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Biocontrol potential of indigenous fungal antagonists from soils naturally suppressive to *Fusarium oxysporum* f. sp. *cubense* tropical race 4

Arnel V. Somolostro*[1], Carolina D. Amper[1], Mellprie B. Marin[1], Darwin M. Apistar[1], Myrna G. Ballentes[2], Ailyn Q. Daniel[1]

[1]*Department of Plant Pathology, College of Agriculture, Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines*
[2]*Department of Entomology, College of Agriculture, Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines*

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ABSTRACT

Worldwide banana production has become increasingly vulnerable to infections from *Fusarium oxysporum* f. sp. *cubense* race 4 (Foc TR4), given the dominance of the Cavendish banana (90%) which is highly vulnerable to the infection. To improve current Foc TR4 disease management used, this study considers alternatives control, like the use of soils that resist the disease along with their beneficial microbiota and ability to limit fungal development. The research involved analysis of soils from plantations in Tulunan, Cotabato, Philippines, where crops remained healthy despite outbreaks nearby and considered it as suppressive while the opposite was conducive. In particular, suppressive soils differed from conducive soils by being characterized as neutral pH, sandy clay loam, higher organic and phosphorous availability while conducive were sandy loam, acidic, and low organic content. A total of 26 microorganisms were extracted from suppressive soils, among which five species showed strong antagonistic activity against Foc TR4: *Phlebiopsis gigantea, Paecilomyces fulvus, Aspergillus niger, Trichoderma harzianum,* and *Talaromyces viridulus*. The results from three assays (dual culture assay, test for volatile organic compounds, and culture filtrate assay) performed showed that, *A. niger* and *T. harzianum* significantly reduced the rate of the pathogen growth by nearly 80% through resource competition; *P. gigantea* inhibited 54% by producing volatiles, and *T. viridulus* by 27% via metabolite production. All these experiments prove suppressiveness is not random but dependent on unique physical, chemical, and biological conditions.

***Corresponding author:** Arnel V. Somolostro ✉ avsomolostro@gmail.com

INTRODUCTION

Bananas represent an important aspect in the lives of millions of farmers worldwide because of the food energy, money, and nutrition provided by the crop. They are considered the world's largest grown fruits. As indicated by FAO (2017) and Maymon (2020), worldwide production of bananas amounts to about 114 million tons yearly, valued at about $8 billion. About 15% of bananas are exported, with almost all of those exportable being from the Cavendish variety. The transition from other varieties to Cavendish was spurred in the 1960s by its ability to resist Race 1 of Panama Disease. Today, a similar historical transformation is taking place in response to an even greater challenge, Tropical Race 4 (TR4). Initially identified in Southeast Asia and Australia in the 1970s, TR4 compromises the immunity of Cavendish and infects most commercial banana varieties. The effect of TR4 is that of vascular blockage, resulting in red discoloration (yellowish), desiccation of stems, and plant death after a few weeks/months (Matos *et al.*, 2023). The management of TR4 has several challenges because of the presence of chlamydospores, which are highly resistant and have several means of spread, for instance, through farming equipment, irrigation water, soil carried by people's shoes, tires, and diseased plants (Matos *et al.*, 2023; FAO, 2023). As of now, TR4 has been reported in more than 21 countries that produce bananas, such as the Philippines, Peru, Venezuela, and others, with crop losses estimated at 80% (Matos *et al.*, 2023; FAO, 2023).

A universal solution remains unavailable. Producing resistant cultivars takes a lot of time and is not always comparable with Cavendish in terms of taste, durability, and productivity. Moreover, fungicides may be ineffective against deep infections, expensive for small farmers, and damaging for the soil and water ecosystems in the long run. And as the emergence of Foc TR4 itself proved, relying on host resistance alone only creates pressure for pathogens to evolve new ways to infect (Dita *et al.*, 2018).

This gap has turned attention toward one of nature's own defenses, soil suppressiveness. This refers to the natural ability of soils to prevent or inhibit disease epidemics in the face of pathogens, susceptible plants, and favorable environmental conditions. Recent studies have revealed that the microbes within the soil are responsible for the phenomenon. These beneficial organisms fight pathogens through many overlapping strategies: they consume nutrients first so nothing is left for invaders, release natural antibiotics and toxins, wrap around and eat pathogenic cells directly, or even trigger the plant's own internal immune responses (Jayaraman *et al.*, 2021; Löbmann *et al.*, 2016). Generally speaking, healthy, biologically active soils are suppressive; degraded, low-diversity soils are conducive, meaning they make it easy for diseases to take hold (Dita *et al.*, 2018).

Although its usefulness cannot be overstated, little is known about what makes the disease suppressive or which microorganisms play the major role in suppressing Foc TR4 infection under Philippine conditions. This study will attempt to answer three main questions. These are: (1) What physical and chemical characteristics differentiate disease suppressive soils from non-suppressive soils. (2) What kinds of fungi found in suppressive soils prevent the development of Foc TR4. (3) By what means do these fungi protect themselves from the pathogen.

MATERIALS AND METHODS

Sites selection and sampling

Samples were collected from commercial banana farms managed by independent growers from Tulunan, Cotabato regions that produce a share of the Philippines' Cavendish crop. To ensure true comparative differences, two distinct types of sites were selected, following standard protocols established by earlier soil disease research (Shen *et al.*, 2013). Suppressive sites were defined as fields that had remained productive with low or no wilt symptoms for at least 10 years, even though neighboring farms had become infested. Conducive

sites, by contrast, were fields with a long history of severe, recurring wilt damage, where disease levels rose steadily year after year. In order to reduce bias and acquire an unbiased sample, a zigzag pattern was used in sampling across the entire field with 20 soil samples being collected from a depth of 15-20 cm where most of the feeder roots of banana were located in each location. These samples were mixed uniformly to form one composite sample for each location and then stored in UV sterilized plastic bags and preserved on ice cooler until transported to the laboratory where samples were maintained at 4°C until analysis.

Physicochemical analysis of soil

All samples obtained were then analyzed by the Soil and Plant Analysis Laboratory in Central Mindanao University, and analysis was done on selected parameters such as texture of soil, which indicates the drainage, aeration, and nutrient content of the soil; pH; organic matter; available phosphorus; and exchangeable potassium.

Isolation and characterization and identification of Foc TR4

Samples were obtained from the inner vascular tissue of Cavendish plants showing advanced symptoms of wilt, yellowing leaves, discolored rhizomes, and rotting lower stems. In the laboratory, 5–10 cm sections were cut from infected bracts, surface dirt was washed off with tap water, and outer tissue was sterilized by dipping in 70% ethanol for 60 seconds, followed by three rinses in sterile distilled water and blotting dry with sterile filter paper. Small pieces of sterile samples were then placed on Potato Dextrose Agar (PDA) plates. After 5–7 days of growth at room temperature, colonies matching the appearance of Fusarium were sub-cultured to obtain pure cultures. Initial identification was done through microscopic observation of hyphal and spore structure; and final confirmation that the strain was indeed Tropical Race 4 was completed using Polymerase Chain Reaction (PCR) followed by genetic sequencing of the 18S rRNA region.

Isolation of native microbes from suppressive soils

All potential biocontrol organisms were isolated solely from suppressive soils previously collected. The stock suspension was made by mixing 10 grams of composite soil with 90 milliliters of distilled water and shaking vigorously for 15 minutes to release microorganisms from the soil. Serial dilutions were then made using this stock suspension. The fungal isolation techniques involved the inoculation of appropriate dilutions in an amount of 0.1mL across PDA plates, while the NA medium was used in case of bacteria. The plates were then incubated at room temperature for a period of seven days. The selected colonies which differed from one another in terms of color, texture, morphology, and growth rate were further purified by transferring to new culture plates until pure cultures could be obtained.

***In vitro* assessment of antagonistic activity of selected antagonists**

Three independent experiments were performed to test the inhibition of Foc TR4 growth by each potential microorganism. The experimental design used was Completely Randomized Design (CRD) using four replicates for each treatment. Statistical analysis was done using ANOVA (STAR) and Tukey's HSD.

Dual culture assay

The experiment conducted involved testing for the effect caused by the interaction between two species which utilize the same source of nutrition (Anith *et al.*, 2021) through dual culture assay. All 26 microorganisms was subjected to this assay by collecting 6 mm plug using cork borer from the four days old antagonist isolate and placed to the new PDA dish with 2 cm distant from the edge of a plate. A separate plug, of the same dimensions, was cut from a newly cultured sample of Foc TR4, and this was also positioned 2 cm away from the other edge of the dish. For the control experiment, plates containing only the pathogen but not any other competing species were used. Plates were left at room temperature for nine days, starting from day

four after inoculation; the inhibition of growth was measured using the following formula as proposed by Anith *et al.* (2021). The growth inhibition percentage is given by [(R1-R2)/R1] x 100 where R1 represents the mean radius of the pathogen colony grown under control plates while R2 represents the radius of the same pathogen colony grown in the presence of antagonist.

Test for volatile organic compounds (VOC)

Some fungi may use VOC as their mode of defense against the pathogens. In such a case, top 15 isolates showed antagonism against Foc TR4 from dual culture assay were subjected to VOC by inoculated antagonists into sterile PDA and incubated it for seven days. A second PDA plate inoculated with a 6 mm plug of Foc TR4 was then taken, turned upside down, and sealed tightly over the plate holding the antagonist. This created a closed system where gas could move freely, but the two organisms could never touch physically. Controls consisted of pathogen plates sealed over plain, uninoculated media. Mycelial growth was measured daily 5 to 7 days after inoculation (DAI). The setup was repeated using a suspension of Foc TR4 spores (102 CFU/mL) instead of a solid plug, to determine if these airborne chemicals stopped germination as well as growth.

Culture filtrate assay

Compounds that dissolve in water and move through soil moisture (Were *et al.*, 2021) were also evaluated. From 15 isolates in VOC, 11 were selected and subjected to culture filtrates test. Eight to ten plugs from each antagonist were transferred into 25 mL of sterile Potato Dextrose Broth, with cultures shaken gently at 27°C for 7–10 days. When they reached complete maturity, cultures were subjected to centrifugation at 10,000×g for 10 minutes to form pellets containing fungal cells, thereby separating them from the remaining liquid media. The resulting filtrate obtained after filtration through a 0.22-micron filter resulted in cell-free and sterile culture filtrates that were mixed with molten PDA, in a ratio of 1:9, and poured as plates to which was placed Foc TR4 inoculum plugs. After seven days of incubation, colony sizes were compared against pathogen growth on untreated PDA to measure inhibition.

Morphological and molecular identification of selected antagonists

From all isolates, only top 5 that showed highest consistency, significant inhibition the three assays performed were selected for morphological characteristics. To confirmed initial morphological identification, pure cultures of antagonist were subjected to molecular analysis. Pure genomic DNA was extracted from each strain, followed by amplification of the 18S rRNA region, as another source of marker for fungal species recognition using established in-house primers Philippine Genome Center as responsible for molecular analysis. Resulting genetic sequences were compared against all published records in the NCBI GenBank database using the BLAST tool to confirm the closest match and assign species names.

RESULT AND DISCUSSION

Differences between suppressive and conducive soils through physicochemical analysis

For every measurement taken, the gap between the two soil groups was clear and consistent, as shown in Table 1. Soils from healthy, suppressive fields fell into the sandy clay loam category textured and soils from conducive sites were sandy loams. The pH readings revealed that suppressive soils obtained neutral at 7.01 and a low pH on conducive soils recording a value of 4.39. The content of organic matter further supported the trend, since in the suppressive soils, the level of organic carbon recorded an average of 3.80%, almost double the amount attained in the conducive soils, which stood at 2.00%. Similarly, the level of available phosphorus was considerably higher in the suppressive soils, recording 139 ppm compared to 120 ppm for the conducive soils. However, exchangeable potassium recorded values that were almost the same, with 1553 ppm in the suppressive soils and 1570 ppm in the conducive soils.

Table 1. Physical and chemical properties of soils differing in susceptibility to *Fusarium* wilt

Parameter	Suppressive soil	Conducive soil
Textural class	Sandy clay loam	Sandy loam
Soil reaction (pH)	7.01	4.39
Organic matter content	3.80%	2.00%
Available phosphorus	139 ppm	120 ppm
Extractable potassium	1553 ppm	1570 ppm

Table 2. Morphological features of the selected fungal antagonists

Species	Cultural and microscopic traits
Phlebiopsis gigantea	Colonies grow flat and spread out with a waxy, smooth surface; key microscopic markers include clamp connections along hyphae, thickened lamprocystidia, and short, asexual spore structures called oidia
Paecilomyces fulvus	Grows rapidly with a powdery, cotton-like texture; produces masses of yellowish-olive spores borne on highly branched, brush-like spore-bearing stalks
Aspergillus niger	Forms dense, velvety colonies that mature from white to deep jet-black; characterized by large, swollen heads that hold rows of round, dark brown spores in two distinct layers
Trichoderma harzianum	Extremely fast-growing; starts soft and white, turning bright green as spores develop; produces narrow, flask-shaped cells that release clusters of tiny, round spores
Talaromyces viridulus	Forms compact, granular colonies with a yellow-green tint; has cross-walled hyphae, round sexual spore sacs, and branching structures resembling a penicillus that bear chains of asexual spores

Fig. 1. Colony morphology on PDA medium (a), macroconidia (b and c), and microconidia measurements (d and e) of R

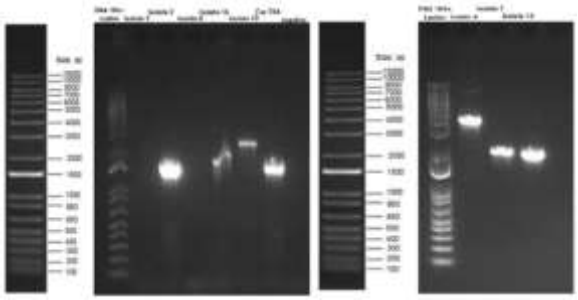

Fig. 2. Agarose gel electrophoresis of post PCR products using in-house NS1/NS24 primers

Identification and characterization of Foc TR4

The isolation of the pathogen from symptomatic Cavendish banana bracts correlates well with descriptions of Foc TR4 infection. The colonies when grown in PDA medium begin as white and cottony in appearance and turn to light pink with a cream border around a violet center. Further, these cultures have a fruity smell and grow fast to completely cover up to a Petri dish of 9 cm diameter in 7 to 10 days (Matos *et al.*, 2023). Under a light microscope at a magnification of 400x, it was noted that there were some oval-shaped microconidia whose mean length was 6.15 µm while their mean width was 2.76 µm. In addition, the elongated and curved macroconidia had a mean length of 31.44 µm while their mean width was 3.74 µm (Fig. 1). The fungal samples' morphological features appear to resemble those of Foc TR4 described by Garcias-Bastidas (2022). Moreover, PCR amplification followed by the sequencing of 18S ribosomal RNA gene further confirms the species and race of the fungus.

Isolation, characterization, and identification of antagonistic fungi

From the composite suppressive soil samples, a total of 26 distinct microbial cultures were grown: 22 were fungi, and 4 were bacteria. After the series of screening tests, five fungal species stood out for inhibiting *Foc* TR4 reliably in every trial conducted. Genetic sequencing (shown in Fig. 2) confirmed identities as *Phlebiopsis gigantea*, *Paecilomyces fulvus*, *Aspergillus niger*, *Trichoderma harzianum*, and *Talaromyces viridulus*. Each displayed a distinct appearance both on culture plates and under 400x magnification, as summarized and visualize in Table 2 and Figs 3-7.

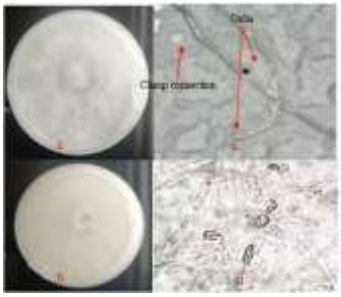

Fig. 3. Characteristics of *Phlebiopsis gigantean*: surface and bottom of growth appearance on PDA (a and b), clamp connection and oidia (c), and lamprocystidia (d)

Fig. 4. Characteristics of *Trichoderma harzianum*: surface and bottom of growth appearance on PDA (a and b), phialides (c), and conidia (d)

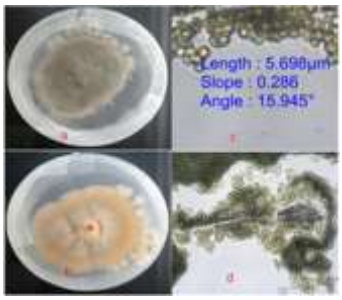

Fig. 5. Characteristics of *Talaromyces viridulus*: surface and bottom growth appearance on PDA (a and b), asci (c), and penicillus-type formation of conidia (d)

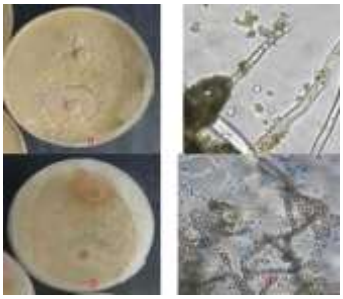

Fig. 6. Characteristics of *Paecilomyces fulvus*: surface and bottom of potato dextrose agar plates (a and b), conidiophores and swelling phialides (c), and conidia in long basipetal chains (d)

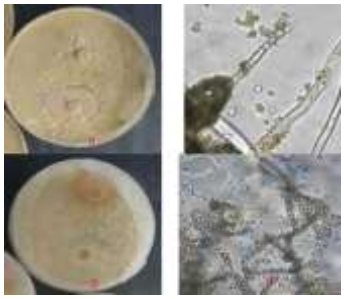

Fig. 7. Characteristics of *Aspergillus niger*: surface and bottom on potato dextrose agar plates (a and b), conidiophores and vesicles with metulae and phialides (c), and conidia in long basipetal chains (d)

Performance in dual culture assay

All the five fungi tested when grown with Foc TR4 were found to inhibit its spread, with the percentage of inhibition getting higher with prolonged incubation to reach a peak on day 9 (Table 3). The highest growth inhibition percentage was observed in *A. niger* with a radius decrease percentage of 79.19%. *T. harzianum* ranked second with a percentage of 77.51%, followed by *T. viridulus* with 77. Even *P. gigantea*, which ranked lowest in this specific test, still reduced pathogen growth by a meaningful 65.08%.

Observations of plate development provided clear clues regarding mechanism: the fastest-growing fungi quickly covered the entire surface of the medium, leaving almost no space or nutrients for the pathogen to establish itself (Bouizgarne, 2013). In many cases, a clear, empty gap opened up between the advancing edge of the antagonist and the pathogen colony, an obvious sign that soluble toxins or growth inhibitors were also released into the surrounding medium (Expósito *et al.*, 2017).

Inhibition through volatile organic compounds

The pattern shifted when isolates were tested without direct physical contact, relying only on gases released into surrounding airspace (Kälvö *et al.*, 2018). Here, *P gigantea* stood far above the rest, holding back Foc TR4 mycelial growth by 54.54% and cutting spore germination by 56.65%, levels no other isolate could match in this setup (Tables 4 and 5). *T. harzianum* and *T. viridulus* followed with moderate effects, while *P. fulvus* and *A. niger* showed only weak activity through volatile emissions alone.

Table 3. Percent inhibition of radial growth of *Fusarium oxysporum* f. sp. *cubense* TR4 treated with fungal antagonists

Fungal isolate	Percent growth inhibition					
	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI	9 DAI
Phlebiopsis gigantea	32.44[b]	44.27[b]	49.99[c]	55.78[b]	59.82[c]	65.08[c]
Paecilomyces fulvus	37.78[b]	51.20[b]	56.59[b]	61.39[b]	66.43[b]	71.38[b]
Aspergillus niger	53.09[a]	63.47[a]	68.04[a]	71.88[a]	75.53[a]	79.19[a]
Trichoderma harzianum	50.21[a]	60.45[a]	65.35[a]	69.48[a]	73.42[a]	77.51[a]
Talaromyces viridulus	50.79[a]	60.65[a]	65.13[a]	69.35[a]	73.39[a]	77.21[a]
F – test	*	*	*	*	*	*
CV (%)	12.53	8.21	7.04	5.83	4.93	4.73

Note: Means marked with the same letter within a single column are not statistically different at $p < 0.05$; DAI - Days After Incubation

Table 4. Percent inhibition of *Fusarium oxysporum* f. sp. *cubense* TR4 mycelial growth due to the volatile organic compounds produced by the fungal antagonists

Fungal isolate	Percent growth inhibition		
	5 DAI	6 DAI	7 DAI
Phlebiopsis gigantea	51.44	52.66[a]	54.54[a]
Paecilomyces fulvus	28.31	30.06[b]	30.89[b]
Aspergillus niger	39.90	36.58[b]	33.89[b]
Trichoderma harzianum	40.60	42.18[ab]	42.54[ab]
Talaromyces viridulus	43.61	40.49[ab]	41.49[b]
F – test	*	*	*
CV (%)	23.77	21.57	20.80

Note: Means marked with the same letter within a column are not statistically different at $p< 0.05$

Table 5. Percent conidial germination of *Fusarium oxysporum* f. sp. *cubense* TR4 due to the volatile organic compounds produced by the fungal antagonists

Fungal isolate	Percent growth inhibition		
	5 DAI	6 DAI	7 DAI
Phlebiopsis gigantea	59.39[a]	58.06[a]	56.65[a]
Paecilomyces fulvus	41.89[b]	41.36[b]	40.30[b]
Aspergillus niger	43.55[b]	43.07[b]	42.74[b]
Trichoderma harzianum	43.25[b]	43.73[b]	43.73[b]
Talaromyces viridulus	45.52[b]	43.48[b]	42.40[b]
F – test	*	*	*
CV (%)	17.33	15.28	20.80

Note: Means marked with the same letter within a column are not statistically different at $p < 0.05$

Table 6. Growth inhibition of *Fusarium oxysporum* f. sp. cubense TR4 by sterile culture filtrates after 7 days of exposure

Fungal isolate	Percent growth inhibition
	7 DAI
Phlebiopsis gigantea	17.03[b]
Paecilomyces fulvus	14.95[b]
Aspergillus niger	13.74[b]
Trichoderma harzianum	14.92[b]
Talaromyces viridulus	27.24[a]
F- test	*
CV (%)	15.67

Note: Means marked with the same letter within a column are not statistically different at $p < 0.05$

The maximum efficiency of control was achieved around day 5, and then followed by an almost steady period up until day 7, after which a slight drop in efficiency could be noticed towards the end of the experiment period. This trend is quite similar to the natural properties of volatiles, which although extremely effective, are prone to breakdown and dispersal over time (Kälvö *et al.*, 2018).

Activity of soluble, diffusible metabolites

When filtered liquid metabolites released into growth medium, stripped of all living cells were tested, a distinct effectiveness pattern emerged. *T. viridulus* was the clear leader, limiting pathogen expansion by 27.24%, a margin significantly higher than every other strain evaluated (Table 6). This indicates the species produces stable, dissolved compounds capable of traveling through soil moisture and inhibiting growth even at a distance, without direct contact with the pathogen.

Isolates dominant in direct competition tests *A. niger* and *T. harzianum* showed only low to moderate activity through liquid filtrates. The difference supports results obtained through plate assay tests, which have found that the effectiveness of the compounds mainly stems from fast growth and dominance in the environment, rather than the production of soluble compounds. Discussion: The consistent differences suggest that the resistance to Fusarium wilt can be predicted based on soil physical and chemical characteristics (Cha *et al.*, 2015). Suppressive soil has the ideal texture that enables water retention and sufficient aeration to provide an environment that allows helpful microbes to develop undisturbed (Obayomi *et al.*, 2018). Soils' pH becomes one of the critical aspects affecting susceptibility to the fungus because neutral soil conditions allow all the necessary microbes to thrive and prevent the development of pathogens. Acidity, conversely, becomes a barrier for beneficial flora but not for harmful organisms (Orr and Nelson, 2018; Jayaraman *et al.*, 2021). The high content of organic matter helps in maintaining microbial life, stabilizing the soil's pH, building healthy soil structure, providing substances needed for protection compounds production (Deng *et al.*, 2021), and ensuring plants' healthy development due to improved immunity (Bouizgarne, 2013). While there are no considerable differences in phosphorus levels both soil groups, adequate phosphorus concentration promotes plant growth and improves plant immunity (Bouizgarne, 2013). Finally, potassium concentration is similar among studied soils, proving its importance for plant health without any additional effect (Jayaraman *et al.*, 2021).

The combination of these microorganisms provides a diverse range of effects leading to the suppression of pathogens' activities in soil (Bubici *et al.*, 2019; Razo-Belman and Ozuna, 2023). In this respect, the contributions made by *A. niger* and *T. harzianum* involve a quick colonization of microhabitats rich in nutrients to ensure resource acquisition (Bouizgarne, 2013). In addition, Trichoderma serves as a mycoparasite due to the degradation of harmful microorganisms by means of targeted enzymatic reactions (Expósito *et al.*, 2017); *Aspergillus* species produce organic acids which prevent pathogen activity (Bouizgarne, 2013). *P. gigantea* utilizes volatile substances produced by the fungus in the air surrounding the pathogen in order to impact the pathogenic activity (Kälvö *et al.*, 2018). Furthermore, *T. viridulus* synthesizes stable molecules endowed with strong antibacterial properties. The application of fungi capable of performing different strategies can be expected to create multiple layers of protection similar to the one observed in natural soil (Bubici *et al.*, 2019; Razo-Belman and Ozuna, 2023).

CONCLUSION

This study demonstrated that soils naturally suppressive to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) possess distinct physicochemical characteristics, including a neutral pH, sandy clay loam texture, higher organic matter content, and greater phosphorus availability. These conditions appear to favor the establishment and activity of beneficial microbial communities that contribute to disease suppression.

A total of 26 indigenous microorganisms were isolated from suppressive soils, of which five fungal species—*Phlebiopsis gigantea*, *Paecilomyces fulvus*, *Aspergillus niger*, *Trichoderma harzianum*, and *Talaromyces viridulus*—exhibited significant antagonistic activity against Foc TR4. The antagonists suppressed pathogen growth through multiple mechanisms, including competition for space and nutrients, production of volatile organic compounds, and secretion of diffusible inhibitory metabolites. Among the isolates, *A. niger* and *T. harzianum* showed the greatest inhibition in dual-culture assays, *P. gigantea* was the most effective producer of inhibitory volatile compounds, and *T. viridulus* exhibited the strongest activity through soluble metabolites.

The findings highlight the important role of indigenous fungal communities in maintaining soil suppressiveness and reducing the development of Fusarium wilt. The complementary modes of action displayed by the selected antagonists suggest their potential for integration into sustainable disease management programs. Harnessing these naturally occurring fungi, together with practices that promote soil health, offers a promising and environmentally sound approach for managing Foc TR4 and improving the long-term sustainability of banana production systems.

RECOMMENDATIONS

Based on the findings of this study, the use of indigenous fungal antagonists, particularly *Trichoderma harzianum*, *Aspergillus niger*, *Phlebiopsis gigantea*, and *Talaromyces viridulus*, should be further explored as potential biological control agents against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4. Maintaining soil conditions that favor microbial diversity, including adequate organic matter and balanced soil pH, is also recommended to enhance natural disease suppression. Furthermore, field-scale evaluations are necessary to validate the effectiveness of these fungal antagonists under commercial banana production systems and to develop practical formulations for their application.

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