



Antagonistic and growth-enhancing effect of native *Trichoderma* spp. on 'Gros Michel' banana under Foc R1 infection

Efecto antagonístico y promotor del crecimiento de especies nativas de *Trichoderma* en el banano 'Gros Michel' bajo infección por Foc R1

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ABSTRACT

Banana production in Ecuador has been severely impacted by *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *ubense* (Foc). This persistent soilborne pathogen remains viable for decades and reduces the effectiveness of conventional control strategies. *Trichoderma* spp. has emerged as a promising biological alternative due to its antagonistic capacity and plant-associated effects. This study evaluated the efficacy of native *Trichoderma* strains as biological control agents against Foc Race 1 (R1) in *Musa* AAA cv. Gros Michel. Dual culture assays and greenhouse experiments were conducted using a completely randomized design, with data analyzed by ANOVA and Tukey's test ($\alpha = 0.05$). *In vitro*, *T. lixii*, *T. asperellum*, and *T. harzianum* inhibited up to 58% of Foc radial growth. Under greenhouse conditions, *Trichoderma* application significantly reduced vascular wilt severity ($p < 0.05$) and was associated with increased pseudostem diameter and root biomass. *T. afroharzianum* and *T. lixii* lowered disease incidence to levels comparable to those of the healthy control. These findings support the potential use of native *Trichoderma* isolates in sustainable management strategies for *Fusarium* wilt in banana.

Keywords: Biological control; fungal diseases; rhizosphere microbiota; integrated disease management; Musaceae.

RESUMEN

La producción de banano en Ecuador se ha visto gravemente afectada por la marchitez por *Fusarium*, causada por *Fusarium oxysporum* f. sp. *ubense* (Foc). Este patógeno persistente del suelo permanece viable durante décadas y reduce la efectividad de las estrategias de control convencionales. *Trichoderma* spp. ha surgido como una alternativa biológica prometedora debido a su capacidad antagónica y efectos asociados a la planta. Este estudio evaluó la eficacia de cepas nativas de *Trichoderma* como agentes de control biológico contra Foc Raza 1 (R1) en *Musa* AAA cv. Gros Michel. Se realizaron ensayos de cultivo dual y experimentos en invernadero utilizando un diseño completamente aleatorio, con datos analizados mediante ANOVA y la prueba de Tukey ($\alpha = 0,05$). *In vitro*, *T. lixii*, *T. asperellum* y *T. harzianum* inhibieron hasta el 58% del crecimiento radial de Foc. En condiciones de invernadero, la aplicación de *Trichoderma* redujo significativamente la severidad de la marchitez vascular ($p < 0,05$) y se asoció con un aumento del diámetro del pseudotallo y la biomasa radicular. *T. afroharzianum* y *T. lixii* redujeron la incidencia de la enfermedad a niveles comparables a los del grupo control sano. Estos hallazgos respaldan el uso potencial de aislados nativos de *Trichoderma* en estrategias de manejo sostenible para la marchitez por *Fusarium* en banano.

Palabras clave: Control biológico; enfermedades fúngicas; microbiota de la rizosfera; manejo integrado de enfermedades; Musaceae.

1. Introduction

Fusarium oxysporum f. sp. cubense Race 1 (Foc R1) is one of the major phytopathogens affecting banana (*Musa* spp., Musaceae) and remains a recurring and evolving threat to global production (Izquierdo-García et al., 2024; Kema et al., 2021). The predominant method for managing phytopathogenic fungi has traditionally been the application of synthetic fungicides (Torres-Rodríguez et al., 2024). However, their intensive use alters the physicochemical and biological properties of soils, reduces fertility, contaminates air and water, and leads to pesticide residues in food with potential risks to human health (Anis Mufida et al., 2024; Torres-Rodríguez et al., 2024; Sánchez-Alarcón et al., 2021). Regarding Foc R1, fungicide-based strategies are largely ineffective because of the pathogen's biology and its long-term persistence in soil (Ploetz, 2015). Consequently, sustainable agriculture has emerged as a key alternative to reduce agrochemical dependence while maintaining productivity and protecting natural resources, human health, and ecosystem balance (Zou et al., 2024).

Beyond the current impact of *Fusarium oxysporum* f. sp. cubense Race 1, banana production in Ecuador faces an increasing phytosanitary risk associated with the potential introduction of Tropical Race 4 (Foc TR4), one of the most destructive pathogens threatening global banana systems. Ecuador, as the world's leading banana exporter, remains particularly vulnerable due to the extensive monoculture of susceptible cultivars and the limited availability of effective curative measures once the pathogen becomes established. Although this study focuses on Foc R1, research on biological control agents and plant-microbe interactions is highly relevant to TR4 preparedness, as preventive strategies based on microbial-mediated disease suppression and enhanced plant resilience are among the few sustainable tools available for long-term management. In this sense, studies evaluating native antagonistic microorganisms contribute not only to current disease control but also to strengthening national biosecurity and risk mitigation frameworks.

Within this framework, the use of antagonistic microorganisms has gained relevance as a biocontrol strategy, given their ability to suppress pathogens and promote plant growth in economically important crops (Zheng et al., 2024). Among them, *Trichoderma* spp. has been widely incorporated into integrated pest and disease

management programs in banana due to its ability to colonize the rhizosphere, enhance nutrient uptake, stimulate plant growth, and activate systemic defense responses (Harman et al., 2021). The mechanisms of action of *Trichoderma* include mycoparasitism, secretion of secondary metabolites, antibiotic production, and competition for nutrients and space (Esquivel-Naranjo et al., 2025; Natsiopoulos et al., 2024; Chávez-Avilés et al., 2024). These properties position *Trichoderma* as a key tool in the transition toward more sustainable production systems by reducing dependence on synthetic fungicides and mitigating their environmental impacts.

Recent studies have reported the antagonistic potential of *Trichoderma* spp. strains against *Fusarium oxysporum*, associated with enzyme production and reduced pathogen growth under *in vitro* conditions (Nguyen Quoc Khuong et al., 2023), as well as with antifungal traits and improvements in plant health reported in cropping systems (Jamil, 2021).

Despite the extensive use of *Trichoderma* spp. as biological control agents, the relationship between direct antagonism observed under *in vitro* conditions and effective disease suppression in planta remains unclear, particularly for *Fusarium* wilt of banana. Many studies rely primarily on *in vitro* assays, which may not adequately capture the complexity of plant-microbe-pathogen interactions occurring in the rhizosphere. Consequently, isolates exhibiting strong *in vitro* antagonism do not always translate into superior performance under greenhouse or field conditions.

Addressing this gap is critical for the rational selection of biological control agents. Therefore, the objective of this study was to evaluate native *Trichoderma* spp. isolates from Ecuador by integrating *in vitro* antagonism assays with greenhouse experiments of disease suppression and plant growth promotion in *Musa* AAA cv. Gros Michel infected with Foc R1.

2. Methodology

The plant material comprised banana plantlets (*Musa* AAA cv. Gros Michel, family Musaceae) produced by *in vitro* propagation and acclimatized for eight weeks under controlled greenhouse conditions. The plantlets were generated and supplied by the Biotechnology Department of the National Institute of Agricultural Research (INIAP), which develops certified propagation protocols for Musaceae.

The plants were grown in plastic pots containing a homogeneous substrate composed of agricultural soil and sand (2:1, v/v). The substrate was steam-sterilized prior to use to reduce background microbial interference and ensure that observed effects were primarily attributable to the inoculated *Trichoderma* isolates and Foc R1. Pots were maintained under greenhouse conditions and irrigated as required to avoid water stress throughout the experimental period.

The biological material consisted of seven native *Trichoderma* spp. isolated from the INIAP microbial collection (Table 1), previously identified at the molecular level and cryopreserved at -80°C . The molecular identification was performed at the Biotechnology Department of the Tropical Experimental Station Pichilingue (INIAP) through ITS and TEF1- α gene sequencing and phylogenetic analysis. These identification results were previously published by our research group (Terrero-Yépez et al., 2025), where sequences showing >99% identity with GenBank reference strains confirmed their taxonomic classification.

Antagonistic activity was assessed through dual culture assays on PDA, following Silva (2018) with modifications. A 5-mm disk of actively growing Foc R1 mycelium was placed at one edge of the plate and a 5-mm disk of the *Trichoderma* isolate on the opposite edge. Each treatment included four replicates.

Plates were incubated at $25 \pm 1^{\circ}\text{C}$ under low-light conditions. Radial growth was measured every 24 h. Percentage of inhibition was calculated as: $\text{PRI} (\%) = 100 \times (R - r) / R$, where R is the pathogen radius in the control and r is the radius in dual culture.

In vivo evaluation under greenhouse conditions

A total of 135 banana plantlets were assigned to nine treatments (15 plants per treatment). *Trichoderma* suspensions (200 mL per plant at 1×10^7 conidia mL^{-1}) were applied at 15-day intervals for three applications. After 90 days sowing (DAS), plants were inoculated with Foc R1

using 5-mm mycelial plugs placed into the root zone.

Evaluated variables: Plant height (cm), Pseudostem diameter (cm) measured 2 cm above the base using a digital caliper, Number of functional leaves, and Root fresh and dry biomass (g) (drying at 70°C for 72 h).

Disease incidence and severity

Severity was assessed monthly using a 6-level external symptom scale (Orjeda, 1998):

- 1 = no symptoms;
- 2 = yellowing of older leaves;
- 3 = yellowing of lower leaves;
- 4 = yellowing of young leaves;
- 5 = severe yellowing;
- 6 = dead plant.

Experimental design and statistical analysis

A completely randomized design (CRD) was used for both *in vitro* and *in vivo* experiments. ANOVA was performed after verifying normality and homoscedasticity. Means were compared using Tukey's test at $\alpha = 0.05$.

Statistical analyses were conducted using R software (version 4.3.1) (packages *stats* and *agricolae*).

3. Results and discussion

In vitro antagonism of Trichoderma spp. against Foc R1

Dual culture assays revealed marked differences among the seven *Trichoderma* isolates, demonstrating that antagonism is a strain-dependent trait. After 48 h of incubation, *T. lixii* (F17) exhibited the strongest suppressive activity, inhibiting the radial growth of Foc R1 by 58%. This substantial reduction indicates an efficient combination of competition for space and nutrients, as well as the possible production of antifungal metabolites. *T. asperellum* (F74) and *T. afroharzianum* (F19) followed with inhibition values of 45% and 38%, respectively, constituting the group of intermediate antagonists.

Table 1
Trichoderma spp. isolates from INIAP collection

	<i>Trichoderma</i> species	Geographic origin	Botanical source	Physical source
F74	<i>T. asperellum</i>	Guayas	Plantain	Soil
F73	<i>T. lentiforme</i>	Santa Elena	Banana	Root
F84	<i>T. harzianum</i>	Los Ríos	Banana	Soil
F78	<i>T. afroharzianum</i>	Los Ríos	Banana	Soil
F17	<i>T. lixii</i>	Manabí	Plantain	Leaf
F19	<i>T. afroharzianum</i>	Santo Domingo	Abacá	Soil
F76	<i>T. asperellum</i>	Los Ríos	Banana	Soil

Moderate inhibition levels were observed for *T. harzianum* (F84; 34%) and another *T. asperellum* isolate (F76; 31%). In contrast, both isolates of *T. afroharzianum* (F78 and F19) displayed inhibition values below 25%, indicating a limited suppressive effect under direct confrontation. This weaker *in vitro* antagonism is consistent with previously reported patterns for some endophytic *Trichoderma* strains, which may rely more on indirect mechanisms, such as the induction of plant defenses, than on direct competition.

The dual-culture assays confirmed that the antagonistic activity of *Trichoderma* against *Fusarium oxysporum* f. sp. *cubense* R1 is strongly strain-dependent, even among isolates within the same species (Rodríguez-García et al., 2020; Kumari et al., 2024). While isolates such as *T. lixii* (F17) exhibited strong direct inhibition, others showed moderate or low suppressive effects under *in vitro* conditions. Importantly, reduced inhibition in dual culture assays should not be interpreted as low biocontrol potential, as increasing evidence indicates that several *Trichoderma* strains rely primarily on indirect mechanisms, including induction of systemic resistance, modulation of plant defense pathways, and efficient root colonization, which are not captured in host-free assays (Chen et al., 2021; Natsiopoulou et al., 2024). Therefore, *in vitro* confrontation tests provide only partial insight into the biocontrol capacity of *Trichoderma*, reinforcing the need to integrate laboratory assays within planta evaluations to achieve a biologically meaningful selection of candidate strains (Fotohiyan et al., 2017; Chávez-Avilés et al., 2024).

Suppression of disease severity, incidence, and infection intensity in biotized banana plants

In the treatment inoculated with Foc R1, plants developed pronounced disease symptoms, reaching a mean severity score of 4.67 on the six-

point scale. In contrast, plants treated with *Trichoderma* showed much lower severity values, ranging from 1.47 to 2.53 ($p < 0.0001$). These reductions indicate that the isolates slowed disease progression and limited typical wilt symptoms, including leaf yellowing and vascular decline.

Disease incidence followed the same trend. Foc R1 infected 100% of the plants in positive control, confirming the pathogen's high aggressiveness. In contrast, incidence in the *Trichoderma*-treated plants was markedly lower, ranging from 6.67% to 66.67%, depending on the isolate. This reduction demonstrates that the antagonistic strains were able to limit infection and prevent the spread of vascular wilt among the plants.

Infection intensity and mean grade further supported these findings. The pathogen control recorded 92% infection intensity and a mean grade of 4.60, reflecting both extensive tissue colonization and severe symptom progression. *Trichoderma* treatments significantly reduced both indicators by 24% – 50%, and F78 achieved a mean grade of only 1.22, confining symptoms to the mildest categories. These results confirm that *Trichoderma* not only limits the number of infected plants but also restricts disease progression within individual plants. As shown in Table 2, all *Trichoderma* isolates significantly reduced disease severity and infection parameters compared with the pathogen control.

Under the experimental conditions evaluated, native *Trichoderma* spp. proved to be a promising biological alternative for the management of *Fusarium* wilt in 'Gros Michel' banana, as evidenced by the significant reductions in disease severity, incidence, and infection intensity compared with the pathogen control. These results support the initial hypothesis that biotization with *Trichoderma* mitigates disease expression and improves plant performance, while also revealing marked strain-specific differences.

Table 2

Disease severity and infection index in plants treated with native *Trichoderma* spp. Isolates

Treatment	Severity (Media ± E.E.)	Tukey Severity	% Infección (Mean ± S.E.)	Tukey Infection index
Foc	4.67 ± 0.25	A	100.00 ± 6.89	A
F74	2.53 ± 0.25	B	66.67 ± 6.89	AB
F19	2.40 ± 0.25	B	66.67 ± 6.89	AB
F17	2.20 ± 0.25	B	53.33 ± 6.89	B
F73	1.73 ± 0.25	BC	40.00 ± 6.89	BC
F84	1.73 ± 0.25	BC	46.67 ± 6.89	B
F76	1.53 ± 0.25	BC	53.33 ± 6.89	B
F78	1.47 ± 0.25	BC	6.67 ± 6.89	CD
Water	1.00 ± 0.25	C	0.00 ± 6.89	D

Means followed by the same letter within a column are not significantly different according to Tukey's test ($p \leq 0.05$).

Such variability is consistent with the broad functional diversity described within the genus *Trichoderma*, which includes mechanisms such as competition, mycoparasitism, antibiosis, and induction of host defenses (Harman et al., 2021; Esquivel-Naranjo et al., 2025). The strong suppression of disease parameters observed across isolates indicates that, under greenhouse conditions, *Trichoderma* can effectively limit both pathogen establishment and symptom progression, reinforcing its role as a disease-modulating agent rather than a strictly antagonistic one.

Integration of *in vitro* and *in vivo* results further highlighted that direct antagonism does not necessarily predict greenhouse performance. While F17 showed the strongest inhibition of Foc R1 in dual-culture assays, F78 emerged as the most effective isolate in planta, achieving near-complete suppression of disease incidence and markedly reducing severity and infection intensity to levels comparable to those of the healthy control. This pattern aligns with previous studies showing that isolates selected for *in vitro* antagonism often exhibit disproportionate or contrasting performance under greenhouse conditions, where indirect mechanisms and plant-mediated interactions play a dominant role (Fotohiyan et al., 2023; Natsopoulos et al., 2024; Chávez-Avilés et al., 2024). The superior performance of F78 suggests that mechanisms such as efficient root colonization, induction of systemic resistance, or enhanced plant tolerance to vascular dysfunction may be more relevant than direct mycoparasitism in suppressing *Fusarium* wilt. These findings emphasize the necessity of combining laboratory and greenhouse evaluations to identify robust biocontrol candidates and underscore the value of native isolates, which may be better adapted to local agroecological conditions and therefore more reliable for integration into sustainable disease management programs.

Plant growth responses under biotic stress from Foc R1

Growth parameters differed significantly among treatments (Table 3). Foc R1 infection substantially compromised plant vigor, as shown by the lowest pseudostem diameter (1.29 cm) and reduced biomass in the pathogen control. In contrast, all *Trichoderma* treatments maintain pseudostem diameters above 2.27 cm, values comparable to those of the healthy control. This parameter, closely associated with structural stability and resource allocation in Musaceae, indicates that *Trichoderma* mitigated vascular obstruction and promoted tissue integrity despite pathogen pressure.

Shoot biomass exhibited pronounced differences among treatments. Plants inoculated only with Foc R1 accumulated the lowest biomass (76.33 g), whereas those treated with the strains F78 and F17 reached 151.67 g and 151.33 g, respectively, approximately double that of the pathogen control. The statistical overlap among most *Trichoderma* treatments highlights the genus's generally strong biostimulatory capacity, often associated with enhanced nutrient uptake, improved root functionality, and phytohormone-like activity.

Root fresh weight followed the same trend. F78 produced the highest biomass (96.67 g), followed by F17 and F73, whereas the pathogen control had the lowest value (57 g). These results indicate that root colonization by *Trichoderma* promotes root proliferation even under pathogen stress, thereby improving water and nutrient acquisition and contributing to plant resilience.

Native *Trichoderma* isolates mitigated the detrimental effects of *Fusarium* wilt on banana plant development. Infection with Foc R1 markedly reduced pseudostem diameter and biomass accumulation, consistent with the vascular obstruction and impaired resource translocation characteristic of *Fusarium* wilt in banana (Ploetz, 2015; Kema et al., 2021).

Table 3
Integrated effects of native *Trichoderma* spp. on growth-related variables of 'Gros Michel' banana under Foc R1 infection

Treatment	Pseudostem diameter (cm)	Tukey	Shoot fresh weight (g)	Tukey	Root fresh weight (g)	Tukey	Plant height (cm)	Tukey
Water	2.38	A	127.33	B	70.33	BC	16.96	AB
F74	2.33	A	144.00	A	85.67	AB	17.22	AB
F17	2.33	A	151.33	A	91.33	AB	16.96	AB
F84	2.31	A	147.33	A	83.33	AB	17.36	AB
F73	2.31	A	148.67	A	88.00	AB	17.64	A
F19	2.31	A	146.33	A	86.33	AB	16.97	AB
F76	2.31	A	125.67	B	70.67	BC	15.98	BC
F78	2.27	A	151.67	A	96.67	A	15.86	BC
Foc R1	1.29	B	76.33	C	57.00	C	15.16	C

In contrast, all *Trichoderma* treatments maintain pseudostem diameters comparable to the healthy control, suggesting preservation of vascular functionality and structural stability. Similar protective effects on plant architecture under *Fusarium* stress have been reported for *Trichoderma asperellum* and *T. koningiopsis* in banana, where treated plants showed improved stem robustness and biomass accumulation despite pathogen presence (Luo et al., 2023; Sarma et al., 2025). These findings support the notion that *Trichoderma* can alleviate the physiological constraints imposed by Foc. The pronounced increases in shoot and root biomass, particularly in plants treated with isolates F78 and F17, further highlight the dual role of *Trichoderma* as a biocontrol and plant growth-promoting agent under biotic stress. Root biomass preservation is especially relevant, as early root deterioration is a key driver of *Fusarium* wilt progression in banana (Kema et al., 2021). Enhanced root development under pathogen pressure has been widely linked to *Trichoderma*-mediated improvements in nutrient mobilization, root system architecture, and modulation of plant hormonal balance (Contreras-Cornejo et al., 2024; Yao et al., 2023). Although plant height differences were less pronounced, several isolates partially offset the growth restriction caused by Foc R1, indicating that vertical development is less sensitive than biomass-related traits but still responsive to fungal biostimulation, rather than merely suppressing symptom expression.

Integration of antagonism and plant growth promotion patterns

Overall, these results demonstrate that in vitro antagonism alone is not a reliable predictor of disease suppression or plant growth promotion under greenhouse conditions, highlighting the importance of host-mediated and indirect mechanisms in the biocontrol performance of *Trichoderma* (Figure 1). Differences among isolates suggest that traits such as efficient root colonization, production of bioactive metabolites, and induction of systemic resistance may play a more prominent role in vivo than direct mycoparasitism observed in dual culture assays. Consequently, strain-specific performance must be evaluated through integrated laboratory and greenhouse approaches to identify robust candidates for *Fusarium* wilt management.

An additional contribution of this study lies in its regional relevance. Although *Trichoderma* spp. has been widely studied worldwide, information on the performance of Ecuadorian native isolates against *Fusarium oxysporum* f. sp. *ubense* R1 remains limited. This work addresses that gap by identifying native strains, particularly *T. afroharzianum* F78, that combine strong disease suppression with plant growth promotion under controlled conditions. The use of local isolates is especially valuable, as they may be better adapted to regional soils, climatic conditions, and banana genotypes, potentially enhancing their stability and effectiveness in production systems.

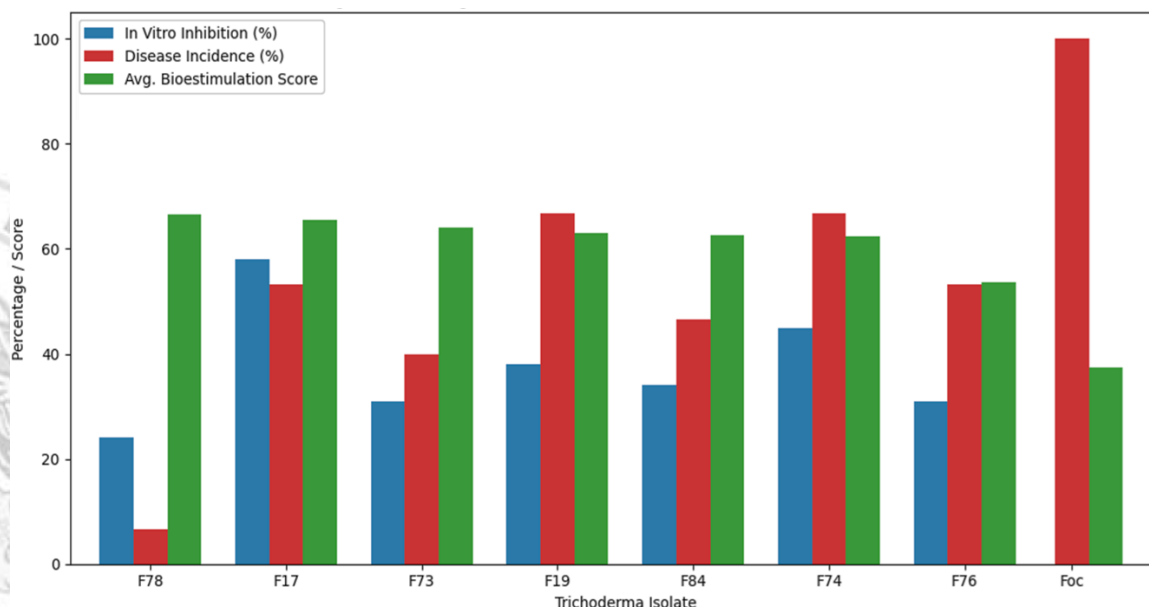


Figure 1. Integrated performance of native *Trichoderma* spp. isolates against Foc R1 in 'Gros Michel' banana. Radar plot summarizing normalized values of in vitro antagonism, disease severity reduction, disease incidence reduction, and plant growth promotion under greenhouse conditions. Higher values indicate superior overall performance.

Despite the promising results obtained, some limitations should be considered when interpreting the findings. The experiments were conducted exclusively under greenhouse conditions using a single banana cultivar ('Gros Michel') and a single *F. oxysporum* f. sp. *cubense* R1 isolate, which may not fully represent the diversity of host-pathogen interactions present under field conditions. In addition, the use of steam-sterilized substrates likely reduced background microbial interactions, potentially amplifying the apparent effects of *Trichoderma* isolates compared with non-sterile soils. Therefore, extrapolation of these results to commercial systems should be made with caution.

Future research should focus on elucidating the mechanisms underlying strain-specific behavior through analyses of root colonization dynamics, expression of defense-related genes in banana, and characterization of antifungal metabolites. Moreover, long-term field evaluations across different agroecological conditions are essential to determine the consistency and practical relevance of these effects. Such efforts will be critical for the development of sustainable, biologically based strategies for *Fusarium* wilt management in Ecuador and other tropical banana-producing regions.

4. Conclusions

Native *Trichoderma* spp. isolates significantly reduced disease severity, incidence, and infection intensity caused by *Fusarium oxysporum* f. sp. *cubense* R1 in 'Gros Michel' banana under greenhouse conditions.

In vitro antagonistic activity did not necessarily predict greenhouse performance, highlighting the importance of indirect mechanisms such as root colonization and induction of plant defense responses.

Selected isolates, particularly F78 and F17, not only suppressed disease but also promoted plant growth, increasing biomass and pseudostem diameter under pathogen pressure.

These findings support the use of native *Trichoderma* isolates as a sustainable strategy for *Fusarium* wilt management and as a preventive tool within integrated biosecurity programs.

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