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Isolation and molecular identification of fungal contaminants of *Digitaria exilis* from five farms in Kwara state, Nigeria

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Abstract

Digitaria exilis (acha/fonio), an important indigenous cereal grain in West Africa, faces significant post-harvest challenges, particularly fungal contamination during storage. Understanding the mycological profile of stored fonio is essential for developing effective quality control measures and ensuring food safety. This study investigated the fungal diversity and contamination levels in *D. exilis* samples collected from five different farms (Guanara, Kosubosu, Kayama, Bakase, and Duduro) across Kwara State, Nigeria. One-kilogram samples were obtained from each location and subjected to mycological analysis. Total fungal counts ranged from 2.93 ± 0.04^{a} to 6.33 ± 0.03 CFU/g, with Kayama samples exhibiting the highest contamination levels and Guanara samples showing the lowest. Twenty fungal isolates were characterized using combined morphological and molecular approaches employing ITS1 and ITS4 primers for amplification and sequencing. Species identification revealed *Penicillium* spp. as the predominant contaminant (26.92%), followed by *Aspergillus fumigatus* (19.23%), *Fusarium* spp. (19.23%), *Aspergillus clavatus* (15.39%), *Aspergillus parasiticus* (11.54%), and *Aspergillus niger* (7.69%). Phylogenetic analysis of the sequences, deposited in NCBI GenBank, demonstrated clustering of isolates primarily within the families Trichocomaceae (*Aspergillus* and *Penicillium* species) and Nectriaceae (*Fusarium solani*). This study provides the first comprehensive molecular characterization of storage fungi associated with *D. exilis* in Nigeria, establishing baseline data crucial for developing targeted post-harvest management strategies and contributing to the broader understanding of cereal grain mycobiota in tropical storage conditions.

Keywords: *Digitaria exilis,* cereal grain, fungal isolates, morphological and molecular approaches

Introduction

Microorganisms shape our world in profound ways; some serve as essential allies in food production and medicine, while others pose significant threats to our health and food security (Bawa and Addai, 2022). Among these threats, toxin-producing fungi, particularly certain *Aspergillus* species, represent a growing concern for global food safety. These microscopic organisms can colonize crops both before and after harvest, producing mycotoxins under favorable environmental conditions. The challenge is daunting: mycotoxins are nearly impossible to prevent entirely, as they emerge naturally when temperature, humidity, and other factors align. These fungal toxins remain a leading cause of contamination and spoilage in staple foods, including grains, oilseeds, cereals, and pulses (Bawa and Addai, 2022; Ogu *et al*., 2023).

For millions of Nigerians, grains form the backbone of daily nutrition, providing essential energy and nutrients. Rice, maize, millet, sorghum, and wheat dominate dining tables across the country. However, fungal infestation of these grains presents both an economic burden and a public health crisis (Ezekiel et al., 2014). Staple crops such as maize, rice, cowpea, wheat, barley, and millet are particularly vulnerable to mycotoxin-producing fungi, threatening food security and livelihoods.

Among West Africa's indigenous cereals, *Digitaria exilis*, commonly known as fonio, acha, or "hungry rice" stands out as a nutritional powerhouse. This small-seeded grain, cultivated for centuries across the region, offers exceptional nutritional value with high levels of methionine and cysteine, amino acids typically deficient in major cereals like rice, wheat, sorghum, barley, and rye (Ohabughiro *et al*., 2020). Beyond its superior amino acid profile, fonio boasts high crude lipid content, excellent digestibility, favorable processing qualities for enzyme production, and impressive shelf stability (Ezekiel *et al*., 2014; Ohabughiro *et al*., 2020).

Despite its promise as a resilient and nutritious crop, fonio faces the same fungal challenges that plague other grains. Toxigenic fungi readily colonize stored fonio, especially when moisture and temperature conditions favor their growth, threatening both the economic viability of this ancient grain and the health of communities that depend on it.

Materials and Methods

Sample Collection

Digitaria exilis samples were collected from five different sampling points at each of five farms (Guanara, Kosubosu, Kayama, Bakase, and Duduromo) in Kwara State, Nigeria. Approximately 1 kg of grain from each farm was placed in sterile zip-lock bags and transported to the laboratory for mycological analysis. The samples were stored in a desiccator containing a desiccant to avoid absorbing moisture until analysis (Maryam, 2017).

Media Preparation

The culture media used in this study are Coconut Agar, Potato Dextrose Agar and Potato Dextrose Broth. They were prepared according to standard guidelines and antibiotics added as a bacteriostatic agent to inhibit the growth of bacteria in the medium. The mixture was homogenously mixed and autoclaved at 121^{o}C, 1.5 psi for 15 minutes and allowed to cool to 45^{o}C before dispensing.

Isolation and Purification of Fungi from Samples

The samples were surface sterilized using 70% ethanol for 2 minutes. Samples were rinsed with distilled water to remove ethanol on the surface. One gram of grounded sample was separately placed in 9 ml of sterile distilled water, shaken using an orbital shaker, and serially diluted up to 10^{-2}. Aliquots 0.1mL were plated on PDA plate and incubated at 28□C for 7 days (Ohabughiro *et al*., 2020). Enumeration of the fungi isolates was done using the formula below:

$$\text{Linear growth} = Po + (k \times T)$$

Where pt: average length of time,

K: constant growth of rate,
T: elapsed time in week from the time zero

The fungi that were visually observed were transferred to a new PDA for the purpose of yielding pure cultures for identification. Continuous sub-culturing of the isolates was done until pure isolates were obtained. Pure fungal cultures were stored in McCartney bottles and placed in a cool incubator at 4 °C to stop further fungal growth.

Identification of Fungi Isolates

Fungi identification was done through both phenotypic and molecular characterization.

Phenotypic characterization of isolated fungi

Fungal isolates were identified following the method described by Ohabughiro *et al*. (2020). A small piece of fungal mycelium was carefully removed and placed on a glass slide with a drop of 70% ethanol. The mycelium was gently teased apart using a sterile inoculation needle to separate the fungal structures. After the ethanol evaporated, the specimen was stained with lactophenol cotton blue (LPCB) and covered with a glass coverslip. Slides were examined under a Leica DM500 Digital Microscope (Leica Microsystems, Switzerland) at 400× magnification. Identification was based on visible fungal characteristics including colony appearance, hyphal structure, arrangement of spore-bearing structures, and spore morphology using standard mycological identification guides.

Molecular Characterization of Fungi Isolates

Extraction of genomic deoxyribonucleic acid (DNA)

Fungal DNA extraction was performed using a fungal DNA mini-extraction kit (Inqaba, South Africa) following the manufacturer's protocol.

A generous amount of pure fungal culture was suspended in 200 µL of isotonic buffer in a Bashing Bead lysis tube, followed by the addition of 750 µL lysis solution. The tubes were processed in a bead beater with a 2 mL tube holder assembly at maximum speed for 5 minutes, and then centrifuged at 10,000×g for 1 minute. The supernatant (400 µL) was transferred to a Zymo-Spin IV spin filter placed in a collection tube and centrifuged at 7,000×g for 1 minute. The filtrate was mixed with 1,200 µL of fungal DNA binding buffer, bringing the total volume to 1,600 µL. This mixture was processed in two 800 µL aliquots through a Zymo-Spin IIC column by centrifugation at 10,000×g for 1 minute, with the flow-through discarded after each spin.

The column was transferred to a fresh collection tube and washed sequentially with 200 µL DNA Pre-wash buffer (10,000×g, 1 minute) and 500 µL fungal DNA Wash Buffer (10,000×g, 1 minute). For DNA elution, the Zymo-Spin IIC column was placed in a sterile 1.5 mL microcentrifuge tube, and 100 µL of DNA elution buffer was applied to the column matrix. Final centrifugation at 10,000×g for 30 seconds yielded ultra-pure DNA, which was stored at -20°C until further use (Ohabughiro et al., 2020).

Polymerase chain reaction amplification protocol

PCR amplification was performed in a 20 µL reaction mixture containing 10 µL OneTaq Quick-Load 2X Master Mix with Standard Buffer (New England Biolabs Inc.), 1 µL each of forward and reverse primers (ITS1 and ITS4), 7 µL nuclease-free water, and 1 µL DNA template. The ITS1 forward primer sequence was 5'-TCC GTA GGT GAA CCT GCG G-3', and the ITS4 reverse primer sequence was 5'-TCC TCC GCT TAT TGA TAT GC-3'. The reaction mixture was gently mixed and transferred to a preheated thermal cycler (Eppendorf Nexus Gradient Mastercycler, Germany). Amplification conditions consisted of an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 50°C for 30 seconds, and extension

at 68°C for 1 minute, with a final extension at 68°C for 10 minutes. PCR products were separated by electrophoresis on 2% agarose gel and visualized using SYBR Gold nucleic acid stain. The amplified products were purified using the ExoSAP-IT protocol prior to sequencing (Ohabughiro *et al*., 2020).

Results

Fungal Count of the Samples

The fungal count of *Digitaria exilis* samples obtained from different farms in Kwara state (Guanara, kosubusu, kayama, Bakase, and Duduro) is shown in Table 1. In each farm, five randomly identified lots in the value chain were selected for sampling. Fugal count of the samples in different farms and lots were between 2.93 ± 0.04 x 10^4 and 6.33 ± 0.03 x 10^4 CFU/g. The highest count was observed in lot A sample from Kayama (A) reaching 6.33± 0.03 x 10^4 CFU/g and the lowest from Guanara (lot E) with 2.93 ± 0.04 x 10^4 CFU/g as shown in Table 4.1. Duduro and Kayama consistently shows higher fungal count while Guanara and Bakase generally showed lower count across most factors, these differences implies environment affects fungal proliferation

Morphological characteristics of the isolates are shown in Table 2. Four fungal isolates identified in Guanara farm include *Aspergillus niger, Aspergillus fumigatus, Fusarium* spp. and *Penicillum* spp. Kosubosu showed the presence of *Aspergillus parasiticus, Penicillium* spp. and *Aspergillus clavatus*. Kayama farm recorded the presence of *Fusarium* spp, *Aspergillus clavatus* and *Aspergillus fumigattus*. In Bakase, there was prevalence of *A. parasiticus*, *Penicillum spp, A. niger, A. fumigatus, and A. clavatu*s. Duduro had the highest number of fungal isolates, dominated by *A. parasiticus, A. fumigatus*, *A. clavatus*, *Fusarium* spp and *Penicillum spp*.

Based on morphological identification, Table 3 shows the percentage frequency of occurrence of various fungal across five farms. *Penicillium* species had the highest frequency occurrence of 26.92%, making it the most dominant fungal isolate while *A. clavatus, A. fumigatus, A. niger, Fusarium* species and *A. parasiticus* had the frequency occurrence of 15.39, 19.23, 7.69, 19.23, and 11.54% respectively.

Table 1: Fungal count obtained from *Digitaria exilis* Samples(x 10^4 cfu/g)

Villages	A	B	C	D	E
Guanara	4.80±0.00^{b}	4.83±0.02^{e}	5.33±0.03^{c}	4.6±0.01^{d}	2.93±0.04^{a}
Kosubosu	4.40±0.04^{ab}	3.77±0.01$_{b}$	4.20±0.00^{b}	4.23±0.03^{b}	3.87±0.07^{c}
Kayama	6.33±0.03^{c}	4.57±0.03^{d}	3.70±0.00^{a}	4.80±0.01^{e}	3.50±0.02^{b}
Bakase	4.30±0.00^{a}	3.63±0.04^{a}	3.73±0.03^{a}	4.50±0.03^{c}	4.37±0.03^{d}
Duduro	6.00±0.00^{c}	4.27±0.05^{c}	5.47±0.08^{c}	4.1±0.04^{a}	5.0±0.001^{e}

Means and Standard deviation with different subscript letter within the same column are significantly different (p<0.05) on Duncan's Multiple Range Test

Keys: A = lot A, B = lot B, C = lot C, D = lot D, E = lot E

Table 2: Morphological characteristics of fungi isolate from different locations

Codes	Morphological characteristics of Fungi Isolates	Suspected/probable Isolates
GU1	Blackish conidial heads	*Aspergillus niger*
GU2	Olive-green, yellowish-green colonies	*Aspergillus fumigatus*
GU3	White colonies with rose pigment in the centre.	*Fusarium spp*
GU4	Bluish green colonies	*Penicillum* spp
KO1	Dark green conidial heads	*Aspergillus parasiticus*
KO2	Heavily sporulating, blue or green colonies	*Penicillum* spp
KO3	Bluish-green to greyish-green with age.	*Aspergillus clavatus*
KO4	Grey-green colonies	*Penicillum* spp
KA1	Snow white colonies with rose pigment in the centre	*Fusarium* spp
KA2	Snow white colonies with rose pigment in the centre.	*Fusarium spp*
KA3	Yellowish-green colonies surrounded by a white circle	*Aspergillus clavatus*
KA4	Olive-green, yellowish-green colonies surrounded by a white circle	*Aspergillus fumigatus*
BA1	Bluish green colonies	*Penicillum* spp
BA2	Dark green conidial heads	*Aspergillus parasiticus*
BA3	Olive-green, yellowish-green colonies	*Aspergillus fumigatus*
BA4	Dark green conidial heads	*Aspergillus niger*
BA5	Bluish green colonies	*Penicillum* spp
BA6	bluish-green to greyish-green	*Aspergillus clavatus*
DU1	Olive-green, yellowish-green colonies	*Aspergillus* parasiticus
DU2	Dark green conidial heads	*Aspergillus fumigatus*
DU3	bluish-green to greyish-green	*Aspergillus clavatus*
DU4	Dark green conidial heads	*Aspergillus fumigatus*
DU5	Snow white colonies with rose pigment in the centre	*Fusarium* spp
DU6	Grey-green colonies	*Penicillum* spp
DU7	Grey-green colonies	*Penicillum* spp
DU8	Snow white colonies with rose pigment in the centre	*Fusarium* spp

Keys: Isolates from GU1-GU4 were from Guanara samples; KO1-KO4 were from Kosubosu samples; KA1-KA4 from Kayama samples; BA1-BA4 from Bakase samples; DU1-DU8 from Duduro samples

Table 3: Percentage Frequency of occurrence of Fungal isolates

Suspected Isolates	Guanara	Kosubosu	Kayama	Bakase	Duduro	Total Occurrence	Frequency (%)
Aspergillus clavatus	0	1	1	1	1	4	15.39
Aspergillus fumigatus	1	0	1	1	2	5	19.23
Aspergillus niger	1	0	0	1	0	2	7.69
Fusarium species	1	0	2	0	2	5	19.23
Penicillium species	1	2	0	2	2	7	26.92
Aspergillus. parasiticus	0	1	0	1	1	3	11.54
Total Occurrences	4	4	4	6	8	26	100

Genotypic identification of the fungi isolates

Figure 1 shows clear and unique bands of amplified DNA of isolates after gel electrophoresis. Unique bands were excised and sequenced. Sequences were extracted using bioEdit application, after which the extracted sequence were blasted in NCBI data base to find the result with the highest percentage similarity. The BLAST result with the highest percentage of similarity was submitted to the NCBI GenBank for accession number.

Table 4.4 presents the fungal strains identified in the study along with their respective NCBI GenBank accession numbers. *Aspergillus niger* coded GU2 and KO1 were assigned PQ686287 and PQ686291 respectively, while *Aspergillus clavatus* coded KO2, DU2, and KA1 were also assigned PQ686289, PV147260 and PV147261 respectively. Other *Aspergillus* species include *A. fumigatus* and *A. parasiticus, and A flavus* coded BA1 and KA2, KA3 and KA4 with accession numbers PQ686292 and PV175343, PV902476 and PV902475 respectively. Also, *Fusarium solani, Penicilium sabarticum* and *Penicillium chrysogenium* coded GU1, BA2 and DU1 were assigned accession numbers PQ686295, PV147258 and PV147259 respectively.

The phylogenetic relationship among the isolates is shown in Figure 2, *Aspergillus clavatus* (PV147260), *Aspergillus clavatus* (PV147261), *Aspergillus fumigatus* (PQ686292), *Aspergillus clavatus* (PQ686289), *Aspergillus niger* (PQ686291) and *Aspergillus niger* (PQ686287) principally cluster with the *Trichocomaceae* family, related to *Asoergillus fumigatus* (NG-242393.1), *Aspergillus fumigatus* (LC485158.1), *Aspergillus clavatus* (Ab002070.1), *Aspergillus clavatus* (NG-081374.1), *Aspergillus niger* (NG-065763*), Aspergillus niger* (MN420840.1) respectively. In Figure 3, *Aspergillus parasiticus* (PV175343) principally cluster with the *Trichocomaceae* family, related to *Aspergillus parasiticus* (HM802959.1). As shown in Figure 4, the *Aspergillus flavus* (PV902475) and *Aspergillus flavus* (PV902476) principally cluster with the *Trichocomaceae* family, related to *Aspergillus flavus* (OM240729) and *Aspergillus flavus* (PQ144089) respectively. *Penicillium subarcticum* (PV147258) and *Penicillium chrysogenium* (PV147259) principally cluster with the *Trichocomaceae* family, related to *Penicillium subarcticum* (MT365125.1), *Penicillium subarcticum* (NG-074919.1), *Penicillium chrysogenium* (MT649559.1) and *Penicillium chrysogenium* (NG-062803.1) respectively as shown in Figure 5. Figure 6 shows that *Fusarium solani* (PQ686295) principally cluster with the *Nectriaceae* family, related to *Fusarium solani* (MH300452.1) and *Fusarium solani* (MT453275.1). The isolates from *Digitaria exilis* (Acha) seed samples form a well-supported clade that is distinct but closely related to known reference strains obtained from NCBI.

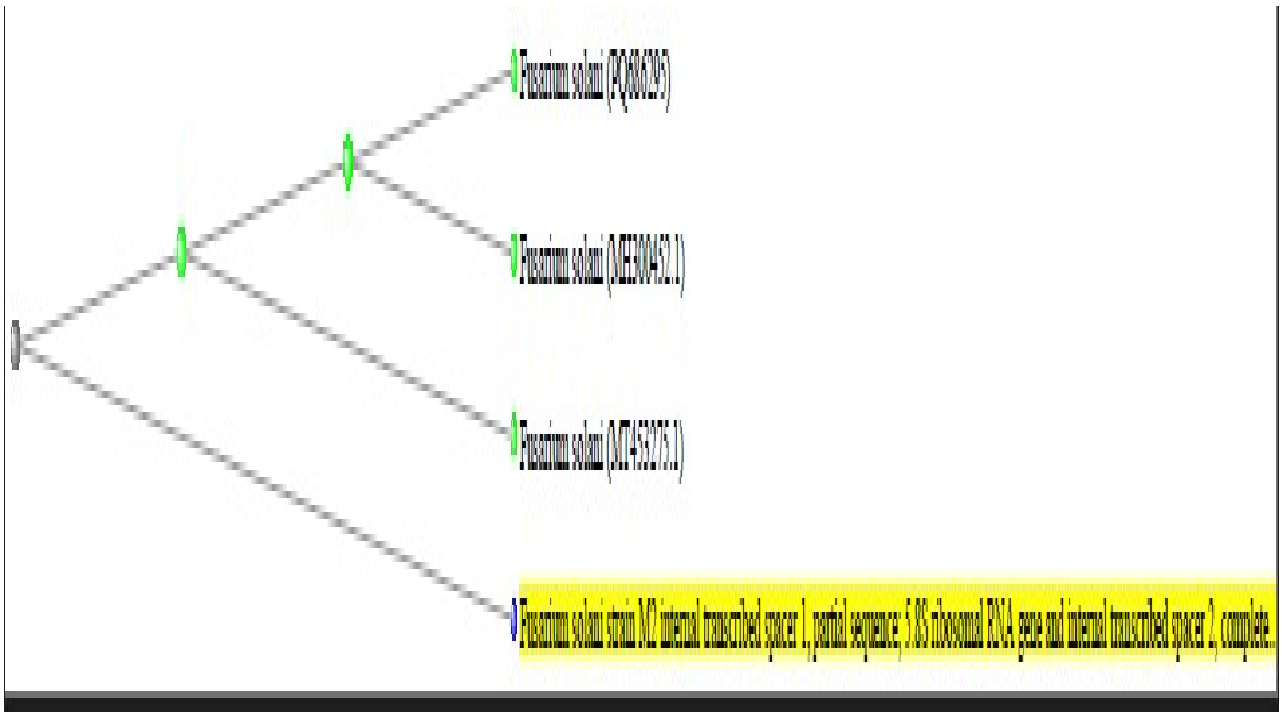

Figure 1: Amplified DNA of the Isolates

Key: M: DNA ladder; lanes 1 – 12 are GU1, GU2, KO1, KO2, BA1, BA2, DU1, DU2, KA1 and KA2 respectively**.** From GU1-GU4 were from Guanara samples; KO1-KO4 were from Kosubosu samples; KA1, KA2, KA3, KA4 from Kayama samples; BA1-BA4 from Bakase samples; DU1-DU8 from Duduro samples.

Table 4.4: Identified Fungal Strain with NCBI GenBank Ascension number

S/N.	Name.	Code	Accession numbers
1	*Fusarium solani*	GU1	PQ686295
2	*Aspergillus niger*	GU2	PQ686287
3	*Aspergillus niger*	KO1	PQ686291
4	*Aspergillus clavatus*	KO2	PQ686289
5	*Aspergillus fumigatus*	BA1	PQ686292
6	*Penicillium subarcticum*	BA2	PV147258
7	*Penicillium chrysogenium*	DU1	PV147259
8	*Aspergillus clavatus*	DU2	PV147260
9	*Aspergillus clavatus*	KA1	PV147261
10	*Aspergillus parasiticus*	KA2	PV175343
11	*Aspergillus flavus*	KA3	PV902476
12	*Aspergillus flavus*	KA4	PV902475

Keys: From GU1-GU2 were from Guanara samples; KO1-KO2 were from Kosubosu samples; BA1-BA2 from Bakase samples; DU1-DU2 from Duduro samples; KA1-KA2 from Kayama samples

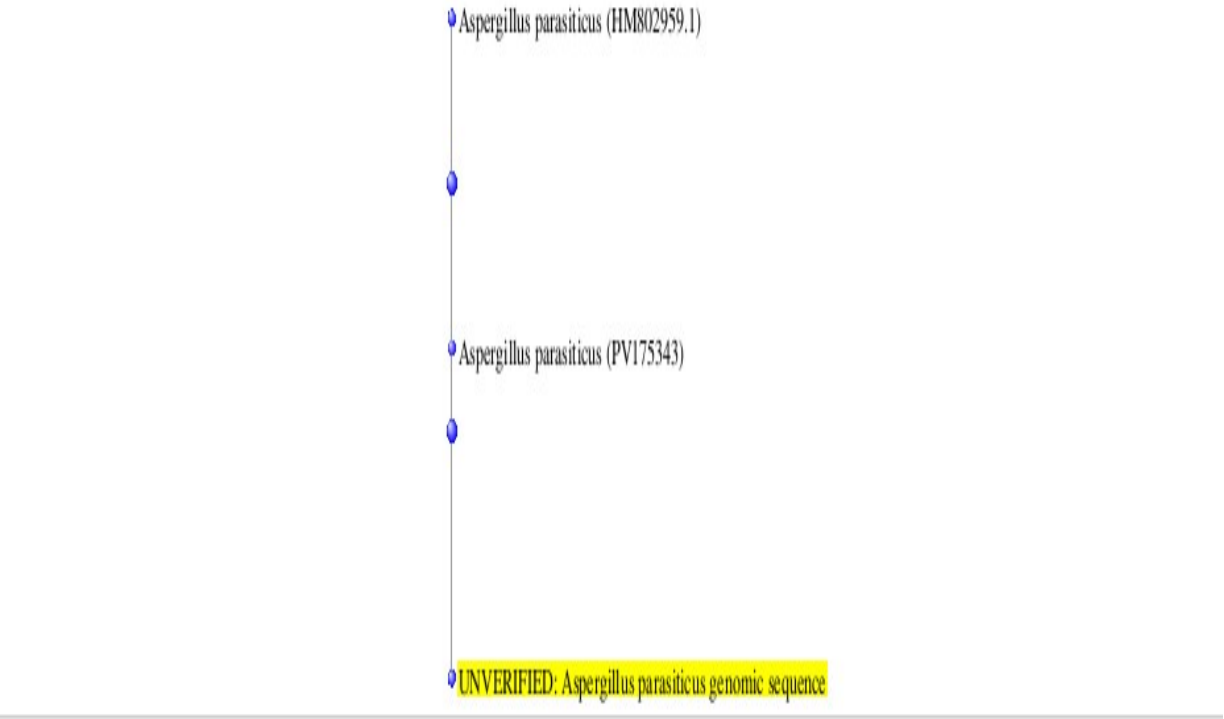

Figure 2: The phylogenetic tree showing the relationship between *Aspergillus clavatus* (PV147260), *Aspergillus clavatus* (PV147261), *Aspergillus fumigatus* (PQ686292), *Aspergillus clavatus* (PQ686289), *Aspergillus niger* (PQ686291) and *Aspergillus niger* (PQ686287) and other closely related species**.**

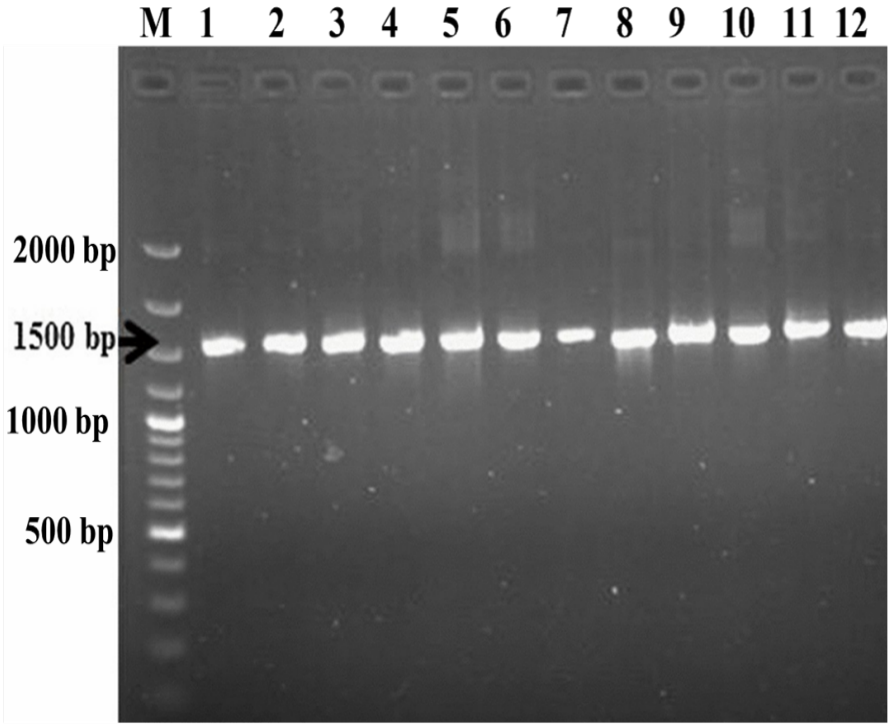

Figure 3: The phylogenetic tree showing the relationship between *Aspergillus parasiticus* (PV175343) and other closely related species.

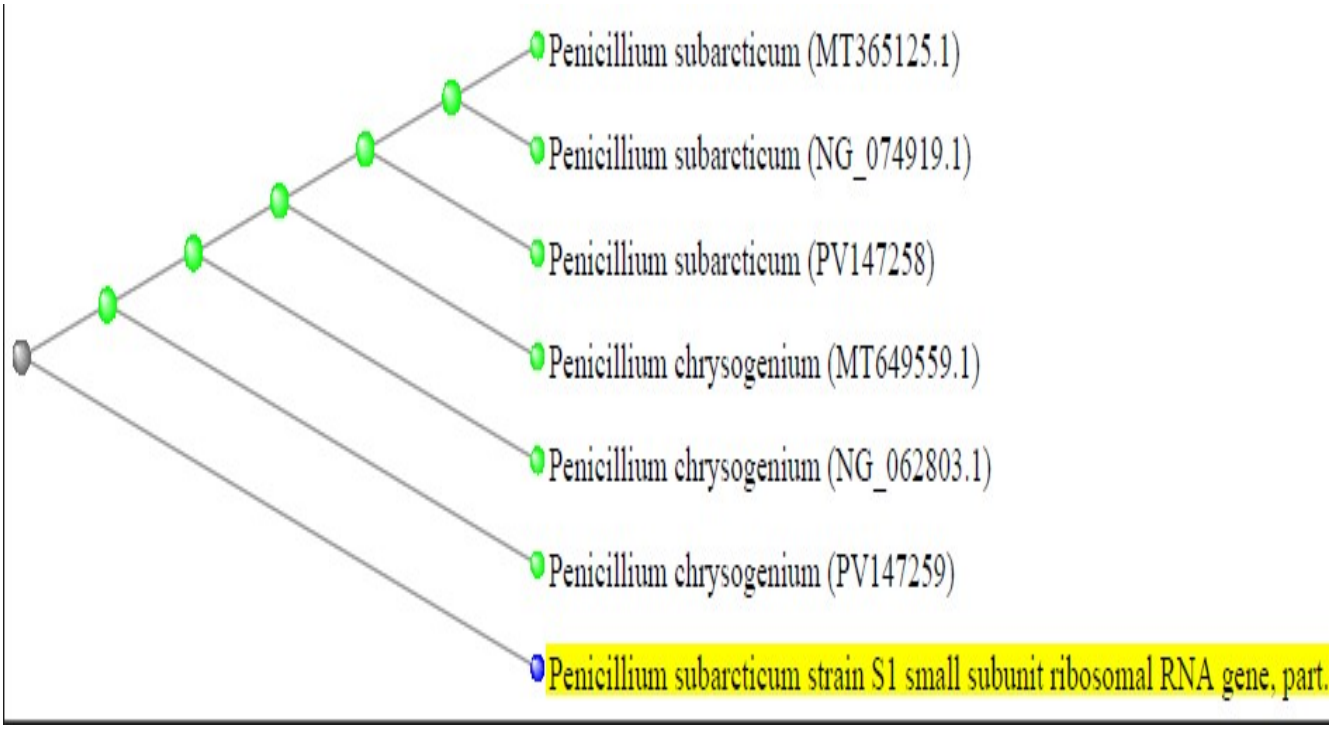

Figure 4: The phylogenetic tree showing the relationship between *Aspergillus flavus* (PV902475) and *Aspergillus flavus* (PV902476) and other closely related species**.**

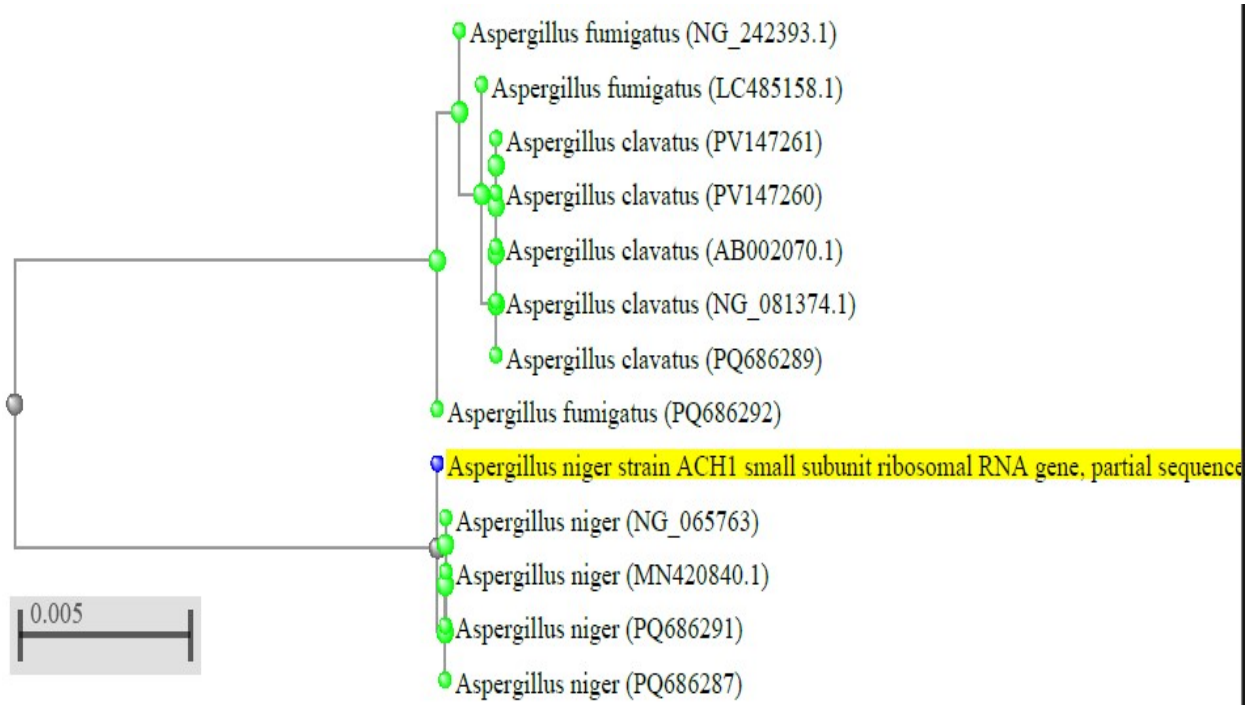

Figure 4.4: The phylogenetic tree showing the relationship between *Penicillium subarcticum* (PV147258) and *Penicillium chrysogenium* (PV147259) and other closely related species.

Figure 4.5: The phylogenetic tree showing the relationship between *Fusarium solani* (PQ686295) and other closely related species.

Discussion

In this study, total fungal count of the samples in Guanara, kosubusu, kayama, Bakase, and Duduro farms and lots were between 2.93±0.04x 10^4 and 6.33±0.03 x 10^4 CFU/g. The highest count was observed in samples from Kayama (A) reaching 6.33±0.03x 10^6 CFU/g and the lowest from Guanara (E) of 2.93±0.04 x 10^4 CFU/g. Audu *et al*. (2025) reported total fungal counts of 14.1 x 10^6 and 2.2 x 10^4 from stored maize grains in warehouses and local markets. Popoola *et al*. (2024) also reported the total fungal load of 7.1 x 10^4 and 9.8 x 10^4 in stored corn. According to Pudasaini (2024), a high fungal count indicates inconsistencies in the handling and storage of grains. According to the morphological identification, a total of 26 isolates were obtained from the different locations, with *Penicillium* species exhibiting the highest occurrence (26.92%), making it the most dominant microorganism which is in agreement with the report of Itlal *et al*. (2023) who also reported high occurrence of *Penicillium* spp (22.26%) in stored maize. Sultan *et al*. (2022) also reported high occurrence of *Aspergillus* sp. (44.8%) in sorghum. The high occurrence of *Penicillium* species in this study suggests that storage conditions in the study locations favour its growth, particularly in high-humidity environments as also confirmed by Kaur and Watson (2024). In this study, *Aspergillus fumigatus* (19.23%) and *Fusarium* species (19.23%) also showed significant prevalence, both of which different researches have been linked to mycotoxin contamination, posing serious food safety risks which is also in agreement with the reports of Audu *et al*. (2025) who reported high occurrence of *Aspergillus* (44.8%) in stored sorghum.

Farm-specific trends indicated that Duduro had the highest number of fungal isolates, implying that environmental conditions in that farm promoted fungal growth. In a similar study, Ezekiel *et al*. (2015) found that *Aspergillus* spp. dominated (46–48%), followed by *Fusarium* spp. (30–42%) and *Penicillium* spp. (11.5%) in *Digitaria exilis* samples. Makun *et al*. (2007) also isolated *Aspergillus, Penicillium*, and *Fusarium* species from *Digitaria exilis* samples in Nigeria.

In the study, after the BLAST search in NCBI data base, it was observed that *Fusarium solani* GU11 had 100% homology with *Fusarium solani* DSM100290 (MT453275) isolated from raw milk cheese in Denmark (Lopez-Gonzales *et al*., 2025). *Aspergillus niger* GU2 had 9.15% homology with *A. niger* ATCC 4356 (CP139575) isolated from stored seed samples in Biology - Plant Ecology department, Free University Berlin, Altensteinstrasse, 6, Berlin 14195, Germany (Lehmann *et al*., 2020). *Aspergillus niger* KO1 had 100% homology with *A. niger* ANN4 (MN420840) isolated from stored barley sample in School of Life Sciences, Forman Christian College, Lahore, Ferozpur road, Lahore 54000, Pakistan (Zahreen, 2019). *Aspergillus clavatus* KO2, *Aspergillus clavatus* DU2 and *Aspergillus clavatus* KA1 have 100% homology with *Aspergillus clavatus* 31MAY88 (OQ581775) isolated from Garri Processing Factory, Ilesha-Owo Expressway, Akure, Nigeria (Olusola-Makinde and Odubade, 2023). *Aspergillus fumigatus* BA1 had 100% homology with *Aspergillus fumigatus* FM324 (CP066505) isolated from stored barley in Institute of Biotechnology, Zhejiang University, 866 Yuhangtang Road, Hangzhou, Zhejiang 310058, China (Xu *et al*., 2020). *Penicillium subarcticum* BA2 had 100% homology with *Penicillium subarcticum* CBS1111719 (MT365125) isolated from stored corn samples in Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, Utrecht, Utrecht 3584CT, Netherlands (Houbraken *et al*., 2020). *Penicillium chrysogenium* DU1 has 100% homology with *Penicillium chrysogenium* PLN (ON490463) isolated from stored wheat sample in Botany Department, Faculty of Science, Mansoura University, Mansoura, Mansoura, Dakahlia 35516, Egypt (Sultan and El-Afify, 2022). *Aspergillus parasiticus* KA2 had 100% homology with *A. parasiticus* CBS 100926 (MF448472) isolated sored maize sample in Institut National de la Recherche Agronomique, 180 Chemin de Tournefeuille, Toulouse 31027, France (Carvajal-Campos *et al*., 2017). *Aspergillus flavus* KA3 and *Aspergillus flavus* KA4 had 100% homology with *A. flavus* MTCHT111 (OM240729) isolated from stored grain sample in Mar Thoma College, Tiruvalla, Kuttapuzha PO, Tiruvalla, Kerala 689103, India (Thomas, 2022).

According to the phylogenic analysis, *Aspergillus fumigatus* (PQ686292), *Aspergillus clavatus* (PQ686289), *Aspergillus niger* (PQ686291) and *Aspergillus niger* (PQ686287) principally cluster with the *Trichocomaceae* family, related to *Aspergillus fumigatus* NG-242393.1 (Il'iushin, 2024), *Aspergillus fumigatus* LC485158.1 (Mikawa *et al*., 2019), *Aspergillus clavatus* Ab002070.1 (Tamura and Sugiyama, 2008), *Aspergillus clavatus* NG-081374.1 (Tamura *et al*., 2022), *Aspergillus niger* NG-065763, and *Aspergillus niger* MN420840.1 (Zahreen, 2019) respectively. *Aspergillus parasiticus* (PV175343) principally cluster with the *Trichocomaceae* family, related to *Aspergillus parasiticus* HM802959.1 (Soares, 2021). *Aspergillus flavus* (PV902475) and *Aspergillus flavus* (PV902476) principally cluster with the *Trichocomaceae* family, related to *Aspergillus flavus* OM240729 (Thomas, 2022) and *Aspergillus flavus* PQ144089 (Pushpa *et al*., 2024) respectively. *Penicillium subarcticum* (PV147258) and *Penicillium chrysogenium* (PV147259) principally cluster with the *Trichocomaceae* family, related to *Penicillium subarcticum* MT365125.1, and *Penicillium subarcticum* NG-074919.1 (Houbraken *et al*., 2021) respectively. *Fusarium solani* (PQ686295) principally cluster with the *Nectriaceae* family, related to *Fusarium solani* MH300452.1 and *Fusarium solani* MT453275.1 (Santos *et al*., 2020).

Furthermore, Vashisht *et al*. (2023) reported that genotypic identification is a reliable method for characterizing microbial strains compared to traditional morphological techniques (Gil *et al*., 2023; Vashisht *et al*., 2023). The Genotypic identification is a reliable method for characterizing microbial strains compared to traditional morphological technique, Huang,G.,& Peng,X. (2024).

Conclusion

This comprehensive study on the characterization of fungal contamination in *Digitaria exilis* (Acha) from five farms in Kwara State, Nigeria, highlights critical food safety concerns that require immediate attention. Significant fungal loads ranging from $2.93 \pm 0.04 \times 10^4$ to $6.33 \pm 0.03 \times 10^4$ CFU/g were detected, dominated by *Penicillium* species (26.92%), *Aspergillus fumigatus* (19.23%), and *Fusarium* species (19.23%), underscoring the vulnerability of this indigenous grain to post-harvest contamination.

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