DOI: 10.1590/1808-1657000612019

Fungicidal potential of essential oils in control of *Fusarium* spp. and *Sclerotinia sclerotiorum*

Potencial fungicida de óleos essenciais no controle de **Fusarium** *spp. e* **Sclerotinia sclerotiorum**

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ABSTRACT: The use of highly toxic pesticides to control soil pathogens, such as *Fusarium* spp. and *Sclerotinia sclerotiorum* has generated concern, due to the irreversible impacts caused on the environment, in addition to selecting resistant isolates. In this way, essential oils appear as an efficient alternative in control of diseases. Facing the problem of soil pathogens control and high antimicrobial fungicide that essential oils present, this work aimed to evaluate the *in vitro* fungicidal potential of essential oils in control of *Fusarium* spp. and *S. sclerotiorum.* A completely randomized design, factorial scheme 2×4×8 was used, with two isolates (*Fusarium* spp. and *S. sclerotiorum*), four essential oils (*Aloysia citriodora, Cymbopogon winterianus, Lippia alba* and *Ocimum americanum*), eight essential oil concentrations (0.0; 0.2; 0.4; 0.6; 0.8; 1.0; 1.2 and 1.4 $\mu L \cdot mL^{-1}$), and ten replicates. The essential oils inhibited mycelial growth of the fungi in different concentrations, being their potential justified by the presence of antifungal chemical compounds. Essential oils of *A. citriodora, C. winterianus, L. alba* and *O. americanum* present high fungicidal potential, being viable alternatives for formulation of commercial products, boosting the pesticides industry.

KEYWORDS: *Aloysia citriodora; Cymbopogon winterianus; Lippia alba; Ocimum americanum*; soil fungi; pesticides.

RESUMO: O uso de pesticidas com alta toxicidade para controlar patógenos do solo, como *Fusarium* spp. e *Sclerotinia sclerotiorum,* tem gerado preocupação, devido aos impactos irreversíveis causados no meio ambiente, além de selecionar isolados resistentes. Dessa forma, os óleos essenciais surgem como uma alternativa eficiente no controle de doenças. Diante da problemática de controle de patógenos do solo e alto potencial antimicrobiano que os óleos essenciais possuem, este trabalho teve como objetivo avaliar o potencial fungicida de óleos essenciais no controle de *Fusarium* spp. e *S. sclerotiorum, in vitro*. Utilizou-se um delineamento inteiramente randomizado, esquema fatorial 2×4×8, com dois isolados (*Fusarium* spp. e *S. sclerotiorum*), quatro óleos essenciais (*Aloysia citriodora, Cymbopogon winterianus, Lippia alba* e *Ocimum americanum*) e oito concentrações de óleo essencial (0,0; 0,2; 0,4; 0,6; 0,8; 1,0; 1,2 e 1,4 $\mu L \cdot mL^{-1}$), com dez repetições. Os óleos essenciais inibiram o crescimento micelial dos fungos em diferentes concentrações, sendo seu potencial justificado pela presença de compostos químicos antifúngicos. Os óleos essenciais de *A. citriodora, C. winterianus, L. alba* e *O. americanum* apresentam alto potencial fungicida, sendo alternativas viáveis para formulação de produtos comerciais, impulsionando a indústria de agrotóxicos.

PALAVRAS-CHAVE: *Aloysia citriodora; Cymbopogon winterianus; Lippia alba; Ocimum americanum*; pesticidas.

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Received on: 07/18/2020. Accepted on: 08/17/2020

INTRODUTION

Conventional agriculture has reduced soil quality, leading to imbalance and serious problems with root and vascular diseases (VAN BRUGGEN; SEMENOV, 2015). Soil-borne diseases can lead to significant yield reductions in various crops (VAN BRUGGEN; FINCKH, 2016), as in the case of *Phaseolus vulgaris* (NASERI; HAMADANI, 2017), *Beta vulgaris* (FREDDO et al., 2016), *Fragaria* × *ananassa* (TOURNAS; KATSOUDAS, 2005), *Glycine max* (WESTPHAL et al., 2008), which are affected by *Fusarium* spp. as agents of root rot. Another soil pathogen that has become a serious problem in commercial crops is *Sclerotinia sclerotiorum*, the causal agent of white mold in soybean crops (FURLAN, 2015).

The management of these diseases has become a challenge in agriculture, since it requires pesticides that are often highly toxic (CARRASCOSA et al., 2015). Chemical pesticides, particularly soil fumigants, have been severely restricted in recent decades due to the potential environmental damage caused by their use (VAN BRUGGEN; FINCKH, 2016), requiring a long period of time for complete degradation (FENG; ZHENG, 2007). In addition, the impact of pesticides on the environment, on human health and on reducing the sensitivity of pathogens has been discussed (LAMICHHANE et al., 2016), due to pressure from society for more sustainable agriculture.

Therefore, new technologies are necessary so that phytosanitary management can be done in a less aggressive manner, both in environmental, economic and social terms (DAYAN et al., 2009). Thus, the use of alternative products, such as medicinal plants that can be exploited in obtaining natural pesticides, their raw extracts and/or essential oils, is shown as a viable option (MORAIS, 2009).

Essential oils are complex substances and act as inhibitors of several phytopathogenic fungi; therefore, they were studied in order to point them out as an alternative to phytosanitary control in plantations, with the objective of replacing the use of synthetic pesticides (ROMERO et al., 2012).

The fungicidal potential of *A. citriodora* essential oil has already been observed for fungi such as *Fusarium* spp. (FREDDO et al., 2016), *Colletotrichum gloeosporioides*, *Penicillium digitatum*, *Botrytis cinerea* (COMBRINCK et al., 2011) and *Moniliophthora roreri* (LOZADA et al., 2012). In the present study, it was observed that the essential oil of *Cymbopogon citratus* has positive effects on the control of microorganisms such as *Micosphaerella fragariae* (SCHNEIDER et al., 2012), *Fusarium equiseti*, *C. gloesporioides*, *Fusarium solani* (DAS et al., 2016) *Corynespora cassiicola* (OLIVEIRA et al., 2017) and *Monilinia fructicola* (PANSERA et al., 2015). The essential oil of *L. alba* was reported with fungicidal potential against *Fusarium* spp., *Penicillium* spp. (GLAMOČLIJA et al., 2011), *Didymella bryoniae, Rhizoctonia solani, Sclerotium rolfsii* (SARMENTO-BRUM et al., 2014) and nematicide potential against *Meloidogyne incognita* (GONÇALVES et al., 2016). *Ocimum basilicum* has also been reported with fungicidal potential in the control of *Phakopsora pachyrhizi* (BORGES et al., 2013).

The objective of this work was to evaluate the *in vitro* fungicidal potential of the essential oils of *Cymbopogon winterianus, A. citriodora, Ocimum americanum* and *L. alba* to control soil pathogens and their high fungicidal potential against *Fusarium* spp. and *S. sclerotiorum.*

METHODOLOGY

Plant material and experimental conditions

The experiment was conducted at the Phytopathology Laboratory of the Federal University of Santa Maria, Campus Frederico Westphalen/RS, Brazil, during 2016. The plants used belong to the medicinal garden of the Laboratory of Tissue Culture and Aromatic Extractives. After collection of the plant material of *A. citriodora, C. winterianus, L. alba* and *O. americanum*, the essential oils were extracted by the hydrodistillation method using Clevenger apparatus. The oils were kept in a freezer until the time of their use for the experiment. Soil fungi *Fusarium* spp. and *S. sclerotiorum* were isolated from symptomatic strawberry and soybean plants, respectively. The microorganisms were previously multiplied in potato-dextrose-agar (PDA) medium, in incubation chamber at 22 and 25 °C, respectively, for further experiments.

Experimental design

The experiment was conducted in a completely randomized design in triple factorial with two species of fungi (*Fusarium* spp. and *S. sclerotiorum*) × four essential oils (*A. citriodora, C. winterianus, L. alba* and *O. americanum*) × eight concentrations of essential oils (0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 $\mu L \cdot mL^{-1}$) *in vitro* with 10 replicates, and the experimental unit considered was a Petri dishes.

The concentrations used were determined from the literature and previous trials. To evaluate the fungicidal activity of the essential oils on the fungal isolates, they were incorporated in the BDA flux at 50 °C and then poured into Petri dishes (90 mm) in a laminar flow chamber. When solidified, mycelial discs were inoculated from the fungal colonies with 7.0 mm diameter to the center of Petri dishes containing their respective treatments. After inoculation, the plates were incubated in a incubation chamber chamber at 22 °C for *S. sclerotiorum* and 25 °C for *Fusarium* spp.

Analysis of variables

The diameter of the colonies was measured in orthogonal position with a digital caliper, until the zero concentration (control) reached the total diameter of the plate. On the sixth day of evaluation for *Fusarium* spp. and on the fifth day for *S. sclerotiorum*, the last measurement was made, determining mycelial growth (MG). From the mean values of the control treatment it was possible to evaluate the variable percentage of inhibition of mycelial growth (PIMG) for each treatment. The concentration required for 50% growth inhibition (EC_{50}) for each essential oil was calculated from linear regression with percent inhibition and logarithmic scale doses.

The variables MG and PIMG were submitted to analysis of variance and, when significant, the regression analysis was carried out, using the statistical program Genes (CRUZ, 2013).

For the variable PIMG, all the essential oils tested in the control of *Fusarium* spp. showed increased inhibition as the dose of the oils increased (Fig. 3). At doses that inhibited mycelial growth (Fig. 2), 100% control was observed (Fig. 3), explaining the dependence between the variables.

All the essential oils tested had fungistatic power against *S. sclerotiorum*, and mycelial growth was reduced as the concentrations of the essential oils increased. The inhibition of mycelial growth was variable with the concentration, requiring 0.8 $\mu L \cdot mL^{-1}$ of the essential oil of *A. citriodora* (Fig. 4a), 1.2 $\mu L \cdot mL^{-1}$ of the *C. winterianus* oil (Fig. 4b), 0.4 $\mu L \cdot mL^{-1}$ of *L. alba* (Fig. 4c) and 1.4 $\mu L \cdot mL^{-1}$ of *O. americanum* (Fig. 4d).

All essential oils presented 100% control of mycelial growth (Fig. 5) in the dosages where they inhibited mycelial growth, as shown in Figure 4.

RESULTS

According to the analysis of variance, all analyzed variables presented significance for the factors essential oils × concentrations, and are presented as a regression, separately by variable. The essential oil of *Al. citriodora* provided the lowest EC_{50}, with approximately 0.11 $\mu L \cdot mL^{-1}$ for *S. sclerotiorum*, whereas 0.28 $\mu L \cdot mL^{-1}$ was required for *Fusarium* spp. For *S. sclerotiorum*, *C. winterianus* oil provided the lowest EC_{50}, with only 0.05 $\mu L \cdot mL^{-1}$ (Fig. 1).

The essential oils promoted reduced growth of *Fusarium* spp. isolated from strawberry, as the concentrations of essential oils increased (Fig. 2). It has been found that inhibition of mycelial growth of *Fusarium* spp. with 0.6 $\mu L \cdot mL^{-1}$ for *A. citriodora* oil (Fig. 2a), 1.0 $\mu L \cdot mL^{-1}$ for *C. winterianus* oil (Fig. 2b), and 1.2 $\mu L \cdot mL^{-1}$ for *O. americanum* oil (Fig. 2d). For *L. alba* oil, the growth inhibition at the concentration was observed with 1.4 $\mu L \cdot mL^{-1}$ of essential oil (Fig. 2c).

DISCUSSION

The essential oil of *A. citriodora* presented high fungicidal power over the isolates of *Fusarium* spp. and *S. sclerotiorum*. The doses of 0.6 and 0.8 $\mu L \cdot mL^{-1}$ were those that completely inhibited their mycelial growth, reaching 100% control, respectively. The results obtained corroborate with other authors who highlight the *Aloysia* genus with antibacterial (ALI et al., 2011), antimicrobial (ESCOBAR et al., 2010) and antifungal potential (LOZADA et al., 2012). The antifungal effect of other *Aloysia* species has already been proven for other pathosystems, such as *Fusarium* spp. isolated from beet seedlings (FREDDO et al., 2016), *Fusarium verticillioides* isolated from corn (LÓPEZ et al., 2004), *C. gloesporioides* isolated from mango and avocado, and *Penicillium* isolated from cactus fruits (COMBRINCK et al., 2011).

The minimum concentration required to inhibit 100% mycelial growth of *A.citriodora* essential oil varies according

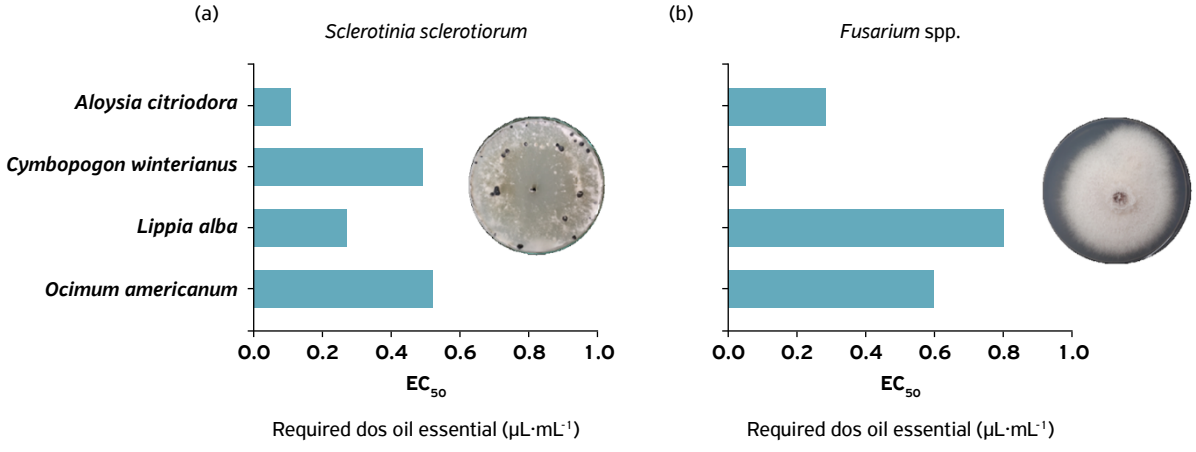

Figure 1. Effective concentration for 50% control of mycelial growth (EC_{50}) of *Sclerotinia sclerotiorum* and for *Fusarium* spp.

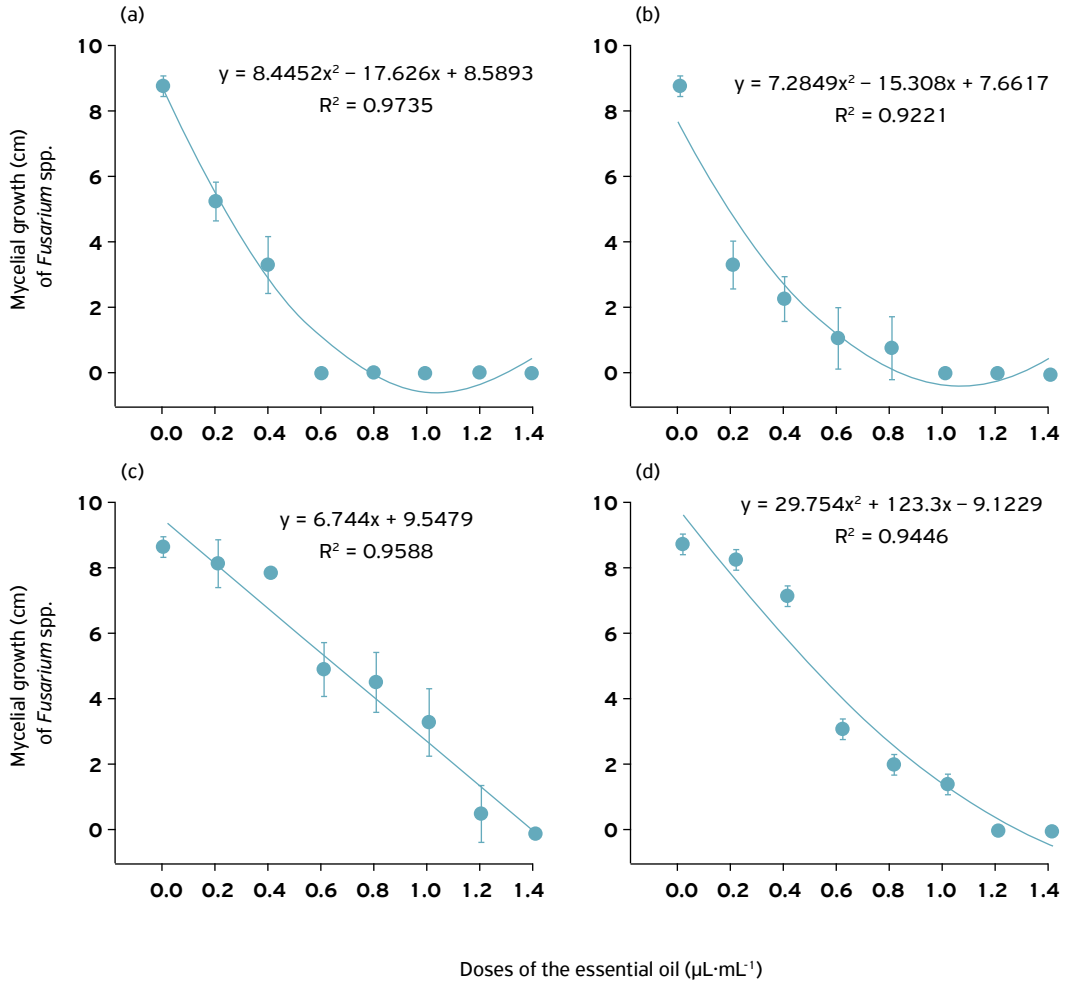

Figure 2. Mycelial growth of *Fusarium* spp., submitted to doses of the essential oil of *Aloysia citriodora* (a), *Cymbopogon winterianus* (b), *Lippia alba* (c) and *Ocimum americanum* (d).

to the pathogens, with 0.2% for *C. gloeosporioides* of avocado and mango and 0.3% for *P. digitatum* (COMBRINCK et al., 2011) and a concentration of 0.25% for the reduction of the incidence of tipping of beet seedlings inoculated with *Fusarium* spp. (FREDDO et al., 2016).

Aloysia citriodora presented an antifungal effect to *S. sclerotiorum*, with 100% fungicidal power on the germination of sclerotia in the concentration of 0.25%, reducing postemergence cucumber seedling troweling from the treatment of seeds, conferring the plants with the highest content of proteins. In this same study, it was verified that the increase of the concentrations of the essential oil of *A. citriodora* promoted increase of the biochemical activity of the enzyme phenylalanine ammonia-lyase (FAL) (FREDDO et al., 2016). In addition, the FAL, together with peroxidase, are considered to be key plant defense enzymes, between primary and secondary metabolism, and the first is important in the formation of phenolic compounds.

The enzymatic activity of β-1,3-glucanases was also affected by the concentration of essential oil of *A. citriodora* as concentration increased in the treatment of cucumber seeds (FREDDO et al., 2016). According to MASSOLA JÚNIOR; KRUGNER (2011), *S. sclerotiorum*, is a true fungus and, like that, have β-glucans and chitin in their cell walls. β-1,3-glucanases are lytic enzymes, which have the potential to hydrolyze the β-glucans present in the cell wall of fungi, classified as "pathogenesis-related proteins" (PR-Proteins) (PASCHOLATI; DALIO, 2011), acting in this way, in the control of *S. sclerotiorum.*

Aloysia citriodora oil has as main compounds limonene (3.2 to 18.5%), β-citral (17.6–24.6%), α-citral (25.1–31.5%), only varying their concentrations during the seasons (PROCHNOW et al., 2017). The components of this essential oil with probable fungitoxic effect to the studied fungi are citral and limonene, corroborating with PAULUS et al. (2013).

The essential oil of *C. winterianus* promoted 100% control at concentrations of 1.0 and 1.2 µL·mL^{-1} for *Fusarium* spp. and *S. sclerotiorum*, respectively. In the present study, it was observed that the growth of *C. citratus* was higher in the genotypes of *Alternaria solani* and *C. cassiicola* (OLIVEIRA et al., 2017), in the inhibition of mycelial growth of *Fusarium oxysporum* at 62.5 ppm and inhibition of spore germination at 125 ppm

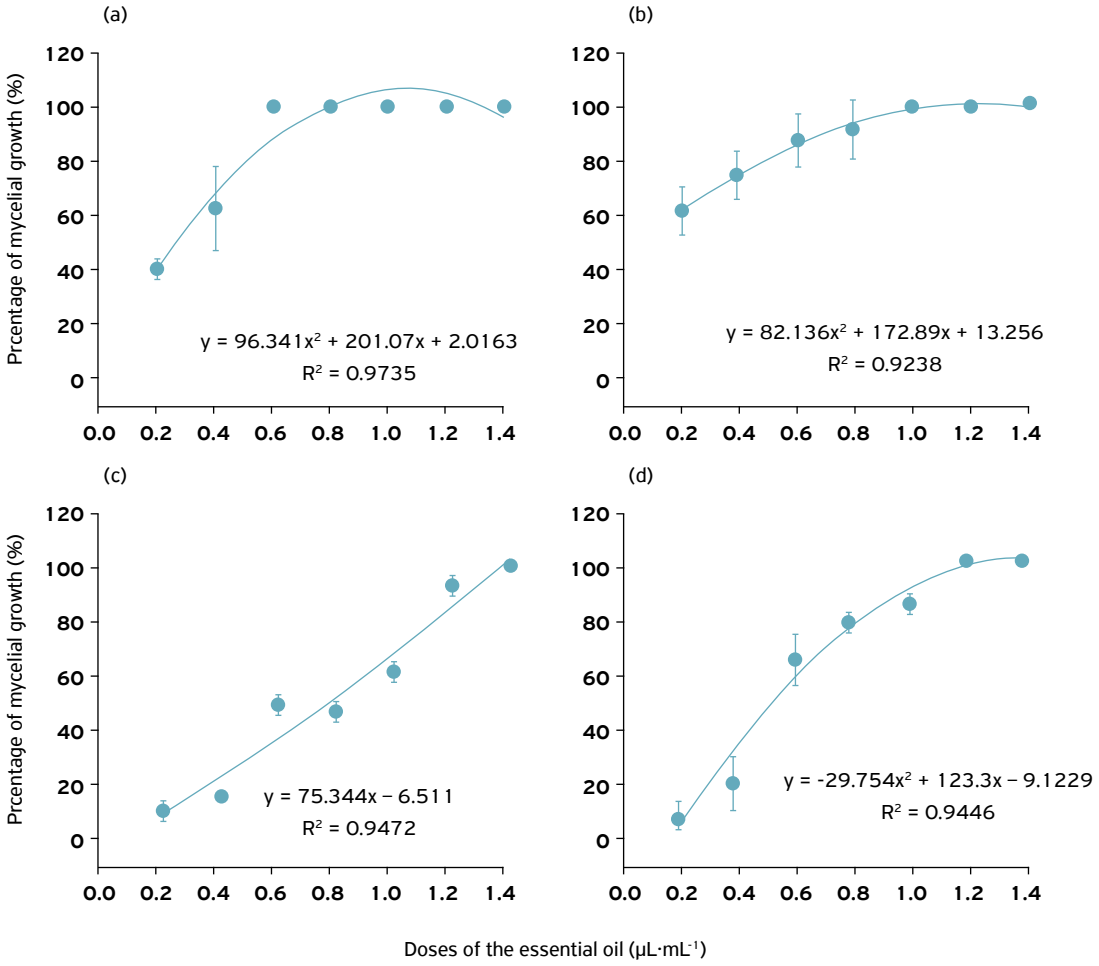

Figure 3. Percentage inhibition of mycelial growth of *Fusarium* spp., submitted to doses of the essential oil of *Aloysia citriodora* (a), *Cymbopogon winterianus* (b), *Lippia alba* (c) and *Ocimum americanum* (d).

(SHARMA et al., 2017), and in postharvest diseases caused by *M. fructicola* and *C. gloesporioides* (PANSERA et al., 2015). In the present study, it is possible to determine the effect of the microorganisms on the growth of the spores. *Cymbopogon nardus* oil also has fungicidal potential against *Fusarium subglutinans* in 25 µl aliquot (SEIXAS et al., 2011).

The essential oil of *C. citratus* at the concentration of 0.01% presented inhibition of mycelial growth and alteration in the morphology of the *S. sclerotiorum* colony, while other essential oils, such as *Salvia officinalis* and *Baccharis trimera*, required a higher dose (PANSERA et al., 2012). Similarly, SHARMA et al. (2017) found that spores of *F. oxysporum* when exposed to clove oil revealed extensive damage and a considerable increase of cell surface roughness in the hypha and spore morphology. It is believed that the lipophilic nature of the essential oils may facilitate penetration into the fungal membrane causing its disruption (LAMBERT et al., 2001).

The fungitoxic effect on *F. subglutinans* isolates is higher when using citronella grass essential oil than when using the isolated citronellal chemical compound (SEIXAS et al., 2011). This relationship may be related to the interaction of various constituent compounds of the essential oil. Thus, the major effect of inhibition of the essential oil is due to the synergism between the essential oil compounds that act together, providing a greater fungistatic effect (SEIXAS et al., 2011).

Cymbopogon winterianus oil has insect repellent action (NERIO et al., 2010), showing activity against *Aedes aegypti* mosquito larvae (MENDONÇA et al., 2005) and antimicrobial activity (OLIVEIRA et al., 2011). The antimicrobial activity of essential oils of the genus *Cymbopogon* is justified by their chemical composition with geraniol, citronellal and citronellol (JIROVETZ et al., 2007; KORDALI et al., 2007), corroborating with the results found.

Lippia alba essential oil concentrations that inhibited the mycelial growth of *Fusarium* spp. and *S. sclerotiorum* in this work were 1.4 and 0.4 µL·mL^{-1}, respectively. *Lippia alba* is a medicinal plant that has high sedative (HOHLENWERGER et al., 2016), antidepressant, digestive, antihemorrhoidal and antiasthmatic potential (HENNEBELLE et al., 2008). In addition to its uses in traditional medicine, this oil has been reported

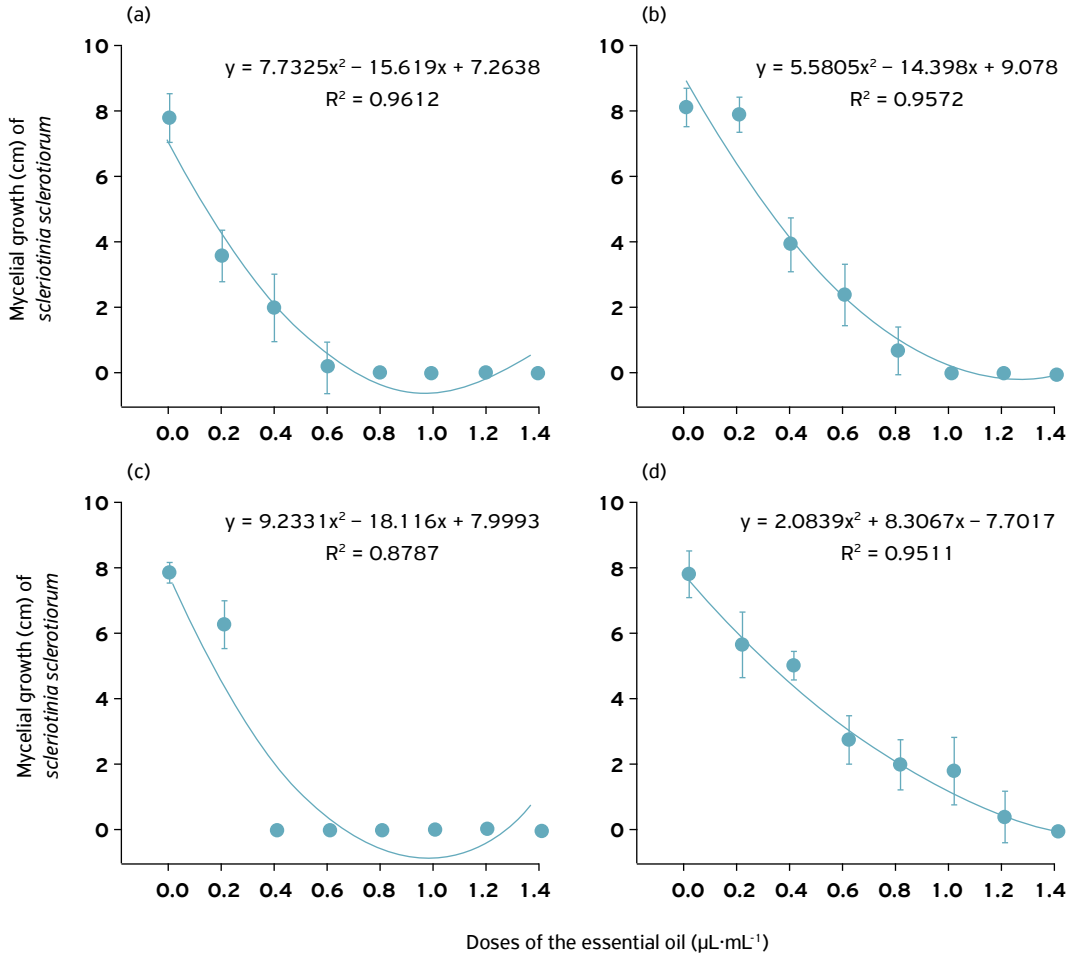

Figure 4. Mycelial growth of *Sclerotinia sclerotiorum*, submitted to doses of the essential oil of *Aloysia citriodora* (a), *Cymbopogon winterianus* (b), *Lippia alba* (c) and *Ocimum americanum* (d).

with insecticidal power against *Tribolium castaneum* in wheat grains (RINGUELET et al., 2014), acaricide against *Rhipicephalus microplus* (PEIXOTO et al., 2015), nematicide (GONÇALVES et al., 2016), and fungicide (AQUINO et al., 2014).

The fungicidal activity of *L. alba* oil was observed for other microorganisms such as *Aspergillus* spp., *Penicillium* spp. and *Trichoderma viride* (GLAMOČLIJA et al., 2011), *D. bryoniae, P. grisea, R. solani* and *S. rolfsii* (SARMENTO-BRUM et al., 2014). The fungicidal activity of the essential oil of *L. alba* is justified by its chemical composition, being linalool (47.66%), β-myrcene (11.02%) and eucalyptol (9.77%) their major components (HOHLENWERGER et al., 2016).

Other species of the genus *Lippia* have also been studied for their antimicrobial effects. For *Aspergilus niger, Penicillium* spp. and *F. oxysporum*, the oil of *L. sidoides* presented inhibition of mycelial growth in the concentration of 3×10^{-1} µL·mL^{-1} (OLIVEIRA et al., 2008). GADELHA et al. (2003) verified the efficiency of *L. sidoides* oil in the control of *Fusarium* spp. during postharvest treatment of the melon peduncle at concentrations of 2.0 µL·mL^{-1} as a preventive, and 4.0 µL·mL^{-1} as a dressing.

The essential oil of *O. americanum* required higher concentrations to inhibit the mycelial growth of the isolates of *Fusarium* spp. and *S. sclerotiorum*, with concentrations of 1.2 and 1.4 µL·mL^{-1}, promoting 100% control of the isolates, respectively. When using the acetic extract of basil (*O. basilicum*) at 25% concentration, it gave 50% inhibition of the mycelial growth of *Fusarium* spp. (CAMATTI-SARTORI et al., 2011). The extract of *Ocimum gratissimum* at the concentration of 30% showed only 3.57% of control in the mycelial growth of *S. sclerotiorum* (GARCIA et al., 2012).

The essential oil of *O. gratissimum* showed reduction in the mycelial growth rate index of *C. gloesporioides* as the concentrations of essential oil increased (AQUINO et al., 2014). Similar results were achieved with this oil in 20, 40 and 60 µL aliquots completely inhibiting the *in vitro* mycelial growth of soil fungi such as *R. solani, S. rolfsii, Phytophthora* spp. (BENINI et al., 2010).

NASCIMENTO et al. (2016) found inhibition of the mycelial growth of *F. solani* f. sp. *glycines* at the concentration

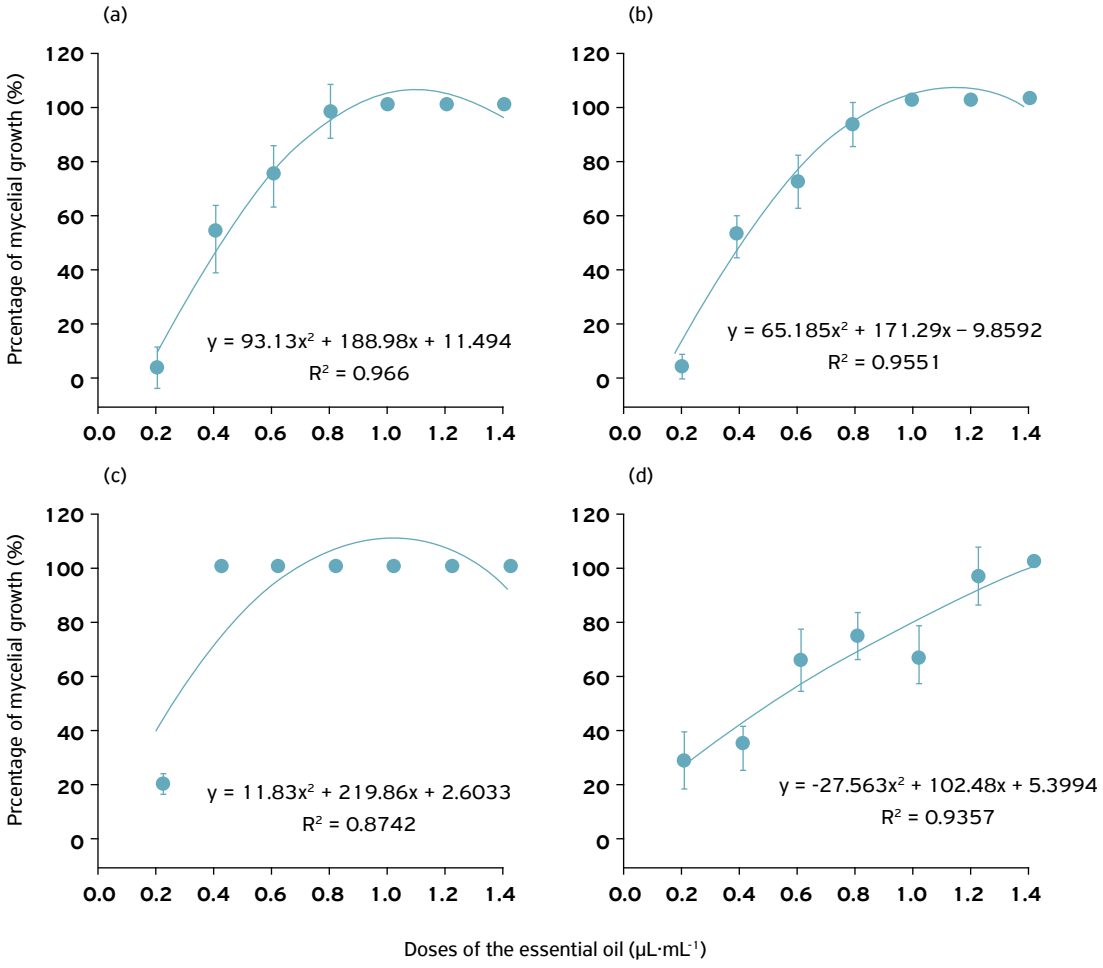

Figure 5. Percentage inhibition of mycelial growth of *Sclerotinia sclerotiorum* isolated from soybean, submitted to doses of the essential oil of *Aloysia citriodora* (a), *Cymbopogon winterianus* (b), *Lippia alba* (c) and *Ocimum americanum* (d).

of 6.0 µL·mL^{-1} of the essential oil of *O. basilicum*, *in vitro*. This concentration is considered higher when compared to the present study, which required 1.2 µL·mL^{-1}, thus characterizing the essential oil of *Oc. americanum* as more potent in fungal control.

The major compound of *O. americanum* essential oil is E-methyl cinnamate, presenting antimicrobial effect (JUIZ et al., 2016). In fact, the species *O. americanum* is rich in essential oils that may have as major constituents: methyl cinnamate, eugenol and methyl-chavicol, depending on the place of harvest (VIEIRA; SIMOM, 2000). Thus, it is assumed that the chemical composition of the essential oil explains the microbial activity found.

Generally, the action attributed to an isolated compound may not be accurate due to possible interactions that may occur between the oil compounds (ZAGO et al., 2009). Thus, the antifungal effect for *Fusarium* spp. and *S. sclerotiorum* verified for the essential oils of *A. citriodora, C. winterianus, O. americanum* and *L. alba* are attributed to the interaction of their chemical compounds, as already discussed by SEIXAS et al. (2011).

Fungi control through essential oils often involves the prevention of hyphal growth and sporulation, interruption of nutrient uptake and metabolism, rupture of the plasma membrane, disruption of the mitochondrial structure and interference with respiratory enzymatic reactions of the mitochondrial membrane (PATEL; JASRAI, 2011). Acting in this way, in several cellular mechanisms of the fungal cell, being potential alternative for use as biopesticide. The results found for the essential oils of *A. citriodora, L. alba, C. winterianus* and *O. americanum* species are innovative for the studied pathosystems.

CONCLUSION

The essential oils of *A. citriodora, C. winterianus, L. alba* and *O. americanum* present high fungicidal potential, since they contain a low EC_{50}, being viable alternatives for the formulation of commercial products, boosting the agrochemical industry.

ACKNOWLEDGEMENTS: The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CNPq).

FUNDING: This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

ETHICAL APPROVAL: Not applicable.

AVAILABILITY OF DATA AND MATERIAL: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHORS' CONTRIBUTIONS: Methodology: Pretto, M.M.; Mariotto, A.B.; Holz, E.; Fontana, D.C. Project administration: Schmidt, D.; Caron, B.O. Supervision: Kulczynski, S.M. Writing – review & editing: Fontana, D.C.; Santos, J.

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