



Integrated use of bacteria and microbial metabolites for management of soil-borne root rot in melon

Tatianne Alves · Ana Paula de Moura · Jarlan Silva · Vitória Souza ·
Silvan Silva Filho · Juliano Fernandes · Diogo Araújo · Luiz Evangelista ·
Vanuza Ávila Filha · Washington da Silva · Márcia Ambrósio

Received: 23 September 2025 / Accepted: 4 March 2026 / Published online: 8 April 2026
© The Author(s) 2026

Abstract Soil-borne pathogens represent a critical constraint to melon (*Cucumis melo* L.) production, calling for the development of sustainable biocontrol strategies. This study evaluated the efficacy of formulations integrating bacterial consortia, *Trichoderma* spp., and microbial metabolites for disease management. A pilot greenhouse study was conducted, and the treatments that provided the lowest disease severity were tested in the field. The field trial followed a randomized complete block design with six treatments and seven replicates: T1 (*Bacillus* consortia + Metabolites 1), T2 (Bacterial consortia + Metabolites 2), T3 (Metabolites 1 + Metabolites 2), T4 (Bacterial consortia + Metabolites 1 + Metabolites 2), T5 (standard farm management), and T6

(untreated control). We assessed plant development (stem diameter and shoot dry mass), disease severity, yield components (fruit number and weight), fruit quality (°Brix), and soil health indicators (enzyme activity). Across two experimental cycles, treatments T1 and T4 consistently reduced disease severity by 50% relative to the control. Specifically, T4 significantly enhanced soil health, evidenced by increased activity of β -glucosidase, arylsulfatase, and acid phosphatase (≈ 47 , 23, and 120 μg p-nitrophenol g^{-1} soil h^{-1} , respectively), while achieving a yield of 3.5 fruits per plant. These results demonstrate that the integrated application of microbial consortia and their metabolites provides an effective and viable alternative to conventional chemical management of soil-borne pathogens in melon.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10658-026-03209-2>.

T. Alves · A. P. de Moura · J. Silva · V. Souza ·
S. Silva Filho · J. Fernandes · D. Araújo · L. Evangelista ·
V. Ávila Filha · W. da Silva (✉) · M. Ambrósio (✉)
Departamento de Ciências Agronômicas e Florestais,
Universidade Federal Rural do Semi-Árido (UFERSA),
Mossoró, RN, Brazil
e-mail: washington.daSilva@ct.gov

M. Ambrósio
e-mail: marciamichelle@ufersa.edu.br

J. Silva · W. da Silva
Department of Plant Pathology and Ecology, The
Connecticut Agricultural Experiment Station (CAES),
New Haven, CT 06511, USA

Keywords Soil-borne pathogens · Biological control · Plant disease · *Cucurbitaceae*

Introduction

The sustainability of melon (*Cucumis melo* L.) cultivation is increasingly threatened by a complex of soil-borne diseases that cause extensive root rot and premature vine decline (Nascimento et al., 2018; Almeida et al., 2025; Nogueira et al., 2023). This challenge is particularly acute in Brazil, one of the world's primary melon exporters. In 2023, 862,000 tons of melon were produced in the country, of which

167,000 tons were exported (HF Brasil, 2024). The Brazilian Northeastern region accounts for 90% of the national production, and the state of Rio Grande do Norte holds more than 70% of this total (IBGE, 2023; HF Brasil, 2024). In this area, the unique edaphoclimatic conditions—marked by high thermal amplitude, low humidity, and intense solar radiation—while ideal for a superior fruit flavor, also facilitate the persistence of specialized pathogens (Rocha, 2009; Figueiredo et al., 2017).

The recurring presence of *Monosporascus cannonballus*, *Macrophomina phaseolina*, *Fusarium* spp., and *Rhizoctonia solani*, which are among the main soil-borne pathogens (SBP) that attack melon plants, in these semi-arid soils creates a significant bottleneck for growers (Dantas et al., 2013; Medeiros et al., 2015; Porto et al., 2016; Stewart et al., 2015). These pathogens produce robust survival structures that withstand the fallow periods typical of the region's climate, leading to severe outbreaks once irrigation resumes (Porto et al., 2016). To make matters worse, there are no chemical fungicides registered for melon field applications in the Ministério da Agricultura, Pecuária e Abastecimento (Brazilian Department of Agriculture) (AGROFIT, 2023). Furthermore, European countries that import Brazilian melons have strict regulations that restrict the use of agrochemicals in melons and other agricultural products, due to environmental issues and risks to human health, which makes it even more difficult to control these pathogens (Nogueira et al., 2023; Borges et al., 2023; European Commission 2022). Therefore, given the limitations and environmental concerns surrounding conventional chemical fumigants, there is an urgent need to validate biological alternatives that are effective under these specific environmental stressors.

Bioprospecting for antagonistic microorganisms, such as *Bacillus* consortia and *Trichoderma* species, has emerged as a promising path for managing these root-infecting complexes (Lahlali et al., 2022; Coêlho et al., 2018; Bedendo, 2018). Recent evidence suggests that the integration of these live agents with their own bioactive microbial metabolites can yield a synergistic effect, not only inhibiting pathogen growth but also stimulating host defenses and improving soil health indicators (Tuzun, 2001; Coêlho et al., 2018; Lorito et al., 2010; Druzhinina et al., 2011).

Despite the potential of these consortia, their performance in the field under the rigorous climate of

the Brazilian Northeast requires detailed assessment. Thus, the objective of this study was to evaluate the impact of formulations based on bacteria, *Trichoderma*, and microbial metabolites on disease severity, plant development, and soil enzyme activity in melon crops. We hypothesized that the combined application of microbial consortia and their secondary metabolites would reduce the severity of soil-borne root rot and stimulate rhizosphere health, indicated by increased soil enzymatic activity. By focusing on these integrated bioprotection strategies, we aimed to provide a scientifically grounded and viable management protocol for high-yield and sustainable melon production.

Material and methods

The experiments were carried out at Fazenda Agrícola Famosa, located in the municipality of Icapuí/CE, from October to December 2024, and the analyses were performed at the Laboratório de Microbiologia e Fitopatologia of the Departamento de Ciências Agrônomicas e Florestais of the Universidade Federal Rural do Semi-Árido, located in Mossoró, Brazil.

A pilot study was conducted with 30 treatments and four replicates arranged in a completely randomized design, composed of six alternative products and their associations. Combinations of up to four products were tested, verifying the interaction between bacteria (*Bacillus*, *Lactobacillus*, and *Enterococcus*), the fungus *Trichoderma*, and fermentation metabolites (produced by bacteria from the genus *Bacillus* and yeasts), on the severity of root system rot. This study was performed in greenhouse conditions, with soil from an area with a history of diseases caused by SBP, including *Fusarium*, *Macrophomina*, *Monosporascus*, and *Rhizoctonia*. The melon Gália, variety SV4974, was used in this pilot study. After 65 days of seedling transplantation, the root rot severity was evaluated, and the data were analysed using the Kruskal–Wallis test ($p \leq 0.05$). Based on the preliminary results from the greenhouse pilot, the five treatments demonstrating the lowest disease severity were selected for further validation under field conditions. The field trials were conducted using a randomized complete block design (RCBD) with six treatments (Table 1) and seven replicates. To ensure the robustness of the

findings, the entire field procedure was performed twice as independent experimental runs (Experiment 1 and Experiment 2).

The area with a history of root diseases in melon plants was cultivated with the Gália melon (variety SV4974). Most treatments were applied once per week beginning with the transplantation of the seedling, totaling five times, except for the treatment T5, which was applied four times, at the dosages recommended by the manufacturers, as mentioned in Table 1.

After preparing the soil, organic compost (cow manure, sorghum, and monoammonium phosphate) was added, and the planting lines were covered with white polyethylene mulch, which helps to reduce the population of weed plants in the area and maintains soil moisture. The seedlings were transplanted 10 days after sowing. Then a non-woven fabric blanket was placed over them, which helps to reduce the attack of pests, such as the leafminer (*Liriomyza huidobrensis* Blanchard). Irrigation was carried out via drip irrigation, with the nutrient solution containing macro and micronutrients needed for crop development. During the period of opening of the first flowers, when the plants were approximately 22 days after transplanting (DAT), the non-woven fabric blanket was removed for pollinators (bees) to have access to the flowers to perform pollination. The crop cycle was 60 DAT, when the following parameters were evaluated:

- o **Stem diameter:** The plant stem diameter was measured using a digital caliper, model MTX-316119.
- o **Shoot dry mass:** The base of the stem was separated from the root, and the shoot was placed in Kraft paper bags and dried in a forced air oven ($\pm 65^\circ\text{C}$) until a constant weight was observed.
- o **Disease severity (SEV):** The roots were separated from the shoot and washed in running water for evaluation using the score scale modified by Ambrósio et al. (2015), where: 0=plant without symptoms; 1=less than 3% of infected tissue; 2=3–10% of infected tissue; 3=11–25% of infected tissue; 4=26–50% of infected tissue; and 5=more than 50% of infected tissue.
- o **Number of fruits per plant:** The number of fruits on each plant of the treatments and replicates was counted.
- o **Fruit weight and °Brix:** The fruits were weighed on a digital scale, model Vonder 3885001010, and the °Brix of each fruit was measured using a digital refractometer, model MA871.
- o **Soil enzymatic activity:** Soil quality and microbial activity were assessed through the quantification of three key enzymes: β -glucosidase, aryl-sulfatase, and acid phosphatase. These activities were determined by measuring the release of p-nitrophenol (pNP) following the incubation of air-dried soil samples with their respective specific substrates. The following substrates were utilized for each enzymatic assay: β -glucosidase: p-nitrophenyl- β -D-glucopyranoside (PNG), Aryl-sulfatase: p-nitrophenyl sulfate (PNS), and Acid phosphatase: disodium p-nitrophenyl phosphate (P-NPP). All determinations were performed in triplicate directly on the soil samples. The enzy-

Table 1 Treatments applied to soil cultivated with Gália melon, aiming at managing root diseases

Treatment	Composition and dosage
T1	<i>B. subtilis</i> and <i>B. licheniformis</i> (1 kg/ha) + Metabolites 1 (natural fermentation of amino acids, microbial metabolites) (1L/ha);
T2	<i>B. subtilis</i> , <i>Lactobacillus plantarum</i> and <i>Enterococcus faecium</i> (1 kg/ha) + Metabolites 2 (natural fermentation of beneficial microorganisms) (1L/ha);
T3	Metabolites 1 (natural fermentation of amino acids enriched with macronutrients) (1L/ha) + Metabolites 2 (natural fermentation of amino acids) (1L/ha);
T4	<i>B. subtilis</i> + <i>B. licheniformis</i> + <i>L. plantarum</i> and <i>E. faecium</i> (1 kg/ha) + Metabolites 1 (natural fermentation of amino acids enriched with macronutrients) (1L/ha) + Metabolites 2 (natural fermentation of amino acids) (1L/ha);
T5	<i>T. asperellum</i> (300 mL/ha), <i>B. subtilis</i> (1L/ha), Metabolites 1 (natural fermentation of amino acids enriched with macronutrients) (1L/ha) + Metabolites 2 (natural fermentation of amino acids) (1L/ha);
T6	Control

matic activities were expressed in μg p-nitrophenol g^{-1} soil h^{-1} (Tabatabai, 1994).

- o **Statistical analyses:** The data were subjected to the Shapiro–Wilk normality test ($p < 0.05$) and Bartlett's homogeneity of variances test ($p \leq 0.05$) to fulfill the ANOVA presumptions. Due to the differences between experiments 1 and 2 in the field, the data from both experiments were analyzed separately. Subsequently, parametric variance analysis was performed, and the means were compared by the Tukey test ($p \leq 0.05$). All statistical analyses were performed on R (R Core Team, 2022) using RStudio as a graphical interface (RStudio Team, 2024).

Results

The preliminary greenhouse pilot study identified four superior treatments for suppressing root rot: T18 (*B. subtilis* + *B. licheniformis* + metabolites 1), T19 (*B. subtilis* + *L. plantarum* + *E. faecium* + metabolites 2), T21 (metabolites 1 + metabolites 2), and T28 (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 1 + metabolites 2). Compared to the control, treatments T18, T19, and T28 reduced disease severity by 62.5%, while T21 achieved a reduction of 68.75% (Supplementary Fig. 1). Microbiological analysis of the symptomatic root systems identified *Fusarium* spp. and *Macrophomina* spp. as the primary causal agents of the observed rot.

In the first field experiment, treatments T2 (*B. subtilis* + *B. licheniformis* + metabolites 1) and T3 (metabolites 1 + metabolites 2) significantly enhanced plant vigor, yielding the largest stem diameters at 10.6 mm and 9.7 mm, respectively. This represents an increase of 23.5% (T2) and 16.5% (T3) relative to the control (8.1 mm). While treatments T1, T4, and T5 performed similarly to T3, no significant statistical differences in stem diameter were observed during the second experimental run (Fig. 1A).

Regarding shoot dry mass, no statistically significant differences were found between treatments in either experiment. However, it is noteworthy that T4 (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 1 + metabolites 2) promoted an approximate 24% increase in aerial dry mass compared to the untreated control (Fig. 1B).

In Experiment 1, treatments T1, T2, T3, and T4 showed no significant statistical variance among themselves. Specifically, T2 and T3 achieved a 50% reduction in disease severity, while T1 and T4 resulted in a 20% reduction, all relative to the control (Fig. 1C).

In Experiment 2, all treatments incorporating microorganisms and their metabolites significantly outperformed the control. Treatments T1 and T4 were the most effective in this trial, both providing a 50% reduction in disease severity compared to the control group (Fig. 1C).

Statistical analysis of the yield data revealed that Treatment T4 (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 1 + metabolites 2) consistently optimized fruit production. In the first experiment, T4 significantly outperformed all other treatments, achieving a mean of 3.4 fruits per plant (Fig. 2A).

In the second experiment, T4 maintained its superior performance (3.4 fruits per plant), differing significantly from most treatments, with the exception of T5 (*T. asperellum* + *B. subtilis* + metabolites 1 + metabolites 2), which yielded 3.3 fruits per plant. Notably, while T5 was statistically comparable to T4, it did not show significant differences from T1, T2, or T3. Across both experimental runs, the untreated control consistently produced the lowest yield (2.8 fruits per plant), while T4 reached the maximum productivity (Fig. 2A).

Regarding fruit quality parameters, no statistically significant differences were observed between treatments for fruit weight (Fig. 2B) or soluble solids content ($^{\circ}\text{Brix}$; Fig. 2C) in either experiment. These results indicate that while the microbial consortia significantly enhance yield by reducing disease pressure, they maintain the standard commercial quality characteristics of the melon fruits.

The application of microbial consortia and metabolites significantly influenced the biochemical activity of the melon rhizosphere, particularly in Treatment T4 (*B. subtilis* + *B. licheniformis* + *L. plantarum* + *E. faecium* + metabolites 1 + metabolites 2). For β -glucosidase activity in Experiment 1, T4 promoted the highest enzymatic rates ($44.3 \mu\text{g}$ p-nitrophenol $\text{g}^{-1} \text{h}^{-1}$), differing significantly only from the untreated control. All other treatments remained statistically comparable to the control. A similar trend was observed in Experiment 2, where T4 again

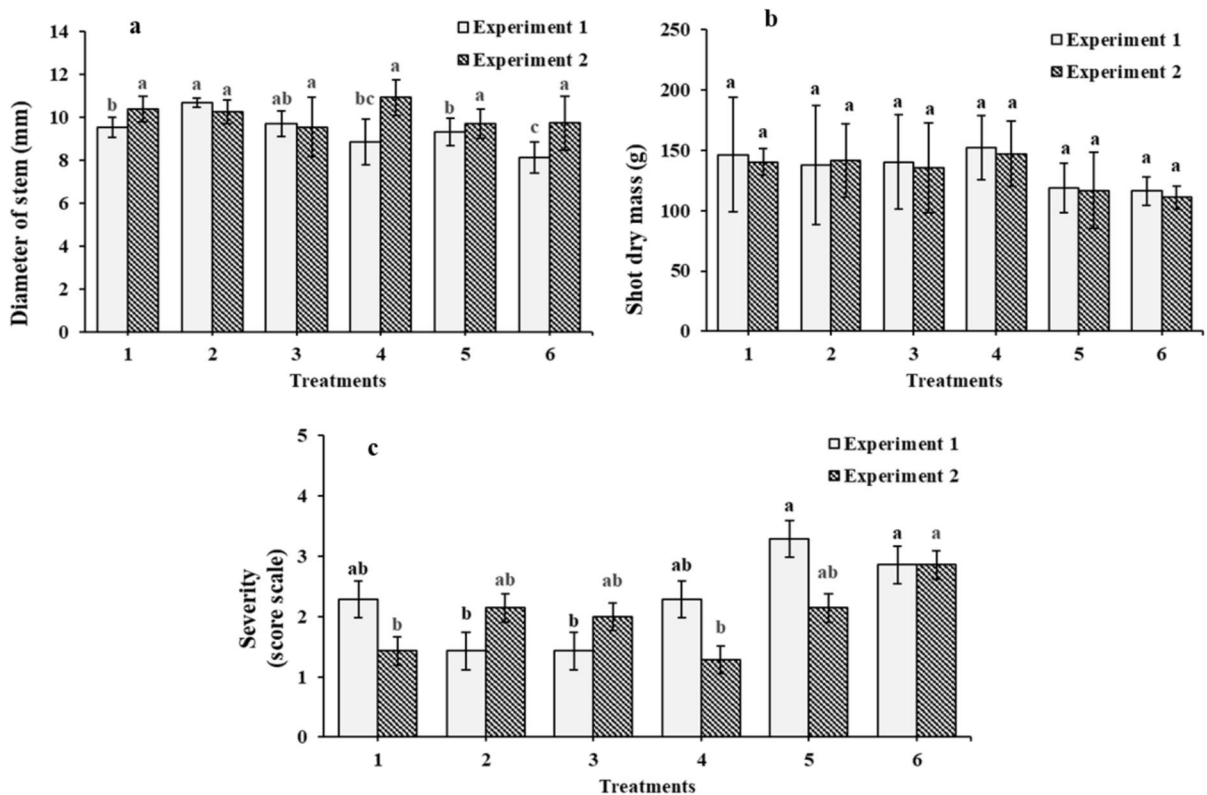


Fig. 1 Stem diameter (a), shoot dry mass (b), and root rot severity (c) in melon plants. Cultivated soils were treated with the mix formulation of alternative products, being T1=(*B. subtilis*+*B. licheniformis*+metabolites 1), T2=(*B. subtilis*+*L. plantarum*+*E. faecium*+metabolites 2),

T3=(metabolites 1+metabolites 2), T4=(*B. subtilis*+*B. licheniformis*+*L. plantarum*+*E. faecium*+metabolites 1+metabolites 2), T5=standard management (*T. asperellum*+*B. subtilis*+metabolites 1+metabolites 2), and T6=Control

demonstrated the greatest activity; however, in this instance, Treatment T3 (metabolites 1+metabolites 2) performed significantly lower than T4, while T4 remained statistically similar to the remaining treatments (Fig. 3A).

Regarding Arylsulfatase activity, T4 exhibited the superior performance across both experiments, differing significantly from all other treatments and the control (Fig. 3B). This suggests a robust impact of the combined bacterial-metabolite consortium on sulfur cycling within the soil.

Finally, the results for acid phosphatase showed no significant statistical variance between treatments in the first experiment. Conversely, in Experiment 2, T4 significantly outperformed the control, reaching a peak activity of $141.2 \mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$. While T4 was superior to the control, its activity level was

statistically consistent with the other evaluated treatments (Fig. 3C).

Discussion

The application of biological products has become a standard practice in Brazilian melon production, often involving on-farm multiplication (on-farm production) of microbial inoculants. These bioproducts are primarily deployed to manage soil-borne pathogens (SBPs), though they are also recognized for their secondary benefits in promoting plant growth and enhancing nutrient bioavailability through microbial activity. Despite their widespread use, the specific mechanisms by which these complex microbial communities interact with the SBPs complex in melon—and their subsequent impact on host

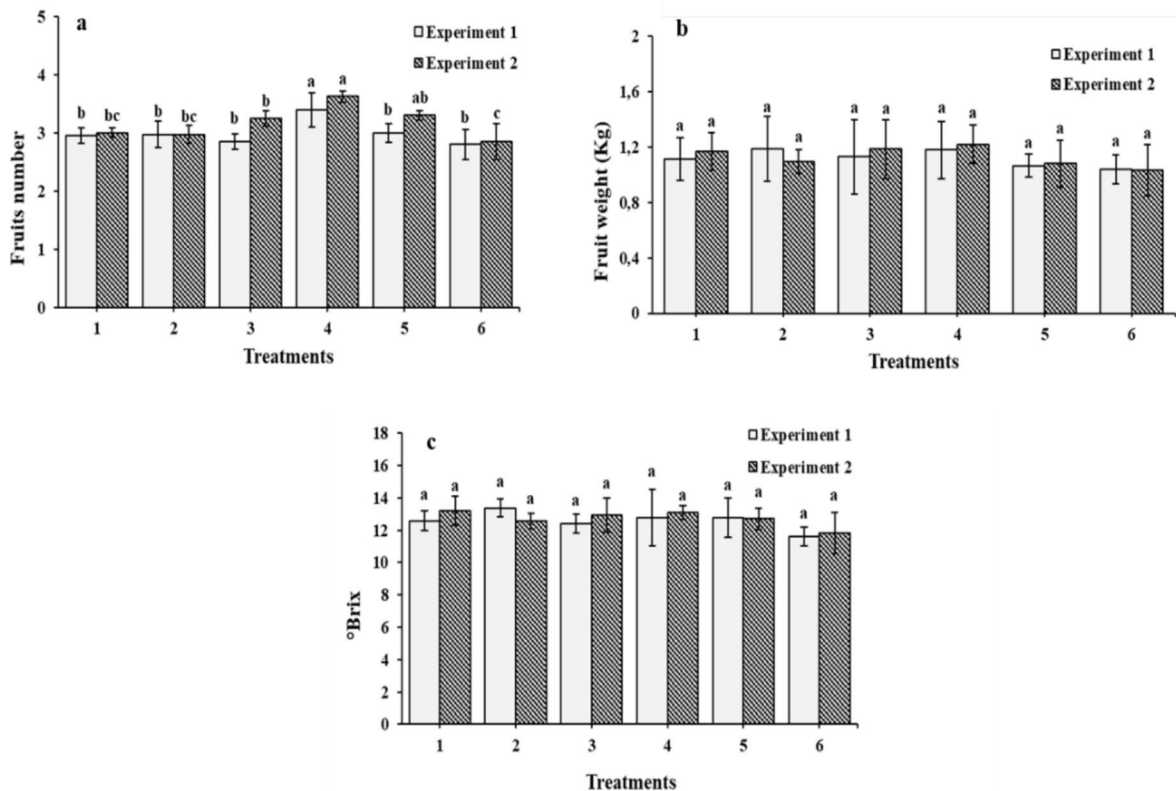


Fig. 2 Weight (a), number of fruits per plant (b), and °Brix (c) of melon fruits. Cultivated soils were treated with the mixed formulation of alternative products. T1=(*B. subtilis*+*B. licheniformis*+metabolites 1), T2=(*B. subtilis*+*L. plantarum*+*E. faecium*+metabolites 2), T3=(metabolites

1+metabolites 2), T4=(*B. subtilis*+*B. licheniformis*+*L. plantarum* and *E. faecium*+metabolites 1+metabolites 2), T5=standard management (*T. asperellum*+*B. subtilis*+metabolites 1+metabolites 2), and T6=Control

development—remain poorly characterized. This study investigated the efficacy of integrated formulations comprising diverse bacterial consortia, *Trichoderma* spp., and their respective metabolites. Our objective was to clarify whether this synergistic combination can effectively mitigate root rot in intensive monoculture systems, where high pathogen pressure and soil degradation often limit the performance of conventional biological control agents.

In both field experiments, no single treatment demonstrated a statistically superior reduction in disease severity. This outcome likely reflects the inherent constraints of the melon crop's short phenological cycle (approximately 60 days). Unlike synthetic fungicides, which typically provide immediate localized protection, biological control agents (BCAs) operate through complex temporal dynamics. The efficacy of

BCAs depends on their ability to successfully establish, compete for niches, colonize the rhizosphere, and engage in multi-trophic interactions that trigger systemic plant defense mechanisms (Compant et al., 2019). Consequently, the evaluation period of a single melon cycle may be insufficient to witness the full transformative potential of microbial treatments on soil suppressiveness. This observation aligns with the findings of Nascimento et al. (2016), who underscored that a single cultivation cycle is often inadequate for detecting significant shifts in the soil microbial community structure or achieving substantial reductions in disease pressure. While the initial reduction in root rot severity (SEV) may appear modest in this study, we hypothesize that the cumulative effect of these beneficial microorganisms will lead to a gradual, year-over-year decline in pathogen

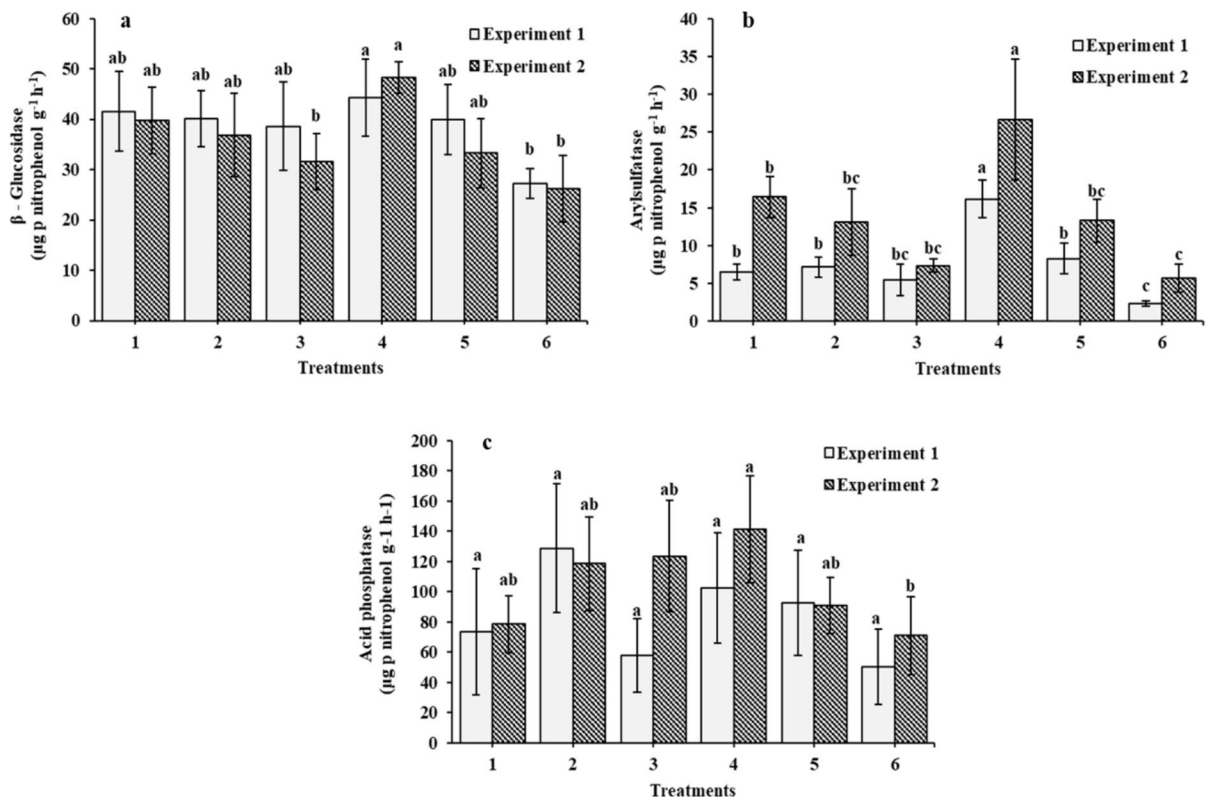


Fig. 3 Activity of the enzyme β -glucosidase (a), Arylsulfatase (b), Acid phosphatase (c) of soils. Cultivated soils were treated with mix formulation of alternative products. T1=(*B. subtilis*+*B. licheniformis*+metabolites 1), T2=(*B. subtilis*+*L. plantarum*+*E. faecium*+metabolites 2), T3=(metabolites

1+metabolites 2), T4=(*B. subtilis*+*B. licheniformis*+*L. plantarum* and *E. faecium*+metabolites 1+metabolites 2), T5=standard management (*T. asperellum*+*B. subtilis*+metabolites 1 and metabolites 2), and T6=Control

inoculum as the consortia stabilize within the soil matrix. Long-term studies designed to monitor these microbial communities over successive years are essential to validate this hypothesis and determine the threshold for stable soil suppressiveness.

Similar challenges have been documented in other systems. For instance, Volynchikova (2022) observed that despite the high *in vitro* antagonistic performance of *Pseudomonas* spp. against *Phytophthora infestans*, their *in vivo* efficacy remained limited by environmental variables. Furthermore, Barbieri (2023) reported that the application of *Trichoderma harzianum*, *T. asperellum*, and *Bacillus amyloliquefaciens* in soybean crops did not yield significant improvements in plant performance under natural conditions. The authors suggested that the time required for microbial establishment may exceed the developmental window

of the host crop, a phenomenon that likely explains the results observed in our melon trials.

Despite the temporal constraints of the melon cycle, a consistent trend emerged across both experiments: treatments integrating bacteria and their metabolites significantly outperformed the untreated control in reducing disease severity. The most effective formulations relied on a complex "mix" of *Bacillus* and lactic acid bacteria (LAB), such as *Lactobacillus*.

The efficacy of *Bacillus* species in the rhizosphere can be largely attributed to their multifaceted antagonistic repertoire. These bacteria produce a diverse array of hydrolytic enzymes (including chitinases, glucanases, cellulases, lipases, and proteases), which actively degrade the structural components of fungal and oomycete cell walls (Miljaković et al., 2020). Beyond enzymatic degradation, *Bacillus* produces

potent secondary metabolites (e.g., lipopeptides) that act as biosurfactants. These biological properties have established *Bacillus*-based products as robust alternatives to synthetic fungicides for sustainable plant protection (Fira et al., 2018; Wang et al., 2018).

Furthermore, our results highlight the potential of non-traditional biocontrol genera, such as *Lactobacillus* and *Enterococcus*, in suppressing soil-borne pathogens (Jaini et al., 2022). Specifically, metabolites derived from *L. plantarum* have demonstrated significant antifungal activity against *Fusarium* species (Zhao et al., 2017). The antimicrobial efficacy of these LAB metabolites is often linked to their surfactant or "detergent-like" properties, which disrupt the integrity of the pathogen's cytoplasmic membranes, leading to cellular leakage and death (Sjogren et al., 2003). The integration of these diverse microbial groups and their metabolic byproducts likely provided a broader spectrum of action, targeting multiple pathogens within the root rot complex simultaneously.

While *Trichoderma*-based products are extensively utilized for managing soil-borne pathogens (SBPs) in Northeastern Brazil, their field performance is contingent upon a complex interplay of soil properties, native microbial competition, pathogen pressure, and environmental variables. High temperatures and specific edaphoclimatic conditions of the semi-arid region can significantly hinder the establishment and saprophytic activity of introduced *Trichoderma* isolates. In this study, such environmental stressors may have limited the biocontrol expression of *Trichoderma*.

The superior performance of bacterial-metabolite consortia (T4) compared to *Trichoderma* treatments is likely due to the faster growth kinetics and rapid rhizosphere colonization characteristic of *Bacillus* and lactic acid bacteria. Given the short phenological cycle of melon (approx. 60 days), the crop demands biological interventions that provide rapid responses. Agile microbial consortia are therefore more suitable for producers facing high SBP pressure, as supported by Mohamed et al. (2020), who demonstrated that *Enterobacter cloacae* was more efficient than *Trichoderma* in controlling potato wilt due to its rapid establishment.

The significant increase in fruit yield observed in T4 (*Bacillus* spp., *L. plantarum*, and *E. faecium*) aligns with previous research on the growth-promoting effects of fermented microbial products

(Sretenović et al., 2024). *Bacillus* species are well-known for synthesizing potent antifungal lipopeptides, such as iturins, bacillomycins, and surfactins (Ongena & Jacques, 2008), which in this study may have contributed to lower disease severity (SEV). Pathogen-induced obstruction of vascular tissues typically limits the transport of water and nutrients, diverting metabolic resources from fruit production to defense responses (Agrios, 2005; Huot et al., 2014). By suppressing these pathogens and enhancing nutrient bioavailability, biocontrol bacteria preserve the plant's physiological integrity, thereby increasing fruit number and ensuring a healthier environment for plant growth.

Furthermore, the potential lack of adaptation of commercial *Trichoderma* strains (often isolated from regions with temperate climates) to the harsh semi-arid conditions of Brazil may explain their delayed performance. Enhancing the efficacy of fungal biocontrol in this region may require the bioprospecting of native *Trichoderma* species already adapted to local temperature, humidity, and pH levels, as suggested by Imran et al. (2022) for the management of tomato late blight.

In the present study, indicators of soil biological activity—specifically β -glucosidase, arylsulfatase, and acid phosphatase—exhibited significantly higher levels in Treatment T4. This enhancement can be directly linked to the robust presence of *Bacillus* species, which utilize these enzymes as core components of their metabolic pathways for substrate degradation and nutrient acquisition (Moreira & Siqueira, 2006). These enzymes are pivotal in agroecosystems, governing the decomposition of organic matter and the mineralization of essential nutrients, such as carbohydrates, sulfates, and phosphates, which are vital for maintaining soil fertility (Burns et al., 2013).

The integration of diverse *Bacillus* strains and their metabolites in T4 appears to have fostered a synergistic environment, accelerating nutrient dynamics in the melon rhizosphere. This observation is consistent with Caballero et al. (2022), who demonstrated that the application of a fermented bioproduct based on *B. licheniformis* significantly stimulated the activities of dehydrogenase, phosphatase, and β -glucosidase in agricultural soils. Similarly, Ng et al. (2022) reported a substantial increase in enzymatic activity following the inoculation of *B. subtilis* and *Pseudomonas fluorescens* compared to untreated controls.

Our results suggest that the "mix" of microorganisms and metabolites in T4 does not merely suppress pathogens but actively reconstructs the soil's biogeochemical potential. By increasing the availability of phosphorus (via acid phosphatase) and sulfur (via arylsulfatase), the consortium potentially mitigates the physiological stress caused by root rot, providing plants with the nutritional resources necessary to sustain higher fruit yields even under pathogen pressure.

Conclusion

While the integrated application of bacterial consortia, microbial metabolites, and *Trichoderma* spp. did not identify a single definitive strategy for the immediate eradication of melon root rot; Treatment T4 (combining diverse bacteria and metabolites) emerged as a highly promising biocontrol candidate. The superior soil enzymatic activity and significantly higher fruit yield associated with this treatment suggest that a multi-component bioprotection approach is more effective than traditional single-agent applications in the intensive melon monoculture systems of Northeastern Brazil. These findings indicate that the synergy between *Bacillus*, lactic acid bacteria, and their metabolic byproducts represents a viable alternative for sustainable disease management, though further longitudinal studies are required to optimize application timing and verify cumulative efficacy over multiple crop cycles. Ultimately, this study advances the understanding of the potentials and constraints of bioproducts in semi-arid agriculture, providing a scientific foundation for future research aimed at consolidating resilient and sustainable management protocols for melon producers.

Acknowledgements The authors thank Fazenda Agrícola Famosa for making the farm available for the experiment. The authors thank Alltech Crop Science for the donation of equipment for the analysis.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). This work was supported through funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Grant/Award Numbers 307860/2022–1 and 140602/2021–6, and from Programa Institucional de Apoio ao Pesquisador (PIAP), Award: 12/2023, Number of process: 23091.005800/2023–42.

This research was partially funded by the USDA Specialty Crop Block Grant Program (SCBGP) – Grant Award #23DAG0087AA and the USDA-NIFA Hatch Grant – Project #1017940.

Data availability The analyzed data that support the conclusions of this study are available in the article.

Declarations

Ethics approval The article does not contain studies with human participants or animals performed by any of the authors, and complies with the Ethical Rules applicable to the 'Journal of Plant Pathology'.

Consent to participate All authors read and approved the manuscript.

Informed consent All authors consented to the publication of this study.

Conflicts of interest The authors declare no conflicts of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Agrios, G. N. (2005). *Plant Pathology* (5th ed). Elsevier Academic Press.
- AGROFIT. (2023). Sistema de Agrotóxicos Fitossanitários Ministério da Agricultura, Pecuária e Abastecimento-. Disponível em: https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons Acessado em: 25 abril de 2024.
- Almeida, B. D. H., Costa, M. H., Evangelista, L. F., Frutuoso, R. M., Dias, A. M., Silva, J. L., ...& Ambrósio, M. M. (2025). Sustainable management of fusarium rots in melons: A dual strategy combining thermotherapy and 1-MCP. *Crop Protection*, 107522. <https://doi.org/10.1016/j.cropro.2025.107522>
- Ambrósio, M. M., Dantas, A. C., Martínez-Perez, E., Medeiros, A. C., Nunes, G. H., & Picó, M. B. (2015). Screening a variable germplasm collection of *Cucumis melo*

- L. for seedling resistance to *Macrophomina phaseolina*. *Euphytica*, 206, 287–300. <https://doi.org/10.1007/s10681-015-1452-x>
- Barbieri, C. R. (2023). *Biológicos no controle de doenças, indução de resistência e promoção de crescimento na cultura da soja*. Universidade Tecnológica Federal do Paraná, Pato Branco.
- Beauregard, P. B., Chai, Y., Vlamakis, H., Losick, R., & Kolter, R. (2013). *Bacillus subtilis* biofilm induction by plant polysaccharides. *Proceedings of the National Academy of Sciences*, 110(17), E1621–E1630. <https://doi.org/10.1073/pnas.1218984110>
- Bedendo, I. P. (2018). Podridões de raiz e colo. In: Amorim, L., Rezende, J. A. M., Bergamin Filho, A. (eds) *Manual de Fitopatologia*, 5ª ed. Agronômica Ceres, p 573.
- Borges, D. F., Nogueira, G. D. A., Cruz, G. D. A., Silva, S. G. D. A., da Silva, W. L., & Ambrósio, M. M. D. Q. (2023). Changes on soil microbiota induced by the use of commercial products and the incorporation of plant materials. *Revista Caatinga*, 36, 300–309. <https://doi.org/10.1590/1983-21252024v37i12099rc>
- Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., Weintraub, M. N., & Zoppini, A. (2013). Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology & Biochemistry*, 58, 216–234. <https://doi.org/10.1016/j.soilbio.2012.11.009>
- Caballero, P., Macías-Benítez, S., Moya, A., Rodríguez-Morgado, B., Martín, L., Tejada, M., Castaño, A., & Parrado Rubio, J. (2022). Biochemical and microbiological soil effects of a biostimulant based on *Bacillus licheniformis*-fermented sludge. *Agronomy*, 12(8), 1743. <https://doi.org/10.3390/agronomy12081743>
- Coelho, I. L., Conceição, C. S., Santos, T. S., Oliveira, M. G., Moraes, A. J. G., Cruz, B. L. S., Laranjeira, D. (2018). Biocontrole de doenças radiculares: uma realidade prática ou apenas utopia? In: Lopes UP, Michereff SJ (eds) *Desafios do Manejo de Doenças Radiculares Causadas por Fungos*. EdUFRPE, Recife, pp 154–160.
- Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*, 19, 29–37. <https://doi.org/10.1016/j.jare.2019.03.004>
- Dantas, A. M. M., Ambrósio, M. M. Q., Nascimento, S. R. C., Senhor, R. F., Cézar, M. A., & Lima, J. S. S. (2013). Incorporation of plant materials in the control of root pathogens in muskmelon. *Revista Agro@mbiente on-Line*, 7(3), 338–344. <https://doi.org/10.18227/1982-8470ragro.v7i3.1257>
- Druzhinina, I. S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B. A., Kenerley, C. M., Monte, E., & Kubicek, C. P. (2011). *Trichoderma*: The genomics of opportunistic success. *Nature Reviews Microbiology*, 9(10), 749–759. <https://doi.org/10.1038/nrmicro2637>
- European Commission. (2022). “Approval of active substances.” Food Safety | European Commission. https://ec.europa.eu/food/plant/pesticides/approval_active_substances_en.
- Figueirêdo, M. C. B., Gondim, R. S., & Aragão, F. A. S. (2017). *Produção de melão e mudanças climáticas: Sistemas conservacionistas de cultivo para redução das pegadas de carbono e hídrica*. Embrapa.
- Fira, D., Dimkić, I., Berić, T., Lozo, J., & Stanković, S. (2018). Biological control of plant pathogens by *Bacillus* species. *Journal of Biotechnology*, 285, 44–55. <https://doi.org/10.1016/j.jbiotec.2018.07.044>
- Gabardo, G., Pria, M. D., Prestes, A. M. C., & da Silva, H. L. (2020). *Trichoderma asperellum* e *Bacillus subtilis* como antagonistas no crescimento de fungos fitopatogênicos *in vitro*. *Brazilian Journal of Development*, 6(8), 55870–55885. <https://doi.org/10.34117/bjdv6n8-123>
- Hortifruti Brasil. (2024). Anuário 2024–2025. <https://www.hfbrasil.org.br/revista/edicao-de-dezembro-anuario-2024-2025.aspx>. Acessado em 28 de abril de 2025.
- Huot, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth–defense tradeoffs in plants: A balancing act to optimize fitness. *Molecular Plant*, 7(8), 1267–1287. <https://doi.org/10.1093/mp/ssu049>
- IBGE – Instituto Brasileiro de Geografia e Estatística. (2023). *Produção de melão no Brasil*. IBGE. <https://www.ibge.gov.br/explica/producao-agropecuaria/melao/br>. Acessado em 25 de abril de 2025.
- Imran, M., Abo-Elyousr, K. A., Mousa, M. A., & Saad, M. M. (2022). Screening and biocontrol evaluation of indigenous native *Trichoderma* spp. against early blight disease and their field assessment to alleviate natural infection. *Egyptian Journal of Biological Pest Control*, 32(1), 40. <https://doi.org/10.1186/s41938-022-00544-4>
- Jaini, M. F. M., Roslan, N. F., Yusof, M. A. T., Saidi, N. B., Ramli, N., Zainudin, N. A. I. M., & Hashim, A. M. (2022). Investigating the potential of endophytic lactic acid bacteria isolated from papaya seeds as plant growth promoter and antifungal agent. *Pertanika Journal of Tropical Agricultural Science*, 45(1), 207–233. <https://doi.org/10.47836/pjtas.45.1.12>
- 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>
- Lima, F. F., Nunes, L. A., Figueiredo, MdoVB., Araújo, FFde, Lima, L. M., & Araújo, ASde. (2011). *Bacillus subtilis* e adubação nitrogenada na produtividade do milho. *Revista Brasileira De Ciências Agrárias*, 6(4), 657–661. <https://doi.org/10.5039/agraria.v6i4a1429>
- Lorito, M., Woo, S. L., Harman, G. E., & Monte, E. (2010). Translational research on *Trichoderma*: From ‘omics to the field. *Annual Review of Phytopathology*, 48(1), 395–417. <https://doi.org/10.1146/annurev-phyto-073009-114314>
- Medeiros, A. C., Melo, D. R. M., Ambrósio, M. M. Q., Nunes, G. H. D. S., & Costa, J. M. D. (2015). Métodos de inoculação de *Rhizoctonia solani* e *Macrophomina phaseolina* em meloeiro (*Cucumis melo*). *Summa Phytopathologica*, 41(4), 281–286. <https://doi.org/10.1590/0100-5405/2083>
- Miljaković, D., Marinković, J., & Balešević-Tubić, S. (2020). The significance of *Bacillus* spp. in disease suppression

- and growth promotion of field and vegetable crops. *Microorganisms*, 8(7), 1037. <https://doi.org/10.3390/microorganisms8071037>
- Mohamed, B. F., Sallam, N. M., Alamri, S. A., Abo-Elyousr, K. A., Mostafa, Y. S., & Hashem, M. (2020). Approving the biocontrol method of potato wilt caused by *Ralstonia solanacearum* (Smith) using *Enterobacter cloacae* PS14 and *Trichoderma asperellum* T34. *Egyptian Journal of Biological Pest Control*, 30(1), 61. <https://doi.org/10.1186/s41938-020-00262-9>
- Moreira, F. M. S., & Siqueira, J. O. (2006). *Microbiologia e bioquímica do solo* (2ª ed). Editora UFLA.
- Nascimento, P. G. M. L. D., Cruz, B. L. S. D., Dantas, A. M. D. M., Freitas, F. C. L. D., Ambrósio, M. M. D. Q., & Sales, R. (2016). Comunidades microbianas em solo cultivado com melão sob diferentes sistemas de manejo. *Revista Brasileira De Ciência Do Solo*, 40(0), e0160130. <https://doi.org/10.1590/18069657rbcS20160130>
- Nascimento, P. G., Ambrósio, M. M., Freitas, F. C., Cruz, B. L., Dantas, A. M., Júnior, R. S., & da Silva, W. L. (2018). Incidence of root rot of muskmelon in different soil management practices. *European Journal of Plant Pathology*, 152(2), 433–446.
- Ng, C. W. W., Yan, W. H., Tsim, K. W. K., San So, P., Xia, Y. T., & To, C. T. (2022). Effects of *Bacillus subtilis* and *Pseudomonas fluorescens* as the soil amendment. *Heliyon*, 8(11), e11674. <https://doi.org/10.1016/j.heliyon.2022.e11674>
- Nogueira, G. A., Costa Conrado, V. S., de Luiz Almeida Freires, A., de Ferreira Souza, J. J., Figueiredo, F. R. A., Barroso, K. A., & Ambrosio, M. M. D. Q. (2023). Aggressivity of different *Fusarium* Species causing fruit rot in melons in Brazil. *Plant Disease*, 107(3), 886–892.
- Ongena, M., & Jacques, P. (2008). *Bacillus* lipopeptides: Versatile weapons for plant disease biocontrol. *Trends in Microbiology*, 16(3), 115–125. <https://doi.org/10.1016/j.tim.2007.12.009>
- Porto, M. A. F., Ambrósio, M. M. Q., Freitas, F. C. L., Nascimento, S. R. D. C., Cruz, B. L. S., & Guimarães, L. M. S. (2016). Feijão-de-porco (*Canavalia ensiformis*) no controle da podridão radicular do meloeiro causada por associação de patógenos. *Summa Phytopathologica*, 42(4), 327–332. <https://doi.org/10.1590/0100-5405/2183>
- R Core Team. (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing.
- Rocha WF (2009) Situação da cobertura vegetal do bioma Caatinga. In: *Mudanças Climáticas e Desertificação no Semiárido Brasileiro*. Embrapa Semiárido/Embrapa Informática Agropecuária, Petrolina-PE e Campinas-SP, pp 41–49.
- RStudio Team. (2024). RStudio: Integrated Development for R. RStudio, PBC, Boston.
- Samaras, A., Roumeliotis, E., Ntasiou, P., & Karaoglanidis, G. (2021). *Bacillus subtilis* MBI600 promotes growth of tomato plants and induces systemic resistance contributing to the control of soilborne pathogens. *Plants*, 10(6), 1113. <https://doi.org/10.3390/plants10061113>
- Sjögren, J., Magnusson, J., Broberg, A., Schnürer, J., & Kenne, L. (2003). Antifungal 3-hydroxy fatty acids from *Lactobacillus plantarum* MiLAB 14. *Applied and Environmental Microbiology*, 69(12), 7554–7557. <https://doi.org/10.1128/AEM.69.12.7554-7557.2003>
- Sretenović, M., Tamaš, N., Zec, G., Stojanoski, M., Tešić, N., Miletić, N., & Djordjević, B. (2024). Productivity, bio-control and postharvest fruit quality of strawberry cultivar ‘Clery’ using plant growth promoting microorganisms. *Cogent Food & Agriculture*, 10(1), 2310896. <https://doi.org/10.1080/23311932.2024.2310896>
- Stewart, J. E., Turner, A. N., & Brewer, M. T. (2015). Evolutionary history and variation in host range of three *Stagonosporopsis* species causing gummy stem blight of cucurbits. *Fungal Biology*, 119(5), 370–382. <https://doi.org/10.1016/j.funbio.2014.12.008>
- Tabatabai, M. A. (1994). Soil enzymes. In R. W. Weaver, S. Angle, & P. Bottomley (Eds.), *Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties* (Vol. 5, pp. 775–833). Soil Science Society of America.
- Tuzun, S. (2001). The relationship between pathogen-induced systemic resistance (ISR) and multigenic (horizontal) resistance in plants. *European Journal of Plant Pathology*, 107, 85–93. <https://doi.org/10.1023/A:1008784417222>
- Volynchikova, E., & Kim, K. D. (2022). Biological control of oomycete soilborne diseases caused by *Phytophthora capsici*, *Phytophthora infestans*, and *Phytophthora nicotianae* in solanaceous crops. *Mycobiology*, 50(5), 269–293. <https://doi.org/10.1080/12298093.2022.2136333>
- Wang, X. Q., Zhao, D. L., Shen, L. L., Jing, C. L., & Zhang, C. S. (2018). Application and mechanisms of *Bacillus subtilis* in biological control of plant disease. *Role of Rhizospheric Microbes in Soil*, 1, 225–250. https://doi.org/10.1007/978-981-10-8402-7_9
- Zhao, H., Vegi, A., & Wolf-Hall, C. (2017). Screening of lactic acid bacteria for anti-Fusarium activity and optimization of incubation conditions. *Journal of Food Protection*, 80(10), 1648–1656. <https://doi.org/10.4315/0362-028X.JFP-17-100>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.