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Application of Biplot Techniques to Evaluate the Potential of *Trichoderma* spp. as a Biological Control of Moniliasis in Ecuadorian Cacao

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Abstract: Cocoa, a crop with great socioeconomic impact in Ecuador, faces continuous challenges that undermine the quality and yield of the crops. Moniliasis, a phytosanitary problem that is difficult to control, is the main limiting factor in cocoa production. This disease is caused by the phytopathogen *Moniliophthora roreri*, which causes premature maturation and necrosis of the ears. In this study, 50 strains of *Trichoderma* spp. in two-culture media, PDA and MEA, were used to evaluate the mycelial characteristics and antagonistic capacity of the strains in individual situations and in circumstances of confrontation against the phytopathogens *Moniliophthora roreri* (MRCP) and *Moniliophtora roreri* (MMCA). The data from the parameters obtained in the in vitro experimental practice were subjected to the multivariate PCA biplot method; the results indicated that five strains exhibited a notable antagonism capacity against the two specific strains of *M. roreri*: strain E22 grown on PDA medium, and the other four (E25, E29, E30, E39) cultivated on MEA medium. The open field trial showed that all treatments based on *Trichoderma* spp. improved productive performance compared to control plantations to which no biopreparation was applied. Significant differences ($p < 0.05$) were reported for all treatments. The efficiency of the biopreparations ranged between 51.26% and 72.46% with yields of 677.86 kg/ha to 976.90 kg/ha, respectively. The *Trichoderma* strain E29 showed the greatest potential for the biological control of *M. roreri* under field conditions. The findings validate the effect of diversified *Trichoderma* biopreparations in the biological control of moniliasis, providing concrete data on the efficacy of the biopreparation under real cultivation conditions and supporting its practical viability.

Keywords: *Theobroma cacao*; *Trichoderma* spp.; *Moniliophthora roreri*; moniliasis; antagonism

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1. Introduction

Cocoa (*Theobroma cacao*) is a plant native to the Upper Amazon basin, between Peru and Ecuador, from where it has since spread to tropical areas of Africa, Asia and the Caribbean [1]. The beans from its fruit are in high demand on a global scale, particularly in developed countries in Europe and North America, where they are used as raw material in the chocolate industry. The work involved in the field contributes to the development of the rural economy and is the source of income for more than 6 million farmers in the world who rely on this activity as their only source of work [2]. Ecuador, a traditionally agricultural territory, is the fourth-largest producer of cocoa in the world and the largest producer of fine aroma cocoa; the contribution to the national Gross Domestic Product (GDP) was 1.98% in 2019, which meant revenues of USD 763,897 million in FOB exports [3].

Phytosanitary problems, such as the invasion of plant tissues by phytopathogenic fungi, are the main factors limiting cocoa production. These pests tend to infect the xylem of the plant and cause a wide spectrum of diseases that undermine crop quality and yield [4].

Moniliophthora roreri is causal fungi of moniliasis in the cocoa crop, with greater relative occurrence in Latin American countries where there is a greater genetic diversity of the species [5,6]. They are strictly asexual-reproducing fungi, producing hyphae, conidia, chlamydospores and conidiophores as survival structures. Infection by *M. roreri* affects only the pods at any stage of development, with young ears being more susceptible, and manifests itself through internal necrosis, irregular brown colonic lesions, premature maturation and external sporulation; it is extremely difficult to manage because it spreads through various forms of dispersal. To control moniliasis, in addition to cultural actions, different strategies have been used, such as genetic and chemical control; however, they have proven to be inefficient and economically unfeasible since phytopathogens develop resistance mechanisms and under stressful circumstances release structures containing genetic material (spores) capable of remaining viable and resisting extreme conditions for long periods [7]. A frequently explored pest management alternative is biological control, a method that uses living organisms as antagonistic agents of the pest organisms to be reduced; this approach is environmentally friendly and contributes to the mitigation of the carbon footprint in agriculture associated with chemical pesticides [8].

In its broadest definition, biological control is a pest management strategy that involves the use of species and their by-products to control phytopathogens and diseases in plantations, through direct colonization reactions or, in turn, through the production of elicitors that induce plant immunity against pathogens and insects [9]. The enzymes secreted by antagonistic agents parasitize the cell wall of pathogens, interfering with metabolic processes; among these biological catalysts, those belonging to the class of hydrolases stand out: proteases, xylanases, cellulases, glucanases, xylanases and pectinases [10]. The high catalytic activity secreted by BCAs favors the hydrolysis of the bonds that unite the structural components of the cell wall of many pest microorganisms, symbiotically affecting the rupture until their decomposition. Each type of enzyme differs in specificities and methods of action and, therefore, is responsible for a different catalysis process. Thus, cellulose degradation is carried out by a complex of cellulases that includes endoglucanases (endo-1,4-p-glucanases), cellobiohydrolases and peroxidases (manganese peroxidases and lignin peroxidase), which produce phenols and alcohols. Once degraded and released, the resulting hydrolytic products can be used by other microorganisms as a source of nutrients [11]. Several investigations adopting this perspective have reported the identification of species of the genus *Trichoderma* that have demonstrated antagonistic activity against a wide range of phytopathogens in different crops [12,13].

Trichoderma spp., because of its easy isolation from soil and mycelial characteristics, has enormous application value and potential in the biocontrol of pest organisms. It has been demonstrated that different species of *Trichoderma* spp. can be manipulated as a natural enemy of *Moniliophthora roreri* in vitro [13]. The success of these species as BCAs is due to the activation of multiple abilities encompassing mycoparasitism, competition for nutrients, deactivation of enzymes of the phytopathogens, and antibiosis. In addition, the growth rate of *Trichoderma* is substantially faster than that of *M. roreri*; consequently, it can effectively disable their growth. This living technology promotes host plant development, controls the density and activity of unwanted organisms, and improves nutrient use efficiency. The direct introduction of *Trichoderma* spp. through fungal spore-based biopreparations into the agricultural environment is subject to a myriad of interactions that may modify the efficacy it initially showed in in vitro trials. For field trials of biocontrol agents (BCA) to be effective, it is necessary to develop biological products that have the greatest compatibility between the three levels (invader, environment and plant) [14]. This situation is complex and difficult to predict due to the constant changes in ecological characteristics and circumstances, as well as other factors that vary the desired impact. In this context, it is essential to examine the performance of *Trichoderma* spp. in individual circumstances as well as in antagonism interactions with *M. roreri*, by means of data mining tools that contribute to the improvement of agricultural systems [15,16].

In recent years, the multivariate statistical analysis of agronomic data has received a great boost because it allows the identification of complex relationships between multiple variables simultaneously. This massive data analysis technology combines mathematical algorithms and big data techniques to broaden the understanding of the interdependence between variables and treatments. The visual representation of existing patterns aids in decision making and, consequently, favors significant advances in agronomic research. There are several algorithms and methodologies that focus on the relationship between different attributes, such as PCA biplot clustering algorithms. The application of these data mining techniques facilitates the understanding of patterns hidden at first sight, synthesizing large amounts of information in a space of smaller dimension [17].

The main objective of this article was to use the multivariate technique PCA biplot to determine the strain of *Trichoderma* spp. that has better mycelial characteristics and greater capacity to induce systemic antagonism in vitro against two exclusive pathogens of cocoa crop: *Moniliophthora roreri* (MRCP) and *Moniliophtora roreri* (MMCA). In addition, the prevalence of moniliasis and the effectiveness of the treatment based on *Trichoderma* spp. were evaluated using the DUNCAN test with a significance level of $p < 0.05$ to compare the treatments based on *Trichoderma* spp., with respect to the control treatment.

2. Materials and Methods

2.1. Acquisition of Microorganisms

In this investigation, 50 strains of *Trichoderma* spp. were used, as were two strains of *Moniliophthora roreri:* (MRCP and MMCA). All isolates were maintained on Potato Dextrose Agar (PDA) Petri plates and deposited in the fungal collection of the Ecuahidrolizados Research and Development Laboratory.

2.2. Culture Media

Two solid culture media (PDA and MEA) were prepared to allow microbial growth, which were prepared individually in different Erlenmeyer flasks. The PDA culture medium was prepared by dissolving 39 g of potato dextrose agar in 1 L of distilled water; the MEA medium was obtained by dissolving 18 g of malt extract with 15 g of bacteriological agar in 1 L of distilled water [18].

The mixtures were sterilized in autoclave at 15 psi (121 °C) for 15 min. After that, they were dispensed in Petri dishes, containing 15 mL each. The media were allowed to solidify for 30 min, and the Petri dishes were sealed and preserved in incubation at 28 °C for 24 h to verify sterility.

2.3. Inoculation of Trichoderma spp. In Vitro

The Petri dishes that did not present contamination were inoculated with the *Trichoderma* spp. strains for mycelium propagation. The purpose of the research of this sowing was to identify the mycelial characteristics of *Trichoderma* spp. in the absence of other microorganisms, as well as the antagonistic capacity against *Moniliophthora roreri* (MRCP) and *Moniliophthora roreri* (MMCA), phytopathogens that cause moniliasis in cocoa.

The experimentation was carried out by cultivating 50 different *Trichoderma* spp. strains individually in Petri dishes of two different culture media (PDA and MEA).

For the in vitro experimentation, the numbering of the strains was carried out in accordance with the following pattern: the letter "E" was used as the prefix, followed by a number from 1 to 50; thus, for example, strain 50 was distinguished as E50.

2.4. Mycelial Growth Evaluation

To calculate the maximum specific growth rate (μ_{max}) and lag time (λ) of the 50 *Trichoderma* spp. strains, the experimental data of mycelial area were fitted to the Baranyi model [19] (Equation (1)):

$$y(t_{max}) = y_{max} + \ln\left(\frac{-1 + e^{\mu max^{\lambda}} + e^{\mu max^{t}}}{\left(-1 + e^{\mu max^{t}}\right) + e^{\left(\mu max^{\lambda} + y_{max} - y_o\right)}}\right) \quad (1)$$

Equation (1). Modified Baranyi Model, where the symbols are defined as follows:

- μ_{max} = specific growth rate; it is monotonic and decreasing;
- y_{max} = microbial population at a given time;
- t = growth time;
- λ = lag phase;
- y_o = initial microbial population.

2.5. In Vitro Antagonism Tests

Discs of 5 mm diameter of *M. roreri* with mycelium that had undergone 7 days of the in vitro inhibitory effect of *Moniliopthora* spp. fungal strains, together and separately against *Trichoderma* spp. isolates, were evaluated using confrontation tests using the dual culture technique. For this, 5 mm diameter discs of the antagonistic fungus (*Trichoderma* spp.) containing 7 days of mycelial growth and discs of the same length of the pathogenic fungus such as *Moniliophthora roreri* (MRCP) and *Moniliophtora roreri* (MMCA) with 4 days of development were placed at the opposite side. The linear growth monitoring of the two opposing colonies was recorded twice daily until the complete colonization of the Petri dish [20].

The percentage of pathogen inhibition (PTA) was calculated based on the radial mycelial growth of the mycelium of the pathogen controls with respect to the *Trichoderma* spp. and pathogen confrontations, using the following formula (Equation (2)) [21]:

$$\mathbf{ATP}(\%) = \mathbf{100} * \left[\mathbf{RPC} - \frac{(\mathbf{RFP} - \mathbf{RIP})}{\mathbf{RCP}}\right] \quad (2)$$

Equation (2). Percentage of pathogen inhibition, where symbols are defined as follows:

- RPC= radial mycelial growth of the pathogen (*Moniliophthora roreri* (MRCP) and *Moniliophtora roreri* (MMCA)) in control culture on the last day;
- RFP = radial mycelial growth of the pathogen in the presence of the antagonist (*Trichoderma* spp.) on the final day;
- RIP = radial mycelial growth of the pathogen on the day the confrontation started.

2.6. Field Experiments

Following the method described in [2], bioformulations based on *Trichoderma* spp. were prepared with a concentration of 1×10^9 conidia per milliliter (conidia/mL). The experiment was carried out at the "Esmeraldas" farm, which hosts cocoa plantations of the CCN-51 variety with 16 years of longevity. The study site, located in the province of Los Ríos at an altitude of 74 m above sea level, had a soil type of clay loam texture and flat topography. The climatic conditions during the research were as follows: an average temperature of 29.75 °C, a humidity ranging from 76% to 88%, and an average annual precipitation of 1173 mm.

The field to be used in the study was subdivided into 22 subplots of 5 m^2 each and consisted of 25 trees. The application of the preparation was foliar and radicular, spreading a dose of 0.3 L per plantation, a process that was repeated every 10 days for 5 months during the entire production cycle from the stage of maximum flowering.

The biopreparations were obtained according to the following formulation:

- Control treatment: no application of *Trichoderma* spp. fungal spores;
- Treatment T1: Use of fungal spores of the *Trichoderma* E22 strain;
- Treatment T2: Use of fungal spores of the strain *Trichoderma* E25;
- Treatment T3: Use of fungal spores of the *Trichoderma* E29 strain;
- Treatment T4: Use of fungal spores of the *Trichoderma* E30 strain;

- Treatment T5: Use of fungal spores of the *Trichoderma* E39 strain.

The prevalence of cocoa fruits with moniliasis symptoms was quantified by means of (Equation (3)) [22].

$$\mathbf{I} = \frac{\mathbf{DP}}{\mathbf{TP}} * \mathbf{100} \quad (3)$$

Equation (3). Prevalence of moniliasis symptoms, where

- I = Incidence (%);
- DP = Number of diseased fruits;
- TP = Total number of harvested pods.

Treatment efficiency was estimated using (Equation (4)) [23]:

$$\mathbf{E} = \frac{\mathbf{FIWoT} - \mathbf{FIWT}}{\mathbf{FIWoT}} * \mathbf{100} \quad (4)$$

Equation (4). Efficiency of biopreparations application, where

- E = Efficiency (%);
- FIWoT = Final percentage of the incidence without the application of *Trichoderma* spp;
- FIWT = Final percentage of incidence with the application of *Trichoderma* spp.

Yield was estimated in kg of dry cocoa beans considering the change in mass quantity during processing, with an equivalent of 40% of dry weight at the end of the drying stage with respect to the fresh weight at harvest.

2.7. Statistical Analysis

The mycelial characteristics of maximum velocity, lag phase and percentage of antagonistic pathogen inhibition capacity were measured in triplicate and the data were subjected to data mining techniques, such as PCA biplot, using R software ver. 4.4.0.

2.7.1. PCA Biplot

Biplot principal component analysis (PCA) is a standard multivariate analysis tool, which focuses on simplifying the information contained in a large number of dimensions and evaluating the behavior of the data in a two-dimensional matrix X[I,P] (two-way table) [24–26].

Singular Values (DVS) are decomposed from a covariance or correlation matrix:

$$\mathbf{A} = \mathbf{U\Sigma V^T}$$

where U and V are orthogonal column matrices containing the left (rows) and right (column) singular vectors, while the matrix Σ contains the non-zero singular values σ_i of X such that $\sigma_1 \geq \sigma_2 \geq \ldots \geq \sigma_r$.

To guarantee the DVS representation, a factorization represented in a set of three matrices is necessary, such that $U \in \mathbb{R}^{Ixr}$, $V \in \mathbb{R}^{Pxr}$ y $\Sigma \in \mathbb{R}^{rxr}$ is a diagonal rectangular matrix of rank r $\leq$ min {I,P}.

The two-dimensional structure details individuals in the rows and variables in the columns, represented as follows [24–26]:

$$\mathbf{X_{[I,P]}} = \left\{ \mathbf{x_{ij}} \middle| \mathbf{i} = 1, \ldots, 1; \mathbf{j} = 1, \ldots, \ldots, \mathbf{P} \right\}$$

2.7.2. Descriptive Statistics

The results of the field experimental test were studied using analysis of variance (ANOVA) to determine the significance of individual differences at the $p < 0.05$ level, cocoa pod yield and treatment efficacy (with or without solution). Subsequently, Duncan's test was applied ($p < 0.05$). For this analysis, the data were submitted to the statistical software (SPSS ver. 26).

3. Results and Discussion

3.1. Statistical Algorithm for Mycelial Characteristics and Antagonistic Capacity of Trichoderma spp. against Moniliopthora roreri

Figure 1 presents the formation of three groups as a function of four variables: lag phase, mycelial growth, antagonistic ability against *M. roreri* (MRCP) and *M. roreri* (MMCA). Figure 1a shows that the cumulative inertness amounted to 85.5%, while Figure 1b shows that the cumulative inertness amounted to 84.8%.

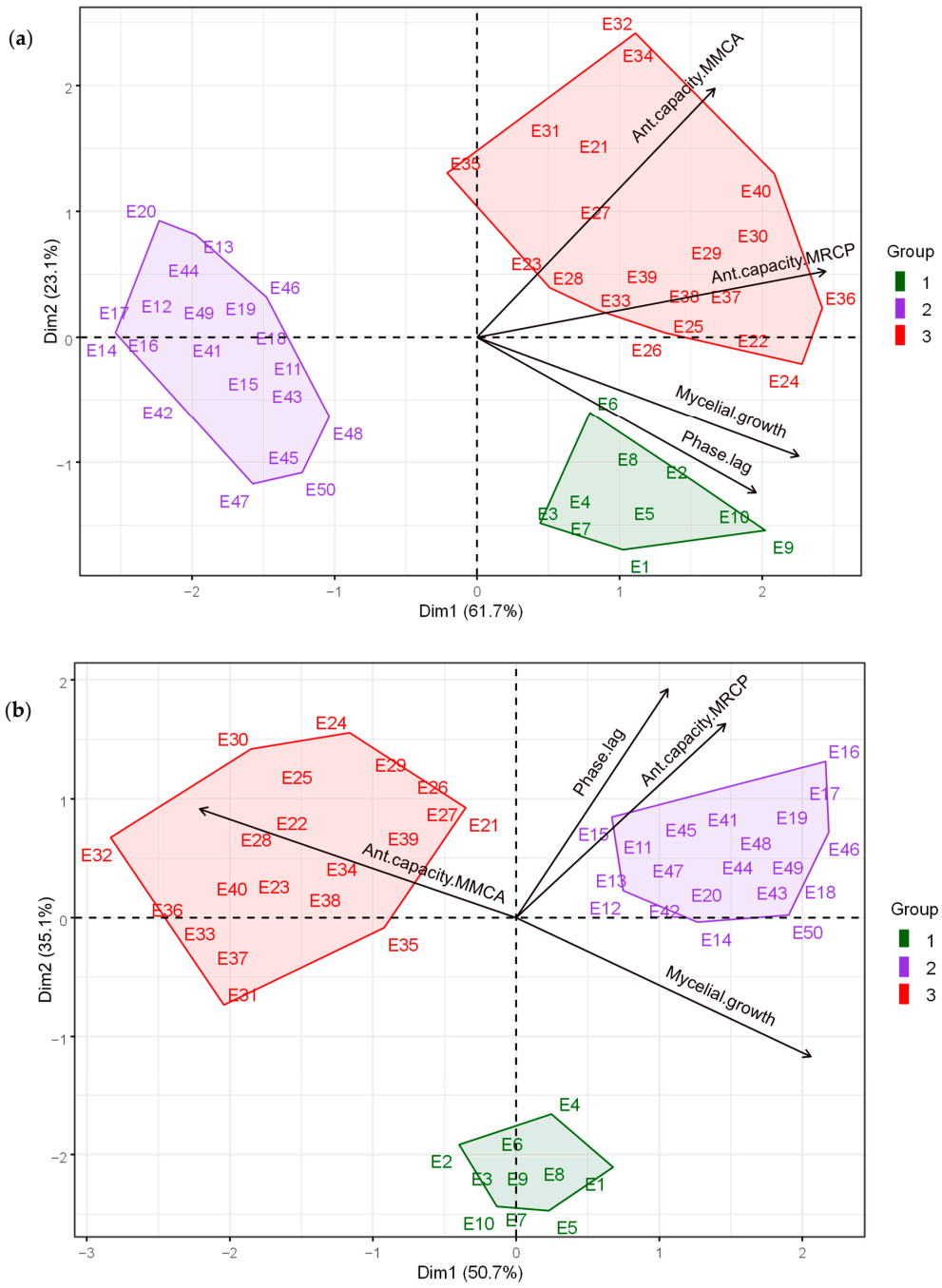

Figure 1. Factorial plots of mycelial characteristics (lag phase and mycelial growth) and antagonistic ability of *Trichoderma* spp. against two strains of *M. roreri* are illustrated. (**a**) PCA biplot for mycelial characteristics and antagonistic ability of *Trichoderma* spp. against *Moniliopthora roreri* grown on PDA medium. (**b**) PCA biplot for mycelial characteristics and antagonistic ability of *Trichoderma* spp. against *Moniliopthora roreri* grown on MEA medium.

Figure 1a indicates the presence of the 50 growths of *Trichoderma* spp. corresponding to the inoculation of the 50 strains on PDA plates. The formation of the groups was as follows: Group 1, "green color", formed by 10 varieties of *Trichoderma* spp. (E1, E2, E3, E4, E5, E6, E7, E8, E9, E10) that indicated the strongest relationship with the mycelial growth characteristic; Group 2, "purple color", formed by 20 varieties of *Trichoderma* spp. (E11, E12, E13, E14, E15, E16, E17, E18, E19, E20, E41, E42, E43, E44, E45, E46, E47, E48, E49, E50), that presented the highest values of antagonistic capacity against *M. roreri* (MCRP) and the lag phase; and Group 3, "red color", was made up of 20 varieties of *Trichoderma* spp. (E21, E22, E23, E24, E25, E26, E27, E28, E29, E30, E31, E32, E33, E34, E35, E36, E37, E38, E39, E40) and showed the relation with antagonistic capacity against *M. roreri* (MMCA). The *Trichoderma* strain E22 represented in the center of Group 3 had a significant contribution in the second dimension of the biplot and was consolidated as the strain grown on PDA with the highest degree of antimicrobial activity against the two specific strains of *M. roreri*.

On the other hand, Figure 1b shows the presence of the 50 growths of *Trichoderma* spp. corresponding to the inoculation of the 50 strains on MEA plates. The groups were distributed as follows: Group 1, "green color", made up of 10 *Trichoderma* spp. (E1, E2, E3, E4, E5, E6, E7, E8, E9, E10), with the highest relationship with the lag phase and mycelial growth; Group 2, "purple color", formed by 20 varieties of *Trichoderma* spp. (E11, E12, E13, E14, E15, E16, E17, E18, E19, E20, E41, E42, E43, E44, E45, E46, E47, E48, E49, E50) with a greater relation to the lag phase; and Group 3, "red color", formed by 20 varieties of *Trichoderma* spp. (E21, E22, E23, E24, E25, E26, E27, E28, E29, E30, E31, E32, E33, E34, E35, E36, E37, E38, E39, E40), which presented the relationship with antagonistic capacity against *M. roreri* (MMCA and MCRP). In this culture medium, the results showed that four strains (E25, E29, E30 and E39) presented the optimal mycelial characteristics, evidencing a close relationship of antagonism against to the two specific strains of *M. roreri.*

Several *Trichoderma* strains have been extensively studied as biological control agents against a wide range of soil and airborne plant pathogenic fungi. The biodiversity of the *Trichoderma* spp. species reveals dynamic characteristics of antagonistic capacity and rapid mycelial growth, which emerge from several attributes, such as the competition for nutrients, the production of antibiotic substances, and resistance to inhibitors and the decomposition of organic substances [27]. The synthesis of antifungal compounds that damage the cell wall of the pathogen causes mycoparasitism, leading to the arrest of its growth and a higher percentage of inhibition [28]. These characteristics can be modified by biotic factors determined by genetic variability or by abiotic conditions related to environmental interactions. Limaco et al. [29] argue that ventilation, humidity and temperature are the main factors influencing fungal evolution. This assertion explains the variations in behavior observed in vitro and ex vitro between each strain of *Trichoderma* spp.

Also, the antagonistic activity of *Trichoderma* to biologically control pests is severely controlled by their individual mycelial characteristics. These interactions influence both the production of antimicrobial compounds and the ability to parasitize other fungi. Hartz first delved into the genetic delimitation of *Trichoderma* spp., emphasizing the relevance of morphological characteristics, especially the presence or absence of specialized structures. In the particular case of *T. viride*, this species' conidiophores are sparsely branched, a condition that reduces the dispersion of conidia, and with it, the effectiveness of *Trichoderma* as agent of biological control. On the other hand, *T. harzianum* develops conidiophores of a verticillate form that propitiate the dispersion of the fungus and the capacity to colonize new substrates [30].

In this analysis, the findings showed that individual mycelial characteristics of *Trichoderma* spp. do not directly correlate with a high index of antagonism when coming into contact with phytopathogens. The *Trichoderma* E9 strain grown in MEA medium developed greater mycelial growth but did not stand out as an antagonist agent. Of the more than 400 recognized species of *Trichoderma*, *T. harzianum*, *T. asperellum*, *T. atroviride*, and *T. atrobrunneum*, among others, have been widely recognized as BCA. However, each species deploys variable mechanisms of action in response to the pathogen to be inhibited

and the surrounding environmental conditions. A frequently underestimated factor is the health and composition of the soil, which, through its chemical and physical properties, whether altered or typical of certain soils, can lead to negative interactions between the biodiversity of beneficial soil microbes, thus minimizing the effectiveness of biopreparations. For this reason, it is important that future studies address a more detailed exploration of the relationships between soil parameters, the mycelial growth of *Trichoderma* spp. and the antagonistic capacity against pathogens.

3.2. Results of Biocontrol with Trichoderma spp. in the Field

The biological treatments made with spores of the *Trichoderma* strains that showed a higher degree of antagonism initially in the in vitro tests also demonstrated an effect on the epidemic intensity of *M. roreri* in the open field, influencing the final incidence and yield (Table 1).

Table 1. Effect of five *Trichoderma* spp. strains on cocoa plantations previously infected with moniliasis.

Strain	Incidence (%) *	Eficience (%) *	Yield (kg/ha) *
Control	22.23 ^{e} ± 1.02	--	630.73 ^{a} ± 9.88
T1	8.33 ^{b} ± 0.20	60.56 ^{c} ± 0.77	831.86 ^{d} ± 16.27
T2	11.26 ^{c} ± 0.40	51.26 ^{a} ± 1.04	677.86 ^{b} ± 40.36
T3	2.50 ^{a} ± 0.40	72.46 ^{e} ± 2.12	976.90 ^{e} ± 25.19
T4	9.23 ^{b} ± 0.75	65.13 ^{d} ± 1.73	761.60 ^{c} ± 27.16
T5	12.60 ^{d} ± 0.78	57.36 ^{b} ± 1.81	673.93 ^{ab} ± 20.99

Control = Treatment without biopreparation; T1 = Treatment based on *Trichoderma* strain E22; T2 = Treatment based on *Trichoderma* strain E25; T3 = Treatment based on *Trichoderma* strain E29; T4 = Treatment based on *Trichoderma* strain E30; T5 = Treatment based on *Trichoderma* strain E39. * Different letters denote significant differences between treatments.

The results indicate that there were significant differences ($p < 0.05$) between treatments in terms of the final incidence of moniliasis, crop yield and efficiency of the biopreparations. The highest incidence of the pest was presented in the control treatment (22.23%) followed by treatment T5 (12.60%) and the lowest incidence was reported with the use of treatment T3 (2.52%); the highest productive yield was manifested in the plantations treated with T3 with a range of 976.90 kg/ha and the lowest in the crops with a control treatment with an average of 630.73 kg/ha of harvest. These findings agree that control treatments without the application of *Trichoderma* spp. are directly correlated with moniliasis proliferation and low crop yield.

In a previous experiment carried out at the same study site, Valenzuela et al. [2] applied treatments based on *Trichoderma* spp., administering a dose of 0.2 mL per plant every 15 days. As findings, a positive impact was observed in cocoa crops treated with antagonistic microorganisms, with yields ranging between 615.20 and 753.10 kg/ha, compared to crops without treatment (533.50 kg/ha). When contrasting these results with those described in the present investigation, a general decrease in the incidence rate of the treated plantations is seen, as well as an increase in the minimum and maximum yields (673.94%, 976.90%, respectively) with respect to the previous research. In addition to the strains of *Trichoderma* spp. used, a relevant difference lies in the amount of dose administered and the frequency of application, because in this new study a higher dose of biopreparation per plant (0.3 mL) was supplied with a reduced frequency (10 days) than previously established. These variations in proportions could represent factors with a direct influence on the incidence of the disease, crop yields and the efficiency of treatments, since it is scientifically proven that inadequate doses contribute to the development of resistance by pathogens and reduce the desired effects.

In a study carried out in Costa Rica, Seng et al. [31] obtained a lower incidence rate in cocoa trees in Treatments 4 and 5 in which *Trichoderma* spp. spores were used (9.15% and 5.78%, respectively). In Peru, Leiva et al. [23] reported that the efficacy of biological treatments ranged from 38.99% to 71.9%. The genetic diversity of each country can also influence the effectiveness of the bioproduct in the soil.

The widespread *Trichoderma* biological control of moniliasis in cocoa crops would greatly increase the production of viable cocoa beans worldwide. Several control strategies have been tried, such as planting cocoa varieties with improved genotypes, chemical treatments and the use of BCA. Breeding programs and chemical treatments have led to the development of varieties with improved genetic resistance to diseases and tolerance to adverse environmental conditions, but they are not effective against *Moniliopthora roreri* because the pathogen evolves in response to genetic improvements. However, the use of *Trichoderma* spp. as a BCA exhibits characteristics that make it a promising tool in the control of this pest since this fungal genus is also constantly evolving, activating defense genes and producing antimicrobial metabolites, and is compatible with other management strategies.

4. Conclusions

The use of *Trichoderma* spp. as a biological alternative to the use of chemical pesticides is of great interest and is at the center of the debate within the scientific community. The PCA biplot statistical technique, as part of data mining, allowed us to identify that the mycelial characteristics of each *Trichoderma* species do not directly correlate with their ability to antagonize the development of pathogenic microorganisms. The variability of mycelial characteristics and the antagonistic capacity of *Trichoderma* spp. cultured in the two-culture media (PDA, MEA) could be attributed to the influence of the nutritional composition on the development of the fungi. On the one hand, the growth of the *Trichoderma* strain E22 in PDA culture medium presented the highest antagonist activity; meanwhile, four *Trichoderma* strains (E25, E29, E30, E39) grown in MEA nutrient medium exhibited the highest inhibitory potential against phytopathogens. The potential of *Trichoderma* as a biological control was evaluated in the open field by applying biopreparations based on the strains with the best in vitro characteristics in cocoa plantations, with which it was obtained that the T3 treatment, formulated with spores of the *Trichoderma* E29 strain, had the higher efficiency rate, a lower incidence of moniliasis and a higher cocoa yield value in the experiment, unlike the control treatment that had a higher incidence with lower yield. The contribution of this research will serve as a basis for evaluating the effect of *Trichoderma* as a biological control agent against moniliasis under different combinations of culture media, from the perspective of multivariate statistics. The present research encountered certain limitations, which led to the identification of future perspectives and aspects that would be advisable to address in subsequent studies in this field.

Limitations:

I. The number of *Trichoderma* strains used in this study was small and may not represent the total diversity that exists in different regions and conditions.
II. The study did not diversify the cultivation conditions of *Trichoderma*, limiting itself to the use of two different culture media (PDA and MEA).
III. The application of biopreparations in the field was conducted on a single farm; thus, these results do not guarantee replicability on farms in different localities under varying agronomic conditions.
IV. The monitoring period for the application of biopreparations in the field was limited to five months; therefore, the sustainability of *Trichoderma* usage was not determined.

Future perspectives:

I. Diversify the *Trichoderma* strains by testing samples from a wide variety of regions to obtain a more accurate understanding of the potential of specific strains as antagonists.
II. Investigate the effects of different culture media and cultivation conditions (temperature, pH, humidity) on the mycelial growth and antagonism of *Trichoderma* strains.
III. Conduct trials in multiple localities and under different cultivation conditions to evaluate the efficacy and consistency of biopreparations on a larger scale.
IV. Perform long-term follow-up studies to confirm the persistence of the antagonism and the influence of biopreparations on soil and plant health over time.

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