Plant Pathology & Quarantine 16(1): 186–200 (2026) ISSN 2229-2217
www.ppqjournal.org

Article
Doi 10.5943/ppq/16/1/17

Diversity, Molecular Identification, and Food Safety Implications of Storage Fungi Associated with Common Bean (*Phaseolus vulgaris* L.) in Western Cameroon

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Akwa TE, Maingi JM, Birgen JK 2026 – Diversity, Molecular Identification, and Food Safety Implications of Storage Fungi Associated with Common Bean (*Phaseolus vulgaris* L.) in Western Cameroon. Plant Pathology & Quarantine 16(1), 186–200, Doi 10.5943/ppq/16/1/17

Abstract

Postharvest fungal contamination remains a major constraint to the storage stability, seed quality, food safety, and economic value of common bean (*Phaseolus vulgaris* L.) in sub-Saharan Africa. Despite the importance of common bean as a staple food crop in Cameroon, information regarding the molecular diversity of storage-associated fungi remains limited. This study employed an integrative morphoculture and internal transcribed spacer (ITS)-based molecular approach to characterize fungal species associated with stored common bean cultivars collected from Menoua Division, Western Cameroon. Fungal isolates were recovered from six commonly cultivated bean types using potato dextrose agar (PDA) and initially grouped based on colony morphology and microscopic characteristics. Representative isolates were subjected to ITS rDNA amplification, sequencing, and phylogenetic analysis. Sequence comparisons against GenBank identified four fungal species with high confidence: *Aspergillus flavus* (99.78%), *Penicillium aethiopicum* (99.59%), *Fusarium oxysporum* (99.51%), and *Xylaria hypoxylon* (98.78%). Fungal diversity analysis revealed moderate to high species diversity among bean cultivars, with Shannon diversity indices ranging from 1.16 to 1.34 and Simpson diversity indices from 0.63 to 0.74. Phylogenetic reconstruction confirmed clear taxonomic separation among isolates and validated species-level identification, including resolution of non-sporulating morphotypes. The predominance of storage-adapted and potentially mycotoxigenic fungi indicates significant deficiencies in prevailing storage systems and highlights potential risks to food safety and seed viability. This study provides the first molecular baseline of storage fungi associated with common bean in Cameroon and underscores the need for improved storage practices, routine fungal surveillance, and integrated postharvest management strategies to minimize losses and reduce mycotoxin-related risks.

Keywords – Food safety – Mycotoxigenic fungi – *Phaseolus vulgaris* – phylogenetic analysis – post-harvest contamination

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes globally and a cornerstone of food and nutritional security in sub-Saharan Africa. In Cameroon, it is widely

Submitted 01 September 2026, Accepted 19 October 2026, Published 29 October 2026
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cultivated across diverse agro-ecological zones due to its adaptability, short growth cycle, and high nutritional value (Akwa et al. 2020). Beyond its role as a dietary staple rich in protein and micronutrients, common bean contributes significantly to rural livelihoods and local economies (Beebe et al. 2012, Kamtchoum et al. 2018).

Despite its importance, the sustainability of common bean production is undermined by substantial post-harvest losses, particularly during storage. Post-harvest handling including drying, transportation, and storage represents a critical control point where both quantitative and qualitative deterioration can occur. In many parts of Cameroon, beans are stored in traditional structures such as granaries and sacks under poorly regulated environmental conditions, which favour microbial colonisation (Akwa et al. 2026). Stored food commodities are highly susceptible to fungal contamination, posing serious threats to food quality and safety (Kumar & Kalita 2017, Njobeh et al. 2009).

Fungal infestation during storage is a major driver of grain deterioration, resulting in reduced seed viability, nutritional losses, and economic damage (Birgen et al. 2020). Of greater concern is the ability of many storage fungi to produce mycotoxins secondary metabolites that are highly toxic to humans and animals. Genera such as *Aspergillus*, *Fusarium*, and *Penicillium* are among the most common contaminants of stored grains and are responsible for the production of aflatoxins, fumonisins, and other harmful compounds (Omotayo et al. 2019, Pitt & Hocking 2009). For instance, *Aspergillus flavus* produces aflatoxins, potent carcinogens strongly associated with liver cancer and food safety crises in developing regions (Omotayo et al. 2019). Similarly, *Fusarium* species produce fumonisins, which have been linked to oesophageal cancer and disruption of sphingolipid metabolism (Kumar & Kalita 2017).

Recent studies have shown that climate variability, particularly increases in temperature and humidity, is exacerbating the prevalence and distribution of mycotoxigenic fungi in stored agricultural commodities, especially in tropical environments (Magan et al. 2020, Jiménez Medina et al. 2021). In Cameroon, emerging evidence highlights the widespread occurrence of toxigenic fungi in staple crops, raising growing concerns about food safety and public health (Njobeh et al. 2009, Ngoko et al. 2008).

Accurate identification of storage-associated fungi is therefore essential for understanding contamination pathways and developing effective mitigation strategies. Conventional morphological identification methods, although widely used, are often limited by phenotypic plasticity and the inability to resolve non-sporulating isolates. In contrast, molecular approaches particularly sequencing of the internal transcribed spacer (ITS) region have significantly improved the accuracy and reliability of fungal identification and are now considered the standard for fungal taxonomy and phylogenetic studies (Schoch et al. 2012, Nilsson et al. 2019). Integrating morphological and molecular techniques provides a robust framework for comprehensive fungal characterisation.

Despite the agricultural importance of common bean in Cameroon, molecular data describing fungal communities associated with stored bean cultivars remain extremely limited. This knowledge gap restricts the development of evidence-based strategies for fungal management and mitigation of mycotoxin contamination during storage.

Therefore, this study aimed to isolate and characterise fungal species associated with stored common bean cultivars in Menoua Division, Western Cameroon, using an integrative approach combining morphological assessment and ITS-based molecular identification. By providing detailed insights into fungal diversity and identity, this study contributes to the foundation for improved post-harvest management practices and enhanced food safety in the region.

Materials & Methods

Study Area

The study was conducted in Menoua Division, located in the West Region of Cameroon, a major agricultural zone characterised by intensive cultivation of common bean (*Phaseolus vulgaris* L.). The region lies within a humid tropical climate with marked wet and dry seasons, conditions

that are conducive to both crop production and post-harvest microbial proliferation (Bechem & Afanga 2017).

Sampling Strategy and Sample Collection

A multistage sampling design was employed to ensure representative coverage of bean-producing areas. In the first stage, six subdivisions; Dschang, Santchou, Nkong-Ni, Fokoué, Penka-Michel, and Fongo-Tongo were purposively selected based on their prominence in common bean production. In the second stage, farmers within each subdivision were randomly selected. Finally, stored bean samples were collected using a convenience sampling approach from farmer storage units.

Samples were obtained from beans stored in gunny bags, which are commonly used for post-harvest storage in the region. To ensure representativeness, grains were collected from three strata (top, middle, and bottom) of each storage bag using a sterile polyvinyl chloride (PVC) scoop. Bulk samples of 500 g per cultivar were prepared and transported to the laboratory for analysis.

Six commonly cultivated bean types were investigated: kidney bean (small red), black turtle bean, navy bean (white), pinto bean (mottled brown), pea bean (mottled red), and large-seeded bean (Fig. 1).

Fig. 1 – Cultivars of common bean collected from Menoua Division, Cameroon. A Black turtle bean (Black bean). B Kidney bean (small sized red bean). C Pea bean (mottled red bean). D Navy bean (White bean). E Large seeded bean (Large red bean). F Pinto bean (mottled brown bean).

Isolation and Detection of Fungi

Surface Sterilisation

Fungal isolation was conducted following standard seed health testing protocols. Subsamples of 50 grains per cultivar were randomly selected and surface-sterilised to eliminate external contaminants while preserving internal fungal structures. Grains were immersed in 70% ethanol for 1 minute, followed by 10% sodium hypochlorite for 1 minute, and then rinsed thoroughly in sterile distilled water for 3 minutes. Sterilised grains were blot-dried on sterile filter paper (Schulz et al. 1993).

Direct Plating Technique

Surface-sterilised grains were plated on potato dextrose agar (PDA) using the agar plate method described by Nega (2014). Ten grains were placed equidistantly per Petri dish, with five replicates per cultivar (Fig. 2). Plates were sealed with parafilm and incubated at 28 ± 2°C for 7

days to promote fungal growth. Emerging fungal colonies were monitored daily and sub cultured to obtain pure isolates.

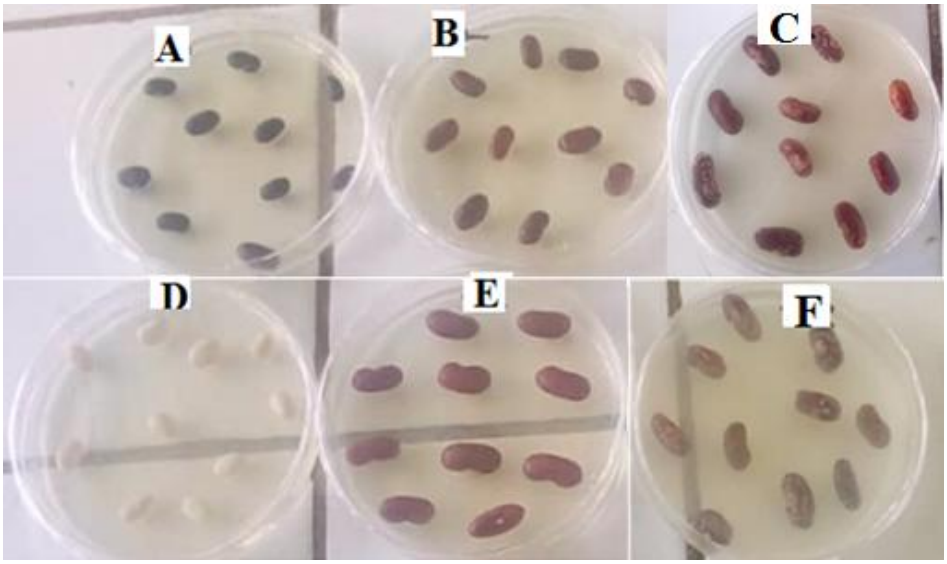

Fig. 2 – Common bean cultivars directly plated on PDA for isolation of fungi. A Black turtle bean (Black bean). B Kidney bean (small sized red bean. C Pea bean (mottled red bean). D Navy bean (White bean). E Large seeded bean (Large red bean). F Pinto bean (mottled brown bean).

Morphological Characterisation of Fungal Isolates

Preliminary identification of fungal isolates was based on macroscopic and microscopic characteristics. Colony morphology including colour, texture, growth rate, and margin characteristics was assessed on PDA following standard taxonomic keys (Klich 2002).

For microscopic examination, fungal mycelia were mounted on glass slides and stained with lactophenol cotton blue. Slides were observed under light microscopy at ×10 and ×40 magnifications to evaluate reproductive structures such as conidiophores, conidia, and hyphal characteristics.

Non-sporulating isolates were classified as sterile mycelia ("mycelia sterilia") and grouped into morphotypes based on colony characteristics (Lacap et al. 2003). Representative isolates from each morphological group were selected for molecular analysis.

Molecular Characterisation

DNA Extraction

Genomic DNA was extracted from actively growing fungal cultures using a modified protocol described by Zhang et al. (2010). Approximately 30 mg of fungal mycelium was harvested and ground in liquid nitrogen. The powdered mycelium was lysed in extraction buffer (Tris-acetate, sodium acetate, EDTA, and SDS), followed by centrifugation and purification steps including RNase treatment and isopropanol precipitation. DNA pellets were washed with 70% ethanol, air-dried, and resuspended in Tris-EDTA buffer.

DNA quality and integrity were assessed using 1.5% agarose gel electrophoresis, and samples were stored at −20°C until further analysis.

PCR Amplification of ITS Region

The internal transcribed spacer (ITS) region of fungal ribosomal DNA was amplified using universal primers ITS1 and ITS4, which are widely used for fungal identification (White et al. 1990, Ferrer et al. 2001).

PCR reactions were carried out in a 50 µL reaction volume containing PCR master mix, primers, template DNA, and nuclease-free water. Amplification was performed in a thermocycler under the

following conditions: initial denaturation at 98°C, followed by 34 cycles of denaturation, annealing at 58°C, and extension at 72°C, with a final extension step.

Amplified products were visualised on 1% agarose gel, and fragment sizes (~600 bp) were estimated using a 200 bp DNA ladder.

DNA Sequencing and Species Identification

PCR products were purified and sequenced using Sanger sequencing technology. Bidirectional sequencing was performed using the same primers employed for amplification.

Obtained sequences were edited and compared against reference sequences in the GenBank database using the BLAST algorithm for species identification. Species assignment was based on ≥98% sequence similarity for species-level identification and ≥95% for genus-level identification (Sampietro et al. 2010, Nilsson et al. 2019).

Phylogenetic and Sequence Analysis

Phylogenetic relationships among fungal isolates were inferred using the neighbour-joining method implemented in MEGA software (Tamura et al. 2006). Bootstrap analysis with 1000 replicates was performed to assess the robustness of tree topology.

Multiple sequence alignment was conducted using ClustalW (Larkin et al. 2007) to evaluate sequence homology and identify conserved and variable regions. Alignment outputs were further analysed to assess nucleotide composition and genetic divergence among isolates (Corpet 1988).

Data Analysis

Fungal specie identification was validated based on sequence similarity, query coverage, and phylogenetic clustering. Species delineation followed established thresholds for ITS-based identification, ensuring reliability of taxonomic classification (Nilsson et al. 2019). To evaluate fungal community structure among bean cultivars, ecological diversity indices were calculated. Species frequency was determined as.

$$Frequency(\%) = \frac{Number\ of\ samples\ containing\ a\ species}{Total\ number\ of\ samples} \times 100$$

Species diversity was estimated using the Shannon–Wiener diversity index.

$$H' = -\sum p_i \ln p_i$$

where *pi* represents the proportion of individuals belonging to species *i*.

Species dominance was further assessed using Simpson's diversity index.

$$D = 1 - \sum p_i^2$$

These indices provided quantitative measures of fungal richness, diversity, and dominance within stored bean cultivars.

Results

Fungal Incidence in Stored Common Bean Cultivars

Fungal growth (Fig. 3) was observed in all plated common bean cultivars following 7 days of incubation at 28 ± 2°C, indicating widespread contamination across all sampled storage systems. Colonisation was evident through visible changes in grain appearance, including discoloration and the emergence of aerial mycelia and reproductive structures.

Notably, individual grains frequently supported multiple fungal colonies, suggesting co-occurrence of different fungal taxa within the same substrate. This pattern reflects a complex storage mycobiota and indicates favourable environmental conditions for fungal proliferation across all bean types.

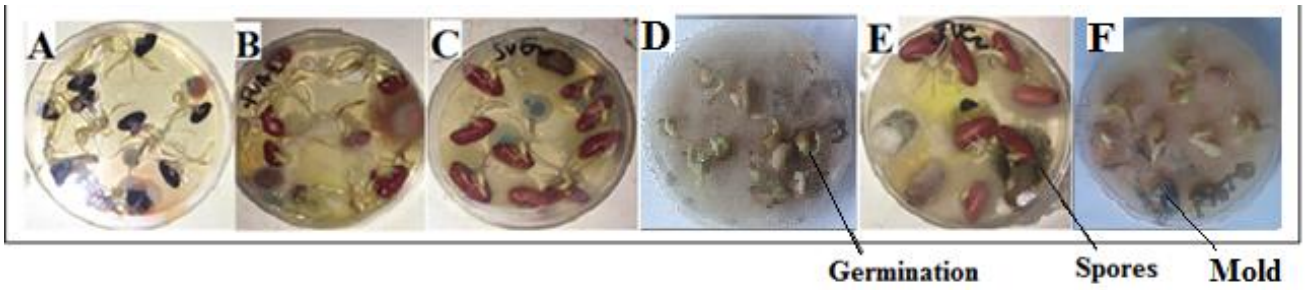

Fig. 3 – Fungal growth on plated stored bean cultivars. A Black turtle bean (Black bean). B Kidney bean (small sized red bean). C- Pea bean (mottled red bean). D Navy bean (White bean). E Large seeded bean (Large red bean). F Pinto bean (mottled brown bean).

Morphological Characterisation of Fungal Isolates

Based on macroscopic colony features and microscopic examination, fungal isolates were grouped into four distinct morphotypes corresponding to the genera *Aspergillus*, *Penicillium*, *Fusarium*, and a sterile morphotype (mycelia sterilia)- Table 1

Colonies of *Aspergillus* species were characterised by rapid growth, with yellow to dark pigmented surfaces and creamy reverse sides. Microscopically, isolates exhibited septate hyphae, rough conidiophores, globose vesicles, and radiating chains of conidia.

Penicillium species displayed green, powdery colonies with a creamy reverse. Microscopic observation revealed characteristic brush-like conidiophores (penicilli) bearing chains of conidia.

Fusarium species colonies were typically fluffy with pink to reddish pigmentation. Microscopy showed curved, multicellular macroconidia and simple phialides.

The sterile morphotype produced white, non-sporulating colonies with no observable reproductive structures under microscopy, necessitating molecular identification.

Colony growth rates varied among isolates, with *Aspergillus* spp. exhibiting the fastest radial expansion (mean diameter: 7.2 cm after 7 days), followed by *Fusarium* spp. (6.6 cm), sterile morphotype (6.4 cm), and *Penicillium* spp. (2.1 cm), indicating differences in growth dynamics and competitive potential under storage conditions.

Table 1 Morphoculture characteristics of fungal isolates recovered from stored common bean cultivars.

Isolate (Genus)	Colony Diameter (cm, 7 days)	Surface Colour	Reverse Colour	Margin	Texture	Key Microscopic Features
Aspergillus spp.	7.2 ± 0.1	Yellow to dark	Cream	Irregular	Cottony to granular	Septate hyphae; rough conidiophores; globose vesicles; radiating conidia
Fusarium spp.	6.6 ± 0.2	Pink to reddish	Light pink to purple	Irregular	Fluffy, woolly	Curved, multicellular macroconidia; simple phialides
Penicillium spp.	2.1 ± 0.1	Green	Cream	Irregular	Powdery to cottony	Brush-like conidiophores (penicilli); chains of conidia

Table 1 Continued

Isolate (Genus)	Colony Diameter (cm, 7 days)	Surface Colour	Reverse Colour	Margin	Texture	Key Microscopic Features
Morphotype (sterile mycelium)	6.4 ± 0.2	White	White	Regular	Fluffy	No observable reproductive structures

Occurrence of fungal species on common bean cultivars

Fungal occurrence differed significantly among common bean cultivars ($P \leq 0.05$), revealing a structured distribution of storage-associated taxa (Fig. 4). *Xylaria hypoxylon* was the most prevalent species across all cultivars, with significantly higher abundance in kidney, black, and large-seeded beans compared to pinto, pea, and navy beans. *Aspergillus flavus* showed a cultivar-dependent pattern, peaking in large-seeded and pea beans, while remaining comparatively low in other cultivars. *Penicillium aethiopicum* was significantly enriched in pinto beans, indicating a distinct niche preference. In contrast, *Fusarium oxysporum* exhibited a restricted distribution, occurring predominantly in navy beans and absent in several cultivars.

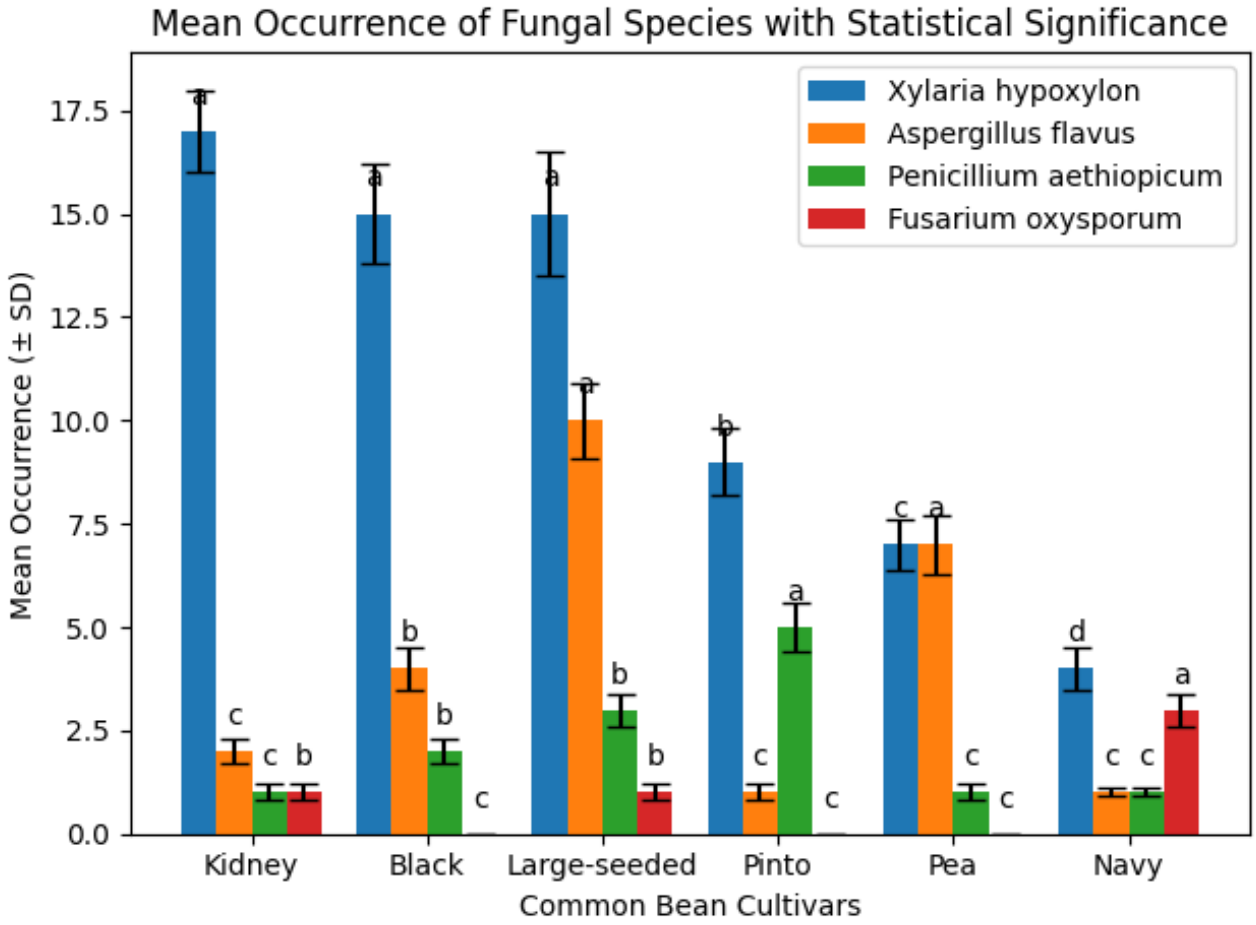

Fig. 4 – Mean occurrence (± SD) of fungal species isolated from stored common bean cultivars. Bars represent mean values from replicate samples (n = 5), with error bars indicating standard deviation. Different letters above bars denote statistically significant differences among cultivars for each fungal species (one-way ANOVA followed by Tukey's HSD test, $p \leq 0.05$).

Fungal Diversity and Distribution

Diversity analysis revealed moderate to high fungal diversity among bean cultivars (Table 2). Shannon diversity values ranged from 1.16 in navy bean to 1.34 in large-seeded bean cultivars, indicating differences in species distribution and dominance patterns. Similarly, Simpson diversity values ranged from 0.63 to 0.74, suggesting relatively balanced fungal communities with moderate species dominance. Evenness values exceeding 0.84 for all cultivars further demonstrated that fungal species were relatively evenly distributed within the storage ecosystem.

The comparatively lower diversity observed in navy bean was associated with the greater predominance of *Aspergillus flavus*, whereas large-seeded and black bean cultivars exhibited more balanced fungal communities.

Table 2 Fungal diversity indices among stored common bean cultivars.

Bean cultivar	Species richness (S)	Shannon index (H′)	Simpson index (1−D)	Evenness (J′)
Kidney bean	4	1.30	0.72	0.94
Black bean	4	1.32	0.73	0.95
Pea bean	4	1.25	0.69	0.90
Navy bean	4	1.16	0.63	0.84
Large-seeded bean	4	1.34	0.74	0.97
Pinto bean	4	1.29	0.71	0.93

PCR Amplification of Fungal ITS Regions

Successful amplification of the internal transcribed spacer (ITS) region was achieved for all representative fungal isolates. Agarose gel electrophoresis (Fig. 5) revealed single, distinct bands of approximately 600 base pairs for each isolate, confirming the purity of cultures and successful targeting of the ITS region. No amplification was observed in the negative control, indicating the absence of contamination during PCR processing.

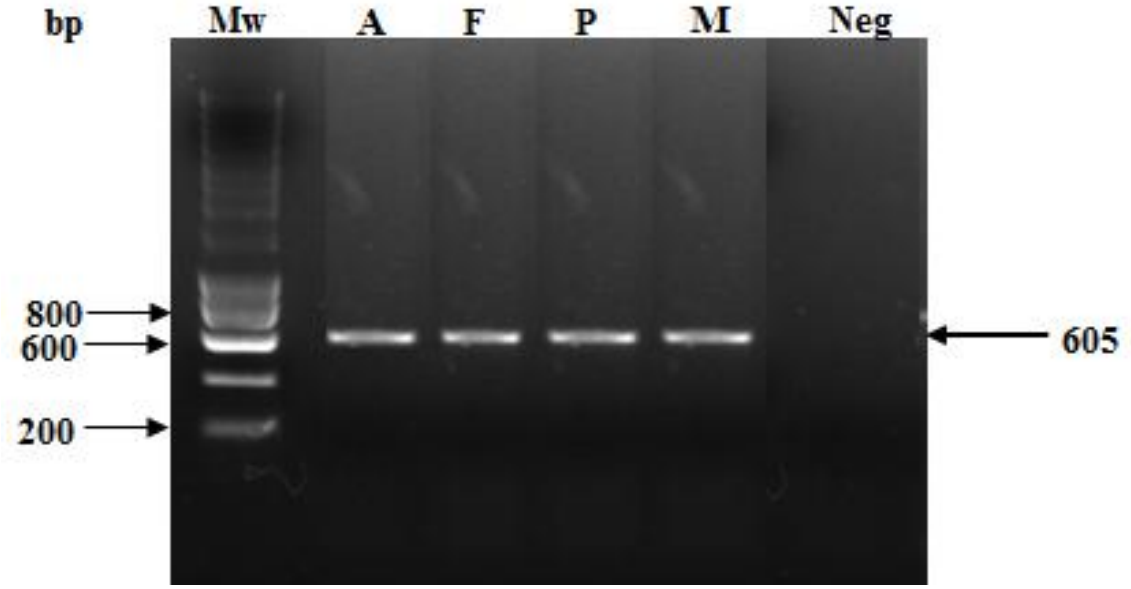

Fig. 5 – 1% agarose gel electrophoresis showing ITS rDNA PCR amplification (~600 bp) of fungal isolates recovered from stored common bean (*Phaseolus vulgaris* L.) cultivars.

Mw (molecular weight) = 200 bp marker A, F, P and M represent the amplicons obtained from various fungi isolate types, Neg: Negative control. A= *Aspergillus sp*., F=*Fusarium sp*., P=*Penicillium sp*., M=Morphotype isolate

Molecular Identification of Fungal Species

BLAST analysis of ITS sequences against the GenBank database enabled species-level identification of all isolates (Table 3). Sequence similarity values exceeded 98% for all samples, confirming high-confidence taxonomic assignments. The isolates were identified as follows: *Aspergillus flavus* (99.78% similarity), *Penicillium aethiopicum* (99.59% similarity), *Fusarium oxysporum* (99.51% similarity) and *Xylaria hypoxylon* (98.78% similarity). Importantly, the previously unresolved sterile morphotype was identified as *Xylaria hypoxylon*, demonstrating the effectiveness of ITS sequencing in resolving non-sporulating fungal taxa. All sequence matches showed E-values of zero and high query coverage (97–100%), further validating species identification.

Table 3 Molecular identification of fungal isolates based on ITS sequence analysis.

Isolate Code	GenBank Accession No.	Identified Species	Closest Reference Strain	Query Coverage (%)	Sequence Identity (%)	E-value
P_ITS	MT331850.1	*Penicillium aethiopicum*	Strain ND22	100	99.59	0.0
A_ITS	MT331851.1	*Aspergillus flavus*	Strain NRRL 3057	99–100	99.78	0.0
F_ITS	MT331852.1	*Fusarium oxysporum*	Strain Ag149 / f.sp. dianthi	100	99.51	0.0
M_ITS	MT331853.1	*Xylaria hypoxylon*	Strain G1045 / Xylaria sp. Ps-31	97–98	98.78	0.0

Phylogenetic Analysis

Phylogenetic relationships inferred using the neighbour-joining method revealed that all isolates clustered with their respective reference sequences from GenBank, confirming their taxonomic placement. The phylogenetic tree exhibited strong bootstrap support values ranging from 86% to 100%, indicating high confidence in branch topology. Each isolate formed a distinct clade corresponding to its species, with clear separation between taxa, reflecting genetic divergence among the identified fungi. Branch length variation further indicated evolutionary distance between species, with no evidence of close clustering beyond species-level grouping.

Multiple Sequence Alignment

Alignment was conducted using MultAlin (Corpet 1988) and visualised for comparative analysis of nucleotide homology across isolates (Figure 6). The total alignment length was approximately 650 bp, covering the ITS1–5.8S–ITS2 rDNA region commonly used for fungal taxonomic identification. Highly conserved regions (≥90% sequence identity across isolates) were observed predominantly within nucleotide positions 39–43, 45–49, and 261–390, indicating strong sequence conservation among fungal taxa in these regions. These conserved blocks reflect evolutionary stability of the ITS rDNA region and support reliable phylogenetic inference. In contrast, variable and poorly conserved regions (≤50–70% identity) were detected primarily between positions 10–38 and 627–650, indicating nucleotide divergence among isolates. These polymorphic regions contribute to species-level discrimination and genetic differentiation. Insertions and deletions (indels), represented as gaps ("–") in the alignment, were observed at multiple loci (e.g., positions 1–10, 76–82, and 85–87**)**.

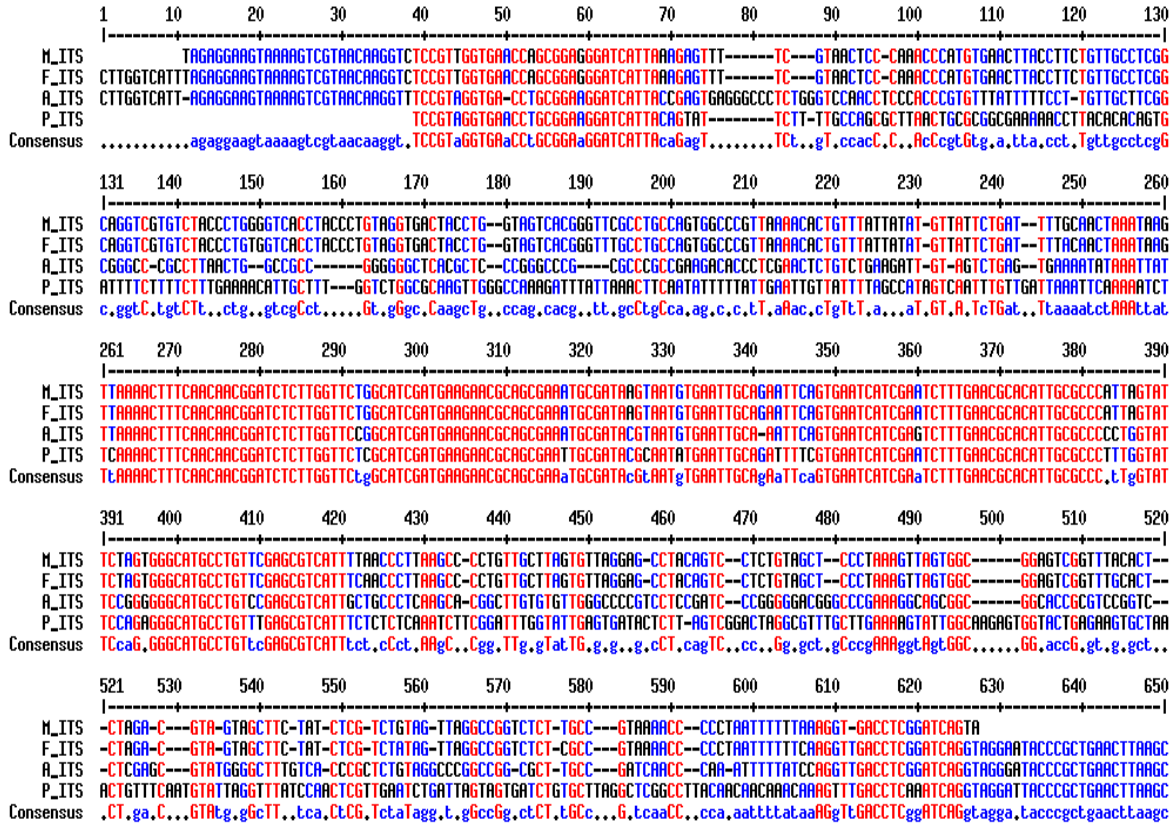

Fig. 6 – Multiple sequence alignment of ITS rDNA sequences showing conserved and polymorphic sites among fungal isolates recovered from stored common bean (*Phaseolus vulgaris* L.) cultivars

Discussion

The present study provides an integrative morphological and ITS-based molecular characterisation of fungi associated with stored common bean (*Phaseolus vulgaris* L.) cultivars in Menoua Division, Cameroon. The combined approach revealed a diverse assemblage of storage-associated fungi, dominated by *Aspergillus flavus*, *Penicillium aethiopicum*, *Fusarium oxysporum*, and *Xylaria hypoxylon*. These findings underscore the vulnerability of traditionally stored legumes to fungal colonisation and highlight significant implications for food safety, seed quality, and post-harvest management.

Fungal diversity and storage ecosystem dynamics

The application of molecular techniques has been extensively used to identify fungi at both levels of taxonomy and phylogeny (Bruns et al. 1991). The single band obtained in this study after DNA amplification of the ITS region of all the fungi isolates confirmed that the isolates were obtained from pure cultures. Results obtained from phylogenetic analysis confirmed that the fungi recovered from the stored cultivars were all of distinct species. This possibly could be due to the nature of the conserved targeted ITS region found between 16S and 23S ribosomal RNA gene that functions in bringing about the identity of fungal species and genera (Dendis et al. 2003). The consistent recovery of fungal isolates across all bean cultivars indicates that storage conditions in the study area provide a conducive microenvironment for fungal establishment and proliferation. The dominance of *Xylaria hypoxylon* across all bean types suggests a high degree of ecological versatility, likely linked to its saprophytic capacity and ability to exploit carbon-limited substrates during prolonged storage. Although typically associated with lignocellulosic material, its persistence in stored legumes indicates opportunistic colonisation under suboptimal storage conditions, consistent with reports of *Xylaria* spp. as facultative endophytes and post-harvest colonisers (Rogers 2000).The predominance of *Aspergillus* and *Penicillium* species aligns with their well-documented ecological role as xerotolerant “storage fungi,” capable of thriving under

low moisture and nutrient-limited conditions typically encountered in stored grains (Pitt & Hocking 2009, Magan et al. 2020). Their rapid growth rates observed in this study further support their competitive advantage in post-harvest ecosystems, where they often outcompete field fungi during prolonged storage periods. During storage, moisture content of stored grains decreases. This lower moisture content creates a favourable medium for the growth and survival of xerotolerant and xerophilic fungi (Iamanaka et al. 2005). During long-term storage, fungi of the genera *Aspergillus* and *Penicillium* ("storage flora") progressively replace the "field flora" such as *Fusarium* and *Alternaria* over a period of several months (Fleurat et al. 2002). The differential abundance of *Aspergillus flavus*, particularly in large-seeded and pea beans, is indicative of substrate-mediated susceptibility. Seed size, coat permeability, and biochemical composition (e.g., lipid and carbohydrate content) are known to influence fungal invasion and aflatoxin biosynthesis (Magan et al. 2020, Pitt & Hocking 2009). Larger seeds may retain more moisture or provide greater nutrient reserves, thereby enhancing colonisation efficiency. This aligns with previous findings that xerotolerant *Aspergillus* species preferentially dominate under warm, low-moisture storage environments typical of tropical regions.

In contrast, the detection of *Fusarium oxysporum* suggests that contamination may also originate from field infection prior to storage. *Fusarium* species are generally classified as "field fungi" requiring relatively higher moisture for growth, and their persistence into storage indicates inadequate drying or incomplete field-to-storage transition control (Girisham et al. 2017, Jiménez Medina et al. 2021). This co-occurrence of field and storage fungi reflects a dynamic and transitional mycobiome shaped by environmental conditions and post-harvest handling practices. The isolation of *Aspergillus flavus* and *Fusarium oxysporum* also shows conformity with work done by Kator et al. (2016) who isolated *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Botryodiplodia theobromae* from samples of common bean obtained from selected markets in Makurdi, Nigeria. The identification of *Fusarium oxysporum* in the present study is consistent with the findings of Zhang et al. (2006), who reported the occurrence of various *Fusarium* species in common bean, including *Fusarium solani* and *Fusarium oxysporum*.

Molecular identification and taxonomic resolution

Morphological identification provided preliminary grouping of isolates; however, its limitations were evident in the classification of sterile mycelia. The inability of some isolates to sporulate under laboratory conditions is a known constraint in classical mycology, often leading to incomplete or ambiguous identification (Lacap et al. 2003). In this study, ITS rDNA sequencing successfully resolved all isolates to species level, including the morphologically unidentifiable isolate, which was identified as *Xylaria hypoxylon*. This confirms the high discriminatory power of ITS-based barcoding in fungal taxonomy and its superiority over morphology alone for non-sporulating or phenotypically plastic taxa (Schoch et al. 2012, Nilsson et al. 2019).

The high sequence similarity values (>98%) and strong bootstrap support obtained from phylogenetic analysis further validate the reliability of molecular identification. Clear clustering of isolates with reference sequences demonstrates robust phylogenetic separation and confirms species-level assignment. These results are consistent with previous studies highlighting ITS as a universal fungal barcode region capable of resolving both closely and distantly related taxa (Sampietro et al. 2010).

Ecological significance of identified fungi

The detection of *Aspergillus flavus* is of particular concern due to its well-established ability to produce aflatoxins, among the most potent naturally occurring carcinogens. Aflatoxin contamination in staple crops remains a major public health challenge in tropical regions, where warm and humid conditions favour fungal growth and toxin biosynthesis (Omotayo et al. 2019, Magan et al. 2020). Similarly, *Fusarium oxysporum* is associated with fumonisin production, which has been implicated in oesophageal cancer and other metabolic disorders through disruption of sphingolipid metabolism.

Penicillium aethiopicum, although less studied than other storage fungi, is increasingly recognised as a contributor to post-harvest spoilage and potential mycotoxin contamination in stored legumes. Its presence in all cultivars examined suggests widespread dissemination within storage environments, likely facilitated by poor aeration and high relative humidity.

The occurrence of *Xylaria hypoxylon* is particularly noteworthy, as members of the genus are typically associated with decaying wood and plant material. Its detection in stored beans may reflect environmental contamination or opportunistic colonisation under prolonged storage conditions. Previous studies have reported *Xylaria* species as endophytes or post-harvest colonisers of plant tissues, supporting their ecological versatility (Rogers 2000). *Xylaria sp.* has also been found to be a post-harvest fungus associated with the deteriolation of *Raphia hookeri* fruits, shea nuts, kernel and germinating common bean seeds (Esiegbuya et al. 2013, Esiegbuya et al. 2014, Parsa et al. 2016).

Implications for post-harvest management

The co-existence of toxigenic and spoilage fungi across all bean cultivars indicates systemic weaknesses in post-harvest handling practices, particularly in drying efficiency, storage hygiene, and moisture control. Traditional storage systems used in the study area likely maintain microclimatic conditions that favour fungal persistence and competition. The observed fungal diversity also suggests that current practices are insufficient to inhibit both field-derived and storage-adapted fungal populations.

Climate variability, particularly increasing temperature and humidity fluctuations, may further exacerbate fungal proliferation and mycotoxin risk in stored legumes (Jiménez Medina et al. 2021). Therefore, improving post-harvest management through controlled drying, hermetic storage systems, and routine fungal monitoring is essential to mitigate these risks.

Study Limitations and Future Perspectives

Although the present study provides important insights into the diversity of storage-associated fungi in common bean, several limitations should be acknowledged. First, fungal identification relied exclusively on ITS rDNA sequencing. While ITS is considered the universal fungal barcode, additional loci such as β-tubulin, calmodulin, translation elongation factor 1-alpha (TEF1-α), and RNA polymerase II genes could provide greater taxonomic resolution for closely related species complexes. Second, fungal abundance was assessed through culture-dependent methods, which may underestimate total fungal diversity by excluding non-culturable taxa. Third, mycotoxin production was not quantified, preventing direct assessment of food safety risks associated with the identified fungi. Finally, environmental parameters such as grain moisture content, relative humidity, storage duration, and temperature were not monitored and therefore could not be correlated with fungal occurrence.

Future studies should integrate metabarcoding, multi-locus phylogenetics, and metabolomic approaches to characterize fungal communities more comprehensively and evaluate their toxigenic potential. Additionally, investigations assessing the effectiveness of hermetic storage systems and other climate-smart postharvest technologies under farmer conditions would contribute substantially to reducing fungal contamination and postharvest losses.

Conclusion

This study provides the first comprehensive integration of morphoculture characterization and ITS-based molecular identification of storage-associated fungi colonizing common bean (*Phaseolus vulgaris* L.) cultivars in Western Cameroon. Four fungal species, namely *Aspergillus flavus*, *Penicillium aethiopicum*, *Fusarium oxysporum*, and *Xylaria hypoxylon*, were identified with high confidence through molecular analyses, demonstrating the effectiveness of ITS sequencing in resolving fungal taxa that could not be reliably identified using morphology alone.

The predominance of storage-adapted fungi, including potentially mycotoxigenic species, reveals significant vulnerabilities within existing postharvest storage systems and raises concerns regarding grain quality, seed viability, and food safety. The occurrence of both field-derived and

storage-associated fungi further suggests that fungal contamination is influenced by a continuum of factors extending from pre-harvest infection to postharvest handling and storage conditions.

These findings establish an important baseline for fungal biodiversity assessment in stored common bean systems in Cameroon and provide critical evidence for developing targeted interventions aimed at minimizing postharvest losses and reducing mycotoxin exposure risks. Adoption of improved drying practices, hermetic storage technologies, and routine fungal monitoring programs will be essential for enhancing food security, protecting public health, and improving the sustainability of common bean value chains in sub-Saharan Africa.

Data Availability Statement

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. The ITS rDNA sequences generated in this study have been deposited in the GenBank database under the accession numbers MT331850.1, MT331851.1, MT331852.1, and MT331853.1. All other data supporting the findings, including culture characteristics, morphological observations, and phylogenetic outputs, are included within the manuscript and its supplementary materials.

Acknowledgements

The authors sincerely acknowledge the farmers of the Menoua Division, West Region of Cameroon, for their cooperation and for providing access to stored common bean cultivars used in this study. The authors also express gratitude to the staff of the Plant Science Laboratory and the Molecular and Biotechnology Laboratory of the University of Buea for technical assistance and laboratory support during fungal isolation, culture, and molecular analyses. Finally, the authors extend their appreciation to the staff of the Department of Biochemistry, Microbiology, and Biotechnology at Kenyatta University for their constructive criticism and valuable corrections during the defense of this work.

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