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STUDY OF THE EFFECT OF A MINERAL FUNGICIDE TO COMBAT *moniliophthora roreri* ON *thebroma* crop

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Abstract: Moniliasis, caused by the fungus *Moniliophthora roreri* (Monilia), represents one of the main threats to cocoa production in various regions of the world, including southern Mexico. In particular, the state of Tabasco has experienced significant losses in yields and crop quality. Given the economic and social impact that moniliasis has on local farmers, it is crucial to identify and evaluate efficient and sustainable management alternatives for its control. The use of mineral fungicides could offer a promising solution, being a less environmentally invasive option compared to conventional chemicals. The study will generate relevant data to improve agricultural practices in Tabasco, reduce crop losses and contribute to the sustainability of cocoa production. The objective of this research was to evaluate the effect of a mineral fungicide made from vegetable ash for the mitigation of monilia in Grijalva cocoa plantations in Tabasco. The methodology consisted of an experiment in which five different mixtures of the fungicide were tested, applied on cocoa plants in an experimental plot by spraying over a period of four months. Samples were collected for observation of findings and a completely randomized block experimental design (CRBED) with ANOVA analysis was used. The study allowed the identification of the fungicide mixture that forms the most effective protective layer on the ear for the treatment of monilia. The work concludes with recommendations to potentiate the effect of the fungicide developed.
Keywords: *Moniliophthora roreri, Theobroma Cacao*, sustainable development, mineral fungicide, mineral fungicide

INTRODUCTION

The cultivation of Theobroma cacao is of great economic, social and cultural relevance in Tabasco, one of the main cocoa-producing regions in Mexico. In particular, Grijalva cacao, a variety native to the region, is known for its superior quality and distinctive flavor profile, which has earned it national and international recognition. This cocoa plays a key role in the local economy, sustaining numerous smallholder families and cooperatives (ICCO, 2020). According to data from the Mexican Ministry of Economy, in 2023 Tabasco stood out as one of the states with the highest international sales of Cocoa Beans, Whole or Broken, Raw or Roasted, and the main destinations for this product were Japan, the United States, Netherlands, New Zealand and the United Kingdom. Thanks to the production of this bean, Mexico placed its name among the main commercial origins of cocoa along with major producing countries such as Ecuador, Dominican Republic, Peru, Ivory Coast and Colombia.

However, the cocoa crop continues to face major challenges due to fungal diseases, which pose a constant threat to the sustainability of production. Among the most devastating diseases affecting this crop are witches' broom (Moniliophthora perniciosa), black rot (Phytophthora palmivora) and moniliasis or monilia (Moniliophthora roreri). Of these, monilia is one of the most destructive and has caused significant losses in cocoa plantations in Latin America, particularly in key regions such as Tabasco, Mexico.

The fungus Moniliophthora roreri, first identified in Ecuador in 1917, has spread rapidly through Central America and the Caribbean, reaching Mexico in 2000. In Tabasco, high humidity and temperature conditions have favored the spread of monilia, which attacks cocoa pods in formation, causing fruit rot and, in severe cases, losses of up to 90% of the crop. In Tabasco plantations, this pathogen has become a significant threat, affecting the profitability of local producers.

The life cycle of the fungus begins with the appearance of white spots on the ears, which eventually progress to the complete decomposition of the fruit. This pathogen has a high dispersal capacity, as its spores can be carried by the wind, which facilitates its spread between nearby plantations. Historically, control measures have included manual removal of infected ears and the use of synthetic fungicides, but these methods are not always effective and can have negative effects on the environment.

In response to the growing need for more sustainable solutions, the use of mineral fungicides has been investigated as a more environmentally friendly alternative. These products, being based on natural minerals, have a low toxicity profile and have been shown to be effective against several fungal diseases. However, information on their specific efficacy against Moniliophthora roreri on Grijalva cacao in Tabasco is limited.

The objective of this study is to evaluate the effect of a mineral fungicide in the mitigation of monilia in Grijalva cocoa plantations in Tabasco. Through an experimental design carried out under real growing conditions, it seeks to determine the efficacy of this alternative to mitigate the disease, with the hope of providing producers with a more efficient and sustainable tool.

MATERIALS AND METHODS

The research was planned in three parts, the first consisted of the preparation of the fungicide in different proportions of ash - lime in one liter of water, then the field tests were conducted in an experimental farm and finally a statistical analysis was applied to select the sample that achieves the highest percentage of mitigation against moniliasis. Each of these stages is described in greater detail below.

EXPERIMENTAL SITE

The field work was conducted at La Hacienda "Granito de Cacao" which is located on the Hermenegildo Highway, Galeana 2nd Section-Chacalapa, in the municipality of Jalpa de Méndez, Tabasco, Fig.1, is located in the Municipality Jalpa de Méndez of the State of Tabasco Mexico and is located at GPS coordinates: Longitude (dec): -93.135556 and latitude (dec): 18.170000, at a medium altitude of 10 meters above sea level, with a total of 1706 inhabitants. The hacienda is located in the Chontalpa subregion of the region that produces cocoa with denomination of origin "Cacao Grijalva".

The experiment was carried out from September to December 2023, during which time the average values of temperature, relative humidity and insolation were as shown in Table 1.

Month	Average value of T (°C)	Average relative humidity value (%)	Average insolation value (h)
September	24.7 - 30.6	82	9.2
October	23.6 - 29.2	82	6.6
November	22 - 27.5	83	6.2
December	20.8 - 26.3	84	6.1

Table 1. Average values of temperature, relative humidity and insolation of the experimental site.

TYPES OF COCOA IDENTIFIED ON THE EXPERIMENTAL FARM

Talking about the types or kinds of cocoa implies an extensive review since its origins, there may be different opinions regarding the number of types that can be found or it is even likely that there are varieties that have not yet been identified or defined, The existence of different types of cocoa is due to a combination of biological, geographical, environmental and human factors. These factors influence the taste, quality and characteristics of cocoa beans

One of the most common classifications is the one that considers three types, which are: Criollo, Forastero and Trinitario. Particularly

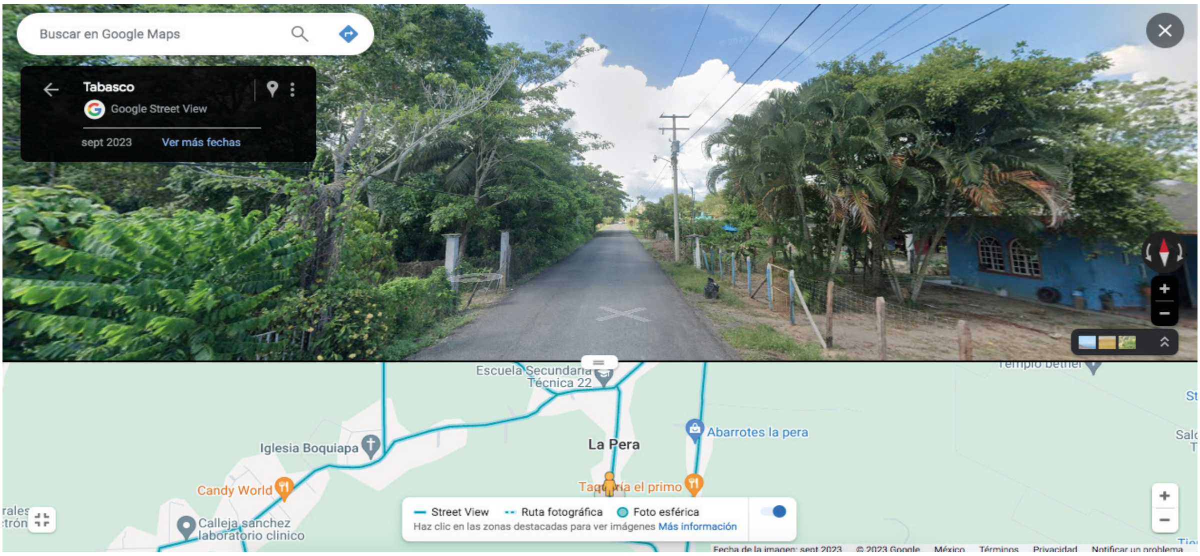

Figure 1. Location of Granito de Cacao Experimental Farm

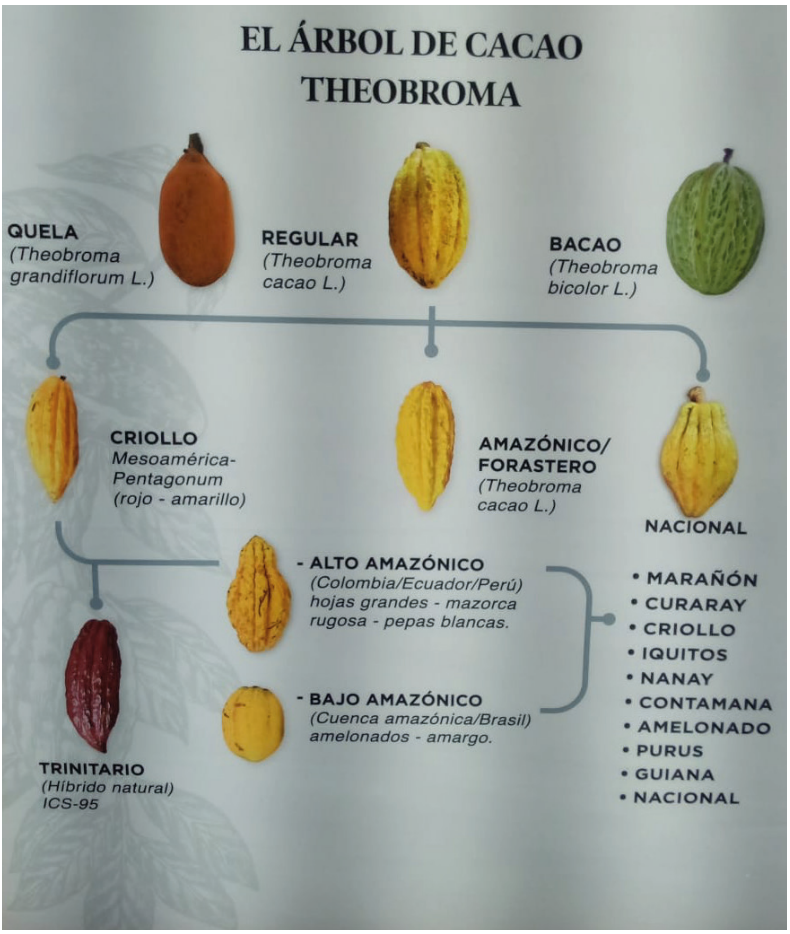

Figure 2. Classification of types of cocoa, photograph obtained from an exhibition at the National Cocoa Museum, Guayaquil, Ecuador.

in this work we have considered to show in two moments the aspect of the identification of the types of fruits of the Granito de cacao farm.

First, it is important to clarify that the bean produced at the hacienda is Grijalva cocoa, a denomination of origin issued by the Mexican Institute of Intellectual Property (IMPI) on August 29, 2016 in which the following natural and human factors that contribute to its peculiar origin were specified,

- Tabasco's geographical location and climate present unique conditions for the growth of cocoa and it has been a producing area since pre-Hispanic times.
- One of the characteristics that gives Cacao Grijalva its identity and quality is that it is produced thanks to the joint action of nature and man in what are called domesticated forests.
- Cocoa flower pollination is mainly and effectively carried out by mosquitoes of the genus Forcipomyia, with greater abundance during rainy periods and in times of peak flowering of cocoa.

Based on the above points and on field work, it was found that the plants are approximately seventy years old and that during that time the geographic and climatological conditions of the region have acted together with pollination to produce the type of fruit that is harvested today. According to a registry of cocoa varieties by the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), it is possible to identify on the farm the Criollo varieties related to Canek, Olmeca and to a lesser extent Tabscoop. The origin of these varieties comes from cloning between Mexican criollo cocoa and genotypes of selections from Ecuador.

On the other hand, a visual comparison has also been made, contrasting a classification found in the National Cocoa Museum located in Guayaquil, Ecuador. See Fig.2 with samples found in the farm Fig.3. It is possible to note that among the varieties can be found criollo, forastero and low Amazonian type.

Figure 3. Samples of cocoa fruit found in the organic cocoa producer "Granito de Cacao".

FINDINGS IDENTIFIED IN THE PLANTATION

The field work made it possible to observe the degree of moniliasis in the plants of the producer farm, see Fig. 4. The total number of plants and the number of plants with cobs infected by the pest gave a percentage of approximately 90% of plants contaminated by the.

Figure 4. Cobs affected by Moniliasis, collected at the Granito de Cacao production farm, showing the degree of disease in the shell, in the beans and in stages from chilillo to cob size.

At least three blooms were also identified of which two were considered as total loss, the third bloom (the most recent) was selected for experimental trials with the different fungicide mixtures. See figure 5.

Figure 5. Flowering plants identified in the producer farm, showing two that are already damaged by the pest and one in a healthy state that was selected for the experiment.

EXPERIMENTAL APPROACH

Based on the diagnosis described above and taking into account the logistical conditions for the field work, it was decided to prepare a set of five mixtures of mineral fungicide whose main component is the residual ash obtained from the combustion of dry biomass combined with lime. The objective of this experiment was to study the effect of this mixture depending on the amount of lime and ash dissolved in a certain amount of water. The sample for the experimental work consisted of five plants for each of the mixtures, and five plants as a control population, forming a plot of 30 plants distributed in 6 rows. Figure 6 shows the sequence of stages of the experiment.

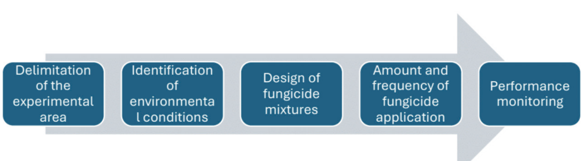

Figure 6. Diagram of the experiment stages.

As mentioned above, five mixtures of the mineral fungicide were prepared, which were named: T1, T2, T3, T4 and T5, see Figure 7, varying among them the amount of vegetable ash and lime, while the amount of water was always the same for all of them. Table 2 shows the proportions of ash and lime used for each treatment.

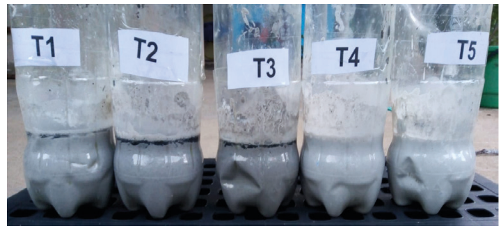

Figure 7. Prepared fungicide treatments

Treatment	Amount of lime (kg)	Amount of vegetable ash (kg)
T1	2	2
T2	1.5	2.5
T3	1	3
T4	2.5	1.5
T5	3	1

Table 2. Fungicide Treatments Applied

From each of the mixtures, one liter of solution was prepared to be poured into 20 liters of water in a manual sprayer pump with which the fumigation was carried out.

FUNGICIDE APPLICATION

Fumigation was initiated at the stage when the cocoa fruits had a diameter between 5 and 6 cm with an approximate length between 3 and 5 cm (measured across the longitudinal centerline), in the first week of their development. From that stage on, spraying was carried out every week for four months during the cocoa growing season, with rigorous monitoring of climatic conditions and the health status of the plants.

Figure 8. Ear of corn in stage ready for fumigation.

Each row was identified with the label of the corresponding treatment (T1, T2, T3, T4, T5), assigning T0 to the control row, which received no treatment. From the first spraying, a record was made of the number of healthy and diseased ears in order to be able to observe any positive effect through variations in the number of healthy and diseased ears, to ensure a correct count from the first to the last spraying with the fungicide, the following measures were applied

- Two people counted and recorded the number of cobs simultaneously in each of the plants, order to obtain an average of the number of cobs.
- Counting was carried out during the hours of the day with the highest illumination in order to have better visibility at the time of counting.

In the following counts, the ears that showed signs of moniliasis, mainly white spots on the husk, were identified, see Figure 4. The climatic conditions that prevailed during the experiment were those shown in Table 1.

RESULTS AND DISCUSSION

Weekly sampling was conducted to evaluate the incidence and severity of Moniliophthora roreri on cocoa pods. The efficacy of each treatment was determined by measuring the percentage of affected pods and the degree of damage compared to the control sample.

EFFECT OF FUNGICIDE TO MITIGATE MONILIASIS

During the period 2021 - 2022 the production of the farm decreased drastically (Figure 9) and the degree of moniliasis infestation reached approximately 98%. A first positive result observed from the third week of spraying was the survival of more than 50% of the fruits of the entire experimental plantation at the first month of development.

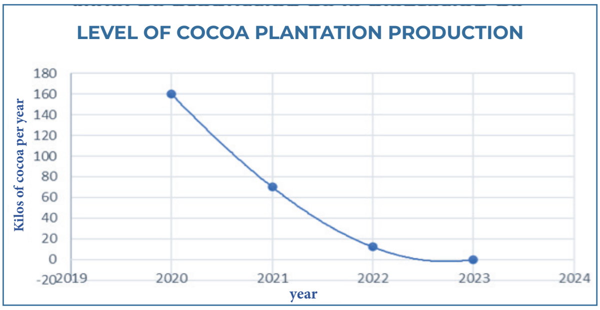

Figure 9. Production of the "Granito de Cacao" production farm

After the sixth week of spraying, the surviving cobs continued to develop and another notable aspect was the size of the cobs of the plants that received the treatments, compared to the plants that did not receive any type of treatment, as in the case of the plants in the control row.

Figure 10. Left: control row cobs, right: cobs from plant with treatment.

It is worth mentioning that each of the five rows (one for each treatment) showed the improvements mentioned above, but in different percentages, so the next step was to analyze which of the rows showed the highest number of healthy fruits, for which an experimental design was applied and a comparison was made with the findings identified in the observational work.

EXPERIMENTAL DESIGN

There are several types of experimental designs, which are classified according to their objectives, the number of factors and the type of effects to be studied. In this research, a completely randomized block design (CRBD) was used with 5 treatments that were applied in an experimental area of 5 rows with four plants each. Each row received one treatment and one more row was included in the design as a control.

The blocks correspond to the spraying periods in which the spraying and the counts of healthy ears were carried out; an average number of ears per treatment applied was recorded. The data collected were subjected to an analysis of variance (ANOVA) to determine if there were significant differences among treatments.

The statistical model for the experiment is written as

$$Y_{ij}=\mu+\tau_i+\gamma_j+\varepsilon_{ij}$$

For

$i = 1,2,3,4,5,6$

$j = 1,2,3,4$

Where,

y_{ij} represents the number of healthy cobs corresponding to treatment i in month j. For each treatment per row, four cocoa plants were available, so that the healthy ears in each row were averaged for the analysis. This calculation was made over the four months of application.

Note that each month represents a block since it is considered that after the first application, a protective layer is formed on the plants that protects each experimental unit, which in some way produces an effect on the experiment.

The arrangement of the data is shown in Table 3, where the data for corresponds to the number of ears of the control, i.e., no treatment was applied.

		Treatments					
		τ_1	τ_2	τ_3	τ_4	τ_5	τ_6
Blocks	γ_1	27	38	26	24	25	28
	γ_2	40	41	42	33	35	37
	γ_3	38	43	35	32	25	36
	γ_4	41	42	38	34	27	41

Table 3. Arrangement of a DBCA for the experiment of mixtures to combat moniliasis in cocoa.

Table 4 shows the doses of the mixtures prepared for each treatment,

	Cal Dose (Kg)	Dose Ash (Kg)
τ_1	2	2
τ_2	2.5	1.5
τ_3	3	1
τ_4	1.5	2.5
τ_5	1	3

Lime and ash levels of mixtures to combat moniliasis in cocoa.

The hypotheses for the treatments to be tested are as follows

$$H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu$$

$$H_0: \mu_1 \neq \mu_j \; para\ algún\ i \neq j$$

To simplify the ANOVA calculations, INFOSTAT software was used. Table 5 shows the following combinations of mean pairs and the conclusion in each case:

μ_i vs μ_j		Common letters?	Conclusion
μ_1	μ_2	No	*Ho* is rejected
μ_1	μ_3	Yes	*Ho* is not rejected
μ_1	μ_4	No	*Ho* is rejected
μ_1	μ_5	No	*Ho* is rejected
μ_1	μ_6	Yes	*Ho* is not rejected
μ_2	μ_3	No	*Ho* is rejected
μ_2	μ_4	No	*Ho* is rejected
μ_2	μ_5	No	*Ho* is rejected
μ_2	μ_6	No	*Ho* is rejected
μ_3	μ_4	No	*Ho* is rejected
μ_3	μ_5	No	*Ho* is rejected
μ_3	μ_6	Yes	*Ho* is not rejected
μ_4	μ_5	Yes	*Ho* is not rejected
μ_4	μ_6	No	*Ho* is rejected
μ_4	μ_6	No	*Ho* is rejected

Note that, from the comparison of means, the mean corresponding to treatment 2 is different from all other treatments, so that the effect of treatment 2 produces on average the highest amount of healthy ears and therefore would be the ideal mix.

It is worth mentioning that the above result coincides with the observations of the field work, which consisted of a continuous inspection in the producer farm, week by week, of the evolution of the fruits that received the treatment. One of the main indications of a correct protective effect is the layer that forms gradually as the fumigations are carried out, see figure 11, this layer was more noticeable on the cobs of the plants fumigated with T2.

Figure 11. Protective layer formed by the fungicide on cob shell

In this experiment, the layer began to be noticeable from the second application. Simultaneously to the treatment with the fungicide, phytosanitary work was also carried out, consisting of pruning the cocoa plants, cleaning the soil and removing the infected pods, which allowed the effect of the applied substance to be enhanced by allowing more sunlight to enter the plants of the experimental plot for a longer period of time.

CONCLUSIONS

The control of monilia in cocoa by means of a fungicide made from vegetable ash in this experiment has allowed obtaining so far a mixture that generates a direct protection to the cocoa pods by means of a protective layer that is formed as spraying is carried out periodically, the proportion that has shown the best results is T2 composed of 2.5 kg of ash and 1.5 kg of lime. Inspection of the samples collected has identified the following three effects of the fungicide on the cobs:

A) This result implies that the fruit received the treatment in a timely manner and that the fumigation was carried out completely on the peel, blocking the entry of the spores.

B) Healthy cob with signs of spores that failed to enter through the shell, healthy almonds in its entirety, this result implies that perhaps due to failures in the spraying technique or rain shortly after application, the protective layer was formed with some slight deficiency, allowing some spores to lodge on the shell, but were eliminated by subsequent applications of fungicide.

C) In this case, the monilia spores were able to implant in the cob and penetrate the shells, causing damage to a percentage of the kernels. However, it was still possible to obtain a portion of healthy almonds. Upon inspection, it was determined that these kernels were located in places where a correct fumigation or

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