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Eco-Friendly Biocontrol of Moniliasis in Ecuadorian Cocoa Using Biplot Techniques

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Abstract: Cocoa is the main crop in Ecuador's agricultural sector and is the most important to the country's economy. This crop is mainly threatened by moniliasis caused by *Moniliophthora roreri* and *Moniliophthora perniciosa*. Monialisis is a disease that causes the watery rot of cocoa beans, causing serious yield losses at crop harvest and great economic losses. In this research, we used 50 *Trichoderma* spp. cultivated in two culture media, PDA and MEA, to demonstrate mycelial growth and antagonistic capacity against two cacao-crop pathogens: *M. roreri* and *M. perniciosa*. Multivariate methods, namely a PCA biplot and a GGE biplot, indicated that four strains of *Trichoderma* spp. (17, 33, 42 and 44) cultivated on the PDA medium had the highest mycelial characteristic values and antagonistic capacities against *Moniliophthora perniciosa.* The experimental test showed that the lowest incidence of moniliasis and highest yield of cocoa occurred when using the treatments based on the *Trichoderma* spp. The results obtained in this study allow the use of strain 42 to control moniliasis in cocoa, avoiding economic losses.

Keywords: biplots; moniliasis; multivariate methods; *Trichoderma*

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1. Introduction

Cocoa (*Theobroma cacao*), originally from South America, has already been domesticated, and is currently being produced in tropical regions of America, Africa and Asia, particularly in the Caribbean and Latin America [1]. This crop requires temperatures between 26 and 27 °C and annual rainfall between 1300 and 2800 mm to achieve higher production and a lower proliferation of pests and diseases [2]. Cocoa production employs around 6 million farmers worldwide [3]. In 2019–2020, Latin America produced 0.9 million tons of cocoa. The Ivory Coast and Ghana are responsible for the highest production (2.1 and 0.8 million tons, respectively), followed by Ecuador (0.32 million tons) [4]. In addition, Ecuador leads in the production and export of fine-aroma cocoa, with 62% of the world market. This product is highly desired due to its unique flavor characteristics [5–7].

Cocoa plantations can be seriously affected by fungal pests, such as *Moniliophthora roreri* and *Moniliophthora perniciosa* [8], also known as moniliasis, which attack the tissues, fruits, floral cushions and buds of the plant. *Moniliophthora perniciosa* infects cocoa when the spores penetrate the meristematic tissues, including pods and flower cushions, leading to the formation of witch's broom and fruit loss [9]. *Moniliophthora roreri* causes frosty pod rot; it is found in most major cocoa-producing areas and causes serious yield losses [10]. Chemical fungicides are used to control moniliasis; however, the chemical pesticides used generate resistance in pathogens, cause damage to the environment in various forms, do not prevent the proliferation of secondary pests, and are not compatible with organic production [11]. Alternative control methods, such as biological control, have been found to reduce plant diseases as effectively as chemical fungicides [12].

Biological control in plants refers to the protection of crops using phytosanitary microorganisms that have antagonistic interactions with the pathogens that cause different diseases [13]. The main mechanisms of action of biological agents against pathogens are indirect and direct methods. Indirect methods use phytopathogenic agents that compete for nutrients and space and, in this way, invade and kill the mycelium of the antagonist. Direct methods use secondary metabolites to inhibit the growth of the pathogen. The success of the direct method is linked to the cultivation of phytosanitary microbes in the correct culture medium to stimulate the production of secondary metabolites and not allow competitive advantage against the antagonist [14].

Endophytic fungi are the most widely used biological agents against different pathogens, such as *Moniliophthora roreri* and *Moniliophthora perniciosa*, as well as bacteria, insects and weeds. Phytopathogens have great benefits, such as their low environmental impact and protecting crops against different pests. This is in contrast to chemical pesticides, which are specific to different plant diseases [15]. *Trichoderma* is the main endophytic fungal species. It is widely known for its ability to antagonize the growth of different plant pathogens through competition for nutrients and space. Additionally, *Trichoderma* spp. produces enzymes that degrade the cell wall, such as chitinases, glucanases and proteases, to parasitize the antagonistic fungi [16]. For these reasons, it is important to evaluate the biological activities of *Trichoderma* spp. against two pathogens that affect cocoa, *M. roreri* and *M. perniciosa,* using data science techniques in order to help farmers make correct decisions.

The databases used in data science can be analyzed using big data techniques and come from different sources: experiments, the Internet or government repositories. Data science uses a series of techniques to analyze multiple variables while providing more precise information by analyzing the structure of data. The most important data science techniques are machine learning modeling, natural language processing, sentiment analysis, neural networks or deep learning analysis used in regression analysis, classification, clustering analysis, association rules, time series analysis, sentiment analysis, behavior patterns, anomaly detection, factor analysis, log analysis and deep learning using the internal structure of data. These numerical methods can be applied to considerable masses of data; they allow data scientists to establish relationships between data and detect the most significant [17]. Conventional statistical techniques have been used in the area of plant biotechnology to improve different commercial properties, such as yield and tolerance of biotic and abiotic stresses; however, reduction methods, such as PCA biplot and GGE biplot, focus on multiple variables and present them in fewer, complex variables [18].

The main goal of this research was to use the multivariate PCA biplot and GGE biplot techniques to determine the strains of *Trichoderma* spp. with the strongest mycelial characteristics and antagonistic capacities against two cocoa-crop pathogens: *Moniliophthora roreri* and *Moniliophthora perniciosa*. Additionally, the incidence of monialisis and the efficiency of the treatment were determined using the DUNCAN test at a significance level of $p < 0.05$ between the treatments with or without biosolution based on the *Trichoderma* spp. used.

2. Materials and Methods

2.1. Fungi Strains

This study used 50 strains of *Trichoderma* spp., 1 strain of *Moniliophthora roreri* and 1 strain of *Moniliophthora perniciosa*. Strains were maintained on PDA dishes and deposited in the fungal collection of the Research and Development Laboratory of Ecuahidrolizados.

2.2. Culture Media

The PDA medium was prepared by combining 39 g of potato dextrose agar in 1 L of distilled water in an Erlenmeyer flask, whereas the MEA medium was prepared by dissolving 18 g of malt extract and 15 g of bacteriological agar in 1 L of distilled water using a flask.

In both cases, the flask was sterilized in an autoclave at 15 psi (121 °C) for 15 min. Subsequently, 10 mL of the sterile medium was poured into Petri dishes. Dishes with solidified medium were placed in plastic bags and incubated at 28 °C for 24 h to verify sterility. Next, the Petri dishes without contamination were used for the propagation of the mycelium of the fungi [19,20].

2.3. Mycelial Growth

The mycelial growth of 50 *Trichoderma* spp. on PDA dishes or MEA plates was determined using the modified Gompertz equation (Equation (1)) [21,22]:

$$\mathbf{log\ N = A + C * exp\{-exp[-B(t - M)]\}} \quad (1)$$

Equation (1). Modified Gompertz model, where:
A, B, C = parameters of the model;
t = days;
M = day with maximum growth rate;
log N = growth kinetics.

Based on the growth kinetics, the maximum growth, μ_{max}, (Equation (2)) and the lag time λ (Equation (3)) were calculated [21,23]:

$$\boldsymbol{\mu_{max} = (B * C)/e} \quad (2)$$

Equation (2). Maximum growth, where:
e = The Euler constant.

$$\boldsymbol{\lambda = [\ln(1 + (\mu_{max}/v)\ ]/\mu_{max}} \quad (3)$$

Equation (3). Lag time, where:
v = $\mu_{max.}$

2.4. Antagonistic Capacity

Discs 5 mm in diameter of *M. roreri* or *M. perniciosa* containing 7 days of mycelial growth were placed at one end of the Petri dishes containing PDA or MEA, 1.5 cm from the edge. Otherwise, 5-millimeter-diameter discs of *Trichoderma* spp. with 4 days of mycelial growth were placed at the opposite end of the Petri dish, and the confrontations were kept 6 cm equidistant from each other. The linear growths of the two colonies facing each other were recorded twice a day until the total colonization of the Petri dish.

The percentage of pathogen inhibition (ATP) was determined based on the radial growth of the mycelium of the pathogen controls with respect to the clashes between Trichoderma spp. and pathogens, using Equation (4) [24]:

$$\mathbf{ATP = 100 * \left[RPC - \frac{(RFP - RIP)}{RPC}\right]} \quad (4)$$

Equation (4). Pathogen inhibition, where:
RPC = growth radius of the control pathogen mycelium on the final day;
RFP = growth radius of the pathogen mycelium in the presence of the antagonist on the final day;
RIP = growth radius of the mycelium of the pathogen on the day the confrontation began, even without the antagonist (culture on the seventh day).

2.5. Experimental Test

The experimentation was carried out on the "Esmeraldas" farm located in the Province of Los Ríos, which has 15-year-old cocoa plantations.

On the farm, 15 subplots were established. Each subplot was made up of 25 trees in a 5 × 5 square. The biosolution was obtained through a dose of 1×10^9 conidia/mL^{-1}, and

0.2 L of the biosolution was sprayed per cocoa tree at intervals of 15 days for 5 months. The experimental design was as follows:

Subplots 1, 2 and 3 = Control treatment without biosolution;

Subplots 4, 5 and 6 = Treatment with biosolution using *Trichordema* strain 17 "T1";

Subplots 7, 8 and 9 = Treatment with biosolution using the *Trichordema* strain 33 "T2";

Subplots 10, 11 and 12 = Treatment with biosolution using the *Trichordema* strain 42 "T3";

Subplots 13, 14 and 15 = Treatment with biosolution using the *Trichordema* strain 44 "T4".

The incidence of cocoa pods with symptoms of monialisis was determined by using Equation (5), while the efficiency of the treatment was calculated using Equation (6) [25]:

$$\mathbf{I} = \frac{\mathbf{DP}}{\mathbf{TP}} \times \mathbf{100} \quad (5)$$

Equation (5). Incidence of cocoa pods, where:

I = Incidence (%);

DP = Number of damaged pods;

TP = Total pods.

$$\mathbf{E} = \frac{\mathbf{FIWoT} - \mathbf{FIWT}}{\mathbf{FIWoT}} \times \mathbf{100} \quad (6)$$

Equation (6). Efficiency of the treatment, where:

E = Efficiency (%);

FIWoT = Percentage of final incidence without application of *Trichoderma* spp.;

FIWT = Percentage of final incidence with application of *Trichoderma* spp.

The yield was estimated in kg of dry cocoa beans, and the dry weight was calculated as 40% of the fresh weight of the cocoa [25].

2.6. Statistical Analysis

The mycelial characteristics of maximum velocity, lag phase and the antagonistic capacity percentage of pathogen inhibition were measured in triplicates and the data were subjected to data mining techniques, such as PCA biplot and GGE biplot, using R software ver. 4.1.1.

The results of the experimental test were studied using one-way analysis of variance (ANOVA) to determine the significance of individual differences at the $p < 0.05$ level, the yield of cocoa pods and the efficiency of the treatment (with or without solution). After these tests, if statistical differences were found, the Duncan test with $\alpha = 0.05$ was applied. The analyses were carried out using statistical software (Statgraphic ver. 16).

2.6.1. PCA Biplot

Principal components analysis is used in cases of continuous variable. Its purpose is to reduce the number of variables by representing the data matrix in a lower dimensionality than the original [26].

In this analysis, the centered and/or scaled matrix of rank r is approximated through its orthogonal projection of lower rank through q linear and independent vectors. These matrices represent the columns of a matrix named T, such that $\mathbf{T}'\mathrm{T} = 1$. The solution is to find T, which should be symmetrical and positively definite, such that it minimizes the mean squared error of the predictions $\|\mathrm{X_c} - \hat{\mathrm{X}}_\mathrm{c}\|$:

$$\mathbf{X_c} \cong \mathbf{X_c}\mathbf{T}\mathbf{T}' = \mathbf{Z}\mathbf{T}'$$

where Z contains the main components.

If T is equal to the previous equation, its columns coincide with the eigenvectors associated with the q largest eigenvalues of the variance–covariance matrix.

In addition, if the DVS of the previous equation is considered, it is found that [27,28]:

$$\mathbf{X_c} \cong (\mathbf{UDV'})\mathbf{VV'} = \mathbf{UDIV'} = \mathbf{UDV'}$$

2.6.2. GGE Biplot

The biplot construction procedure consists in finding the best matrix X* of rank 2. The term GGE is the contraction of G+GE. A biplot representing the GGE of a MET dataset is called a GGE biplot. The GGE biplot model was used for phenotypic stability analysis [29]. The above equations can be written as [30]:

$$\mathbf{Y_{ijk}} = \boldsymbol{\mu} + \boldsymbol{\propto_i} + \boldsymbol{\beta_j} + \boldsymbol{\theta_{ij}}$$

or as:

$$\mathbf{Y_{ijk}} - \boldsymbol{\mu} - \boldsymbol{\beta_j} = \boldsymbol{\propto_i} + \boldsymbol{\theta_{ij}}$$

where:

$\mathbf{Y_{ijk}}$ = Expected yield of genotype i in environment j;
$\boldsymbol{\mu}$ = Global mean of all observations;
$\boldsymbol{\propto_i}$ = Main effect of genotype i;
$\boldsymbol{\beta_j}$ = Main effect of environment j;
$\boldsymbol{\theta_{ij}}$ = Interaction between genotype i and environment j.

3. Results and Discussion

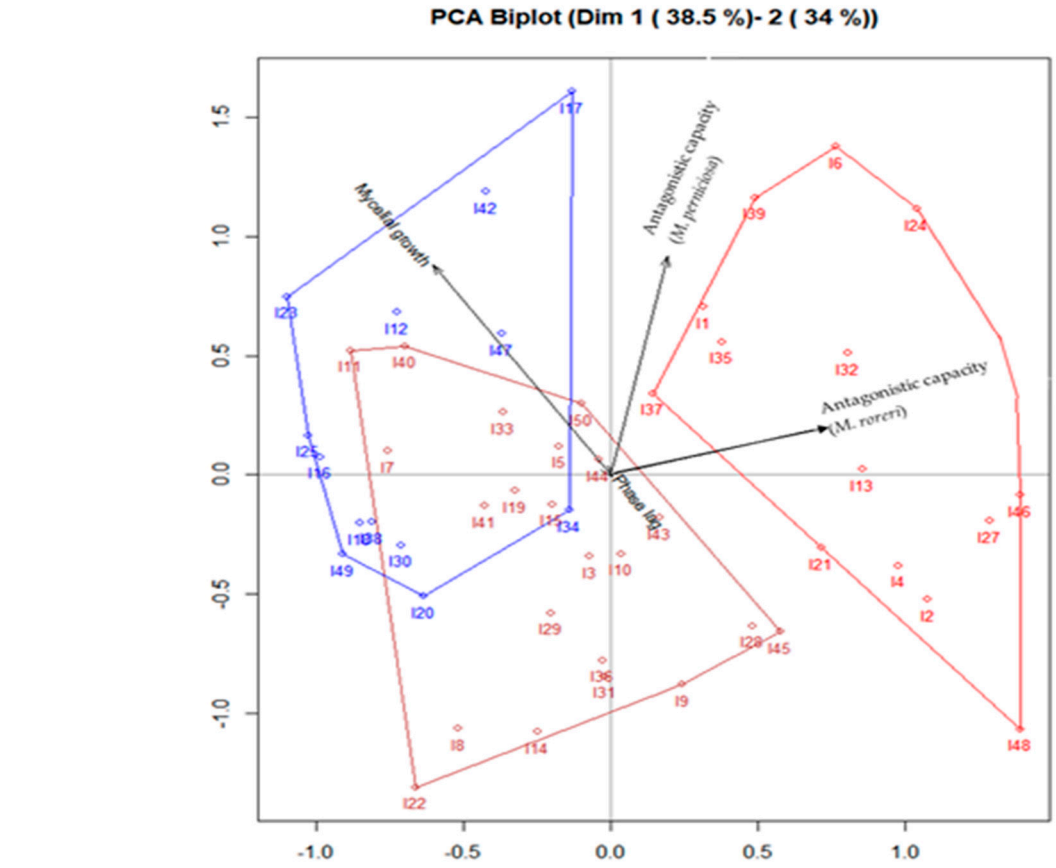
The focus of this research was to identify the *Trichoderma* spp. cultivated on two media (PDA or MEA dishes) with the highest mycelial characteristics (maximum velocity and lag phase), as well as the antagonistic capacity against two cocoa-crop pathogens: *Moniliophthora roreri* and *Moniliophthora perniciosa*. The incidence of monialisis and the efficiency of the treatment with or without biosolution based on the *Trichoderma* spp. were calculated.

The numeration of the strains was conducted using the following distribution: 1–50: *Trichoderma* spp. cultivated on PDA dishes or *Trichoderma* spp. growth on MEA plates.

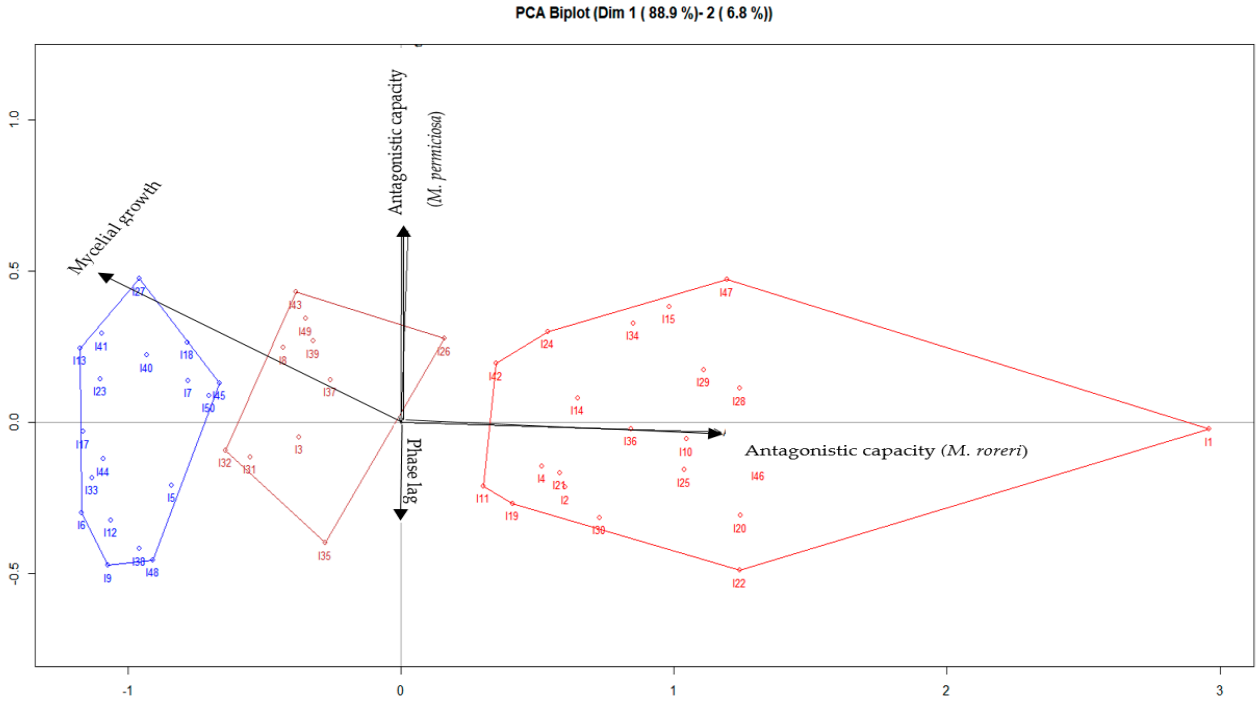
3.1. PCA Biplot Algorithm for Mycelial Characteristics and Antagonistic Capacity of Trichoderma spp.

Figure 1 presents the factorial graph of planes 1–2 (PCA Biplot). Figure 1a shows that the accumulated inertia amounted to 72.5%, whereas Figure 1b shows that the accumulated inertia amounted to 95.7%. Additionally, the groups were determined using the biplot coordinates based on three variables: mycelial growth, the antagonistic capacity against *Moniliophthora roreri* and the antagonistic capacity against *Moniliophthora perniciosa*.

Figure 1a shows the presence of 50 strains of *Trichoderma* cultivated on PDA plates. Cluster 1 (color red) shows the presence of 14 *Trichoderma* spp. with the strongest relation to the antagonistic capacity against *Moniliophthora roreri*. Clusters 2 and 3 (blue and brown colors, respectively) denote the presence of the 21 strains of *Trichoderma* with the highest mycelial growth. Cluster 3 (brown color) indicates the presence of the 15 *Trichoderma* spp. with the highest phase lag. On the other hand, Figure 1b shows the growth of 50 *Trichoderma* spp. on MEA dishes. Cluster 1 (red color) indicates the presence of the 20 *Trichoderma* spp. with the strongest relation to the antagonistic capacity against *Moniliophthora roreri*. Cluster 2 (brown color) shows the presence of the 10 strains of *Trichoderma* with a direct relation to the antagonistic capacity against *Moniliophthora perniciosa.* Cluster 3 (blue color) indicates the presence of the 20 *Trichoderma* spp. with the highest mycelial speed. Arzate-Vega [31] indicated that the antagonistic fungus presents greater aggressiveness towards the resistance of the phytopathogen if there are fewer days of contact.



(**a**)



(**b**)

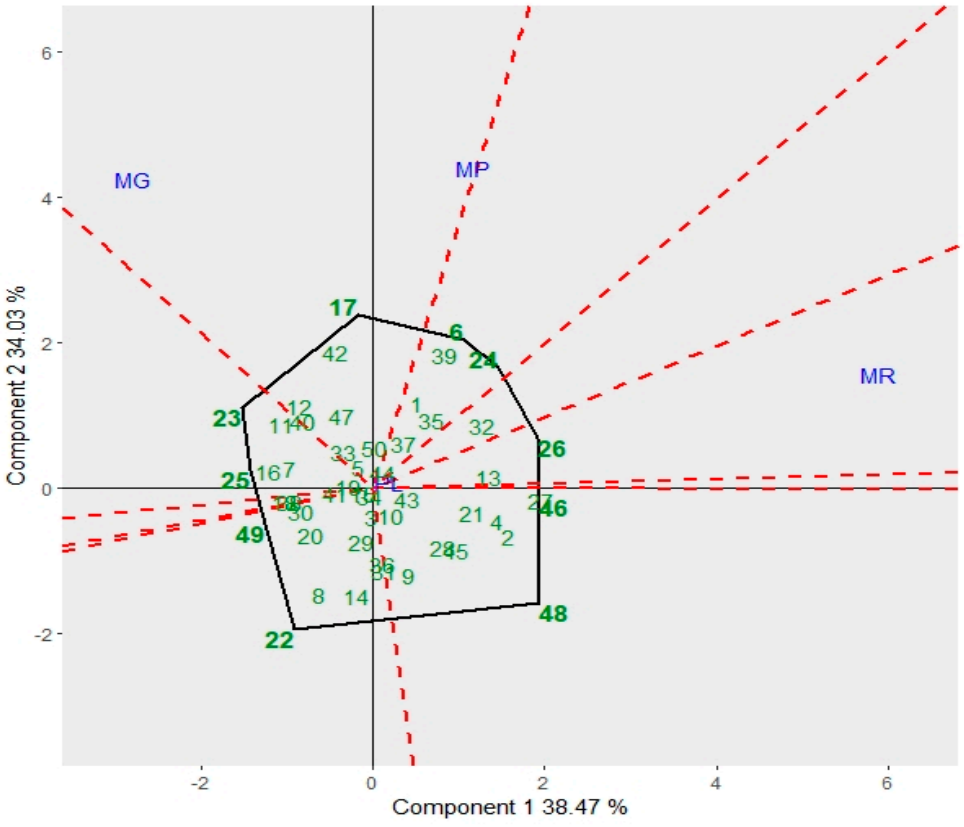
Figure 1. (**a**) PCA biplot for mycelial characteristics and antagonistic capacity of *Trichoderma* spp. cultivated on PDA medium. (**b**) PCA biplot for mycelial characteristics and antagonistic capacity of *Trichoderma* spp. growth on MEA medium.

The antagonistic activities of *Trichoderma* fungi against *Moniliophthora roreri* and *Moniliophthora perniciosa* have been studied in different research works; these activities include the inhibition of the growth of these pathogens and a reduction in the symptoms of moniliasis. *Trichoderma* fungi presented an inhibition percentage of 23% for *M. roreri* and 27% for *Moniliophthora perniciosa* [32], whereas other studies showed that endophytic biocontrol agents, such as *Trichoderma* spp., are antagonists of *Moniliophthora roreri* because they germinate and penetrate the surface of the crop to establish their systems in infection, demonstrating a 23.8% reduction in the presence of moniliasis in cocoa crops [33]. In another study, seven microorganisms were isolated from the cocoa rhizosphere and their antagonism capacities were studied against the pathogen *Moniliophthora roreri*. *Trichoderma* spp. presented the highest percentage of inhibition and sporulation of *Moniliophthora roreri* [34].

Trichoderma spp. use several biological control mechanisms that can encourage plant growth and induce defense responses [35]. *Trichoderma* spp. are mainly used for bioremediation in soils with high herbicide or pesticide contents, helping to reduce these contents [36]. The implementation of this genus of fungi removes the brooms produced by *Moniliophthora perniciosa*, which prevents the spread of the disease in healthy ears and reduces the incidence of infection [37]. *Trichoderma* provides good control relative to taking no action, reducing the effects on the Ecuadorian economy of infection with this crop.

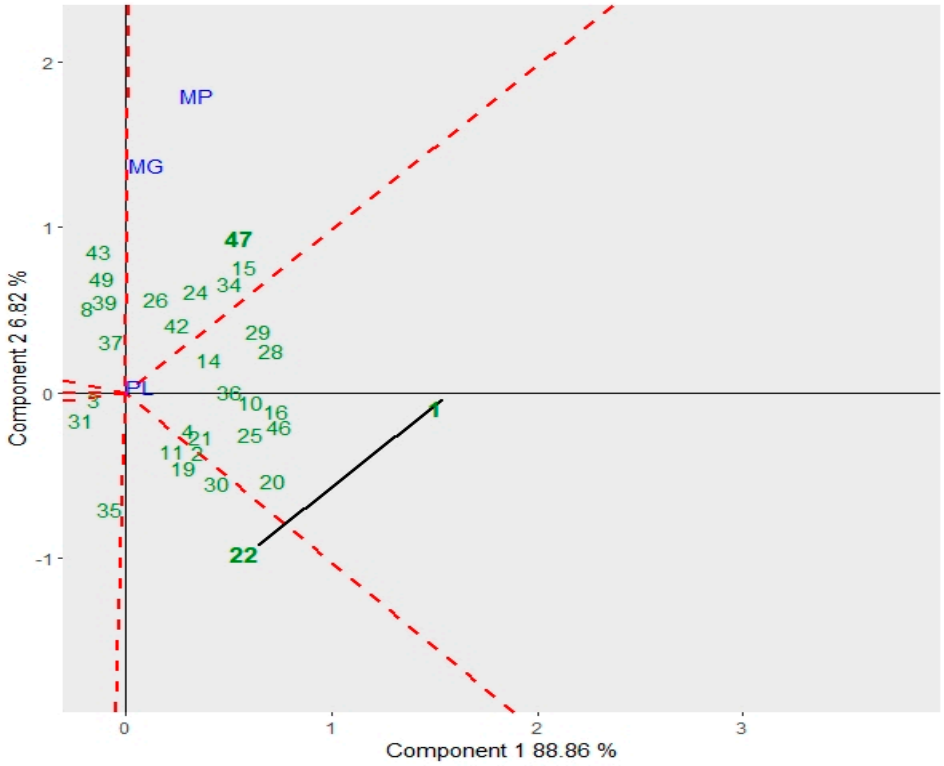
3.2. GGE Biplot for Mycelial Characteristics and the Antagonistic Capacity of Trichoderma spp.

Figure 2 presents the factorial graph of planes 1–2 (GGE biplot). Figure 2a shows that the accumulated inertia amounted to 72.5%, while Figure 2b indicates that the accumulated inertia amounted to 95.7%. The GGE biplot shows the *Trichoderma* strains (green color) that presented the highest values of mycelial growth and antagonistic capacity.



(**a**)

Figure 2. *Cont.*



(**b**)

Figure 2. (**a**) GGE biplot of mycelial characteristics and antagonistic capacity of *Trichoderma* spp. cultivated on PDA plates. Groups of *Trichoderma* spp. showed similar characteristics (G1: 18, 30, 38); (G2: 28, 45); (G3: 15, 19, 34); (G4: 31, 36). (**b**) GGE biplot for mycelial characteristics and antagonistic capacity of *Trichoderma* spp. growth on MEA dishes. MG = mycelial growth, MP = antagonistic capacity (*M. permiciosa*), MR = antagonistic capacity (*M. roeri*), PL = phase lag. Note: MR is not presented in Figure 2b.

Figure 2a shows the 50 strains of *Trichoderma* cultivated on the PDA plates. It was observed that nine of the *Trichoderma* strains presented a stronger relation to mycelial growth, six presented a stronger relation to the antagonistic capacity against *Moniliophthora perniciosa* and two showed a higher relation to the antagonistic capacity against *Moniliophthora roeri*. Figure 2b indicates the growth of 50 *Trichoderma* spp. on the MEA dishes. Six of these strains showed a stronger relation to mycelial growth and to the antagonistic capacity against *Moniliophthora perniciosa*. In conclusion, the PCA biplot and GGE biplot indicated that the strains 17, 33, 42 and 47 cultivated on the PDA plates presented higher values of mycelial growth and antagonistic capacity against *Moniliophthora perniciosa* in comparison with the *Trichoderma* spp. grown on the MEA dishes. The most commonly used medium in the propagation of different kinds of fungi is PDA due to its nutritional composition [38]. Different authors have indicated that fungi strains grown in this medium presented the strongest mycelial characteristics [39]. However, the use of MEA medium in the propagation of *Trichoderma* spp. was studied to obtain a low degree of sporulation. *Trichoderma* spores obtained in liquid media lose their viability due to physical factors, such as their thinner cell walls and greater number of organelles [40]. The highest spore production is obtained in solid media with the presence of a source of sugars [41]; for this reason, it is important to generate spores in solid media cultures.

Spore production is stimulated by the following factors: a solid culture medium with a high lignin content, the incubation temperature, the moisture content, the incubation period, and the inoculum concentration. The lignin present in the culture medium generates a physical–chemical barrier against microbial attack. The optimal temperature for the

maximum production of *Trichoderma* spores is 25 °C. High temperatures affect microbial growth due to alterations in the membrane structure and protein degradation, while low temperatures affect the production of spores [42]. The optimal moisture content in the culture for spore production is 65%. A lower moisture content in the culture medium reduces the solubility of nutrients and increases the surface tension of the water, whereas a higher moisture content hinders gas exchange, reducing the efficiency of the medium [43]. The maximum spore density occurs after 31 days of incubation. Finally, the optimal inoculum concentration is 5%. Low inoculum concentrations delay the lag phase, while high concentrations increase the competition for nutrients in the substrate [44]. The reduction in the useful life of the *Trichoderma* spores in the conservation process is influenced by the temperature. The optimal storage temperature is 4 °C, while high temperatures affect the viability of *Trichoderma* spores [45]. An adequate dose of spores of *Trichoderma* strains makes it possible to obtain an adequate amount for use in biopesticides that enhance plant health in different horticultural crops [46].

The use of two culture media in the propagation of the fungi makes it possible to obtain strains with different characteristics and, through the use of statistical techniques such as PCA biplot and GGE biplot, we can determinate the specific strain of *Trichoderma* with the highest values of mycelial growth and antagonistic capacity against cocoa-crop pathogens, with the aim of controlling monialisis infection. The cultivation of *Trichoderma* spp. on PDA allows us to obtain strains with better characteristics than those cultivated on MEA plates.

3.3. Field Responses of Trichoderma spp. *against Monialisis Caused by Moniliophthora perniciosa*

The use of the biosolution based on the *Trichoderma* spores of the strains 17, 33, 42 and 44 cultivated on the PDA plates showed effects on the final incidence of cocoa monialisis, the efficiency of the treatments and the crop yield (Table 1).

Table 1. Effect of treatments with *Trichoderma* spp. against monialisis caused by *Moniliophthora perniciosa*.

Treatment	Incidence (%)	Efficiency (%)	Yield (kg/ha)
Control	18.2 ^{a}	-	533.50 ^{c}
T1	5.8 ^{c}	70.4 ^{b}	721.80 ^{a}
T2	10.1 ^{b}	54.3 ^{c}	615.20 ^{b}
T3	3.1 ^{d}	75.1 ^{a}	753.10 ^{a}
T4	9.7 ^{b}	60.8 ^{c}	630.50 ^{b}

Control = Treatment without biosolution; T1 = Treatment with biosolution using *Trichordema* strain 17; T2 = Treatment with biosolution using *Trichordema* strain 33; T3 = Treatment with biosolution using *Trichordema* strain 42; T4 = Treatment with biosolution using *Trichordema* strain 44. Different letters in each column indicate significant differences between the effects of five treatments based on *Trichoderma* spp. against monialisis at level $p < 0.05$, according to Duncan's test, n = 3.

Treatment 3 presented the lowest incidence of monialisis, of 3.1%, while the control treatment showed the highest incidence (18.2%). On the other hand, the highest efficiencies were presented by treatments 1 and 3, which demonstrated values of 70.4% and 75.1%, respectively; the lowest efficiencies were presented by treatments 2 and 4, with values between 54.3 and 60.8%, respectively. The highest cocoa yields were obtained using treatments 1 and 3, which showed values of 721.80 and 753.10 Kg/ha, respectively; the lowest yield value was obtained with the control treatment, which presented a value of 533.50 Kg/ha.

Other authors have presented similar results. Leiva et al. [25] used five treatments based on *Trichordema* spp. biosolutions against frosty pod rot on cocoa trees, showing incidence values between 5.62 and 20.32%, efficiencies ranging from 38.99 to 71.99% and yields from 787 to 1115 Kg/ha. Loguercio et al. [47] showed incidence values against *Moniliophthora perniciosa* from 5 to 78.89% by using two biosolutions based on *Trichoderma* spp. Krauss and Soberanis [48] and obtained higher yields of cocoa when using treatments

based on *Trichoderma* spp. formulations than when using the control treatment (without the *Trichoderma* spp. formulation). *Trichoderma* spp. strains have the capacity to be possible biocontrol agents against phytopathogenic fungi, since they can produce secondary metabolites, antibiotics and mycotoxins that are capable of degrading the resistant structures of pathogenic fungi [49].

Biological formulations based on cells or spores need to pass through a product-stabilization process in prolonged storage in order to maintain the viability of the biological compounds and the functionality of the product [50]. The propagation, growth and sporulation of fungi are dependent on different environmental conditions, such as the culture medium, pH, temperature, light, humidity and mixture of atmospheric gases [51]. The use of solutions based on *Trichoderma* spores has presented important results in the control of various plant diseases [52]. The application of biopesticides based on *Trichoderma* spores is an economical and environmentally friendly method against various pests. Additionally, it makes it possible to obtain higher yields in different crops, encouraging the production of healthy and safe organic crops [53]. The application of this biological formulation in the crop has shown that it improves the antioxidant machinery and tolerates water stress [54]. This is an important predictor because the correct choice of *Trichoderma* strain can control moniliasis infection in cocoa and does not generate economic losses for farmers.

4. Conclusions

The cultivation of *Trichoderma* spp. on two culture media allowed us to obtain strains with different specific characteristics for use in different industrial applications.

The use of the PDA medium in the propagation of *Trichoderma* spp. allowed us to obtain strains with better characteristics than those cultivated in the MEA medium.

The multivariate techniques, PCA biplot and GGE biplot, indicated that the four strains of *Trichoderma* cultivated on the PDA medium presented the highest values of mycelial growth and antagonistic capacity against cocoa-crop pathogens.

The use of treatment 3 on the cacao trees produced the lowest incidence of moniliasis and the highest cacao yields.

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