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Reduction of the Survival of the Soilborne Pathogen *Phytophthora palmivora* on *Theobroma cacao* Pods Through the Application of *Trichoderma* spp.
Reducción de la Supervivencia del Patógeno edáfico *Phytophthora palmivora* en Mazorcas de *Theobroma cacao* Mediante el uso de *Trichoderma* spp.

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SUMMARY

In Ecuador, *Phytophthora palmivora* is an important pathogen affecting the cultivation of *Theobroma cacao*. The survival of this microorganism depends largely on its ability to reproduce in pods left on the soil. This study evaluated fourteen native isolates of *Trichoderma* spp. with the aim of reducing the pathogen's survival and thereby decreasing the potential inoculum present in the soil. The research was conducted in the Microbiology area of the Rumiología laboratory at the Universidad Técnica Estatal de Quevedo (Ecuador). *Trichoderma* and *P. palmivora* strains were obtained from institutional collections and cultured on Potato Dextrose Agar and V8 Juice Agar media, respectively. Dual cultures of biocontrol agents and the pathogen were performed to calculate the pathogen's growth inhibition percentage. Additionally, the isolates were classified according to their antagonistic activity using standard scales. The two best-performing biocontrol strains were selected to conduct pod assays to evaluate their effect on infection and reproduction of artificially inoculated *P. palmivora*. Moreover, the impact of these strains on the reproductive capacity of the pathogen was assessed in naturally infected pods. Strains TR8-N and TR19-N demonstrated superior results, exhibiting over 90% inhibition and high antagonistic capacity. Microscopic observations revealed alterations in pathogen hyphae and evidence of mycoparasitism. In pod assays, those treated with TR8-N showed no symptoms following artificial pathogen inoculation. Both TR19-N and TR8-N reduced pathogen inoculum production on the surface of naturally infected pods. These results indicate that at least two native isolates evaluated could be applied on affected pods to reduce pathogen inoculum in cacao cultivation soils.

Index words: *antagonism, biocontrol, infection, inoculum, zoospores.*

RESUMEN

En Ecuador, *Phytophthora palmivora* es un patógeno de importancia en el cultivo de *Theobroma cacao*. La supervivencia del microorganismo depende de la capacidad para reproducirse en mazorcas dejadas en el suelo. En esta investigación, se evaluaron catorce aislamientos nativos de *Trichoderma* spp. con el propósito de disminuir la supervivencia del patógeno y reducir el inóculo potencial en los suelos del cultivo. El estudio se desarrolló en el área de Microbiología del laboratorio de Rumiología de la Universidad Técnica Estatal de Quevedo (Ecuador). Las cepas de *Trichoderma* y *P. palmivora* se obtuvieron a partir de colecciones institucionales y fueron cultivadas en medios Papa Dextrosa Agar y Agar Jugo V8. Se realizaron cultivos duales de los biocontroladores y del patógeno para calcular el porcentaje de

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inhibición del crecimiento. Adicionalmente, los aislamientos se clasificaron de acuerdo con su actividad antagonista utilizando escalas estándar. Las dos cepas del biocontrolador con mejor desempeño fueron empleadas para realizar ensayos en mazorcas y evaluar el efecto sobre la infección y reproducción de *P. palmivora* inoculada artificialmente. Por otra parte, en mazorcas infectadas naturalmente se evaluó el impacto de las cepas sobre la capacidad reproductiva del patógeno. Las cepas TR8-N y TR19-N mostraron mejores resultados, con porcentajes de inhibición superiores al 90% y alta capacidad antagonista. Observaciones microscópicas permitieron identificar alteraciones en las hifas del patógeno y evidencias de micoparasitismo. En los ensayos en mazorcas, aquellas tratadas con TR8-N no presentaron síntomas tras la inoculación artificial del patógeno. Tanto TR19-N como TR8-N redujeron la producción de inóculo del patógeno en la superficie de mazorcas infectadas naturalmente. Estos resultados demuestran que al menos dos de las cepas nativas de *Trichoderma* evaluadas podrían ser aplicadas sobre mazorcas afectadas para reducir el inóculo del patógeno en los suelos del cultivo de cacao.

Palabras clave: *antagonismo, biocontrol, infección, inóculo, zoosporas.*

INTRODUCTION

Theobroma cacao is one of the most socioeconomically important crops worldwide, valued both for its organoleptic properties and its significance in the food industry (Kongor *et al*., 2024). With a global production exceeding 5.8 million tons annually, cacao represents a critical source of income for smallholder farmers in Africa, Central America, and South America (Osorio-Zambrano, Castillo, Rodriguez, and Terán, 2021). In Ecuador, the cacao production is concentrated mainly in the Coastal and Amazon regions, where cacao also holds social, environmental, and cultural significance (Paredes *et al*., 2022).

Despite its importance, cacao trees face serious challenges due to diseases that cause significant losses. Among these, black pod rot caused by *Phytophthora palmivora* is recognized as one of the most devastating pathology, with estimated losses of up to 30% worldwide (Legavre *et al*., 2015; Solis, Peñaherrera, and Vera, 2021). This pathogen can infect more than 200 plant species and critically affects smallholder farmers who account for over 80% of global cacao production (Surujdeo-Maharaj *et al*., 2016; Perrine-Walker, 2020). The pathogen's viability and the severity of the disease in cacao plantations are closely linked to its ability to survive in crop residues, especially those deposited on the soil. Currently, disease control largely relies on agrochemicals, which pose negative impacts on human health, biodiversity, and the environment, and promote pathogen resistance (Salous, Marcillo, Vargas, and Alcivar, 2020).

Phytopathogens are present in the soil and, under favorable environmental conditions, can infect plants and cause significant economic losses; however, beneficial microorganisms such as Trichoderma spp. play a fundamental role in controlling these pathogens (Torres-Rodríguez *et al*., 2025). In this context, biological control emerges as a sustainable alternative for managing cacao diseases. Among biological control agents (BCAs), fungi of the genus *Trichoderma* stand out for their efficacy against various phytopathogens due to rapid growth, competition for nutrients and space, and ability to exclude pathogens from the same habitat (Herrera-Téllez *et al*., 2019; Sharma, Singh, and Sharma, 2012). Furthermore, biocontrol microorganisms have demonstrated positive outcomes in controlling diseases caused by soilborne and aerial pathogens in various plant species (Nascimento *et al*., 2022; Yao *et al*., 2023; Wang *et al*., 2024).

Within this framework, the present study aimed to evaluate the effect of native strains of *Trichoderma* spp. on the development of *P. palmivora* both *in vitro* and on cacao pod tissues with artificial and natural infections. The hypothesis was that at least one of these strains holds potential as a biological control agent to reduce pathogen growth and viability in affected pods, thereby contributing to more sustainable cacao management and reducing agrochemical dependence in production systems.

MATERIALS AND METHODS

The study was conducted in the Microbiology area of the Rumiología Laboratory at the Technical State University of Quevedo (Ecuador), Experimental Campus "La María." The laboratory environmental conditions were maintained at an average temperature of 25 ± 2 °C and relative humidity of 70%.

The research was conducted using an experimental approach with a completely randomized design (CRD) to determine the antagonistic capacity of fourteen endophytic strains of *Trichoderma* against *P. palmivora*. The native and endophytic isolates of *Trichoderma* spp. used as treatments are part of the Endophytic Microorganism Collection of UTEQ and were previously selected for their activity against other cacao pathogens (Guerrero, Palma, Cevallos y Mónaco, 2025). Colonies for each isolate were obtained on Potato Dextrose Agar (39 g Difco® dehydrated PDA/L distilled water). Ten Petri dishes per strain were inoculated, sealed with paraffin tape (Parafilm®), and incubated in darkness at 25 °C for eight days.

The pure strain of the phytopathogen used as inoculum in this study belongs to the Plant Pathogen Collection of the Microbiology area (UTEQ). For *in vitro* and pod assays, *P. palmivora* colonies were obtained on AV8 media (200 mL V8 vegetable juice, 18 g agar, 3 g calcium carbonate, distilled water to 1 L, pH adjusted to 6.5).

In vitro Inhibition Percentage of Native *Trichoderma* spp. Isolates Against *P. palmivora*

In vitro inhibitory and antagonistic effects of native *Trichoderma* spp. isolates against *P. palmivora* were assessed by dual culture technique on 90 mm Petri dishes containing AV8 culture media. At one end, a 5 mm mycelial disc of *P. palmivora* was placed 2 cm from the edge; at the opposite end, a 5 mm disc of *Trichoderma* spp. was inoculated 5 cm away. Plates were incubated at 25 ± 2 °C. Incubation time was determined by the pathogen growth in the control (without biocontrol). Incubation was stopped when the pathogen completely colonized the control plate surface.

Twelve days after incubation, the diameters of pathogen colonies in the biocontrol treatment plates were measured using a Vernier caliper to obtain accurate values from each Petri dish.

Using the collected data, the inhibition percentage was calculated for each replicate, applying the formula proposed by Morais *et al*. (2022):

$$\% I = \frac{(\mathrm{DC} - \mathrm{DT})}{\mathrm{DC}} * 100 \quad (1)$$

Where: % I corresponds to the pathogen growth inhibition percentage, DC to the diameter of the control colony, and DT to the diameter of the treatment colony.

Evaluation of the Antagonistic Capacity and Effect of *Trichoderma* spp. Isolates on the Development of *P. palmivora*

The antagonism of the strains was evaluated using the scale proposed by Bell *et al*. (1982), which allows assessment of the interaction between *Trichoderma* spp. and phytopathogenic fungi in dual cultures by classifying the outcome according to the area of the medium colonized by each organism. This scale consists of five levels: at level 1, *Trichoderma* completely overgrows the pathogen, while at level 5 the opposite occurs, with the pathogen dominating the surface.

According to this classification, isolates that achieve levels 1 or 2 are considered highly antagonistic, demonstrating an effective capacity to inhibit the pathogen, as shown in Table 1. The evaluation was performed through individual observations in each replicate (Petri dishes), assigning the corresponding scale value based on the relative growth of *Trichoderma* spp. and the pathogen.

Table 1. Scale of Bell *et a*l. (1982) used to assess the level of antagonism.

Scale	Interaction	Interpretation
1	*Trichoderma* spp. grows completely over the pathogen and the culture medium	Highly antagonistic
2	*Trichoderma* spp. grows over two-thirds of the culture medium	Highly antagonistic
3	*Trichoderma* spp. and the pathogen each colonize approximately half of the medium surface (more than one-third and less than two-thirds), and neither dominates the other	Moderately antagonistic
4	The pathogen colonizes at least two-thirds of the medium	Weakly antagonistic
5	The pathogen grows completely over Trichoderma spp. and covers the surface of the medium	Not antagonistic

An isolate of *Trichoderma* spp. will be considered highly antagonistic if its average score is ≤ 2, while higher grades indicate lower or no inhibition.

The antagonistic activity was evaluated using an adaptation of the scale proposed by Soytong (1988) for biocontrol agents of cacao pathogens, as modified by Guerrero *et al*. (2025). This scale rates the antagonism of *Trichoderma* spp. strains based on the degree of inhibition and overgrowth on the pathogen, considering both the reduction of the pathogen colony and the colonization of the medium by the biocontrol agent. The previously calculated inhibition percentages were used for this application, allowing classification of the antagonistic capacity of each strain and selection of the most effective ones according to the ranges established in Table 2.

Effect of Biocontrol Agents on the Vegetative Structures of the Pathogen

The microscopic observations conducted in dual cultures revealed the interaction between *Trichoderma* spp. and the pathogen. Samples of mycelium from the growth zones of the pathogen were extracted using a sterile needle, mounted, stained with methylene blue, and examined under an Olympus CX-22 binocular microscope with a 40X objective. The aim was to identify potential alterations in hyphal structures and evidence of mycoparasitism induced by *Trichoderma* spp. In each biocontrol treatment, five observations were made, along with five controls containing pure cultures of the pathogen. The results were categorized into two types: cellular deformities or alterations in the hyphae, and mycoparasitism, evidenced by wrapping and perforation of the *P. palmivora* hyphae.

Effect of *Trichoderma* spp. Application on Infection and Symptom Development of *P. palmivora* in *Theobroma cacao* Pods

To evaluate the impact of *Trichoderma* spp. application on infection development in cacao pods, the pathogen was artificially inoculated onto pods previously treated with the biocontrol agents. The *Trichoderma* strains selected for this study were chosen based on their performance in inhibition and antagonism bioassays conducted on culture media. The two best-performing isolates were cultured on PDA medium ten days prior to inoculation. Similarly, pathogen cultures aged fifteen days were prepared.

Thirty healthy cacao pods of the CCN-51 variety, approximately four months old, were collected on the first day of the experiment. They were transported to the laboratory in a thermal container, washed initially with tap water and then twice with sterile distilled water, ensuring no epidermal injuries occurred. For *Trichoderma* inoculation, spore suspensions were prepared in sterile distilled water for each isolate. Spores were harvested from ten-day-old PDA cultures using a Drigalsky loop and suspended in sterile distilled water with 0.1% (v/v) Tween 20. Each suspension's concentration was adjusted to 1×10^7 spores per milliliter using a Neubauer chamber.

Ten pods were sprayed to drip point per treatment, applying an average volume of 5 mL per pod. Treated pods were individually placed in polypropylene bags (14" × 9"), containing filter paper moistened with sterile water, creating a humid chamber to favor the growth of both the biocontrol agents and the pathogen.

For inoculation of *P. palmivora*, a 10 mm diameter filter paper disc was previously soaked in a zoospore suspension of the pathogen at a concentration of 1×10^7 zoospores per milliliter of sterile water. The zoospore suspension was prepared from fifteen-day-old cultures grown on AV8 medium and adjusted with a Neubauer counting chamber, following the methodology of Delgadillo-Durán *et al*. (2020). The control consisted of pods inoculated with sterile distilled water. In total, thirty pods were inoculated, with ten per biocontrol treatment and ten for the control. The inoculated pods were incubated in plastic bags for eight days at 25 ± 2 °C, under a twelve-hour daily natural light cycle.

Table 2. Adaptation of Soytong's (1988) scale used to evaluate the antagonistic activity of *Trichoderma* spp. strains.

Interpretation	Inhibition (%)	Symbol
High antagonistic activity	>75 %	++++
Moderate antagonistic activity	61-75 %	+++
Moderate antagonistic activity	51-60 %	++
Low antagonistic activity	<50 %	+
No antagonistic activity		-

Eight days after pathogen inoculation, symptom evaluation was performed by observing visible signs on the pod surface as well as internal symptoms beneath the inoculation site. External evaluation recorded the percentage of pods showing necrosis at the inoculation site, along with presence or absence of characteristic pathogen signs such as mycelium and reproductive structures. In pods with symptoms, cross-sections were made with a scalpel to confirm that necrosis originated directly beneath the inoculated area. The results were compiled into a summary table.

Effect of *Trichoderma* spp. on the Production of Inoculum of *P. palmivora* in Naturally Infected Pods

The isolates evaluated in the previous assay were also used to apply treatments on CCN-51 cacao pods exhibiting natural infection symptoms and signs caused by *P. palmivora*. Selected pods had an external infection percentage between 50 and 60% and fruit length ranging from 12 to 15 cm. All visible signs of the pathogen on the pod surfaces were removed by washing with sterile distilled water, carefully avoiding damage to the pod epidermis. Each pod was then dried using sterile paper towels.

The biocontrol agents were inoculated and incubated in polypropylene bags following the same methodology as the previous assay. Ten pods were treated per biocontrol agent, with an additional ten pods sprayed with sterile distilled water serving as controls.

Evaluations were conducted eight days post-inoculation by collecting reproductive structures of the pathogen present on the symptomatic surface of each pod. For each pod, five samples were collected by scraping signs within a 1 cm² area and suspending each in 10 mL of sterile distilled water. Prior to zoospore counting, suspensions were refrigerated at 5 °C for three hours. Following incubation, counts of reproductive structures were performed using a Neubauer counting chamber, and an average zoospore count per cm² was obtained for each pod.

The zoospore production inhibition percentages (% IZ) were calculated using the following formula:

$$\% IZ = \frac{(\mathrm{ZC} - \mathrm{ZT})}{\mathrm{ZC}} * 100 \quad (2)$$

Where: % IZ corresponds to the percentage of inhibition of zoospore production, ZC to the number of zoospores in the control, and ZT to the number of zoospores in the treatment.

Statistical Analysis

Quantitative variables related to the percentage inhibition of mycelial growth of the pathogen and zoospore production were subjected to statistical analysis to verify the assumptions of residual normality, using the Kolmogorov-Smirnov test with Lilliefors correction at a significance level of 0.05. Additionally, homogeneity of variances was assessed through Levene's test (0.05). Upon confirmation of the fulfillment of both assumptions, an analysis of variance (ANOVA) was performed, followed by mean comparisons using the Scott-Knott test at a 5% significance level for the pathogen's mycelial growth inhibition percentages, and Tukey's test (5%) for zoospore production inhibition percentages. All statistical analyses were conducted using R software, version 4.3.2 (R Core Team, 2023).

RESULTS AND DISCUSSION

The fourteen *Trichoderma* isolates evaluated exhibited variability in their capacity to inhibit the mycelial growth of *P. palmivora*, confirming the antagonistic potential of at least five strains. This finding supports the existence of a functionally active group within the isolate collection, underscoring the biocontrol potential of the genus *Trichoderma*. The Scott-Knott test (0.05) grouped treatments into seven homogeneous clusters, reflecting differences in inhibitory efficacy among the strains (Table 3).

Strains TR8-N and TR19-N stood out for their inhibitory ability, showing the highest percentages of mycelial growth inhibition of *P. palmivora*, with values of 92.63% and 91.92%, respectively. These results were statistically similar, suggesting both strains have significant biocontrol potential. Previous research has documented that *Trichoderma* strains possess mycoparasitic abilities and produce secondary metabolites contributing to the control of pathogens such as *P. palmivora* (Ben M'henni *et al*., 2022). In this study, dual culture assays revealed two distinct behaviors: one group of strains grew parallel to the pathogen without significant interference, and another showed active competition, invading and limiting pathogen development. This behavior aligns with scientific literature highlighting *Trichoderma*'s competitive capacity against other microorganisms.

Table 3. Analysis of the percentages of mycelial growth inhibition of *P. palmivora* according to the Scott-Knott test.

Treatment	Trichoderma strain	Growth Inhibition
		%
T1	TR4-N	22.07 e
T2	TR6-N	16.75 g
T3	TR7-N	23.93 e
T4	TR8-N	92.63 a
T5	TR11-N	88.05 b
T6	TR14-N	20.20 f
T7	TR17-N	85.80 c
T8	TR18-N	80.58 d
T9	TR19-N	91.92 a
T10	TR21-N	81.25 d
T11	TR22-N	21.22 e
T12	TR23-N	23.97 e
T13	TR27-N	89.60 b
T14	TR36-Q	23.22 e
CV		5.94 %

Different letters indicate significant differences among treatments according to the Scott–Knott test ($P \leq 0.05$)..

Other strains, such as TR27-N and TR11-N, grouped in category "b," also showed high inhibition levels with percentages of 89.60% and 88.05%, respectively. These strains performed similarly to the most active isolates, marking them as viable options for biological control of *P. palmivora*. In groups "c" and "d," strains TR17-N, TR21-N, and TR18-N exhibited inhibition percentages between 80% and 85.8%, indicating considerable efficacy, although somewhat lower than the leading strains. Strains TR4-N, TR6-N, TR7-N, TR14-N, TR22-N, TR23-N, and TR36-N exhibited lower inhibition on *P. palmivora* growth, with values below 25%. These results placed these strains in groups "e," "f," and "g" in the Scott-Knott test, indicating limited biocontrol potential against the evaluated pathogen.

This comprehensive evaluation highlights promising *Trichoderma* spp. isolates for future biocontrol applications targeting *P. palmivora* in cacao cultivation. The notable activity of strains TR8-N and TR19-N can be attributed to their highly competitive capacity, supported by mechanisms such as accelerated growth, resource competition, mycoparasitism, antibiosis, and the production of antifungal compounds. These abilities have been widely recognized in previous studies that emphasize the role of *Trichoderma* in the biological control of fungal diseases (Alfiky, Abou, De Vrieze, Haridon y Weisskopf, 2024).

The analysis of antagonism between *Trichoderma* spp. strains and *P. palmivora* using the scales proposed by Bell *et al*. (1982) and Soytong (1988), allowed the classification of strains based on their ability to inhibit pathogen growth. Results were evaluated based on interactions between the biocontrol agent and the pathogen, as well as the percentage inhibition of mycelial growth.

According to Bell *et al*. (1982), the most effective treatments in terms of antagonism against *P. palmivora* were TR19-N (mean value of 1.4), TR8-N (1.8), and TR11-N, TR17-N, TR21-N, and TR14-N, each with a mean value of 2 (Table 4). The remaining strains had values above 2, indicating lower antagonistic capacity. These findings demonstrate that five out of fourteen evaluated strains have high antagonism against *P. palmivora*, highlighting their potential as biocontrol agents. The Soytong (1988) scale confirmed these results, showing seven of the fourteen strains, with inhibition exceeding 75%, were classified as having high antagonistic activity. These highly inhibitory strains included TR19-N, TR8-N, TR27-N, TR11-N, and TR17-N, displaying notable potential to reduce pathogen growth (Table 4).

Table 4. Analysis of the antagonism of Trichoderma spp. strains against P. palmivora according to the scales of Bell *et al*. (1982) and Soytong (1988).

Treatment	Trichoderma strains	Antagonism Bell Scale	Antagonistic capacity Soytong Scale
T1	TR4-N	3.5	+
T2	TR6-N	3.0	+
T3	TR7-N	3.7	+
T4	TR8-N	1.8	++++
T5	TR11-N	2.0	++++
T6	TR14-N	2.0	+
T7	TR17-N	2.0	++++
T8	TR18-N	2.4	++++
T9	TR19-N	1.4	++++
T10	TR21-N	2.0	++++
T11	TR22-N	3.2	+
T12	TR23-N	3.1	+
T13	TR27-N	2.9	++++
T14	TR36-Q	2.7	+

According to Bell's Scale, an isolate of *Trichoderma* spp. is considered highly antagonistic if its average score is ≤ 2, whereas higher scores indicate lower or no inhibition. In Soytong's Scale: (++++) High antagonistic activity, (+) Low antagonistic activity.

Integrating both analyses narrowed the number of strains showing clear potential for pathogen control, consistently highlighting strains TR19-N, TR8-N, TR27-N, TR11-N, and TR17-N. These strains not only exhibited significant inhibition in *in vitro* conditions but also demonstrated notable effects on pathogen development at the microscopic level. Microscopic observations revealed that the most effective strains caused structural damage in pathogen cells, including cytoplasmic disorganization and visible hyphal deformities. Furthermore, a characteristic mycoparasitic phenomenon was observed, such as coiling and perforation of *P. palmivora* hyphae by *Trichoderma* spp. hyphae, indicating a clear mycoparasitic interaction, as documented by Sood *et al*. (2020). These findings support the hypothesis that mycoparasitism mechanisms are fundamental to the biocontrol action of *Trichoderma*.

In addition to mycoparasitism, a combined antibiosis action was observed, manifested as cellular collapse of pathogen structures. This phenomenon aligns with dual functions attributed to enzymes like chitinase, which possess both destructive and antimicrobial properties (Sood *et al*., 2020). The presence of antimicrobial metabolites, such as pentylols, together with these hydrolytic enzymes, facilitates degradation of the phytopathogen's cell wall, as suggested by Kubicek and Harman (1998). These results are consistent with previous studies, such as Prismantoro *et al*. (2024), who demonstrated that *Trichoderma yunnanense* inhibits the growth of *P. oryzae* and *R. solani* through a combination of competition, mycoparasitism, and antibiosis. Moreover, observations of sporangia colonization and hyphal coiling, reported by Sarria *et al*. (2021), reinforce the notion of a direct, specific, and aggressive interaction between *Trichoderma* and *P. palmivora*.

The results from this study provide robust evidence of the biocontrol potential of the evaluated *Trichoderma* spp. strains. Strains TR19-N, TR8-N, TR27-N, TR11-N, and TR17-N (Table 5) were the most effective in inhibiting *P. palmivora* growth in both dual culture assays and microscopic observations. These strains exert their effects through combined mechanisms of mycoparasitism and antibiosis, making them promising candidates for biological control of fungal diseases in cacao crops.

The *in vivo* evaluation of the action of *Trichoderma* spp. strains TR8-N and TR19-N on cacao pods artificially inoculated with *P. palmivora* demonstrated varying levels of effectiveness in pathogen inhibition. Strain TR8-N completely inhibited fungal development under experimental conditions, whereas TR19-N reduced disease incidence to a lesser extent. These results confirm a positive correlation between the effectiveness observed *in vitro* and the capacity of these strains to reduce visible rot symptoms under controlled conditions, underscoring their potential as biocontrol agents in the field.

Table 5. Analysis of the vegetative structures (hyphae) of *P. palmivora* in dual cultures with *Trichoderma* spp. strains.

Treatment	*Trichoderma* strain	Hyphae alterations	Mycoparasitism
T1	TR4-N	-	-
T2	TR6-N	-	-
T3	TR7-N	-	-
T4	TR8-N	*	+
T5	TR11-N	*	+
T6	TR14-N	*	-
T7	TR17-N	*	+
T8	TR18-N	*	-
T9	TR19-N	*	+
T10	TR21-N	*	+
T11	TR22-N	-	-
T12	TR23-N	-	-
T13	TR27-N	-	-
T14	TR36-Q	*	-

Mycelium with altered cell structures and cytoplasm, disorganization of cellular contents, +: mycoparasitism.

The high competitiveness of *P. palmivora* is attributed to its ability to produce reproductive structures that persist in soil, serving as an inoculum reservoir. These structures can germinate directly on plant surfaces or release motile zoospores that, in the presence of water, seek entry points to infect plant tissues (Carella, Gogleva, Tomaselli, Alfs y Schornack, 2018; Mohamed, Sundram y Ramachandran, 2019). Such traits, combined with high humidity and warm temperatures, favor pathogen spread. In this context, the efficacy of TR8-N and TR19-N lies in their ability to create an inhospitable environment for the pathogen, significantly reducing disease incidence. In contrast, untreated pods exhibited higher infection levels, consistent with findings by Ndoungué *et al*. (2021), who highlighted disease spread primarily via zoospores entering through wounds, facilitated by conducive environmental conditions.

The results in Table 6 clearly illustrate these differences: pods treated with TR8-N showed no necrosis symptoms or pathogen signs, while those treated with TR19-N exhibited 20% internal and external necrosis incidence. In the control inoculated with *P. palmivora* and treated with sterile water, infection reached 50%, displaying visible mycelium and pathogen reproductive structures.

This comprehensive assessment highlights the promise of TR8-N and TR19-N strains as effective biocontrol agents against *P. palmivora* in cacao crops, aligning with sustainable disease management goals.

The isolates TR8-N and TR19-N demonstrated the ability to inhibit zoospore production in cacao pods naturally infected with *P. palmivora*. Both *Trichoderma* spp. strains significantly reduced the production of the pathogen's reproductive structures by 59.47% (TR8-N) and 62.11% (TR19-N), as shown in Table 7.

Table 6. Effect of the application of two *Trichoderma* spp. strains on the development of P. *palmivora* in cacao pods.

Treatments	Pods with external necrosis	Pods with internal necrosis	Pathogen signs in necrotic areas
	- % -		
Trichoderma spp. strain TR8-N	0	0	-
Trichoderma spp. strain TR19-N	20	20	+
Control (sterile water)	50	50	+

+ Presence of signs, mainly mycelium and some reproductive structures; - Absence of signs.

Table 7. Effect of the application of two *Trichoderma* spp. strains on the development of P. *palmivora* inoculum in cocoa pods with natural infection.

Treatments	Inhibition of zoospore production
	%
Trichoderma spp. strain TR8-N	59.47 a
Trichoderma spp. strain TR19-N	62.11 a
Control (sterile water)	0 b

Different letters indicate significant differences among treatments according to the Tukey test ($P \leq 0.05$).

The outstanding performance of strains TR8-N and TR19-N suggests that these isolates intervene in key stages of the infection cycle through mechanisms such as nutrient competition, mycoparasitism, and production of antifungal metabolites. Recent studies document these mechanisms: Youassi *et al*. (2024) demonstrated that extracts from *T. asperellum* significantly reduce necrosis in cacao fruits infected by *P. megakarya* by inducing defense enzymes, while Sofiana, Kuswinanti, Rosmana, and Ubaidillah (2025) reported up to a 77% reduction in fruit rot due to secondary metabolites of *T. asperellum*. These findings reinforce the potential of *Trichoderma* spp. as a tool in integrated cacao disease management, especially regarding its effect on pathogen survival and inoculum production, which are closely related to infected pod residues and the soil where they are deposited.

The variability in effectiveness between strains observed in this study underlines the need for rigorous isolate selection in biocontrol programs. Manzar *et al*. (2022) emphasize that *Trichoderma* spp. efficacy depends on both biological characteristics and environmental conditions during application. Moreover, the fact that native strains achieved comparable or superior results to well-documented foreign strains highlights the importance of leveraging local biodiversity in biological control strategies for diseases caused by pathogens capable of surviving in crop soils. Beyond direct pathogen antagonism, *Trichoderma* spp. offers agroecological sustainability benefits by enhancing nutrient uptake and stimulating plant growth. This effect is linked to enzymes such as proteases, cellulases, and chitinases that degrade complex soil compounds and release nutrients usable by plants (Harman, 2006; Turaeva *et al*., 2020). The present study's results demonstrate the potential of native *Trichoderma* spp. strains to reduce pathogen inoculum such as *P. palmivora*, preventing its accumulation in soil and thereby reducing future disease incidence. By inhibiting the pathogen's reproductive structures and activity on these residues, the biocontrol strains help to lower the overall disease pressure in the plantation ecosystem, supporting sustainable disease management and reducing crop losses. This finding underscores the importance of using effective native strains in integrated disease control programs for cacao with a focus on soil health.

CONCLUSION

The inhibition percentages of *P. palmivora* development by endophytic *Trichoderma* spp. strains used in this study showed that half of the isolates inhibited more than 80% of the pathogen's development, indicating that these native organisms have significant potential to suppress this cacao pathogen. From the analysis of antagonistic capacity and the effect of *Trichoderma* spp. isolates on *P. palmivora* growth *in vitro*, it is inferred that at least five of the fourteen evaluated strains possess potential for reducing *P. palmivora* growth: TR19-N, TR8-N, TR27-N, TR11-N, and TR17-N, with TR19-N and TR8-N standing out.

When evaluating the effect of applying TR19-N and TR8-N strains on infection and symptom development in *T. cacao* pods under controlled conditions, TR8-N completely inhibited pathogen development, highlighting its efficacy among the thirteen strains tested. Both TR19-N and TR8-N also considerably inhibited pathogen inoculum production in pods with natural oomycete infection. The application of the two biocontrol strains, TR8-N and TR19-N, demonstrated potential to reduce the quantity of inoculum that can be produced in cacao pods left on the soil in cacao plantations. This reduction in inoculum production is critical because pods remaining on the ground serve as reservoirs of *P. palmivora* inoculum, which can lead to future infections in cacao crops.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Data are however available from the authors upon reasonable request and with permission of UTEQ.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

Conceptualization and Formal analysis: R.G.; Methodology: R.G. and C.M.; Funding acquisition: R.G.; Investigation: R.G. and D.V.S.; Data curation: R.P.P. and M.A.F.; Writing – original draft preparation: R.G. and D.V.S.; Writing – review and editing: R.P.P. and C.M.

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