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Microbiological Analysis of Stored Groundnuts in Yenagoa, Bayelsa State, with Emphasis on Aflatoxin-Producing *Aspergillus* Species

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Abstract

Groundnuts (*Arachis hypogaea*), an important dietary protein source and economic crop in Nigeria, are highly susceptible to fungal contamination during storage, particularly by *Aspergillus* species that produce aflatoxins. Aflatoxin B_1, the most potent of these toxins, is a Group 1 carcinogen associated with liver cancer, immune suppression, and growth retardation. Despite the widespread consumption of groundnuts in Yenagoa, Bayelsa State, limited data exist on microbial and aflatoxigenic contamination in the local supply chain. This study assessed the microbiological quality of stored groundnuts with emphasis on aflatoxin-producing *Aspergillus* species across three major markets—Swali, Opolo, and Tombia. Thirty samples (10 per market) were analyzed using serial dilution, culturing on Potato Dextrose Agar (PDA) and *Aspergillus flavus* and *parasiticus* agar (AFPA), and microscopic identification with lactophenol cotton blue staining. Aflatoxin production was screened qualitatively using the Ammonia Vapor Test, and moisture content was determined by the oven-dry method. Results showed that 80% of samples were contaminated with *Aspergillus* spp., predominantly *A. flavus* (50%) and *A.*

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parasiticus (23.3%). Aflatoxin detection was 60% overall, highest in Tombia (70%), followed by Swali (60%) and Opolo (50%). Tombia samples also recorded the highest mean moisture content (11.1%), exceeding the safe threshold (<8%) for fungal inhibition. Additional fungi, including *Penicillium*, *Fusarium*, and *Rhizopus* spp., were also isolated, indicating poor storage hygiene. These findings underscore a serious food safety concern in Yenagoa, where high aflatoxigenic fungal prevalence, driven by elevated moisture and inadequate storage, poses significant health risks. The study recommends routine surveillance, vendor education, and affordable moisture-proof storage interventions to reduce aflatoxin exposure and protect public health in Bayelsa and similar tropical contexts.

Keywords: Aflatoxins; *Aspergillus flavus*; *Aspergillus parasiticus*; Moisture Content; Fungal Contamination; Yenagoa Markets

INTRODUCTION

Groundnuts (*Arachis hypogaea*), commonly known as peanuts, are an essential staple in many Nigerian households, providing a rich source of protein, healthy fats, vitamins, and minerals (Carvalho et al., 2025; Chen et al., 2025; Pirotta et al., 2025; Saleh et al., 2025). In Bayelsa State, particularly in Yenagoa, groundnuts are widely consumed roasted, boiled, processed into snacks like kulikuli, or used in soups and sauces. They also serve as a means of livelihood for local farmers, traders, and street vendors. However, despite their nutritional and economic importance, groundnuts are highly vulnerable to microbial contamination during storage, especially by fungal species of the genus *Aspergillus*, some of which produce aflatoxins—among the most potent naturally occurring toxins known to science (Ezekiel et al., 2020; Sserumaga et al., 2021; Zhang et al., 2025).

Aflatoxins, particularly aflatoxin B1, are toxic secondary metabolites primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Bata-Vidács et al., 2025; Pal et al., 2025). These fungi thrive in warm, humid environments, making tropical regions like the Niger Delta highly conducive to their growth. When groundnuts are not properly dried or are stored in poorly ventilated, moisture-prone conditions—common challenges in rural and urban markets in Yenagoa—they become susceptible to fungal invasion and subsequent aflatoxin contamination. The consumption of aflatoxin-contaminated food poses serious public health risks, including acute liver toxicity, immunosuppression, growth retardation in children, and increased risk of hepatocellular carcinoma (Mahmoud et al., 2025; Pal et al., 2025). The International Agency for Research on Cancer (IARC) classifies aflatoxin B1 as a Group 1 carcinogen, indicating its established link to cancer in humans (Zuo et al., 2025).

Despite the widespread consumption and commercial importance of groundnuts in Yenagoa, there is limited local data on the microbiological safety of stored groundnuts sold in the area. Many vendors store groundnuts in conditions that promote fungal growth, such as in plastic containers, jute bags, or on bare floors without moisture control (Okparavero et al., 2024; Swathi et al., 2023). There is also a general lack of awareness among sellers and consumers regarding the risk of aflatoxin contamination. Moreover, food safety enforcement in informal markets remains weak, and regular screening for aflatoxigenic fungi is rarely carried out. This situation raises concerns about chronic dietary exposure to aflatoxins among residents, especially given the limited diagnostic infrastructure for detecting related health conditions. The absence of localized studies makes it difficult to quantify the extent of contamination and develop region-specific interventions. Therefore, there is an urgent need to assess the prevalence of Aspergillus species, particularly those capable of producing aflatoxins, in stored groundnuts available in Yenagoa markets and households.

Although advanced techniques like polymerase chain reaction (PCR) and high-performance liquid chromatography (HPLC) are used globally to detect aflatoxins and their fungal producers, these methods are often unavailable in many Nigerian undergraduate laboratories due to cost and resource limitations. Therefore, this study employs conventional microbiological techniques—such as serial dilution, culture on selective media, and microscopic identification—to isolate and identify fungi, with special emphasis on *Aspergillus* species from stored groundnut samples collected within Yenagoa metropolis.

The aim of this research is to assess the microbiological quality of stored groundnuts in selected locations across Yenagoa and determine the presence and frequency of aflatoxin-producing *Aspergillus* species. The findings are expected to contribute baseline data for future monitoring programs, raise public awareness on proper food storage practices, and encourage food safety interventions by local health authorities. This study underscores the importance of integrating microbiological surveillance into public health and food security efforts in Bayelsa State and Nigeria at large.

MATERIALS AND METHODS

Study Area

This study was conducted in Yenagoa, the capital city of Bayelsa State, located in the Niger Delta region of southern Nigeria. Yenagoa experiences a humid tropical climate with high rainfall and warm temperatures, which favour fungal proliferation and increase the risk of mycotoxin contamination in stored food commodities (Nagbi et al., 2021). Groundnut samples were collected from selected markets and vendors across different parts of the city, including Swali, Opolo, and Tombia markets.

Study Design

This was a laboratory-based cross-sectional study aimed at isolating, identifying, and characterizing fungal contaminants, with particular attention to aflatoxigenic *Aspergillus* species, in stored groundnuts sold in Yenagoa.

Sample Collection

A total of 30 groundnut samples were randomly collected from various storage sources including open-market vendors and retail shops. Approximately 250g of each sample was aseptically collected using sterile polythene bags, labelled with date and location, and transported to the Microbiology Laboratory for immediate processing.

Sample Preparation

Each groundnut sample was sorted to remove visibly damaged or mouldy seeds. For microbial analysis, 25g of each sample was weighed and homogenized in 225 mL of sterile distilled water using a blender. The homogenate was then subjected to serial dilution up to 10^{-5} using sterile normal saline (0.85% NaCl).

Fungal Isolation

Fungi were isolated using the pour plate and spread plate techniques on two types of media namely Potato Dextrose Agar (PDA) (Suleiman1 et al., 2024), a general-purpose fungal medium supplemented with chloramphenicol (0.05g/L) to inhibit bacterial growth and *Aspergillus Flavus* and *Parasiticus* Agar (AFPA), a selective medium specifically designed to enhance the growth and detection of aflatoxigenic *Aspergillus* species (Tesfaye et al., 2024). Plates were incubated at 30°C for 7 days. Colonies with characteristic *Aspergillus* morphology were sub-cultured to obtain pure isolates for identification.

Macroscopic and Microscopic Identification

Fungal isolates were examined macroscopically for colony morphology, pigmentation, texture, and reverse coloration. Microscopic examination was done using Lactophenol Cotton Blue (LPCB) staining. A small portion of each fungal colony was mounted on a clean slide, stained, and observed under a light microscope at x40 magnification. Identification was based on standard taxonomic keys (Peterson, 2003; Samson et al., 2014), with a focus on identifying *Aspergillus flavus*, *A. parasiticus*, and other common storage fungi (*Penicillium, Fusarium, Rhizopus*).

Screening for Aflatoxin Production (Qualitative)

Presumptive *Aspergillus* isolates were screened for aflatoxin production using the Ammonia Vapor Test (Musangi et al., 2024). Pure cultures of *Aspergillus* spp. were grown on Coconut Agar Medium (CAM) for 5 days at 28°C. Plates were inverted and exposed to ammonia vapours by placing a few drops of concentrated ammonia solution (NH_4OH) on the inner lid. A colour change to pink, red, or orange indicates potential aflatoxin production, as per modified protocols from the International Mycological Association (IMA) (Rajarajan et al., 2021). This qualitative method serves as a rapid and cost-effective screening tool suitable for undergraduate labs.

Moisture Content Determination

Since moisture is a key factor influencing fungal growth, the moisture content of each sample was determined using the oven-dry method (Dabhi et al., n.d.; Musangi et al., 2024). About 5g of groundnut was weighed into a pre-weighed crucible and placed in a hot air oven at 105°C for 24 hours. The final dry weight was recorded, and moisture content (%) was calculated as:

$$\text{Moisture Content (\%)} = \left(\frac{\text{Initial Weight - Dry Weight}}{\text{Initial Weight}}\right) \times 100$$

Data Analysis

All experiments were conducted in triplicate. Descriptive statistics such as frequency, percentage, and mean values were used to analyse fungal occurrence and moisture content. Results were presented using tables. Microsoft Excel in Microsoft 365 was used for descriptive statistics.

Ethical Considerations

Although the study involved non-human samples, verbal consent was obtained from groundnut vendors before sample collection.

RESULTS

The average pH and moisture content of the groundnut samples varied slightly among the three sampling locations. As shown in Table 1, all moisture values exceeded the safe threshold for storage that is typically <8% (Kimario et al., 2024), indicating a high risk for fungal growth and aflatoxin development. Fungal isolates were identified based on colony morphology and microscopic features. Table 2 summarizes the diagnostic features of the predominant fungal isolates recovered from the groundnut samples. The distribution of fungal species in the groundnut samples across Swali, Opolo, and Tombia is presented in Table 3. *Aspergillus flavus* had the highest occurrence, particularly in Tombia samples. Aflatoxigenic potential was confirmed in 60% of the samples. Tombia had the highest number of aflatoxin-positive samples, consistent with its higher moisture content and fungal burden.

Table 1: Mean pH and Moisture Content of Groundnut Samples by Location

Sample Location	Mean pH	Mean Moisture Content (%)
Swali	6.2	9.8
Opolo	6.5	10.2
Tombia	6.3	11.1
Overall Mean	6.3	10.4

Table 2: Cultural and Morphological Characteristics of Fungal Isolates

Fungal Isolate	Colony colour	Texture	Reverse colouration	Microscopic Feature
Aspergillus flavus	Yellow-green	Powdery	Golden-yellow	Rough conidiophores, radiate heads
Aspergillus parasiticus	Dark green	Granular	Brown	Globose vesicles, phialides on metulae
Penicillium spp.	Bluish-green	Velvety	Pale-yellow	Branched conidiophores, brush-like appearance
Fusarium spp	Pinkish-white	Cottony	Red	Sickle-shaped macroconidia

Fungal Isolate	Colony colour	Texture	Reverse colouration	Microscopic Feature
Rhizopus spp	Grayish-black	Fluffy	None	Sporangiophores with dark round sporangia

Table 3: Occurrence (%) of Fungal Isolates in Groundnut Samples by Location (n = 10 per site)

Fungal Isolate	Swali (%)	Opolo (%)	Tombia (%)	Overall mean occurrence (%)
Aspergillus flavus	50	40	60	50.0
Aspergillus parasiticus	20	30	20	23.3
Penicillium spp.	30	20	40	30.0
Fusarium spp.	10	10	20	13.3
Rhizopus spp.	10	10	10	10.0

Table 4: Aflatoxin Detection by Ammonia Vapor Test

Sample location	Total samples	Aflatoxin-Positive Samples	Aflatoxin Detection Rate (%)
Swali	10	6	60
Opolo	10	5	50
Tombia	10	7	70
Total/Mean	30	18	60

DISCUSSION

This study revealed a significant level of fungal contamination in stored groundnuts sold in Yenagoa, Bayelsa State, with an emphasis on aflatoxigenic *Aspergillus* species. The findings corroborate concerns raised in prior studies (Ezekiel et al., 2020; Sserumaga et al., 2021; Pal et al., 2025) regarding the microbial safety of food products in tropical, resource-limited environments. Multiple fungal species were isolated, with *Aspergillus flavus* emerging as the most prevalent, followed by *A. parasiticus, Penicillium, Fusarium*, and *Rhizopus* species.

Environmental and Storage-Related Risk Factors

The climatic and market conditions in Yenagoa — marked by high humidity, rainfall, and warm temperatures — create an ideal environment for fungal proliferation (Nagbi et al., 2021). The study recorded mean moisture contents above the critical 8%

safety threshold across all three sampling sites (Swali, Opolo, Tombia), with Tombia showing the highest (11.1%). Elevated moisture is a primary predisposing factor for mycotoxin contamination (Kimario et al., 2024). Poor market storage practices, such as keeping groundnuts in polythene bags, open sacks, or directly on the floor without ventilation or desiccants, further exacerbate this risk, as noted in similar Nigerian contexts (Okparavero et al., 2024).

Fungal Diversity and Morphology

Cultural and microscopic identification revealed typical characteristics of known storage fungi. *A. flavus* colonies presented as yellow-green with powdery texture and golden-yellow reverse coloration, while *A. parasiticus* showed darker green granular colonies — features that align with taxonomic descriptions by Samson et al. (2014). The presence of other genera like *Penicillium* and *Fusarium* is noteworthy due to their ability to spoil food and produce other mycotoxins, such as ochratoxins and fumonisins (Swathi et al., 2023). These organisms not only threaten food safety but also affect market value and export potential of groundnut products.

Aflatoxigenic Potential and Public Health Implications

The Ammonia Vapor Test detected aflatoxin production in 60% of the samples, with Tombia showing the highest detection rate (70%). This finding is consistent with the fungal profile of the area and its higher recorded moisture content. The presence of aflatoxigenic strains of *A. flavus* and *A. parasiticus* is especially concerning given that aflatoxin B_1, their primary metabolite, is classified as a Group 1 carcinogen by the IARC (Zuo et al., 2025).

Chronic dietary exposure to aflatoxins has been linked to hepatocellular carcinoma, immune suppression, and stunted growth in children (Mahmoud et al., 2025). In a region with limited access to health screening and diagnostics, such contamination poses a latent but serious threat to public health.

Local Comparisons and Context

The findings of this study are in line with earlier works conducted in Nigeria and other sub-Saharan African countries. Ezekiel et al. (2020) reported similar prevalence rates of aflatoxigenic fungi in groundnut and maize samples across southwestern Nigeria. Pal et al. (2025) also emphasized the lack of aflatoxin surveillance and poor enforcement of food safety standards in informal markets, which mirrors the situation in Yenagoa. While high-end detection techniques like PCR and HPLC are used globally (Bata-Vidács et al., 2025), this study shows that effective baseline screening can still be conducted using cost-effective conventional methods such as the Ammonia Vapor Test and basic microscopy.

Socioeconomic and Policy Dimensions

The implications of these findings stretch beyond health concerns. Groundnuts are an economic staple in Bayelsa, serving as a source of income for farmers and traders. Repeated fungal contamination could lead to economic losses, reduced consumer trust, and diminished marketability. Moreover, the current regulatory environment is insufficient, with poor market inspections, lack of aflatoxin awareness, and no routine fungal screening at the point of sale. According to Pal et al. (2025), addressing food safety challenges in such contexts requires a multi-sectoral approach involving government, academia, and civil society.

CONCLUSION

This study has demonstrated a high prevalence of fungal contamination in stored groundnuts in Yenagoa, with *A. flavus* and *A. parasiticus* being the dominant species responsible for aflatoxin production. The high moisture content of samples and informal storage practices contributed to fungal proliferation. The detection of aflatoxigenic strains in 60% of samples underscores the significant public health risks posed by routine consumption of contaminated groundnuts. The results call for urgent attention to food storage practices, market hygiene, and aflatoxin awareness among stakeholders in the groundnut value chain.

Recommendations

- Community outreach should focus on sensitizing vendors and consumers about aflatoxins, proper storage, and moisture control.
- Introduction of affordable and moisture-proof storage containers at local markets will help reduce fungal contamination.
- Local government health departments should implement regular microbial surveillance using cost-effective methods.

- Food safety authorities should intensify market inspections and enforce minimum hygiene standards in food vending.
- Equipping undergraduate and community laboratories with basic media and test kits for aflatoxin detection can promote early intervention.
- Longitudinal studies on aflatoxin levels and health outcomes should be encouraged, along with dialogue with policymakers to strengthen food safety regulations.

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