Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252023v36n206rc

Essential oils in the management of *Alternaria alternata* f. sp. *citri* in 'Dancy' tangerine fruits

Óleos essenciais no manejo de *Alternaria alternata* f. sp. *citri* em frutos de tangerineira 'Dancy'

Mirelly M. Porcino[1]*, Valdeir de S. Oliveira[1], Hilderlande F. da Silva[1], Mileny dos S. de Souza[2], Luciana C. do Nascimento[1]

[1]Department of Plant Science and Environmental Sciences, Universidade Federal da Paraíba, Areia, PB, Brazil. [2]Department of Agronomy, Universidade Federal de Alagoas, Maceió, AL, Brazil.

ABSTRACT - Tangerines and their hybrids are affected by economically essential diseases. The Alternaria brown spot (*Alternaria alternata* f. sp. *citri*) deserves to be highlighted, as it is present in all tangerine production areas. This study aimed to determine the effect of essential oils on *A. alternata* f. sp. *citri*. The experiments were carried out in the Laboratories of Phytopathology and Biology and Technology of Post-Harvest of the Federal University of Paraíba, Areia, PB. Three pathogen isolates were used, and ten essential oils from grape, sunflower, eucalyptus, ginger, copaiba, mint, fennel, citronella, clove, and linseed at a concentration of 1%, diluted in potato dextrose agar for *in vitro* tests. The oils were diluted in distilled water for the fruit test, and the fruits were immersed in the treatments for 5 min. In addition, the fungicide Thiabendazole (400 mL/100L) and sterile distilled water were used as the control treatments. The fruits were inoculated with a conidia suspension of the pathogen (105 conidia/mL). Mycelial Growth Rate Index, colony diameter, spore production and dimensions, fruit disease severity, and enzymatic activity were evaluated. A completely randomized design was used, with 12 treatments and four replications *in vivo*. The results indicate that the essential oils of mint, eucalyptus, fennel, and citronella were efficient in the *in vitro* control of the pathogen, ultimately inhibiting the growth of fungal colonies. Eucalyptus oil efficiently managed *A. alternata* f. sp. *citri* on 'Dancy' tangerine fruits. Essential oils did not influence the enzymatic activity of the fruits.

Keywords: Fungitoxic activity. *Citrus tangerine* hort. ex Tanaka. Alternative control. Alternaria brown spot.

RESUMO - As tangerineiras e seus híbridos são acometidos por doenças economicamente importantes. Merece destaque a mancha marrom de Alternaria (*Alternaria alternata* f. sp. *citri*), por estar presente em todas as áreas de produção de tangerinas. Esse trabalho objetivou determinar o efeito de óleos essenciais sobre *A. alternata* f. sp. *citri*. Os experimentos foram conduzidos no Laboratório de Fitopatologia, e no laboratório de Biologia e Tecnologia Pós-Colheita da Universidade Federal da Paraíba, Areia, PB. Foram utilizados três isolados do patógeno, e dez óleos essenciais de sementes de uva, girassol, eucalipto, gengibre, copaíba, menta, erva-doce, citronela, cravo e linhaça na concentração de 1%, diluídos em meio de cultura batata-dextrose-ágar, para os testes *in vitro*. Para o teste nos frutos os óleos foram diluídos em água destilada e os frutos imersos nos tratamentos por 5 min. Além do fungicida Tiabendazol (400 mL/100L) e água destilada estéril consistiram nas testemunhas. Os frutos foram inoculados com suspensão de conídios do patógeno (10^5 conídios/mL). Avaliou-se o índice de velocidade de crescimento micelial, diâmetro de colônia, produção e dimensões de esporos, severidade da doença em frutos e atividade enzimática. Utilizou-se o delineamento inteiramente casualizado, com 12 tratamentos e quatro repetições *in vivo*. Os resultados indicam que, os óleos essenciais de menta, eucalipto, erva-doce e citronela foram eficientes no controle *in vitro* do patógeno, inibindo totalmente o crescimento das colônias fúngicas. O óleo de eucalipto foi eficiente no manejo de *A. alternata* f. sp. *citri* em frutos de tangerineira 'Dancy'. Os óleos essenciais não influenciaram na atividade enzimática dos frutos.

Palavras-chave: Atividade fungitóxica. *Citrus tangerina* hort. ex Tanaka. Controle alternativo. Mancha marrom de alternaria.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: September 1, 2021.
Accepted in: September 29, 2022.

***Corresponding author:**
<mirellyagroufpb@hotmail.com>

INTRODUCTION

Brazil is considered the largest producer of citrus in the world, with a planted area of 7.9 million hectares. In the world production of tangerines, Brazil occupies the seventh position, with approximately 984.9 thousand tons cultivated in 52.8 thousand hectares (LANDAU et al., 2020). Tangerines are specially intended for the fresh fruit market and are used to produce concentrated juice. The Southeast region concentrates the largest national production of tangerines with 61%, producing approximately 603 thousand tons in more than 25 thousand hectares, with emphasis on the state of São Paulo, considered the main producer pole of tangerine (LANDAU et al., 2020).

The Northeast region produced 31,545 tons with an average yield of 8.45 t/ha. In Paraíba state, tangerine production was 13,219 thousand tons and a yield of 7.26 t/ha in 2019 (SILVA et al., 2019). However, the national citrus industry faces serious health problems due to the action of pathogens in the production and post-harvest phases. The most important disease of tangerines and their hybrids is Alternaria brown spot (ABS), which causes limitations in crop

production and is found in all producing states in the country (CHEN et al., 2014).

The causal agent of ABS is the fungus *Alternaria alternata* f. sp. *citri*, which, when colonizing plant tissue, releases a specific toxin for the tangerine pathotype, causing cell death at the site of the attack, which can occur in all plant organs (PORCINO et al., 2017). The control of ABS is carried out mainly through registered fungicides; however, this practice has been a matter of concern due to the various risks offered to humans and the environment (XU et al., 2014).

As a result of the search for alternative sources in managing plant diseases, essential oils show promising results in controlling phytopathogens and avoiding their infection (SHARIFI-RAD et al., 2017). Several studies related to managing diseases through essential oils proved to be efficient in inhibiting mycelial growth, 87 sporulation, and spore germination in *in vitro* tests. Scheuermann et al. (2020) reported that when using *Cymbopogon martinii* and *C. citratus* oils at a concentration of 0.075%, they inhibited the mycelial growth of *Rhizoctonia solani*.

Within this context, this study aimed to determine the effect of essential oils on mycelial growth and sporulation of *A. alternata* f. sp. *citri in vitro*, and 'Dancy' tangerine fruits, in addition to verifying the induction of resistance of the treated fruits to the pathogen.

MATERIAL AND METHODS

The experiments were carried out at the Laboratories of Phytopathology (LAFIT) and Biology and Technology of Post-Harvest (LBTPC) of the Agricultural Sciences Center, Federal University of Paraíba, Areia, PB. Three isolates of *A. alternata* f. sp. *citri*, from Massaranduba - Paraíba (I-8) (7° 10′ 15″ S and 35° 51′ 14″ W), Pratânia - São Paulo (I-28) (22° 48' 35" S and 48° 39' 57" W), and Trás dos Montes - Portugal (I-30) (41° 83' 56" S 41° 50′ 8″ W), belonging to the LAFIT fungi collection, preserved by the Castellani (1939) method, genetically identified by the ISSR method (UPGMA).

Experiment I - *In vitro* test

The essential oils of grape seed (*Vitis Vinifera*), sunflower (*Helianthus annus* L.), eucalyptus (*Eucaliptus globulus* Labill.), ginger (*Zingiber officinale* Roscoe), copaiba (*Copaifera langsdorffii* Desf.), mint (*Mentha arvensis* L.), fennel (*Pimpinella anisum* L.), citronella (*Cymbopogon nardus* Rendle), clove (*Syzygium aromaticum* L. MERRILL & PERRY), and linseed (*Linum usitatissimum* L.), purchased commercially, were used.

The oils were incorporated at a concentration of 1% in a semi-solid potato-dextrose-agar (PDA) medium with pH 6.0, plus two drops of Tween 80 (dispersant), then poured into Petri dishes (9 cm). Plates containing only PDA were used for the control, and the fungicide Thiabendazole (400 mL/100L) was incorporated into the culture medium. After seven days of growth of the pathogen, a disk (5 mm in diameter) of the colony was transferred to the center of each plate and placed in a Biochemical Oxygen Demand (B.O.D) incubation chamber under a 12-hour photoperiod at 25±2 °C for seven days.

The diameter of the mycelial colony (DC) was measured daily, with the aid of a millimeter ruler, in two orthogonal directions until the plate containing the control treatment was filled. As well as the measurement of the mycelial growth rate index (MGRI), according to Oliveira (1991), IVCM= (D-Da)/N, where: MGRI =mycelial growth rate index; D=current average colony diameter; Da=average diameter of the colony from the previous day; N=number of days after inoculation in the Petri dish.

To quantify conidia (spore) production, 10 mL of sterile distilled water (SDW) was added to the plates after ten days of incubation, and spores were removed using a soft-bristled brush. The 10^5 suspensions were filtered through a double layer of sterile gauze, and conidia were quantified in a Neubauer chamber (ALFENAS; MAFIA, 2016). The size of the spores was determined by measuring the length and width of 25 spores of each treatment on microscope slides stained with methylene blue visualized under an optical microscope.

Experiment II - Inoculation of the pathogen in fruits

For the *in vivo* test, tangerine fruits, cultivar 'Dancy' were harvested in a commercial orchard in the city of Remígio - PB (6° 54′ 10″ S and 35° 50′ 2″ W), at C2 maturation stage (SILVA et al. al., 2019). Initially, the fruits were washed with water and neutral soap, then disinfested in a 1% sodium hypochlorite solution for 5 minutes, followed by triple washing in distilled water and placed on paper towels to dry. The fruits were immersed for 5 minutes in the same treatments with essential oils previously described diluted at a concentration of 1% in SDW, plus Tween 80.

After 24 hours of treatment application, minor wounds (1 mm deep) were made in the equatorial region on opposite sides of the fruit peel. The fruits were inoculated with a conidia suspension at a concentration of 1 x 10^5 spores mL^{-1} with a manual sprayer and placed in polyethylene trays covered with moistened transparent plastic bags to form a humid chamber, which remained for 24 hours. Renaud et al. (2008) measured disease severity based on the diagrammatic scale Figure 1. The area under the disease progress curve (AUDPC) (SHANER; FINNEY, 1977) and the protection of the fruits by the oils were calculated by the formula: Protection = (AUDPC Treatments x 100/AUDPC Control).

For the extraction of peroxidase (POX), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) enzymes, 3.0 g of fruit peel homogenized in 10 mL of 0.1M phosphate extraction buffer, pH 6.0 were used. The suspension was centrifuged for 15 minutes at 12000 g, collecting the supernatant. The enzymatic analyzes were carried out in three evaluation periods, 5, 15, and 25 days after the application of the treatments.

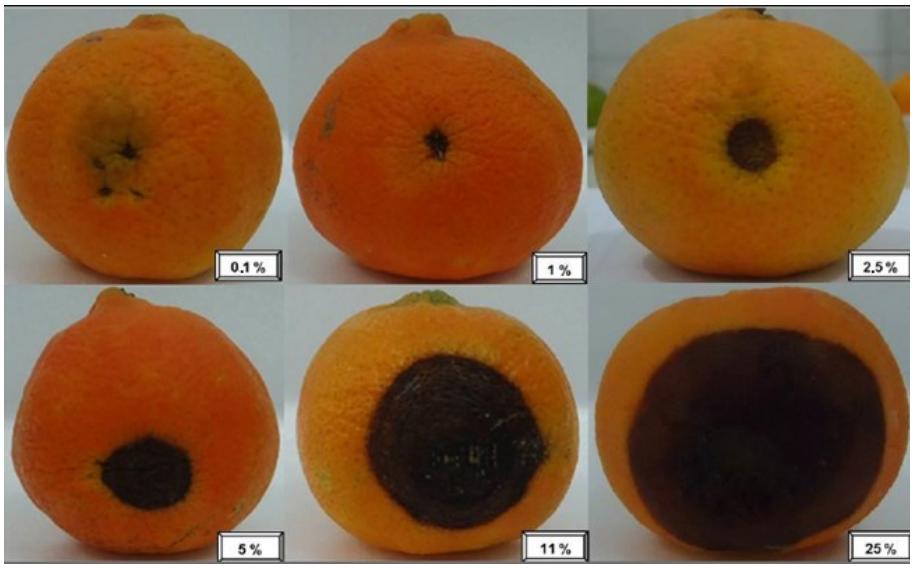

Figure 1. Diagrammatic scale described by Renaud et al. (2008), for the evaluation of Alternaria brown spot (*Alternaria alternata* f. sp. *citri*) in 'Dancy' tangerine (*Citrus tangerina*) fruits.

The reactions to determine the enzymatic activity of POX were prepared by adding 0.25 mL of the supernatant to the reaction medium containing 0.25 mL of 1.7% guaiacol, 0.75 mL of 0.1 M phosphate buffer, pH 6.0 and 0.25 mL of 1.8% H_2O_2. The reactions were monitored with the aid of a spectrophotometer, observing the variation of absorbance at a wavelength of 470 nm, at 25 °C, immediately after mixing and the activity expressed in absorbance units (AU) $min^{-1}mg^{-1}$ of protein.

To determine the PPO activity, 0.5 mL of the supernatant was added to the reaction medium containing 0.25 mL of 0.6 M S-methyl-catechol and 0.75 mL of 0.1 M phosphate buffer pH 6.8. The solution was incubated for 15 minutes at 40 °C, and the reaction stopped with the addition of 800 µL of 2N perchloric acid. The reactions were monitored with the aid of a spectrophotometer, observing the variation in absorbance, at a wavelength of 395 nm, at 25 °C, immediately after removal from the incubator and the PPO activity expressed in absorbance units (AU). $min^{-1}.mg^{-1}$ of protein.

To determine PAL activity, 0.5 mL of the supernatant was transferred to test tubes, and 1.5 mL aliquots of 0.01M TRIS-EDTA buffer solution, pH 8.8, 0.5 mL of phenylalanine solution (30 µM) and 0.5 mL of distilled water were added. After incubation in a water bath at 40 °C for one hour, the reaction was stopped with 2 mL of 5M hydrochloric acid, and spectrophotometric readings were performed at 290 nm at 25 °C. Results were expressed in Absorbance Units (AU) $min^{-1}.mg^{-1}$ of protein.

The blank II was prepared using 1.5 mL of 0.01M TRIS buffer solution pH 8.8, 0.5 mL of enzyme extract, and 1.0 mL of distilled water for each treatment tested. Analyzes were performed in triplicate. The determinations of the amount of protein present in the referred extracts were carried out by the method of Bradford (1976).

The design was completely randomized. Analysis of variance was performed for the *in vitro* test, consisting of four replicates of three plates (12 plates per treatment). The means were compared by the Tukey test up to 5% probability. The R® program version 2017 was used (R CORE TEAM, 2017).

The design was entirely randomized for the *in vivo* test, and the test on the fruits was represented by four replications of three fruits (12 fruits per treatment). The enzymatic analysis was carried out in a 7x3 factorial scheme (seven treatments and three evaluation periods), and these were performed in triplicate. The variables data were submitted for variance analysis, and the Scott-Knott test compared the means up to 5% of probability using the ASSISTAT® program.

RESULTS AND DISCUSSION

Experiment I

In the *in vitro* test, the *Alternaria alternata* f. sp. *citri* differed statistically between treatments in mycelial growth. Isolate I8 (PB) showed a higher mycelial growth rate index (MGRI) compared to the others; their averages ranged from 0 to 2.63 cm (Figure 2A). This result can be justified due to the similarity between the temperature conditions of the *in vitro* culture and the conditions in the field where the isolate was collected.

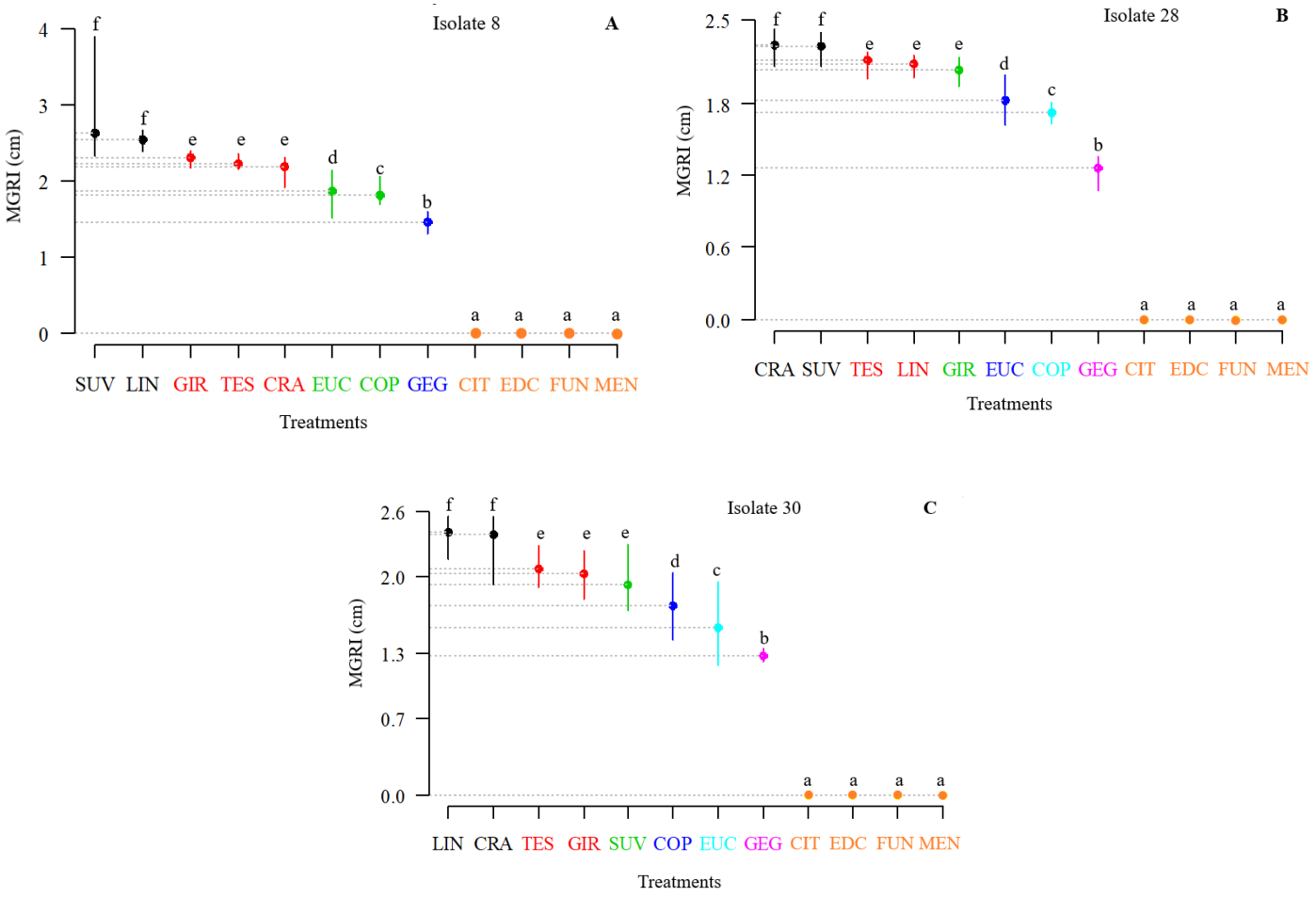

Figure 2. Mycelial growth rate index (MGRI) of *Alternaria alternata* f. sp. *Citri.* Isolate: I8-Paraíba, I28-São Paulo, and I30-Portugal. Treatments: TES-Control, Essential oils of SUV- Grape Seeds, CRA- Clove, EUC- Eucalyptus, LIN- Linseed, GEG- Ginger, CIT- Citronella, COP- Copaiba, EDC- Fennel, MEN- Mint, GIR- Sunflower, diluted at 1% in ADE, FUN- Thiabendazole Fungicide (400 mL/100L.

The essential oils (EOs) of citronella, fennel, and mint prevented the *in vitro* growth of the pathogen, presenting fungitoxic action and equating to the fungicide effect, confirming its direct action in the suppression of the pathogen in the tested experimental conditions.

All isolates showed statistical differences in the diameter of the pathogen colonies. The EOs of ginger, citronella, copaiba, fennel, and mint reduced the mycelial growth of the three isolates, similar to the fungicide treatment (Figure 3).

The EOs present chemical compounds responsible for the efficiency in the management of phytopathogens and may present two or more main components. These compounds are considered the majority and may be responsible for the fungitoxic effect exerted by essential oils. However, some oils present these compounds in small amounts, called trace components, which may have the same effect as the major compounds (BAPTISTA et al., 2015; YU et al., 2017).

Inhibition of mold growth is a standard method of reducing mold growth. Studies pointed out that the antifungal activity against *A. alternata* could be attributed to eugenol and eugenol acetate, which showed excellent results (SHARMA et al., 2017; JINGA et al., 2018). Perina et al. (2014), observed that the EO of *Thymus vulgaris* inhibited the growth of *A. alternata*, in addition to delaying the beginning of the infection process, preventing its penetration. Essential oils act on the cell wall disrupting the plasma membrane that can attack specific organelles in the cytoplasm of pathogens (JINGA et al., 2018).

For spore production, a significant difference was observed between the isolates. All treatments were efficient in inhibiting I8 sporulation (Figure 4A). EOs of eucalyptus, grape seed, copaiba, and linseed induced sporulation of isolates 28 and 30 (Figures 4B and C). There was no statistical difference between treatments and isolates analyzed for the variable conidia diameter of *A. alternata* f. sp. *citri*.

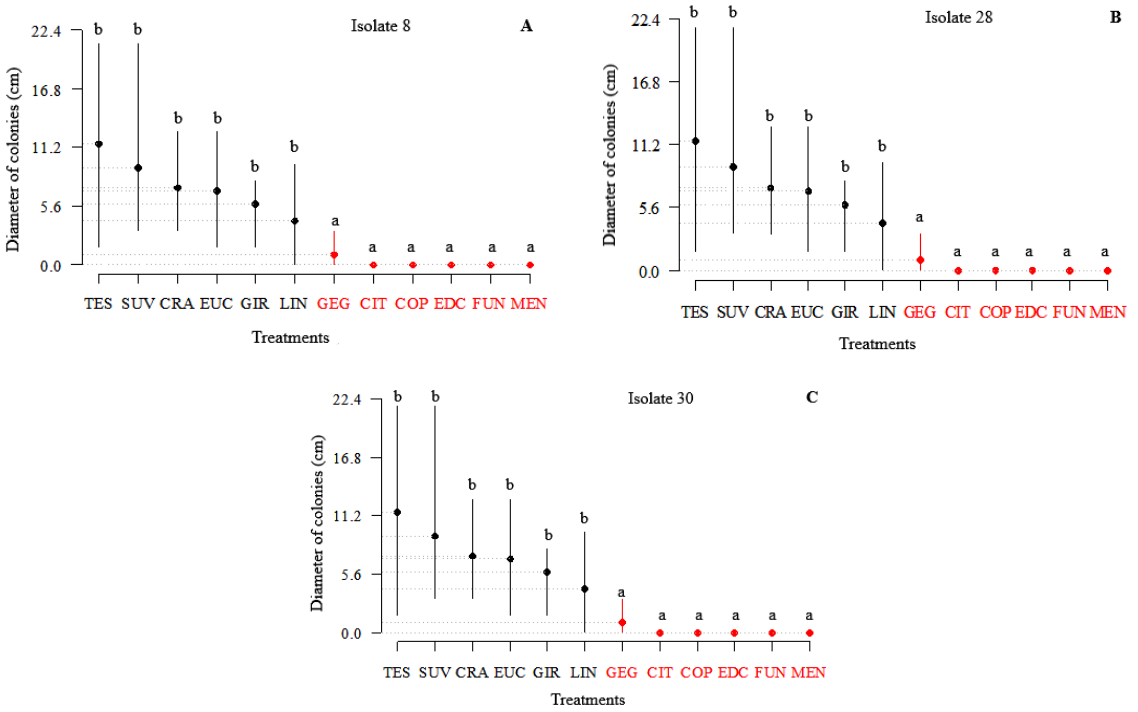

Figure 3. Diameter of colonies of *Alternaria alternata* f. sp. *citri* Isolate: I8-Paraíba, I28-São Paulo, and I30-Portugal. Treatments: TES-Control, Essential oils of SUV- Grape Seeds, CRA- Clove, EUC- Eucalyptus, LIN- Linseed, GEG- Ginger, CIT- Citronella, COP- Copaiba, EDC- Fennel, MEN- Mint, GIR- Sunflower, diluted at 1% in ADE, FUN- Thiabendazole Fungicide (400 mL/100L).

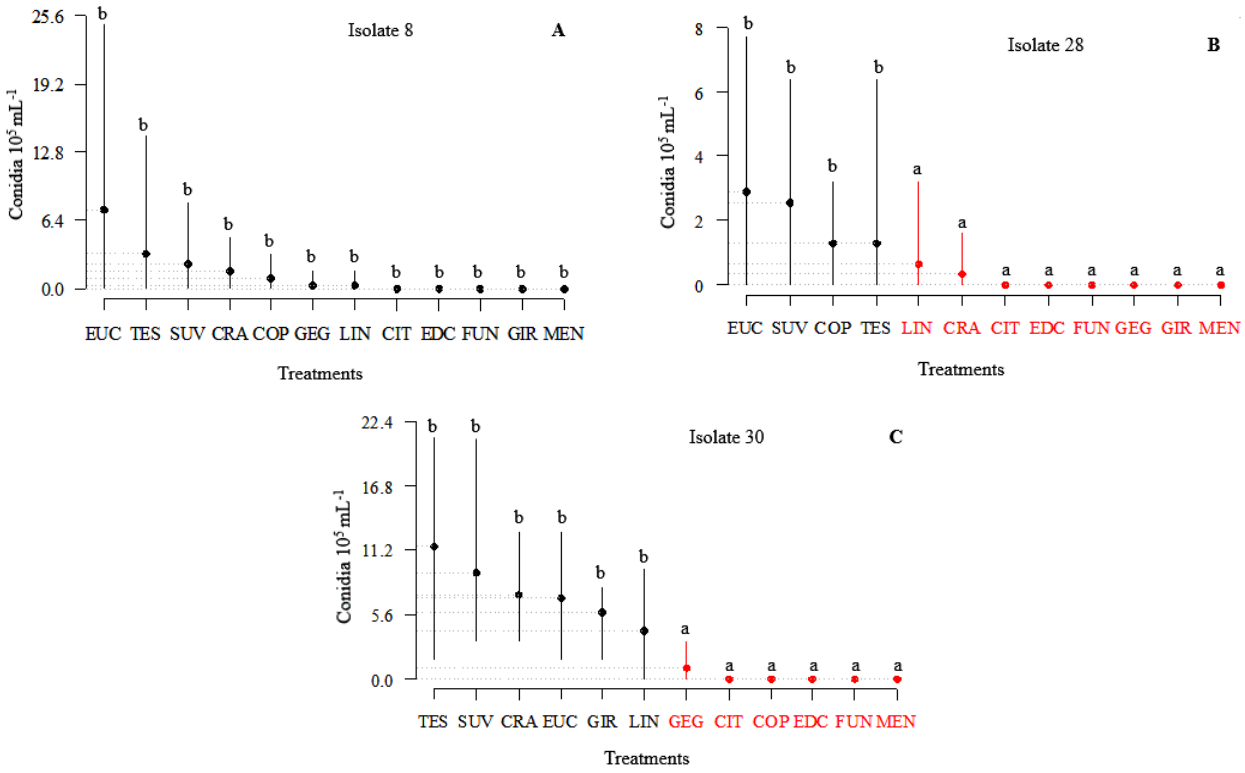

Figure 4. Conidia production of *Alternaria alternata* f. sp. *citri* Isolate: I8-Paraíba, I28-São Paulo, and I30-Portugal. Treatments: TES-Control, Essential oils of SUV- Grape Seeds, CRA- Clove, EUC- Eucalyptus, LIN- Linseed, GEG- Ginger, CIT- Citronella, COP- Copaiba, EDC- Fennel, MEN- Mint, GIR- Sunflower, diluted at 1% in ADE, FUN- Thiabendazole Fungicide (400 mL/100L).

It is important to emphasize that greater growth of the fungal colony does not reflect in greater spore production due to factors intrinsic to the pathogen. Studies describing the antimicrobial action of EOs from *Cinnamomum zeylanicum* and *Eugenia caryophyllus* were effective *in vitro* and *in vivo* in inhibiting the development of *A. alternata* in the epidermis of *Hylocereus undatus* fruits (CASTRO et al., 2017).

Experiment II

Regarding the area under the disease progress curve (AUDPC), there was a statistical difference between the treatments evaluated for I-8 (Figure 5). The other treatments did not differ statistically and did not promote fruit protection compared to the control.

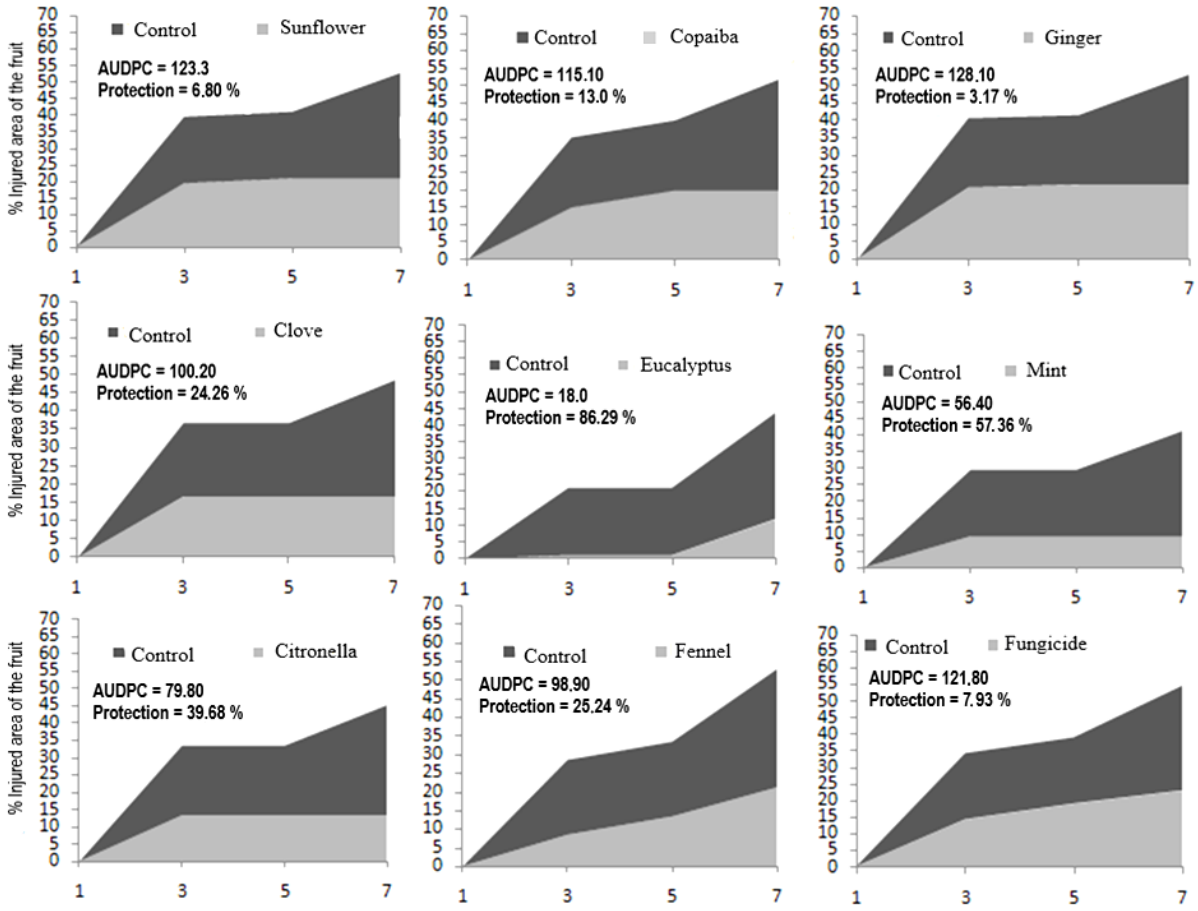

Figure 5. Area under the disease progress curve (AUDPC) and percentage of protection (%) in 'Dancy' tangerine fruits (*Citrus tangerina* Hort. ex Tanaka) submitted to treatments* with essential oils.

An ecological alternative of great potential is the use of EOs in the control of phytopathogens, which is associated with other disease management practices, results in greater efficiency in the control of these microorganisms, in addition to minimizing the emergence of new races of phytopathogens, collaborating in the production of organic products (KACEM et al., 2016; SCHEUERMANN et al., 2020).

According to Xu et al. (2014), *Laurus nobilis* oil (500 µg mL^{-1}) was effective in protecting cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) fruits from *A. alternata* infection, with an inhibition rate of 33.9%. Chen et al. (2014), found that citronella oil showed vigorous inhibition activity on *A. alternata* at a concentration of 1.5 µL mL^{-1} in tomato fruits (*S. Lycopersicum* L.) with a 52% reduction in disease severity, corroborating with the present research.

The antimicrobial action exerted by essential oils is due to the substances present in their composition, which, once in contact with microorganisms, the lipophilic capacity can allow their penetration into fungal membrane structures, causing damage to it, such as the leakage of ions and other cellular components, affecting the integrity of cell membranes (JINGA et al., 2018).

The results obtained in the *in vitro* and *in vivo* tests showed the potential of treatments with essential oils to

reduce mycelial growth, sporulation, and the severity of Alternaria brown spot in tangerine fruits and confirm the importance of OEs in the management of *A. alternata*. f. sp. *citri* in organic production fields since it is an economically viable alternative, easy to apply, compatible with other disease control strategies, and environmentally safe (YU et al., 2017).

Regarding enzymatic activity, no significant differences were observed between the treatments used (Table 1). However, there was a significant difference ($p<0.01$) between the evaluation periods, with an increase in enzymatic activity in the second period (Table 1).

The increase in enzymatic activity in the second evaluation period (15 days after harvest) can be explained by the increase in fruit respiration due to senescence and starch degradation, causing physiological changes, increasing the respiratory capacity of the fruits, and providing greater enzymatic action (MENEGASSI et al., 2017).

Table 1. Activity of peroxidase (POX), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) enzymes in 'Dancy' tangerine fruits treated with essential oils (1%) and thiabendazole fungicide (400 mL/100L), in different periods.

Treatments	Enzymatic activity		
	Periods*		
	1	2	3
	Peroxidase (POX)		
Control	4.83 bB	8.57 cA	8.53 aA
Copaiba Oil	4.91 bC	8.84 cA	6.41 bB
Ginger Oil	4.72 bA	9.30 bB	5.33 cB
Mint Oil	4.06 bC	9.39 bA	5.35 cB
Citronella Oil	6.19 aC	7.90 dA	4.63 dB
Fennel Oil	4.41 bB	7.86 dA	4.35 dB
Fungicide	4.92 bB	11.92aA	4.31 dB
CV= 19.95%			
	Polyphenol oxidase (PPO)		
Control	1.50 bB	2.40 aA	2.18 aA
Copaiba Oil	1.55 bB	2.41 aA	1.86 aB
Ginger Oil	1.59 bB	2.81 aA	1.63 aB
Mint Oil	1.49 bB	2.76 aA	1.58 aB
Citronella Oil	1.82 bB	2.58 aA	1.54 aB
Fennel Oil	1.42 aA	1.94 bA	1.49 aA
Fungicide	1.63 bB	2.90 aA	1.40 aB
CV= 20.62%			
	Phenylalanine ammonia-lyase (PAL)		
Control	1.21 aB	8.57 aA	1.64 aB
Copaiba Oil	1.20 aA	1.66 cA	1.43 aA
Ginger Oil	1.24 aA	1.87 cA	1.30 aB
Mint Oil	1.18 aB	1.73 cB	1.28 aB
Citronella Oil	1.38 aB	1.68 cB	1.25 aB
Fennel Oil	1.19 aA	1.54 cA	1.21 aA
Fungicide	1.29 aB	2.04 bA	1.19 aB
CV= 13.32%			

*Evaluations performed at 5 (1st period), 15 (2nd period), and 25 (3rd period) days after the application of treatments. Means followed by the same lowercase letter in the column and uppercase letter in the lines belong to the same cluster by the Scott-Knott test up to 5% significance.

Enzymes related to plant defense, such as peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, ß-1,3-glucanase, and chitinase are activated through biochemical mechanisms, which are present in plants in an inactive form (MARTINS et al., 2015). Complex changes in fruit metabolism are due to increased enzymatic activity, which is associated with changes in respiratory activity and ethylene biosynthesis. With maturation, the senescence processes continue, which will go through the synthesis processes, resulting in tissue death (DEMARTELAERE et al., 2017).

The environment may influence some oils and therefore have no systemic effect on the plant (SHARMA et al., 2017). This effect can often be related to the morphological characteristics of the plants, such as the presence of wax on the surface of leaves, which hinders the penetration and action of essential oils on the tissue. Essential oils are highly volatile, especially under high temperatures. These factors may be related to low enzyme activation (GUIMARÃES et al., 2015), as observed in this research.

CONCLUSION

The essential oils of mint, citronella, and fennel are efficient in the *in vitro* control of *A. alternata* f. sp. *citri.* Eucalyptus, mint, and citronella oils efficiently reduce the severity of *A. alternata* f. sp. *citri* on 'Dancy' tangerine fruits. The tested oils did not induce the greater activity of the enzymes peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase in 'Dancy' tangerine fruits.

REFERENCES

ALFENAS, A. C.; MAFIA, R. G. **Métodos em fitopatologia**. 2. ed. Viçosa, MG: 2016. 516 p.

BAPTISTA, E. B. et al. Chemical composition and antifungal activity of essential oil from *Eucalyptus smithii* against dermatophytes. **Revista da Sociedade Brasileira de Medicina Tropical**, 48: 746–752, 2015.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of micrograms quantities for proteins utilizing the principle of protein-dye binding. **Analytical Biochemistry**, 72: 248-254, 1976.

CASTELLANI, A. Viability of some pathogenic fungi in distilled water. **Journal of Tropical Medicine and Hygiene**, 42: 225-226, 1939.

CASTRO, J. C. et al. Bioactivity of essential oils in the control of *Alternaria alternate* in dragon fruit (*Hylocereus undatus* Haw.). **Industrial Crops and Products**, 97: 101–109, 2017.

CHEN, Q. et al. Effect of citronella essential oil on the inhibition of postharvest *Alternaria alternata* in cherry tomato. **Journal of the Science of Food and Agriculture**, 94: 2441–2447, 2014.

DEMARTELAERE, A. C. F. et al. Elicitors on the control of anthracnose and post-harvest quality in papaya fruits. **Pesquisa Agropecuária Tropical**, 47: 211-217, 2017.

GUIMARÃES, F. C. M. et al. Enzimas marcadoras de indução de resistência diferencialmente reguladas em soja resistente e suscetível à ferrugem asiática da soja. **Pesquisa Agropecuária Brasileira**, 47: 163-172, 2015.

JINGA, C. et al. Essential oil of *Syringa oblata* Lindl. as a potential biocontrol agent against tobacco brown spot caused by *Alternaria alternata*. **Crop Protection**, 104: 41–46, 2018.

KACEM, N. et al. Chemical composition of the essential oil from Algerian Genista quadriflora Munby and determination of its antibacterial and antifungal activities. **Crop Production**, 90: 87–93, 2016.

LANDAU, E. C. et al. **Dinâmica da produção agropecuária e da paisagem natural no Brasil nas últimas décadas: cenário histórico, divisão política, características demográficas, socioeconômicas e ambientais**. Brasília, DF: Embrapa, 2020. p. 1273-1300.

MARTINS, J. J. A. et al. Caracteristicas fisico-quimicas de tangerina 'cravo' cultivadas organicamente no brejo paraibano. In: SIMPÓSIO NORDESTINO DE QUÍMICA, 1., 2015, **Anais...** Natal: UFRN, 2015. p. 1-3.

MENEGASSI, T. et al. Extrato de *Lentinula edodes* no controle da antracnose do feijoeiro. **Brazilian Journal of Applied Technology for Agricultural Science**, 10: 75-81, 2017.

OLIVEIRA, J. A. **Efeito do tratamento fungicida em sementes no controle de tombamento de plântulas de pepino (Cucumis sativus L.) e pimentão (Capsicum annum L.)**. Lavras, MG: Universidade Federal de Lavras, 1991.

PERINA, F. J. et al. *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, 2014.

PORCINO, M. M. et al. Caracterização morfogenética e reação de resistência de genótipos de tangerineira a *Alternaria alternata* f. sp. *citri*. **Summa Phytopathologica**, 43: 118-123, 2017.

R CORE TEAM. **R: A languageandenvironment for statisticalcomputing**. 2017. R Foundation for Statistical Computing, Vienna, Austria. Disponível em: https://www.r-project.org/. Acesso em: 5 Jan. 2018.

RENAUD, M. S. A. et al. Diagrammatic scale for assessment

of Alternaria Brown Spot of citrus. **Summa Phytopathologica**, 34: 270-271, 2008.

SCHEUERMANN, K. K. et al. Majoritary compounds identified in essential oils of *Cymbopogon* species inhibits *Rhizoctonia solani*, causal agent of rice sheath blight. **Brazilian Journal of Animal and Environmental Research**, 3: 562-574, 2020.

SHANER, G.; FINNEY, R. E. The effects of nitrogen fertilization on the expression of slow mild wing in knox wheat. **Phytopathology**, 67: 1051-1055, 1977.

SHARIFI-RAD, J. et al. Biological activities of essential oils: from plant chemoecology to traditional healing systems. **Molecules**, 22: 1-55, 2017.

SHARMA, A. et al. Antifungal activities of selected essential oils against *Fusarium oxysporum* f. sp. *lycopersici* 1322, with emphasis on *Syzygium aromaticum* essential oil. **Journal of Bioscience and Bioengineering**, 123: 308–313, 2017.

SILVA, A. F. et al. Quality, bioactive compounds and antioxidante activity during maturation of oranges produced in the Borborema territory. **Revista Caatinga**, 32: 526-536, 2019.

XU, S. et al. *In vitro* and in vivo control of *Alternaria alternata* in cherry tomato by essential oil from *Laurus nobilis* of Chinese origin. **Journal of the Science of Food and Agriculture**, 94:1403–1408, 2014.

YU, Y. Y. et al. An improved strategy for stable biocontrol agents selecting to control rice sheath blight caused by *Rhizoctonia solani*. **Microbiological Research**, 203: 1-9, 2017.