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Temporal Dynamics of Fungal Load and Species Diversity in Stored Maize from Sokoto Metropolis, Nigeria (2021–2024)

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Abstract **Original Research**

Maize stored in warehouses is highly susceptible to fungal colonization, with implications for food safety and aflatoxin exposure. This study evaluated the fungal load and species diversity of fungi isolated from stored maize harvested between 2021 and 2024 in Sokoto Metropolis, Nigeria. Fungal load declined from 2022 to 2024, with the highest load recorded in 2022 (5×10^3 cfu) and the lowest in 2023–2024 (3×10^3 cfu each), though this trend was not statistically significant (Spearman's $\rho = -0.866$, $p = 0.333$). A total of 49 fungal isolates were identified, dominated by Aspergillus niger (38.78%), followed by Scopulariopsis candida (14.29%), Aspergillus flavus (12.24%), and Aspergillus oryzae (12.24%). Chi-square analysis revealed no significant association between fungal species distribution and harvest year ($\chi^2 = 13.96$, df = 21, $p = 0.871$). Other isolates included Aspergillus fumigatus, Aspergillus parvisclerotigenus, Aspergillus aflatoxiformans, and Talaromyces islandicus. The predominance of Aspergillus species, particularly aflatoxigenic taxa, underscores the need for improved post-harvest storage practices and routine mycotoxin surveillance in northern Nigeria.

Keywords: stored maize; fungal load; Aspergillus spp.; Sokoto; mycotoxins; food safety.

Introduction

Maize (*Zea mays* L.) is a staple cereal in Nigeria, supporting food security and livelihoods across agroecological zones. However, post-harvest losses due to fungal contamination remain a major challenge, particularly under suboptimal storage conditions common in smallholder warehouses (Atehnkeng *et al.,* 2021; Multi-mycotoxin Contamination in Maize from Niger State, Nigeria, 2025). Fungal colonization not only reduces grain quality but also predisposes maize to mycotoxin contamination, notably aflatoxins produced by *Aspergillus flavus* and related species, which are carcinogenic and immunosuppressive in humans and livestock (Atehnkeng *et al.*, 2021; Bandyopadhyay & Ojiambo, 2022; Ojiambo *et al.*, 2025).

In Nigeria, several studies have documented high fungal diversity in stored maize, with *Aspergillus* species consistently reported as dominant (Profiling and Molecular Identification of Fungi Isolated from

Maize Across Nigeria's Agroecological Zones, 2025; Epidemiology and Genetic Relationship of Toxigenic Fungi in Maize Grains From North Central Nigerian States, 2024; Prevalence and Environmental Determinants of Aflatoxin Contamination in Maize Sold in Open Markets Across Nasarawa State, Nigeria, 2025). Recent surveys across northern Nigeria highlight the Sahel and Sudan savanna zones as hotspots for fungal load and aflatoxin risk, attributed to high temperature, low humidity control, and prolonged storage durations (Profiling and Molecular Identification of Fungi Isolated from Maize Across Nigeria's Agroecological Zones, 2025; Ojiambo *et al.*, 2025). Despite this, temporal data on fungal load and species composition from specific storage hubs such as Sokoto remain limited.

This study characterizes the fungal load and morphological/microscopic diversity of fungi isolated from stored maize harvested between 2021 and 2024 in Sokoto Metropolis. The findings provide baseline data for understanding temporal trends in fungal contamination and inform targeted interventions for mycotoxin risk reduction.

Materials and Methods

Sample Collection

Stored maize samples were collected from warehouses in Sokoto Metropolis, northwestern Nigeria. Samples were grouped according to year of harvest: 2021, 2022, 2023, and 2024. All samples were transported in sterile containers and processed within 24 h of collection.

Fungal Load Determination

Fungal load was determined using the serial dilution and spread plate method on Potato Dextrose Agar (PDA). Plates were incubated at 28 ± 2°C for 5–7 days, and colony-forming units (cfu) were counted and expressed per gram of maize sample.

Fungal Isolation and Identification

Fungi were isolated based on distinct colony morphology on PDA. Pure cultures were obtained by sub-culturing and examined microscopically using lactophenol cotton blue staining. Identification was based on standard morphological and microscopic criteria, including hyphal septation, conidiophore structure, vesicle shape, phialide arrangement, and conidial ornamentation (Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2024; Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2025).

Statistical Analysis

Data were analyzed using descriptive and inferential statistical methods. Fungal load values were expressed as colony-forming units (cfu) per gram of maize sample. Temporal trends in fungal load across harvest years (2021–2024) were assessed using Spearman's rank-order correlation coefficient (ρ), a non-parametric measure suitable for small sample sizes and non-normally distributed data (Field, 2018).

Frequency distributions of fungal isolates across harvest years were summarized as counts and percentages. To test whether the distribution of fungal species differed significantly across years, a chi-square (χ^2) test of independence was performed on the contingency table of isolate counts by year (McHugh, 2013). Expected frequencies were calculated under the null hypothesis of no association between isolate type and harvest year.

All statistical analyses were conducted at a significance level of $\alpha = 0.05$. Due to the exploratory nature of this study and the limited number of replicates per year, statistical power was constrained; therefore, results should be interpreted with caution, particularly where p-values exceed 0.05. Descriptive statistics (mean, standard deviation, median, interquartile range) were computed for isolate frequencies to characterize central tendency and dispersion. Analyses were performed using Python (SciPy library v1.11) (Virtanen *et al.*, 2020).

Results

Fungal Load by Harvest Year

Fungal load varied across harvest years (Table 1). The highest fungal load was recorded in 2022 (5×10^3 cfu), followed by 2023 and 2024 (3×10^3 cfu each). The 2021 sample had the lowest recorded load (7×10^{-3} cfu). Spearman's rank correlation indicated a negative but non-significant trend in fungal load from 2022 to 2024 ($\rho = -0.866$, $p = 0.333$), suggesting a possible decline in contamination over time, though this trend did not reach statistical significance with the current sample size.

Table 1. Fungal load of maize samples according to year of harvest

Year of Harvest	Fungal Load (cfu)
2021	7×10^{-3}
2022	5×10^3
2023	3×10^3
2024	3×10^3

Morphological and Microscopic Characteristics

Eight fungal taxa were identified based on colony and microscopic features (Table 2). *Aspergillus niger* produced black, granular colonies with rough black conidia on long smooth conidiophores. *Aspergillus flavus* formed yellow-green to olive granular colonies with rough spherical conidia. *Aspergillus fumigatus* was distinguished by blue-green velvety colonies and flask-shaped vesicles with uniseriate phialides. *Aspergillus oryzae*, *A. parvisclerotigenus*, and *A. aflatoxiformans* showed variations in colony color, sclerotia production, and phialide arrangement. *Scopulariopsis candida* produced white to cream powdery colonies with annellides and truncate conidia, while *Talaromyces islandicus* exhibited yellow-green to reddish colonies with red pigment diffusion and penicillus-type conidiophores.

Table 2. Morphological and microscopic characteristics of fungi isolated from stored maize

S/No	Isolates	Colony Morphology on PDA	Microscopic Features
1	*Aspergillus niger*	Black, granular colonies; pale reverse	Septate hyphae; long smooth conidiophores; globose vesicles; rough black conidia
2	*Aspergillus flavus*	Yellow-green to olive, granular colonies; yellowish reverse	Septate hyphae; rough conidiophores; globose vesicles; rough spherical conidia
3	*Aspergillus fumigatus*	Blue-green to gray-green, velvety colonies; pale reverse	Septate hyphae; short smooth conidiophores; flask-shaped vesicles; uniseriate phialides;

S/No	Isolates	Colony Morphology on PDA	Microscopic Features
			smooth conidia
4	*Aspergillus oryzae*	White to yellow-green, floccose colonies; pale yellow reverse	Septate hyphae; rough conidiophores; globose vesicles; biseriate phialides; smooth to finely rough conidia
5	*Aspergillus parvisclerotigenus*	Yellow-green granular colonies; small sclerotia present	Septate hyphae; rough conidiophores; globose vesicles; biseriate phialides; rough conidia
6	*Aspergillus aflatoxiformans*	Yellow-green to dark green colonies; abundant sclerotia	Septate hyphae; rough conidiophores; globose vesicles; biseriate phialides; rough spherical conidia
7	*Scopulariopsis candida*	White to cream, powdery colonies; pale reverse	Septate hyphae; annellides; chains of rough, truncate conidia
8	*Talaromyces islandicus*	Yellow-green to reddish colonies; red pigment diffusion	Septate hyphae; penicillus-type conidiophores with metulae and phialides; smooth ellipsoidal conidia

Frequency of Occurrence

A total of 49 isolates were obtained across the four harvest years (Table 3). *Aspergillus niger* was the most frequent isolate (19 isolates; 38.78%), followed by *Scopulariopsis candida* (7 isolates; 14.29%), *Aspergillus flavus* (6 isolates; 12.24%), and *Aspergillus oryzae* (6 isolates; 12.24%). *Aspergillus fumigatus* accounted for 5 isolates (10.20%), while *A. parvisclerotigenus*, *A. aflatoxiformans*, and *T. islandicus* were less frequent (3, 2, and 1 isolates respectively).

The chi-square test of independence revealed no significant association between fungal species distribution and harvest year ($\chi^2 = 13.96$, df = 21, p = 0.871), indicating that the relative proportions of isolates remained stable across the study period despite the decline in total isolate count from 21 in 2021 to 7 in 2024. The grand total of isolates declined progressively across years, with 2021 contributing 42.86% of all isolates, followed by 2022 (22.45%), 2023 (20.41%), and 2024 (14.29%).

Descriptive statistics for isolate frequencies showed a mean of 6.13 ± 5.62 isolates (median = 5.5, IQR = 2.75–6.25). *Aspergillus niger* emerged as the dominant species, accounting for nearly 39% of all isolates, followed by *S. candida* (14.29%) and *A. flavus* (12.24%).

Table 3. Frequency of occurrence of fungi isolated from stored maize samples obtained from Sokoto Metropolis between 2021–2024

Isolates / Harvest Year	2021	2022	2023	2024	Total
Aspergillus niger	8 (42%)	3 (16%)	5 (26%)	3 (16%)	19 (100%)
Aspergillus flavus	1 (17%)	2 (33%)	2 (33%)	1 (17%)	6 (100%)
Aspergillus fumigatus	2 (40%)	1 (20%)	1 (20%)	1 (20%)	5 (100%)
Aspergillus oryzae	1 (16.6%)	3 (50%)	1 (16.6%)	1 (16.6%)	6 (100%)
Scopulariopsis candida	3 (43%)	2 (29%)	1 (14%)	1 (14%)	7 (100%)
Talaromyces islandicus	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
A. parvisclerotigenus	3 (100%)	0 (0%)	0 (0%)	0 (0%)	3 (100%)
A. aflatoxiformans	2 (100%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
Grand Total	**21 (42.86)**	**11 (22.45)**	**10 (20.41)**	**7 (14.29)**	**49 (100)**

Discussion

The fungal load pattern observed in this study peaking in 2022 and declining thereafter may reflect variations in pre-harvest infection pressure, drying practices, or warehouse microclimates across years. Similar temporal fluctuations in fungal load have been reported in other Nigerian maize surveys, where environmental conditions and storage duration strongly influence colonization levels (Profiling and Molecular Identification of Fungi Isolated from Maize Across Nigeria's Agroecological Zones, 2025; Ojiambo *et al.*, 2021). The non-significant downward trend from 2022 to 2024 ($\rho = -0.866$, $p = 0.333$) warrants further investigation with larger, replicated datasets to determine whether this pattern reflects genuine improvements in post-harvest management or random variation.

The dominance of *Aspergillus niger* (38.78%) aligns with recent findings from Maiduguri and other northern Nigerian markets, where *A. niger* consistently emerges as the most frequent isolate from stored maize (Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2024; Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2025). Although *A. niger* is less frequently associated with aflatoxin production than *A. flavus*, it contributes to grain spoilage and can produce other mycotoxins such as ochratoxin A (Atehnkeng *et al.*, 2021; Multi-mycotoxin Contamination in Maize from Niger State, Nigeria, 2025). The high frequency of *A. flavus* (12.24%) is of particular concern given its role as the primary producer of aflatoxin B_1, a potent hepatocarcinogen linked to liver cancer in high-exposure populations (Bandyopadhyay & Ojiambo, 2022; Ojiambo *et al.*, 2025; Prevalence and Environmental Determinants of Aflatoxin Contamination in Maize Sold in Open Markets Across Nasarawa State, Nigeria, 2025).

The presence of *A. parvisclerotigenus* and *A. aflatoxiformans*, both recognized aflatoxigenic species, further elevates the mycotoxin risk profile of the stored maize (Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2024; Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2025). These species are morphologically similar to *A. flavus* but are increasingly identified through molecular methods in Nigerian maize (Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2024). Their detection in this study, even at lower frequencies, underscores the need for molecular confirmation in future work to accurately assess aflatoxin risk.

The lack of significant association between fungal species distribution and harvest year ($\chi^2 = 13.96$, $p = 0.871$) suggests that the mycobiota of stored maize in Sokoto remains relatively stable over time, despite fluctuations in total fungal load (Profiling and Molecular Identification of Fungi Isolated from Maize Across Nigeria's Agroecological Zones, 2025; Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2024). This stability may reflect consistent environmental conditions and storage practices across the study period, though the limited sample size reduces statistical power to detect subtle temporal shifts.

Scopulariopsis candida and *Talaromyces islandicus*, though less commonly reported, have been documented in Nigerian stored maize and rice, where they contribute to spoilage and potential mycotoxin co-contamination (Laut *et al.,* 2023; Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2024; Studies on Aflatoxin-Producing Fungi in Stored Maize (Zea mays L.) from Maiduguri, Nigeria, 2025). The isolation of *T. islandicus* exclusively in 2021 samples suggests possible year-specific contamination events or differences in storage conditions.

Overall, the fungal diversity and load patterns observed here mirror broader trends in northern Nigeria, where Sahel and Sudan savanna zones exhibit elevated fungal and mycotoxin risks due to climatic and storage factors (Profiling and Molecular Identification of Fungi Isolated from Maize Across Nigeria's Agroecological Zones, 2025; Ojiambo *etal.,* 2025; Multi-mycotoxin Contamination in Maize from Niger State, Nigeria, 2025). The declining isolate count from 2021 to 2024 may indicate improved storage practices or reduced initial contamination, but continued surveillance is essential to confirm this trend.

Conclusion

This study reveals a high diversity of fungi in stored maize from Sokoto Metropolis, with *Aspergillus* species dominating the mycobiota. The presence of aflatoxigenic taxa, particularly *A. flavus*, *A. parvisclerotigenus*, and *A. aflatoxiformans*, highlights the potential for aflatoxin contamination in stored maize. The temporal decline in fungal load and isolate frequency from 2021 to 2024 warrants further investigation to determine whether this reflects improved post-harvest handling or environmental variability. Routine mycotoxin monitoring, coupled with improved drying and storage technologies, is recommended to mitigate food safety risks in northern Nigeria.

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