Plant growth-promoting and protective potential of root fungal endophytes associated with a pioneer grass, *Saccharum spontaneum* L., from a lahar-affected area

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Abstract
Root-associated mycobiota can improve nutrient assimilation and induce resistance in their host plants. In this study, we isolated root fungal endophytes (RFE) from *Saccharum spontaneum*, a pioneer grass thriving in a lahar-strewn environment. Seventeen root fungal endophytes identified as belonging to the genera *Talaromyces*, *Penicillium*, *Fusarium*, *Trichoderma*, *Cladosporium*, *Epicoccum*, *Purpureocillium*, and *Rhizoctonia* were screened for plant growth-promoting and protective properties. The colorimetric assay revealed significantly high levels of the phytohormone indole-3-acetic acid (20.13–159.89 µg/ml), which was produced by eight notable RFE isolates. Seven of the root fungal endophytes displayed phosphate solubilizing activities with a solubilization index (SI) ranging from 1.04–1.22 on Pikovskaya's agar. Nine RFE isolates significantly suppressed the growth of the phytopathogen, *Fusarium oxysporum*, exhibiting > 50% inhibition rates in a dual culture experiment. Our study highlighted the beneficial traits of root fungal endophytes, which might be responsible for the successful colonization of lahar areas by the pioneer grass *S. spontaneum*.

Introduction

The root system and rhizosphere house diverse fungal symbionts that support the growth of plants by improving nutrient assimilation and inducing resistance to various environmental stresses. Among these plant growth-promoting fungi (PGPF) associated with the roots are endophytic, mycorrhizal, and rhizospheric fungi. The first two fungal groups invade the root endosphere, with the endophytes being facultative biotrophs, and may complete their life cycles within or outside plant tissues, while mycorrhizas are mostly obligate mycobionts that develop synchronously with their hosts (Domka et al. 2019). Unlike mycorrhizas, which are restricted to the roots, endophytes are ubiquitous and can colonize above-ground parts like stems, leaves, flowers, and seeds. On the other hand, rhizospheric fungi reside in the narrow region of the soil that is closely adhering to or in

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direct proximity to the roots (McNear 2013). All these fungal inhabitants mediate specific processes that are essential to the functioning and overall fitness of plants (Bahram & Netherway 2022).

Several studies have isolated and identified a wide array of fungal symbionts from different plant hosts, e.g., in mangroves (Apurillo et al. 2019, Jacob et al. 2023), rice (Airin et al. 2023), tea shrubs (Nath et al. 2015), cycads (Pecundo et al. 2021), and other plant taxa (Wolfe et al. 2022). However, little is known about those associated with plants growing and thriving in primary successional habitats. These areas are characterized by newly developed lands formed from natural calamities like volcanic eruptions and floods or by climatic changes such as receding glaciers (Kolaříková et al. 2017, Wolfe et al. 2022). Pioneering plant species like weeds, grasses, and shrubs may harbor a unique assortment of fungal species that could assist in their growth promotion, adaptation, and disease tolerance (Triolet et al. 2022, Bi et al. 2024). These plant-fungal consortia may play a crucial role in shaping the ecosystem during the stages of primary succession.

As the earliest and most common symbiosis in plants, the role of mycorrhizal fungi in vegetation succession has been well documented. Studies have shown their effects on plant reproduction patterns, demography, competition, herbivory, and other biotic interactions and their contribution to the establishment of plant species composition, diversity, and successional dynamics (Hartnett & Wilson 2002, Jumpponen & Egerton-Warburton 2005). However, the impacts of endophytic and rhizospheric fungi on the succession of plants remain underexplored. Some studies conducted thus far have reported fungal associations with plant species in post-mining sites, such as *Phialocephala fortinii*, *Crocicreas cyathoideum, Lecythophora* sp.*, Myrothecium* sp., *Exophiala* sp., and *Tetracladium* sp. (Elhottová et al. 2009, Kolaříková et al. 2017). In coasts affected by post-glacial rebound, *P. fortinii, Mollisia minutella,* and *Cortinarius callisteus* have been reported (Tejesvi et al. 2010). In karst landscapes, fungal communities belonging to *Ascomycota* and *Basidiomycota* have been observed (Xu et al. 2021), and *Arthrinium* sp. and *Pseudoplectania* sp. have been found in volcanic substrates (Wolfe et al. 2022). Their role in the invasiveness of plants in arid and semi-arid environments has also been documented, although much research is needed, encompassing other climatic zones (Elsheikh et al. 2021). Further investigation of these fungal assemblages may uncover their exact influence on plant colonization, invasion success, and their potential application in rehabilitating disturbed ecosystems.

One of the largest volcanic events on Earth was the cataclysmic eruption of Mt. Pinatubo in the Philippines in 1991. It was notable for massive lahars or mudflows, with a total bulk volume of 8.4 to 10.4 km^3 spewed during and following its explosive climatic activity (Pierson 1996). These lahars — of which primary sediment sources were loose volcanic clasts like coarse and fine-grained tephra and pyroclastic-flow deposits — caused extensive damage to natural resources burying about 18,000 hectares of forest lands and clogging nearby river systems (Mercado 1996, Pierson, 1996). In just a couple of years after the catastrophic eruption, lahars have accumulated in more than 0.7 mi³ (3 km³, ≈300 million dump-truck loads) (Newhall 1997). The frequency of mudflows declined over time but persisted during typhoon periods for more than a decade (Marler & del Moral 2011), eventually resulting in water- and nutrient-deficient soil. These barren substrates created by lahar deposits were colonized and inhabited by living organisms; hence, served as venues for investigating the complex processes of primary succession and ecosystem recovery.

Fifteen years later, ecological succession led to the emergence of vegetation, with *Saccharum spontaneum* (large rhizomatous grass) and *Parasponia rugosa* (woody tree) reportedly dominating the landscapes of the recovering ecosystems of Mt. Pinatubo (Marler & del Moral 2011). Known for its adaptive characteristics, *S. spontaneum* ('wild sugarcane' in English, 'talahib' in Filipino), as a perennial plant of the grass family (Poaceae), is ubiquitous in various types of habitats. This raises the question—what accounts for its colonization and invasion success? To our knowledge, microorganisms associated with *S. spontaneum* thriving in primary successional habitats have not yet been studied. Hence, our study aimed to isolate root fungal endophytes from *S. spontaneum* exhumed from a lahar-laden environment. The plant growth-promoting and protective properties of

these RFEs, specifically the indole-3-acetic acid (IAA) production, phosphate solubilization, and antagonistic activities against a common plant pathogen, were investigated to gain insights into the possible role of these fungi on the grass' success in colonizing lahar substrates. We hypothesized that the beneficial effects of RFEs can support the growth of *S. spontaneum* amidst exposure to lahar, thereby contributing to its colonization and invasion success in this harsh environment. The data also provided evidence of the ecological importance of RFEs in restoring ecosystems affected by natural disasters such as volcanic eruptions. Isolates demonstrating plant growth-promoting (PGP) effects unlocked the possibilities of harnessing these endosymbionts as potential sustainable sources of bioinoculants to enhance crop productivity in the agricultural sector, particularly those farm areas affected by natural and man-made disturbances.

Materials & Methods

Sampling site and the host plant

Healthy plant samples of *Saccharum spontaneum* were collected from three sampling points [(1) 15.21429622°N, 120.52902323°E; (2) 15.21419626°N, 120.52905749°E; (3) 15.21422988°N, 120.52895463°E] in a grassland ecosystem along the Sacobia River in Brgy, Calumpang, Mabalacat, Pampanga, Central Luzon, Philippines (Fig. 1). Sacobia River is located northeast of Mt. Pinatubo and was one of the eight major river systems clogged by lahar sediments during the volcano's cataclysmic eruption. Lahar deposits of approximately 1×10^8 m^3 and 7×10^7 m^3 were emplaced in the Sacobia-Bamban River in 1991 and 1992, respectively (Martinez 1999). About 90 km^2 of its alluvial plain and fan were covered with lahar by the end of 1992 (Martinez 1999).
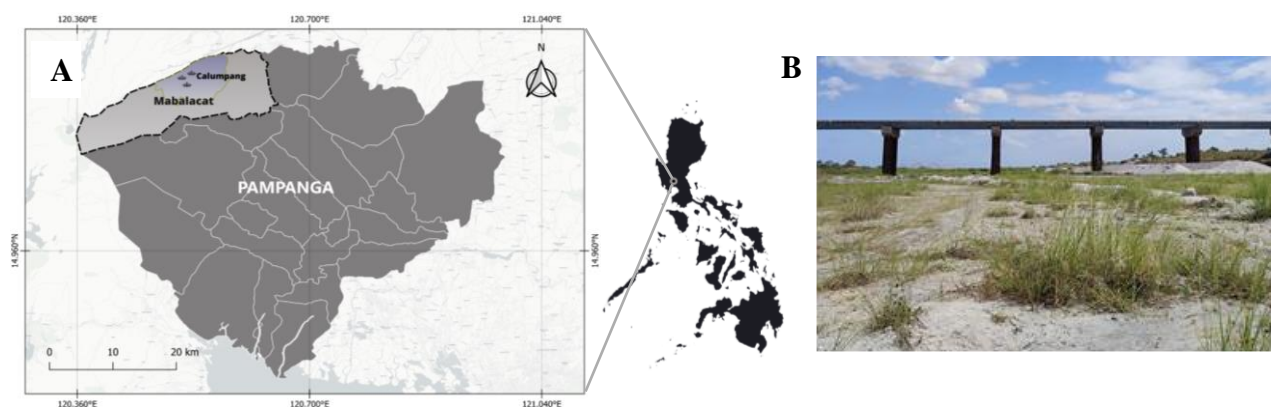
Fig. 1 – (A) Location of host plant collection at Brgy. Calumpang, Mabalacat, Pampanga, Philippines with three sampling points (~10m apart). Map created using QGIS 3.32 Lima. (B) Photo of the actual site with the host plant *S. spontaneum*.

Sampling was carried out in February 2023 during the cool, dry season. Three tussocks of *S. spontaneum* about 10 m apart were randomly excavated at a 30 cm depth. From each tussock, approximately 4–6 pieces of roots with lengths of around 9–12 cm were excised and placed in separate, properly labelled zip-locked polyethylene bags. These were kept in a portable cooler (4 °C), conveyed to the laboratory, and processed within 24 hours. To identify the host plant, the vegetative and reproductive parts of *S. spontaneum* were photographed following the online identification request protocol and later forwarded to Jose Vera Santos Memorial Herbarium (PUH), Institute of Biology, University of the Philippines, Diliman, for species identification and authentication.

The root fungal endophytes

Collected root samples were rinsed thoroughly to remove attached soil debris. The isolation of fungal endophytes started with the surface sterilization of roots by serial rinsing with 70%

alcohol for 1 minute, 5% sodium hypochlorite for 2 minutes, and 70% alcohol for 30 seconds, followed by three washes with sterile distilled water (Apurillo et al. 2019). Under aseptic conditions, the explants were dried on sterile filter paper and cut into small fragments (~5 mm). Root fragments were plated on Dichloran-Rose Bengal Chloramphenicol (DRBC, Condalab, Spain) agar in sextuplicate per sample, with each plate containing 5 explants (total number of explants = 30 per sample). Aliquots (0.1 ml) from the final washed water were plated to assess the efficiency of the surface sterilization. All plates were incubated at 25 °C in the darkness for 3–7 days or until fungal growth was seen. Emerging hyphae from all plated samples were sub-cultured in freshly prepared Potato Dextrose Agar (PDA, HiMedia, India) plates to obtain pure cultures. Stock cultures were maintained in agar slants and stored at 4°C in the Mycology Laboratory, Research Center for the Natural and Applied Sciences, University of Santo Tomas in Manila, Philippines.

Seventeen RFE isolates were selected based on their differences in colony morphologies (= morphospecies) and were grown on potato dextrose agar (PDA, Hi-media, India), Czapek Dox agar (CDA, Condalab, Spain), and malt extract agar (MEA, Hi-Media, India) for observation of colonial and spore morphologies. To confirm identities, fungal cultures were sent to Macrogen, South Korea for sequencing the ITS region. Identification was confirmed following the construction of phylogenetic trees with related taxa. More details on the identities of the root fungal endophytes used in this study are presented in Cruz & dela Cruz (2024). The isolated fungal endophytes used in this study were as follows: *Talaromyces brevis* (RFE1.1.2), *T. pinophilus* (RFE1.4.4B, RFE1.5.3, RFE2.3.4A), *T. aspriconidius* (RFE1.2.1A), *Fusarium suttonianum* (RFE1.3.2), *F. fujikuroi* (RFE1.5.5B, RFE2.6.2), *Penicillium griseopurpureum* (RFE1.13), *Epicoccum sorghinum* (RFE2.2.3A, RFE2.2.3B), *Trichoderma afroharzianum* (RFE1.4.5, RFE1.6.5, RFE1.6.2), *Purpureocillium lilacinus* (RFE3.2.1B), *Cladosporium colocasiae* (RFE2.2.4A)*, and *Rhizoctonia solani* (RFE3.1.1).

Assessment of beneficial traits

The 17 selected RFEs underwent a series of analyses to determine their plant growth-promoting effects and protective potential. These were initially sub-cultured onto freshly prepared PDA plates and incubated for 10 days at 25–28 °C. The fungal inocula used for the succeeding bioassays were obtained from these 10-day-old pure cultures.

IAA production

Fungal isolates were subjected to batch fermentation to facilitate the production of extracellular indole-3-acetic acid (IAA). Two 6 mm agar plugs from each 10-day-old isolate were inoculated into a 250 ml flask containing 100 ml of Czapek-Dox broth amended with 0.1% (w/v) L-tryptophan, and then incubated at 30 °C for 10 days without agitation. After incubation, the broths were filtered using Whatman filter paper No.1 to separate the fungal mycelia from the culture filtrates (CF). Each CF (1 ml) was mixed in 2 mL of Salkowski's indicator (150 ml H_2SO_4, 250 ml distilled water, 7.5 ml 0.5 M $FeCl_3.H_2O$) and incubated in the dark for 30 minutes. The formation of pink to red color in the solution indicated IAA production, while the formation of yellow color indicated a low to negative IAA yield. The color intensity of the mixture was quantified using a UV-VIS spectrophotometer set at 530 nm wavelength. The resulting IAA concentration (µg/ml) of the samples was compared to the standard calibration curve obtained from a series of pure IAA standard (Merck, Germany) solutions (100 ppm, 50 ppm, 25 ppm, 12.5 ppm, 6 ppm, 3.125 ppm; R^2= 0.8322). All tests were done in triplicates. The fungal mycelia were also oven-dried at 60 °C for two days and weighed to determine the production of IAA per unit dry mycelia weight (µg/mg) (Pablo 2020).

Phosphate solubilization

The ability of RFE to solubilize phosphate was observed on Pikovskaya's agar medium (PKV, Hi-Media, India) following the method described by Elias et al. (2016) and Jacob et al. (2023). Under aseptic conditions, 6 mm agar discs from 10-day-old cultures were placed at the

center of PKV agar plates in triplicates and incubated at 25–28 °C for seven days. Unisoculated PKV agar plates served as the control group. After incubation, a clear zone around a growing colony indicated phosphate solubilization. Comparative solubilization index measurement was done by measuring the clear zone and colony diameters in millimeters. The phosphate solubilization index (PSI) was computed as the ratio of the total diameter to the colony diameter (Elias et al. 2016, Jacob et al. 2023).

$$Phosphate\ Solubilization\ Index\ (PSI) = \frac{colony\ diameter + halo\ zone\ diameter}{colony\ diameter}$$

Antagonistic assay

The antagonistic activities of the RFEs were determined using the dual culture method as described by dela Cruz et al. (2021). Initially, RFEs and the test fungal pathogen (*Fusarium oxysporum*, generously provided by Edison Jay Pagoso, University of the Philippines - Manila) were inoculated on potato dextrose agar (PDA) and incubated for 10 days at 25–28 °C. After incubation, 6 mm mycelial discs were cut from the colony margin and then placed at opposite ends on petri plates (in triplicates, the total number of dual culture plates = 51) pre-filled with PDA. Plates inoculated with the pathogen in the absence of antagonist RFE isolates served as the control group. All plates were incubated at 25–28 °C for a week. The radial colony diameters of the RFE isolates and the pathogen were measured daily until the 7th day by a vernier caliper, and the percent inhibition of *F. oxysporum* was calculated as follows (dela Cruz et al. 2021):

$$\%\ Growth\ inhibition = \frac{r\text{-}r'}{r} \times 100$$

where: r = mean colony diameter of the control
       r' = mean colony diameter of treatment

All plates were incubated until the 14th day to observe the types of interaction (A–E) following the descriptions provided by Wicklow et al. (1980). The observed antagonistic activity of each RFE against *F. oxysporum* was later re-evaluated after an extension of incubation for two weeks (observation done on the 30th day). We developed subcategories for each type of interaction depending on which fungus overgrew or outcompeted the other (Table 1).

Table 1 Category scheme used to describe antagonistic activity between RFE and the phytopathogen *F. oxysporum* on dual culture plates following the extended incubation (original categories modified from Wicklow et al. 1980).

Type of Interaction/*Description*	Code
Mutual intermingling of two organisms	A
Neutral interaction between two organisms characterized by mycelial fusion. After some time, the boundary between the two organisms becomes unrecognizable.	
Mutual inhibition upon mycelial contact	B
Antagonistic interaction where the point of contact or border between two organisms remains recognizable after some time.	
• No changes with the initial interaction	B0*
• Fungal endophyte eventually outgrew the pathogen	B1*
• Pathogen eventually outgrew the fungal endophyte	B2*

Table 1 Continued.

Type of Interaction/*Description*	Code
Mutual inhibition at a distance	C
Antagonistic interaction between two organisms where no mycelial contact is observed.	
• No changes with initial interaction	C0*
• Fungal endophyte eventually outgrew the pathogen	C1*
• Pathogen eventually outgrew the fungal endophyte	C2*
Inhibition of one organism upon mycelial contact	D
One fungus outgrows the other upon mycelial contact.	
• Fungal endophyte eventually outgrew the pathogen after some time.	D1*
• Pathogen eventually outgrew the fungal endophyte after some time.	D2*
Inhibition of one organism at a distance	E
One fungus outgrows the other without mycelial contact.	
• Fungal endophyte eventually outgrew the pathogen after some time.	E1*
• Pathogen eventually outgrew the fungal endophyte after some time.	E2*

*Inhibition mechanisms observed after an extended incubation period of 30th day.

Data analysis

Data was analyzed statistically using one-way Analysis of Variance (ANOVA). As a post hoc test, the significant differences in the computed mean values were compared by Tukey's Honest Significant Difference (HSD) at $p \leq 0.05$. A simple correlation analysis was done to describe the relationship between IAA concentration and fungal biomass.

Results

IAA production

The 17 RFEs reacted to Salkowski's reagent and showed the production of extracellular IAA at varying concentrations, i.e., 20.13 – 159.89 µg/ml (Fig. 2). Among these root fungal endophytes, the formation of pinkish to red coloration, indicating the presence of IAA, was observed from the culture filtrates of seven notable isolates, namely *Purpureocillium lilacinum* (RFE3.2.1B), *Trichoderma afroharzianum* (RFE1.4.5, RFE1.6.2), *Penicillium griseopurpureum* (RFE1.1.3), *F. fujikuroi* (RFE1.5.5B, RFE2.6.2), and *F. suttonianum* (RFE1.3.2). Comparison between the different IAA concentrations as produced by the isolated RFE showed that these seven notable isolates produced significantly higher values than the others ($p<0.05$). We also determined and correlated the dry weight of the mycelia formed during batch fermentation in CDB and the concentrations of the IAA produced by the root fungal endophytes. Our simple correlation analysis showed that IAA production is inversely proportional to mycelial dry weight. Therefore, the fungal biomass is not directly related to the extracellular production of IAA (Fig. 3).
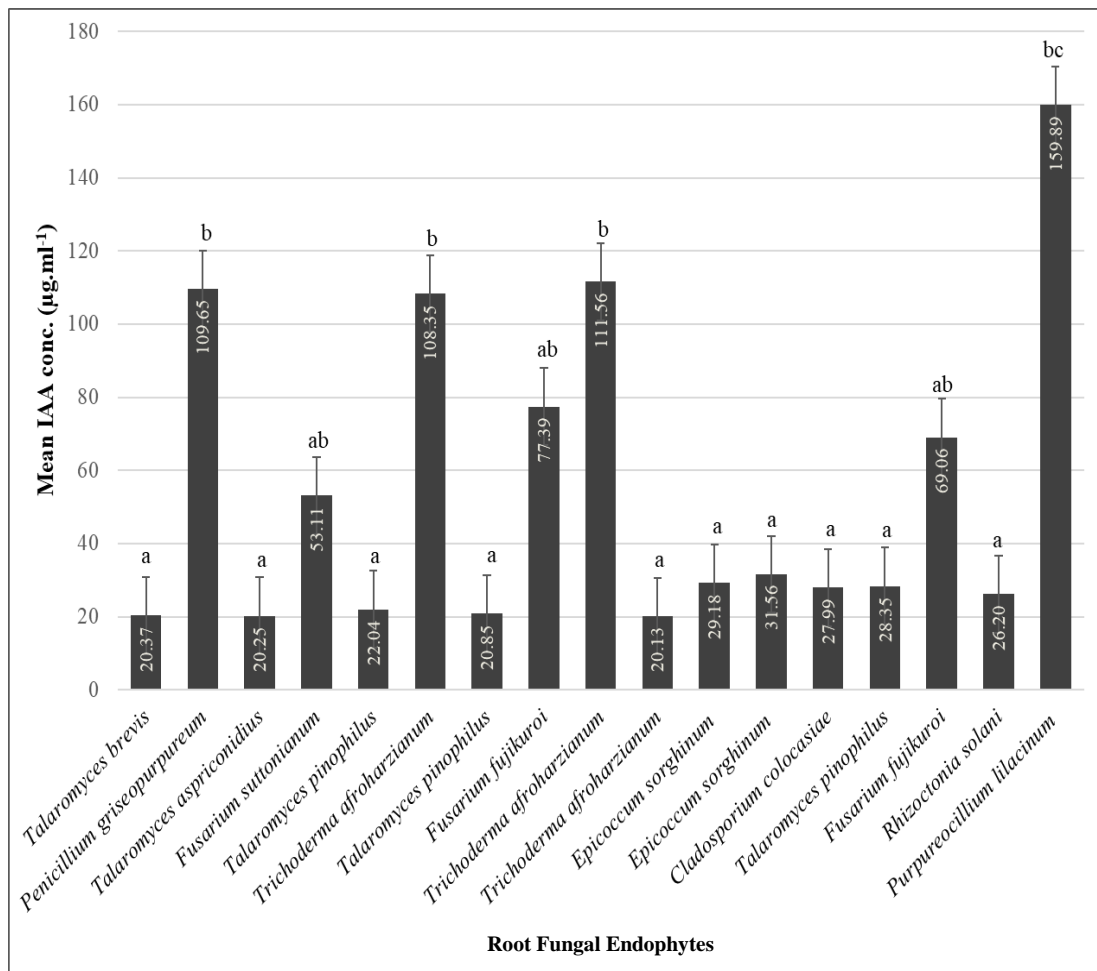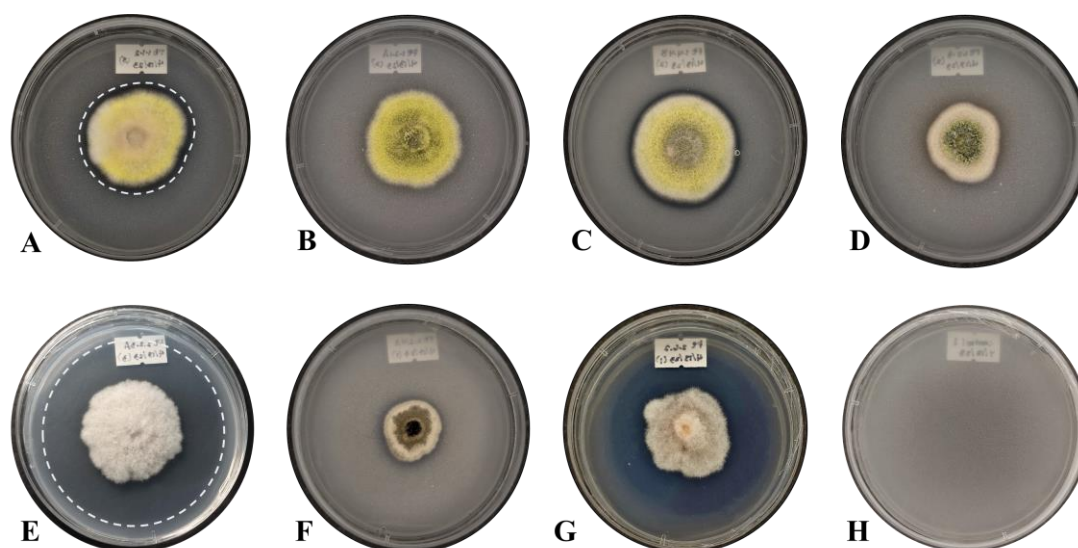
Fig. 2 – IAA production of RFE per ml of Czapek-Dox broth (CDB). Error bars having different letters are significantly different from each other as statistically computed using Tukey's HSD test ($p<0.05$).
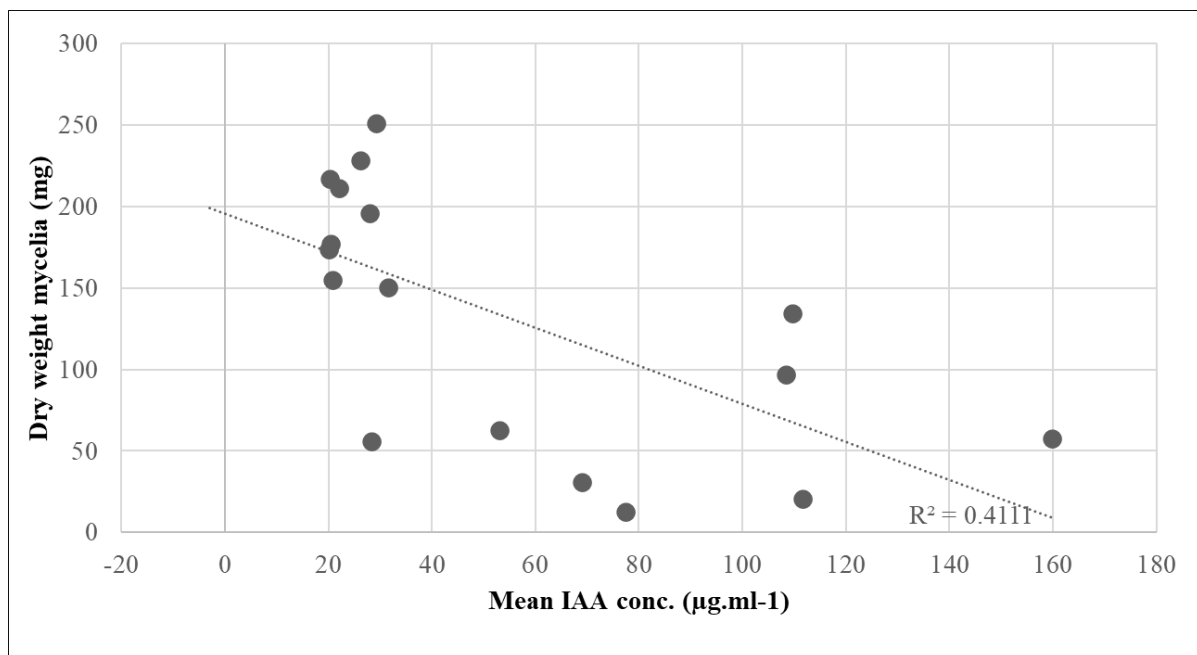

Fig. 3 – Scatterplot showing the intermediate inverse correlation between fungal biomass and IAA production by selected RFEs (r=-0.641, $p<0.05$).

Phosphate solubilization

As one of the macronutrients essential for the growth and development of host plants, phosphorus (P) is absorbed by plants from the soil in the form of phosphate (PO_4^{-2}). Root-associated fungi can acquire P and convert it to labile forms, thereby enhancing the host plants' accessibility to these soluble phosphates. In this study, seven notable fungal isolates, namely, *Talaromyces brevis* (RFE1.1.2), *T. aspriconidius* (RFE1.2.1A), *T. pinophilus* (RFE1.4.4B, RFE1.5.3), *E. sorghinum* (RFE2.2.3A), *C. colocasiae* (RFE2.2.4A), and *F. jujikuroi* (RFE2.6.2) showed the ability to break down phosphate through the formation of clearing zones around the colonies on Pikovkayas Agar (Fig. 4). The computed phosphate solubilization indices (PSI) of these RFEs are shown in Fig. 5.

Fig. 4 – Phosphate solubilizing activities of seven RFEs on PKV agar. A *Talaromyces brevis* (RFE1.1.2), B *Talaromyces aspriconidius* (RFE1.2.1A), C *Talaromyces pinophilus* (RFE1.4.4B), D *Talaromyces pinophilus* (RFE1.5.3), E *Epicoccum sorghinum* (RFE2.2.3A), F *Cladosporium colocasiae* (RFE2.2.4A), G *Fusarium fujikuroi* (RFE2.6.2). H Uninoculated PKV plate (control).
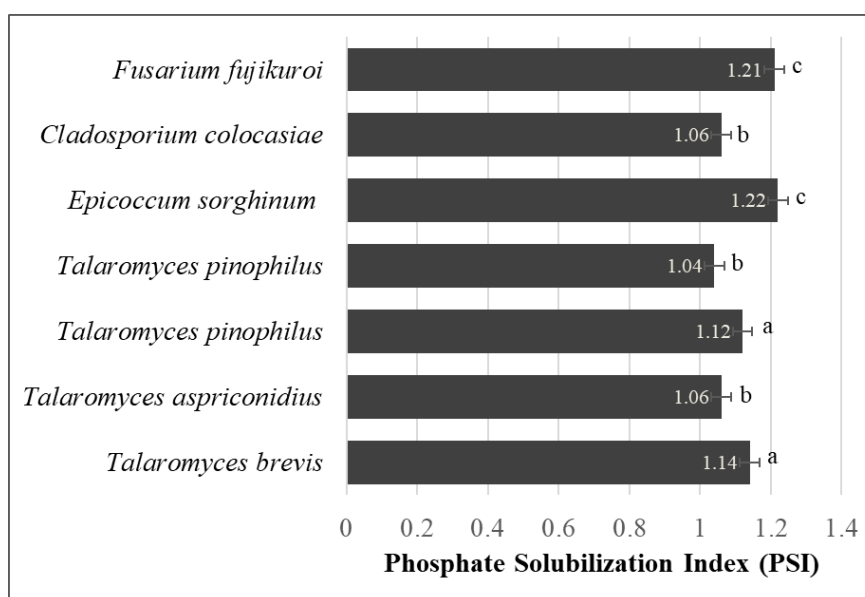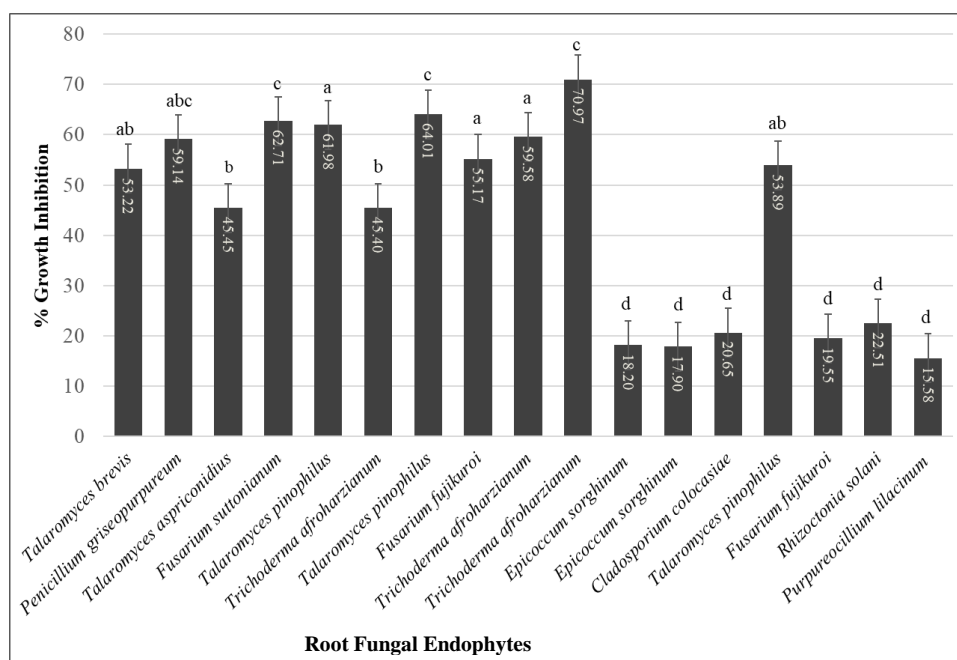

Fig. 5 – Phosphate solubilization indices of seven RFEs isolated from *S. spontaneum*. Error bars having different letters are significantly different from each other as statistically computed using Tukey's HSD test ($p < 0.05$).

Antagonistic Interaction

The 17 RFEs isolated from *S. spontaneum* were also tested for their antagonistic activities against and interaction with *Fusarium oxysporum*, a soil-borne pathogenic fungus that is well known for causing severe wilt disease and root rots in plants. A dual culture experiment was employed to observe the antagonistic action of the RFEs after a 7-day incubation period, which was extended to the 30th day of incubation to describe further their interaction. We devised a new category based on the type of interaction by Wicklow et al. (1980) to illustrate fungi-fungi interaction.

Results of the dual culture assay revealed that nine of the 17 RFEs significantly suppressed the growth of *F. oxysporum* showing > 50% inhibition rates on the 7th day of incubation (Fig. 6). These fungal isolates were identified as *Trichoderma afroharzianum* (RFE1.6.5), *Talaromyces pinophilus* (RFE1.5.3), *F. suttonianum* (RFE1.3.2), *Talaromyces pinophilus* (RFE1.4.4B), *Trichoderma afroharzianum* (RFE1.6.2), *Penicillium griseopurpureum* (RFE1.1.3), *F. fujikuroi* (RFE1.5.5B), *Talaromyces pinophilus* (RFE2.3.4A), *Talaromyces brevis* (RFE1.1.2), arranged with highest to lowest inhibitory rates. The growth of the pathogen was moderately suppressed by *Talaromyces aspriconidius* (RFE1.2.1A) and by another *Trichoderma afroharzianum* (RFE1.4.5), with only 45% radial growth inhibition. Statistical analysis revealed a significant difference in inhibition rates between the 17 RFEs after a 7-day incubation period ($p<0.05$) (Fig. 6).

Fig. 6 – Inhibition rates of *F. oxysporum* by the 17 RFEs in dual culture plates. Error bars bearing different letters are significantly different from each other as statistically computed using Tukey's HSD test ($p<0.05$).

Between the tested RFEs, the pathogen *F. oxysporum* showed relatively low susceptibility to *E. sorghinum* (RFE2.2.3A, RFE2.2.3B), *C. colocasiae* (RFE2.2.4A), *F. fujikuroi* (RFE2.6.2), *R. solani* (RFE3.1.1), and *Purpureocillium lilacinum* (RFE3.2.1B) in dual cultures observed on the 7th day of incubation. After the 2nd week of incubation, the different types of interactions between the RFEs and the pathogen were also observed. The highest number of dual cultures plates (21) was observed to exhibit inhibition of one organism at a distance (Type E), while 19 showed inhibition of one organism upon contact (Type D). Mutual inhibition upon mycelial contact (Type B) was observed in seven dual cultures, while mutual inhibition at a distance (Type C) was displayed by four dual culture plates (Table 2). Figure 7 shows the dual culture between RFE and *F. oxysporum*.
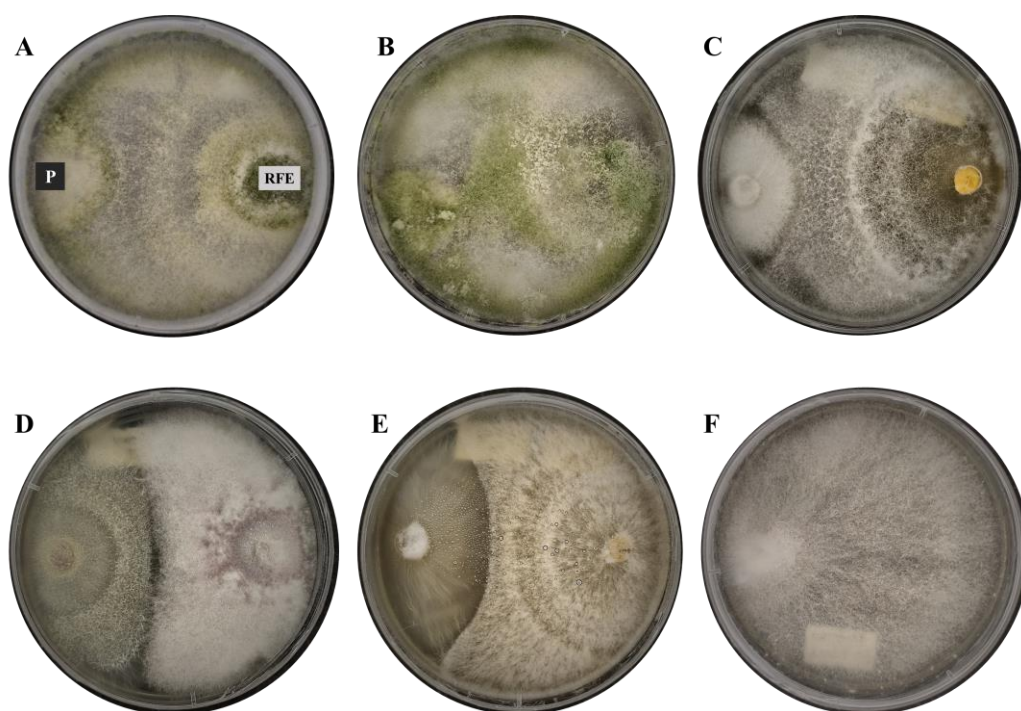
Fig. 7 – Growth inhibition of *F. oxysporum* (**P**, on the left corner of plates) by the root fungal endophytes (RFE, on the right corner of plates). A–C *Trichoderma afroharzianum* isolates: RFE1.4.5, RFE1.6.2, RFE1.6.5, D *Epicoccum sorghinum* RFE2.2.3B, E *Rhizoctonia solani* RFE3.1.1., F Plate inoculated with *F. oxysporum* mycelial discs only (control) showing the pathogen's normal growth in the absence of the antagonistic RFE. All plates were incubated for 14 days.

Table 2 Combative interactions between RFE and *F. oxysporum* following 14 and 30 days of incubation.

Type of Interaction	No. of dual cultures (Day 14)	Type of Interaction	No. of dual cultures (Day 30)
Type A	0	A	0
Type B	7	B0	7
		B1	0
		B2	0
Type C	4	C0	B (2)
		C1	0
		C2	2
Type D	19	D0	B (1)
		D1	13
		D2	5
Type E	21	E0	D2 (8)
		E1	0
		E2	13
Total	51		51

To clearly depict which organism (the fungal endophyte versus the phytopathogen) outgrows or outcompetes the other, subtypes for interactions B to E were devised after an extended incubation up to the 4th week (Day 30). This means that the type of interaction was reassessed

following a prolonged incubation. Table 2 shows the changes in the interactions between the RFE and the phytopathogen, *F. oxysporum*, following a 14- and 30-day incubation period. For instance, from mutual inhibition at a distance on Day 14, two of the four dual cultures that initially displayed Type C interaction changed to Type C2 on Day 30, where the pathogen eventually outcompeted the RFE. The other 2 dual culture plates progressed to Type B, where mutual inhibition upon mycelial contact was observed between the competing organisms. Eight of the 21 dual culture plates showing the outgrowth of the pathogen at a distance (Type E) on Day 14 were later found to exhibit Type D2 interaction on Day 30, where the pathogen overgrown the RFE upon hyphal contact. Meanwhile, the other 13 dual cultures persisted in exhibiting Type E interaction from Day 14, with the pathogen outcompeting the fungal endophytes at a distance. Likewise, mutual inhibition upon mycelial contact (Type B) that was initially displayed by seven dual cultures continued after a 2-week extended period of incubation (Type B0). Furthermore, 11 dual culture plates displayed neutral interactions, 14 showed that the RFE were able to outcompete *F. oxysporum*, and 26 displayed the pathogen outgrew the RFE following a 14-day incubation. These values changed after the 30th day of observation, where 13 dual cultures showed the RFE outcompeting the plant pathogen, 26 showed the pathogen outgrowing the RFE, and 12 displayed mutual intermingling between competing organisms.

Discussion

Plant growth-promoting fungi (PGPF) are known to induce plant growth and development by producing phytohormones (e.g., auxins like IAA and gibberellic acid), by increasing the availability of soluble phosphorus in the soil, and by conferring protection against phytopathogens. A previous study reported the production of IAA by root fungal endophytes, and these included *Aspergillus versicolor, Aspergillus* sp., *Cladosporium cladosporioides, Trichoderma harzianum, Chaetosphaeria* sp., *Microascus murinus, and Hypocrea lixii,* all isolated from *Diket red*, a traditional Cordillera rice landrace (Pablo 2020). Metal-spiked soil inoculated with the endophytic fungus, *Paecilomyces lilacinus* isolated from the shrub *Justicia adathoda,* was also able to promote the growth of *Solanum lycopersicum* through the production of IAA at 40.20 µg/mL (Musa et al. 2023), a lower concentration compared to 109.65 µg/mL produced by the RFE in our study. Earlier greenhouse experiments revealed an increase in the growth of *Lycopersicum esculentum* (Cavello et al. 2015), maize, and soybean (Baron et al. 2020) through IAA that were produced by *Purpureocillium lilacinum* (= *Paecilomyces lilacinus*) which were isolated from soil. Promising IAA production was observed by different species of *Fusarium* derived from the seeds of *Cassia alata, Zingiber roseum*, and *Desmodium pulchellum* (Pradhan 2019). This study also reported that the supplementation of L-tryptophan, carbon, and nitrogen sources in different culture media influenced the growth of and enhanced IAA production of fungal isolates. Root elongation and biomass of the experimental plant *Arabidopsis thaliana* were enhanced through the production of IAA by fungal endophytes, including *Fusarium* sp. that were isolated from different parts of the medicinally important plant, *Sophora flavescens* (Turbat et al. 2020). These studies showed the ability of plant-associated fungi to produce phytohormones that can benefit their host plants. Other related studies that reported the production of phytohormones such as IAA by different fungi included the endophytic strains of *Trichoderma* (Tseng et al. 2020, Zin & Badaluddin 2020), *Aspergillus* (Nath et al. 2015, Mehmood et al. 2019, Pablo 2020), *Talaromyces* (Sahu et al. 2019, Patel et al. 2021, Airin et al. 2023), *Cladosporium* (Pablo 2020), *Rhizoctonia* (Furukawa & Syono 1998), and *Epicoccum* (Li et al. 2022). Root fungal endophytes like these beneficial fungal taxa were also isolated from the pioneering grass species *S. spontaneum* in this study, and these RFEs showed the ability to produce IAA, indicating their potential role of promoting plant growth in nutrient-poor environments such as those affected by lahar flows.

Phosphate solubilizing activities were also reported from *Talaromyces* taxa isolated from soil (Ma et al. 2022, Sembiring & Sabrina 2022) and from the genera *Pestalotiopsis*, *Neopestalotiopsis* and *Pseudopestalotiopsis*-associated with mangroves (Jacob et al. 2023). The phosphate solubilization indices displayed by our isolates *Talaromyces pinophilus* RFE1.5.3 (PSI=1.04) and

T. pinophilus RFE1.4.4B (PSI=1.12) were slightly lower than the PSI (= 2.8) exhibited by *T. pinophilus* derived from andisol soil affected by volcanic eruption (Sembiring & Sabrina 2022). Nevertheless, these showed that *T. pinophilus* was an ideal fungus for biofertilizers as other studies also supported its promising activity. For example, different strains of *T. pinophilus* from the soil displayed different levels of phosphate solubilization in PKV agar (Majumder et al. 2019). Likewise, co-inoculation of *T. pinophilus* and mycorrhizal fungi in oil palm seedlings increased plant dry weight and P uptake by the roots (Sembiring et al. 2018).

Other RFEs were also reported to have significantly higher PSI indices, e.g., *Epicocccum sorghinum* (RFE2.2.3A) and *F. fujikuroi* (RFE2.6.2), indicating a more efficient phosphate solubilizing potential of these two RFE isolates. Interestingly, both fungal species were reported as pathogens of plants and valuable crops. *Fusarium fujikuroi* is a causative agent of bakanae or foot rot disease in rice (Shakeel et al. 2023, Tadasanahaller et al. 2023), while *Epicoccum sorghinum* is a ubiquitous phytopathogen known for causing ring and leaf spots in a variety of plants, including rice, sugarcane, wheat, and sorghum (Oliveira et al. 2018, Laurel et al. 2021). While the mentioned fungi are pathogenic to some plants, they were also reported to be beneficial to other plant hosts, for instance, *S. spontaneum*, as reported in this study, promotes growth through the increase of the bioavailability of phosphorus. This indicates that phosphate solubilization is strain specific.

The members of the genus *Trichoderma* have also been well-studied and widely used in agriculture as effective, low-cost, and eco-friendly biocontrol agents (Zin & Badaluddin 2020, dela Cruz et al. 2023, Yao et al. 2023) in addition to their application in environmental clean-up (De Padua & dela Cruz 2021). Displaying the highest percentage of radial growth inhibition (71%) *in vitro*, this study reports the effective antagonistic activity of the root fungal endophyte *Trichoderma afroharzianum* against *F. oxysporum*. Similarly, the promising biocontrol potential of *T. afroharzianum* isolated from wood chips of grapevine *Vitis vinifera* L. and different soil samples were reported in other recent studies, which also employed the dual culture test. These studies included the inhibitory activities of *T. afroharzianum* against *Fusarium culmorum* (Bouanaka et al. 2021), *Aphanomyces cochlioides*, *Botryosphaeria dothidea*, *Diaporthe eres*, *Diplodia seriata*, *Eutypa lata*, *Neofusicoccum parvum*, and *Pythium acantophoron* (Kovács et al. 2021). However, other fungal endophytes were also reported to show antagonistic activities against other fungi (De Mesa et al. 2020, Pecundo et al. 2021). The endophytic *T. pinophilus* from onion umbels inhibited the growth of *Botrytis cinerea* through mycoparasitism (Abdel-Rahim & Abo-Elyousr 2018), and of *Pythium aphanidermatum* and *Rhizoctonia solani* (Kazerooni et al. 2019).

Dual cultures observed on the 7th day of incubation showed relatively low susceptibility of *F. oxysporum* to *Epicoccum sorghinum*, *Cladosporium colocasiae*, *Fusarium fujikuroi*, *Rhizoctonia solani,* and *Purpureocillium lilacinum* isolates. This could be related to the varying growth rates of culturable fungi using agar-based method. The members of the genus *Fusarium*, including the pathogen *F. oxysporum*, are known to display fast-growing colonies in potato dextrose agar (Cighir et al. 2023), which was the medium used in the conduct of dual-culture assay. Additionally, fungi may have different strategies for acclimatizing under competitive conditions that affect their speed of growth. For instance, instead of dedicating their energy to growing and reproducing, some strains may produce secondary metabolites like antioxidants and antimicrobials, allowing them to thrive and compete effectively for resources available in a given environment (Gonzalez & Aranda 2023). Hence, we deemed it necessary to extend the incubation of dual-culture plates for 14–30 days to observe possible changes in the growth behavior of the competing fungal isolates after a prolonged period. It is noteworthy that the RFE and the test pathogen were viable during the incubation extension, which allowed the continued observation of their combative interactions—some dual culture plates retained the initially observed antagonistic activity while the others progressed to a different type of inhibitory interaction, as depicted in Table 2. For instance, some RFEs that were initially suppressed by the pathogen eventually outcompeted the pathogen or vice versa, and mutual inhibitory interactions progressed to inhibition of one organism by the other after a prolonged period. These observed changes may be due to various reasons, and the dual-culture technique is limited only to measuring the radial growth of competing organisms under controlled

conditions, providing semi-quantitative measurements of inhibitory activity (Bodil et al. 2021). Some drawbacks in employing these classic agar-based methods must be considered, such as the fact that the test microorganisms are restricted to the available space for growth and the nutrients present in a specific agar medium used (Colombo et al. 2019, Bodil et al. 2021, Kashyap et al. 2023). Additionally, the volatile compounds produced by the individual isolates may have influenced their antagonistic activities (Bodil et al. 2021, Kashyap et al. 2023). Nevertheless, our study highlighted the importance of prolonged incubation in the dual culture method as a strategy to evaluate antagonistic interaction between fungi. The beneficial traits observed in our isolated root fungal endophytes also support our hypothesis that fungal endophytes could assist their host plant in colonizing under low nutrient availability or stressful conditions and subsequently restore areas affected by natural and man-made calamities.

Accessibility of data

Supplementary data are available from the corresponding author upon request.

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