Colegio de Postgraduados

Fungicides obtained from plant extracts for the management of *Moniliophthora roreri* Cif. in *Theobroma cacao* L.

Ramírez-González, Sandra I.[1]; López-Báez, Orlando[1]; Espinosa-Zaragoza, Saúl[2*]; Wong-Villarreal, Arnoldo[3]; Romero-Tirado, Rodrigo[2]; Aguilar-Fuentes, Javier[2]; Moreno Basurto, Guillermo[2]; Escobar de la Cruz Salma[2]

[1] Universidad Autónoma de Chiapas, Sistemas Agroalimentarios. Campus IV. Tapachula, Chiapas, México. C.P. 30700.
[2] Universidad Autónoma de Chiapas, Facultad de Ciencias Agrícolas, Campus IV. Huehuetán, Chiapas, México. C.P. 30660.
[3] Universidad Tecnológica de la Selva, División Agroalimentaria. Ocosingo, Chiapas, México, C. P. 29950
* Correspondence: saulez1@gmail.com

ABSTRACT

Objective: The goal of this research is to develop plant extracts and determine their characteristics with effectiveness in the regulation of *M. roreri*, which can be integrated into a management plan for cocoa production in Mexico

Design/Methodology/Approach: The regulatory effect of extracts of: *Pimenta dioica*, *Zingiber officinale*, *Syzygium aromaticum*, *Origanum vulgare*, *Tradescantia spathacea* and *Cinnamomum zeylanicum* on *M. roreri*, using four forms of extraction, carrying out effectiveness tests in the laboratory, and the best extracts were evaluated under field conditions.

Results: The hydrolates of *S. aromaticum* and *C. zeylanicum* at 20% (v/v) were established to be efficient, recording incidences of *M. roreri* in cocoa fruits of 1.18% and 1.08% respectively, with 69.6% in the absolute control. In the *C. zeylanicum* hydrolate, 17 compounds were identified. The hydrolates of *S. aromaticum*, and *C. zeylanicum* at 20% are efficient in the management of cocoa moniliasis *M. roreri*.

Study Limitations/Implications: The production of extracts has specific technical needs, requires investment in producing the extracts in volume and must be made more accessible to producers.

Findings/Conclusions: The use of these extracts on *M. roreri* in traditional or organic production systems is technically and economically feasible.

Keywords: *Cocoa moniliasis*, Cocoa, Plant extracts, Disease management.

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a tropical tree from whose fruits we obtain the seeds from which chocolate is made, as well as the cocoa butter used in the pharmaceutical and food industries. It is estimated that worldwide more than 20 million people depend directly on this crop for subsistence and 90% of the production is harvested from smallholdings (less than 5 hectares). It is mainly grown in 13 countries, of which Ivory Coast, Cameroon,

Ghana, Malaysia, Indonesia, and Brazil obtain 80% of the production. For Mexico, this crop is of great historical, cultural, social, productive, and environmental importance, since it is grown in biodiverse agroforestry systems, which provide multiple ecosystem benefits. Currently, 52,449 hectares are planted in Mexico, located in Tabasco, Chiapas, and Oaxaca, generating more than eight million wages per year, with a contribution of 28,119 tons (SIAP, 2023). Cocoa moniliasis caused by the fungus *Moniliophthora roreri* affects all stages of fruit development. This disease has its first damage reports in Colombia and to date has affected cocoa plantations in 11 different countries in South and Central America. It entered Mexico in 2005, causing production losses, destruction and abandonment of the crop by thousands of producers, causing negative effects on the residents who depended on this crop, a shortage of cocoa, and adverse effects on the ecosystems, since the destruction of biodiverse plantations caused changes in land use, mainly for livestock, sugarcane, African palm, mango, among other monoculture systems (Phillips & Wikinson, 2007; Ramírez, 2008). The production statistics indicate that in 2006, one year after the entry of *M. roreri*, production reached a historical decrease of 50.95% and currently there is a reduction in production and planted hectares of 36% compared to the year prior to its entry to Mexico (SIAP, 2023). Various research works have shown that the management of moniliasis requires precise cultural management, and that the applications of chemically synthesized fungicides can allow a certain degree of control of the pathogen, but with a considerable increase in production costs and this causes environmental deterioration (Ochoa *et al.*, 2017). The use of plant extracts has been shown in various research works to be a highly effective input to manage various phytosanitary problems (Ranasinghe, 2002; Matan & Matan, 2007; Gupta *et al.*, 2008; Tamayo *et al.*, 2016), but it is necessary carry out further research that generates efficient and economically viable alternatives that can be easily carried out by the producer, as well as carry out studies to determine the secondary metabolites that exert activity on phytopathogenic microorganisms and that contribute to the advancement of knowledge of this control alternative (Ramírez *et al.*, 2006). This work aimed to develop and characterize plant extracts that are effective in the management of *M. roreri* which can be incorporated into an organic cocoa production system, for which it was proposed to establish the regulatory effect on *M. roreri* of six plant extracts: *Pimenta dioica* L., *Zingiber officinale* Roscoe, *Syzygium aromaticum* L., *Origanum vulgare* L., *Tradescantia spathacea* Swartz, and *Cinnamomum zeylanicum* Nees.

MATERIALS AND METHODS

Location of the study area. For the development of the research, three phases were proposed: the first in the laboratory, to carry out the selection and determination of the minimum inhibitory concentration of the extracts obtained from six plants with four forms of extraction. The second phase was developed in the field in a monoclonal cocoa plantation in Tapachula, Chiapas, Mexico, where the best extracts selected in the first phase were evaluated and an economic analysis was carried out. In a third phase, the possible components of the extracts that showed the best action against the pathogen were determined, using gas chromatography coupled to mass spectrometry (GC-MS).

Biological material. *Cinnamomum zeylanicum* Nees (leaf and bark), *Syzygium aromaticum* L. (Leaf and flowers), *Pimenta dioica* L. (Seed and Leaf) from the municipality of Pichucalco, Chiapas, Mexico, *Origanum vulgare* L. (Aerial part), *Tradescantia spathacea* Swartz (Leaves), and *Zingiber officinale* Roscoe (Rhizome) from Tapachula, Chiapas. Pathogen evaluated: *Moniliophthora roreri* 'Pichucalco' strain, isolated by the Biotechnology Laboratory of the AUDES Cacao/UNACH.

Extraction methods

Every plant was used in the following four extraction methods:

- **Distillation**: a distiller adapted to obtain extracts was used, for which the plant material was chopped to 1 cm. together with the solvent, they were placed in the distiller kettle and the system was hermetically closed so that the continuous and constant extraction process could be carried out by applying heat and pressure up to 15 psi. With a cooling system, a liquid called hydrolate was obtained.
- **Pressurized**: A kettle with a pressure indicator was used, inside which the 1 cm-chopped plant material was placed together with the solvent and they were subjected to temperature and pressure. Upon reaching 15 psi it was maintained for 10 min, and once it cooled down, it was filtered and the liquid was obtained.
- **Aerobic fermentation**: The 1 cm-chopped plant material was placed in a glass container adapted as a biofermenter and a liter of sterile distilled water was added. It was stirred every day to induce oxygenation and fermentation. On day 14, it was filtered to obtain the liquid.
- **Anaerobic fermentation**: A hermetically closed glass container was used, adapted for the biofermentation process with a safety valve for gas outlet; the 1 cm-chopped plant material was introduced along with sterile distilled water. The fermentation process lasted 14 days; the extract was later obtained by filtration of the mixture.
- **Pathogen strain**: The strain was isolated by the Biotechnology Laboratory of the AUDES Cacao/UNACH, from infected material from the municipality of Pichucalco, Chiapas. The strain was reactivated under laboratory conditions, in Petri dishes with culture medium composed of Difco® brand potato-dextrose-agar (PDA) at 23 °C ± 2 °C.

Phase one. Three effectiveness evaluation methods were used

Agar diffusion method. Test setup at 50% concentration. Each extract was added individually to the culture medium at a concentration of 50% (volume/volume). Once the medium with the extract was prepared, a disk of the fungus was inoculated in the center of the box. They were maintained in a culture chamber under controlled conditions of 23 °C ± 2 °C. The following were included as controls: an absolute control in which the fungus was grown in the PDA medium without any control and another mineral control (10% calcium polysulfide). The total number of treatments was 38. The inhibitory effect was quantified by: the growth of the pathogen by measuring the diameter of the mycelium within each Petri dish every 24 hours, for 12 days, and the production of spores with the support of a

Neubauer chamber (methodology described by Ramírez, 2011). The experimental unit was a Petri dish, and the treatments were distributed in a completely randomized design with five replicates. To determine the effects of the 38 treatments studied, an analysis of variance and Tukey's comparison of means test (5%) were performed on the data obtained.

Determination of the minimum inhibitory concentration: The extracts that showed total inhibition of the growth and development of the pathogen from the previous test at 50% were determined for the minimum inhibitory concentration; this was done by evaluating concentrations of 40, 30, 20, and 10% (volume/volume). The culture medium was prepared with PDA to which each of the extracts was added at the concentrations to be studied, then the inoculation of the pathogen was carried out. The variables evaluated were the same as those of the previous trial.

Paper disc method. It is a qualitative test; 100-mm Petri dishes with PDA medium were used. A disk of the fungus previously grown in PDA medium and with an incubation time of eight days was sown in the center of each box. At a distance of three cm from the disc with the fungus, 9 mm thick and 7 mm in diameter cellulose discs, previously impregnated with each of the 36 extracts, were placed. The four extraction methods for each plant and a chemical control were placed in each Petri dish. They were left to incubate in a culture chamber under controlled conditions of 23 °C ± 2 °C and the inhibition zones that appeared around the paper discs containing the samples were read. Four replicates were done per treatment. The chemical control with i.a. zinc dithiocarbamate, 7 mm diameter Whatman No. 2 paper discs and 50 mm Petri dishes were used.

Kirby-Bauer paper disc method: The PDA culture medium was prepared and kept in a water bath at 45 °C, the homogenized solution of the fungus *M. roreri* was inoculated and 15 mL was poured into 100 mm Petri dishes. Once the medium had solidified, 9 mm thick and 7 mm in diameter cellulose discs impregnated with the extracts were placed on the medium at a radius of 30 mm from the center of the dish. The chemical control was i.a. zinc dithiocarbamate. The samples were subsequently incubated at 23 °C; after 24 hours of incubation each dish was evaluated. For the interpretation of the results regarding the activity of the extract (proposed by Monks *et al*., 2002), the following interpretive categories are established for the diameters of the inhibition zones: (−) No activity, (+) Mild or weak activity (diameter between 7-11 mm), (++) Moderate activity (diameter between 11-16mm), (+++) Strong activity (diameter greater than 16 mm).

Work under field conditions: The tests were carried out on a monoclonal (Clone UNACH 130) cocoa plantation, located at 14° 52' 33.4" NL and 92° 21' 28.8" WL, at an altitude of 47 masl, in Tapachula, Chiapas, Mexico.

Effect of extracts on germination inhibition. In order to obtain fruits of the same age and without incidence of monilia, artificial pollination was carried out on flowers of Clone UNACH 130 and then a plastic device was placed to isolate the flowers and waited for their development. Subsequently, the inoculum of *M. roreri* was prepared and each of the extracts and calcium polysulfide were sprayed on 70-day-old fruits and covered with a polyethylene bag and a damp towel was placed in each for three days. Then, they were uncovered and inoculated with *M. roreri*, using dry conidia attached to the tip of a dissecting needle (9×10^4 conidia/mL), which were deposited in an area of two square

centimeters of the area previously marked with enamel and moistened with sterile water. After inoculation, the young fruits were protected in a humid chamber (according to the methodology described by Merchán, 1991). Another group of fruits was inoculated with *M. roreri* with the same methodology and one day later the treatments were sprayed, keeping the chamber humid.

Treatments: The extracts obtained in the form of hydrolates of *C. zeylanicum* (bark), *Pimenta dioica* (seeds), *S. aromaticum* (flowers), and calcium polysulfide were tested, applied before and after inoculation of the fungus; an uninoculated control and one inoculated with *M. roreri* were also included.

Evaluation: To finish the test, 80 days after inoculation with *M. roreri*, destructive sampling of the ears of each treatment was carried out in order to measure the following variables: incidence, external severity (ES), and internal severity (IS).

- **Incidence**: percentage of diseased fruits in relation to the total of inoculated fruits.
- **External severity**: based on the external appearance of the fruit and the signs of the pathogen, using the scale: grade 0=healthy fruit, 1=humpback, 2=mosaic, 3=spot (necrosis), 4=mycelium up to 25% of the spot, 5=mycelium in more than 25% of the spot (adapted from Brenes, 2003).
- **Internal severity**: it is the percentage of internal necrosis observed in the fruit when it is cut longitudinally and measured with a scale developed by Sánchez *et al.* (1982).

Experimental design: A completely randomized design was used with four treatments and 10 replicates, with the experimental unit being one fruit for a total of 40 fruits for the test.

Statistical analysis: The Internal Severity Index (ISI) and External Severity Index (ESI) data were transformed using the formula $(value + 0.5)^{1/2}$. To determine if there were significant differences in each of the treatments, the analysis of variance was performed and Tukey's mean comparison test was applied at the 5% significance level, when a significant difference existed. The data were processed in the SAS program for Windows 9.0.

Effect of extracts on the development of the disease in cocoa trees

Conditioning of the Experimental site: With the best extracts obtained from the laboratory phase, field tests were carried out in a commercial plantation (Clone UNACH 130) and in the organic certification process, the conditioning of the trees was carried out with cleaning, pruning work, elimination of fruits diseased by moniliasis and other diseases, and layout of the experimental plot.

Application of extracts: Each of the extracts was mixed with water to complete the volume corresponding to the concentration of each hydrolate. The applications were done every 15 days with a manual sprayer to all the trees in each of the plots, in the morning hours.

Treatments: The best extracts from Phase 1 were evaluated, hydrolates of: *S. aromaticum* 20% (v/v), *C. zeylanicum* (30% (v/v), and *P. dioica* 30% (v/v), and calcium polysulfide (10%)

as chemical control. The fruits were sprayed every 15 days using spray pumps intended exclusively for this test; two controls were included: cultural management and absolute control to which no handling was done in order to appreciate the natural behavior of the disease in the test plot.

Experimental design: A completely randomized design was used with five replicates per treatment and six treatments. The experimental unit was one tree for a total of 30 trees per treatment throughout the evaluation period.

Evaluation: Evaluations were taken every 15 days from each of the trees in each treatment. The variables quantified were:

- **Incidence of the disease**: evaluating: number of: healthy young fruits, young fruits diseased by *M. roreri*, healthy ears, ears diseased by *M. roreri*.
- **External severity** (**ESI**) and **Internal severity** (**ISI**): the scale developed by Sánchez *et al.* (1982) was used.
- **Production**: every eight days, the number and total weight of the healthy fruits harvested and the dry weight of the grains were determined.

Statistical analysis: The incidence data obtained were transformed using the arcsine formula $(percentage/100)^{1/2}$ and ISI and ESI using the formula $(value+0.5)^{1/2}$. An analysis of variance and Tukey's mean comparison test at 5% were performed. The data were processed in the SAS program for Windows 9.0. An economic analysis was carried out establishing the cost of production and the value of the sale of cocoa.

Phytochemical characterization of the extracts: Using gas chromatography coupled to mass spectrometry (GC-MS), the extracts were analyzed, characterizing their compounds, as well as their contents. The analyses were carried out at the National Laboratory for Prevention and Control of Doping-CONADE-Mexico. For the GC-MS study, a Gas Chromatograph (model 6890 N) coupled to a Mass Spectrometer (Model 5973N) was used, both from Agilent Technologies, manufactured in China, equipped with a slip/splitless (12:1) injection port. Agilent 19091A-002 column, Methyl Siloxane, capillary, with the following characteristics: length 25 m, diameter 200.0 µm with a particle size of 0.11 μm, carrier gas, Helium, with initial flow of 1 mL/min and then constant flow. Injection volume 1 μL. Initial temperature 60 °C, final temperature 325°C, Run time 114.67 min. For the identification of the compounds, they were compared with the NIST MS 2.0 database.

RESULTS AND DISCUSSION

The results show that all the plants evaluated contain metabolites that are capable of inhibiting the development of the pathogen to a greater or lesser extent. *In vitro*, the most effective were the hydrolates of the flower buds of *S. aromaticum*, leaves and bark of *C. zeylanicum*, and leaves of *P. dioica* at 20% (V/V), as well as the pressurized flower buds of *S. aromaticum*, followed by hydrolates of seeds from *P. dioica*, *C. zeylanicum* bark, and *Z. officinale*, and the aerobic fermentation of *S. aromaticum* flower buds at 30% (V/V). Regarding the best way to obtain extracts, hydrolate turned out to be the method that showed the

best regulatory effect on *M. roreri*, followed by pressurization and aerobic fermentation. Of the three methods evaluated (agar diffusion, modified paper discs, and Kirby-Bauer) to determine the in vitro effectiveness of the extracts on *M. roreri*, the hydrolates of *S. aromaticum*, *C. zeylanicum*, and *P. dioica* were the extracts that showed the greatest inhibition under the three methods evaluated. Table 1 shows the main results.

Under field conditions, both for the artificial inoculation test and for application in a monoclonal cocoa plantation, the hydrolates of *S. aromaticum* and *C. zeylanicum* at 20% showed their high effectiveness in the management of cocoa moniliasis, since even with a high natural incidence of *M. roreri* (69.6%), the reduction values in the disease's impact when applying the hydrolates of *S. aromaticum* and *C. zeylanicum* in the field was 98% (Table 2), and cocoa production increased between 800 and 1000% (Table 3) with respect to the control. This allows obtaining a Benefit/Cost ratio greater than 2 and a profitability of 127.95% and 138% for the hydrolates of *S. aromaticum* and *C. zeylanicum* respectively (Table 4), indicating a high technical and economic feasibility of these alternatives within a management plan for cocoa moniliasis, whether in a traditional system or with organic management.

Regarding the *S. aromaticum* hydrolate, the phytochemical analysis recorded six peaks, of which peak four and six are not natural products, being considered impurities. The majority compound being Eugenol Acetate, the percentage of this compound removing the impurities would be 58.95%, the next compound under these same circumstances would correspond to Eugenol with 15.96%, 2-propenal, 3-(4-hydroxy-3-methoxyphenyl) with 14.84%, and finally peak two corresponding to vanilla with 10.23%. These compounds have been reported by several authors as components of the essential oil of *S. aromaticum*, although in different percentages since they report eugenol as the majority compound with 70 to 85%, and other compounds such as αand αcaryophyllenes, isoeugen, acetate eugenol, and small amounts of esters, ketones, and alcohols. There are also reports that clove essential oil contains 85 - 95% eugenol and acetyleugenol (Mazzafera, 2003; Matan & Matan, 2007; Omidbeygi, 2007; Padrón, 2010; Kumar *et al*., 2012). Achimon *et al.* (2021) reported only 9 constituents for the essential oil, eugenol being the main component (88.70%), followed by β-caryophyllene (6.55%).

The effect of the *S. aromaticum* hydrolate on *M. roreri* may be due to eugenol, which is a phenolic compound with antiseptic action with a recognized inhibitory effect on various organisms. Cai and Wu (1996) verified the antimicrobial potential of the non-polar extracts of *S. aromaticum* against Gram-negative oral pathogens, bacteria such as *E. coli*, and *Salmonella* strains (Di Pascua *et al*., 2006). Dorman and Deans (2000) established its effect on 25 gram-positive and gram-negative pathogenic bacteria in cultures and humans. Mazzafera (2003) also reported its effect on nematodes and insects, and as an antiviral.

Padrón (2010) found that fungi were the most susceptible microorganisms to the fraction with antimicrobial activity of the hexane extract of *S. aromaticum*, whose main constituent was eugenol. It was also observed that the most susceptible fungi were the filamentous ones, mainly *Trichophyton tonsurans* and *Sporotrix schenckii*. These antifungal qualities of *S. aromaticum* on filamentous strains have also been described

Table 1. Comparison of three methods for evaluating the regulatory effectiveness of plant extracts and chemical synthesis products on the growth of *M. roreri*.

Extract	Dilution method		Paper Disc Methods (Degrees of inhibition)	
	50 %	CMI	Modified Kirby-Bauer	Paper discs
C. zeylanicum leaf hydrolate	I	20	–	+++
C. zeylanicum leaf pressurized	I	40	–	+
C. zeylanicum leaf Aerobic F.	I	50	–	+++
C. zeylanicum leaf Anaerobic F.	C	50	–	++
C. zeylanicum bark hydrolate	I	30	++	++
C. zeylanicum bark pressurized	C		–	+
C. zeylanicum bark Aerobic F.	C		–	–
C. zeylanicum bark Anaerobic F.	C		–	–
P. dioica leaf hydrolate	I	20	–	–
P. dioica leaf pressurized	I	50	–	–
P. dioica leaf Aerobic F.	C		–	–
P. dioica leaf Anaerobic F.	C		–	–
P. dioica seed hydrolate	I	30	+	++
P. dioica seed pressurized	I	40	–	
P. dioica seed Aerobic F.	C		–	–
P. dioica seed Anaerobic F.	C		–	–
S. aromaticum leaf hydrolate	I	50	–	–
S. aromaticum leaf pressurized	C		–	–
S. aromaticum leaf Aerobic F.	C		–	–
S. aromaticum leaf Anaerobic F.	C		–	–
S. aromaticum seed hydrolate	I	20	+	+++
S. aromaticum seed pressurized	I	20	–	++
S. aromaticum seed Aerobic F.	I	30	–	+
S. aromaticum seed Anaerobic F.	I	40	–	++
O. vulgare hydrolate	I	50	–	–
O. vulgare pressurized	C		–	–
O. vulgare Aerobic F.	C		–	–
O. vulgare Anaerobic F.	C		–	–
T. spathacea hydrolate	I	50	–	–
T. spathacea pressurized	C		–	–
T. spathacea Aerobic F.	C		–	–
T. spathacea Anaerobic F.	C		–	–
Z. officinale hydrolate	I	30	–	–
Z. officinale pressurized	C		–	–
Z. officinale Aerobic F.	C		–	–
Z. officinale Anaerobic F.	C		–	–
CHEMICAL				
i.a, Zn dithiocarbamate 5 g/L	I		++	+

C=Pathogen growth I=Growth inhibition + Light inhibition ++ Moderate +++ Strong

Table 2. Comparison of means by Tukey's Multiple Range test of the treatment effect of *S. aromaticum*, *C. zeylanicum*, and *P. dioica* hydrolates on the incidence of *M. roreri* in a cocoa plantation.

Treatment	% Total incidence	Tukey*	% Incidence in young fruit	Tukey*	% Incidence in ears	Tukey*
Calcium polysulfide	0.5375	a	0.0763	a	0.4612	a
C. zeylanicum Hydrolate	1.0841	b	0	a	1.0841	b
P. dioica Hydrolate	1.1593	b	0.2637	b	0.8956	b
S. aromaticum Hydrolate	1.1852	b	0.0806	a	1.1046	b
Cultural control	21.023	c	5.2295	c	15.7939	c
Absolute control	69.641	d	19.1607	d	50.4805	d

* Means with the same letter are not statistically different.

Table 3. Comparison of means by Tukey's Multiple Range test of the effect on dry cocoa production of the application of hydrolates of S. aromaticum, *C. zeylanicum*, and *P. dioica* for the management of *M. roreri*, in a commercial plantation.

Treatment	Kg ha^{-1}	Tukey*
Absolute control	89.05	a
Cultural control	280.91	a
P. dioica Hydrolate	760.35	b
S. aromaticum Hydrolate	780.90	b
C. zeylanicum Hydrolate	917.90	b
Calcium polysulfide	928.35	b

* Means with the same letter are not statistically different.

Table 4. Economic analysis of each of the treatments applied to Clone UNACH 130.

Treatment	Total costs USD	Total Income USD	B/C	Net benefit	Profitability %
C. zeylanicum Hydrolate	1887.50	4302.66	2.3	2415.16	127.95
S. aromaticum Hydrolate	1537.50	3660.47	2.4	2122.97	138.1
P. dioica Hydrolate	1887.50	3564.14	1.9	1676.64	88.8
Calcium polysulfide control	1337.50	4351.64	3.3	3014.14	225.4
Cultural control	868.75	1316.48	1.5	447.73	51.5
Absolute control	543.75	417.42	0.8	−126.32	−23.2

B/C=Benefit:Cost ratio.

by authors such as Amiri *et al*. (2008), who mention eugenol as a possible active agent in formulations to prevent the development of post-harvest diseases of fruits such as apples. Likewise, Costa (2011) reports the effect of *S. aromaticum* on *F. oxysporum*, and *R. solani*, and also cites Amaral & Bara (2005) who report an effect on the reduction of mycelial growth of the essential oil of *S. aromaticum* on *Rhizopus stolonifer*. Ranasinghe *et al*. (2002) demonstrate the inhibitory action of *S. aromaticum* on *Lasiodiplodia theobromae*,

C. musae, and *C. proliferatum*, *Fusarium*, and *Colletotrichum* in banana. Acedo-Zegarra *et al.* (2020), demonstrated 100% total antifungal activity against *Fusarium oxysporum* for a 5% concentration of *S. aromaticum* extract.

Gómez-López *et al.* (2020) report the *in vitro* effect of total inhibition of the growth 1of *M. roreri* from bio-oils at 500 μL L^{-1} of *O. vulgare*, *S. aromaticum*, *T. vulgaris*, and *C. verum*, results similar to those of Tamayo *et al.* (2016). However, in the present work, greater activity was found both *in vitro* (using three analysis methods: agar diffusion, modified paper discs, and Kirby-Bauer) and in the field of *S. aromaticum*, *C. zeylanicum*, and *P. dioica* hydrolates, which managed to reduce the incidence of *M. roreri* by more than 98% and increase production and thus profitability, thus being the first reports of the regulatory effect in the laboratory and in the field of the use of vegetable extracts in the control of *M. roreri*.

The antifungal activity of the *S. aromaticum* essential oil is related to its hydrophobicity, which allows it to interact with the lipid wall, the cell membrane, and the permeability of the mitochondria, altering them and causing disturbances in these structures. Oil components can bind to ions and molecules (hormones) of other cells. Dorman & Deans (2000) report that natural antifungals cause damage to the cell membrane of cells exposed to them, leaving them extremely soluble and fractures that ultimately expose the cellular contents, including the nucleus. According to Padrón (2010), essential oils have strong antibacterial properties because they contain a high percentage of phenolic compounds such as eugenol. The above suggests that its mechanism of action is similar to that of other phenolic compounds, by altering the cytoplasmic membrane, interrupting the proton motive force (PMF), the flow of electrons, active transport, and coagulation of cellular contents. It has been found that sublethal concentrations of eugenol inhibit the production of amylase and proteases of *B. cereus*, deteriorating the cell wall, which causes cell lysis. The hydroxyl group of eugenol, to which certain proteins bind, is believed to prevent enzymatic action in *E. aerogenes*.

The effectiveness of the hydrolates of *S. aromaticum*, *C. zeylanicum*, and *P. dioica* is possibly due to their preventive effect by inhibiting the germination and multiplication of conidia, as well as the fungicide effect by destroying the conidia of *M. roreri*, which together causes the reduction of the incidence and external and internal severity of the disease. For the *C. zeylanicum* hydrolate, 17 compounds were identified, the majority being cinnamic aldehyde with 74.08%, eugenol with 6.8%, and cinnamyl acetate with 5.18%. Meanwhile for the *S. aromaticum* hydrolate, the majority compounds are eugenol acetate (58.95%) and eugenol (15.96%), which have reports of activity on various organisms, and these compounds may be the ones that exert inhibitory action on *M. roreri*.

CONCLUSIONS

The hydrolates of *S. aromaticum*, and *C. zeylanicum* at 20% are efficient in the management of cocoa moniliasis *M. roreri*; these are technically and economically viable to incorporate into a *T. cacao* production system in Mexico.

The best way to obtain the extracts is the hydrolate, since in the evaluated plants, *P. dioica*, *Z. officinale*, *S. aromaticum*, *O. vulgare*, *T. spathacea*, and *C. zeylanicum*, this turned

out to be the method that showed the best regulatory effect on *M. roreri*, followed by pressurization and aerobic fermentation.

Even with a high natural incidence of *M. roreri* (69.6%) in a monoclonal cocoa plantation, the reduction values in the disease's impact when applying the hydrolates of *S. aromaticum*, *C. zeylanicum*, and *P. dioica*, and calcium polysulfide in the field allowed an increase in cocoa production and with a Benefit/Cost ratio greater than 2. This indicates a high technical and economic feasibility of these alternatives within a management plan for cocoa moniliasis.

The effectiveness of hydrolates from *S. aromaticum*, *C. zeylanicum*, and *P. dioica* is possibly due to the preventive effect by inhibiting the germination and multiplication of conidia, as well as the fungicide effect by destroying the conidia of *M. roreri*, which together causes the reduction of the incidence and external and internal severity of the disease.

In the case of the hydrolate, 17 compounds were identified, the majority being cinnamic aldehyde, eugenol, and cinnamil acetate; while for the hydrolate of *S. aromaticum*, they were eugenol acetate and eugenol, which have reports of activity on various organisms, and these compounds may be the ones that exert inhibitory action on *M. roreri*.

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