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FOOD MICROBIOLOGY - RESEARCH PAPER

Essential oils on the control of fungi causing postharvest diseases in mango

Elke Simoni Dias Vilela[1] · Daniel Terao[2] · Sonia Claudia do Nascimento de Queiroz[2] · Adriane Maria da Silva[3] · Aline de Holanda Nunes Maia[2] · Juliana Aparecida Fracarolli[3] · Caroline Dorta[4] · Larissa da Silva dos Santos[5]

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Abstract

The use of fungicides in the postharvest treatment of mangoes has been widespread due to the incidence of pathogens, but awareness of the health risks arising from their use has increased, driving the search for more sustainable treatments. This study aimed to evaluate the activity of antifungal treatment of seven essential oils (EO) against four fungi that cause postharvest diseases in mangoes and define the minimum inhibitory concentration (MIC) and chemical composition, analyzed by gas chromatography (GC-MS). The results showed that the EOs of oregano, rosemary pepper, cinnamon bark, and clove inhibited 100% of the mycelial growth of the studied pathogens, with MIC ranging from 250 to 2000 μL.L^{-1}. The main compound found in oregano was carvacrol (69.1%); in rosemary and pepper oil, it was thymol (77.2%); cinnamaldehyde (85.1%) was the main constituent of cinnamon bark, and the eugenol (84.84%) in cloves. When evaluating the antifungal activity of these compounds, thymol and carvacrol showed greater inhibitory activity against fungi. Therefore, this study showed the great potential of oregano, clove, rosemary pepper, and cinnamon bark essential oil as alternative treatments to synthetic fungicides in controlling postharvest diseases in mangoes.

Keywords Alternative treatment · Fruit · MIC · Carvacrol · Thymol · Mango postharvest disease-causing fungi

Introduction

Mango (*Mangifera indica L.*) is one of the most appreciated fruits in the world due to its exotic flavor and high nutritional value. In addition, it is a rich source of bioactive compounds such as vitamins A and C and carotenoids, which have antioxidant, anti-inflammatory, and anticancer activity [1]. Brazil is a major producer and exporter of mango. The yield in 2021 was around 1.5 million tons in an area of 71.8 thousand hectares, exporting 272.6 thousand tons mainly to the American and European markets [2].

Mango is a climacteric fruit usually harvested when it is still immature and ripens even after harvesting. Its ripening involves physiological and biochemical changes, accompanied by a sharp increase in respiration and ethylene production. Due to their perishable nature, fruits are susceptible to attack by pathogens, especially during storage and transport, causing severe losses of fruit lots [3]. Fungi are the most relevant regarding quality and productivity losses in fruits, making it challenging to supply quality fruits to consumers [4]. Anthracnose is the most common postharvest disease in mango, and *Colletotrichum* spp. is the causal agent [5]. There are about 18 species of *Colletotrichum* spp. associated with mango [6], and currently, *C. siamense* has been frequently reported [7]. *Alternaria alternata*, *Lasiodiplodia theobromae*, and *Botryosphaeria dothidea* are other species that compromise the postharvest quality of the fruit [8].

Currently, agrochemicals have been used indiscriminately to control these postharvest diseases, resulting in chemical

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✉ Elke Simoni Dias Vilela
elke.vilela@embrapa.br

[1] Embrapa Meio Ambiente, Raquel Ghini Environmental Microbiology Laboratory, Jaguariúna, SP, Brazil

[2] Embrapa Meio Ambiente, Research Sector, Jaguariúna, SP, Brazil

[3] UNICAMP, Universidade Estadual de Campinas, Agricultural engineering, Campinas, SP, Brazil

[4] UFSCar, Universidade Federal de São Carlos, Agroecology, Araras, SP, Brazil

[5] UFSCar, Universidade Federal de São Carlos, Biology, Araras, SP, Brazil

contamination of the fruits [9]. However, the low availability of registered fungicides on the market to control postharvest disease in mangoes has led to the emergence of resistant pathogen strains through the frequent use of the same active principle [10].

As an alternative to reducing the use of fungicides to control postharvest pathogens in agricultural products, the use of essential oils (EO) has been studied. Despite having a complex composition, EO is widely used due to its broad effectiveness against more than one microorganism. It is important to know its composition and biological properties to help study its effectiveness in controlling fungal diseases [11, 12].

There are studies in the literature showing the activity of EOs and their constituents against a wide range of fungi that cause postharvest diseases in fruits, such as *Botrytis cinerea* [13], *Fusarium avenaceum* [14], and *Geotrichum citri-aurantii* [15], among others. It has been characterized that the mechanism of antifungal action of EOs is attributed to their constituents, which act synergistically or additively in exerting their effects. These constituents are believed to induce enzymatic inhibition, interfere with growth, and seriously damage the hyphal structure [16]. Effects such as inhibition of mycelial growth and spore germination have already been obtained using these individual constituents [17]. The knowledge of the antifungal activity of the constituents can help to understand the antifungal activity of EO [18]. Therefore, the analysis of the constituents of EOs and their antifungal activity is essential to monitoring the quality of the product.

The present study aims to evaluate the antifungal activity of seven EOs on the mycelial growth of fungi: *C. siamense*, *A. alternata*, *L. theobromae*, and *B. dothidea* from mango, to determine the minimum inhibitory concentration (MIC). Moreover, it analyzes the selected oils' chemical composition and evaluates the main constituents' antifungal activity.

Materials and methods

Essential oils

The EOs evaluated were rosemary of field (*Baccharis dracunculifolia*), rosemary pepper (*Lippia sidoides*), clove basil (*Ocimum gratissimum*), oregano (*Origanum vulgare*), tea tree (*Melaleuca alternifolia*), cinnamon bark (*Cinnamomum cassia*), and Himalayan cinnamon (*Cinnamomum glaucescens*).

Mango phytopathogens

The fungal isolates of *C. siamense* (CMAA 1129), *A. alternata* (CMAA 1130), *L. theobromae* (CMAA 1131), and *B. dothidea* (CMAA 1133) were provided by the Collection of Microorganisms of Agricultural and Environmental Importance – CMAA, located at Embrapa Meio Ambiente, in Jaguariúna, SP, Brazil.

Antifungal activity evaluation of the essential oils

Discs of 5 mm in diameter of each fungus, taken from the margins of active growth colonies, were placed in the center of 90 mm Petri dishes with potato-dextrose-agar medium (PDA) supplemented with 1 µL EOs mL^{-1} [19]. To quantify the influence of the solvents used to dilute the EOs, hexane and ethanol were evaluated. The check consisted of Petri dishes containing only PDA medium without EOs. Petri dishes were sealed with parafilm and incubated at 23 ± 2 °C. The experimental design was completely randomized with four replications, considering one Petri dish as an experimental unit. The trial was repeated once. The evaluations were carried out by daily measurements of fungal colony diameter (in two orthogonal directions) using a digital caliper until the check colony reached the total diameter. EOs that exhibited greater inhibition of mycelial growth of pathogens, compared to check, were selected for the following essays.

Determination of the minimum inhibitory concentration (MIC) of selected essential oils

In order to evaluate the MIC of the EOs that had shown the best inhibitory activity [19], 5-mm mycelium discs, taken from the margins of the area with active pathogen growth, were placed in the center of 60 mm Petri dishes containing 7 mL of PDA added with 0.05% tween 80, supplemented with EOs. Previously, selected EOs were incorporated into the PDA medium with concentrations adjusted to 170 $\mu L.L^{-1}$, 250 $\mu L.L^{-1}$, 500 $\mu L.L^{-1}$, 1000 $\mu L.L^{-1}$, or 2000 $\mu L.L^{-1}$. Check plates consisted of PDA added with 0.05% tween. Plates were sealed and incubated at a temperature of 23 ± 2 °C, and the measurements of mycelial diameter were performed daily in two orthogonal directions. The evaluations were carried out until the complete mycelial growth of the pathogens in the check plates. The statistical design was completely randomized, with six replications. The lowest concentration that completely inhibited mycelium growth was defined as the MIC. The experiment was repeated once.

Chemical analysis of selected essential oils by gas chromatography (GC)

The chemical composition of the selected oils was analyzed by gas and mass chromatography (GC-MS) using an Agilent Gas Chromatograph (GC) equipment, 7890B (Agilent Technologies, Palo Alto, CA, USA), installed on the HP-5MSui

column (30m × 0.25 mm i.d., 0.25 um pore). The oven temperature was initially programmed at 50 °C for 2 min and a ramp of 3 °C/min to 230 °C for 20 min and 30 °C for a minute to 300 °C for 10 min. Helium was used as a carrier gas with a flow rate of 1,2 mL.min^{-1}. The injector temperature was 220 °C in splitless mode, and the injection volume was 1 µL. The mass spectrometer, coupled to the GC Agilent 5977B (MSD) with electron ionization source (70eV), was used in scan mode (40–500 m.z^{-1}). The source temperature was 280 °C, and the transfer line was 300 °C. Retention indices were calculated using a homologous series of n-Alkanes C7-C40 injected under the same conditions as the sample. EOs were characterized by calculating the retention index (RI) and comparing the values felt [20]. Using the Agilent MassHunter Workstation Quantitative Analysis Unknowns Analysis software (version 10.0-2016-2018), the spectra of each peak of the chromatogram were compared against mass spectra from the NIST17 database – Mass Spectral Search Program (version 2.3, 2017).

Evaluation of the antifungal activity of constituents

The assessment of the antifungal activity of the major constituents present in the selected oils was carried out according to SANTOS & MELO [21] using a sterilized filter paper disc (6 mm) containing 10 µL of the constituent diluted in a specific solvent at a distance of 2 cm from the 5-mm mycelium disc placed on the plate Petri dish (90 mm in diameter), containing PDA medium. The solvents used were ethanol and ethyl acetate, according to the solubility coefficient of the constituent. The concentration evaluated for thymol and caryophyllene was 0.1 µg.µL^{-1} and, for the other constituents, 0.2 µl.µL^{-1}. The plates were incubated at 23 ± 2 °C, and the diameter of the inhibition halo was measured daily with a digital caliper. Two checks were used: filter paper discs containing 10 µL of ethyl acetate and 10 µL of ethanol. A completely randomized experimental design with three replications (Petri dishes) was adopted. The experiment was repeated once.

Data analysis

The inhibitory effect of essential oils on mycelial growth for each of the four phytopathogenic fungi studied was evaluated by the area under the disease progress curve [AUCMG; [22]]. The progress curve for *C. siamense*, *A. alternata*, *L. theobromae*, and *B. dothidea* was defined as the temporal variation of the means diameter of mycelial growth during five, ten, three and four days of evaluation ($n = 5$, $n = 10$, $n = 3$, $n = 4$).

The AUCMG for each treatment was calculated by summing ($n-1$) areas under each progress curve segment between the ordinates (Y_i,Y_{i+1}), which correspond to the mean diameter of the lesion (mm) for each pair of consecutive evaluation times in days, (t_i,t_{i+1}), respectively, multiplied by the length of the (t_i,t_{i+1}) interval (Eq. (1)).

$$AUCMG = \sum_{i=1}^{n-1} \left[\left(\frac{Y_{i+1} + Y_i}{2} \right) * \left(t_{i+1} - t_i \right) \right] \quad (1)$$

AUCMG data were submitted for analysis of variance (ANOVA). Tukey's test was used to compare means at a 95% confidence level ($p < 0.05$), using the R software, version 4.2. 1. The progress curves were displayed in line graphs (x = evaluation days and y = average mycelial growth, in mm), and the mean AUCMG for each treatment with their respective standard errors were displayed in bar graphs.

The percentage of mycelial growth inhibition (PMGInib; 22) was determined using the mycelial diameter data of the last evaluation day (Eq. (2)), in which CD denotes the mean diameter of the colonies on the check dishes, and TD denotes the mean diameter of the colonies on the treatment dishes:

$$\text{PMGInib} = \left[(\text{CD}–\text{TD})/\text{CD} \right] \times 100\% \quad (2)$$

Results

Antifungal activity of essential oils on mycelial growth of the mango phytopathogens

The higher inhibitory performances among the seven EOs tested on the four studied mango phytopathogens were observed for oregano, clove basil, rosemary pepper, and cinnamon bark. Those EOs completely inhibited (100%) the four pathogens, except for cinnamon bark that partially inhibited (48%) *C. siamense* (Fig. 1).

However, cinnamon bark did not completely control the mycelial growth of *C. siamense*; a delay in growth was observed, showing growth only from the third day of evaluation.

In the second trial, the mycelial growth progress curves and the AUCMG (Fig. 2) performed similarly to the first trial, standing out the EOs: oregano, clove basil, rosemary pepper, and cinnamon bark on the control of the pathogens. The results revealed that the solvents hexane and ethanol have no inhibitory effect on the mycelial growth of the four evaluated mango pathogens, presenting mycelial growth similar to the check, indicating that the presence of the solvents does not interfere with the antifungal activity of oils. According to the mycelial growth progress curves, it is observed that each pathogen presented specific growth characteristics essential to determining the evaluation times. Whereas *L. theobromae* completely covered 90 mm of Petri dishes, on the third day, *A. alternata* took

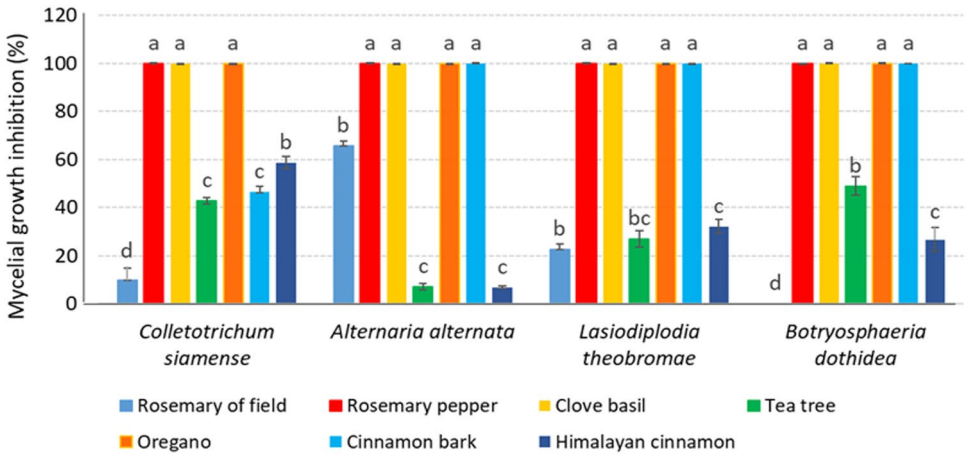

Fig. 1 Inhibitory effect of essential oils (EOs): rosemary of field (*Baccharis dracunculifolia*), rosemary pepper (*Lippia sidoides*), clove basil (*Ocimum gratissimum*), oregano (*Origanum vulgare*), tea tree (*Melaleuca alternifolia*), cinnamon bark (*Cinnamomum cassia*), and Himalayan cinnamon (*Cinnamomum glaucescens*), on mycelial growth (mm) of the fungal mango phytopathogens *Colletotrichum siamense*, *Alternaria alternata*, *Lasiodiplodia theobromae*, and *Botryosphaeria dothidea* from mango. The mycelial growth was measured by the mycelial diameter (mm) on the last evaluation day (mm). The mean diameter with different letters at the top of the bar denotes a significant difference in the inhibitory effect of the compared EOs on each of the phytopathogens studied according to the Tukey test at a significance level of 0.05 ($p < 0.05$). The vertical lines at the top of the bars correspond to the respective standard errors of the mean diameter estimates for each treatment (OE)

10 days to reach the edge of the plate. There was no fungal development in the treatments of oregano, clove basil, and rosemary pepper EOs. Cinnamon bark EO presented the same performance as those previous OEs, except for *C. siamense*.

Minimum inhibitory concentration (MIC) of selected essential oils

The MIC of the selected EOs (oregano, clove basil, rosemary pepper, and cinnamon bark), which exhibited higher mycelial growth inhibition of the four pathogens in previous essays, was determined.

Oregano EO generally showed higher antifungal activity against the four studied pathogens. The MIC to obtain 100% inhibition of mycelial growth was 250 $\mu L.L^{-1}$ for *C. siamense*, *L. theobromae*, and *B. dothidea* and 500 $\mu L.L^{-1}$ for *A. alternata* (Table 1).

While cinnamon bark presented MIC of 250 $\mu L.L^{-1}$ for *A. alternata* and 500 $\mu L.L^{-1}$ for *L. theobromae* and *B. dothidea*, 1000 $\mu L.L^{-1}$ was required to inhibit *C. siamense.*

Whereas rosemary pepper completely inhibited *L. theobromae* and *B. dothidea* at 500 $\mu L.L^{-1}$, 1000 $\mu L.L^{-1}$ was necessary to inhibit *C. siamense* and *A. alternata.*

Comparing the selected EOs, higher MICs were required to inhibit the pathogens by clove basil EO, 1000 $\mu L.L^{-1}$ for *L. theobromae* and 2000 $\mu L.L^{-1}$ for *C. siamense*, *A. alternata*, and *B. dothidea* (Table 1).

Chemical composition of selected EOs

GC-MS analysis revealed nine chemical constituents in oregano EO, ten constituents in rosemary pepper EO (Table 2), five in cinnamon bark EO (Table 3), and eight in clove basil EO (Table 4).

According to the results, carvacrol (69.1%) and thymol (77.2%) were the major constituents in oregano and rosemary EOs, respectively. The constituent ρ-cymene was a secondary compound, representing 18.8% of the total composition in oregano and 14.2% in rosemary pepper EOs. The EO of cinnamon bark presented the constituent cinnamaldehyde (85.1%) as the most abundant, followed by o-methoxy cinnamaldehyde (9.3%). Clove basil EO showed a high concentration of eugenol (84.84%) and several other constituents with a composition from 1 to 3%, and among them, the ρ-cymene compound showed the highest concentration (2.43%).

The retention index (IR_{exp}) was similar to the retention index in the literature (IR_{lit}) [20].

Evaluation of the antifungal activity of constituents

The constituents selected for evaluating antifungal activity against the four pathogens were α-pinene, terpineol, thymol, l-limonene, carvacrol, p-cymene, linalool, and caryophyllene.

According to Fig. 3, thymol and carvacrol were the constituents that showed greater efficiency in inhibiting pathogens.

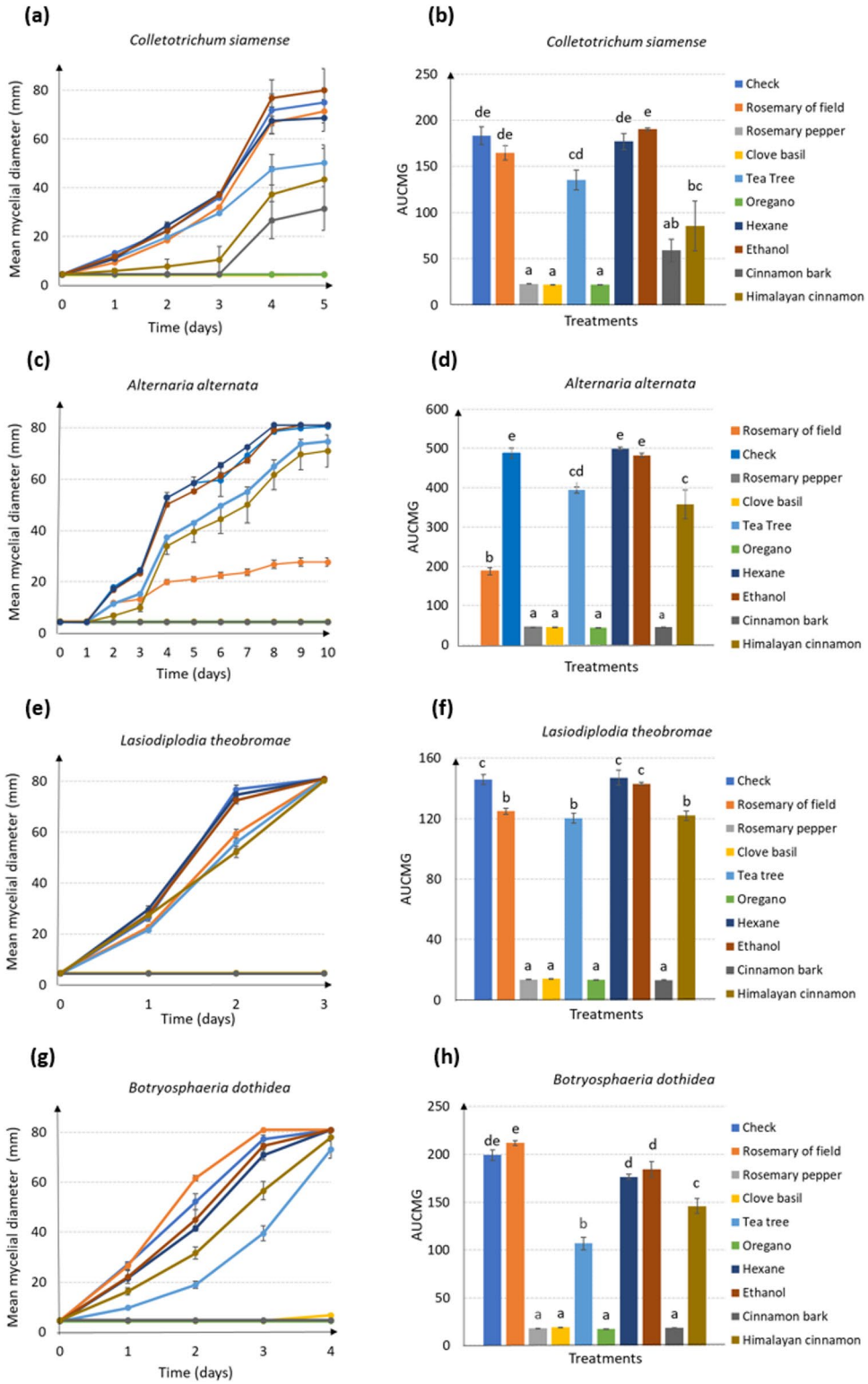

Fig. 2 Mycelial growth progress curve of the mango phytopathogens *Colletotrichum siamense* (**a**), *Alternaria alternata* (**c**), *Lasiodiplodia theobromae* (**e**), and *Botryosphaeria dothidea* (**g**) and respective areas under the curves (AUCMG, mm. day) (**b**, **d**, **f**, and **h**). The essential oils evaluated were field rosemary (*Baccharis dracunculifolia*), pepper rosemary (*Lippia medoides*), clove (*Ocimum gratissimum*), oregano (*Origanum vulgare*), tea tree (*Melaleuca alternifolia*), cinnamon bark (*Cinnamomum cassia*), and Himalayan cinnamon (*Cinnamomum glaucescens*). Hexane was used to dilute rosemary, pepper, and oregano. The remaining EOs were diluted with ethanol. The vertical bars of the mycelial growth curves and AUCMGs correspond to the standard error of the mean estimates. The means of AUCMGs with different letters at the top of the respective bars denote a significant difference in the effect of EOs on each of the phytopathogens studied according to the Tukey test at the significance level of 0.05 ($p < 0.05$)

Thymol provided 90% inhibition of mycelial growth for *C. siamense*, 93% for *A. alternata*, and 95% for *B. dothidea* and provided complete inhibition (100%) for *L. theobromae*. Carvacrol was effective in inhibiting *C. siamense* (89%), *A. alternata* (93%), and *B. dothidea* (90%). For *L. theobromae*, the second constituent with the highest antifungal activity was α-pinene (84%), present in the composition of oregano and rosemary pepper EOs (Table 2). The constituent linalool showed control ranging from 70 to 85% for *A. alternata*, *L. theobromae*, and *B. dothidea* and 40% inhibition for *C. siamense.*

Table 1 Percentage of mycelial growth inhibition and respective standard errors (± S.E) for different concentrations of essential oils from oregano (*Origanum vulgare*), cinnamon bark (*Cinnamomum cassia*), rosemary pepper (*Lippia sidoides*), and clove basil (*Ocimum gratissimum*) against *Colletotrichum siamense*, *Alternaria alternata*, *Lasiodiplodia theobromae*, and *Botryosphaeria dothidea* from mango

Mango phytopathogens	Essential oil (EO)	Mycelial growth inhibition (%) for different EO concentrations ($\mu L.L^{-1}$)				
		170	250	500	1000	2000
Colletotrichum siamense	Oregano	12.9 ± 2,2	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Cinnamon bark	*	60.0 ± 9.1^{c}	86.7 ± 3.7^{b}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Rosemary pepper	*	75.7 ± 3.7^{b}	82.5 ± 1.5^{b}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Clove basil	*	5.4 ± 0.8^{d}	65.1 ± 3.8^{c}	86.1 ± 2.8^{b}	100 ± 0.0^{a}
Alternaria alternata	Oregano	5.0 ± 3.4^{b}	65.5 ± 6.6^{b}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Cinnamon bark	12.7 ± 2.4^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Rosemary pepper	*	51.7 ± 1.5^{c}	87.5 ± 3.3^{b}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Clove basil	*	13.2 ± 1.2^{d}	34.4 ± 1.2^{c}	88.8 ± 0.7^{b}	100 ± 0.0^{a}
Lasiodiplodia theobromae	Oregano	70.7 ± 1,6	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Cinnamon bark	*	62.0 ± 1.6^{c}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Rosemary pepper	*	85.6 ± 0.9^{b}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Clove basil	*	15.8 ± 7.1^{e}	56.0 ± 1.0^{d}	100 ± 0.0^{a}	100 ± 0.0^{a}
Botryosphaeria dothidea	Oregano	37.8 ± 4.1	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Cinnamon bark	*	36.9 ± 2.9^{c}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Rosemary pepper	*	80.4 ± 1.6^{b}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	Clove basil	*	37.7 ± 6.6^{e}	57.4 ± 2.1^{b}	73.9 ± 2.3^{b}	100 ± 0.0^{a}

*Concentration not evaluated

Mean inhibition (%) after complete growth check ± standard error. Means followed by different letters in the column denote a significant difference for each phytopathogen, according to Tukey's test ($p < 0.05$)

Table 2 Chemical composition of oregano essential (*Origanum vulgare*) and rosemary pepper oils (*Lippia sidoides*) analyzed by gas chromatography (GC-MS)

Compound	T_{ret} (min)	Oregano oil		Rosemary pepper oil		IR_{lit}
		IR_{exp}	A%	IR_{exp}	A%	
α-Pinene	11.7	934	1.3	934	0.4	932
Myrcene	14.1	991	0.9	991	1.5	998
ρ-Cymene	15.7	1026	**18.8**	1025	**14.2**	1020
γ-Terpinene	17.2	1060	3.1	*	*	1054
Linalool	19.1	1100	2.0	1100	0.21	1098
Thymol methyl ether	25.2	*	*	1236	1.12	1232
Thymoquinona	25.9	*	*	1252	0.56	1248
Thymol	27.7	1292	3.9	1293	**77.2**	1289
Carvacrol	28.1	1305	**69.1**	1301	0.4	1298
Caryophyllene	33.3	1425	0.1	1425	3.4	1417
Caryophyllene oxide	39.7	1589	0.8	1589	0.8	1582

t_{ret}, retention time; IR_{exp}, retention index determined using a series of C7-C40 n-alkanes; IR_{lit}, retention index from literature; A%, relative percentage from peak area

HP5-MSUi capillary column (30 m × 0.25 mm i.d., 0.25 um). Components with high A% were presented in bold

*Missing compounds

Discussion

The effectiveness of EOs on phytopathogenic fungi has been demonstrated in the literature, being a trend in the control of postharvest diseases in fruits. However, the results of this study have shown that the action of EOs varies according to the pathogen. Among the seven EOs evaluated, they highlighted oregano, clove basil, rosemary

Table 3 Chemical composition of cinnamon bark essential oil (*Cinnamomum cassia*) analyzed by gas chromatography (GC-MS)

Compound	T_{ret} (min)	Cinnamon bark oil		IR_{lit}
		IR_{exp}	A%	
Benzaldehyde	12.8	960	0.9	952
Cinnamaldehyde	27.0	1274	**85.1**	1267
Coumarin	33.9	1439	2.0	1432
Cinnamyl acetate	34.2	1446	2.7	1443
***o*-Methoxy cinnamaldehyde**	37.6	1533	**9.3**	1527

t_{ret}, retention time; IR_{exp}, retention index determined using a series of C7-C40 n-alkanes; A%, relative percentage from peak area; IR_{lit}, retention index from literature

HP5-MSUi capillary column (30 m × 0.25 mm i.d., 0.25 um). Components with high A% were presented in bold

*Missing compounds

Table 4 Chemical composition of clove basil essential oil (*Ocimum gratissimum*) analyzed by gas chromatography (GC-MS).

Compound	T_{ret} (min)	Clove basil oil		IR_{lit}
		IR_{exp}	A%	
2-Butanol,2,3-dimethyl	10.4	907	1.04	*
2,5-Dimethyl-4,hydroxy-3-hexanone	11.1	923	1.16	*
p-Cymene	15.5	1025	**2.43**	1020
Cis-O-cymene	16.1	1038	1.10	1032
Eugenol	30.6	1361	**84.84**	1356
α Copaene	31.4	1380	1.58	1374
Caryophyllene	33.2	1425	2.11	1417
Naphthalene1, 2, 3, 4	37.3	1528	2.00	*

t_{ret}, retention time; IR_{exp}, retention index determined using a series of C7-C40 n-alkanes; A%, relative percentage from peak area; IR_{lit}, retention index from literature

HP5-MSUi capillary column (30 m × 0.25 mm i.d., 0.25 um). Components with high A% were highlighted

*Missing compounds

pepper, and cinnamon bark EOs on the control of postharvest pathogens of mango.

The MIC varied according to the EOs and the target fungus. Oregano EO stood out for its inhibition effect on the pathogens *C. siamense*, *L. theobromae*, and B. *dothidea* at a concentration of 250 $\mu L.L^{-1}$, demonstrating excellent antifungal activity at the lowest concentration tested. Oregano oil also showed excellent results in controlling *B. cinerea* in grapes [23, 24] after exposure to the MIC of 800 $\mu L.L^{-1}$ and orange *G. citri-aurantii* at a concentration of 500 $\mu L.L^{-1}$ [25].

A. alternata was more sensitive to cinnamon bark EO than oregano EO, with an MIC of 250 $\mu L.L^{-1}$. Andrade-Ochoa et al. [26] also reported the same effect evaluating cinnamon oil and oregano in the control of *A. alternata* isolated from the pepper. Even at a low concentration of 66 $\mu g.mL^{-1}$, cinnamon EO inhibited the growth of the pathogen, while 200 $\mu g.mL^{-1}$ of oregano oil was required for complete inhibition.

Regarding rosemary pepper EO, the MIC ranged from 500 $\mu L.L^{-1}$ to *L. theobromae* and *B. dothidea* to 1000 $\mu L.L^{-1}$ to *C. siamense* and *A. alternata.* Oliveira et al. [27] observed total inhibition of mycelial growth of *C. acutatum* of strawberry at the MIC of 250 $\mu l.L^{-1}$ with deformation of the hyphae. The authors attributed the antifungal activity of rosemary pepper EO to the active principle of its major compounds, thymol, and p-cymene, representing 49% and 11% of the composition. They demonstrated the action of these compounds on fungal cell walls [28].

On the other hand, clove basil oil showed an MIC higher than the other EOs studied and corroborated the results presented by Haddout [29] and Vilaplana [30]. Furthermore, the authors reported that complete inhibition of *Fusarium* sp. of grape and *Colletotrichum musae* in banana was only achieved using clove basil EO at concentrations greater than 1000 $\mu L.L^{-1}$.

The MIC also varies according to the fungal species. Danh [31] worked with 1600 $\mu L.L^{1}$ of cinnamon bark EO concentration and 4000 $\mu L.L^{-1}$ of clove basil EO and completely inhibited *C. acutatum* in mango. In this work, the MIC for inhibition of *C. siamense* was 1000 $\mu L.L^{-1}$ and 2000 $\mu L.L^{-1}$, respectively.

The analysis by GC-MS revealed specificity in the composition of the EOs and their relation to the antifungal activity. The compounds found in the EO of oregano, rosemary pepper, cinnamon bark, and clove basil and their respective antifungal activity corroborate the results presented in the literature.

Regarding the antifungal activity of the constituents in the evaluated EOs, carvacrol, thymol, linalool, and α-pinene stood out. Carvacrol and thymol, major constituents of the EO of oregano and rosemary pepper, respectively, have presented the best results, showing the significant inhibitory effect of the fungi *C. siamense*, *A. alternata*, *L. theobromae*, and *B. dothidea*. According to the literature, thymol and carvacrol exhibit potential antifungal activity against several pathogens. Abbaszadeh [17] demonstrated that carvacrol was effective against 11 food-decomposing fungi. Nunes [32] observed inhibition of *Aspergillus niger* and *Penicillium expansum* using different concentrations of Thymus vulgaris EO, which contains carvacrol as one of the main constituents. Silvaran [33] reported that thymol is effective against a wide range of fungi, such as *A. alternata*, *Aspergillus flavus*, *Penicillium Italicum*, *Aspergillus ochraceus*, and *Rhizopus oryzae*. Zhang [34] observed that the mycelia of *B. cinerea*, isolated from tomato, were completely inhibited with 120 $\mu L.L^{-1}$ of thymol and 140 $\mu L.L^{-1}$ of carvacrol. Furthermore,

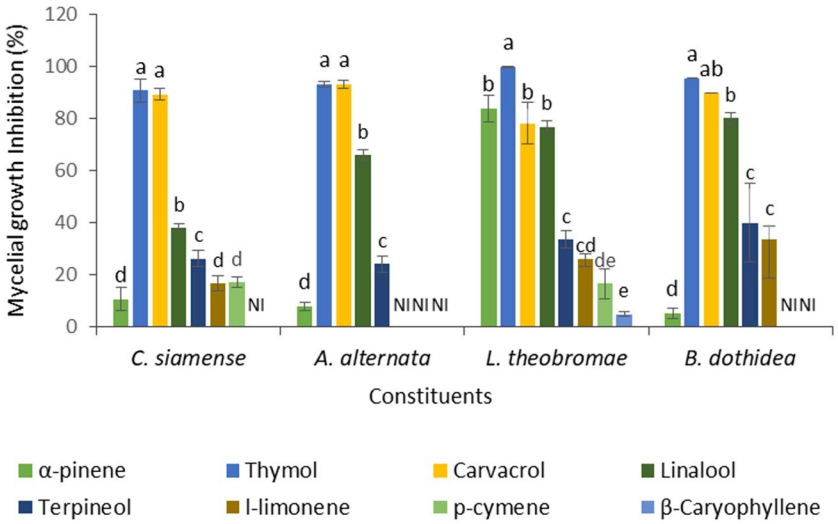

Fig. 3 Percentage of mycelial growth inhibition of *Colletotrichum siamense*, *Alternaria alternata*, *Lasiodiplodia theobromae*, and *Botryosphaeria dothidea* from mango, by significant compounds present in essential oils: α-pinene, terpineol, thymol, l-limonene, carvacrol, p-cymene, linalool, and caryophyllene. The vertical bars correspond to the standard error of the estimated means. NI = there was no inhibition. Means followed by different letters denote a significant difference between the essential oils evaluated for each phytopathogen, according to Tukey's test ($p < 0.05$)

two days after the treatment, the hyphae presented considerable deformation in the morphology and severe damage of the plasma membrane was observed, which was more severe after thymol treatment.

Linalool, despite being present in low concentration (Table 2) in the composition of oregano EO (2%) and rosemary pepper EO (0.21%), has shown high fungicidal action, higher than 80%, against *L. theobromae* and *B. dothidea*. Similarly, α-pinene in low concentration in rosemary pepper EO (0.4%) and oregano (1.3%) have shown 84% inhibition for *L. theobromae*. Generally, the antifungal effect is attributed to the main constituent [35]. However, the inhibition capacity of oregano and rosemary pepper EOs may have been enhanced by the synergistic presence of these secondary constituents with high antifungal activity. Spadaro and Gullino [36] mentioned that carvacrol and p-cymene constituents of thyme EO are more efficient in controlling fungal and bacterial pathogens when applied together.

There are no effective methods to control the mango postharvest fungi evaluated in the present research. The results indicate that using essential oils is a sustainable, efficient, and clean way to control these pathogens. EOs and their compounds have been applied in fruits supplementing edible coat formulations [37]. Further studies are needed to apply the selected EOs in mangoes, evaluating their antimicrobial and fruit quality effect and, consequently, the increase in shelf life.

Conclusions

Oregano (*O. vulgare*), cinnamon bark (*C. cassia*), rosemary pepper (*L. sidoides*), and clove basil (*O. gratissimum*) presented higher antifungal activity against *C. siamense*, *A. alternata*, *L. theobromae*, and *B. dothidea* of mango.

The MIC varied according to the fungi and the EOs. The MIC of oregano EO was 250 $\mu L.L^{-1}$ for *C. siamense*, *L. theobromae*, and *B. dothidea* and 500 $\mu L.L^{-1}$ for *Alternata.* For cinnamon bark EO, the MIC varied from 250 $\mu L.L^{-1}$ to *Alternata*, 500 $\mu L.L^{-1}$ to *L. theobromae* and *B. dothidea*, and 1000 $\mu L.L^{-1}$ to *C. siamense.* Rosemary pepper EOs MIC ranged from 500 $\mu L.L^{-1}$ to *L. theobromae* and *B. dothidea* to 1000 $\mu L.L^{-1}$ to *C. siamense* and *A. alternata.* On the other hand, clove basil EO needed a higher concentration to completely inhibit the fungi, presenting MIC of 1000 $\mu L.L^{-1}$ to *L. theobromae* up to 2000 $\mu L.L^{-1}$ to *C. siamense*, *A. alternata*, and *B. dothidea.*

The major constituents of the EOs were carvacrol (69.1%), cinnamaldehyde (85.1%), thymol (77.2%), and eugenol (84.84%) for oregano, cinnamon bark, rosemary pepper, and clove basil EOs, respectively.

Thymol and carvacrol presented the highest antifungal activity against the mango postharvest fungi evaluated.

The results provide subsidies for developing technologies using EOs to control *C. siamense*, *A. alternata*, *L. theobromae*, and *B. dothidea* of mango as an alternative synthetic fungicide.

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Data availability Data will be made available upon request.

Declarations

Conflict of interest The authors declare no competing interests.

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