



MICROBIOLOGY

Essential oils on the control of postharvest diseases of papaya

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Abstract: Papaya is susceptible to fungal deterioration and the use of essential oils (EOs) emerges as a promising alternative to fungicides, which causes environmental and human health problems. To evaluate the antifungal activity of essential oils and their major constituents in controlling papaya pathogens, the following *in vitro* assessments were conducted: I) screening of seven EOs regarding the antifungal activity; II) determination of the minimum inhibitory concentration (MIC) of the most promising EOs; III) analysis of chemical composition of the most effective oils; and IV) evaluation of the antifungal potential of the major constituents both individually or in combination. The results showed that the EOs of cinnamon bark, oregano, clove basil, and rosemary pepper exhibited high antifungal activity against all studied fungi, with MIC values from 0.50 to 2.00 $\mu\text{L mL}^{-1}$. The major constituents found in oregano EO were carvacrol, *p*-cymene, and thymol; in cinnamon bark EO, cinnamaldehyde, *o*-methoxy, and cinnamyl; in rosemary pepper EO, thymol, *p*-cymene, and caryophyllene; and in clove basil EO, eugenol, *p*-cymene, and caryophyllene. Regarding the antifungal activity of the EO constituents, the mixture of the three major demonstrated greater efficiency against the studied fungi. Using constituents represents an alternative for controlling postharvest diseases in papaya.

Key words: antifungal activity, fruit, alternative treatments, chemical constituents.

INTRODUCTION

Papaya is a climacteric fruit that continues the ripening process after harvest, undergoing a series of biochemical changes that affect its texture, flavor, and aroma. This transition makes the fruit more vulnerable to pathogen attacks, as ripening creates an environment conducive to the proliferation and growth of pathogens (Karagiannis 2024). The main diseases that affect and reduce the postharvest shelf life of papaya are caused by *Phoma caricae-papayae*, *Alternaria alternata*, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, and *Fusarium solani* (Rodrigues et al. 2021). It is estimated that the volume of losses caused by the proliferation of these pathogens reaches 50% of production

(Vinod et al. 2023). Synthetic fungicides have been used as the only method to combat these infections. However, the use of fungicides has proven to be increasingly less effective, due to the selection of resistant strains due to the continued use of the same active ingredient, in addition to causing environmental and human health problems. Therefore, developing effective postharvest fruit preservation methods that have less impact on the environment and human health is a topic of great technological and economic relevance. Postharvest technologies such as essential oil coatings are safe approaches to retaining fruit quality and managing disease (El Khetabi et al. 2022).

Essential oils (EOs) derived from plant roots, leaves, bark, and seeds stand out for their antifungal activity against various pathogens (Allagui et al. 2023, He et al. 2023, Khorram et al. 2018, Wu et al. 2023). Cinnamon bark (*Cinnamomum cassia*) EO, for example, is widely recognized for its high concentration of cinnamaldehyde, responsible for its inhibitory effect against fungi such as *Penicillium digitatum* of orange (Zulu et al. 2023). Rosemary pepper (*Lippia sidoides*) EO also has strong antifungal action, acting on the cell membrane of pathogens such as *C. gloeosporioides* of avocado (Antonia et al. 2024) and *F. pallidoroseum* of melon (Sousa et al. 2023). Similarly, clove basil (*Ocimum gratissimum*) and oregano (*Origanum vulgare*) EO were reported to be active against *Aspergillus niger* (Brandão et al. 2023) and *Botrytis cinerea* (Kosakowska et al. 2024). The use of EOs has gained highlight due to growing consumer awareness about the benefits of natural products over synthetic alternatives (Yang et al. 2023). Furthermore, EOs are considered safe and have potential applications in food (Wu et al. 2023). The chemical composition of EOs varies. Therefore, it is important to understand their chemical composition to predict their mode of action (Falleh et al. 2020). There are usually dominant compounds in the composition of EOs. For example, carvacrol is the major constituent of oregano EO (*Origanum vulgare*), and eugenol is the major compound of clove basil EO (*Ocimum gratissimum*) (Mediouni et al. 2020, Ugbogu et al. 2021). However, the activity of the majority constituents can be enhanced by constituents presenting in small amounts in EOs, thus improving the effectiveness of the majority constituents (Baptista-Silva et al. 2020).

Therefore, this study aimed to screen seven EOs regarding their inhibitory activity on the mycelial growth of five pathogens causing diseases in papaya, namely *P. caricae-papayae*,

A. alternata, *L. theobromae*, *C. gloeosporioides* and *F. solani* of papaya, as well as to determine the minimum inhibitory concentration (MIC) of the selected EOs with higher antifungal potential against the pathogens studied. Furthermore, the chemical composition of the selected EOs and their main constituents combined and individual effects on the control of papaya postharvest pathogens were analyzed.

MATERIALS AND METHODS

Reagents

The reagents are carvacrol (purity $\geq 98\%$), p-cymene (purity 99%), thymol (purity $\geq 98.5\%$), o-methoxy cinnamaldehyde (purity 98%), cinnamyl acetate (purity 99%), caryophyllene (purity $\geq 80\%$), and eugenol (purity 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cinnamaldehyde was purchased from Quinari Fragrancias (Ponta Grossa, Paraná, Brazil), with purity $\geq 98\%$, Dimethyl sulfoxide (DMSO), with purity $\geq 99.5\%$, from the manufacturer Merck KGaA (Darmstadt, Hesse, Germany), and tween 80 (purity 99%) was purchased from Dinamica Química (Indaiatuba, São Paulo, Brazil).

Papaya fungi

The papaya fungal isolates: *P. caricae-papayae* (CMAA 1483), *A. alternata* (CMAA 1487), *L. theobromae* (CMAA 1488), *C. gloeosporioides* (CMAA 1490) and *F. solani* (CMAA 1491), was provided by Collection of Microorganisms of Agricultural and Environmental Importance – CMAA (Embrapa Meio Ambiente, Brazil). The pathogens were cultivated on potato dextrose agar (PDA) and maintained in an incubation room at $22 \pm 2^\circ\text{C}$.

Effect of essential oils on mycelial growth of papaya pathogens

The EOs were obtained commercially from essential oil producers. EOs of 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*) were evaluated using the agar dilution method (Parikh et al. 2021). Each EO in a concentration of $1 \mu\text{L mL}^{-1}$ was incorporated into a previously autoclaved and semi-fluid PDA medium containing 0.05% tween 80 (v/v). Discs measuring 5 mm in diameter, removed from the margin of active pathogen growth, were transferred to the center of the Petri dishes with solidified PDA+EO (about 20 mL of PDA+EO medium). The check was prepared only with PDA + 0.05% tween 80. The sealed plates were incubated at $22 \pm 2^\circ\text{C}$, without photoperiod control. Colony diameter was measured with a digital caliper. This trial was carried out to select the EOs with the best antifungal activity against the five pathogens mentioned.

The average of two daily measurements of mycelial diameter in each experimental unit were used to construct mycelial growth progress curves for each treatment, for each pathogen. The area under each curve segment was calculated (AUMGC, Broetto et al. 2014) (Equation 1).

$$\text{AUMGC}_{jk} = \sum_{i=1}^{n-1} \left[\left(\frac{Y_{(i+1)jk} + Y_{ijk}}{2} \right) \times (t_{(i+1)jk} - t_{ijk}) \right] \quad (1)$$

In Equation 1, AUMGC_{jk} is the Area Under the Mycelial Growth Curve for the replication j ($j=1, 2, 3, 4$) and treatment k ($k=1, 2, \dots, 7$); Y_{ijk} is the mean mycelial diameter measured in two orthogonal directions in the replication j (plate j) of the treatment k , in the evaluation corresponding to the time i ($i=1, 2, \dots, 10$).

The mycelial growth inhibition (%) in the treatment k (MGI_{ik}) ($k=1, 2, 3, \dots, 6$) is the mycelial diameter on the last day of evaluation ($i=2$ for *L. theobromae*; $i=3$ for *P. caricae-papayae*;

$i=9$ for *F. solani* and *A. alternata*; $i=10$ for *C. gloeosporioides*) the treatment k (MD_k) expressed as percentage of the mycelial growth in the check treatment (MD_c) (Equation 2, Al-Reza et al. 2010).

$$\text{MGI}_{ik} (\%) = \left(\frac{\text{MD}_c - \text{MD}_k}{\text{MD}_c} \right) \times 100 \quad (2)$$

Mycelial growth can be defined as the variation in mycelial diameter throughout the evaluation period (mm day^{-1}). The EOs that exhibited the highest inhibitory potential on the mycelial growth of *P. caricae-papayae*, *A. alternata*, *L. theobromae*, *C. gloeosporioides* and *F. solani* were selected for subsequent tests. The experimental design was completely randomized with four replications.

Determination of minimum inhibitory concentration (MIC) of EOs

To determine the MIC of the selected EOs, a precise amount of EO was introduced into the PDA medium sterilized with tween 80 (0.05%) to obtain the final concentration of 0.10, 0.50, 1.00, 2.00 and $2.50 \mu\text{L mL}^{-1}$. After that, about 20 mL of PDA was poured into Petri dishes and disks of pathogen mycelium (5 mm in diameter) were deposited in the center of the dishes with the solidified medium (Parikh et al. 2021). Plates were incubated at $22 \pm 2^\circ\text{C}$. The diameter of the fungal colony was measured daily in an orthogonal position until the colony on the check plate reached the total diameter of the Petri dish. The percentage of growth inhibition was determined using the average diameter obtained on the last day of evaluation (Al-Reza et al. 2010). The MIC is the lowest concentration at which mycelial growth did not occur. The trial was conducted in a completely randomized design with four replications for each treatment.

Chemical characterization of EOs and identification of constituents

The chemical composition of the EOs was analyzed using a GC-MS system (Gas Chromatography/Mass Spectrometry), as described by Vilela et al. (2024). The EOs were dissolved in Hexane at a concentration of 0.1%, except Himalayan cinnamon which was dissolved in acetone. Both solvents were obtained from Scharlab S.L. (Barcelona, Spain), with purity 99%. The analysis was conducted using an Agilent 7890B gas chromatograph (Agilent Technologies, USA) connected to an Agilent 5977B mass detector. The GC had an HP-5MSui column (30m x 0.25mm I.D., film thickness 0.25µm). Helium served as the carrier gas at a flow rate of 1.2 mL min⁻¹. The injector temperature was maintained at 220°C. The oven temperature program began at 50°C (held for 2 minutes), increased at a rate of 3°C/min to 230°C (held for 20 minutes), and then increased at a rate of 30°C/min until reaching 300°C (held for 10 minutes). The injection volume was 1 µL. Mass spectra were recorded at 70 eV and scanned across of 40-500 *m/z* range. The constituents of the EOs were identified by comparing their retention indices (RI) with those of C7-C40 *n*-alkanes injected under the same conditions and by referencing the values reported by Adams (2007). Data acquisition and analysis were performed using the Agilent MassHunter Workstation software (version 10.0). Each chromatogram peak's spectrum was compared to spectra in the NIST17 database (version 2.3). The results were expressed as percentages, quantitatively measuring each constituent's presence within the EOs.

Antifungal activity of EOs constituents

To evaluate the antifungal activity of the major constituents of the selected EOs, the compounds at a concentration of 0.1 µg µL⁻¹ (for thymol and caryophyllene) and 0.2 µL µL⁻¹ (for the other

constituents) were dissolved in ethanol or ethyl acetate (according to the solubility coefficient of each product). A volume of 10 µL of the solution was deposited onto sterile paper discs (6 mm in diameter). The discs were then placed on the surface of Petri dishes containing PDA medium at a distance of 2 cm from the fungal colony disc (fungal disc of 5 mm in diameter) (Ostrosky et al. 2008). The plates were incubated at 22 ± 2°C, and the inhibition distance (mm) between the fungus and the paper disc was measured using a caliper until the fungal colony in the check plate reached the paper disc. The experimental design was completely randomized with four replications. Plates with paper discs containing ethyl acetate or ethanol were used as a check.

The percentage of mycelial growth inhibition of each treatment *k* (PMGI_{*k*}) is the ratio between the inhibition distance of the paper disc and the fungal disc on the last day of evaluation (*f*=2 for *L. theobromae*; *f*=3 for *P. caricae-papayae*; *f*=5 for *F. solani*; *f*=8 for *A. alternata*; *f*= 4 for *C. gloeosporioides*) of each treatment (*D*_{*fk*}) expressed as a percentage of the initial distance between the paper disc and the fungus for each treatment (*D*_{*ik*}) (Equation 3).

$$\text{PMGI}_k (\%) = \left(\frac{D_{fk}}{D_{ik}} \right) \times 100 \quad (3)$$

Antifungal activity of the mixture of major EOs constituents

The mixture of the major constituents of the EOs followed the same proportions as present in the GC-MS analysis. Dimethyl sulfoxide (DMSO) was used to complete the volume and aid in the solubility of the constituents. The mixture, at a concentration of 1 µL mL⁻¹, was added to the PDA medium, which had been previously autoclaved and semi-melted, containing 0.05% tween 80 (Parikh et al. 2021). Pathogen mycelium discs (5 mm in diameter), taken from the actively growing

margin of the pathogens, were transferred to the center of Petri dishes containing the PDA medium with the mixture. Plates containing PDA + tween and PDA + DMSO were used as checks. The sealed plates were incubated at $22 \pm 2^\circ\text{C}$, and the colony diameter was measured with a digital caliper. The average diameter of two replicates per plate was used to calculate the mycelial growth inhibition percentage (MGI %). The experimental design was completely randomized with four replications.

Determination of the MIC of the mixture of major EOs constituents

Based on previous results, the minimum inhibitory concentration (MIC) of the mixture of major constituents of the selected EOs was determined. The mixture of constituents was added to a PDA medium supplemented with tween 80 (0.05%), resulting in final concentrations of 0.10, 0.25, 0.50, 0.75, 1.00, and $1.50 \mu\text{L mL}^{-1}$ (Parikh et al. 2021) and then poured into Petri dishes. Pathogen mycelium discs (5 mm in diameter) were placed on the solidified PDA medium, and the plates were incubated at $22 \pm 2^\circ\text{C}$. The fungal colony diameter was measured daily in two orthogonal positions until the colony in the check plate reached the total diameter of the Petri dish. The percentage of growth inhibition was determined using the average diameter measured on the last day of evaluation (Al-Reza et al. 2010). The lowest concentration at which 100% of the pathogens were inhibited was considered the minimum inhibitory concentration (MIC). The experiment was conducted in a completely randomized design, with four replications for each treatment.

Statistical analysis

The data for the area under the mycelial growth curve (AUMGC), mycelial growth inhibition (MGI %), and percentage of mycelial growth

inhibition (PMGI %) were analyzed using the non-parametric Kruskal-Wallis test (Conover 1999), followed by Dunn's test with Bonferroni correction to compare the mean diameter of fungal colonies on the last day of evaluation. The ordinary linear ANOVA model was not suitable due to violations of normality and homogeneity of variance assumptions. Significance in all analyses was assessed at $p \leq 0.05$. The data were analyzed using the "rstatix" package in the R statistical software (R Core Team 2023).

RESULTS AND DISCUSSION

Antifungal activity of EOs

Among the seven EOs analyzed, the EOs of oregano, cinnamon bark, rosemary pepper, and clove basil demonstrated higher inhibitory activity against the fungi *P. caricae-papayae*, *A. alternata*, *L. theobromae*, *C. gloeosporioides*, and *F. solani* of papaya (Figure 1). Therefore, these EOs were selected for subsequent assays. The rosemary pepper EO completely inhibited the mycelial growth of all fungi. The oregano and clove basil EOs completely inhibited the mycelial growth of the fungi, except for *F. solani*, with inhibition percentages of 90% and 57%, respectively. The cinnamon bark EO completely inhibited *P. caricae-papayae* and *A. alternata* partially *C. gloeosporioides* (44% inhibition), and *F. solani* (34% inhibition) while completely inhibiting the remaining pathogens.

Other studies have also highlighted the potential of rosemary pepper EO in pathogen control. Antonia et al. (2024) evaluated the *in vitro* effect of rosemary pepper EO at a concentration of $0.125 \mu\text{L mL}^{-1}$ on *C. gloeosporioides*, isolated from avocado and observed complete inhibition of the fungus' mycelial growth, with no visible fungal growth after 12 days of assessment. Yuan et al. (2024) reported that under normal conditions, the mycelium of *C. gloeosporioides*

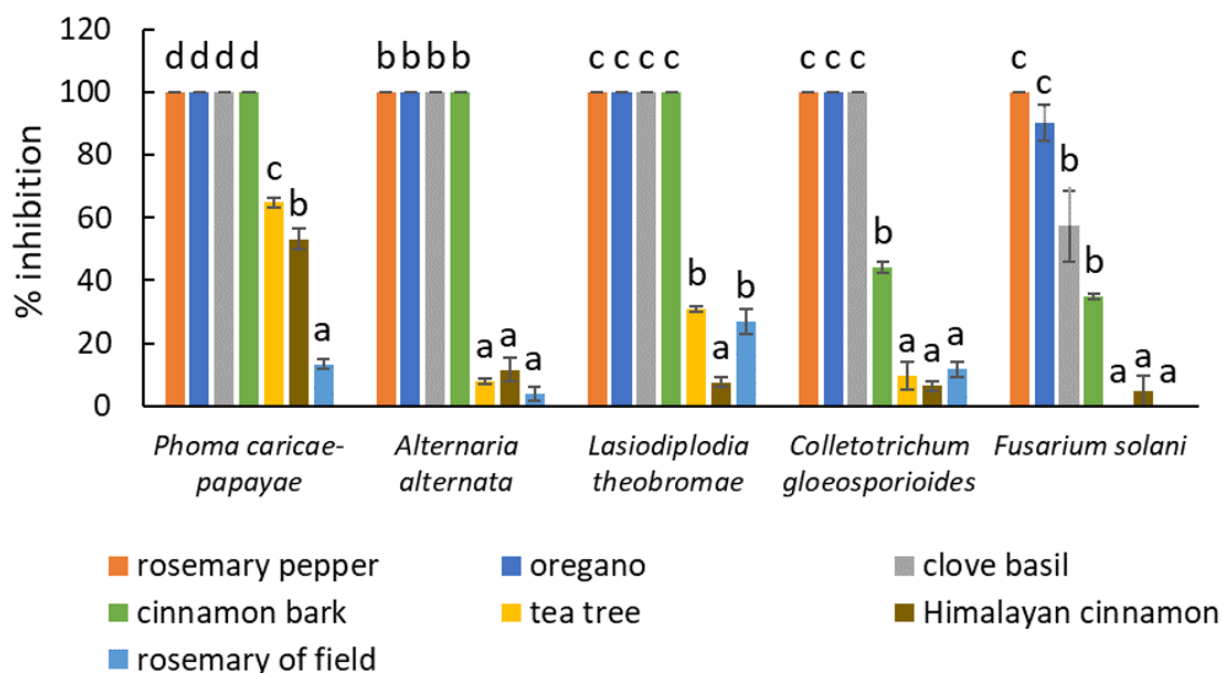


Figure 1. Percentage of inhibition of essential oils of cinnamon bark (*Cinnamomum cassia*), oregano (*Origanum vulgare*), rosemary pepper (*Lippia sidoides*), clove basil (*Ocimum gratissimum*), rosemary of field (*Baccharis dracunculifolia*), Himalayan cinnamon (*Cinnamomum glaucescens*), and tea tree (*Melaleuca alternifolia*) on mycelial growth of *Phoma caricae-papayae*, *Alternaria alternata*, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, and *Fusarium solani*. The percentages were calculated using the mycelial diameter on the last day of evaluation of each treatment by the respective one of the check treatments. Vertical bars on the top of each column correspond to the respective standard errors of the mean percentage estimates. Different lowercase letters on the top of the columns denote significant differences among treatment means, based on Dunn's post hoc test at the 0.05 significance level.

is smooth, but after treatment with rosemary EO, the fungal mycelium became wrinkled and severely deformed. In the study by Oliveira et al. (2024), rosemary EO at a concentration of $0.125 \mu\text{L mL}^{-1}$ completely inhibited the mycelial growth of *Botrytis cinerea* isolated from strawberries and induced structural changes in the hyphae. In the work of Melo et al. (2022), the authors noted that rosemary EO at a concentration of $0.50 \mu\text{L mL}^{-1}$ was able to inhibit the mycelial growth of *L. theobromae* isolated from mango.

Our study also showed differences in growth rates of each papaya pathogen species. According to the mycelial growth progress curves and the AUMGC (Figure 2a-b and 3a-b), while *P.*

caricae-papayae and *L. theobromae* completely covered the Petri dish (90 mm in diameter) within 3 days, *A. alternata*, *C. gloeosporioides*, and *F. solani* delayed 10 days to reach the edge of the dish (Figure 2c and d and 3c, d, e and f).

Minimum Inhibitory Concentration (MIC) of selected EOs

The MIC of oregano, cinnamon bark, rosemary pepper, and clove basil EOs was determined for *P. caricae-papayae*, *A. alternata*, *L. theobromae*, *C. gloeosporioides*, and *F. solani* (Table I).

The oregano EO demonstrated an MIC of $0.50 \mu\text{L mL}^{-1}$ for all tested fungi, except for *F. solani*, with an MIC of $1.00 \mu\text{L mL}^{-1}$. The cinnamon

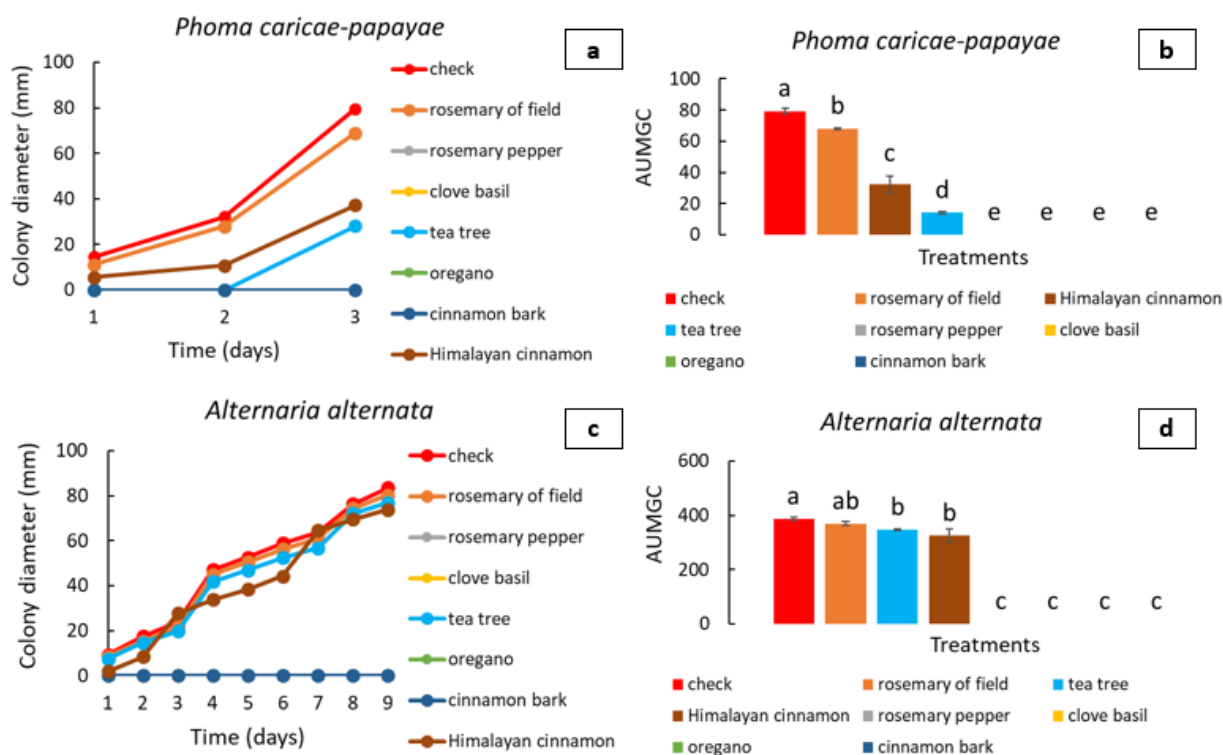


Figure 2. Mycelial growth progress curve of the *Phoma caricae-papayae* (a) and *Alternaria alternata* (cc) and the respective Area Under the Mycelial Growth Curve (AUMGC, mm day⁻¹) (b, d). The essential oils evaluated were cinnamon bark (*Cinnamomum cassia*), oregano (*Origanum vulgare*), rosemary pepper (*Lippia sidoides*), clove basil (*Ocimum gratissimum*), rosemary of field (*Baccharis dracunculifolia*), Himalayan cinnamon (*Cinnamomum glaucescens*), and tea tree (*Melaleuca alternifolia*). The vertical bars on the top of each column correspond to the respective standard error of the mean AUMGC estimates. Different letters at the top of the columns denote significant differences in the AUMGC means, according to Dunn's post hoc test at the 0.05 significance level.

bark EO exhibited a MIC of 0.50 $\mu\text{L mL}^{-1}$ for *P. caricae-papayae*, and *A. alternata*, 1.00 $\mu\text{L mL}^{-1}$ for *L. theobromae*, and 2.00 $\mu\text{L mL}^{-1}$ for *C. gloeosporioides*, and *F. solani*. The rosemary pepper EO presented a MIC of 0.50 $\mu\text{L mL}^{-1}$ for *A. alternata*, *L. theobromae*, *C. gloeosporioides*, and *F. solani*, and 1.00 $\mu\text{L mL}^{-1}$ for *P. caricae-papayae*. As for clove basil EO, the MIC was 0.50 $\mu\text{L mL}^{-1}$ for *L. theobromae*, 1.00 $\mu\text{L mL}^{-1}$ for *P. caricae-papayae*, *A. alternata*, and *C. gloeosporioides*, and only at a concentration of 2.00 $\mu\text{L mL}^{-1}$ *F. solani* was completely inhibited.

Melo et al. (2022) and Silva et al. (2009) have also demonstrated the efficacy of rosemary pepper EO in controlling *L. theobromae* and

C. gloeosporioides. According to Santos et al. (2024), the antimicrobial activity of rosemary pepper EO is attributed to the joint effect of its chemical composition, with terpenes and phenolic compounds, such as thymol and p-cymene, being identified as the main compounds acting by disrupting the cell membrane of microorganisms, increasing the permeability and leading to the loss of essential ions. The results obtained in the study by Mirmajlessi et al. (2024) are consistent with this study. The authors observed that *Fusarium* spp. showed similar sensitivity to oregano EO, with a MIC of 0.84 $\mu\text{L mL}^{-1}$. Jiang et al. (2013) also demonstrated the efficacy of cinnamon bark

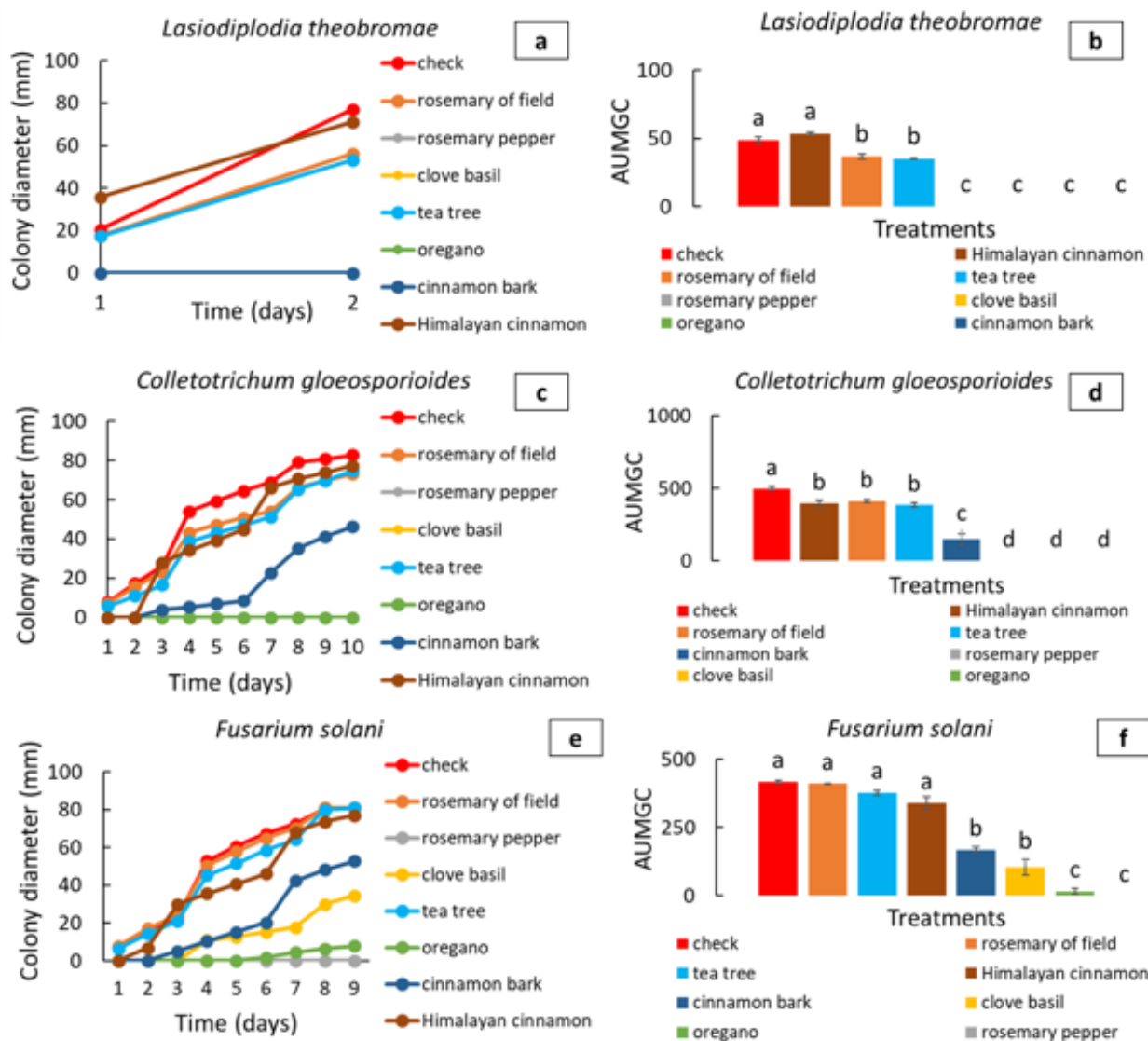


Figure 3. Mycelial growth progress curve of the *Lasiodiplodia theobromae* (a), *Colletotrichum gloeosporioides* (c), and *Fusarium solani* (e) and the respective Area Under the Mycelial Growth Curve (AUMGC, mm day⁻¹) (b, d, f). The essential oils evaluated were cinnamon bark (*Cinnamomum cassia*), oregano (*Origanum vulgare*), rosemary pepper (*Lippia sidoides*), clove basil (*Ocimum gratissimum*), rosemary of field (*Baccharis dracunculifolia*), Himalayan cinnamon (*Cinnamomum glaucescens*), and tea tree (*Melaleuca alternifolia*). The vertical bars on the top of each column correspond to the respective standard error of the mean AUMGC estimates. Different letters at the top of the columns denote significant differences in the AUMGC means, according to Dunn's post hoc test at the 0.05 significance level.

EO against the fungus *Sclerotinia sclerotiorum*, with MIC ranging from 0.128 to 0.256 $\mu\text{L mL}^{-1}$. These variations in MIC are due to the specific sensitivity of each pathogen and the presence of different chemical constituents in different

proportion profiles between EOs (Mutlu-Ingok et al. 2020).

Chemical analysis of selected EOs

GC-MS analysis revealed high variability in the chemical composition of EOs. Oregano EO

Table I. Percentage of inhibition of the mycelial growth of *Phoma caricae-papayae*, *Alternaria alternata*, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, and *Fusarium solani* treated with different concentrations of essential oils of oregano (*Origanum vulgare*), cinnamon bark (*Cinnamomum cassia*), rosemary pepper (*Lippia sidoides*), and clove basil (*Ocimum gratissimum*).

Pathogens	Concentration ($\mu\text{L mL}^{-1}$)	Percentage of inhibition of the essential oils											
		oregano			cinnamon bark			rosemary pepper			clove basil		
<i>Phoma caricae-papayae</i>	0.10	66.18	±	0.55 a	18.61	±	1.88 a	7.26	±	1.19 a	0.00	±	0.19 a
	0.50	100.00	±	0.00 b	100.00	±	0.00 b	80.85	±	1.80 b	0.00	±	0.23 a
	1.00	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b
	2.00	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b
	2.50	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b
<i>Alternaria alternata</i>	0.10	14.23	±	1.28 a	26.15	±	1.74 a	41.54	±	0.38 a	21.89	±	2.72 a
	0.50	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 b	33.68	±	1.70 b
	1.00	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c
	2.00	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c
	2.50	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c
<i>Lasiodiplodia theobromae</i>	0.10	34.39	±	1.21 a	20.39	±	1.50 a	2.14	±	1.01 a	2.44	±	1.24 a
	0.50	100.00	±	0.00 b	80.24	±	1.41 b	100.00	±	0.00 b	100.00	±	0.00 b
	1.00	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 b
	2.00	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 b
	2.50	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 b
<i>Colletotrichum gloeosporioides</i>	0.10	28.82	±	2.74 a	8.99	±	1.42 a	26.09	±	2.08 a	32.89	±	1.50 a
	0.50	100.00	±	0.00 b	11.40	±	1.00 a	100.00	±	0.00 b	56.95	±	0.60 b
	1.00	100.00	±	0.00 b	45.00	±	0.40 b	100.00	±	0.00 b	100.00	±	0.00 c
	2.00	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 c
	2.50	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 c
<i>Fusarium solani</i>	0.10	0.00	±	0.74 a	19.04	±	0.44 a	14.91	±	1.18 a	10.99	±	2.09 a
	0.50	10.00	±	0.50 a	80.45	±	1.73 b	100.00	±	0.00 b	47.91	±	1.83 b
	1.00	100.00	±	0.00 b	83.20	±	0.10 b	100.00	±	0.00 b	58.56	±	1.64 c
	2.00	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 d
	2.50	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 d

The results are expressed as the percentage inhibition $\pm$ standard error. Means followed by different lowercase letters are significantly different according to Dunn's post hoc test at the 0.05 significance level. The percentages were calculated by expressing the mycelial diameter on the last day of evaluation of each treatment by the respective one of check treatment. The minimum inhibitory concentration (MIC) of each essential oil for each pathogen is highlighted.

had a high percentage of carvacrol (69.1%), *p*-cymene (18.8%), and thymol (3.9%) in its composition (Figure 4). In the cinnamon bark EO, cinnamaldehyde (85.1%), *o*-methoxy cinnamaldehyde (9.3%), and cinnamyl acetate (2.7%) were the constituents with the highest percentage (Figure 5). In the EO of rosemary pepper, thymol (77.2%), *p*-cymene (14.2%), and caryophyllene (3.4%) were the major constituents (Figure 6). And finally, in the EO of

clove basil, eugenol (84.84%), *p*-cymene (2.43%), and caryophyllene (2.11%) were found as the major constituents (Figure 7). The complete result of the chemical composition of EOs with the description of all constituents, data on retention time and percentage of peak area are available in Table SI (Supplementary Material). For complementary studies, the three major constituents of EOs were selected.

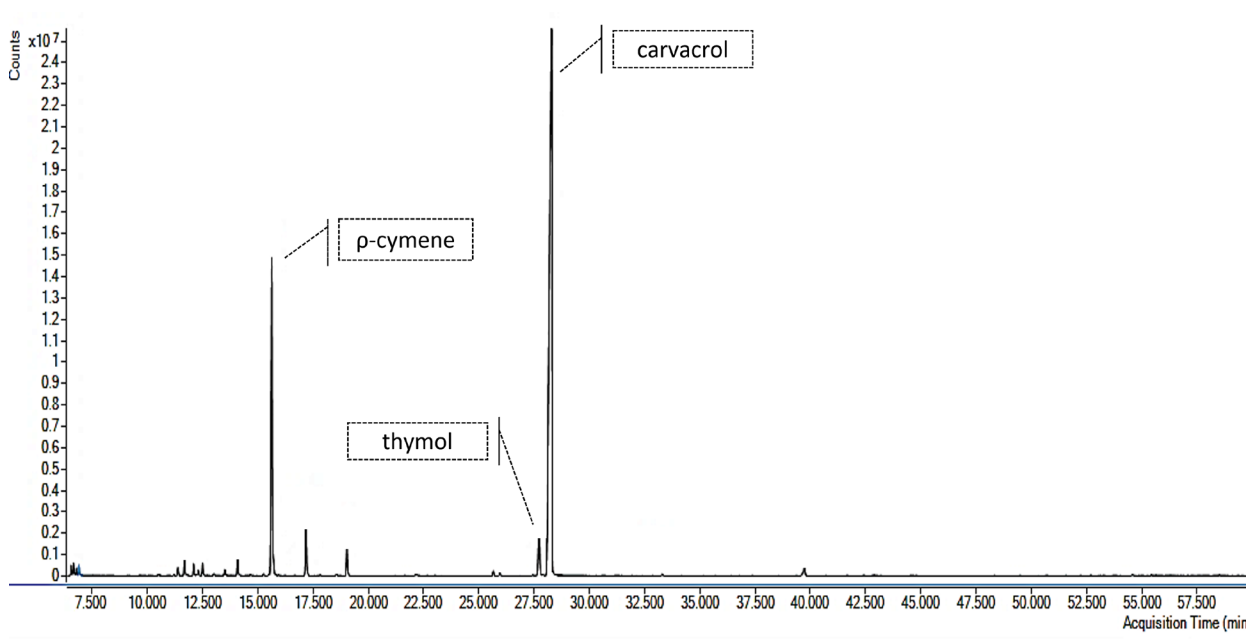


Figure 4. Chromatogram of oregano essential oil analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The major constituents were carvacrol, *p*-cymene, and thymol.

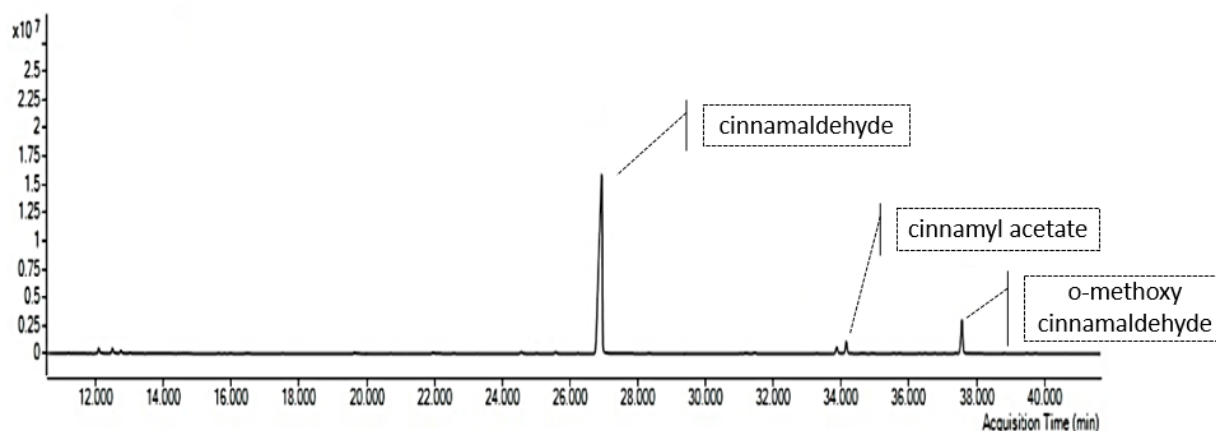


Figure 5. Chromatogram of cinnamon bark essential oil analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The major constituents were cinnamaldehyde, *o*-methoxy cinnamaldehyde, and cinnamyl acetate.

Several studies report similar chemical composition for the EOs studied (oregano, cinnamon bark, rosemary pepper, and clove basil), with differences in the percentage and variety of some constituents (Hao & Quoc 2024, Oliveira et al. 2024, Saffarian et al. 2024, Silva et al. 2024). Differences in the chemical composition of EOs can be explained by the influence of the season or environment of plant cultivation or EO extraction method (Zillo et al. 2018). Thus, to obtain the same composition of EOs, they have to be extracted from the same plant organ,

the plant must grow in the same soil, under the same climate, and harvested in the same year's season (Baptista-Silva et al. 2020).

Antifungal activity of the major constituents of EOs

The major constituent's thymol, carvacrol, eugenol, cinnamaldehyde, o-methoxy cinnamaldehyde, caryophyllene, p-cymene, and cinnamyl present in rosemary pepper, oregano, clove basil, and cinnamon bark EO were selected to evaluate antifungal activity. The chemical

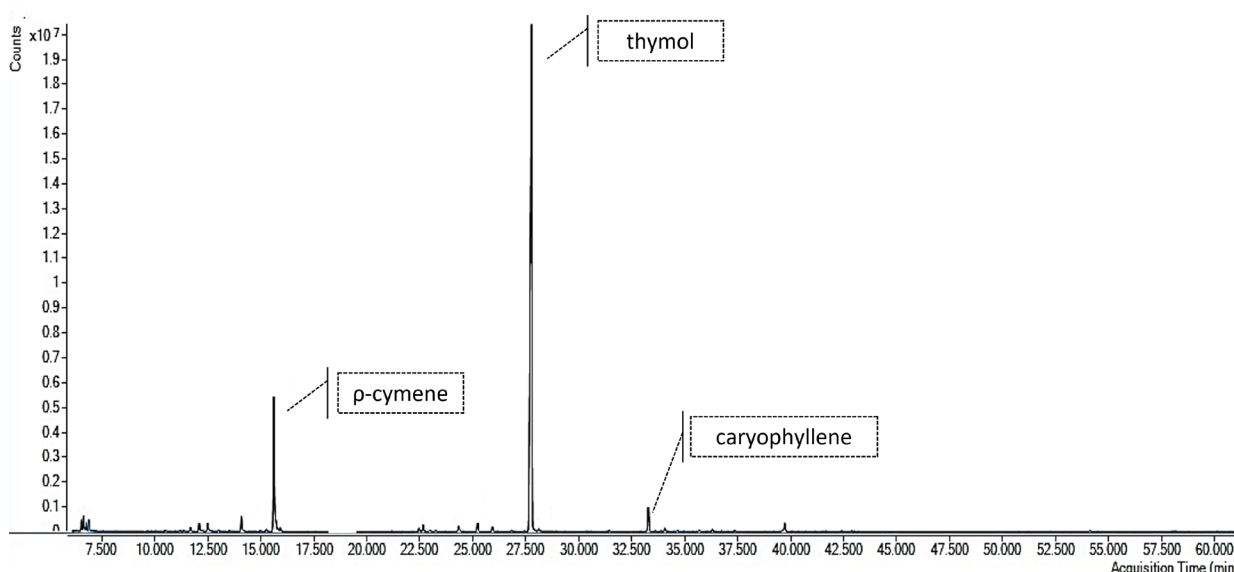


Figure 6. Chromatogram of rosemary pepper essential oil analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The major constituents were thymol, p-cymene, and caryophyllene.

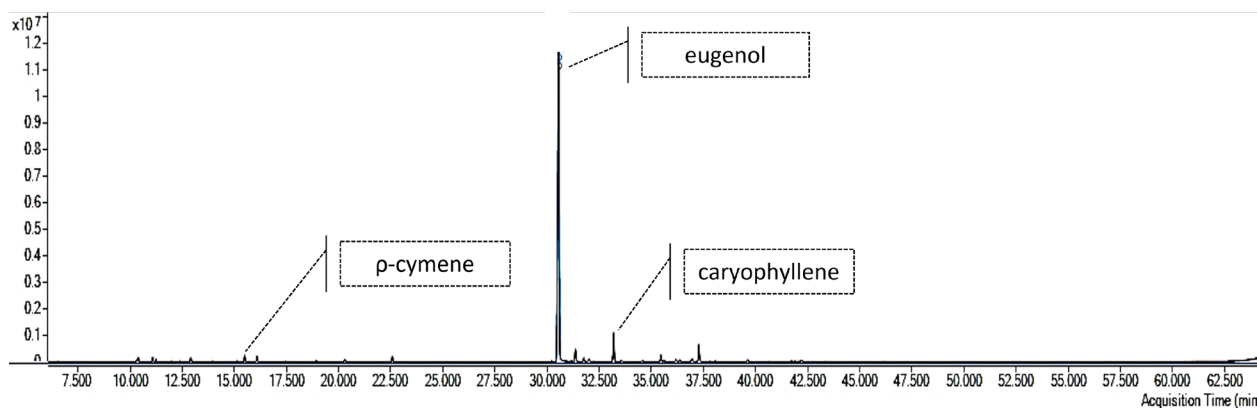


Figure 7. Chromatogram of clove basil essential oil analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The major constituents were eugenol, p-cymene, and caryophyllene.

structures of these constituents are shown in Figure 8.

Thymol and carvacrol, present in high concentration in the EOs of rosemary pepper and oregano, respectively, showed a higher percentage of inhibition compared to other constituents (Figure 9). Expressive effects of thymol and carvacrol were observed for *P. caricae-papayae* with 96% inhibition using both constituents, *A. alternata* with 95% inhibition for thymol and 92% inhibition for carvacrol, *C. gloeosporioides* 94% for thymol and 86% for carvacrol, and *F. solani* with 88% of inhibition for both constituents. Pérez-Alfonso et al. (2012) presented that both thymol and carvacrol effectively inhibiting the mycelial growth of *Penicillium digitatum* and *Penicillium Italicum*.

A lower was observed against *L. theobromae*, showing 70% inhibition for both constituents. Eugenol has a similar effect close to that of thymol and carvacrol, showing around 77% inhibition of *L. theobromae* mycelial growth (Figure 9). According to Sun et al. (2023) eugenol has a significant effect on mycelial growth inhibition and spore germination of *L. theobromae*. Furthermore, observed that the

integrity of the fungal was affected by hyphae disruption after eugenol treatment.

Antifungal activity of the mixture of the major constituents of EOs

The mixture of the three respective major constituents of EOs of oregano (carvacrol, p-cymene, and thymol), cinnamon bark (cinnamaldehyde, o-methoxy cinnamaldehyde, and cinnamyl acetate), rosemary pepper (thymol, p-cymene, and caryophyllene), and clove basil (eugenol, p-cymene, and caryophyllene) respectively promoted 100% inhibition of mycelial growth of *P. caricae-papayae*, *A. alternata*, *L. theobromae*, *C. gloeosporioides*, and *F. solani* (Figure 10, Figure S1). This study revealed that the antifungal activity of the constituents was enhanced when secondary and tertiary compounds were combined, demonstrating a synergistic effect among the constituents. In the study carried out by Sousa et al. (2022), the combined effect of the constituents carvacrol, γ-terpinene and p-cymene increased the antimicrobial activity in relation to the individual constituents. According to Tian et al. (2018), this synergistic effect enhances the antifungal

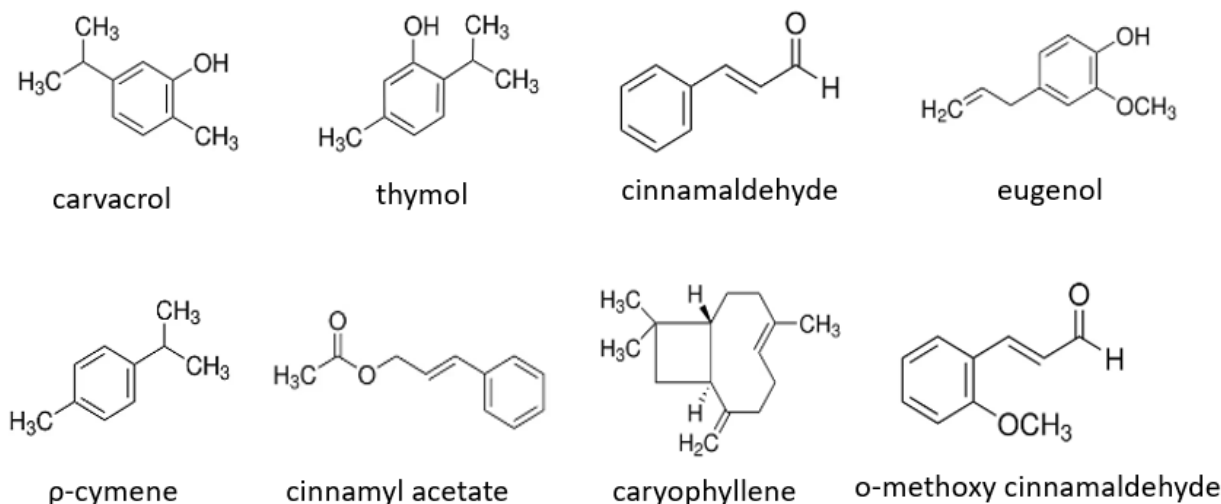


Figure 8. Chemical structure of the major constituents of the essential oils of oregano, cinnamon bark, rosemary pepper, and clove basil: carvacrol, thymol, cinnamaldehyde, eugenol, p-cymene, cinnamyl acetate, caryophyllene, and o-methoxy cinnamaldehyde.

activity by damaging the pathogen's membranes more effectively than when used individually. Similarly, Sousa et al. (2022) observed that the combination of carvacrol, γ -terpinene, and p-cymene in thyme EO enhanced antimicrobial activity compared to the individual constituents. Another study conducted by Heckler et al. (2021) also indicated that antimicrobial efficacy was amplified when carvacrol and thymol were used together, further reinforcing the synergistic potential of these mixtures.

MIC of the mixture of the three major constituents of EOs

Based on the previous results, the MIC of the mixture of the three major constituents of the EOs of oregano (carvacrol, p-cymene, and thymol), cinnamon bark (cinnamaldehyde, o-methoxy cinnamaldehyde, and cinnamyl acetate), rosemary pepper (thymol, p-cymene,

and caryophyllene) and clove basil (eugenol, p-cymene, and caryophyllene) were determinates (Table II, Figures S2, S3, S4, S5 and S6).

The combination of major constituents demonstrated lower MIC compared to the use of pure EOs. The constituents of EOs generally have greater antifungal efficacy due to their high concentration and purity. Unlike EO, which is a complex mixture of compounds, the mixture of the three constituents acts in a more targeted and effective manner, without the interference of other components that can dilute or antagonize its effect. Thus, because they are in their pure form, the constituents have greater ease in acting on the cell membrane of the fungus, disrupting its functions and inhibiting essential enzymes, resulting in a lower MIC. This superiority of the pure constituents was observed by Dias et al. (2020) investigating the EO of *Citrus reticulata*.

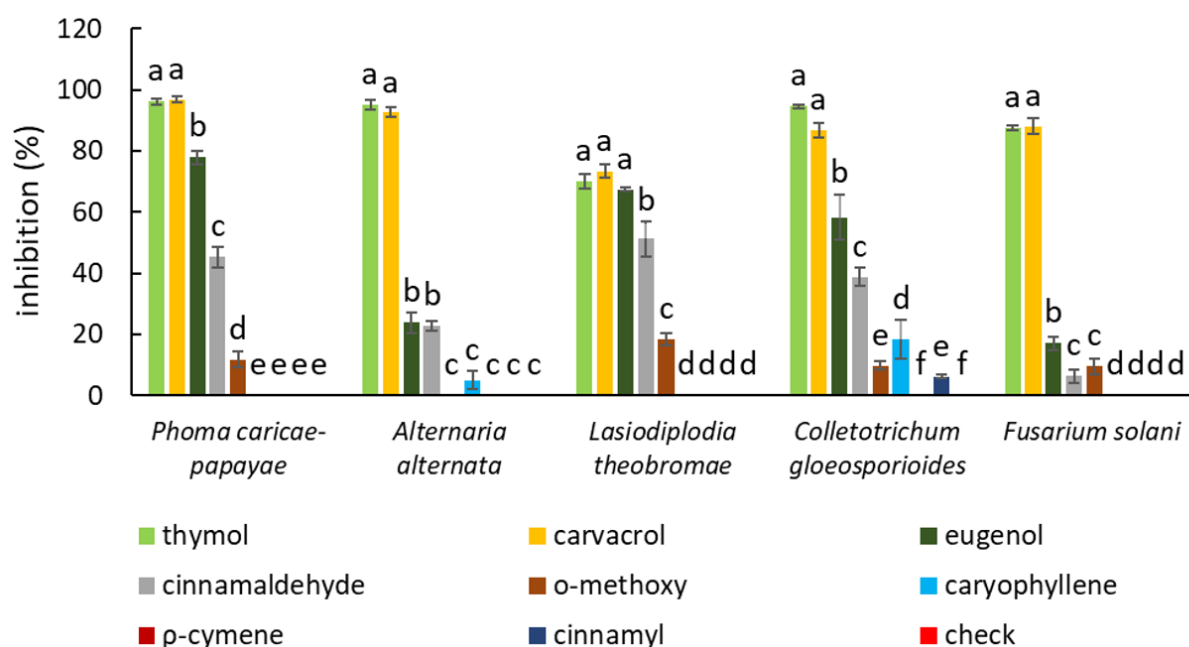


Figure 9. Percentage of inhibition of the mycelial growth of *Phoma caricae-papayae*, *Alternaria alternata*, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, and *Fusarium solani* by major compounds: thymol, carvacrol, eugenol, cinnamaldehyde, o-methoxy cinnamaldehyde, caryophyllene, p-cymene, and cinnamyl present in the essential oils of oregano, cinnamon bark, rosemary pepper, and clove basil. The vertical bars at the top of the columns correspond to the standard error of the estimated means. Different lowercase letters on the top of the columns denote a significant difference in the effect of the constituents, according to Dunn's post hoc test at the 0.05 significance level.

The study showed that the EO inhibited the mycelial growth of *S. sclerotiorum* by 82.91%, while its major constituent was even more effective, completely inhibiting the growth of the fungus.

In this study, the mixture of major constituents of oregano EO presented MIC of $0.25 \mu\text{L mL}^{-1}$ for *P. caricae-papayae*, *A. alternata*, *L. theobromae*, and *F. solani*, and $0.50 \mu\text{L mL}^{-1}$ for *C. gloeosporioides*. For cinnamon bark, the MIC of the combination was $0.25 \mu\text{L mL}^{-1}$ for *F. solani*, and $0.50 \mu\text{L mL}^{-1}$ for *P. caricae-papayae*, *A. alternata*, *L. theobromae*, and *C. gloeosporioides*. The rosemary pepper major constituents exhibited at a MIC of $0.25 \mu\text{L mL}^{-1}$ *P. caricae-papayae* and $0.50 \mu\text{L mL}^{-1}$ *A. alternata*, *L. theobromae*, *C. gloeosporioides*, and *F. solani* and for clove basil constituents, at a MIC of $0.25 \mu\text{L mL}^{-1}$ for *F. solani*, $0.50 \mu\text{L mL}^{-1}$ for *P. caricae-papayae* and *C. gloeosporioides*, and *A. alternata* and *L. theobromae* from $0.75 \mu\text{L mL}^{-1}$, was observed

complete of mycelial growth inhibition. As shown in previous results presented in Table 1, the MIC for *F. solani* using pure EOs ranged from 0.50 to $2.00 \mu\text{L mL}^{-1}$, whereas for the mixture ranged from 0.25 to $0.50 \mu\text{L mL}^{-1}$ (Table II), revealing a synergistic effect of the combination of the major constituents of EOs in the inhibition of phytopathogens. The results of this study are in agreement with the observations of Et-tazy et al. (2023) evaluating oregano EO in the control of *A. niger*. The EO presented a MIC of $0.781 \mu\text{L mL}^{-1}$, while its constituents demonstrated a lower MIC of only $0.098 \mu\text{L mL}^{-1}$, highlighting the superior effect of the constituent compared to the crude EO. These results indicate that the mixture of constituents can be an effective approach in the control of fungi in fruits, once adequate combinations enhance the effects.

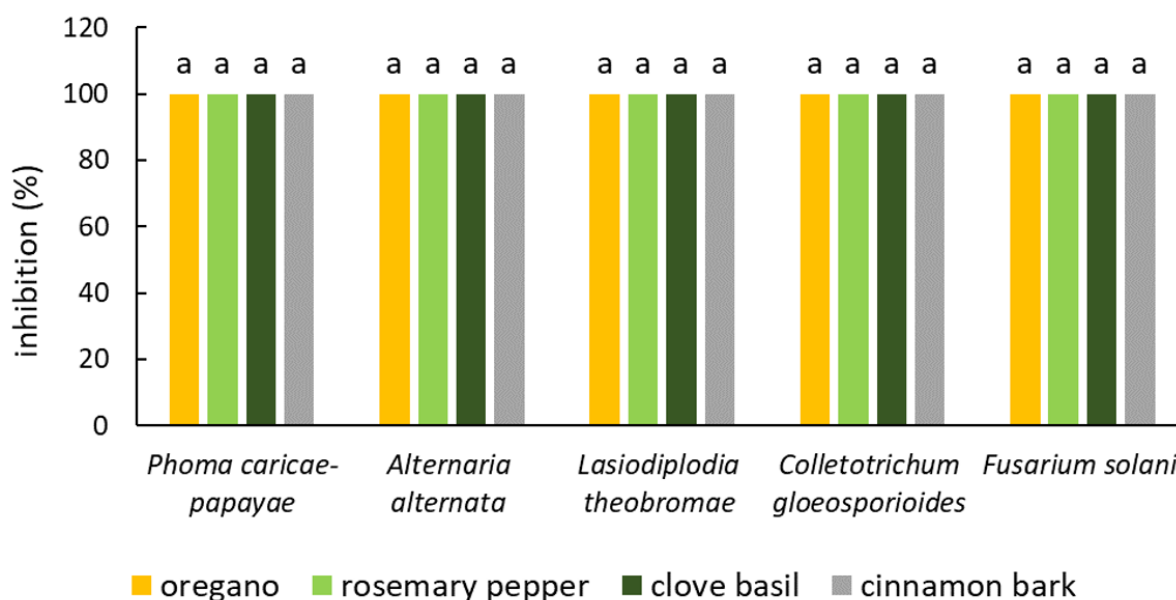


Figure 10. Percentage of mycelial growth inhibition of *Phoma caricae-papayae*, *Alternaria alternata*, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, and *Fusarium solani* after treatment with a mixture of the major constituents of oregano (carvacrol, p-cymene, and thymol), cinnamon bark (cinnamaldehyde, o-methoxy cinnamaldehyde, and cinnamyl acetate), rosemary pepper (thymol, p-cymene, and caryophyllene) and clove basil (eugenol, p-cymene, and caryophyllene). The vertical bars at the top of the columns correspond to the standard error of the estimated means. The different letters at the top of the columns denotes a significant difference in the effect of the constituents, according to Dunn's post hoc test at the 0.05 significance level.

Table II. Inhibition of the mycelial growth (%) of *Phoma caricae-papayae*, *Alternaria alternata*, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, and *Fusarium solani* treated with different concentrations of a mixture of the three major constituents of essential oil of oregano (*Origanum vulgare*), cinnamon bark (*Cinnamomum cassia*), rosemary pepper (*Lippia sidoides*), and clove basil (*Ocimum gratissimum*).

Pathogens	Mixture concentration ($\mu\text{L mL}^{-1}$)	Inhibition of mycelial growth (%) of the mixture of the major constituents of the EOs											
		carvacrol + ρ -cymene + thymol (oregano)			cinnamaldehyde + o-methoxy + cinnamyl acetate (cinnamon bark)			thymol + ρ -cymene + caryophyllene (rosemary pepper)			eugenol + ρ -cymene + caryophyllene (clove basil)		
<i>Phoma caricae-papayae</i>	0.10	46.99	±	0.73 a	23.11	±	0.52 a	86.60	±	0.32 a	15.54	±	0.92 a
	0.25	100.00	±	0.00 b	52.74	±	0.88 b	100.00	±	0.00 b	85.68	±	2.43 b
	0.50	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 c
	0.75	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 c
	1.00	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 c
	1.50	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b	100.00	±	0.00 c
<i>Alternaria alternata</i>	0.10	17.34	±	1.07 a	8.56	±	1.30 a	12.52	±	3.54 a	0.00	±	0.00 a
	0.25	100.00	±	0.00 b	35.83	±	0.17 b	45.40	±	3.44 b	38.50	±	1.61 b
	0.50	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 c	93.13	±	0.53 c
	0.75	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 d
	1.00	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 d
	1.50	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 d
<i>Lasiodiplodia theobromae</i>	0.10	81.32	±	0.95 a	26.64	±	1.27 a	49.10	±	1.92 a	29.67	±	1.41 a
	0.25	100.00	±	0.00 b	59.97	±	1.56 b	90.36	±	0.60 b	65.12	±	0.98 b
	0.50	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 c	88.06	±	1.23 c
	0.75	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 d
	1.00	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 d
	1.50	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 d
<i>Colletotrichum gloeosporioides</i>	0.10	6.06	±	0.48 a	3.96	±	0.60 a	11.17	±	0.56 a	0.01	±	0.00 a
	0.25	70.57	±	6.76 b	15.21	±	0.71 b	17.37	±	0.54 b	43.15	±	0.78 b
	0.50	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 c
	0.75	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 c
	1.00	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 c
	1.50	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 c	100.00	±	0.00 c
<i>Fusarium solani</i>	0.10	22.77	±	0.36 a	20.63	±	1.23 a	0.02	±	0.02 a	7.70	±	0.40 a
	0.25	100.00	±	0.00 b	100.00	±	0.00 b	21.19	±	0.47 b	100.00	±	0.00 b
	0.50	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b
	0.75	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b
	1.00	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b
	1.50	100.00	±	0.00 b	100.00	±	0.00 b	100.00	±	0.00 c	100.00	±	0.00 b

The results are expressed as the percentage inhibition $\pm$ standard error. Means followed by different lowercase letters are significantly different according to Dunn's post hoc test at the 0.05 significance level. The mixture of constituents was prepared following the same proportion found in essential oils by Gas Chromatography-Mass Spectrometry (GC-MS). Dimethyl sulfoxide (DMSO) was used to complete 100% of the mixture proportion. The minimum inhibitory concentration (MIC) of each essential oil for each pathogen is highlighted.

CONCLUSIONS

The EOs of oregano, cinnamon bark, clove basil, and rosemary pepper were most efficient in controlling *P. caricae-papayae*, *A. alternata*, *L. theobromae*, *F. solani*, and *C. gloeosporioides* of papaya. The MIC of oregano and rosemary pepper EO ranged from 0.50 $\mu\text{L mL}^{-1}$ to 1.00 $\mu\text{L mL}^{-1}$ and of cinnamon bark and clove basil EO from 0.50 $\mu\text{L mL}^{-1}$ to 2.00 $\mu\text{L mL}^{-1}$. Among the evaluated pathogens, *F. solani* is shown to be more resistant to being controlled by EOs.

The major constituents of oregano EO were carvacrol (69.1%), p-cymene (18.8%) and thymol (3.9%), and cinnamaldehyde (85.1%), o-methoxy cinnamaldehyde (9.3%) and cinnamyl acetate (2.7%) were presented in higher concentration in cinnamon bark EO. For rosemary pepper EO, thymol (77.2%), p-cymene (14.2%), and caryophyllene (3.4%) were the principal constituents, and for clove basil EO were eugenol (84.84%), p-cymene (2.43%), and caryophyllene (2.11%).

Thymol and carvacrol showed higher antifungal activity against the fungi studied, and the combination of the three major constituents of each EO demonstrated a synergistic effect, presenting better inhibition performance of the pathogens than the pure EOs, and the MIC ranged from 0.25 to 0.50 $\mu\text{L mL}^{-1}$.

Therefore, the mixture of major constituents of oregano, cinnamon bark, clove basil, and rosemary pepper EOs presented to be a promising alternative for controlling *P. caricae-papayae*, *A. alternata*, *L. theobromae*, *F. solani*, and *C. gloeosporioides* of papaya, to be included in postharvest treatment.

Acknowledgments

This project had financial support from the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP 2018/25318-7 and the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq 407421_2021-1 and doctoral scholarship (CNPq

140679/2022-7). The authors express their gratitude to Mr. Marley Mendonça Tavares and Mrs. Debora Renata Cassoli de Souza Dutra for their assistance in the GC-MS analysis.

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SUPPLEMENTARY MATERIAL

Figures S1, S2, S3, S4, S5 and S6.
Table S1.

How to cite

DA SILVA AM, TERAPO D, VILELA ESD, DE QUEIROZ SCN, MAIA AHN & FRACAROLLI JA. 2025. Essential oils on the control of postharvest diseases of papaya. An Acad Bras Cienc 97: e20240770. DOI 10.1590/0001-3765202520240770.

Manuscript received on July 16, 2024;
accepted for publication on November 19, 2024

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ADRIANE MARIA DA SILVA was responsible for data collection, conducting experiments, planning, statistical analyses, and drafting the manuscript. DANIEL TERAÓ contributed through supervision, financial support, and project planning. ELKE SIMONI D. VILELA participated in data collection, experimental execution, and project planning. SONIA CLAUDIA N. DE QUEIROZ provided material resources and contributed to project planning. ALINE H.N. MAIA assisted with statistical analyses. JULIANA APARECIDA FRACAROLLI oversaw supervision. All authors actively participated in reviewing and editing the manuscript.

