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Fungi Associated with the Deterioration of *Zea Mays* L. (Maize) From Market Stores in Abeokuta, Ogun State, Nigeria.

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ABSTRACT

This study addressed the characterization of fungi associated with fungal contamination of maize. The diversity of fungal species responsible for maize spoilage necessitates effective control measures; however, their efficacy remains uncertain. Molecular identification of fungal populations provides essential data for predicting disease incidence and assessing potential risks. Accurate characterization of fungal species is crucial for identifying pathogenic strains in maize samples. Stored maize samples were obtained from four markets in Abeokuta- Kuto, Lafenwa, Olomore, and Osiele. Samples were labeled according to their locations. Fungal isolations were performed using the serial dilution method on Potato Dextrose Agar (PDA). Microscopic identification was based on colony morphology. molecular and macroscopic analyses confirmed 100% fungal contamination in all maize samples. The predominant fungal genera identified included *Aspergillus, Fusarium, Rhizopus, Penicillium, Mucor,* and *Cladosporium* species. The prevalence of these fungi was consistent across all the sampled locations, highlighting their widespread occurrence in stored maize from Abeokuta markets. The significance of these results were discussed.

Keywords: Food safety, Zea mays, contamination, molecular identification, fungi

INTRODUCTION

Food safety concerns have heightened awareness of fungal infections and their detrimental impact on food quality (Asemoloye *et al.*,2017). Maize (*Zea mays*), a staple food for humans and a key ingredient in animal feed, is highly susceptible to fungal contamination, leading to economic losses and health risks (Sobowale *et. al.*,2007;Serena-Saldivar, 2016 ;Jonathan,2019). Due to its widespread consumption and diverse applications in food production, maize is a vital agricultural commodity in the global food market. However, maize is highly susceptible to microbial contamination, primarily caused by fungi, bacteria, yeast, and insect infestations, which contribute significantly to post-harvest spoilage and economic losses (Akinfemi *et..al,*.2009; Nelson *et al.,* 2019; Jonathan *et. al*.,2023).

Fungal contamination of maize poses serious health risks due to the production of mycotoxins—secondary metabolites synthesized by specific fungal species. Mycotoxins such as aflatoxins, fumonisins, and ochratoxins are highly toxic to humans and animals, leading to various health disorders (Pitt & Hocking, 2002). The presence of spoilage fungi in stored maize can result in significant deterioration, including discoloration, fermentation, acidification, and structural degradation, rendering the grains unsuitable for consumption (Jonathan *et. al*.,2025; Pitt & Hocking, 2009).

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Maize is widely valued for its high nutritional content and adaptability, making it an essential crop for ensuring food security. However, fungal contamination threatens both its quality and economic value. The prevalence of spoilage fungi, including *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria*, has been well documented, highlighting the need for effective monitoring and management strategies (IARC, 2002).

Spoilage fungi have posed a persistent threat to food safety throughout agricultural history. These fungi manifest in various forms, including fuzzy growths, powdery residues, and slimy coatings, exhibiting a wide range of colors from white and black to green, orange, red, and brown. Their proliferation leads to biochemical changes in maize, compromising its sensory and nutritional qualities (Pitt & Hocking, 2002, Jonathan *et.,al*.,2025). Unchecked fungal growth contributes to food acidification, fermentation, and decomposition, ultimately reducing the market value of maize and increasing health risks for consumers.

Since the 1960s, increased awareness of pathogenic fungi has reinforced the significance of fungal contamination as a major agricultural concern. Fungal spoilage is no longer considered a minor issue but a critical challenge in food storage and public health. *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* species are among the most commonly identified fungal contaminants in maize, with their occurrence frequently linked to storage conditions, humidity levels, and temperature variations (IARC, 2002; Gbolagade *et. al.*,2006).

The deterioration of maize due to fungal contamination presents significant agricultural and economic challenges, necessitating the development of effective prevention and control measures. However, the efficacy of existing strategies remains uncertain. To address this issue, it is essential to investigate the isolation, identification, and characterization of fungi associated with maize spoilage. Understanding the diversity and pathogenic potential of fungal species affecting stored maize is crucial for implementing targeted control strategies that minimize contamination risks.

Therefore, this study aims to characterize the fungal species responsible for maize spoilage, providing valuable insights into their prevalence, distribution, and potential mitigation strategies. By identifying the factors contributing to fungal contamination, targeted management approaches can be developed to safeguard maize quality and ensure its safety as a critical agricultural commodity.

MATERIALS AND METHODS

Sample collection and preparation

Stored maize (*Zea mays*) grain samples were obtained from four different vendors across four distinct markets in Abeokuta, Ogun State, Nigeria. The collected samples were systematically separated and labeled according to their respective locations: Kuto (7.1384°N, 3.3499°E), Lafenwa (7.0914°N, 3.3265°E), Olomore (7.1485°N, 3.3509°E), and Osiele (7.1933°N, 3.4533°E).

Preparation of Fungi culture media

Thirty-nine grams of Potato Dextrose Agar (PDA) was weighed and transferred into a conical flask containing 1000 mL of distilled water. The mixture was homogenized in the water bath, and then autoclaved at 121°C at pressure of $1.02 kgcm^{-2}$ for 15 min. After autoclaving, the PDA was allowed to cool to 45^0C before it was dispensed into 100mm Petri dishes. Streptomycin sulphate $5mg/1000cm^3$ was added to inhibit bacterial growth. The laboratory bench used for media preparation was sterilized with cotton wool soaked 95% ethyl alcohol (Jonathan *et. al*.,2023).

The prepared PDA plates were used for fungal isolation from maize samples. Serial dilution was employed for fungal isolation following the method described by Jonathan *et al.* (2016). The inoculated plates were sealed and incubated at room temperature for five days. All experiments were conducted in triplicate to ensure reproducibility.

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Isolation of fungi from Corn samples

Fungal isolation from maize samples was performed using the serial dilution method. A 1 g portion of the maize sample was suspended in a test tube containing 9 mL of sterile distilled water, thoroughly mixed, and appropriately labeled. Subsequently, 1 mL of the suspension was transferred into a second test tube containing 9 mL of sterile distilled water to obtain a 10^{-1} dilution. This process was repeated sequentially to prepare further dilutions of 10^{-2}, 10^{-3}, and 10^{-4}. A 1 mL aliquot from each dilution was aseptically inoculated onto solidified Potato Dextrose Agar (PDA) plates. The plates were then sealed and incubated at room temperature for five days, with daily monitoring for fungal growth. All inoculations were performed in triplicate to ensure experimental reliability (Jonathan *et al.*, 2016).

Identification of Fungal isolates and contamination

Macroscopic identification of fungal isolates was conducted based on colony characteristics observed on Potato Dextrose Agar (PDA). Microscopic examination was performed using the wet mount technique with lactophenol cotton blue staining. A sterile inoculating needle was used to transfer a portion of mycelial from the culture plate onto a clean, grease-free glass slide containing a drop of saline water. The fungal mycelia were carefully teased apart to ensure proper separation. A drop of lactophenol cotton blue stain was then added to the preparation, which was subsequently covered with a clean cover slip. The stained preparation was examined under a microscope to assess morphological characteristics, including colony color, shape, texture, pigmentation, and hyphal arrangement (Jonathan,2019).

Fungal isolates purification

Morphologically distinct fungal colonies observed on the PDA plates were carefully selected using a sterilized inoculating needle and transferred onto fresh PDA plates for further culturing. The inoculated plates were incubated at room temperature for five days to promote fungal growth.Purification of fungal isolates was achieved through subculturing by excising the mycelial tips and transferring them onto fresh PDA plates. This process was repeated as necessary until pure cultures were obtained (Okpewho,*et al*.,2024) .

DNA extraction and polymerase chain reaction

DNA extraction and PCR analysis for genetic sequencing were conducted at Inqaba Biotech, Ibadan, Oyo State, following the standard protocols established by the facility. Fungal isolates were identified using the Internal Transcribed Spacer (ITS) region. Amplification of the ITS target region was performed using the universal primers ITS1 and ITS4, following the method described by White, Bruns, Lee, and Taylor (1960). The obtained sequences were analyzed through BLAST searches in the GenBank database to determine their homology with previously identified fungal sequences.

Molecular identification of fungi isolates

The extracted DNA was sequenced in both forward and reverse directions. The resulting DNA sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) database to facilitate the identification of individual fungal isolates.

Fungal DNA extraction, gene application and sequencing

The fungal ITS1-4 region was amplified using the primer set ITS1 (TCCGTAGGTGAACCTGCGG, forward) and ITS4 (TCCTCCGCTTATTGATATGC, reverse) through polymerase chain reaction (PCR). Each reaction contained 4 µL of a 10–50 ng DNA sample, 25 µL of master mix (comprising DNA polymerase, dNTPs, $MgCl_2$, and reaction buffers at optimal concentrations for efficient DNA amplification), 0.5 µL of each primer, and 20 µL of nuclease-free water to achieve a total reaction volume of 50 µL.

Amplification was carried out in thermal cycling tubes placed in a C1000 Touch thermocycler (Bio-Rad, USA) using the following cycling conditions: an initial denaturation at 95°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, primer annealing at 58°C for 30 seconds, and elongation at 72°C for 45

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seconds. A final extension step was performed at 72°C for 10 minutes. The PCR amplicons were separated by gel electrophoresis on a 1.5% agarose gel and visualized under UV light. Purified DNA fragments were analyzed for quantity using an ABI PRISM 3700XL Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, Wilmington, DE, USA). DNA purity was determined by measuring the ratio of UV absorbance at 260 nm to 280 nm, while DNA quality was assessed using gel electrophoresis on a 1% agarose gel.

Number of visible fungal isolates

The total number of visible fungal colonies isolated from each medium was calculated using the formula:

Total Colony Count= (Number of Colonies× Dilution Factor) ÷Volume of Inoculum

RESULTS

1 **2**

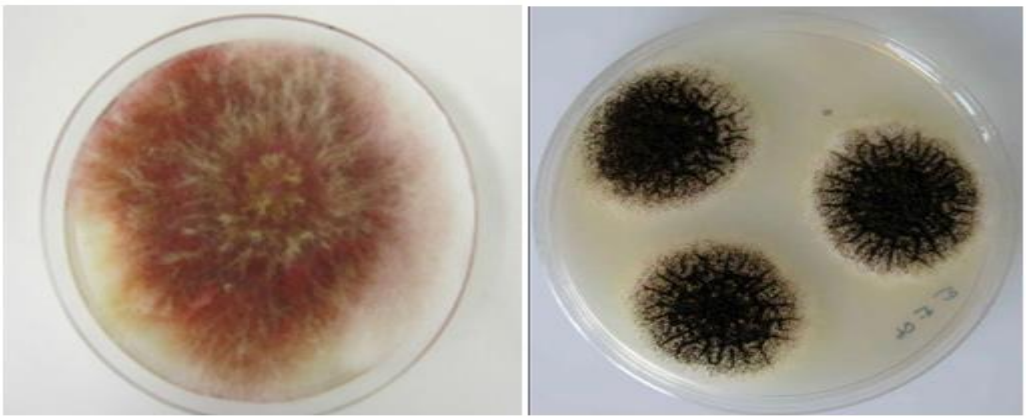

Plate 1: Fusarium graminearum obtained from Corn sample on PDA

Plate 2: Aspergillus niger obtained from Corn sample on PDA

Table 1: Morphological characteristics of isolated Fungus on PDA

Isolates	Cultural Characteristics	Morphological Characteristics
Aspergillus spp.	Black sporulation and white colour of colony.	Slow flat growth with white regular margin and may have a white border. Conidia borne in 360 arrangements covering the upper surface of the conidiophore.
Fusarium spp.	Rapidly growing wooly to yellow and lemon colour	Multicellular distinctive sickle shaped macroconidia.
Rhizopus spp.	Large fluffy white milky colonies which end up turning black as the culture age	Presence of stolon, Dark pear-shaped sporangeium on hemispherical columella.
Penicillium spp.	Fluffy white large colonies almost covering the entire surface.	Brush-like appearance, non-septate branched hyphal enlarge at the apex to form conidiospore.
Mucor spp.	Cream white fluffy colonies almost covering the surface	Sporangium comes out directly from the hyphal without stolon.
Cladosporium	Olive-green to brown colonies covering the surface	Dark pigmented conidiophores with separate hyphae, unicellular walled

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Table 2: Showing frequency of visible Isolates

Isolates	% Frequency
Aspergillus niger	20%
Aspergillus flavus	10.55%
Aspergillus fumigates	11.11%
Fusarium verticiliodes	10.75%
Fusarium graminearum	16.66%
Rhizopus sp.	5.55%
Penicillium citrinium	11.11%
Cladosporium	5.55%
Mucor sp.	11.11%
Mucor sp.	7.50%
	100

Table 3: Putative Taxonomic affinities of sequence types inferred from BLAST search of ITS sequences

Isolates ID	Taxonomic affinity (Gene bank no)	Percentage Similarity
Abk KT 1	*Aspergillus niger* (MT236835)	99.88%
ABK LAN 2	*Aspergillus flavus* (KT415779)	97.64%
ABK OL 2	*Aspergillus fumigatus* (MT547032)	98.58%
ABK OS 1	*Fusarium verticiliodes* (MN629646)	97.56%
ABK OS 2	*Rhizopus* sp.(KT159918)	96.50%
ABK LAN 2	*Fusarium graminearum* (MN658375)	99.26%
ABK KT 3	*Penicillium citrinium* (KT159919)	99.62%
ABK OL 4	*Mucor* sp. (KT426730*)*	100%
ABK KT 5	*Cladosporium* (KI467090)	98.73%
ABK LAN 6	*Mucor* sp.(MT267351)	99.23%

Table 4: Showing counts of isolated species present and their location

S/N	ISOLATES	KUTO	LAFENWA	OLOMORE	OSIELE
1	*Aspergillus niger*	+	-	+	+
2	*Aspergillus flavus*	-	-	+	-

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3	*Aspergillus fumigates*	+	+	-	-
4	*Fusarium graminearum*	+	+	-	-
5	*Fusarium verticillioides*	+	+	-	+
6	*Rhizopus* spp.	-	+	-	-
7	*Penicillium citrinium*	+	-	-	-
8	*Cladosporium*	+	-	-	-
9	*Mucor* sp.	-	-	+	+
10	*Mucor* sp.	+	-	-	-

KEY WORDS: present= +

Absent= -

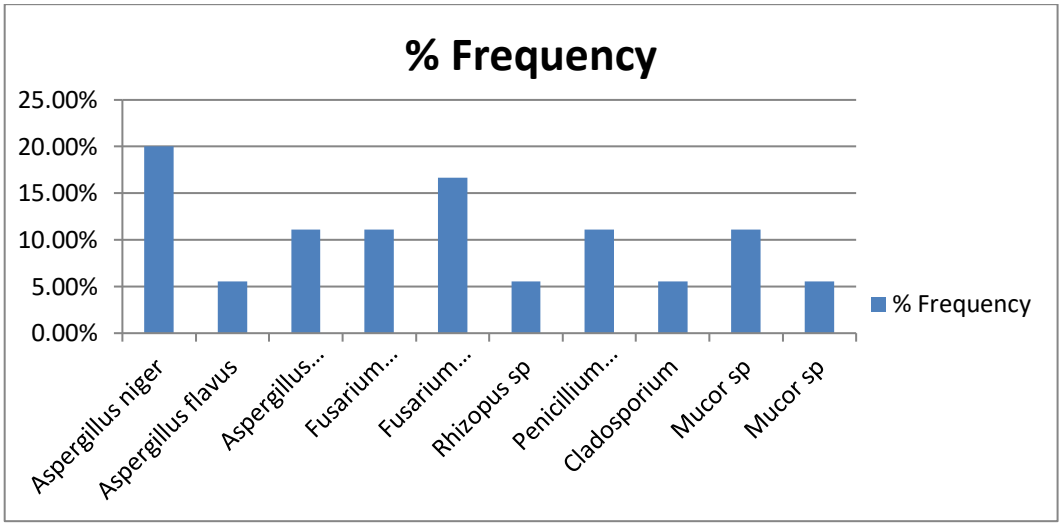

Figure 1: Frequency of Isolates

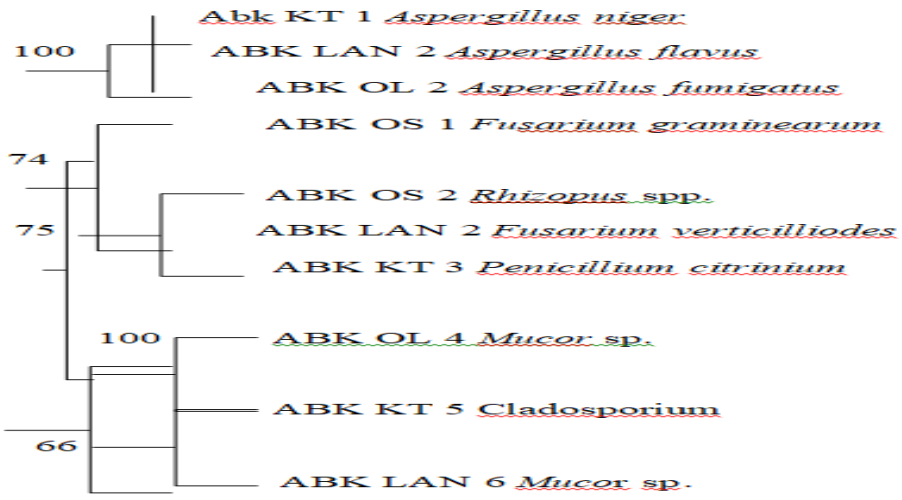

Figure 2: Dendogram showing phylogenetic tree of isolated strains of fungi.

The Internal Transcribed Spacer (ITS) polymerase chain reaction (PCR) analysis performed on the 10 fungal isolates using ITS1 and ITS4 primers confirmed the presence of fungal contamination in all samples. A total of

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10 fungal species were isolated from Potato Dextrose Agar (PDA) cultures and identified using both macroscopic and molecular methods from the four maize samples collected from various market locations. The 10 fungal isolates belonged to six genera: *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium*, *Cladosporium*, and *Mucor* spp. Among the sampled markets, *Kuto* exhibited the highest fungal diversity compared to the other locations, as presented in Table 4. The frequency of maize contamination was highest in *Kuto* market (90%), followed by *Lafenwa* market (80%), *Osiele* market (60%), and *Olomore* market (40%). Table 1 provides the morphological characteristics of *Aspergillus* and *Fusarium* spp.

DISCUSSION

This study identified *Aspergillus* as the dominant fungal genus in the studied maize samples, followed by *Fusarium*, *Penicillium*, *Rhizopus*, *Cladosporium*, and *Mucor*. The prevalence of these fungal genera was consistent across all sampling locations. The successful amplification of all 10 fungal isolates using ITS1 and ITS4 primers confirmed their fungal identity. Identification based on colony characteristics, such as growth pattern, shape, and pigmentation, provided valuable insights into distinguishing the different fungal species (Okpewho, 2024)

The presence of diverse fungal species in stored maize across the four market locations highlights a critical concern regarding fungal contamination. This contamination is primarily attributed to inadequate and improper storage conditions, exposure to fungal spores from the environment, and contamination from storage facilities, transportation, and handling practices. These factors influence the rate and extent of fungal infection in maize grains.

The findings of this study align with the work of Conrad *et al.* (2012), who reported that the Internal Transcribed Spacer (ITS) region has the highest probability of successful fungal identification across a broad range of species. The use of cultural and morphological characteristics for fungal identification, including colony growth patterns, conidial morphology, and pigmentation, is consistent with the findings of Tafinta (2013), who demonstrated that these features are reliable for fungal classification.

Furthermore, the fungal species identified in the maize samples, predominantly *Aspergillus*, *Fusarium*, and *Penicillium*, corroborate the findings of Chulze *et al.* (2003), who reported the presence of *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme*, and *Penicillium* species in spoiled maize in Argentina. Similarly, Dalcero et al. (2004) identified *Fusarium verticillioides*, *Fusarium proliferatum*, and *Aspergillus flavus* as major contributors to kernel rot and fumonisin contamination in stored maize in Argentina.

Additionally, this study aligns with the findings of Wang *et al.* (2019), who identified *Aspergillus*, *Fusarium*, and *Penicillium* as dominant fungal species contributing to fungal community dynamics and mycotoxin accumulation in stored maize in China. Li *et al.* (2020) also characterized fungal communities in stored maize kernels from different regions of China and reported the dominance of *Aspergillus*, *Fusarium*, and *Penicillium* genera.

CONCLUSION AND RECOMMENDATIONS

This study confirmed that stored maize in the investigated market locations is significantly contaminated with fungal species, with *Aspergillus*, *Fusarium*, and *Penicillium* being the most predominant genera. The successful amplification of fungal DNA using ITS1 and ITS4 primers validated the fungal identities, while morphological and molecular analyses provided complementary insights into species characterization. The presence of these fungi indicates that inadequate storage conditions, environmental exposure, transportation, and handling practices contribute to fungal contamination.These findings emphasize the urgent need for improved post-harvest management to mitigate fungal infections, which can lead to spoilage and potential mycotoxin production. To minimize contamination, proper storage measures such as moisture control and aeration should be implemented. Training programs for farmers and handlers on best agricultural and storage practices are essential. Routine monitoring of fungal contamination and mycotoxin levels should be conducted to ensure food safety. Additionally, the use of antifungal treatments, including natural and synthetic agents, can help suppress fungal growth. Strengthening public awareness and enforcing food safety regulations are also critical steps.

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Finally, further research should explore the use of biocontrol agents and alternative storage methods to prevent fungal proliferation and mycotoxin accumulation.

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