



Effective control of anthracnose (*Colletotrichum gloeosporioides*) in postharvest tomato under different storage temperatures using essential oils from eucalyptus (*Eucalyptus globulus*) and lemongrass (*Cymbopogon citratus*)

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ABSTRACT

Anthracnose is a postharvest disease of tomato fruit caused by the pathogenic fungus *Colletotrichum gloeosporioides*, which causes significant losses during storage. Nowadays, it is necessary to search for new environmentally and health friendly alternatives to prevent the disease, being essential oils (EOs) a tool with great antifungal potential. In this work, EOs were obtained from eucalyptus (*Eucalyptus globulus*) and lemongrass (*Cymbopogon citratus*) leaves by hydrodistillation, which were characterized physicochemically, presenting mainly the compounds eucalyptol (1,8-cineole) and carveol, respectively. The *in vitro* antifungal activity of these EOs was analyzed by inhibition of micellar growth and inhibition of conidial germination in *C. gloeosporioides*. Both EOs inhibited mycelial growth and conidial germination of the pathogen, but the one from lemongrass showed a higher antifungal capacity. Lemongrass EOs were also applied to tomato fruits stored at room temperature (25 °C) and cold (8 °C), infected with *C. gloeosporioides*, reporting a significant reduction in the incidence (until 63 %) and severity (until 9 %) of anthracnose developed at room temperature (in 7 days), and an absolute elimination of the disease (0 % incidence and severity) in cold (in 40 days), due to the antimicrobial action of the EOs. Therefore, EOs obtained from lemongrass leaves by hydrodistillation are an effective tool in the control of anthracnose in tomato fruits, with absolute effectiveness in cold storage.

1. Introduction

Global food security is one of the major scientific-technical and social concerns, due to the exponential increase of the world population, climate change and the increase in food demand (Mogale et al., 2020). Precisely, food insecurity, malnutrition and the increase in world hunger are the major challenges of the zero hunger sustainable development goal by 2030 (Arora & Mishra, 2022). In addition, annual food losses (1.3 billion tons; 1/3 of total production) increase the pressure on global food security (Mogale et al., 2020). In this regard, postharvest diseases of fresh produce cause significant reductions in the amount of available marketable fruit and vegetables. The percentage of losses is very different depending on the vegetable product and the use (or not) of cold storage systems (Li et al., 2024). In particular, the main pathogens

involved in postharvest plant losses belong to the fungal genera *Penicillium*, *Botrytis*, *Rhizopus*, *Monilinia*, *Mucor*, *Alternaria*, *Colletotrichum* and *Geotrichum* (Díaz-Urbano et al., 2023).

Tomato (*Lycopersicon esculentum*, Miller) is the third most important industrially and economically important vegetable crop in the world, behind potato and sweet potato (Kumar et al., 2020). In 2022, world tomato production exceeded 186 million tons, produced on an area of almost 5 million hectares; with China being the world's leading producer (~68 million tons), followed by India (~21 million tons) (FAOSTAT, 2024). Most of the tomato consumed worldwide (80 %) is in processed foods, such as sauce, juice or soup. Both fresh and processed, tomatoes represent an important nutritional contribution to the human diet, providing potassium, iron, folate, lycopene, vitamin C, β -carotene and different phenolic compounds (Collins et al., 2022). Cultivated

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tomatoes have a low genetic diversity, as a consequence of the breeding and selection processes developed since its domestication. For this reason, tomato, both in cultivation and postharvest, can be affected by more than 200 different pathogens (Panno et al., 2021). Specifically in postharvest, the main fungi causing rot losses belong to the *Alternaria*, *Rhizopus* and *Colletotrichum* genera (Yadav et al., 2022).

Anthraco-nose is a tomato fruit disease caused by fungi of the genus *Colletotrichum*, mainly *C. gloeosporioides*, causing losses of up to 30 % in postharvest storage (Peralta-Ruiz et al., 2023). The symptomatology of anthracnose in tomato is characterized by the appearance of black lesions, which grow until the mycelium colonizes and rots the fruit completely (Peralta-Ruiz et al., 2023). Traditionally, physical treatments (steam heat, forced-air dry heat or hot water dips of) and synthetic fungicides have been used to prevent disease occurrence in stored tomatoes. However, the need for new, safer alternatives has led to the development of effective biological strategies in recent years. For example treatment with essential oils (EOs), plant extracts, chitosan, or the use of microbial biological control agents (bacteria and fungi) (Ciofini et al., 2022; Peralta-Ruiz et al., 2023), such as *Wickerhamomyces anomalus*, which has demonstrated efficacy against gray mold in post-harvest cherry tomatoes (Raynaldo et al., 2021).

EOs are complex mixtures of volatile oils, produced in plant secondary metabolism, with biocidal capacity (Assadpour et al., 2024), also against plant pathogens (Sánchez-Gómez et al., 2024). Recent studies have also highlighted their antibacterial properties, including effectiveness against multidrug-resistant foodborne pathogens (Sobhy et al., 2025). Specifically, against the plant pathogenic fungus *C. gloeosporioides*, several EOs with *in vitro* antifungal capacity have been described, however, few have been studied *in vivo* for their ability to prevent anthracnose (Radice et al., 2024).

Different EOs have been applied to post-harvest tomato fruits in order to reduce or prevent decay caused by different pathogens. For example, cassia, cinnamon, baobab, or cypress EOs against *Alternaria alternata* or *Botrytis cinerea*, with a reduction in decay of up to 50 % (Antunes & Cavaco, 2010; El Khetabi et al., 2022; Sivakumar & Bautista-Baños, 2014). However, the effectiveness of these EOs needs to be improved for real application in post-harvest storage, which could be achieved by combining them with other techniques such as designed packaging, lower temperature, or controlled atmosphere storage (Antunes & Cavaco, 2010). In addition, EOs can have negative effects on the tomatoes to which they are applied. Although EOs obtained from oregano and thyme are effective against the pathogens *B. cinerea* and *Alternaria arborescens*, they cause phytotoxicity in tomato fruits (Antunes & Cavaco, 2010), which leads to a reduction in fruit quality, such as a lower vitamin C content (Namiota & Bonikowski, 2021). Therefore, the concentrations and adverse effects of EOs must be considered for commercial application.

Forest and wild plant species represent an interesting source of bioactive EOs with potential industrial exploitation (Abate et al., 2021). Eucalyptus (genus: *Eucalyptus*; family: Myrtaceae) is an industrially cultivated tree worldwide for the production of paper pulp, and its EO is the world's first traded oil. The EO obtained from eucalyptus leaves has mainly monoterpenes, oxygenated monoterpenes and oxygenated sesquiterpenes. In the specific case of *E. globulus*, about 50 different compounds have been described in its EO, highlighting the oxygenated monoterpene 1,8-cineole (70 %), the monoterpene α -pinene (10 %) and the sesquiterpene globulol (3 %) (Dhakad et al., 2018). On the other hand, lemongrass or citronella, *Cymbopogon citratus*, is a perennial grass (family: Poaceae) used for its therapeutic properties in the tropic regions. In its leaves, *C. citratus* accumulates EOs with bioactive compounds of industrial interest, such as citral (mixture of terpenoids and geranial), myrcene, genariol, citronellol (cymbopogonol and cymbopogone) and α -oxobisabolene (Oladeji et al., 2019).

In this context, the objective of the present work was to characterize and study the use of EOs obtained from eucalyptus and lemongrass leaves by hydrodistillation as antifungal treatments against

C. gloeosporioides in tomato fruits. To this objective, the physicochemical characterization of the EOs, the study of their *in vitro* antifungal capacity and their capacity to prevent anthracnose in fruits stored at room temperature and at 4 °C were carried out.

2. Materials and methods

2.1. Plant and fungal material

Eucalyptus (*E. globulus*) and lemongrass (*C. citratus*) leaves collected in the municipality of Tepetitla de Lardizábal (southern state of Tlaxcala, Mexico) were used to obtain the EOs. Healthy trees, approximately 3 m in diameter and 50 m tall, were selected to obtain the eucalyptus leaves. Healthy leaves of 10–15 cm were collected at 3 m height. On the other hand, lemongrass leaves were collected from healthy plants, 1–1.5 m tall.

The pathogen used in this study was the fungus *C. gloeosporioides* conserved and molecularly characterized by the Centro de Investigación en Biotecnología Aplicada (CIBA) (Mexico) (GenBank: MH156758.1 and KF907247.1). The fungus was maintained on PDA culture medium, with monthly reseeded.

For *in vivo* infection studies with the pathogen, plum tomatoes (Roma VF variety) were used, which were acquired from local plantations. The fruits were used immediately after purchase.

2.2. Obtaining the EOs

Once the leaves were harvested and washed, they were dried by surface evaporation for 5 days at 25–27 °C and constant aeration in the shade. The extraction of EOs was carried out using the hydrodistillation technique, with an 8 L distillation tank (Chuyuan, China), from 2L of sterile distilled water and 2 kg of dry leaf biomass.

The yield of the process of obtaining EOs was determined from the quotient between the mass of EOs obtained and the initial leaf biomass. For the eucalyptus EOs, a yield of 0.72 ± 0.04 was obtained, while for the lemongrass EOs it was 0.57 ± 0.03 .

2.3. EOs physicochemical analysis

The density of the EOs obtained was determined using the methodology described in ISO 279:1998, which relates the mass/volume of the EO to the mass/volume of water, both at 20 °C (ISO 279, 1998). The refractive index was taken with a HI 96800 digital refractometer (HANNA Instruments, Spain). Solubility in ethanol was performed according to NMX-K-081-1976, adding 96 % ethanol at 20 °C and in a 1:10 ratio (EO:ethanol) and observing the presence/absence of turbidity (NMX-K-081-1976, 1976). In addition, the EOs were visually analyzed for the qualitative parameters color, transparency, presence/absence of particles and layer separation. All analyses were performed in quadruplicate on all EOs obtained.

The chemical analysis of the EOs obtained was performed by gas chromatography-mass spectrometry (GC-MS), according to the methodology described by Bai et al. (2021), using an Agilent 7200 GC-QTOF system paired with an HP-5MS fused silica capillary column (30 m \times 0.25 mm i.d., 0.25- μ m film thickness). The gas chromatography was run under programmed temperature conditions, starting at 60 °C for 4 min and increasing to 220 °C at a rate of 4 °C/min, resulting in a total duration of 44 min. Helium was utilized as the carrier gas at a flow rate of 0.8 mL/min. The injector was maintained at 250 °C and operated in split mode with a ratio of 1:50. A sample volume of 0.6 μ L was injected. To prevent damage to the ion source filament, a solvent delay of 3 min was implemented. The temperatures for the interface, ion source, and quadrupole were set to 290 °C, 230 °C, and 150 °C, respectively. The analyses were performed in scanning mode across a mass range of m/z 40–550 using electron ionization at an energy of 70 eV. Volatile components were identified by comparing their retention indices (RIs) with

those found in existing literature, calibrated against a series of n-alkanes (C7-C30) under identical operating conditions on the HP-5MS column. Subsequent identification involved comparing the acquired mass spectra to standard spectra from the NIST 17. Likewise, the identification was corroborated by comparing the calculated retention indices with values reported in the literature.

2.4. Antifungal evaluation of EOs in vitro against *C. gloeosporioides*

The potential antifungal activity of EOs obtained from eucalyptus and lemongrass leaves was analyzed by two different methodologies: inhibition of mycelial growth and inhibition of conidial germination. For the analysis of mycelial growth inhibition, the methodology previously described by Eugui et al. (2023) was used, with several modifications. EOs were dissolved in 10 % (v/v) dimethyl sulfoxide (DMSO) and applied on potato dextrose agar (PDA) medium in 9 cm Petri dishes at different concentrations: 0.01, 0.05, 0.075, 0.10, 0.125 and 0.15 % (v/v). Plates with PDA and with PDA + DMSO 0.01 % were used as controls. On each of these plates, a 6 mm agar plug with *C. gloeosporioides* mycelium was deposited in the center. The plates were incubated at 25 °C in the dark for 6 days, quantifying the pathogen growth area every 2 days using ImageJ software (NIH, USA). These data were used to calculate the inhibition ratio (IR) and the minimum inhibitory concentrations at 50 and 90 % (MIC50 and MIC90). The entire assay was performed in triplicate with 6 plates per treatment.

The IR was calculated according to the following formula (1):

$$IR = (AC - AT) / AC \times 100 \quad (1)$$

where AC represent the colony area of the pathogenic fungus in the control treatment, and AT means the colony area of the pathogenic fungus in EOs treatments (Menéndez-Cañamares et al., 2024). For the calculation of MIC50 and MIC90, the values obtained from IR and a linear regression equation between the neperian logarithms of the concentrations of the abscissae and the percentage of growth inhibition of the ordinates ($C_i = f(\ln C_i)$) were used (Ambang et al., 2021).

On the other hand, the inhibition of the germination of *C. gloeosporioides* conidia was studied as another approach to the antifungal activity of the different EOs. The methodology described by Morcuende et al. (2024) was followed, with some modifications. In 1.5 mL Eppendorf tubes, 1 mL of potato dextrose broth (PDB) and 10^7 conidia of *C. gloeosporioides* (quantified with a hemocytometer) were poured, keeping the tubes at 25 °C and 60 rpm. In addition to the control treatment with PDB, another EO diluent control was performed with 0.01 % (v/v) DMSO and treatments with different concentrations of the EOs (0.01, 0.05, 0.075, 0.10, 0.125, 0.15 and 0.175 %; v/v) dissolved in 10 % (v/v) DMSO. The number of germinated conidia at 12 and 24 h of incubation was quantified. The complete assay was performed with 6 tubes per treatment and in triplicate.

2.5. In vivo tests at different storage temperatures

The study of anthracnose control on tomato fruits was carried out with EOs obtained from lemongrass leaves using the methodology described by Poveda et al. (2022), with modifications. The fruits were washed with soap and water for 3 min and rinsed with distilled water. Subsequently, the fruits were immersed for 5 min in 3 % sodium hypochlorite, rinsing 3 times with sterile distilled water. The fruits were surface sprayed to full coverage with the MIC50 and MIC90 doses calculated in the *in vitro* study (0.082 and 0.097 %, v/v, respectively). A 3 × 3 mm cross-shaped wound was made in the epidermis of the fruits where 10 µL of 1×10^7 conidia/mL of *C. gloeosporioides* were deposited. The fruits were kept at 25 °C for 7 days in the dark. The control treatment consisted of covering the fruits with distilled water. All treatments were carried out on 15 fruits and the entire assay was performed in triplicate (= three independent replicates with 15 fruits each).

At 3, 5 and 7 days of incubation, disease incidence and severity data were collected according to the methodology described by Eugui et al. (2025). Disease incidence (DI) was evaluated as the percentage of infected fruits over the total. Disease severity (DS) was evaluated using a qualitative scale: 0 = fruit with no visible damage; 1 = 1–25 % of the fruit damaged; 2 = 26–50 % of the fruit damaged; 3 = 51–75 % of the fruit damaged; 4 = 76–100 % of the fruit damaged. The DS index was then calculated with formula (2):

$$DS = \frac{\sum (N^\circ \text{ infected fruits in each scale} \cdot \text{scale value})}{\text{Total fruits} \cdot \text{highest scale value}} \cdot 100 \quad (2)$$

Similarly, the anthracnose control capacity of lemongrass EOs in cold storage was analyzed. For this purpose, the same assay was carried out in triplicate, but in storage at 8 °C and taking the DI and DS data at 16, 24, 32 and 40 days.

2.6. Statistical analysis

Statistical analysis of the data was carried out with STATISTICA 5.5 software. The Student's *t*-test was used for a comparison of means at $P < 0.05$; significant differences are denoted using an asterisk. One-way ANOVA using Tukey's multiple range test at $P < 0.05$ was used for pairwise comparisons; the different letters indicate significant differences.

3. Results

3.1. Physicochemical characteristics of eucalyptus and lemongrass EOs

Physical analysis of the EOs obtained by hydrodistillation of eucalyptus and lemongrass leaves reported very similar characteristics in both (Table 1). Both oils were light yellow, transparent, soluble in ethanol, free of suspended particles and without different phases. Small non-significant differences were reported between eucalyptus and lemongrass EOs in density (0.9 and 0.84, respectively) and refractive index (1.46 and 1.48, respectively).

With respect to the chemical analysis by GC-MS of the EOs, seven different compounds were obtained in the eucalyptus EO and three in the lemongrass EO (Fig. 1, Table 2). In the eucalyptus EO the components identified were eucalyptol (51 %), γ -terpinene (16 %), p-cymene (11 %), α -phellandrene (10 %), D-limonene (6 %), 3-carene (5 %) and m-cymene (1 %). While in lemongrass EO the components identified were carveol (43 %), trans-verbenol (39 %) and pseudo limonene (17 %) (Table 2).

3.2. In vitro antifungal capacity of eucalyptus EO against *C. gloeosporioides*

To analyze the *in vitro* antifungal capacity of EO obtained from eucalyptus leaves, two different methodologies were used: micellar growth on a plate and conidial germination in liquid medium. Neither solvent control (DMSO) nor 0.01–0.075 % EO concentrations reduced the growth of *C. gloeosporioides* compared to the control. However, concentrations between 0.1 % and 0.175 % inhibited pathogen growth (Fig. 2a). After IR calculation, it was determined that concentrations 0.1 % and 0.125 % inhibited pathogen growth by 35–47 %. In addition, the 0.15 % and 0.175 % concentrations inhibited growth significantly more than the other concentrations used, up to 94 % (at the 0.175 % concentration) and 70 % (at the 0.15 % concentration) (Fig. 2b). After performing the linear regression of the IR data, we obtained a MIC50 of 0.127 % and a MIC90 of 0.194 %.

On the other hand, the effect of different concentrations of eucalyptus EO on conidial germination of *C. gloeosporioides* was studied. The organic solvent DMSO control did not report inhibition of conidial germination. However, all concentrations inhibited pathogen germination significantly against the control, between 84 and 89 % (Fig. 3).

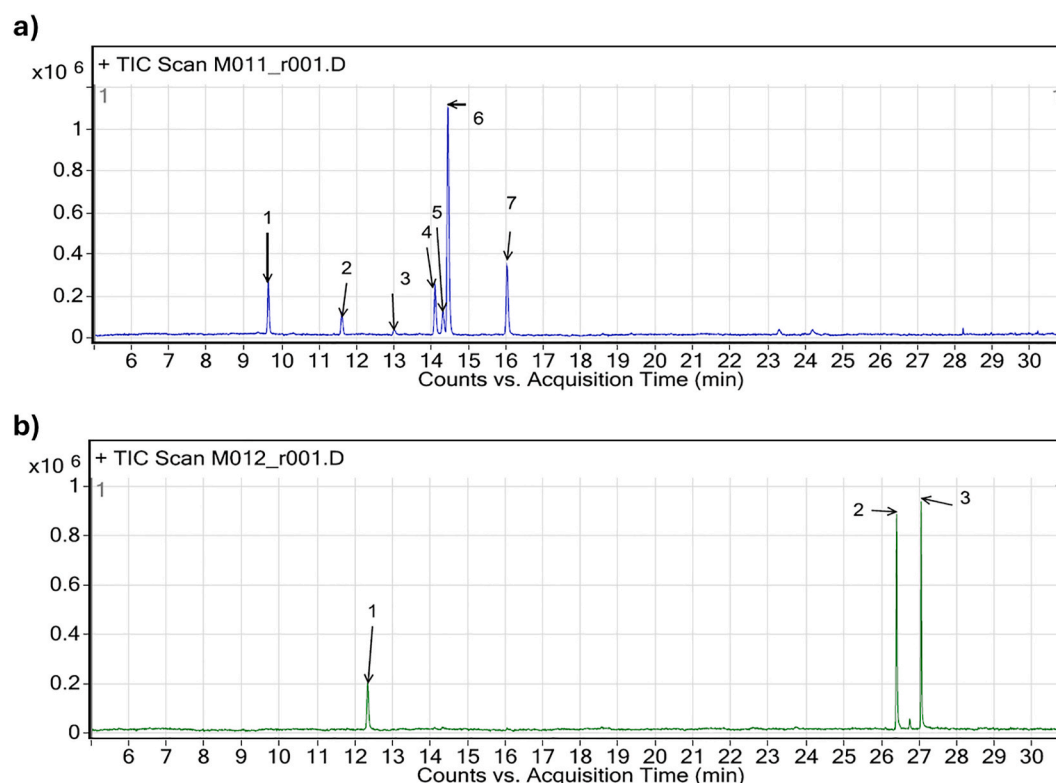
Table 1

Physical characteristics of eucalyptus and lemongrass EOs: density, refractive index, solubility in ethanol and visual characteristics.

EO	Density (g/ml)	Refractive index	Solubility in ethanol	Color	Transparency	Suspended particles	Layer separation
Eucalyptus	0.90 ± 0.005	1.46 ± 0.02	Soluble	Light yellow	Total	Negative	Negative
Lemongrass	0.84 ± 0.01	1.48 ± 0.06	Soluble	Light yellow	Total	Negative	Negative

Data represent the mean of four technical replicates, together with their standard deviation.

No significant differences were obtained between the EOs.

**Fig. 1.** GC-MS chromatograms of eucalyptus (a) and lemongrass (b) essential oils. The identification of the numbered peaks is given in Table 2: a) 1: α -phellandren, 2: 3-carene, 3: m-cymene, 4: p-cymene, 5: D-limonene, 6: eucalyptol, 7: γ -terpinene; b) 1: pseudo limonene, 2: carveol, 3: trans verbenol.**Table 2**

Identity, retention time (RT; min) and percentages of the components of eucalyptus and lemongrass EOs.

EOs	Pike	RT	Identity	Percentage
Eucalyptus	1	9.60	α -phellandrene	10
	2	11.60	3-carene	5
	3	13.10	m-cymene	1
	4	14.10	p-cymene	11
	5	14.30	D-limonene	6
	6	14.45	Eucalyptol	51
	7	16.03	γ -terpinene	16
Lemongrass	1	12.35	Pseudo limonene	17
	2	14.40	Carveol	43
	3	27.05	Trans verbenol	39

3.3. *In vitro* antifungal capacity of lemongrass EO against *C. gloeosporioides*

As with eucalyptus EO, the antifungal capacity of lemongrass EO was analyzed by inhibition of mycelial growth and conidial germination. With respect to mycelial growth, no inhibition of *C. gloeosporioides* growth was observed in the control with DMSO, nor with the application of EO at concentrations 0.01–0.075 %. However, concentrations above 0.10 % clearly inhibited pathogen growth (Fig. 4a). The IR calculation

reported that the 0.01–0.075 % concentrations inhibited mycelial growth between 10 and 28 %, being a significantly lower inhibition than with the 0.10–0.15 % concentrations (of 98 % IR) (Fig. 4b). Linear regression of the different IRs allowed the calculation of a MIC₅₀ of 0.082 % and a MIC₉₀ of 0.097 %.

With respect to pathogen conidial germination, all concentrations used significantly inhibited fungal germination. The 0.01 and 0.05 % concentrations inhibited significantly less (79–80 % IR) conidial germination of *C. gloeosporioides* than the 0.1–0.175 % concentrations (99–100 % IR) (Fig. 5).

Since the EO that reported the most antifungal activity *in vitro* was that from lemongrass leaves, this will be the one used in the *in vivo* assays under different storage temperatures.

3.4. *In vivo* anthracnose control under different storage temperatures

In the control of anthracnose in tomato fruits by means of EOs, those from lemongrass were used, as they were the most potent against *C. gloeosporioides* *in vitro*. Different storage temperatures were tested (25 and 8 °C). In storage at room temperature (25 °C) the MIC₅₀ and MIC₉₀ of lemongrass EO significantly inhibited disease development (Fig. 6a), both with respect to incidence (Fig. 6b) and severity (Fig. 6c). At 7 days of storage, the controls reached the maximum anthracnose incidence (87 %) and severity (67 %), significantly reduced with the application of EO MIC₅₀ (63 % and 9 %, respectively). In addition, anthracnose

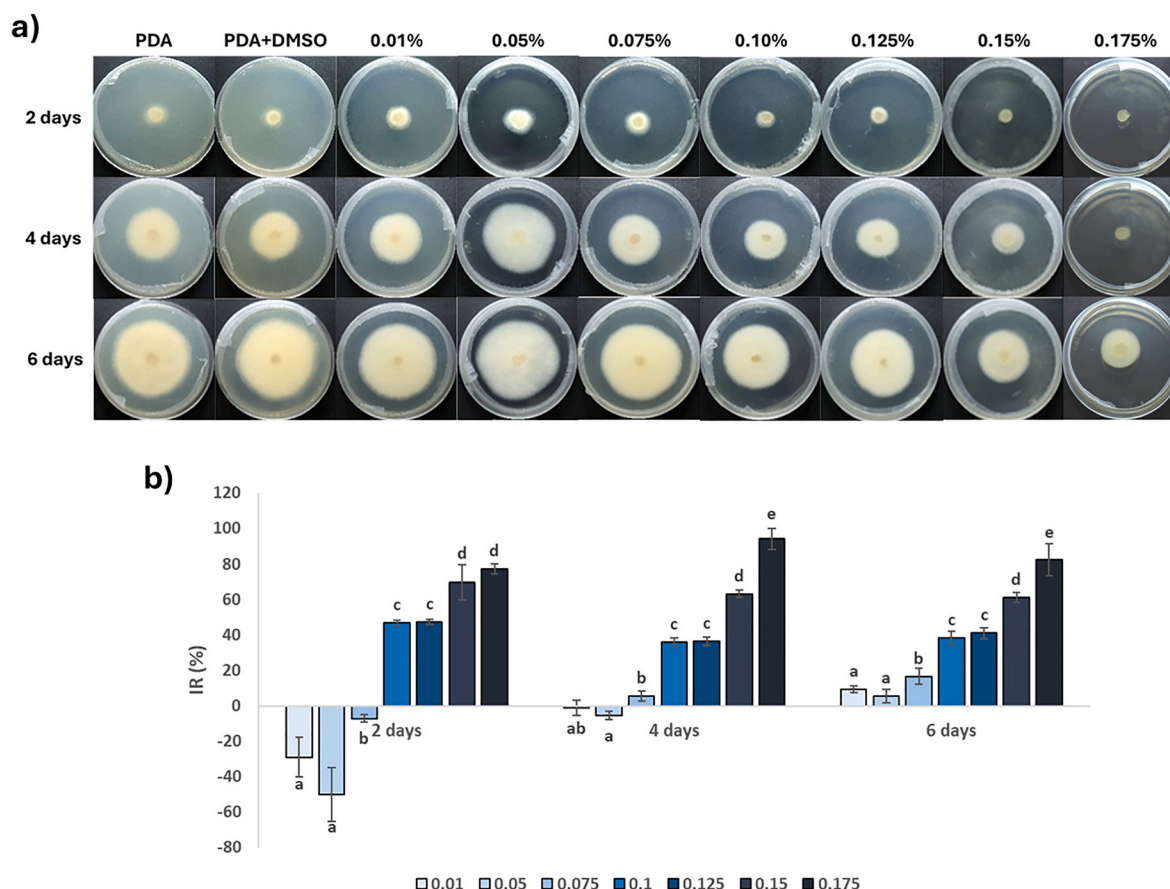


Fig. 2. Effect of different concentrations of eucalyptus EO (0.01, 0.05, 0.075, 0.10, 0.125, 0.15 and 0.175 %) on the growth of the pathogen *C. gloeosporioides* *in vitro*. Representative photographs of plates (a) and inhibition ratio (IR) at 2, 4 and 6 days of EO-pathogen contact. Data are the mean of three replicates with six plates in each one. One-way analysis of variance (ANOVA) was performed, followed by the Tukey's test. Different letters represent significant differences between the different concentrations on the same day ($P < 0.05$).

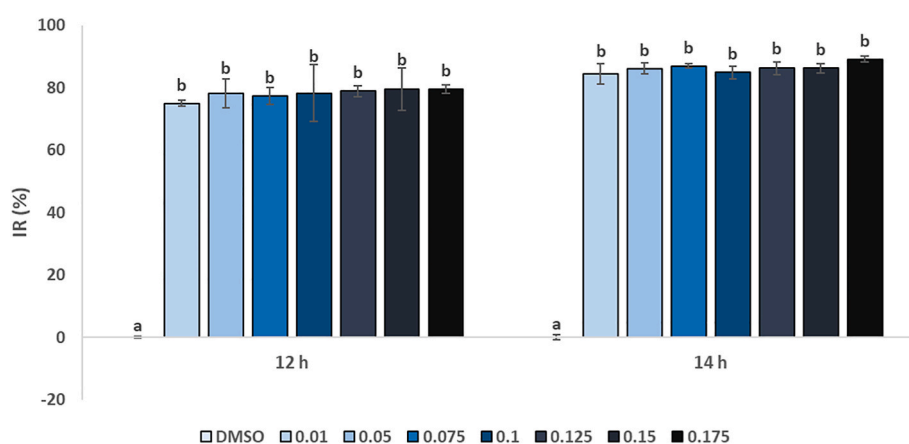


Fig. 3. Effect of different concentrations of eucalyptus EO (0.01, 0.05, 0.075, 0.10, 0.125, 0.15 and 0.175 %) on the conidial germination of the pathogen *C. gloeosporioides* *in vitro*. Inhibition ratio (IR) at 12 and 24 h of EO-pathogen contact. Data are the mean of three replicates with six tubes in each one. One-way analysis of variance (ANOVA) was performed, followed by the Tukey's test. Different letters represent significant differences between the different concentrations on the same incubation time ($P < 0.05$).

incidence and severity were significantly lower than MIC50 with the application of MIC90 (2 % and 1 %, respectively) (Fig. 6b and c).

On the other hand, in cold storage (8 °C), no disease was reported in tomato fruits treated with the different concentrations of lemongrass EO (Fig. 7a). At 40 days of storage, anthracnose incidence in the controls was 82 % and severity was 27 %. However, tomatoes treated with MIC50 and MIC90 of lemongrass EO reported no disease incidence (0 %) and no

disease severity (0 %) (Fig. 7b and c).

4. Discussion

The first phase of the work consisted of obtaining EOs from eucalyptus and lemongrass leaves using an efficient methodology. Hydro-distillation was used and the different physicochemical parameters that

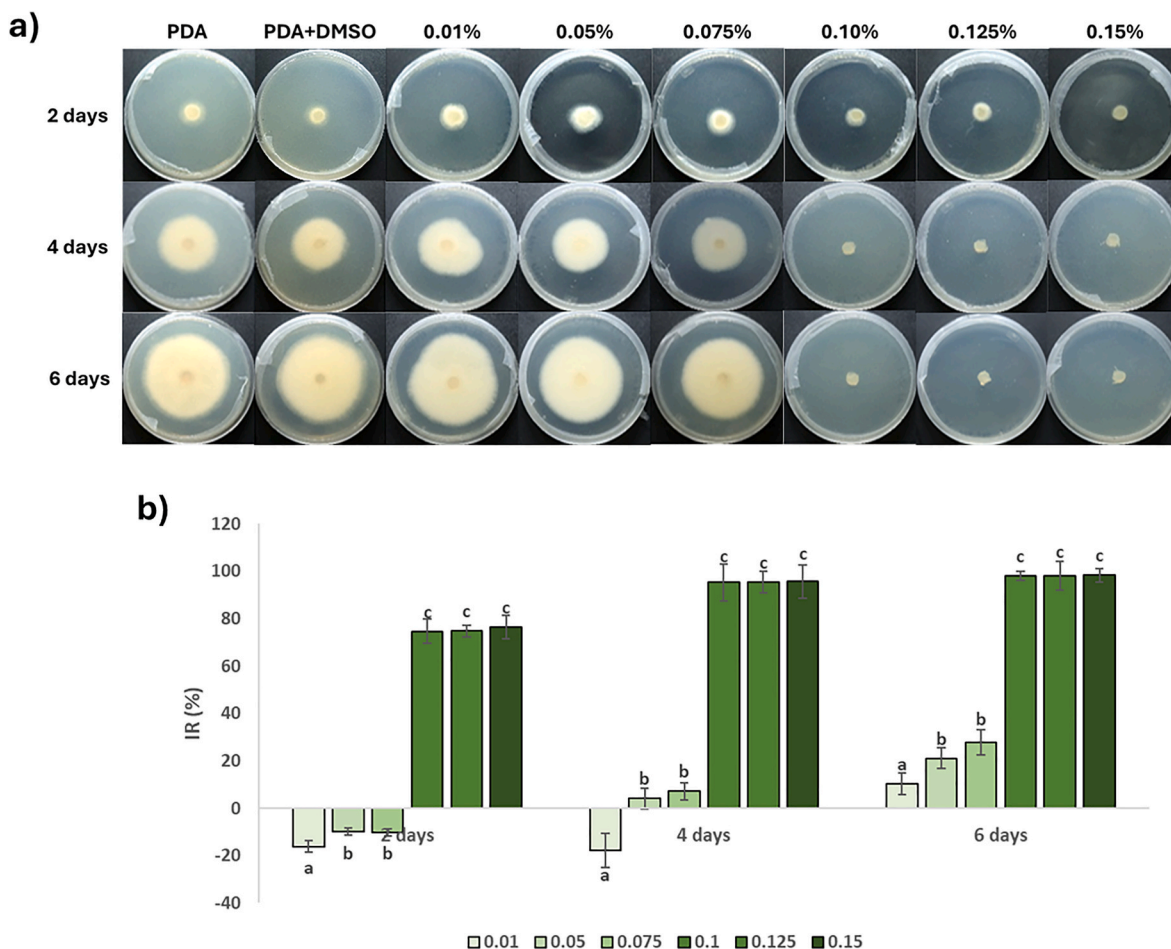


Fig. 4. Effect of different concentrations of lemongrass EO (0.01, 0.05, 0.075, 0.10, 0.125 and 0.15 %) on the growth of the pathogen *C. gloeosporioides* *in vitro*. Representative photographs of plates (a) and inhibition ratio (IR) at 2, 4 and 6 days of EO-pathogen contact. Data are the mean of three replicates with six plates in each one. One-way analysis of variance (ANOVA) was performed, followed by the Tukey's test. Different letters represent significant differences between the different concentrations on the same day ($P < 0.05$).

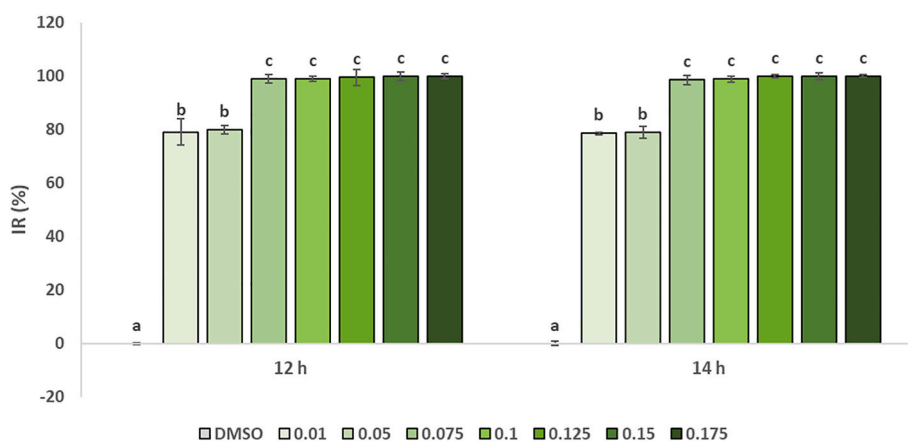


Fig. 5. Effect of different concentrations of lemongrass EO (0.01, 0.05, 0.075, 0.10, 0.125, 0.15 and 0.175 %) on the conidial germination of the pathogen *C. gloeosporioides* *in vitro*. Inhibition ratio (IR) at 12 and 24 h of EO-pathogen contact. Data are the mean of three replicates with six tubes in each one. One-way analysis of variance (ANOVA) was performed, followed by the Tukey's test. Different letters represent significant differences between the different concentrations on the same incubation time ($P < 0.05$).

determine the adequacy of the EOs to the different international standards were analyzed.

Eucalyptus EOs presented a density within the ISO 3065, 2021 standard (0.918–0.928 g/ml) and very similar to that reported in other

works with hydrodistillation and with other extraction methodologies, such as the use of organic solvent and the use of microwave-assisted hydrodistillation (Alarcón et al., 2019; Cedeño et al., 2019). Similarly, the refractive index obtained conformed to the NOM-K-129, 1979

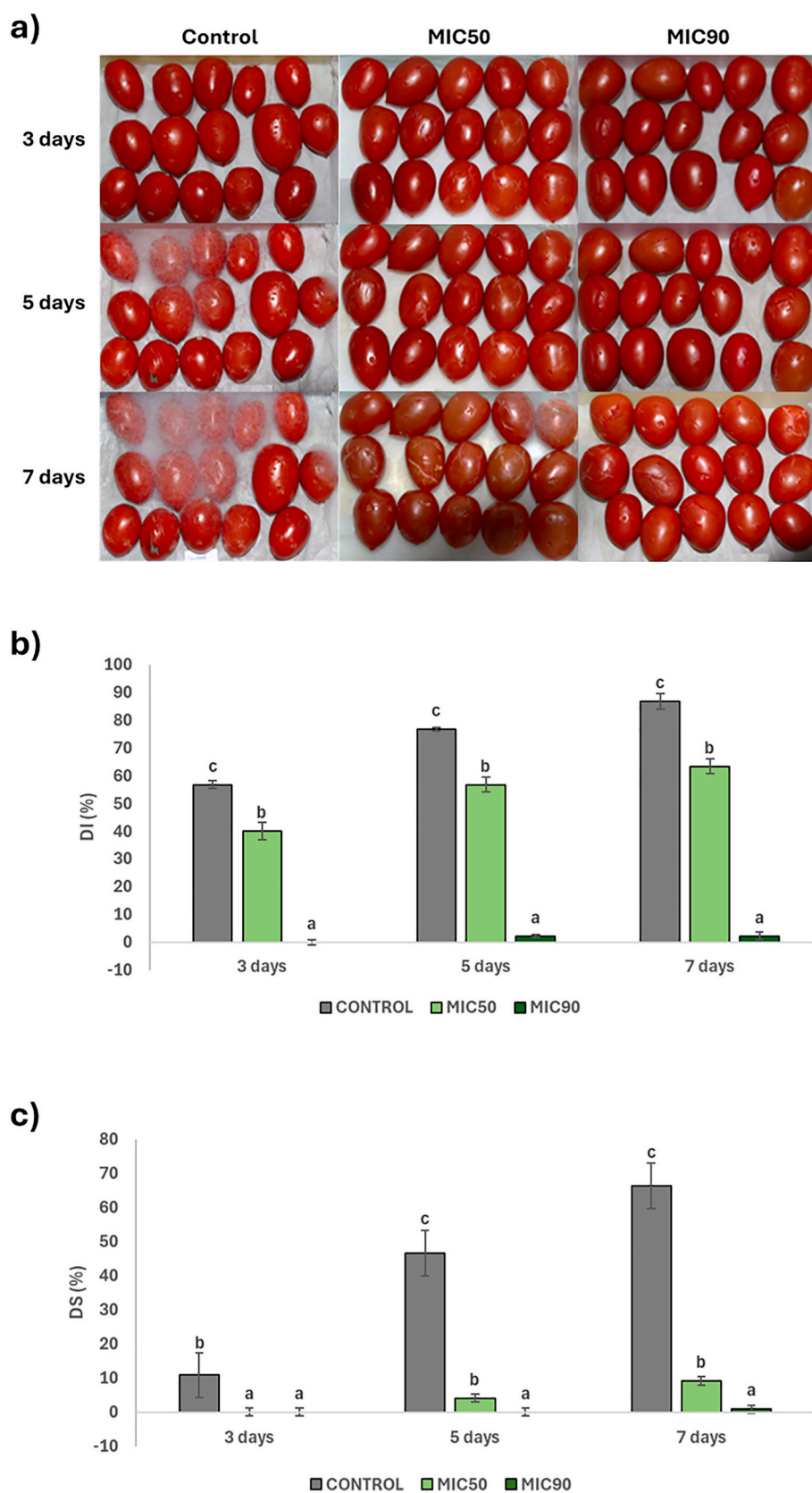


Fig. 6. Control of anthracnose on tomato fruit stored at 25 °C by application of lemongrass EOs at MIC50 and MIC90. Representative photographs of fruits (a), disease incidence (DI) (b) and severity (DS) (c) at 3-, 5- and 7-days post pathogen infection. Data are the mean of three replicates with fifteen fruits in each one. One-way analysis of variance (ANOVA) was performed, followed by the Tukey's test. Different letters represent significant differences between treatments on the same day ($P < 0.05$).

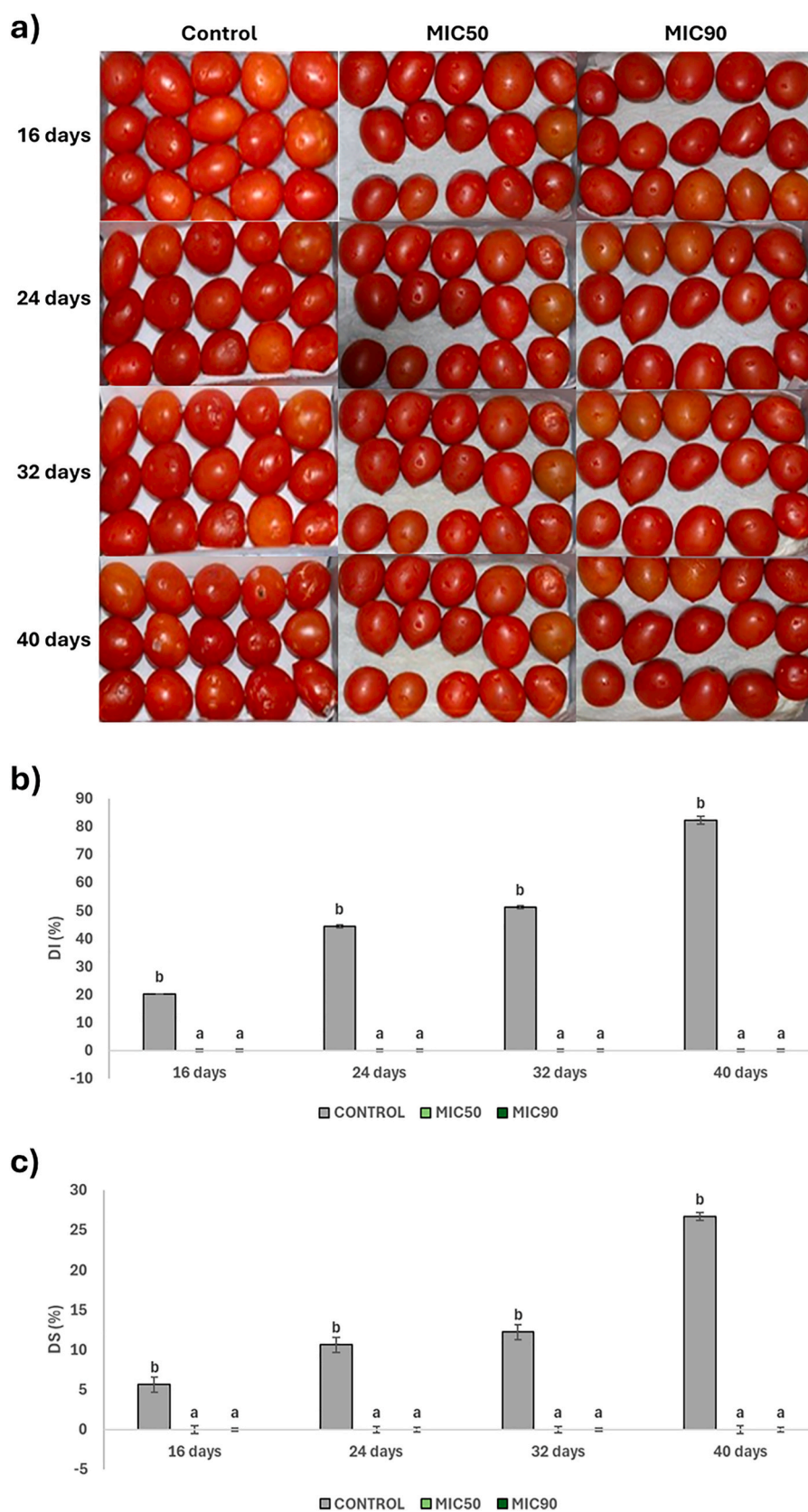


Fig. 7. Control of anthracnose on tomato fruit stored at 8 °C by application of lemongrass EOs at MIC50 and MIC90. Representative photographs of fruits (a), disease incidence (DI) (b) and severity (DS) (c) at 16-, 24-, 32- and 40-days post pathogen infection. Data are the mean of three replicates with fifteen fruits in each one. One-way analysis of variance (ANOVA) was performed, followed by the Tukey's test. Different letters represent significant differences between treatments on the same day ($P < 0.05$).

standard (1.45–1.48), being similar to that obtained by other authors with the same methodology and with the use of ultrasound (Harkat-Madouri et al., 2015; Solano et al., 2022).

On the other hand, lemongrass EO also conformed to NMX-F366-S-1980, 1980 standards in terms of density (0.869–0.894 g/ml), as did other works that used hydrodistillation or organic solvents (Mirzaie et al., 2020; Vázquez-Briones & Guerrero-Beltrán, 2017). The refractive index was also adjusted to the NOM-K-129 standard (1.45–1.48), just like other works that used hydrodistillation (Ajayi et al., 2016; Dangkulwanich & Charaslertrangsi, 2020).

The solubility of EOs in alcohol is a very important characteristic for their use in perfumes, lotions, creams or as flavoring agents in toiletries and hygiene products. Therefore, this solubility parameter is used as a quality indicator for the elaboration of these products (Jugreet et al., 2020). In this sense, both eucalyptus and lemongrass EOs obtained in this work were soluble in ethanol and, as a consequence, potentially useable in different industries.

Chemically, the EOs obtained by hydrodistillation of eucalyptus and lemongrass leaves reported a composition similar to other previous studies. In our work, most of the chemical compounds identified in the eucalyptus EO were monoterpenes, with only one oxygenated monoterpene (eucalyptol). This chemical profile coincides with that obtained for different eucalyptus species (*E. angulosa*, *E. cladocalyx*, *E. diversicolor*, *E. microcoryx*, *E. ovata*, *E. resinifera*, *E. saligna* and *E. sargentii*) by hydrodistillation, with 1,8-cineole (eucalyptol) always being the major compound present. The rest of the compounds identified in our EOs were also present in the oils of the different eucalyptus trees (Ayed et al., 2023). Specifically in *E. globulus*, other authors obtain compound profiles very similar to the one obtained in our work, although with small differences in quantity and number of compounds identified (Čmiková et al., 2023). These chemical differences may be a consequence of the environmental conditions of growth of plant resources (soil, climate, altitude and sun exposure), even reporting differences in quantity and profile of compounds in EOs of eucalyptus between different seasons of the year (Shala & Gururani, 2021; Usman et al., 2020).

In the case of the chemical profile of the EO obtained from lemongrass, most of the compounds obtained in our work were oxygenated monoterpenes (carveol and trans verbenol), and in smaller proportion monoterpenes (pseudo limonene). This profile coincides with that obtained by other authors (Zaman et al., 2022), however, other authors obtained a higher proportion of monoterpene hydrocarbons versus oxygenated monoterpenes (Sherif et al., 2023). These chemical differences in the EOs obtained by hydrodistillation could be due to environmental conditions and/or to the genetic variability of the plant material used (Bassolé et al., 2011; Madi et al., 2021; Valková et al., 2022).

In this work both eucalyptus and lemongrass EO had an antifungal effect against mycelial growth and germination of *C. gloeosporioides* *in vitro*. In the case of eucalyptus, this *in vitro* antifungal capacity against the pathogen had been described in previous work (España et al., 2017; Silué et al., 2023). This antifungal capacity is probably a consequence of the presence of the oxygenated monoterpene 1,8-cineole (eucalyptol), a component of eucalyptus EO identified in our work and widely described as a potent antifungal (Pries et al., 2023). Specifically against *C. gloeosporioides*, eucalyptol causes malformations in hyphae and conidia, as well as a decrease in mycotoxin production (Peralta-Ruiz et al., 2023; Pol et al., 2023).

In the case of lemongrass EO, the highest *in vitro* antifungal capacity against *C. gloeosporioides* was reported in this work. Previous work also obtained up to 100 % inhibition of pathogen growth *in vitro* with different lemongrass EOs (Cissé et al., 2020; Itako et al., 2021). This antifungal effect could be a consequence of the carveol and trans-verbenol components previously identified in the EO, which have been described as potent biocides, even superior to chemical fungicides such as copper exichloride (Sawadogo et al., 2022; Tzortzakis & Economakis, 2007). Both compounds act against pathogenic fungi by

inhibiting cellular respiration, affecting membrane permeability and the synthesis of cell wall components (Athayde et al., 2016; Di Francesco et al., 2022; Tao et al., 2014).

The real applicability of EO as a controller of anthracnose in post-harvest implies its study *in vivo* and under real storage conditions of tomato fruits. In storage at ambient temperature (25 °C), lemongrass EOs achieved effective control of the disease caused by *C. gloeosporioides* in tomato fruit, with MIC90 being the most effective. These results are in agreement with those obtained against this pathogen on other fruits in postharvest using EOs from lemongrass, such as apple, mango, guava and papaya (Antonoli et al., 2020; Oliveira et al., 2018). However, to our knowledge, this is the first work where anthracnose control has been obtained in postharvest tomato fruits by the application of EOs from lemongrass.

Compared to previous studies, the MIC values obtained in this work show a remarkable antifungal efficacy of EOs from *C. citratus*. For example, in research conducted by Cissé et al. (2020) and Itako et al. (2021), the MIC90 of lemongrass OEs against *C. gloeosporioides* was reported to be between 0.15 % and 0.30 % (v/v), while in our study the MIC90 was 0.097 % (v/v), indicating greater inhibitory potency. Likewise, in the review by Radice et al. (2024), EOs from various species showed MIC90 values generally higher than 0.1 %. These differences could be attributed to factors such as the agroclimatic conditions of the plants used, the extraction method applied, or the specific chemical profile of the oils obtained in this work, characterized by high contents of carveol and trans-verbenol. Therefore, our results reinforce the potential of EOs from *C. citratus* as an effective and sustainable alternative for the control of *C. gloeosporioides* in post-harvest tomatoes.

On the other hand, in cold storage (8 °C) the application of lemongrass EOs in this work obtained absolute control of anthracnose in tomato fruits, up to 40 days post-pathogen infection. Effective disease control in cold storage with the use of lemongrass EOs has also been reported previously in banana, papaya and avocado (Maqbool et al., 2011; Mpho et al., 2013), but not in tomato. However, in our work we used storage temperatures of 8 °C, which can reduce the flavor and aroma of tomatoes, making them more difficult to sell (Siddiq & Uebersax, 2018). The most widely used cold storage temperature for tomatoes is 12 °C (Siddiq & Uebersax, 2018), so this should be used in future work with EOs.

Although the results of this study show complete control of anthracnose during cold storage, it is essential to consider some key factors that affect the commercial viability of applying these EOs. The cost of producing the oils can vary depending on the availability of raw materials, the extraction method used, and the scale of production. However, techniques such as hydrodistillation, especially in regions where *C. citratus* is grown locally, allow them to be obtained at competitive costs, as pointed out by Antonoli et al. (2020). In terms of scalability, the incorporation of EOs into coating, fogging, or active packaging systems is a feasible strategy for industrial implementation. For instance, the use of EO-loaded bio-nanocomposite films has been successfully applied for fruit preservation, such as with *Litsea cubeba* oil in mangoes (Yang et al., 2023). Solid nanoliposome encapsulation has also been reported to enhance the stability of *Eucalyptus citriodora* EO, suggesting a promising approach for oils from *E. globulus* as well (Lin et al., 2019). Regarding the possible presence of residues, given that EOs are highly volatile and biodegradable compounds, residual levels in treated fruits are expected to be minimal or even undetectable, a situation supported by their recognition as GRAS substances by the FDA (Choi et al., 2024). However, the need for further studies to specifically evaluate the persistence of residues under real commercial conditions is recognized.

Despite the results obtained on the control of anthracnose in post-harvest tomato the use of EOs, such as from lemongrass, presents important disadvantages, such as their low solubility and the instability of their components, which can photodegrade (Odak et al., 2018). Encapsulation strategies, such as liposome entrapment, have proven

effective in enhancing EO stability, as shown for lemongrass oil in dairy matrices like cheese (Cui et al., 2016). However, the use of lemongrass EOs for the control of anthracnose in tomato could help reduce the use of chemical fungicides, their environmental impact and health problems (Peralta-Ruiz et al., 2023). In addition, cold storage combined with lemongrass EO reported absolute control of the disease, so its antifungal components seem not to degrade under these postharvest storage conditions.

Furthermore, despite the results reported in this work, both in the physical-chemical characterization and in the biocidal capacity of lemongrass EO, it is important to study the effectiveness of these EOs over time. It is known that the stability of EOs is particularly affected by temperature, exposure to light, and oxygen, which causes isomerization, oxidation, or dehydrogenation of their bioactive components (Turek & Stintzing, 2013). It is important to evaluate the biocidal effectiveness of EOs over time and according to the storage conditions used, aspects to be considered in future work.

On the other hand, a single strain of *C. gloeosporioides* was used in this work, which may not allow the conclusions obtained to be generalized. The pathogen used in this work is representative of postharvest diseases of tomato in Mexico, but may not be representative of other agricultural systems worldwide. Future studies should investigate the effectiveness of these EOs in controlling other strains of the pathogen in other tomato varieties during postharvest.

Finally, it is important to evaluate the safety of these EOs in their actual application as a postharvest treatment. Although they are effective fungicides, their use in food requires a thorough biosafety analysis before the development of a marketable product. Future work should analyze the cytotoxicity of these EOs, as is done with other plant-derived products in their biocidal use (Eugui et al., 2023). In addition, other aspects related to consumer perception should be evaluated, such as the morphological, visual, compositional, and organoleptic characteristics of tomatoes treated with these EOs. Future research should evaluate these aspects in post-harvest tomatoes, in the absence and presence of the pathogen.

As conclusions, hydrodistillation allows obtaining EOs from eucalyptus and lemongrass leaves with physicochemical characteristics within international standards and with similar work done previously. Both EOs obtained from eucalyptus and lemongrass showed *in vitro* antifungal capacity against the pathogen *C. gloeosporioides*, however, lemongrass EO had greater controlling potential. Furthermore, in storage at room temperature (25 °C) and in cold storage (8 °C) lemongrass EOs were able to reduce the incidence and severity of anthracnose, and even eliminate the disease completely in cold storage.

CRediT authorship contribution statement

Maribel Flores: Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Jorge Poveda:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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