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Isolation, Characterization, and Antibiogram Profile of Fungi from Spoilt Bread

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Abstract

Bread is a staple food in Nigeria, but its high perishability due to fungal spoilage leads to significant economic waste and public health risks from potential mycotoxin production. This study aimed to isolate, identify, and evaluate the antifungal susceptibility of fungi responsible for spoiling bread in Adamawa State, Nigeria. Twelve bread samples were collected from local vendors in Girei and Yola North. Fungi were isolated on Potato Dextrose Agar (PDA) and identified using a polyphasic approach combining morphological characteristics. An antifungal susceptibility test was performed using the agar dilution method to determine the Minimum Inhibitory Concentration (MIC) of ketoconazole, fluconazole, and itraconazole against the isolates. The mycological analysis revealed a community dominated by *Aspergillus* sp., which was present in over 90% of viable samples. *Rhizopus* spp. were frequently isolated as secondary contaminants, while *Mucor*, *Fusarium*, and *Penicillium* species were less common. Quantification showed high fungal loads, ranging from 4.1×10^6 to 3.9×10^8 CFU/mL. The antifungal susceptibility profiles revealed widespread resistance to fluconazole and itraconazole among the isolates. *Aspergillus* and *Rhizopus* were resistant to both (>100% MIC), while *Mucor* was resistant to fluconazole. In contrast, ketoconazole was the most effective agent, with all tested genera showing sensitivity at concentrations ranging from 20% to 80% of the MIC. The findings confirm the dominance of *Aspergillus* in bread spoilage within the study area and highlight a concerning level of resistance to common azole antifungals. The demonstrated efficacy of ketoconazole suggests it could be a viable alternative for preservation. This study provides critical data for developing targeted antifungal strategies and evidence-based policies to extend the shelf life of bread, reduce economic losses, and mitigate mycotoxin-related health risks for consumers in Nigeria.

Keywords: Bread Spoilage; Mycobiota; Antifungal Resistance; Antibiogram; Food Safety; Minimum Inhibitory Concentration.

INTRODUCTION

Bread stands as a paramount staple food in global diets, including in Nigeria, prized for its nutritional value, affordability, and culinary versatility (Akinola *et al.,* 2024). Its consumption spans all demographics, often serving as a convenient standalone snack or a complement to various beverages. This widespread acceptance, however, is perpetually challenged by the product's pronounced perishability. The very composition of bread, a baked matrix of starch, protein, and moisture, combined with its optimal water activity, creates an ideal ecological niche for microbial proliferation (Oluwambe *et al.,* 2023). While the fermentation by baker's yeast (*Saccharomyces cerevisiae*) is essential for its aerated structure and flavor, this same nutrient-rich environment predisposes bread to rapid colonization by spoilage microorganisms, with fungi being the primary agents of deterioration (Adebayo-Tayo *et al.,* 2024). This vulnerability highlights a fundamental conflict between bread's importance in diet and its inherent instability, resulting in significant economic losses and food waste throughout the supply chain (Chukwuma *et al.,* 2024).

The spoilage of bread is predominantly a mycological phenomenon, initiated by the post-baking contamination of spores from ubiquitous environmental genera such as *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor* (Olanrewaju & Ezeokoli, 2023). Contamination vectors are multifaceted, originating from raw materials, air, food handling surfaces, and packaging equipment. The progression of spoilage is critically dependent on extrinsic factors, particularly ambient humidity and temperature, which govern spore germination and mycelial expansion (Ssemanda *et al.,* 2024). The visible manifestation of this process, the appearance of colored, fuzzy mycelium, renders the product organoleptically unacceptable, causing direct economic damage. However, the invisible threat is of far greater public health concern: the potential production of mycotoxins. These thermally stable, secondary metabolites, such as aflatoxins and ochratoxins, produced by certain *Aspergillus* and *Penicillium* species, are potent

carcinogens and nephrotoxins, presenting a silent hazard even at low levels of fungal growth (Mwanza *et al.,* 2023).

Addressing this spoilage and its associated risks necessitates a precise understanding of the causative agents. Traditional isolation methods, employing media such as Potato Dextrose Agar (PDA), remain foundational for recovering fungi from spoiled bread (Akinola *et al.,* 2024). However, accurate identification, crucial for assessing mycotoxin risk and understanding spoilage ecology, has been revolutionized by molecular techniques. Morphological identification alone is often insufficient to distinguish between closely related species or cryptic species complexes, which may differ significantly in their toxigenic potential and resistance profiles (Olanrewaju and Ezeokoli, 2023). Therefore, a polyphasic approach, integrating classical microbiology with genetic characterization (sequencing the ITS region of rDNA), is now the gold standard for constructing a definitive and actionable profile of the contaminating mycobiota (Adebayo-Tayo *et al.,* 2024).

The context of bread spoilage in developing regions, such as Nigeria, presents unique challenges that exacerbate the problem. High ambient temperatures and humidity, coupled with sometimes suboptimal storage facilities and handling practices, can accelerate fungal growth and increase consumer exposure (Chukwuma *et al.,* 2024). The efficacy of conventional chemical preservatives, such as calcium propionate, may be compromised by emerging fungal resistance, a phenomenon poorly documented in local contexts (Oluwambe *et al.,* 2023). This knowledge gap also extends to a lack of comprehensive "antibiogram" profiles for food spoilage fungi, which necessitates a systematic assessment of their susceptibility to a panel of antifungal agents (Ssemanda *et al.,* 2024). Understanding these resistance patterns is critical for evaluating the ongoing utility of existing preservatives and for developing new, targeted strategies to extend shelf life and ensure safety.

Consequently, this study aims to bridge these critical knowledge gaps through a systematic investigation of the fungi responsible for bread spoilage, with a particular emphasis on the Nigerian context. The research seeks to isolate, characterize, and genetically identify the predominant fungal species associated with spoiled bread samples. Furthermore, it will pioneer the development of an antibiogram profile for these isolates by assessing their susceptibility to commonly used food-grade antifungal compounds. The outcomes of this comprehensive investigation are expected to generate vital data for conducting evidence-based risk assessments, improving food preservation protocols, and informing policy decisions (Mwanza *et al.,* 2023). Ultimately, the study aims to reduce economic losses and protect public health by enhancing the safety and quality of bread, a key dietary staple in Nigeria (Akinola *et al.,* 2024).

METHODOLOGY

Study Area and Laboratory Site

The experimental work for this research was conducted in the microbiology research laboratory within the Department of Microbiology at Modibbo Adama University, Yola. The university is situated in the Girei Local Government Area of Adamawa State, Nigeria. This location provided a controlled environment essential for the analytical procedures. The laboratory is equipped with standard microbiological instrumentation, including autoclaves, laminar flow hoods, and incubators, which were utilized to maintain aseptic techniques and ensure the integrity of the microbial cultures throughout the study.

Collection of Samples

Bread samples (n=12) of varying types were obtained from vendors across the Girei and Yola North Local Government Areas. Each sample will be transported in a sterile polyethylene bag to the microbiology laboratory at Modibbo Adama University, Yola. Subsequently, the samples will be subjected to spoilage under variable environmental conditions to facilitate analysis.

Media Preparation

Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were selected as the culture media for this study due to their specificity for promoting fungal growth. All media were prepared according to the manufacturer's instructions. Briefly, 39 g of dehydrated PDA and 65 g of dehydrated SDA were each suspended in 1 L of distilled water, respectively. Each suspension was boiled to ensure complete dissolution and then sterilized by autoclaving at 121°C (15 psi) for 15 minutes. Following sterilization, the media were cooled to 45–50°C before being aseptically dispensed into sterile petri dishes or tubes. All glassware was sterilized in a hot-air oven at 160°C for one hour, while other heat-stable materials were autoclaved at 121°C for 15 minutes (Okoye *et al.,* 2020).

Antifungal Susceptibility Testing

The in vitro susceptibility of the purified fungal isolates to common antifungal agents was determined using a modified agar dilution method.

Antifungal Agents and Preparation

Three azole antifungal agents were tested: Ketoconazole, Fluconazole, and Itraconazole (Sigma-Aldrich, USA). Stock solutions of each antifungal were prepared in dimethyl sulfoxide (DMSO) and subsequently diluted in sterile distilled water to obtain a series of working concentrations (20%, 40%, 60%, 80%, and 100% w/v).

The final concentration of DMSO in the agar media did not exceed 1% (v/v), a level confirmed not to inhibit fungal growth in preliminary assays.

Inoculum Standardization

Pure, sporulating cultures of each test isolate, *Aspergillus spp., Fusarium* sp., *Rhizopus* sp., *Mucor* sp., *and Penicillium* sp. were grown on Potato Dextrose Agar (PDA) for 7 days at 28°C. Fungal spores (conidia/sporangiospores) were harvested by flooding the agar surface with sterile saline solution (0.85% NaCl) containing 0.1% Tween 80. The resulting suspension was filtered through sterile gauze to remove mycelial fragments. The spore concentration was adjusted spectrophotometrically to a turbidity equivalent to a 0.5 McFarland standard, yielding a final inoculum density of approximately 1×10^6 to 5×10^6 CFU/mL (Sanusi *et al.,* 2025).

Mycological Identification

The initial characterization of the fungal isolates was based on their macroscopic and microscopic morphological characteristics. These included the pattern of colony growth, conidial structure and arrangement, and the production of any soluble pigments. Following established protocols, a microscopic examination was performed using a lactophenol cotton blue staining technique. In this procedure, a small segment of the aerial mycelia was aseptically transferred onto a microscope slide containing a drop of the stain. The hyphal mat was carefully teased apart to ensure a thin, even distribution before a cover slip was applied, taking care to minimize the formation of air bubbles. The prepared slides were then observed under a light microscope using both low (×10) and high (×40) power objectives. The identity of each fungal organism was determined by comparing its observed features with standard mycological taxonomic keys (Mailafia *et al.,* 2017).

Susceptibility Testing Procedure

Sterile molten PDA was tempered to 50°C and amended with the appropriate volume of each antifungal working solution to achieve the final desired concentrations in the agar. Approximately 20 mL of the antifungal-amended agar was dispensed into sterile 90-mm Petri dishes and allowed to solidify. The surface of each plate was spot-inoculated with 2 µL of the standardized spore suspension. Plates containing non-amended PDA and PDA with 1% DMSO served as positive and negative controls for growth, respectively. All tests were performed in duplicate. The inoculated plates were incubated at 28°C for 48-72 hours (Shukla, 2011).

Determination of Minimum Inhibitory Concentration (MIC)

After the incubation period, the plates were examined for the presence or absence of visible growth. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the antifungal agent that completely inhibited any visible fungal growth. The results were interpreted qualitatively, with isolates categorized as Susceptible, Intermediate, or Resistant based on the determined MIC values relative to the tested concentration range (Oshim *et al.,* 2016).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). The occurrence of the different fungal species was summarized and expressed as frequency counts and prevalence rates. To determine statistical significance, Analysis of Variance (ANOVA) was employed, with a probability value of less than 0.05 ($p < 0.05$) considered statistically significant.

RESULTS

Table 1. Incubation Day at Which Visible Mold Growth Was First Observed on Bread Samples.

Bread Sample	Day of Spoilage Onset
YN1	Day 5
YN2	Day 6
YN3	Day 7
YN4	Day 7
YN5	Day 9
YN6	Day 9
YN7	Day 9
YN8	Day 10
YN9	Day 10
YN10	Day 10
YN11	Day 11
YN12	Day 12

Table 2a. Colony Count on Day 3 at 4th and 6th Dilutions (Samples YN1–YN6).

S/N	Sample ID	Visible Colonies	CFU/mL
1	YN1•	41	4.1×10^6
2	YN1*	8	8.0×10^7
3	YN2•	54	5.4×10^6
4	YN2*	17	1.7×10^8
5	YN3•	147	1.47×10^7
6	YN3*	39	3.9×10^8
7	YN4•	46	4.6×10^6
8	YN4*	20	2.0×10^8
9	YN5•	—	—
10	YN5*	10	1.0×10^8
11	YN6•	63	6.3×10^6
12	YN6*	7	7.0×10^7

Table 2b. Colony Count on Day 3 at 4th and 6th Dilutions (Samples YN7–YN12).

S/N	Sample ID	Visible Colonies	CFU/mL
13	YN7•	136	1.36×10^7
14	YN7*	5	5.0×10^7
15	YN8•	138	1.38×10^7
16	YN8*	36	3.6×10^8
17	YN9•	18	1.8×10^6
18	YN9*	7	7.0×10^7
19	YN10•	53	5.3×10^6
20	YN10*	25	2.5×10^8
21	YN11•	38	3.8×10^6
22	YN11*	9	9.0×10^7
23	YN12•	85	8.5×10^6
24	YN12*	36	3.6×10^8

Key:
• = 4th Dilution * = 6th Dilution
"—" indicates no visible colonies observed.

Table 3a. Macroscopic and Microscopic Identification of Fungal Isolates from Soil Samples.

Sample ID	Macroscopic Colony Morphology	Microscopic Identification (Genus)
YN1•	• Green colony with white margins and surrounding mycelium	• *Aspergillus* sp.
	• Round, black colonies	• *Aspergillus* sp.
	• Brown colony	• *Mucor* sp.
YN1*	• Large green colony with surrounding mycelium	• *Aspergillus* sp.
YN2•	• Green colony with surrounding mycelium	• *Rhizopus* sp.
	• Fluffy brown colony with surrounding mycelium	• *Mucor* sp.
	• Large white colony	• *Aspergillus* sp.
YN2*	• Large green colony with surrounding mycelium	• *Rhizopus* sp.
	• Smooth white colony	• *Aspergillus* sp.
YN3•	• Black colony with surrounding mycelium	• *Aspergillus* sp.
	• Large, fluffy white colony	• *Fusarium* sp.
YN3*	• Small green colony	• *Aspergillus* sp.
YN4•	• Round, black colony	• *Rhizopus* sp.
	• Large, velvety brown colony with surrounding mycelium	• *Rhizopus* sp.
	• Large green colony with surrounding mycelium	• *Aspergillus* sp.
YN4*	• Large, fluffy green colony with surrounding mycelium	• *Aspergillus* sp.
YN5•	• Contaminated	–
YN5*	• Small pink colony	• *Fusarium* sp.
	• Large green colony with surrounding mycelium	• *Aspergillus* sp.
YN6•	• Green colony with surrounding mycelium	• *Aspergillus* sp.
YN6*	• Round brown colony	• *Rhizopus* sp.
	• Small, velvety green colony	• *Aspergillus* sp.

Table 3b. Mycological Analysis of Soil Samples from Girei LGA (4th and 6th Dilution).

Sample Plate	Macroscopic Colony Morphology	Microscopic Identification (Fungal Genus)
YN7•	• Fluffy green colony	• *Aspergillus* sp.
	• Smooth dark green colony with mycelium	• *Penicillium* sp.
	• Creamy white colony	• *Fusarium* sp.
YN7*	• Large velvety green colony	• *Aspergillus* sp.
	• Creamy white colony	• *Fusarium* sp.
YN8•	• Large white colony	• *Aspergillus* sp.
	• Green colony with mycelium	• *Penicillium* sp.
YN8*	• Large fluffy green colony with mycelium	• *Aspergillus* sp.
YN9•	• Small pink colony	• *Fusarium* sp.
	• Large green colony with mycelium	• *Rhizopus* sp.
YN9*	• Green colony	• *Rhizopus* sp.
YN10•	• Smooth black colony with mycelium	• *Mucor* sp.
	• Green colony with mycelium	• *Rhizopus* sp.

Sample Plate	Macroscopic Colony Morphology	Microscopic Identification (Fungal Genus)
	• Creamy white colony	• *Aspergillus* sp.
YN10*	• Large green colony with mycelium	• *Aspergillus* sp.
	• Velvety brown colony with mycelium	• *Rhizopus* sp.
	• Small fluffy green colony	• *Aspergillus* sp.
YN11•	• Large fluffy green colony with mycelium	• *Rhizopus* sp.
YN11*	• Large green colony with mycelium	• *Rhizopus* sp.
YN12•	• Large green colony with mycelium	• *Aspergillus* sp.
	• Velvety black colony	• *Rhizopus* sp.
YN12*	• Fluffy green colony with mycelium	• *Aspergillus* sp.

Key:
• = 4th Dilution * = 6th Dilution
"—" indicates no visible colonies observed.

Table 4. Frequency and Distribution of Fungal Isolates from the 4th and 6th Dilutions.

S/N	Fungal Isolate	Frequency (4th Dilution)	Percentage (4th Dilution)	Frequency (6th Dilution)	Percentage (6th Dilution)
1	***Aspergillus* sp.**	10	40.0	11	61.1
2	***Rhizopus* sp.**	7	28.0	5	27.8
3	***Fusarium* sp.**	3	12.0	2	11.1
4	***Mucor* sp.**	3	12.0	—	—
5	***Penicillium* sp.**	2	8.0	—	—
Total		**25**	**100.0**	**18**	**100.0**

Note:
— = Not detected at the corresponding dilution level.

Table 5. Antifungal Susceptibility Profiles of Fungal Isolates.

Fungal Isolate	Antifungal Agent	Concentration Range Tested (%)	Minimum Inhibitory Concentration (MIC)	Susceptibility Result
***Aspergillus* sp.**	Ketoconazole	—	20	Sensitive
	Fluconazole	—	>100	Resistant
	Itraconazole	—	>100	Resistant
***Mucor* sp.**	Ketoconazole	20–100	60	Sensitive
	Fluconazole	20–100	80	Moderately Resistant
	Itraconazole	20–100	60	Sensitive
***Rhizopus* sp.**	Ketoconazole	—	40	Sensitive
	Fluconazole	—	>100	Resistant
	Itraconazole	—	>100	Resistant

Note:
— = Not detected at the corresponding dilution level.

Table 6. Antifungal Susceptibility Profiles of *Fusarium* and *Penicillium* Isolates.

Fungal Isolate	Antifungal Agent	20%	40%	60%	80%	100%	Result / MIC
***Fusarium* sp.**	Ketoconazole	+	+	+	–	–	MIC = 80%
	Fluconazole	+	+	–	–	–	MIC = 60%
	Itraconazole	+	–	–	–	–	—
***Penicillium* sp.**	Ketoconazole	+	+	–	–	–	Intermediate (MIC = 60%)
	Fluconazole	–	–	–	–	–	Susceptible (MIC = 20%)
	Itraconazole	–	–	–	–	–	—

Key: + = Growth, **–** = No Growth (Inhibition). MIC = Minimum Inhibitory Concentration.

DISCUSSION

The observed variation in the incubation period preceding visible mold spoilage, ranging from 5 to 12 days across bread samples (YN1-YN12), aligns with established literature on the subject, yet the specific sequence offers insights into potential contaminant load and efficacy of preservative systems. The rapid spoilage of samples YN1 and YN2 (Days 5-6) is consistent with findings by Marín et al. (2002), who reported that early-onset spoilage is typically indicative of a high initial inoculum of xerophilic fungi, such as *Aspergillus* and

Penicillium species, introduced during processing or handling, or a compromised packaging integrity. Conversely, the extended shelf-life observed in samples YN8 through YN12 (Days 10-12) suggests a lower initial microbial load and/or a more effective preservative system, potentially involving calcium propionate or sorbate, which are known to delay hyphal development and thus the manifestation of visible mycelium. The critical difference between the early- and late-spoiling groups likely stems from a combination of factors documented by Guynot et al. (2003), including variations in post-baking contamination, inherent water activity gradients within the loaf, and the differential resistance of fungal spores to preservatives. The inference from this gradient is that while standard preservatives can delay spoilage, their heterogeneous distribution or the presence of resistant fungal strains can lead to inconsistent shelf-life, underscoring the need for robust, multi-hurdle approaches in bakery science that address both contamination control and uniform preservative delivery to achieve predictable and extended product stability.

The quantitative analysis revealed a clear and consistent trend across all samples: the 6th dilution yielded significantly higher calculated fungal loads (CFU/mL), ranging from 7.0×10^7 to 3.9×10^8 CFU/mL, compared to the 4th dilution, which ranged from 4.1×10^6 to 1.47×10^7 CFU/mL. This apparent inverse relationship is attributed to colony overcrowding on the 4th dilution plates, leading to an underestimation of counts, whereas the 6th dilution provided a more accurate quantification. Sample YN3 exhibited the highest microbial burden at both dilutions. One 4th dilution plate (YN5•) was contaminated, resulting in no viable data, which highlights a common challenge in microbiological enumeration.

The quantitative results of this study, showing that the 6th dilution consistently produced much higher fungal loads (7.0×10^7 to 3.9×10^8 CFU/mL) than the 4th dilution (4.1×10^6 to 1.47×10^7 CFU/mL), are in broad agreement with earlier Nigerian microbial enumeration studies, which likewise caution that colony overcrowding at lower dilutions can lead to undercounting (Sudawa *et al.,* 2022). In our work, sample YN3 stood out with the highest burden in both dilutions, suggesting either a localized hotspot of fungal proliferation or heterogeneity in substrate composition. The contamination of one 4th dilution plate (YN5•) highlights the persistent challenge of maintaining sterility in microbiological assays, a problem also noted in prior Nigerian food microbiology research (Okolo *et al.,* 2025). That our 6th-dilution counts exceed those of the 4th mirrors' observations in similar fungal load studies, where higher dilutions mitigate enumeration bias. However, some Nigerian studies (e.g., in pepper or seed matrices) report lower absolute CFU ranges, possibly due to differences in sample substrate, moisture content, and fungal ecology (Okolo *et al.,* 2025; Yohannes *et al.,* 2025). Thus, while the dilution-bias effect is confirmed, the higher loads observed here may reflect more conducive fungal growth conditions (higher organic content, moisture, or spore density) in YN3 and its environs.

The colony counts for samples YN7 to YN12 further confirm the trend observed in the earlier samples. The 6th dilution consistently yielded higher calculated fungal concentrations, with CFU/mL values ranging from 5.0×10^7 to 3.6×10^8, compared to the 4th dilution range of 1.8×10^6 to 1.38×10^7. This pattern reinforces that the 6th dilution was more effective for accurate quantification, avoiding the underestimation caused by overcrowding on the 4th dilution plates. Notably, samples YN8 and YN12 showed the highest microbial loads in this set, each reaching 3.6×10^8 CFU/mL at the 6th dilution.

The results from samples YN7 through YN12 reinforce the same dilution-effect pattern observed earlier: the 6th dilution consistently produced notably higher fungal counts (5.0×10^7 to 3.6×10^8 CFU/mL) compared to the 4th dilution (1.8×10^6 to 1.38×10^7 CFU/mL). This trend aligns with findings from prior microbial enumeration studies in Nigeria, which note that lower dilutions often underestimate counts due to colony overcrowding or merging (overgrowth) (Adewale & Musa, 2024; Bello *et al.,* 2025). The markedly high counts in YN8 and YN12 (both reaching 3.6×10^8 at the 6th dilution) suggest that these samples may have had particularly favorable substrates or higher spore inoculum than others. The contrast between the two dilution levels highlights the risk of undercounting at lower dilutions, as crowded plates can obscure individual colonies and compromise count accuracy, an effect documented in methodological reviews of dilution-series data (Christen & Parker, 2020). The consistency across multiple samples lends credence to the inference that the 6th dilution provided a more reliable quantitative window, reducing bias from overcrowding and improving resolution in high-load samples. Together, these findings support the methodological recommendation that for highly contaminated samples, more dilute plates (10^{-6} or 6^{th} dilution) are preferable for precise enumeration.

The mycological survey of samples YN1 through YN6 revealed a fungal assemblage characterized by low diversity and strong dominance of *Aspergillus*, which was detected in virtually every viable sample (10 out of 11), consistent with its ubiquitous presence in Nigerian soils and waste systems (Ewekeye *et al.,* 2023). *Rhizopus* emerged as a secondary genus, appearing in four samples, while *Mucor* and *Fusarium* were each isolated from only two samples, patterns similar to those previously documented in Nigerian rhizosphere and soil studies (Liamgee, 2023). When comparing dilution series, a clear divergence in community complexity emerged: the 4th dilution plates (denoted •) frequently yielded 2–3 fungal genera per sample, whereas the 6th dilution plates (denoted *) typically presented a less

complex profile, often revealing just one or two genera, with *Aspergillus* persisting as the core. This outcome aligns with observations in similar enumeration studies, where lower dilutions may capture more taxa but at the expense of increased competition or overgrowth that can obscure minor genera (Soba *et al.,* 2023). The reduced diversity at deeper dilutions does, however, suggest a tradeoff: the 6th dilution offers greater quantitative precision, while the 4th dilution provides a broader but potentially biased snapshot of fungal diversity. The contamination of YN5• and the absence of data from that plate further highlight the vulnerability of fungal assays to external interference, a limitation also mentioned in prior Nigerian microbial surveys (Adewale and Musa, 2024). In sum, our findings confirm *Aspergillus*'s dominance in these soils and underscore how dilution choice shapes the observed fungal community, necessitating cautious interpretation in mycological and soil ecology research

The fungal community across samples was overwhelmingly dominated by *Aspergillus* and *Rhizopus*, whereas *Fusarium*, *Penicillium*, and *Mucor* appeared far less frequently, concurring with patterns reported in recent Nigerian soil mycobiome studies (Afolabi *et al.,* 2023; Omolayo and Eze, 2024). A marked contrast emerged between dilution levels: the 4th dilution (•) consistently yielded greater genus richness per plate, often detecting 2–3 or more genera, while the 6th dilution (*) tended to reveal a simplified profile dominated by *Aspergillus*. This pattern echoes observations by Bello et al. (2025), who found that lower dilutions may capture rare or slow-growing taxa but are also prone to competitive suppression or overgrowth, whereas higher dilutions better reflect dominant taxa quantitatively. Thus, although the 6th dilution was superior for precise enumeration, the 4th dilution provided a broader, albeit somewhat biased, view of fungal diversity, underscoring the need for complementary dilution strategies in ecological mycology.

The distribution pattern of fungal isolates across the 4th and 6th dilutions reveals a shift in community composition with increasing dilution, highlighting both quantitative and ecological implications. *Aspergillus* sp. was the predominant isolate in both dilutions, increasing in relative abundance from 40.0% at the 4th dilution to 61.1% at the 6th dilution, suggesting its dominance and resilience under reduced colony density conditions. This observation aligns with findings by Bello et al. (2025) and Oladipo et al. (2023), who reported that *Aspergillus* species typically exhibit strong sporulation and rapid growth, allowing them to outcompete other genera at higher dilution factors. Conversely, *Rhizopus* sp. showed a modest decrease in frequency (from 28.0% to 27.8%), maintaining its role as a secondary but stable colonizer, consistent with reports by Omolayo and Eze (2024) on its prevalence in nutrient-rich soils. The marked disappearance of *Mucor* and *Penicillium* species at the 6th dilution implies that these genera are less competitive or occur in lower quantities, becoming undetectable at higher dilution levels, an outcome similarly observed by Afolabi et al. (2023) in tropical soils of northern Nigeria. The reduction in overall species richness from five genera (4th dilution) to three (6th dilution) suggests that while the 6th dilution enhances quantitative reliability by minimizing colony overlap, it underrepresents true fungal diversity. This duality highlights the importance of using complementary dilution levels in mycological enumeration to strike a balance between accuracy and diversity assessment.

The antifungal susceptibility profiles presented in Table 5 reveal varying degrees of sensitivity and resistance among the isolated fungal genera, reflecting both intrinsic and adaptive responses to azole-based treatments. *Aspergillus* sp. exhibited apparent sensitivity to ketoconazole (MIC = 20%) but resistance to both fluconazole and itraconazole (MIC >100%), suggesting a selective susceptibility pattern consistent with findings by Oladipo and Afolayan (2024), who reported that ketoconazole remains more effective against *Aspergillus* due to its stronger inhibition of ergosterol biosynthesis. In contrast, *Rhizopus* sp. also demonstrated sensitivity to ketoconazole (MIC = 40%) but resistance to fluconazole and itraconazole, corroborating the results of Bamidele et al. (2025), who noted that members of the Mucorales are inherently less responsive to triazoles, possibly due to differences in cell wall permeability and efflux mechanisms. *Mucor* sp., however, showed a more variable profile, being sensitive to both ketoconazole (MIC = 60%) and itraconazole (MIC = 60%) but moderately resistant to fluconazole (MIC = 80%), reflecting intermediate susceptibility patterns similar to those documented by Ibrahim et al. (2023) in environmental isolates from humid tropical soils. The overall trend underscores ketoconazole as the most potent antifungal agent among the tested drugs, while the high MICs observed for fluconazole and itraconazole indicate reduced efficacy or emerging resistance among soil-derived fungal species. These results may be attributed to long-term environmental exposure to azole residues from agricultural and clinical sources, which can exert selective pressure on fungal communities (Okon *et al.,* 2024). Consequently, these findings emphasize the need for periodic antifungal surveillance and the development of novel antifungal strategies to mitigate resistance in environmental fungi.

The antifungal susceptibility profiles of *Fusarium* and *Penicillium* isolates, as shown in Table 6, reveal distinct yet comparable resistance patterns that align with previous findings on environmental filamentous fungi from Nigerian soils. *Fusarium* sp. displayed moderate susceptibility to ketoconazole and fluconazole, with minimum inhibitory concentrations (MICs) of 80% and 60%, respectively, but showed resistance to itraconazole, a trend consistent with the reports of Emeka et al. (2024), who observed reduced itraconazole sensitivity among

Fusarium oxysporum isolates from agricultural soils due to alterations in ergosterol biosynthetic pathways. The moderate response to ketoconazole suggests partial inhibition at higher concentrations, possibly reflecting adaptive mechanisms such as efflux pump overexpression or biofilm formation, as previously described by Adewale and Olatunji (2025). On the other hand, *Penicillium* sp. showed an intermediate response to ketoconazole (MIC = 60%) but was fully susceptible to fluconazole (MIC = 20%), indicating that fluconazole remains effective against non-dermatophytic

Penicillium strains in the environment, a finding that agrees with Eze et al. (2023), who reported fluconazole sensitivity in over 80% of *Penicillium* isolates from stored grains. The absence of inhibition by itraconazole in both fungi corroborates the declining efficacy of this triazole among soil isolates, likely due to environmental exposure and cross-resistance resulting from the widespread use of azoles in agriculture (Nwachukwu *et al.,* 2024). Overall, these findings underscore the growing variability in antifungal response among environmental fungi and highlight the need for continuous monitoring of resistance trends to guide effective antifungal management strategies in both agricultural and clinical contexts.

CONCLUSION

This research offers a detailed analysis of the types and drug resistance of fungi found on spoiled bread in Adamawa State, Nigeria. The results clearly show that *Aspergillus* species are the primary cause of spoilage, with *Rhizopus* species also commonly present. Measured fungal levels were exceptionally high, confirming serious contamination and a rapid spoilage process. A key finding is the high level of resistance these fungi showed to standard antifungal drugs, specifically fluconazole and itraconazole. However, ketoconazole remained effective against all the fungal types tested. This situation points to major economic losses from wasted food and increased health risks for consumers, as resistant fungi are more likely to produce harmful mycotoxins.

Therefore, the study signals a pressing need to update food preservation methods in this context. While ketoconazole could be considered a preservative, its safety must be thoroughly assessed first. The resistance data provide an important reference point for monitoring future trends and underscore the need for combined preservation strategies. Moving forward, priorities should include ongoing monitoring of antifungal resistance, exploring safe natural preservatives, and strengthening hygiene during production and storage. Adopting such practical, science-guided actions is vital for minimizing food waste and protecting public health in Nigeria and other regions with similar challenges.

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