



EFFECT OF NATIVE TRICHODERMA STRAINS ON MONILIOPHTHORA RORERI INFECTION IN THEOBROMA CACAO L.

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ABSTRACT

Objective: To determine the effect of the application of native *Trichoderma* strains on the infection caused by *Moniliophthora roreri* in *Theobroma cacao*.

Theoretical Framework: Cacao has global importance due to its direct relation to chocolate production. Each year, in various countries, cacao cultivation faces issues such as moniliasis (*Moniliophthora roreri*), which reduces crop yields. Traditional disease control methods are ineffective, necessitating new alternatives, such as the application of *Trichoderma* spp.

Method: The antagonistic capacity of forty native endophyte *Trichoderma* isolates against *M. roreri* was evaluated. The ten best-performing strains were sprayed on healthy cacao pods. The number of pods affected by moniliasis, healthy pods, and the internal and external severity of infected pods were assessed.

Results and Discussion: 32.5% of the *Trichoderma* strains inhibited more than 90% of the radial growth of *M. roreri*. All *Trichoderma* isolates showed high antagonistic activity. The evaluated isolates increased the number of healthy pods, while six strains reduced moniliasis by up to 35%. The severity of *M. roreri* was lower in treated pods compared to the untreated control pods.

Research Implications: The study results highlight the need for continued evaluation of *Trichoderma* strains to develop a future tool for reducing *M. roreri* infections in plantations of small and medium-sized farmers.

Originality/Value: Few studies on local endophytic strains of *Trichoderma* spp. have been conducted in the study area.

Keywords: *Trichoderma*, cacao, biocontrol, *Moniliophthora*, endophyte.

EFEITO DE CEPAS NATIVAS DE TRICHODERMA NA INFECÇÃO POR MONILIOPHTHORA RORERI EM THEOBROMA CACAO L.

RESUMO

Objetivo: Determinar o efeito da aplicação de cepas nativas de *Trichoderma* sobre a infecção causada por *Moniliophthora roreri* em *Theobroma cacao*.

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Teórico: O cacau tem importância mundial devido à sua relação direta com a produção de chocolate. A cada ano, em diversos países, seu cultivo enfrenta problemas como a monilíase (*Moniliophthora roreri*), que reduz as colheitas. O controle tradicional de doenças não é eficaz, o que exige novas alternativas, como a aplicação de *Trichoderma* spp.

Método: Avaliou-se a capacidade antagônica de quarenta isolados nativos de *Trichoderma* contra *M. roreri*. As dez cepas com melhor desempenho foram aplicadas em vagens saudáveis, e foram avaliados o número de vagens com monilíase, o número de vagens saudáveis e a severidade interna e externa nas vagens infectadas.

Resultados e Discussão: 32,5% das cepas de *Trichoderma* inibiram mais de 90% do crescimento radial de *M. roreri*. 100% dos isolados do agente de biocontrole apresentaram alta atividade antagônica. Os isolados avaliados aumentaram o número de vagens saudáveis, enquanto seis cepas reduziram a incidência de monilíase em até 35%. A severidade de *M. roreri* foi menor nas vagens tratadas em comparação com as vagens do controle sem aplicação.

Implicações da Pesquisa: Os resultados do estudo destacam a necessidade de continuar avaliando cepas de *Trichoderma* para desenvolver futuramente uma ferramenta que reduza as infecções por *M. roreri* em plantações de pequenos e médios agricultores.

Originalidade/Valor: Poucos estudos com cepas endofíticas locais de *Trichoderma* spp. foram realizados na área de estudo.

Palavras-chave: *Trichoderma*, cacau, biocontrole, *Moniliophthora*, endófito.

EFFECTO DE CEPAS NATIVAS DE TRICHODERMA SOBRE LA INFECCION POR MOLINIOPHTHORA RORERI EN THEOBROMA CACAO L.

RESUMEN

Objetivo: Determinar el efecto de la aplicación de cepas nativas de *Trichoderma* sobre la infección producida por *Moniliophthora roreri* en *Theobroma cacao*.

Marco Teórico: El cacao tiene importancia mundial por su relación directa con la producción del chocolate. Cada año en varios países su cultivo se enfrenta a problemas como la moniliasis (*Moniliophthora roreri*) que reduce sus cosechas. El control tradicional de enfermedades no es efectivo por lo que se requieren nuevas alternativas como la aplicación de *Trichoderma* spp.

Método: Se evaluó la capacidad antagónica de cuarenta aislamientos nativos de *Trichoderma* frente a *M. roreri*. Las diez cepas con mejor desempeño se asperjaron en mazorcas sanas y se evaluó el número de mazorcas con moniliasis, sanas y severidad interna y externa en las mazorcas con Moniliasis.

Resultos y Discusión: El 32,5% de las cepas de *Trichoderma* inhibieron más del 90% del desarrollo radial de *M. roreri*. El 100% de los aislamientos del biocontrolador fueron altamente antagonistas. Los aislamientos evaluados incrementaron el número de mazorcas sanas, mientras que seis cepas disminuyeron la Moniliasis hasta en un 35%. La severidad de *M. roreri* fue menor en las mazorcas tratadas que en las del control sin aplicación.

Implicaciones de la investigación: Los resultados del estudio señalan la necesidad de seguir evaluando cepas de *Trichoderma* para poder obtener a future una herramienta para disminuir las infecciones de *M. roreri* en las plantaciones de pequeños y medianos agricultores.

Originalidad/Valor: Pocos estudios con cepas endofitas locales de *Trichoderma* spp. se han realizado en la zona de estudio.

Palabras clave: *Trichoderma*, Cacao, Biocontrol, *Moniliophthora*, endófito.

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1 INTRODUCTION

Cacao (*Theobroma cacao*) is one of the species closely tied to the history and culture of many populations in Central and South America. Its production has transcended borders and expanded across various tropical regions in countries of South America, Central America, the Caribbean, Africa, and Asia (Daymond & Bekele, 2022).

From cacao seeds, chocolate is produced—one of its most well-known and popular derivatives—whose confectionery trade generates approximately 200 billion dollars (Zhang & Motilal, 2016). For this reason, highly sought-after cacao beans have become a major export product for several countries worldwide, generating billions of dollars in sales annually (Beg et al., 2017).

In 2023, Ecuador produced approximately 375,750 metric tons of dried cacao beans (Statista, 2024), ranking as the third-largest producer in the world. Although cacao cultivation plays a significant role in the international market and Ecuador's economy, the cacao sector faces productivity challenges due to several factors. The main causes include high disease incidence, high production costs, inadequate plantation management, low productivity, the occurrence of natural phenomena (droughts, floods, among others), and, in certain areas, insect pests (Ploetz, 2016; Tosto et al., 2023).

The main strategies developed and studied to control diseases such as frosty pod rot, and others include cultural control, chemical control, genetic resistance, and to a lesser extent, biological control. However, despite efforts to reduce disease incidence, these remain the leading cause of crop losses (Bailey & Meinhardt, n.d.; ten Hoopen & Krauss, 2016a).

2 THEORETICAL FRAMEWORK

Trichoderma spp. is globally recognized as a genus of antagonistic fungi. Antagonistic interactions between *Trichoderma* and other organisms have been classified as antibiosis, mycoparasitism, and competition for space and nutrients (Hjeljord & Tronsmo, 1998; Saldaña-Mendoza et al., 2023).

The phytopathogens control using *Trichoderma* spp. strains has demonstrated various combinations of these antagonistic interactions. In some cases, isolates with high production of secondary metabolites (acting through antibiosis) have also been reported to function as mycoparasites (Chen et al., 2016). Mycoparasitism studies have shown results where pathogen growth inhibition primarily occurs through competition for space and nutrients. Ferreira &



Musumeci (2021) assert that the versatility of the genus enables it to perform effectively in different environments, thanks to its control mechanisms, particularly antagonism.

The most frequently reported species include *T. harzianum*, *T. asperellum*, and *T. viride*, particularly against soil-borne pathogens. Results from studies with these three species show that they can inhibit the growth of phytopathogens by 40% to 90% (de França et al., 2015; Harman et al., 2004; Rosmana et al., 2015; Samuels & Hebbar, 2015; Wang et al., 2021). Lesser-known species have also demonstrated significant performance in studies (Hammad et al., 2021; Piegza et al., 2021).

Research conducted on cacao (since the early 2000s) indicates that native isolates of *Trichoderma* spp. exhibit inhibitory effects and mycoparasitic activity against pathogens. According to ten Hoopen & Krauss (2016a), most attention has been directed at three specific pathogens: *M. roreri*, *M. pernicioso*, and *Phytophthora* spp. Antagonism studies against these pathogens have been carried out with species such as *T. ovalisporum*, *T. harzianum*, *T. koningiopsis*, *T. stromaticum*, and *T. asperellum*, among others.

In Ecuador, studies on antagonism with endophytes in cacao began with research by Evans et al. in 2003, who collected numerous mycoparasite candidates of *M. roreri* from the western region of the country (Evans et al., 2003). However, over the past two decades, there has been little documentation regarding the diversity of endophytic species and their effects on *Moniliophthora* species, which significantly impact cacao health in Ecuador.

For this reason, this research presents the results of antagonism tests conducted between endophytic isolates from cacao farms and the two main cacao pod pathogens. Tests included radial growth inhibition, analysis of antagonistic capacity using Bell's (1982) and Soyong (1988) scales, and examination of the interactions between biocontrol agents and pathogens at *in vivo* level.

3 METHODOLOGY

The present research was conducted in two stages: *in vitro* and *in vivo*. The *in vitro* assays were performed in the Microbiology Laboratory of the Quevedo State Technical University (UTEQ), located at the La María Campus (km 7 on the Quevedo – El Empalme road, Ecuador). The *in vivo* experiment was carried out in a cacao plantation (EET-103) in the locality of El Lechugal, situated in Quevedo, Los Ríos, Ecuador. The selected microorganism collection (forty strains of *Trichoderma* spp. and an isolate of *M. roreri*) used in the study is part of the organisms obtained from previous studies at UTEQ, which were acquired through direct



isolation from the internal tissues of cacao tree trunks.

3.1 ANTAGONISTIC ACTIVITY OF *Trichoderma* spp. AGAINST *M. Roreri*

The objective of the antagonism test was to select the ten most effective isolates. The pathogen cultures were obtained through subculturing on Petri dishes (9 cm in diameter) containing malt extract agar (MEA), prepared from dehydrated medium (33.6 g/L Malt Extract Agar BD Difco™). The subcultures were incubated for fifteen days in a Memmert® incubator at a temperature of 25 °C. The same procedure was followed for the *Trichoderma* spp. colonies.

Three days prior to the confrontation, active culture discs of the pathogen (5 mm in diameter) were placed at one end of the Petri dishes containing MEA medium. This pre-inoculation step was necessary due to the pathogen's slower growth in culture media compared to *Trichoderma* spp. For each *Trichoderma* spp. isolate, ten plates were inoculated, along with ten plates for the control. The biocontrol agents were inoculated by placing 5 mm diameter discs at the opposite end of the Petri dish, 5 cm away from the pathogen inoculum disc. Additionally, ten plates of each pathogen were incubated without biocontrol agents to serve as controls.

The incubation lasted for twelve days, after which the radius of the pathogen cultures (calculated as the average of four segments per colony) and the biocontrol agents in both the treatments and the control were measured. These data were then used to determine the inhibition percentages based on formula (1) from Morais et al. (2022):

$$\% IR = \frac{(CR-TR)}{CR} * 100 \quad (1)$$

donde:

% IR = Inhibition Radio Percent

RC= Control Radio

RT= Treatment Radio

The Bell et al. (1982) scale was used to evaluate antagonism, assigning values from 1 to 5 based on the type of interaction between the organisms, where:

1. *Trichoderma* spp. grows completely covering the pathogen and the culture medium.
2. *Trichoderma* spp. grows over two-thirds of the culture medium.



3. *Trichoderma* spp. and the pathogen each colonize approximately half of the culture medium surface (more than one-third but less than two-thirds), with neither dominating the other.
4. The pathogen colonizes at least two-thirds of the medium.
5. The pathogen grows completely over *Trichoderma* spp. and covers the entire surface of the medium.

A *Trichoderma* spp. isolate was considered antagonistic if its mean score was ≤ 2 , whereas it was classified as non-highly antagonistic if its mean score was ≥ 3 . Furthermore, percentage inhibition data were used to evaluate antagonistic activity based on an adaptation of the scale proposed by Soyong (1988):

- ++++ = High antagonistic activity ($> 75\%$ inhibition)
- +++ = Strong antagonistic activity (61–75% inhibition)
- ++ = Moderate antagonistic activity (51–60% inhibition)
- + = Low antagonistic activity ($< 50\%$ inhibition)
- e = No antagonistic activity

The experiment was conducted using a completely randomized design (CRD) with forty treatments and ten observation units per treatment. Additionally, a control group consisting of the pathogen alone was included.

To verify the assumptions of residual normality, the Kolmogorov-Smirnov normality test with Lilliefors correction (0.05) was applied, while Levene's test (0.05) was used to assess the homogeneity of variances. An analysis of variance (ANOVA) was performed, and mean comparisons were conducted using the Scott-Knott test at a 0.05 probability level.

All statistical analyses were carried out using R software, version 4.3.2 (R Core Team, Vienna, Austria). Informative tables were generated based on the antagonism evaluation results obtained using the Bell and Soyong scales.

3.2 EFFECT OF *TRICHODERMA* SPP. APPLICATION ON *M. RORERI* INFECTION IN CACAO PODS UNDER FIELD CONDITIONS

The experiment was conducted in an eight-year-old EET-103 cacao plantation, which was preconditioned to ensure uniform growing conditions. The preparation involved light pruning, including tip trimming of branches, removal of orthotropic branches and basal shoots, as well as the elimination of pods or branches exhibiting disease symptoms. Additionally, soil



fertilization with macronutrients (NPK 10-30-10) and foliar application of micronutrients (B, Zn, Mg) were performed.

The pods used in the study were obtained through artificial pollination, with an average of fifteen floral cushions pollinated per tree. To prevent natural infection by pathogens, pollinated flowers were covered with plastic bags immediately after pollination. Once the pods reached three weeks of age, ten pods per tree were labeled for each treatment. Additionally, trees were designated as controls. In each *Trichoderma* spp. treatment, ten pods per tree were inoculated, along with an additional ten pods per tree in the control group, which received an application of sterile distilled water.

The spore suspension of the biocontrol agents was prepared using spores obtained from ten-day-old cultures grown on PDA medium (Potato Dextrose Agar, BD Difco™). Spores were collected from the surface of the medium using a Drigalsky loop and suspended in sterile distilled water with 0.01% Tween 80. For each treatment, 250 mL of suspension was prepared and adjusted to a concentration of 1×10^7 to 1×10^8 spores per milliliter by counting the concentration of the stock suspension using a Neubauer chamber.

The spore suspensions were transported to the field at 16:00 in 300 mL plastic manual sprayers. The application was performed on each experimental pod until runoff, with an average of 2 mL of suspension applied per pod. A 5 mL sample of the spore suspension was taken to the laboratory after application to conduct a germination test. This test was performed using five Petri dishes containing 20 mL of PDA medium (Potato Dextrose Agar, BD Difco™). Each plate was inoculated with 100 microliters of the suspension, which was evenly spread over the surface. Spore germination was assessed at 16 and 24 hours post-inoculation (incubated at 25 °C in darkness), and the germination percentage was estimated based on the average of the five inoculated units.

The experiment followed a completely randomized design (CRD) with ten replicates (trees) per treatment. Each tree contained ten pre-labeled pods. Over the four months following antagonist inoculation, monthly evaluations were conducted to record the number of healthy pods and the presence of symptoms associated with *M. roreri* and other pathogens. At the end of the fourth month, the obtained data were used to calculate the percentages of healthy pods and incidence rates of moniliasis.

Once the control pods had reached physiological maturity (last week of the fourth-month evaluation) external and internal disease severity was assessed in symptomatic pods (associated with *M. roreri*) from each treatment. Disease severity was evaluated using the scale proposed by Sánchez (1987), as presented in Table 1.

**Table 1***Severity scale of M. roreri infection in cacao pods.*

Scale	External Severity Symptom	Internal Infection Severity (%)
0	Healthy fruit	0
1	Presence of hydrosis	1 - 20
2	Presence of swelling and/or yellowing	24 – 40
3	Presence of brown spots	41 - 60
4	Presence of mycelium covering up to one-fourth of the brown spot	61 - 80
5	Presence of mycelium covering more than one-fourth of the brown spot	80

Source: Sánchez (1987)

Data on healthy pods, frosty pod rot, witches' broom disease, other pathologies, and cherelle wilt incidence were analyzed using the Shapiro-Wilk normality test (0.05) to assess residual normality and Levene's test (0.05) to evaluate homogeneity of variances. For variables that met these assumptions, an analysis of variance (ANOVA) was performed, followed by the Scott-Knott test to differentiate treatment means. For variables that did not meet the assumptions, logarithmic transformations were applied when necessary. If normality and homoscedasticity were still not achieved, the non-parametric Kruskal-Wallis test was conducted. In cases where significant differences were detected, multiple comparisons were performed using Dunn's post hoc test with Bonferroni correction.

External severity values of pods infected with *M. roreri* were analyzed using the Shapiro-Wilk and Levene tests, followed by ANOVA and Tukey's multiple comparison test at a 0.05 probability level. Since internal severity data did not follow a normal distribution, they were analyzed using the Kruskal-Wallis test. When significant differences were observed, multiple comparisons were conducted using Dunn's post hoc test with Bonferroni correction. All statistical analyses were performed using R software, version 4.3.2 (R Core Team, Vienna, Austria).

4 RESULTS AND DISCUSSIONS

The dual growth assay of *M. roreri* strains alongside native *Trichoderma* spp. isolates demonstrated that all biocontrol strains exhibited the ability to inhibit pathogen development to varying degrees. The analysis of variance (ANOVA) for *M. roreri* inhibition percentages indicated statistically significant differences among the analyzed *Trichoderma* spp. strains (p -value < 0.0001). Table 2 presents the inhibition percentages achieved by each isolate, where 32.5% of the strains were capable of inhibiting more than 90% of the pathogen's radial growth.



According to the Scott-Knott test, inhibition percentage means were grouped into eleven statistically distinct clusters. Notably, 25% of the native *Trichoderma* spp. strains belonged to the group with the highest inhibition rates against *M. roreri*. Within this subset, ten isolates were statistically similar to each other yet distinct from the remaining isolates. The most effective isolates were: TR23-CCN (92.49%), TR1-N (91.53%), TR3-N (91.89%), TR5-N (91.74%), TR29-CCN (91.65%), TR4-N (91.63%), TR31-CCN (91.55%), TR8-N (91.54%), TR21-N (91.51%), and TR11-N (91.47%). These inhibition values are considered high in most available scientific reports.

For instance, Hammad et al. (2021) observed that when *T. atroviride* and *T. brevicompactum* were tested against *Botrytis cinerea*, mycelial growth inhibition ranged between 62% and 65%. Similarly, Taribuka et al. (2017) reported inhibition rates of 50% and 55% when evaluating antagonistic *F. oxysporum* subspecies *cubense* using *T. asperellum* and *T. koningiopsis*, respectively. An et al. (2021) documented inhibition rates ranging from 31.48% to 75.37% for *Fusarium* spp., *Alternaria alternata*, and *Rhizoctonia solani*.

Table 2

Inhibition Percentages and In Vitro Antagonism of Trichoderma spp. Strains Against M. roreri

<i>Trichoderma</i> Strains	% Inhibition		Bell Antagonism	Soytong Antagonism
TR1-N	91,53	a	1	****
TR2-N	88,29	c	1	****
TR3-N	91,89	a	1	****
TR4-N	91,63	a	1	****
TR5-N	91,74	a	1	****
TR6-N	88,59	c	1	****
TR7-N	88,79	c	1	****
TR8-N	91,54	a	1	****
TR9-N	88,55	c	1	****
TR10-N	87,93	d	1	****
TR11-N	91,47	a	1	****
TR12-N	87,41	d	1	****
TR13-N	87,83	d	1	****
TR14-N	88,82	c	1	****
TR15-N	90,02	b	1	****
TR16-N	88,64	c	1	****
TR17-CCN	88,90	c	1	****
TR18-CCN	88,02	d	1	****
TR19-CCN	90,69	b	1	****
TR20-CCN	83,32	g	1,2	****
TR21-CCN	91,51	a	1	****
TR22-CCN	84,79	f	1,1	****
TR23-CCN	92,49	a	1	****
TR24-CCN	79,50	i	1,3	****
TR25-CCN	87,61	d	1	****
TR26-CCN	81,38	h	1	****



TR27-CCN	90,19	b	1	****
TR28-CCN	78,96	i	1,2	****
TR29-CCN	91,65	a	1	****
TR30-CCN	86,04	e	1,1	****
TR31-CCN	91,55	a	1	****
TR32-Q	82,64	g	1	****
TR33-Q	89,20	c	1	****
TR34-Q	77,32	j	1,3	****
TR35-Q	86,07	e	1	****
TR36-Q	85,49	f	1,1	****
TR37-Q	86,38	e	1	****
TR38-Q	75,66	k	1,4	****
TR39-Q	87,38	d	1	****
TR40-N	88,33	c	1	****

Means sharing the same letter within each data group do not differ statistically, according to the Scott-Knott test at a 5% significance level.

The application of the Bell scale (Table 2) revealed that all *Trichoderma* spp. isolates exhibited high antagonistic activity against *M. roreri*, with antagonism values below 2. However, certain strains achieved the lowest possible value (1), indicating slightly higher antagonistic potential compared to the others. The Soyong scale results showed that 100% of the *Trichoderma* isolates demonstrated strong antagonistic activity, with inhibition rates exceeding 75% against *M. roreri*.

The dominance of *Trichoderma* spp. strains on the culture medium surface is attributed to their rapid growth, often leading to overgrowth of the biocontrol agent onto pathogen colonies (Tseng et al., 2020). This phenomenon aligns with findings by Valetti et al. (2022), who reported that *Trichoderma* exhibited rapid growth and inhibited *Valsa ceratosperma* by 75%. Similarly, Panth et al. (2020) suggested that the initial inhibition effect is due to the presence of *Trichoderma* metabolites, which may or may not be followed by parasitic interactions. The *Trichoderma* spp. isolates used in this study also demonstrated the ability to grow over the pathogen, producing mycelium and spores that covered its colonies. According to Dubey et al. (2007), this is a characteristic trait of hyperparasitic organisms. Hyperparasitism in *Trichoderma* spp. is one of the mechanisms that could significantly contribute to reducing pathogen population density (Rubayet & Bhuiyan, 2023).

ANOVA results for the percentage of healthy pods revealed a significant effect (p -value < 0.0001), suggesting statistical differences among treatments. The Scott-Knott test further confirmed significant differences among treatment means, demonstrating that all ten biocontrol treatments were statistically different from the untreated control, which exhibited only 25% healthy pods. Eight *Trichoderma* spp. treatments formed group "a," which included treatments T9, T6, T10, T8, T5, T2, T4, and T7, with the highest percentage of healthy pods, ranging from



43% to 53%. The second treatment group ("b") included T1 and T3, which were statistically similar to each other but significantly different from the control, with a mean healthy pod percentage of 37% (Table 3).

Table 3

Percentage of healthy pods at the fourth month of evaluation in plots treated with native Trichoderma spp. strains.

Treatment	Strain	Healthy pods (%)	
T1	TR 23-CCN	37	b
T2	TR1-N	44	a
T3	TR3-N	37	b
T4	TR5-N	43	a
T5	TR29-CCN	45	a
T6	TR4-N	50	a
T7	TR31-CCN	43	a
T8	TR21-CCN	47	a
T9	TR19-CCN	53	a
T10	TR15-N	48	a
T11 (Control)		25	c

Means sharing the same letter within each data group do not differ statistically, according to the Scott-Knott test at a 5% significance level.

The application of different *Trichoderma* strains effectively protected the fruits of treated trees. The ability of native biocontrol strains to protect cacao pods is a characteristic inherent to endophytic strains. Chaverri & Samuels (2013) suggest that findings from endophyte research can contribute to the development of new disease control strategies. Endophytic *Trichoderma* spp. species are considered more effective in triggering localized or systemic defense mechanisms compared to non-endophytic strains (Chaverri & Samuels, 2013; Natsiopoulos et al., 2024; ten Hoopen & Krauss, 2016b).

The number of cacao pods exhibiting symptoms associated with *M. roreri* infection at the fourth month of evaluation was used to calculate the disease incidence percentages. The number of pods affected by moniliasis was higher than those damaged by other diseases. The results of the Kruskal-Wallis test indicated a statistically significant difference among groups (p -value = 0.001, $\chi^2 = 45.459$), suggesting that at least one treatment group differed significantly from the others in terms of moniliasis incidence. According to the Bonferroni post hoc test, significant differences were observed (Table 4).

Treatments T5, T6, T7, T8, T9, and T10 showed statistically significant differences from the control (T11). No significant differences were found in other pairwise comparisons, as their adjusted p -values exceeded 0.05. Therefore, the observed differences are concentrated in the identified treatment pairs. The distribution of moniliasis incidence across treatments shows that



the control (T11) has the highest median value with the greatest variability, indicating the highest disease incidence. Conversely, treatments T6 and T9 appear to have lower incidence rates and reduced data dispersion.

Tabla 4

Pairwise Comparisons of Treatments Showing Statistical Differences According to the Bonferroni Test (0.05 Probability Level) for the Variable: Incidence of Moniliasis in Cacao Pods.

Treatment	Strain	p-value
T3 - T9	TR3-N	0.0118*
T5 - T11	TR29-CCN	0.0144*
T6 - T11	TR4-N	0.0025*
T7 - T11	TR31-CCN	0.0002*
T8 - T11	TR21-CCN	0.0001*
T9 - T11	TR19-CCN	0.0000*
T10 - T11	TR15-N	0.0006*

Data pairs marked with an asterisk (*) do not differ statistically according to the Bonferroni test at a 5% significance level.

At the end of the fourth-month evaluation, external and internal disease severity was assessed across treatments. The analysis of variance (ANOVA) was performed, revealing statistically significant differences among treatments, with a coefficient of variation of 13.26%. Treatment means were further analyzed using Tukey's test at a 0.05 probability level, which identified significant differences between *Trichoderma* spp. treatments and the untreated control (T11). All *Trichoderma* spp. strains evaluated had a measurable effect on external disease severity, with mean severity scores at least one point lower than the untreated control (3.621). Considering that a severity score of 3 corresponds to the presence of brown spots, while a score of 4 indicates brown spots with spore production, the severity range observed in *Trichoderma* spp. treatments (2.2 to 2.5) suggests that no spore formation was observed in the treated groups.

Tabla 5

*External severity values in cacao pods infected with *M. roreri* in plants treated with native *Trichoderma* spp. strains.*

Treatment	Strain	Frosty Pod Rot External Severity	
T1	TR 23-CCN	2,37	b
T2	TR1-N	2,40	b
T3	TR3-N	2,52	b
T4	TR5-N	2,39	b
T5	TR29-CCN	2,43	b



T6	TR4-N	2,31	b
T7	TR31-CCN	2,31	b
T8	TR21-CCN	2,53	b
T9	TR19-CCN	2,27	b
T10	TR15-N	2,21	b
T11 (Control)		3,62	a

Means sharing the same letter within each data group do not differ statistically according to Tukey's test at a 5% significance level.

Internal severity values were analyzed using the non-parametric Kruskal-Wallis test. The results ($p\text{-value} < 0.001$, $\chi^2 = 41.783$) indicated that at least one treatment group differed significantly from the others in terms of internal moniliasis severity. The Bonferroni test, identified significant differences, which are presented in Table 6. Statistically significant differences were observed between the untreated control (T11) and treatments T2, T4, T5, T6, T7, T8, T9, and T10. The untreated control (T11) exhibited the highest internal severity of moniliasis, with a value of 3.63. According to Sánchez's scale (1987), this severity level corresponds to an infection covering between 41% and 60% of the pod's internal tissue. In contrast, treatments with the lowest internal severity values, such as T10 (2.23), T9 (2.25), and T7 (2.31), exhibited infection levels covering less than 40% of the internal tissue in cacao pods.

Tabla 6

Pairwise Comparisons of Treatments Showing Statistical Differences According to the Bonferroni Test (0.05 Probability Level) for the Variable: Internal Severity of Moniliasis in Cacao Pods.

Treatment	Strain	p-value
T2 – T11	TR1-N	0,0229*
T4 – T11	TR6-N	0.0103*
T5 – T11	TR29-CCN	0.0023*
T6 – T11	TR4-N	0.0021*
T7 – T11	TR31-CCN	0.0001*
T8 – T11	TR21-CCN	0.0001*
T9 – T11	TR19-CCN	0.0000*
T10 – T11	TR15-N	0.0000*

Data pairs marked with an asterisk (*) do not differ statistically according to the Bonferroni test at a 5% significance level.

Findings from the present study indicate that both external and internal disease severity levels were lower in pods treated with *Trichoderma* spp. compared to the untreated control. These results suggest that even in infected pods, the extent of infection could be mitigated by the antagonist's action. According to Tseng et al. (2020), this effect may be attributed to the continued activity of biocontrol agents, even under stress conditions. Furthermore, Tchameni et al. (2017) suggested that the protective response of cacao plants treated with *Trichoderma*



spp. may be influenced by the production of phenolic compounds and amino acids, which play a critical role in plant defense mechanisms.

The Kruskal-Wallis test yielded p -values greater than 0.05 for incidence percentages of cacao pods affected by witches' broom disease, other pathologies, and cherelle wilt, indicating no statistically significant differences in median values among treatments. These results suggest that none of the applied *Trichoderma* spp. strains had a significant effect compared to the untreated control.

5 CONCLUSIONS

The findings of this study demonstrate that all *Trichoderma* spp. isolates evaluated exhibited antagonistic activity against *M. roreri*, with varying degrees of inhibition. The statistical analyses confirmed significant differences among the strains, highlighting their potential as biocontrol agents. The ten most effective isolates demonstrated inhibition rates between 91.47% and 92.49%, values that align with or exceed those reported in previous studies on fungal antagonism. All *Trichoderma* spp. isolates were classified as highly antagonistic, with Bell scale values below 2, and 100% of the strains exhibited strong antagonistic activity (>75% inhibition) according to the Soyong scale.

The application of *Trichoderma* spp. resulted in significantly higher percentages of healthy cacao pods compared to the untreated control. Statistical analysis confirmed that biocontrol treatments significantly reduced the incidence of moniliasis. The incidence of other cacao pod diseases, including witches' broom, cherelle wilt, and other pathologies, was not significantly affected by the application of *Trichoderma* spp.

This study provides strong evidence supporting the use of *Trichoderma* spp. as a biological control agent against *M. roreri* in cacao cultivation. The high inhibition rates observed, combined with the ability of certain isolates to promote healthier pods, highlight their potential for integration into sustainable disease management strategies. Future research should focus on optimizing application methods, understanding strain-specific interactions, and evaluating their efficacy in different agroecosystems.

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