Eur J Plant Pathol (2025) 172:373–389
https://doi.org/10.1007/s10658-025-03010-7

Antifungal activity of essential oils in *Colletotrichum lindemuthianum* and alternative control of bean anthracnose

Julián Mauricio Agredo Hoyos · Adriano Francis Dorigan · Patrícia Ricardino da Silveira · Claudia Regina Gontijo Labory · Pedro Martins Ribeiro Júnior · Rafael Fernandes · Eduardo Alves

Accepted: 22 January 2025 / Published online: 3 February 2025

Abstract The major disease of the common bean, anthracnose, is caused by the hemibiotrophic fungus *Colletotrichum lindemuthianum* and results in significant economic losses. In the present study, we evaluated: (i) the antifungal activity of 26 essential oils (EOs) in the germination and mycelial growth of two isolates of *C. lindemuthianum*, one of race 65 (LV 136) and the other of race 73 (LV 99), (ii) ultrastructural changes of *Cymbopogon citratus*, *Eugenia caryophyllata* and *Cymbopogon martini* EOs in fungal conidia, (iii) the effect of these EOs on anthracnose control, applied before and after inoculation, and (iv) the effect of *C. citratus* EOs on the activity of defense enzymes Chitinase (CHI), Polyphenol oxidase (PPO), Guaiacol peroxidase (POX) and Phenylalanine ammonia-lyase (PAL) on bean plants. Of the 26 EOs, 95% of the conidial germination was inhibited by *C. citratus*, *C. martini*, *Cinnamomum* sp., *T. vulgaris*, *E. caryophyllata*, and *M. recutita* at concentrations lower than 0.1%. Mycelial growth was completely inhibited by *C. citratus*, *E. caryophyllata* and *C. martini* at 0.1% for both races. There was little mycelial growth in the fungicide treatment 4 day after infection, DAI. Transmission electron microscopy (TEM) ultrastructure showed that *C. citratus*, *C. martini* and *E. caryophyllata* had direct antifungal action against *C. lindemuthianum*. These EOs damaged the conidia's ultrastructure by causing vacuolization, cytoplasmic leakage, and plasma membrane invagination. The activity of plant defense enzymes increased in the treatment with *C. citratus*. Moreover, *Cymbopogon citratus* and *E. caryophyllata* decreased anthracnose severity in both the preventive and the curative experiment by 29% and 21%, respectively. Essential oils demonstrated potential for the management of anthracnose in bean crops caused by *C. lindemuthianum*.

Keywords *Phaseolus vulgaris* · Defense enzymes · Transmission electron microscopy · Alternative control of plant diseases

J. M. A. Hoyos · A. F. Dorigan · P. R. da Silveira · C. R. G. Labory · R. Fernandes · E. Alves (✉)
Department of Plant Pathology, Federal University of Lavras, Lavras, Minas Gerais, Brazil
e-mail: ealves@ufla.br

A. F. Dorigan
School of Agronomy, Phytosanitary Department, Federal University of Goiás, Avenida Esperança, S/N, Goiânia, Brazil

P. M. R. Júnior
Embrapa Semiárido, Rodovia BR-428, Km 152, Petrolina, PE CEP 56302-970, Brazil

Introduction

According to the FAO (2024), common bean (*Phaseolus vulgaris* L.) is a significant grain legume for direct human consumption worldwide. Brazil produced 2.84 million tons of beans in 2022, grown on 2.607.616 hectares (Faostat, 2024). Anthracnose caused by the

hemibiotrophic fungus *Colletotrichum lindemuthianum* is the major disease of common bean and causes significant yield loss in Latin America and Africa (De Lima Castro et al., 2017; Padder et al., 2017; Pandey et al., 2023). According to da Silva et al. (2020), Chakraborty et al. (2022) and Kaur et al. (2023), legume losses in the field caused by this disease can reach 60 to 100%.

C. lindemuthianum is difficult to control, mostly because of its high pathogenic diversity and tendency to spread through infected seeds (Gadaga et al., 2020; Padder et al., 2017). In Brazil, races 65 and 73 are the most frequently observed in bean crops (Pinto et al., 2012). Currently, chemical control with fungicides like Strobilurin fungicides, fluazinam, and thiophanate-methyl and resistant cultivars are the major methods to control anthracnose on beans (Boersma et al., 2020). The use of fungicides can favor the selection of resistant plant pathogens and cause adverse effects on human health and the environment (Blazquez, 2014; Fisher et al., 2018). Disease management alternatives could take the place of fungicides and support modern, sustainable agriculture (Ballaré, 2014). Essential oils (EOs), can work both directly on the pathogen and indirectly on the plant, triggering defensive mechanisms against the disease (Jamiołkowska, 2020; Stenger et al., 2021).

EOs of medicinal and aromatic plants have the property of inhibiting microbial and fungal development (Álvarez-Martínez et al., 2021; Sarto & Zanusso Júnior, 2014; Wang et al., 2019). Moreover, oil compounds are eco-friendly, biodegradable and are less toxic to mammals, which makes them of great potential for use in integrated pest management (Zaker, 2016). EOs have been reported to be effective against some pathogenic fungi. Oil from *Thymus* spp. has shown strong antifungal activities against *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Phomopsis*, *Trichoderma*, *Trichophyton*, *Microsporon*, and *Epidermophyton* spp. (Soković et al., 2009). Oregano and thyme oils inhibit the mycelial growth of *C. lindemuthianum*, *Didymella rabiei*, *F. solani*, *Botrytis cinerea*, *Alternaria* spp., *Phytophthora infestans*, *Verticillium fungicola*, and more (Daferera et al., 2003; Lopez-Reyes et al., 2013; Pane et al., 2013; Regnier et al., 2014; Soković & van Griensven, 2006; Soylu et al., 2006). Lemongrass and basil oils demonstrate significant protection against *P. capsici*, *P. drechsleri*, and *P. melonis* (Amini et al., 2016). Ultra structural studies of the effects of EOs on phytopathogenic fungi show that EOs may damage the fungal membrane and promote electrolyte leakage, resulting in fungal death (Perina et al., 2014; Wu et al., 2023). Moreover, a high oil concentration *in vitro* may cause ultrastructural changes such as nucleolar segregation, lipid degeneration and damaged mitochondria (André et al., 2017). Therefore, EOs may act either as fungitoxic substances (Wang et al., 2019) or induce plant resistance (Jamiołkowska, 2020; Stenger et al., 2021).

Few studies have been published on the antifungal activity and mechanism of action of EOs in *C. lindemuthianum*, including *Rosmarinus officinalis*, *Origanum vulgare*, and *Bunium persicum* (Khaledi & Hassani, 2018; Vaz et al., 2022), with the intention of using these oils in integrated disease management. The objectives of this study were to (i) assess antifungal activity of 26 EOs on the germination and mycelial growth of pathogen races 65 and 73, (ii) evaluate ultrastructural changes of oils of *Cymbopogon citratus*, *Eugenia caryophyllata* and *Cymbopogon martini* in fungal conidia, (iii) study the effect of oils on controlling anthracnose, applied before and after inoculation, and (iv) evaluate the effect of *C. citratus* oil on the activity of defense enzymes on bean plants.

Materials and methods

Fungal isolates

Two single-spore isolates of *C. lindemuthianum*, one of race 65 (LV 136) and the other of race 73 (LV 99) characterized in previous studies were used (Damasceno & Silva et al., 2007). These isolates were deposited in the collection of the Laboratory of Plant Resistance to Diseases, Department of Biology, Federal University of Lavras, Brazil. The isolates were maintained in culture medium M3 (Junqueira et al., 1984) and incubated in a growth chamber at 22 ± 2 °C under a 12 h photoperiod for 15–20 days.

Essential oils

EOs extracted from 26 medicinal, aromatic, or forestry plants of the families Apiaceae (*Pimpinella anisum* L.), Asteraceae (*Baccharis dracunculifolia* DC. *Matricaria recutita* L.), Boraginaceae (*Cordia verbenacea* DC.), Cupressaceae (*Chamaecyparis pisifera* (Siebold & Zucc.) Endl., *Chamaecyparis plumosa* Hort. ex Beissn.), Lamiaceae (*Lavandula officinalis* ChaixexVill.,*Mentha arvensis* L., *Ocimum*

basilicum L., *Ocimum selloi* Benth., *Origanum vulgare* L., *Rosmarinus officinalis L., Thymus vulgaris* L.), Lauraceae (*Cinnamomum* sp., *Laurus nobilis* L.), Myrtaceae (*Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson, *Eucalyptus globulus* Labill., *Eugenia caryophyllata* Thunb., *Melaleuca alternifolia* Cheel), Poaceae (*Cymbopogon citratus* DC) Stapf., *Cymbopogon martini* (Roxb.)., *Cymbopogon nardus* (L.) Rendle), Rutaceae (*Citrus limon* (L.) Burm.f., *Citrus sinensis* (L.) Osbeck.), Verbenaceae (*Lippia citriodora* Kunth), and Zingiberaceae (*Zingiber officinale* Roscoe) were used in this study.

EOs of *Lavandula*, *Ocimum*, *Origanum,* and *Cinnamomum* were extracted by hydrodistillation in a modified Clevenger-type apparatus under continuous heating, for three hours, after the appearance of the first drop resulting from the condensation of the entrained water vapor (Oreopoulou et al., 2019). The first three plants were collected in the Garden of Medicinal Plants of the Federal University of Lavras, Minas Gerais, Brazil, and kept in a refrigerator at 4 °C until extraction. The *Cinnamomum* bark was bought at a local store. The other oils were supplied by Chamel Natural Products Industry and Trade Ltd., Paraná, Brazil. All oils were kept in a refrigerator at 4 °C and protected from light in amber vials.

The effect of EOs on conidial germination of *C. lindemuthianum*

All 26 oils were diluted in sterile distilled water containing Tween 20, 0.1%; the final concentrations were 0.02, 0.1, and 0.5%. Forty microliters aliquots of diluted oil and 40 µL of conidial suspension of *C. lindemuthianum* 1.2×10^6 conidia/mL (concentration in all experiments) were placed in polypropylene 96-well plates. The control treatment received 40 µL of conidial suspension and 40 µL of sterile distilled water.

Germination was halted after 12 h incubation at 22 ± 2ºC with 20 µL lactoglycerol cotton blue. For each treatment, fifty conidia were evaluated under a stereo microscope. The conidia were considered to have germinated when the germ tube length was longer than their diameter. The experimental design was completely randomized. Six replicates were prepared for each treatment. The effect of the EOs on conidial germination was assessed using the factorial arrangements $26\times3\times2$ (oil × concentrations × races) and a control treatment. Each plate well was an experimental unit. The plates were incubated for 12 h.

Data analysis was performed by generalized linear models assuming a logit link and a binomial distribution. The linear predictor is represented by $\log[p_i / (1- p_i)]=\beta_{0i}+\beta_{1i}\cdot\sqrt{c}$, where p_i = probability of inhibition of fungal germination when using the EO i ($i=1, 2,\ldots, 26$) at concentration c ($c=0.02$, 0.1 and 0.5%). β_{0i} and β_{1i} are parameters that shape the relationship. Inference was conducted by considering Pearson residuals' estimated over dispersion parameter (φ).

The effect of EOs on mycelial growth of *C. lindemuthianum*

Before the experiment, the pH of each treatment was measured [PDA medium (42 g/L potato-dextrose-agar, KASVI, India) plus products]: with EOs of *C. citratus* pH 5.7; *E. caryophyllata* pH 5.68*; C. martini* pH 5.68; Azoxystrobin fungicide (250 g/L a.i., Syngenta, Basel, Switzerland) 5.65; ASM (acibenzolar-S-methyl) pH 5.36; sterile distilled water + Tween 20, 0.1% pH 5.66; sterile distilled water pH 5.63.

Each race of *C. lindemuthianum* received 0.1% oil of *C. citratus*, *E. caryophyllata*, and *C. martini* since oils inhibited 95% germination at this concentration or less. The test was performed in Petri dishes 9 cm in diameter with PDA medium. The oils were added to the medium before being poured onto Petri dishes after the temperature fell to 50 °C. The control treatments comprised sterile distilled water and sterile distilled water plus Tween 20, 0.1%. Azoxystrobin fungicide at 0.4 g L^{-1} was used as an inhibition pattern, and Bion (acibenzolar-S-methyl, ASM) at 0.08 g L^{-1} was used as the standard induced resistance in seven treatments.

Fungal plugs of 5 mm containing young mycelia were placed in the center of each plate, which was incubated at 22 ± 2 °C and 12 h photoperiod until the end of the evaluation. The experimental design was completely randomized. Eight replicates were prepared for each treatment, and each plot consisted of a plate. The diameter of the colonies was evaluated every two days from inoculation until the mycelium of the control occupied the entire medium surface. Using these data, we calculated the mycelial growth rate (MGR) according to the formula proposed by Maguire (1962).

The effect of EOs on the ultra structure of the *C. lindemuthianum* conidia

This study was performed only with race 65 of *C. lindemuthianum*, which has greater geographical spread and high virulence in Brazil (Balardin & Kelly, 1997; Pinto et al., 2012). The oils were *C. citratus, E. caryophyllata* and *C. martini* 0.1%. Azoxystrobin fungicide 0.4 g L^{-1} and sterile distilled water with Tween 20, 0.1% were used as control treatments.

Conidial masses were fixed in a modified Karnovsky solution (glutaraldehyde 2.5%, formaldehyde 2.5% in sodium cacodylate buffer 0.05 M pH 7.2, and calcium chloride ($CaCl_2$) 0.001 M). The material was prepared for transmission electron microscopy (TEM) as described by Rozwalka et al. (2010). Each treatment was replicated twice. Observations were performed in a transmission electron microscope Zeiss EM 109, 80 kV.

The chemical composition of EOs

EOs showing antifungal activity higher than 97% at 0.1%, *C. martini* (0.144 mg), *M. chamomilla* (0.127 mg), *E. caryophyllata* (0.200 mg), *T. vulgaris* (0.147 mg), *C. verbenaceae* (0.179 mg), *C. citratus* (0.160 mg) and *Cinnamomum* sp. (0.118 mg) were dissolved in 0.5 mL dichloromethane. One microliter of diluted EOs was injected into a gas chromatograph coupled to a mass spectrometer (GCMS-QP2010 Plus, Shimadzu). The chromatograph used ultrapure helium as carrier gas at a flow rate of 1.8 ml min^{-1}. Separation was performed on a capillary column Equity-5 (30 m×0.22 mm ID×0.25 µm) with column oven at 60 °C for 2 min, heating at 3 °C min^{-1} to 240 °C and remaining at 240 °C for 15 min. The injector temperature was 220 °C, with a manual split injection at 1:15 and a running time of 77 min. The mass spectrometer used interface GC–MS at 250 °C, with an ion source at 200 °C and an impact ionization mode at 70 eV. Oil constituents were identified by testing the mass spectral similarity between the relative area (%) and GCMS library data (Wiley 8).

The effect of the EO of *Cymbopogon citratus* in the activity of defense enzymes

Bean plants were obtained and inoculated as described in the preventive experiment. Treatments comprised ASM, oil of *C. citratus* and, and one control in plants inoculated with race 65 of *C. lindemuthianum* and non-inoculated plants. For enzyme analysis, leaf samples were collected at 3, 4, and 5 days after spraying, DAS. The inoculation occurred at 3 DAS. After each collection, leaves were frozen in liquid nitrogen and stored at −80 °C until maceration.

One gram of each powder sample was placed in tubes. Five milliliters of potassium phosphate buffer 100 mM pH 7.0 was added per sample and tubes were homogenized for 10 s. The suspension was centrifuged at 12.000 rpm for 15 min (4 °C), and the supernatant was used as an enzyme source.

Enzymatic activity

The total protein of the enzyme extract was determined using the Bradford method (1976), using the bovine serum albumin (BSA) standard curve. Chitinase (CHI; EC 3.2.1.14) activity was evaluated according to Wirth and Wolf (1990), using substrate CM-Chitin-RBV. CHI activity was expressed in activity units per milligram of protein per minute. Polyphenol oxidase (PPO; EC 1.10.3.1) activity was measured by converting catechol to quinone (Gauillard et al., 1993), using the molar extinction coefficient of 3,450 M^{-1} cm^{-1} and expressed in quinone formation per milligram of protein per minute. Guaiacol peroxidase (POX; EC 1.11.1.7) activity was calculated based on the method by Urbanek et al. (1991), using the molar extinction coefficient of 26.6 M^{-1} cm^{-1} and expressed in µmol of oxidized H_2O_2 per milligram of protein per minute. Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) activity was measured according to the method of Mori et al. (2001). The PAL activity values were expressed in µmol of trans-cinnamic acid per milligram of protein per minute using the molar extinction coefficient of 30.5 M^{-1} cm^{-1}. The experimental design was randomized complete block. Four replicates were prepared for each treatment, and experimental plots comprised four plants per collection. Enzymatic activities were compared using the Duncan test at 5% probability at each collection time.

The effect of the EOs on bean anthracnose control

Two experiments, one preventive and one curative, were conducted in a greenhouse to measure the effect of EOs on anthracnose control. In the preventive experiment, plants of the bean cultivar Perola were grown in a 3 L plastic pot containing a 2:1:1 ratio of Plantmax, soil and sand. The plants were grown in a

Table 1 Estimation of coefficients of logistic regression model for races 65 and 73 of *Colletotrichum lindemuthianum* treated with essential oils *in vitro*

	β0	β1	c95%i	p0.5	β0	β1	c95%i	p0.5
Melaleuca alternifolia	−1,31	4,52	0,89	0,87	−0,97	0,44	78,62	0,34
Chamaecyparis pisifera	−1,15	6,65	0,38	0,97	−0,36	0,15	457,63	0,44
Pimpinella anisum	−1,23	8,40	0,25	0,99	−0,03	0,60	24,54	0,60
Ocimum selloi	−1,84	7,16	0,45	0,96	−0,65	1,57	5,22	0,61
Eucalyptus globulus	−4,96	31,51	0,06	1,00	−1,06	2,17	3,40	0,62
Corymbia citriodora	−2,34	9,32	0,32	0,99	−1,19	2,48	2,78	0,64
Citrus limon	−2,14	13,04	0,15	1,00	−0,04	1,00	8,85	0,66
Lippia citriodora	0,79	5,86	0,14	0,99	−1,09	3,18	1,61	0,76
Mentha arvensis	−1,60	8,60	0,28	0,99	−1,63	3,98	1,32	0,77
Ocimum basilicum	−1,43	8,16	0,29	0,99	−0,73	3,81	0,93	0,88
Baccharis	−1,91	8,91	0,30	0,99	−1,79	5,65	0,70	0,90
Rosmarinus officinalis	−0,97	5,86	0,45	0,96	−2,70	7,63	0,55	0,94
Lavandula officinalis	−2,48	8,59	0,40	0,97	−2,02	7,14	0,48	0,95
Cymbopogon nardus	−1,84	6,94	0,48	0,96	−1,81	7,05	0,45	0,96
Chamaecyparis	−1,25	6,82	0,38	0,97	−1,17	6,24	0,43	0,96
Origanum vulgare	-	117,15	0,03	1,00	−1,60	6,58	0,48	0,96
Cordia verbenacea	−0,94	6,89	0,32	0,98	−0,96	6,23	0,39	0,97
Laurus nobilis	-	115,83	0,03	1,00	−1,35	9,00	0,23	0,99
Zingiber officinale	−1,91	7,21	0,45	0,96	-	117,39	0,03	1,00
Citrus sinensis	−5,20	34,46	0,06	1,00	−2,43	11,53	0,22	1,00
Matricaria recutita	−2,82	21,11	0,07	1,00	−4,32	27,26	0,07	1,00
Eugenia caryophyllata	-	121,23	0,03	1,00	−4,68	29,33	0,07	1,00
Thymus vulgaris	-	113,49	0,03	1,00	−2,06	20,80	0,06	1,00
Cinnamomum sp.	-	116,48	0,03	1,00	-	117,23	0,03	1,00
Cymbopogon martini	-	106,49	0,02	1,00	−2,93	27,30	0,05	1,00
Cymbopogon citratus	23,63	0,00	*	1,00	−7,26	91,66	0,01	1,00

*1,835,004,501,896,020,000,000.00

c95%i represents the concentration of essential oil that inhibits 95% of conidial germination, was calculated as $p = \exp(\beta_0 + \beta_1.\sqrt{c}) / 1 + \exp(\beta_0 + \beta_1.\sqrt{c})$

p 0.5 is the percentage of germination inhibition estimated for a concentration of 0.5%

greenhouse under natural conditions (25 ± 5ºC) over the experimental period. In the V3 stage, plants were hand-sprayed with 3 ml EO of *C. citratus*, *E. caryophyllata*, and *C. martini* 0.1% in distilled water with Tween 20, 0.1%; ASM 0.08 g L^{-1}; azoxystrobin 0.4 g L^{-1} and distilled water up to the point of runoff. At 7 days after spraying (DAS), the plants were spray-inoculated with a conidial suspension of *C. lindemuthianum* and placed in a growth chamber for 48 h.

The experimental design was a randomized complete block. Twelve plants were used for each treatment. Four replicates were prepared for each treatment, and each experimental plot consisted of one pot containing three plants. Anthracnose severity assessment was carried out on four consecutive dates at weekly intervals starting at 5 DAI. The diagrammatic scale proposed by Godoy et al. (1997) was used. The disease severity values from the 4 anthracnose assessments were integrated as area under the disease progress curve (AUDPC), following the method proposed by Shaner and Finney (1977). When *F* test was significant, means were compared by the Scott Knott test ($p \leq 0.05$).

In the curative experiment, bean plants obtained as in the previous experiment were inoculated at the

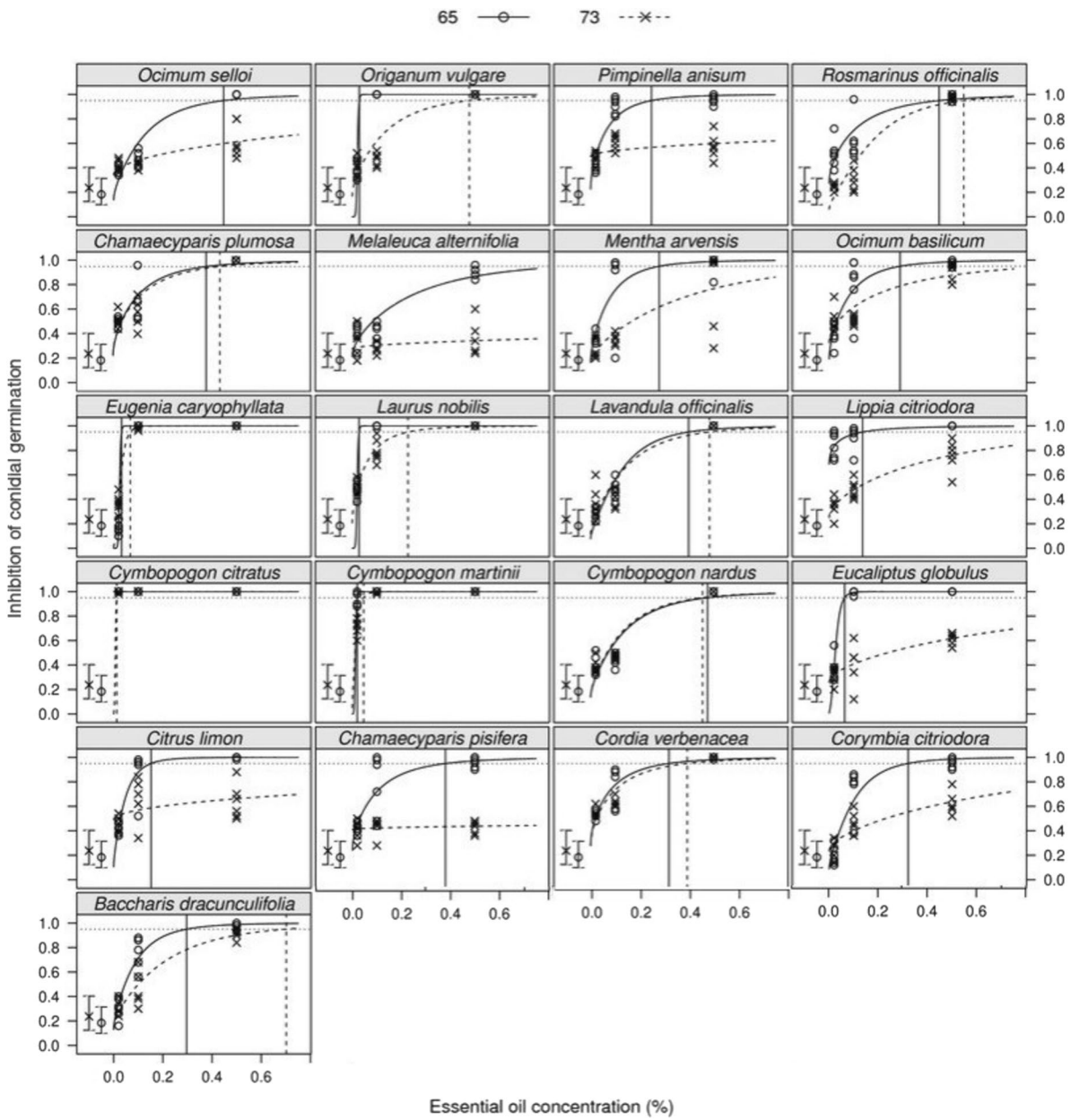


Fig. 1 Regression models fitted to data of number of germinated conidia. Vertical dashed lines represent the concentration that causes 95% germination inhibition in races 65 () and 73 () of *Colletotrichum lindemuthianum*. Confidence intervals at 95% for germination rate in control treatments are shown near the regression curves. Dots stand for germination rate

V3 stage. At 7 DAI, all treatments from the previous experiment were sprayed except for the ASM treatment. The experimental design, anthracnose assessments, AUDPC determination, and data analysis were performed as described for the previous experiments.

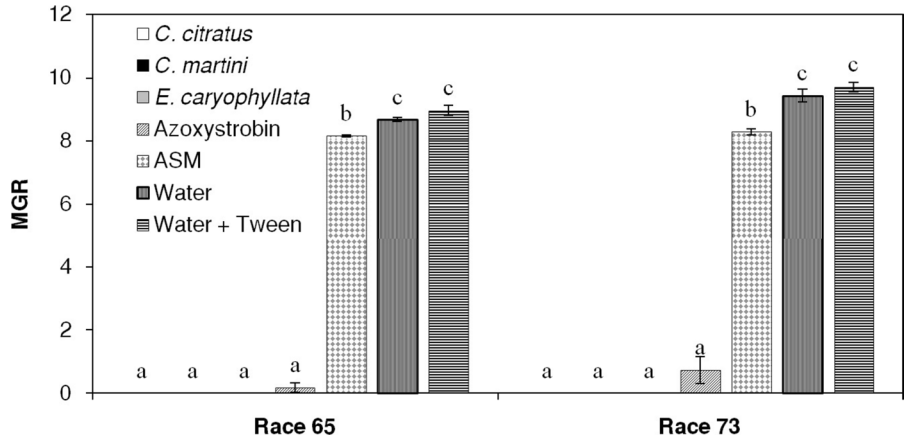


Fig. 2 Effect of *C. citratus, C. martini* and *E. caryophyllata* oils at 0.1%, azoxystrobin fungicide 0.4 g L^{-1}, acibenzolar-S-methyl (ASM) 0.08 g L^{-1}, distilled water and distilled water plus Tween 20, 0.1% in mycelial growth rate (MGR) in two races of *Colletotrichum lindemuthianum*. Means followed by the same letter in each race do not differ by *Scott Knott test* ($p \leq 0.05$). Error bars represent standard error

Results

The effect of EOs on conidial germination of *C. lindemuthianum*

Analysis of deviance results demonstrated a significant interaction, indicating that differences occurred among treatments for germination percentage. All 26 oils inhibited conidial germination 87–100% in pathogen race 65 at a concentration of 0.5%, whereas 17 oils inhibited conidia germination in race 73. The oils of *C. citratus, C. martini, Cinnamomum sp., T. vulgaris, E. caryophyllata,* and *M. recutita* inhibited germination by 95% in both races of *C. lindemuthianum* at the lowest concentrations, 0.01–0.07% (Table 1 and Fig. 1).

According to the estimated logistic regression coefficients, an increase in the concentration of *C. sinensis*, *L. nobilis*, *C. verbenacea*, and *O. vulgare* from 0.1 to 0.22, 0.23, 0.39, and 0.48%, respectively, inhibits conidial germination by 95% in race 73. The increased concentration of most EOs promotes increased antifungal activity against both races of *C. lindemuthianum*, and the conidial germination of race 65 was more inhibited (Table 1).

The effect of EOs on mycelial growth of *C. lindemuthianum*

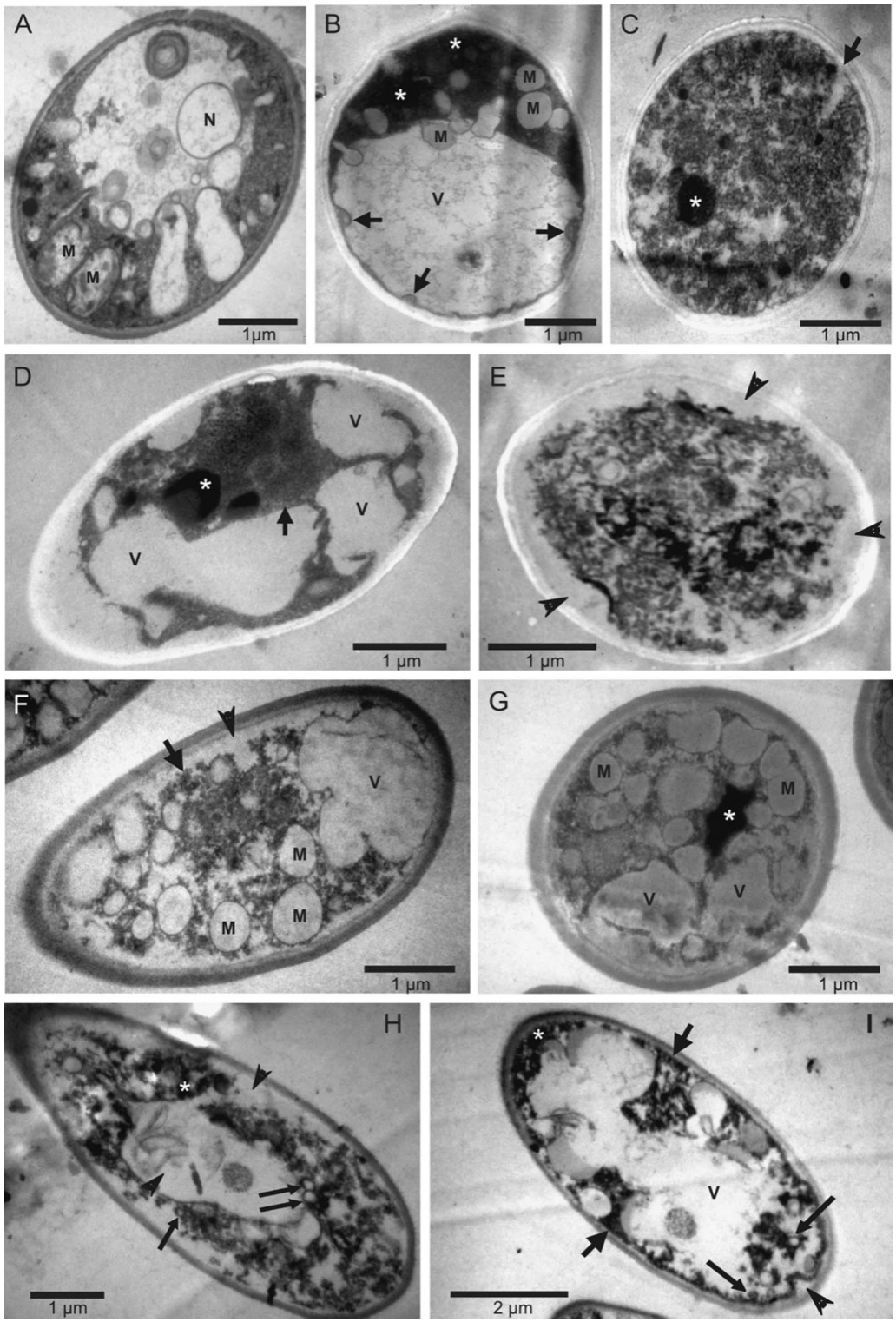
The treatment showing the highest mycelial growth rate in both races was sterile distilled water + Tween, followed by the treatment with sterile distilled water alone.. The ASM treatment differed from the two others in that it slightly inhibited fungal growth. The other treatments had no growth. The oils of *C. citratus*, *E. caryophyllata*, and *C. martini* at a concentration of 0.1% completely inhibited mycelial growth in both races. Fungicide treatment demonstrated little growth after 4 days and was not statistically different from the EOs (Fig. 2).

The effect of EOs on the conidial ultra structure of *C. lindemuthianum*

The EOs of *C. citratus*, *E. caryophyllata*, and *C. martini* were selected for their potential to inhibit conidial germination in *C. lindemuthianum* at a concentration of 0.02, 0.1, and 0.5%. When conidia of race 65 of *C. lindemuthianum* were treated with sterile distilled water (control), the treatment did not affect the integrity of the cell wall, plasmalemma, or cytoplasm. Some organelles such as the nucleus and the mitochondria showed well-defined envelopes. Contrary to this, conidia treated with the fungicide and oils at a concentration of 0.1% exhibited ultrastructural changes (Fig. 3A).

The treatment of conidia with the fungicide caused cytoplasmic vacuolization, invagination of the plasmalemma, and disruption of the mitochondrial internal structure with decreased cristae (Fig. 3B). Accumulation of electro-dense material covered portions of the cytoplasm, which was condensed as a result of contact with osmium. This may indicate penetration of a fungitoxic agent, in this case of synthetic origin (Fig. 3B and C). Moreover, some conidia were very degenerated and had ruptures of the plasmalemma (Fig. 3C).

Conidia treated with the EO of *C. citratus* showed significant changes, including intense vacuolization


A
N
M
M
1µm
B
*
*
M
M
M
V
1 µm
C
*
1 µm
D
V
*
V
V
1 µm
E
1 µm
F
V
M
M
M
1 µm
G
M
M
*
V
V
1 µm
H
*
1 µm
I
*
V
2 µm

◂**Fig. 3** Transmission electron micrographs of the effect of essential oils (EOs) 0.1% on conidial ultrastructure of *Colletotrichum lindemuthianum* race 65. **A**: Control, mitochondria (M), nucleus (N). **B** and **C**: fungicide treatment. **B**: some mitochondria (M), a large vacuole (V), invagination of the plasmalemma (arrows) and electro-dense material (asterisks). **C**: severely damaged conidia with broken plasmalemma (arrow) and electro-dense material (asterisks). **D** and **E**: treatment with essential oil of *Cymbopogon citratus*. **D**: intense vacuolization (V), cytoplasmic condensation (arrows) and electro-dense material (asterisks). **E**: very degenerate conidia, probably not feasible, with plasmolysis and voids (arrowheads). **F** and **G**: treatment with essential oil of *Eugenia caryophyllata*. **F**: cytoplasmic condensation (arrows), voids (arrowheads), vacuoles (V) and several mitochondria (M). **G**: some mitochondria (M), vacuoles (V) and electro-dense material (asterisks). **H** and **I**: treatment with essential oil of *Cymbopogon martini*. **H**: altered conidia with vesicles (arrows) and voids (arrowheads). **I**: invagination of plasma membrane (arrowheads), vesicles (thin arrows), vacuoles (V), electro-dense material (asterisks) and dispersed cytoplasm mixed with voids or vacuoles (larger arrows)

and condensation of the cytoplasm (Fig. 3D). The accumulation of electro-dense clusters covering parts of the condensed cytoplasm indicated the penetration of a fungitoxic agent of biotic origin (Fig. 3D and E). Plasmolysis of conidia, large voids produced by previous cytoplasm leakage, and lysis of membranous organelles were observed. In addition, the cell wall was thinner (Fig. 3E), and there was electro-dense material (Fig. 3D and E).

The EO of *E. caryophyllata* caused vacuolization, fusion of vacuoles, cytoplasm condensation, and disruption of the mitochondrial internal structure with decreased cristae (Fig. 3F and G). Cytoplasm leakage and electro-dense compounds covered parts of the aggregated cytoplasm (Fig. 3F and G). Conidia exposed to the oil of *C. martini* had voids (Fig. 3H), intense vacuolization, shrinkage, and invagination of the plasmalemma (Fig. 3I), cytoplasmic condensation, electron-dense aggregates and vesicles (Fig. 3H and I).

The chemical composition of the EOs

The identification and composition of the main chemical constituents of the EOs reveal a broad diversity. Cinnamaldehyde (92.36%) in *Cinnamomum* sp., eugenol (91.94%) in *E. caryophyllata*, geraniol (75.92%) in *C. martini*, pulegone (68.96%) in *C. verbenaceae*, geranial (58.89%) and neral (38.50%) in *C. citratus*, thymol (33, 72%) in *T. vulgaris*, and -b-farnesene (31.17%) in *M. recutita* were found.

The effect of the EO of *Cymbopogon citratus* in the activity of defense enzymes

The activity of chitinase (CHI) was higher in the ASM treatment at 3 DAS without inoculation with *C. lindemuthianum*. The treatment with the EO of *C. citratus* (Cc) and the control (Te) did not differ; however, they differed from the ASM treatment (Fig. 4A). After plant inoculation (after the first collection), at 4 DAS, all treatments had an activity similar to the activity of CHI. At 5 DAS, the ASM treatment and the treatment inoculated with *C. citratus* oil (Cc) had equal CHI activity, differing from the other treatments (Fig. 4A).

Polyphenol oxidase (PPO) activity at 3 DAS was statistically similar for all treatments. At 4 DAS, PPO activity was higher in plants treated with ASM without *C. lindemuthianum* inoculation. At 5 DAS, which was the last assessment time at 2 DAI, the ASM treatment inoculated with *C. lindemuthianum* was the most active, followed by the treatment inoculated with *C. citratus* oil, differing from the other treatments (Fig. 4B). After this period, there could be an increase in enzyme activity when plants recognized the pathogen.

Peroxidase activity (POX) remained similar in all treatments at 3 and 4 DAS. At 5 DAS, enzyme activity largely increased in plants treated with ASM and inoculated with *C. lindemuthianum*, differing from the other treatments (Fig. 5A). Regarding the activity of phenylalanine ammonia-lyase (PAL) at 3 to 5 DAS, treatments had no significant effect on both inoculated and non-inoculated plants (Fig. 5B).

The effect of EOs on bean anthracnose control

When assessing the protective effect on bean plants against anthracnose, the treatments using ASM, fungicide and the EO of *C. citratus* showed lower disease severity than the control (water) treatment. The ASM treatments had the most significant disease reduction, up to 62%, followed by the fungicide and *C. citratus* oil treatment, which reduced the disease by 44% and 29%, respectively. These treatments did differ significantly but differed from the ASM and the control treatments. Other treatments did not reveal a significant decrease in the disease severity or differ from the control (Table 2). Nevertheless, all treatments had a

curative effect by reducing anthracnose severity compared to the control. The treatment did not differ from each other by the Scott-Knott test ($p < 0.05$). The most significant reduction was caused by the fungicide (28.5%), followed by the EOs of *C. citratus*, *E. caryophyllata*, and *C. martini*, at 21%, 18.7%, and 13.5%, respectively (Table 2).

The EOs used in these experiments at a concentration of 0.1% did not produce any phytotoxicity symptoms on beans. Thus, at 7 DAS, the EOs could not have had a proper antimicrobial effect against *C. lindemuthianum*, which had been inoculated seven days after oil application. Instead, oils could have caused induced resistance in beans. Both preventive and curative experiments with *C. citratus* decreased the severity of anthracnose by 29% and 21%, respectively. Moreover, the treatment did not differ statistically from the fungicide treatment, which reduced disease by 44% and 28.5% in the two experiments, respectively (Table 2).

Discussion

Natural plant products like essential oils (EOs) have the potential to be a viable substitute for synthetic pesticides and reduce the negative impacts of pesticides on the environment and human health. Bean crops face biotic stresses, especially fungal diseases like anthracnose caused by *C. lindemuthianum*, the major disease of common beans. Natural products, especially EOs, offer an alternative for disease management and have other advantages as well. Moreover, EOs may fit Organic Materials Review Institute (OMRI) standards for certification for pest and plant disease control in crop fields (OMRI, 2024).

Firstly, some of the EOs in this study were effective against *C. lindemuthianum*. For example, the Eos of *C. citratus*, *C. martini*, *Cinnamomum* sp., *T. vulgaris*, *E. caryophyllata*, *M. recutita*, and *C. sinensis at a concentration of* 0.1% possess antifungal activity, being able to inhibit 95% conidial germination of *C. lindemuthianum.* Not surprisingly, the broad efficacy of the EOs occurs because plants with a 0.01 to 5% content of the EOnaturally use their oil components to defend themselves against plant diseases (Arshad et al., 2014; Butnariu & Sarac, 2018). Mycelial growth of *C. lindemuthianum* was entirely inhibited by *C. citratus*, *E. caryophyllata*, and *C. martini* at a concentration of 0.1%, which indicates that EOs affect fungal development in their initial stage. Inhibition of germination and/or mycelium growth caused by pure EOs or their constituents may occur in different concentrations and forms of application (Khan & Ahmad, 2011). In previous studies, the volatile geraniol identified as the main constituent of *C. citratus* has been demonstrated to strongly inhibit mycelial growth of *Colletotrichum falcatum* and *Botrytis cinerea* (Singh et al., 2002; Yan et al., 2021).

Secondly, in the present study, the effect of the Eos on membrane permeability was confirmed by transmission electron microscopy causing germination inhibition of the conidia of *C. lindemuthianum*. A discontinuous wavy plasma membrane and the degeneration of inner mitochondrial structure were found in *C. lindemuthianum* conidia exposed to EOs. According to reports from earlier research, two races of *Pseudocercospora griseola* experienced significant damage to their conidial ultrastructure, and *B. cinerea's* plasma membrane cells treated with EOs also experienced damage (Hoyos et al., 2012; Yan et al., 2021). Most of these damages are irreversible, and ultrastructural changes such as disruption and cytoplasmic leakage may be caused by the loss of membrane integrity, leading to cell death.

The complex composition of EOs suggests multiple mechanisms of action and different invasion targets (Sil et al., 2020). In our study, conidia of *C. lindemuthianum* treated with oil of *C. citratus* showed plasmolysis, large voids produced by previous cytoplasm leakage, and lysis of membranous organelles. Moreover, the Eos from *E. caryophyllata* caused vacuolization, a fusion of vacuoles, cytoplasm condensation, and disruption of the mitochondrial internal structure with decreased cristae, while *C. martini* cause voids, intense vacuolization, shrinkage, and invagination of the plasmalemma, cytoplasmic condensation, electron-dense aggregates and vesicles. It has been suggested that the Eos from *C. citratus, E. caryophyllata and C. martini* may cause severe ultra structural damage in *C. lindemuthianum* conidia. The membrane damage leads to the leakage of intracellular components to the extracellular matrix (Li et al., 2018). Cell death may occur by loss of cell content, followed by autolysis. Moreover, permeabilization of outer and inner mitochondrial membranes leads to cell death by apoptosis and necrosis (Yapryntseva et al., 2024). Studies on membrane permeability show that certain EO compounds, which are generally lipophilic substances, can penetrate

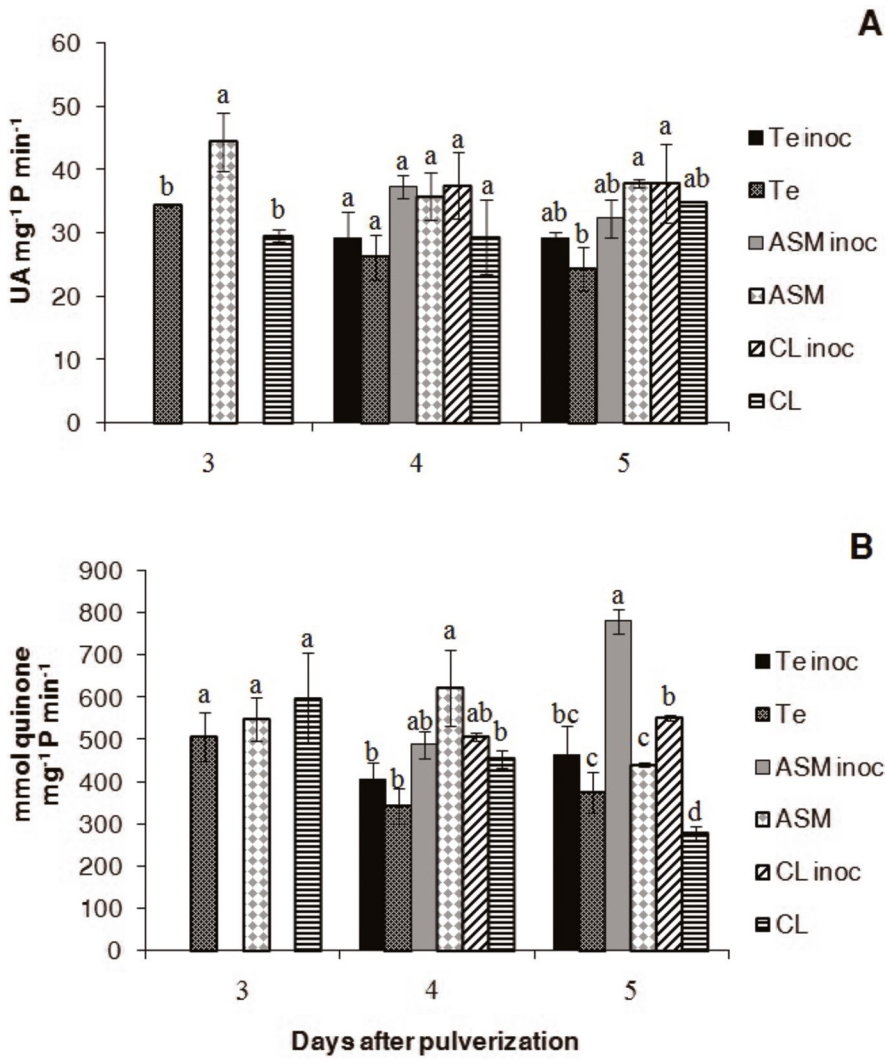


Fig. 4 Activity of chitinase (CHI) (**A**) and polyphenol oxidases (PPO) (**B**) in leaves of bean cv. Pérola after spraying with water (Te), water inoculated with *C. lindemuthianum* (Te inoc), acibenzolar S-methyl (ASM), acibenzolar S-methyl inoculated with *C. lindemuthianum* (ASM inoc), *C. citratus* (Cc) and *C. citratus* inoculated with *C. lindemuthianum* (Cci-noc). Inoculation with *C. lindemuthianum* occurred at 3 days after spraying. Bars with the same letter do not differ by *Duncan test* ($p \leq 0.05$). Error bars indicate the standard error

the cytoplasma membrane and wall (Dwivedy et al., 2016). Such compounds affect the structure of different layers of polysaccharides, fatty acids, and phospholipids, thus permeabilizing them. In eukaryotes, these oils promote depolarization of mitochondrial membranes by lowering the membrane potential, thus affecting the ion cycle of Ca^{2+} (Bakkali et al., 2005). The distortion of the cellular structure could cause expansion and disruption of the membrane, thus increasing its passive permeability and fluidity.

Cytotoxic activity of EOs is mainly due to the presence of phenols, aldehydes, and alcohols (Bajpai et al., 2012; Bakkali et al., 2008). The components of EOs that have the strongest antimicrobial activity are phenols, aldehydes, ketones, alcohols, ethers, and hydrocarbons in ascending order (Sadgrove & Jones, 2015). The composition of the main chemical constituents of EOs was determined and revealed a broad diversity. We identified the major components of the three EOs give the largest germination inhibition: geranial and neral (aldehydes) in *C. citratus*; eugenol (phenol) in *E. caryophyllata* and geraniol (alcohol) in *C. martini*. Previous studies of the antifungal components of EOs revealed that the oils consist of 20–60 components at various concentrations. The main constituents studied here may have a strong antimicrobial effect, which may indicate that these molecules have synergistic antimicrobial activity. The synergistic effects of EOs' major and minor constituents may be taken into consideration for their antimicrobial activity. Oils could be activating

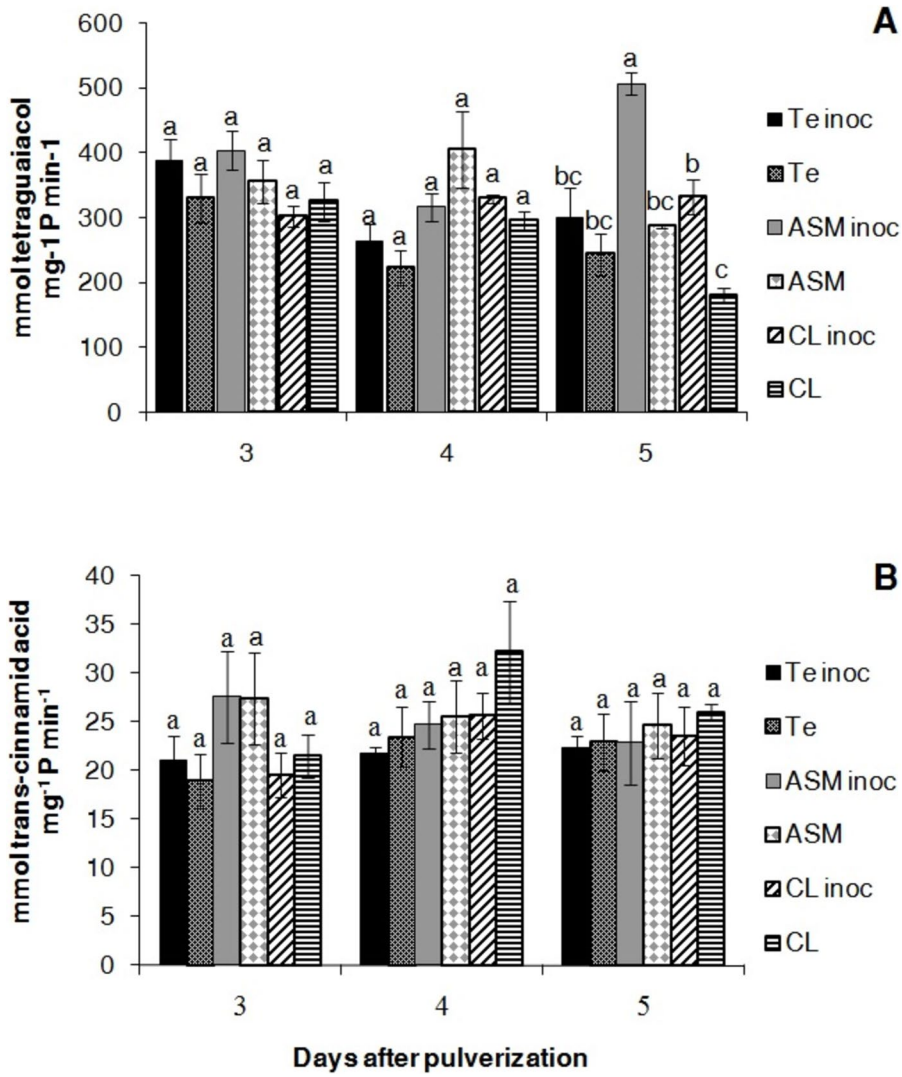


Fig. 5 Activity of peroxidase (POX) (**A**) and phenylalanine ammonia lyase (PAL) (**B**) in leaves of bean cv. Pérola after spraying with water (Te), water inoculated with *Colletotrichum lindemuthianum* (Te inoc), acibenzolar S-methyl (ASM), acibenzolar S-methyl inoculated with *C. lindemuthianum* (ASM inoc), *C. citratus* (Cc) and *C. citratus* inoculated with *C.lindemuthianum* (Cc-inoc). Inoculation with *C. lindemuthianum* occurred three days after spraying. Bars with the same letter do not differ by *Duncan test* ($p \leq 0.05$). Error bars indicate the standard error

Table 2 Area under the disease progress curve (AUDPC) and percentage of anthracnose control according to treatments for preventive and curative experiments in greenhouse beans

	Preventive Experiment		Curative Experiment	
Treatments	AUDPC	Control (%)	AUDPC	Control (%)
Water	127c	0	118b	0
C. martini	124c	2	102a	13.5
E. caryophyllata	103c	19	96a	18.7
C. citratus	89b	29	93a	21
Fungicide: Azoxystrobin	71b	44	85a	28.5
ASM: Acibenzolar-S-Methyl	48a	62	-	-
C.V. (%)	21.21	-	9.34	-

Means with same letter in the column do not differ by *Scott-Knott test* ($p < 0.05$)

defense mechanisms in plants, given that many antimicrobial oil constituents either volatilize quickly or do not stay long in the treated tissues (Bakkali et al., 2008).

Thirdly, concerning the effect of the EO of *C. citratus* on the activity of defense enzymes, *C. citratus* and the ASM treatment had equal chitinase activities, at 5 DAS. However, higher chitinase activity was found in plants inoculated with *C. lindemuthianum* 16 days after phosphite treatment, followed by plants treated with the ASM treatment(Costa et al., 2018). Our results for PPO activity demonstrated that the ASM treatment inoculated with *C. lindemuthianum* was the most active, followed by the treatment inoculated with the EO of *C. citratus*, at 5 DAS. On tomato leaves, an increase in PPO activity induced by the EO of *C. citratus* was observed when tomato was inoculated with *Alternaria solani*, at 3 DAS (Hendges et al., 2023). Previous studies have reported that an increase in enzyme activity may cause a high concentration of toxic oxidation products and, thus, a higher level of resistance to pathogens (Zheng et al., 1999).

In our study, all treatments possessed similar POX activity at 3 and 4 DAS. On the other hand, an increase of peroxidase activity was observed in the second and third pairs of tomato leaves with the treatment of the EO of *C. citratus* on tomato inoculated with *Alternaria solani* at 96 h, with a maximum response at 144 h (Hendges et al., 2023). Our findings revealed no significant effect on PAL activity of inoculated and non-inoculated plants at 3 to 5 DAS. Godinez-Vidal et al. (2007) also reported little differences in PAL activity on chilli plants at 7 and 14 DAI with *Nacobbus aberrans*. *Phaseolus vulgaris* and *Musa* spp. showed delayed PAL activity at 6, 8, and 12 days following treatment with biotic and abiotic inducers (Saravanan et al., 2004).

As observed by Walling (2001), volatiles are potent signals for induced resistance, but must be carefully evaluated. Many constituents of EOs are volatile, have antimicrobial action, and are synthesized immediately after pathogen infection by the plant's defense systems (Brilli et al., 2019). These volatiles are described as defense signals recognized by membrane receptors that respond by amplifying the signal. Such response amplification mechanisms generally requires low protein concentrations and can generate more effective and less transient responses than the synthesis of induced resistance enzymes.

Fourthly, regarding the protective effect on bean plants against anthracnose, *C. citratus* and *E. caryophyllata* oils were effective and reduced the disease severity by 21% and 18.7%, as shown in the curative experiment and by its high cytotoxicity to *C. lindemuthianum* conidia (Fig. 3D and E). Additionally, as was the case in the preventative experiment, *C. citratus* oil had an indirect effect, presumably as a result of the plant's defense mechanisms being induced. These findings are similar to those reported in other studies. For example, the use of EOs to control Asian soybean rust reduced the severity of disease on soybean plants by between 52 and 80%, even with a single application treated before inoculation (Da Silva et al., 2012).

EOs are effective against fungal and bacterial pathogens (Álvarez-Martínez et al., 2021; Parikh et al., 2021; Sarto & Zanusso Júnior, 2014; Wang et al., 2019). Oil compounds have a lot of potential for use in integrated management of plant diseases since they are economically viable, effective against wild kinds and pesticide-resistant strains, and have low mammalian toxicity (Brilli et al., 2019). Moreover, they can be a good alternative for anthracnose control, as they provide direct toxicity to fungi and can also induce defense responses in plants. In addition to EOs, another important strategy is to adopt integrated management strategies, such as biological control of *C. lindemuthianum* using endophytic *Induratia* spp., *Pseudomonas chlororaphis*, *P. fluorescens*, *Streptomyces bacillaris*, *Trichoderma* spp., systemic induction, and microbial volatile organic compounds (Bardas et al., 2009; Figueira et al., 2020; Kim et al., 2024; Martins et al., 2019; Mota et al., 2021; Xue et al., 1998). In conclusion, the fungitoxic potential is related to both chemical composition of oil and pathogen sensitivity to one or more constituents in different amounts. Direct fungitoxic action of EOs of *C. citratus*, *E. caryophyllata* and *C. martini* causes severe ultra structural damage in *C. lindemuthianum* conidia, thus invalidating germination. Our data suggests the potential application of three EOs as an alternative method for pathogens of bean crops, especially *C. lindemuthianum*. Therefore, they can be used in integrated management of anthracnose with less risk to human health and the environment.

Acknowledgements The authors wish to thank the Coordination of Improvement of Higher Education Personnel (CAPES) for financial support and doctoral scholarship, the National Council for Scientific and Technological Development (CNPq 33/2021) for productivity grant for the last author, and the

Foundation for Research Support of Minas Gerais (FAPEMIG) and Studies and Projects Financing Brazilian Agency (FINEP) for financial support to the Laboratory of Electron Microscopy and Ultra structural Analysis of the Federal University of Lavras, Minas Gerais, Brazil.

Data availability The data that supports the findings of this study are available upon request.

Declarations

Research involving human participants and/or animals This study does not include experiments with either human participants or animals.

Conflict of interest The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

Álvarez-Martínez, F. J., Barrajón-Catalán, E., Herranz-López, M., & Micol, V. (2021). Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. *Phytomedicine, 90*, 153626. https://doi.org/10.1016/j.phymed.2021.153626

Amini, J., Farhang, V., Javadi, T., & Nazemi, J. (2016). Antifungal Effect of Plant Essential Oils on Controlling Phytophthora Species. *The Plant Pathology Journal, 32*, 16–24. https://doi.org/10.5423/ppj.oa.05.2015.0091

André, W. P. P., Cavalcante, G. S., Ribeiro, W. L. C., dos Santos, J. M. L., Macedo, I. T. F., Paula, H. C. B., & de Bevilaqua, C. M. L. (2017). Anthelmintic effect of thymol and thymol acetate on sheep gastrointestinal nematodes and their toxicity in mice. *Revista Brasileira De Parasitologia Veterinária, 26*, 323–330. https://doi.org/10.1590/S1984-29612017056

Arshad, Z., Asif Hanif, M., Waseem Khan Qadri, R., and Mumtaz Khan, M. (2014). Role of essential oils in plant diseases protection - A review. *IJCBS*, *6*, 11-17.

Bajpai, V. K., Baek, K.-H., & Kang, S. C. (2012). Control of *Salmonella* in foods by using essential oils: A review. *Food Research International, 45*, 722–734. https://doi.org/10.1016/j.foodres.2011.04.052

Bakkali, F., Averbeck, S., Averbeck, D., Zhiri, A., & Idaomar, M. (2005). Cytotoxicity and gene induction by some essential oils in the yeast Saccharomyces cerevisiae. *Mutation Research/genetic Toxicology and Environmental Mutagenesis, 585*, 1–13.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils - A review. *Food and Chemical Toxicology, 46*, 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Balardin, R. S., & Kelly, J. D. (1997). Re-characterization of *Colletotrichum lindemuthianum* races. *Bean Improvement Cooperative Annual Report, 40*, 126–127.

Ballaré, C. L. (2014). Light Regulation of Plant Defense. *Annual Review of Plant Biology, 65*, 335–363. https://doi.org/10.1146/annurev-arplant-050213-040145

Bardas, G. A., Lagopodi, A. L., Kadoglidou, K., & Tzavella-Klonari, K. (2009). Biological control of three *Colletotrichum lindemuthianum* races using *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas fluorescens* WCS365. *Biological Control, 49*, 139–145. https://doi.org/10.1016/j.biocontrol.2009.01.012

Blazquez, M. A. (2014). Role of natural essential oils in sustainable agriculture and food preservation. *Journal of Scientific Research and Reports, 3*, 1843–1860. https://doi.org/10.9734/JSRR/2014/11376

Boersma, S. J., Depuydt, D. J., Vyn, R. J., & Gillard, C. L. (2020). Fungicide efficacy for control of anthracnose of dry bean in Ontario. *Crop protection, 127*, 104979. https://doi.org/10.1016/j.cropro.2019.104979

Brilli, F., Loreto, F., & Baccelli, I. (2019). Exploiting plant volatile organic compounds (VOCs) in agriculture to improve sustainable defense strategies and productivity of crops. *Frontiers in Plant Science, 10*, 264. https://doi.org/10.3389/fpls.2019.00264

Butnariu, M., and Sarac, I. (2018). Essential oils from plants. *Journal of Biotechnology and Biomedical Science*, 1, 35–43. https://doi.org/10.14302/issn.2576-6694.jbbs-18-2489

Chakraborty, N., Sarkar, A., Dasgupta, A., Paul, A., Mukherjee, K., & Acharya, K. (2022). In planta validation of nitric oxide mediated defense responses in common bean against *Colletotrichum gloeosporioides* infection. *Indian Phytopathology, 75*, 15–24. https://doi.org/10.1007/s42360-021-00425-0

Costa, B. H. G., de Resende, M. L. V., Monteiro, A. C. A., Ribeiro Júnior, P. M., Botelho, D. M. D. S., & Silva, B. M. D. (2018). Potassium phosphites in the protection of common bean plants against anthracnose and biochemical defence responses. *Journal of Phytopathology, 166*(2), 95–102. https://doi.org/10.1111/jph.12665

Da Silva, A. C., de Souza, P. E., Pinto, J. E. B. P., da Silva, B. M., Amaral, D. C., & de Arruda Carvalho, E. (2012). Essential oils for preventative treatment and control of Asian soybean rust. *European Journal of Plant Pathology, 134*, 865–871.

Da Silva, L. L., Moreno, H. L. A., Correia, H. L. N., Santana, M. F., & de Queiroz, M. V. (2020). Colletotrichum: Species complexes, lifestyle, and peculiarities of some sources of genetic variability. *Applied Microbiology Biotechnology, 104*, 1891–1904. https://doi.org/10.1007/s00253-020-10363-y

Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2003). The effectiveness of plant essential oils on the growth of Botrytis cinerea, Fusarium sp. and Clavibacter michiganensis subsp. michiganensis. *Crop Protection, 22*, 39–44. https://doi.org/10.1016/S0261-2194(02)00095-9

Damasceno e Silva, K. J., De Souza, E. A., & Ishikawa, F. H. (2007). Characterization of Colletotrichum lindemuthianum isolates from the state of Minas Gerais, Brazil. Journal of Phytopathology, 155(4), 241-247.https://doi.org/10.1111/j.1439-0434.2007.01226.x

de Lima Castro, S. A., Gonçalves-Vidigal, M. C., Gilio, T. A. S., Lacanallo, G. F., Valentini, G., da Silva Ramos Martins, V., & Pastor-Corrales, M. A. (2017). Genetics and

mapping of a new anthracnose resistance locus in Andean common bean Paloma. BMC genomics, 18, 1-12. https://doi.org/10.1186/s12864-017-3685-7

Dwivedy, A. K., Kumar, M., Upadhyay, N., Prakash, B., & Dubey, N. K. (2016). Plant essential oils against food borne fungi and mycotoxins. *Current Opinion in Food Science, 11*, 16–21. https://doi.org/10.1016/j.cofs.2016.08.010

FAO (2024) Statistical database. Food and Agriculture Organization of the United Nations. Retrieved June 12, 2024, from https://www.fao.org/faostat/en/#home

Faostat. (2024, June 12). Food and agriculture organization of the united nations statistics. Retrieved June 12, 2024, from http://www.fao.org/faostat/en/#data/QC

Figueira, E. P. P., Kuhn, O. J., Martinazzo-Portz, T., Stangarlin, J. R., Pereira, M. D. P., & Lampugnani, C. (2020). Histochemical changes induced by *Trichoderma* spp. and potassium phosphite in common bean (*Phaseolus vulgaris*) in response to the attack by *Colletotrichum lindemuthianum*. *Semina: Ciências Agrárias*, 41, 811. https://doi.org/10.5433/1679-0359.2020v41n3p811

Fisher, M. C., Hawkins, N. J., Sanglard, D., & Gurr, S. J. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science, 360*, 739–742. https://doi.org/10.1126/science.aap7999

Gadaga, S. J. C., Siqueira, C. S., & Machado, J. C. (2020). Transmission potential of *Colletotrichum lindemuthianum* (race 65) in association with bean seeds under controlled conditions. *Journal of Seed Science, 42*, e202042025. https://doi.org/10.1590/2317-1545v42231421

Gauillard, F., Richardforget, F., & Nicolas, J. (1993). New spectrophotometric assay for polyphenol oxidase activity. *Analytical Biochemistry, 215*, 59–65. https://doi.org/10.1006/abio.1993.1554

Godinez-Vidal, D., Rocha-Sosa, M., Sepúlveda-García, E. B., Lara-Reyna, J., Rojas-Martínez, R., & Zavaleta-Mejía, E. (2007). Phenylalanine ammonia lyase activity in chilli CM-334 infected by *Phytophthora capsici* and *Nacobbus aberrans*. *European Journal of Plant Pathology, 120*, 299–303. https://doi.org/10.1007/s10658-007-9215-8

Godoy, C. V., Carneiro, S. M. T. P. G., Iamauti, M. T., Dalla Pria, M., Amorim, L., Berger, R. D., & Bergamin Filho, A. (1997). Diagrammatic scales for bean disease: Development and validation. *Zeitschrift Für Pflanzenkrankheiten und Pfanzenschutz, 104*, 336–345.

Hendges, C., Stangarlin, J. R., Zamban, V. C., Kuhn, O. J., Tartaro, E. L., & Carmelo, D. B. (2023). Antifungal activity against *Alternaria solani* and control of early blight in tomato by essential oil of citronella. *Rev Ceres, 70*, 101–111. https://doi.org/10.1590/0034-737X202370020012

Hoyos, J. M. Á., Alves, E., Rozwalka, L. C., Souza, E. A., & Zeviani, W. N. (2012). Antifungal activity and ultrastructural alterations in *Pseudocercospora griseola* treated with essential oils. *Ciência e Agrotecnologia, 36*, 270–284. https://doi.org/10.1590/S1413-70542012000300002

Jamiołkowska, A. (2020). Natural compounds as elicitors of plant resistance against diseases and new biocontrol strategies. *Agronomy, 10*, 173. https://doi.org/10.3390/agronomy10020173

Junqueira, N. T. V., Chaves, G. M., Zambolim, L., Romeiro, R. S., & Gasparotto, R. (1984). Isolamento, cultivo e esporulação de *Microcyclus ulei*, agente etiológico do mal das folhas da seringueira. *Revista Ceres, 31*, 322–331.

Kaur, S., Gonçalves-Vidigal, M. C., Davidson, J., Mysore, K. S., & Pandey, A. K. (2023). Disease and pest resistance in legume crops. *Frontier Plant Science, 14*. https://doi.org/10.3389/fpls.2023.1166387

Khaledi, N., & Hassani, F. (2018). Antifungal activity of *Bunium persicum* essential oil and its constituents on growth and pathogenesis of *Colletotrichum lindemuthianum*. *Journal of Plant Protection Research*, *58*, 431–441. https://doi.org/10.24425/jppr.2018.124646

Khan, M. S. A., & Ahmad, I. (2011). *In vitro* antifungal, anti-elastase and anti- keratinase activity of essential oils of *Cinnamomum*, *Syzygium* and *Cymbopogon*-species against *Aspergillus fumigatus* and *Trichophyton rubrum*. *Phytomedicine, 19*, 48–55. https://doi.org/10.1016/j.phymed.2011.07.005

Kim, D. R., Jeon, C. W., & Kwak, Y. S. (2024). Antifungal properties of *Streptomyces bacillaris* S8 for biological control applications. *Plant Pathology Journal, 40*, 322–328. https://doi.org/10.5423/ppj.nt.01.2024.0021

Li, W., Yuan, S., Sun, J., Li, Q., Jiang, W., & Cao, J. (2018). Ethyl *p*-coumarate exerts antifungal activity *in vitro* and *in vivo* against fruit *Alternaria alternata* via membrane-targeted mechanism. *International Journal of Food Microbiology, 278*, 26–35. https://doi.org/10.1016/j.ijfoodmicro.2018.04.024

Lopez-Reyes, J. G., Spadaro, D., Prelle, A., Garibaldi, A., & Gullino, M. L. (2013). Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits *in vivo*. *Journal of Food Protection, 76*, 631–639. https://doi.org/10.4315/0362-028x.jfp-12-342

Maguire, J. D. (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science, 2*, 176–177. https://doi.org/10.2135/cropsci1962.0011183X000200020033x

Martins, S. J., de Faria, A. F., Pedroso, M. P., Cunha, M. G., da Rocha, M. R., & de Medeiros, F. H. V. (2019). Microbial volatiles organic compounds control anthracnose (*Colletotrichum lindemuthianum*) in common bean (*Phaseolus vulgaris* L.). *Biological Control, 131*, 36–42. https://doi.org/10.1016/j.biocontrol.2019.01.003

Mori, T., Sakurai, M., & Sakuta, M. (2001). Effects of conditioned medium on activities of PAL, CHS, DAHP synthase (DS-Co and DS-Mn) and anthocyanin production in suspension cultures of *Fragaria ananassa*. *Plant Science, 160*, 355–360. https://doi.org/10.1016/S0168-9452(00)00399-X

Mota, S. F., Pádua, P. F., Ferreira, A. N., Gomes, L. D. B. W., Dias, M. A., Souza, E. A., & Cardoso, P. G. (2021). Biological control of common bean diseases using endophytic *Induratia* spp. *Biological Control, 159*, 104629. https://doi.org/10.1016/j.biocontrol.2021.104629

OMRI. 2024. Products list, Crop products. 1–379. Retrieved June 30, 2024, from https://www.omri.org/sites/default/files/opl_pdf/CompleteCompany-NOP-EN.pdf

Oreopoulou, A., Tsimogiannis, D., & Oreopoulou, V. (2019). Extraction of polyphenols from aromatic and medicinal plants: an overview of the methods and the effect of extraction parameters. Polyphenols in plants, 243-259. https://doi.org/10.1016/B978-0-12-813768-0.00025-6

Padder, B. A., Sharma, P. N., Awale, H. E., & Kelly, J. D. (2017). *Colletotrichum lindemuthianum*, the causal agent of bean anthracnose. *Journal of Plant Pathology, 99*, 317–330. https://doi.org/10.4454/jpp.v99i2.3867

Pandey, A. K., Kumar, A., Mbeyagala, E. K., Barbetti, M. J., Basandrai, A., Basandrai, D., Nair, R. M., & Lamichhane, J. R. (2023). Anthracnose resistance in legumes for cropping system diversification. *Critical Reviews in Plant Sciences, 42*, 177–216. https://doi.org/10.1080/07352689.2023.2228122

Pane, C., Villecco, D., Roscigno, G., Falco, E. D., & Zaccardelli, M. (2013). Screening of plant-derived antifungal substances useful for the control of seedborne pathogens. *Archives of Phytopathology and Plant Protection, 46*, 1533–1539. https://doi.org/10.1080/03235408.2013.771458

Parikh, L., Agindotan, B. O., & Burrows, M. E. (2021). Antifungal activity of plant-derived essential oils on pathogens of pulse crops. *Plant Disease*, PDIS–06–20–1401. https://doi.org/10.1094/PDIS-06-20-1401-RE

Perina, F. J., Amaral, D. C., Fernandes, R. S., Labory, C. R., Teixeira, G. A., & Alves, E. (2014). Thymus vulgaris essential oil and thymol against *Alternaria alternata* (Fr.) Keissler: Effects on growth, viability, early infection and cellular mode of action. *Pest Management Science, 71*, 1371–1378. https://doi.org/10.1002/ps.3933

Pinto, J. M. A., Pereira, R., Mota, S. F., Ishikawa, F. H., & Souza, E. A. (2012). Investigating phenotypic variability in *Colletotrichum lindemuthianum* populations. *Phytopathology, 102*, 490–497. https://doi.org/10.1094/phyto-06-11-0179

Regnier, T., Combrinck, S., Veldman, W., & Du Plooy, W. (2014). Application of essential oils as multi-target fungicides for the control of *Geotrichum citri-aurantii* and other postharvest pathogens of citrus. *Industrial Crops and Products, 61*, 151–159. https://doi.org/10.1016/j.indcrop.2014.05.052

Rozwalka, L. C., Alves, E., & Amaral, D. C. (2010). Ultrastructural study of conidia of *Colletotrichum gloeosporioides* and *Colletotrichum musae* treated with essential oils. *Interciencia, 35*, 912–915.

Sadgrove, N., & Jones, G. (2015). A Contemporary introduction to essential oils: Chemistry, bioactivity and prospects for Australian agriculture. *Agriculture, 5*, 48–102. https://doi.org/10.3390/agriculture5010048

Saravanan, T., Bhaskaran, R., & Muthusamy, M. (2004). *Pseudomonas fluorescens* induced enzymological changes in banana roots (Cv. *Rasthali*) against *Fusarium* wilt. *Plant Pathollgy Journal, 3*, 72–80. https://doi.org/10.3923/ppj.2004.72.80

Sarto, M. P. M., & Zanusso Júnior, G. (2014). Atividade antimicrobiana de óleos essenciais. *Revista UNINGÁ Review, 20*, 98–102.

Shaner, G., & Finney, R. F. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. *Phytopathology, 67*, 1051–1056. https://doi.org/10.1094/Phyto-67-1051

Sil, A., Pramanik, K., Samantaray, P., Mondal, Md. F., & Yadav, V. (2020). Essential oils: A boon towards ecofriendly management of phytopathogenic fungi. *Journal of Entomology and Zoology Studies, 8*, 1884–1891.

Singh, G., Singh, O. P., Rao, G. P., & Sharma, S. R. (2002). The vapour action of essential oils and monoterpenoids against pathogenic fungi. *Sugar Tech, 4*, 69–71. https://doi.org/10.1007/BF02956885

Soković, M. D., Vukojević, J., Marin, P. D., Brkić, D. D., Vajs, V., and van Griensven, L. J. L. D. (2009). Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities. *Molecules*, 238–249. https://doi.org/10.3390/2Fmolecules14010238

Soković, M., & van Griensven, L. J. L. D. (2006). Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. *European Journal of Plant Pathology, 116*, 211–224. https://doi.org/10.1007/s10658-006-9053-0

Soylu, E. M., Soylu, S., & Kurt, S. (2006). Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent *Phytophthora infestans*. *Mycopathologia, 161*, 119–128. https://doi.org/10.1007/s11046-005-0206-z

Stenger, L. D., Libardoni, G., Junior, A. W., Zanela, J., et al. (2021). Essential oils in pathogen resistance induction of *Eucalyptus benthamii* Maiden et Cambage. *Ciência Rural, 51*, e20190915. https://doi.org/10.1590/0103-8478cr20190915

Urbanek, H., Kuznial-Gebarowska, E., & Herk, H. (1991). Elicitation of defence responses in bean leaves by *Botrytis cinerea* polygalacturonase. *Acta Physiololgy Plant, 13*, 43–50.

Vaz, P. D. da S., Souza, W. R. N. de., Wendland, A., Paz, A. T. S.., Cortes, M. V. C. B., Bara, M. T. F. (2022). Antifungal activity of *Origanum vulgare* and *Rosmarinus officinalis* phenolics-containing extracts against *Colletotrichum lindemuthianum* and in the suppression of anthracnose in common beans. *Research, Society and Development*, 11, e47911932145. https://doi.org/10.33448/rsd-v11i9.32145

Walling, L. L. (2001). Induced resistance: From the basic to the applied. *Trends Plant Science, 6*, 445–447. https://doi.org/10.1016/S1360-1385(01)02046-5

Wang, D., Zhang, J., Jia, X., Xin, L., & Zhai, H. (2019). Antifungal effects and potential mechanism of essential oils on *Colletotrichum gloeosporioides in vitro* and *in vivo*. *Molecules, 24*, 3386. https://doi.org/10.1016/j.indcrop.2022.115975

Wirth, S. J., & Wolf, G. A. (1990). Dye-labelled substrates for the assay and detection of chitinase and lysozyme activity. *Journal of Microbiological Methods, 12*, 197–205. https://doi.org/10.1016/0167-7012(90)90031-Z

Wu, T., Zhang, B., Luo, X., Li, A., Zhang, S., An, J., Zhang, Z., & Liu, Y. (2023). Antifungal efficacy of sixty essential oils and mechanism of oregano essential oil against *Rhizoctonia solani*. *Industrial Crops and Products, 191*, 115975. https://doi.org/10.1016/j.indcrop.2022.115975

Xue, L., Charest, P. M., & Jabaji-Hare, S. H. (1998). Systemic induction of peroxidases, 1,3-β-Glucanases, chitinases, and resistance in bean plants by binucleate *Rhizoctonia* species. *Phytopathology, 88*, 359–365. https://doi.org/10.1094/phyto.1998.88.4.359

Yan, J., Wu, H., Chen, K., Feng, J., & Zhang, Y. (2021). Antifungal activities and mode of action of *Cymbopogon citratus*, *Thymus vulgraris*, and *Origanum heracleoticum* essential oil vapors against *Botrytis cinerea* and their potential application to control postharvest strawberry gray mold. *Foods, 10*, 2451. https://doi.org/10.3390/foods10102451

Yapryntseva, M. A., Zhivotovsky, B., & Gogvadze, V. (2024). Permeabilization of the outer mitochondrial membrane: Mechanisms and consequences. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* 167317. https://doi.org/10.1016/j.bbadis.2024.167317

Zaker, M. (2016). Natural plant products as eco-friendly fungicides for plant diseases control- a review. *The Agriculturists, 14*, 134. https://doi.org/10.3329/agric.v14i1.29111

Zheng, C. M., Teng, B., Gao, F. L., & Wu, Z. P. (1999). Studies on the changes of superoxido dismutase, peroxidase and poliphenol oxidase in seed coat of soybeans after infection with soybean mosaic virus. *Scientia Agricultura Sinica, 32*, 99–101.