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Comparative analysis of beneficial fungi in the management of moniliasis (*Moniliophthora roreri*) in cocoa (*Theobroma cacao* L.) at in vitro level

Análisis comparativo de hongos benéficos en el manejo de moniliasis (Moniliophthora roreri) en cacao (Theobroma cacao l.) a nivel in vitro

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Palabras claves:
Hongos, moniliasis. Cacao, comparación, edafoclimáticos, agricultura, agronomía

Resumen

Introducción. en los últimos años, se ha demostrado que el uso de hongos beneficiosos puede ser una alternativa sostenible y eficaz para el control de la moniliasis en el cacao. Sin embargo, aún existe un conocimiento limitado sobre la interacción entre los hongos beneficiosos y los factores edafoclimáticos en el manejo de la moniliasis en el cacao. Por lo tanto, es necesario profundizar esta investigación para desarrollar estrategias de manejo más efectivas y sostenibles para el control de esta enfermedad. **Objetivo.** Realizar un análisis comparativo de hongos benéficos en el manejo de la moniliasis en cacao a nivel in vitro. **Metodología.** El método de investigación propuesto para el estudio combinó métodos cuantitativos y cualitativos. Esto nos permitió recopilar datos numéricos y cualitativos para comprender tanto las características biológicas de los hongos beneficiosos como su comportamiento en la incidencia de moniliasis en el cacao. **Resultados.** A partir del muestreo realizado en el cultivo de cacao de la Ciudad Universitaria campus Dr. Jacobo Bucaram Ortiz Milagro se identificaron varios géneros de hongos, siendo los predominantes Aspergillus, Penicillium, Trichoderma y Fusarium, entre otros. Se sabe que estos géneros se encuentran comúnmente en suelos agrícolas tropicales. **Conclusión.** La interacción entre los factores climáticos y la acción antagónica de los hongos beneficiosos sobre la moniliasis es compleja y multifactorial en la agricultura. Es fundamental considerar no solo las condiciones ambientales para el desarrollo de estos hongos, sino también factores como el tipo de suelo, la competencia microbiana y las prácticas agrícolas. **Área de estudio general:** Agronomia. **Tipo de estudio:** Artículos originales

Keywords:
Fungi, moniliasis. Cocoa, comparison, soil and climate, agriculture, agronomy

Abstract

Introduction. In recent years, it has been shown that the use of beneficial fungi can be a sustainable and effective alternative for the control of moniliasis in cocoa. However, there is still limited knowledge about the interaction between beneficial fungi and edaphoclimatic factors in the management of moniliasis in cocoa. Therefore, it is necessary to deepen this research to develop more effective and sustainable management strategies for the control of this disease. **Objective.** Conduct a comparative analysis of beneficial fungi in the management of moniliasis in cocoa at an in vitro level. **Methodology.** The research method proposed for the study combined quantitative and qualitative methods. This allowed us to collect numerical and qualitative data to understand both the biological characteristics of beneficial fungi and their behavior on the incidence of moniliasis in cocoa. **Results.** Several genera of fungi were identified from the sampling conducted in the cocoa cultivation of the Ciudad Universitaria Dr. Jacobo Bucaram Ortiz Milagro campus, the predominant ones being *Aspergillus, Penicillium, Trichoderma and Fusarium,* among others. These genera are known to be commonly found in tropical agricultural soils. **Conclusion.** The interaction between climatic factors and the antagonistic action of beneficial fungi on moniliasis is complex and multifactorial in agriculture. It is essential to consider not only the environmental conditions for the development of these fungi, but also factors such as soil type, microbial competition, and agricultural practices.

Introduction

Cocoa is a tropical fruit; its crops are found mostly on the coast and in the Amazon. It is a tree with small flowers that are seen on the branches and produce an ear that contains grains covered with a pulp rich in sugar. Cocoa production is concentrated in the provinces of Los Ríos, Guayas, Manabí, and Sucumbíos (Guerrero, 2024).

Cocoa cultivation is currently one of the greatest economic potentials in Ecuador, enabling improvement in tropical areas under regenerative practices and profitable production; with the presence of clonal CCN-51 cocoa, and cocoa above with quality of origin designation; even as an emblem at an international level (Vargas et al, 2024).

In 2023, the area planted with cocoa nationwide was 609,750 hectares (Instituto Nacional de Estadísticas y Censos [INEC], 2023).

Between January and March 2024, Ecuador exported 101,000 tons of cocoa and processors, which represents an increase of 30% compared to the same period in 2023, according to the ECB. Meanwhile, the value of cocoa exports increased by 145% or USD 302 million compared to the first three months of the previous year, for a total amount of shipments abroad of USD 510.8 million (Primicias, 2024).

Ecuador is one of the countries that make up the center of origin of cocoa, this factor has been decisive in placing the country as the main supplier of fine aroma cocoa worldwide. However, there are various microorganisms that usually have a negative impact on production, the genetic degeneration of clones, the poor use of agrochemicals and the adaptability of pathogens, added to favorable climatic conditions for their proliferation, can cause considerable losses, including the main diseases caused by fungal pathogens are monilla, witch's broom, and black cob, with the particularity that the first two mentioned are endemic and if optimal management is not carried out, production can decrease considerably.

The disease is present in 13 Latin American countries (Belize, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Peru, Panama, Bolivia, and Venezuela) with estimated losses of 80% of the annual harvest. Of every three cocoa fruits (called pods) affected by various diseases, two pods are injured by moniliasis. In the northern Amazon region of Ecuador, which includes Sucumbíos, Orellana and Napo, there are environmental conditions of high humidity, where more than 40% of production is lost, that is, 8,000 tons of cocoa (CropLife Latin America, 2024).

Moniliasis is a disease that affects the leaves, branches, and fruits of cocoa, causing them to rot and significantly reducing the crop's yield. The control of moniliasis is conducted using several control strategies and eventually one of them is the use of synthetic fungicides, which are not only expensive, but can also have a negative impact on the environment and health of workers (Reatigue, 2021).

In recent years, it has been shown that the use of beneficial fungi can be a sustainable and effective alternative for the control of moniliasis in cocoa. However, there is still limited knowledge about the interaction between beneficial fungi and edaphoclimatic factors in the management of moniliasis in cocoa. Therefore, it is necessary to deepen this research to develop more effective and sustainable management strategies for the control of this disease. These fungi, which are part of the soil microbiota, can function as biocontrol agents by colonizing the cocoa roots and protecting the plant from infection by *Moniliophthora roreri* (Companioni et al., 2019).

Likewise, it is necessary to emphasize that when using beneficial fungi, it is important to consider the edaphoclimatic factors that can influence the development of moniliasis. These factors can include temperature, humidity, and soil composition, among others (Arvelo et al., 2017).

The objective of this work is to perform a comparative analysis of beneficial fungi in the management of moniliasis in cocoa at the in vitro level. Among the specific objectives are: to identify the genera of the endemic fungal microbiota present in the laboratory, to estimate the action of beneficial fungi in the control of moniliasis at the in vitro level, to analyze the influence of climatic factors on the antagonistic action of beneficial fungi and moniliasis.

Methodology

The research method proposed for the study combined quantitative and qualitative methods. This allowed us to collect numerical and qualitative data to understand both the biological characteristics of beneficial fungi and their behavior on the incidence of moniliasis in cocoa.

The most appropriate research modality for this study was an experimental approach. An experiment was designed under controlled conditions in which optimal climatic conditions were provided for the development of both the beneficial microorganism (*Trichoderma spp*) and the pathogen (*Moniliophthora roreri*). These conditions were temperature of 28°C and relative humidity of 80%. These were achieved through the implementation of a humid chamber, the control of these controlled conditions was conducted using a hygrometer, with the objective of evaluating its impact on the development of moniliasis and the effectiveness of beneficial fungi to control the disease. In parallel, field samples were studied to obtain information on the presence and activity of beneficial fungi on fungal pathogens.

Identification of beneficial fungi

The process of identifying beneficial fungi begins with the collection of soil samples, which were obtained from the experimental national cocoa plot of the Ciudad Universitaria Milagro using simple and cold cooked rice in glass containers with a canvas lid, buried at a depth of 15-20 cm in strategic points of the crop for 4 days. Subsequently, the inoculation was carried out in PDA medium (Potato Dextrose Agar), 39g of PDA was dosed in 1000 ml of distilled water following the manufacturer's recommendations, this solution was placed in an autoclave at 121° C for 20 minutes, subsequently using the Using a laminar flow chamber, the culture medium was dispensed into Petri dishes, to sow the collected fungal materials, which were incubated at a temperature of 28 º C. The fungi were identified according to the dichotomous keys specified in the “Dichotomous Key Manual for the Identification of Fungi” (Sociedad Español de Microbiología [SEM@foro], 2024).

Degree of antagonism (GA)

It was measured according to the scale proposed by Bell et al. (1982) and Askew & Laing (1994), which describes the growth of the bio controller against the pathogenic fungus as outlined below:

Class 1: Trichoderma grows completely on the pathogen and covers the entire surface of the medium.

Class 2: Trichoderma grows on at least two-thirds of the surface of the medium.

Class 3: Trichoderma colonizes 50% of the environment and no organism appears dominated by the other.

Class 4: the pathogen colonizes at least two thirds of the surface of the medium

Class 5: the pathogen grows completely on the surface of the medium.

Percent growth inhibition (PIC)

Every 24 h, the pathogen colony radius was measured in all Petri dishes and the following formula was applied:

PIC = [(Ctest-Ctrat)/ Ctest] x 100 **(1)**

Ctest = control growth

Ctrat= treatment growth

The data obtained were analyzed using Anova and Tukey's test ($p<0.05$).

Population and sample

Population. – The national cocoa plants from the Ciudad Universitaria plot of Dr. Jacobo Bucaram Ortiz Milagro

Sample. – Soil samples were taken from 10 plants, which were conducted using zigzag sampling, which is a combination of systematic and random sampling to guarantee greater coverage of the experimental plot.

Data collection techniques

Data were collected from petri dishes. All growths and respective isolations were performed in PDA medium (Potato Dextrose Agar, Acumedia) in 9 cm diameter Petri dishes and incubated at 28 ° C and 80% relative humidity for 5 days in dark conditions. For staining, a solution of Lactophenol Blue was used.

The degree of antagonism (GA) was established by measuring according to the scale proposed by Bell et al. (1982) and Askew & Laing (1994), which describes the growth of the biocontroller against the mycopathogen as described in the table.

Table 1

Degree of antagonism (GA) according to the scale proposed by Bell, Well and Markham and Askew and Laing

Category	Description
Class 1	Trichoderma grows completely on the pathogen and covers the entire surface of the medium
Class 2	Trichoderma grows on at least two-thirds of the surface of the medium
Class 3	Trichoderma colonizes 50% of the environment and no organism appears dominated by the other
Class 4	The pathogen colonizes at least two thirds of the surface of the medium
Class 5	The pathogen grows completely on the surface of the medium

To determine the percentage of growth inhibition (PIC): Every 24 h, the radius of the pathogen colony was measured in all Petri dishes applying the following formula:

$PIC = [(Ctest-Ctrat)/ Ctest] \times 100$ **(2)**

Ctest = control growth

Ctrat=treatment growth

The climatic data were controlled using a hygrometer to provide the ideal conditions for the development of the microorganisms under study.

Descriptive and inferential statistics

The presentation of the data was presented in a descriptive manner and was expressed in percentage and UPM/g as appropriate.

Experimental design

To conduct the experimental laboratory phase of this research, a completely randomized design was used within which three treatments were evaluated, the same ones indicated in table 2. Each treatment was evaluated through 10 repetitions, generating a total of 30 units experimental. Each experimental unit will be represented by Petri dishes.

Treatments to evaluate

In table 2 you can see the distribution of the treatments where two commercial formulas of *Trichoderma* were evaluated: Biotrich with the identified species (*T. harzianum) and Tricomix, which is a product that is formulated with the species T. harzianum and T viridis* and a third treatment was with a strain collected in the field and that was identified at the genus level as belonging to Trichoderma spp. The quantities used were 1 x 109 spores/mL per Petri dish.

Table 2

Treatments evaluated in the trial

	Commercial formula of *T. harzianum* confronted with *M. roreri*	Commercial formula of (*T. harzianum and T viridis*) confronted with *M. roreri*	*Trichoderma spp.* (collected) confronted with *M. roreri*
Petri dishes	10	10	10

Variance analysis

For the statistical assessment of the response variables, analysis of variance (ANOVA) and Tukey's mean comparison test were used, both at 5% type I error. The ANOVA model is detailed in table 3.

Table 3

Anova of the experiment

FV	df
Treatments (t-1)	2
Experimental Error t(r-1)	27
Total tr-1	29

It should be noted that these analyzes were conducted after adjusting the data to a normal distribution where required.

Results

Identification of the genera of the endemic fungal microbiota present at the experimental site

Several genera of fungi were identified from the sampling conducted in the cocoa cultivation of the Ciudad Universitaria Dr. Jacobo Bucaram Ortiz Milagro campus, the predominant ones being *Aspergillus, Penicillium, Trichoderma and Fusarium*, among others. These genera are known to be commonly found in tropical agricultural soils.

Estimation of the action of beneficial fungi in the control of moniliasis at in vitro level

Action of beneficial fungi in the control of moniliasis at in vitro level:

- The fungus *Trichoderma harzianum* was evaluated for its ability to inhibit the growth of *Moniliophthora roreri*.

- A significant inhibition of the growth of the pathogenic fungus was observed in the presence of the *T. harzianum* strain, showing a great capacity for the biological control of moniliasis at the in vitro level.

Table 4 shows the percentage of PIC growth inhibition (Log10 adjusted) of the pathogen *M. roreri* by the action of commercial *T. harzianum* (Biotrich) and *T. harzianum* and *T viridis* (Tricomix) and *Trichoderma spp* collected in the tests. antagonism where no significance was detected (p>0.05) between the treatments being equal. However, the highest value corresponded to the trademark Biotrich (27.80), followed by *Trichoderma spp* (26.23) and the other trademark Tricomix with 25.31. The calculated and adjusted coefficient of variation was 25.68%.

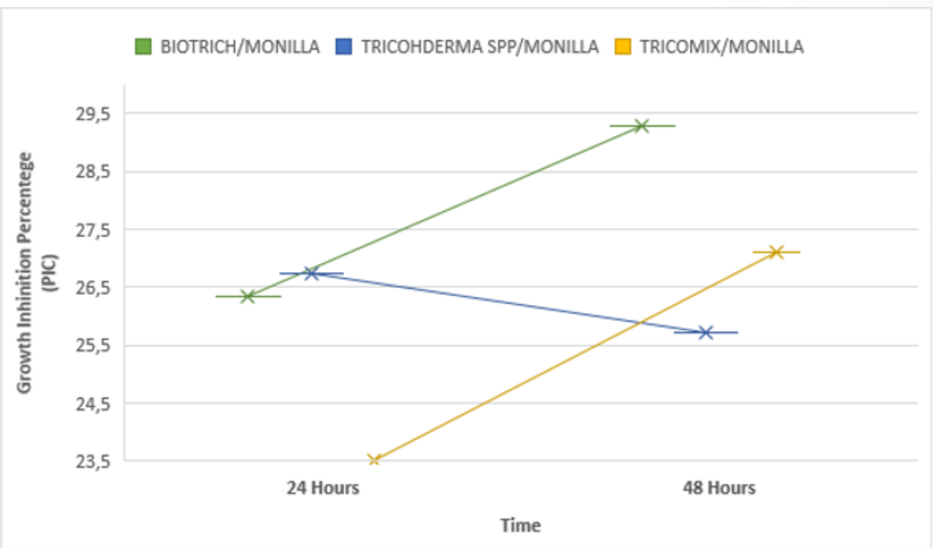
Table 4

Percentage of PIC growth inhibition of the pathogen M. roreri by the action of commercial T. harzianum (Biotrich and Tricomix) and Trichoderma spp. in antagonism tests

Nº.	Treatments	Average
1	Tricomix	25.31 a
2	Biotrich	27.80 a
3	*Trichoderma spp.*	26.23 a
CV (%)		25.68

Figure 1

Percentage of growth inhibition over time



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In figure 1 it can be distinguished that the treatment with BIOTRICH presented a geater inhibition in the growth of *M. roreri*, although this distinction was not significantly different from the other treatments. Likewise, the treatment based on Trichoderma spp did not provide evidence of inhibition, rather it had growth in favor of *M. roreri* after 48 hours.

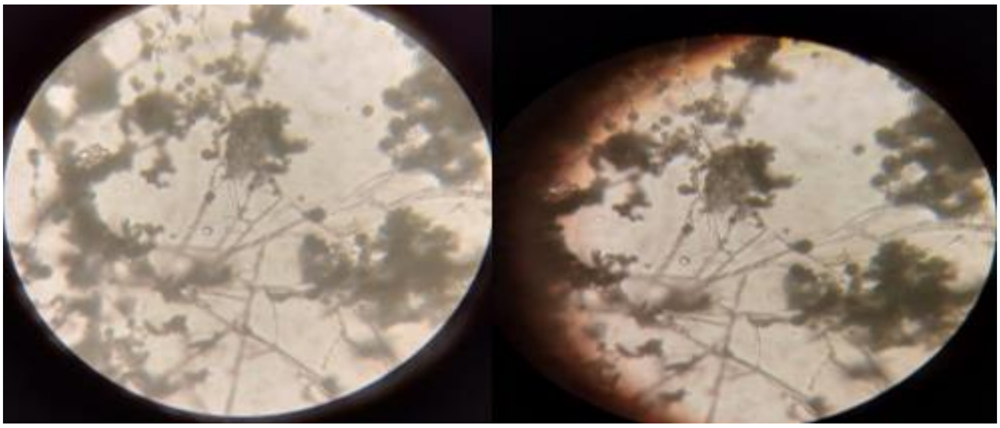
Figure 2

Evidence of antagonism between M. roreri and Trichoderma strains

Figure 2, shows the preparation of the fungal samples in petri dishes; denoting of antagonism between *Moniliophthora roreri* and *Trichoderma* strains, evidencing the scope of the beneficial fungus in the management of the pathogen in the treatment of moniliasis.

Figure 3

M. roreri stained with lactophenol blue



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In Figure 3, the fungus *Moniliophthora roreri* can be seen more clearly with the help of a dye, lactophenol blue; in turn amplifying the resolution of the microscope lens; in cultures of the pathogen under controlled conditions.

Analysis of the influence of climatic factors on the antagonistic action of beneficial fungi and moniliasis

Table 5 shows the climatic data of the Milagro area, where the mushrooms were collected for the experimental work. Annual precipitation was 1,200 - 1,400 mm, soil pH was 6.5 to 8.0 which is considered slightly acidic to neutral. The calculated relative humidity was between 70% - 80%, which is considered high, while the Heliophany detected reached values between 2,500 - 3,000 hours/year, being high.

Table 5

Edafoclimatic data for the Milagro area, 2023

Edaphoclimatic parameter	Value
Annual precipitation	1200-1400 mm
Soil pH	6.8-8.0
Relative Humidity	70-80%
Heliophany	2500-3000 hours/ligth/year

Source: Ingenio Valdez (2018)

The aforementioned information is indicative of the environmental conditions where the pathogen *Moniliophthora roreri* and Trichoderma spp (strain isolated and identified at the genus level) coexist. Likewise, in the laboratory, the influence of climatic factors, temperature, and relative humidity on the mycelial growth. Determining that at a temperature of 28° C and 80% relative humidity the development of microorganisms is efficient, reaching a percentage of inhibition in the case of Tricomix (*T. harzianum* and *T viridis*) of up to 67% in the evaluations carried out and On average, the data analysis indicates that the average inhibition with this commercial product was 25.31, likewise Biotrich reached the maximum inhibition value in one of the replicates 67.7% and an average of 27.80, finally the strain isolated from the experimental plot record maximum inhibition value 59.5% and average 26.2%. It can be corroborated by these data that if *Trichoderma spp* is subjected to adequate climatic conditions it has the capacity to inhibit the development of *Moniliophthora roreri*, however it is important to analyze other climatic factors that affect its capacity as a biocontroller.

Discussion

The fungi identified in the laboratory were Aspergillus, Penicillium, Trichoderma and Fusarium, among others that could not be identified. The genera found are common in tropical soils dedicated to agricultural exploitation. Mendoza & Pazmiño (2021) reported the presence of eight genera of fungi in cocoa soils on the Ecuadorian coast, including *Trichoderma*, *Fusarium*, *Aspergillus*, *Penicillium*, *Paecilomyces*, *Beauveria*, *Cladosporium* and *Bipolaris*. This information coincides with the fungal report obtained. In this research, the first four genres were identified.

Native fungal species affect the maintenance of soil health and plant productivity, emphasizing the need to further explore endemic fungal communities in agricultural environments, in this regard Mészárošová et al. (2024) state that Soil microbial communities are structured by sampling site and show significant spatial patterns that are partially driven by soil chemistry. The influence of the focal plant on the soil microbiome is low but tends to increase with plant succession and diversity. In contrast, root communities, especially bacterial ones, are strongly structured by native plant species.

The results presented show a comparison of the percentage growth inhibition (PIC) of the pathogen *M. roreri* by Trichoderma strains, both commercial and harvested. Despite not finding significant differences between the treatments, a slight trend towards greater effectiveness was observed in the commercial strain Biotrich (27.80%). The results obtained in this research are consistent with the research carried out by Quispe (2023), in which three strains of *Trichoderma spp* were evaluated and the inhibition of mycelial growth of *M. roreri* was reported by 45.02% (strain TH2), 44.89% (strain TL3) (strain and a PIC of 42.11% (strain NSHS3T).

The interaction of climatic factors can have a complex effect on the antagonistic action of beneficial fungi on moniliasis. In general, an optimal pH of the culture medium (3.5), high relative humidity, and a temperature between 28 and 35° C can favor the antagonistic action of beneficial fungi. These environmental conditions agree with those implemented by Fraga (2023), in his research entitled Evaluation and multiplication of *Trichoderma spp*. In three types of substrates, it was determined that under the controlled conditions there was optimal development of *Trichoderma spp*.

Conclusion

- In the experimental national cocoa plot owned by the Dr. Jacobo Bucaram Ortiz – Milagro University City. The genera Fusarium spp. *Trichoderma spp*, *Aspergillus* and *Penicillium* as part of the accompanying fungal microbiota of *M. roreri*, which provides an important basis for understanding the dynamics of the microbial community in the cocoa ecosystem and its implication in the control of diseases such as moniliasis.

- The antagonism of *T. harzianum* could be observed in the control of moniliasis at the in vitro level, which significantly inhibited *Moniliophthora roreri*, colonizing it completely. The use of Trichoderma spp. is presented as a promising strategy for the control of cocoa pod rot caused by *M. roreri*.
- The interaction between climatic factors and the antagonistic action of beneficial fungi on moniliasis is complex and multifactorial in agriculture. It is essential to consider not only the environmental conditions for the development of these fungi, but also factors such as soil type, microbial competition, and agricultural practices.

Conflict of interest

The authors declare that there is no conflict of interest in relation to the article presented.

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