A. flavus* isolates when exposed to Ammonia vapour (Right to Left: Plum Red to Red)

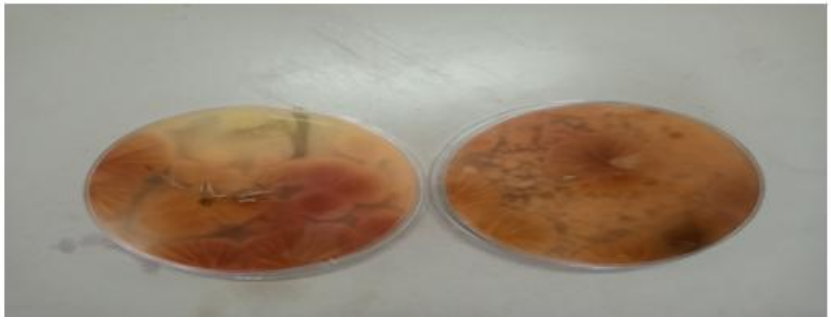


Plate 4. Change of colour developed on *A. flavus* isolates when exposed to Ammonia vapour (Right to Left: Pink to Red).

4. DISCUSSION

From the research conducted on *Zea mays* in storage, four genera of fungi were isolated and identified as *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus*. This is in line with the research of Onyeze et al*.* [14] and Amadi and Adeniyi [15]. The most common species causing spoilage of maize grains in storage is *Aspergillus* sp. which showed the highest occurrence of 42.86% in this research and this agrees with the work of Egwurochi et al*.* [16]. Also, *A. flavus* isolated in this study has been found to produce aflatoxins which are potentially harmful to humans and animals [17]. A similar study by Hussein et al*.* [18] reported that when *A. flavus* isolates were cultured, they produced aflatoxins at different rates and according to a study carried out by Shekhar et al*.* [10] which revealed that the colour change of cultures from plum red, red and pink is connected to aflatoxin concentrations assessed by Enzyme-linked immunosorbent assay (ELISA). With this, the use of ammonia vapour to identify *A. flavus* isolates from highly poisonous to least poisonous is a valuable and rapid procedure.

Usually, fungi that cause spoilage are believed to be pathogenic or toxigenic [19]. It has been reported by Amadi and Adeniyi [15] that toxigenic fungi and the mycotoxins they produce pose a threat to human health and the economy. This is in line with the position of Cielger and Bennett [20], who noticed that mycotoxins have been linked to a variety of human and animal health problems. On the other hand, pathogenic fungi can also cause infections or allergies [21].

5. CONCLUSION

The findings from this study indicated that *Aspergillus* spp. are mostly responsible for spoilage of maize grains in storage and contamination with *A. flavus* can lead to poison production which implies that maize in storage can be infected by a variety of fungi that create harmful mycotoxins, putting the consumer's health at risk. Therefore, maize should be thoroughly dried before storage to prevent fungi infestation, which is dangerous to both humans and animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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