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***Research Article***

# Molecular Identification and Distribution of Fungal Species Associated with Stored Pearl Millet (*Pennisetum glaucum (L.) R. Br.*) in Ogbomoso, Oyo State, Nigeria

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**ABSTRACT**
Pearl millet (*Pennisetum glaucum* L. R. Br.), a member of the Poaceae family, is the sixth most important cereal crop globally and a major staple in northern Nigeria due to its high nutritional values and gluten-free properties. However, fungal contamination during storage poses serious economic and public health concerns, particularly due to the production of mycotoxins. This study investigated the occurrence and diversity of fungal species associated with stored pearl millet seeds collected from five major markets in Ogbomoso, Oyo State, Nigeria. Pearl millet seeds (480g) were collected in each market, surface-sterilized, moistened with sterile distilled water, and stored for seven days to induce spoilage. Seeds were plated on Potato Dextrose agar amended with streptomycin and incubated for three days at 25±2°C. The emerging fungi were sub-cultured to obtain pure isolates and identified using morphological characteristics and molecular analysis targeting the Internal Transcribed Spacer (ITS) region. Sequence analysis through BLAST confirmed five fungal species: *Aspergillus tamarii, Fusarium arcuatisporum, Penicillium griseofulvum, Curvularia lunata,* and *Pseudopestalotiopsis theae*. *A. tamarii* had the highest frequency of occurrence (29.73%), while *F. arcuatisporum* had the lowest frequency (13.51%). Idi-Abebe market recorded the highest fungal distribution (40.54%), likely due to poor storage infrastructure. The predominance of mycotoxigenic species underscores the need for improved post-harvest handling, routine mycological surveillance, mycotoxin risk assessment, and storage hygiene to enhance the safety and shelf life of stored pearl millet within local value chains.

**Keywords:** Molecular analysis; Morphological characteristics; Mycotoxins; Pearl millet; *Pseudopestalotiopsis theae*

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## INTRODUCTION

Pearl millet (*Pennisetum glaucum* L. R. Br.), a member of the family poaceae, is the sixth important cereal crop after maize, wheat, rice, barley, and sorghum (Satyavathi *et al*., 2021). It is known by different names across regions and cultures, reflecting its broad geographical importance: bajra in Hindi, bulrush or spiked millet in English, "mil chandelles" in Arabic, and ''dukhnin'' in French (Kumar *et al*., 2012). In Nigeria, pearl millet is a major staple crop, particularly in the Northern regions, where it is commonly called ''gero'', "maiwa'', or "dauroin'' in Hausa language (Jika *et al*., 2017; Adegbola *et al*., 2023). The origin of pearl millet can be traced to the western region, specifically the present-day Mauritania, from where it spread to other part of the world (Zarnkow, 2014). Today, it is extensively cultivated on 30-million-ha area in the arid and semi-arid tropical regions of Asia and Africa (Satyavathi *et al*., 2021).

Pearl millet is highly versatile and can be processed through milling, decortication, germination,

fermentation, cooking and extrusion to produce products such as flours, biscuits, snacks, pasta and non-dairy probiotic beverages (Dias-Martins *et al*., 2018). It is also deeply embedded in traditional diet, being used to prepare foods including couscous, flatbreads, porridges, and various local drinks. In Northern Nigeria, pearl millet serves as a key ingredient in popular foods and drinks like ''kunu'' ''zaki'', ''ogi'', "pito'', and ''masa'' (Ukwuru *et al*., 2018; Triki *et al*., 2022). Nutritionally, pearl millet is a valuable source of vitamins A and B, calcium, iron, zinc. It provides essential minerals such as potassium, phosphorus, magnesium, copper, and manganese, and has also been reported to contain phenolic acids, flavonoids, and tannins, which contribute positively to human health (Hassan *et al*., 2021). Furthermore, as a gluten-free grain, pearl millet is a suitable and safe dietary alternative for people with celiac disease or gluten intolerance (Meena *et al*., 2021).

Despite the nutritional and health benefits derived from pearl millet grains, they are highly prone to fungal contamination during storage, particularly by the fungi in the genera of *Aspergillus*, *Penicillium*, *Fusarium*, and some xerophytic species that compromise the quality and safety of the grains and produce harmful mycotoxins that pose serious food safety risks (Thierry *et al*., 2025). Mycotoxins such as aflatoxins, ochratoxins, and fuminosins are carcinogenic, hepatotoxic, nephrotoxic and possess immunosuppressive properties (Olahan *et al*., 2024). In Nigeria, pearl millet grains are commonly stored and traded in open markets where grains are often exposed to high humidity, fluctuating temperature, and poor storage hygiene, conditions that favour fungal colonization and proliferation. However, there is limited information on the identity and distribution of fungi associated with stored pearl millet seeds in Nigeria. The previous studies available rely on morphological identification which often result in misidentification of fungal isolates. Therefore, this study employed both morphological characteristics and molecular analysis targeting the Internal Transcribed Spacer (ITS) region of the DNA to identify fungal species associated with stored pearl millet seeds collected from five major markets in Ogbomoso, Oyo State, Nigeria.

## MATERIALS AND METHODS

### Collection of Pearl Millet Seeds

Pearl millet seeds were collected from five major markets known for sale of agricultural produce in three local governments within Ogbomoso, and the global position coordinates of each of the markets was recorded using the GPS coordinates application, version 6.25. Iresa Pupa Market (Surulere LGA) (Latitude 8°5'51"N, Longitude 4°23'43" E), Odo-oba Market (Ogbomoso South LGA) (Latitude 8° 3'13"N, Longitude 4° 8'54"E) and Sabo (Latitude 8° 9'0" N, Longitude 4° 14'18"E), Idi-abebe (Latitude 8°8'43"N, Longitude 4° 14'0" E) and Tapa Markets (Latitude 8° 2'41" N, Longitude 4° 8'55" E) (Ogbomoso North LGA). In each of the markets, three vendors selling pearl millet were randomly selected. From each vendor, 160 g of pearl millets were purchased and measured into a sterile polythene bag. Three bags from a market, each containing 160g of pearl millet were then mixed in an appropriately labeled sterile bag to form 480 g per market. The samples were analysed at Biology Laboratory, Department of Plant Biology, University of Ilorin.

### Preparation of Potato Dextrose Agar Culture Medium

Potato Dextrose Agar (PDA) was used for the isolation of fungi and was prepared according to the manufacturer's instruction. Thirty-nine grams of PDA powder were dissolved in one liter of distilled water. The mixture was gently agitated to ensure full dissolution of the powder and sterilized in an autoclave at 121°C and 15 P.S.I for 15 minutes. After cooling to 47°C, it was amended with streptomycin BP to prevent bacterial growth as described by Ajadi and Olahan (2023).

### Isolation of Fungi from Pearl millets Seeds

Isolation of fungi from pear millet seeds was carried out using the direct plating methods described by Popoola *et al*. (2024) with a slight modification. One gram of each sample (in triplicate) was weighed using a sensitive balance and surface-sterilized in 1% sodium hypochlorite solution for one minute. Subsequently, they were rinsed three times with sterilized distilled water and placed on blotting filter papers to dry. The dried samples were later moistened with sterilized distilled water and stored in an air tight container for seven days to induce spoilage and instigate fungal growth. After seven days, the dried samples were individually plated on disposable Petri-dishes containing 15 ml of freshly prepared PDA amended with streptomycin and then

incubated at 25 ± 2ºC for three days. By the third day, mixed cultures observed on each plate were sub-cultured using sterilized inoculating needles on PDA culture medium amended with streptomycin in disposable Petri-dishes. The sub-culturing procedures were repeated until pure fungal isolates were obtained.

**Morphological Identification of Fungi from Pearl millets Seeds**

Macroscopic and microscopic characteristics were tentatively used for the identification of the fungal isolates. Macroscopic identification of the pure isolates was based on visual observation of the mycelia, including an assessment of colony colour, texture, and the appearance of the reverse sides of the culture plates.

Microscopic identification was carried out following the procedure of Osamwonyi and Wakil (2012). A pin head size of mycelia from 72 hours old culture was placed onto a drop of lactophenol cotton blue on a clean grease-free slide. The mycelia were properly teased apart using sterile inoculating needle and covered with a cover slip. The slide was then observed under 40× objective lens using a photographic microscope (Amscope) attached to a light microscope. These observations focused on evaluating the thickness and morphology of the hyphal wall, the color of the hyphae, the presence or absence of septa, and the presence or absence of spores in accordance with the guidelines provided by Fawole and Oso (2007). The identity of each fungal isolate was confirmed by comparing observed characteristics with standard identification manuals of Navi *et al*. (1999) and Kidd *et al*. (2016; 2023).

**Molecular Characterization of Fungi from Pearl Millets Seeds**

The Genomic DNA of the fungal isolates from the spoilt pearl millet grains were extracted following the protocol of Quick-DNA™ Fungal/Bacterial MiniPrepKit (Zymo Research Group, California, USA). The quantity and concentration of the extracted DNA was measured using Nano-Drop 2000c spectrophotometer (Thermo Fisher Scientific Inc. Wilmington, Delaware, USA) and the extracted DNA was sent to Inqaba Biotec, Ibadan, Nigeria, for amplification and sequencing in both directions. The primers used to amplify the nuclear ribosomal DNA (rDNA) fragments of the isolates were the Internal Transcribed Spacer 1 (ITS1): 5′ (TCCGTAGGTGAACCTGCGG) 3′ and ITS4: 5′ (TCCTCCGCTTATTGATATGC) 3′. Following PCR amplification and purification, the DNA fragments were sequenced in both forward and reverse directions to ensure accuracy. After sequencing the DNA, Seqtrace software (Seqtrace-win-0.9.0) was used to align and merge the forward and reverse sequences into a single consensus sequence, following the method describe by Stucky (2012). The consensus sequences were then compared with reference sequences available in the National Center for Biotechnology Information (NCBI) database using the BLASTN tool for species identification. Phylogenetic tree was subsequently constructed by aligning the query sequences with reference sequences retrieved from NCBI, using Molecular Evolutionary Genetic Analysis (MEGA version 11. 0) and applying the maximum likelihood method as described by Tamura *et al*. (2021).

**Determination of Frequency of Occurrence of the Pure Fungal Isolates**

The frequency of occurrence of each of the fungal isolates and distribution of the fungal species across the pearl millet grains on the basis of the markets from which they were collected was calculated according to the formula proposed by Ilondus (2011), i.e.

$$\text{Frequency of occurrence} = \frac{Number\ of\ \text{times a fungus was encountered}}{\text{Total fungal isolated}} \times 100$$

## RESULTS AND DISCUSSION

Five fungal species belonging to five genera were isolated from stored pearl millet, and they were labeled as Isolates A to E, which were morphologically identified tentatively as *Pseudopestalotiopsis theae, Penicillium griseofulvum, Curvularia lunata, Fusarium arcuatisporum* and *Aspergillus tamarii* based on their observed macroscopic and microscopic features.

The fungal species isolated from stored pearl millet were accurately identified in this study. In lieu of relying on traditional morphological methods, which often depend on spore and mycelia characteristics and are prone to misidentification, molecular techniques provide more reliable results by enabling precise DNA sequence comparisons (Bhunjun *et al*., 2021).

The phylogenetic tree based on the maximum likelihood (ML) method resolved into four major

clades, corresponding to the genera *Curvularia*, *Aspergillus*, *Penicillium*, and *Fusarium*, with all sequences clustering within these well-supported taxonomic groups (Fig.1).

Phylogenetic analysis of the ITS sequences revealed that the fungal isolates isolated clustered into four well-supported clades corresponding to the genera *Curvularia*, *Aspergillus*, *Penicillium*, and *Fusarium*. Isolate ABB PW clustered strongly with *Curvularia lunata*, supported by high bootstrap values (≥96), indicating a robust evolutionary relationship. Similarly, isolate ABB GW clustered within the *Aspergillus tamarii* / *A. flavus* complex supported by bootstrap value >81%. Isolate ABB BW and ABB W both clustered within the *Fusarium incarnatum–equiseti* species complex (FIESC) with bootstrap values of 65–99%.

*Aspergillus tamarii* had the frequency of occurrence (29.73%), followed by *Penicillium griseofulvum* (24.32%), and followed by *Pseudopestalotiopsis theae* (16.22%) *and Curvularia lunata* (16.22%), while *Fusarium arcuatisporum* (13.51%) had the least (Table 3).

Table 4 showed the frequency of occurrence of the fungal isolates across the markets from where the pearl millet seeds were collected. The highest frequency of occurrence of the fungal isolates was observed in the samples collected from Idi-Abebe market (40.54%), followed by the samples from Odo-Oba market (24.32%), followed by Iresa-Pupa market (16.22%), Tapa market (13.51), while the samples collected from Sabo Market had the lowest frequency of occurrence of fungal isolates (5.41%).

**Table 1: Morphological identification of Fungi associated with Pearl millet**

| Fungal Isolates | A | B | C | D | E |
|---|---|---|---|---|---|
| **Colony colour** | Pink-white circular colony | Blue-green to gray-green surface with a slightly wrinkled texture | Dark to ash-like colour | White cottony-like colony. | Greenish to brown colonies surrounded by white mycelium |
| **Hypha type** | Septate | Septate | Septate | Septate | Septate |
| **Spore type** | Conidia | Conidia | Conidia | Macroconidia | Conidia |
| **Tentative Identity** | *Pseudopestalotiopsis theae* | *Penicillium griseofulvum* | *Curvularia lunata* | *Fusarium arcuatisporum* | *Aspergillus tamarii* |

**Table 2: Species Identified Through Blast Searches of the DNA Sequences**

| Isolates | Organisms (Accession numbers) | Query Cover (%) | Percentage Identity (%) |
|---|---|---|---|
| **A** | *Pseudopestalotiopsis theae* (MH472583.1) | 99 | 99.64% |
| **B** | *Penicillium griseofulvum* (PQ176809.1) | 100 | 99.61% |
| **C** | *Curvularia lunata* (KY806118.1) | 100 | 99.47% |
| **D** | *Fusarium arcuatisporum* (MZ363845.1) | 100 | 99.10% |
| **E** | *Aspergillus tamarii* (MN128231.1) | 100 | 98.35% |

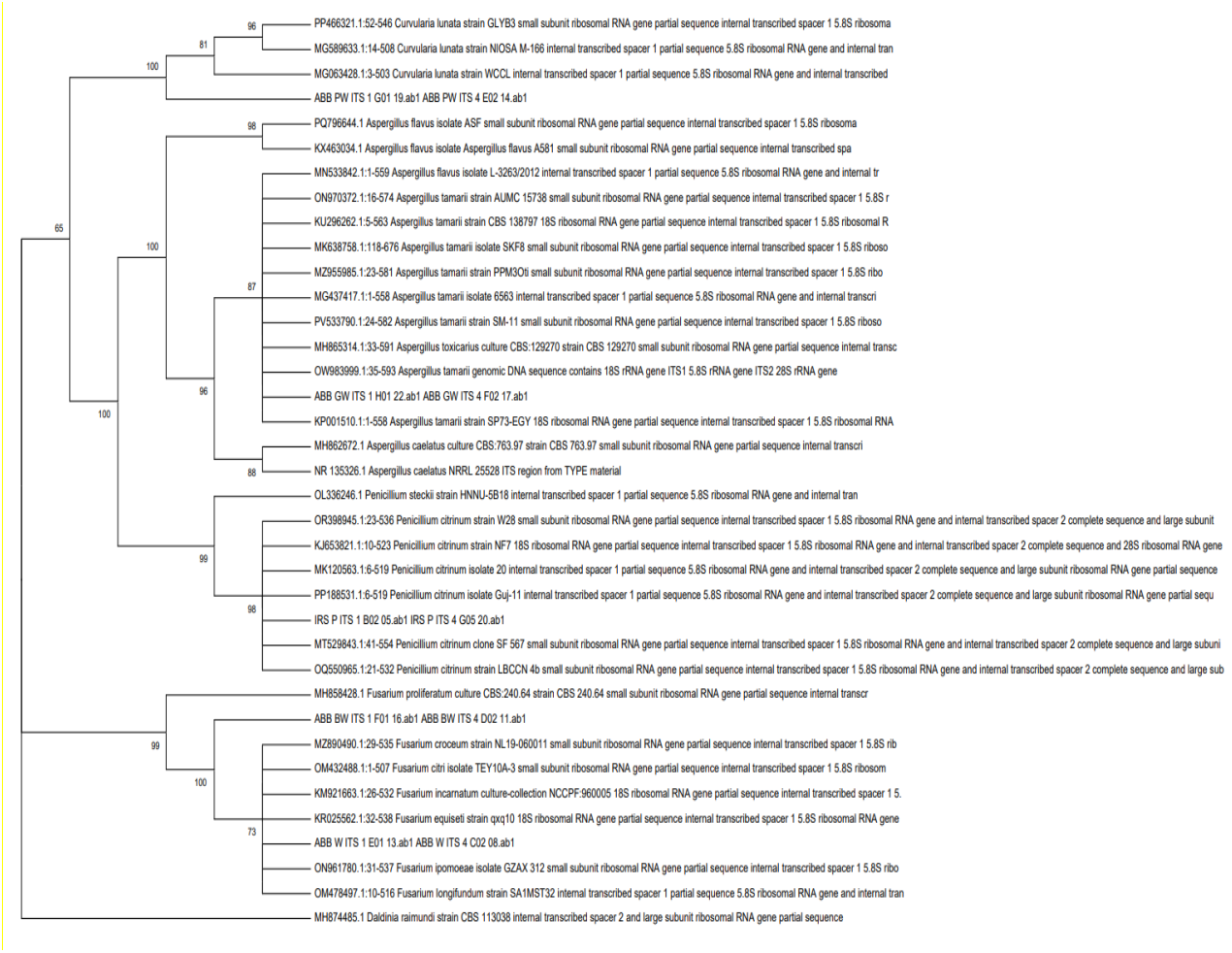
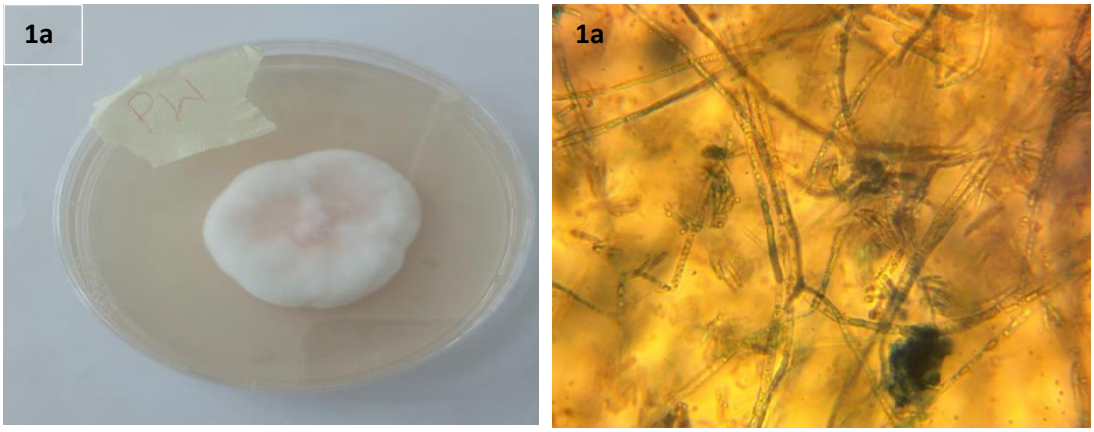



**Fig.1. Phylogenetic analysis of fungi isolated from stored pearl millets by maximum likelihood method**



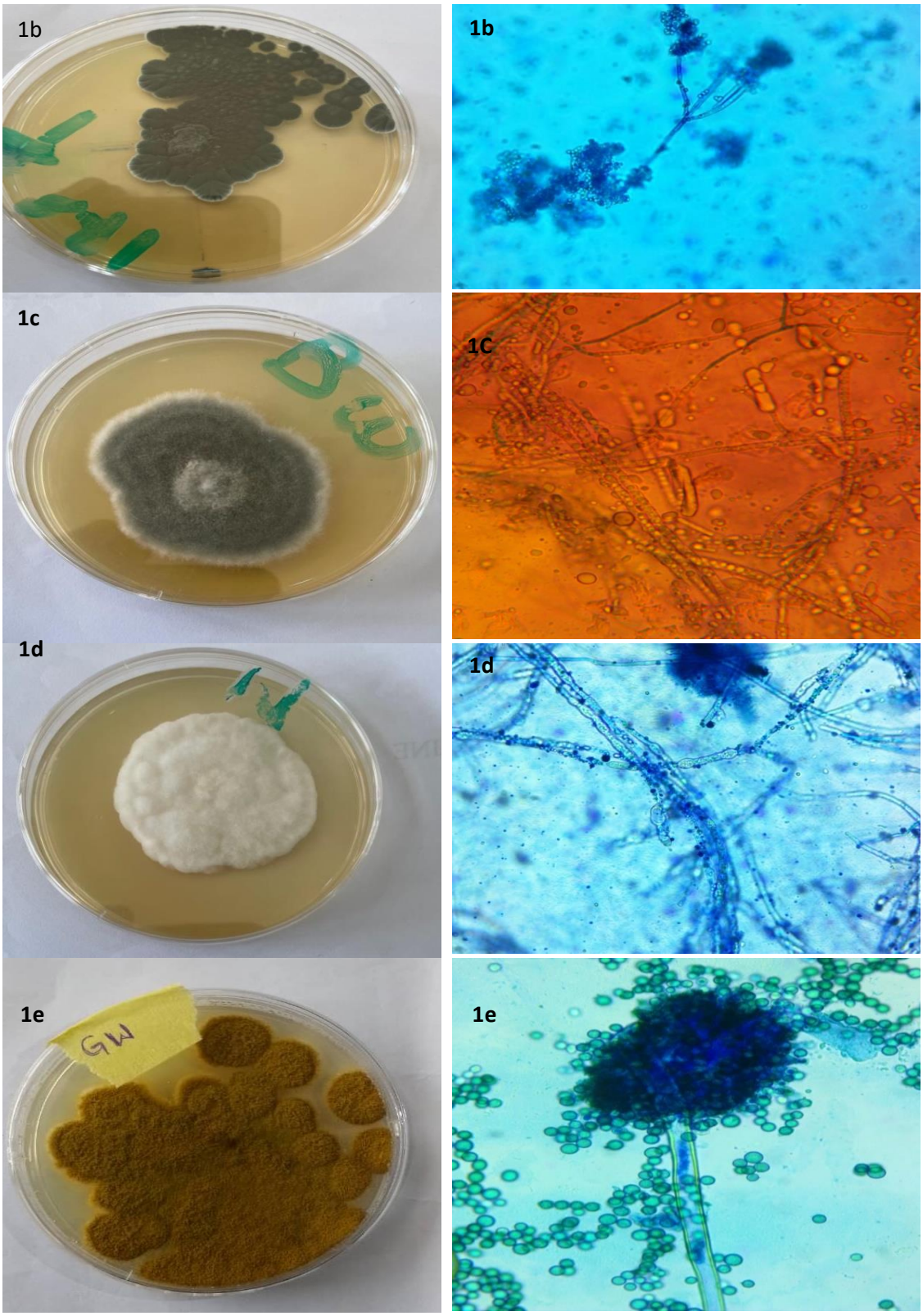



**Plate 1a–1e: Shows the macroscopic and microscopic views of pure fungal cultures isolated from stored pearl millet seeds grown on PDA plates**

Plates 1a is identified as *Pseudopestalotiopsis theae*; 1b as *Penicillium griseofulvum*; 1c as *Curvularia lunata*; 1d as *Fusarium arcuatisporum*; 1e as *Aspergillus tamarii*

**Table 3: Occurrences of Fungal Isolates on pearl millet seeds from ogbomoso**

| Fungal Isolates | Number of times encountered | Frequency (%) |
|---|---|---|
| *Pseudopestalotiopsis theae* | 06 | 16.22 |
| *Penicillium griseofulvum* | 09 | 24.32 |
| *Curvularia lunata* | 06 | 16.22 |
| *Fusarium arcuatisporum* | 05 | 13.51 |
| *Aspergillus tamarii* | 11 | 29.73 |
| **TOTAL** | **37** | **100** |

**Table 4: Frequency of Occurrence of the Fungal Isolates and Total Fungi across the Markets**

| **Location** | ***P. theae*** | ***P. griseofulvum*** | ***C. lunata*** | ***F. arcuatisporum*** | ***A. tamarii*** | **TFC** | **F (%)** |
|---|---|---|---|---|---|---|---|
| Iresapupa | 0 | 5 | 0 | 0 | 1 | 06 | 16.22 |
| Odo-Oba | 0 | 0 | 1 | 0 | 8 | 09 | 24.32 |
| Sabo | 0 | 1 | 0 | 0 | 1 | 02 | 5.41 |
| Idi-Abebe | 5 | 1 | 4 | 5 | 0 | 15 | 40.54 |
| Tapa | 1 | 2 | 1 | 0 | 1 | 05 | 13.51 |
| **TOTAL** | **06** | **09** | **06** | **05** | **11** | **37** | **100** |

**Keys**: TFC= Total Fungi Count, F (%) = Frequency of occurrence

In this study, the fungal species isolated were *Pseudopestalotiopsis theae*, *Penicillium griseofulvum*, *Curvularia lunata*, *Fusarium arcuatisporum*, and *Aspergillus tamarii* from pearl millet seeds collected from five markets in Ogbomoso. Molecularly, all the identifications showed a query cover of no less than 99% and a percentage identity of at least 98%, confirming the accuracy of species identification. As noted by Raja *et al*. (2017), Hofstetter *et al*. (2019), and Ajadi and Olahan (2024), reliable molecular identification requires a minimum of 80% query cover and 97% percentage identity when comparing sequences of isolated fungi with those in the NCBI database, using the BLASTN tool.

The fungal species isolated in this study have also been reported in various places in Nigeria and abroad. Hussain *et al*. (2009) isolated *Aspergillus sp., Penicillium spp., Curvularia lunata*, *Fusarium* spp, *Bipolaris* spp*., Helminthosporium* spp, *Drechslera* spp *Alternaria alternate,* and *Rhizopus* spp from seeds of different varieties of pearl millet in Pakistan. Naqvi *et al*. (2013) reported the presence of *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus species* among fungi isolated from farmer-saved pearl millet seeds. Bamkefa *et al*. (2020) also reported *Aspergillus* spp*, Aspergillus fumigatus, Curvularia* spp, *Fusarium* spp, and *Rhizopus stolonifer* as mycoflora associated with millet (*Pennisetum glaucum* L.) purchased from major markets in Ibadan metropolis, Nigeria. Kumari *et al.* (2022) isolated *Aspergillus* spp *Curvularia lunata Penicillum* spp*, Fusarium* spp*, Mucor* spp*, Rhizopus* spp*, Pyricularia* spp*, Alternaria* spp, *and Helminthosporum* spp from pearl millet seeds in their study on the incidence and detection of seedborne mycoflora of pearl millet and their deteriorative effects on plant health. Similarly, Kator *et al*. (2025) conducted a study in Makurdi, Benue State, on pearl millet and reported the isolation of *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer*, as well as *Fusarium* species from the seeds. These fungi are probably the vehicles responsible for the transmission of pathogenic foodborne diseases (Aladhadh, 2023). These findings were similar except for *Pseudopestalotiopsis theae* which was identified in this study. The isolation of *Pseudopestalotiopsis theae,* a producer of pesthetoxin, in this study is unique because it has not been frequently reported in millet. However, this fungus has been reported to cause tea gray blight disease (Kimura *et al*., 1998) and leafspot on Aleo vera in Bangladesh (Ahmmed *et al*., 2022). Its occurrence in millet points to either opportunistic colonization under favourable conditions or an expanded ecological adaptability beyond what has been previously documented.

The phylogenetic analysis revealed that the fungal isolates associated belong to the genera *Curvularia***,** *Fusarium***,** *Aspergillus***,** and *Penicillium*; all of which contain species historically documented as important contaminating fungi of the pearl millet **(***Pennisetum glaucum***)**. The strong clustering of one isolate with *Curvularia lunata* indicate that *Curvularia* species are among the most destructive pathogens of pearl millet, responsible for leaf spot, grain discoloration, and

foliar blight as reported by Little *et al*. (2024). C. lunata have been reported for its ability to infect millet leaves under humid conditions (Kumar *et al*., 2013). The identification of this isolate therefore confirms it as a potential pearl millet pathogen and aligns with earlier taxonomic studies on *Curvularia*-associated cereals (Manamgoda *et al*., 2014).

The isolation of a *Fusarium incarnatum-equiseti* species complex indicates the possibility of cross-kingdom interactions affecting pearl millet. Fusarium incarnatum-equiseti species complex (FIESC) have been repeatedly associated with grain mold, stalk rot, and seedling blight in pearl millet and other small cereals (Leslie and Summerell, 2006; Chastukhina *et al*., 2025). These fungi often act as opportunistic pathogens, exploiting wounds, senescing tissues, or abiotic stress conditions for their infection. Their detection in the sample may suggest potential production of mycotoxins such as trichothecenes and zearalenone that compromise grain quality (Ramdial *et al*., 2017).

Similarly, the isolation of another species in the *Aspergillus flavus–A. tamarii* species group suggest infection during post-harvest handling and storage (Raghavender *et al*., 2007). Same as the isolate identified as *Penicillium citrinum* which aligns with reports that *Penicillium* species colonize stored millet grain and can produce compounds such as citrinin, which reduce grain quality and present food safety concerns (Houbraken *et al*., 2014).

The highest frequency of occurrence of *Aspergillus tamarii* in our study aligns with the general findings of Aspergillus dominance, as reported by Bamkefa *et al*. (2020) who also found *Aspergillus fumigatus* as the most frequently isolated fungal species associated with pearl millet seeds (*Pennisetum glaucum* L.) purchased from major markets in Ibadan Metropolis, Nigeria. The dominance of *Aspergillus tamarii* is of serious concern because the fungus secretes cyclopiazonic acid (CPA), a mycotoxin, that is noxious to man and animals (Homa *et al*., 2019). The greater frequency of *Aspergillus tamarii* compared to other fungi in this study may be linked to its bioecology as a soil-borne pathogen. According to Nji *et al*. (2023), *Aspergillus* species have been isolated from a wide range of environments, with soil remaining their primary reservoir. This suggests that soil and plant debris serve as viable sources of inoculum, infecting grains in the field and subsequently contaminating harvested pearl millet seeds during storage.

The variation in the frequency of fungi species observed across the sampled markets can be attributed largely to differences in post-harvest handlings and the poor storage facilities of grains sold in the markets. Factors such as high humidity, poor ventilation, and prolong storage promote fungal growth in pearl millet, while handling during transportation and sale can further introduce contaminants. This agrees with Baidhe *et al*. (2024), who noted that inappropriate storage infrastructures and handling protocols pose food safety and health-related risks.

## CONCLUSION

The isolation of *Pseudopestalotiopsis theae*, *Penicillium griseofulvum*, *Curvularia lunata*, *Fusarium arcuatisporum*, and *Aspergillus tamarii* from pearl millet seeds obtained from five markets in ogbomoso, Oyo State, Nigeria underscores the fact that fungi can contaminate and spoil agricultural produce during storage. The dominance of *A. tamarii,* a toxigenic fungus that secretes a mycotoxin called cyclopiazonic acid (CPA), further highlight mycotoxins risks associated with stored millet.

Improved post-harvest handling practices and proper storage conditions should be prioritized to limit fungal contamination. Regular mycological surveillance and mycotoxins risk assessment are necessary to monitor grain safety. Maintaining good storage hygiene and promoting best practices along the local value chain will help to enhance the safety and shelf-life of stored pearl millet.

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