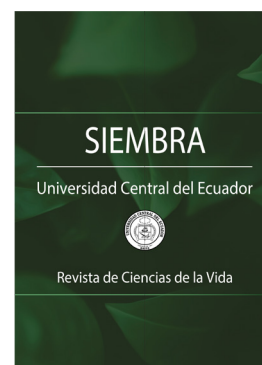


Trichoderma spp. and their influence on the resilience of plantain plants to *Ralstonia solanacearum* (Smith) phylotype II

Trichoderma spp. y su influencia en la resiliencia de plantas de plátano ante *Ralstonia solanacearum* (Smith) filotipo II

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Abstract

Ralstonia solanacearum Smith, the causal agent of bacterial moko, represents one of the main phytosanitary threats to Musaceae crops due to its high aggressiveness and wide distribution. To evaluate biological management strategies, the effect of *Trichoderma* spp. strains on disease severity and agronomic development of plantain plants (*Musa AAB*) obtained by tissue culture technique and inoculated with *R. solanacearum* was determined. The experiment was established under a completely randomized design with nine treatments, including a non-inoculated control (T8) and a control inoculated only with the pathogen (T9). Results showed that T6 (*T. lentiforme* F19) and T2 (*T. harzianum* F73) significantly reduced disease severity ($p < 0.0001$). Additionally, T6, T5 (*T. lixii* F17), and T4 (*T. afroharzianum* F78) promoted plant growth, with significant increases in plant height and pseudo-stem diameter. No significant differences were observed in leaf number ($p > 0.05$). These results confirm the potential of *Trichoderma* spp. as biocontrol agent and biostimulant in Musaceae, constituting a viable alternative within integrated management strategies for the mitigation of *R. solanacearum* in plantain crops.

Keywords: *Ralstonia solanacearum*, *Trichoderma* spp., biocontrol, bacterial Moko, agricultural sustainability.

Resumen

Ralstonia solanacearum Smith, agente causal del moko bacteriano, representa una de las principales amenazas fitosanitarias para cultivos de musáceas debido a su elevada agresividad y amplia distribución. Con el objetivo de evaluar estrategias de manejo biológico, se determinó el efecto de cepas de *Trichoderma* spp. sobre la severidad de la enfermedad y el desarrollo agronómico de plantas de plátano (*Musa AAB*) obtenidas mediante técnica de cultivo de tejidos inoculadas con *R. solanacearum*. El experimento se estableció bajo un diseño completamente al azar con nueve tratamientos, incluyendo un control sin inoculación (T8) y un control inoculado únicamente con el agente patógeno (T9). Los resultados evidenciaron que T6 (*T. lentiforme* F19) y T2 (*T. harzianum* F73) redujeron significativamente la severidad de la enfermedad ($p < 0.0001$).

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Asimismo, T6, T5 (*T. lixii* F17) y T4 (*T. afroharzianum* F78) promovieron el crecimiento de las plantas, con incrementos significativos en la altura y el diámetro del pseudotallo. No se observaron diferencias significativas en el número de hojas ($p > 0,05$). Estos resultados confirman el potencial de *Trichoderma* spp. como agente de biocontrol y bioestimulante en musáceas, constituyendo una alternativa viable dentro de estrategias de manejo integrado para la mitigación de *R. solanacearum*.

Palabras clave: *Ralstonia solanacearum*, *Trichoderma* spp., biocontrol, Moko bacteriano, sostenibilidad agrícola.

1. Introduction

Plantain (*Musa* AAB.) is a crop of great economic and social relevance in Ecuador. With a planted area of approximately 152,654 ha, the country has established itself as one of the world's leading exporters of fresh plantain, reaching sales of 363,000 t and generating revenues of US\$ 213 million in 2023 (Sistema de Información Pública Agropecuaria del Ecuador [SIPA], 2017). The provinces of Manabí and Guayas concentrate around 50% of national production, creating approximately 65,000 direct and indirect jobs in the sector (Mendoza Saltos, 2023). In addition, plantains are an essential component of the Ecuadorian diet, with per capita consumption of 40 kg per year, representing 70% of production directed toward the domestic market (García et al., 2019; Mendoza Saltos, 2023).

One of the main factors contributing to the reduction of agricultural yields in *musaceae* is its high incidence of diseases. These include *Mycosphaerella fijiensis* Morelet, responsible for the disease known as black sigatoka; water rots caused by *Dickeya chrysanthemi* Burkholder and *Pectobacterium carotovorum* Jones; and viral diseases such as Banana Streak Virus [BSV] and Cucumber Mosaic Virus [CMV]. In addition to the presence of the bacterial pathogen *Ralstonia solanacearum* phylum II Smith, which causes the bacterial Moko (Clough et al., 2024).

Bacterial moko is one of the main phytopathological threats globally and represents a critical risk for the sustainability of banana cultivation (Agrocalidad, 2013; Delgado et al., 2014). This phytopathogenic bacterium attacks the vascular system, obstructing water and nutrient transport, leading to wilting, and eventual death of infected plants (López-Alvarez et al., 2020). The economic losses associated with this disease are alarming, especially in tropical regions such as Ecuador, where the economic income of a large number of producers depends on this crop (Gómez-Calvo, 2004; Pardo et al., 2019). In addition, the disease affects food security in regions where plantain is a fundamental component of the daily diet (Goszczynska et al., 2000).

Traditionally, management strategies for *R. solanacearum* have been based on cultural practices, crop rotation and chemical applications (Pardo et al., 2019). However, these measures are often costly, unsustainable and, in many cases, unable to completely eradicate the pathogen due to its persistence in the soil and its wide host range. Therefore, the search for sustainable and effective alternatives, such as the use of biological control agents [BCAs], has intensified (Sun et al., 2023).

Among these agents, *Trichoderma* spp. have been noted for their ability to reduce disease incidence, induce systemic resistance in plants and promote plant growth (Sharma et al., 2022). This beneficial microorganism acts through various mechanisms, such as mycoparasitism, production of secondary metabolites with antimicrobial properties, and stimulation of natural plant defenses. Previous studies have demonstrated its efficacy in the suppression of soil pathogenic microorganisms, including *R. solanacearum*, thus representing a promising tool for integrated disease management. (Shashitu, 2021).

In this context, the present study aimed to evaluate the effect of *Trichoderma* spp. on banana plants obtained by tissue culture inoculated with *R. solanacearum*. This approach not only seeks to explore the potential of this fungus as a biological control agent, but also to analyze its impact on plant growth and development under controlled conditions.

2. Materials and Methods

2.1. Preparation of *Trichoderma* spp. inoculum

The strains of *Trichoderma* spp. proceed from the collection of microorganisms of the Banana, Plantain and

other *Musaceae* Program of INIAP, and were obtained from samples of banana, plantain and abaca plants from the main producing areas of Ecuador (Table 1). To inoculate the potential biocontrol agents, the *Trichoderma* spp. strains were seeded for activation in Petri dishes with PDA. The suspensions to be inoculated were obtained by adding 5 ml of sterile water in each Petri dish, and with a Drigalsky glass spatula the mycelium was gently removed to detach the conidia. The suspensions were filtered with gauze in order to obtain the conidial suspension of each isolate, and their concentration was determined by counting in the Neubauer chamber, the inoculum being adjusted to a concentration of 1×10^7 conidia ml^{-1} (Ramos Martínez, 2006).

Table 1. Origin of *Trichoderma* spp. strains.

Isolation	Botanical Origin	Geographical Origin	Provenance
<i>Trichoderma asperellum</i> F74	Plantain	Guayas – Guayaquil	Soil
<i>Trichoderma harzianum</i> F73	Banana	Santa Elena – Santa Elena	Root
<i>Trichoderma koningiopsis</i> F84	Banana	Los Ríos – Babahoyo	Soil
<i>Trichoderma afroharzianum</i> F78	Banana	Los Ríos – Valencia	Soil
<i>Trichoderma lixii</i> F17	Banana	Manabí – El Carmen	Leaf
<i>Trichoderma lentiforme</i> F19	Abaca	Santo Domingo – La Concordia	Soil
<i>Trichoderma azevedoi</i> F76	Banana	Los Ríos – Vinces	Soil

The *Trichoderma* spp. strains used in this study corresponded to isolates from soil, roots and leaves, previously cultivated on PDA medium for propagation. Each strain has distinctive macroscopic morphological characteristics, considered at the time of their selection as biocontrol agents (Figure 1). Strain F74 (*T. asperellum*, soil) is characterized by distinct concentric growth, cottony mycelium, and intense green coloration in the center, accompanied by dark exudates. F84 (*T. koningiopsis*, soil) exhibits a hairy appearance, with a pink center and light green halo, and high sporulation capacity. F78 (*T. afroharzianum*, soil) shows compact texture and blue-green shades arranged in a radial pattern. Strain F73 (*T. harzianum*, root) has a homogeneous mycelium of white color, without central pigmentation. F19 (*T. lentiforme*, soil) shows uniform growth, with a light green center and regular margins. F17 (*T. lixii*, leaf) is distinguished by its cottony mycelium, with a transition from whitish in the center to pale green shades at the margins. Finally, F76 (*T. azevedoi*, soil) is recognized by its prominent concentric pattern, dark green center, white margins, granular texture and production of dark exudates.

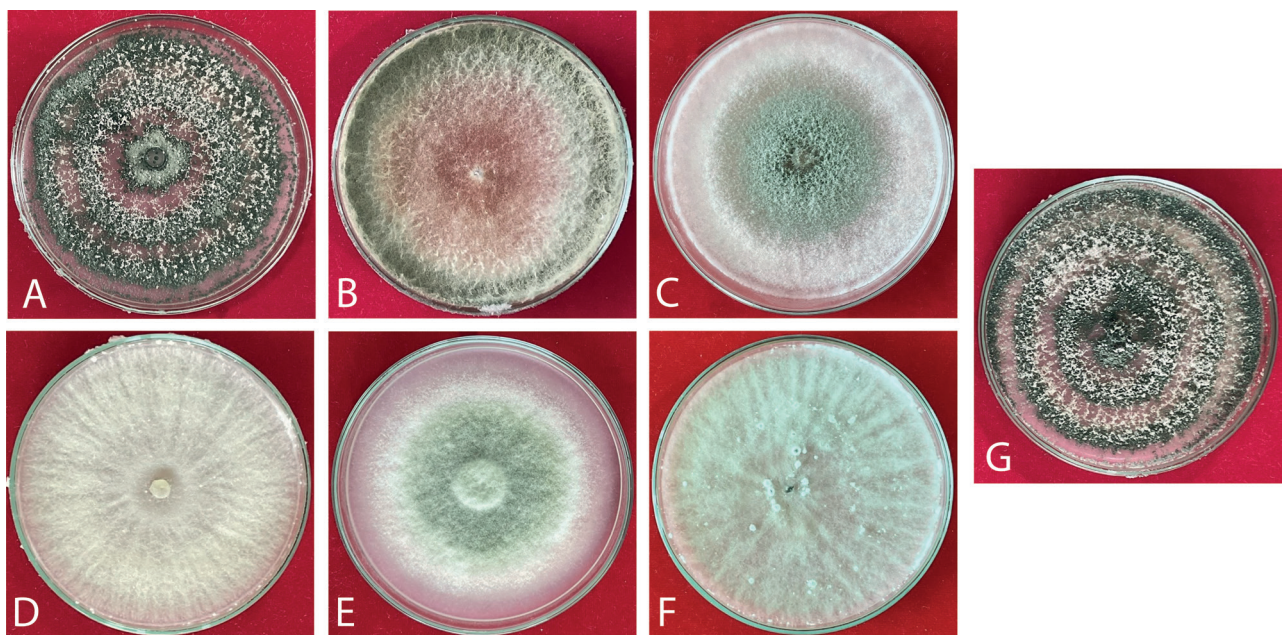


Figure 1. Cultural characteristics of *Trichoderma* spp. strains on PDA medium. (A) F74 soil, (B) F84 soil, (C) F78 soil, (D) F73 root, (E) F19 soil, (F) F17 leaf, (G) F76 soil.

2.2. Molecular confirmation of the identity of the strains of Trichoderma spp.

Molecular identification of *Trichoderma* spp. strains was carried out by amplification and sequencing of the ITS (Internal Transcribed Spacer), and TEF1- α (elongation factor 1-alpha) genes. Subsequently, PCRs were performed using primers ITS1/ITS4 for the ITS region and EF1-728F/EF1-986R for TEF1- α . The amplified products were sequenced and compared with NCBI databases through BLASTn. Phylogenetic analyses confirmed the identity of the following species: *Trichoderma asperellum* F74, *Trichoderma harzianum* F73, *Trichoderma koningiopsis* F84, *Trichoderma afroharzianum* F78, *Trichoderma lixii* F17, *Trichoderma lentiforme* F19 and *Trichoderma azevedoi* F76. The sequences obtained showed identity percentages greater than 99% with reference sequences deposited in GenBank.

2.3. Treatment of vitroplants with isolates of Trichoderma spp.

Inoculation of the biocontrol agents [BCAs] was carried out eight days after transplanting with doses of 200 mL per plant at a concentration of 1×10^7 conidia mL⁻¹. The application of the treatments (Table 2) was carried out in drench at a fortnightly frequency, until completing three applications.

The bacterium *R. solanacearum* phylotype II, from the pathogen collection of the Banana, Plantain and other *Musaceae* Program of INIAP, was inoculated 15 days after the last application of *Trichoderma* spp. The pathogen was prepared from Petri dishes containing the bacteria, and was diluted in sterile distilled water until reaching the turbidity degree 0.5 MacFarlan scale (1×10^8 CFU mL⁻¹), verifying by means of a spectrophotometer with the objective that the optical density at 550-660 nm is 0.7-1.0, which was inoculated by puncture to the pseudostem. Disease progress was evaluated weekly by determining the severity of the plants per treatment until 80% of the control plants showed wilt symptoms. For this purpose, the arbitrary severity scale from 0 to 4 described by Bakar et al. (2018) with modifications was used, where 0 corresponds to healthy plant with no visible symptoms; 1 to mild chlorosis in older leaves; 2 to pronounced chlorosis with mild necrosis and incipient wilting; 3 to evident wilting in several leaves accompanied by progressive necrosis and leaf drop; and 4 to severe wilting of the whole plant, collapse of the pseudostem, and generalized necrosis.

Table 2. Treatment description with *Trichoderma* spp. and *R. solanacearum*.

Treatment	Description
T1	<i>Trichoderma asperellum</i> F74 + <i>Ralstonia solanacearum</i>
T2	<i>Trichoderma harzianum</i> F73 + <i>Ralstonia solanacearum</i>
T3	<i>Trichoderma koningiopsis</i> F84 + <i>Ralstonia solanacearum</i>
T4	<i>Trichoderma afroharzianum</i> F78 + <i>Ralstonia solanacearum</i>
T5	<i>Trichoderma lixii</i> F17 + <i>Ralstonia solanacearum</i>
T6	<i>Trichoderma lentiforme</i> F19 + <i>Ralstonia solanacearum</i>
T7	<i>Trichoderma azevedoi</i> F76 + <i>Ralstonia solanacearum</i>
T8	Water only (negative control)
T9	Inoculation with moko only (positive control)

2.4. Experimental Design

The experiment was designed under a completely randomized scheme, with nine treatments (T1-T9) and three replicates per treatment. The experimental units consisted of groups of five plantain (*Musa AAB*.) plants obtained by tissue culture techniques, thus ensuring genetic and physiological uniformity.

The data obtained were subjected to analysis of variance [ANOVA] in order to determine significant differences between treatments. Tukey’s test ($p < 0.05$) was used for comparison of means. All analyses were carried out using the statistical software Infostat (2020).

3. Results

The statistical analysis of severity showed significant differences between treatments ($p < 0.0001$), with an adjusted R^2 of 0.96, reflecting the efficacy of the treatments in the observed variability. The negative control (T8) confirmed the absence of disease (mean: 0.00), while the positive control (T9) presented the highest severity (mean: 3.82), highlighting the impact of *R. solanacearum* without any management.

Figure 2 shows the plantain plants biotized with *Trichoderma* spp. applied only with water (negative control), and controlled only with *R. solanacearum* (positive control). A better vegetative development is observed in the plants treated with *Trichoderma* spp. compared to the positive and negative control.

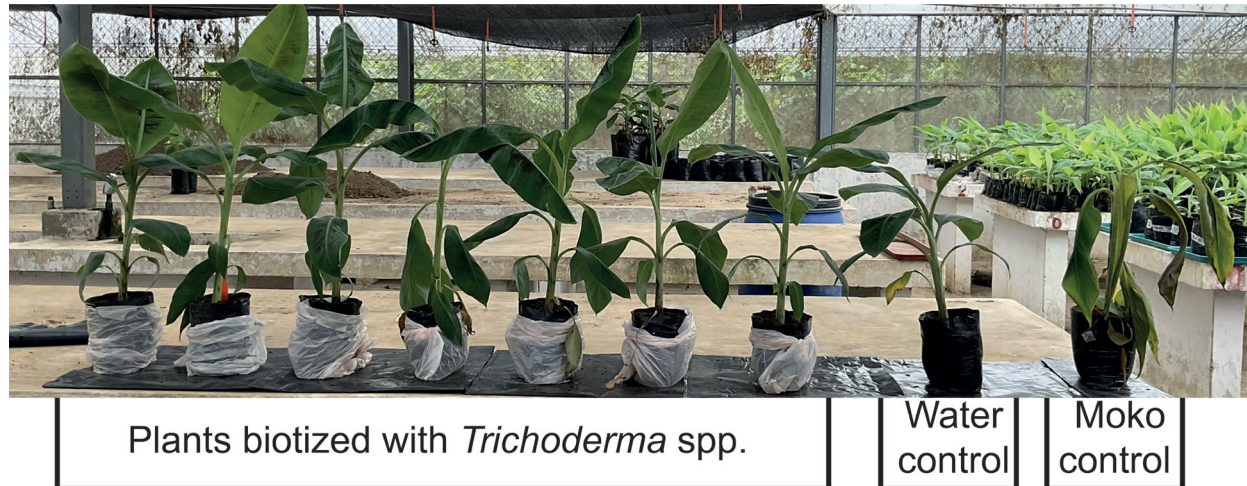


Figure 2. Comparison of vegetative development in plantain plants treated with *Trichoderma* spp., versus negative and positive controls.

The treatments (T1-T7), constituted by different strains of *Trichoderma* spp. significantly reduced the severity of the disease compared to treatment T9, in which the plants were inoculated only with *R. solanacearum*, registering the highest severity (3.82 ± 0.11) (Table 3). Treatments T6 (*T. lentiforme* F19) and T2 (*T. harzianum* F73) presented the lowest means (2.20 ± 0.11), indicating greater efficacy in suppressing the pathogen. In contrast, T7 (*T. azevedoi* F76) showed a less pronounced reduction (2.80 ± 0.11), while T3 (*T. koningiopsis* F84), T4 (*T. afroharzianum* F78), T5 (*T. lixii* F17) and T1 (*T. asperellum* F74) presented intermediate values (2.23 to 2.59 ± 0.11), reflecting a variable response in severity reduction. The absence of symptoms in the negative control (T8, without inoculation; 0.00 ± 0.11), confirms that the differences observed in the treatments are exclusively due to the presence of the pathogen.

Table 3. Severity analysis in treatments showing statistically different groups.

Treatments	Mean	EE	N	Group*
T9	3.82	0.11	3	A
T7	2.80	0.11	3	B
T1	2.59	0.11	3	BC
T5	2.50	0.11	3	BC
T4	2.48	0.11	3	BC
T3	2.23	0.11	3	C
T2	2.20	0.11	3	C
T6	2.20	0.11	3	C
T8	-0.00	0.11	3	D

* Means with a common letter are not significantly different ($p > 0.005$).

The results showed significant differences as to plant height among the treatments ($p = 0.0007$), with T6 (*T. lentiforme* F19), T5 (*T. lixii* F17) and T4 (*T. afroharzianum* F78) standing out, with means of 25.49 cm, 25.10 cm and 24.52 cm, respectively, therefore indicating a positive effect on the promotion of plant growth (Table 4). In contrast, treatment T9 (*R. solanacearum* without *Trichoderma*), with 19.5 cm, presented the lowest average height, followed by T8 (negative control, without inoculation) with 20.59 cm, highlighting the absence of the biostimulant effect observed in the treatments with *Trichoderma* spp. The treatments T1 (*T. asperellum* F74), T7 (*T. azevedoi* F76) and T3 (*T. koningiopsis* F84) presented intermediate values (22.74 cm, 21.76 cm and 21.55 cm, respectively), without significant differences with the most effective treatments.

Table 4. Plant height analysis by treatment with statistically different groups.

Treatment	Mean	EE	N	Group*
T6	25.49	0.85	3	A
T5	25.10	0.85	3	A
T4	24.52	0.85	3	AB
T1	22.74	0.85	3	ABC
T7	21.76	0.85	3	ABC
T3	21.55	0.85	3	ABC
T2	21.35	0.85	3	ABC
T8	20.59	0.85	3	BC
T9	19.5	0.85	3	C

* Means with a common letter are not significantly different ($p > 0.005$).

Pseudostem diameter was significantly influenced by treatments ($p < 0.005$), with the highest values observed in T6 (*T. lentiforme* F19) and T5 (*T. lixii* F17), both with 1.72 ± 0.06 cm, significantly exceeding treatment T9 (*R. solanacearum* without *Trichoderma*), which presented the lowest average diameter (1.35 ± 0.06 cm) (Table 5). Although T4 (*T. afroharzianum* F78) obtained a diameter of 1.67 ± 0.06 cm, its response did not differ statistically from the most effective treatments. Treatments T1 (*T. asperellum* F74), T3 (*T. koningiopsis* F84), T7 (*T. azevedoi* F76), and T2 (*T. harzianum* F73) presented intermediate values, with diameters between 1.54 and 1.45 cm, with no significant differences among them. Treatment T8 (negative control, without inoculation), showed a diameter of 1.41 ± 0.06 cm, with no differences compared to T2, T3 and T7.

Table 5. Pseudostem diameter analysis by treatment showing statistically different groups.

Treatment	Mean	EE	N	Group*
T6	1.72	0.06	3	A
T5	1.72	0.06	3	A
T4	1.67	0.06	3	AB
T1	1.54	0.06	3	ABC
T3	1.50	0.06	3	ABC
T7	1.47	0.06	3	ABC
T2	1.45	0.06	3	ABC
T8	1.41	0.06	3	BC
T9	1.35	0.06	3	C

* Means with a common letter are not significantly different ($p > 0.005$).

The number of leaves did not show significant differences between treatments ($p = 0.958$), which could be attributed to the low sensitivity of this variable in the initial stages of the experiment. Despite this, the treatments with *Trichoderma* spp. maintained values comparable to the controls, indicating that there was no detriment in

leaf development. (Tabla 6).

Table 6. Leaf number analyses per Treatment showing statistically different groups.

Treatment	Mean	EE	N	Group*
T6	8.33	1.19	3	A
T8	8.33	1.19	3	A
T2	8.00	1.19	3	A
T7	8.00	1.19	3	A
T1	8.00	1.19	3	A
T5	7.67	1.19	3	A
T3	7.33	1.19	3	A
T9	6.67	1.19	3	A
T4	6.66	1.19	3	A

* Means with a common letter are not significantly different ($p > 0.005$).

The statistical model applied showed high explanatory power for severity, height and diameter, with significant adjusted R^2 values. However, the low R^2 in the number of leaves underscores the need to interpret this variable with caution in future research.

4. Discussion

The use of *Trichoderma* spp. in agriculture has proven to be an effective strategy for both disease management and plant growth improvement. This has encouraged its adoption in sustainable production systems. In the present study, plants inoculated with different *Trichoderma* species showed a significant reduction in the severity of *R. solanacearum*, with T6 (*T. lentiforme* F19) and T2 (*T. harzianum* F73), respectively, standing out as the most effective treatments ($p < 0.0001$). These results are consistent with those reported by Ahmad et al. (2024), who documented reductions of up to 50% in the incidence of *Ralstonia* spp. in horticultural crops treated with *Trichoderma* strains. The observed efficacy could be attributed to mechanisms such as niche competition, production of antimicrobial metabolites and induction of systemic resistance.

Under controlled conditions, the application of metabolites derived from T2 (*T. harzianum*) has demonstrated to significantly reduce soil bacterial load and reduce bacterial wilt symptoms in tomato (*Solanum lycopersicum* L.) crops (Yan and Khan, 2021). Similarly, in this study, *Trichoderma* treatments not only mitigated disease severity, but also promoted plant growth. These findings reinforce the biostimulant capacity of *Trichoderma*, which has been associated with phytohormone production and improved availability of essential nutrients, as documented by Ramírez-Valdespino et al. (2019).

Pseudostem diameter was also positively influenced by *Trichoderma* inoculation. This increase could be related to a greater deposition of cellulose and lignin in the cell wall, strengthening the stem structure and providing greater mechanical resistance to the plant (Manzar et al., 2022). In contrast, the number of leaves did not show significant differences among treatments ($p > 0.05$), which coincides with previous studies where it has been reported that this variable is less sensitive in early stages of the crop (Damodaran et al., 2020).

5. Conclusions

The results obtained confirm the dual potential of several *Trichoderma* spp. strains as integrated management agents of *Ralstonia solanacearum* and plant growth promoters. Specifically, strains F19 (*T. lentiforme*) and F73 (*T. harzianum*) significantly reduced the severity of bacterial wilt ($p < 0.0001$), while F19, F17 (*T. lixii*) and F78 (*T. afroharzianum*) increased plant height up to 25.49 cm and pseudostem diameter by 1.72 cm, with no significant differences in leaf number ($p > 0.05$), thus indicating low sensitivity of this variable at early stages.

Nevertheless, the efficacy demonstrated in vitro requires validation under field conditions, where interaction with the native microbiota and edaphoclimatic variables can modify the performance of the biocontrollers. In this sense, the optimization of doses and formulations, as well as the combination of these strains with other integrated management strategies, will be essential in order to maximize their impact within commercial production systems of *musaceae*, and to guarantee the long-term stability of biostimulant effects and the containment of possible adaptations of *R. solanacearum*.

Contributor roles

- Pedro Isaías Terrero Yépez: conceptualization, methodology, project administration, supervision, writing - original draft.
- Nicole Factos: research, formal analysis, data curation, writing - original draft.
- Paola Rodulfo: methodology, resources, research, writing – review & editing.
- Karina Solis: funding acquisition, validation, supervision, writing – review & editing.
- Carlos Molina: supervision, project administration, resources, writing – review & editing.
- Karen Rafaela Mayorga: investigation, visualization, writing – review & editing.

Ethical implications

Ethics approval not applicable.

Conflict of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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