Bioactive management alternatives for *Sclerotinia minor* Jagger in lettuce (*Lactuca sativa*)

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ABSTRACT

Objective: To determine the effect of biological control agents and plant extracts on the sclerotia of *Sclerotinia minor* through *in vitro* and *in vivo* trials.

Design/Methodology/Approach: Through *in vitro* and *in vivo* trials, analyses were performed on sclerotia treated with bioactive products, including the percentage of germination, parasitic colonization, disease incidence, and fresh plant weight.

Results: *Trichoderma harzianum* completely inhibited the growth of *S. minor in vitro* through mycoparasitism and enzymatic degradation. *In vivo*, it reduced disease incidence to 20% and resulted in a lower percentage of germination and sclerotia produced post-trial, whereas the control treatments reached 100% infection. Sclerotial germination only occurred in the presence of the host plant. *Trichoderma* maintained a stable population in the soil (10^4 CFU/g), demonstrating high persistence. In contrast, *Bacillus* spp. showed lower efficacy, with high percentages of sclerotial germination (92.5-97.5%). Organic matter slightly increased disease incidence by creating favorable conditions for the pathogen.

Limitations/Implications: Due to the biology of the pathogen, it is necessary to determine more precisely the biological interactions occurring in the plant rhizosphere to better understand the action of the biological agents.

Findings/Conclusions: *Trichoderma harzianum* establishes itself as a viable alternative for the management of *S. minor* in lettuce, reducing sclerotia viability through mycoparasitism and competition. Its implementation allows for a reduction in the dependence on synthetic fungicides and contributes to more sustainable production, minimizing the development of fungicide resistance.

Keywords: Biocontrol, sclerotia, white mold, mycoparasitism, variability.

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) is cultivated worldwide. By 2022, Mexico ranked as the ninth largest producer of this vegetable, with a production volume of 558,032.88 tons (FAO, 2022). According to the Agri-Food and Fisheries Information Service, as of November 2024, Mexico reported a production of 494,710.85 tons, with the principal producing states being Guanajuato (138,668.54 tons), Zacatecas (78,524.67 tons), Puebla (73,835.59 tons), Aguascalientes (57,657 tons), and Baja California (30,422.50 tons) (SIAP, 2024).

Lettuce is a crop affected by numerous organisms, particularly phytopathogens such as *Botrytis cinerea* (gray mold), *Bremia lactucae* (downy mildew), *Fusarium*, *Rhizoctonia*, *Phytophthora* (root diseases), *Sclerotinia sclerotiorum*, and *Sclerotinia minor* (white mold or soft rot) (INIA, 2016). *S. sclerotiorum* and *S. minor* are regarded as highly destructive pathogens in this production system (Arias *et al.*, 2007; Macioszek *et al.*, 2023). Owing to the pathogenesis of the fungus and the nature of the crop, the concept of disease severity is not applicable; rather, disease incidence predominates, since once the pathogen initiates its pathogenic process, the infection can no longer be halted.

The morphology of *S. minor* has been described as tuberculoid sclerotia with a black sclerotic rind composed of two to six deep layers and a prismatic texture, originating from medullary cells, with irregular contours and dimensions ranging from 0.5 to 2 mm (Rakesh *et al.*, 2016; Petkova *et al.*, 2024). These structures are rough in texture and angular in shape (Laemmlen, 2001). This pathogen has been reported to infect approximately 400 hosts (Wu *et al.*, 2008).

Disease onset occurs during the eruptive phase of the *S. minor* sclerotium through the myceliogenic mode. The mycelium comes into contact with lettuce stems and senescent leaves, penetrating the plant tissue. Subsequently, through the action of hydrolytic enzymes, degradation of the vascular bundle takes place, resulting in impaired water and nutrient transport, which ultimately causes desiccation and complete wilting of the plant (Hao *et al.*, 2007; Arias *et al.*, 2007; Hayes *et al.*, 2010).

Its management is considered particularly challenging because sclerotia can survive for up to 20 years (Bullock *et al.*, 1980; Blancard, 2002; Crutcher *et al.*, 2017; Petkova *et al.*, 2024). For the preventive management of diseases caused by *Sclerotinia* species, synthetic fungicides are used in Mexico and worldwide, including fluazinam, fluopyram, fluxapyroxad, trifloxystrobin, pyraclostrobin, and boscalid. Combinations of these active ingredients have been reported to reduce disease incidence by up to 60% (Matheron and Porchas, 2009). However, their efficacy may be affected by pathogen pressure, climatic conditions, application methods, and, above all, by pathogen-acquired resistance resulting from the excessive use of these fungicides (Petkova *et al.*, 2024). To date, no lettuce varieties resistant to these pathogens have been identified (Chitrampalam *et al.*, 2008; Mamo *et al.*, 2019; Petkova *et al.*, 2024), although partial resistance has been reported among different cultivated lettuce varieties (Grube and Aburomia, 2004).

This situation complicates the management of these pathogens; therefore, investigating the effect of natural extracts and biological control agents could provide an ecologically sound strategy to reduce the resistant propagules present in the soil and diminish disease incidence. Accordingly, the objective of this study was to determine the effect of biological control organisms and plant extracts on the sclerotia of *Sclerotinia minor*.

MATERIALS AND METHODS

In vitro assay

Inoculum Collection and Reproduction

The inoculum was collected from fields in the municipality of San Francisco Putla (latitude: 19° 07′54” N, longitude: 99° 37′50” W), in the Toluca Valley. Sclerotia were

extracted from plants exhibiting the characteristic symptoms and signs of the disease, surface-disinfested with 70% ethanol for 10 s, and rinsed three times with distilled water. Subsequently, they were placed on sterile blotting paper and transferred in groups of four sclerotia to Petri dishes containing PDA culture medium (4.0 g potato starch, 20.0 g dextrose, and 15.0 g agar per liter of solution) for inoculum reproduction.

Treatments

Eight commercial products, including biological organisms and natural extracts (Table 1), were used. Of these, only Baktillis, Apolo, and Timorex Gold are authorized on the label for use in lettuce. These products were evaluated under *in vitro* conditions in order to biologically assess the capacity of each treatment to inhibit fungal growth and development.

In 52-mm-diameter Petri dishes containing PDA, two sclerotia were placed 1 cm apart from each other; the sclerotia exhibited fungal mycelium on their surface. For treatments 1 to 5 (Table 1), the products were directly inoculated onto the sclerotia. For treatments 6, 7, and 8 (Table 1), since they consisted of natural extracts, the active ingredient was incorporated into the PDA culture medium before the sclerotia were placed onto this substrate. The highest dose recommended on the manufacturers' labels for each product was used. Sterile distilled water was sprayed onto the positive control. After inoculation, observations were made every 3 days for 15 days to monitor *S. minor* mycelial growth. Each product included five experimental units with two sclerotia each, for a total of 10 replicates per product. It should be noted that none of these products is labeled for the management of *Sclerotinia minor* Jagger; however, they are commonly used in vegetable production systems.

Transfer of Sclerotia After Treatment Application

Fifteen days after inoculation, the experimental units were opened, and each sclerotium was transferred to a new Petri dish containing PDA to observe, for an additional 15 days, the effect of the biological control organisms or the residual activity of the plant extracts on sclerotial germination capacity. Thirty days after the initial sclerotial inoculation, a qualitative assessment was conducted by evaluating mycelial growth and sclerotial production.

Table 1. Treatments and doses of bioactive products used in the *Sclerotinia minor-Lactuca sativa* pathosystem under *in vitro* conditions.

Treatment	Active ingredient	Product	Concentration (CFU spores mL^{-1} or g^{-1})	Dose (g or mL L^{-1})
1	*Bacillus amyloliquefaciens* strain MBI	Serifel	5.5×10^{10}′	5
2	*Bacillus subtilis*	Baktillis	1×10^{12}	7.5
3	*Trichoderma harzianum* strain KRL-AG2	PHC T-22	1×10^{7}	2.27
4	*B. subtilis*, *T. harzianum*, *Streptomyces lydicus*	Apolo	CC*	5
5	*Melaleuca alternifolia* oil	Timorex Gold	0.2225 g	3.75
6	*Swinglea glutinosa* extract	Ecoswing	868 g a.i.	5
7	*Larrea tridentata* extract	Org-fung	40 g a.i.	5
8	*Bacillus subtilis* strain SP 83	Fungi free	5.5×10^{9}′	5

*Combination of concentrations.

In vivo assay

Preparation of Experimental Units

Field soil was subjected to double pasteurization at 15 psi for 4 h each cycle and subsequently mixed with peat moss at a 4:1 ratio (pasteurized field soil:peat moss). Pots measuring 30 × 30 cm were filled with approximately 0.625 kg of the soil mixture, and one lettuce seedling of the Great Lake variety was transplanted into each pot.

Using the sclerotium-trap method (Stazzonelli *et al.*, 2017), as adapted by Arrúa Alvarenga and Aquino Jara (2013) with modifications, 10 sclerotia of *S. minor* were placed into a microporous fabric bag, which was then buried in each pot. A total of 240 bags were prepared and placed individually in each pot at a depth of 5 cm. From the sclerotia reproduced during the *in vitro* stage, four randomly selected sclerotia were placed in each Petri dish containing PDA culture medium, with a total of two Petri dishes. These were incubated at 28 °C.

Application of Bioactive Products and Organic Matter

For product application, a 1000 mL suspension was prepared for each treatment. Subsequently, 25 mL of each suspension was applied to each pot as a drench. For the organic matter treatment, pasteurized bat guano (previously registered for commercial sale) was used at a dose of 25 mL of active ingredient per liter of water and incorporated into the substrate. Both the bioactive products and the organic matter were applied at 25 mL per experimental unit every 7 days for one month.

All experimental units were fertilized every 5 days with a 20-30-10 (N-P-K) fertilizer at a dose of 3 g L^{-1} of water, beginning on day 1 after transplanting and continuing until 45 days after transplanting (head formation stage). The experimental units were maintained under open-field conditions in order to simulate field conditions and naturally expose the pathogen to the environment. Irrigation was applied every 2 days, and temperature and relative humidity were monitored using a HOBO PRO data logger, model U23-00A, programmed to record data every 30 min.

Experimental Design

The assay was established under a completely randomized design with three factors (treatments, plant, and organic matter) in order to determine the effect of each on the epidemiological system of *L. sativa* L.-*S. minor* and to identify the physical, biological, or environmental conditions influencing disease incidence. The treatment factor included six levels (five products and an untreated control), the plant factor had two levels (with or without plant), and the organic matter factor also had two levels (with or without organic matter), resulting in a total of 24 possible combinations. Each combination consisted of 10 replicates, for a total of 240 experimental units.

Post-assay analysis of sclerotial germination

Eighty days after lettuce transplantation, the plants were weighed, and five bags were selected from each of the established combinations as a representative sample of the 10 experimental units per combination. These were labeled and processed in the laboratory.

From each bag, four sclerotia were selected and placed into 2 mL Eppendorf tubes containing 1 mL of sterile distilled water. Sclerotial washing was performed by vortexing for two periods of 30 s each, after which the sclerotia were decanted onto Whatman® grade 1 filter paper (90 mm diameter) placed in a glass funnel, and excess water was removed with the aid of burners. Subsequently, the four sclerotia were transferred to Petri dishes containing PDA culture medium. Sclerotial germination was evaluated 11 days after sowing (DAS).

Colonization by biological organisms

For the recovery of organisms from the soil, samples were taken from five pots out of the 10 experimental units of each combination to avoid excessive sample processing, except for those receiving treatment 5 (*Melaleuca alternifolia* oil). PDA culture medium was used for samples containing *Trichoderma* spp., whereas nutrient agar was used for samples containing bacteria. Extraction was performed using 1 g of soil sample in 10 mL of sterile distilled water, followed by serial dilutions to a concentration of 10^{-4} for *Bacillus* spp. strains and 10^{-2} for *Trichoderma* spp. strains. Finally, 100 μL of each dilution was plated onto the respective culture medium and spread with a bacteriological loop. The plates were incubated at 28 °C. To obtain CFU g^{-1} of soil, the standard colony-forming unit counting equation was used as follows:

$$\frac{UFC}{g} = \frac{N \times D}{V} \times weight\ factor$$

where: N=number of colonies; D=dilution factor; V=volume plated on the dish (mL).

The count was adjusted by the weight factor (0.1 g mL^{-1}), since the initial preparation consisted of 1 g of soil mixed with 10 mL of water. Because only a single dilution was performed, the results should be interpreted as point estimates. Since the bacteria were extracted from a previously sterilized medium, each of the products containing the bacterial strains used was plated at a concentration of 10^{-6} so that colony counts could be performed based on their morphology.

Determination of *Trichoderma* parasitism

To determine the mode of action, Petri dishes containing nutrient agar were used, and two points were marked at a distance of 5 cm from the center of each dish. On one side, a PDA fragment containing mycelium and sclerotia of *S. minor* was inoculated, while on the opposite equidistant point, a PDA fragment bearing a colony of each of the two *Trichoderma* strains was placed under sterile conditions. After seven days, observations were made of the effect of *Trichoderma* mycelium on the mycelium of *S. minor* under light microscopy.

Statistical analysis

The principal response variable used to evaluate treatment effects was fresh lettuce weight. Since fresh weight data did not conform to a normal distribution (Shapiro-Wilk,

$p<0.05$), a nonparametric Scheirer-Ray-Hare test was applied for a factorial design with two factors: "Treatment" (6 levels) and "Organic Matter" (2 levels). This test is a useful statistical tool when nonparametric data analysis is required, that is, when the assumptions of normality, homogeneity of variance, and independence are not met. The test was used to analyze the independent effects of the factors as well as their interaction. All data analyses were performed in R v4.3.1 (R Core Team, 2025).

RESULTS AND DISCUSSION

In vitro assay

It was observed that treatments 1, 2, 3, 4, and 8 (Table 2; Figure 1) showed no mycelial growth from the sclerotia upon exposure to the products, nor after sclerotial transfer (Figure 3). In contrast, treatments 5, 6, and 7 (Table 2; Figure 2), which corresponded to natural extracts, exhibited the presence of cottony mycelium as well as the formation of new sclerotia even before transfer was performed. The treatments that showed no mycelial growth were selected for the *in vivo* assay.

In vivo assay

Four days after sclerotial sowing, germination and mycelial growth were observed in the viability test, confirming that the sclerotia placed in the bags retained the capacity to germinate and initiate the infection cycle.

In the assay, a maximum disease incidence of 30% was observed, whereas the absolute control exhibited 100% incidence (Figures 4 and 5).

Through the Scheirer-Ray-Hare test, a highly significant effect of treatments on fresh lettuce weight was detected ($H=42.215$, $df=5$, $p<0.0001$). However, neither organic matter ($H=0.002$, $df=1$, $p=0.964$) nor the interaction between treatments and organic matter ($H=1.086$, $df=5$, $p=0.955$) showed significant effects. Dunn's *post hoc* test with Holm adjustment revealed that treatment T6 (absolute control) differed significantly from all other treatments (T1-T5) ($p<0.001$ in all cases), whereas no statistically significant differences were found among treatments T1 to T5 ($p>0.05$ for all comparisons). These results indicate that treatment T6 (absolute control) produced a significantly lower fresh

Table 2. Germination of *Sclerotinia minor* sclerotia after exposure to different biocontrol products.

Treatment	Active ingredient	15 days after sclerotial sowing	
1	*Bacillus amyloliquefaciens* strain MBI	–	–
2	*Bacillus subtilis*	–	–
3	*Trichoderma harzianum* strain KRL-AG2	–	–
4	*B. subtilis*, *T. harzianum*, *S. lydicus*	–	–
5	*Melaleuca alternifolia* oil	+	+
6	*Swinglea glutinosa* extract	+	+
7	*Larrea tridentata* extract	+	+
8	*Bacillus subtilis* strain SP 83	–	–

Positive germination (+); negative germination (–).

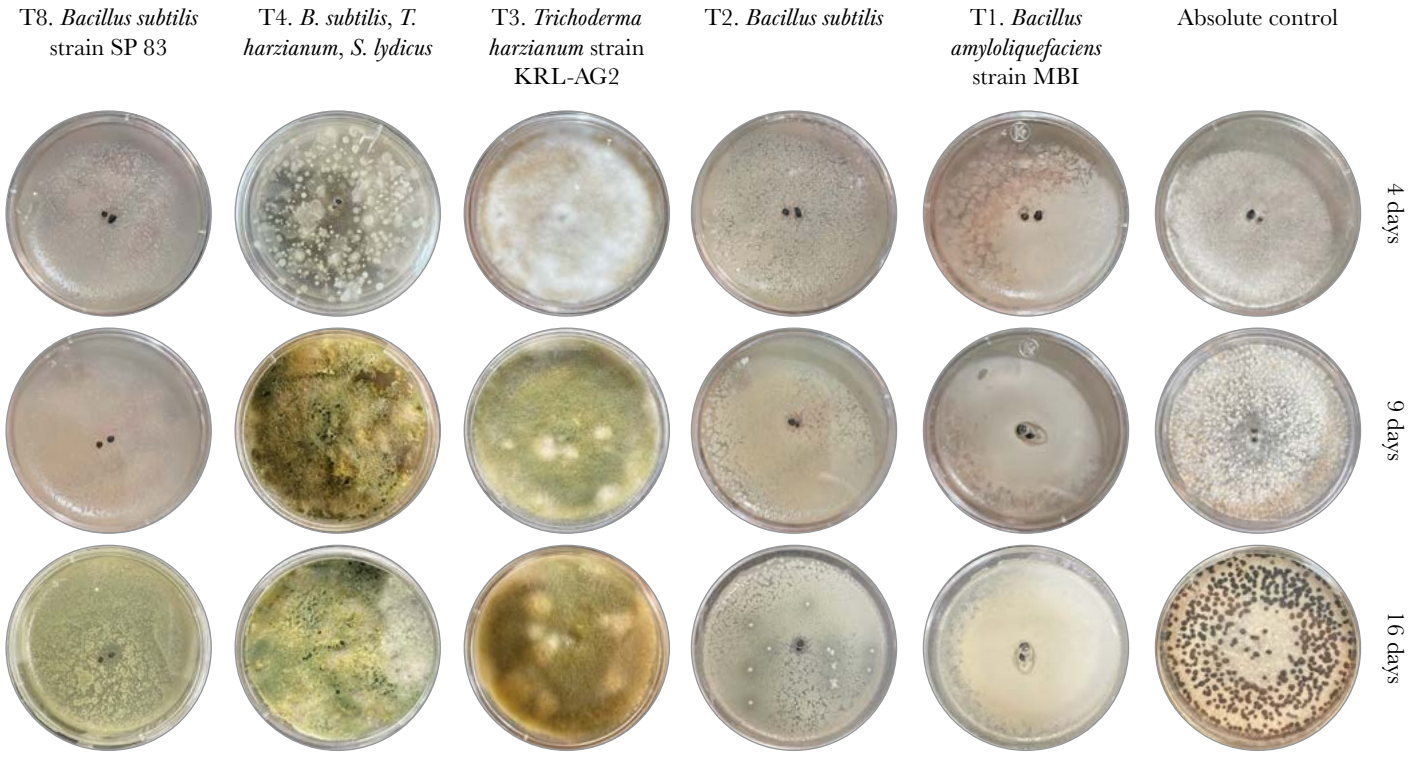

Figure 1. Growth after sowing of treatments composed of biological organisms.

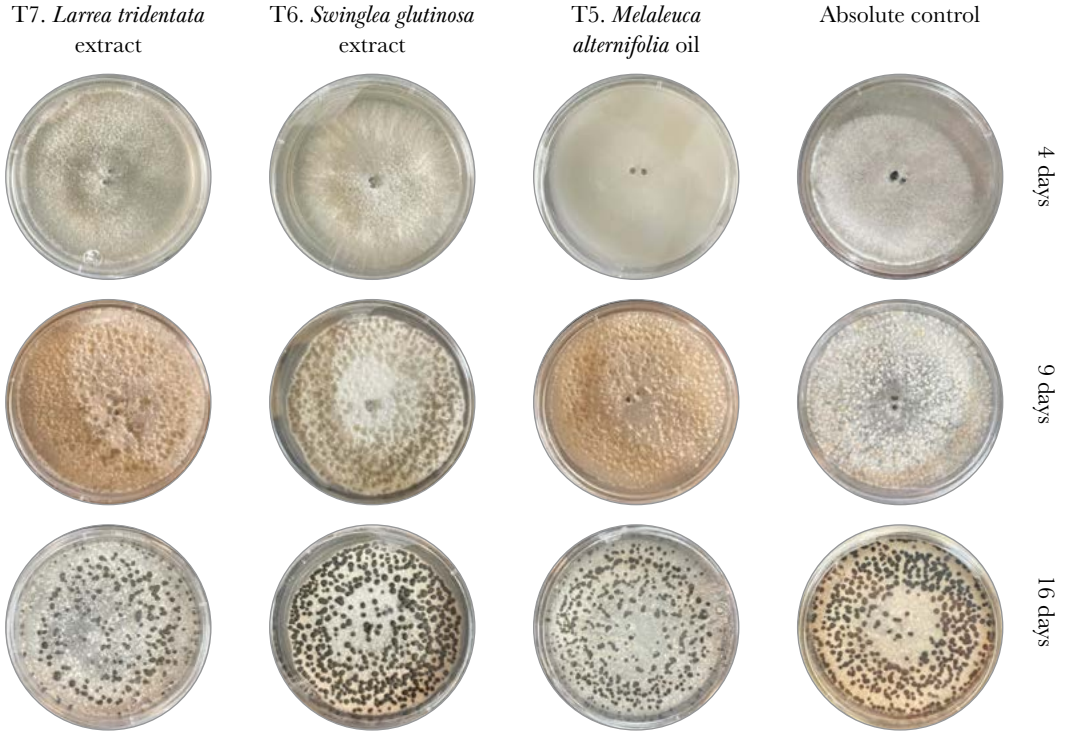

Figure 2. Growth after sowing of treatments composed of natural extracts and oils.

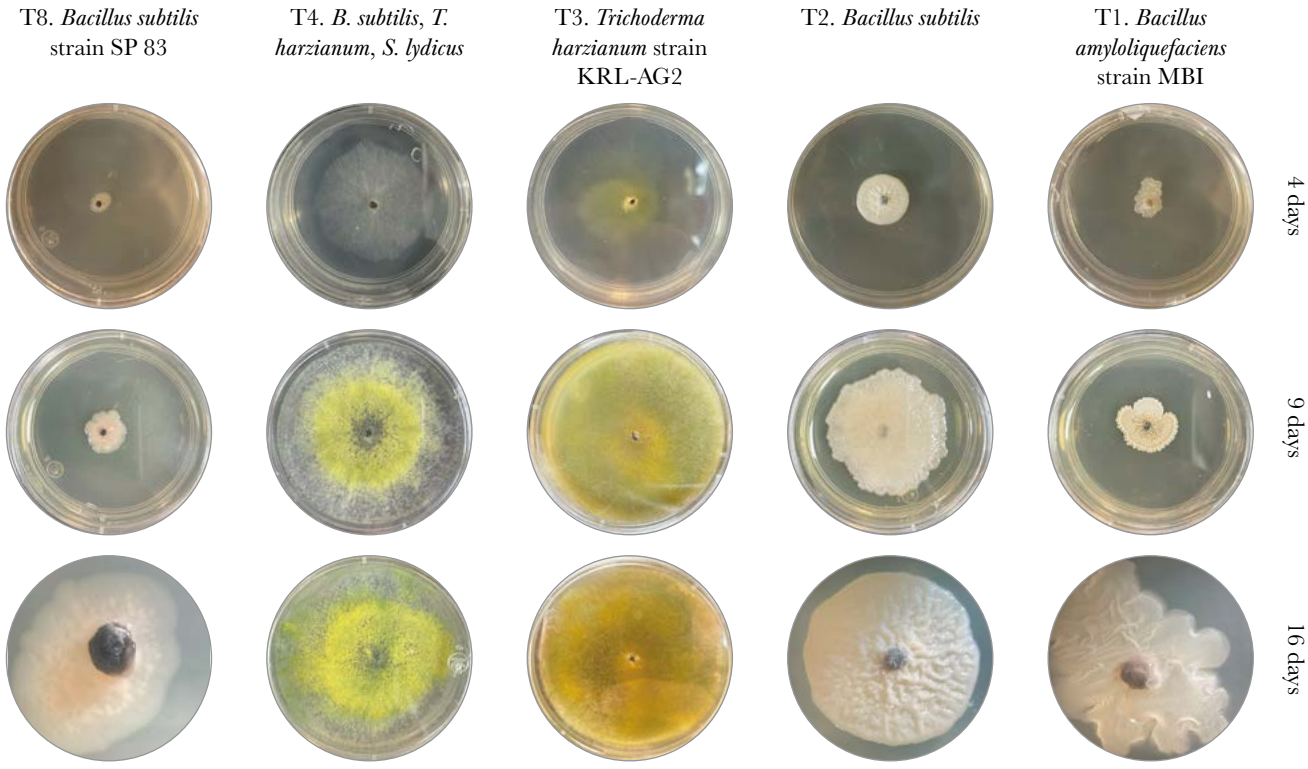

Figure 3. Sclerotia exposed to bioactive products that were reseeded without germination.

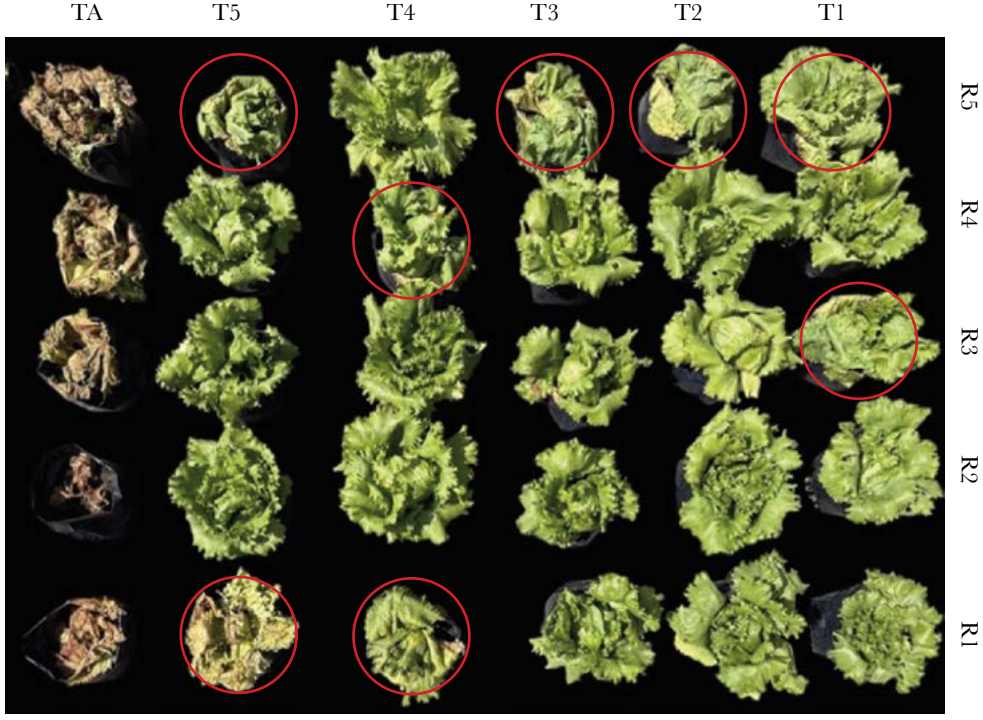

Figure 4. Incidence of white mold in a five-treatment factorial trial, without application of organic matter and with the presence of plants 80 days after transplanting. Absolute control (TA), *Bacillus amyloliquefaciens* (T1), *Bacillus subtilis* (T2), *Trichoderma harzianum* strain KRL-AG2 (T3), *B. subtilis*, *T. harzianum*, *S. lydicus* (T4), *Melaleuca alternifolia* oil (T5).

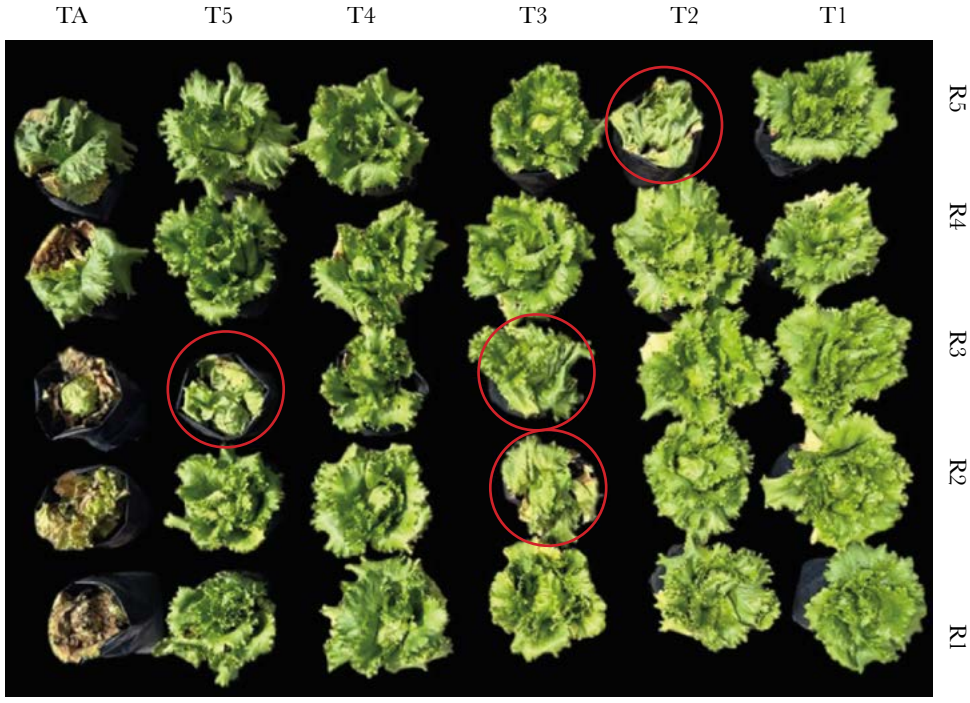

Figure 5. Incidence of white mold in a five-treatment factorial trial, without application of organic matter and with the presence of plants 80 days after transplanting. Absolute control (TA), *Bacillus amyloliquefaciens* (T1), *Bacillus subtilis* (T2), *Trichoderma harzianum* strain KRL-AG2 (T3), *B. subtilis*, *T. harzianum*, *S. lydicus* (T4), *Melaleuca alternifolia* oil (T5).

weight than all other treatments (T1-T5). The absence of significant differences among treatments T1 to T5 suggests that all of them exerted a similar effect on fresh lettuce weight, which was superior to that of control T6. Likewise, the lack of a significant interaction between treatments and organic matter indicates that treatment effects were consistent regardless of whether organic matter was applied; nevertheless, given the biology of this pathosystem, this factor may contribute to the establishment of plant-pathogen or bioactive organism-pathogen interactions. Studies conducted by Abawi and Grogan (1979) indicate that *S. minor* may benefit from soils with high organic matter content, as this contributes to greater moisture retention and nutrient availability, which may explain the high incidence observed in the experimental units supplied with organic matter. This finding is consistent with reports on strains of *Bacillus amyloliquefaciens* used for the control of *Sclerotinia* species, in which *B. amyloliquefaciens* did not produce an additional increase in the normal growth variables of lettuce, even though it had the capacity to produce auxins (Sabaté *et al.*, 2018). The absence of interaction may also be associated with the number of experimental units, suggesting that a larger sample size may be required than that used in this assay.

Post-Assay Germination and Sclerotial Production

It is worth noting that no germination was observed in sclerotia located in experimental units where no plant was present, thereby reinforcing the findings of Abawi and Grogan (1979), Hao *et al.* (2007), and Arias *et al.* (2007), who reported that germination of *S. minor* requires the microclimate generated between the lettuce canopy and the soil surface.

For the sclerotial germination variable, germination percentage data were also analyzed using a nonparametric approach because the assumptions of normality were not met (Shapiro-Wilk, $p<0.05$). The Scheirer-Ray-Hare analysis revealed a highly significant effect of treatments ($H=89.65$, $df=4$, $p<0.001$), whereas no significant effects were detected for organic matter ($H=29.34$, $df=114$, $p=1$) or for the interaction between these factors. Multiple comparisons using Dunn's test with Holm adjustment revealed significant differences among treatment groups ($p<0.001$). Specifically, T3 (*B. subtilis*, *T. harzianum*, *S. lydicus*) and T4 (*Trichoderma harzianum* strain KRL-AG2) showed a significant reduction in sclerotial germination compared with the other treatments. In particular, T4 exhibited the lowest germination values and was statistically different from T1, T2, T5, and the absolute control (T6). Treatments T1, T2, T5, and T6 did not differ significantly from one another ($padj>0.05$ in all comparisons), maintaining high germination percentages (>75%).

Trichoderma (T3 and T4) was able to reduce germination to below 10% in most replicates, and this effect was independent of organic matter addition ($p>0.05$). The absence of significant differences between T3 and T4 suggests that both treatments constitute viable alternatives for inhibiting the germination of *S. minor* sclerotia, although T4 showed a tendency toward greater consistency in this effect. These findings are in agreement with Rivera-Méndez *et al.* (2015), who studied the interaction between *Sclerotium cepivorum* and *Trichoderma* to determine the potential of this organism as a biological control agent and to reduce disease incidence. Their results showed lower mortality in blocks where *Trichoderma* was applied, associating this outcome with its antagonistic capacity against soilborne propagules.

Regarding the number of sclerotia produced (Table 3), the Scheirer-Ray-Hare test revealed highly significant differences among treatments in sclerotial production ($p<0.0001$). However, no significant effects were detected for the presence or absence of organic matter ($p=0.276$) or for the interaction between treatments and organic matter ($p=0.291$), indicating that treatment efficacy was independent of the organic matter factor. Dunn's post hoc analysis with Holm adjustment showed the same trend observed for germination percentage, in which treatments T3 (*B. subtilis*, *T. harzianum*, *S. lydicus*) and T4 (*Trichoderma harzianum*) were the most effective ($padj=0.115$). Treatments T3 and T4 did not differ significantly from each other ($padj=0.115$), while both exhibited a favorable contrast with the absolute control T6. In contrast, treatments T2 (*Bacillus amyloliquefaciens*) and T5 (*Melaleuca alternifolia* extract) were significantly inferior to the absolute control. Treatment T1 (*Bacillus subtilis*) showed an intermediate performance, being superior to T2 and T5 ($padj<0.0001$), but without significant differences relative to the most effective treatments or the control ($padj>0.05$). These results indicate that only the treatments containing *Trichoderma* (T3 and T4) were capable of significantly reducing sclerotial production, with an effect independent of organic matter addition.

Colonization of Biological Organisms

The presence of the applied organisms was confirmed, and their persistence varied according to the product as well as the presence or absence of plants and organic matter (Figures 6 and 7). *Bacillus subtilis* and *Bacillus amyloliquefaciens* showed low concentrations

Table 3. Number of sclerotia cm^2 from sclerotia exposed to bioactive ingredients.

Treatment	Number of sclerotia per cm^2			
	a	b	c	d
Witness	19.20±0.96	20.20±1.80	20.40±1.28	21±1.58
B. subtilis	17.60±1.16	16±1.48	18.80±0.86	18.60±1.28
B. amyloliquefaciens	21.40±1.20	13.20±1.15	19.40±1.20	15.40±0.74
B. subtilis, *T. harzianum*, *S. lydicus*	0	2.80±1.74	1.0±1.0	0
T. harzianum strain KRL-AG2	0	1.20±1.20	0	5.20±2.26
Melaleuca argentifolia oil	18.80±0.73	14.16±0.87	19±0.94	18.40±1.07

a: treatments with plant and organic matter, b: treatments with plant without organic matter, c: treatments without plant and with organic matter, d: treatments without plant and without organic matter.

Figure 6. Colonies of *Bacillus* spp. extracted from the soil with application of bioactive products.

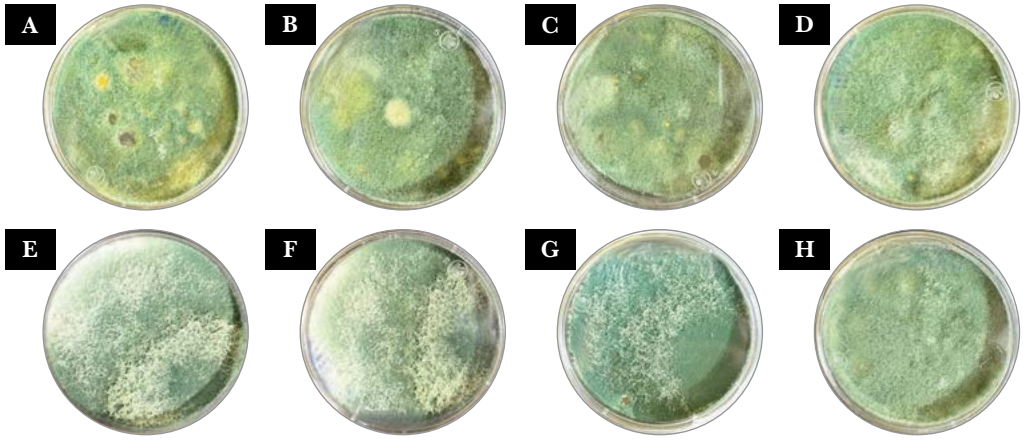

Figure 7. Colonies of *Trichoderma* spp. extracted from soil with application of bioactive products. A) Trichoderma harzianum + M.O. + plant. B) *Trichoderma harzianum* + plant. C) *Trichoderma harzianum* + M.O. D) *Trichoderma harzianum*. E) *Trichoderma harzianum* strain KRL-AG2 + M.O. + plant. F) *Trichoderma harzianum* strain KRL-AG2 + plant. G) *Trichoderma harzianum* strain KRL-AG2 + M.O. H) *Trichoderma harzianum* strain KRL-AG2.

in treatments with plants, ranging from 9.3×10^6 to 1.09×10^7 CFU g^{-1} and 10^7 CFU g^{-1}, respectively, and these values declined even further in the absence of plants. This phenomenon may be explained by the findings of Larena and Melgarejo (1996), who reported that *Bacillus* species exhibit low efficiency in soils under continuous lettuce production, as such soils commonly display a neutral to alkaline pH.

In contrast, *Trichoderma* strains showed remarkably stable concentrations (10^4 CFU g^{-1}) across all scenarios, thereby demonstrating their strong saprophytic capacity. This observation is consistent with the findings of Singh *et al*. (2024), suggesting a marked ability to adapt to the environment and exploit organic matter efficiently. *Trichoderma* exhibited superior establishment in the soil of each experimental unit, whereas the concentration of *Bacillus* species was the most adversely affected. This pattern may also be explained by the observations of Larena and Melgarejo (1996), who described the low efficiency of *Bacillus* species in soils where lettuce is continuously cultivated, due to the fact that such soils tend to exhibit a neutral to slightly acidic pH. The opposite appears to occur with *Trichoderma*, since this organism thrives within that pH range, which enables it to establish, develop, and exert a protective effect on the plant (Martínez *et al*., 2013), a response that was not observed for the *Bacillus* species.

Parasitism of *Trichoderma*

With respect to the parasitism exerted by *Trichoderma* species, observations under light microscopy revealed that the two strains used in this assay were capable of penetrating, infecting, and coiling around the mycelium of *S. minor* (Figure 8). Together with their faster growth rate relative to *S. minor*, this prevented the development of both mycelium and sclerotia. The parasitism of *S. minor* sclerotia observed in this study has been extensively documented in the scientific literature by several authors (Arias *et al*., 2007; Petkova *et al*., 2024; Kishan *et al*., 2017), who describe *Trichoderma* spp. as biological control agents with the capacity to induce mycoparasitism, a process in which *Trichoderma* spp. grows chemotropically toward the host, adheres to its hyphae, coils around them, and in some cases penetrates them (Carsolio *et al*., 1999). In other words, it can attack both the sclerotia and the mycelium of the pathogen (Chitrampalam *et al*., 2008).

In light of the foregoing, it is evident that *Trichoderma* exerts a direct effect on the biology of the fungus, since once it infects the pathogen and consumes its cellular contents, structural damage occurs that prevents proper germination and reproduction. When incorporated into an integrated disease management strategy, *Trichoderma* may act on the sclerotia and reduce inoculum levels in the field, particularly when combined with appropriate cultural practices such as the removal of diseased plants. Future research should consider the pH factor in order to more precisely determine the mycoparasitic activity of these organisms and their functional effect on the pathogen.

Trichoderma harzianum (strains KRL-AG2 and harzianum) demonstrated the capacity to act as an effective biological control agent against *Sclerotinia minor* in lettuce, primarily through mycoparasitism, by reducing sclerotial viability and limiting disease progression under the conditions evaluated. Although no significant differences were observed among treatments for the fresh weight of lettuce, the statistical analysis revealed significant

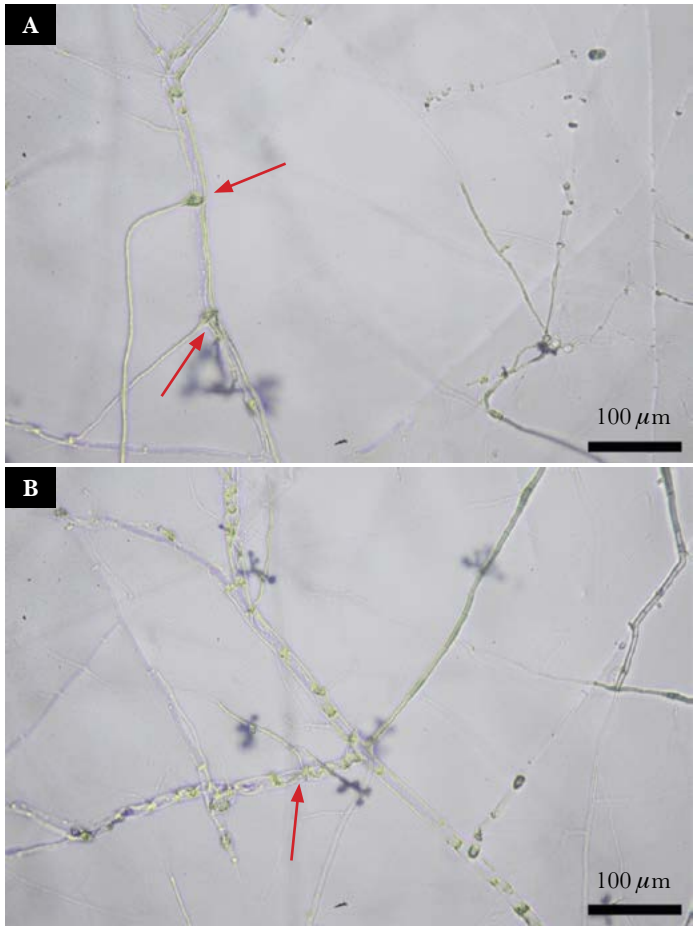

Figure 8. Parasitism of *Trichoderma* strains on *S. minor* mycelium. A) Mycelium penetration. B) Mycelium coiling.

differences between the evaluated treatments and the absolute control for this variable, indicating disease mitigation at varying levels depending on the product applied. From a biological standpoint, the treatments that showed the greatest efficacy in terms of disease incidence reduction and suppression of the biological capacities of *S. minor* were those based on *T. harzianum* and *T. harzianum* strain KRL-AG2, whereas the *Bacillus*-based treatments and the plant extract exhibited lower effectiveness.

It was also determined that the presence of the host plant is essential for the germination of *Sclerotinia minor* sclerotia, and that the incorporation of organic matter did not exert a statistically significant effect on disease incidence. Nevertheless, from a biological perspective, greater pathogen aggressiveness was observed in those experimental units amended with organic matter, which also appeared to exert a positive effect on both the pathogen and the biological control organisms within the *S. minor-Lactuca sativa* L. epidemiological system.

Based on the methodology employed and the results obtained, it is recommended that *Trichoderma* applications be carried out after crop transplantation and before the first senescent leaves collapse, at 7-day intervals, with a maximum of four drench applications.

For future research, it is important to evaluate soil factors such as pH, since this variable influences both pathogen behavior and the performance of biological control agents. Likewise, the study of organic matter within this epidemiological system is of considerable importance, as it will help clarify its role in the different microbial interactions involved and its impact on disease development.

CONCLUSIONS

These findings support the use of *Trichoderma* as an environmentally sound tool that directly affects the germination capacity of sclerotia, reduces the inoculum present in the field, and lowers disease incidence over time. In addition, these products are relatively low in cost, which may facilitate their adoption by a greater number of producers, particularly small- and medium-scale growers, thereby strengthening their integrated disease management strategies.

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