Multidisciplinary Collaborative Journal
ISSN: 3073-1356

DOSO EDITORIAL CIENTÍFICA

Article

Efficacy of *Trichoderma* sp. and *Bacillus subtilis* as biocontrol agents against *Pseudocercospora fijiensis* in the cultivation of banana (*Musa* × *paradisiaca*)

Angel Virgilio Cedeño Moreira [1], Kimberly López Cedeño [2], Mauricio Renato Morejón Centeno [3], Juan Antonio Torres Rodríguez [4], Ketty Vanessa Arellano Ibarra [5], Jorge Alberto Alejandre Rosas [6] and Alejandra Alvarado Mávil [7]*

[1] Facultad de Ciencias Pecuarias y Biológicas, Universidad Técnica Estatal de Quevedo, Av. Quito km 1.5 vía a Santo Domingo, Quevedo 120501, Ecuador; https://orcid.org/0000-0002-6564-5569; acedenom@uteq.edu.ec
[2] Instituto Superior Tecnológico La Maná, Av. Amazonas entre Miguel Iturralde y Héroes del Cenepa, La Maná 050202, Ecuador; https://orcid.org/0000-0002-6838-1474; klopez@istlm.edu.ec
[3] Facultad de Ciencias Agrarias y Forestales, Universidad Técnica Estatal de Quevedo, Av. Quito km 1.5 vía a Santo Domingo, Quevedo 120501, Ecuador; https://orcid.org/0000-0002-2621-2306; mmorejonc@uteq.edu.ec
[4] Facultad de Ciencias Agrarias y Forestales, Universidad Técnica Estatal de Quevedo, Av. Quito km 1.5 vía a Santo Domingo, Quevedo 120501, Ecuador; https://orcid.org/0000-0003-3326-4371; jatorres@uteq.edu.ec
[5] Facultad de Ciencias Pecuarias y Biológicas, Universidad Técnica Estatal de Quevedo, Av. Quito km 1.5 vía a Santo Domingo, Quevedo 120501, Ecuador; https://orcid.org/0000-0001-7168-7485; Ketty.arellano2017@uteq.edu.ec
[6] Facultad de Ciencias Químicas de Orizaba, Universidad Veracruzana, Oriente 6 No. 1009, Colonia Rafael Alvarado, CP. 94340 Orizaba, Mexico; https://orcid.org/0000-0002-1252-4966; jalejandre@uv.mx
[7] Facultad de Ciencias Químicas de Orizaba, Universidad Veracruzana, Oriente 6 No. 1009, Colonia Rafael Alvarado, CP. 94340 Orizaba, Mexico; https://orcid.org/0009-0009-3041-8997; aalvarado@uv.mx

* Correspondence: aalvarado@uv.mx

Citation: Cedeño Moreira, A. V., López Cedeño, K., Morejón Centeno, M. R., Torres Rodríguez, J. A., Arellano Ibarra, K. V., Alejandre Rosas, J. A., & Alvarado Mávil, A. (2026). Eficacia de Trichoderma sp. y Bacillus subtilis como agentes de control biológico contra Pseudocercospora fijiensis en el cultivo del plátano (Musa × paradisiaca). *Multidisciplinary Collaborative Journal*, *4*(2), 1-15. https://doi.org/10.70881/mcj/v4/n2/145

Received: 02/03/2026
Revised: 06/04/2026
Accepted: 09/04/2026
Published: 14/04/2026

https://doi.org/10.70881/mcj/v4/n2/145

Abstract: This study evaluated the efficacy of *Trichoderma* sp. and *Bacillus subtilis* as biocontrol agents against *Pseudocercospora fijiensis*, the fungus responsible for Black Sigatoka in bananas (*Musa* × *paradisiaca*). Under laboratory conditions, the inhibition of ascospore germination and radial growth was evaluated by applying *Trichoderma* sp. and *B. subtilis* metabolites at concentrations of 5% and 10 %. At a 10 % concentration, *B. subtilis* achieved 100% inhibition of ascospore germination and a 90 % reduction in radial growth of *P. fijiensis*, outperforming *Trichoderma*, which achieved 60 % inhibition in both tests at the same concentration. In greenhouse trials, disease incidence and severity were measured in Cavendish banana seedlings inoculated with *P. fijiensis* and treated with weekly foliar applications of the biocontrol agents. At a 10 % concentration, *B. subtilis* reduced disease severity to 10 %, while *Trichoderma* at 10 % achieved a 30 % reduction in severity, compared to the control, which maintained a constant severity of around 81 %. These results highlight the potential of *B. subtilis* as a robust biocontrol agent against *P. fijiensis*, providing a sustainable and effective alternative for managing Black Sigatoka in agricultural systems, thus reducing dependency on chemical fungicides and promoting environmentally responsible cultivation practices.

Palabras clave: biocontrol, ascospore germination, incidence, metabolites, radial growth

Resumen: Este estudio evaluó la eficacia de *Trichoderma* sp. y *Bacillus subtilis* como agentes de biocontrol contra *Pseudocercospora fijiensis*, el hongo responsable de la Sigatoka Negra en banano (*Musa* × *paradisiaca*). Bajo condiciones de laboratorio, se evaluó la inhibición de la germinación de ascosporas y el crecimiento radial mediante la aplicación de metabolitos de *Trichoderma* sp. y *B. subtilis* en concentraciones de 5% y 10%. A una concentración de 10%, *B. subtilis* logró una inhibición del 100% de la germinación de ascosporas y una reducción del 90% en el crecimiento radial de *P. fijiensis*, superando a *Trichoderma*, que logró una inhibición del 60% en ambas pruebas a la misma concentración. En ensayos de invernadero, se midió la incidencia y severidad de la enfermedad en plántulas de banano Cavendish inoculadas con *P. fijiensis* y tratadas con aplicaciones foliares semanales de los agentes de biocontrol. Con una concentración del 10 %, *B. subtilis* redujo la gravedad de la enfermedad al 10 %, mientras que *Trichoderma*, al 10 %, logró una reducción del 30 % en la gravedad, en comparación con el control, que mantuvo una gravedad constante de alrededor del 81 %. Estos resultados resaltan el potencial de *B. subtilis* como un potente agente de biocontrol contra *P. fijiensis*, ofreciendo una alternativa sostenible y eficaz para el manejo de la Sigatoka Negra en sistemas agrícolas, reduciendo así la dependencia de fungicidas químicos y promoviendo prácticas de cultivo respetuosas con el medio ambiente.

Keywords: Biocontrol, germinación de ascosporas, incidencia, metabolitos, crecimiento radial

1. Introduction

Banana (*Musa* × *paradisiaca*) cultivation represents one of the most important economic and nutritional pillars in tropical and subtropical regions worldwide (Sau et al., 2023). This crop is a fundamental source of income and employment for millions of farmers and rural communities, in addition to being an important source of carbohydrates and other nutrients in human diets (Alonso et al., 2020). However, banana crops are severely threatened by various diseases, among which Black Sigatoka, caused by the fungus *Pseudocercospora fijiensis*, is particularly prominent (Esguera et al., 2024).

Despite its agricultural and socioeconomic importance, banana cultivation is highly susceptible to foliar fungal diseases, which impair the plant's photosynthetic capacity and significantly affect fruit yield and quality (Da Silva et al., 2023). Among these, leaf spot diseases caused by the fungus *Pseudocercospora fijiensis* (formerly *Mycosphaerella fijiensis*) represent the most serious threat to the banana industry, as this phytopathogen has been the principal constraint on banana production over the past fifty years (Arango Isaza et al., 2016; Strobl & Mohan, 2020). This phytopathogen causes yield losses estimated at 33% to 70% in banana and plantain crops, and its management requires substantial investment in phytosanitary inputs (Arango Isaza et al., 2016; Chang et al., 2016).

This disease causes premature leaf necrosis, drastically reducing the plant's photosynthetic capacity and thereby affecting fruit yield and quality (Da Silva et al., 2023). Farmers facing Black Sigatoka are often forced to increase the use of chemical fungicides to control the disease, which, in the long term, leads to environmental, economic, and public health consequences (Esguera et al., 2024). Moreover, the excessive use of these products has fostered the emergence of resistant strains of *P. fijiensis*, thereby reducing the effectiveness of traditional chemical control methods (Palmieri et al., 2022).

In recent years, sustainable agriculture has gained prominence as a preferred approach to disease management, emphasizing the importance of reducing dependence on chemical products and promoting biological alternatives that enhance the natural resistance of plants (Torres-Rodriguez et al., 2021; Collinge et al., 2022; Elnahal et al., 2022). In this context, biocontrol agents such as *Trichoderma* spp. and *B. subtilis* have emerged as promising solutions because of their antagonistic properties and their ability to inhibit a wide range of phytopathogens (Lahlali et al., 2022).

The genus *Trichoderma* includes fungi that have demonstrated efficacy in controlling fungal phytopathogens through mechanisms such as competition for space and nutrients, mycoparasitism, and the production of antifungal compounds (Tyśkiewicz et al., 2022). Similarly, *B. subtilis*, a beneficial bacterium, has shown great potential for disease control through the production of metabolites that inhibit phytopathogen growth and stimulate systemic resistance in plants (Dimkić et al., 2022).

The use of *Trichoderma* spp. and *B. subtilis* not only represents an effective strategy to reduce the incidence of Black Sigatoka, but also promotes environmentally responsible and cost-effective management (Dadrasnia et al., 2020). Previous studies have documented that both biocontrol agents can adapt well to diverse conditions, persist in the environment, and provide continuous control of phytopathogens (Lahlali et al., 2022). However, the effectiveness of these species as biocontrol agents against *P. fijiensis* in banana cultivation requires thorough analysis under both laboratory and field conditions in order to evaluate their benefits and limitations in a real agricultural context (Cuellar et al., 2021).

The objective of the present study is to evaluate the efficacy of *Trichoderma* spp. and *B. subtilis* as biocontrol agents for the management of *P. fijiensis*. Through this research, we aim to generate useful knowledge about the potential of these microorganisms in the biological control of Black Sigatoka, offering a sustainable and safe alternative that contributes to reducing the use of chemical fungicides in banana cultivation. This approach could improve the sustainability of agricultural production, reduce disease management costs, and contribute to the ecological and socioeconomic well-being of banana-producing regions.

2. Methodology

Strain Acquisition

The *Bacillus subtilis* and *Trichoderma* sp. strains used in this study were obtained from the strain bank of the Biology and Microbiology Laboratory at the Technical State University of Quevedo (UTEQ). These strains had been previously isolated, characterized, and preserved under controlled conditions to ensure their viability and purity. The *Trichoderma* sp. strain was inoculated on potato dextrose agar (PDA; Difco, 39 g L^{-1}) and incubated at 25 °C in darkness for 7 days. *B. subtilis* was inoculated on nutrient agar (NA) and incubated at 30 °C for 24 h.

On the other hand, the *P. fijiensis* strains used in this study were isolated from a commercial banana plantation located in Valencia, Ecuador. The *P. fijiensis* strains were cultured on potato dextrose agar (PDA) and maintained at 25 °C in darkness for 15 days. Each strain was purified using the hyphal tip method, which allowed the acquisition of pure cultures by selecting and transferring only the tips of actively growing hyphae. This

purification process ensured the elimination of potential contaminants, thereby guaranteeing the purity of the strains for use in the inhibition assays.

Molecular identification of *Pseudocercospora fijiensis*

For molecular identification, an rDNA fragment encompassing the ITS region (ITS1–5.8S–ITS2) and extending toward the large subunit (28S/LSU) was amplified using the specific primers MF137 (5′-GGCGCCCCCGGAGGTCTCCTT-3′) and R635 (5′-GGTCCGTGTTTCAAGACGG-3′), as described by Johanson and Jeger (1993), which generate an expected amplicon of approximately 1.0 kb (~1018 bp). The PCR reaction was performed in a final volume of 25 µL containing 1× buffer, $MgCl_2$ (1.5–2.5 mM), dNTPs (0.2 mM each), primers (0.2–0.5 µM each), Taq DNA polymerase (approximately 1 U), and template DNA (approximately 20–50 ng).

Amplification was carried out with an initial denaturation at 94 °C, followed by 35 cycles of 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. The amplified products were separated by electrophoresis on a 1.5% agarose gel in 1× TAE buffer at 90–110 V for 40–60 min and visualized by staining with ethidium bromide or SYBR Safe under a UV transilluminator. Amplicon size was estimated by comparison with a 100 bp DNA ladder. A negative control without template DNA (C0) was included to rule out contamination.

Determination of the incidence and severity of *Pseudocercospora fijiensis* in banana plantlets

The incidence and severity of *P. fijiensis* were assessed under controlled greenhouse conditions. Cavendish banana plantlets obtained through in vitro propagation, approximately 30 cm in height, were used in the experiment. The plantlets were transplanted into 26-cm-diameter pots at a density of one plant per pot and allowed to acclimatize for 14 days. The experimental design included an inoculated treatment and a non-inoculated control, with 10 replicates per treatment.

For inoculation, plantlets assigned to the inoculated treatment were sprayed with a spore suspension of *P. fijiensis* adjusted to 1×10^6 spores mL^{-1} and uniformly applied to both adaxial and abaxial leaf surfaces. Immediately after inoculation, the plants were maintained in a humid chamber for 48 h to promote infection. Control plants were sprayed with sterile distilled water only and kept under the same environmental conditions (Torres-Rodriguez et al., 2025). Disease incidence was evaluated six weeks after inoculation and expressed as the percentage of infected plantlets relative to the total number of evaluated plants, according to the following equation:

$$DI\ (\%) = (IP / TP) \times 100$$

where IP represents the number of infected plants and TP the total number of evaluated plants.

Disease severity was determined in each of the 10 replicates by recording the total number of functional leaves and estimating the percentage of leaf area affected by Black Sigatoka symptoms on each leaf. Severity was scored using a seven-class visual scale (0–6) adapted from Orjeda (1998), where 0 = no symptoms; 1 = less than 1% infected leaf area; 2 = 1–5%; 3 = 6–15%; 4 = 16–33%; 5 = 34–50%; and 6 = 51–100%. The

disease severity index (DSI) was then calculated according to Craenen (1998) using the following equation:

$$DSI\ (\%) = [\Sigma(nb) / ((N - 1)\ T)] \times 100$$

where n is the number of leaves at each severity grade, b is the numerical value assigned to each grade, N is the total number of categories in the scale (7), and T is the total number of leaves evaluated per plant.

Inhibition of *Pseudocercospora fijiensis* ascospore germination by metabolites produced by *Trichoderma* sp. and *Bacillus subtilis*

The inhibition of *P. fijiensis* ascospore germination was assessed under laboratory conditions using metabolites produced by *Trichoderma* sp. and *B. subtilis*. Four treatments were evaluated: two concentrations of *Trichoderma* sp. metabolites (5% and 10%) and two concentrations of *B. subtilis* metabolites (5% and 10%). The metabolites were obtained from liquid cultures and filtered through Whatman No. 1 filter paper.

For each treatment, 20 µL of an *P. fijiensis* ascospore suspension (1×10^6 spores mL^{-1}) was mixed with 20 µL of the corresponding metabolite solution in a sterile Petri dish. The negative control consisted of 20 µL of the ascospore suspension mixed with 20 µL of sterile distilled water.

Each treatment, including the control, was replicated ten times, and the plates were incubated at 25 °C in darkness for 48 h. Following incubation, ascospore germination was assessed by light microscopy at 40× magnification. For each replicate, five microscopic fields were randomly selected, and at least 100 spores were counted per treatment. Germination inhibition was calculated according to the following equation:

$$AGI\ (\%) = (UA / TA) \times 100$$

where AGI represents the percentage of ascospore germination inhibition, UA is the number of ungerminated ascospores, and TA is the total number of ascospores observed.

Radial Growth Inhibition of *Pseudocercospora fijiensis*

The inhibitory effect of metabolites produced by *Trichoderma* sp. and *B. subtilis* on the radial growth of *P. fijiensis* colonies was evaluated under controlled laboratory conditions. Two concentrations of each metabolite (5% and 10%) were assessed, using dilutions prepared from liquid culture extracts of both microorganisms.

In each Petri dish containing potato dextrose agar (PDA), a mycelial disc of *P. fijiensis* was placed at the center, and 50 µL of each metabolite solution was applied at equidistant points surrounding the disc. Plates were incubated at 25 °C in darkness, and colony radial growth was measured after 7 days. Radial growth inhibition was expressed as a percentage relative to the untreated control. The percentage of radial growth inhibition was calculated according to the following equation (Torres-Rodriguez et al., 2024):

$$RGI\ (\%) = [(R1 - R2) / R1] \times 100$$

where RGI denotes the percentage of radial growth inhibition, R1 is the mycelial growth of *P. fijiensis* in the control treatment, and R2 is the mycelial growth of *P. fijiensis* in the presence of metabolites from *Trichoderma* sp. or *B. subtilis.*

Application of *Trichoderma* sp. and *Bacillus subtilis* for the biological control of *Pseudocercospora fijiensis*

The experiment was conducted under controlled greenhouse conditions to evaluate the effect of *Trichoderma* sp. and *B. subtilis* on the severity of *P. fijiensis* in banana plantlets. The *B. subtilis* strain was cultured in selective medium at 28 °C for 24–48 h until reaching a concentration of 1×10^8 CFU mL^{-1}, whereas the *Trichoderma* sp. strain was cultured in selective medium at 28 °C for 5–7 days until reaching a concentration of 1×10^8 conidia mL^{-1}. The inoculum of *P. fijiensis* was obtained from cultures grown on PDA and incubated at 25 °C for 7 days, and the spore suspension was adjusted to 1×10^6 spores mL^{-1}.

The experiment was arranged in a completely randomized design with five treatments: (1) a positive control inoculated only with *P. fijiensis* and sprayed with sterile distilled water, (2) *B. subtilis* at 5%, (3) *B. subtilis* at 10%, (4) *Trichoderma* sp. at 5%, and (5) *Trichoderma* sp. at 10%. Each treatment included five replicates, with five plantlets per replicate (n = 25 per treatment). Inoculation was performed by foliar spraying of the *P. fijiensis* spore suspension, ensuring uniform coverage of the entire leaf surface. Subsequently, the plantlets were placed in a humid chamber at 90% relative humidity for 48 h to promote infection.

The biocontrol agents were applied by foliar spraying until complete leaf coverage was achieved, and applications were repeated weekly for 30 days. Greenhouse conditions were maintained at a mean temperature of 28.2 °C and 82.4% relative humidity. Disease severity was assessed at the end of the experimental period using the scale and formula previously described in the section entitled “Determination of the incidence and severity of *Pseudocercospora fijiensis* in banana plantlets”.

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using RStudio software. Differences among treatment means were determined using Tukey’s multiple comparison test at $p < 0.05$. Before conducting ANOVA, the assumptions of normality and homogeneity of variances were verified using the Shapiro–Wilk and Bartlett’s tests, respectively.

3. Results

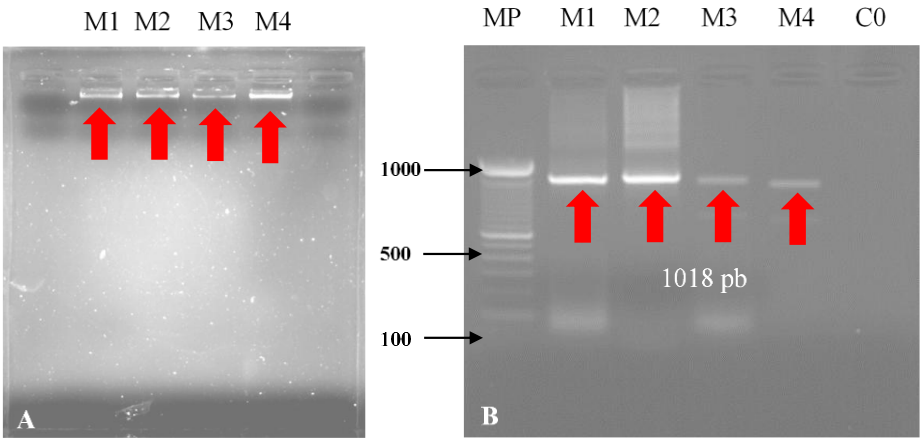
Molecular Identification of *Pseudocercospora fijiensis*

Amplification with *P. fijiensis*-specific ITS primers (Figure 1B) produced a band of 1018 base pairs (bp), confirming the molecular identification of the phytopathogen. In samples M1, M2, M3, and M4, the successful amplification of the 1018 bp fragment, indicated by red arrows, demonstrates the presence of *P. fijiensis* in all analyzed samples. The molecular weight marker (MP) shows the reference band positions (100 bp, 500 bp, and 1000 bp), allowing verification that the amplified fragments correspond to the expected size for *P. fijiensis* identification. The negative control (C0) showed no amplification,

confirming both the specificity of the primers and the absence of contamination in the assay.

Figure 1

Molecular identification of Pseudocercospora fijiensis by PCR amplification of the ITS region



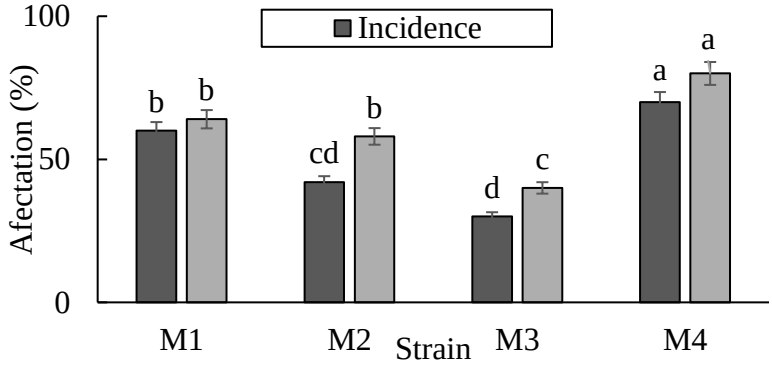
Note: (A) Genomic DNA extracted from *Pseudocercospora fijiensis* isolates (M1–M4). (B) PCR amplification of the ITS region using the species-specific primers MF137 and R635, showing the expected ~1018 bp amplicon (red arrows) in isolates M1–M4. MP, DNA ladder; C0, no-template negative control.

Infectivity of *Pseudocercospora fijiensis* in Banana Seedlings

The incidence and severity of *P. fijiensis* varied significantly among the evaluated strains (Figure 2). M4 was the most virulent strain, showing the highest incidence (70%) and severity (80%), whereas M3 exhibited the lowest values for both variables, with 30% incidence and 40% severity. M1 showed intermediate but relatively high levels of affectation, reaching 60% incidence and 64% severity, while M2 presented 42% incidence and 58% severity. These findings reveal substantial variability in pathogenicity among the tested strains and identify M4 as the most aggressive isolate in banana seedlings.

Figure 2

Incidence and severity of Pseudocercospora fijiensis in four strains (M1, M2, M3, and M4) evaluated in banana seedlings



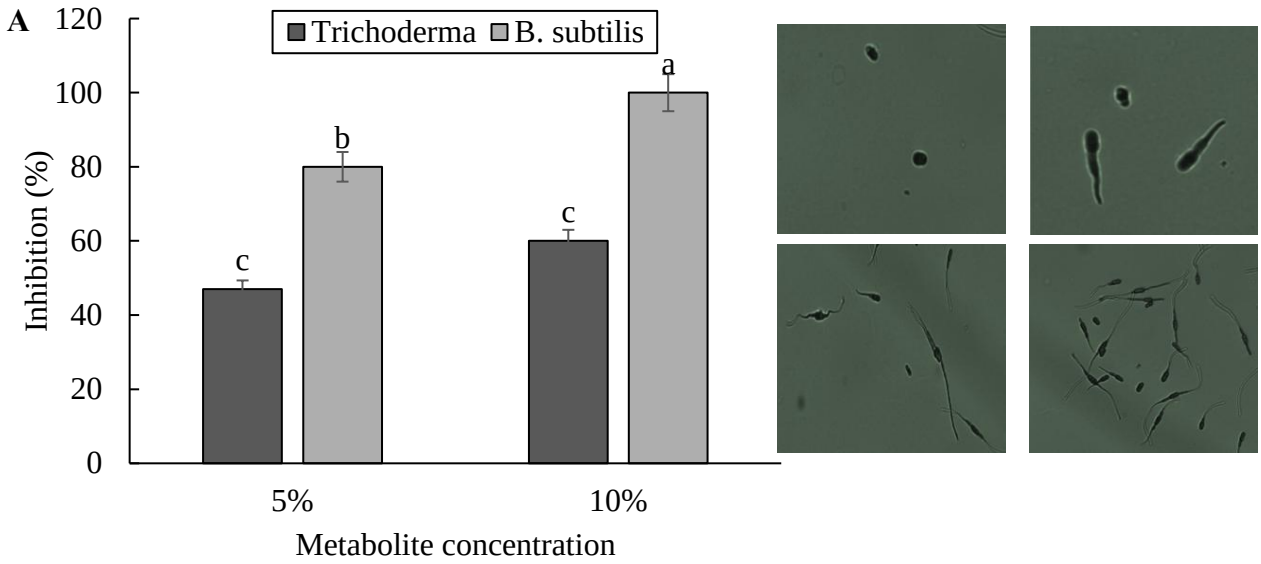
Note: Dark bars represent incidence, and light bars represent severity. Different letters above the bars indicate significant differences among strains within each variable according to Tukey's test ($P < 0.05$).

Ascospore Germination Inhibition

Ascospore germination of *P. fijiensis* was significantly affected by the microbial metabolites evaluated (Figure 3). *B. subtilis* showed the highest inhibitory activity at both concentrations, reaching 80% inhibition at 5% and complete inhibition (100%) at 10%. In contrast, metabolites from *Trichoderma* sp. produced lower inhibition values, with 47% at 5% and 60% at 10%. In both microorganisms, inhibition increased with metabolite concentration; however, *B. subtilis* consistently outperformed *Trichoderma* sp., indicating a stronger suppressive effect on ascospore germination.

Figure 3

Inhibition of Pseudocercospora fijiensis ascospore germination by microbial metabolites



Note: (A) Percentage of ascospore germination inhibition induced by metabolites produced by *Trichoderma* sp. and *B. subtilis* at 5% and 10%. Different letters above the bars indicate significant differences among treatments according to Tukey's multiple comparison test ($P < 0.05$). (B) Representative microscopic images showing the effect of each treatment on ascospore germination. B1, *B. subtilis* at 10%; B2, *B. subtilis* at 5%; B3, *Trichoderma* sp. at 10%; and B4, *Trichoderma* sp. at 5%.

Radial Growth Inhibition

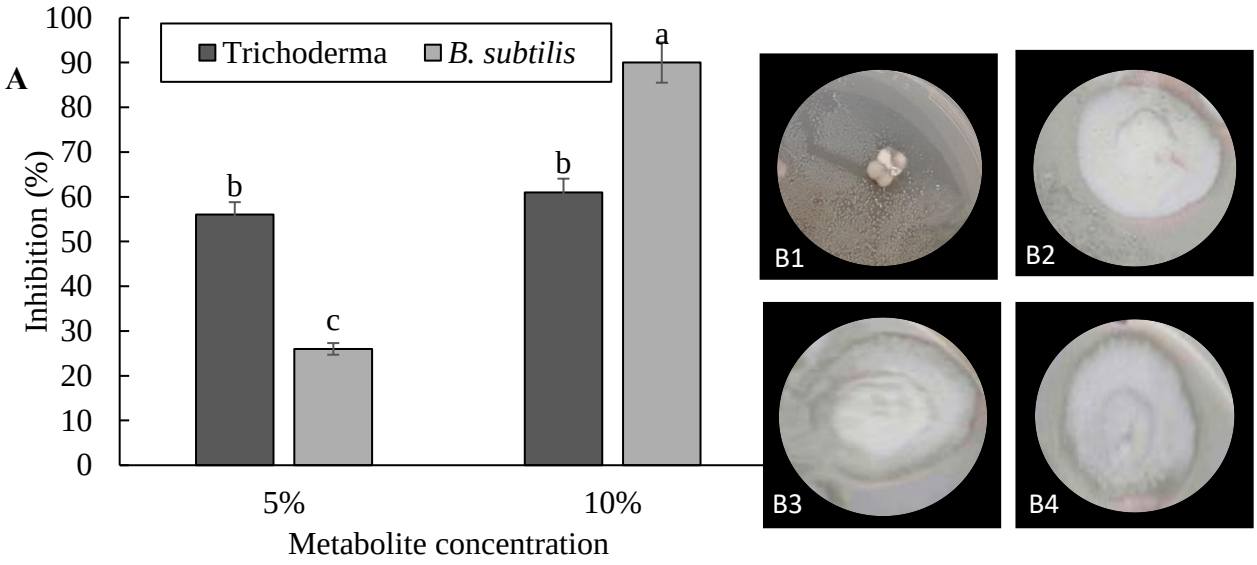
Radial growth of *P. fijiensis* differed significantly among the evaluated treatments (Figure 4). The strongest inhibition was observed with metabolites from *B. subtilis* at 10%, which reduced colony growth by 90% relative to the control. Metabolites from *Trichoderma* sp. at the same concentration produced 61% inhibition. At 5%, *Trichoderma* sp. showed moderate inhibitory activity (56%), whereas *B. subtilis* exhibited the lowest effect (26%). Overall, inhibition increased with concentration for *B. subtilis*, while *Trichoderma* sp. maintained intermediate inhibition at both concentrations. These results indicate that *B.*

subtilis, particularly at 10%, was the most effective treatment for suppressing mycelial growth of *P. fijiensis*.

Representative images further showed that metabolite application induced evident morphological changes in *P. fijiensis* colonies, including irregular colony development and altered pigmentation at the margins. Such responses are consistent with fungal stress and reinforce the inhibitory activity of the evaluated microbial metabolites against the phytopathogen.

Figure 4

Effect of microbial metabolites on the radial growth of Pseudocercospora fijiensis



Note: (A) Radial growth inhibition (%) of *P. fijiensis* colonies treated with metabolites produced by *Trichoderma* sp. and *B. subtilis* at 5% and 10%. Different letters above the bars indicate significant differences among treatments according to Tukey's multiple comparison test ($P < 0.05$). (B) Representative colony morphology of *P. fijiensis* under each treatment. B1, *B. subtilis* at 10%; B2, *B. subtilis* at 5%; B3, *Trichoderma* sp. at 10%; and B4, *Trichoderma* sp. at 5%.

Reduction of Disease Severity in Banana Seedlings

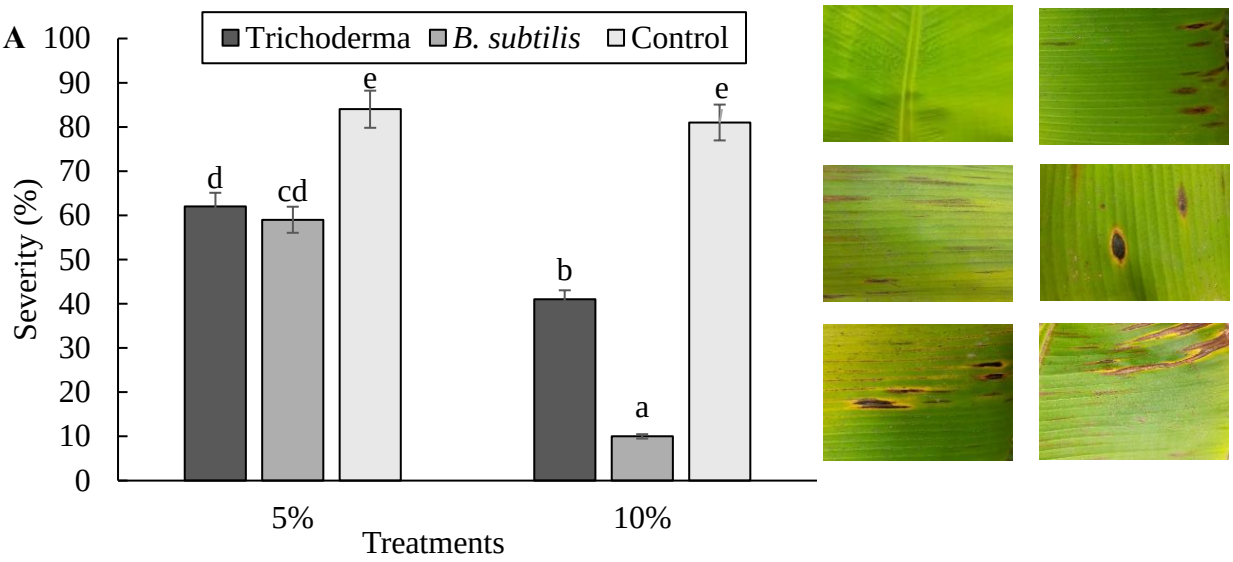
The severity of *P. fijiensis* in banana seedlings was significantly reduced by the application of microbial treatments, although the magnitude of the effect depended on both the biological agent and the concentration used (Figure 5). At the 5% concentration, *Trichoderma* sp. reduced disease severity to 62%, whereas *B. subtilis* reduced it to 59%. Both treatments showed lower severity than the phytopathogen-inoculated control, which reached 84%.

A stronger reduction in disease severity was observed at the 10% concentration. *B. subtilis* was the most effective treatment, reducing severity to 10%, while *Trichoderma* sp. reduced it to 41%. In contrast, the control maintained a high severity value of 81%. Statistical analysis showed significant differences among treatments ($P < 0.05$), with *B. subtilis* at 10% showing the greatest suppressive effect on disease development. Overall, these results indicate that both *B. subtilis* and *Trichoderma* sp. have biocontrol potential

against *P. fijiensis* in banana seedlings, although *B. subtilis*, particularly at 10%, was the most effective treatment.

Figure 5

Severity of Pseudocercospora fijiensis in banana seedlings treated with Trichoderma sp. and Bacillus subtilis



Note: (A) Severity (%) of *P. fijiensis* in banana seedlings treated with *Trichoderma* sp. and *Bacillus subtilis* at 5% and 10%, relative to the phytopathogen-inoculated control. Different letters above the bars indicate significant differences among treatments according to Tukey's multiple comparison test ($P < 0.05$). The control is shown in both concentration groups for comparative purposes, although it represents a single treatment. (B) Representative leaf symptoms of *P. fijiensis* under each treatment. B1, *B. subtilis* at 10%; B2, *Trichoderma* sp. at 10%; B3, *B. subtilis* at 5%; B4, *Trichoderma* sp. at 5%; and B5–B6, phytopathogen-inoculated control treated only with sterile water.

4. Discussion

The variability in incidence and severity observed among *P. fijiensis* strains is likely associated with differences in virulence-related traits among isolates. Previous studies have shown that *P. fijiensis* exhibits substantial genetic diversity, which enables rapid adaptation to different environmental conditions and host defense responses, thereby influencing its pathogenicity (Souleymane et al., 2022; Esguera et al., 2024). This genetic variability may be reflected in differences in the production of cell wall-degrading enzymes, toxins, and other infection-related compounds that facilitate host colonization and tissue damage (Noar et al., 2022).

Previous reports have indicated that some *P. fijiensis* isolates may have a greater capacity to produce effector molecules that suppress host defenses and increase disease severity (Pinheiro et al., 2022). In particular, highly virulent isolates, such as M4 in the present study, may possess specific genes involved in the regulation of pathogenicity-related compounds and enzymes that promote tissue colonization and lesion development (Olivares et al., 2021). In contrast, less aggressive isolates, such as

M3, may express these virulence-related factors at lower levels, which could explain their reduced ability to infect host tissues and cause severe symptoms.

The results of the present study are consistent with previous research reporting the effectiveness of *B. subtilis* as a biocontrol agent against phytopathogens through the production of secondary metabolites such as surfactins, iturins, and fengycins, which directly inhibit spore germination and pathogen growth (Zhu et al., 2020; Mahmood et al., 2022). These antimicrobial compounds disrupt cell membrane integrity, which may explain the strong inhibitory effect against *P. fijiensis* observed in this study. At the higher concentration tested (10%), *B. subtilis* achieved complete inhibition, likely due to the increased availability of these bioactive metabolites, further supporting its potential as an effective biocontrol agent (Dimkić et al., 2022).

Metabolites produced by *Trichoderma* sp. also showed inhibitory activity against the pathogen, although their effect was less pronounced than that of *B. subtilis*. Previous studies have shown that *Trichoderma* spp. produce hydrolytic enzymes, such as glucanases and chitinases, which weaken phytopathogen cell walls and reduce their capacity to infect host tissues (Konappa et al., 2020; Dutta et al., 2023). Although this mode of action may not result in complete inhibition as efficiently as the antibiotic compounds produced by *B. subtilis*, it can still substantially reduce pathogen development, as observed in the present study, where inhibition reached 60% at the 10% concentration.

The greater efficacy of *B. subtilis* compared with *Trichoderma* sp. in reducing disease severity in banana seedlings may be explained by differences in their modes of action. *B. subtilis* is widely recognized for its ability to produce antimicrobial metabolites, including surfactins, iturins, and fengycins, which directly affect pathogen cells by altering membrane permeability and suppressing mycelial growth (Elsharkawy et al., 2022; Ajuna et al., 2024). In addition, *B. subtilis* may activate induced systemic resistance in plants, thereby enhancing host defense against subsequent infections (Rabari et al., 2023; Jinal et al., 2024). The combined effect of direct antagonism and host defense stimulation likely explains the marked reduction in disease severity observed at the 10% concentration, where *B. subtilis* reduced severity to 10%.

In contrast, *Trichoderma* sp. acts mainly through the production of hydrolytic enzymes and through competition for space and nutrients, which can limit phytopathogen establishment and development. In addition, some *Trichoderma* strains are known to promote plant defense responses and contribute indirectly to disease suppression (Contreras et al., 2020; Poveda et al., 2020). These mechanisms may explain the reductions in severity observed at both 5% and 10%, although their effect was lower than that achieved with *B. subtilis*, particularly at the higher concentration.

5. Conclusions

The results demonstrated significant variability in virulence among the evaluated *Pseudocercospora fijiensis* strains, with M4 showing the highest incidence and severity in banana seedlings. Among the microbial treatments, *Bacillus subtilis* exhibited the strongest antagonistic activity, particularly at the 10% concentration, where it achieved complete inhibition of ascospore germination, the highest radial growth inhibition, and

the greatest reduction in disease severity. In contrast, *Trichoderma* sp. also showed inhibitory activity, although its effectiveness was consistently lower than that of *B. subtilis*.

In addition to suppressing pathogen development, the microbial metabolites induced visible morphological alterations in *P. fijiensis* colonies, suggesting a direct antifungal effect. Under greenhouse conditions, the marked reduction in disease severity observed in banana seedlings treated with *B. subtilis*, especially at 10%, confirms its high potential as a biocontrol agent against Black Sigatoka. Overall, these findings support the use of *B. subtilis* as a promising biological alternative for the management of *P. fijiensis* in banana production systems.

Author Contributions: Conceptualization, A.V.C.M. and J.A.T.R.; methodology, A.V.C.M., J.A.A.R and A.A.M.; formal analysis, M.R.M.C., K.L.C. and K.V.A.I.; investigation, A.V.C.M., K.V.A.I. and J.A.T.R.; resources, A.V.C.M. and K.V.A.I.; writing—original draft preparation, A.A.M.; writing—review and editing, J.A.A.R. and A.A.M.; visualization, M.R.M.C. and K.L.C.; supervision, J.A.T.R.; All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive external funding.

Acknowledgments: We would like to thank the Universidad Técnica Estatal de Quevedo (UTEQ) and the UTEQ Research Department for their ongoing support. Special thanks to the entire team at the UTEQ microbiology laboratory.

Data availability statement: The data are available upon reasonable request from the corresponding author at: aalvarado@uv.mx

Conflicts of interest: The authors declare no conflict of interest.

References

Ajuna, H. B., Lim, H. I., Moon, J. H., Won, S. J., Choub, V., Choi, S. I., ... & Ahn, Y. S. (2024). The prospect of antimicrobial peptides from *Bacillus* species with biological control potential against insect pests and diseases of economic importance in agriculture, forestry and fruit tree production. *Biotechnology & Biotechnological Equipment, 38*(1), 2312115. https://doi.org/10.1080/13102818.2024.2312115

Alonso-Gómez, L. A., Solarte-Toro, J. C., Bello-Pérez, L. A., & Cardona-Alzate, C. A. (2020). Performance evaluation and economic analysis of the bioethanol and flour production using rejected unripe plantain fruits (*Musa paradisiaca* L.) as raw material. *Food and Bioproducts Processing, 121*, 29-42. https://doi.org/10.1016/j.fbp.2020.01.005

Arango Isaza, R. E., Diaz-Trujillo, C., Dhillon, B., Aerts, A., Carlier, J., Crane, C. F., ... & Kema, G. H. (2016). Combating a global threat to a clonal crop: banana black Sigatoka pathogen Pseudocercospora fijiensis (synonym Mycosphaerella fijiensis) genomes reveal clues for disease control. PLoS Genetics, 12(8), e1005876.

Chang, T. C., Salvucci, A., Crous, P. W., & Stergiopoulos, I. (2016). Comparative genomics of the Sigatoka disease complex on banana suggests a link between parallel evolutionary changes in Pseudocercospora fijiensis and Pseudocercospora eumusae and increased virulence on the banana host. PLoS Genetics, 12(8), e1005904.

Collinge, D. B., Jensen, D. F., Rabiey, M., Sarrocco, S., Shaw, M. W., & Shaw, R. H. (2022). Biological control of plant diseases–What has been achieved and what is the direction. *Plant Pathology, 71*(5), 1024-1047. https://doi.org/10.1111/ppa.13555

Contreras-Cornejo, H. A., Macías-Rodríguez, L., del-Val, E., & Larsen, J. (2020). Interactions of *Trichoderma* with plants, insects, and plant pathogen microorganisms: chemical and molecular bases. In J. M. Mérillon & K. G. Ramawat (Eds.), *Co-evolution of secondary metabolites* (pp. 263-290). Springer. https://doi.org/10.1007/978-3-319-96397-6_23

Craenen, K., 1998. Technical Manual on Black Sigatoka Disease of Banana and Plantain. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, p. 60.

Cuellar-Gaviria, T. Z., González-Jaramillo, L. M., & Villegas-Escobar, V. (2021). Role of *Bacillus tequilensis* EA-CB0015 cells and lipopeptides in the biological control of black Sigatoka disease. *Biological Control, 155*, 104523. https://doi.org/10.1016/j.biocontrol.2021.104523

Da Silva Magalhães, D., de Jesus Matos Viegas, I., da Silva Barata, H., Costa, M. G., da Silva, B. C., & de Lima Mera, W. Y. W. (2023). Deficiencies of nitrogen, calcium, and micronutrients are the most limiting factors for growth and yield of smell pepper plants. *Revista Ceres, 70*(3), 125–135. https://doi.org/10.1590/0034-737x202370030013

Dadrasnia, A., Usman, M. M., Omar, R., Ismail, S., & Abdullah, R. (2020). Potential use of *Bacillus* genus to control of bananas diseases: Approaches toward high yield production and sustainable management. *Journal of King Saud University-Science, 32*(4), 2336–2342. https://doi.org/10.1016/j.jksus.2020.03.011

Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., & Fira, D. (2022). Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms—A review. *Physiological and Molecular Plant Pathology, 117*, 101754. https://doi.org/10.1016/j.pmpp.2022.101754

Dutta, P., Mahanta, M., Singh, S. B., Thakuria, D., Deb, L., Kumari, A., ... & Pandey, A. K. (2023). Molecular interaction between plants and *Trichoderma* species against soil-borne plant pathogens. *Frontiers in Plant Science, 14*, 1145715. https://doi.org/10.3389/fpls.2023.1145715

Elnahal, A. S. M., El-Saadony, M. T., Saad, A. M., Desoky, E. S. M., El-Tahan, A. M., Rady, M. M., & El-Tarabily, K. A. (2022). The use of microbial inoculants for biological control, plant growth promotion, and sustainable agriculture: A review. *European Journal of Plant Pathology, 162*(4), 759–792. https://doi.org/10.1007/s10658-021-02393-7

Elsharkawy, M. M., Elsawy, M. M., & Ismail, I. A. (2022). Mechanism of resistance to Cucumber mosaic virus elicited by inoculation with *Bacillus subtilis* subsp. *subtilis*. *Pest Management Science, 78*(1), 86–94. https://doi.org/10.1002/ps.6580

Esguera, J. G., Balendres, M. A., & Paguntalan, D. P. (2024). Overview of the Sigatoka leaf spot complex in banana and its current management. *Tropical Plants, 3*(1), 1–15. https://doi.org/10.3390/tropicalplants3010001

Jinal, N. H., & Amaresan, N. (2020). Evaluation of biocontrol *Bacillus* species on plant growth promotion and systemic-induced resistant potential against bacterial and fungal wilt-causing pathogens. *Archives of Microbiology, 202*(7), 1785–1794. https://doi.org/10.1007/s00203-020-01891-2

Johanson, A., & Jeger, M. J. (1993). Use of PCR for detection of Mycosphaerella fijiensis and M. musicola, the causal agents of Sigatoka leaf spots in banana and plantain. Mycological research, 97(6), 670-674. https://doi.org/10.1016/S0953-7562(09)80145-7

Konappa, N., Krishnamurthy, S., Dhamodaran, N., Arakere, U. C., Chowdappa, S., & Ramachandrappa, N. S. (2020). Opportunistic avirulent plant symbionts *Trichoderma*: Exploring its potential against soilborne phytopathogens. In C. Manoharachary, H. B. Singh, & A. Varma (Eds.), *Trichoderma: Agricultural Applications and Beyond* (pp. 219–255). Springer. https://doi.org/10.1007/978-3-030-54758-5_11

Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., ... & Barka, E. A. (2022). Biological control of plant pathogens: A global perspective. *Microorganisms, 10*(3), 596. https://doi.org/10.3390/microorganisms10030596

Mahmood, T., Fatima, R., & Maalik, S. (2022). Lipopeptides: Powerful antifungal weapons produced by *Bacillus* species. *Plant Bulletin, 1*(1), 51–64. https://doi.org/10.3390/plants11070948

Noar, R. D., Thomas, E., & Daub, M. E. (2022). Genetic characteristics and metabolic interactions between *Pseudocercospora fijiensis* and banana: Progress toward controlling black Sigatoka. *Plants, 11*(7), 948. https://doi.org/10.3390/plants11070948

Olivares, B. O., Rey, J. C., Lobo, D., Navas-Cortés, J. A., Gómez, J. A., & Landa, B. B. (2021). Fusarium wilt of bananas: A review of agro-environmental factors in the Venezuelan production system affecting its development. *Agronomy, 11*(5), 986. https://doi.org/10.3390/agronomy11050986

Orjeda, G., 1998. Evaluation of Musa Germplasm for Resistance to Sigatoka Diseases and Fusarium Wilt. INIBAP Technical Guidelines 3. International Plant Genetic Resources Institute, Rome, Italy. International Network for the Improvement of Banana and Plantain, Montpellier, France; ACP-EU Technical Centre for Agricultural and Rural Cooperation, Wageningen, The Netherlands.

Palmieri, D., Ianiri, G., Del Grosso, C., Barone, G., De Curtis, F., Castoria, R., & Lima, G. (2022). Advances and perspectives in the use of biocontrol agents against fungal plant diseases. *Horticulturae, 8*(7), 577. https://doi.org/10.3390/horticulturae8070577

Pinheiro, T. D. M., Rego, E. C. S., Alves, G. S. C., Fonseca, F. C. D. A., Cotta, M. G., Antonino, J. D., ... & Miller, R. N. G. (2022). Transcriptome profiling of the resistance response of *Musa acuminata* subsp. *burmannicoides*, var. Calcutta 4 to *Pseudocercospora musae*. *International Journal of Molecular Sciences, 23*(21), 13589. https://doi.org/10.3390/ijms232113589

Poveda, J., Eugui, D., & Abril-Urias, P. (2020). Could *Trichoderma* be a plant pathogen? Successful root colonization. In M. Zeilinger, I. Druzhinina, & G. Singh (Eds.),

Trichoderma: Host Pathogen Interactions and Applications (pp. 35–59). Springer. https://doi.org/10.1007/978-3-030-54761-5_3

Rabari, A., Ruparelia, J., Jha, C. K., Sayyed, R. Z., Mitra, D., Priyadarshini, A., ... & Mohapatra, P. K. D. (2023). Articulating beneficial rhizobacteria-mediated plant defenses through induced systemic resistance: A review. *Pedosphere, 33*(4), 556–566. https://doi.org/10.1016/S1002-0160(23)60038-0

Sau, S., Bhattacharjee, P., Kundu, P., & Mandal, D. (2023). Banana. In D. Mandal, U. Wermund, L. Phavaphutanon, & R. Cronje (Eds.), *Tropical and Subtropical Fruit Crops* (pp. 1–62). Apple Academic Press. https://doi.org/10.1201/9781003305033-1

Souleymane, S., Gnenakan, Y., Brahima, C., Leonard, O. S., & Daouda, K. (2022). Alternative strategy to the chemical control of *Mycosphaerella fijiensis* Morelet, causative agent of banana trees black Sigatoka by the use of biopesticides. *American Journal of BioScience, 10*(3), 106–117. https://doi.org/10.11648/j.ajbio.20221003.14

Strobl, E., & Mohan, P. (2020). Climate and the global spread and impact of bananas' black leaf Sigatoka disease. Atmosphere, 11(9), 947.

Torres-Rodriguez, J. A., Reyes Pérez, J. J., Ramos, L. T. L., Gonzalo-Matute, L., Rueda-Puente, E. O., & Hernandez-Montiel, L. G. (2025). Chitosan as a Postharvest Alternative for the Control of Phytophthora capsici in Bell Pepper Fruits. Sci, 7(2), 37.

Torres-Rodriguez, J. A., Reyes-Pérez, J. J., Carranza-Patino, M. S., Gaibor-Fernández, R. R., Rivas-García, T., & Rueda-Puente, E. O. (2024). Chitosan: Biocontrol agent of *Fusarium oxysporum* in tomato fruit (*Solanum lycopersicum* L.). Emirates Journal of Food and Agriculture, 36, 1-9.

Torres-Rodriguez, J. A., Reyes-Perez, J. J., Castellanos, T., Angulo, C., Quinones-Aguilar, E. E., & Hernandez-Montiel, L. G. (2021). A biopolymer with antimicrobial properties and plant resistance inducer against phytopathogens: Chitosan. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49(1), 12231-12231.

Tyśkiewicz, R., Nowak, A., Ozimek, E., & Jaroszuk-Ściseł, J. (2022). *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *International Journal of Molecular Sciences, 23*(4), 2329. https://doi.org/10.3390/ijms23042329

Zhu, M. L., Wu, X. Q., Wang, Y. H., & Dai, Y. (2020). Role of biofilm formation by *Bacillus pumilus* HR10 in biocontrol against pine seedling damping-off disease caused by *Rhizoctonia solani*. *Forests, 11*(6), 652. https://doi.org/10.3390/f11060652