

Antifungal action of essential oils against Fusarium rot in melon

Ação antifúngica de óleos essenciais contra podridão de Fusarium em melão

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ABSTRACT - The objective of this work was to determine the composition and evaluate in vitro and in vivo effects of essential oils (*Lippia sidoides* Cham., *Ocimum gratissimum* L., *Cymbopogon citratus* Stapf., *Ocimum selloi* Benth., *Citrus sinensis* L., *Ocimum micranthum* Willd., *Ocimum* sp., and *Piper aduncum* L.) on the control of Fusarium rot in melon fruits, caused by the fungus *Fusarium pallidoroseum*. The essential oils were obtained by hydro-distillation and their chemical composition was determined by GC-MS and GC-FID. The effect of each essential oil (concentrations of 0, 500, 1500, and 3000 μ L L⁻¹) on the fungal mycelial growth was evaluated in in vitro experiment. The effective concentration that inhibited 50% of mycelial growth (EC₅₀) was determined through the probit method; mycelial growth index (MGI) was also calculated. The essential oils with higher potential for inhibiting mycelial growth of *F. pallidoroseum* were evaluated for their inhibitory effect on the fungus spore germination in in vitro and in vivo experiments using melon fruits (variety Galia). Chemical composition analysis of essential oils enabled the identification of varying amounts of chemical compounds, with predominance of monoterpenes. The essential oils of *L. sidoides*, *O. gratissimum*, *C. citratus*, and *O. micranthum* presented higher inhibiting effects on *F. pallidoroseum* mycelial growth and spore germination, therefore, they are promising raw materials for the development of commercial fungicides, mainly for controlling postharvest rot caused by *F. pallidoroseum*.

Keywords: *Cucumis melo*. *Fusarium pallidoroseum*. Essential oil.

RESUMO - Objetivou-se com esse trabalho determinar a composição e avaliar, 'in vitro' e 'in vivo', o efeito dos óleos essenciais de alecrim-pimenta (*Lippia sidoides* Cham.), alfavaca-cravo (*Ocimum gratissimum* L.), capim-limão (*Cymbopogon citratus* Stapf.), elixir-paregórico (*Ocimum selloi* Benth.), laranja (*Citrus sinensis* L.), manjerição (*Ocimum micranthum* Willd.), *Ocimum* sp. e pimenta-de-macaco (*Piper aduncum* L.) no controle da podridão por Fusarium causada pelo fungo *Fusarium pallidoroseum* em frutos de melão. Os óleos essenciais foram obtidos por hidrodestilação e a composição química determinada por GC-MS e GC-FID. No ensaio 'in vitro', foi testado o efeito de cada óleo essencial (concentrações de 0, 500, 1500 e 3000 μ L/L) sobre o crescimento micelial do patógeno. Determinou-se a concentração efetiva que inibiu o crescimento micelial em 50% (EC₅₀) pelo método de probit e o Índice de Crescimento Micelial (ICM). Os óleos essenciais com maior potencial inibidor no crescimento micelial do *F. pallidoroseum* foram avaliados quanto ao efeito inibitório na germinação de esporos do fungo no ensaio 'in vitro' e 'in vivo' com frutos de melão 'Galia'. A análise da composição química possibilitou a identificação de quantidades variáveis de compostos químicos, com predominância dos compostos monoterpênicos. Os óleos essenciais de alecrim-pimenta, alfavaca-cravo, capim-limão e manjerição, apresentaram melhores efeitos na inibição do crescimento micelial e germinação dos esporos do fungo, mostrando-se promissores como matéria-prima para o desenvolvimento de fungicidas comerciais, em especial no controle de podridão causada por *F. pallidoroseum*, em pós-colheita.

Palavras-chave: *Cucumis melo*. *Fusarium pallidoroseum*. Óleo essencial.

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INTRODUCTION

Melon (*Cucumis melo* L.) is one of the most important fruits grown in Brazil. The national export of this fruit was 245 thousand Mg in 2020 (FAOSTAT, 2020). However, melon growers usually report that, on average, 30% of the exported melon present Fusarium rot and are discarded by international buyers, resulting in rejection and large financial losses to growers.

Fusarium pallidoroseum (Cooke) Sacc (synonymy: *Fusarium semitectum*) is the causal agent (pathogen) of Fusarium rot. The pathogen infection can occur in the field soon after harvesting, or during the packaging procedures, mainly in the step of cutting the excess peduncle. The pathogen can continue its pathogenesis even under refrigeration, leading to complete fruit destruction or causing lesions that decrease the marketing potential of fruits.

Few active ingredients are registered in the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA) for the control of postharvest diseases in fruits. Currently, imazalil is the only active ingredient registered (MAPA registration no. 3498) for postharvest application on melon fruits; but

Fusarium pallidroseum is not included in the registration recommendations. Additionally, the use of pesticides has been restricted in the national and international markets due to their potential carcinogenic and teratogenic actions, high toxicity, and side effects on human health, as well as they can cause environmental pollution and require long time for complete degradation (SUÁREZ-QUIROZ et al., 2013).

The use of natural products with bioactive compounds can be an alternative for the integrated management of postharvest diseases. In this context, the use of plant essential oils has been the subject of several studies that have validate their effectiveness as an alternative method for controlling postharvest diseases in fruits.

The scientific literature has shown the effectiveness of essential oils from a wide variety of botanical species in inhibiting the development of several fungal phytopathogens (ZNINI et al., 2013; SIVAKUMAR; BAUTISTA-BAÑOS, 2014; BRITO et al., 2015; VIEIRA et al., 2018; NABILA; SOUFIYAN, 2019; DAS et al., 2021; ZHANG et al. 2021). However, the commercially available plant essential oils and extracts are largely used as preharvest fungicides for biological agriculture (ISMAN, 2020; RAVEAU; FONTAINE; LOUNÈS-HADJ SAHRAOUI, 2020; RGUEZ et al., 2020). Few studies have evaluated the postharvest application of essential oils (COMBRINCK; REGNIER; KAMATOU, 2011; CHEN et al., 2014; FONTANA et al., 2021).

Essential oils are products of the secondary metabolism of plants, composed mainly of terpenes, aldehydes, ketones, and phenolic compounds (SIVAKUMAR; BAUTISTA-BAÑOS, 2014). They affect the membranes of microorganisms, modulating functions connected to cell permeability and signaling, among others, causing destabilization and even cell death (ZORE et al., 2011).

Postharvest applications of essential oils on melon fruits can be an alternative for decreasing *Fusarium* rot. Therefore, the objective of this work was to determine the composition and evaluate in vitro and in vivo effects of different essential oils on the control of rot disease in melon fruits, caused by the fungus *Fusarium pallidroseum*.

MATERIAL AND METHODS

Plant material - The essential oils were extracted from plants from the Medicinal Plant Garden at the Brazilian Agricultural Research Corporation (Embrapa Agroindústria Tropical), Fortaleza, CE, Brazil. The plant material used were: leaves of *Lippia sidoides* Cham., *Cymbopogon citratus* Stapf. and *Piper aduncum* L.; inflorescences and leaves of *Ocimum gratissimum* L., *Ocimum selloi* Benth., *Ocimum micranthum* Willd., and *Ocimum* sp., and peels of *Citrus sinensis* L.

Obtaining of essential oils - 200 g fresh plant material in distilled water were subjected to hydrodistillation for four hours in a modified Clevenger apparatus (Coperglass®) for essential oil extraction, according to the method described by Tigrine-Kordjani et al. (2012).

Essential oil composition - Qualitative and quantitative analyses of essential oils were carried out using gas chromatography coupled to mass spectrometry (GC-MS) and to flame ionization detector (GC-FID), respectively, according to Ribeiro et al. (2015).

Antifungal action of essential oils (in vitro) - A *Fusarium pallidroseum* (MN652629) isolate from the microbial bank of the Postharvest Pathology Laboratory at Embrapa Agroindústria Tropical was reactivated and used in the experiments. Aliquots of each essential oil were added to culture medium containing potato-dextrose-agar (BDA) (45 °C) and Tween 20 (1:1) for obtaining the following essential oil concentrations: 0, 500, 1500, and 3000 µL L⁻¹. Each mixture was poured into Petri dishes (90 mm in diameter). Then, PDA discs of 5 mm in diameter containing mycelium of the fungus were transferred to the center of each plate. The control consisted of BDA disc containing fungus mycelium with no addition of essential oil to the culture medium. The plates were sealed and incubated for eight days in biochemical oxygen demand (BOD) chamber at temperature of 25 °C under 12-hour photoperiod. The experiment was conducted in a completely randomized design, with five replications, consisting of one plate per replication distributed randomly on the shelves of the BOD chamber. The colony diameter (mm) was measured in two orthogonal axes when the colonies of the control plots reached the edge of the plates. The effect of essential oil concentrations on mycelial growth inhibition was determined through regression analysis (FINNEY, 1951). The effective concentration of essential oil that inhibits 50% of mycelial growth (EC₅₀, µL L⁻¹) was determined by survival analysis using the probit method (MACEDO et al., 2013). Mycelial growth index (MGI) was calculated using the formula: $(C_1/D_1) + (C_2/D_2) + (C_n/D_n)$, where *C* is the mycelium size and the *D* indicates the day on which the mycelium was measured (ELIZEI et al., 2014).

The four essential oils (*Lippia sidoides*, *Ocimum gratissimum*, *Cymbopogon citratus*, and *Ocimum micranthum*) that showed the best performance for inhibition of fungal mycelial growth (lower MGI) were used for the experiment of spore germination inhibition.

Spore suspension was obtained by adding 20 mL of sterile distilled water to fungal culture in Petri dish and scraping using a Drigalski spatula. The spore suspension was filtered through gauze and then its concentration was adjusted to 2.0×10² spores mL⁻¹ using a Neubauer chamber under an optical microscope. Each essential oil concentration (500, 1500, and 3000 µL L⁻¹) was added to BDA culture medium (45 °C) containing Tween 20 (1:1). Subsequently, 0.1 mL of spore suspension was added to Petri dishes containing the BDA culture medium with the different essential oil concentrations, spreading it evenly on the surface of the culture medium using a Drigalski spatula. The control consisted of a spore suspension grown in BDA culture medium without addition of essential oil. The plates were sealed and incubated for eight days in a BOD chamber at temperature of 25 °C under 12-hour photoperiod. The experiment was conducted in a completely randomized

design, with five replications, consisting of one plate per replication distributed randomly on the shelves of the BOD chamber. The percentage of spore germination inhibition was calculated, the data obtained were subjected to analysis of variance, and the means were compared by the Tuckey's test at 5% probability level (Statistic 8.0).

Antifungal action of essential oils (in vivo) - Melon (*Cucumis melo* L.) fruits (variety Galia) from the exporting agriculture company Agricola Famosa Ltd., at Icapui, CE, Brazil, were harvested and sent to the Postharvest Pathology Laboratory at Embrapa on the same day. The fruits were disinfested in a solution containing sodium hypochlorite at 1.5% for a minute, washed in distilled water, and left to dry naturally. Subsequently, they were inoculated with a suspension of spores of the fungus *F. pallidroseum*, grown under similar process to that described for the in vitro spore germination inhibition experiment using the essential oils. The spore suspension concentration was adjusted to 1.0×10^6 spores mL^{-1} .

Two different inoculations were carried out: discs of filter papers soaked in the spore suspension were placed at four equidistant points on the surface of fruits without lesions; a pair of scissors was dipped in the spore suspension and immediately used to cut the fruit peduncles. Inoculated fruits were stored at room temperature (29 ± 2 °C) and relative air humidity of $65 \pm 2\%$ for approximately 24 hours. Subsequently, the fruits were immersed in solutions containing Tween 20 (1:1), distilled water, and different concentrations (0, 500, 1500, and $3000 \mu\text{L L}^{-1}$) of essential oils of *L. sidoides*, *O. gratissimum*, *C. citratus*, and *O. micranthum*. Then, the fruits were transferred to an incubation

room at daily temperature of 30 ± 2 °C and relative humidity of $65 \pm 5\%$ for six days. Disease incidence was evaluated using a scale of grades varying from 0 (absence) to 1 (presence of fungal mycelium); disease severity was determined using a scale of grades varying from 0 to 5, where 0 = absence of lesion; 1 = sum of lesions of up to 10 mm; 2 = sum of lesions from 11 to 20 mm; 3 = sum of lesions from 21 to 40 mm; 4 = sum of injuries from 41 to 60 mm; and 5 = sum of injuries larger than 60 mm (TERAO et al., 2009). The experiment was conducted in a completely randomized design, in a 4×4 factorial arrangement (four essential oils and four concentrations), with three replications for each treatment and one fruit per plot. The data obtained for disease incidence were subjected to analysis of variance and the means were compared through orthogonal contrasts. The data obtained for disease severity was subjected to analysis of variance and the means were compared by the Tukey's test at 5% probability level.

RESULTS AND DISCUSSION

The analysis of essential oil (EO) composition showed varying amounts of chemical compounds in the different EO evaluated (Table 1). A total of 152 compounds were identified. The highest numbers of compounds identified were found in EO of *Ocimum selloi* (28) and *Ocimum micranthum* (25). However, nine compounds were identified in EO essential oil extracted from leaves of *Cymbopogon citratus* and only six compounds were identified in EO from peels of *Citrus sinensis*.

Table 1. Number of chemical compounds, and predominant compounds and their respective concentrations in the essential oils evaluated.

Essential oil	Number of compounds	Predominant compounds	RT	%	KI _c
<i>Lippia sidoides</i>	22	thymol	13.1	63.8	12.9
		β -caryophyllene	17.4	10.7	14.19
<i>Ocimum gratissimum</i>	22	eugenol	15.10	57.7	1355
		1,8 cineole	5.8	17.8	1040
<i>Cymbopogon citratus</i>	9	geraniol	12.4	46.4	1271
		neral	11.5	34.0	1241
		β -myrcene	4.7	8.91	992
<i>Ocimum selloi</i>	28	linalool	7.4	46.8	1102
		estragole	10.3	25.7	1200
<i>Citrus sinensis</i>	6	limonene	5.7	96.4	1036
		linalool	7.4	1.5	1101
		β -myrcene	4.8	1.1	993
<i>Ocimum micranthum</i>	25	eugenol	15.0	42.7	1355
		elemicin	21.0	13.2	15.47
<i>Ocimum</i> sp.	22	(ϵ)-methyl cinnamate	16.17	45.9	1387
		1,8 cineole	5.8	29.3	1040
<i>Piper aduncum</i>	18	dillapiole	23.1	89	16.2

RT = Retention time (minute); % = Relative concentration; KI_c: calculated Kovats index.

The predominant compounds identified in EO extracted from leaves of *Lippia sidoides* were thymol (63.8%) and β -caryophyllene (10.7%). Eugenol was the predominant compound identified in EO extracted from leaves of *Ocimum gratissimum* (57.7%) and *O. micranthum* (42.7%). EO of *C. citratus* presented higher citral (geraniol and neral: 80.4%) and β -myrcene (8.9%) concentrations. The predominant compounds identified in EO *O. selloi* were linalool (46.8%) and estragole (25.7%). Limonene (96.4%), linalool (1.5%), and β -myrcene (1.1%) were the predominant compounds in EO of *C. sinensis*. EO from leaves of *Ocimum* sp. presented predominantly (ϵ)-methyl cinnamate (45.9%) and 1,8-cineole (29.3%). Dillapiole (89%) was the main component identified in EO of *Piper aduncum* (Table 1).

The chemical compounds found in the EO evaluated are, mainly, from the class of terpenes; 76% of the predominant compounds (Table 1) are monoterpenes: citral (geraniol and neral), β -myrcene, thymol, p -Cymene, limonene, linalool, eugenol, 1,8-cineole, and estragole; the other compounds are sesquiterpenes (β -caryophyllene) and phenylpropanoids (dillapiole, elemicin, and (ϵ)-methyl cinnamate).

The abundance of monoterpenes in essential oils extracted from medicinal plants is reported in several works (MORAIS et al., 2012; DEHSHEIKH et al., 2020). However, the chemical composition of oils extracted from similar botanical species can vary qualitatively and quantitatively, mainly regarding predominant compounds (MORAIS et al., 2012). Fontenelle et al. (2007) identified thymol (isomer of carvacrol) as the main compound in EO of *L. sidoides*, as found in the present study. However, other studies found carvacrol and 1,8-cineole as the predominant compounds in *L. sidoides* (MORAIS et al., 2012; GUIMARÃES et al., 2014). Similarly, eugenol was found as the predominant compound

in EO of *O. micranthum* (Table 1), as well as in the study of Saliu et al. (2011); however, Oussou (2010) found predominance of thymol in a EO of this same species.

The variability in the chemical composition of essential oils is determined by plant genetic factors; however, the grown environment affects the presence and amounts of secondary metabolites (FUMAGALI et al., 2008). Stimuli from the environment can change the metabolic pathway, causing the biosynthesis of different compounds (GOBBO-NETO; LOPES, 2007).

Regression analysis resulted in equations with a quadratic trend for all treatments with essential oils (Table 2). The regression coefficient (R^2) ranged from 0.71 to 0.99, denoting greater inhibition of fungal mycelial growth as the essential oil concentration was increased (Figure 1). The EO of *L. sidoides*, *O. micranthum*, and *C. citratus*, which presented the highest inclination angles (a) (22.9, 22.1, and 21.9 respectively) and angular coefficients (b) (94.4, 92.8, and 90.3, respectively) in the equations, were those that presented the highest inhibition of *F. pallidorozeum* (Table 2).

The EO of *L. sidoides*, *O. micranthum*, and *C. citratus* were the most efficient in inhibiting mycelial growth of *F. pallidorozeum*, among the EO evaluated. These oils required lower effective concentration to inhibit 50% of mycelial growth (EC_{50}): 283.6 $\mu\text{L L}^{-1}$ for *L. sidoides*, 412.2 $\mu\text{L L}^{-1}$ for *O. micranthum*, and 453.4 $\mu\text{L L}^{-1}$ for *C. citratus*; these EC_{50} were lower than the the minimum EO concentration (500 $\mu\text{L L}^{-1}$) tested in the present study.

The inhibition of mycelial growth was also evaluated through the mycelial growth index (MGI). Similarly, the lowest MGI were found for the EO of *L. sidoides*, *O. micranthum*, and *C. citratus* (5.5, 8.5, and 10.9, respectively), as well as for *O. gratissimum* (15.4). The mycelial growth speed was slower when using these essential oils (Table 2).

Table 2. Parameters of the quadratic regression (ax^2+bx+Y_0) as a function of concentration, effective concentration for 50% inhibition of mycelial growth ($EC_{50}, \mu\text{L L}^{-1}$), lower confidence interval (LCI), and mycelial growth index (MGI).

Essential oil	Y_0	a	b	R^2	EC_{50}	LCI	MGI*
<i>Lippia sidoides</i>	78.9	22.9	94.4	0.88	283.6	501.2-66.0	5.5 e
<i>Ocimum gratissimum</i>	89.1	20.2	90.3	0.99	680.3	866.2-494.5	15.4 cd
<i>Cymbopogon citratus</i>	83.0	21.9	92.8	0.95	453.4	657.4-249.4	10.9 de
<i>Ocimum selloi</i>	85.6	-0.3	15.7	0.94	2418.1	2695.1-2141.1	33.1 a
<i>Citrus sinensis</i>	87.7	2.6	10.8	0.71	7506.9	8283.4-6730.3	29.4 ab
<i>Ocimum micranthum</i>	81.9	22.1	93.2	0.94	412.2	619.4-204.9	8.5 e
<i>Ocimum</i> sp.	94.5	13.5	72.4	0.98	1027.6	1148.4-906.8	25.3 b
<i>Piper aduncum</i>	84.5	15.3	63.6	0.94	1382.2	1792.5-971.9	18.4 c
Mean							18.3
CV(%)							15.5

*Means followed by the same letter are not statistically different from each other by the Tukey's test at 1% and 5% probability for MGI. CV = coefficient of variation.

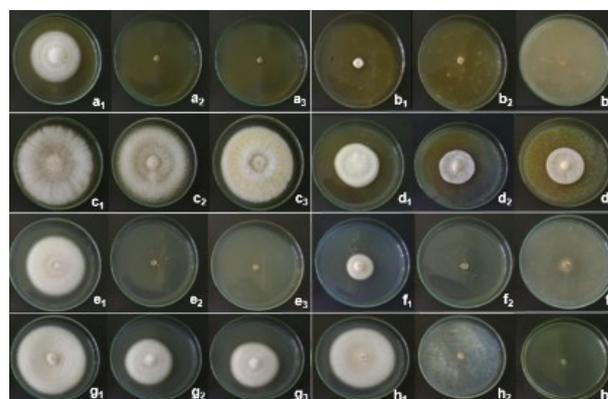
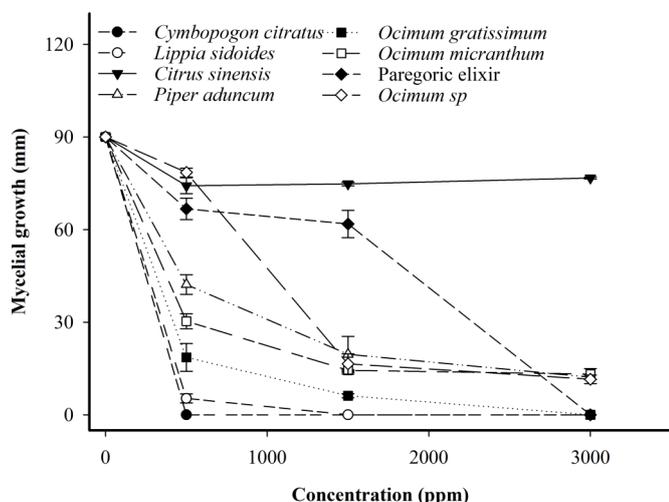


Figure 1. Mycelial growth of *Fusarium pallidoroseum* subjected to application of different concentrations (1: 500 $\mu\text{L L}^{-1}$, 2: 1500 $\mu\text{L L}^{-1}$, and 3: 3000 $\mu\text{L L}^{-1}$) of essential oils of *Cymbopogon citratus* (a), *Lippia sidoides* (b), *Citrus sinensis* (c), *Piper aduncum* (d), *Ocimum gratissimum* (e), *Ocimum micranthum* (f), *Ocimum selloi* (g), and *Ocimum sp.* (h).

The antifungal activity of these oils probably is due to a synergic interaction of all the molecules they contain or only those found at higher levels in GC-MS and GC-FID analyses. Most studies have evaluated only the main compounds in essential oils (COMBRINCK; REGNIER; KAMATOU, 2011; ZORE et al., 2011; MAREI; RASOUL; ABDELGALEIL, 2012), as evaluating the interaction of all compounds present in essential oils and its effect on a pathogen is complex. Regarding the present study, thymol (EO of *L. sidoides* and *C. citratus*) and eugenol (EO of *O. micranthum*) may have been the compounds responsible for the antifungal activity of these EO against *F. pallidoroseum*.

Most compounds identified in the EO evaluated in the present study are monoterpenes, including thymol and eugenol. The action of monoterpenes on microorganisms involves cytoplasmic granulation, membrane rupture, and inactivation or inhibition of the synthesis of extracellular and intracellular enzymes, such as pectin methylesterase (PME), cellulases, and polyphenol oxidases (PFO) (MAREI; RASOUL; ABDELGALEIL, 2012).

The EO of *C. sinensis* and *O. selloi* presented higher EC_{50} (7506.9 and 2418.1 $\mu\text{L L}^{-1}$, respectively) and MGI (29.4 and 33.1, respectively) (Table 2). These results indicate that higher concentrations are necessary to cause greater inhibition of mycelial growth of *F. pallidoroseum*. Considering the EO from peels of *C. sinensis*, the EC_{50} required to inhibit the fungus growth was approximately three-fold (7506.9 $\mu\text{L L}^{-1}$) the highest EO concentration tested (3000 $\mu\text{L L}^{-1}$). Similarly, Velázquez-Nuñez et al. (2013) evaluated the effect of EO extracted from peels of *C. sinensis* and found limonene as the predominant compound (97%) and that a EO concentration of 16000 $\mu\text{L L}^{-1}$ was needed to inhibit *Aspergillus flavus*.

Regarding spore inhibition evaluation, the EO of *L. sidoides*, *O. micranthum*, *C. citratus*, and *O. gratissimum* presented the best results in decreasing the fungal mycelial growth (lower MGI).

According to the analysis of variance for germination of spores of *F. pallidoroseum*, significant difference (at 1% and 5% probability) between the treatments with EO and the control (without EO) was found only for the EO concentration of 500 $\mu\text{L L}^{-1}$. EO of *L. sidoides*, *C. citratus*, and *O. gratissimum* inhibited 100% the germination of *F. pallidoroseum* spores, differing from the EO of *O. micranthum* (Figure 2).

Total inhibition of spore germination was found for all the four EO evaluated at concentrations of 1500 and 3000 $\mu\text{L L}^{-1}$ (data not shown).

These results denote that spores of *F. pallidoroseum* are susceptible to these essential oils than mycelia. Silva and Bastos (2007) evaluated the activity of essential oils of ten *Piper* species (*P. dilatatum*, *P. hostmannianum*, *P. calosum*, *P. cyrtopodon*, *P. turbeculatum*, *P. divaricatum*, *P. nigrispicum*, *P. hispidum*, *P. marginatum*. var. *anisum*, and *P. enkea*) on the mycelial growth and spore germination of *Moniliophthora perniciosa*, which causes witches' broom disease in cocoa trees. They also found higher sensitivity for spores than for mycelia. This result is highly important, as spores are the main infectious structures of *F. pallidoroseum*.

The essential oils selected for the spore germination in vitro experiment were used for the in vivo experiment to evaluate the control of postharvest rot caused by *F. pallidoroseum* in fruits of Galia melon. However, determining the incidence and severity of the disease in the fruits was not possible, as the essential oils, at all concentrations tested, caused phytotoxicity, resulting in injuries (burns and depressions) in the epidermis of the fruits within the first twelve hours of incubation (Figure 3). Phytotoxicity caused by application of essential oils on fruits was also reported for guavas treated with EO of *Eugenia caryophyllus* and *C. citratus* (ROZWALKA et al., 2008) and for papaya fruits treated with EO of *Schinus terebinthifolius* (GRIPPA et al., 2010).

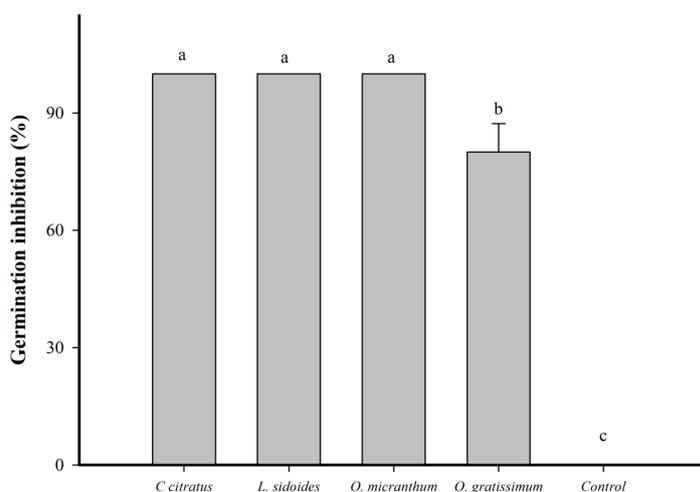


Figure 2. Inhibition of germination of spores (%) of *Fusarium pallidoroseum* subjected to application of essential oils ($500\mu\text{L L}^{-1}$) of *Lippia sidoides*, *Ocimum gratissimum*, *Cymbopogon citratus*, and *Ocimum micranthum*.

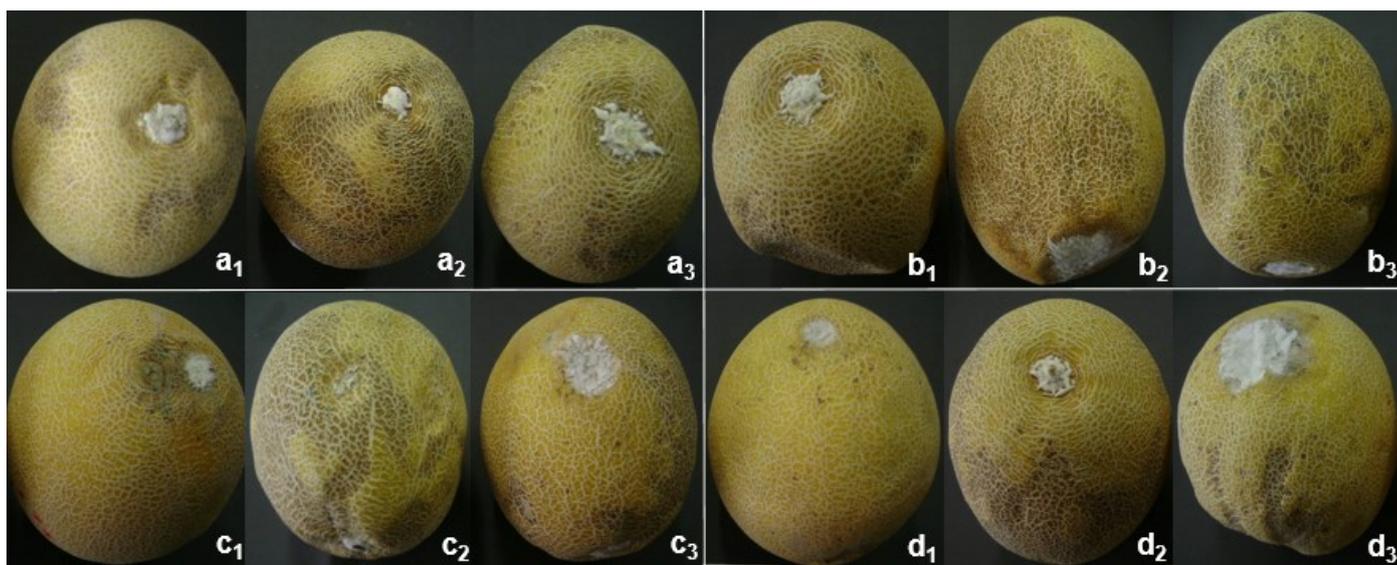


Figure 3. Galia melon fruits subjected to application of different concentrations (1: $500\mu\text{L L}^{-1}$, 2: $1500\mu\text{L L}^{-1}$ and 3: $3000\mu\text{L L}^{-1}$) of essential oils of *Lippia sidoides* (a), *Ocimum gratissimum* (b), *Cymbopogon citratus* (c), and *Ocimum micranthum* (d).

This result denotes the need for further studies using lower concentrations of these essential oils, or even applying the same or higher concentrations only to the region of the peduncles of melon fruits. Lesions caused by *F. pallidoroseum* are more frequent in the peduncular abscission area, whereas other forms of infection occur through wounds in the fruits. Melon growers have used the method of applying synthetic products through the fruit peduncle to prevent diseases.

Considering the potential to inhibit mycelial growth of *Fusarium pallidoroseum* and, mainly, the germination of the main infectious structures (spores) of this fungus, the use of essential oils of *Lippia sidoides*, *Ocimum micranthum*, and *Cymbopogon citratus* are promising for the development of

commercial fungicides, mainly for the control of rot disease caused by *F. pallidoroseum*. Furthermore, the main international buyers of melon from Brazil are increasingly less tolerant of the use of synthetic fungicides and presence of chemical residues in melon fruits.

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