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Identification of antifungal essential oils for control of brown spot pathogens of 'Rocha' pear

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Summary. Brown spot of pear (BSP) is a major concern in 'Rocha' pear production. Essential oils were identified with *in vitro* antifungal properties against fungi isolated from BSP, and encapsulated formulations were developed via spray-drying. Four fungal isolates from symptomatic leaf and fruit tissues of 'Rocha' pears were identified as *Stemphylium vesicarium* (isolates MUM 26.01 and MUM 26.02) and *Alternaria arborescens* (isolates MUM 26.03 and MUM 26.04) through multilocus sequencing. Sensitivity of these isolates was assessed to five fungicides commonly used in Portuguese orchards. Tebuconazole and cyprodinil were the most effective across isolates, while trifloxystrobin gave limited inhibition, indicating low sensitivity of these isolates. To assess sustainable alternatives, 18 essential oils (EOs) were screened, showing that lemongrass and lemon thyme EOs were the most promising candidates, completely inhibiting mycelium growth and spore germination of the fungi at low concentrations. Encapsulation of these two EOs on HI-CAP® 100, using spray-drying, preserved their antifungal activity. Chemical characterization showed that lemongrass and lemon thyme EOs consisted primarily of oxygenated monoterpenes, which were probably responsible for the high antifungal activity observed. These results highlight the potential of lemongrass and lemon thyme EOs as promising candidates for *in vivo* validation

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as sustainable alternatives to synthetic fungicides for control of *S. vesicarium* and *A. arborescens*, and management of BSP in 'Rocha' pear production.

Keywords. *Alternaria arborescens*, *Stemphylium vesicarium*, fungicide sensitivity, *in vitro* screening.

INTRODUCTION

'Rocha' pear (*Pyrus communis* L. 'Rocha') is a Portuguese pear variety recognized with Protected Designation of Origin (PDO) status. 'Rocha' pear is an important fruit crop in Portugal, with extensive cultivation area and significant economic value (Lomba-Viana *et al*., 2022). Production is primarily concentrated in Portugal's western region, where the disease brown spot of pear (BSP) has become a major concern, resulting in substantial yield reductions and consequent economic losses (Loebler *et al*., 2020).

Stemphylium vesicarium (Wallr.) E. Simmons is a plant pathogen particularly problematic for pear production, causing BSP, and is responsible for significant losses of production in Italy (where it was first reported in the mid-1970s), Spain, France, Belgium, Poland, and the Netherlands (Głos *et al*., 2023; Cavina *et al*., 2024). Although BSP mainly affects European pear production regions, it has recently been reported in Japan and Argentina (Temperini *et al*., 2022; Tudela *et al*., 2023a).

Stemphylium vesicarium has a dual lifecycle, with a saprophytic and a pathogenic phase (Llorente and Montesinos, 2006). During the saprophytic phase, the pathogen overwinters as pseudothecia on orchard debris. These structures develop throughout autumn and winter, and reach maturity at optimal temperatures of 10-15 °C, releasing ascospores of *Pleospora allii* (Rabenh.) Ces & De Not.. These ascospores act as primary sources of the asexual conidia of *S. vesicarium* (Llorente *et al*., 2010; Cavina *et al*., 2024). In the subsequent pathogenic phase, occurring during spring and summer (the pear growing season), these conidia are the principal inoculum responsible for infections, with optimal disease development at temperatures of 20 to 25 °C (Llorente and Montesinos, 2006; Tudela *et al*., 2023a). Virulence of *S. vesicarium* is largely attributed to production of host-specific toxins, which induce leaf vein necroses and disrupt plasma membrane integrity of plant cells (Singh *et al*., 1999). BSP symptoms manifest from pear flowering to fruit harvest, with necrotic lesions on leaves, twigs, petioles, and mainly on fruits as circular brown spots, often with red haloes. These symptoms can expand due to invasion by saprophytic fungi, including *Alternaria* spp., causing fruit rots (Cavina *et al*., 2024).

Synthetic fungicides are commonly used for management of BSP, initially providing low-cost and temporary solutions (Toledo *et al*., 2023). However, due to the high disease pressures and continuous infection cycles through growing seasons, high numbers of fungicide applications at 7 to 14 d intervals may be required to achieve low disease levels in orchards with a high disease incidence (Llorente *et al*., 2012b). The dithiocarbamate, strobilurin, succinate dehydrogenase inhibitor (SDHI), dicarboximide, and triazole fungicide groups are most commonly used against *S. vesicarium* in pear orchards (Llorente *et al*., 2012b; Luz *et al*., 2018; Tudela *et al*., 2023b). In Portugal, several of these active ingredients (including tebuconazole, trifloxystrobin, kresoxim-methyl, dithianon, cyprodinil and copper) are currently authorized for pear protection against BSP, according to the technical guidelines of the Portuguese Directorate-General for Food and Veterinary (DGAV), and the official phytopharmaceutical database SIFITO (DGAV, 2024). However, their excessive and repeated use may lead to pathogen resistance (Yin *et al*., 2023). These products may also pose environmental and public health concerns, due to their toxicity, non-biodegradable nature, and long residual activity adversely affecting non-target organisms and the environment (Corkley *et al*., 2022; Dorjee *et al*., 2023; Szczygieł *et al*., 2024).

Natural compounds, including essential oils (EOs), are gaining attention as sustainable alternatives to conventional agrochemicals for control of fungal diseases. EOs are complex mixtures of hydrophobic, fragrant, and volatile compounds synthesized by plants through metabolic pathways (Parikh *et al*., 2021; Dorjee *et al*., 2023). The main biosynthetic pathways include the mevalonate (MVA), methylerythritol phosphate (MEP), and shikimic acid processes, responsible for the two major groups of EOs: terpenoids and phenylpropanoids (de Sousa *et al*., 2023). These compounds have broad antifungal spectrum (Nazzaro *et al*., 2017), inhibiting fungal growth and preventing spore germination. Their modes of action include disrupting cell membranes, damaging cell wall integrity, and interfering with mitochondrial activity (Dorjee *et al*., 2023; Fincheira *et al*., 2023), and their antifungal activity has been extensively demonstrated *in vitro* conditions (Raveau *et al*., 2020). Increasing *in vivo* evidence also demonstrates their ability to suppress disease development in treated plants (Soylu *et al*., 2010; Antonioli *et al*., 2020; Xylia *et al*., 2021; Akhtari *et al*., 2022).

Despite the potential benefits of using EOs in crop protection, the European Union (EU) currently lacks a comprehensive approval framework for these substances as plant protection products. However, some naturally occurring components found in EOs (thymol, geraniol, 1,8-cineole), have been recognized as "low-risk" substances, and are included on the list of approved active substances outlined in Commission Implementing Regulation (EU) No. 540/2011. Particular EO constituents (e.g. citral and linalyl acetate) have received authorization as flavouring agents under Commission Implementing Regulation (EU) No. 872/2012, and are deemed to be of low risk for consumers. Despite these regulatory constraints, the physicochemical properties of EOs, including their high volatility, rapid degradation, low environmental persistence, and broad-spectrum antifungal activity, make them promising candidates for new biofungicide development. These characteristics align with EU initiatives including the Sustainable Use of Pesticides Directive, which is aimed at reducing reliance on synthetic fungicides and fostering sustainable crop protection.

The same traits that benefit environmental compatibility may limit practical EO application in the field, due to challenges related to their stability and bioavailability. Nano- and micro-encapsulation techniques, including spray-drying, have been increasingly employed to enhance stability, enable controlled release, and improve bioactivity of EOs (Antonioli *et al.*, 2020; Altay *et al.*, 2024). While previous studies have documented applications of the EOs against *Alternaria* spp., particularly *A. alternata* (Minozzo *et al.*, 2023; Bajac *et al.*, 2025), their effectiveness in addressing BSP pathogens in pear orchards, specifically *S. vesicarium*, has yet to be investigated. Building on this background, the present study aimed to identify EOs with antifungal activity, and, among the most effective candidates, to validate their *in vitro* efficacy by developing encapsulated formulations through spray-drying. These formulations are intended for use in controlling pathogenic fungi isolated from 'Rocha' pears exhibiting BSP symptoms. This study hypothesized that encapsulating EOs will enhance their stability while preserving antifungal activity. Eighteen EOs were screened *in vitro* against *S. vesicarium* isolates from 'Rocha' pear, to select those with the greatest antifungal potential. The three most active EOs were then encapsulated by spray-drying with HI-CAP® 100, and these formulations were assessed for inhibition of mycelium growth and conidium germination of *S. vesicarium* and *A. arborescens* isolated from 'Rocha' pear.

MATERIALS AND METHODS

Isolation of fungi

Fungus isolates were obtained from leaf and fruit samples collected from western Portuguese pear orchards between September 2023 and November 2024. Samples of pears and leaves were surface-sterilized with 1% sodium hypochlorite for 3 min, followed by three rinses with sterile distilled water. Necrotic tissues were then plated on Potato Dextrose Agar (PDA; Liofilchem) supplemented with chloramphenicol (Sigma-Aldrich) to inhibit bacterial growth, and were incubated for 4 d at 25 °C. Three emerging isolates were isolated from fruit and one from leaf, and were transferred to PDA plates to establish pure cultures. Mycelium agar blocks (5 × 5 mm) from pure cultures were preserved at room temperature in vials containing sterile distilled water. The isolates used in this study were deposited in Micoteca da Universidade do Minho (MUM, Braga, Portugal). For inoculum preparation, isolates were each cultured in PDA at 25 °C for 10 d.

Identification of isolates

Fungi were cultured in Potato Dextrose Broth (PDB; Liofilchem) in Erlenmeyer flasks that were incubated for 7 d at room temperature at 150 rpm. Resulting biomass of each isolate was collected by filtration and stored at -20 °C until further use. Genomic DNA was extracted using MagaBio Fungal Genome DNA Purification Kits (Bioer), following the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific), and samples were subsequently stored at -20 °C. PCR amplification was carried out targeting the ITS region, *gpd* gene and *Alt-a1* gene, and PCR products were sent for Sanger sequencing. The PCR conditions and primer sequences used are summarized in Table S1. The quality of the resulting sequences was verified using Geneious 11.1.5 software (Biomatters Limited), and the trimmed sequences were compared against the NCBI nucleotide database (nr/nt) using the Megablast program to determine closest related taxa. The sequences of the three loci were individually aligned in MEGA 12 (Kumar *et al.*, 2024), and ambiguous regions were removed using Gblocks (Castresana, 2000; Dereeper *et al.*, 2008). The cured alignments were concatenated, and a Maximum Likelihood (ML) phylogenetic tree was constructed in MEGA 12. Reference sequences used for tree construction are listed in Tables S2 and S3.

Fungus isolation and conidium production

Isolation and production of conidia from *S. vesicarium* and *A. arborescens* were carried out using previously described methods (Puig *et al*., 2015; Loebler *et al*., 2020), with some adaptations. The isolates were grown in tomato pulp agar plates, containing 1.25% (w/v) tomato pulp (Sumol+Compal), 3 g L^{-1} calcium carbonate (Merck), and 20 g L^{-1} agar (Liofilchem). The plates were kept at room temperature for 30 d with a 16 h daily photoperiod. Conidia were then harvested by scraping the plates with 10 mL of 0.01% (v/v) Tween 80 (Merck-Schuchardt). The resulting suspensions were each filtered through glass wool and cleaned by centrifugation for 10 min at 3,600 rpm. To determine conidium concentrations, serial dilutions were prepared, and 100 µL of each dilution was spread onto PDA plates. After 3 d incubation at 25 °C, the numbers of colony-forming units (CFUs) were counted, and were then used to estimate initial conidium concentrations. The suspensions were adjusted to obtain final conidium concentrations of 1 to 3×10^3 mL^{-1}, and were stored at 4 °C until further use.

Fungicide sensitivity assessments

To assess fungus isolate sensitivity, five commercial fungicides (copper complex, tebuconazole, trifloxystrobin, fluxapyroxad, cyprodinil) were evaluated against the *S. vesicarium* and *A. arborescens* isolates, using the "poisoned food" technique (Nene and Thapliyal, 1979). Each fungicide was added to sterilized molten PDA at concentrations that corresponded to the recommended application dose for field use (Table 1). The mixed PDA was then distributed into Petri plates. After complete solidification, mycelium squares (0.5 cm × 0.5 cm) were cut from 10-d-old cultures of the fungi, and placed in the centres of the PDA plates, with mycelia facing downwards. The experimental controls assessed growth of *S. vesicarium* or *A. arborescens* isolates on PDA medium without added fungicides. Colony diameters of the fungi were measured over the following 13 d period at 25 °C, and growth inhibition factors were calculated using the following formula (1):

$$Growth\ inhibition\ factor\ (\%) = \frac{D_C - D_T}{D_C} \times 100, \quad (1)$$

where:
D_C = diameter (cm) of control colony without treatment;
D_T = diameter (cm) of colony with treatment.

The diameter of the initial colony (0.5 cm) was subtracted from all measurements.

This experiment was carried out using biological triplicates.

Antifungal effects of essential oils against pathogenic fungi isolated from 'Rocha' pear material with BSP symptoms

Essential oils

A total of 18 EOs were used in this study. For some EOs, more than one production batch from the same plant species was tested. These batches were identified as batch 1, batch 2, or batch 3 (Table 2). The EO samples were stored in sealed vials at 4 °C until used. The EOs and their respective sources are listed in Table 2.

Antifungal activity of free essential oils

Antifungal activity of 18 EOs was evaluated using the "poisoned food" technique (Nene and Thapliyal, 1979). The tested oils were each added to sterilized PDA containing 0.01% (v/v) Tween 80, at concentrations of 1, 5, 10 or 50 µL mL^{-1}. The mixed PDA was then distributed into Petri plates, and a 0.5 × 0.5 cm mycelium square of *S. vesicarium* was placed in the centre of each PDA plate. Experimental controls included *S. vesicarium* iso-

Table 1. Fungicides and concentrations tested against fungi isolated from symptomatic material from 'Rocha' pears

Type of fungicide	Active ingredient	Group name[1]	FRAC[2] group code	FRAC level of resistance risk	Concentration tested
Copper based product	Copper complex	Inorganic	M01	Low	150 mL hL^{-1}
Triazole	Tebuconazole	DMI	3	Medium	75 g hL^{-1}
Strobilurin	Trifloxystrobin	QoI	11	High	10 g hL^{-1}
Carboxamide	Fluxapyroxad	SDHI	7	Medium-high	30 mL hL^{-1}
Anilinopyrimidine	Cyprodinil	AP	9	Medium	50 g hL^{-1}

[1] DMI, demethylation inhibitions; QoI, quinone outside inhibitor; SDHI, succinate-dehydrogenase inhibitor; AP, anilinopyrimidine.
[2] FRAC, Fungicide resistance action committee (FRAC, 2025).

Table 2. Essential oils (EOs) assessed against fungi isolated from symptomatic material of 'Rocha' pears

EO common designation	Source plant species
Basil	*Ocimum basilicum*
Eucalyptus (batch 1)	*Eucalyptus globulus*
Eucalyptus (batch 2)	*Eucalyptus globulus*
Eucalyptus (batch 3)	*Eucalyptus globulus*
Laurel	*Laurus nobilis*
Lavender	*Lavanda viridis*
Lemon eucalyptus (batch 1)	*Corymbia citriodora*
Lemon eucalyptus (batch 2)	*Corymbia citriodora*
Lemongrass	*Cymbopogon citratos*
Lemon mint	*Monarda citriodora*
Lemon thyme (batch 1)	*Thymus citriodorus*
Lemon thyme (batch 2)	*Thymus citriodorus*
Mutiati	*Colophospermum mopone*
Orange essence oil	*Citrus sinensis*
Oregano	*Oregano viridans*
Rosemary (batch 1)	*Rosmarinus officialis*
Rosemary (batch 2)	*Rosmarinus officialis*
White eucalyptus	*Eucalyptus alba*

lates on PDA containing 0.01% (v/v) Tween 80, without EO. Colony diameters were measured over a 13 d period at 25 °C, and the growth inhibition factor was calculated using formula 1 (above). This experiment was carried out using biological triplicates.

Essential oil encapsulation

The three EOs exhibiting the greatest antifungal activity against the *S. vesicarium* isolates, as identified in the EO screening, were encapsulated with modified maize starch using a spray-drying technique (HI-CAP® 100) as the capsule wall material, following the methods described by Baranauskiene and Venskutonis (2009), with some modifications. The emulsions were prepared by homogenizing each EO (at concentrations of 15% or 30% w/w) in previously prepared aqueous solutions of HI-CAP® 100 (30% w/w), using an Ultra-Turrax mixer at 1,600 rpm for 5 min. Spray-drying was carried out using a Büchi B-290 Mini Spray Dryer, with an inlet air temperature of 140 °C and a feed pump rate of 8%. Detailed study of the encapsulation efficiency, formulation optimization and release profiling were described in a complementary manuscript by Fernandes *et al.* (2026). The resulting microcapsules were stored in a desiccator protected from light until further use.

Effects of three essential oils on mycelium growth

A stock solution for each of the three EOs encapsulated with HI-CAP® 100 (with 15% or 30% of EO) was prepared by diluting each atomized sample in sterile distilled water. The assessed essential oils were mixed with PDA enriched with 0.01% (v/v) Tween 80 from the prepared stock solution to obtain the following encapsulated EO concentrations: 1, 10, 20, 40, or 80 mg mL^{-1}. The mixed PDA media were transferred to Petri plates, and a 0.5 × 0.5 cm mycelium square of each *S. vesicarium* or *A. arborescens* isolate was placed into each PDA plate. Resulting colony diameters were measured over a 13 d period at 25 °C, and the growth-inhibition factors were calculated using formula 1 (above). This experiment was carried out using biological triplicates.

Effects of two essential oils on conidium germination

The two most effective encapsulated EOs against mycelium growth of the four fungi were used to evaluate their effectiveness for inhibiting germination of *S. vesicarium* or *A. arborescens* conidia.

Each atomized sample with 15 or 30% of EO was diluted in sterile distilled water and mixed with PDA containing 0.01% (v/v) Tween 80, to obtain EO concentrations of 20 or 10 mg mL^{-1}, respectively. The mixed PDA medium was transferred to Petri plates and 10 µL of previously prepared conidium suspension (1 to 3×10^3 mL^{-1}) was inoculated in the centre of each plate. Resulting colony diameters were measured over a 13 d period at 25 °C, and growth inhibition factors were calculated using formula 1 (above). This experiment was carried out using biological triplicates.

Chemical characterization of the three most active essential oils

The chemical compositions of the three EO exhibiting the greatest antifungal activity were analysed using gas chromatography–mass spectrometry (GC–MS) analysis. This was carried out using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 N mass selective detector (Agilent Technologies). Samples (each of 1 µL) were injected into a fused-silica capillary column (HP-5MS; 30 m × 0.32 mm internal diameter, 0.25 µm film thickness; 5% diphenyl, 95% dimethylpolysiloxane; Agilent Technologies) via a vaporization injector maintained at 250 °C in the spitless mode (2 min). The oven temperature programme started at 45 °C (held for 1 min), then increased at 5 °C min-

^{1} to 250 °C, and this temperature was then maintained 5 min, resulting in a total run time of 47 min. Helium was used as the carrier gas, at a linear velocity of 30 cm s^{-1}. The temperature of the transfer line was set at 280 °C, the ion source at 230 °C, and the quadrupole analyser at 150 °C, and a turbo molecular pump (10-5 torr) was used. Ionization was achieved by electron impact at 70 eV, and mass spectra were acquired in full-scan mode over a mass range of 40–400 Da. A solvent delay of 3 min was applied. Data acquisition and instrument control were carried out using MSD ChemStation software (G1701CA, version C.00.00; Agilent Technologies). Compound identification was based on comparisons of calculated retention indices, relative to a standard mixture of n-alkanes, and by matching the obtained mass spectra with those available in the Wiley spectral library (G1035B, Rev D.02.00; Agilent Technologies). Semi-quantitative analyses were carried out using normalized peak area percentages without the application of correction factors.

Statistical analyses of data

The analyses were each carried out with three replicates. A two-way analysis of variance (ANOVA) was used to determine whether there were statistically significant differences between experimental group means, using Prism software packages (GraphPad Software version 9.5.0). *Post hoc* tests for assessment of statistically significant differences of factors were carried out using Tukey's and Šídák's multiple comparisons tests. The experiments were carried out with a confidence level of 95%, and statistical significance was assumed at $P < 0.05$.

RESULTS

Fungi isolated from 'Rocha' pear material with symptoms of BSP

Four fungus isolates were obtained from symptomatic 'Rocha' pear tissues, three from pear fruits, and one from a leaf. The multilocus phylogenetic analysis based on ITS and *gpd* sequences (Figure 1A) showed that isolates MUM 26.01 and MUM 26.02 clustered consistently with reference isolates of *S. vesicarium* (CBS 133914, CBS 274.31, CBS 191.86T e CBS 192.86T), forming a clade with strong bootstrap support (99%), confirming identification of both isolates as *S. vesicarium*. Figure 1B shows the phylogenetic tree for *Alternaria* based on concatenated sequence analysis of the ITS region, and *gpd* and *alt-a1* genes. Isolates MUM 26.03 and MUM 26.04 grouped together with reference isolates of *A. arborescens* (CBS 109730, CBS 102605T), forming a highly supported clade with bootstrap values of 96%. This strong support confirmed identification of MUM 26.03 and MUM 26.04 as *A. arborescens.*

Assessments of sensitivity of fungi to commercial fungicides

The inhibitory efficacy of the five tested commercial fungicides on mycelium growth depended on fungus species and isolates, as show in Figure 2A. *Alternaria arborescens* MUM 26.03 was more resistant to copper complex and tebuconazole, with mean percent inhibition of 22.6 ± 2.8% for copper complex and 49.6 ± 2.8% for tebuconazole, whereas isolate MUM 26.04 had greater tolerance to the three other fungicides assessed. For *S. vesicarium*, isolate MUM 26.02 was more sensitive to copper complex and fluxapyroxad, reaching mean percent inhibition of 39.7 ± 2.8% for copper complex and 60.9 ± 10.8% for fluxapyroxad. Cyprodinil was most effective against *S. vesicarium* MUM 26.01, achieving mean percent inhibition of 88.7 ± 1.0%.

Among the five fungicides assessed, tebuconazole and cyprodinil gave the greatest levels of inhibition for all four isolates. In contrast, trifloxystrobin and copper complex were the least effective treatments, mean percent inhibitions less than 33.1 ± 3.8%. Fluxapyroxad showed variable effectiveness. While this fungicide had moderate activity against *S. vesicarium* isolate MUM 26.02, its effect on *S. vesicarium* MUM 26.01 was minimal (mean inhibition = 12.1 ± 2.0%), and its inhibition of the *Alternaria* isolates was also comparatively low, ranging from a mean reduction of 18.6 ± 2.1% to 36.8 ± 6.6%.

The fungicides also induced visible alterations in the colony morphologies compared to the experimental controls. Representative examples are shown in Figure 2. For *S. vesicarium* MUM 26.01, exposure to copper complex, tebuconazole, or cyprodinil produced whitish colonies, in contrast to the characteristic pigmentation observed in the controls (Figure 2 B). In contrast, *S. vesicarium* MUM 26.02 showed no evident morphological alterations under the tested conditions. For A. *arborescens* MUM 26.04, the copper complex produced colonies that were dark brown, whereas cyprodinil led to noticeably lighter coloured colonies (Figure 2 C). Similarly, *A. arborescens* MUM 26.03 also had darkened colonies when exposed to copper complex.

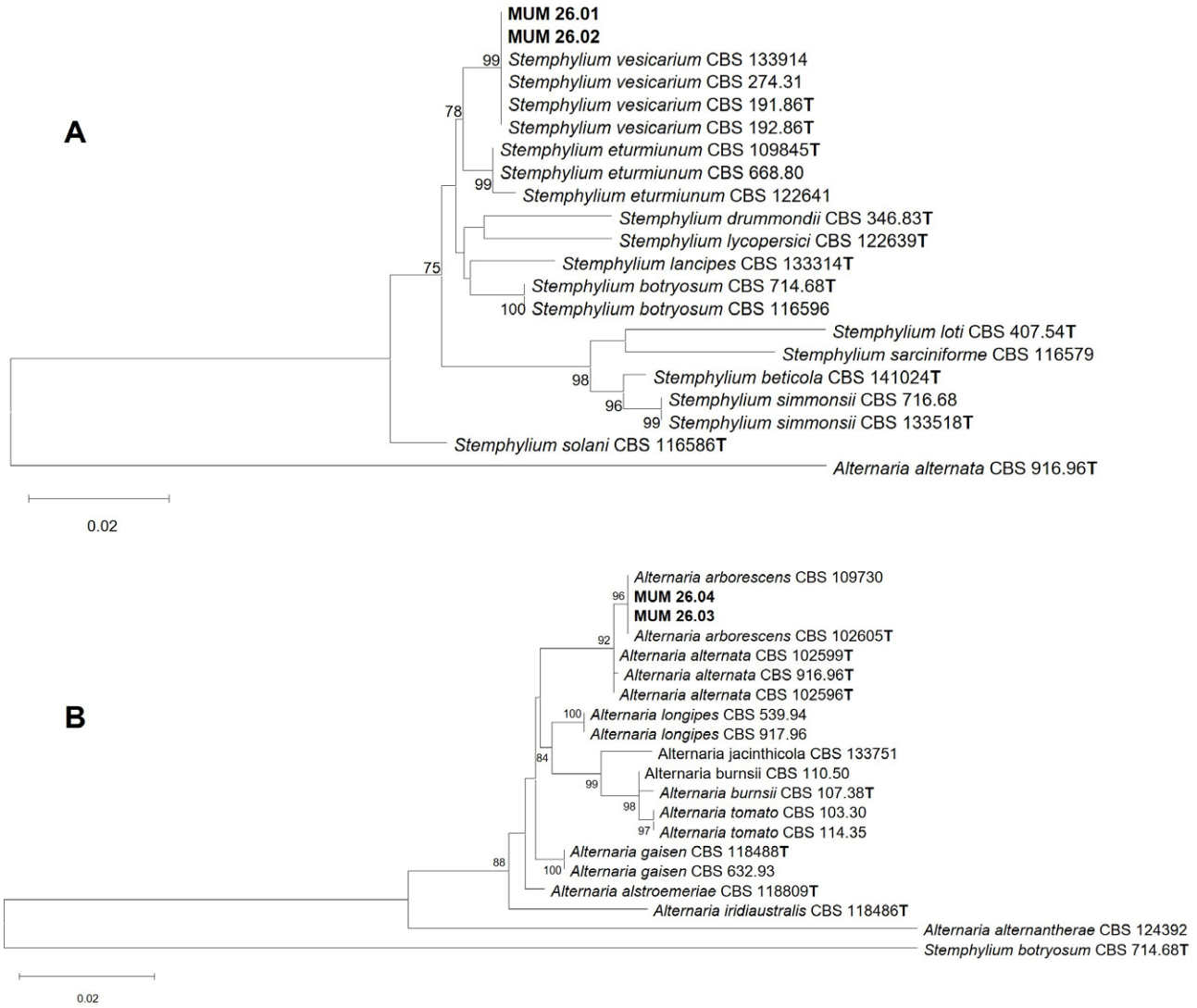

Figure 1. Identifications of the fungi isolated from 'Rocha' pear material with BSP. A, Phylogenetic tree of *Stemphylium*. B, Phylogenetic tree of *Alternaria*. Bootstrap values less than 70% were omitted from the figure.

Antifungal effects of essential oils against pathogenic fungi isolated from 'Rocha' pear with BSP symptoms

Antifungal activity of free essential oils

Eighteen EOs, at concentrations of 1, 5, 10 or 50 µL mL^{-1}, were evaluated against two *S. vesicarium* isolates. All the EOs at 50 µL mL^{-1} inhibited *S. vesicarium*, after 13 d of incubation (Table 3). However, lemon eucalyptus (batch 1), mutiati or orange EOs did not fully inhibit mycelium growth. At 10 µL mL^{-1}, most of the EOs were less inhibitory to mycelium growth of the two *S. vesicarium* isolates. Lemon eucalyptus (batch 2), lemongrass, and lemon thyme (batch 2) EOs gave complete growth inhibition at concentrations of 10 and 0.5 µL mL^{-1}. At 1 µL mL^{-1}, these three EOs had different effects, depending on the *S. vesicarium* isolate: lemongrass and lemon thyme (batch 2) prevented growth of *S. vesicarium* MUM 26.02, but were less effective against *S. vesicarium* MUM 26.01. Among these three EOs, lemon eucalyptus (batch 2) had the least antifungal activity at the lowest concentration assessed.

Based on the results of this initial screening, three EOs – lemon eucalyptus (batch 2), lemongrass, and lemon thyme (batch 2) – were selected for encapsulation assays (Fernandes *et al.*, 2026) and subsequently for the other experiments.

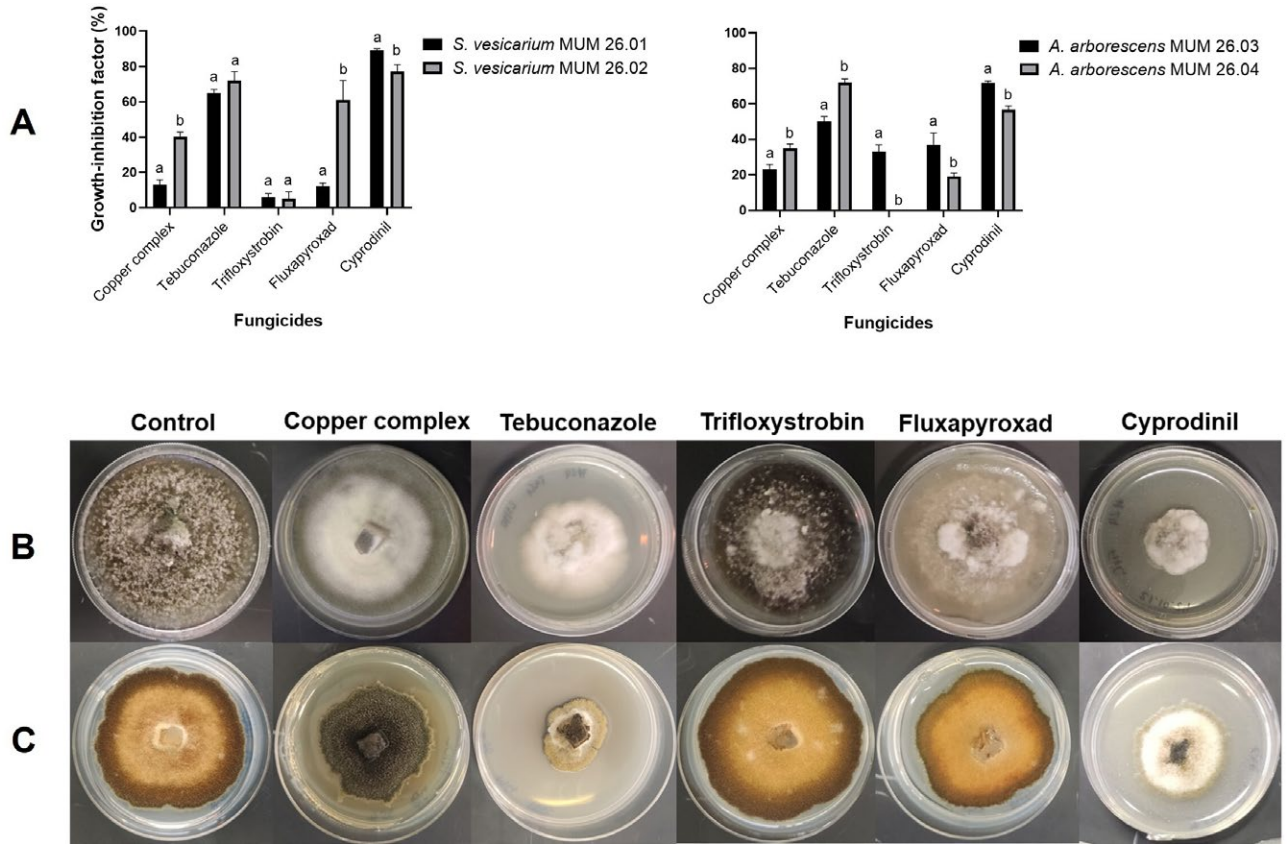

Figure 2. Effects of commercial fungicides on mycelium growth of fungi isolated from 'Rocha' pear material with BSP. A, Mean growth inhibition (%) of *Stemphylium vesicarium* or Alternaria arborescens isolates in the presence of the five assessed fungicides, after 13 d incubation at 25 °C (different letters indicates differences ($P < 0.05$) within each fungicide. B and C, Images of Petri plates containing colonies of *S. vesicarium* isolate MUM 26.01 (B) or *A. arborescens* MUM 26.04 (C) in the presence of two tested fungicides, after 13 d of incubation at 25 °C.

Effects of essential oils encapsulated with HI-CAP® 100 on mycelial growth

From the initial screening, the three most effective EOs (lemon eucalyptus, lemongrass, and lemon thyme) were encapsulated by spray-drying using HI-CAP® 100, with oil concentrations of 15 and 30%. The six EO formulations were tested against the two *S. vesicarium* isolates at different concentrations in PDA. As shown in Figure 3, all the formulations maintained their antifungal activity, inhibiting growth of the two *S. vesicarium* isolates. Lemon eucalyptus EO formulations did not fully prevent mycelium growth. The greatest observed mean inhibition from the 15% formulation was 70.4 ± 3.8% for *S. vesicarium* MUM 26.01 and 59.5 ± 1.1% for *S. vesicarium* MUM 26.02. For the 30% formulation and at the greatest concentration of 80 mg mL^{-1}, mean inhibition was 57.0 ± 2.1% for *S. vesicarium* MUM 26.01 and 71.8 ± 1.1% for *S. vesicarium* MUM 26.02. In contrast, the lemongrass and lemon thyme EO formulations gave greater isolate inhibition. Minimum mean effective inhibitory concentration for the lemongrass and lemon thyme EO formulations was observed at 20 mg mL^{-1} for both *S. vesicarium* isolates from 15% of EO encapsulated (Figure 3 A), and at 10 mg mL^{-1} for both isolates at 30% of encapsulated EO (Figure 3 B). At the 15%, both formulations completely inhibited growth of *S. vesicarium* MUM 26.02 at 10 mg mL^{1}, whereas, at the same concentration against strain MUM 26.01, lemongrass and lemon thyme EOs reduced growth by, respectively, 3.0 ± 1.0% and 37.0 ± 13.1%. The encapsulating agent HI-CAP® 100 was also tested in the absence of EOs against the two *S. vesicarium* isolates, and caused less than 3% inhibition of mycelium growth (Figure S1).

Figure 4 shows effects of encapsulated lemon eucalyptus, lemongrass, or lemon thyme EOs using HI-CAP® 100 at 15 or 30% (respectively, 20 or 10 mg mL^{-1}) on the two *A. arborescens* isolates. The inhibitory effects were similar to those observed against *S. vesicarium*. The formulations of lemongrass and lemon thyme EOs with HI-CAP® 100 completely inhibited both *A. arborescens* iso-

Table 3. Proportions (%) of mycelium growth inhibition (after 13 d in culture at 25 °C) for two *Stemphylium vesicarium* isolates (MUM 26.01 or MUM 26.02) exposed to four concentrations of different essential oils

Essential oil	Essential oil concentration (μL mL^{-1})							
	50		10		5		1	
	MUM 26.01	MUM 26.02	MUM 26.01	MUM 26.02	MUM 26.01	MUM 26.02	MUM 26.01	MUM 26.02
Basil	100%	100%	60%	50%	-	-	-	-
Eucalyptus (batch 1)	100%	100%	60%	33%	-	-	-	-
Eucalyptus (batch 2)	100%	100%	100%	88%	-	-	-	-
Eucalyptus (batch 3)	100%	100%	86%	50%	-	-	-	-
Laurel	100%	100%	86%	83%	-	-	-	-
Lavander	100%	100%	100%	40%	-	-	-	-
Lemon eucalyptus (batch 1)	83%	86%	-	-	-	-	-	-
Lemon eucalyptus (batch 2)	100%	100%	100%	100%	100%	100%	47%	34%
Lemongrass	100%	100%	100%	100%	100%	100%	61%	100%
Lemon mint	100%	100%	57%	38%	-	-	-	-
Lemon thyme (batch 1)	100%	100%	54%	30%	-	-	-	-
Lemon thyme (batch 2)	100%	100%	100%	100%	100%	100%	61%	100%
Mutiati	80%	86%	-	-	-	-	-	-
Orange essence oil	50%	40%	-	-	-	-	-	-
Oregano	100%	100%	86%	73%	-	-	-	-
Rosemary (batch 1)	100%	100%	86%	88%	-	-	-	-
Rosemary (batch 2)	100%	100%	80%	63%	-	-	-	-
White eucalyptus	100%	100%	51%	33%	-	-	-	-

lates while the lemon eucalyptus EO formulation did not fully inhibit the two isolates. For the MUM 26.03 isolate, the mean inhibition percentages were 38.7 ± 1.2% for the 15% formulation and 33.6 ± 1.2% for the 30% formulation. In contrast, for the MUM 26.04 isolate, the mean inhibition percentages were 49.6 ± 2.1% for the 15% formulation and 44.5 ± 0.0% for the 30% formulation.

Effect of EO encapsulated with HI-CAP® 100 on conidium germination

The antifungal activity of lemongrass and lemon thyme EO HI-CAP® 100 formulations encapsulated with 15 or 30% of each oil were also evaluated for effects on germination of *S. vesicarium* and *A. arborescens* conidia, at concentrations of 20 or 10 mg mL^{-1} on PDA. Figure 5 shows that all these formulations completely inhibited conidium germination and subsequent growth of each isolate of both fungi.

Chemical characterization of three active essential oils

The chemical compositions of the lemongrass, lemon thyme, and lemon eucalyptus EOs were analysed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The chemical compositions of the lemongrass, lemon thyme, and lemon eucalyptus EOs are shown in Table 4. Gas chromatography showed that lemongrass EO was mainly composed of citral isomers, with trans-citral and cis-citral together constituting greater than 88% of the total composition. Lemon thyme EO consisted predominantly (72.6%) of oxygen-containing monoterpenes, among which geraniol, 1,8-cineol, and thymol were the most abundant components. The citral isomers, geranial and neral, were also detected in the lemon thyme EO, but accounted for less than 8% of total composition. The chemical composition of lemon eucalyptus EO showed a predominance of monoterpene hydrocarbons, with α-pinene as the major constituent, accounting for 51.6% of the total composition. Eucalyptol (1,8-cineole) (12.9%) and ρ-cymene (10.7%) were the second and third most abundant compounds in this oil.

DISCUSSION

Brown spot of pear, caused by *S. vesicarium*, has become a major concern for 'Rocha' pear production in Portugal (Loebler *et al*., 2020; Cavina *et al*., 2024). In the present study, *S. vesicarium* was isolated from symptomatic 'Rocha' pear tissues, and *A. arborescens* was also

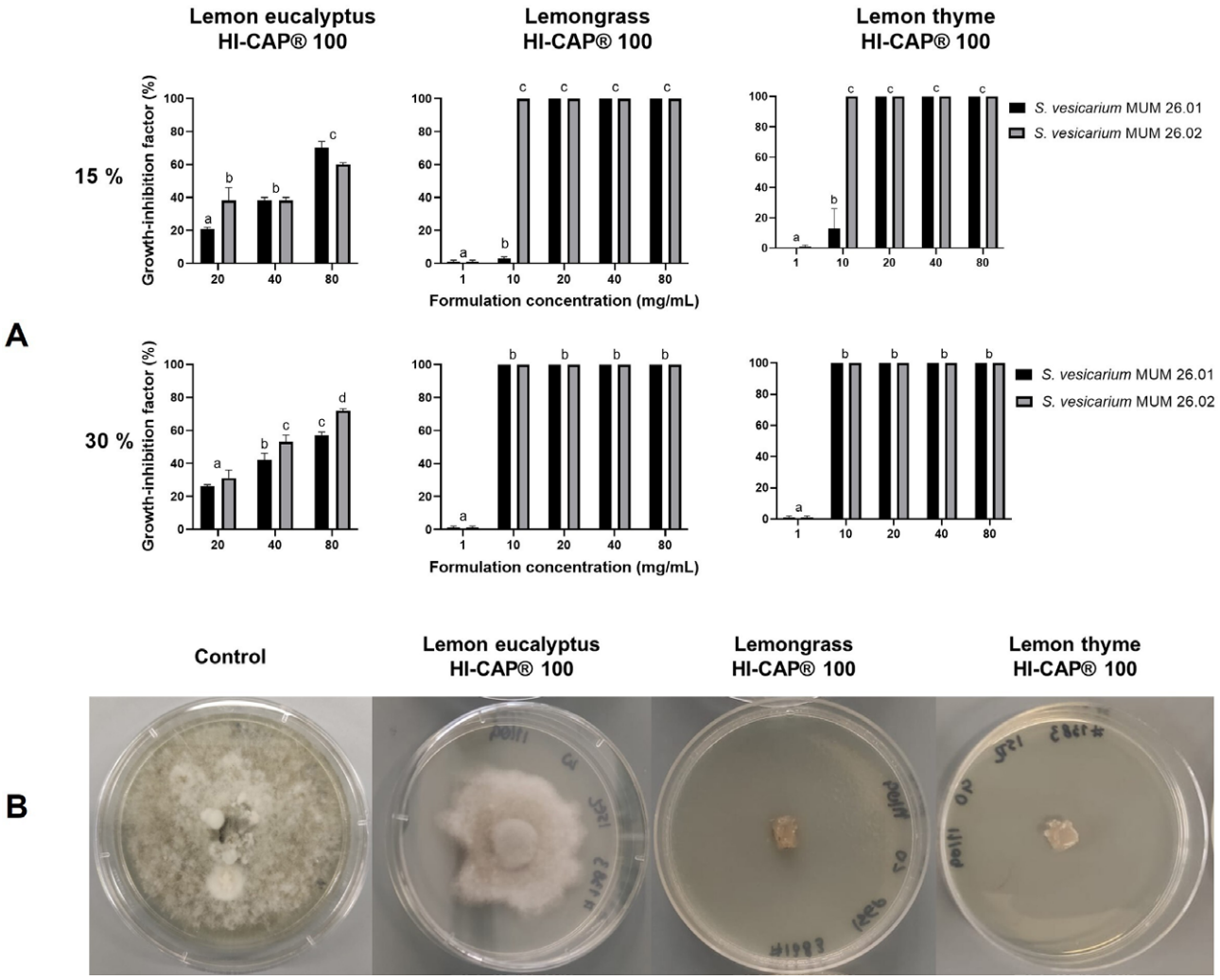

Figure 3. A) Mean percentages of growth inhibition in cultures of two *Stemphylium vesicarium* isolates (MUM 26.01 and MUM 26.02) exposed for 13 d at 25 °C to different concentrations of three essential oils encapsulated with HI-CAP® 100 at 15 or 30% of each oil. Means accompanied by different letters are different ($P < 0.05$). B) Images of cultures of *S. vesicarium* isolate MUM 26.02 with (left) no additive to the culture medium, or with lemon eucalyptus (second left), lemongrass (second right) or lemon thyme (right) EO HI-CAP® 100 formulations (20 mg mL^{-1}) after 13 d at 25 °C.

isolated, indicating that both fungi may contribute to this disease. The presence of *A. arborescens* in 'Rocha' pear had previously been reported (Sobreiro and Batalha Neto, 2024), supporting the potential involvement of this fungus in the BSP disease complex.

Since fungicides remain the primary method for managing BSP (Toledo *et al.*, 2023), the sensitivity of particular pathogen isolates was evaluated for commercial fungicides commonly applied by Portuguese 'Rocha' pear producers. The results have shown that fungicide efficacy varied depending on active ingredient, fungal species, and on the specific isolates involved. This probably shows why discussions within the technical community based on observable incidence of the disease have not yielded conclusive results.

Among the assessed fungicides, tebuconazole and cyprodinil had greatest effectiveness, achieving incidence reductions of, respectively, 70% and 88%. In contrast, trifloxystrobin had no or little inhibitory activity (0% to 33%). Fluxapyroxad showed a variable pattern among the four pathogen isolates. Inhibition percentages of the two *S. vesicarium* isolates were approx. 12% and 60%. These results indicate that sensitivity of BSP fungal pathogens to specific fungicides may be diminishing, and that continued use of these products could increase losses of pathogen sensitivity.

Efforts to reduce the number of fungicide active ingredients used, in response to standards imposed by major retail chains, have aimed to minimize the num-

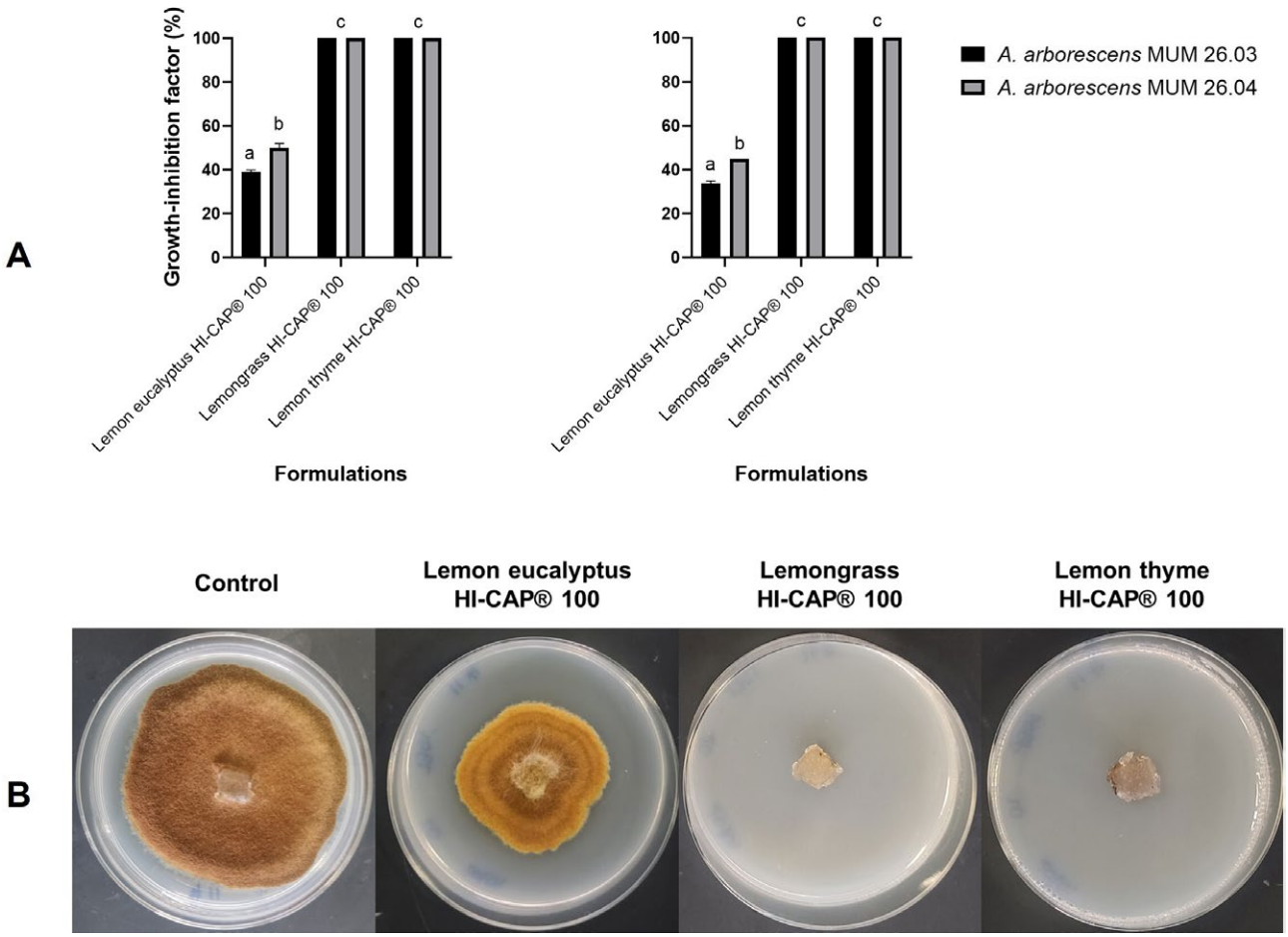

Figure 4. A) Mean percentages of growth inhibition in culture of two *Alternaria arborescens* isolates (MUM 26.03 and MUM 26.04) exposed for 13 d at 25 °C to three essential oils encapsulated with HI-CAP® 100 at 15% (20 mg mL^{-1}) and 30% (10 mg mL^{-1}) of each oil. Means accompanied by different letters are different ($P < 0.05$). B) Images of cultures of *A. arborescens* MUM 26.03 with (left) no additive to the culture medium, or with lemon eucalyptus (second left), lemongrass (second right) or lemon thyme (right) EO HI-CAP® 100 formulations (20 mg mL^{-1}) after 13 d at 25 °C.

ber of detectable pesticide substances, and this may have contributed to shifts in fungicide sensitivity, as a result of repeated applications of particular chemicals (Paušič *et al*., 2023; Olita *et al*., 2024). Previous studies have reported variable sensitivity profiles for different fungicide classes in *Stemphylium* and *Alternaria* isolates obtained from fruit crops. Strobilurin fungicides, including trifloxystrobin, have shown low efficacy against isolates of *Alternaria* (Camiletti *et al*., 2022) and *S. vesicarium* (Collina *et al*., 2007; Alberoni *et al*., 2010). In contrast, DMI fungicides, including tebuconazole, have exhibited lower risks for resistance development, and remain effective for inhibiting *Alternaria* spp. (He *et al*., 2019; Camiletti *et al*., 2022). *Alternaria* isolates have also demonstrated sensitivity to cyprodinil, although resistance to some SDHI fungicides (e.g. fluxapyroxad) has also been observed (Yang *et al*., 2015; Beg *et al*., 2025).

Copper-based products are recognized as effective contact fungicides, primarily used for preventative disease management since they reduce inoculum accumulation on plant surfaces (Lamichhane *et al*., 2018; La Torre *et al*., 2018). These products lack translaminar or systemic activity, so their curative efficacy against established fungal structures is limited compared to systemic fungicides, which can penetrate host tissues to control ongoing infections (McGrath, 2009), as shown by Donne *et al.* (2020) and Koka *et al.* (2021). This distinction was reflected in the present study, where the copper complex formulation assessed showed limited effectiveness against the *S. vesicarium* and *A. arborescens* isolates evaluated.

Beyond influencing pathogen mycelium growth, presence of fungicides also altered pigment production and colony structure of the two pathogens assessed. Exposure to copper complex fungicide consistently

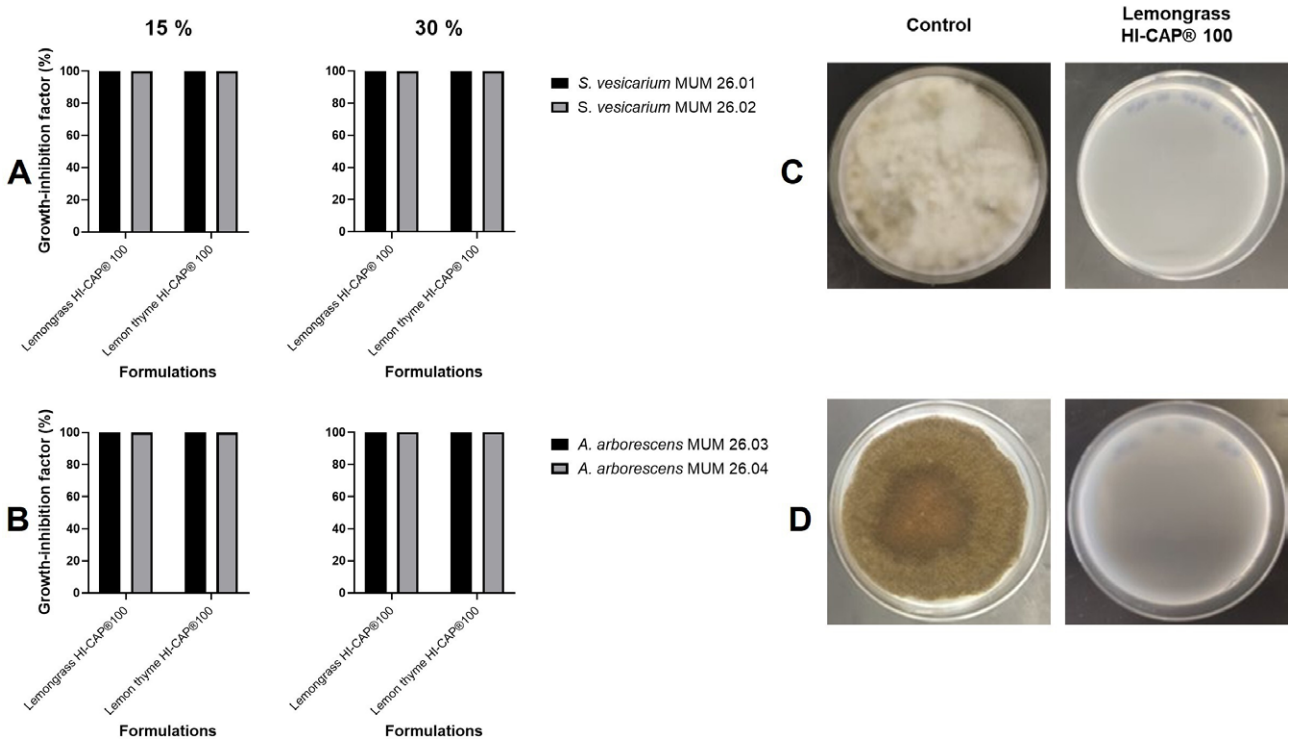

Figure 5. A) Mean percentages of growth inhibition in cultures of two *Stemphylium vesicarium* isolates (MUM 26.01 and MUM 26.02) grown from conidia and exposed for 13 d at 25 °C to two essential oils encapsulated with HI-CAP® 100 at 15% (20 mg mL^{-1}) or 30% (10 mg mL^{-1}) of each oil. No significant differences were observed ($P > 0.05$). B) Mean percentages of growth inhibition in cultures of two *Alternaria arborescens* isolates (MUM 26.03 and MUM 26.04) grown from conidia and exposed for 13 d at 25 °C to two essential oils encapsulated with HI-CAP® 100 at 15% (20 mg mL^{-1}) or 30% (10 mg mL^{-1}) of each oil. No significant differences were observed ($P > 0.05$). C) Images of cultures of *S. vesicarium* isolate MUM 26.01 with (left) no additive to the culture medium or with (right) lemongrass EO HI-CAP® 100 formulation at 15% (20 mg mL^{-1}) after 13 d at 25 °C. D) Images of cultures of *A. arborescens* isolate MUM 26.04 with (left) no additive to the culture medium or with (right) lemongrass EO HI-CAP® 100 formulation (20 mg mL^{-1}) after 13 d at 25 °C.

induced formation of dark brown colonies in both the *A. arborescens* isolates. This hyper-pigmentation may indicate an adaptative defence response to copper exposure, whereby increased melanin synthesis sequesters copper ions within the fungus cell walls and reduces oxidative stress associated with copper-induced free radicals (Fogarty and Tobin, 1996; Borkow and Gabbay, 2005). Conversely, copper, tebuconazole and cyprodinil all triggered an opposite response, resulting in whitish appearance in *S. vesicarium* MUM 26.01. This suggests that these three fungicides in contact with the *S. vesicarium* isolate probably disrupted biosynthesis of secondary metabolites, including the melanin pigments, which play important roles for cell protection (Cordero and Casadevall, 2017). *Stemphylium vesicarium* MUM 26.02 produced no visible changes in morphology, highlighting that the macroscopic shifts observed were isolate and treatment dependent.

Similar effects have been documented elsewhere. Changes in *Aspergillus flavus* pigmentation from green to white, and darkened colonies in *Cladosporium cladosporioides*, when exposed to different fungicides, have been previously reported (Llorente *et al*., 2012a; Akbari Dana *et al*., 2019). In addition, previous studies have shown that copper-based compounds can induce formation of dark pigments in fungi (Griffith *et al*., 2007), indicating that fungicides can trigger physiological stress responses manifested as morphological alterations. These results are consistent with the variable pathogen sensitivity patterns described elsewhere, and highlight the ongoing challenges for identifying fungicide efficacy against BSP pathogens.

To address this problem, focus is shifting towards exploring alternative biobased disease management strategies, which are environmentally friendly and less likely to inducing pathogen resistance. EOs are promising biocontrol agents due to their broad spectrum antifungal properties and natural origins (Nazzaro *et al*., 2017). Therefore, the present study aimed to identify plant-derived EOs able to control growth of pathogens associated with BSP. From 18 EOs assessed against the

Table 4. Chemical characterizations of lemongrass, lemon thyme, and lemon eucalyptus essential oils

Essential oil	Components	Content (%)
Lemongrass	Trans-citral (geranial)	46.8
	Cis-citral (neral)	41.6
	Nerol	4.0
	2-methyl-3-butene-2-ol	1.7
	6-methyl-5-heptene-2-one	1.4
	Cis-carveol	1.1
	Linalyl acetate	0.9
Lemon thyme	Oxygen-containing monoterpenes, including:	72.6
	Geraniol	27.5
	1,8-Cineol	16.3
	Thymol	9.2
	Geranial	4.2
	Neral	3.4
	α-Terpineol	2.2
	Sesquiterpenes hydrocarbons, including:	9.5
	E-Caryophyllene	3.3
	ρ-Cymene	3.7
	γ-Terpinene	2.1
Lemon eucalyptus	α-Pinene	51.6
	Eucalyptol (1,8-Cineol)	12.9
	ρ-Cymene	10.7
	Aromadendrene	5.6
	γ-Terpinene	4.7
	Limonene	3.4
	α-Phellandrene	1.6
	Bicyclogermacrene	1.0

S. vesicarium isolates, those from lemon eucalyptus, lemongrass, or lemon thyme had high antifungal activities, completely inhibiting *S. vesicarium* growth at 5 μL mL^{-1}. Lemongrass and lemon thyme also controlled growth of *S. vesicarium* MUM 26.02 at 1 μL mL^{-1}.

This strong antifungal activity aligned with previous reports. High inhibition rates against phytopathogenic fungi using encapsulated and non-encapsulated lemongrass EO have been reported (Antonioli *et al.*, 2020; Sattary *et al.*, 2020; Gangavarapu and Palwai, 2022). Although studies on the fungicidal effects of lemon thyme EO against plant pathogens are limited, the strong antimicrobial effects have been reported against *Cutibacterium acnes* (Oliveira *et al.*, 2022). Geraniol, the main constituent of lemon thyme EO, has also shown inhibitory activity against *Aspergillus* spp. (Tang *et al.*, 2018). Lemon eucalyptus EO has also been reported to reduce growth of *A. alternata*, *Colletotrichum gloesporioides* and *Fusarium oxysporum*, which are key pathogens associated with postharvest rot in apples (Oli *et al.*, 2019). Despite the strong antifungal activity exhibited by the EO in the present study, the treated plants were not free from disease. For instance, *Golovinomyces biocellatus* has been reported to cause powdery mildew on lemon thyme (*Thymus citriodorus*) in Italy (Garibaldi *et al.*, 2016). This highlights that, despite the antimicrobial properties associated with secondary aromatic plant metabolites, natural *in planta* concentrations may not always be sufficient to prevent pathogen establishment. However, concentrated EO extracts can still have significant antifungal activity when applied exogenously against phytopathogens.

To mitigate the common challenges of volatility and degradation of chemicals containing EOs, encapsulation techniques have been proposed for improving EO stability, potentially contributing to enhanced bioactivity (Antonioli *et al.*, 2020; Altay *et al.*, 2024). Therefore, based on their high antifungal activities in the initial screening of the present study, lemon eucalyptus, lemongrass, and lemon thyme EOs were subsequently encapsulated by spray-drying using a modified maize starch (HI-CAP® 100) and EO concentrations of 15% or 30%, to evaluate how the transition from free liquid to solid microcapsule may affect their bioactivity (Fernandes *et al.*, 2026). Both lemongrass and lemon thyme EO formulations completely inhibited *S. vesicarium* growth, at a minimum concentration of 20 mg mL^{-1} for the 15% formulations, and 10 mg mL^{-1} for the 30% formulations. This showed that spray-drying did not compromise the active constituents, and demonstrated the effectiveness of the polymer matrices.

Lemon eucalyptus EO gave decreased efficacy after encapsulation, and did not fully inhibit fungal growth at the concentrations tested, with inhibition percentages ranging between 30% and 70% among all conditions assessed. Similar results were obtained for the two *Alternaria* isolates. These results indicate that the antifungal activity of lemongrass and lemon thyme EOs was not compromised by encapsulation, validating their selection from the initial screening, and supporting their potential as encapsulated formulations for EO-based products. Previous studies have compared antifungal activities of free and encapsulated EOs. Soltanzadeh *et al.* (2021) reported lower antifungal activity of encapsulated lemongrass EO against *A. flavus*, *Penicillium notatum* and *F. solani* compared with the free oil, which completely inhibited fungal growth. Antonioli *et al.* (2020) stated that lemongrass EO containing poly(lactic acid) nanocapsules efficiently reduced *C. acutatum* and *C. gloesporioides*, but a high concentration was required compared to free EO. Formulations of *Cinnamomum cassia* EO,

both free and spray-dried encapsulated, were strongly antifungal against *A. alternata*, *A. flavus* and *P. crustosum* (Minozzo *et al*., 2023). Sattary *et al.* (2020) showed that antifungal effects of encapsulated lemongrass and clove EOs was greater than from the free EOs.

Stemphylium vesicarium and *A. arborescens* conidia are important in the crop diseases they cause, affecting pome fruit and vegetables (Fontaine *et al*., 2021; Gossen *et al*., 2021; Habib *et al*., 2021; Głos *et al*., 2023). For the BSP complex, *S. vesicarium* primarily overwinters in pear orchards as pseudothecia, releasing ascospores of *P. allii* under favourable climate conditions in the spring (Llorente *et al*., 2010). These sexual spores contribute to saprophytic colonization of plant residues, which become the principal sources of conidia as the main inoculum responsible for infections during pear growing seasons (Cavina *et al*., 2024). Given the central role of conidia in disease initiation and spread, controlling conidium germination is essential for lowering disease pressure and managing BSP. In the present study, effects of lemongrass and lemon thyme EO HI-CAP® 100 formulations were evaluated against *S. vesicarium* and *A. arborescens* conidium germination. Under the concentrations assessed (20 mg mL^{-1}, for the 15% formulations, and 10 mg mL^{-1}, for the 30% formulations), the EOs completely inhibited conidium germination. These results further confirm the antifungal potential of these selected EOs, demonstrating their effectiveness for inhibiting pathogen mycelium growth and conidium germination.

The chemical characterizations of the lemongrass, lemon thyme, and lemon eucalyptus EOs showed distinct chemical profiles. The lemongrass and lemon thyme EOs mainly contained oxygenated monoterpenes, including citral, geraniol, 1,8-cineole, and thymol. For plant protection, geraniol, the main constituent (27.5%) of lemon thyme EO, is of particular interest. This compound reduced mycelium growth of *Drechslera oryzae*, *F. oxysporum* and *Sclerotium rolfsii* (Kaur *et al*., 2019). Beyond plant protection, recent trials have demonstrated that incorporating geraniol into biodegradable composite coatings reduced incidence and lesion severity caused by *Monilinia fructicola* in plum fruit during cold storage (Asgarian *et al*., 2023). Geraniol is also listed as an approved active substance under Commission Implementing Regulation (EU) No. 540/2011, highlighting its safety and efficacy for use in plant protection products.

Citral was the main compound (88.4%) present in lemongrass EO. Citral has demonstrated protective activity in fruit systems by inhibiting mycelium growth, while also being recognized as a food flavouring agent in Commission Implementing Regulation (EU) No. 872/2012. Citral application successfully controlled mycelium growth and reduced lesion diameter caused by *A. alternata* in pitaya fruit storage (Luo *et al*., 2024), and on pears (Chen *et al*., 2025). Tao *et al.* (2014) also reported that this compound inhibited mycelium growth of *P. italicum*, an important postharvest citrus pathogen. In contrast, lemon eucalyptus EO was composed mostly of monoterpene hydrocarbons, including α-pinene (51.6%), along with lower proportion of 1,8-cineole (12.9%). Aldehydes and alcohols, including citral and geraniol, interact strongly with fungal cell membranes due to their high lipophilicity (Bakkali *et al*., 2008). Upon entering the phospholipid bilayers, they enhance membrane fluidity and permeability, potentially causing the leakage of ions and essential metabolites (Trombetta *et al*., 2005). Furthermore, aldehydes, including citral, are chemically active, and can create Schiff bases or other covalent compounds with membrane proteins. This may disrupt enzyme activities and disturb cellular equilibria (Hyldgaard *et al*., 2012). Additionally, disruption of production of ergosterol, an essential sterol for fungal membranes, can be important. Monoterpenes such as citral, geraniol, and thymol, may interfere with this process, resulting in compromised integrity of cell membranes and reduced fungal growth (Contreras Martínez *et al*., 2022). Low abundance of these oxygenated compounds in lemon eucalyptus EO may therefore account for the reduced antifungal activity against *S. vesicarium* and *A. arborescens*, compared to lemongrass and lemon thyme EOs, as was observed in the present study.

Overall antifungal efficacy cannot be solely attributed to the major EO compounds. Minor elements, while in limited amounts, can modify plant and pathogen membrane permeability or assist substance penetration, enhancing their antifungal properties (Burt, 2004). For example, 1,8-cineole has been identified as a permeabilizing agent; it improves movement of other monoterpenes across lipid bilayers (Trombetta *et al*., 2005). Synergy has been shown between citral and geraniol, which collectively improve membrane disruption and respiratory inhibition in different fungal pathogens. These connections clarify why EOs often exert stronger influences than individual compounds, as observed in previous research (Shukla *et al*., 2012; Soares *et al*., 2016).

Ongoing debate supported by an increasingly body of evidence, suggests that the antifungal activity is primarily driven by the major active compounds. Studies on *C. cassia* and *Cymbopogon martini* EOs have concluded that high-content components including trans-cinnamaldehyde and trans-geraniol accounted for the majority of inhibitory effects against toxigenic fungi (Wang *et al*., 2018). Similarly, research on EO from *Thymus vulgaris* has shown that the phenolic compound thymol outper-

formed the whole EO for control of *A. alternata* growth (Perina *et al*., 2015; Andrade-Ochoa *et al*., 2023). From a product development perspective, this duality is important, since identification of individual active compounds may facilitate product regulation. Further research is required to clarify the relative contributions of major and minor compounds to antifungal efficacy, and to determine the most suitable approaches for future development of plant protection products.

CONCLUSIONS

This study aimed to identify EOs with antifungal activity against pathogenic fungi isolated from 'Rocha' pears exhibiting BSP symptoms, and to validate their efficacy as encapsulated formulations developed through spray-drying. Of the 18 EOs assessed, low concentrations of lemongrass and lemon thyme EOs completely inhibited mycelium growth and conidium germination of *S. vesicarium* and *A. arborescens* isolates with different pesticide sensitivity profiles, which had been isolated from 'Rocha' pear material exhibiting BSP symptoms. Their antifungal activities were retained after encapsulation, indicating their potential for practical disease management applications. These results highlight that EOs are potential alternatives to conventional fungicides, with advantages in biodegradability and reduced risk of development of pathogen resistance. As existing disease management solutions have proven ineffective, and fungicide resistance is increasingly compromising crop viability and profitability, EOs may be promising tools to mitigate decline of 'Rocha' pear cropping. Nevertheless, since the present study was conducted exclusively under *in vitro* conditions, further *in vivo* and field studies are required to validate the efficacy of these EO formulations. Future research should first assess their antifungal effectiveness and potential phytotoxic effects directly on 'Rocha' pear fruit, before progressing to field-scale assessments. Subsequent studies should also evaluate formulation persistence, stability, and performance after application, considering the influence of environmental factors on antifungal activity.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Inês Mendonça: conceptualization, investigation, formal analyses, and writing of the original draft manuscript. Beatriz Fernandes: conceptualization, investigation, formal analyses. Carmo Serrano: Conceptualization, writing, review, and manuscript editing. Ana C. Marques: writing, review, and manuscript editing. Ricardo Oliveira: Investigation, formal analysis, writing, review and manuscript editing. Carina Almeida: writing, review and manuscript editing, funding acquisition. Miguel Leão de Sousa: writing, review, and manuscript editing. Armando Venâncio: writing, review, and manuscript editing, funding acquisition. Sónia Silva: conceptualization, writing, review and manuscript editing, funding acquisition.

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