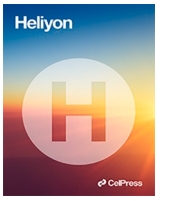
Heliyon 10 (2024) e31443

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Chemical, antioxidant, and antifungal analysis of oregano and thyme essential oils from Ecuador: Effect of thyme against *Lasiodiplodia theobromae* and its application in banana rot

Glenda Pilozo [a,b], Mirian Villavicencio-Vásquez [b], Ivan Chóez-Guaranda [b], Damon Vera Murillo [c], Cynthia Duarte Pasaguay [c], Christofer Tomalá Reyes [c], Maria Maldonado-Estupiñán [c], Omar Ruiz-Barzola [c], Fabián León-Tamariz [d], Patricia Manzano [a,b,c,*]

[a] *Facultad de Ciencias de la Vida, ESPOL Polytechnic University, ESPOL, Campus Gustavo Galindo, Km. 30.5 Vía Perimetral, Guayaquil, 090902, Ecuador*

[b] *Centro de Investigaciones Biotecnológicas del Ecuador, ESPOL Polytechnic University, ESPOL, Campus Gustavo Galindo, Km. 30.5 Vía Perimetral, Guayaquil, 090902, Ecuador*

[c] *Facultad de Ciencias Naturales y Matemáticas, ESPOL Polytechnic University, ESPOL, Campus Gustavo Galindo, Km. 30.5 Vía Perimetral, Guayaquil, 090902, Ecuador*

[d] *University of Cuenca, Universidad de Cuenca, Departamento de Biociencias, Facultad de Ciencias Químicas, Campus Central Av. 12 de Abril, Cuenca, Ecuador*

ARTICLE INFO

Keywords:
Bioactivity
Botanical fungicide
Carvacrol
Phytosanitary control
Postharvest pathogen management
Thymol

ABSTRACT

The objective of this study was to evaluate the antioxidant capacity by spectrophotometric methods, the *in vitro* and *in vivo* antifungal effect against *Lasiodiplodia theobromae* and the constitution of the essential oils (EO) of oregano and thyme in comparison with their commercial counterparts. The results showed by the EOs of extracted thyme (T-EO), commercial thyme (CT-EO), extracted oregano (O-EO) and commercial oregano (CO-EO), demonstrated antioxidant profiles with a radical neutralizing potential (DPPH•) of IC_{50}: 1.11 ± 0.019; 1.08 ± 0.05; 40.56 ± 0.227 and 0.69 ± 0.004 mg/mL, respectively. They also revealed a ferric ion reducing capacity (FRAP) of 93.05 ± 0.52; 97.72 ± 0.42; 21.85 ± 0.57 and 117.24 ± 0.64 mg Eq Trolox/g. A reduction in β-carotene degradation of 65.71 ± 0.04; 51.97 ± 0.66; 43.58 ± 1.56 and 57.46 ± 1.56 %. A total phenol content (Folin-Ciocalteu) of 132.97 ± 0.77; 141.89 ± 2.56; 152.04 ± 0.10 and 25.66 ± 0.40 mg EGA/g. Chemical characterization performed by gas chromatography mass spectrometry (GC-MS) showed that the respective major components of the samples were thymol (T-EO: 45.78 %), thymol (CT-EO: 43.57 %), alloaromadendrene (O-EO: 25.17 %) and carvacrol (CO-EO: 62.06 %). Regarding antifungal activity, it was evident that at the *in vitro* level, both commercial EOs had a MIC of 250 ppm while the extracted thyme EO had a MIC of 500 ppm; *In vivo* studies demonstrated that the application of thyme EO had a behavior similar to the synthetic fungicide, slowing down rot in bananas under storage conditions. Finally, partial least squares discriminant analysis (PLS-DA) and heat maps suggest *p*-cymene, carvacrol, linalool, eucalyptol, 4-terpineol, (z)-β-terpineol, alkanhol, caryophyllene, β-myrcene, D-limonene, α-terpinene, α-terpineol, D-α-pinene, camphene, caryophyllene oxide, δ-cadinene, terpinolene and thymol as

* Corresponding author. Centro de Investigaciones Biotecnológicas del Ecuador and Facultad de Ciencias Naturales y Matemáticas, ESPOL Polytechnic University, ESPOL, Campus Gustavo Galindo, Km. 30.5 Vía Perimetral, Guayaquil, 090902, Ecuador.
E-mail address: pmanzano@espol.edu.ec (P. Manzano).

https://doi.org/10.1016/j.heliyon.2024.e31443
Received 1 August 2023; Received in revised form 10 May 2024; Accepted 15 May 2024
Available online 18 May 2024

relevant biomarkers associated with the assessed bioactive properties demonstrating the potential of extracted essential oils for the development of a botanical biofungicide.

1. Introduction

Agriculture plays a fundamental role in Ecuador's economy, representing 7.81 % of the gross domestic product (GDP) [1], and banana production represents an important contribution to this item. Only in 2022 banana cultivation generated around 3267.6 million dollars of incomes. For this reason, the control and proper management of its production is crucial, generally follows a strict phytosanitary control to avoid an economic decrease, as reported in 2021 that reached 6.3 % [2]. As a means of phytosanitary control, producers generally use synthetic pesticides such as imazalil, thiabendazole, pyrimethanil, and fludioxonil, among others [3]. These types of substances are applied indiscriminately and regularly, causing a residual accumulation in the environment and food, negatively affecting the ecosystem and human health [4,5].

However, the industry is forced to use them to protect crop production at all stages, since several microorganisms can cause total or partial loss of the crop. This is the case with bananas, which can suffer from anthracnose, fusarium, black sigatoka, or Diplodia rot, among others [3,6,7]. The main causal agent is the multi-infectious fungus *Lasiodiplodia theobromae* [8], which is also one of the pathogens responsible for causing brown rot in the rind of several tropical fruits [9,10].

The banana presents a high vulnerability to the attack of this fungus because it provides favorable conditions for this pathogen to develop due to its nutritional and chemical attributes [3]. The presence of fungal symptoms in fruits negatively affects their quality and price in the market causing a devaluation [11]. In recent years, numerous research studies have supported the efficacy of using natural and sustainable compounds as an alternative to synthetic pesticides [4,12,13], to meet the constantly increasing needs of the market, which currently prioritizes the use of efficient, selective, and safe substances for both crops and humans, such as essential oils applied as active components in foods [14,15].

Essential oils are suitable extracts to combat *L. theobromae*. Some outstanding examples are the essential ils of *Myrcia lundiana*, *Varronia curassavica*, *Lippia rehmannii*, *Thymus vulgaris*, *Origanum vulgare* species, which at *in vitro* level present antifungal activity against this pathogen [16–20]. In addition, *in vivo* trials on tropical fruits demonstrate the potential of essential oils from species such as *Cymbopogon winterianus*, *Monarda fistulosa* to replace synthetic fungicides in the control of postharvest pathogens such as *Lasiodiplodia theobromae* and *Colletotrichum musae*, since they delay the signs of rot caused by these agents and extend the useful life of these foods [3,21].

Despite the relevant findings, information on the antifungal potential of essential oils obtained from *Thymus vulgaris* and *Origanum vulgare* against *Lasiodiplodia theobromae* remains insufficient, especially for species from the Andean region of Ecuador. Therefore, the objective of this study is to evaluate the antifungal and antioxidant capacity of these oils compared to their commercial counterparts through *in vitro* and *in vivo* assays. In addition, identifying relevant biomarkers with the combination of partial least squares discriminant analysis (PLS-DA) and heat maps to explore and understand the complex interactions between variables, with the premise of pointing out patterns and trends of molecules that are more associated with bioactive properties and obtaining valuable information about possible mechanisms of action of essential oils.

In this context, the purpose of this study is to present a sustainable alternative with the potential capacity to combat the growth of *L. theobromae in vitro* and *in vivo* studies. Consequently, to sustainably increase the shelf life of bananas and their commercial value [13,15,22]. In this way, a contribution is made to the fulfillment of Goal 12 of the 2030 Agenda for Sustainable Development [23] and to the National Biodiversity Strategy 2015–2030, providing an alternative to reduce food losses by carrying out responsible management of agroindustrial waste and replacing the use of chemical products that produce harmful effects on both the ecosystem and health [24,25].

2. Material and methods

2.1. Plant material

Thymus vulgare was collected in San Antonio de Pasa (−1.265626, −78.741699) and *Origanum vulgare* from Patate (−1.307191, −78.503035), Tungurahua-Ecuador. A sample of plant material was authenticated by an expert from the Herbarium of the Universidad del Azuay (Ecuador) with one specimen of each species, and they were deposited with vouchers No. 14192 and 14191, respectively. Samples were collected according to the methodology indicated in the contract for access to genetic resources with the Ministry of Environment of Ecuador with the code (MAATE-CMARG-2022-0569).

2.2. Extraction of the essential oil

The extraction was carried out using a series of steam distillations performed in cycles of 60 min each. The ratio of dry plant matter (250 g) to water was 1:10 (w/v). The oil was separated from the hydrolate in a Clevenger trap, and the EOs were treated with anhydrous sodium sulfate, refrigerated, and frozen for subsequent analysis [26]. The extraction of essential oils was carried out at different times of the year, but the antioxidant and antifungal evaluation were performed with EOs obtained in mid-2022 during the dry season.

2.3. Determination of the physical properties of essential oils

The guidelines recommended by the Argentine Food Code were followed, under the quality parameters specified therein to measure the physical properties of the essential oils, such as relative density, and polarimetric deviation using an Anton Paar polarimeter and refractive index measured by an Abbemat 300 refractometer [27].

2.4. DPPH• antioxidant activity assay

The ability to scavenge the DPPH• radical was determined by the procedure described by Viteri et al. (2021). Briefly, 50 μL of the sample was mixed with 150 μL of a solution of DPPH• (0.15 mM) dissolved in methanol with DMSO at 0.5 % in the dark for 30 min. Subsequently, absorbance was measured at 517 nm in a microplate reader (Cytation™ 5, BioTek). A calibration curve with Trolox was used, and the results were expressed in mg Trolox equivalent/g extract, calculated according to the equation $y = 0.6467X + 31.252$ ($R^2 = 0.9939$) adding the blank [28]. To calculate the IC_{50} of the essential oils, a serial dilution (4000 at 31.5 ppm) was performed, plotting a curve with the logarithm of the concentration versus the percentage of antioxidant activity. This value determines the necessary concentration of the oil to stabilize at 50 % to DPPH•, and allows a more precise measurement of the antioxidant efficacy of the sample, facilitating the interpretation of the results by being able to compare them with previous studies.

2.5. Ferric reducing antioxidant power (FRAP) assay

Antioxidant capacity was measured using the iron reduction method described by Viteri et al. [28]. The FRAP complex was prepared using 300 mM acetate buffer (pH 3.6), a solution of TPTZ (10 mM) dissolved in 40 mM hydrochloric acid, and a solution of ferric chloride (20 mM), in a 10:1:1 ratio (v/v/v). Twenty μl of each sample dissolved in methanol with DMSO at 0.5 % was taken and mixed with 180 μl of the FRAP solution, incubated at 25 °C for 30 min and its absorbance was measured at 595 nm (Cytation™ 5, BioTek). The calibration curve was constructed with Trolox previously adding the blank, and the results were expressed in mg Trolox equivalent/g of the essential oil, calculated according to the equation $y = 0.0096X + 0.0118$ ($R^2 = 0.9996$).

2.6. β-carotene bleaching assay

For this, 1 mL of β-carotene chloroform solution (1 mg/5 mL) was added to the flask containing 10 μL of linoleic acid and 100 μL tween 20. The mix was roto-evaporated at 40 °C for 6 min until the elimination of chloroform. Then, 25 mL of hydrogen peroxide was added. Briefly, 2.5 mL of the previously prepared solution was mixed with 100 μL of the sample diluted in methanol, incubated at 50 °C taking readings of the optical density at 470 nm at 0, 30, 60, 90, and 120 min. Butylhydroxytoluene (BHT) was used as a positive control at a concentration of 2000 ppm to observe a greater difference in degradation compared to the negative control. To determine the rate of β-carotene bleaching, the difference in spectral absorbance between the initial reading and the last bleaching reading that remains essentially linear divided by time was calculated. The antioxidant index was the ratio of the bleaching rate of the negative control (system with no test compound added) to the bleaching rate when a test compound was in the system. This was called the percentage of inhibition of the degradation of β-carotene (%AI) [29], applying the following formula:

$$\%AI = \frac{DR\ control - DR\ sample}{DR\ control} * 100$$

Where DR control is the degradation rate or bleaching rate of the control is calculated:

$$\text{DR control} = \ln(Aci / Act) / t$$

Aci = is the absorbance of the emulsion with the control or standard at 0 min.
Act = is the absorbance of the control at time t.
t = is the incubation time.
DR Sample is the degradation rate or bleaching rate of the control calculated:

$$\text{DR sample} = \ln(Asi / Ast) / t$$

Asi = is the absorbance of the emulsion with the extract or sample at 0 min.
Ast = is the absorbance of the sample at time t.
t = is the incubation time.

2.7. Determination of total phenolic content (TPC)

It was analyzed by the Folin-Ciocalteu method described by Viteri et al. [28]. Twenty μL of the sample was mixed with 100 μL of Folin-Ciocalteu reagent (1:10 v/v) and 80 μL of a Na_2CO_3 solution (7.5 %), incubated for 60 min at room temperature, and the absorbance of the resulting blue solution was measured at 760 nm in a microplate reader (Cytation™ 5, BioTek). The total phenol content was expressed as mg of gallic acid equivalent per liter of sample according to the equation $y = 0.0059X\text{-}0.0257$ ($R^2 = 0.997$) obtained from the standard graph of gallic acid.

2.8. Chemical characterization

The composition of the essential oils was analyzed two times a year, at the end of 2021 during the rainy season and in mid-2022 during the dry season. They were compared against the composition of their commercial counterparts using Agilent Technologies gas chromatography mass spectrometry equipment (GC 7890A system and MSD XL inert 5975C with triple-axis detector). An HP-5MS capillary column (30 m × 0.25 mm) with phenyl methylpolysiloxane as stationary phase (film thickness 0.25 μm) and ultrapure helium as carrier gas (1.2 mL/min) was used. Injection of 2.0 μL of the sample (10 mg/mL) was performed at 250 °C in splitless mode, and the oven temperature was maintained at 70 °C for 2 min, then increased to 285 °C at 5° per min. The temperature of the MSD transfer line was 280 °C and the ion source was 230 °C. Electron ionization of 70 eV was used, and data compounds were collected with the full scan mode (40–1000 *m/z*) on the quadrupole mass analyzer. Finally, the compounds were identified by comparing the mass spectra with the information available in the NIST 11, Wiley 9 database and the retention index using a series of n-alkanes (C7–C40) [30].

2.9. Determination of in vitro antifungal activity

The active culture of the fungus *L. thobromae* was obtained from the Culture Collection of Microorganisms of Biotechnology Research Center of Ecuador (CCM-CIBE) [31], and the assay was carried out by the poisoned medium method. For the essential oils, 5 concentrations were used (2000, 1000, 500, 250, and 100 ppm) dissolved in PDA with 1 % DMSO. A 4 mm diameter disc was seeded with the 7-day-old test pathogen and incubated at 28 °C until mycelial growth completely covered the surface of the negative control box. Five replicates per concentration were performed to obtain a statistically sound conclusion [32]. To calculate the area, the ImageJ program (NIH) was used, taking 4 different points, the percentage of mycelial growth inhibition (PIC) was determined by the following formula [16]:

$$\%\ PIC = \frac{\text{control diameter} - \text{treatment diameter}}{\text{control diameter}} * 100$$

2.10. In vivo antifungal capacity assay

Freshly harvested untreated banana fruits (cv. Poovan) were obtained from Grupo Agrícola Prieto located in El Oro, Ecuador. The *in vivo* study was carried out when the skin color of the fruits was light green, approximately 23 cm and about 40 mm in diameter. The fruits were disinfected with 70 % alcohol and air-dried. Spore suspensions of the fungal pathogen from a 30-day culture were prepared with distilled water and 0.05 % (v/v) Tween-80 and the culture surface was gently scraped with a rod. Suspensions were filtered through a six-layer gauze for removal of residual mycelium and the spore concentration was adjusted to 1×10^6 spores mL^{-1} using a Neubauer chamber [3,33,34].

For inoculation, fruits were uniformly wounded (5 mm deep and 4 mm wide) with a sterile corkscrew at the crown end, followed by inoculation using spore suspensions (20 μL) of *L. theobromae*. The respective treatments were then applied (Table 1), the inoculated samples were kept in plastic bags to preserve humidity and their initial weight was recorded [3,34,35]. The groups were: 1) Negative control group: fruits inoculated and treated with distilled water; 2) Positive control: fruits inoculated and treated with synthetic fungicide Mertect (0.15 %); 3) Control group: uninoculated and untreated fruits. The other two groups were inoculated and treated with essential oils (Table 1), and the concentration was calculated based on the minimum inhibitory concentration obtained in the *in vitro* assay.

The experiment was carried out twice simulating export conditions (14 °C and H: 80 %) for 28 days and shelf conditions for 4 more days (25 °C and H: 60 %), with 5 banana clusters, a total of 35 fruits for each group, the same ones that started the trial at the A1 (green) ripening stage [35]. The damage produced, and banana maturity was evaluated at 0, 7, 14, 21, 28, and 32 days; determined by visual quality index (1–6), peel color (1–7), crown rot disease severity (0–7), crown rot reduction (%), weight loss (%) [34].

2.11. Weight loss (%)

It was estimated by weighing the fruit on a balance (Isolab) on days 0, 7, 14, 21, 28, and 32. PP was determined by the formula: PP (%) = ((Pi-Pf)/Pi) x 100. Where Pi represents the initial weight and Pf is the final weight [34,36].

Table 1
Randomized experimental design of the formulation of treatments for *in vivo* antifungal analysis.

Treatment	Commercial EO (μL)	Extracted EO (μL)	Tween 80 (μL)	Mertect (μL)
CT (−)	–	–	50	–
CT (+)	–	–	–	150
CWI	–	–	–	–
T1	25	–	50	–
T2	–	50	50	–

*CWI: Control without inoculum; CT (−): negative control; CT (+): positive control with the commercial fungicide.

2.12. Visual quality index

It was graded according to external appearance [34], according to the existence of marks or lesions categorized on a scale of 1–6, where: 1: imperfections or serious lesions (inedible); 2: edibility limit; 3: marketability limit; 4: regular (moderate damage); 5: good (minor lesion); 6: excellent clean skin (no lesion).

2.13. Peel color

It was determined by a graphic scale scored from 1 to 7 to separate fruits according to their maturity stage (58), where: 1: dark green; 2: light green; 3: more green than yellow; 4: more yellow than green; 5: yellow with green tips; 6: all yellow; 7: yellow with spots [34].

2.14. Severity of crown rot disease

It was determined using the scale employed by Ref. [34], where: 0: no discoloration or mycelial growth on the crown; 1: limited discoloration or mycelial growth on the cut crown surface; 2: discoloration or mycelial growth less than 10 % of the crown area; 3: 11–40 % discoloration or mycelial growth on the crown area; 4: 41–70 % discoloration or mycelial growth in crown area; 5: 71–100 % discoloration or mycelial growth in crown area; 6: discoloration or mycelial growth advanced to finger stalks; 7: finger stalk rot occurs, causing fingers to fall off when handled.

2.15. Correlation analysis

To identify significant differences between groups of samples based on metabolites and the biological activities of the samples, partial least squares discriminant analysis (PLS-DA) was used, while to visualize expression patterns of these parameters in the different samples heatmap cluster analysis (HCA) was used. Both analyzes were performed in MetaboAnalyst 5.0.

2.16. Statistical analysis

A minimum of three records were taken for each analysis, represented as the mean ± standard deviation. These experimental data were subjected to one-way analysis of variance (ANOVA): The difference between means was compared with the HSD Tukey post-hoc test, using R version 4.3.2. The "AovBay" R package was also used for the application and visualization of the analysis [37]. The multivariate analysis and the Hotelling test were performed in InfoStat version September 29, 2020. Differences were considered significant at a probability of 5 %.

3. Results and discussion

3.1. Physical characterization of essential oils

Density, polarimetric deviation, and refractive index of essential oils were evaluated to estimate their quality and purity. As can be seen in Table 2, the essential oils obtained were compared with the commercial essential oils, and it can be seen that the values for both thyme and oregano were similar to their commercial counterparts. Most of the samples comply with the range established by the Argentine food code (AFC), except for the oregano EO extracted in this study, whose density was lower, suggesting that the quality of this oil was not that required at the established values [27].

These results obtained were compared with other studies, whose values are similar for both species; oregano reports 1.4727 nD, 0.923 g/mL, and 6° of rotation; while thyme has 1.4875 nD, 0.939 and 1° of optical rotation. In this same work, it is mentioned that the variation of the optical rotation is due to the degree of humidity of the raw material and the weather season at the moment of the extraction of the essential oils, since they observed variation between winter and spring. This allows us to deduce that differences in the climatic conditions in which the species of this study were cultivated influenced their quality attributes [38,39].

These results give us a premise of the antifungal potential that these essential oils can present, since this property is inherent to the purity and quality of these substances, it is known that they are directly related to the concentration of their components, such as in the

Table 2
Physical parameters of the essential oils, CAA (1995).

Parameters	T-EO			O-EO		
	Extracted	Commercial	Rank AFC	Extracted	Commercial	Rank AFC
nD	1.4966	1.4923	1.4900–1.5080	1.5028	1.5108	1.5020–1.5080
DR	0.9073[b]	0.9142[b]	0.890–0.945	0.8691[a]	0.9490[c]	0.938–0.963
DP	0	−4.6	0	0	−1.4	−2 a +3

T-EO: Essential oil of thyme; O-EO: Essential oil of oregano; nD: Refractive index at 20 °C; DR: Relative density (g/mL); DP: Polarimetric deviation (°); AFC: Argentine Food Code; a-c: Statistically significant difference ($P < 0.05$).

case of oxygenated terpenes that enhance the pharmacological activities, including the antifungal effect [40].

3.2. Free radical scavenging capacity (DPPH•)

The essential oils were compared with their commercial counterparts. We can appreciate in Fig. 1 that significantly higher activity was exhibited by the commercial oregano EO (IC_{50}: 0.69 ± 0.004 mg/mL), followed by the commercial EO (IC_{50}: 1.08 ± 0.05 mg/mL), and the extracted EO (IC_{50}: 1.11 ± 0.019 mg/mL) of thyme. The latter two being statistically different from each other ($P < 0.05$). Finally, we can say that the extracted oregano EO is very different from the other samples, with an IC_{50} of 40.56 ± 0.227 mg/mL.

As reported in the literature, there are IC_{50} values lower than 0.2 mg/mL for other species of the genus *Origanum*, as well as values up to 4.7 mg/mL [41,42]. On the other hand, the antioxidant activity of thyme EOs is similar to species from various locations in China (IC_{50} = 0.512–0.931 mg/mL). This variation can be attributed to the different climatic conditions of plant cultivation, among other factors [43].

Due to the above described, it can be highlighted that commercial EOs present higher activity than those extracted in this study. This may be because the industry has standardized processes in their production. However, the results showed that all the samples were moderately antioxidants, except the extracted oregano EO, which showed low activity. This difference can be attributed to the cultivation conditions, processing techniques, the genetic and chemical composition between oregano species affecting the quality of the EO. As we observed in the previous section, this sample did not meet the quality rank of the AFC [39,41,42].

The difference between species which grown in different areas is influenced at an epigenomic level, since under certain climatic conditions, plants can express their genes to increase the production of specific compounds in order to respond to environmental stimuli [44]. This is the case of oregano, that cultivated at a high altitude presents a decline in the main constituents, showing a considerable loss in the concentration of carvacrol, one of the molecules responsible for the antioxidant activity of this species [45,46].

3.3. Ferric ion reducing potential (FRAP)

The Fe-reducing power of the essential oils ranged from 21.85 to 117.24 mg Eq Trolox/g essential oil, with the highest activity of the commercial oregano essential oil, while the oil of the same species extracted in this study had the lowest activity. On the other hand, thyme essential oils showed good activity, but with a lower value than the commercial oregano EO. It is worth mentioning that all samples are significantly different ($P < 0.05$) (Fig. 2).

The antioxidant activity of the oregano essential oil samples in this study were lower than those reported by previous studies of the same species, but of Italian and Chilean origin [46,47]. On the other hand, the thyme samples were slightly lower than the species from Italy (126.87 mg Eq Trolox/g) [47], but higher than other species of Spanish origin, such as *T. vulgaris* (12.69 mg Eq Trolox/mL), and other species of this genus such as *T. mastichina* (19.26 mg Eq Trolox/mL), *T. zygis* (49.56 mg Eq Trolox/mL) and *T. capitatus* (58.12 mg Eq Trolox/mL) [48].

Also, it can be noted that the reducing power of commercial thyme EO (391.01 mmol Eq Trolox/L) extracted in this study (372.33 mmol Eq Trolox/L) was higher than the range reported in another study, where the effect of different drying conditions of this species was evaluated against the antioxidant activity, where a range of 32.49–108.48 mmol Eq Trolox/L was obtained [49].

3.4. Antioxidant activity measured by β-carotene bleaching

The oxidation of fatty acids present in foods is an indication of their deterioration, representing a challenge faced by the food industry. Although currently there are substances that can prevent this oxidation, efforts for natural alternatives have increased [50].

To evaluate the ability of the samples to prevent fatty acid oxidation, the color intensity of β-carotene was measured during an oxidation reaction in the presence of the samples and a reference antioxidant (BHT) at a wavelength of 470 nm [29].

In Fig. 3A–B we can observe the behavior of the essential oils to BHT at a concentration of 2000 ppm, where the thyme essential oil extracted in this study was an inhibitor of linoleic acid oxidation significantly higher to the rest with 65.71 ± 0.4 %, followed by oregano EO (57.46 ± 1.6 %), commercial thyme EO (51.97 ± 0.66 %), and commercial oregano EO (43.58 ± 1.56 %).

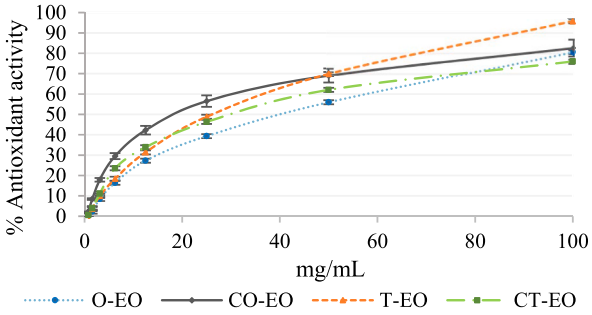


Fig. 1. Mean inhibitory concentration (IC_{50}) of essential oils. A: Curve formed by antioxidant activity versus concentration; CO-EO: commercial oregano essential oil; CT-EO: commercial thyme essential oil; T-EO: essential oil extracted from thyme; O-EO: essential oil extracted from oregano.

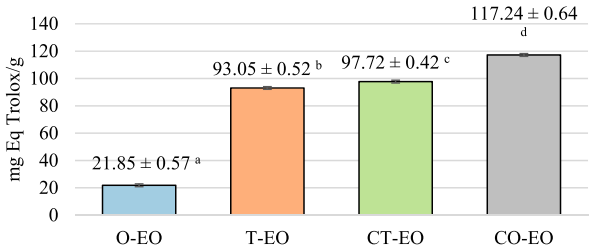


Fig. 2. Ferric ion reducing potential of essential oils mg Eq Trolox/g: Milligrams Trolox equivalent per gram of essential oil; A: FRAP-reducing activity of the samples; CO-EO: commercial oregano essential oil; CT-EO: commercial thyme essential oil; T-EO: essential oil extracted from thyme; O-EO: essential oil extracted from oregano * Different letters show a statistically significant difference ($P < 0.05$).

The results for Thyme EO were lower than those reported in a previous study (>75 %) that evaluated the antioxidant capacity by the carotenoid bleaching method for six months, finding that it tends to increase its potential during storage [51].

On the other hand, the antioxidant potential of the oregano EOs studied was lower than that of the *vulgare* subspecies (99.89 ± 0.04 %), but higher than that presented by the *hirtum* subspecies (23.54 ± 1.24 %). This implies that the variability of genetics among the species could be related to their effectiveness in preventing lipid oxidation [52].

These results suggest that both samples of essential oil revealed the potential use in the food industry, due to their ability to inhibit the oxidation of lipids such as linoleic acid. This effect is associated with the individual and synergistic presence of unsaturated terpenes and phenolic compounds such as thymol and carvacrol, for their ability to transfer electrons or hydrogen atoms [53,54].

3.5. Total phenolic content

To measure the total phenolic content, the Folin-Ciocalteu method is widely used to identify the reducing capacity of a matrix. However, this technique is not specific for detecting phenolic substances, since the result is affected by the presence of other structures [46].

Essential oils, we can observe (Fig. 4) that the following order can be distinguished: Extracted oregano EO and thyme EO of the commercial brand followed by the extracted thyme EO in this study present a high content in total polyphenols, and with a great difference is the commercial oregano EO, according to the statistical analysis there is a significant difference in the mean of all samples. Comparing these data with those reported in the literature, we can highlight that the first three samples have a high phenolic content, even higher than that of *O. vulgare* and *T. Vulgaris* species from Morocco (up to 54.38 mg GAE/g) [55].

By counteracting the high antioxidant activity of the commercial oregano sample against a low equivalent of total phenols by the Folin-Ciocalteu technique, we can determine that the latter should not be an individual quality parameter, but rather which must be complemented with more antioxidant techniques, since it does not discriminate the matrix of the sample, that is, it can reflect higher values as the number of metabolites increases, so a sample may contain fragmented molecules resulting from the degradation of its main compounds and yield a high result by this method of analysis [46,56,57].

3.6. Chemical characterization of essential oils

To compare the results obtained in the chemical characterization by GC-MS, the main components were considered to be those that exceeded 0.1 % concentration in the samples; the rest were considered trace components. To identify them, compounds with a similarity in the retention index (±10 %) to the calculated retention index and a match greater than 700 were chosen.

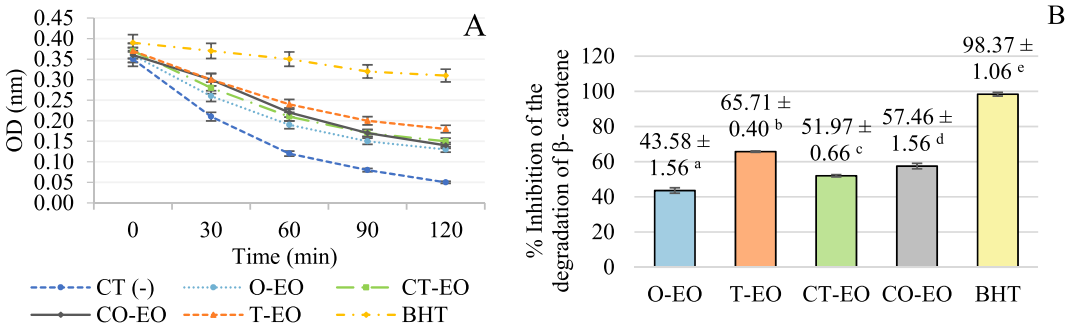


Fig. 3. The absorbance of β-carotene in the presence of essential oils, BHT and negative control. A: Behavior of the samples with respect to time; B: Percentage of antioxidant activity; OD: Optical desity; CO-EO: commercial oregano essential oil; CT-EO: commercial thyme essential oil; T-EO: essential oil extracted from thyme; O-EO: essential oil extracted from oregano; CT (+): Positive control BHT 2000 ppm; CT (−): Negative control. * Different letters show a statistically significant difference ($P < 0.05$).

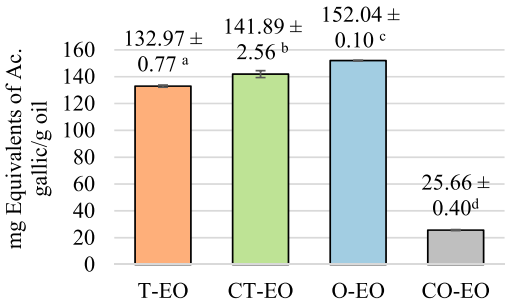


Fig. 4. Total phenolic content of the essential oils. A: Values of the phenolic content of the samples; B: Bayesian graph; CO-EO: commercial oregano essential oil; CT-EO: commercial thyme essential oil; T-EO: essential oil extracted from thyme; O-EO: essential oil extracted from oregano. * Different letters present a statistically significant difference ($P < 0.05$).

Table 3 shows that 28 compounds were identified for commercial thyme EO, representing 97.69 % of the total composition, with thymol (43.57 %), *p*-cymene (17.37 %), γ-terpinene (8.83 %), linalool (7.10 %) and carvacrol (5.25 %) as the major compounds.

In the sample of thyme EO extracted in 2021, 96.07 % of its constitution was recognized with 37 compounds, of which the most abundant were thymol (30.85 %), *p*-cymene (17.67 %), γ-terpinene (17.35 %), and caryophyllene (5.58 %). In the essential oil of this species, obtained in 2022, 23 compounds were recognized, corresponding to 92.09 % of the total content, with thymol (45.78 %), aloaromadendrene (20.53 %) and β-cadinene (6.03 %) standing out.

On the other hand, the commercial oregano essential oil consists of 35 components, representing 97.11 % of the essential oil. The major components were carvacrol (62.06 %), linalool (6.87 %), and *p*-cymene (4.64 %). In the case of the sample extracted from this species in 2021, the identity of 48 molecules consisting 87.99 % of the essential oil was obtained, highlighting *p*-cymene (15.17 %), γ-terpinene (12.79 %), *trans*-β-ocimene (8.85 %), thymol (7.32 %), and thymol methyl ether (7.04 %) with the highest concentration. While from the extracted oregan, 23 compounds were identified that constitute 96.93 % of its totality. We can highlight as the majority components alloaromadendrene (25.17 %), γ-gurjunene (20.79 %), β-cadinene (14.81 %), β-bisabolene (6.93 %), and γ-elemene (6.69 %). When comparing the constitution of the essential oils extracted from *O. vulgare* species, we can highlight that the commercial oil has a higher percentage of phenolic monoterpenes (carvacrol and thymol), 35 times higher than the extracted EO.

This variation is attributable to the influence that climatic conditions have on the biosynthesis of secondary metabolites in this species. Previous studies have shown that the yield of carvacrol in regions of high altitude is lower. In this sense, it is reported that in the arid region of Chile at 3000 m above sea level (m.a.s.l.) they obtained a percentage of relative abundance of 5.6 % [46], while in the one carried out in India at various altitudes (1650–3200 m.a.s.l.) 1.1 % of carvacrol was detected at the maximum altitude [58], in both references we can observe that the thymol content is higher than 15 % (Table 3).

In our case the samples that reflect a lower content of carvacrol were grown in the Andean region of Ecuador at 2577 m.a.s.l., and the yield was lower in both climatic periods of the year compared to the commercial sample. The literature also relates that the lower the proportion of the main phenolic monoterpenes, the higher the concentration of their precursors (*p*-cymene and γ-terpinene), in our case this is only reflected in the sample obtained in 2021 [59].

In the case of the essential oils obtained from the species *Thymus vulgaris*, the phenolic monoterpenes count was higher in the extracted sample (49.70 %) while the commercial sample was 48.82 %. It is known that in low humidity environments, thyme is capable of producing in greater quantity the most complex metabolites such as thymol and carvacrol. Besides genetic diversity is also related to the chemical polymorphism that is presented in thymol, as shown in a study of differentiation of the chemotypes of this species, which depending on the variety and location produce its major metabolites (Table 3) [60].

3.7. Determination of in vitro antifungal activity

The evaluation was carried out over 3 days, where the antifungal activity of the samples was compared with the control. The results showed that the most effective treatments were commercial oregano essential oil, commercial thyme EO, and extracted thyme EO with an inhibition percentage of 100 %, while extracted oregano essential oil only showed 27.28 % (Fig. 5A–E).

The most effective samples were then diluted and evaluated at 100, 250, 500, 1000, and 2000 ppm to find the minimum inhibitory concentration. The order according to their effectiveness values were as follows: Commercial oregano > Commercial thyme > Thyme. Note that for commercial oils the MIC is 250 ppm while for thyme EO it is 500 ppm (Fig. 6 and Table 4).

Comparing these results with literature reports of antifungal effects against *L. thobromae* of various essential oils, we can evidence a high antifungal potential in the EOs of this study. For example, the EO of *Varronia curassavica* species had only 78.3 % of activity at a concentration of 30000 ppm [17]; while the total inhibition of the pathogen produced by South African thyme is reported between 500 and 1000 ppm, and another species of the genus *Origanum* is obtained at 2000 ppm [18].

The high antifungal activity observed can be explained by the inactivation of the conidia of the fungus when they were in contact with the volatile compounds of the essential oils. The vapor is stronger than the liquid EO, and when it comes into contact with the structure of the fungus it delays the formation of the spores, and induces damage to their coating because their lipophilic properties. This phenomenon facilitates the integration of these substances into the lipid layer to the internal part of the biological membrane,

Table 3
Composition of essential oils obtained by GC-MS.

Compounds	RI	Thyme			Oregano		
		C	2021	2022	C	2021	2022
β-Thujene	873	0.77	1.84			0.80	
Camphene	943	0.89	0.40		0.16		
L-β-Pinene	943	0.29					
β-Pinene	943		0.27				
3-Carene	948		0.15				
d-α-Pinene	948	1.14	0.80		0.43	0.35	
α-Pinene	948						0.73
3-Octanone	952					0.22	
β-Myrcene	958	1.64	1.81		1.01	1.67	
Morillol	969		0.20		0.17	0.39	
α-Phellandrene	969	0.16					
trans-β-Ocimene	976					8.85	
β-Ocimene	976					1.32	
α-Terpinene	998	1.68	2.90		0.98	0.60	
γ-Terpinene	998	8.83	17.35	1.87	0.45	12.79	1.71
D-Limonene	1018	0.67	0.63		0.39	0.55	
p-Cymene	1042	17.37	17.67	1.37	4.64	15.17	0.43
Terpinolene	1052		0.13	0.16	3.28		
Eucalyptol	1059	0.33	0.97		0.34		
Linalol	1082	7.10	2.92		6.87	0.18	
2.6-Dimethyl-3,5,7-octatriene-2-ol, E, E	1090					0.26	
α-Pinene oxide	1095					0.15	
trans-2-Caren-4-ol	1136					0.21	
4-Terpineol	1137	1.66	0.75		1.61	0.21	
L-4-terpineol	1137	0.81					
2-Camphanol	1138		0.39				
Camphol	1138	0.91			1.44		
Isoborneol	1138		0.37				
α-Terpineol	1143	0.15	0.18		0.32		
(Z)-β-Terpineol	1158	0.36	1.39		0.34		
Desconocido	1158	0.31					
Geranial	1174					4.49	
Neral	1174					3.56	
p-Menth-8-en-2-one, trans	1179		0.11				
Carvone	1190				0.31		
p-Cymenol-8	1197		0.10				
6-Nonynoic acid, methyl ester	1200					0.22	
trans-*p*-mentha-1(7),8-dien-2-ol	1201					0.18	
Epoxy-linalooloxide	1224					0.20	
Isothymol methyl ether	1231	0.32		0.15	0.40		0.58
Thymol methyl	1231						3.48
Thymol methyl ether	1231	0.20		0.13		7.04	
1,4-dihydroxy-*p*-menth-2-ene	1259					0.29	
Carvacrol	1262	5.25	2.93	3.92	62.06	0.14	
Thymol	1262	43.57	30.85	45.78	2.92	7.32	1.84
Acetic acid, 1,7,7-trimethyl-bicyclo [1,2,2]	1277			0.12			
Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)	1280					0.20	
Isoaromadendrene epoxide	1281				0.23		
Cuminol	1284			0.41			
Bicyclo (3,1) hexane-6-methanol	1322				0.31		
Propanoic acid, 2-methyl, 3-hydroxy-2,4,4-trimethylpentyl ester	1331		0.90				
3-Methyl-2-((2E)-2-penteny)-2-cyclopenten-1-one	1338				0.15		
β-Bourbonene	1339		0.21	0.63		1.30	1.83
β-Cubebene	1339					0.12	
Thymoquinone	1340			0.32			
Geranic acid	1342					1.05	
Neric acid	1342					1.55	
α-cubebene	1344						0.58
2,2-Dimethyl-1-(2-hydroxypropyl)	1347		0.88				
Methyleugenol	1361				0.13		
δ-Eiemene	1377			1.46			
1H-Cycloprope-azulene, decahydro-1,1,7-trimethyl-4-methylene	1386	0.10					
Alloaromadendrene	1386			20.53			25.17
Aromadendrene	1386				0.19	0.14	
β-Elemene	1398						0.13
β-Gurjunene	1403						2.68
Ledene	1419				0.12		

(*continued on next page*)

Table 3 (*continued*)

Compounds	RI	Thyme			Oregano		
		C	2021	2022	C	2021	2022
Carvacrol acetate	1421				0.25		
α-Bergamotene	1430		0.12				
Humulene	1431	0.16					
γ-Elemene	1431			1.01			6.69
γ-Cadinene	1435		0.19	0.79			
γ-Muurolene	1435		0.54			0.10	
α-Muurolene	1440		0.11	0.32			
β-Cadinene	1440			6.03			14.81
2-Tridecanone	1449					0.32	
γ-Gurjunene	1461			1.00			20.79
δ-Cadinene	1469	0.12	0.40		0.24	0.10	
Eremophilene	1474						0.27
β-Vatirenene	1489						0.52
Caryophyllene	1494	2.40	5.58		1.62	2.75	
Isocaryophyllene	1494		0.21				
γ-Himachalene	1499						0.50
β-Bisabolene	1500			0.41	3.83	1.04	6.93
Caryophylene oxide	1507	0.40	0.91	2.55	0.65	3.94	
Epoxycaryophyllene	1507					0.13	
ᴅ-Germacrene	1515		0.52				
α-Bisabolene (Z)	1518						0.13
β-Guaiene	1523			1.05		0.11	
Globulol	1530					0.41	
Viridiflorol	1530					0.13	
trans-Z-α-Bisabolene epoxide	1531					0.13	
(−)-Spathulenol	1536	0.10	0.15		0.40	6.09	2.49
Ethynyl-1, 4a-dimethyl-4a,5,6,7,8, 8a-hexahydro-2(1H)-naphtjalenone	1548						3.08
Durohzdroquinone	1574			1.95			
Cubenol	1580				0.11	0.18	
T-Cadinol	1580				0.47		
α-Cadinol	1580				0.16		
Humulene oxide II	1592					0.81	
α-acorenol	1598					0.13	
γ-Eudesmol	1626		0.24				
1-(2-(3-Methyl-3-(5-methyl-2-furyl) butyl)-2-oxiranyl) ethanone	1643						1.31
4.4-Dimethyl-3-(3-methylbut-2-enylidene) octane-2,7-dione	1670					0.10	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2045						0.23
Androst-5-en-3-one, 4,4-dimethyl	2085			0.11			

RI: Retention index; C: Commercial.

affecting its integrity, increasing the penetration of hydrogen and potassium ions and through an interaction with membrane proteins causing an alteration by unspecified means [20,61–63]. Various studies also mention possible mechanisms of action of EOs against phytopathogens. Poonam et al. pointed out that the presence of thymol led to an apotopsis of *Candida* and Cryptococcus related to an alteration of the membrane due to the low level of ergosterol [64]. Kowalczyk et al. highlights the increase in oxidative stress due to the generation of reactive oxygen species. The decrease in the extracellular polymeric matrix and the capsular polysaccharide, caused by the interference of thymol in the metabolite of fatty acids [63,65].

3.8. Determination of antifungal activity in vivo

In the analysis of the *in vitro* antifungal activity, greater efficacy was evidenced by the thyme EO. These results served as the basis for the *in vivo* assay on healthy banana fruits of export category previously sterilized and inoculated with the test pathogen. From their macroscopic characteristics, we can highlight that the symptoms of infection in bananas appear on day 7 after inoculation. However, when observing the state of the crown in the spectroscope, the formation of mycelial structures around the inoculation orifice can be appreciated in some of the biological units of all the groups (Fig. 7).

On day 14 of the trial, a clear differentiation between the treatments was evident, which was able to group them into two different categories. In the first category, bananas showed discoloration or mycelial growth in the crown area of about 41 % and 70 %. The positive control proved to be the most effective, while groups T1, and T5 presented similar characteristics to each other. Finally, the negative control presented the most advanced infection effects, showing that the antifungal effect of the treatments depends directly on the concentration (%) of the extracts, which confirms what is found in the literature that postulates the activity of the EOs as dose-dependent [3,22] (Table 5).

By Day 21, the signs of the disease were progressing, but the behavior of the samples was similar to that of the previous week. From Day 28 onwards, the negative control showed an accelerated advance of the disease, which was even evident in the finger stalks; this shows that the Treatments mentioned were not able to stop the development of the disease during storage under simulated export

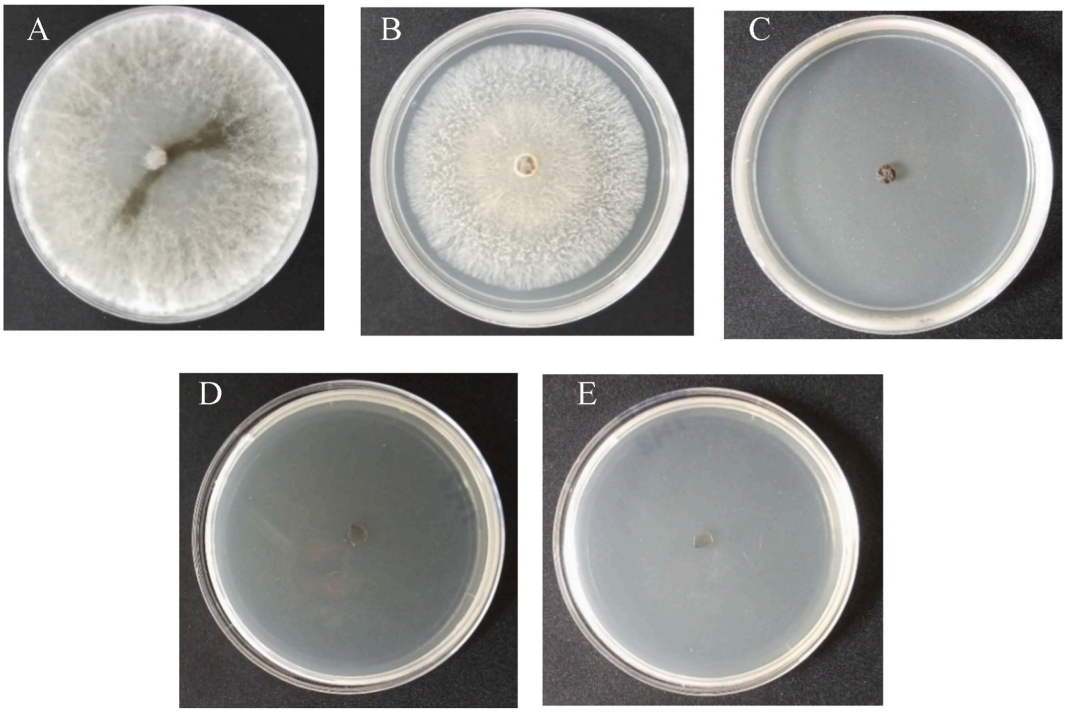


Fig. 5. Effect of oregano and thyme essential oils at 2000 ppm against mycelial growth of *L. theobromae*. A: Negative control; B: Oregano EO; C: Thyme EO; D: Commercial oregano EO: E: Commercial thyme EO.

conditions. During the last four days of testing under simulated marketing conditions, we can see that the least signs of the disease were shown in the positive control, followed by the control without inoculum, T2, and T1. In terms of weight loss, the best results were obtained in the groups treated with the commercial fungicide (Mertect), T1 and T2, which were statistically similar. This means that the application of commercial essential oil (250 ppm) and extracted essential oil (500 ppm) have a promising effect in preventing weight loss in bananas (Fig. 8).

Another parameter evaluated was the visual quality of the fruit on a scale of 1–6, with 6 being the best. At the beginning of the trial, most of the bananas had a score of 5, that is, a good appearance with a minimal lesion. After the application of the Treatments, a clear differentiation between the groups was observed, as can be seen in Fig. 9.

We can observe that at the end of the trial, the fruits of the negative control showed severe lesions and were considered inedible, which is less than 2; while the quality of groups T1, T2, and the control without inoculation were statistically equal to the positive control, they were at the limit of edibility. On the other hand, the control without inoculum showed a quality close to the limit of marketability. These effects on the final quality of the fruit can be attributed not only to the inoculation of the pathogen but also to the change in storage conditions, starting on day 28, when they went from storage at 14–25 °C, a temperature close to that at which the pathogen presents its maximum development. We can also point out that the Treatments that presented the least loss of quality were also the ones with the greatest active effect, as evidenced in the crown rot index.

In addition, using means of the color of the peel, we were able to show the level of maturity of the fruits, with accelerated maturity being one of the main signs of infection by *L. theobromae*. The results indicated that the control without inoculum and the positive control presented the lowest maturity rating, with the skin tone of the fruits being considered more yellow than green (Fig. 10).

The treatment T2 presented fruit with a yellow surface and green tips, while the T2 treatment had the same color as the negative control, had a yellow surface with spots. However, the statistical analysis showed that there were no significant differences between the groups ($P < 0.05$).

Finally, Table 6 shows the summary of banana fruit quality after 32 Days of testing under simulated export conditions (14 °C and H: 80 %) and shelf conditions (25 °C and H: 60 %), expressed as the area of the curve formed by the elapsed Days versus the values of each parameter [6].

When performing the multivariate analysis and contrasting the different factors as a whole, it became evident that the positive control was the treatment that reported the best characteristics in all evaluations, with statistically similar effects to T1 and T2. This shows that globally Treatments with essential oils serve to delay the rotting of the banana crown. This is in agreement with literature reports in which essential oils report antifungal effects in the postharvest stage. Esquivel demonstrated that thyme EO vapor inhibits mango decay by controlling the development of *C. gloeosporioides* [20]. Zarla et al. studied the behavior of *B. cinerea* on strawberries treated with *Daucus carota* subsp. EO at a concentration (%) of 0.01 mL/L, showed a preventive antifungal effect of 100 % and a protective effect of 80 % for 7 days [66].

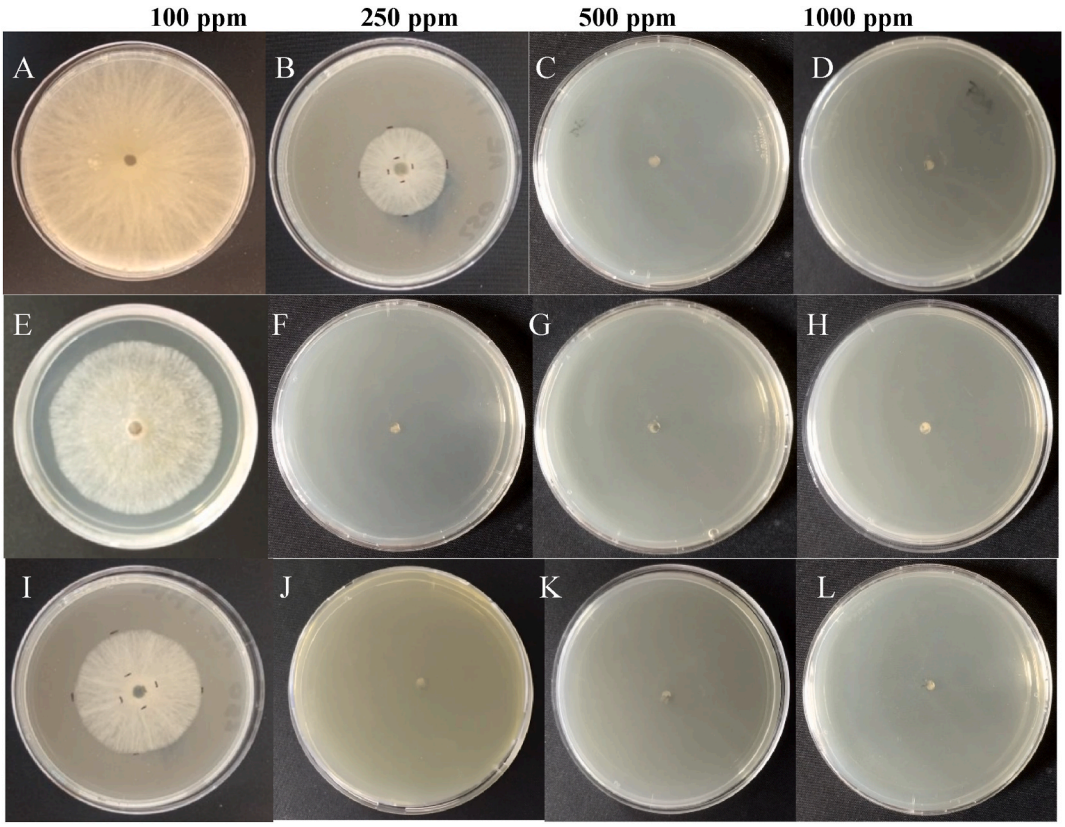


Fig. 6. Effect of essential oils on mycelial growth of *L. theobromae*. A–D: thyme EO; E–H: commercial thyme EO; I–L: commercial oregano EO; of which were used in concentrations of 100 (A, E & I), 250 (B, F & J), 500 (C, G & K) and 1000 (D, H & L) ppm.

Table 4
Minimum inhibitory concentration (MIC) in oregano and thyme essential oil samples.

Samples	Inhibition of pathogen growth (%)				
	100 ppm	250 ppm	500 ppm	1000 ppm	2000 ppm
CO-EO	64.48 ± 1.65^{a}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
CT-EO	52.99 ± 3.00 ^{ab}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
T-EO	0.08 ± 0.50^{c}	41.54 ± 2.10^{b}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}

* Different letters present a statistically significant difference in the non-parametric Kruskal-Wallis test ($P < 0.05$).
CO-EO: commercial oregano essential oil; CT-EO: commercial thyme essential oil; T-EO: essential oil extracted from thyme.

In bananas, studies have also been conducted against *L. theobromae*, Kulkarni showed that EO vapors from *Monarda fistulosa* caused a significant reduction of the crown rot in banana fruits inoculated with LT and *C. musae*, during the 7 Days trial. Renganathan reported that thyme EO was able to reduce the disease by 81.1 % compared to the control during the 12-day trial [67].

On the other hand, the use of beneficial microorganisms such as *Trichoderma viride* demonstrated to reduce up to 65.06 % of the rot in artificially inoculated bananas. Also showed better results when applied before the inoculation with *L. theobromae*, indicating its protective effect against the diplodia [68].

Also, the capacity of other types of extracts to inhibit the development of *L. theobromae* and *C. musae* in banana fruits has been tested with an aqueous extract of Zimmu, which prolonged the useful life of banana up to 64 days stored in cold at 14 °C. This effect was reduced to an inhibition of crown rot of 86 % for 12 days when changing the storage conditions to 28 °C [69].

These effects may be because Treatments interact with the pathogen, one of the typical mechanisms of destruction of the fungal membrane by EOs is the inhibition of chitin synthase, an enzyme responsible for the formation of chitin, an important component of the fungal wall [3].

In addition, they generate an increase in the defense mechanism of bananas. Sangeetha's team found that when bananas were immersed in an aqueous extract of Zimmu, there was an increase of phenolic compounds, the elevation of the action of the enzymes chitinase, β-1,3-glucanase and phenylalanine ammonia-lyase. It is presumed that in this way the banana acquires resistance against pathogens [70].

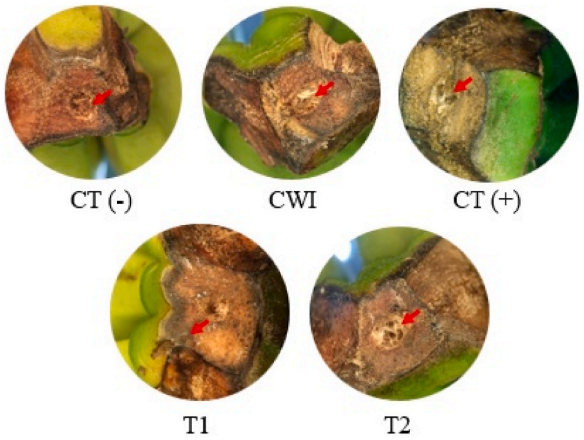


Fig. 7. Effect of essential oils on mycelial growth of *L. Theobromae* CSI: control without inoculum; CT (−): negative control; CT (+): positive control; T1: Essential oils of Commercial Thyme at 250 ppm; T2: Essential oil extracted of Thyme at 500 ppm.

Table 5
Effect of treatments applied to bananas inoculated with *L. theobromae*, measured by the banana crown rot index (0–7).

Treatments	Day 0	Day 7	Day 14	Day 21	Day 28	Day 32	Groups
CWI	0 ± 0.00	0 ± 0.00	4.8 ± 0.20	4.8 ± 0.20	5.2 ± 0.20	5.4 ± 0.24	ab
CT (−)	0 ± 0.00	0 ± 0.00	5.4 ± 0.24	5.4 ± 0.24	6.4 ± 0.24	6.4 ± 0.24	bc
CT (+)	0 ± 0.00	0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.6 ± 0.24	4.8 ± 0.20	a
T1	0 ± 0.00	0 ± 0.00	4.6 ± 0.24	4.4 ± 0.24	5.2 ± 0.20	5.6 ± 0.24	ab
T2	0 ± 0.00	0 ± 0.00	4.6 ± 0.24	4.6 ± 0.24	5.6 ± 0.20	5.4 ± 0.40	abd

CWI: control without inoculum; CT (−): negative control; CT (+): positive control; T1: Essential oils of Commercial Thyme at 250 ppm; T2: Essential oil extracted of Thyme at 500 ppm.
*Different letters present a statistically significant difference ($P < 0.05$).

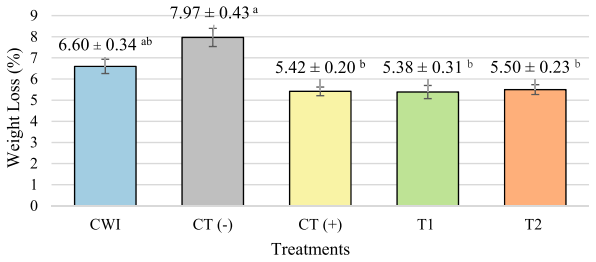


Fig. 8. Total weight loss of treated bananas. CWI: control without inoculum; CT (−): negative control; CT (+): positive control; T1: Essential oils of Commercial Thyme at 250 ppm; T2: Essential oil extracted of Thyme at 500 ppm. *Different letters present a statistically significant difference ($P < 0.05$).

Finally, it is worth mentioning that several studies reported that the design of coatings and active packaging with microencapsulation of metabolites has served to control the severity and incidence of postharvest diseases in fruits such as mango and Okra [20, 71]. This is an alternative to redesigning the formula of the treatments used in this research and enhancing their resulting effect [72].

3.9. Correlation

To analyze the behavior of the essential oils, a partial least squares discriminant analysis was performed, identifying their constituents and antifungal (*in vitro*) and antioxidant (DPPH•) activity as discriminating characteristics, since they present a $P < 0.05$ and a VIP score>1, in addition the *P* value obtained from the permutation is also less than 0.05 (Fig. 11).

Fig. 11, shows us that the concentration (%) of (−)-spathulenol, β-bisabolene, γ-gurjunene is a differentiating characteristic in oregano EO; whereas, for the extracted thymol EO it is thymol and δ-cadinene, for the commercial EO of this species it is *p*-cymene, camphene, γ-terpinene, d-α-pinene, and thymol methyl ether.

What we can highlight from these observations is that thyme EOs contain differentiating compounds reported in the literature to have high antioxidant and antifungal activity. On the other hand, Commercial oregano EO also shows a high representation of this type

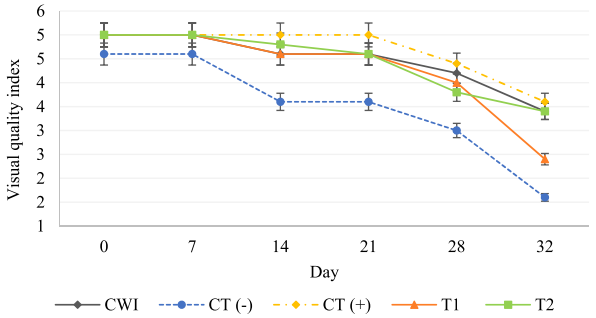


Fig. 9. Degradation of the visual quality of banana fruits. CSI: control without inoculum; CT (−): negative control; CT (+): positive control; T1: Essential oils of Commercial Thyme at 250 ppm; T2: Essential oil extracted of Thyme at 500 ppm. *Different letters present a statistically significant difference ($P < 0.05$).

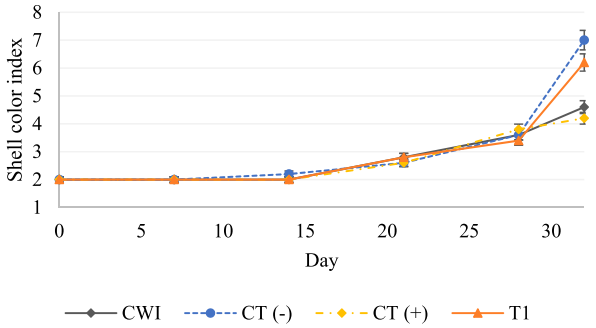


Fig. 10. *Progress of fruit ripening as evidenced by skin color*. CWI: control without inoculum; CT (−): negative control; CT (+): positive control; T1: Essential oils of Commercial Thyme at 250 ppm; T2: Essential oil extracted of Thyme at 500 ppm.

Table 6
A general summary of the effect of the Treatments in the *in vivo* study.

Treatments	CRI	WL	VQ	SC	Groups
CWI	2.73 ± 0.08	10.93 ± 2.03	18.25 ± 3.08	31.9 ± 5.19	a
CT (−)	3.21 ± 0.13	13.81 ± 0.35	24.03 ± 0.46	41.06 ± 0.93	e
CT (+)	2.36 ± 0.07	10.36 ± 1.29	16.92 ± 1.84	29.64 ± 3.19	c
T1	2.68 ± 0.09	12.28 ± 0.74	21.16 ± 1.10	36.11 ± 1.86	cd
T2	2.76 ± 0.17	12.16 ± 1.73	20.32 ± 2.63	35.24 ± 4.46	cd

CWI: control without inoculum; CT (−): negative control; CT (+): positive control; CRI: Crown rot index; WL: weight loss; VQ: visual quality; SC: Skin color; T1: Essential oils of Commercial Thyme at 250 ppm; T2: Essential oil extracted of Thyme at 500 ppm.
*Different letters present a statistically significant difference ($P < 0.05$).

of structure [22,66].

The mechanisms related to its antifungal action highlight the ability of monoterpernes such as *p*-cymene, carvacrol and thymol to suppress the synthesis of ergosterol, the brlA, abaA and wetA genes, regulators of fungal development, in addition to the negative regulation of the aflD, aflK, aflQ and aflR genes. This causes an inhibition of mycelial growth, spore production and toxin production of *Aspergillus flavus* [73]. Something interesting to note is that apparently *p*-cymene is not effective against filamentary fungi in previous studies against *Rhizopus oryzae* and *Aspergillus niger*. However, being in a majority concentration but combined with other monoterpernes, it exhibits a high antifungal power against *A. flavus*, *Saccharomyces cerevisiae* and *A. niger*, possibly due to a synergistic effect of the EO components [74].

These results allow us to highlight the differentiating parameters in the essential oil samples because it can be seen the concentration (%) and the differential effect of the metabolites and their antioxidant as well as their antifungal effects. Finally, to demonstrate the relationship between the EO metabolites and the properties evaluated, Pearson's correlation was used utilizing heat maps, where the positively correlated results are colored red, and the inversely proportional parameters are colored blue (Fig. 12).

We can appreciate that the antifungal activity is highly related to the antioxidant activity measured by FRAP, DPPH• and total phenolic content. As for its direct dependence on the concentration (%) of secondary metabolites, we can highlight that the highest relationship is with *p*-cymene, carvacrol, linalool, eucalyptol, 4-terpineol, (z)-β-terpineol, camphol, caryophyllene, β-myrcene, D-limonene, α-terpinene, α-terpineol, D-α-pinene, camphene and caryophyllene oxide. In addition, it is also moderately related to the

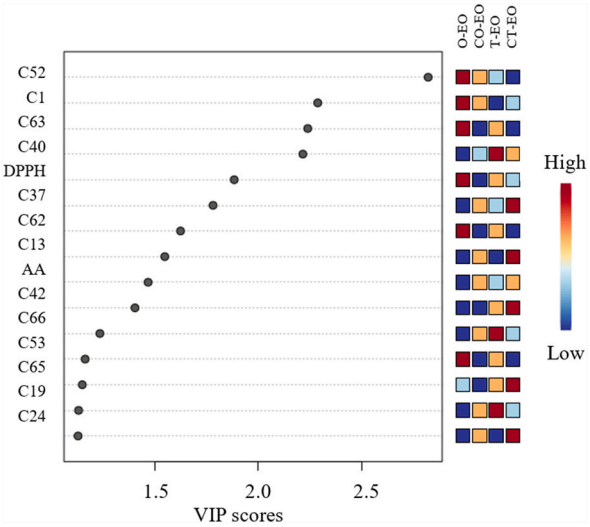


Fig. 11. Partial Least Squares Discriminant Analysis (PLS-DA) C1: (−)-Spathulenol; C13: Camphene; C19: Caryophyllene oxide; C24: d-α-Pinene; C37: *p*-Cymene; C40: Thymol; C42: Thymol methyl ether; C52: β-Bisabolene; C53: β-Bourbonene; C62: γ-Elemene; C63: γ-Gurjunene; C65: γ-Terpinene and C66: δ-Cadinene; AA: antifungal activity; CO-EO: commercial oregano essential oil; CT-EO: comercial thyme essential oil; T-EO: essential oil extracted from thyme; O-EO: essential oil extracted from oregano.

percentage of δ-cadinene, terpinolene, and thymol.

To be precise, the essential oils that were shown to effectively inhibit the growth of *L. theobromae* were constituted of these molecules. The EO of *M. fistulosa* was composed mainly of thymol, carvacrol and cinnamyl carbanilate and achieved its action at 66.66 ppm (3); the EO of thyme, which also contained thymol, achieved its effect at 500 ppm [75].

From what is reported in the literature, it is known that the greatest antifungal activity of essential oils is presented by terpenes and terpenoids. This effect is enhanced by their phenolic structure and the addition of the hydroxyl group. This explains why thymol, carvacrol, and L-α-terpineol are related to effective antifungal activity. Other structures presenting phenolic rings that have promising effects were δ-cadinene, L-4-terpineol, γ-terpinene [3,66].

Thymol and carvacrol are generally related to the level of antioxidant and antifungal effect of oregano and thyme EOs [76]. This relationship has been proven by *in vivo* and *in silico* studies, the latter was performed demonstrating the interaction of these molecules with the enzyme responsible for the production of an important component of the fungal wall [3,72].

Another important observation to note, is that there is a highly inverse relationship between the antifungal effect in proportion to the following metabolites: alloaromadendrene, β-elemene, β-gurjunene, β-cadinene, α-bisabolene, (Z) thymol methyl ethynyl-1,4a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1H)-naphtjalenone, α-cubebene, β-vatirenene, eremophilene, 1-(2-(2-(3-methyl-3-(5-methyl-2-furyl) butyl) -2-oxiranyl) ethanone, α-pinene, γ-himachalene, β-bourbonene, γ-gurjunene and 3,7, 11,15-tetramethyl-2-hexadecen-1-ol.

In the literature we can appreciate that the EO of *V. curassavica* Jacq contains a high percentage of alloaromadendrene, but a low antifungal potential since it is necessary to use 30000 ppm to obtain an inhibition of this pathogen of 78.35 %, coinciding with the results of this correlation [17]. In addition to the individual effect of these molecules, it is known that essential oils are generally greater than the action of their compounds, inducing the possibility of a synergy between their constituents that could potentiate their biological activities [22,66]. These results allow us to identify the metabolite profile that influence on the effect of essential oils against *L. theobromae*, thus establishing the relative concentration (%) as quality parameters of the samples.

4. Conclusion

The relationship between the antifungal and antioxidant activity of essential oils (EO) was revealed a strong association between these bioactivities and certain secondary metabolites such as *p*-cymene, carvacrol, γ-terpinene and thymol. It is evident that the EOs that had a higher content of these molecules able to inhibit *L. theobromae* under *in vitro* conditions and their antioxidant potential was greater. Furthermore, antifungal activity is inversely related to certain metabolites such as alloaromadendrene and α-pinene. On the other hand, thyme EO stands out for its antifungal potential, since in the *in vivo* test it demonstrated a performance analogous to the positive control, in terms of crown rot index, weight loss, visual quality and color of the fur. Furthermore, a possible synergy between the constituents of EOs is suggested that could enhance their biological activity. Thus, establishing quality parameters for the use of thyme EO as active ingredients in antifungal formulations, and consolidating it as a promising raw material. Based on these findings, we recommend carrying out new studies that deepen the understanding of the interactions between the components of EOs and their response in terms of biological activity. These additional evaluations would be essential to expand our knowledge of this topic and its implications.

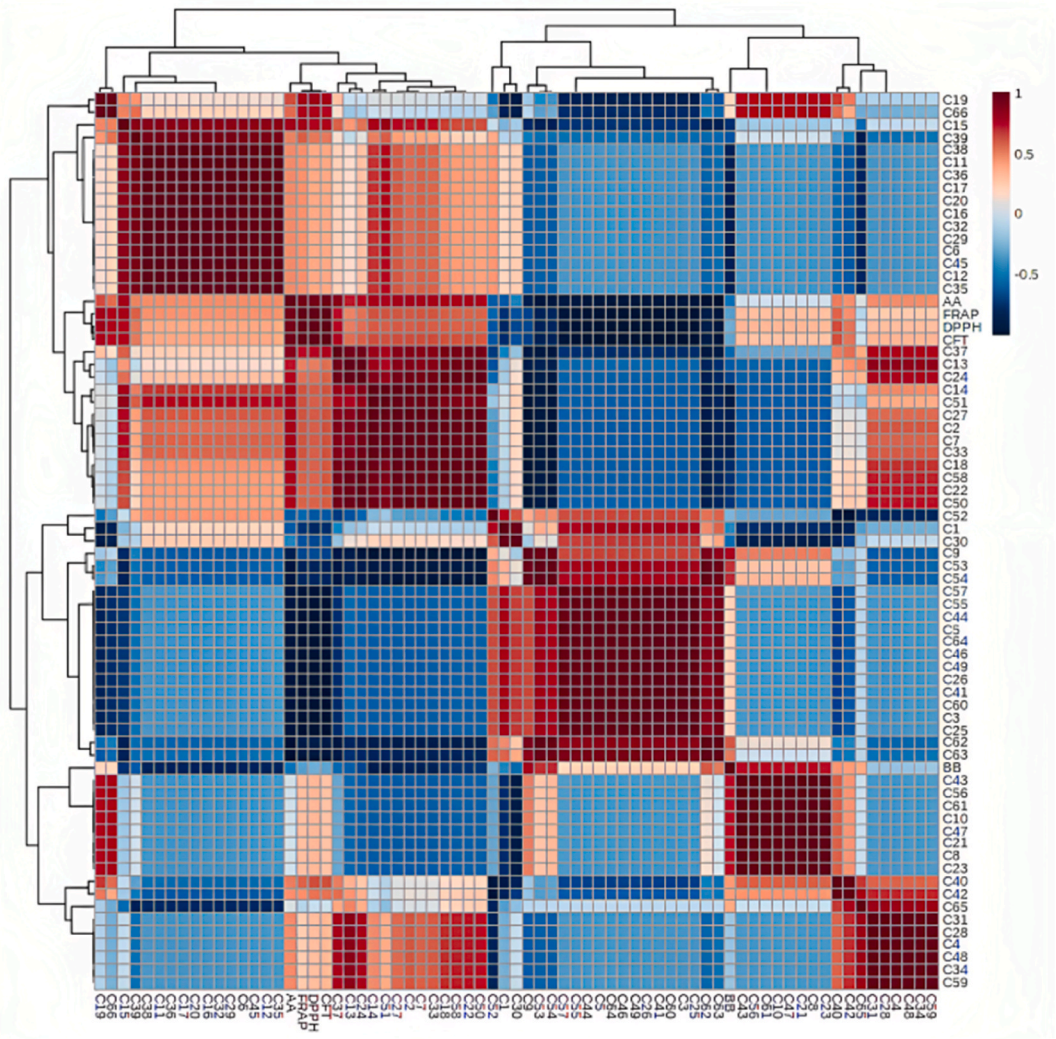


Fig. 12. Heat map of the existing correlation between variables. AA: activity antifungal; BB: technique by bleaching β-carotene.

Funding statement

This work was supported by ESPOL Polytechnic University and VLIR NETWORK Ecuador.

Data availability statement

Research data is not openly published due to intellectual property considerations.

Additional information

During the preparation of this work, the authors used CHATGPT to improve the readability of the text. After using this tool, the authors reviewed and edited the content as necessary and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Glenda Pilozo: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Writing – original draft, Supervision, Methodology, Investigation. **Mirian Villavicencio-Vásquez:** Writing – original draft, Methodology, Investigation, Formal analysis. **Ivan Chóez-Guaranda:** Writing – original draft, Methodology, Investigation, Formal analysis. **Damon Vera Murillo:** Writing – original draft, Resources, Formal analysis. **Cynthia Duarte Pasaguay:** Writing – original draft, Resources, Formal analysis. **Christofer Tomalá Reyes:** Writing – original draft, Resources, Formal analysis. **Maria Maldonado-Estupiñán:** Writing – original

draft, Resources, Formal analysis, Conceptualization. **Omar Ruiz-Barzola:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Fabián León-Tamariz:** Writing – review & editing, Supervision, Methodology. **Patricia Manzano:** Writing – review & editing, Supervision, Methodology.

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.

References

[1] G.-M. Carrión-Loaiza, Análisis del producto interno bruto agrícola ecuatoriano y sus principales productos en el periodo 2002 – 2019, Dom Cien 6 (2020) 940–952.
[2] BCE, Evolución de la Balanza Comercial por Productos Enero - Diciembre 2022, 2023.
[3] S.A. Kulkarni, P.S. Sellamuthu, S.K. Nagarajan, T. Madhavan, E.R. Sadiku, Antifungal activity of wild bergamot *(Monarda fistulosa)* essential oil against postharvest fungal pathogens of banana fruits, South Afr. J. Bot. 144 (2022) 166–174, https://doi.org/10.1016/j.sajb.2021.08.019.
[4] A. Deknock, N. De Troyer, M. Houbraken, L. Dominguez-Granda, I. Nolivos, W. Van Echelpoel, et al., Distribution of agricultural pesticides in the freshwater environment of the Guayas river basin (Ecuador), Sci. Total Environ. 646 (2019) 996–1008, https://doi.org/10.1016/j.scitotenv.2018.07.185.
[5] J.E.E. Olalla, F.C.F. Lugo, M.A.M. Montanares, Uso de organofosforados por agricultores de la comunidad de Guaslán-Ecuador y los cambios hematológicos, Rev Cienc y Agric 17 (2020) 31–50.
[6] J. Yan, J. Zhang, C. Hu, L. Deng, M.A. Ritenour, Use of carvacrol and thymol in shellac coating to control stem-end rot on 'Ruby Red' grapefruit and maintain fruit quality during simulated storage and marketing, Sci. Hortic. (Amst.) 272 (2020) 109606, https://doi.org/10.1016/j.scienta.2020.109606.
[7] C.A. Brühl, M. Arias Andres, S. Echeverría-Sáenz, M. Bundschuh, A. Knäbel, F. Mena, et al., Pesticide use in banana plantations in Costa Rica – a review of environmental and human exposure, effects and potential risks, Environ. Int. 174 (2023) 107877, https://doi.org/10.1016/j.envint.2023.107877.
[8] Viana T. Lopes, W. Moya Peres, David G. Queiroz, D. Lopes de Matos, O. Ribeiro Campos, Mitsuo Yamashita P. Oscar, et al., Efect of essetial oils on the "*in vitro*" micelial growth of the fungus Lasiodiplodia theobromae, South Am J Basic Educ Tech Technol 7 (2020) 301–310.
[9] J.T.K. Kwembe, O. Onautshu, P.T. Mpiana, P. Vermeir, G. Haesaert, Chemical composition of essential oil of ageratum conyzoides with antifungal activity on the *Lasiodiplodia theobromae* strain in the region of kisangani and DR Congo, Arch Curr Res Int (2021) 12–23.
[10] A.B. Perumal, P.S. Sellamuthu, R.B. Nambiar, E.R. Sadiku, Effects of essential oil vapour treatment on the postharvest disease control and different defence responses in two mango (*Mangifera indica L.*) cultivars, Food Bioprocess Technol. 10 (2017) 1131–1141, https://doi.org/10.1007/s11947-017-1891-6.
[11] G. He, H. Matsuura, T. Yoshihara, Isolation of an α-methylene-γ-butyrolactone derivative, a toxin from the plant pathogen *Lasiodiplodia theobromae*, Phytochemistry 65 (2004) 2803–2807, https://doi.org/10.1016/j.phytochem.2004.08.011.
[12] E.M. Deresa, T.F. Diriba, Phytochemicals as alternative fungicides for controlling plant diseases: a comprehensive review of their efficacy, commercial representatives, advantages, challenges for adoption, and possible solutions, Heliyon 9 (3) (2023) e13810.
[13] F.A.R. León, L.B.C. Benítez, R.M.B. Narváez, La percepción de los costos de producción del banano orgánico en el Cantón Machala, Ecuador, Gestión En El Terc Milen 20 (2018) 45–50.
[14] B. Xu, Y. Gan, S. Zhang, J. Zhang, Identification of the antifungal activity of *Trichoderma longibrachiatum* T6 and assessment of bioactive substances in controlling phytopathgens, Pestic. Biochem. Physiol. (2018), https://doi.org/10.1016/j.pestbp.2018.02.006.
[15] D. da C. Gonçalves, W.R. Ribeiro, D.C. Gonçalves, L. Menini, H. Costa, Recent advances and future perspective of essential oils in control *Colletotrichum spp.*: a sustainable alternative in postharvest treatment of fruits, Food Res. Int. 150 (2021) 110758, https://doi.org/10.1016/j.foodres.2021.110758.
[16] M.F. Alves, DA. de C. Nizio, T.S. Sampaio, AF do Nascimento, F. de A. Brito, JO de Melo, et al., *Myrcia lundiana* Kiaersk native populations have different essential oil composition and antifungal activity against *Lasiodiplodia theobromae*, Ind. Crops Prod. 85 (2016) 266–273, https://doi.org/10.1016/j.indcrop.2016.03.039.
[17] DA. de C. Nizio, F. de A. Brito, T.S. Sampaio, J. de O. Melo, FLS da Silva, P.R. Gagliardi, et al., Chemical diversity of native populations of *Varronia curassavica* Jacq. and antifungal activity against *Lasiodoplodia theobromae*, Ind. Crops Prod. 76 (2015) 437–448, https://doi.org/10.1016/j.indcrop.2015.07.026.
[18] S. Combrinck, T. Regnier, G.P.P. Kamatou, *In vitro* activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit, Ind. Crops Prod. 33 (2011) 344–349, https://doi.org/10.1016/j.indcrop.2010.11.011.
[19] J.H. Linde, S. Combrinck, T.J.C. Regnier, S. Virijevic, Chemical composition and antifungal activity of the essential oils of *Lippia rehmannii* from South Africa, South Afr. J. Bot. 76 (2010) 37–42, https://doi.org/10.1016/j.sajb.2009.06.011.
[20] F. Esquivel-Chávez, C. Colín-Chávez, J.J. Virgen-Ortiz, M.Á. Martínez-Téllez, R. de J. Avena-Bustillos, G. Peña-Madrigal, et al., Control of mango decay using antifungal sachets containing of thyme oil/modified starch/agave fructans microcapsules, Futur Foods 3 (2021) 100008, https://doi.org/10.1016/j.fufo.2020.100008.
[21] M.A.A. Mangoba, D. de G. Alvindia, Fungicidal activities of *Cymbopogon winterianus* against anthracnose of banana caused by Colletotrichum musae, Sci. Rep. 13 (2023) 6629, https://doi.org/10.1038/s41598-023-33396-5.
[22] C. Brito, H. Hansen, L. Espinoza, M. Faúndez, A.F. Olea, S. Pino, et al., Assessing the control of postharvest gray mold disease on tomato fruit using mixtures of essential oils and their respective hydrolates, Plants 10 (2021), https://doi.org/10.3390/plants10081719.
[23] PNUD. Programa de las Naciones Unidas para el Desarrollo n.d. https://www1.undp.org/content/undp/es/home/sustainable-development-goals/goal-12-responsible-consumption-and-production.htmL.
[24] Ministerio del Ambiente del Ecuador, Estrategia Nacional de Biodiversidad 2015-2030 (2016). Quito, Ecuador, http://maetransparente.ambiente.gob.ec/documentacion/WebAPs/EstrategiaNacionaldeBiodiversidad2015-2030-CALIDADWEB.pdf.
[25] PNUD, Programa de las Naciones Unidas para el Desarrollo, 2021. https://www1.undp.org/content/undp/es/home.htmL.
[26] T.I.M. Ragab, Gendy ANG. El, I.A. Saleh, M.A. Esawy, Chemical composition and evaluation of antimicrobial activity of the *Origanum majorana* essential oil extracted by microwave-assisted extraction, conventional hydro-distillation and steam distillation, J Essent Oil Bear Plants 22 (2019) 563–573, https://doi.org/10.1080/0972060X.2019.1611486.
[27] Código Alimentario Argentino (CAA), Capitulo XVI. Correctivos Y Coadyuvantes, 1995.
[28] R. Viteri, A. Giordano, G. Montenegro, F. Zacconi, Eucryphia cordifolia extracts: phytochemical screening, antibacterial and antioxidant activities, Nat. Prod. Res. (2021) 1–5, https://doi.org/10.1080/14786419.2021.1960525.
[29] K. Chebbac, A.E.L. Moussaoui, M. Bourhia, A.M. Salamatullah, A. Alzahrani, R. Guemmouh, Chemical analysis and antioxidant and antimicrobial activity of essential oils from *Artemisia negrei* L. Against drug-resistant microbes, Evidence-Based Complement Altern Med 2021 (2021) 5902851, https://doi.org/10.1155/2021/5902851.
[30] I. Chóez-Guaranda, A. Ortega, M. Miranda, P. Manzano, Chemical composition of essential oils of Passiflora edulis f. flavicarpa agroindustrial waste, Emir. J. Food Agric. (2017) 458–462.
[31] M.G. Maridueña-Zavala, A. Quevedo, K. Aguaguiña, L. Serrano, D. Sosa del Castillo, The active culture of the fungus L. thobromae was obtained from the Culture Collection of Microorganisms of Biotechnology Research Center of Ecuador (CCM-CIBE), Bionatura 6 (2021) 1664–1668.

[32] J.E.R. Guimarães, B. de la Fuente, M.B. Pérez-Gago, C. Andradas, R. Carbó, B.-H. Mattiuz, et al., Antifungal activity of GRAS salts against *Lasiodiplodia theobromae in vitro* and as ingredients of hydroxypropyl methylcellulose-lipid composite edible coatings to control Diplodia stem-end rot and maintain postharvest quality of citrus fruit, Int. J. Food Microbiol. 301 (2019) 9–18, https://doi.org/10.1016/j.ijfoodmicro.2019.04.008.

[33] T. Odetayo, L. Sithole, S. Shezi, P. Nomngongo, S. Tesfay, N.Z. Ngobese, Effect of nanoparticle-enriched coatings on the shelf life of Cavendish bananas, Sci. Hortic. (Amst.) 304 (2022) 111312, https://doi.org/10.1016/j.scienta.2022.111312.

[34] D.G. Alvindia, An integrated approach with hot water treatment and salt in the control of crown rot disease and preservation of quality in banana, Int. J. Pest Manag. 59 (2013) 271–278, https://doi.org/10.1080/09670874.2013.845927.

[35] T. Odetayo, L. Sithole, S. Shezi, P. Nomngongo, S. Tesfay, N.Z. Ngobese, Effect of nanoparticle-enriched coatings on the shelf life of Cavendish bananas, Sci. Hortic. (Amst.) 304 (2022) 111312, https://doi.org/10.1016/j.scienta.2022.111312.

[36] J. Li, Q. Sun, Y. Sun, B. Chen, X. Wu, T. Le, Improvement of banana postharvest quality using a novel soybean protein isolate/cinnamaldehyde/zinc oxide bionanocomposite coating strategy, Sci. Hortic. (Amst.) 258 (2019) 108786, https://doi.org/10.1016/j.scienta.2019.108786.

[37] J.J. Pambabay Calero, M.J. Rojas-Campuzano, S.A. Bauz-Olvera, O.H. Ruiz-Barzola, AovBay: an R package for application and visualization of parametric non-parametric and bayesian ANOVA, Rev. Colomb. Estadística 47 (2024) 87–109, https://doi.org/10.15446/rce.v47n1.108065.

[38] B. Cáceres, V. Rozo, E. García, Estudio de la calidad de aceites esenciales de orégano, tomillo y romero cultivados en Severino (El Carmen, Jujuy) recolectados en invierno y primavera, Rev Científica FCA 14 (2021) 7–18.

[39] Joint FAO, WHO Food Standards Programme Codex Committee on Spices and Culinary Herbs, 3rd Sess, 2017.

[40] Salha G. Ben, R. Herrera Díaz, O. Lengliz, M. Abderrabba, J. Labidi, Effect of the chemical composition of free-terpene hydrocarbons essential oils on antifungal activity, Molecules 24 (2019), https://doi.org/10.3390/molecules24193532.

[41] P.N. Paudel, P. Satyal, R. Satyal, W.N. Setzer, R. Gyawali, Chemical composition, enantiomeric distribution, antimicrobial and antioxidant activities of *Origanum majorana* L. Essential oil from Nepal, Molecules 27 (2022), https://doi.org/10.3390/molecules27186136.

[42] A. Khadhri, I. Bouali, C. Aouadhi, M. Lagel, E. Masson, A. Pizzi, Determination of phenolic compounds by MALDI–TOF and essential oil composition by GC–MS during three development stages of *Origanum majorana*L, Biomed. Chromatogr. 33 (2019) e4665.

[43] T. He, X. Li, X. Wang, X. Xu, X. Yan, X. Li, et al., Chemical composition and anti-oxidant potential on essential oils of *Thymus quinquecostatus* Celak. from Loess Plateau in China, regulating Nrf2/Keap1 signaling pathway in zebrafish, Sci. Rep. 10 (2020) 11280, https://doi.org/10.1038/s41598-020-68188-8.

[44] C. Köhler, N. Springer, Plant epigenomics—deciphering the mechanisms of epigenetic inheritance and plasticity in plants, Genome Biol. 18 (2017) 132, https://doi.org/10.1186/s13059-017-1260-9.

[45] D. Bisht, C.S. Chanotiya, M. Rana, M. Semwal, Variability in essential oil and bioactive chiral monoterpenoid compositions of Indian oregano (*Origanum vulgare* L.) populations from northwestern Himalaya and their chemotaxonomy, Ind. Crops Prod. 30 (2009) 422–426.

[46] M.J. Simirgiotis, D. Burton, F. Parra, J. López, P. Muñoz, H. Escobar, et al., Antioxidant and antibacterial capacities of *Origanum vulgare* L. essential oil from the arid Andean Region of Chile and its chemical characterization by GC-MS, Metabolites 10 (2020) 414.

[47] M. Pellegrini, A. Ricci, A. Serio, C. Chaves-López, G. Mazzarrino, S. D'Amato, et al., Characterization of essential oils obtained from *Abruzzo autochthonous* plants: antioxidant and antimicrobial activities assessment for food application, Foods 7 (2018) 19.

[48] C. Ballester-Costa, E. Sendra, J. Fernández-López, J.A. Pérez-Álvarez, M. Viuda-Martos, Assessment of antioxidant and antibacterial properties on meat homogenates of essential oils obtained from four *Thymus* species achieved from organic growth, Foods 6 (2017) 59.

[49] J. Rodriguez, E.C. Melo, A. Mulet, J. Bon, Optimization of the antioxidant capacity of thyme (*Thymus vulgaris* L.) extracts: management of the convective drying process assisted by power ultrasound, J. Food Eng. 119 (2013) 793–799, https://doi.org/10.1016/j.jfoodeng.2013.07.016.

[50] A. El Moussaoui, F.Z. Jawhari, A.M. Almehdi, H. Elmsellem, K. Fikri Benbrahim, D. Bousta, et al., Antibacterial, antifungal and antioxidant activity of total polyphenols of *Withania frutescens* L, Bioorg. Chem. 93 (2019) 103337, https://doi.org/10.1016/j.bioorg.2019.103337.

[51] C. Kirkin, G. Gunes, Quality of thyme (*Thymus vulgaris* L.) and black pepper (*Piper nigrum* L.) during storage as affected by the combination of gamma-irradiation and modified atmosphere packaging, South Afr. J. Bot. 150 (2022) 978–985, https://doi.org/10.1016/j.sajb.2022.09.005.

[52] C. Sarikurkcu, G. Zengin, M. Oskay, S. Uysal, R. Ceylan, A. Aktumsek, Composition, antioxidant, antimicrobial and enzyme inhibition activities of two *Origanum vulgare* subspecies (subsp. *vulgare and subsp. hirtum*) essential oils, Ind. Crops Prod. 70 (2015) 178–184, https://doi.org/10.1016/j.indcrop.2015.03.030.

[53] E.L. Chávez-Delgado, D.A. Jacobo-Velázquez, Essential oils: recent advances on their dual role as food preservatives and nutraceuticals against the metabolic syndrome, Foods 12 (2023) 1079.

[54] M. Olszowy, A.L. Dawidowicz, Essential oils as antioxidants: their evaluation by DPPH, ABTS, FRAP, CUPRAC, and β-carotene bleaching methods, Monatshefte Für Chemie - Chem Mon 147 (2016) 2083–2091, https://doi.org/10.1007/s00706-016-1837-0.

[55] H. Ait Bouzid, S. Oubannin, M. Ibourki, L. Bijla, A. Hamdouch, E.H. Sakar, et al., Comparative evaluation of chemical composition, antioxidant capacity, and some contaminants in six Moroccan medicinal and aromatic plants, Biocatal. Agric. Biotechnol. 47 (2023) 102569, https://doi.org/10.1016/j.bcab.2022.102569.

[56] G.R. Martins, A.F. Monteiro, F.R.L. do Amaral, A.S. da Silva, A validated Folin-Ciocalteu method for total phenolics quantification of condensed tannin-rich açaí (Euterpe oleracea Mart.) seeds extract, J. Food Sci. Technol. 58 (2021) 4693–4702, https://doi.org/10.1007/s13197-020-04959-5.

[57] J. Pico, R.Y. Pismag, M. Laudouze, M.M. Martinez, Systematic evaluation of the Folin–Ciocalteu and Fast Blue BB reactions during the analysis of total phenolics in legumes, nuts and plant seeds, Food Funct. 11 (2020) 9868–9880, https://doi.org/10.1039/D0FO01857K.

[58] D. Bisht, C.S. Chanotiya, M. Rana, M. Semwal, Variability in essential oil and bioactive chiral monoterpenoid compositions of Indian oregano (Origanum vulgare L.) populations from northwestern Himalaya and their chemotaxonomy, Ind. Crops Prod. 30 (2009) 422–426.

[59] K. Mechergui, W. Jaouadi, J.P. Coelho, M.L. Khouja, Effect of harvest year on production, chemical composition and antioxidant activities of essential oil of oregano (Origanum vulgare subsp glandulosum (Desf.) Ietswaart) growing in North Africa, Ind. Crops Prod. 90 (2016) 32–37.

[60] Z. György, N. Incze, Z. Pluhár, Differentiating *Thymus vulgaris* chemotypes with ISSR molecular markers, Biochem Syst Ecol 92 (2020) 104118, https://doi.org/10.1016/j.bse.2020.104118.

[61] A. Marchese, C.R. Arciola, E. Coppo, R. Barbieri, D. Barreca, S. Chebaibi, et al., The natural plant compound carvacrol as an antimicrobial and anti-biofilm agent: mechanisms, synergies and bio-inspired anti-infective materials, Biofouling 34 (2018) 630–656, https://doi.org/10.1080/08927014.2018.1480756.

[62] Z.E. Suntres, J. Coccimiglio, M. Alipour, The bioactivity and toxicological actions of carvacrol, Crit. Rev. Food Sci. Nutr. 55 (2015) 304–318, https://doi.org/10.1080/10408398.2011.653458.

[63] M.R. Khan, C. Huang, H. Zhao, H. Huang, L. Ren, M. Faiq, et al., Antioxidant activity of thymol essential oil and inhibition of polyphenol oxidase enzyme: a case study on the enzymatic browning of harvested longan fruit, Chem Biol Technol Agric 8 (2021) 61, https://doi.org/10.1186/s40538-021-00259-y.

[64] P. Kumari, N. Arora, A. Chatrath, R. Gangwar, V. Pruthi, K.M. Poluri, et al., Delineating the biofilm inhibition mechanisms of phenolic and aldehydic terpenes against Cryptococcus neoformans, ACS Omega 4 (2019) 17634–17648.

[65] A. Kowalczyk, M. Przychodna, S. Sopata, A. Bodalska, I. Fecka, Thymol and thyme essential oil—new insights into selected therapeutic applications, Molecules 25 (2020) 4125.

[66] A. Tabet Zatla, M.E.A. Dib, N. Djabou, F. Ilias, J. Costa, A. Muselli, Antifungal activities of essential oils and hydrosol extracts of *Daucus carota* subsp. sativus for the control of fungal pathogens, in particular gray rot of strawberry during storage, J. Essent. Oil Res. 29 (2017) 391–399, https://doi.org/10.1080/10412905.2017.1322008.

[67] P. Renganathan, R. Karan, S. Dhaarani, K.R. Saravanan, S. Devi, Effect of plant oils and plant extracts for the management of crown rot of banana caused by *Lasiodiplodia theobromae*, Turkish Online J Qual Inq 11 (2020) 266–276.

[68] M.G. Mortuza, L.L. Ilag, Potential for biocontrol of *Lasiodiplodia theobromae* (pat.) griff. & maubl. In banana fruits by *Trichoderma* species, Biol. Control 15 (1999) 235–240, https://doi.org/10.1006/bcon.1999.0716.

[69] G. Sangeetha, R. Thangavelu, S. Usha Rani, A. Muthukumar, Antimicrobial activity of medicinal plants and induction of defense related compounds in banana fruits cv. Robusta against crown rot pathogens, Biol. Control 64 (2013) 16–25, https://doi.org/10.1016/j.biocontrol.2011.12.013.

[70] G. Sangeetha, R. Thangavelu, S. Usha Rani, A. Muthukumar, Antimicrobial activity of medicinal plants and induction of defense related compounds in banana fruits cv. Robusta against crown rot pathogens, Biol. Control 64 (2013) 16–25, https://doi.org/10.1016/j.biocontrol.2011.12.013.
[71] Q. Zhang, Z. Wang, Y. Li, X. Liu, L. Liu, J. Yan, et al., Thymol edible coating controls postharvest anthracnose by regulating the synthesis pathway of Okra lignin, Foods 12 (2023) 395.
[72] A. Culmone, G. Mirabile, I. Tinebra, M. Michelozzi, A. Carrubba, M.G. Bellardi, et al., Hydrolate and EO application to reduce decay of *Carica papaya* during storage, Horticulturae 9 (2023) 204.
[73] F. Tian, S.Y. Woo, S.Y. Lee, H.S. Chun, p-Cymene and its derivatives exhibit antiaflatoxigenic activities against Aspergillus flavus through multiple modes of action, Appl Biol Chem 61 (2018) 489–497, https://doi.org/10.1007/s13765-018-0382-4.
[74] A. Marchese, C.R. Arciola, R. Barbieri, A.S. Silva, S.F. Nabavi, A.J. Tsetegho Sokeng, et al., Update on monoterpenes as antimicrobial agents: a particular focus on p-cymene, Mater (Basel, Switzerland) 10 (2017) E947, https://doi.org/10.3390/ma10080947.
[75] T.K. Mendy, A. Misran, T.M.M. Mahmud, S.I. Ismail, Antifungal properties of *Aloe vera* through *in vitro* and *in vivo* screening against postharvest pathogens of papaya fruit, Sci. Hortic. (Amst.) 257 (2019) 108767, https://doi.org/10.1016/j.scienta.2019.108767.
[76] T. Novoa, B.S.E. Brito, Análisis comparativo de las propiedades fisicoquímicas y capacidad antioxidante de un morfotipo de orégano (*Origanum vulgare* L.) cultivado en dos localidades de la sierra ecuatoriana, Siembra 8 (2021).