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Characterization of Fungal Isolates Associated with Rhizospheric Indigenous Arbuscular Mycorrhizal Fungi (AMF) from Different Plant Species in Mwea, Mitunguu, and Juja in Central Kenya

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are associated with plant roots and exhibit beneficial impacts such as stress tolerance, better nutrient uptake, and water absorption. These functions have opened the door to research opportunities for considering other fungi associated with AMF on the root rhizosphere. This study aimed to characterize fungal isolates associated with rhizospheric indigenous AMF from different plant species, i.e., banana, grass, and papaya, in three agroecologies, i.e., Mwea, Mitunguu, and Juja in Central Kenya. A total of 30 fungal isolates were isolated from soil samples through the pour plate technique. The isolates had diverse microscopic morphological characteristics, describable in form, margin, color, size, and surface. Physiochemical parameters showed varied growth at different pH values, temperatures, and salinity levels. Optimal growth appeared at pH 7.0, 30-35 °C, and 0-0.5 M NaCl salinity. The internal transcribed spacer (ITS) and AMF subunit sequences showed a level of diversity similar to *Aspergillus spp. Ajellomyces spp., Fusarium spp., Trichoderma spp., Penicillium spp., Glomus spp.,* and *Diversispora spp*. In conclusion, there was a symbiotic relationship between AMF and other fungal isolates.

Introduction

In agriculture, fungi are among the significant essential pathogens of crops as well as bio-control agents to avert and control plant diseases (Costa et al., 2012), control terrestrial weeds (Machado et al., 2012), reduce aquatic weeds, insects, and pests (Rangel et al., 2018).

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs and require active plant roots as hosts to accomplish their life stages. Due to this nature, mass cultivation of AMF through scientific techniques such as hydroponic and aeroponic

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cultivation becomes challenging as the plant roots, the host, become necessary for completing the AMF life cycle (Ghorui et al., 2023). Additionally, AMF can create arbuscular organs, vesicles, hyphae, and spores in and out of the root cortex within the roots of living plants (Prasad et al., 2017). The role of AMF in agriculture includes improvement of salinity and drought tolerance of the host plant through the increase of uptake of nutrients, organic solutes accumulation, and reduction of oxidative stress due to the intensified activity of dismutase, calmodulin, peroxidase, superoxide, catalase, and ascorbate peroxidase (Huang et al., 2014). Mycorrhizal fungi are also known to block the leaching of base cations and abate the toxic effects of heavy metals such as aluminum (Finlay et al., 2009).

Trichoderma, non-mycorrhizal fungi, arbitrate stress reactions in plants. They have the potential to parasitize plant pathogenic fungi by stimulating the defense response, thus escalating the growth of the plant (Sharma et al., 2019). On the other hand, *Piriformospora indica* species can withstand salt stress, prompt disease resistance, and support crop growth (Waller et al., 2005).

According to Photita et al. (2001), fungal diversity emanates significantly from endophytic fungi, and the distribution is massive in temperate and tropical rainforests. Endophytic fungi are associated with many plant species (Rana et al., 2019). They have established an evenness with their plant host during evolution and are a latent source of bioactive secondary metabolites (Sonaimuthu et al., 2010). They enable the plant host to adapt to biotic and abiotic stresses. Plant growth increases to make the plant better resist stressors, and secondary metabolites contribute to this tolerance (Rastegari et al., 2020). These secondary metabolites are a source of insecticidal, immunosuppressive, anticancer, antidiabetic, and biocontrol compounds (Wekesa et al., 2022). They are a crucial solution to the health of humans, animals, and plants (Yadav et al., 2020). Plant hosts benefit mainly from an asymptomatic relationship; rare cases may exhibit pathogenic effects (Neubert et al., 2006).

Biofertilizers occur from living microbes and are vital nutrients for plant growth and development. Apart from their environmentally friendly and inexpensive nature, they enhance soil fertility and crop productivity (Kour et al., 2020). Fungal biofertilizers improve plant growth and development by improving phosphorus uptake. The most common fungi with phosphate solubilizing properties are *Candida montana*, *Trichosporon beigelii*, *Cryptococcus luteolus,* *Kluyveromyces walti*, *Zygoascus hellenicus*, *Rhodotorula aurantiaca*, *Penicillium purpurogenum* (var. rubrisclerotium), and *Saccharomycopsis schoenii* (Gizaw et al., 2017). *Aspergillus*, *Fusarium*, and *Penicillium sp*. are also found in the rhizosphere of numerous plants, while the genera *Aspergillus*, *Penicillium,* and *Chaetomium* are widespread (Yadav et al., 2018). Rhizospheres occupied with *Trichoderma* species can parasitize with other species of fungi. These species increase plant productivity, improve crop nutrition and nutrient acquirement, and are generally engaged in biofertilizer production, primarily in agricultural soils. Moreover, metabolites produced by *Trichoderma* species work as a fungicide against disease-causing fungal pathogens (Harman et al., 2008).

Penicillium spp. has extensive distribution in nature through various soil environments such as cultivated, forest, or desert locations (Chandanie et al., 2006). Plant hormones such as indole-3-acetic acid (IAA), cytokinin, and gibberellins (GA) become secreted by *Penicillium* fungus, thus promoting plant growth, and are also involved in phosphate solubilization (Radhakrishnan et al., 2013). They also secrete antibiotics, insecticides, herbicides, anticancer compounds, antioxidants, extracellular enzymes, and mycotoxins (Munns and Tester, 2008).

A well-known plant-microbe interaction is the mutualism between AMF and host plants (Verma et al., 2017). However, plants form associations with endophytic fungi under natural occurrences (Dastogeer and Wylie, 2017). Plants and microbes interaction has a crucial influence on the function of the plant and their community ecology (Vimal et al., 2017). Most farmers in Mwea, Mitunguu, and Juja in Central Kenya focus on papaya production. There, papaya orchards appear amid banana trees and grasses. Studies conducted by Muiruri et al. (2022) indicated that grass and banana rhizospheric soils had the most abundant AMF spores compared to papaya rhizospheric soils. However, other fungi that come in conjunction with AMF within the rhizospheric portion of soil profiles surrounding these plant roots have not been subject to previous research. Therefore, molecular characterization of fungal isolates alongside mycorrhizal fungi is of significant interest for the resolve of sustainable agriculture through maximal utilization of beneficial fungi in the rhizospheric zones of plants.

Materials and Methods

Study area and sample collections

Soil samples were collected from the Mwea, Mitunguu, and Juja areas in Central Kenya from the rhizosphere of papaya, grass, and banana plants. Using random sampling methods, we collected soil samples from different points on the farms. The global positioning system (GPS) registered the collection area for documentation, publication, and recollection if necessary. The samples were packaged in sterile bags and stored in a cool box before being transported to the Institute for Biotechnology Research (IBR) at Jomo Kenyatta University of Agriculture and Technology in Kenya for further analysis.

Isolation of mycorrhizal fungal spores

Arbuscular Mycorrhiza Fungal spores were isolated from 50 g air-dried soil samples by wet sieving method, described by Boyno et al. (2023), with a few modifications. In brief, spores were obtained by wet sieving and decanting techniques. Approximately 250 mL of soil was suspended at a ratio of 1:1 water. Heavier particles were allowed to settle for a few seconds, and the liquid became decanted through a 2 mm sieve. The suspension was saved and stirred to re-suspend all particles and decanted through a 5 µm sieve to obtain pure spores (Fig. 1). The spores were cultured on modified potato dextrose agar (PDA) (Himedia, India) and incubated at 30 °C for five days.

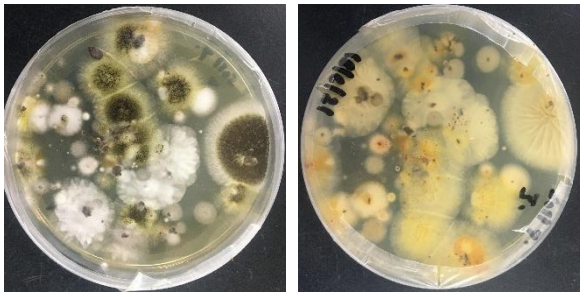

Fig. 1. Unpurified cultures of the fungal spores from the rhizospheric soil of papaya, grass, and banana plants from Mwea, Mitunguu, and Juja areas.

Morphological characterization of isolated fungi

The cultures were grown on PDA plates for 7–10 days at 25 °C to observe the morphology of the isolated fungi. Glass slides and coverslips were cleaned using 95% ethanol to begin the morphological identification process. Then, a drop of lactophenol cotton blue was placed onto a clean microscope slide, and a tiny pinch of the fungus was taken from the culture plate. It was placed onto the dye, spread using a needle, and covered with a cover slip. Then, it was examined under a microscope for imaging (Lange, 2014).

Physiochemical characterization of fungi from the rhizospheric soil of papaya, grass, and banana plants from the Mwea, Mitunguu, and Juja areas

Growth at different sodium chloride concentrations

According to the manufacturer, the fungal isolates were cultured on PDA (Himedia, India) at various NaCl concentrations per liter (0.0 M, 0.5 M, 1.0 M, 1.5 M, and 2.0 M NaCl). The growth of the fungal isolates was then observed and recorded as either excellent (++++) average (+++), satisfactory growth (++), minimum growth (+), and no growth (-).

Growth at various temperatures

The fungal isolates were cultured on PDA media at varying temperatures of 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, and 50 °C (Barcenas-Moreno and Baath, 2009) and incubated at various temperatures for seven days. The growth of the fungal isolates was then observed and recorded as either excellent growth (++++), average growth (+++), satisfactory growth (++), minimum growth (+), or no growth (-).

Effect of pH on the growth of the isolates

The fungal isolates were cultured on PDA media at varying pH of 5.0, 7.0, 8.5, and 10.0 (Rousk et al., 2010). The plates were incubated at 30 °C for seven days. The growth of the fungal isolates was then observed and recorded as either excellent growth (++++), average growth (+++), satisfactory growth (++), minimum growth (+), or no growth (-).

Molecular identification of isolated fungi

DNA extraction from spores

The spores from the most represented groups were selected, and the DNA was extracted from single spores. Each spore was crushed under a stereomicroscope in 10 µL volume (5 µL 5× GoTaq PCR buffer and five µL distilled water) and then boiled for 15 min. Centrifugation at 14000 rpm followed. Two µL of supernatant was used as a template for the PCR.

DNA extraction from mycelium

Molecular identification of the most active endophytic fungi was done according to the CTAB protocol described by Qadri et al. (2013). The fungal mycelia were freeze-dried, and the cells were lysed in 10 mL of extraction buffer. Afterward, the lysate was extracted by adding an equal volume of isopentanol/chloroform (1:24), followed by centrifugation at 10,000 rpm for 10 min at 4 °C. The genomic DNA was precipitated from the aqueous phase in chilled isopropanol by centrifugation at 10,000 rpm for 10 min at 4 °C.

PCR amplification of ITS and AML genes

Genomic DNA from each spore and mycelium was used as a template to amplify the ITS and AML genes. A pair comprised AML1 (5'-ATC AAC TTT CGA TGG TAGGAT AGA-3') and AML2 (5'-GAA CCC AAA CAC TTT GGT TTC C-3') (Lee et al., 2008). The ITS4 and ITS5 regions of the genomic DNA were PCR amplified using universal ITS primers, ITS4 (5'TCCTCCGCTTATTGATATGC3') and ITS5 (5'GGAAGTAAAAGTCGTAACAA3'). The amplification was performed using Peqlab Primus 96 PCR equipment. It was amplified in a 40 µL mixture comprising 20 µL of Master mix, 18.2 µL of PCR water, 0.4 µL of AML1/ITS4 forward primer, 0.4 µL of AML2/ITS5 reverse primer, and 1 µL of template DNA (100 ng L-1) DNA. The following temperature cycling profiles were applied for the reaction mixtures: A 10 min enzyme activation at 96 °C for a single cycle, followed by 35 cycles of 45 s of denaturation at 95 °C, 45 s of primer annealing at 53 °C, 1 min of the chain of elongation at 72 °C, and 10 min of the chain of final extension at 72 °C. The presence and size of PCR amplicon were verified on 1.2% agarose gel and visualized under UV light. PCR amplicons were purified using the QIAquick PCR amplification kit protocol (Qiagen) according to the manufacturer's instructions. The PCR amplicon was sent to Macrogen for sequencing.

Editing and phylogenetic analyses

BLAST, provided by GenBank, determined sequence similarities. The BLAST findings selected ITS and AML gene sequences with the highest similarity index. Sequence editing was performed manually using MEGA 7.0 (Tamura et al., 2007) and Chromas 2.5. MEGA 7.0 software assessed the Kimura-2 parameter model as a distance method. One thousand replicates of non-parametric bootstrapping were used for constructing a Neighbor-Joining (NJ) consensus tree.

Data analysis

Soil samples were replicated three times per plant and region. A total of 30 fungal isolates were visually characterized based on their growth at different pH, sodium chloride, and temperatures. The growth of the fungal isolates was recorded as either excellent growth (++++), average growth (+++), satisfactory growth (++), minimum growth (+), or no growth (-). The data from the morphological characterization of the isolates were analyzed using Minitab.

Results

Isolation and morphological characterization of fungi from rhizospheric soil

In this study, 30 fungi were isolated from the selected soil samples. Colony morphology characteristics included forms ranging from circular to rhizoid, as well as various elevations ranging from flat, raised, umbonate, and convex (Table 1). The isolates also exhibited differences in their margins, ranging from entire, undulate, filamentous, lobate, and filiform. The color of the isolates ranged from white to black, cream-white, and yellow. Sizes ranged from small to medium and large. The surface ranged from smooth, filiform, dull, glistening, rough, and wrinkled, and they were all opaque (Fig. 2).

The PC analysis of the morphological characteristics in PCA 1 and 2 showed varied correlations. There was diversity in color compared to opacity, form, and other morphological parameters. The margin, surface, size, and elevation parameters became clustered as one (Fig. 3).

Table 1. Morphological characterization of the fungi isolated from the rhizospheric soil of papaya, grass, and banana plants from the Mwea, Mitunguu, and Juja areas in Central Kenya.

	Colony morphology						
Isolate	Form	Elevation	Margin	Size	Color	Surface	Opacity
S1-1	Circular	Flat	Entire	Medium	White	Smooth	Opaque
S1-2	Irregular	Raised	Undulate	Small	Black	Filiform	Opaque
S1-3	Circular	Flat	filamentous	Large	Yellow	Dull	Opaque
S1-5	Circular	Flat	Entire	Medium	White	Smooth	Opaque
S1-7	Filamentous	Flat	Lobate	Medium	White	Dull	Opaque
S1-8	Circular	Flat	Entire	Medium	White	Glistening	Opaque
S2-1	Rhizoid	Flat	Entire	Large	Black	Glistening	Opaque
S2-2	Rhizoid	Raised	Entire	Medium	Cream	Smooth	Opaque
S2-3	Circular	Raised	Entire	Small	White	Smooth	Opaque
S2-4	Irregular	Flat	Entire	Medium	Cream-white	Smooth	Opaque
S2-5	Irregular	Umbonate	Undulate	Small	Cream	Smooth	Opaque
S2-6	Irregular	Flat	Filiform	Medium	Black	Rough	Opaque
S2-7	Rhizoid	Flat	Filiform	Small	Black	Dull	Opaque
S3-1	Irregular	Raised	Lobate	Large	Black	Dull	Opaque
S3-3	Rhizoid	Raised	Lobate	Small	Cream	Rough	Opaque
S3-4	Irregular	Raised	Lobate	Small	Cream	Rough	Opaque
S3-6	Irregular	Raised	Filiform	Small	Cream	Rough	Opaque
S4-2	Irregular	Raised	Entire	Medium	Cream	Dull	Opaque
S4-4	Rhizoid	Raised	Undulate	Medium	Black	Dull	Opaque
S4-3	Irregular	Raised	Undulate	Small	Cream	Wrinkle	Opaque
S5-2	Irregular	Umbonate	Lobate	Small	Black	Wrinkle	Opaque
S5-1	Irregular	Convex	Entire	Large	White	Wrinkle	Opaque
S6-2	Irregular	Raised	Entire	Medium	Black	Wrinkle	Opaque
S6-1	Irregular	Raised	Lobate	Small	Cream	Smooth	Opaque
S7-7	Circular	Raised	Lobate	Large	Cream	Smooth	Opaque
S7-3	Circular	Raised	Entire	Medium	Green	Dull	Opaque
S7-4	Rhizoid	Flat	Entire	Small	Black	Glistening	Opaque
S8-4	Irregular	Flat	Filamentous	Medium	Black	Glistening	Opaque
S9-2	Irregular	Raised	Filamentous	Small	Cream	Dull	Opaque
S10-3	Circular	Flat	Entire	Medium	Cream	Rough	Opaque

S1 = Mwea papaya, S2 = Mwea banana, S3 = Mwea grass, S4 = Mitunguu papaya, S5 = Mitunguu banana, S6 = Mitunguu grass, S7 = Juja papaya, S8 = Juja banana, S9 = Juja grass, S10 = control.

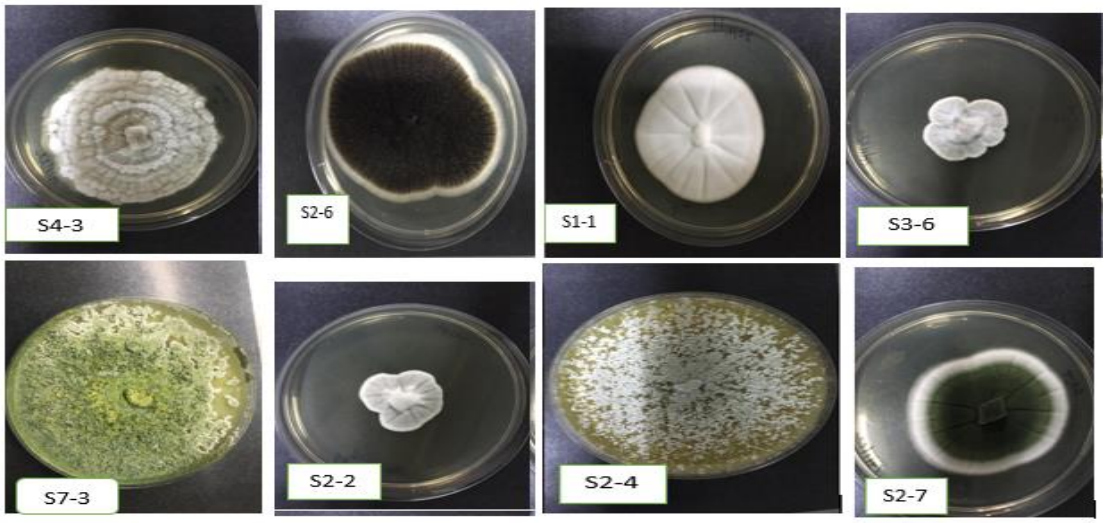

Fig. 2. Morphology of selected fungi isolated from the rhizospheric soil of papaya, grass, and banana plants from the Mwea, Mitunguu, and Juja areas: S1 = Mwea papaya, S2 = Mwea banana, S3 = Mwea grass, S4 = Mitunguu papaya, S7 = Juja papaya.

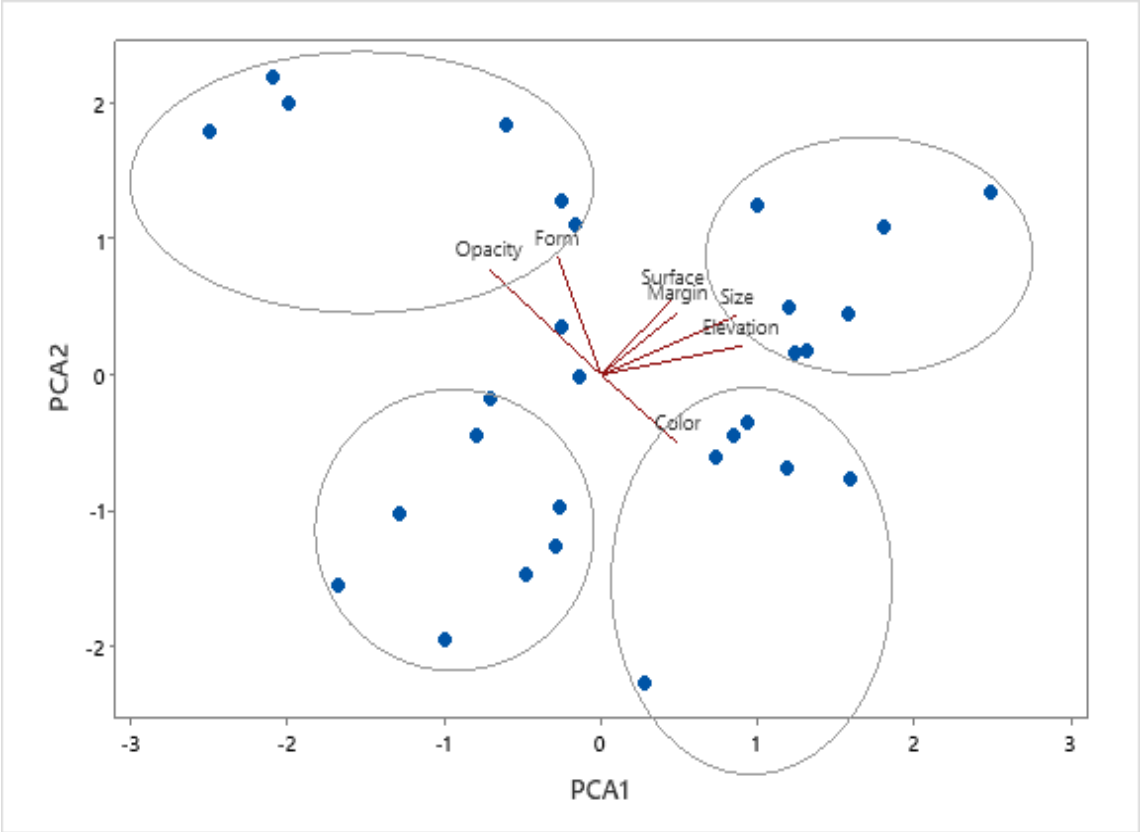

Fig. 3. Principal component analysis of the morphological characteristics of the fungal isolates from the rhizospheric soil of papaya, grass, and banana plants from the Mwea, Mitunguu, and Juja areas in Central Kenya.

Growth at different pH

Table 2 shows a varied growth of fungal isolates at different pH. Although the isolates were obtained from a typical environment, they could grow at pH 8.5 and 10.0. Optimum growth was recorded at pH 7.0, with most of the isolates having average growth compared to pH 5.0, pH 8.5, and pH 10.0. The growth trend was lower at pH 5.0. It increased at pH 7.0 but decreased from pH 8.5 to 10.0.

Table 2. pH-dependent growth of fungal isolates from the rhizospheric soil of papaya, grass, and banana plants from the Mwea, Mitunguu, and Juja areas in Central Kenya.

Isolate	pH 5.0	pH 7.0	pH 8.5	pH 10.0
S1-1	-	++	++	+
S1-2	+	+++	++	++
S1-3	-	+	-	-
S1-5	-	++	+	-
S1-7	++	+++	++	+
S1-8	+	+	-	+
S2-1	+	+	+	+
S2-2	++	++	+	-
S2-3	+	+	-	-
S2-4	-	++	+	+
S2-5	-	+++	-	-
S2-6	-	+	+	-
S2-7	-	+	++	+
S3-1	+	+	-	-
S3-3	-	+	+	+
S3-4	+	+	++	-
S3-6	+	++	-	-
S4-2	++	+++	+	+
S4-4	-	++	-	-
S4-3	-	+	+	+
S5-2	+	++	+	+
S5-1	-	++	+	+
S6-2	++	+++	++	++
S6-1	-	+++	-	+
S7-7	++	+++	+	-
S7-3	-	+	-	-
S7-4	-	++	++	+
S8-4	+	+	+	-
S9-2	-	+++	-	+
S10-3	+	++	+	-

Growth rate at various pH levels: ++++ excellent growth, +++ average growth, ++ satisfactory growth, + minimum growth, and – no growth.

Growth at different temperatures

Table 3 shows varied growth rates of fungal isolates at different temperatures. The isolates grew at temperatures ranging from 20 °C to 50 °C. Optimal growth was recorded at temperatures ranging from 30 °C to 35 °C. The lowest growth rates were observed at temperatures 20 °C and 50 °C.

Table 3. Temperature-dependent growth of fungal isolates from the rhizospheric soil of papaya, grass, and banana plants from the Mwea, Mitunguu, and Juja areas in Central Kenya.

Isolate	20 °C	30 °C	35 °C	40 °C	45 °C	50 °C
S1-1	-	++	++	+	-	-
S1-2	-	+++	+++	++	+	+
S1-3	-	+	++	+	-	-
S1-5	-	++	+	-	+	-
S1-7	+	+++	++	+	-	-
S1-8	+	++	+	+	+	-
S2-1	+	+++	++	++	+	-
S2-2	-	++	++	+	-	-
S2-3	+	++	+	-	-	-
S2-4	-	+++	++	++	+	-
S2-5	-	+++	+	-	-	-
S2-6	-	++	++	+	-	-
S2-7	-	++	+++	++	+	+
S3-1	-	++	+	-	-	-
S3-3	-	++	++	+	-	-
S3-4	+	+++	+	-	-	-
S3-6	+	+++	++	+	+	+
S4-2	+	+++	++	++	+	-
S4-4	-	++	++	+	-	-
S4-3	-	++	++	+	+	+
S5-2	+	+	++	++	+	-
S5-1	-	+++	++	+	-	-
S6-2	+	++	+	+	+	+
S6-1	-	++	+	+	-	-
S7-7	-	++	++	+	+	+
S7-3	-	++	+	-	-	-
S7-4	-	+	++	+	-	-
S8-4	-	++	++	+	-	+
S9-2	-	++	+	-	-	-
S10-3	-	+++	++	+	+	-

Growth rates at various temperatures: ++++ excellent growth, +++ average growth, ++ satisfactory growth, + minimum growth, and – no growth.

Growth at different sodium chloride (NaCl) concentrations

The fungal isolates showed varied growth rates at different concentrations of NaCl. Salt concentrations of 0.0 M and 0.5 M caused the highest growth rates in all isolates. The growth trend decreased in response to an increase in NaCl concentration (Table 4).

Phylogenetic analysis of fungal isolated from soils of papaya, grass, and banana farms

From the partial sequence, ten isolates from AMF species were analyzed. Among them were *Glomus etunicatum*, *Diversispora varaderana, Diversispora gibbose, Diversispora peridiata, Glomus clarum,* and *Glomus lamellosum* (Fig. 4). Three isolates (10.7%) belonged to *Aspergillus* genus, with a 99.74% to 100% similarity index, and they were *Aspergillus niger*, *Aspergillus sp,* and *Aspergillus cervinus*.

One isolate (3.5%) was obtained from *Ajellomyces,* with a similarity index of 100%. *Fusarium* genera were also isolated, with a similarity index of 99.65% to 100%. They included *Fusarium solani*, *Fusarium oxysporum,* and other *Fusarium spp*. Four isolates (14.3%) belonging to *Trichoderma* were isolated, with a similarity percentage of 100%. They included *Trichoderma harzianum*, *Trichoderma virens* (2), and *Trichoderma atroviride*. Six isolates (21.4%) from the *Penicillium* genus were also isolated. The isolate had a similarity percentage of 99.30% to 100%. They included *Penicillium brasilianum*, *Penicillium onobense, Penicillium aethiopicum, and Penicillium brocae*. No novel isolates were identified since all isolates had a similarity percentage above 98%, using reference sequences from the NCBI database (Table 5).

Table 4. NaCl-dependent growth rates of isolated fungi from the rhizospheric soil of papaya, grass, and banana plants from the Mwea, Mitunguu, and Juja areas in Central Kenya.

Isolate	0 M	0.5 M	1.0 M	1.5 M	2.0 M
S1-1	+++	++	+	+	-
S1-2	++	++	++	++	+
S1-3	+	++	+	+	-
S1-5	++	+++	+	-	-
S1-7	+++	++	+	+	-
S1-8	++	++	+	+	+
S2-1	+++	+	+	++	+
S2-2	+	++	+	+	-
S2-3	++	++	-	-	-
S2-4	++	++	+	++	+
S2-5	+++	+++	++	-	-
S2-6	++	++	+	+	-
S2-7	+	+++	-	-	-
S3-1	++	++	-	-	-
S3-3	++	+++	+	+	-
S3-4	+++	++	+	-	-
S3-6	++	++	++	+	+
S4-2	++	++	+	++	+
S4-4	+	+++	++	+	-
S4-3	+++	++	+	+	-
S5-2	++	++	+	+	+
S5-1	++	++	+	+	-
S6-2	++	+++	++	+	+
S6-1	++	+	+	+	-
S7-7	+++	++	+	+	-
S7-3	++	+	+	-	-
S7-4	+++	+	+	+	-
S8-4	++	+++	++	+	-
S9-2	+++	+	+	-	-
S10-3	++	++	++	+	+

Growth rates at varied salt concentrations: ++++ excellent growth, +++ average growth, ++ satisfactory growth, + minimum growth, and – no growth.

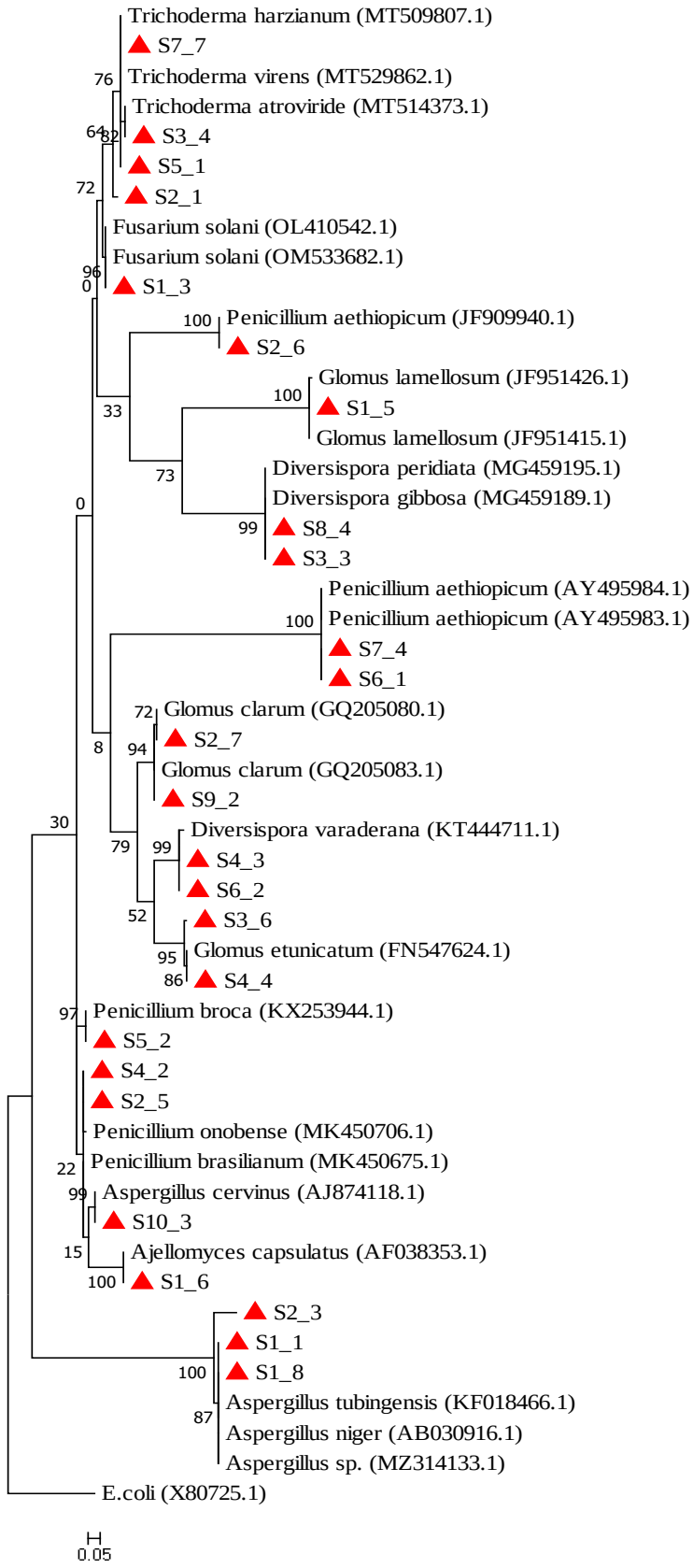

Fig. 4. Phylogenetic tree of fungal isolates from the Mwea, Mitunguu, and Juja areas, Central Kenya. Rhizospheric soils were based on ITS, AML1, and AML2 sequences. S1 = Mwea papaya, S2 = Mwea banana, S3 = Mwea grass, S4 = Mitunguu papaya, S5 = Mitunguu banana, S6 = Mitunguu grass, S7 = Juja papaya, S8 = Juja banana, S9 = Juja grass, S10 = control (non-rhizospheric soil).

Table 5. Blast results of fungal isolates and their respective closest relatives.

Isolate code	Max Score	Total Score	Query Coverage	Acc No.	Next Neighbor in Blast	%ID
S1_1	909	909	100%	AB030916.1	*Aspergillus niger*	100%
S1_6	2198	2198	100%	AF038353.1	*Ajellomyces capsulatus strain UAMH 7141*	100%
S1_8	2800	2800	100%	MZ314133.1	*Aspergillus sp. isolate 7F2*	99.74%
S1_3	1471	1471	100%	OM533682.1	*Fusarium solani strain LZ09-07*	100%
S1_4	1038	1038	100%	EU750681.1	*Fusarium sp. 14012*	99.65%
S2_1	1092	1092	100%	GU048879.1	*Fusarium oxysporum isolate CAFO-IHBT*	100%
S2_3	481	481	100%	LC534254.1	*Fusarium sp. NBRC*	100%
S2_4	1112	1112	100%	MT509807.1	*Trichoderma harzianum strain KSRCT-BT-MS2*	100%
S5_1	1020	1020	100%	MT529862.1	*Trichoderma virens clone SF_586*	100%
S7_7	1020	1020	100%	MT529862.1	*Trichoderma virens clone SF_586*	100%
S3_4	1014	1014	100%	MT514373.1	*Trichoderma atroviride strain LSK_7*	100%
S2_5	1088	1088	99%	MK450675.1	*Penicillium brasilianum strain CMV002C3*	99.83%
S4_2	1554	1554	99%	MK450706.1	*Penicillium onobense strain CMV006B5*	99.30%
S6_1	798	798	100%	AY495983.1	*Penicillium aethiopicum strain CBS*	100.00%
S7_4	784	784	100%	AY495984.1	*Penicillium aethiopicum strain CBS 270.97*	100.00%
S2_6	1814	1814	100%	JF909940.1	*Penicillium aethiopicum strain CBS 484.84*	100.00%
S5_2	965	965	100%	KX253944.1	*Penicillium brocae strain S4*	99.81%
S3_6	1000	1000	100%	FN547624.1	*Glomus etunicatum*	99.64%
S4_4	1007	1007	100%	FN547624.1	*Glomus etunicatum*	99.82%
S4_3	2756	2756	99%	KT444711.1	*Diversispora varaderana isolate 7*	98.90%
S6_2	2756	2756	99%	KT444711.1	*Diversispora varaderana isolate 7*	98.90%
S3_3	1205	1205	99%	MG459189.1	*Diversispora gibbosa isolate 3-1*	99.25%
S8_4	1282	1282	100%	MG459195.1	*Diversispora peridiata isolate 5-1*	100.00%
S9_2	928	928	100%	GQ205083.1	*Glomus clarum strain DAOM 234281*	100.00%
S2_7	902	902	100%	GQ205080.1	*Glomus clarum strain DAOM 234281*	99.20%
S1_8	1051	1051	100%	JF951426.1	*Glomus lamellosum*	100.00%
S1_5	1055	1055	100%	JF951415.1	*Glomus lamellosum*	100.00%
S10_3	1068	1068	100%	AJ874118.1	*Aspergillus cervinus*	100%

Discussion

In this study, the blast analysis showed ten isolates were arbuscular mycorrhizae fungi, as the main focus of the study, and had an identity percentage between 98.90% and 100%. Among the isolates in this study, members of the phylum *Glomeromycota*, which contained all known arbuscular mycorrhizal fungi, had an identity percentage between 98.90% and 100%. It contained ten isolates as compared to the phylum Ascomycota, which had 18 isolates, comprising *Aspergillus spp., Ajellomyces spp, Fusarium spp.*, *Trichoderma spp.* and *Penicillium spp.*

Most of the isolates had an average growth at pH 7.0. However, at lower and higher pH (5.0, 8.5, and 10.0), the growth rate of the isolates decreased. This study also revealed that despite obtaining the isolates from temperatures ranging between 25 °C and 30 °C, they could grow at temperature ranges of 30 °C to 50 °C in the cultured PDA. Nonetheless, lower growth rates were recorded at very low temperatures of 20 °C and high temperatures of 50 °C. The genomic DNA was

heavily loaded and could not move further, thus appearing approximately near the loading well. Moreover, all the genomic DNA from the fungal isolates had similar sizes, which explains why they are linear.

A similar research on fungal isolates characterization conducted by Wang et al. (2017) concluded that the abundance of the soil fungi and the fungal communities differed between the bulk soils and the rhizospheric soils. Fungal phyla that dominated all soil samples, from the highest to the lowest, were Ascomycota (68.7%), Zygomycota (13.3%), and Basidiomycota (4.1%). Roots normally release organic composites due to variations in rhizospheric fungal groups, which depend on plants. Thus, an exceptional nutrient pool is created on the rhizosphere and is reachable to microorganisms in the soil (Han et al., 2016). In a different study, Penicillium spp. (*P. raistrickii*, *P. funiculosum*, P. janthinellum, and *P. erythromellis*) and *Trichoderma spp*. (*T. pseudokoningii* and *T. konengii*) dominated the rhizospheric area of established tea plants (Pandey et al., 2001). The majority of the fungal isolates accompanying the tea rhizospheric area displayed an extensive range of temperature and pH tolerance, with properties indicating superior adaptation and survival in the rhizospheric area of the soil (Pandey et al., 2001). Fungi appear in three groups according to their function. They include the lichens, the saprotrophs, and the mycorrhizas (Aislabie, 2013). Due to their symbiotic roles, fungi are key to the operations of the ecosystems of the terrestrial arctic vegetation, which actively grow close to the ground, i.e., plants such as herbs, dwarf shrubs, graminoids, and mosses. These plants depend highly on mutualistic relationships with arbuscular mycorrhizal fungi to survive these harsh environments (Bjorbaekmo et al., 2010). Endophytic fungi are also abundant in the roots and some parts above the ground of arctic-alpine plants (Newsham et al., 2009).

The composition of the microbial community on the rhizosphere is affected by plant growth, depending on their different life cycle and responses due to environmental variations (Baetz and Martinoia, 2014). Fungi in the rhizosphere contribute to effective plant growth and health while acting against pathogens, helping toward nutrient provision and plant residue decomposition (Ehrmann and Ritz, 2014).

The soil texture substantially affects the organic carbon content, and this issue, in turn, determines the microbial community on the plant rhizosphere (Wang et al., 2009), Enzyme activities in soils come in intense association with fungal rhizosphere communities (Welc et al., 2014). Thus, the chemical and physical properties of the soil interfere with the fungal community found on the rhizosphere (Schappe et al., 2017). Arbuscular mycorrhizal fungi species are distributed and vary depending on climate, land use, and edaphic environments. *Glomus spp*. are the most widely distributed. *Sclerocystis spp* and *Gigaspora spp*. are commonly found in tropical soils (Singh, 2000). Thus, variations in fungal isolates in this study were describable.

In this study, some fungi continued growth at 20 °C and 50 °C, indicating survival at harsh temperatures. However, the ideal temperature range for most fungal isolates was between 30 °C and 50 °C in the cultured PDA.

Pandey et al. (2001) observed that many fungi linked with the tea bush roots displayed a mesophilic temperature requirement. *Paecilomyces arioti* and *Aspergillus terreus* grew at 50 °C while *Penicillium janthinellum* and *Penicillium lanosum* did at 5 °C. Fungal growth and activities were higher at temperatures, i.e., between 25 °C and 30 °C for both forest-humus agricultural soils. However, the fungal activity decreased rapidly above 40 °C (Pietikainen et al., 2005). Interestingly, fungi dominated in high-altitude soils at low temperatures (Ley and Schmidt, 2002). The main causes of soil fungistasis, inhibition of fungal growth, and germination, are soil microorganisms, limited carbon, production of antifungal compounds, and fungal community composition (Barcenas-Moreno and Baath, 2009).

This study further revealed that an environment with too much acidity or alkalinity was not ideal for most fungal isolates. However, few isolates could continue to grow at pH values of 5.0, 8.5, and 10.0.

In a different research, the soil pH ranged between 4.3 and 6.1 on rhizospheric soils of different tea zone samples while having pH values of 5.1 to 6.2 on non-rhizospheric soils (Chen et al., 2006). AMF species are reportedly associated with specific soil characteristics. For example, *Acaulospora spp*. are better adapted to soils with lower pH of less than 5, i.e., acidic soils, while *Glomus mosseae* occur mostly in soils with high pH and fine textures. *Gigaspora spp*. is better adapted to sand and dune soils (Nayak et al., 2019). Nevarez et al. (2009) observed that the soil pH determined the composition of the fungal community, which could have been due to the phylogenetic alterations between the fungal communities. Additionally, the pH values between pH 5 and 9 did not inhibit the growth of various fungal species. The highest vegetative growth of *Schizophyllum commune*, a basidiomycete

bracket fungus, occurred at an optimum pH of 5.5 (Adejoye et al., 2007).

Soil microbes, fungi, archaea, and bacteria are essential in the ecosystem for their varied and critical roles. Unlike archaea or bacteria, fungi are closer to animals and plants since they are eukarya, i.e., their cells encompass membrane-bounding nuclei with chromosomes that contain DNA (Aislabie, 2013). Due to their highly diverse nature, fungi serve various roles in the ecosystem, such as mutualists, predators, pathogens, plants, endophytes, and decomposers (Aislabie, 2013). Microbes can occur throughout the soil profile; nonetheless, they are plentiful in rhizospheric soils where plants exist and near the macropores (Fierer et al., 2007). This study sought to identify and characterize other fungal isolates along with arbuscular mycorrhizal fungi (AMF) isolated from the rhizospheric soils of papaya, grass, and banana plants from Mwea, Mitunguu, and Juja areas.

Arbuscular mycorrhizal fungi (AMF) display plenty of benefits to the plant hosts. The current research proved that the AMFs do not rely on their neighboring fungi to perform their duties since they are more abundant compared to other fungi in the rhizospheric region. However, other fungal isolates, such as *Trichoderma spp*. have previously been established as bio-control agents against some plant diseases. *Penicillium spp*. feeds on decaying matter in the ecosystem and is essential for producing antibiotics, organic acids, and cheese (Solomon et al., 2019). However, the overall benefits of AMF in the soil surpass the combined importance of other beneficial fungi that coexist with AMF. The coexistence of *Fusarium spp*. with AMF within the context of this study confirms that AMF can fight soil-borne pathogens, as demonstrated by Devi et al. (2022), who observed the decline in severity of *Fusarium* wilt of tomato caused by *Fusarium oxysporum* in media containing *Glomus fasciculatum* and *Funneliformis mosseae* (AMF species) in pots and field conditions.

Similar studies conducted by Muiruri et al. (2022) revealed the abundance of AMF in the rhizospheric soils of papaya, banana, and grass from similar agroecological zones. The said research characterized them morphologically based on their color, Melzer's reaction on the spore color, shape, size, and spore surface. This study has further characterized all the rhizospheric fungal isolates based on their morphological, physiochemical, and molecular characteristics.

Conclusion

This study added to the current knowledge of rhizosphere fungal communities and verified the coexistence of AMF with other fungal communities. Such a coexistence could be harmful or beneficial to their surrounding organisms, depending on the circumstances. Notably, the AMF population was more abundant than individual species of other fungal isolates, thereby overcoming and masking most of the adjoining harmful effects of undesirable soil-borne fungi.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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