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Control of coffee rust (*Hemileia vastatrix*) with *Trichoderma* spp.: quantitative assessment using the Pliman package

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ABSTRACT

Background/Objective. Coffee rust (*Hemileia vastatrix*) reduces coffee production and profitability. Alternatives for its control (chemical and genetic) have not been fully effective; therefore, the use of microorganisms such as *Trichoderma* has been proposed. Currently, tools like the Pliman package provide a quantitative alternative for assessing disease severity. The objective was to evaluate the effectiveness of *Trichoderma* strains applied to the soil in reducing the severity of *H. vastatrix* through digital image analysis using the Pliman package.

Materials and Methods. The effect of *Trichoderma harzianum* (Th), *T. asperellum* (Ta), and their consortium (Th_Ta) was evaluated at two concentrations (1×10^{8} and 1×10^{12} conidia mL^{-1}) on 42 'Catuaí Amarillo' coffee plants, both asymptomatic and symptomatic. Soil applications were performed every two months for one year. Disease severity was quantified using the Pliman package in RStudio. The data were analyzed with a factorial ANOVA and Tukey's tests ($p<0.05$).

Results. *Trichoderma* treatments significantly ($p < 0.05$) reduced disease severity compared with the control. Treated asymptomatic plants showed the lowest severity (*Th_Ta*=5.99%, *Th*=5.61%, *Ta*=8.61%). In symptomatic plants, *T. asperellum* (*Ta*) was the most effective, reducing severity to 8.25%, a value not significantly different from that of treated asymptomatic plants. No significant differences were found between the concentrations used (1×10^{8} and 1×10^{12} conidia mL^{-1}). Severity showed a clear seasonal variation, being lower during dry months (February=4.89%, August=4.71%, and April=5.23%) and higher during rainy months (June=15.29%, October=10.79%, and December=23.39%).

Conclusion. Soil application of *Trichoderma* spp. significantly reduces the severity of coffee rust. The most effective treatments were the consortium of *T. harzianum* and *T. asperellum* in asymptomatic plants, and *T. asperellum* alone in symptomatic plants, with the concentration of 1×10^{8} conidia mL^{-1} being the most advisable due to its efficacy and economic feasibility. Finally, the Pliman package proved to be an effective tool for the objective quantification of the disease.

Keywords: Biological control, Integrated management, Coffee cultivation, Quantitative assessment

INTRODUCTION

Coffee cultivation in Mexico is a strategic activity due to its economic, sociocultural, and environmental importance (Escamilla, 2021). The cultivated area, trade volume, and number of producers dedicated to coffee growing have given rise to regions recognized nationally and internationally—such as Chiapas, Veracruz, Oaxaca, Puebla, and Guerrero (SIAP, 2022)—which have gained reputation, tradition, and identity (Nava-Tablada, 2012). However, the sector faces significant challenges, with coffee rust (*Hemileia vastatrix*) being its main phytosanitary threat. The 2012–2013 crisis caused severe losses, affecting more than 100,000 hectares and reducing national production by 50% (Boudrot *et al.*, 2016). For this reason, coffee rust is a disease of international, national, and regional importance (Chambe, 2020), and in Mexico it is subject to official control (Haddad *et al.*, 2009).

The infection manifests as yellow spots on the underside of the leaves, causing premature defoliation, branch desiccation, and even plant death (Quispe-Apaza *et al.*, 2021). This negatively affects bean quality and reduces yields by up to 40% (Alvarado-Castillo *et al.*, 2017; Escamilla, 2021). Disease control has relied mainly on systemic and contact fungicides formulated with copper (Avelino *et al.*, 2015; Haddad *et al.*, 2009; Ramírez-Rodríguez *et al.*, 2020), which, although effective, pose risks to human health and the environment due to direct handling, residual effects, and contamination of soil, air, and water. In addition, they involve high costs and the risk of resistance development (Ramírez-Rodríguez *et al.*, 2020).

Given this situation, a promising strategy is the use of antagonistic microorganisms such as *Lecanicillium lecanii* and *Trichoderma* spp. (Michel-Aceves *et al.*, 2019), recognized for their ability to act as beneficial agents in agriculture (Alfonso *et al.*, 2005). The latter is particularly effective due to its rapid colonization of the rhizosphere, promotion of plant growth, and multiple mechanisms of action, including parasitism, competition, induction of systemic resistance (ISR), and nutrient absorption, especially phosphorus (Harman *et al.*, 2004; Aswani *et al.*, 2022; Yao *et al.*, 2023). It is worth noting that although the pathogen is foliar, applying *Trichoderma* to the soil can trigger systemic responses that modulate severity in the aerial part, making this organism a promising candidate for the biocontrol of rust (Aswani *et al.*, 2022; Sharma *et al.*, 2022).

In this regard, early and accurate detection of the disease is crucial for the proper application of phytosanitary agents, as well as for predicting losses and forecasting epidemics. In the case of rust, diagrammatic scales based on visual estimates have been developed to assess disease severity, serving as important tools for decision-making (Barbedo, 2014). For example, CENICAFÉ in Colombia developed diagrams with 15 severity levels ranging from 0.05 to 80.0% (López-Vásquez *et al.*, 2018); Brazil developed six levels (2.5, 5, 10, 20, 40, and 80%) (Capucho *et al.*, 2011); and in Mexico, SENASICA established seven damage categories ranging from 0 to 70% (Calderón, 2016). However, these estimates can be subjective and depend on the evaluator's experience, leading to inaccuracies (Avelino *et al.*, 2015).

For this reason, disease assessment has advanced toward the use of digital image analysis, streamlining the process and providing precise estimates (Bock *et al.*, 2020; Gallego-Sánchez *et al.*, 2020; Mutka and Bart, 2015). Some of the software used includes ImageJ with the rust script (Schneider *et al.*, 2012), Quant (Bock *et al.*, 2020), and RStudio with the Pliman package (Olivoto *et al.*, 2022). These programs analyze the red, green, and blue (RGB) color values of digital photographs to objectively quantify the leaf area affected by diseases (Bock *et al.*, 2020). However, their application for quantifying coffee rust has not been reported in the literature. Therefore, the objective of this study was to determine the effect of soil-applied *Trichoderma* spp. on the severity of *H. vastatrix* in coffee, using the Pliman package in RStudio as a tool for its quantification.

MATERIALS AND METHODS

Study area. The experiment was conducted at Los Barreales farm, located in the municipality of Teocelo, Veracruz (19°23'37.3" N, 96°59'01.0" W; 19°23'39.7" N, 96°59'12.4" W), from April 2022 to February 2023.

Biological material. The strains *T. harzianum* (IE-996) and *T. asperellum* (TA-3) were obtained from the culture collections of the Instituto de Ecología A.C. (INECOL) and the Universidad Veracruzana, Tuxpan campus, respectively. They were grown on Potato Dextrose Agar (PDA) (Bioxon®) under aseptic and dark conditions for five days at a temperature of 26 °C. The coffee plants (*C. arabica*) used were of the 'Catuaí Amarillo' variety, eight years old, and managed under conventional practices.

Inoculum production. Mass production of *Trichoderma* spp. was carried out using rice as a substrate, following the method of Michel-Aceves *et al.* (2008) with modifications. For this, 3 kg of grain were washed with running water and soaked for 20 minutes in 5 L of water containing 1.25 g of chloramphenicol (Lebrocetin®) to prevent bacterial contamination. After draining the excess water, 350 g portions were weighed and packed in polypropylene bags for sterilization under conventional conditions for 30 minutes. Once the substrate cooled to room temperature, it was inoculated with a conidial suspension obtained from PDA cultures, previously adjusted to a concentration of 1×10^{7} conidia mL^{-1} using a Neubauer chamber. Under sterile conditions, 20 mL of this suspension were injected into each bag. The bags were incubated at 25 ± 2 °C for 21 days, shaken every 72 hours to promote uniform sporulation. Finally, the colonized rice was washed with sterile distilled water containing 0.01% TWEEN 80, and the liquid was filtered through a No. 20 mesh sieve (0.850 mm). From this suspension, final concentrations were adjusted for the field treatments: 1×10^{8} (C1) and 1×10^{12} (C2) conidia mL^{-1}. The solutions were stored in 500 mL containers until application (Martínez *et al.*, 2008; Yáñez-Hernández *et al.*, 2023).

Experimental design. A randomized block design with a 3×2×2 factorial arrangement was used, with the following factors: (1) Strains: *T. harzianum* (Th), *T. asperellum* (Ta), and a consortium (Th_Ta) (prepared by mixing equal volumes of Th and Ta suspensions at the corresponding concentration); (2) Concentrations: 1×10^{8} (C1) and 1×10^{12} (C2) conidia mL^{-1}; and (3) Initial plant health status: Symptomatic (with *H. vastatrix* pustules) and Asymptomatic (without visible pustules), resulting in 12 treatments. Additionally, two controls were included: a negative control (T), which received sterile water, and a conventional control (B), corresponding to the grower's standard management, totaling 14 treatments. Each treatment had three replicates (coffee plants), for a total of 42 experimental units. To minimize interference, one row of uninoculated plants was left between treatments. Each plant received 1 L of the corresponding suspension, applied directly to the soil in the drip zone every two months for one year (six applications in total). Since the trial was conducted under field conditions without physical barriers, natural pathogen dispersal was allowed. This condition made it possible to assess treatment efficacy under realistic inoculum pressure, although the health status of some plants could change throughout the study. The response variable was the percentage of rust severity, determined through image analysis using the Pliman package (Enríquez-López *et al.*, 2025).

Digital analysis of rust severity using the Pliman package. The evaluation was conducted through digital image analysis using the Pliman package in RStudio. The capture and analysis protocol was previously established and validated by Enríquez-López *et al.* (2025) for the same coffee variety and pathosystem. The protocol standardizes the process through four validation stages with samples of increasing precision, allowing the selection of the normalized green-red difference index (NGRDI) with a threshold of 0.045 as the most accurate parameter for distinguishing *H. vastatrix*-infected tissue from healthy tissue. In the present study, this protocol was applied to each treatment at every application period, collecting three leaves per plant (one from the upper, middle, and lower canopy strata) from the 42 experimental units. The leaves were photographed against a white background under diffuse natural light using a Sony A6400 camera with a Sigma 30 mm lens, following the defined technical parameters (Enríquez-López *et al.*, 2025). The images were automatically processed in RStudio using the predefined index and threshold (NGRDI = 0.045) to quantify the percentage of affected leaf area (severity) in each treatment over time.

Data analysis. The data obtained were organized into a database, verifying the assumptions of normality, homoscedasticity, and independence. A repeated-measures ANOVA was then applied to evaluate the different factors and their interactions, as well as the temporal evolution of rust severity throughout the study year, followed by Tukey's mean comparison test ($p < 0.05$). All analyses were performed using RStudio version 4.3.2.

RESULTS AND DISCUSSION

Effect of *Trichoderma* on rust severity. Significant differences (Tukey, $p < 0.05$) were found among treatments. The application of *Trichoderma* spp. reduced the severity of *H. vastatrix* compared with the controls. The most effective treatments were the consortium (Th_Ta; 8.10%^{a}) and *T. harzianum* (Th; 8.43%^{a}), followed by *T. asperellum* (Ta; 9.2%^{a}). All these values were significantly lower than those of the conventional or blank treatment (13.58%^{b}) and the untreated control (20.66%^{c}). These results indicate the biocontrol potential of *Trichoderma* against a foliar pathogen, even when applied to the soil, as documented in other systems, such as the control of *Cercospora beticola* in beet (Galletti *et al.*, 2008) and *Exserohilum turcicum* in maize (Limdolthamand *et al.*, 2023), suggesting the activation of induced systemic resistance mechanisms in the plant.

Effect of conidial concentration. No significant differences (Tukey, $p < 0.05$) were found between the two concentrations evaluated, with C1 (8.40%^{a}) being as effective as C2 (8.76%^{a}) in reducing severity. This finding is of great practical relevance, as the lower dose is more economical. The effectiveness of concentrations around 1×10^{8} conidia mL^{-1} has been reported for reducing and controlling foliar diseases in various plants and is commonly used in greenhouse studies (Galletti *et al.*, 2008; Limdolthamand *et al.*, 2023), to promote growth in tomato (Celis-Perera *et al.*, 2023), control damping-off in *Capsicum chinense* (Larios *et al.*, 2019), and improve seed germination in passion fruit (*Passiflora* *edulis* var. *flavicarpa*), supporting the feasibility of its field use (Cubillos-Hinojosa *et al.*, 2009).

Influence of the initial plant health status. This factor was highly significant (Tukey, $p < 0.05$). Plants initially classified as asymptomatic showed a significantly lower final average severity (8.06%^{a}) than those initially symptomatic (13.37%^{b}). This indicates that the treatments were more effective under preventive than curative conditions. Asymptomatic plants, having a better physiological condition, likely had a greater capacity to generate a robust defense response induced by *Trichoderma* (Aswani *et al.*, 2022). This result reinforces the importance of implementing biocontrol strategies preventively within integrated rust management.

Interaction between *Trichoderma* and plant health status. A significant interaction effect (Tukey, $p < 0.05$) was detected in the effectiveness of the treatments according to the initial condition of the plant. In asymptomatic plants, the consortium Th_Ta (5.99%^{ab}) and *T. harzianum* (Th; 5.61%^{a}) formed the statistical group with the lowest severity, followed by *T. asperellum* (Ta; 8.61%^{abc}). In contrast, in symptomatic plants, *T. asperellum* (Ta; 8.25%^{abc}) was the most effective strain, showing a severity statistically equal to that of treated asymptomatic plants and significantly lower than that of the consortium (Th_Ta; 10.2%^{cd}) and *T. harzianum* (Th; 12.8%^{de}) in this group (Figure 1A).

This result suggests a specialization in the mechanisms of action of the strains. While the consortium (Th_Ta) appears to be ideal for preventive protection in asymptomatic ("healthy") plants, possibly due to a synergistic effect that enhances defense induction, *T. asperellum* (Ta) showed a greater ability to induce a curative or containment response in symptomatic ("diseased") plants. This systemic effect, mediated from the rhizosphere to the foliage, strongly supports the hypothesis that *Trichoderma* activates systemic resistance mechanisms in the plant. This phenomenon, widely documented (Shoresh *et al.*, 2005), is manifested in protection against various foliar pathogens such as *Cercospora beticola* (Galletti *et al.*, 2008), *Exserohilum turcicum* (Limdolthamand *et al.*, 2023), and *Phytophthora palmivora* (Sirikamonsathien *et al.*, 2023). Such activation involves the expression of defense genes in tissues distant from the site of pathogen infection, which is a hallmark of induced systemic resistance (Mukherjee *et al.*, 2012).

Interaction between *Trichoderma* and concentration. The interaction effect between these factors was not significant ($p > 0.05$). This confirms that the effectiveness of both concentrations (1×10^{8} and 1×10^{12} conidia mL^{-1}) was consistent across the different strains, regardless of whether the plants were initially asymptomatic or symptomatic, reinforcing the recommendation to use the lower dose (Figure 1D).

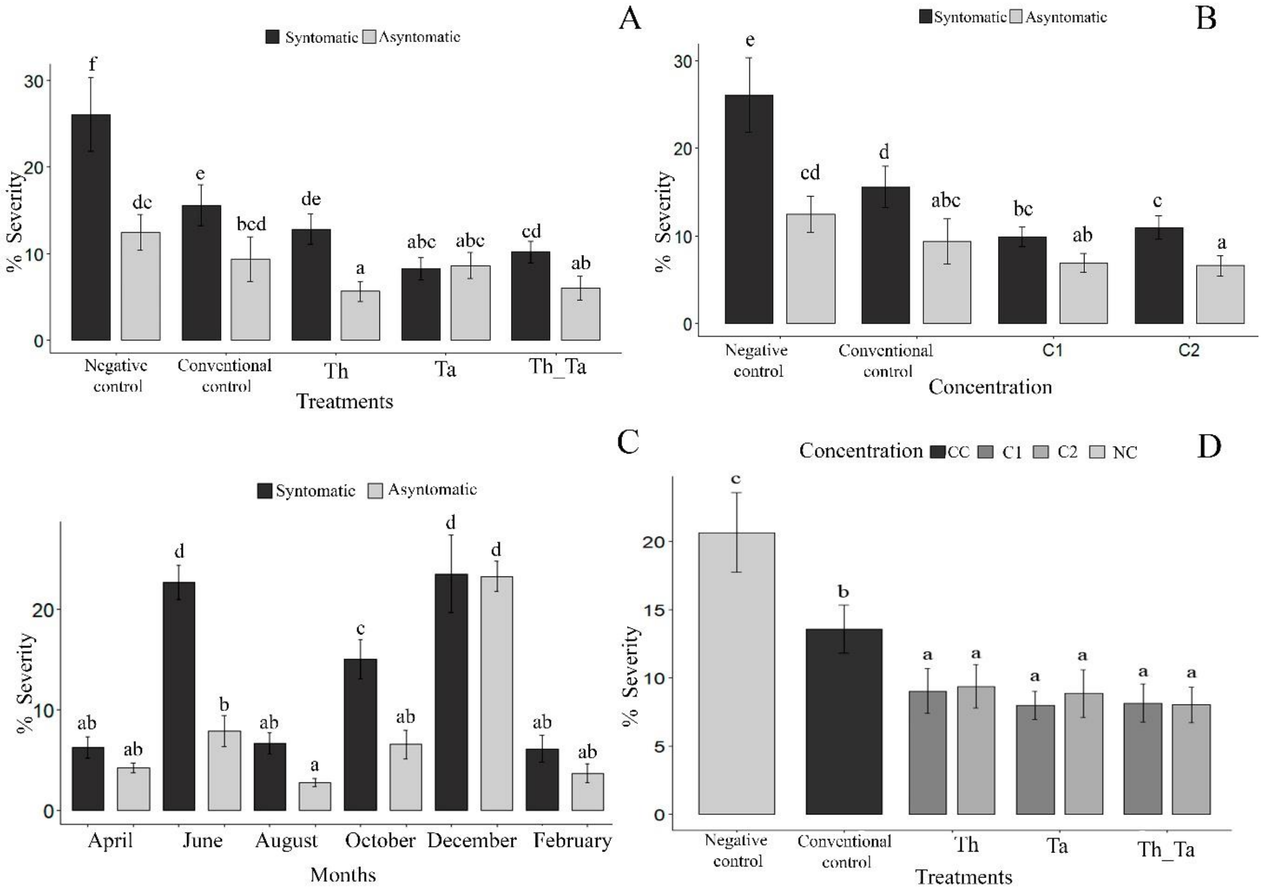

Figure 1. Effect of treatments with *Trichoderma* spp. on the severity of coffee rust (*Hemileia vastatrix*) in symptomatic and asymptomatic plants. (**A**) Interaction between plant type (symptomatic and asymptomatic) and treatments; (**B**) Interaction between plant type and concentration (C1 = 1×10^{8} conidia mL^{-1}, C2 = 1×10^{12} conidia mL^{-1}); (**C**) Variation in severity throughout the year; (**D**) Interaction between treatments and concentrations. Bars indicate mean ± standard error. Significant differences were determined by repeated-measures ANOVA and Tukey's test ($p < 0.05$).

Interaction between concentration and plant health status. Significant differences were found (Tukey, $p < 0.05$). The greatest benefit of applying *Trichoderma* was observed in asymptomatic plants, where both concentrations, C2 ($6.57\%^{a}$) and C1 ($6.90\%^{ab}$), achieved the lowest severity levels, forming the statistical group of highest efficacy. In contrast, symptomatic plants showed higher overall severity. However, *Trichoderma* treatments at both concentrations significantly reduced the disease compared with the conventional or blank treatment ($16.69\%^{d}$) and especially with the untreated control ($27.76\%^{e}$) (Figure 1B). This pattern demonstrates that although the initial health status is a determining factor, the application of *Trichoderma* provides significant control in both scenarios. The ability to maintain the lowest severity levels in asymptomatic plants reinforces the concept that a plant with an intact immune system responds more robustly to microbial elicitors, maximizing the expression of induced systemic resistance (Aswani *et al.*, 2022; Shoresh *et al.*, 2005). Even in symptomatic plants, the reduction in severity suggests that *Trichoderma* can modulate plant physiology to slow pathogen progression. The absence of a clear difference between C1 and C2, even within each plant type, further supports the robustness and reliability of the lower dose (1×10^8 conidia mL^{-1}) for practical field implementation, whether in preventive or curative strategies.

Temporal variation in severity. A highly significant seasonal fluctuation was observed (F = 18.92; df = 5; $p < 0.001$), directly influenced by the climatic conditions of the region. The lowest values were recorded during the dry months: August (4.71%^{a}), February (4.89%^{a}), and April (5.23%^{a}), when high solar radiation and low precipitation naturally limit the germination of urediniospores (Toniutti *et al.*, 2017). In contrast, the highest disease peaks occurred during the humid months: June (15.29%^{c}), October (10.79%^{b}), and especially December (23.39%^{d}). The increase in severity during these months coincides with periods of higher rainfall (Parada-Molina *et al.*, 2020) and the concurrence of high humidity and moderate temperatures, which create optimal conditions for fungal germination and dispersal (Avelino *et al.*, 2006; Hinnah *et al.*, 2020). It is noteworthy that the type of rainfall is a determining factor: heavy rain can wash away spores, whereas light or splashing rain favors their germination and dispersal on the underside of the leaf (Lasso *et al.*, 2020; Li *et al.*, 2023). Additionally, the sharp peak in December may be attributed to the mechanical dispersion of spores facilitated by harvest activities (Moreira-Morrillo *et al.*, 2023).

Effectiveness of treatments over time. The analysis of the treatment-by-month interaction revealed that the most effective combinations were *T. harzianum* in February (1.86%^{a}), *T. asperellum* in February (2.14%^{a}), the Th_Ta consortium in August (2.93%^{ab}), and *T. harzianum* in August (3.55%^{ab}), which formed the statistical group with the lowest severity. In contrast, the highest severity values corresponded to the untreated control in December (49.11%^{j}) and October (23.45%^{i}). All *Trichoderma* treatments maintained the disease significantly controlled during these critical months (October–December) (Figure 2). This control under high inoculum pressure suggests that *Trichoderma* induced a state of systemic resistance in the plant. It has been demonstrated that the genus *Trichoderma* acts as an elicitor, activating systemic defense responses in the host plant (Woo *et al.*, 2006). Mechanisms such as the expression of genes encoding chitinases and β-1,3-glucanases (Shoresh & Harman, 2008), as well as the involvement of jasmonic acid and ethylene signaling pathways (Mamani-Huayhua *et al.*, 2021), create an enhanced "early warning" state that enables the plant to respond more effectively to subsequent infections by foliar pathogens, as observed in systems such as bean (Yedidia *et al.*, 2001) and cucumber (Yedidia *et al.*, 2003).

The ability of the soil-applied strains to reduce the severity of a foliar pathogen strongly supports that this same induced systemic resistance mechanism (Pocurull *et al.*, 2019) was operating in the coffee crop, being sufficiently potent to mitigate infection even under the most favorable environmental conditions for *H. vastatrix*.

Influence of initial plant health status on temporal dynamics. The analysis of the interaction between initial plant health status and evaluation month revealed a significant pattern (Tukey, $p < 0.05$). Throughout most of the year, asymptomatic plants maintained significantly lower severity than symptomatic plants.

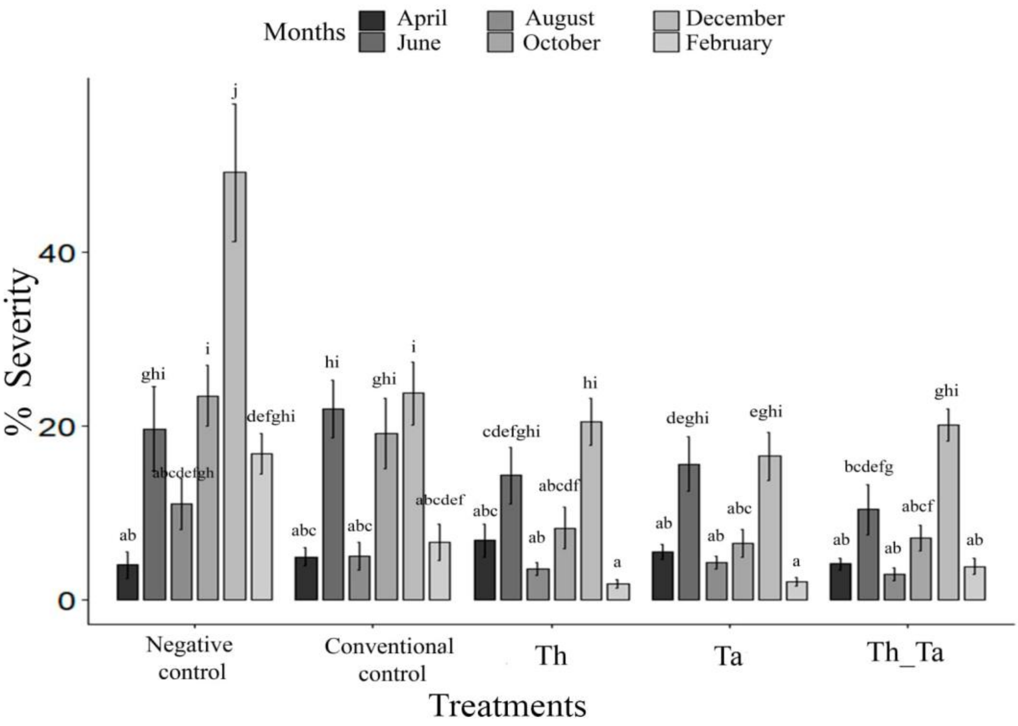

Figure 2. Interaction between *Trichoderma* spp. treatments and evaluation months on the severity of coffee rust. The figure shows the temporal evolution of severity in plants treated with *T. harzianum* (Th), *T. asperellum* (Ta), their consortium (Th_Ta), the conventional management (blank), and the untreated control. Bars represent mean ± standard error. Significant differences were determined by repeated-measures ANOVA ($p < 0.05$).

This was particularly evident in June, when asymptomatic plants (7.91%^{b}) showed moderate severity, while symptomatic plants reached one of their highest values (22.67%^{d}). In October, asymptomatic plants remained in an intermediate group (6.55%^{ab}), whereas symptomatic plants reached 15.03%^{c} (Figure 1C). However, the most revealing finding of this interaction was observed during the epidemic peak in December. In that month, both asymptomatic and symptomatic plants converged within the same statistical group of maximum severity (^{d}). This result may indicate that under conditions of extremely high environmental inoculum pressure, the initial advantage of being healthy is overcome by the intensity of the epidemic.

Nevertheless, *Trichoderma* treatments successfully modulated disease severity throughout most of the year. This indicates that the application of the biocontrol agent can buffer the loss of physiological advantage in initially healthy plants while also providing a significant level of protection to already infected plants, possibly through the activation of induced systemic resistance mechanisms previously documented (Pocurull *et al.*, 2019). Taken together, these results demonstrate that soil application of *Trichoderma* spp., particularly at a concentration of 1×10^{8} conidia mL^{-1}, represents a reliable and economically viable strategy for the integrated management of coffee rust, offering both preventive and curative protection and proving especially valuable during periods of highest potential economic loss.

CONCLUSIONS

Soil application of *Trichoderma* spp. is an effective strategy for managing coffee rust. Effectiveness was consistent among the evaluated strains, with the consortium of *T. harzianum* and *T. asperellum* standing out in asymptomatic plants for preventive protection, and *T. asperellum* in symptomatic plants for its curative action. The concentration of 1×10^{8} conidia mL^{-1} proved to be as effective as higher doses, making it the most viable option due to its lower cost. Objective quantification with the Pliman package confirmed that the highest biocontrol efficacy occurs during the epidemic months of October to December, when rust severity is naturally highest. Therefore, the incorporation of *Trichoderma* into integrated management schemes is justified as a reliable, economical, and biologically based practice to reduce the severity of *H. vastatrix* under field conditions.

Limitations

This study did not include the measurement of in situ microclimatic variables to quantitatively correlate them with disease severity, nor was root colonization by *Trichoderma* quantified; therefore, its establishment and persistence are inferred from the effects observed on the foliage.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Sandra Lizveth Enríquez López: article writing, data analysis, and experimental execution. Gerardo Alvarado Castillo: project supervision, interpretation of results, and critical review of the manuscript. Carlos Roberto Cerdán Cabrera: guidance in result interpretation and methodological support. Rosalba Argumedo Delira: advisory support and critical analysis of the project. Esteban Escamilla Prado: collaboration in the interpretation of results.

Supplementary material

No supplementary material was included for this article.

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