Chemistry & Biodiversity

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CHEMISTRY & BIODIVERSITY

RESEARCH ARTICLE

Direct and Volatile Potential Applications of Essential Oils for Post-Harvest Fungal Control

Ygor G. P. Osti[1] | Larissa G. R. Duarte[2] | Conny W. T. Fukuyama[1] | Isadora C. Pedrino[1] | Higor V. Santos[3] | Josemar G. de Oliveira Filho[2] | Maria Eduarda A. Astolfo[3] | Maria Eduarda M. Martins[4] | Stanislau Bogusz Junior[3] | Marcos David Ferreira[2]

[1]Department of Biotechnology, Federal University of São Carlos, Sao Carlos, Brazil | [2]Brazilian Agricultural Research Corporation, Embrapa Instrumentation, Sao Carlos, Brazil | [3]Institute of Chemistry (IQSC), University of São Paulo (USP), Sao Carlos, Brazil | [4]Postgraduate Program in Food Science and Engineering, IBILCE, São Paulo State University 'Julio de Mesquita Filho', UNESP, São José do Rio Preto, Brazil

Correspondence: Larissa G. R. Duarte (larissagraziele@gmail.com)

Received: 29 December 2024 | **Revised:** 8 March 2025 | **Accepted:** 10 March 2025

Funding: This research was funded by FAPESP (#2022/10686-6), CNPq (#383138/2023-0, 138584/2023-0), CAPES (# 001), Empresa Brasileira de Pesquisa Agropecuária (#20.19.03.0124.00.00)—Embrapa, Rede Agronano, CNPq/MCTI Sisnano (#442575/2019-0), and M. D. Ferreira CNPq Research Productivity fellowship (#307141/2022-5).

Keywords: *Alternaria alternata* | antifungal agents | biological activity | *Fusarium solani* | *Lasidiodiplodia theobromae*

ABSTRACT

Around one-third of food production is lost globally, significantly impacting food security, primarily due to post-harvest deterioration from phytopathogenic fungi. This study aimed to assess the antifungal properties of essential oils (EOs) from *Syzygium aromaticum*, *Origanum vulgare L.*, *Cymbopogon martinii*, *C. citratus*, *Mentha spicata*, and *Mentha piperita* against three fungi affecting tropical fruits: *Lasiodiplodia theobromae*, *Alternaria alternata*, and *Fusarium solani*. The antifungal efficacy was evaluated using direct contact and volatilization methods, determining the minimum inhibitory concentration (MIC) needed to completely inhibit fungal growth. Scanning electron microscopy was employed to observe the effects of EOs on fungal cells. Among the tested oils, *O. vulgare* showed the most promise, with MIC values ranging from 40 to 200 µL/L for volatile exposure and from 125 to 500 µL/L for direct contact. The results indicated that vapor exposure was more effective, requiring lower concentrations for fungal control. Thus, these EOs, particularly that of *O. vulgare*, present a viable alternative to synthetic fungicides for managing post-harvest fungal infections in tropical fruits, promoting sustainable agricultural practices.

1 | Introduction

Post-harvest losses resulting from natural degradation in agricultural products represent a significant challenge to food security and economic sustainability. The Food and Agriculture Organization of the United Nations (FAO) has been putting great efforts into developing an indicator model to monitor food loss at a global level, based on surveys produced by each country. The most recent report showed that fruits and vegetables are the most affected group by post-harvest losses, representing around 31.2%, globally (FAO). Despite increasing collaboration, these data are still scarce and underrepresented, which could mean even more significant losses worldwide, mainly due to fungal spoilage [1].

Filamentous fungi encompass a vast array of species capable of thriving in diverse environments, making them widely distributed in nature. Their ability to proliferate poses a threat not only to agricultural crops but also to post-harvest management. The contamination by these fungi leads, globally, to significant economic losses and compromises the quality of fresh

products [2]. In tropical fruits, the fungi *Lasiodiplodia theobromae, Alternaria alternata*, and *Fusarium solani* are growing interest among the primary agents due to the increase in incidence and severity of post-harvest diseases caused by them.

L. theobromae is a phytopathogen fungus that can affect a wide variety of tropical crops and has been reported in a rising number of tropical fruits, such as mango, mangosteen, passion fruit, papaya, grapes, avocados, and cocoa ([3; Rusin et al. 4; 5; Quin et al. 6]). Recent studies have shown that this fungus is quite virulent, causing extensive damage just a few days after its inoculation, and showing resistance to some fungicides applied on papaya [7]. The infection happens mostly through injuries in the fruit skin that can occur at any point during harvest, transport, or storage and the disease initially manifests itself as brown spots on the skin around the wound and softening of the fruit in contaminated areas. Over time, it's possible to observe the growth of a grayish-white mycelium over the fruit skin, with gradual darkening, reaching a dark gray color. Additionally, there is rot inside the affected fruit as the fungus penetrates the inner tissues [5].

A. alternata is known as a broad-spectrum pathogen, affecting numerous plant species, including tropical fruits such as kiwi, mango, persimmon, and dragon fruit [8–10]. The contamination usually happens through spores present in the air or by direct contact with other contaminated fruits. Dark necrotic spots or superficial discoloration are often observed symptoms and, in some cases, the proliferation of mycelium on the surface of contaminated fruits may also be visible under favorable conditions such as high humidity and temperature. This species is also known for producing mycotoxins which, in high quantities, can be harmful to human health [7].

Fusarium sp. are mostly soil saprophytes and some species can also cause post-harvest diseases in many fruits such as avocado, mango, papaya, and pineapple, being easily spread generally from the production field. *F. solani* seems to be the main species that affects tropical fruits [11]. The infection caused by this fungus presents some characteristic signs, such as brown or necrotic lesions, causing wilting and gradual softening of the fruits, resulting in an aged appearance and loss of quality [12]. Controlling this pathogen is also difficult because the use of traditional fungicides can cause negative impacts on the local ecosystem [13].

Traditionally, synthetic fungicides have been used to control fruit postharvest pathogens. However, its possible negative effects on human health and the environment have raised concerns about its use. Furthermore, the selection and emergence of strains resistant to a notable number of fungicides have led to attempts to find biological resources to replace these synthetic products [14]. In this sense, plant essential oils (EOs) and their constituent molecules have received special attention as it is recognized as Generally Recognized as Safe by the FDA since 2008 ([Panwar et al. 2024 15; 16, de Oliveira Filho et al. 17]). EOs are volatile oils with low water solubility, extracted from various parts of plants using both traditional and innovative methods (Mendonça et al. [18]). They are widely used in the fragrance and perfume industry [19]. The use of EOs as antifungal agents has a solid theoretical basis, based on the bioactive properties of these natural compounds, which is why it has been shown to be a safe and effective alternative ([20]; Vilela et al. [21]). The presence of EOs appears to compromise the cell's structural integrity, leading to reduced growth. Treated cells often show a more segmented appearance with clumped growth and increased unidentified material on their surface, indicating cell wall disruption and cytoplasmic leakage, which results in oxidative damage [1].

This study aims to investigate the antifungal potential of *Syzygium aromaticum* (clove), *Origanum vulgare L*. (oregano), *Cymbopogom martinii* (palmarosa), *Cymbopogon citratus* (lemongrass), *Mentha spicata* (green mint) and *Mentha piperita* (peppermint) EOs against three species of phytopathogenic fungi (*L. theobromae, A. alternata*, *and F. solani*) to determine the minimum concentrations capable of completely inhibiting the growth of these microorganisms to mitigate post-harvest impacts. The relevance of this investigation transcends the scientific field, directly impacting the agricultural sector by providing promising alternatives for post-harvest preservation. By contributing to the understanding of the effectiveness of different EOs, by different methods, against phytopathogenic fungi, this study offers a safe and sustainable alternative to reduce economic losses and improve food security in a challenging post-harvest scenario.

2 | Results and Discussion

2.1 | EO Composition

Based on the chromatographic results, the EOs were divided into two groups: the first group consisted of EOs containing alcohol as the main compound and the second group contained aldehyde and ketone as the main compounds [22]. Compounds corresponding to peaks of relative area less than 1% were not listed.

Group 1: The clove (*S. aromaticum*) EO showed Eugenol (90.75%) and β-Caryophyllene (7.81%) as the main components, results similar to those reported in the literature, with the same major compounds (82.4% and 14.0%, respectively) by Kacániová et al. 2021 [23] and 89.73% eugenol by Fukuyama et al., [24]. The antifungal activity of clove EO has been reported against the fungus *Aspergillus sp*. due to its major compounds such as eugenol in the work of Haro-González et al. [25]. As for the oregano (*O. vulgare*) EO, the composition obtained was Carvacrol (67,89%), p-Cymene (8,69%), Thymol (8.13%), γ-Terpinene (6.15%), Caryophyllene (1.86%), β-Myrcene (1.45%) and α-Terpinene (1.22%). Very close to the results reported by Lombrea et al. 2020 [26] In the palmarosa (*C. martinii*) EO, the main components were Geraniol (86.83%), Nerol acetate (7.78%), β-Linalool (1.41%), and Caryophyllene (1.00%). These results are corroborated by Dangol et al. [27] who found geraniol (76.6%–87.9%) and geranyl acetate (4.4%–15.2%) as the main components (Figure 1).

Group 2: The lemongrass (*C. citratus*) EO showed (E)-Geranial (46.56%), Citral (36.47%), Geraniol (7.19%), β-Myrcene (4.17%), and β-Geraniol (1.40%). Other studies have already shown Geranial (55.2%) and Citral (38.34%) as the main components [28, 29]. The mint (*M. spicata*) EO showed Carvone (66.67%), Limonene (24.00%), and Pulegone (1.29%) as the main compounds, also within a reasonable variation (Carvone representing 41.1% and

TABLE 1 | Percentages of growth inhibition of the fungus *Lasiodiplodia theobromae* in direct contact with different essential oils at different concentrations.

Concentration (µL/L)	*Syzygium aromaticum*	*Origanum vulgare*	*Cymbopogon martinii*	*Cymbopogon citratus*	*Mentha spicata*	*Mentha piperita*
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
62.5	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
125	0 ± 0.00	42.78 ± 8.67	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
250	0 ± 0.00	82.06 ± 1.16	0 ± 0.00	4,20 ± 6.54	8.65 ± 7.01	0 ± 0.00
500	32.76 ± 2.62	100 ± 0.00	0 ± 0.00	100 ± 0.00	56.66 ± 9.75	33.30 ± 5.68
750	72.34 ± 6.07	100 ± 0.00	90.84 ± 7.96	100 ± 0.00	25.00 ± 8.42	60.53 ± 1.49
1000	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
MIC	750 <MIC< 1000	250 <MIC<500	750 <MIC<1000	250<MIC<500	750 <MIC<1000	750<MIC<1000

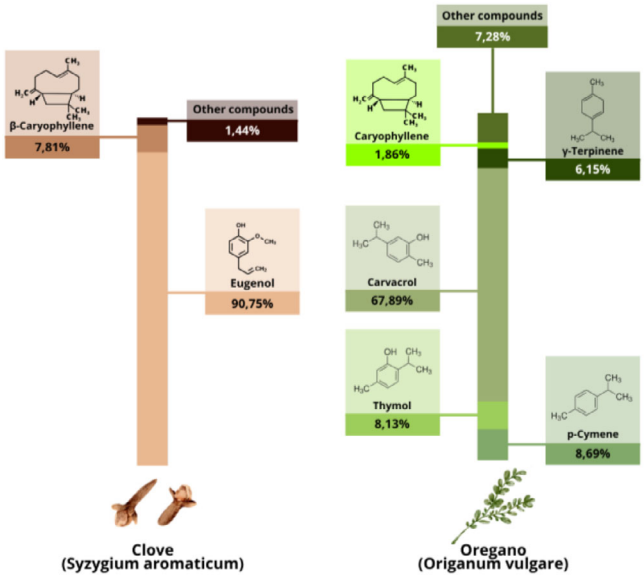

FIGURE 1 | Schematic composition of the two most active essential oils (Clove and Oregano) against the tested fungi. The figure shows the major molecules and their respective percentages found in the essential oils (EOs).

limonene, 14.4%) as described by Giménez-Santamarina et al., [30]. In the peppermint (*M. piperita*) EO, the main components identified were Menthol (37.62%), Menthone (7.57%), Isomenthol (8.25%), Eucalyptol (6.90%), Cyclohexanol (3.77%), and Caryophyllene (2.19%) which are within the range of variation found in other studies, as described by Hudz et al., [31].

2.2 | Antifungal Activity Evaluation

2.2.1 | Direct Contact Method

All EOs inhibited the mycelial growth of *L. theobromae, A. alternata*, and *F. solani* by the direct contact method in a dose-dependent manner (Tables 1–3). The highest antifungal activity against *L. theobromae* was provided by the EOs of *O. vulgare* and *C. citratus*, both with total inhibition of mycelial growth between 250 and 500 µL/L (Table 1). The other studied EOs showed lower antifungal activity, with total inhibition of mycelial growth between concentrations of 750 and 1000 µL/L (Table 1).

For the fungus *A. alternata* (Table 2), the highest antifungal activity was provided by the EOs of *O. vulgare* and *C. martinii*, with total inhibition of mycelial growth between concentrations of 125 and 250 µL/L, and 250 and 500 µL/L, respectively. The EOs of *M. spicata* and *M. piperita* showed lower antifungal activity, being unable to completely inhibit the mycelial growth of the fungus *A. alternata* up to the maximum tested concentration of 1000 µL/L (Table 2).

Against the fungus *F. solani* (Table 3), the EOs of *O. vulgare* and *S. aromaticum* showed high antifungal activity, with MIC values between 250 and 500 µL/L and 500 and 750 µL/L, respectively. The other studied EOs were not able to completely inhibit the growth of *F. solani* up to the maximum tested concentration of 1000 µL/L (Table 3).

Among the three fungi studied, it was found that their sensitivity to EOs differed, with *F. solani* being the most resistant to the action of most of the EOs tested, requiring high concentrations for *C. martinii*, *C. citratus*, *M. spicata*, and *M. piperita* to inhibit its development, however as mentioned *O. vulgarae* and *S. aromaticum*, respectively, were effective in controlling on lower concentrations than the maximum (Table 3). This high resistance of *F. solani* to the antimicrobial action of those EOs can be attributed to a combination of factors such as cell wall thickness, adaptive capacity, and genetic variability. The fungus *F. solani*, when in stressful situations, such as when subjected to antimicrobial agents, can increase the synthesis of chitin, a component of the cell wall, and thus acquire greater protection against external agents [32]. Furthermore, the genetic variability of this fungus may result in some strains being more resistant than others with different levels of resistance to EOs (Zabka & Pavela [33]).

Our results demonstrated that the EO of *O. vulgare* has high inhibitory activity against *L. theobromae*, corroborating the literature that highlights the antifungal potential of bioactive compounds present in this oil ([20]; Vilela et al. [21]). Similarly, Motelica et al. [34] demonstrated the efficacy of zinc oxide nanoparticles loaded with EOs against several pathogens,

TABLE 2 | Percentages of growth inhibition of the fungus *Alternaria alternata* in direct contact with different essential oils at different concentrations.

Concentration (µL/L)	*Syzygium aromaticum*	*Origanum vulgare*	*Cymbopogon martinii*	*Cymbopogon citratus*	*Mentha spicata*	*Mentha piperita*
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
62.5	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
125	0 ± 0.00	0 ± 0.00	10.37 ± 3.25	19.65 ± 0.99	0 ± 0.00	0 ± 0.00
250	22.61 ± 1.55	100 ± 0.00	36.62 ± 7.10	23.83 ± 3.46	18.97 ± 0.63	4.70 ± 7.33
500	85.65 ± 13.17	100 ± 0.00	100 ± 0.00	48.95 ± 7.24	20.32 ± 0.26	37.87 ± 9.76
750	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	20.53 ± 0.32	50.26 ± 9.66
1000	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	44.49 ± 9.78	83.42 ± 6.89
MIC	500<MIC<750	125 <MIC< 250	250 <MIC< 500	500<MIC<750	MIC > 1000	MIC > 1000

TABLE 3 | Percentages of growth inhibition of the fungus *Fusarium solani* in direct contact with different essential oils at different concentrations.

Concentration (µL/L)	*Syzygium aromaticum*	*Origanum vulgare*	*Cymbopogon martinii*	*Cymbopogon citratus*	*Mentha spicata*	*Mentha piperita*
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
62.5	16.70 ± 3.68	13.52 ± 3.26	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
125	20.36 ± 3.41	24.80 ± 0.77	0 ± 0.00	0 ± 0.00	0 ± 0.00	16.44 ± 0.44
250	39.87 ± 2.64	88.27 ± 1.15	0 ± 0.00	0 ± 0.00	0 ± 0.00	20.93 ± 1.86
500	83.92 ± 8.29	100 ± 0.00	0 ± 0.00	0 ± 0.00	14.44 ± 5.28	27.26 ± 5.26
750	100 ± 0.00	100 ± 0.00	13.16 ± 0.34	0 ± 0.00	29.09 ± 3.82	58.73 ± 1.22
1000	100 ± 0.00	100 ± 0.00	27.57 ± 4.34	14.01 ± 3.17	47.56 ± 7.40	86.93 ± 1.75
MIC	500<MIC<750	250 <MIC< 500	MIC > 1000	MIC > 1000	MIC > 1000	MIC > 1000

highlighting the importance of exploring different formulations to optimize post-harvest disease control. Additionally, the use of nanocomposites based on chitosan, ZnO and Ag nanoparticles, and citronella EO, as demonstrated by Motelica et al. [35], presents a promising alternative for fruit coating, aiming at controlling pathogens and increasing the shelf life of products. These innovative approaches, which combine different control strategies, may be particularly useful in mitigating the development of resistance by fungi and reducing dependence on synthetic fungicides. The search for effective and sustainable alternatives is crucial to ensure food security and reduce environmental impacts associated with post-harvest disease control.

2.2.2 | Exposure to Volatiles Method

An important property of EOs is their antifungal activity in the vapor phase, which allows their application in fresh stored products that are sensitive to direct contact preservation treatments. In addition to being less impactful on the integrity of the product, this method can minimize the negative effects of EOs on the sensory properties of the fruits, such as changes in odor and flavor [36]. Tables 4–6 show the percentage inhibition of mycelial growth of *L. theobromae, A. alternata*, and *F. solani* and the minimum inhibitory concentration (MIC) of EOs in the vapor phase.

According to Table 4, the most potent antifungal activity against *L. theobromae* was exhibited by *O. vulgare* with MIC values ranging between 40 and 100 µL/L of air. Following closely, *C. martinii* and *C. citratus* EOs proved to be the second most effective, completely inhibiting fungal growth at concentrations between 100 and 200 µL/L. *S. aromaticum* showed a MIC ranging from 200 to 400 µL/L. In contrast, *M. spicata* and *M. piperita* EOs demonstrated less significant antifungal activity, with complete inhibition observed only at concentrations exceeding 1000 and 800 µL/L of air, respectively (Table 4).

For *A. alternata* (Table 5), the EOs of *O. vulgare* and *S. aromaticum* showed higher antifungal activity, with total inhibition of mycelial growth between concentrations of 40 and 100 µL/L. The other EOs also showed good antifungal activity, being able to completely inhibit the mycelial growth of the fungus at low concentrations: between 100 and 200 µL/L of air for *C. martinii* and *C. citratus*, 200 and 400 µL/L of air for *M. spicata*, and between 400 and 600 µL/L of air for *M. piperita*.

Again, the EOs of *S. aromaticum* and *O. vulgare* exhibited the highest antifungal activity against *F. solani* (Table 6), completely inhibiting its mycelial growth at concentrations of 200-400 µL/L and 100-200 µL/L of air, respectively. The other EOs tested did not achieve complete inhibition in the concentration range tested (Table 6). Just like in the direct contact test (Table 3), the fungus

TABLE 4 | Percentages of growth inhibition of the fungus *Lasiodiplodia theobromae* exposed to volatiles of different essential oils at different concentrations.

Concentration (μL/L of air)	*Syzygium aromaticum*	*Origanum vulgare*	*Cymbopogon martinii*	*Cymbopogon citratus*	*Mentha spicata*	*Mentha piperita*
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0,00
20	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0,00
40	0 ± 0.00	59.64 ± 4.82	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
100	1.00 ± 1.73	100 ± 0.00	35.87 ± 30.5	31.12 ± 24.74	0 ± 0.00	0 ± 0.00
200	33.86 ± 29.03	100 ± 0.00	100 ± 0.00	100 ± 0.00	0 ± 0.00	0 ± 0.00
400	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	0 ± 0.00	3.35 ± 3.05
600	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	41.23 ± 4.25	78,93 ± 2.43
800	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	77.83 ± 1.81	95.90 ± 7.09
1000	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	82.70 ± 2.45	100 ± 0.00
MIC	200<MIC<400	40<MIC<100	100<MIC<200	100<MIC<200	1000<MIC	800<MIC<1000

TABLE 5 | Percentages of growth inhibition of the fungus *Alternaria alternata* exposed to volatiles of different essential oils at different concentrations.

Concentration (μL/L of air)	*Syzygium aromaticum*	*Origanum vulgare*	*Cymbopogon martinii*	*Cymbopogon citratus*	*Mentha spicata*	*Mentha piperita*
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
20	12.41 ± 1.47	0 ± 0.00	23.63 ± 3.07	16.86 ± 9.64	38.49 ± 0.93	49.61 ± 5.06
40	96.11 ± 3.38	39.9 ± 4.29	41.60 ± 3.55	36.26 ± 7.02	48.19 ± 0.45	52.70 ± 1.60
100	100 ± 0.00	100 ± 0.00	82.63 ± 16.34	65.64 ± 8.41	57.63 ± 0.46	62.55 ± 2.65
200	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	70.67 ± 0.67	86.29 ± 0.61
400	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	85.93 ± 24.38
600	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
800	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
1000	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
MIC	40<MIC<100	40<MIC<100	100<MIC<200	100<MIC<200	200<MIC<400	400<MIC<600

TABLE 6 | Percentages of growth inhibition of the fungus *Fusarium solani* exposed to volatiles of different essential oils at different concentrations.

Concentration (μL/L of air)	*Syzygium aromaticum*	*Origanum vulgare*	*Cymbopogon martinii*	*Cymbopogon citratus*	*Mentha spicata*	*Mentha piperita*
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
20	19.17 ± 1.20	25.74 ± 2.53	17.28 ± 0.28	13.28 ± 0.92	18.55 ± 1.44	10.15 ± 3.16
40	20.94 ± 0.51	47.97 ± 10.00	19.05 ± 2.31	17.39 ± 2.42	21.9 ± 1.91	13.15 ± 2.99
100	58.10 ± 8.35	86.21 ± 2.83	19.12 ± 5.05	18.5 ± 0.88	26 ± 3.02	27.37 ± 1.29
200	84.50 ± 1.38	100 ± 0.00	29.26 ± 1.61	21.61 ± 3.66	32 ± 4.52	45.67 ± 5.10
400	100 ± 0.00	100 ± 0.00	43.46 ± 3.22	25.67 ± 5.27	55.76 ± 5.42	48.74 ± 7.67
600	100 ± 0.00	100 ± 0.00	47.50 ± 2.11	29.71 ± 1.03	77.85 ± 2.11	61.41 ± 12.61
800	100 ± 0.00	100 ± 0.00	53.47 ± 5.62	33.67 ± 2.22	83.05 ± 1.09	77.63 ± 2.82
1000	100 ± 0.00	100 ± 0.00	52.93 ± 11.53	34.03 ± 2.09	71.06 ± 23.55	83.75 ± 2.51
MIC	200<MIC<400	100<MIC<200	1000<MIC	1000<MIC	1000<MIC	1000<MIC

TABLE 7 | Comparison between minimal inhibitory concentrations found by direct contact and exposure to volatiles for each combination oil/fungus.

	Lasiodiplodia theobromae		*Alternaria alternata*		*Fusarium solani*	
Essential oil	**Volatiles exposure**	**Direct contact**	**Volatiles exposure**	**Direct contact**	**Volatiles exposure**	**Direct contact**
Syzygium aromaticum	200<MIC<400	1000<MIC	40<MIC<100	500<MIC<750	200<MIC<400	500<MIC<750
Origanum vulgare	40<MIC<100	250<MIC<500	40<MIC<100	125<MIC<250	100<MIC<200	250<MIC<500
Cymbopogon martinii	100<MIC<200	750<MIC<100	150<MIC<200	250<MIC<500	1000<MIC	1000<MIC
Cymbopogon citratus	100<MIC<200	250<MIC<500	150<MIC<200	500<MIC<750	1000<MIC	1000<MIC
Mentha spicata	1000<MIC	750<MIC<100	200<MIC<400	1000<MIC	1000<MIC	1000<MIC
Mentha piperita	800<MIC<1000	750<MIC<100	400<MIC<600	1000<MIC	1000<MIC	1000<MIC

F. solani was the least sensitive to the action of EOs by the method of exposure to volatiles.

2.2.3 | Comparison between both Methods

Overall, the EO of *O. vulgare* was the most efficient in inhibiting the growth of the studied tropical fungi (*L. theobromae, A. alternata*, and *F. solani*) by both direct contact and exposure to volatiles methods (Table 7). The strong antifungal activity of *O. vulgare* EO has been reported by other authors and seems to be mainly related to the presence of Carvacrol in its composition [7, 37]. These results support the hypothesis about the antifungal activity of Carvacrol, suggesting that it creates a stressful environment that can cause structural damage, damaging proteins, altering morphology and even leading to apoptosis [38].

The *S. aromaticum* EO has also shown great results, especially by the exposure to volatiles method, in which the MICs obtained were between 40 and 100 µL/L of air for *A. alternata* and between 200 and 400 µL/L of air for both *L. theobromae* and *F. solani*. The major compound in the EO of *S. aromaticum* is eugenol, which appears to inhibit the plasma membrane H+ ATPase, causing increased cell permeability. It also causes an increase in lipid peroxidation levels and reactive oxygen species (ROS) formation, leading to oxidative stress and cell death [39].

Studies involving *C. citratus* and its main components, such as citral, have demonstrated effectiveness in combating post-harvest spoilage microorganisms. The antimicrobial action is through the inhibition of the biosynthesis of ergosterol, a crucial component of the fungal cell membrane, thus affecting the cellular integrity of these microorganisms ([40]; OuYang et al. [41]).

The volatile exposure method showed better efficiency compared to the direct contact method. The method by exposure to volatiles may have been more effective than the direct contact method due to the volatiles dispersing and reaching a larger area and meeting a greater quantity of mycelia, in addition to persisting in the air inside the plate for a longer period. The direct contact method also has the disadvantage of needing to use solvents or emulsifiers for better availability and diffusion of the individual active compounds in the culture medium which can affect the inhibition results [42].

In other studies, such as that by Oliveira and collaborators (2019) [43], the direct contact method against *Colletotrichum acutatum* achieved better control. Therefore, it is important to highlight that the antimicrobial potential depends on the sensitivity of each species, the structural and morphological characteristics of each microorganism, and the composition and proportion of each individual compound in EOs [44].

2.2.4 | Scanning Electron Microscopy

The results obtained in the tests confirm the compounds present in EOs have great application potential. To better understand the inhibitory effects and their possible applications, it is necessary to investigate these compounds' mechanisms of action and the susceptibility of fungi to the possible mechanisms. The most widespread antimicrobial mechanism of EOs is the ability of compounds to penetrate the cell, causing damage to its wall and cytoplasmic membrane, oxidative damage, and ultimately cell lysis ([1]).

To observe differences in the effects of EOs on cellular structure, the fungus *L. theobromae* was tested with two EOs with different major compounds: *S. aromaticum* (eugenol—alcohol) and *C. citratus* (geranial—aldehyde) and visualized by scanning electron microscopy at MIC concentrations and in higher concentrations (Figures 2 and 3).

The two most effective EOs against *L. theobromae* were *O. vulgare* (alcohol) and *C. citratus* (aldehyde and ketone). However, *S. aromaticum* and *C. citratus* EOs were chosen for the images as *S. aromaticum* EO (alcohol) was the second one for fungi control and has been reported to be effective for inhibiting *L. theobromae* (Vilela et al. [21]; [5]). Additionally, clove EO has been pointed as an alternative sanitizer for fruit packing-house (Soraggi et al.2021 [45]). The images of EO action are at the MIC value to identify the effects of the lowest concentration of EO necessary to inhibit fungal growth and at concentrations above the MIC to intensify the visible effects and also observe whether there is a relationship between dose and susceptibility of the fungus or if the compounds are only effective in high concentrations (García-Salinas et al. [46]).

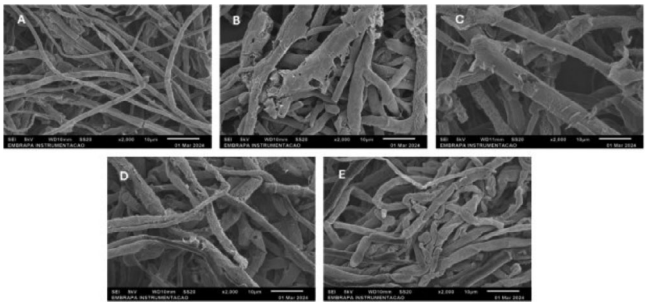

FIGURE 2 | Scanning electron microscopy (SEM) of *Lasiodiplodia theobromae* hyphae in contact with clove (*Syzygium aromaticum*) essential oil. (A) control (B) in direct contact with an oil concentration of 1250 μL/L (C) in direct contact with an oil concentration of 1750 μL/L (D) by volatiles in an oil concentration of 400 μL/L air (E) by volatiles at an oil concentration of 1000 μL/L air.

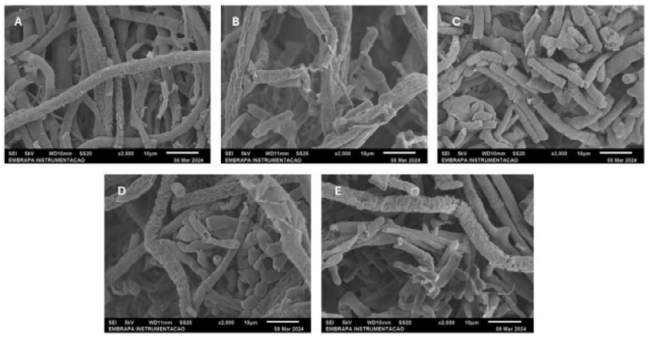

FIGURE 3 | Scanning electron microscopy (SEM) of *Lasiodiplodia theobromae* hyphae in contact with lemongrass (*Cymbopogon citratus*) essential oil. (A) control (B) in direct contact with an oil concentration of 400 μL/L (C) in direct contact with an oil concentration of 1000 μL/L (D) by volatiles in an oil concentration of 400 μL/L air (E) by volatiles at an oil concentration of 1000 μL/L air.

It is possible to observe damage caused to cell walls in all samples treated with *S. aromaticum* EO (Figure 2). The cell surfaces have a much more irregular texture and size (Figure 2B,D) and some signs of wall disruption (Figure 2C) when compared to the untreated control (Figure 2A). Some of the treated cells also have a completely shriveled appearance (Figure 2D), possibly due to cell lysis and extravasation of cytoplasmic material, confirming the previously mentioned mechanism. The growing pattern also seems to have been affected by the EOs: control cells have a more uniform pattern of growth, with elongated and intact hyphae, while treated cells have a more segmented appearance and agglomerated growth (Figure 2E) and with more unidentified material adhered to the cell surface (Figure 2B,D), similarly to results found by Stringaro et al. [38].

It is also possible to notice this when compared to the untreated control (Figure 3A). The cell surfaces have a modified texture with some debris (Figure 3B,D,E). The cells also appear shorter and with clustered growth (Figure 3C,E). Lemongrass EO damages the cell membranes of fungi, so the mycelia are fragmented into smaller parts due to damage to structural integrity ([47]; Mukarram et al. 2021 [48]). Figure 4 below shows a schematic of

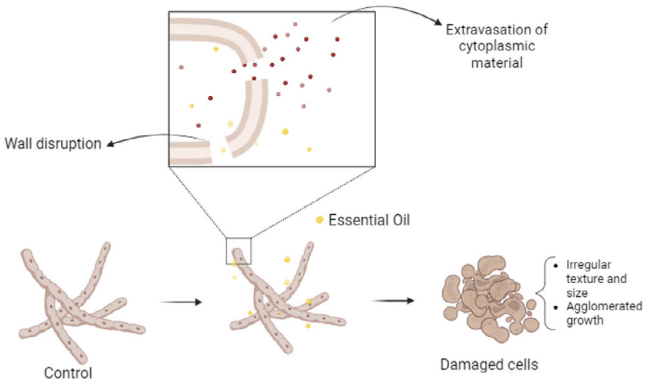

FIGURE 4 | Schematic of the action of essential oils on the fungi tested.

the effects observed on the treated cells compared to the untreated control.

All tested EOs showed potential application: the oregano (*O. vulgare*) EO proved to be the most efficient, being the only one that achieved 100% inhibition for all fungi tested, both by direct contact and exposure to volatiles methods. The *S. aromaticum* EO had the second-best activity, not being able to completely inhibit fungal growth only against *L. theobromae* by direct contact method while *A. alternata* and *F. solani* were both completely inhibited within the tested range by both methods. Despite being quite efficient, these oils have an intense aroma, especially *O. vulgare*, which can be unfavorable for application and must be taken into consideration as it may have an effect on the acceptance of the treated products by the final consumer [49].

The *C. citratus* EO presented excellent results against *L. theobromae* and *A. alternata* by both methods but did not reach complete inhibition against *F. solani*. Also, *C. martinii* had excellent results against *A. alternata* by both methods and against *L. theobromae* by the volatile exposure method. When it comes to sensorial perception, *C. citratus* and *C. martinii* EOs may be less disturbing than the *O. vulgare* EO, with softer aromas, bringing a certain freshness.

The *M. spicata* and *M. piperita* were the less effective ones, showing good activity against *A. alternata* by volatile exposure, but were not as efficient by direct contact method. For *L. theobromae*, the complete inhibition happened only at higher concentrations and *F. solani* was the least affected.

3 | Conclusions

The results of this study demonstrated that the EOs tested have strong antifungal potential against *L. theobromae*, *A. alternata*, and *F. solani*, with exposure to volatiles proving to be more effective than direct contact, requiring lower concentrations for total inhibition of the fungi. The EO of *O. vulgare* stood out as the most efficient, with MIC ranging from 40 to 200 μL/L in exposure to volatiles and between 125 and 500 μL/L in direct contact, while *S. aromaticum* also exhibited high activity, especially against *A. alternata* and *F. solani*. Electron microscopy analyses revealed severe structural changes in the treated fungal cells, suggesting

mechanisms of action associated with cell wall degradation and plasma membrane destabilization.

In addition to confirming the effectiveness of EOs, this study provides concrete information for the practical application of these substances in the post-harvest conservation of tropical fruits, contributing to the development of natural alternatives to the use of synthetic fungicides. In the industrial context, the use of controlled release systems in modified atmosphere packaging (MAP) may be a promising approach, allowing volatile compounds to be released gradually, ensuring prolonged protection against fungi and reducing sensory impacts on fruits. Another possibility is the incorporation of EOs into edible coatings applied directly to the surface of the fruits, creating a physical and chemical barrier that hinders fungal growth.

Future studies should investigate the stability of volatile compounds during storage, as well as the interaction of these treatments with the sensory characteristics of the fruits, ensuring the viability of industrial use. Thus, this work not only reinforces the potential of EOs in controlling post-harvest fungi, but also paves the way for their practical application, promoting more sustainable and safe solutions for the conservation of tropical fruits.

4 | Experimental

4.1 | Materials

Essential oil of *S. aromaticum* (clove), *O. vulgare L.* (oregano), *C. martinii* (palmarosa), *M. piperita* (peppermint) was obtained from Harmonie Aromaterapia (Florianopolis, SC, Brazil); *M. spicata* (green mint) EO was acquired from Terraflor Aromaterapia (Alto Paraiso de Goias, GO, Brazil); and *C. citratus* (lemongrass) EO was acquired from Mundo dos Óleos (Brasilia, DF, Brazil). The potato-dextrose agar (PDA) medium was obtained from KASVI (Madrid, Spain) and Tween 80 from Synth (Diadema, SP, Brazil). The fungal strains tested were *L. theobromae* CMF 0657, *A. alternata* DSMZ 12633 CCT 7152, *F. solani* IMI 314228 CCT 2876.

4.2 | EO Composition

The EOs of Clove (*S. aromaticum*), Oregano (*O. vulgare*), Palmarosa (*C. martini*), Lemongrass (*C. citratus*), Green mint (*M. spicata*) and peppermint (*M. piperita*) were initially diluted in dichloromethane (1:10) and stored in 1.5 mL vials at −28°C. For analysis, 1 µL of the diluted samples (10% v/v) was injected into a Shimadzu GC-MS model GCMS-QP2010 Plus, equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm), under the following chromatographic conditions: injector at 250°C operating in split mode 1:20 by 1.0 min; helium carrier gas at 1.0 mL min^{-1} oven temperature ramp: 60°C (1 min), increasing 3°C min^{-1} up to 240°C; interface temperature: 240°C, ionization source electrons +70 eV, scanning mode between 35 and 350 m/z. A solution of n-alkanes (C8-C20) was injected into the GC-MS under the same conditions as the sample to obtain the programmed temperature retention indices (LTPRI—linear temperature programmed retention index) of volatile compounds. The identification of the analytes was carried out by comparing the LTPRI and the mass obtained for the sample with mass spectra and LTPRI from the literature (NIST, 2011 [50]), with similarity of at least 85% for mass spectra, and maximum variation in LTPRI of ± 10.

4.3 | Antifungal Activity by the Direct Contact

PDA culture medium was prepared according to the manufacturer's instructions (KASVI, Madrid, Spain) at a concentration of 39 g/L of distilled water. For the direct contact method, the culture media is added with a Tween 80 emulsifier (0.05% v/v) for better incorporation of EOs into the culture medium. After sterilization and sufficient cooling, different volumes of EOs are added to obtain final concentrations equal to 0, 62.5, 125, 250, 500, 750, and 1000 µL/L of PDA, performed in triplicate. The whole manipulation of the plaques was done in a sterilized environment. The fungal inoculation was done by transferring 10 mm diameter plugs containing the fungus of interest from an actively growing colony to the center of each plate with 20 mL of the culture medium containing the EOs at the concentrations mentioned above. The incubation temperature was 28°C until the control plaques reached 100% of growth, monitored every 24 h.

The evaluation of the antifungal activity of EOs was carried out by measuring the inhibition of the growth of the fungi of interest in different concentrations of oil and comparing them to controls without oil, containing only Tween 80 (0.05% v/v). After the incubation, the diameter of the colonies was measured using a digital caliper in two perpendicular directions. The inhibition of fungal growth in different concentrations of EOs was calculated by Equation (1):

$$\mathrm{PI}\,(\%) = [(\mathrm{CONTROL}_{\mathrm{growth}} - \mathrm{TREATED}_{\mathrm{growth}})/\mathrm{CONTROL}_{\mathrm{growth}}] \times 100 \quad (1)$$

4.4 | Antifungal Activity by Exposure to Volatiles

The PDA culture medium was prepared at a concentration of 39 g/L of distilled water. This was then followed by sterilization using moist heat in an autoclave. After sufficient cooling, 20 mL of sterile PDA medium was transferred to each plate in a sterilized environment.

The fungal inoculation was done by transferring 10 mm diameter plugs containing the fungus of interest from an actively growing colony to the center of each plate with 20 mL of sterile culture medium and 50 mL of air. After inoculation, different volumes of EOs were applied to a sterile disc of filter paper (r = 10 mm) fixed in the center of the inner part of the Petri dish lid. The resulting concentrations tested were 0, 20, 40, 100, 200, 400, 600, 800, and 1000 µL/L of air. The plates were then sealed with parafilm and kept at 28°C until the Control colonies occupied the entire area of the plate (100% growth), monitored every 24 h. After the incubation, the average diameter of the colonies was calculated, using a digital caliper in two perpendicular directions. The inhibition of fungal growth in different concentrations of EOs was also calculated by Equation (1).

4.5 | Scanning Electron Microscope

The morphological changes of fungi structure caused by the antimicrobial activity of EOs and their volatiles were analyzed by scanning electron microscopy (SEM JEOL JSM-6701F; Tokyo, Japan) to visualize the caused damage at a microscopic level. For this process, the sample preparation was based on Yu et al. [51], with necessary modifications.

Petri plaques containing PDA medium were inoculated with 10 mm diameter PDA discs containing the fungus of interest cut from an active colony and then incubated at 28°C for 4 days. After this period, the colonies were exposed to equivalent concentrations to the previously achieved MIC for each fungus/oil combination for both methods. For the Direct Contact experiment, different volumes of an EO:DMSO (1:1) solution were added directly on top of the hyphae, for the Volatiles Exposure, the EOs were added to a filter paper disc on the plaque lid, as used in the previous assays. The protocol was run in triplicate and the control group consisted of samples without oil addition.

After more 24-h incubation, a PDA disc (10 mm diameter) containing the cells was cut and left in glutaraldehyde (3%, v/v) overnight, and then immersed in phosphate buffer (0.05 M, pH 6.8). The samples were then dehydrated in a graded series of acetone solution (30%, 50%, 70%, and 90%; v/v), and later dried in liquid carbon dioxide at the critical point. After that, the dried disks of samples were coated with gold.

Author Contributions

Ygor G. P. Osti: conceptualization, data curation, investigation, and writing – original draft. **Larissa G. R. Duarte**: conceptualization, methodology, supervision, visualization, and writing – review and editing. **Conny W. T. Fukuyama**: investigation and formal analysis. **Isadora C. Pedrino**: investigation and formal analysis. **Higor V. Santos**: investigation and formal analysis. **Josemar G. de Oliveira Filho**: conceptualization, methodology, supervision, visualization, and writing – review and editing. **Maria Eduarda de A. Astolfo**: investigation and formal analysis. **Maria Eduarda Martins**: formal analysis. **Stanislau Bogusz Junior**: methodology and supervision. **Marcos D. Ferreira**: conceptualization, funding acquisition, methodology, project administration, resources, supervision, and writing – review and editing.

Acknowledgments

This research was funded by FAPESP (# 2022/10686-6), CNPq (# 383138/2023-0, 138584/2023-0), CAPES (# 001), Empresa Brasileira de Pesquisa Agropecuária (# 20.19.03.0124.00.00)—Embrapa, Rede Agronano, CNPq/MCTI Sisnano (# 442575/2019-0), and M. D. Ferreira CNPq Research Productivity fellowship (# 307141/2022-5).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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