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Positive effect of *lippia sidoides* essential oil associated with carboxymethylcellulose in the control of anthracnose in avocado

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Abstract

The fungus *Colletotrichum gloeosporioides*, which is the causal agent of anthracnose disease in green-skinned avocados, is responsible for significant postharvest fruit losses. In this context, strategies should be considered to avoid this problem. The use of essential oil (EO) can represent an alternative to contribute to antifungal activity, avoiding the use of chemical products, as their indiscriminate use can have harmful effects on human health. It is known that essential oil (EO) may exhibit antifungal activity and can be used as an alternative to chemical products. Therefore, the potential of using *Lippia sidoides* EO to control this fungus was investigated through *in vitro* evaluation (MIC and EC_{50}) on *C. gloeosporioides* isolated from avocados. Furthermore, the potential of incorporating this oil with carboxymethylcellulose (CMC) for postharvest treatment in avocados was assessed *in vivo* to control anthracnose and maintain their physicochemical and sensory quality. The EO from *L. sidoides* demonstrated a MIC of 125 µl L^{-1} and an EC_{50} of 46.83 µl L^{-1} against this pathogen. The results indicated that the CMC edible coating associated with *L. sidoides* EO exhibited a positive effect on fruit quality during cold storage. In terms of sensory aspects, avocados treated with *L. sidoides* EO associated with CMC showed improved appearance compared to the control treatment. These findings suggest that *L. sidoides* EO has potential in the postharvest treatment of avocados. Additionally, these results are significant and unprecedented for this crop, as research on the postharvest effects of incorporating this EO with edible coatings in avocados is still limited.

Keywords *Persea americana*, *Colletotrichum gloeosporioides*, Postharvest, Edible coatings, Alternative control

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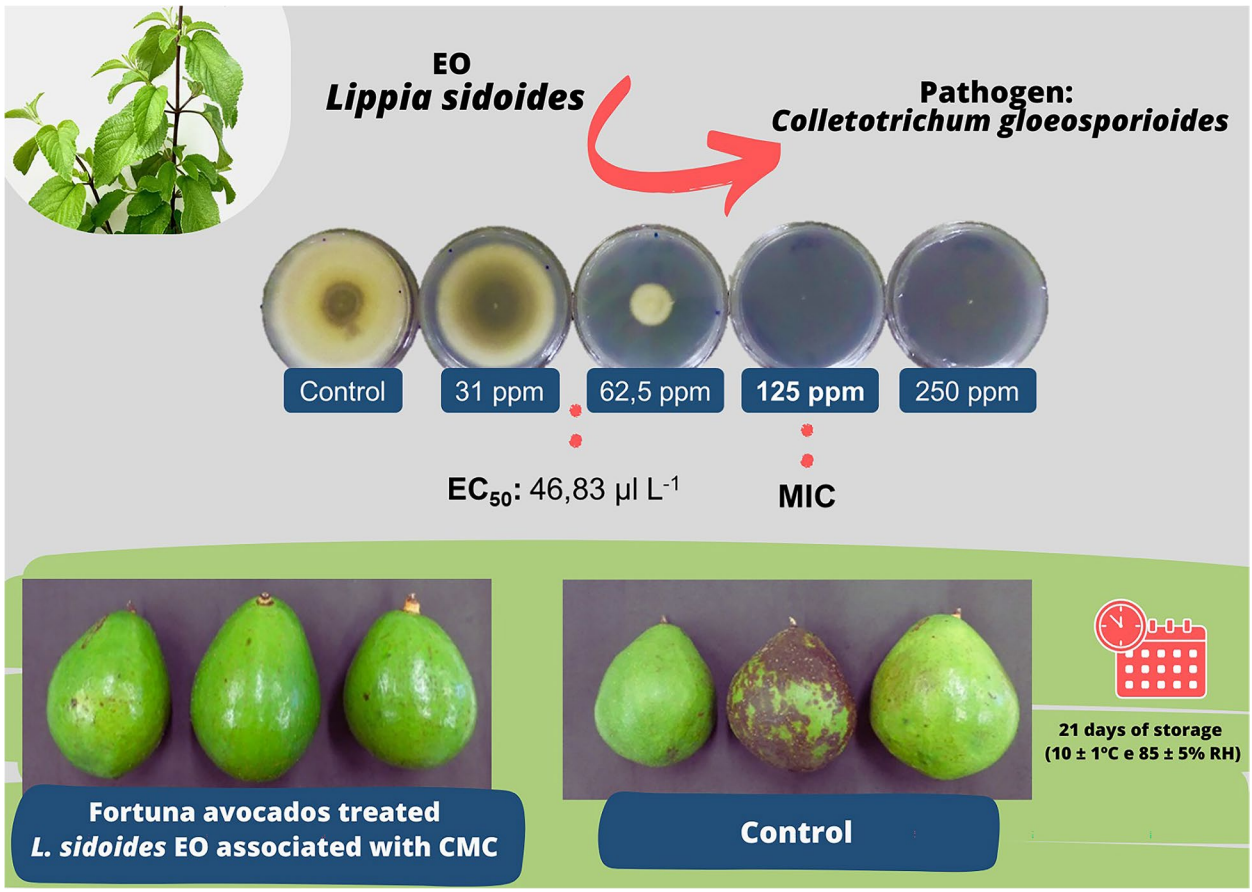
Graphical Abstract



Introduction

The demand for avocados (*Persea americana* Mill.) has intensified over the years due to their versatility in consumption and associated health benefits. The fruit is seen as an immune-boosting food, as it has a large amount of nutrients beneficial to human health, such as lipids, especially monounsaturated fatty acids, vitamin E, dietary fiber, minerals, and phytosterols (Dreher & Davenport 2013; Viera et al. 2023; Stephen et al. 2023).

Avocado production and consumption have grown significantly in the last decade, and according to the FAO (FAO 2023), it is estimated that avocados will become the most commercially traded tropical fruit in the world by 2030. In Brazil, the most commonly cultivated avocado varieties are the green-skinned types, such as Fortuna. This fruit has smooth, green skin and is a hybrid of West Indian and Guatemalan varieties (CEAGESP 2023).

Nevertheless, avocados have a reduced shelf life due to their high respiratory rate and elevated ethylene production after harvest. As a result, the fruit's firmness decreases, making them more susceptible to mechanical injuries and, consequently, invasion by deteriorating microorganisms. In the case of avocados, anthracnose is one of the major postharvest diseases, causing significant damage to production and supply chains (Kimaru et al. 2020).

The causal agents of anthracnose in avocados are fungi from the genus Colletotrichum, with *C. gloeosporioides* being one of the most prevalent. These fungi can infect the fruit directly, causing body rotting, or through harvest bruising, leading to stem rotting (Campos-Martínez et al. 2016; Fuentes-Aragón et al. 2020).

Usually, anthracnose is controlled using synthetic fungicides, with prochloraz, a fungicide from the imidazole family, being the most effective inhibitor of *C. gloeosporioides* mycelial growth postharvest (Bill et al. 2014). Also, many avocado exporting countries apply imidazole (i.e. prochloraz) as a commercial fungicide to control

anthracnose and stem rot during storage and transportation. However, the maximum residue level (MRL) allowed for avocados is becoming increasingly restricted. For example, some importing countries in Europe require fruits with an MRL below 2 mg/kg due to the negative impact of fungicide residues on human health. As a result, the limitations in the application of prochloraz have led to the search for alternatives, mainly natural treatments, that are environmentally friendly and safe, with the ability to replace the use of synthetic fungicides (Obianom et al. 2019).

The application of chemical fungicides poses risks to human health and the environment. It also creates phytosanitary barriers in countries with strict regulations on pesticide maximum residue limits in fruits. In Brazil, the use of such compounds has been prohibited by a resolution from the National Health Surveillance Agency (Brasil 2016).

Therefore, avocado producers have been searching for alternative methods to control anthracnose, with essential oils (EOs) emerging as a promising option. EOs, derived from plant materials, possess antimicrobial properties and offer the advantage of low risk in selecting resistant pathogens (Lin et al. 2022). Studies have already highlighted the relevance of using essential oils (EOs) as a source of natural bioactive substances for the control and prevention of food-borne diseases and post-harvest crop contamination (Pan et al. 2020). However, the use of EOs in fruits may be limited due to application costs and potential disadvantages, such as intense aroma and potential toxicity. Thus, incorporating these compounds into edible coatings represents a viable alternative to overcome these challenges.

Among the various EOs studied, *Lippia sidoides* EO has gained attention as a potential source of active biological compounds, including thymol (Saraiva et al. 2020) and carvacrol (de Morais et al. 2012). In some countries, such as Brazil, the species has been cultivated in medicinal plant gardens and is part of the list of plants selected by the Government of the State of Ceará and the Unified Health System as a phytotherapeutic (Batista et al. 2013). According to Hernandes et al. (2017) who evaluated the toxicity of essential oil from Lippia species, the compound has the potential to be a preservative, as it has been shown to be safe from a toxicological point of view, with no allergic or mutagenic potential. The results suggested that Lippia essential oil could safely replace currently marketed preservatives in the preservation of orange juice, syrup, and shampoo. Andrade et al. (2014) also evaluated the acute and chronic toxic effects of Lippia oil administered orally to rats and found that the compound has a wide margin of safety, with no detectable toxic effects in rats treated with doses up to 120 mg/kg. Thymol, the major component of *Lippia sidoides* essential oil, is approved by the US Food and Drug Administration (FDA) as "Generally Recognized as Safe" (GRAS) and can be used as a food additive (Marchese et al. 2016).

Several biomolecules have been used as matrices for edible coatings on fruits. Polysaccharides, such as carboxymethylcellulose (CMC), are often preferred due to their low cost, high production, biodegradability, and edibility (Nascimento et al. 2022). Studies have demonstrated the control of phytopathogenic fungi in postharvest fruits through the application of *L. sidoides* EO combined with edible coatings (Araújo et al. 2018; Oliveira et al. 2019a, b). However, there is a scarcity or absence of research on the antifungal activity of this EO combined with CMC, specifically regarding the postharvest quality of avocados. Furthermore, no studies have been conducted on West Indian-Guatemalan hybrid cultivars, such as Fortuna.

Therefore, the aim of this study was to investigate the *in vitro* antifungal activity of *Lippia sidoides* EO against the fungus *Colletotrichum gloeosporioides* isolated from avocados. Additionally, an *in vivo* protocol was used to assess the potential of incorporating this EO with CMC into an edible film for postharvest treatment of Fortuna avocado in relation to its physicochemical and sensory quality.

The novelty of this work is the revelation of the efficacy of the essential oil (EO) from *L. sidoides* as a potential natural antifungal agent for use on Antillean-Guatemalan hybrid avocado cultivars *in natura*. This EO is considered a low-cost and high-yield source of antifungal activity. Our findings provide benefits to producers and consumers, as they will have access to healthier and longer-lasting avocados that are protected by a natural antimicrobial coating made from biodegradable, abundant, and low-cost raw materials.

Material and methods

Extraction of Lippia sidoides EO and isolation of Colletotrichum gloeosporioides

The essential oil obtained from *L. sidoides* leaves through hydrodistillation was prepared and analyzed for its chemical composition using gas chromatography-mass spectrometry (Oliveira et al. 2019a, b). This EO exhibited a predominant composition of thymol (49%), followed by cimene and iso-caryophyllene. More information about the preparation and composition of the EO has already been presented in a previous study by Oliveira et al. (2019b).

The pathogen *C. gloeosporioides* was isolated directly from an avocado exhibiting typical anthracnose bruise and identified as AV03 through morphological and molecular characterization (Tozze Júnior et al. 2015). The fungus was cultivated on Potato Dextrose Agar (PDA)

and maintained for 15 days in a growth chamber at 25 °C with a 12-hour photoperiod.

In vitro assessment of antifungal efficacy of Lippia sidoides EO against Colletotrichum gloeosporioides

The antifungal activity of *L. sidoides* EO against *C. gloeosporioides* was assessed by determining its Minimum Inhibitory Concentration (MIC) using the agar dilution method (Plaza et al. 2004). For MIC determination, the direct contact method was employed, where the inoculum was exposed to Potato Dextrose Agar (PDA) containing EO at concentrations of 31, 62.5, 125, and 250 µl L^{-1}, selected based on preliminary experiments. To ensure proper homogenization of the EO with the PDA medium, soy lecithin was used as an emulsifier (0.2% w/v in ethanol). A control treatment consisting of an emulsifier and culture medium was also included. Each treatment (EO concentration) was evaluated with 5 replicates.

The pathogen was inoculated at the center of each Petri dish using a suspension containing 10^5 spores per milliliter. The dishes were then incubated in growth chambers at 25 °C with a 12-hour photoperiod. The experiment was concluded when the fungus in the control treatment reached its maximum growth within the experimental plot, corresponding to the total diameter of the dish.

Measurements of colony mycelial growth were taken every 2 days in two perpendicular directions (diameter in centimeters). The percentage of inhibition (PI) of mycelial growth for each concentration was calculated using the following equation (Plaza et al. 2004):

$$\text{PI (\%)} = \frac{\text{Growth of the control} - \text{Growth of the treatment}}{\text{Growth of the control}} \times 100$$

In vivo assessment of *Lippia sidoides* EO in association with CMC

Fortuna avocados were harvested from a conventional commercial plantation located in Timburi, São Paulo, Brazil (23°14′4.86″S; 49°34′42.71″W). The fruits were visually inspected for plant health and appearance, and then washed in running water. Sanitization was carried out by immersing the fruits in a sodium hypochlorite solution (200 µl L^{-1}) for 10 minutes, followed by washing and air-drying at room temperature.

Two treatments were employed: control fruits (C), immersed in sterilized distilled water, and fruits treated with an emulsion of CMC and *L. sidoides* EO (EO), with a concentration 10 times higher than the minimum inhibitory concentration (MIC) determined through *in vitro* testing (1250 µl L^{-1}).

The CMC used had a purity of 99.83%, moisture content of 7.5%, pH of 6.9, and viscosity of 310 cP, measured in a 1% solution at 25 °C. The degree of substitution was 0.71. The emulsion was prepared at a concentration of 1.5% (w/v) in distilled water, heated to 60 °C, and continuously stirred at 2000 rpm for 20 minutes using a 3-blade naval propeller stirrer (Fisatom – 713D). Subsequently, 0.5 mL of glycerol (50% w/w of the dry weight of CMC) was added as a plasticizer, followed by an additional 15 minutes of stirring. The EO was pre-emulsified with Tween-80 at a ratio of 2:1 v/v. The treatment was applied by immersing the avocados in the CMC suspension containing *L. sidoides* EO for 2 minutes. The fruits were then stored in a cold chamber at 10 ± 1 °C and 85 ± 5% Relative Humidity (RH).

Physicochemical analyses and disease incidence evaluations were conducted every 7 days during storage, starting from the first evaluation after fruit treatment and continuing up to 28 days. Each treatment consisted of four replicates, with each replicate comprising four avocado units (averaging 703.35 g per fruit). The experimental design followed a randomized 2 × 5 factorial scheme, with two treatments (C and EO) and five evaluation periods (0, 7, 14, 21, and 28 days).

Physicochemical and disease incidence analyses

The accumulated weight loss (WL %) was calculated as the difference between the initial mass and the mass at the end of the storage period (Zillo et al. 2018). Pericarp coloration was determined by measuring the parameters of luminosity (L*), hue angle (°Hue), and chromaticity (C*) using a Minolta Chroma Meter (CR-400) with illuminant "C" (Konica Minolta Sensing 2003). Four readings were taken on the fruit peel, 90° apart, in the equatorial region of each fruit.

Firmness (Fm, N) was determined using a penetrometer (Fruit Pressure Test - FDN1 0.1–1 N) with an 8 mm tip. Four readings were taken, 90° apart, in the middle third of each fruit. Soluble solids (°Brix) were measured using a refractometer (Krüss Optronic – DR 201–95), and titratable acidity (%) was determined by titration (AOAC Association of Official Agricultural Chemists 2015). These results were represented by the Palatability Index (Pi), calculated as the ratio of soluble solids to titratable acidity.

The disease index (DI) in the pericarp, indicating visible fungal growth on the fruit surface, was evaluated using a scale (Regnier et al. 2010) ranging from "Score 0" corresponding to healthy fruit (0% of symptoms) to "Score 5" (71 to 100% of the surface area with symptoms).

For the analysis of respiratory activity (RA, mL CO_2 $kg^{-1}h^{-1}$) and ethylene production (EP, µL of C_2H_4 $kg^{-1}h^{-1}$), avocados were stored individually in hermetically sealed glass vials and maintained at 10 °C for 1 hour. Subsequently, carbon dioxide (CO_2) and ethylene (C_2H_4) concentrations were quantified. A gas sample of 1 mL was

collected using a syringe through a septum in the vial lid. Gas chromatography was performed using a Thermo Finnigan Trace 2000 GC equipped with a flame ionization detector (FID), with nitrogen as the carrier gas at a flow rate of 33.3 mL min^{-1} and a column temperature of 200 °C.

The analyses were conducted on all fruits within each replicate, and the results are reported as the mean for each replicate (based on 4 units per replicate).

Sensory evaluation

The simple ranking test was used to compare sensory distinctions among treatments concerning the intensity of specific attributes (Meilgaard et al. 2006). However, to meet the minimum requirement of three samples for conducting the test, an additional treatment was included for the sensory analysis. This treatment involved immersing the fruits only in CMC. Consequently, the avocados subjected to sensory evaluation comprised three treatment groups: CMC-treated avocados, avocados treated with *L. sidoides* EO and CMC, and nontreated avocados (control). The sensory evaluation was performed after 24 hours of refrigerated storage.

The test was conducted in individual cabins under standardized white light. Each panelist received three samples randomly presented and uniquely coded with a three-digit number. A total of 33 nontrained panelists participated in the sensory panel. The panelists evaluated the samples based on attributes of appearance, specifically brightness and green color, as well as aroma. The samples were classified on a scale, with scores from 1 (the least intense attribute) to 3 (the most intense attribute). Each panelist assigned only one sample for each classification.

The present study was approved by the Committee on Ethics in Research of the "Luiz de Queiroz" College of Agriculture, University of São Paulo (Piracicaba, Brazil), under the protocol number CAAE: 55100316.8.0000.5395.

Statistical analyses

Statistical Analysis System (SAS) software (version 9.4) was used to perform the statistical analyses. The results of the percentage of inhibition (PI %) in the *in vitro* evaluation of antifungal capacity were subjected to analysis of variance (ANOVA) using the F test, and the statistical significance of the mean differences was determined by Tukey's test. For the *in vivo* experiment, the PI % values were used as a dependent variable to calculate the Median Effective Concentration (EC_{50}) and its confidence limits using the probit analysis method. The physicochemical and disease incidence data were analyzed using Principal Components Analysis (PCA). The sensory analysis data were interpreted by calculating the sum of classifications and using the Friedman test.

Results

Extraction of Lippia sidoides EO and isolation of Colletotrichum gloeosporioides

L. sidoides EO exhibited dose-dependent antifungal activity and effectively inhibited the growth of the fungus *C. gloeosporioides* starting from a concentration of 62.5 µl L^{-1} (Table 1). There were no significant differences ($P < 0.05$) among the concentrations of 62.5, 125, and 250 µl L^{-1} of EO in terms of their action on the fungus. Therefore, these EO concentrations demonstrated comparable effectiveness based on the statistical analysis. However, the MIC of the EO was determined to be 125 µl L^{-1}, as it completely inhibited mycelial growth (100%) without any visible growth (Fig. 1). The EC_{50} of *L. sidoides* EO on *C. gloeosporioides*, along with its confidence limits at 95%, was calculated to be 46.83 µl L^{-1} (45.29–48.38 µl L^{-1}).

In vivo assessment of *Lippia sidoides* EO in association with CMC

Principal Components Analysis (PCA) was conducted on the total dataset (Table 2), resulting in the extraction of two main components, which accounted for 81.16% of the total variance. The first principal component (PC1) explained 60.44% of the statistical variance and exhibited positive correlations with weight loss (WL), luminosity (L*), and disease index (DI), while displaying negative correlations with hue angle (°Hue) and firmness (Fm). The second principal component (PC2) explained 20.72% of the statistical variance and showed positive correlations with respiratory activity (RA) and chromaticity (C*). Ethylene production and palatability index were not correlated with the principal components due to their limited relevance in the scientific interpretation of the results (Fig. 2).

Table 1 Inhibition parameters of *Colletotrichum gloeosporioides* using *Lippia sidoides* essential oil

	Concentration (µl L^{-1})				
MG% †	**0**	**31**	**62.5**	**125**	**250**
	0.0 ± 0.0^{c}	8.21 ± 6.03^{b}	83.97 ± 9.71^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
MIC	125 µl L^{-1}				
EC_{50} ‡	46.83 µl L^{-1} (45.29–48.38 µl L^{-1})				

† Results are mean ± SD, $n = 5$; values followed by different letters in the row indicate significant difference ($P < 0.05$). The original results were transformed by the equation indicated by the Shapiro-Wilk test ($\hat{y} = \log 10\ y$). ‡ EC_{50} respective 95% confidence limits. Abbreviations: MG%, mycelial growth inhibition; MIC, minimum inhibitory concentration; EC_{50}, median effective concentration

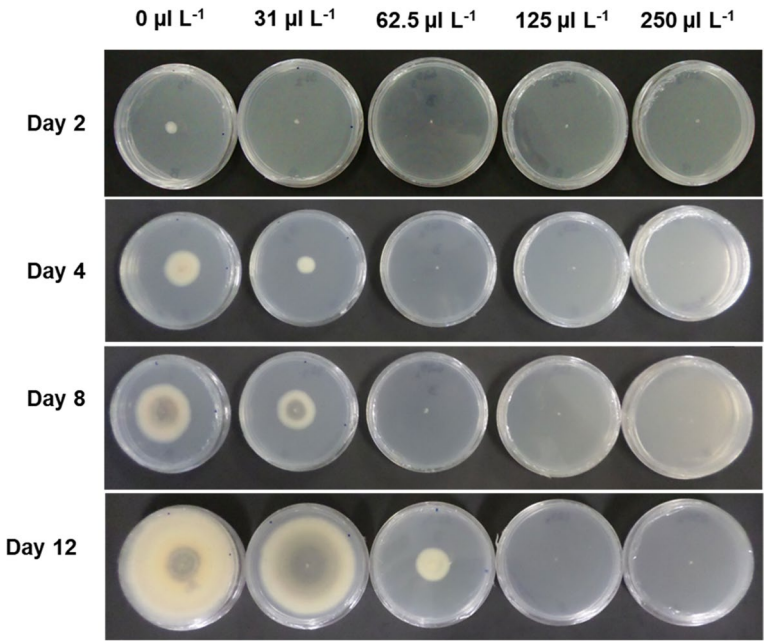


Fig. 1 Mycelial growth progression of *Colletotrichum gloeosporioides* isolated from avocados with the addition of different concentrations of the essential oil from *Lippia sidoides*, stored at 25 °C and under a photoperiod of 12 hours for 12 days

Among the variables associated with avocado pericarp color, °Hue and L* were represented in PC1, while C* was represented in PC2 (Fig. 2). In general, it was observed that the initial color of avocados was characterized by a dark yellowish-green hue (120°) with lower saturation. As the refrigerated storage progressed, the color tone transitioned toward a greenish-yellow shade (106–115°) with increased saturation and luminosity. Avocados treated with a CMC coating and *L. sidoides* EO demonstrated better color retention compared to the control. From day 14 of storage, both coated and noncoated avocados exhibited similar coloration. However, the control fruits displayed a more pronounced color change until the 28th day of storage.

The disease index (DI) analysis revealed the presence of fungal mycelial growth in the pericarp of avocados during storage, with the highest DI values observed at 28 days in the control fruit. Throughout storage, it was observed that the avocados treated with CMC and *L. sidoides* EO exhibited a maximum of 41.3% pericarp affected by diseases on the final analysis, while the untreated fruit showed 73.8% pericarp affected. The changes in color and fungal growth observed in the pericarp (Fig. 3) clearly demonstrated the preservation of the visual aspect of the avocados with CMC and *L. sidoides* EO until the 21st day of storage.

The avocados exhibited similar mass loss, regardless of whether they were treated with EO or not, with an increase observed from the 7th day of storage. The mass loss was approximately 1% initially and reached approximately 4% by the end of storage.

The firmness of the avocado pulps, whether coated or not, decreased throughout storage. The control fruit consistently displayed lower firmness compared to the avocados coated with CMC in association with *L. sidoides* EO, starting from the 7th day. The most significant decline in firmness occurred between storage days 7 and 14, with the firmness values of the control decreasing approximately 30 times, while the values of the treated fruit decreased approximately 15 times. From the 21st day of storage, the firmness of the avocados became similar between the control and treated fruit. The coating demonstrated a notable positive effect on maintaining avocado quality for at least 14 days of cold storage.

The nontreated avocados (control) exhibited higher respiratory activity (RA) compared to the coated ones from 7 to 21 days of storage. However, avocados treated with CMC and *L. sidoides* EO displayed higher RA on the first day of analysis, which can be attributed to the increased handling of these fruits during the application and drying of the treatment.

The PCA (Fig. 2) also demonstrated correlations between variables. Variables positioned at an angle close to 0° (PI and DI, C* and L*, EP and C*) indicated a high positive correlation. Conversely, variables positioned at an angle close to 180° (°Hue and WL, Fm and L*) showed a high negative correlation, indicating that when a sample had a high value for one parameter, the value would be low for the other.

Table 2 Physicochemical and physiological parameters of avocados treated or not with *L. sidoides* essential oil

Samples	Storage (days)				
	0	7	14	21	28
Chromaticity					
C	30.89 ± 3.19	30.81 ± 2.45	37.74 ± 3.18	41.40 ± 3.92	36.01 ± 6.66
EO	29.40 ± 3.37	28.33 ± 2.20	29.99 ± 4.13	36.99 ± 3.55	35.29 ± 4.45
Luminosity					
C	42.39 ± 2.62	41.27 ± 1.92	46.24 ± 2.78	49.84 ± 3.53	47.12 ± 6.74
EO	40.22 ± 1.92	40.74 ± 1.14	42.06 ± 2.49	46.99 ± 2.38	46.34 ± 2.94
Hue angle (°)					
C	120.96 ± 2.00	120.76 ± 1.34	117.89 ± 1.56	114.89 ± 1.87	106.73 ± 3.65
EO	121.95 ± 1.49	122.00 ± 0.90	121.38 ± 1.80	118.14 ± 1.71	114.35 ± 2.74
Weight loss (%)					
C	0.0 ± 0.00	0.98 ± 0.10	1.99 ± 0.21	2.94 ± 0.32	3.94 ± 0.61
EO	0.0 ± 0.00	1.12 ± 0.17	2.13 ± 0.35	3.16 ± 0.50	4.15 ± 0.55
Disease index					
C	0.0 ± 0.00	0.0 ± 0.00	1.25 ± 2.50	18.75 ± 15.48	73.75 ± 22.50
EO	0.0 ± 0.00	0.0 ± 0.00	1.25 ± 2.50	11.25 ± 13.15	41.25 ± 21.75
Firmness (N)					
C	134.75 ± 7.25	87.59 ± 29.50	2.84 ± 1.44	2.00 ± 0.44	1.47 ± 0.39
EO	133.64 ± 3.69	105.13 ± 32.69	6.48 ± 5.74	3.40 ± 1.00	2.66 ± 0.88
Palatability index					
C	50.31 ± 14.87	30.58 ± 6.85	42.76 ± 7.33	63.25 ± 6.77	117.98 ± 19.26
EO	66.16 ± 11.50	40.54 ± 7.39	86.69 ± 15.64	102.09 ± 16.71	111.86 ± 10.16
Respiratory activity (mL CO_2 kg^{-1} h^{-1})					
C	13.92 ± 0.18	14.16 ± 2.11	17.61 ± 1.74	18.22 ± 2.37	8.39 ± 1.93
EO	18.50 ± 4.63	12.94 ± 0.86	14.01 ± 2.11	16.78 ± 1.49	14.32 ± 2.39
Ethylene production (µL C_2H_4 kg^{-1} h^{-1})					
C	0.61 ± 1.00	114.54 ± 60.29	59.11 ± 27.58	114.01 ± 36.79	69.04 ± 42.24
EO	21.09 ± 42.08	13.11 ± 6.21	39.76 ± 14.78	27.75 ± 5.02	53.48 ± 21.59

Results are mean ± SD, $n = 16$. *C* control treatment, *EO* fruit treated with *L. sidoides* essential oil (1250 µl L^{-1}) associated with carboxymethylcellulose

The sensory evaluation (Table 3) revealed the beneficial effect of applying the CMC edible coating in association with *L. sidoides* EO on the sensory quality of the avocados, particularly in terms of appearance.

For the attribute of brightness, the sums assigned to the coated fruit, with or without *L. sidoides* EO, were statistically similar ($P < 0.05$) and higher than those of the control, which exhibited a statistically significant difference. The statistical analysis of the green color attribute showed a similar pattern to brightness; however, the fruit treated with CMC and EO displayed the most pronounced green color among the samples. Regarding the aroma, the sums of the samples with CMC in association with EO and the control did not differ statistically. However, the panelists indicated that the avocados treated with CMC and *L. sidoides* EO exhibited a mild aroma reminiscent of cleaning products, eucalyptus, citrus, and fruity sweetness. On the other hand, the control-treated avocados had an aroma resembling green peel, wood, and leaves.

Discussion

Extraction of Lippia sidoides EO and isolation of Colletotrichum gloeosporioides

The tests *in vitro* evidenced the dose-dependent antifungal action of *L. sidoides* EO on *C. gloeosporioides*, with total inhibition of fungal development at 125 µl L^{-1}. This value is above that found by Zillo et al. (2018), who observed a MIC of 75.3 µl L^{-1} for *L. sidoides* against *C. gloeosporioides* isolated from papaya. In contrast, Oliveira et al. (2019a, b) observed a MIC similar to this study, whose values were between 62.5 and 125.0 µl L^{-1} for *L. sidoides* against *Rhizopus stolonifer* from strawberry.

The antifungal action of *L. sidoides* EO derives from its chemical composition, as presented in Oliveira et al. (2019a, b), whose major compounds were thymol (49.46%) and cymene (11.40%). Likewise, Saraiva et al. (2020) analyzed the composition of *L. sidoides* EO extracted from leaves and identified thymol as its main constituent. A similar result was obtained for thyme EO in the control of

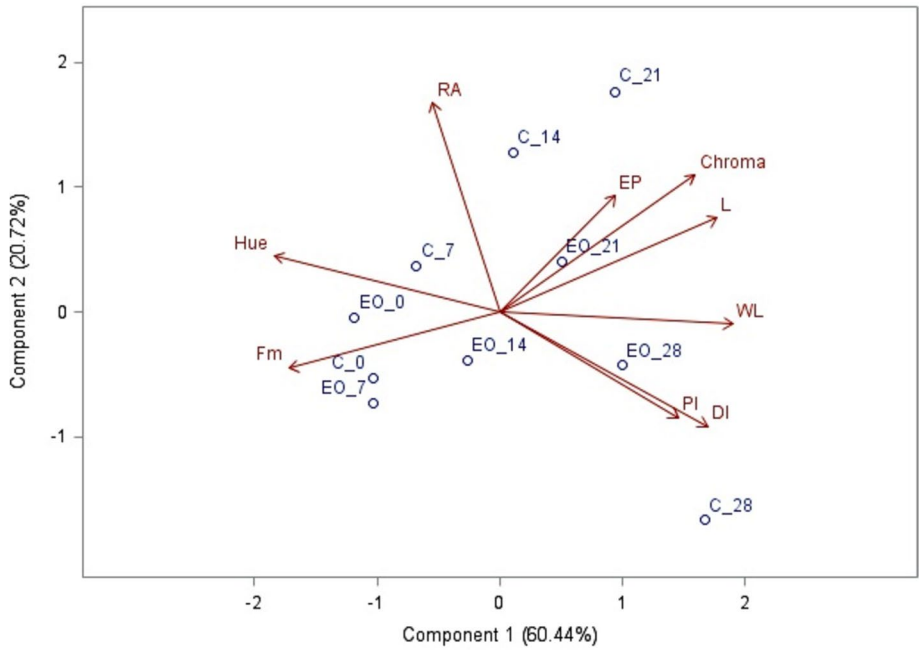


Fig. 2 Distribution of the variables and observations obtained in the PCA of avocado postharvest parameters. *Notes:* C_0; C_7; C_14; C_21 and C_28: control treatment analyzed on the 0, 7, 14, 21 and 28 days after treatment application. EO_0; EO_7; EO_14; EO_21 and EO_28: fruit treated with *L. sidoides* EO (1250 µl L^{-1}) associated with CMC analyzed on the 0, 7, 14, 21 and 28 days after treatment application. Abbreviations: PCA: Principal Component Analysis. Component 1, principal component 1; Component 2, principal component 2; C*, chromaticity; L*, luminosity; °Hue, hue angle; WL, weight loss; DI, disease index; Fm, firmness; PI, palatability index; RA, respiratory activity; EP, ethylene production

anthracnose in avocados, which is also composed mainly of thymol (Sarkhosh et al. 2018).

Thymol and cymene are compounds found in several EOs and belong to the class of monoterpenes, which are biochemically modified terpenes to which enzymes can add oxygen molecules and remove or move a methyl group (Eslahi et al. 2017). Usually, monoterpenes, which are lipophilic molecules, induce the biological activity of the EO and are responsible for its characteristic aroma (Brusotti et al. 2014). In this sense, the main targets of thymol are the fungal cell wall and membrane, altering the permeability of microbial cells with consequent loss of macromolecules (Bennis et al. 2004; López-Malo et al. 2005), which results in a decrease in the effectiveness of infection establishment and, consequently, in the progression of the disease caused by the pathogen.

On the other hand, cymene (or *p*-cymene), the second major compound of *L. sidoides* EO, displays a similar structure to thymol, but the hydroxyl group is absent in the aromatic ring (Games et al. 2016). *P*-cymene has inhibitory action on the enzymes pectin methyl esterase and cellulase of filamentous fungi. These enzymes are the main target of conventional fungicides, which could be substituted by EO, with the advantage of the compound *p*-cymene being "Generally Recognized As Safe" (GRAS) by the U.S. Food and Drug Administration (Marei et al. 2018).

The antifungal potential of *L. sidoides* was demonstrated in several studies. da Silva et al. (2009) and Aquino et al. (2012) observed that the mycelial growth and conidia germination in C. *gloeosporioides* isolated from passion fruit were affected by the same EO. The inhibition of other filamentous fungi, such as *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp. and *Fusarium oxysporum*, was also proven with the use of *L. sidoides* EO (de Oliveira et al. 2008).

This study was the first to investigate the EC_{50} (46.83 µl L^{-1}) of *L. sidoides* EO on *C. gloeosporioides* extracted from green-skinned avocados. This result is relevant because, with a low dose, which corresponds to 37.5% of the MIC, 50% of the growth of the fungal population was inhibited. The value of the EC_{50} found in this work is lower than those found in studies performed with several EOs against fungi from the genus *Colletotrichum*, demonstrating once more the antifungal potential of *L. sidoides* EO.

Sarkhosh et al. (2017) found a EC_{50} value of 858.4 µl L^{-1} for savory EO against *C. gloeosporioides*. Against the fungus *C. acutatum*, Prieto et al. (2011) found EC_{50} value of 153.9 µl L^{-1} for *Zanthoxylum fagara* EO. Nonetheless, Hoseini et al. (2019), studying several EOs against *Colletotrichum nymphaeae,* found EC_{50} values close to ours for *Lavandula angustifolia* EO (lavender; 49.6 µl L^{-1}), as well as values lower than ours for *Cymbopogon citratus*

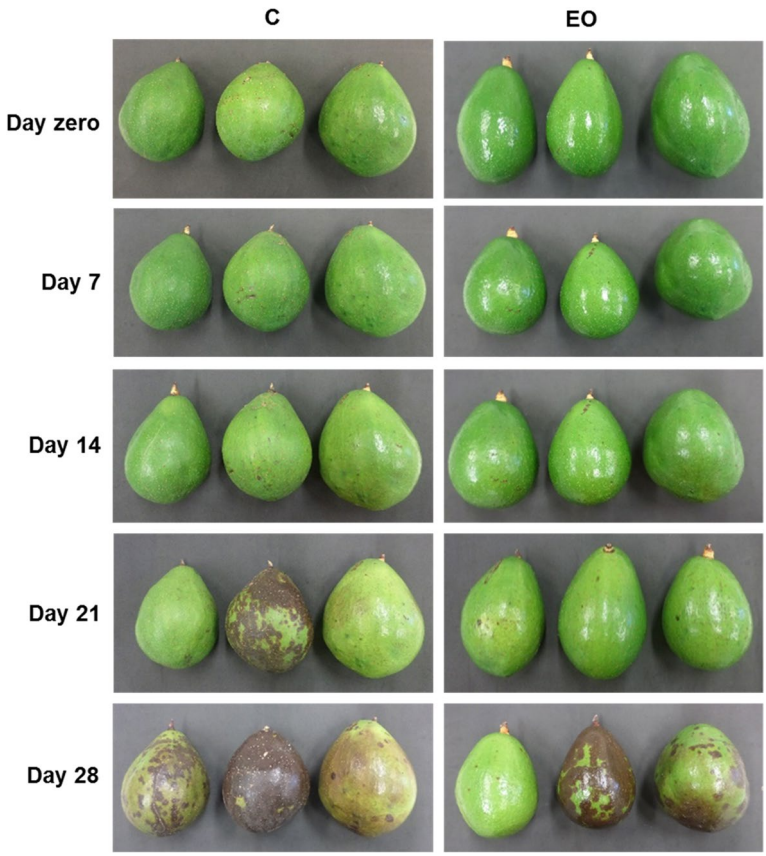


Fig. 3 Appearance of avocados stored for up to 28 days (10 ± 1 °C and 85 ± 5% RH) treated or not with *L. sidoides* essential oil associated with carboxymethylcellulose. Abbreviations: C, control treatment; EO, fruit treated with *L. sidoides* essential oil (1250 µl L^{-1}) associated with carboxymethylcellulose

Table 3 Simple ranking test related to the sensory attributes of the different avocado's treatments

Treatments	Attributes		
	Appearance: brightness[2]	Appearance: green color[2]	Aroma[2]
C	44 [b]	47 [b]	64 [a]
EO	75 [a]	85 [a]	73 [a]
CMC	79 [a]	66 [ab]	43 [b]

Values followed by different letters in the same column indicate significant difference ($P < 0.05$).

C treatment control, *EO* avocados treated with *L. sidoides* essential oil (1250 ppm) associated with carboxymethylcellulose, *CMC* avocados treated only with carboxymethylcellulose

EO (lemon grass; 12.79 µl L^{-1}). The variations observed among the EC_{50} values are mainly due to EO type and the fungal species studied, in addition to the analytical method used.

Therefore, these results suggest the effectiveness of *L. sidoides* EO in the prevention of anthracnose in avocados. Additionally, this discussion is relevant from a scientific perspective since the *in vitro* action of *L. sidoides* EO on the fungus isolated from green-skinned avocados has not yet been studied.

In vivo assessment of *Lippia sidoides* EO in association with CMC

The results obtained in the postharvest evaluation indicated that the application of CMC edible coating in association with *L. sidoides* EO (1250 µl L^{-1}) had a positive effect on the postharvest quality maintenance of the avocados during refrigerated storage, resulting in an extended shelf life of 7 days compared to the control. This finding is significant and unprecedented for avocados, as research on the postharvest effects of incorporating this EO into edible coatings for fruits is limited. In this study, the association of *L. sidoides* EO with CMC was reported for the first time by Zillo et al. (2018), who demonstrated the effectiveness of *L. sidoides* EO in controlling fungal rot and maintaining postharvest quality in papayas, which supports the findings of this study in avocados.

Among the postharvest quality parameters analyzed in avocados, the occurrence index of pericarp diseases

demonstrated the antifungal capability of *L. sidoides* EO when applied in association with CMC, confirming the results obtained in *in vitro* assays. The incorporation of EO in polysaccharide coatings can enhance the shelf life of fresh produce by facilitating the release and surface concentration of antimicrobial compounds from the EO, thereby increasing the antimicrobial activity of the coatings (Sivakumar & Bautista-Baños 2014). This may be attributed to the composition of *L. sidoides* EO, which contains 1.8-cineole, thymol, or carvacrol as the most abundant compounds known for their antifungal, antibacterial, and insecticidal activities (de Morais et al. 2012; Guimarães et al. 2015).

Corroborating the present study, other research has demonstrated the efficacy of applying EOs in combination with edible coatings during the postharvest period of avocados. Bill et al. (2014) recommended the combined treatment of chitosan and thyme EO for controlling anthracnose in 'Hass' avocados during storage. Correa-Pacheco et al. (2017) found that edible coatings based on chitosan-thyme EO nanoparticles (55%) reduced the incidence of *C. gloeosporioides* in 'Hass' avocados by up to 60% after 8 days at 27 °C. Kubheka et al. (2020) concluded that the combination of edible gum arabic (15%) and CMC-containing moringa extract (1%) suppressed *C. gloeosporioides* mycelial growth in 'Maluma' avocados during storage at 5.5 °C for 21 days, followed by 7 additional days at 21 °C.

The application of the edible coating formed by the association of CMC and *L. sidoides* EO had minimal influence on the water vapor loss of avocados. This was due to the high solubility of CMC in water, which mitigated the expected effects of the lipophilic EO compound. As a result, the coating acted as a semipermeable barrier against fruit moisture loss (Sivakumar & Bautista-Baños 2014). Similar observations were made for pulp firmness, accumulated mass loss, and respiratory activity.

Firmness and mass loss are important parameters for consumers because they are associated with fruit shelf life. The decrease in firmness observed during storage, indicative of tissue softening, is a natural consequence of fruit ripening and senescence. However, the nontreated avocados exhibited lower firmness, indicating greater softening due to cell wall weakening, loss of membrane integrity, hydrolysis of cellulose and hemicellulose, and depolymerization of pectin and starch (Defilippi et al. 2018).

Avocados stored under environmental conditions without any treatment are considered climacteric fruits, exhibiting rapid increases in respiratory rate and ethylene production. This results in a rapid climacteric peak, changes in lipid content, altered texture, degradation of mesocarp cells, reduction in starch content, and increased levels of glucose and fructose (Payasi & Sanwal 2010). In this study, the average respiratory rate of the control avocados (16.6 mL CO_2 $kg^{-1} h^{-1}$) was higher than that of the fruit treated with CMC and *L. sidoides* EO (14.57 mL CO2 $kg^{-1} h^{-1}$). This difference may be attributed to the application of EO, which has a hydrophobic nature that affects O_2 diffusion through the formed coating, thereby influencing fruit respiration. Similar results were observed by Junior et al. (2018), where 'Hass' avocados coated with a combination of chitosan-propolis exhibited reduced respiration and ethylene production due to modifications in the atmosphere surrounding the fruit epidermis. Tesfay et al. (2017) also demonstrated that avocados coated with CMC and moringa extract showed lower respiration rates compared to the control.

The peel color of nontreated avocados exhibited a more rapid lightening of the green color and an approximation to yellow tones, indicating accelerated ripening. The reduction in green coloration, or yellowing, of the avocado peel occurred due to chlorophyll degradation reactions, resulting in the formation of pheophorbide, the immediate precursor of colorless decomposition products (Queiroz Zepka et al. 2019). Therefore, it can be inferred that the application of *L. sidoides* EO in association with CMC delayed the pigment transformation in the avocado peel, which is indicative of fruit ripening. Similar observations have been made in studies (Bill et al. 2014; de Cenobio-Galindo et al. 2019; Fischer et al. 2018), where avocados coated with edible coatings in association with EOs retained green coloration of the peel, while nontreated avocados exhibited a color closer to yellow.

From a sensory perspective, avocados treated with *L. sidoides* EO in association with CMC exhibited improved appearance (brightness and green color) compared to the control fruit. In this sense, one of the limitations of CMC use is the potential for a sticky texture (Akbarian et al. 2015). However, in the present study, panelists did not report this perception. Although the aroma of the treated fruit was similar to the control, panelists associated it with aromas of cleaning products and others. This observation may be directly related to the composition of *L. sidoides* EO, which is derived from an aromatic plant with a spicy flavor that gives it the common name of "alecrim-pimenta" ("pepper-rosemary") (Guimarães et al. 2015).

Similarly, other studies have demonstrated that edible coatings associated with EOs or leaf extracts can be used to extend the shelf life and improve the quality of avocados, with satisfactory sensory acceptance (flavor, texture, aroma, or overall) (Kubheka et al. 2020; Mpho et al. 2013; Sellamuthu et al. 2013).

Conclusions

The study concluded that *L. sidoides* essential oil (EO) exhibited *in vitro* antifungal activity against *C. gloeosporioides* at a concentration of 62.5 µl L^{-1}. Besides that, avocados treated with *L. sidoides* EO and carboxymethyl cellulose (CMC) showed enhanced postharvest quality in comparison to the control treatment. This formulation extended the fruit's shelf life, particularly by reducing disease incidence.

In terms of sensory attributes, the use of EO associated with CMC contributed to improvements in certain aspects, mainly related to the fruit's appearance. However, some limitations, such as an undesirable aroma, still require further investigation and improvement.

This study not only demonstrates the efficacy of *L. sidoides* essential oil as a natural antifungal agent for Antillean-Guatemalan hybrid avocados, but also offers a cost-effective, high-yield solution that benefits both the economic and environmental sectors, ensuring healthier, longer-lasting produce for producers and society. Therefore, encouraging studies that explore the use of different coating matrices in conjunction with essential oils is relevant.

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Authors' contributions

BD, J, PPM, AM: conceptualization, methodology, investigation and writing of the manuscript.
NMV: writing, review and editing.
EM, MHF: supervision and study conceptualization.
All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were per the institutional and national research committee's ethical standards. The present study was approved by the Committee on Ethics in Research of the "Luiz de Queiroz" College of Agriculture, University of São Paulo (Piracicaba, Brazil), under the protocol number CAAE: 55100316.8.0000.5395. Written informed consent was obtained to participate in the study.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interest.

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