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Morphological and Molecular Characterization of Fungal Species Associated with *Fusarium oxysporum* f.sp. cubenese (Foc) in Gatundu North, Kenya

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ABSTRACT

Banana (*Musa* spp.) is the most produced fruit globally, serving as a vital food security crop and a rich source of nutrients. It is a staple food and a significant source of income for millions of people in tropical and subtropical regions. However, the current and future production of bananas is severely threatened by Fusarium wilt, also known as Panama disease, caused by *Fusarium oxysporum* f.sp. cubense (Foc). The banana rhizosphere hosts various fungal species that may be associated with the pathogenicity of Foc. This study characterized the fungal microbiome of bananas from three wards in Gatundu North, Kiambu County, Kenya. A total of 612 soil samples exhibiting symptoms of Fusarium wilt were collected from the sampling sites. Ninety-eight fungal species were isolated using serial dilution on potato dextrose agar (PDA) and subsequently characterized morphologically. DNA extraction from each species was performed using the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) (ITS1 and ITS4), followed by sequencing via Sanger sequencing. The sequences were compared to the GenBank database using the Basic Local Alignment Search Tool (BLAST), and a phylogenetic tree was constructed using the neighbor-joining method in MEGA X software (version 6.1). The characterized isolates belonged to the following genera: Fusarium, Penicillium, Paecilomyces, Rhizoctonia, Trichoderma, Simplicillium, Epicoccum, Curvularia, Alternaria, Bipolaris, Exserohilum, Setosphaeria, Cochliobolus, Syncephalastrum, and several unidentified species. This study identifies fungal species associated with the pathogenesis of Foc in bananas in Gatundu North, Kiambu County, Kenya, and highlights the potential for developing biocontrol strategies for managing Panama disease.

Abbreviations: Biocontrol Agent (BCA), Cetyltrimethylammonium bromide (CTAB), *Fusarium oxysporum f.sp. cubenese* (*Foc*), Fusarium Wilt of Banana (FWB), Institute of Biotechnology Research (IBR), Internal Transcribed Spacers (ITS), Molecular Evolutionary Genetic Analysis (MEGA), Polymerase Chain Reaction (PCR), Potato Dextrose Agar (PDA)

Introduction

Bananas (*Musa* spp.), also known as plantains, are the most popular and widely produced fruits globally, with an output of 155 million tons (FAO, 2020). They are rich in carbohydrates, vitamins A,

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B6, and C, and serve as an excellent source of potassium, calcium, magnesium, and dietary fiber (SHEP PLUS et al., 2019; Banana and Plantains, 2022). In the tropics and subtropics, bananas are a staple food consumed both cooked and fresh (Banana and Plantains, 2022), providing approximately 10-20% of the daily caloric intake for millions worldwide (Nayar, 2009). Beyond their role as a staple, bananas are a cash crop that generates essential income, contributing significantly to the economy (FAO, 2018). While the majority of the fruit is sold and consumed locally, an estimated 15% of global production is traded internationally (Banana and Plantains, 2022). A survey of smallholder farmers across at least 11 banana-producing countries indicated that the crop accounts for 75% of farmers' monthly earnings (FAO, 2018).

Diseases and pests have a devastating impact on bananas globally, affecting both pre- and post-harvest stages (Ploetz, 1998). Fusarium wilt of banana (FWB), also known as Panama disease, is the most damaging banana disease worldwide. It is caused by the soilborne hyphomycete fungus *Fusarium oxysporum* f.sp. *cubense* (Foc) (Maymon et al., 2020). Chlamydospores, the most critical survival structures of the pathogen, persist in the soil, where they can overwinter for many years or initiate the disease cycle immediately if conditions are favorable and a susceptible host is present; thus, the disease is polycyclic in nature (Ploetz and Pegg, 2000). FWB results in annual losses of 60-90% (Niwas et al., 2022) and an incidence rate exceeding 80% in the Gros Michel variety, which is popular in Eastern and Central Kenya (Njau et al., 2010). This disease threatens food security and income generation in affected areas, particularly in the tropics and subtropics. In some instances involving Foc Tropical Race 4 (TR4), entire farms may be abandoned, resulting in total yield loss and subsequent job loss, disruption of local markets, and supply chain instability (Montiflor et al., 2019; Viljoen et al., 2020).

Foc attacks the vascular tissues of bananas, leading to wilt, chlorosis, collapse, discoloration of the xylem, and eventual necrosis of the plant (Pegg et al., 2019). Species within the Fusarium genus are known to produce mycotoxins in crops, posing serious health risks to humans and animals (Vismer et al., 2019). The disease spreads both internationally and locally through infected and asymptomatic planting materials, surface water and irrigation runoff, contaminated tools, and the clothing and footwear of banana plantation workers, as well as via animals, infected dead leaves, and dust (Pegg et al., 2019). Due to its persistence in the soil, managing Foc is particularly challenging. The costs and complexities associated with various management strategies pose significant threats to the livelihoods of many poor, rural, smallholder farmers, who often lack the necessary resources and expertise (Acuña et al., 2022). Quarantine and exclusion measures are essential in areas free from *Foc* (Ploetz, 2015). Cultivating resistant varieties remains the most effective control method, though biocontrol, orchard sanitation, soil amendments, suppressive soils, and other cultural practices can help reduce disease spread (Ploetz, 2015).

This study aims to identify and characterize *Foc*-associated microbes that either enhance or inhibit its ability to cause Fusarium wilt of banana (FWB). Identifying all microorganisms involved is crucial for the diagnosis and management of diseases caused by *Foc*. Accurate diagnosis of *Foc*-associated fungal species is vital for finding and recommending appropriate solutions to farmers, as misdiagnosis can have severe consequences for both crops and farmers' livelihoods. Characterizing all fungal species associated with FWB may reveal beneficial fungi with antagonistic properties against *Foc*. Proper identification of these fungal pathogens is essential for developing new control strategies for the disease.

Materials and Methods

Sampling sites and sample collection

Soil samples were collected from various sites across Chania, Gituamba, and Mang'u wards in Gatundu North, Kiambu County, Kenya. Purposive sampling was employed to select farms and collect samples. The samples were primarily taken from the rhizosphere of bananas exhibiting symptoms of Fusarium wilt (FWB) as well as from healthy banana plants, at a depth of 25 cm, as outlined by Pérez-Vicente et al. (2014). Bananas affected by FWB were identified based on their external symptoms. A total of 200 g of soil samples were collected and stored separately in sterilized paper bags, which were labeled with the farmer's name, the village, and the ward of origin. The samples were kept in a cool box at 4 °C during field collection and were subsequently transported to the Institute for Biotechnology Research (IBR) laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT) for further analysis.

Fungal isolation and morphological characterization

Fungal isolation was done using potato dextrose agar (PDA) (Himedia; composition: Potatoes (200 g L^{-1}), Dextrose (20 g L^{-1}), and Agar(15 g L^{-1})

media supplemented with an antibiotic, streptomycin (100 mg L^{-1}) which suppressed the growth of bacteria (Gil-Serna et al., 2009). One g of each sample was placed in individual universal bottles containing 9 mL of distilled water and serially diluted to dilution to 10^6. Each dilution was pipetted onto the PDA medium in a quantity of 20 µL and spread using a sterilized glass spreader. They were placed in an incubator at a temperature of 28 °C for 7–10 d before fungal colonies formed. These colonies were subcultured on isolation media for a further 14 d and then colony morphology and conidial patterns were observed under a simple light microscope (40X) according to Leck (1999). Morphological traits were subsequently evaluated in accordance with prior assessments made by Leslie and Summerell (2006) and Woudenberg et al. (2013).

Molecular characterization

Single spore isolation in preparation for the extraction of fungal DNA was done in accordance with a protocol described by Pérez-Vicente et al. (2014).

DNA extraction

The CTAB protocol, as described by Qadri et al. (2013), was employed for fungal genomic DNA extraction using mycelium from individual fungal isolates. A total of 500 mg of fungal mycelium was freeze-dried and lysed in 10 mL of extraction buffer (2X buffered CTAB solution: 2% CTAB (w/v), 100 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl). The mycelium was ground using the melted tip of a 1 mL Eppendorf tube. The samples were incubated in a thermostatic water bath at 65°C for 1 hour. Following lysis, the lysate was extracted with an equal volume of chloroform:isoamyl alcohol (24:1), and centrifuged at 3,500 rpm for 5 minutes at 4°C. The chloroform phase was discarded, and 400 µL of the upper aqueous phase containing nucleic acids was transferred to a sterile 1.5 mL microcentrifuge tube. Genomic DNA was precipitated with chilled isopropanol and centrifuged at 3,500 rpm for 20 minutes at 4°C. The resulting DNA pellet was rinsed twice with 70% ethanol, air-dried for 25 minutes, and resuspended in Tris-EDTA buffer.

Polymerase chain reaction (PCR) amplification

PCR amplification of fungal DNA was performed using the internal transcribed spacer (ITS) rDNA regions with two primer sets: ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR mixture consisted of 1× PCR buffer, 0.5 mM $MgCl_2$, 2.5 U Taq DNA polymerase (QIAGEN, Germantown, MD, USA), 0.25 mM dNTPs, 0.5 µM of each primer, and approximately 5 ng of extracted genomic DNA. PCR was carried out using a DNA Engine Thermal Cycler (PTC-200, BIO-RAD, USA) under the following conditions: an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR products were visualized on a 1% agarose gel, and the expected product sizes were confirmed. The samples were subsequently sent to Macrogen (The Netherlands) for Sanger sequencing (Clark et al., 2019).

Data analysis

The Basic Local Alignment Search Tool (BLAST) was used to compare the ITS sequences to those in the GenBank database to identify similarities. Sequence alignment was performed using the ClustalX 1.8 software program (http://www.clustal.org/clustal2). Phylogenetic tree construction was carried out using MEGA X v6.1 software (Molecular Evolutionary Genetic Analysis), employing the neighbor-joining method with confidence levels assessed through bootstrap analysis (1,000 replicates). The hierarchical clustergram of the different isolates was generated using R software (version 4.3.2).

Results

Isolation of fungi per ward

A total of 612 samples were collected from three wards of Gatundu North during the period of August – September 2023 (Fig. 1). As shown in Figure 2, Gituamba (81%) has the highest amount of banana rhizospheric samples as compared to Mang'u (14%) and Chania (5%).

Morphological Characteristics of the Fungal Isolates

A total of 98 species were isolated. The microbes associated with *Fusarium oxysporum* f. sp. *cubense* (Foc) were identified both macroscopically and microscopically, following the protocols of Leslie and Summerell (2006) and Woudenberg et al. (2013). Observations were made at 40X magnification using a light microscope. Ninety-eight (98) isolates were recovered from 612 soil samples collected from the three wards of Gatundu North, Kiambu County: Gituamba (62%), Mang'u (24%), and Chania (14%). The cultural and microscopic characteristics of the isolates were observed after

culturing and subculturing on PDA media, as shown in Table 1.

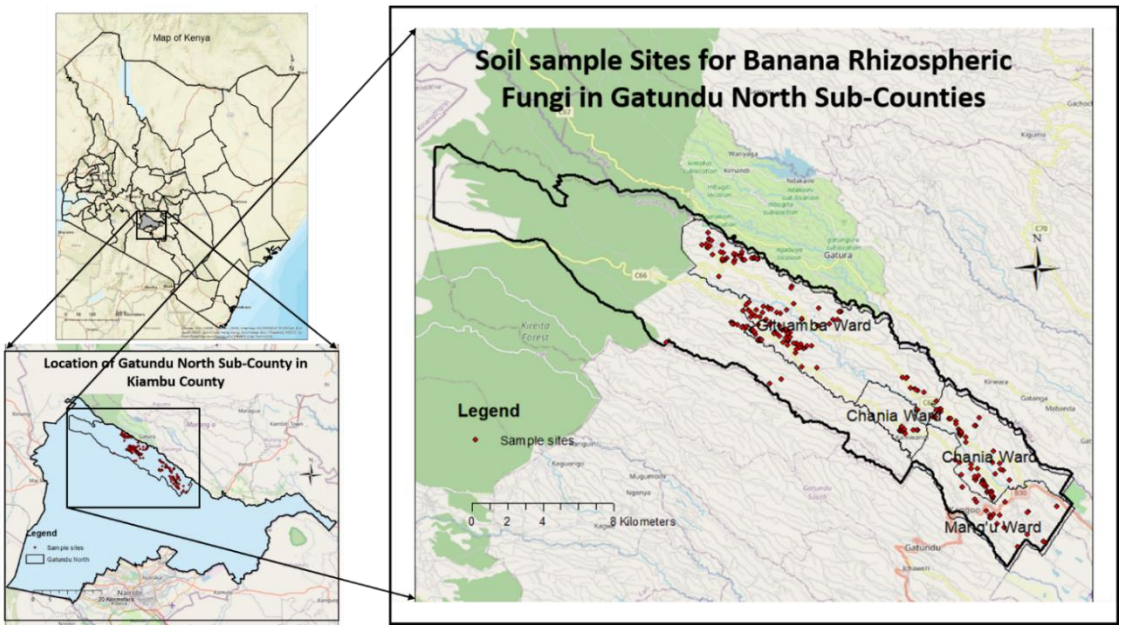

Fig. 1. Map of Gatundu North, Kiambu County, Kenya depicting Sites of Soil Samples Collection.

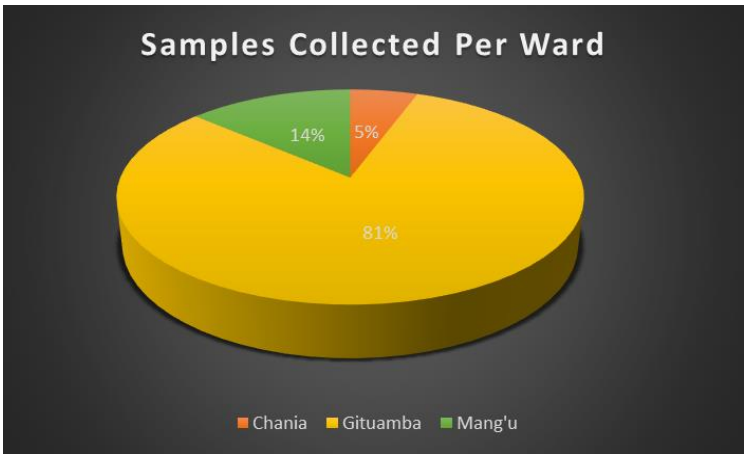

Fig. 2. The composition of number of samples obtained from banana soil farms per ward.

Culturally, the dominant colony colors included gray (29), pink (17), grayish-brown (15), white (11), grayish-black (8), and whitish-gray (8) (Table 1, Fig. 3). Table 1 also highlights variations in elevation and margin among the isolates. The observed margins included entire, rhizoid, undulate, filiform, and ciliate, while the elevations were raised, flat, and umbonate. Five common conidial shapes were identified: globose (31), cylindrical (24), round (16), elliptical (15), and irregular (12). Some isolates had smooth conidial surfaces, while others were rough. The conidial patterns were used to generate the hierarchical clustergram (Fig. 4). Fungal isolates exhibiting similar macroscopic and conidial characteristics were grouped into the same genus (Table 1).

However, relying solely on morphological characterization carries a high risk of misidentification and incorrect genus assignment. Therefore, molecular characterization of the isolates is essential to confirm their identities.

Table 1. Morphological characteristics of isolates.

		Mycelial Features					
Isolate Codes	**Conidia patterns**	**Colours**	**Elevation**	**Margin**	**Closet Relative in BLAST**	**Homology (%)**	**Accession numbers**

F-1	G	Dark grey	Raised	Filiform	*Fusarium sp.*	99	
F-2	G	Black	Flat	Filiform	*Syncephalastrum sp.*	99	
F-3	G	White	Raised	Rhizoid	*Alternaria sp.*	92	
F-4	R	White	Raised	Filiform	*Alternaria sp.*	92	
F-5	G	Whitish grey	Flat	Undulate	*Fusarium sp.*	95	
F-6	G	White	Raised	Entire	*Exserohilum sp.*	92	
F-7	E	Greyish brown	Umbonate	Filiform	*Simplicilium sp.*	92	
F-8	C	White	Raised	Undulate	*Curvularia sp.*	97	
F-9	G	Greyish brown	Flat	Undulate	*Curvularia sp.*	97	
F-10	G	Grey	Raised	Entire			
F-11	G	Pink	Raised	Entire	*Fusarium annulatum*	95	FJ527683.1
F-12	G	Greyish brown	Raised	Filiform	*Epicoccum sp.*	92	
F-13	G	Pink	Raised	Ciliate			
F-14	I	Greyish brown	Raised	Undulate	*Epicoccum sp.*	92	
F-15	G	Grey	Flat	Filiform	*Cochilobolus sp*	99	KM246063.1
F-16	E	Grey	Raised	Filiform	*Curvularia cymbopogonis*	99	LT631387.1
F-17	R	Greyish brown	Raised	Undulate			
F-18	C	Grey	Raised	Entire			
F-19	I	Whitish grey	Raised	Undulate	*Fusarium sp.*	95	
F-20	R	Pink	Raised	Undulate			
F-21	C	Pink	Raised	Undulate			
F-22	I	Pink	Raised	Rhizoid	*Fusarium verticillioides*	95	MF348253.1
F-23	R	Grey	Raised	Entire			
F-24	I	Grey	Raised	Entire			
F-25	I	Grey	Raised	Entire			
F-26	R	Purplish	Raised	Entire	*Fusarium oxysporum*	99	MN449465.1
F-27	R	White	Raised	Entire	*Fusarium sp.*	95	
F-28	I	Grey	Raised	Entire	*Fusarium sp.*	95	
F-29	I	Greyish brown	Raised	Entire	*Curvularia sp.*	97	
F-30	E	Brown-green	Raised	Entire	*Trichoderma sp.*	97	HE820756.1
F-31	G	Greyish brown	Flat	Entire	*Paecilomyces dactylrthromorphus*	99	NR_149330.1
F-32	R	Black	Raised	Entire	*Alternaria alstromariae*	92	NG0698882.1
F-33	C	Whitish grey	Raised	Entire	*Curvularia sp.*	97	
F-34	R	Pink	Raised	Entire			
F-35	G	White	Raised	Rhizoid	*Curvularia sp.*	97	
F-36	E	Grey	Raised	Entire			
F-37	R	White	Raised	Rhizoid	*Fusarium oxysporum f.sp. spinacia*	99	FJ972801.1
F-38	C	Pink	Raised	Undulate			
F-39	C	Greyish black	Flat	Entire	*Curvularia sp.*	97	
F-40	C	Greyish black	Raised	Entire	*Setosphaeria turcica*		LT883410.1
F-41	G	White	Raised	Entire	*Trichoderma sp.*	97	
F-42	C	Whitish grey	Raised	Entire	*Fusarium napiforme*	95	MH862670.1
F-43	C	Whitish grey	Raised	Entire	*Trichoderma atroviride*	97	EU715667.1
F-44	C	Whitish grey	Raised	Entire	*Curvularia lunata*	99	MT516307.1
F-45	C	Greyish brown	Raised	Entire	*Simplicillium sp.*	92	
F-46	G	Greyish brown	Raised	Entire	*Curvularia lunata*	99	MT516304.1
F-47	G	Grey	Raised	Filiform			
F-48	I	Grey	Raised	Entire	*Simplicillium sp.*	92	
F-49	E	Greyish black	Raised	Entire	*Curvularia sp*	99	MH885323.1
F-50	E	Greyish black	Raised	Entire			
F-51	E	Greyish black	Raised	Entire	*Curvularia sp.*	97	
F-52	E	Greyish black	Raised	Entire	*Fusarium sp.*	99	
F-53	E	Grey	Raised	Entire			
F-54	E	Black	Flat	Entire	*Epicoccum sp.*	92	
F-55	C	Grey	Raised	Entire	*Epiccocum latusicollum*	99	MT023664.1

F-56	G	Grey	Raised	Entire	*Fusarium sp.*	99	
F-57	G	Greyish brown	Raised	Filiform	*Simplicillium sp.*	92	
F-58	I	Grey	Raised	Entire	*Penicillium sp.*	99	
F-59	G	Greyish black	Raised	Entire	*Alternaria sp.*	92	MT555067.1
F-60	R	Pink	Raised	Entire	*Paecilomyces sp.*	99	MZ890180.1
F-61	C	Greyish brown	Raised	Filiform	*Penicillium sp.*	99	
F-62	R	Pink	Raised	Ciliate	*Fusarium oxysporum f.sp. alli*	99	FJ985495.1
F-63	C	Greyish brown	Raised	Undulate	*Bipolaris pluriseptata*	97	MH876119.1
F-64	E	Grey	Raised	Entire	*Exserohilum rostratum*	97	MT416021.1
F-65	R	Grey	Raised	Filiform	*Curvularia australis*	99	LT715587.1
F-66	C	Greyish brown	Raised	Undulate	*Alternaria burnsii*	92	NG069257.1
F-67	G	Grey	Raised	Entire			
F-68	C	Whitish grey	Raised	Undulate			
F-69	C	Pink	Raised	Undulate			
F-70	C	Pink	Raised	Undulate			
F-71	G	Pink	Raised	Rhizoid	*Fusarium oxysporum f.sp. cyclaminis*	99	KU128992.1
F-72	C	Grey	Raised	Entire			
F-73	G	Pink	Raised	Entire	*Fusarium oxysporum f.sp.cucumerinum*	99	AB106052.1
F-74	G	White	Raised	Entire	*Penicillium menonorum*	94	NR_137063.1
F-75	G	Grey	Raised	Rhizoid	*Simplicillium lamellicola*	92	MH871532.1
F-76	I	White	Raised	Entire	*Trichoderma atroviride*	97	MG309711.1
F-77	E	Green	Flat	Undulate	*Trichoderma atroviride*	92	MH398583.1
F-78	G	White	Raised	Filiform	*Simplicillium lamellicola*		MH866307.1
F-79	R	Grey	Raised	Entire	*Rhizoctonia sp*	93	JQ859886.1
F-80	C	Greyish black	Raised	Entire	*Syncephalastrum racemosus*	99	AF043516.1
F-81	R	Grey	Raised	Undulate	*Penicillium menonoruminternal*	99	HQ646591.1
F-82	C	Grey	Raised	Undulate			
F-83	E	Grey	Raised	Entire	*Exserohilum rostratum*	97	MT416021.1
F-84	R	Pink	Raised	Entire	*Fusarium pseudonygamai*	95	NG_069846.1
F-85	C	Greyish brown	Raised	Filiform	*Bipolaris secalis*	97	MH876123.1
F-86	G	Pink	Raised	Ciliate	*Penicillium vinaceum*	94	NR_121242.1
F-87	C	Greyish brown	Raised	Undulate			
F-88	R	Grey	Raised	Entire			
F-89	C	Grey	Raised	Filiform	*Curvularia akaiiensis*	99	MW644950.1
F-90	E	Greyish brown	Raised	Undulate	*Bipolaris secalis*	97	KU554626.1
F-91	C	Grey	Raised	Entire			
F-92	G	Whitish grey	Raised	Undulate	*Rhizoctonia fusispora*	93	MT775823.1
F-93	G	White	Flat	Undulate	*Epicoccum thailandicum*	99	NG_069435.1
F-94	G	Pink	Raised	Undulate			
F-95	G	Green	Raised	Undulate	*Trichoderma harzianum*	97	MK913350.1
F-96	I	Pink	Raised	Rhizoid			
F-97	G	Grey	Raised	Entire			
F-98	E	Purplish	Raised	Entire	*Epicoccum sorghinum*	99	MK516207.1

Key: (G) Globose; (R) Round; (E) Elliptical; (C) Cylindrical; (I) Irregular.

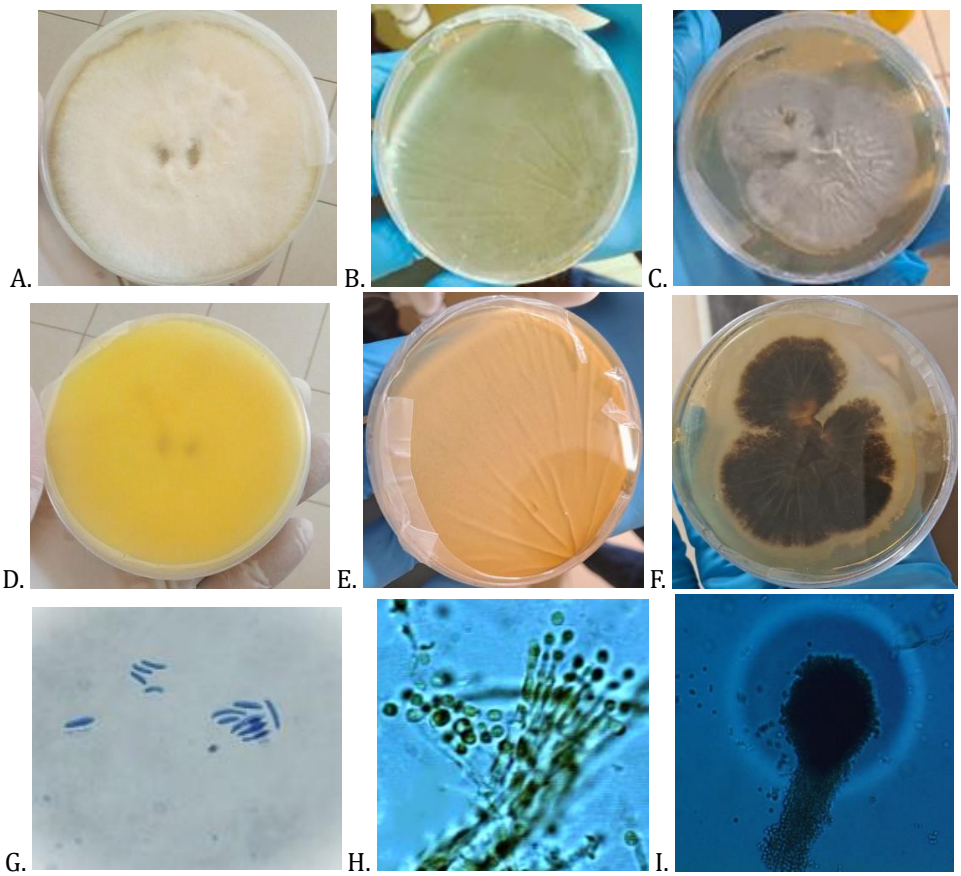

Fig. 3. Description of mycelial features and conidia: A. White, entire and raised, (D) obverse: yellow; B. Green, undulate and raised, (E) obverse: brown; C. Grey, filiform and flat, (F) obverse: black; G. Oval/kidney-shaped microconidia; H. Branched conidiophore with globose conidia; I. Round conidia.

Molecular characterization of fungal isolates

Ninety-eight fungal isolates were used to extract genomic DNA for molecular analysis and sequencing. Molecular characterization of the fungal isolates was performed through direct sequencing by Macrogen (The Netherlands), using PCR-amplified ITS fragments from each isolate. The ITS region sequences showed 94% to 99% similarity with sequences in GenBank (Fig. 5). The fungal isolates were classified into three divisions: *Ascomycota*, *Basidiomycota*, and *Zygomycota*. Isolates from the *Ascomycota* division were categorized into the classes *Sordariomycetes*, *Eurotiomycetes*, and *Dothideomycetes*. Isolates from *Basidiomycota* and *Zygomycota* were classified into the classes Agaricomycetes and Zygomycetes, respectively.

The isolates were subsequently grouped into 14 genera and identified as belonging to the following species: *Fusarium oxysporum* sp., *Fusarium oxysporum, Fusarium oxysporum* f. sp. *spinaciae, Fusarium oxysporum* f. sp. *alli, Fusarium oxysporum* f. sp. *cyclaminis, Fusarium napiforme, Fusarium annulatum, Fusarium verticillioides, Fusarium pseudonygamai; Penicillium vinaceum, Penicillium menonorum, Penicillium citrinum, Penicillium* sp.; *Paecilomyces dactylethromorphus, Paecilomyces* sp.; *Rhizoctonia fusispora, Rhizoctonia* sp.; *Trichoderma harzianum, Trichoderma atroviride; Simplicillium lamelicola; Epicoccum sorghinum, Epicoccum tatuslicccum, Epicoccum thailandicum; Curvularia australis, Curvularia lunata, Curvularia cymbopogonis, Curvularia* sp., *Curvularia akaiiensis; Alternaria* sp., *Alternaria burnsii, Alternaria alstroemriae; Bipolaris pluriseptata, Bipolaris secalis; Exserohilum rostratum; Setosphaeria turcica; Cochliobolus* sp.; and *Syncephalastrum racemosum.*

Discussion

This study aimed to characterize fungal species associated with *Fusarium oxysporum* f. sp. *cubense* (Foc), the causal agent of Fusarium wilt of banana (FWB). Identifying and characterizing these fungal species is crucial for developing effective management strategies for FWB.

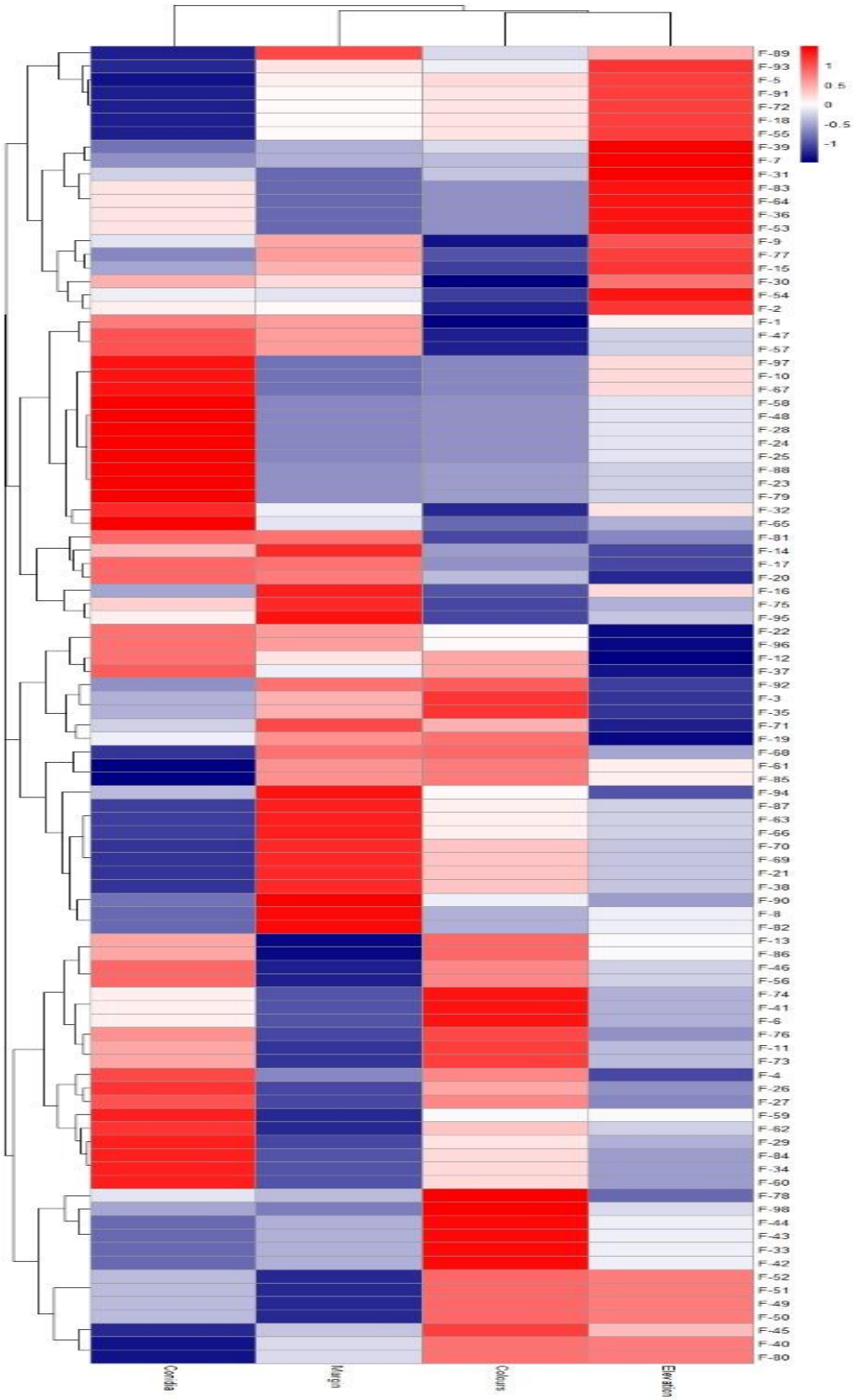

Fig. 4. Heatmap (Hierarchical clustering) showing the morphological characteristics (conidial pattern, margin, colors and elevation) of the 98 isolates.

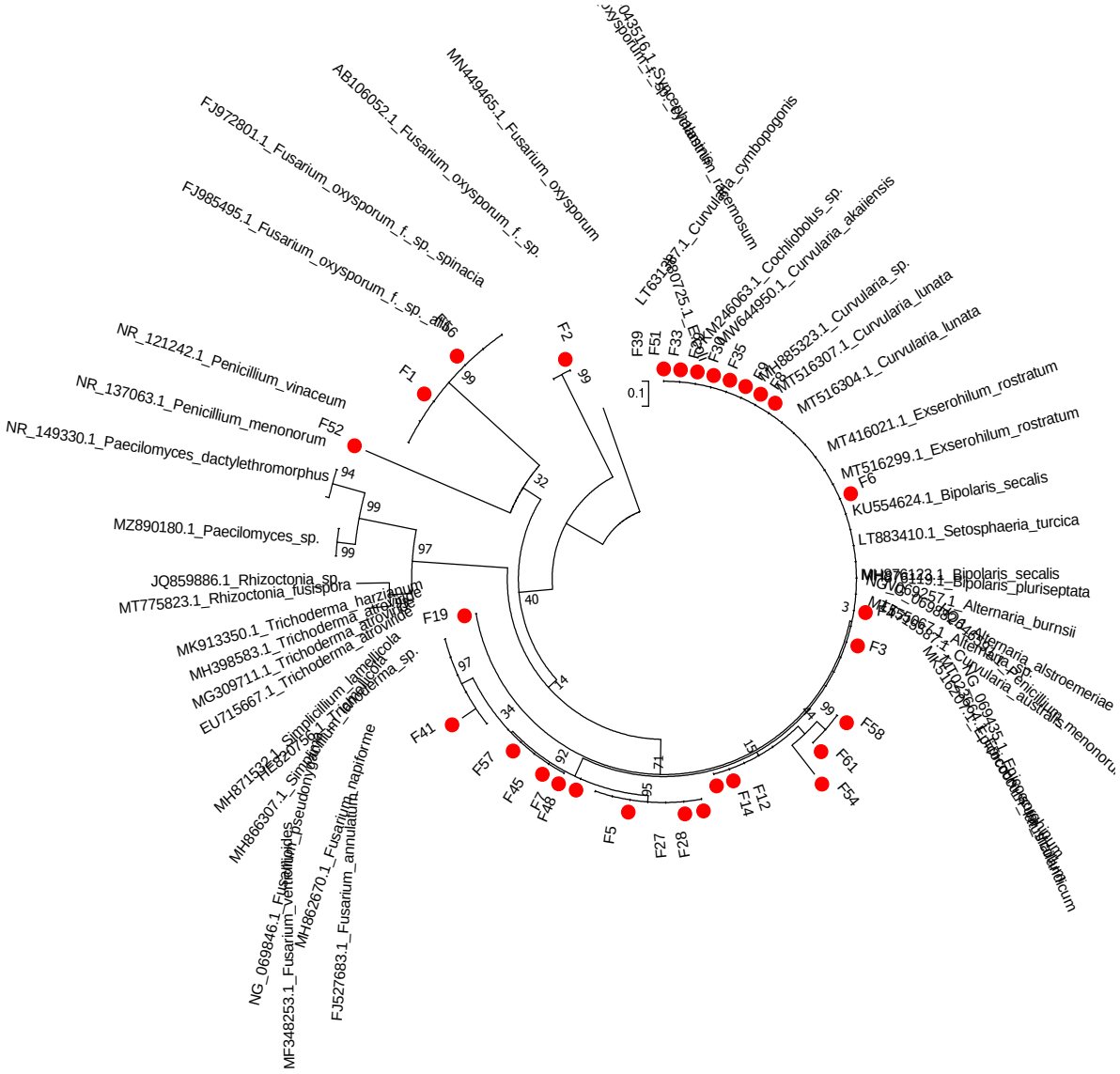

Fig. 5. The evolutionary history of tested fungal based on ITS sequences using the maximum likelihood method based on the Kimura-2 parameter model.

Morphologically, 98 fungal species were characterized, and genomic DNA was extracted using the ITS regions amplified by the primers ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Molecular analysis revealed 43 fungal species coexisting with Foc in producing FWB, along with 28 additional genera (unidentified at the species level, but identified by their proximity to known species and genera). A total of 27 unidentified species were also recovered during the study.

Fusarium is a fungal genus (Division: *Ascomycota*; Class: *Sordariomycetes*; Family: *Nectriaceae*) comprising over 1,500 species. Members of this genus are ubiquitous, soilborne pathogens responsible for diseases that pose significant challenges to global food production and are costly to manage (Arie, 2019). Certain *Fusarium* species are known to produce mycotoxins, which contaminate grains such as millet, rendering them unsuitable for human consumption (Vismer et al., 2019). *Fusarium* species thrive in diverse climates and soils, with a wide range of host plants including bananas, tomatoes, cabbage, onions, and eggplants (Arie, 2019).

In this study, the following *Fusarium* species were identified: *F. napiforme*, *F. annulatum*, *F. pseudonygamai*, *F. verticillioides*, *F. oxysporum*, *F. oxysporum* f. sp. *alli*, *F. oxysporum* f. sp. *spinaciae*, and *F. oxysporum* f. sp. *cyclaminis* (along with six unidentified *Fusarium* species).

Fusarium annulatum (previously known as *F. proliferatum*) is a morphologically and genetically diverse species within the *Fusarium fujikuroi* species complex (FFSC) and is documented as a pathogen for over 200 plant hosts (Yilmaz et al., 2021). *F. verticillioides* is a significant fungal pathogen of maize globally (Omotayo and Babalola, 2023). *F. pseudonygamai* has been linked to mycotoxin production in maize, millet, and sorghum (Vismer et al., 2019). *F. oxysporum* f. sp. *spinaciae* causes Fusarium wilt of spinach, severely limiting vegetable production in tropical and acidic soils (Batson et al., 2023).

With approximately 75 species, the genus *Curvularia* (Division: Ascomycota; Class: Dothideomycetes; Family: Pleosporaceae) comprises endophytes, pathogens, and saprophytes, most of which are facultative phytopathogens that cause yield losses in commercially important agricultural crops at both pre- and post-harvest stages. These fungi produce mycotoxins and can occasionally cause infections in vertebrates (Manamgoda et al., 2012). In this study, *Curvularia lunata*, a ubiquitous and damaging phytopathogen, was the most frequently isolated species. Although it is a rare cause of human infections, *C. lunata* is known to cause leaf spot in maize and banana (Chowhan and Chakraborty, 2023; Zhang et al., 2023). This is plausible, given that maize was cultivated near the banana plants. Other *Curvularia* species associated with Fusarium wilt of banana (FWB) identified in this study included *C. australis*, *C. akaiiensis*, *C. cymbopogonis*, and eight unidentified species.

C. cymbopogonis, a fungal pathogen of crops in the Andropogoneae tribe (Family: Poaceae), has a global distribution, though it is most commonly found across Africa, the Americas, the Indian subcontinent, and Asia (CAB International, 1998; Manamgoda, 2015). It is the causal agent of leaf spots on citronella (*Cymbopogon nardus*) and lemongrass (*C. citratus*), as well as seed and seedling blights (CAB International, 1998). Sari et al. (2023) identified *C. akaiiensis* as the causal agent of leaf spot disease on vetiver (*Vetiveria zizanoides*).

With around 350 species, the genus *Penicillium* (Class: Eurotiomycetes; Family: Aspergillaceae) consists of globally distributed soil-dwelling fungi that play a significant role in agriculture, acting as both decomposers and causal agents of various crop diseases (Visagie et al., 2014). Species within this genus are also well-known for their ability to produce commercially valuable secondary metabolites, including hormones, antibiotics, alkaloids, and mycotoxins (Shahid et al., 2020). In this study, *Penicillium vinaceum* and *P. menorum*, the latter a non-pathogenic species with plant growth-promoting properties (Babu et al., 2015), were isolated, along with two unidentified *Penicillium* species.

The genus *Paecilomyces* (Class: Eurotiomycetes; Family: Thermoascaceae), comprising over 100 species, is another soil-borne ascomycete. Many species within this genus are phytopathogenic, though some have also been implicated in causing infections in humans and other vertebrates (Senthilkumar et al., 2020). *Paecilomyces* is saprobic and nematophagous, frequently isolated from soil, decaying plant material, insects, nematodes, and laboratory air (as a contaminant). It is morphologically distinguished from *Penicillium* by its lack of green colonies and its phialides' cylindrical base and elongated neck (Senthilkumar et al., 2020). Certain *Paecilomyces* species have been employed as biocontrol agents for pathogens and pests of agricultural crops, particularly in the control of plant-parasitic nematodes (FAO, 2024; Senthilkumar et al., 2020).

Rhizoctonia (Division: Basidiomycota; Class: Agaricomycetes; Family: Ceratobasidiaceae) is a ubiquitous soil-dwelling genus comprising opportunistic phytopathogens with a broad host range (Roberts, 1999). The most well-known species, *Rhizoctonia solani*, causes sheath blight in rice (Senapati et al., 2022), as well as root rots, crown rots, seed rots, and stem canker in crops from several plant families, including Fabaceae, Solanaceae, Poaceae, Amaranthaceae, Brassicaceae, and Rubiaceae (Ajayi-Oyetunde and Bradley, 2017). In this study, *Rhizoctonia fusispora* and an unidentified *Rhizoctonia* species were isolated.

The genus *Trichoderma* (Division: Ascomycota; Class: Sordariomycetes; Family: Hypocreaceae) is globally distributed and plays an important role as a biocontrol agent (BCA) against various phytopathogens (Woo et al., 2023). Besides controlling soil-borne diseases, *Trichoderma* species also enhance plant resilience, promote faster growth, improve nutrient uptake, and mitigate environmental contamination from pesticides (FAO, 2024). In this study, *Trichoderma harzianum*, *T. atroviride*, and three unidentified *Trichoderma* species were isolated. *T. harzianum* is well known for its mycoparasitic properties and effectiveness as a BCA against several phytopathogenic fungi, including *Fusarium* species (Pani et al., 2021), while *T. atroviride* is employed in the biocontrol of both soil- and airborne phytopathogenic fungi (Brunner et al., 2005).

The genus *Epicoccum* (Division: Ascomycota;

Class: Dothideomycetes) consists of cosmopolitan, ubiquitous fungi found in wood, soil, and on plants, where they interact with both endophytes and pathogens (Del Frari et al., 2019). Several species of *Epicoccum* are associated with grapevine trunk diseases (GTD). In this study, *Epicoccum sorghinum*, *E. thailandicum*, *E. latusicollum*, and three unidentified *Epicoccum* species were isolated. *E. sorghinum* is a facultative phytopathogen of grasses (Family: Poaceae), particularly those cultivated in tropical regions, and is known to cause leaf spot in various plants (Oliveira et al., 2018). *E. thailandicum* has been identified as the causal agent of leaf disease on *Amomum villosum* in China (Wang et al., 2023).

The genus *Simplicillium* (Family: Cordycipitaceae; Division: Ascomycota) includes widely distributed species with a broad host range. Certain species play a role in biological control, being mycoparasitic and associated with rusts and other phytopathogenic fungi (Wei et al., 2019). In this study, *Simplicillium lamellicola*, along with four unidentified species, was recovered. Initially misclassified as *Acremonium strictum*, *S. lamellicola* was reclassified based on molecular data (Le Dang et al., 2014). It has been used as a BCA against Fusarium head blight caused by *Fusarium graminearum* in wheat (Abaya et al., 2021) and has been shown to reduce the severity of tomato late blight, wheat leaf rust, and barley powdery mildew (Le Dang et al., 2014).

Alternaria is a globally distributed genus of fungi (Division: Ascomycota; Class: Dothideomycetes; Family: Pleosporaceae) commonly recovered from the outdoor environment, air, plants, and soils. Species in this genus exhibit pathogenic, endophytic, and saprobic lifestyles, with many serving as pathogens of both plants and animals, including humans, and acting as allergens (Sánchez et al., 2022). Plant diseases caused by *Alternaria* species affect a variety of grains, vegetables, and fruit-producing plants by infecting their seeds, leaves, flowers, or fruits (Fernandes et al., 2023). In addition to causing pre- and post-harvest losses in agriculture, many *Alternaria* species produce secondary metabolites that function as phytotoxins, playing a crucial role in plant pathogenesis (Patriarca et al., 2014).

In this study, *Alternaria alstroemeriae*, *A. burnsii*, and three unidentified *Alternaria* species were identified. *A. alstroemeriae* has been previously reported as the causal agent of black spot on the perennial ornamental plant *Alstroemeria* in Japan and Colombia (Yamagishi et al., 2009; Valdés et al., 2014), and this study likely represents the first report of its association with banana. Additionally, *Alternaria jacinthicola* was reported by Wang et al. (2021) as the causal agent of a new leaf blight disease of Robusta banana (*Musa acuminata* cv. Giant Cavendish, AAA Group), while *Alternaria alternata* is recognized as the cause of leaf spot and leaf blight disease in bananas. *A. burnsii*, the most prevalent and damaging fungal pathogen of cumin, causes cumin blight, a seedborne disease of significant economic importance in cumin-growing regions worldwide (Varma et al., 2022).

The genus *Bipolaris* (Division: Ascomycota; Class: Dothideomycetes; Family: Pleosporaceae) consists of over 100 species, most of which are saprobes and plant pathogens found in soil and plant debris. Some species are also capable of infecting humans and animals (Sivanesan, 1987). *Bipolaris* is culturally similar to other anamorphic genera such as *Drechslera*, *Curvularia*, and *Exserohilum*. Species in this genus are distributed globally and are known to cause diseases in grasses, cereals, and grains, including leaf spot, head blight, seedling blight, root rot, and black point in wheat and barley (Kumar et al., 2002; Yadav and Thrimurty, 2006). In this study, *Bipolaris secalis* and *B. pluriseptata* were identified. *B. secalis* has been reported in Rwanda as the causative agent of leaf spot disease in *Brachiaria* (syn. *Urochloa*), an alternative fodder crop (Uzayisenga et al., 2023). *B. pluriseptata*, a seedborne fungal pathogen, has been associated with finger millet (*Eleusine coracana*) and grain sorghum (*Sorghum bicolor L.*) in India, Saudi Arabia, and Zambia (Manamgoda et al., 2014). Other species, such as *Bipolaris sacchari* and *B. oryzae*, are recognized as causal agents of leaf spot on banana in Brazil and China, respectively (Silva et al., 2008; Zhao et al., 2023).

Exserohilum (Division: Ascomycota; Class: Dothideomycetes; Family: Pleosporaceae) is a terrestrial, cosmopolitan genus of fungi that predominantly inhabit mild tropical and subtropical climates. They are commonly found in soil, decomposing wood, and plant material, particularly grasses (Therese and Madhaven, 2011). The genus is distinguished from its close relatives *Bipolaris* and *Drechslera* by its conidia formation and extending hilum. In this study, *Exserohilum rostratum* was the only species identified. This species is not only a plant pathogen but has also been documented as a human pathogen (Therese and Madhaven, 2011; Sharma et al., 2014). *E. rostratum* has been reported as the causal agent of banana leaf spot disease (known as Exserohilum leaf spot) in China (Lin et al., 2011).

The genus *Setosphaeria*, closely related to *Exserohilum*, is considered the sexual state of *Exserohilum* (Therese and Madhaven, 2011). Many plant species are affected by *Setosphaeria rostrata* (syn. *Exserohilum rostratum*), a widespread plant pathogen that is common among grasses and causes leaf spot disease (Kusai et al., 2016). *S. turcica*, the reproductive stage and anamorph of *Exserohilum turcicum*, causes northern leaf blight (NLB) in maize, sorghum, and other grasses. It is prevalent in regions where these crops are grown and has been responsible for significant yield losses in maize, particularly in Uganda (Martin et al., 2011).

Cochliobolus, along with the morphologically similar genera *Bipolaris* and *Curvularia*, is a fungal genus whose species are well-known pathogens of approximately 60 plant genera, particularly grasses, grains, and cereals within the Family Poaceae (Manamgoda et al., 2012). Many species previously placed within *Cochliobolus* have since been reassigned to either *Bipolaris* or *Curvularia* (Manamgoda et al., 2012). In this study, an unidentified *Cochliobolus* species was identified.

Syncephalastrum, a zygomycete genus (Family: Syncephalastraceae; Order: Mucorales), is commonly found in tropical soils and environments and is an occasional pathogen in human infections (Irshad et al., 2020). In this study, *Syncephalastrum racemosum* and an unidentified species of *Syncephalastrum* were identified. An unnamed species of *Syncephalastrum* was previously isolated and characterized from the banana rhizosphere in Côte d'Ivoire (Ouina et al., 2020). *S. racemosum* has been mentioned in antagonistic interactions with the cyst nematode *Heterodera oryzicola* in banana crops in India (Charles et al., 2000).

Conclusion

This study identified fungal species associated with *Fusarium oxysporum* f.sp. *cubense* (Foc) in the banana rhizosphere of three wards in Gatundu North, Kiambu County. Some of these fungal species act as symbionts with Foc in causing Fusarium wilt disease (FWB) in bananas, while others exhibit natural antagonism. The relationships of additional species with Foc warrant further investigation. Notably, some of these fungi produce mycotoxins, infect humans, and generate secondary metabolites.

A total of fourteen genera representing three divisions, five classes, and eight families of fungi were characterized. The diverse fungal species isolated from these banana microbiomes actively contribute to the pathogenicity of FWB. To our knowledge, this study marks the first documentation of certain characterized fungal species associated with banana. Key genera identified include *Curvularia*, *Bipolaris*, *Setosphaeria*, *Alternaria*, *Penicillium*, *Epicoccum*, *Fusarium*, *Simplicillium*, *Rhizoctonia*, and *Syncephalastrum*, all of which are significant contributors to the decline and death of banana plantlets.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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