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Evaluation of fungal contaminants in stored grains and their pathogenic potentials

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ABSTRACT

Stored cereal grains such as rice, maize, and millet are highly susceptible to fungal contamination, which compromises food quality and poses significant public health risks. This study evaluated the microbiological quality and pathogenic potential of three commonly consumed grains rice, maize, and millet, retailed in three major markets within the Port Harcourt Metropolis (Mile 1, Mile 3, and Rumuokoro). Specifically, it quantified the fungal load and assessed the biofilm-forming capability of the isolates as an indicator of virulence. Nine grain samples were analyzed, and Total Fungal Plate Counts (TFPC) were determined using standard serial dilution and plating techniques. Biofilm formation was assessed using the Brain Heart Infusion (BHI), Congo Red Agar method. Statistically significant differences were observed in fungal loads across both markets and grain types ($p < 0.001$). Mean fungal counts ranged from 1.5×10^5 CFU/g in millet from Mile 1 to 3.6×10^5 CFU/g in rice from Rumuokoro, values that exceeded the acceptable safety limit of 1×10^4 CFU/g. The predominant fungal contaminants were *Rhizopus* spp. and yeasts (26.7% each), followed by *Fusarium*, *Penicillium*, and *Mucor* (10% each), *Aspergillus* and *Cladosporium* (6.7% each), and Alternaria (3%). Zygomycetes demonstrated the highest virulence potential, with 50% of *Rhizopus* and 66.7% of Mucor isolates exhibiting strong biofilm formation. All Alternaria isolates were biofilm-positive. Moderate biofilm production was observed in *Fusarium* (33.3%) and yeast isolates (25%), whereas *Aspergillus*, *Penicillium*, and *Cladosporium* isolates did not produce biofilms. The high fungal burden, coupled with the presence of biofilm-forming isolates, underscores the need for improved post-harvest handling practices, including rapid drying, adequate storage conditions, strict sanitation, and periodic microbial surveillance. These measures are essential to safeguard grain quality and mitigate potential health risks to consumers.

Keywords: Biofilm, fungal contaminants, food safety, pathogenic, public health, stored grains, total fungal plate count.

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INTRODUCTION

Cereal grains are vital to national and global food security, serving as the primary source of calories for billions of people and forming the foundation of animal feed systems. However, they are highly vulnerable to fungal contamination, which represents one of the most significant biological threats to post-harvest grain quality. Fungal genera such as *Aspergillus*, *Fusarium*, and *Penicillium* can infect grains during both pre-harvest and storage phases. These fungi produce highly active secondary metabolites, including mycotoxins, and can form biofilms, features that enhance their pathogenicity and compromise food safety, human health, and animal health. The presence of pathogenic fungi in cereal grains is a major global food-safety concern, particularly in tropical and subtropical regions where climatic conditions favour fungal growth (Pedroso et al., 2023).

In Nigeria, fungal contamination of stored grains is especially severe due to inadequate storage facilities, high relative humidity, fluctuating temperatures, and prolonged storage periods common in smallholder and subsistence agricultural systems. Excessive fungal loads, biofilm formation, and elevated mycotoxin levels have been repeatedly documented across various regions. For example, Ekpakpale et al. (2021) reported *Aspergillus*

flavus and *Aspergillus parasiticus* as predominant contaminants of rice and maize in Ondo State, with aflatoxin concentrations frequently exceeding regulatory limits set by the European Union and Codex Alimentarius. Similarly, Orole and Chongs (2024) demonstrated the widespread occurrence of toxigenic fungi in maize from North-Central Nigeria, attributing contamination to poor storage conditions, the use of unventilated bags, and inadequate drying before storage.

Beyond fungal colonization, Nigerian stored grains are also prone to infestation by bacterial pathogens, insect pests, and chemical contaminants, contributing to a multifaceted post-harvest quality challenge. Of these, fungal infestation remains the most economically and medically significant because mycotoxins persist even after visible mould removal. The socioeconomic implications are considerable: contaminated grains lead to revenue losses for farmers, rejection of export consignments, and increased public health expenditures associated with mycotoxin-related illnesses (Ezekiel et al., 2021).

Biofilms, complex assemblies of microbial cells encased in an extracellular matrix, further enhance fungal survival and virulence (Biswas et al., 2023). Post-harvest fungal contamination arises from an interplay of biological, physical, and socio-economic factors. Environmental variables such as temperature and water activity (aw) strongly influence fungal community composition and determine whether colonization results in biofilm synthesis. Notably, the optimal conditions for fungal growth are not always identical to those for biofilm formation, complicating predictions and control strategies. Poor drying, high grain moisture, mechanical grain damage, and inadequate storage structures (e.g., jute bags and open sheds) create micro-environments conducive to fungal proliferation and biofilm synthesis over prolonged storage periods (Mannaa and Kim, 2017).

Biofilm formation not only amplifies fungal pathogenicity but also contributes to both acute and chronic health risks associated with mycotoxin exposure. At the commodity level, the combined effects of fungal infection and biofilm contamination result in quantitative and qualitative grain losses, reduced market value, restricted trade, and significant financial burdens for producers (Pedroso et al., 2023).

Local assessments and molecular profiling have increasingly revealed the diversity, toxigenic capacity, and biofilm-forming potential of grain-associated mycobiota in Nigeria. Recent studies using morphological and molecular approaches have identified diverse fungal assemblages in stored and retailed cereals and have reported toxin accumulation during storage (Orole and Chongs, 2024; Ekpakpale et al., 2021). The demonstrated genetic potential for biofilm biosynthesis in locally circulating fungal strains is concerning, as even moderately conducive storage conditions can result in substantial biofilm and toxin accumulation over typical storage durations.

Despite the growing body of epidemiological and laboratory evidence, awareness and mitigation practices remain limited, particularly among households and small-scale traders. Surveys across Nigerian states indicate low consumer and producer awareness of mycotoxin and biofilm risks, with studies showing increased toxin and biofilm concentrations after storage compared to harvest levels, highlighting the significant impact of poor post-harvest management (Ezekiel et al., 2021). Preventive interventions such as moisture reduction, improved aeration, hermetic storage, and efficient sorting or decontamination methods can substantially reduce fungal colonization, biofilm formation, and mycotoxin buildup. However, adoption is hindered by cost constraints, knowledge gaps, and inadequate post-harvest infrastructure (Pedroso et al., 2023; Mannaa and Kim, 2017).

Given these challenges, systematic examination of fungal contaminants in stored grains and assessment of their pathogenic potential remains essential. This study integrates classical mycological approaches by quantifying fungal loads in grains and evaluating the biofilm-forming ability of fungal isolates. Such data are crucial for developing targeted mitigation strategies, guiding risk communication to vulnerable populations, and supporting evidence-based policy interventions (Ekpakpale et al., 2021; Orole and Chongs, 2024; Pedroso et al., 2023).

The present research therefore seeks to address critical knowledge gaps through a contemporary, evidence-driven evaluation of fungal contamination and pathogenicity in stored grains sold in major markets within the Port Harcourt Metropolis.

MATERIALS AND METHODS

Study area

Grain samples were purchased from three major markets within the Port Harcourt Metropolis, Rivers State: Mile 1 Market (4°47′24″ N, 6°59′36″ E), Mile 3 Market (4°46′12.796″ N, 6°58′24.4866″ E), and Rumuokoro Market (4.80173° N, 6.99292° E) (Figure 1).

Sample collection

A total of nine (9) grain samples were collected from the three markets (three samples per market). Each sample was placed in a sterile bag, labeled appropriately, and transported to the laboratory within 1–2 hours for microbiological analysis.

Sample preparation

Ten (10) grams of each grain sample were added to 90

Figure 1. Map showing the sample locations (Source: Google Maps: accessed 19th of November, 2025).

mL of 0.9% sodium chloride (NaCl) solution to obtain a 1:10 dilution. From this stock, 1 mL was transferred into a test tube containing 9 mL of 0.9% NaCl to prepare a 10^{-1} dilution. Serial dilutions were then prepared up to 10^{-6} following the method of Meynell and Meynell (1970).

Preparation of Brain Heart Infusion–Congo Red Agar (BHI–CRA)

Brain Heart Infusion–Congo Red Agar (Tmedia, Titan Biotech Ltd.) was prepared by supplementing the medium with 5% sucrose and 0.8 g/L Congo red dye. The mixture was thoroughly dissolved and sterilized by autoclaving at 15 psi (121°C) for 15 minutes. After cooling to approximately 50°C, the Congo red solution was mixed with the sterile BHI agar, dispensed into sterile Petri dishes, and allowed to solidify, following the method of Arciola et al. (2018).

Enumeration of Total Fungal Plate Count (TFPC)

Total fungal counts were determined on Potato Dextrose Agar (PDA) using the spread plate technique. Triplicate plates were inoculated with 0.1 mL of the 10^{-4} dilution. The inoculum was evenly distributed using a sterile hockey stick and allowed to absorb into the agar. Plates were incubated in an inverted position at 37°C for 3–5 days. After incubation, colonies were counted and recorded as described by Cheesbrough (2010).

Isolation and identification of fungi

Fungal isolation was carried out using the spread plate method on PDA supplemented with chloramphenicol (500 mg/L). The medium was prepared by dissolving 39 g of dehydrated PDA in 1000 mL of distilled water, followed by sterilization at 15 psi (121°C) for 15 minutes. After cooling to approximately 50°C, the medium was dispensed into sterile Petri dishes. Plates were inoculated with 0.1 mL of sample dilution, spread evenly, and incubated at room temperature for 5–7 days.

Distinct fungal colonies were selected and sub-cultured repeatedly on fresh PDA to obtain pure isolates. The purified isolates were incubated at room temperature for 3 days before further analysis. Identification of fungal isolates was based on cultural and microscopic characteristics, including colony morphology, pigmentation, conidial structures, and hyphal features, following Cheesbrough (2010).

Microscopic slide preparation and observation

A drop of lactophenol cotton blue stain was placed on a grease-free slide. A sterile loop was used to transfer a small portion of the pure fungal colony onto the stain, and the smear was covered with a coverslip. Slides were examined under ×100 and ×400 magnification, and observed microscopic features were recorded.

Yeast isolates were further confirmed using Gram staining. A thin smear was prepared by emulsifying the colony in distilled water on a slide, air-dried, and stained with crystal violet for 1 minute. The slide was rinsed, treated with Lugol's iodine for 1 minute, decolorized with alcohol for 30 seconds, and counterstained with safranin for 2 minutes. The stained smear was examined using the ×100 oil immersion objective, as described by Cheesbrough (2010).

Biofilm detection

Qualitative biofilm production was assessed using the BHI–Congo Red Agar (CRA) method (Arciola et al., 2018). Fungal isolates were streaked onto the prepared BHI–CRA plates and incubated at room temperature for 24–48 hours. Biofilm-producing isolates appeared as black or brown colonies, while non-biofilm producers formed smooth red or pink colonies, following the description by Harika et al. (2020).

Data analysis

Statistical analysis was conducted using one-way ANOVA, followed by Tukey's HSD post-hoc test, to determine significant differences between market locations. A p-value < 0.05 was considered statistically significant.

RESULTS

Table 1 presents the fungal counts (CFU/g) of the nine grain samples analyzed. All samples recorded fungal loads exceeding 1.0×10^5 CFU/g, surpassing the acceptable safety limit. Significant differences were observed among contamination levels across markets and grain types ($p < 0.001$). Rice from Rumuokoro Market had the highest mean fungal load (3.6×10^5 CFU/g), which was significantly higher than that of rice from Mile 1 Market (1.50×10^5 CFU/g). Mile 1 Market consistently showed the lowest fungal contamination in both rice and maize. Conversely, for millet, Mile 3 Market recorded the highest fungal load (3.53×10^5 CFU/g), significantly exceeding values from Rumuokoro Market (1.80×10^5 CFU/g).

Table 1. Total fungal plate count of grain samples in three major markets in Port Harcourt Metropolis, Rivers State.

Sample code	Rep 1	Rep 2	Rep 3	Mean ± SD	CFU/g	Significant group (p < 0.05)
M1-RC	15	14	16	15.00 ± 1.00	1.5 X 10^5	a
M3-RC	20	20	17	19.00 ± 1.73	1.9 X 10^5	a
RM-RC	34	32	42	36.00 ± 5.29	3.6 X 10^5	b
M1-MZ	18	16	17	17.00 ± 1.00	1.7 X 10^5	a
M3-MZ	34	36	35	35.00 ± 1.00	3.5 X 10^5	b
RM-MZ	32	31	29	30.67 ± 1.53	3.0 X 10^5	b
M1-ML	23	24	27	24.67 ± 2.08	2.4 X 10^5	b
M3-ML	34	35	37	35.33 ± 1.53	3.5 X 10^5	c
RM-ML	20	16	18	18.00 ± 2.00	1.8 X 10^5	a

Key: M1=Mile 1 Market; M3=Mile 3 Market; RM=Rumuokoro Market; -RC=Rice; -MZ=Maize; -ML=Millet.
Different letters across column denotes significant difference at p < 0.05.

Table 2 summarizes the fungal isolates obtained from the grain samples across the three markets. Identification based on cultural and microscopic characteristics revealed the presence of the following fungal genera: *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor*, Rhizopus, *Alternaria*, *Cladosporium*, and Yeasts.

Table 3 details the distribution of fungal species within individual grain samples. *Aspergillus* spp. were isolated from Mile 1 maize and Rumuokoro rice samples. *Fusarium* spp. were found in Mile 3 millet and Rumuokoro maize. *Penicillium* spp. appeared only in Mile 3 maize and millet. *Mucor* spp. occurred in Mile 1 rice and Rumuokoro rice samples. *Rhizopus* spp. were the most widely distributed, occurring in Mile 1 and Mile 3 rice and maize, as well as Rumuokoro maize and millet. *Alternaria* spp. were detected exclusively in Mile 3 millet, while *Cladosporium* spp. were present only in Mile 1 maize and millet. Yeasts were isolated from Mile 1 rice and maize, Mile 3 rice, and Rumuokoro millet.

Table 4 shows the frequency and percentage occurrence of all isolates. *Rhizopus* spp. and Yeasts were the most dominant, each accounting for 8 isolates

Table 2. Macroscopic and microscopic features of fungal species identified from grain samples.

Isolate code	Macroscopic features	Microscopic features	Species identified
A	Yellow-green to olive colonies with a cream reverse. Blue-green colonies, suede-like texture	Conidia arrangement covering the conidioshores.septate hyphae	*Aspergillus* sp.
B	Colonies are woolly to cottony, white to pink, violet, or reddish. Reverse often shows a distinct pigmentation (pink to purple).	Septate hyphae	*Fusarium* sp.
C	Colonies are velvety to powdery, typically blue-green with a white margin; reverse is pale to yellow.	Septate hyphae, hyaline.Branched conidiophores with characteristic brush-like (penicillus) arrangement.	*Penicillium* sp.
D	Fluffy, cotton-like colonies, white becoming grey to brown. Rapid growth, filling plates quickly.	Broad, aseptate hyphae. Sporangiophores branched or unbranched, with spherical sporangia.	*Mucor* sp.
E	Large fluffy white milky colonies which later dark when culture ages	Broad Aseptate hyphal with upright sporangiosphere connected by stolon and rhizoids, dark pear-shaped sporaregium.	*Rhizopus* sp.
F	Colonies are grey to dark brown or black, with a suede-like to woolly texture. Reverse is darkly pigmented.	Septate, pigmented hyphae. Conidia are large, brown, club-shaped with both transverse and longitudinal septa, often in chains.	*Alternaria* sp.
G	Olive-green to black colonies, velvety to powdery. Reverse is black.	Darkly pigmented, septate hyphae. Conidiophores branched, bearing chains of oval to lemon-shaped conidia with distinct scars.	*Cladosporium* sp.
H	Milk colony, round, smooth edge	Retains primary stain, crystal violet	Yeast

Table 3. Distribution of fungal species isolated from the grain samples from three major markets in Port Harcourt Metropolis, Rivers State.

Sampling location	Samples	*Aspergillus* sp.	*Fusarium* sp.	*Penicillium* sp.	*Mucor* sp.	*Rhizopus* sp.	*Alternaria* sp.	*Cladosporium* sp.	Yeast
Mile 1 Market	RC	-	-	-	+	+	-	-	+
	MZ	+	-	-	-	+	-	+	-
	ML	-	-	-	-	-	-	+	+
Mile 3 Market	RC	-	-	-	-	+	-	-	+
	MZ	-	-	+	-	+	-	-	-
	ML	-	+	+	-	-	+	-	-
Rumokoro Market	RC	+	-	-	+	-	-	-	-
	MZ	-	+	-	-	+	-	-	-
	ML	-	-	-	-	+	-	-	+

Key: RC=Rice, MZ=Maize, ML=Millet, - = Absent.

(26.7%). *Fusarium*, *Penicillium*, and *Mucor* each contributed 3 isolates (10%), while *Aspergillus* and *Cladosporium* appeared less frequently (2 isolates; 6.7% each). *Alternaria* spp. were the least represented (1 isolate; 3%).

Table 5 presents the qualitative biofilm assay results on BHI–Congo Red Agar. *Mucor* spp. showed strong biofilm-forming ability, with 2 out of 3 isolates testing positive. *Rhizopus* spp. also displayed substantial biofilm production, with 4 out of 8 isolates positive. The single *Alternaria* isolate (100%) was also biofilm-positive. In contrast, only 1 out of 3 *Fusarium* isolates and 2 out of 8 yeast isolates produced biofilm. *Cladosporium*, *Aspergillus*, and *Penicillium* isolates showed no evidence

Table 4. Frequency and percentage occurrences of fungal isolates from grain samples from the three major markets in Port Harcourt Metropolis.

Isolates	Frequency	% Occurrence
Aspergillus sp.	2	6.7
Fusarium sp.	3	10
Penicillium sp.	3	10
Mucor sp.	3	10
Rhizopus sp.	8	26.7
Alternaria sp.	1	3
Cladosporium sp.	2	6.7
Yeast	8	26.7
Total	**30**	**100**

Table 5. Biofilm results of fungi isolated from grain samples from three major markets in Port Harcourt Metropolis on Congo Red Agar.

No. of isolates	Sample code	Isolates	Biofilm test
1	M1-RC: A	Yeast	+
2	M1-RC: B	*Rhizopus* sp.	-
3	M1-RC: C	Yeast	-
4	M1-RC: D	*Mucor* sp.	+
5	M3-RC: A	*Rhizopus* sp.	-
6	M3-RC: B	Yeast	-
7	M3-RC: C	Yeast	-
8	RM-RC: A	*Mucor* sp.	+
9	RM-RC: B	*Mucor* sp.	-
10	RM-RC: C	*Aspergillus* sp.	-
11	M1-MZ: A	*Aspergillus* sp.	-
12	M1-MZ: B	*Rhizopus* sp.	-
13	M1-MZ: C	*Cladosporium* sp.	-
14	M3-MZ: A	*Rhizopus* sp.	+
15	M3-MZ: B	*Penicillium* sp.	-
16	M3-MZ: C	*Penicillium* sp.	-
17	RM-MZ: A	*Rhizopus* sp.	+
18	RM-MZ: B	*Rhizopus* sp.	-
19	RM-MZ: C	*Fusarium* sp.	-
20	M1-ML: A	Yeast	-
21	M1-ML: B	Yeast	+
22	M1-ML: C	*Cladosporium* sp.	-
23	M3-ML: A	*Alternaria* sp.	+
24	M3-ML: B	*Penicillium* sp.	-
25	M3-ML: C	*Fusarium* sp.	-
26	M3-ML: D	*Fusarium* sp.	+
27	RM-ML: A	*Rhizopus* sp.	+
28	RM-ML: B	*Rhizopus* sp.	+
29	RM-ML: C	Yeast	-
30	RM-ML: D	Yeast	-

Key: M1=Mile 1 Market; M3=Mile 3 Market; RM=Rumuokoro Market; -RC=Rice; -MZ=Maize; -ML=Millet.

of biofilm formation.

Table 6 provides the percentage distribution of biofilm-positive isolates. *Mucor* spp. showed the highest proportion of biofilm formers (66.7%), followed by *Rhizopus* spp. (50%). The single *Alternaria* isolate was biofilm-positive (100%). Biofilm formation was less common in *Fusarium* (33.3%) and yeast isolates (25%). No biofilm formation was observed in *Cladosporium*, *Aspergillus*, and *Penicillium* isolates, suggesting differences in adhesion mechanisms or reduced Congo-red interaction.

Figure 2 illustrates the overall prevalence of biofilm formation among all fungal isolates. A total of 33.30% were biofilm-positive, whereas 66.70% showed no biofilm activity.

Table 7 shows the distribution of biofilm-producing

Table 6. Percentage of biofilm-positive fungal isolates from grain samples.

Fungal species	No. of isolates	Biofilm+ (n)	Biofilm- (n)	% Positive
Alternaria sp.	1	1	0	100%
Mucor sp.	3	2	1	66.7%
Rhizopus sp.	8	4	4	50%
Fusarium sp.	3	1	2	33.3%
Yeast	8	2	6	25%
Aspergillus sp.	2	0	2	0%
Penicillium sp.	3	0	3	0%
Cladosporium sp.	2	0	2	0%

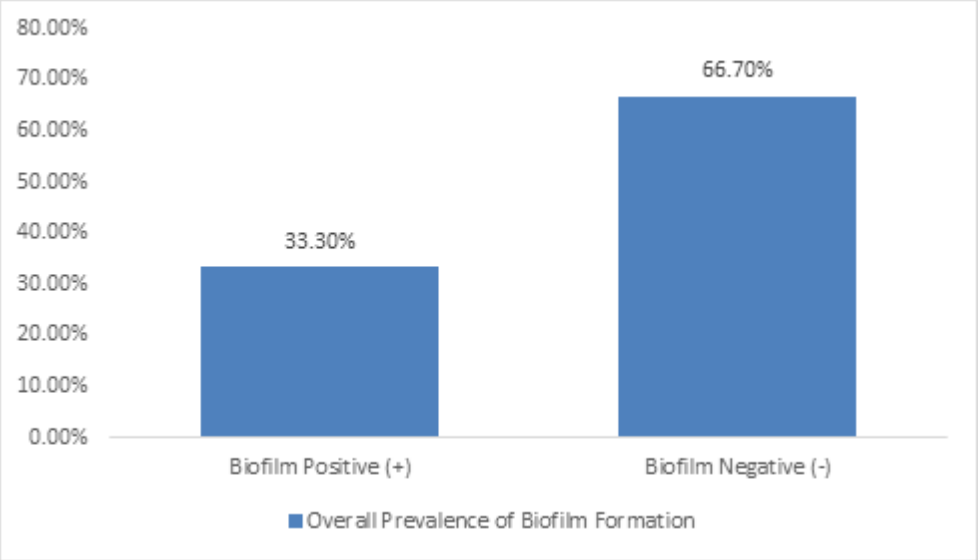

Figure 2. Overall prevalence of biofilm formation.

Table 7. Percentage of biofilm-positive isolates across the three major markets in Port Harcourt Metropolis, Rivers State.

Market	Total isolates	Biofilm+ (n)	Biofilm-(n)	% Positive
Rumuokoro (RM)	10	4	6	40%
Mile 1 (M1)	10	3	7	30%
Mile 3 (M3)	10	3	7	30%

isolates across markets. Rumuokoro Market exhibited the highest proportion of biofilm-producing fungi (40%), whereas Mile 1 and Mile 3 each recorded 30%. Local environmental factors, such as temperature, humidity, storage duration, and sanitation, likely influenced fungal adhesion and biofilm development.

DISCUSSION

The fungal counts obtained from rice, maize, and millet sold in Mile 1, Mile 3, and Rumuokoro markets provide clear evidence of substantial mould contamination, with all values far exceeding the internationally accepted safety limit of 1×10^4 CFU/g for ready-to-eat cereals (ICMSF, 1996). These elevated levels indicate significant postharvest fungal proliferation, driven by humid storage environments, inadequate drying practices, and extensive handling typical of open Nigerian markets. Rice exhibited the widest variability in fungal burden, aligning with the findings of Ekpakpale et al. (2021), who reported similarly high loads in open-market rice from Ondo State. Maize counts were markedly higher than those recorded in Zamfara by Kaura (2025), whereas millet counts were lower than values reported in Ilorin by Banso and Banso (2023). These differences likely reflect variations in marketplace microclimates, grain turnover rates, and retailer storage practices.

The grains harboured a wide diversity of storage fungi, including *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor*, *Rhizopus*, *Alternaria*, *Cladosporium*, and yeasts. *Rhizopus* spp. and yeasts were the most frequently isolated groups (26.7% each), confirming their ecological advantage under the warm, fluctuating humidity characteristic of markets in Rivers State. The lower frequencies of *Fusarium*, *Penicillium*, and *Mucor* (10% each), and the rarer occurrence of *Aspergillus* and *Cladosporium* (6.7% each), as well as *Alternaria*, resemble patterns previously reported, where fast-growing zygomycetes dominate early colonization, followed by slower sporulators such as *Penicillium* and *Alternaria* (Hell et al., 2000; Atanda et al., 2013; Ekpakpale et al., 2021). Market-specific differences in fungal diversity were notable: Mile 1 showed the lowest contamination, likely due to rapid stock turnover; Mile 3 recorded the highest diversity, consistent with prolonged storage and frequent handling; Rumuokoro was dominated by water-tolerant zygomycetes, suggesting intermittent wetting or poor covering during rainfall.

The detection of well-known mycotoxin-producing genera, *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria*, even at modest frequencies is a significant food-safety concern. These fungi are associated with aflatoxins, fumonisins, ochratoxins, and alternariol, all of which are common in West African food systems (Bankole and Adebanjo, 2003). Fungal loads at or above 10^5 CFU/g, as found in this study, substantially increase the likelihood of mycotoxin accumulation, thereby heightening the risk of chronic health outcomes such as hepatocellular carcinoma, immune suppression, and childhood stunting (Keta et al., 2019).

Analysis of virulence traits showed that 33.3% of all isolates were capable of forming biofilms, with the highest prevalence in millet (45.5%), followed by rice (30%) and maize (22.2%). Biofilm formation was most common among zygomycetes, *Mucor* and *Rhizopus*, which produced dense extracellular polysaccharide matrices that enhance adhesion and environmental resilience. This is consistent with patterns reported in food-processing and grain-storage environments (Fanning and Mitchell, 2012). A subset of *Alternaria* and *Fusarium* isolates also formed robust biofilms, demonstrating their ability to produce pigmented extracellular matrices that support survival in humid and nutrient-limited environments. In contrast, *Aspergillus*, *Penicillium*, and *Cladosporium* showed no detectable biofilm formation, a result consistent with their known reliance on invasive hyphal penetration rather than cohesive surface biofilm formation (Srey et al., 2013). Despite their biofilm-negative phenotype, these genera remain significant hazards due to their high mycotoxigenic capacity.

The coexistence of biofilm-forming zygomycetes (e.g., *Rhizopus*) with potent toxin-producing fungi (e.g., *Aspergillus*) in some market environments suggests the possibility of synergistic interactions. Biofilm matrices from *Rhizopus* or *Mucor* may enhance the survival or persistence of other fungi by providing physical protection against desiccation, UV exposure, or cleaning efforts. Such multi-species colonization can amplify food-safety risks, especially in settings characterized by poor hygiene, intermittent dampness, and inadequate storage infrastructure.

Variations in fungal load, composition, and virulence across markets reflect differences in grain handling, packaging, moisture regulation, and storage duration. Mile 1's relatively lower fungal load suggests improved turnover and potentially better handling. Rumuokoro's elevated rice counts point to longer open-air exposure or insufficient drying prior to sale, while Mile 3 reflects the cumulative effects of prolonged storage and repeated handling, leading to greater fungal diversity and more advanced colonization.

CONCLUSION

Stored grains marketed in Rivers State are heavily contaminated with spoilage and potentially pathogenic fungi, with fungal loads consistently exceeding established safety limits. Rice, maize, and millet obtained from the Mile 1, Mile 3, and Rumuokoro markets exhibited widespread colonization by diverse fungal groups, dominated by *Rhizopus* species and yeasts. The high prevalence of biofilm-forming zygomycetes, alongside biofilm-positive *Fusarium* and *Alternaria* isolates, highlights not only the risk of rapid spoilage but also the enhanced resilience of these organisms to routine cleaning, washing, and antifungal treatments. The detection of opportunistic pathogens and fungi capable of producing hazardous mycotoxins further amplifies public-health concerns, particularly in environments where grains are stored under warm, humid, and poorly regulated conditions. Overall, these findings underscore the urgent need for improved postharvest handling, effective drying, hermetic storage, and routine microbial surveillance to safeguard grain quality and protect consumer health in Rivers State.

Recommendations

To mitigate the risks associated with fungal contamination and improve the safety of stored grains, the following measures are recommended:

- Implement regular sanitation protocols with specific focus on biofilm disruption—to reduce the persistence of resilient fungal species on storage surfaces.
- Strengthen moisture-management practices, including thorough drying of grains before storage and the use of moisture barriers during marketing.
- Adopt improved storage technologies, such as hermetic

bags and well-ventilated, covered storage structures, to limit fungal proliferation.

- Enhance hygiene during handling and transportation, particularly in open-market settings where cross-contamination is common.
- Conduct periodic microbial surveillance to monitor fungal loads, identify emerging contaminants, and guide evidence-based interventions.
- Train grain retailers and marketers on best practices for storage, sanitation, and moisture control to reduce contamination risks.

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