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Biological control of moniliosis (*Moniliophthora roreri*) in national cocoa (*Theobroma cacao L.*) using indigenous microorganisms

Control biológico de moniliosis (*Moniliophthora roreri*) en cacao nacional (*Theobroma cacao L.)* mediante microorganismos autóctonos

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ABSTRACT

This study evaluated the use of microorganisms native to Mocache, Ecuador, for the biological control of frost sheath disease. A Completely Randomized Block Design (CSBD) with six treatments and three replicates was used: T1 (Control), T2 (chemical Cuprofix), T3 (Mucilage + molasses), T4 (MN-rich substrate + molasses + water + infected cocoa pod), T5 (MN-rich substrate + molasses + water + infected cocoa pod + mucilage) and T6 (Mucilage + molasses + cocoa pod frost sheath). Data were analyzed by General Linear Models (GLM) and comparison of means with Tukey's test (p<0.05). T5 presented the lowest incidence of M. roreri at 30 days (20.42 %), 60 days (20.84 %) and 90 days (14.89 %). For internal severity, it showed values of 2.00, 2.37 and 2.50 at 30, 60 and 90 days, respectively. For external severity, T6 obtained the best result at 60 days (3.13). In terms of productivity, T5 achieved the highest yield with 410.11 kg ha-1 yr-1.

Keywords: biopreparations; *Moniliophthora roreri*; production parameters; substrate; *Theobroma cacao*

RESUMEN

Este estudio evaluó el uso de microorganismos nativos de Mocache, Ecuador, para el control biológico de la enfermedad de vaina helada. Se empleó un Diseño de Bloques Completamente al Azar (DBCA) con seis tratamientos y tres repeticiones: T1 (Testigo), T2 (Cuprofix químico), T3 (Mucílago + melaza), T4 (Sustrato rico en MN + melaza + agua + vaina de cacao infectada), T5 (Sustrato rico en MN + melaza + agua + vaina de cacao infectada + mucílago) y T6 (Mucílago + melaza + vaina de escarcha de la vaina de cacao). Los datos fueron analizados mediante Modelos Lineales Generales (GLM) y comparación de medias con la prueba de Tukey (p<0,05). El T5 presentó la menor incidencia de M. roreri a los 30 días (20,42 %), 60 días (20,84 %) y 90 días (14,89 %). En cuanto a la severidad interna, mostró valores de 2,00, 2,37 y 2,50 a los 30, 60 y 90 días, respectivamente. Para la severidad externa, el T6 obtuvo el mejor resultado a los 60 días (3,13). En términos de productividad, el T5 alcanzó el mayor rendimiento con 410,11 kg ha^{-1} $año^{-1}$.

Palabras clave: biopreparados; *Moniliophthora roreri*; parámetros productivos; sustrato; *Theobroma cacao*

1. INTRODUCTION

Cocoa (*Theobroma cacao* L) belongs to the Sterculiaceae family and the genus *Theobroma*. It occurs in more than 70,000 km2 in the world between 20° north and south latitude. It is one of the most commercially important crops due to the economic value of its seeds, which has great significance in the development of the economy of several countries within the equatorial area (Antolinez Sandoval et al., 2020). In Ecuador, it is considered one of the most relevant non-oil sectors, which represents a social capital generating income and fame for the country for its chocolate quality (Mendoza Vargas et al., 2021).

Worldwide, Ecuador is one of the largest producers of fine cocoa, thus producing 60% of global production. The country exports to different parts of the world such as Europe and the United States, the largest production is found on the Ecuadorian coast, the varieties that predominate in cocoa plantations are: criollos, trinitario and forastero (Revilla Escobar et al., 2024). According to the International Cocoa Organization (ICCO), in 2022 it had a production of 4.7 million tons (Vega Vega et al., 2023).

In general, the country's cocoa plantations are operated in an integral way, which includes sanitary work which, if not treated in time, compromises production by 90% when contracting diseases. The common ones are moniliasis (*Moniliophthora roreri Cif* and *Par*), witches' broom (*Moniliophthora perniciosa Aime* and *Phillips-Mora*), phytoptora (*Phytophthora spp*.), and to a lesser extent machete disease (*Ceratocystis cacaofunesta Engelbrecht* and *Harrington*). In relation to all the diseases that attack cocoa production, moniliasis is considered the most aggressive (Sánchez-Mora & Garcés-Fiallos, 2012).

The decomposition of the cob caused by moniliasis is considered asymptomatic in its initial stage, this condition changes from the third month when the stage begins where the tissue begins to die (Bailey et al., 2018). On the other hand, Zhang & Motilal (2016) mention that the conditions where it is grown such as the geographical location (climate and humidity) promote diseases, which are added to nature in perennial crops in which the ears remain on the tree, which is why the frozen pod directly affects it.

Chemical fungicides are commonly used to control moniliasis, however, the pesticides used generate resistance, additionally causing environmental damage. The recent European Directive on "Sustainable Use of Pesticides" encouraged the development of alternative control methods (Aiello et al., 2019). There are studies in which they mention that alternative biological methods reduce diseases, biological control refers to protection through the use of microorganisms (Valenzuela-Cobos et al., 2023).

Therefore, global programs have focused on the isolation of biological control agents (BCAs), which include pathogenic fungi (Parafati et al., 2017), studies on microorganisms have been developed mainly to determine their quantities in the soil, so the objective of this research was to evaluate the biological control of moniliosis (*Moniliophthora roreri*) in national cocoa (*Theobroma cacao* L.) by means of native microorganisms.

2. MATERIALS AND METHODS

2.1. Plant material

The study was carried out on National cocoa cultivars in the "Dolores" farm located in the Maculillo area, belonging to the Mocache canton – Ecuador, geographically located at 01° 09' 512'' south latitude and 79° 36' 909'' west longitude, with an average altitude of 65 m.a.s.l. The intervention was carried out in the months of July to October 2016.

2.2. Research design

A field study with six treatments and three replications (Table 1) was conducted to evaluate the effect of four mucilage-based biopreparations and cocoa pods on the control of *Moniliophthora roreri*. The data were analyzed using General Linear Models (GLM) and the mean differences were compared with Tukey's test ($p<0.05$).

Table 1. Study Treatments

Treatments	Description
T1	Witness.
S2	Chemical cuprofix.
S3	Mucilage + molasses.
S4	Substrate rich in MN + molasses + water + M. infected cocoa pod.
S5	Substrate rich in MN + molasses + water + infected cocoa pod + mucilage.
S6	Mucilage + molasses + cocoa pod frost pod.

2.3. Experimental procedure

Mucilage + molasses (T3) biopreparations

The T3 treatment consisted of 480 ml of mucilage and 50 ml of molasses.

Biopreparation of substrate rich in MN + molasses + water + ear infected with *M. roreri*

For the T4 treatment, 1 kg of substrate or soil rich in microorganisms was selected, that is, the soil that has been saturated with fermented cocoa mucilage for a considerable time, plus 0.5 kg of parts of cocoa pods infected with *M. roreri* and crushed into small particles, then placed in the jars with hermetic closure (anaerobic). previously labeled, along with 50 ml of molasses and 1480 ml of distilled water.

Biopreparations of substrate rich in MN + molasses + water + ear infected with *M. roreri* + mucilage

For the elaboration of the T5 treatment, 1 kg of substrate, plus 50 ml of molasses, plus 1000 ml of distilled water, plus 0.5 kg of previously crushed contaminated pods and 480 ml of cocoa mucilage were used, which was extracted by the gravity obtaining method, following the procedure of treatment 1.

Biopreparations of Mucilage + molasses + ear infected with *M. roreri*

The T6 treatment used 480 ml of mucilage together with 50 ml of molasses and 0.5 kg of contaminated cob previously crushed, then it was stored in a dark and cool place for a period of 7 days, once this period had elapsed, it was filtered and diluted in a ratio of 1:20 (1: product and 20: water) to be applied in the plantation.

The pH of the biopreparations was measured in order to obtain products that do not affect the small fruits and therefore the final production, within a pH range of 4.50 to 5.50.

2.4. Experimental measurements

2.4.1. Health variables

Incidence of diseased ears (IME): This was obtained by the total count of harvested ears infected by *M. roreri*. For this, the following formula was used:

$$\text{IME}=\frac{n}{N}*100 \text{ (Ecua 1)}$$

Where:

IME = Incidence of diseased cobs.

n = Number of diseased fruits.

N = Total number of fruits harvested.

Degree of severity: At the time of harvest, the cobs were classified according to the internal and external damage they presented. The symptom classification scale presented in Table 2 was used to perform the evaluations.

Table 2. Symptom Rating Scale

Value	Internal (% of grains affected)	External (Classification of symptoms)
0	0	Healthy fruit
1	1-20	Presence of oily spots (hidrosis)
2	21-40	Presence of swelling and/or premature maturity
3	41-60	Presence of chocolate stain
4	61-80	Presence of mycelium covering up to a quarter of the brown spot
5	>80	Presence of mycelium covering more than a quarter of the chocolate stain

Source: Aranzazu Hernández & Jaimes Suárez (2010)

2.5. Productive variables

Production of healthy ears (DM): Healthy fruits that reached maturity during the evaluation process were counted in order to determine the effect of biopreparations to control the disease.

Yield: It was carried out by carrying out the weight of the ripe fruits that were harvested during the evaluation period, expressed in (Kg/plant).

3. RESULTS AND DISCUSSION

3.1. Incidence of diseased ears (IME)

Table 3 shows significant differences in the incidence of diseased ears at 30, 60 and 90 days. Among the treatments evaluated, T5 (NM-rich substrate + molasses + water + infected cocoa pod + mucilage) had the lowest incidence with 20.42 % at 30 days, 20.84 % at 60 days and 14.89 % at 90 days, the latter being lower than T1 (control). These results suggest that microorganisms influenced the reduction of the disease in the treatments applied.

Table 3. Incidence of monilla disease in cocoa

Treatments	Incidence of Diseased Cobs (IME)		
	30 days	60 days	90 days
T1	73.33±2.04 E	48.74±0.50 D	40.76±0.87 D
S2	32.92±0.54 BC	34.05±0.63 C	26.45±0.34 C
S3	42.68±1.21 D	25.30±0.86 AB	25.71±0.51 C
S4	30.83±0.65 B	28.18±0.59 AB	21.35±0.15 B
S5	20.42±0.38 A	20.84±0.53 A	14.89±0.62 A
S6	36.67±1.35 C	28.40±0.78 AB	26.04±1.06 C
C.V. (%)	16,78	11,34	12,35
S.E.M	0,18	0,11	0,11

Note: CV: Coefficient of variation. SEM: Standard error of the mean. Different letters show significant difference according to Tukey's test (P<0.05)

For their part, Bolaños Ortega et al. (2020) carried out a study on the use of the fungus *T. harzianum* refer to the fact that using biological material helps control the diseases contracted by cocoa, in addition to improving production obtaining 3161.6 Kg $^{Ha-1}$. Other studies have evaluated the incidence of moniliasis according to the months, where they have reported that in the months of June and December its incidence increases by 22%, compared to the month of September that presented with the 2% lower occurrence (Anzules Toala et al., 2022).

3.2. Degree of severity of internal damage to the cob

Regarding the variable of the degree of severity of internal damage in the cobs (Table 4), it was found that T5 (MN rich substrate + molasses + water + infected cocoa pod + mucilage), presented the best results, obtaining 2.00, 2.37 and 2.50, for days 30, 60 and 90 respectively, values that were lower compared to the control treatment. This indicates that the internal damage of the cobs can be reduced.

Table 4. Degree of severity of internal damage in ears affected by monilla

Treatments	Degree of severity of internal damage to the cob		
	30 days	**60 days**	**90 days**
T1	2.84±0.56 A	3.43±0.16 C	3.84±0.31 BC
S2	2.38±0.48 A	3.04±0.52 AB	3.56±0.43 B
S3	3.01±0.73 A	3.00±0.00 AB	3.55±0.64 B
S4	2.72±0.50 A	3.33±0.24 AB	4.00±0.41 C
S5	2.00±0.82 A	2.37±0.48 A	2.50±0.58 A
S6	3.08±0.83 A	3.00±0.41 AB	4.00±0.41 C
C.V. (%)	26,04	9,65	11,23
S.E.M	0,12	0,05	0,07

Note: CV: Coefficient of variation. SEM: Standard error of the mean. Different letters show significant difference according to Tukey's test ($P<0.05$)

Moniliasis is based on integrated management, Villamil et al. (2012) in their study of the effect of using the fungus *Trichoderma spp* against moniliasis presented that after 12 days it obtained 1.00 growth, which indicates that it did influence to stop its spread. Similarly, Valenzuela-Cobos et al. (2023), experimented with controlling the lower incidence when using *Trichoderma spp*, obtaining favorable results which allow the use of 42 strains for its control, thus obtaining 3.1 effectiveness.

3.3. Degree of severity of external damage to the ear

The data obtained in Table 5 showed that T5 (NM-rich substrate + molasses + water + infected cocoa pod + mucilage) obtained the best results at 30 and 90 days, while T6 (Mucilage + molasses + cocoa pod frost pod), had a lower incidence in the 60 days with 3.13. According to Soto-Chochocca et al. (2022), they demonstrated that the use of biological treatments for the control of moniliasis can be reduced, in their study of the addition of *Aloe barbadensis* and *Trichoderma sp*, where they obtained values between 14.9 – 20.5%, in the same way Joya-Dávila et al. (2015), decreased the incidence through the use of *Zingiber* Officinale hydrodistillate obtained percentages between 88 -100%, in other studies they obtained between 40 – 50 % (Tamayo et al., 2016). The antifungal or antimicrobial effect of *Z. officinale* corresponds to gingerol, zingerone, and paradol (bioactive compounds) that contain high flavonoid, phytochemical, and pharmacological content (Nortaa Kunedeb Sowley & Kankam, 2020).

Table 5. Degree of severity of external damage in ears affected by monilla

Treatments	Degree of severity of external damage to the ear		
	30 days	**60 days**	**90 days**
T1	3.75±0.62 B	3.93±0.17 C	4.39±0.13 C
S2	3.13±0.25 AB	3.71±0.21 AB	3.79±0.25 B
S3	3.40±0.49 AB	3.50±0.58 AB	4.30±0.48 C
S4	3.38±0.48 AB	3.35±0.29 AB	4.00±0.41 BC
S5	2.75±0.50 A	3.38±0.48 AB	3.00±0.82 A
S6	3.79±0.25 A	3.13±0.25 A	3.38±0.48 AB
C.V. (%)	13,51	10,53	13,11
S.E.M	0,08	0,06	0,08

Note: CV: Coefficient of variation. SEM: Standard error of the mean. Different letters show significant difference according to Tukey's test (P<0.05)

3.4. Productive variables

Production of healthy ears

Table 6 shows the values of the production of healthy ears, in which a significant difference was observed (p<0.05), demonstrating that T5 obtained a greater number of healthy ears (3.11, 2.69 and 2.88) compared to controls (3.11, 2.69 and 2.88). In this way, it is emphasized that the application of microorganisms reduces the incidence of diseases and increases the health of the fruits. A wide variety of bacterial genera, including *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Stenotrophomonas*, *Streptomyces,* and *Xanthomonas*, have protective activity against plant diseases against fungal and bacterial pathogens (Bolaños Ortega et al., 2020). According to Anzules Toala et al. (2022), the use of fungicides (chemical and biological) decreases the incidence of moniliasis (*Moniliophthora roreri*), which allowed a greater number of healthy ears to be obtained in the plantations.

Table 6. Average number of mature, healthy ears

Treatments	Production of healthy ears		
	30 days	**60 days**	**90 days**
T1 (Witness)	3.11±0.41 A	2.69±0.14 A	2.88±0.24 A
S2	2.88±0.24 A	4.06±0.35 BC	6.77±0.64 C
S3	3.17±0.35 A	3.79±0.47 B	6.39±0.72 C
S4	2.81±0.21 A	5.20±0.66 C	5.94±0.70 BC
S5	5.41±0.46 C	6.14±0.20 D	8.26±0.54 D
S6	3.42±0.30 B	2.88±0.24 A	4.89±0.56 B
C.V. (%)	10,63	10,10	9,53
S.E.M	0,06	0,07	0,09

Note: CV: Coefficient of variation. SEM: Standard error of the mean. Different letters show significant difference according to Tukey's test (P<0.05)

Yield

In yield (Table 7) a significant difference was determined (p<0.05), denoting that treatment 5 (substrate rich in MN + molasses + water + infected ear + mucilage) obtained a higher average with 493.57 KgHa -1 year-1 of dried cocoa, followed by treatments 6 (Mucilage + molasses + infected cob) and treatment 3 (Mucilage + molasses) with 410.11 KgHa -1 year-1 and 405.88 KgHa -1 year-1 respectively. This allowed us to assert that the application of microorganisms can control monilla disease, reducing its incidence and therefore greater production. Quintana Herrera (2021), who used three methods (cultural work, chemical control and biological control) for the control of moniliasis, where he demonstrated that the highest yield was obtained when using *Trichoderma spp* (240 kg) and ethanolic cinnamon extract (213 kg). On the other hand, it is mentioned that 43.2% of producers have reported the attack of more than one disease, decreasing up to 50% of the production of cobs (Sánchez-Mora et al., 2015).

Table 7. Cocoa plantation yield applied different biological controls

Treatment	Yield (KgHa-1year-1)
T1 (Witness)	343.60 ± 21.23 A
S2	367.32 ± 26.30 AB
S3	405.88 ± 3.53 C
S4	392.32 ± 5.59 B
S5	493.57 ± 8.57 D
S6	410.11 ± 3.66 C
C.V. (%)	3,74
S.E.M	2,51

Note: CV: Coefficient of variation. SEM: Standard error of the mean. Different letters show significant difference according to Tukey's test (P<0.05)

CONCLUSIONS

The results indicate that the biological control composed of substrate rich in native microorganisms, molasses, water, microorganisms from infected cob and cocoa mucilage rich in groups of lactic acid bacteria corresponding to treatment five (T5) significantly decreased the incidence of monilla and degree of severity of external and internal damage in the cob. It also allowed for greater production of healthy pods and higher cocoa yields. In this way, it is concluded that biological control is an effective and sustainable alternative or complement to conventional pesticides for the management of fungal and bacterial diseases of plants.

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CONFLICT OF INTEREST

There is no conflict of interest related to the subject matter of the work.

AUTHOR CONTRIBUTION

Conceptualization: Bastidas-Ruiz, V. E. and Paredes-Toala, L. A.
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Formal analysis: Bastidas-Ruiz, V. E.
Acquisition of funds: Bastidas-Ruiz, V. E., Paredes-Toala, L. A. and Jácome-López, G. To
Research: Bastidas-Ruiz, V. E., Paredes-Toala, L. A. and Jácome-López, G. To
Methodology: Bastidas-Ruiz, V. E.
Appeals: Bastidas-Ruiz, V. E., Paredes-Toala, L. A. and Jácome-López, G. To
Supervision: Jácome-López, G. To
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Writing - revision and editing: Bastidas-Ruiz, V. E., Paredes-Toala, L. A. and Jácome-López, G. To

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