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Molecular Identification of Fungal Complex Associated with Stored Maize Grains Vended in Some Local Government Areas of Adamawa State, Nigeria

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Abstract

Maize is a crucial global crop but remains highly vulnerable to fungal contamination, which poses serious threats to food safety and agricultural productivity. This study aimed to identify fungal species associated with maize samples from Adamawa State, Nigeria, using morphological and molecular techniques. Morphological analysis facilitated genus-level identification, while rDNA ITS sequencing provided precise species-level classification. The identified fungal species included *Lichtheimia ramosa*, *Aspergillus latus*, *Aspergillus flavus*, *Amesia atrobrunnea*, and *Cladosporium cladosporioides*. Among them, *Aspergillus flavus* was the most prevalent (48.1%), followed by *A. latus* (22.1%), *L. ramosa* (14.3%), *C. cladosporioides* (12.9%), and *A. atrobrunnea* (2.6%). Yola North recorded the highest level of fungal contamination. Growth rate analysis showed that *L. ramosa* exhibited the fastest growth, while *A. atrobrunnea* had the slowest. Molecular identification confirmed the fungal species, with ITS sequences displaying 81% to 95% similarity to reference strains. Phylogenetic analysis further clarified the evolutionary relationships among the isolates. The dominance of *Aspergillus* species, particularly *A. flavus*, raises concerns due to their mycotoxin-producing capabilities, which pose health risks and compromise food safety. While these findings align with previous reports on fungal contamination in stored grains, they differ from studies highlighting *Fusarium* and *Penicillium* as dominant contaminants in other regions. This study emphasizes the need for accurate fungal identification, improved storage techniques, and advanced molecular tools to mitigate contamination. These insights are essential for enhancing food security, promoting agricultural sustainability, and safeguarding public health in maize-reliant regions such as Nigeria.

Keywords: Maize grain; fungal contamination; *Aspergillus flavus*; Morphological and Molecular identification.

INTRODUCTION

Nigeria is the second-largest maize producer in Africa, with an annual output exceeding 12 million metric tons (MT), second only to South Africa. Maize is a staple crop in the country, with approximately 80% of production consumed directly or used as animal feed, while the remaining 20% supports industrial applications (Wossen *et al.,* 2023). As a vital component of food security in sub-Saharan Africa, maize is commonly consumed alongside legumes (Benjamin *et al.,* 2024).

The top maize-producing states, contributing around 64% of national output, include Borno, Niger, Plateau, Katsina, Gombe, Bauchi, Kogi, Kaduna, Oyo, and Taraba. In 2017, the International Institute of Tropical Agriculture (IITA) valued Nigeria's maize industry at $6 billion (approximately N2.5 trillion).

Maize (*Zea mays* L.), an annual cereal from the Poaceae family, plays a crucial role in global food security (Ahmad *et al.,* 2024). The term "maize" originates from ancient Greek and Taino languages, with "Zea" signifying "sustaining life" and "mays" meaning "life-giver" (Saleh *et al.,* 2019). Ranking third in global importance after rice and wheat, maize thrives in temperate, tropical, and subtropical climates (Benjamin *et al.,* 2024). However, significant losses occur at pre- and post-harvest stages, necessitating global efforts to mitigate these challenges. In Nigeria, maize is the most widely consumed staple, occupying over 27% of cereal farmland across multiple African nations, including Kenya, Malawi, and Zimbabwe (Akanmu *et al.,* 2023).

Post-harvest storage is critical, as maize is hygroscopic and prone to moisture absorption, leading to deterioration (Baidhe *et al.,* 2024). Even well-dried kernels can reabsorb moisture, increasing spoilage risks (Jimoh *et al.,* 2023). Proper storage techniques are essential to protect maize from adverse weather, microbial contamination, and insect damage (Okparavero *et al.,* 2024). Fungal growth, particularly under hot and humid conditions, further threatens grain quality, underscoring the importance of effective storage practices (Dadlani *et al.,* 2023).

Plant diseases significantly impact agricultural productivity and economic efficiency (Ekwomadu & Mwanza, 2023). Food safety concerns arise from microbial contamination and toxin production, notably mycotoxins, which cause mycotoxicosis in humans and animals (Kolawole *et al.,* 2024). Fungal genera such as *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* are major mycotoxin producers, with some species affecting crops in the field and others during storage (Fashola, 2023). Environmental factors and fungal species specificity influence mycotoxin production, highlighting the need for effective harvesting, processing, and early detection strategies (Hamad *et al.,* 2023).

Accurate identification of mycotoxigenic fungi is crucial, as closely related species can produce different toxin profiles (Dey *et al.,* 2023). Molecular diagnostic tools offer faster and more reliable detection than conventional methods, which are often labor-intensive, costly, and less specific (Fang *et al.,* 2024). By integrating molecular techniques, researchers can enhance fungal detection, improve food safety, and promote sustainable agricultural practices (Alameri *et al.,* 2023).

MATERIALS AND METHODS

Source of maize grain and site selection

Maize grain samples were collected from four local government areas in Adamawa State: Ganye, Gombi, Yola North, and Fufore. From each market in these areas, three replicates of maize grains were gathered. The samples were carefully placed into sterile bags and transported to the laboratory for analysis. The study's primary goal was to isolate and identify fungal species associated with the maize grains.

The experimental design followed a Completely Randomized Design (CRD), with treatments applied across the four local government areas. Each treatment was replicated three times, resulting in 12 experimental units for the study. This approach ensured a systematic and unbiased evaluation of fungal presence in the maize samples.

Media Preparation and Plating

Potato Dextrose Agar (PDA) supplemented with 0.25 g of chloramphenicol was used in the experiment, following the method outlined by Tanveer et al. (2011). The addition of chloramphenicol, an antibiotic, was intended to inhibit bacterial growth in the culture medium. From each sample, four to five maize grains were placed onto the PDA plates and incubated for 3 to 5 days. Sterilized forceps, dipped in 100% ethanol and flamed before use, were employed to transfer the grains into Petri dishes containing the PDA medium. The study was conducted using a Completely Randomized Design (CRD) and was replicated three times to ensure the reliability and accuracy of the results.

Morphological identification of fungi isolated from maize grain

Morphological characteristics are essential for identifying fungal cultures, encompassing features such as spore shape and size, the presence or absence of micro- and macroconidia, chlamydospore formation, the arrangement of spores on conidiophores, septation of conidia, and the color of both mycelium and spores. The presence or absence of aerial false heads is also a key consideration. For the morphological identification of fungi, reference materials, such as those provided by Ikechi-Nwogu et al. (2023), were consulted.

For molecular identification, pure cultures of each fungal species were obtained by sub-culturing spores onto potato dextrose agar (PDA) using the single spore isolation technique. These cultures were incubated at 25 °C under blue/black light with a 16/8-hour photoperiod for 7 days, after which DNA extraction was performed. To preserve the isolated fungal species for future research, they were stored in a 15% glycerol solution at -80 °C. This combined approach ensures precise identification and long-term preservation of fungal cultures, facilitating further research and analysis.

Molecular Identification of Fungal Isolates

Extraction of genomic DNA

To extract genomic DNA from fungal mycelia, representative fungal isolates were first cultured on Potato Dextrose Agar (PDA) for 7 days. Approximately 100 grams of fresh mycelia were collected and ground using a sterile pestle. The genomic DNA extraction was performed using the Quick DNATM Fungal and Bacterial Miniprep Kit. The procedure began with lysing the fungal cells using a bead beater, followed by centrifugation to separate the supernatant. The supernatant was filtered and combined with Genomic Lysis Buffer before passing through a Zymo-spin™ column. After a series of washing steps, the DNA was eluted using DNA Elution Buffer, yielding high-purity DNA suitable for subsequent applications. This method ensures efficient and reliable extraction of fungal genomic DNA for further analysis.

Quantification of extracted DNA quality

The quality and quantity of the extracted genomic DNA were assessed using a Nanodrop ND-1000 spectrophotometer. DNA purity was evaluated by measuring the absorbance ratios at 260/280 nm. To determine the DNA concentration, the samples were analyzed on a 0.80% agarose gel, which was run at 75 V/cm for 40 minutes. A 1 kb DNA ladder (PROMEGA) was used as a reference to estimate the size of the genomic DNA. The gel was stained with Midori Green Advanced DNA Stain (NG Japan) and visualized under a

transilluminator (BIO-RAD), with images captured for documentation. Additionally, gel electrophoresis was performed using a 1000 bp ladder at 70 V/cm for 45 minutes to confirm further the DNA size and integrity (Ismail, 2017; Simbolo, 2013). This comprehensive approach ensured an accurate assessment of DNA quality and concentration for downstream applications.

Polymerase chain reaction (PCR)

Amplification of the partial ribosomal DNA (rDNA) region was performed using the primer pair ITS-5 forward (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS-2 reverse (5'-GCTGCGTTCTTCATCGATGC-3'), as outlined by White et al. (1990). The total reaction volume of 25 µl consisted of 12.5 µl of PCR master mix, 0.5 µl of each primer, 9.5 µl of sterile distilled water, and 2 µl of DNA template specific to each fungal isolate. The PCR amplification was carried out in a thermal cycler with the following program: initial denaturation at 94 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 1 minute, and extension at 72 °C for 2 minutes. A final extension step at 72 °C for 5 minutes concluded the process (Ismail, 2017). This protocol ensured efficient amplification of the target rDNA region for subsequent analysis.

Agarose gel electrophoresis

5 µl of each PCR product was loaded onto a 1% (w/v) agarose gel and separated at 100 V using 1X TAE buffer for gel electrophoresis. The agarose powder (CSL-AG100LE Multi-Purpose Agarose, Cleaver Scientific) was mixed with TAE buffer and heated in a microwave until completely dissolved. The solution was then cooled to 55°C, after which three drops of DNA stain were added. The mixture was poured into a gel cast containing a comb and allowed to solidify. Once the gel was set, the PCR products were loaded, and electrophoresis was performed. A 1 kb DNA ladder was used as a size reference for the PCR products. After separation, the gel was visualized and photographed using a UV gel documentation system. Digital images were processed using Quantity One software provided with the transilluminator and saved in JPEG format for further analysis, following the methods described by Nirmaladevi et al. (2016). This procedure ensured accurate visualization and documentation of the amplified DNA fragments.

DNA sequencing

Fungal species identification was performed automatically sequencing ITS PCR products using a DNA analyzer at Inqaba Biotec West Africa, Ibadan, Nigeria. The resulting nucleotide sequences were assembled and edited using BioEdit software (Isuosuo *et al.,* 1999). Identification was carried out using the National Center for Biotechnology Information (NCBI) BLAST tool, which compared the obtained sequences with those in the NCBI database to identify regions of local similarity (Madden, 2013).

Data Analysis

Following a Completely Randomized Design (CRD), all numerical data were analyzed using ANOVA (version 9.4). Mean differences were assessed using the Least Significant Difference (LSD) test at a 5% significance level.

RESULTS

Table 1. Fungal counts of isolates associated with stored maize grains vended in Yola North, Ganye, Fufore, and Gombi Local Government Areas of Adamawa State.

		Organisms				
LG	**Location**	***A. l***	***A. f***	***L. r***	***A. a***	***C. c***
YOLA N	**1**	+	+	+	-	+
	2	+	+	+	-	+
	3	+	+	+	-	+
GAN	**1**	-	+	+	+	+
	2	+	+	-	-	+
	3	-	+	-	-	-
FU	**1**	+	+	+	+	+
	2	+	+	+	-	+
	3	-	+	+	-	+
GOM	**1**	-	+	-	-	-
	2	+	+	-	-	-
	3	+	+	+	-	+

Keys:
YN = Yola North Gan = Ganye Fu = Fufore Gom = Gombi
A. l = Aspergillus latus
A. f = Aspergillus flavus
L. r = Lichtheimia ramosa
A. a = Amesia atrobrunnea
C. c = Cladosporium cladosporioides
+ = present
- = absent

Table 2. Frequency of Occurrence and Percentage Frequency of Occurrence of Different Fungal Isolates Identified in Samples Obtained from Ganye, Yola North, Gombi, and Fufore in Adamawa State.

Isolated Fungi	Local Government Areas				Total	% Frequency
	Yola/North	Ganye	Fufore	Gombi		
Aspergillus latus	7	1	6	3	17	22.1
Aspergillus flavus	12	8	9	8	37	48.1
Lichtheimia ramosa	5	1	4	1	11	14.3
Amesia atrobrunnea	NP	1	1	NP	2	2.6
Cladosporium cladosporioides	3	2	4	1	10	12.9
Total	27	13	24	13	77	100

NP = Not present

Table 3. Mean Fungal Growth Rate (mm) of isolates from Maize Grains.

Isolate	Isolates' Growth Rate (mm)							
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
A. flavus	26.33^{b}	45.00^{b}	54.00^{b}	70.67^{b}	76.33^{c}	83.33^{b}	90.00^{a}	90a
L. ramosa	33.67^{a}	69.33^{a}	76.67^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90a
A. latus	27.67^{b}	46.67^{b}	55.33^{b}	72.67^{b}	84.00^{b}	90.00^{a}	90.00^{a}	90a
A. atrobrunnea	15.00^{c}	23.33^{c}	34.67^{c}	42.67^{c}	50.67^{d}	64.00^{c}	76.67^{b}	90a
C. cladosporioides	31.33^{a}	45.33^{b}	54.33^{b}	75.33^{a}	90.00^{b}	90.00^{a}	90.00^{a}	90a
Mean	26.80	45.93	55.00	70.268	78.20	83.47	87.20	90.00
SE (+) (-)	1.797	2.903	2.623	1.634	1.743	2.715	1.418	0.000
CV (%)	8.582	7.620	8.723	6.020	6.164	3.498	6.296	0.00
Significance level	*	*	*	*	*	**	*	NS

Key: * = Significant difference, ** = highly significant, Coefficient of variation (CV), Standard error (SE), Level of significance (using ANOVA) and least significant difference (LSD), NS= Not significant. P<0.05. Means carrying the same letter (s) in the same column are significantly equal at the 5 % significance level (p = 0.05 %).

Table 4. Plates of the Morphological Characterization of Fungal Isolates Obtained from Maize Grains Vended in Yola Markets.

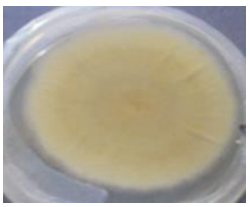
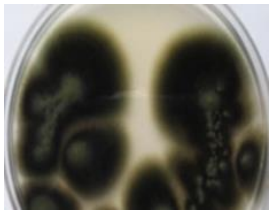
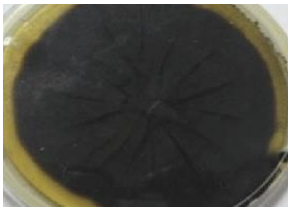
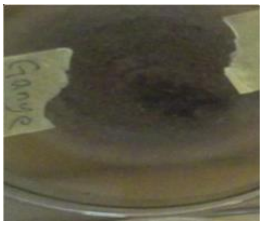
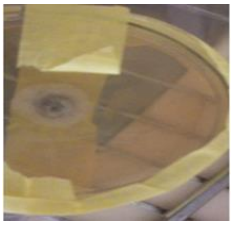
S/N	Name of isolates	Front side	Reverse side	Conidia image
1	***A. flavus***			
2	***C. cladosporioides***			
3	***A. atrobrunnea***			

Table 4. *Cont.*

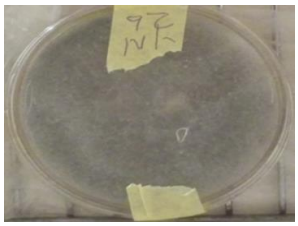
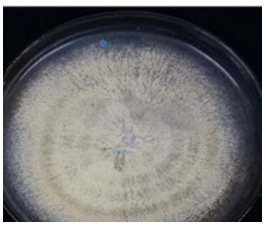
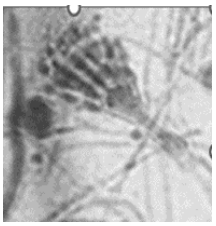
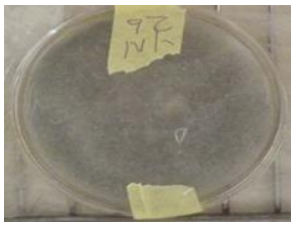
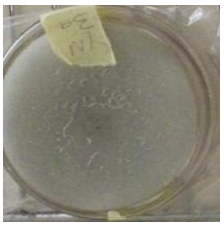
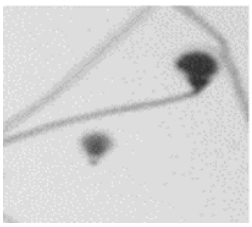
S/N	Name of isolates	Front side	Reverse side	Conidia image
4	***A. latus***			
5	***L. ramosa***			

Table 5. Morphological Description of the Fungal Isolates.

Isolates	Colony description	Conidia shape/size
Aspergillus flavus	The mycelia color is white, followed by olive-green conidia formation that dominated the colony color appearance, the reverse is cream. It grows moderately on PDA and covers the 90 mm petri dish in 7 days.	Colonies are flat at the borders while raised in the middle. The size ranged between 250 µm and 450 µm in diameter with thin walls and rough texture.
Cladosporium Cladosporioides	On PDA medium, colonies are olive-grey to dull green, velvety, and tufted, the colony edge is olive-grey and feathery and grows moderately on PDA covering 90 mm dish in 5-7 days. Mycelia forms mats and on the colony surface, it grows upward.	Single-celled, oval-shaped, and smooth-walled. Conidia are numerous, forming terminal branches with up to ten conidia per branch. Cladosporium cladosporioides is smaller in size ranging from 4-8 µm long, 2-4 µm wide.
Amesia atrobrunnea	The colony is black to brown color and has flexuous and septate hairs. it grows slowly on PDA and matures within 7-8 days to cover the 90 mm petri dish.	The ascospores are fusiform and some are elongate, they turn dark brown at maturity. The size is 7.5 - 10x 4 -5.5 µm.
Aspergillus latus	The colonies were moderately deep, had white mycelia, no soluble pigment, light brown sporulation, the colony surface had woolly hairs (floccose) and grew to maturity within 6-7 days covering 90 mm petri dish.	Conidia were observed to be globose (spherical) to sub-globose (almost spherical) and smooth. The size is 3 µm – 4 µm.
Lichtheimia ramosa	Cultures are fast growing, pale white, turning grey with age, and they display a greater growth rate and mature within 4 days covering the 90 mm petri dish.	The sporangiospores are hyaline to light black, round to oval in shape. It is 3µm - 4.5µm in diameter.

Polymerase chain reaction (PCR)

The five representatives of fungal isolates from maize grains sold in the selected local governments were morphologically studied and identified, then further subjected to molecular identification to confirm their identities. The PCR amplification of the ITS genes was defined clearly with all five (5) isolates with the expected size of about 600 bp with ITS primers shown.

DNA *sequence of fungal isolates*

ITS nucleotide sequences of the identified fungi isolated from maize grains vented in some selected markets in Yola, Adamawa State

>Lichtheimia ramosa

TCGGAAAGRAAAAAAYSTGGMWTCGATGAAAA
CGCATCATCSACAGGACTCCCCACAGAA
AACCCTCCTATGTTTGRATAYCCGGAACKTTGAA
TCTCGTCTKCGTGCTTGAACCCAGMA
TTGGCCACCTAAAAACTTTCCTTACSATCGTCTA
ACAAACAATATTGWKATGGGAAAAAC
TTTTAAAAGGAGTGTCCTGTGACCCATAACCCRA
ATCACAAMAAAGGGGGAMCCCCTTKG
GGGGCCCCACTTTSCATAYCCCTGGGGTAAATTT
TMRGGGGGAAAACACCCCAAWCTARG
GWTTTTTTAAACTTCTCWWAAAAAACWCCTCC
GRAGGTYCACCTACGGAACCCTTKGTMA
CAAATAAAAACTCCCAAAATCG

>Aspergillus latus
AAACWGGGGMGGGGACATGACAGAAGGGGGG
GWTGCCTKWAAACGCCAACCTCCTAAMAATGA
TAWACAACCAGAAGCRTCGGTGACGGCCGCGA
CCCCCAACCCGCCGGAGACCACTGK
ACTTCATGGCGGAGAGGGATGCMGCCTMASCCT
GAATACMAATCAGAAAAAACTTTCAAC
RATGGATCTCTCGGTTCCCGTWTWAATAAASAW
CCCTCCATKAA

>Aspergillus flavus
CAWTGYGTKAAAGGTTATTACCGATTGTAAGGG
ATCTASTCAGCCCTACCTCCCACCCAW
GRWTWCTGCCCCGTAATTGGTTCGGSGGGGCCC
CCATTSTTGGMCCCCCCCGGGGGTCTT
TAATCCGGGGCCCSSCCCAARCRAAAAAAMCAA
CAAAAATKSYGGAGGGAAGGTAGGKTT
MSTTAGATYCCCTCAATTCGKTAAAAATCTTTCC
CAKGGTTCCCTTKGGRAACCKTGTTA
AAAAATTTAACTTCACACGA

>Amesia atrobrunnea
CAGTTAATTGAATTAATTTGGMTAAAATAAATA
AACAATGGTTTATAGGAAATATAAKTR
GGGAATCACCGGGCGGGGTCCTGGGAAGGTCCG
AGGGGGGCCAAACACCCGCCMGCGAAA
CCAMKGTTTAAGGGAAACTTAACCATTGTTTAA
TTATTTTTACTCTMTAAAATAATCCCT
CCACTGSTCCACCAKTGGARACCTTRTTAATTTT
TTTTTTTACAA

>Cladosporium cladosporioides
CATCGATGAAGAACGCAGCATCGATGAAGAACG
CAGCATCGATGAAGAACGCAGCATCGATGAAGA
ACGCAGCATCGATGAAGAACGCAGCATCGATGA
AGAACGC

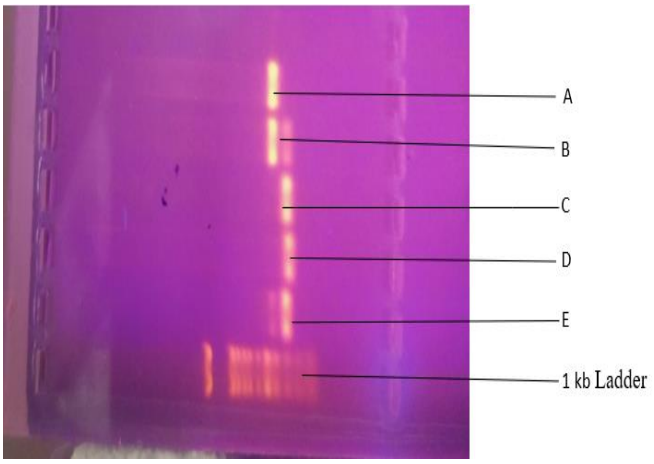


Figure 1. (Plate 5) Gel Electrophoresis Image of the PCR Products of DNAs of the Representative Isolates on 1 % agarose, showing bars of about 400 bp on 1 kb DNA molecular weight ladder, (A) *Lichtheimia ramosa*, (B) *Aspergillus latus*, (C) *Aspergillus flavus*, (D) *Amesia atrobrunnea,* and (E) *Cladosporium cladosporioides*. Showing Amplified Region of ITS.

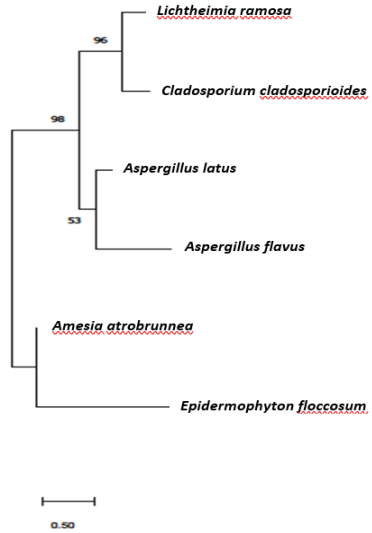


Figure 2. (Plate 6) Internal Transcribed Spacer (ITS) Phylogeny of fungal Isolates obtained from maize grains vented in Yola markets, using a Maximum Livelihood Analysis. Bootstrap Values were Represented at each node with *Epidermophyton floccosum* as an outer layer.

Table 6. Identification of representative fungal isolates from the maize grains based on ITS sequence.

S/N	Identified species	% Similarity with GenBank strains	GenBank reference strain number
1	*Lichtheimia ramosa*	85.32	LK023322.1
2	*Aspergillus latus*	86.32	OR501409.1
3	*Aspergillus flavus*	95	MZ357882.1
4	*Amesia atrobrunnea*	81.24	MH864203.1
5	*Cladosporium cladosporioides*	87.83	JF796748.1

DISCUSSION

Maize is a vital crop globally and provides numerous substrates that support fungal growth (Burlakoti *et al.,* 2024). This study employed both morphological and molecular techniques to identify five fungal species from the maize samples using rDNA ITS sequence analysis. Morphological methods are helpful for identification up to the genus level, but molecular analysis is needed for species-level identification.

Five fungal species were isolated and identified at the species level through rDNA ITS sequence analysis from maize grain samples collected from four major markets in Adamawa State: Ganye, Gombi, Yola North, and Fufore. The study found that most maize samples from these markets were contaminated with varying levels of fungal growth, while a few showed no fungal presence. The identified fungal species included *Lichtheimia ramosa*, *Aspergillus latus*, *Aspergillus flavus*, *Amesia atrobrunnea*, and *Cladosporium cladosporioides*, all exhibiting morphological traits consistent with previous studies (Bensch *et al.,* 2012).

Among these species, *Aspergillus flavus* is particularly notable due to its ability to produce mycotoxins, which pose significant health risks to humans, animals, and plants, as well as contribute to food spoilage (Awuchi *et al.,* 2021). These fungi can contaminate grains both preharvest and postharvest, especially under inadequate drying and storage conditions, leading to increased aflatoxin levels (Gachara *et al.,* 2024). *Aspergillus* and *Cladosporium*, both common in stored grains, were the predominant genera in this study (Mato *et al.,* 2024).

The study further quantified fungal prevalence across the collected maize samples. A total of 36 fungal isolates were identified, with *Aspergillus flavus* being the most prevalent (48.1%), followed by *Aspergillus latus* (22.1%), *Lichtheimia ramosa* (14.3%), *Cladosporium cladosporioides* (12.9%), and *Amesia atrobrunnea* (2.6%). *Amesia atrobrunnea* was the least common species detected. Yola North had the highest fungal contamination among the sampled markets, with 27 occurrences recorded.

The growth rates of the five fungal species isolated from maize samples were recorded daily until each species fully covered the Petri dish. Lines were drawn across the center of each plate to assess growth, and the mycelial mat diameter was measured along these lines. Among the isolates, *Lichtheimia ramosa* exhibited the fastest growth, covering the 90 mm Petri dish within four days. *Cladosporium cladosporioides* displayed moderate growth, reaching full coverage in five days. *Aspergillus latus* and *Aspergillus flavus* followed similar growth patterns, maturing in six and seven days, respectively, while *Amesia atrobrunnea* exhibited the slowest growth, taking eight days to develop fully.

The colonization of stored grains by fungi is influenced not only by their growth rates but also by factors such as moisture content, temperature, storage duration, and initial fungal contamination. Some maize samples exhibited no fungal growth, likely due to storage under safe moisture conditions, which inhibited fungal proliferation. A summary of the growth rates for all species is presented in Table 3.

Recent studies have confirmed the rapid growth of *Lichtheimia ramosa*. When cultured on potato dextrose agar (PDA), it forms grey-white colonies with numerous hyphae, reaching an average diameter of 6.9 cm within four days (Imade *et al.,* 2020). Its rapid expansion enables it to cover entire plates within one to seven days. The genomic sequences of *Lichtheimia ramosa* and its closely related species, *Lichtheimia corymbifera*, have been published (Shen *et al.,* 2023).

Regarding *Aspergillus latus*, which was identified in maize grains in this study, taxonomic literature suggests that *Aspergillus sublatus* and *A. latus* are closely related, with *A. sublatus* holding taxonomic priority (Chen *et al.,* 2016). *A. sublatus* has also been recognized as a significant causative agent of aspergillosis (Chrenkova *et al.,* 2018).

Amesia atrobrunnea, another species identified in this study, is characterized by dark brown to black ascomata, flexuous septate hairs, and a maturation period of seven to eight days. Previously classified as *Chaetomium atrobrunneum*, it was later reassigned to the *Amesia* genus based on phylogenetic analysis (Wang *et al.,* 2016).

Isolates of *Cladosporium cladosporioides* have been associated with systemic infections in humans. When cultured on PDA, it forms olive-grey to dull-green colonies with a velvety texture and feathery edges. This species primarily spreads across the medium rather than growing vertically and occasionally produces characteristic hyphal exudates (Bensch *et al.,* 2012). As an asexual fungus, *C. cladosporioides* reproduces by producing vegetative spores, or conidia.

As for *Aspergillus flavus*, the colonies appeared in shades of olive-green, yellowish-green, or dark green, often surrounded by a white ring covered with conidia over time. The colonies were typically velvety in texture, sometimes woolly, and often produced exudates. Additionally, most isolates of *A. flavus* produced sclerotia, in line with descriptions by Gautam and Bhadauria, (2019).

All numerical data in the study, based on a Completely Randomized Design (CRD), were analyzed using ANOVA version 9.4, with significant means separated by LSD at a 5% significance level. The growth rates of fungi showed significant differences on Days 1 through 5, with Day 6 showing highly significant differences. On Day 8, there was no significant difference ($p = 0.05$) since all plates were fully covered by fungal growth.

DNA extraction and molecular identification were performed for all fungal isolates. Genomic DNA from each fungus, which had been cultured for 7 days on PDA, was extracted using the Quick-DNATM Fungal/Bacterial Miniprep kit following the manufacturer's protocol. DNA quality and quantity were assessed using a Nanodrop ND-1000 spectrophotometer, and DNA integrity was verified by electrophoresis on a 0.80% agarose gel, run at 75 V/cm for 40 minutes. Plate 6 illustrates the DNA bands for the fungi.

Molecular identification of the five fungal isolates was conducted using DNA barcoding, focusing on sequencing the ITS region. The ITS rDNA sequences

were amplified with primers ITS5 and ITS2, and the sequences were compared to those in the NCBI-BLAST database. The results showed varying fungal biodiversity across different locations. Aspergillus flavus was the most prevalent, present in all sampled locations, while *Amesia atrobrunnea* was only found in two locations.

The sequencing of the five amplified samples was carried out by Inqaba Biotec West Africa in Ibadan, Nigeria. Sequence analysis revealed significant similarities with known fungal strains in the GenBank database. Cladosporium cladosporioides showed 87.83% similarity (GenBank reference JF796748.1), *Aspergillus latus* had 86.32% similarity (OR501409.1), and *Aspergillus flavus* demonstrated a 95% match (MZ357882.1). *Lichtheimia ramosa* exhibited 85.32% similarity (LK023322.1), and *Amesia atrobrunnea* had 81.24% similarity (MH864203.1). These findings are summarized in Table 6.

The phylogenetic tree presented in Plate 7 illustrates the evolutionary relationships among the fungi isolated in the study. The ITS sequences of the five fungal species were compared with reference sequences from the NCBI nucleotide database. These sequences were aligned using MAFFT (Multiple Alignment for Sequence) version 6.0, and the phylogenetic tree was generated through maximum likelihood (ML) analysis using the Hasegawa-Kishino-Yano (HKY) model in MEGA 7 software. The optimal phylogenetic tree was identified by selecting the one with the highest likelihood score, using 1000 bootstrap replicates to assess the support for each clade.

The ITS sequences obtained in this study had a similarity range of 81% to 90% with reference sequences from previous studies. In addition to the sequences generated in the research, other reference sequences were retrieved from GenBank and aligned with MAFFT. The alignment was further refined manually using BioEdit software. Phylogenetic congruency was tested using the 70% reciprocal bootstrap criterion, confirming the relationships among the five fungi, which belong to four genera. The bootstrap values supported the major groups and branching clusters in the tree. *Epidermophyton floccosum* was included as an outgroup to root the tree and clarify the relationships.

These results align with previous findings that *Aspergillus flavus* is the most commonly isolated fungal species from contaminated grains (Katati *et al.,* 2024). *Aspergillus*, particularly from the *flavi* group, is a major contaminant of maize during storage, as observed in this study, where *A. flavus* was the most frequently isolated fungus. This agrees with reports that *Aspergillus* species are highly prevalent across various environments such as soil, water, air, and food (Nji *et al.,* 2023), and matches the findings from previous studies in Nigeria where *Aspergillus aflatoxiformans* was predominant in grains like maize and rice (Ezekiel *et al.,* 2021).

The dominance of *Aspergillus* species in the current study is of concern due to their association with invasive aspergillosis, a disease affecting humans, with species like *A. flavus*, *A. terreus*, *A. niger*, *A. ustus*, and *A. versicolor* being implicated (Abdel-Azeem *et al.,* 2019). Accurate and timely identification of these fungi is essential for managing their impact and conducting proper surveillance. The global distribution of *Aspergillus* species, capable of growing in various climates, underscores their importance in contamination and human health.

This study aligns with the findings of Chilaka et al. (2012), who identified Aspergillus and Cladosporium as dominant fungal genera in stored grains, reinforcing the results observed here. Likewise, Wu et al. (2012) recognized Aspergillus as the most frequently occurring fungal species (Olugbenga & Chongs, 2024). However, these findings contradict those of Joshi et al. (2022), who reported Fusarium as the most prevalent genus. Notably, no Penicillium species or other typical storage fungi were detected in this study, differing from previous research that frequently cites Penicillium as a common contaminant of maize grains.

CONCLUSION

This study identified five fungal species from four genera associated with maize grains obtained from markets in Gombi, Ganye, Yola North, and Fufore Local Government Areas of Adamawa State. The species detected included *Aspergillus flavus*, *Aspergillus latus*, *Amesia atrobrunnea*, *Lichtheimia ramosa*, and *Cladosporium cladosporioides*. The prevalence of these fungi varied, with occurrence rates ranging from 2.6% to 48.1%. The *Aspergillus* genus was the most dominant, representing 70.2% of the total fungal isolates.

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