

# **Essential oils for the postharvest control of blue mold and quality of 'Fuji' apples**

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**Abstract** – The objective of this work was to evaluate the effect of rosemary, cinnamon, citronella grass, and clove essential oils on the in vitro growth of *Penicillium expansum*, on the diameter of blue mold lesions, and on the physical and chemical attributes of 'Fuji' apples stored under refrigeration for different periods of time. The compositions of the essential oils were determined, and their effects on the growth inhibition, number, and viability of fungal spores in vitro were evaluated at 0, 100, and 1,000  $\mu\text{L L}^{-1}$  oil concentrations. At postharvest, evaluations were performed for fruit treated with 0, 50, 100, and 500  $\mu\text{L L}^{-1}$  essential oil and kept under refrigeration for 30 days, and for fruit treated with 0, 100, and 500  $\mu\text{L L}^{-1}$  oil and kept under refrigeration for two days. The essential oils – eucalyptol (rosemary), eugenol (cinnamon), citronellal (citronella), and eugenol (clove) – reduce the growth, number, and viability of *P. expansum* spores 24 hours after the induction to germination, as well as the diameter of blue mold lesions in apples, except for citronella oil at 500  $\mu\text{L L}^{-1}$  after 30 days of cold storage. There are no changes in the physicochemical attributes of apples, treated with different concentrations of the oils after refrigeration for five months, followed by seven days at room temperature.

Index terms: *Malus domestica*, *Penicillium expansum*, *Rosmarinus officinalis*, fruit rot.

## **Óleos essenciais para o controle pós-colheita do mofo-azul e a qualidade de maçãs 'Fuji'**

**Resumo** – O objetivo deste trabalho foi avaliar o efeito de óleos essenciais de alecrim, canela, citronela e cravo-da-índia sobre o crescimento de *Penicillium expansum* in vitro, o diâmetro de lesões de mofo-azul e os atributos físico-químicos em maçã 'Fuji', armazenada sob refrigeração por diferentes períodos. As composições dos óleos essenciais foram determinadas, e seus efeitos sobre a inibição do crescimento, o número e a viabilidade de esporos do fungo in vitro foram avaliados quanto às concentrações de 0, 100 e 1.000  $\mu\text{L L}^{-1}$ . No período pós-colheita, avaliaram-se os frutos tratados com 0, 50, 100 e 500  $\mu\text{L L}^{-1}$  de óleo essencial e armazenados sob refrigeração por 30 dias e os frutos tratados com 0, 100 e 500  $\mu\text{L L}^{-1}$  de óleo essencial e mantidos sob refrigeração por dois dias. Os óleos essenciais – eucaliptol (alecrim), eugenol (canela), citronelal (citronela) e eugenol (cravo) – reduzem o crescimento, o número e a viabilidade de esporos de *P. expansum* 24 horas após o estímulo à germinação, bem como o diâmetro de lesões de mofo-azul em maçãs, à exceção do óleo de citronela a 500  $\mu\text{L L}^{-1}$ , após 30 dias de armazenamento refrigerado. Não há alteração dos atributos físico-químicos de maçãs tratadas com diferentes concentrações dos óleos, após a refrigeração por cinco meses, seguida de sete dias à temperatura ambiente.

Termos para indexação: *Malus domestica*, *Penicillium expansum*, *Rosmarinus officinalis*, podridão de fruto.

## **Introduction**

Long periods of storage time and exposure on market shelves can cause 'Fuji' apples to have high loss rates, from 12 to 20%, depending on the time of year and region of cultivation in Brazil (Argenta et al.,

2015). *Penicillium expansum* Link, 1809 (blue mold) and *Cryptosporiopsis perennans* (Zeller & Childs) Wollenweber, 1939 (bull's eye rot) are among the most significant causes for rot in 'Fuji' apples. According to Medeiros (2015), losses caused by blue mold during cold storage can reach 13%.



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The use of postharvest fungicides is not always efficient to control the major rots, and their use in pre and postharvest may constitute environmental and toxicological risks (Neri et al., 2006). In addition, some countries, such as Italy, reported the occurrence of *P. expansum* strains resistant to fungicides of the benzimidazole group (Neri et al., 2006).

The need to find alternative solutions for the chemical control of diseases results from the growing concern about the environment, the purchase of pesticide-free fruits, and the demand for healthy products by consumers. In addition, the food production industry is concerned about cases of food contamination caused by bacteria such as *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* spp., and fungi such as *Colletotrichum* spp. and *Penicillium* spp. in countries such as New Zealand and USA (Popa et al., 2007; Miller et al., 2013).

Therefore, the positive effects of essential oils of plant species, or their components, on the control of fungi, bacteria, and viruses, have been reported in the literature. Tao et al. (2014) observed that the essential oil of *Citrus reticulata* inhibited the growth of *Penicillium italicum* and *P. digitatum* in vitro, and altered the morphology of hyphae and the integrity of the fungal plasma membrane. Rosemary oil caused extravasation of cellular fluid and inhibited the growth of *Fusarium verticillioides* (Bomfim et al., 2015). Essential oils of thyme, oregano, and lemon grass incorporated into the culture medium completely inhibited the in vitro growth of *Botrytis cinerea* and *Alternaria arborescens*; the vaporization of thyme and oregano essential oils inhibited the growth of *Rhizopus stolonifer* by 69 and 64%, respectively (Plotto et al., 2003). Sun et al. (2014) observed a reduced growth of *Escherichia coli* and *P. digitatum* both in vivo and in vitro, in the presence of carvacrol, cinnamaldehyde, and trans-cinnamaldehyde, major compounds of some essential oils such as cinnamon, clove, oregano, and thyme. However, the inhibitory effect on growth, morphology alteration of hyphae, and rupture of microorganism membranes can be different depending on the essential oil, the concentration used, and the pathogen under study (Plotto et al., 2003; Droby et al., 2008; Lorenzetti et al., 2011; Sun et al., 2014; Bomfim et al., 2015).

Despite the publication of several reports on the action of essential oils on microorganisms in vitro, little is known about their effects on the development

of postharvest pathogens in fruits and on what changes occur in their physicochemical characteristics.

The objective of this work was to evaluate the effect of rosemary, cinnamon, citronella grass, and clove essential oils on the in vitro growth of *Penicillium expansum*, on the diameter of blue mold lesions, and on the physical and chemical attributes of 'Fuji' apples stored under refrigeration for different periods of time.

## Materials and Methods

Three experiments were carried out – one in vitro and two in vivo, with essential oils of rosemary (*Rosmarinus officinalis* L.), cinnamon (*Cinnamomum zeylaciun* Blume), citronella grass (*Cymbopogon winterianus* Jowitt), and clove (*Syzygium aromaticum* L.).

The aromatic compounds present in the essential oils were quantified at the chemistry laboratory of natural products of Embrapa Agroindústria Tropical, located in the municipality of Fortaleza, CE, Brazil. These compounds were analyzed through gas chromatography/mass spectrometry using the GC-7890B/MSD-5977A quadrupole (Agilent, Santa Clara, CA, USA), with identification by the Kovats index in a HP-5 column and calculation of the relative area of each compound (Ribeiro et al., 2015).

In the first experiment, essential oils at 100 and 1,000  $\mu\text{L L}^{-1}$  were volatilized in Petri dishes with the fungus *P. expansum*. Adhesives containing the essential oils were placed in the internal part of the lid of each dish containing a PDA culture medium. The amount of essential oil needed to reach the concentrations was determined according to the formula:  $C_1 \times V_1 = C_2 \times V_2$ , in which  $C_1$  is the desired concentration of essential oil ( $\mu\text{L L}^{-1}$ );  $V_1$  is the free volume in the Petri dish, obtained by subtracting the volume of culture medium placed in the Petri dish of 0.006 L from the total volume of the Petri dish of 0.012 L;  $C_2$  is the concentration of the oil (100%); and  $V_2$  is the value to be calculated – the volume of oil needed to reach the concentration desired inside the Petri dish. An adhesive containing sterilized distilled water was used in the control treatment. Discs of 0.7 cm diameter containing PDA medium with pure *P. expansum* isolate from ten-day old colonies were placed on each dish. The Petri dishes were placed in growth chambers at 23°C and subjected to a 12-hour photophase. After ten days, the number

and viability of spores were evaluated. On the sixth and tenth day, the colony diameters were measured to obtain growth inhibition, calculated by the formula IC (%) = [(Dc - Dt) / Dc] × 100, in which Dc (cm) is the colony diameter of the control treatment; and Dt (cm) is the diameter of colony with the essential oil treatments.

To determine the number of spores, 5 mL of a Tween 20 solution (0.05%) and sterile distilled water were placed on the *P. expansum* colonies. The number of spores was counted using a Neubauer chamber (hemacytometer) and an optical microscope, and the result was multiplied by 10,000 to obtain the number of spores per microliter of suspension (Fernandez, 1993).

The conidia viability (%) was evaluated after 24 or 48 hours, in Petri dishes with agar-water, using 0.5 mL of the spore suspension. The dishes were sealed and incubated in a growth chamber at 23°C and subjected to a 12-hour photophase. Spores were considered viable when they had germ tubes equal to or greater than their size.

For the experiments 2 and 3, 'Fuji' apples were harvested at the ideal maturation point in a commercial orchard in the municipality of Vacaria, in the state of Rio Grande do Sul, Brazil, and placed in a specific tray to hold 100 apples. Excessively large or small fruit were removed by visually selecting those of uniform size. In order to ensure that all fruit were similar in all treatments, after the homogenization of the samples, they were disinfested in a sodium hypochlorite solution (1%, v v<sup>-1</sup>) for 3 min, and washed with sterile distilled water to remove the solution residues. Then, they were dried for 2 hours at room temperature (23±1°C). The fruit were drilled – 2 mm diameter and 4 mm deep – in the equatorial region, using an electronic texturometer (TAXT-plus, Stable Micro System, Surrey, UK), and 10 µL of a suspension of 10<sup>6</sup> spores mL<sup>-1</sup> of *P. expansum* were placed in the holes.

In the experiment 2, the fruit were treated under refrigeration at 0±0.5°C and 90±2% relative humidity (RH) for 30 days, using the essential oils at 0, 50, 100, and 500 µL L<sup>-1</sup>. The essential oils were applied by volatilization in plastic containers with 4.1 L capacity. Ten fruit, weighing approximately 140 g each, were placed in each container. Then the volume of essential oil necessary to reach the desired concentrations was added, according to the formula previously described for the experiment 1. The containers were sealed with

moldable polyvinyl chloride (PVC) plastic film to ensure that there was no change in the internal atmosphere of the containers. After the treatment with the essential oils, the fruit infected prior to treatment were removed from the refrigerator and the diameter of the blue mold lesions (cm) were evaluated daily, for five days, at room temperature (22±4°C and 70.5% RH).

In the experiment 3, the fruit remained under atmosphere containing 0, 100, or 500 µL L<sup>-1</sup> of the volatilized essential oils, at 0±0.5°C and 90±2% RH, for two days. The infected fruit were stored for a further 43 days without the essential oils, totaling 45 days of cold storage, and then the diameter of the blue mold lesions (cm) were evaluated for six days at room temperature (22±4°C and 70.5% RH).

In the experiments 2 and 3, after the treatment with essential oils, noninfected fruit were stored for five months under refrigeration, followed by seven days storage at room temperature. After this period, pulp firmness, soluble solids (SS), titratable acidity (AT), and background color of the epidermis (L=brightness, C=color, and h°=hue angle hue) were evaluated according to the methodology described by Silveira et al. (2014).

All experiments were conducted in a completely randomized design. In the experiment 1, ten Petri dishes were used per treatment, and each one represented a replicate. In the experiments 2 and 3, four replicates were used, and each ten-fruit container represented one replicate. Data were subjected to the analysis of variance, and the means were compared by the Tukey's test, at 5% probability, using the SAS program (SAS Institute, Inc., Cary, NC, USA). To estimate the rate at which the rot evolved in the experiments 2 and 3, data on the lesion diameter (cm) were subjected to the linear regression analysis, and the angular coefficients of the lines (b) were compared two-by-two by the Student's t-test. The data percentages were transformed by the equation sine arc (y/100)<sup>1/2</sup>.

## Results and Discussion

The analysis of the chemical composition of the essential oils showed a broad range of components, in varying amounts (Table 1). A total of 5 components were found in clove essential oil, and 23 components in citronella grass essential oil. The essential oils have a complex chemical composition, represented mainly by

hydrocarbons (terpenes and terpenoids) and phenolic compounds (Tao et al., 2014). Clove and cinnamon

essential oils showed 87% phenolic compounds (phenylpropanoids) in their composition, and eugenol

**Table 1.** Chemical composition of rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum zeylaczium*), citronella grass (*Cymbopogon winterianus*), and clove (*Syzygium aromaticum*) essential oils.

Compound <sup>(1)</sup>	KI	Relative area (%)				Class
		Rosemary	Cinnamon	Citronella grass	Clove	
$\alpha$ -pinene	939	13.6	0.81	-	-	Monoterpene
Camphene	954	2.94	0.25	-	-	Monoterpene
$\beta$ -pinene	979	7.08	0.21	-	-	Monoterpene
$\beta$ -myrcene	990	0.72	-	-	-	Monoterpene
$\alpha$ -phellandrene	1,002	0.14	0.96	-	-	Monoterpene
$\alpha$ -terpinene	1,017	0.28	-	-	-	Monoterpene
$\rho$ -cimene	1,024	1.27	0.85	-	-	Monoterpene
Limonene	1,029	2.43	-	3.20	-	Monoterpene
Eucalyptol	1,031	48.15	-	-	-	Monoterpenoid
$\gamma$ -terpinene	1,059	0.41	-	-	-	Monoterpene
Terpinolene	1,088	0.17	-	-	-	Monoterpene
Linalol	1,098	0.77	1.59	0.52	-	Monoterpene
Camphor	1,146	15.44	-	-	-	Monoterpenoid
Isopulegol	1,149	-	-	0.87	-	Monoterpene
Citronellal	1,153	-	-	36.18	-	Monoterpenoid
Iso-isopulegol	1,159	-	-	0.53	-	Monoterpene
Borneol	1,169	1.67	-	-	-	Monoterpenoid
Terpinen-4-ol	1,177	0.32	-	-	-	Monoterpenoid
$\alpha$ -Terpineol	1,188	0.83	-	-	-	Monoterpenoid
Citronellol	1,225	-	-	12.82	-	Monoterpene
Geraniol	1,252	-	-	18.85	-	Monoterpenoid
Geranial	1,267	-	-	0.44	-	Monoterpenoid
E-cinnamaldehyde	1,270	-	0.94	-	-	Phenylpropanoid
Safrole	1,287	-	1.09	-	-	Phenylpropanoid
Bornyl acetate	1,288	0.28	-	-	-	Phenylpropanoid
Citronellol acetate	1,352	-	-	3.60	-	Monoterpene
Eugenol	1,359	-	82.68	0.68	85.27	Phenylpropanoid
Geranyl acetate	1,381	-	-	2.87	-	Monoterpene
$\beta$ -elemenone	1,390	-	-	3.00	-	Sesquiterpene
Trans- $\beta$ -caryophyllene	1,419	3.52	3.42	-	11.47	Sesquiterpene
Trans-cinnamyl acetate	1,446	-	1.17	-	-	Phenylpropanoid
$\alpha$ -caryophyllene	1,454	-	-	-	1.31	Sesquiterpene
Allo-aromadendrene	1,460	-	0.53	-	-	Sesquiterpene
Germacrene-D	1,484	-	-	2.13	-	Sesquiterpene
$\alpha$ -muurolene	1,500	-	-	0.92	-	Sesquiterpene
Germacrene-A	1,509	-	-	0.60	-	Sesquiterpene
$\gamma$ -cadinene	1,513	-	-	0.91	-	Sesquiterpene
Eugenol acetate	1,522	-	2.25	-	-	Phenylpropanoid
$\delta$ -cadinene	1,523	-	-	3.52	0.25	Sesquiterpene
Elemol	1,549	-	-	4.24	-	Sesquiterpene
Germacrene-D-4-ol	1,575	-	-	0.70	-	Sesquiterpene
Caryophyllene oxide	1,583	-	-	-	0.28	Sesquiterpene
$\gamma$ -eudesmol	1,632	-	-	0.52	-	Sesquiterpenoid
Muurolol	1,646	-	-	0.93	-	Sesquiterpenoid
$\beta$ -eudesmol	1,650	-	-	0.51	-	Sesquiterpenoid
$\alpha$ -eudesmol	1,653	-	-	1.45	-	Sesquiterpenoid
Benzyl benzoate	1,760	-	3.24	-	-	Phenylpropanoid

<sup>(1)</sup>Compounds listed in increasing order of Kovats index. KI, Kovats index on an HP-5 column.

was the main one (86.6% and 82.7%, respectively). Bakkali et al. (2008) also found phenolic compounds in clove, cinnamon, and nutmeg essential oils as their main composition, and cinnamaldehyde was the major phenolic compound in cinnamon essential oil. However, cinnamon essential oil used in the present work had more than 80% eugenol and less than 1% cinnamaldehyde in its composition. Some studies have reported that the extraction method (Fisher & Phillips, 2008), climatic and agronomic factors – such as fertilization, irrigation, and plant development phase at the time of extraction (Masotti et al., 2003) –, as well as the plant part used for extraction (roots, fruits, leaves, or stems) could affect the composition of essential oils (Varughese et al., 2016).

Terpenoids may be the main components of some essential oils, as observed for citronella grass and rosemary oils (Table 1). In citronella grass oil, approximately 60% of terpenoids and 40% of terpenes were identified, and citronellal, geraniol, and citronellol were the major compounds with relative proportions of 36.2, 18.9, and 12.8, respectively. Castro et al. (2007) identified 15 compounds in citronella grass essential oil, with the same major compounds in relative proportions, similarly to those identified in the present study. In rosemary essential oil, 18 compounds, such as eucalyptol, camphor, and  $\alpha$ -pinene were identified as the main components, with relative concentrations of 48.2, 15.4, and 13.6%, respectively. Bomfim et al. (2015) identified 24 compounds in rosemary essential oil, with the same main components at similar relative concentrations.

It is important to note that the composition of all the essential oils analyzed here showed one to three major compounds and several others, at concentrations equal to or less than 1% (Table 1). The efficiency of some compounds isolated from essential oils of different plant species has been reported for the control of fungi, such as octane (Tao et al., 2014), trans-2-hexanal (Neri et al., 2006), eugenol, cinnamaldehyde, and carvacrol (Sun et al., 2014), which are major compounds in essential oils.

Various components present in essential oils could show synergism in the control of microorganisms (Bakkali et al., 2008). The activity of the major compounds is probably articulated by minority molecules (Hoet et al., 2006). Many of these minor compounds are responsible for the definition of

aroma, density, texture, and color of oils. In addition, there are important characteristics of these minor compounds for the control of microorganisms, such as cell penetration, lipophilic and hydrophilic action, attachment to cell wall and membrane, and distribution of the compounds of fungicidal or fungistatic action in the fungal cell (Bakkali et al., 2008).

All essential oils had a reducing effect on the growth of *P. expansum*, both in vitro (Table 2) and in vivo, except for rosemary oil at 100  $\mu\text{L L}^{-1}$  (Figure 1), but this effect varied depending on the oil, concentration, and exposure time. Despite the nonsignificant result of the rosemary essential oil at 100  $\mu\text{L L}^{-1}$  for the inhibition of *P. expansum* growth, it significantly reduced the number of fungal spores at this concentration (Table 2). However, the viability did not differ from the control after 24 and 48 hours of induction to germination. Cinnamon, citronella grass, and clove oils reduced spore viability from 58 to 63%, after 24 hours of induction to germination; however, after 48 hours of induction, viability did not differ from that of the

**Table 2.** Growth inhibition (GI) at six and ten days after incubation (PDA at 23°C and 12-hour photophase), and number and viability of *Penicillium expansum* spores, 24 and 48 hours after induction to germination, incubated for ten days (PDA, 23°C, and 12-hour photophase), depending on volatilized essential oils at 100 and 1,000  $\mu\text{L L}^{-1}$  concentrations<sup>(1)</sup>.

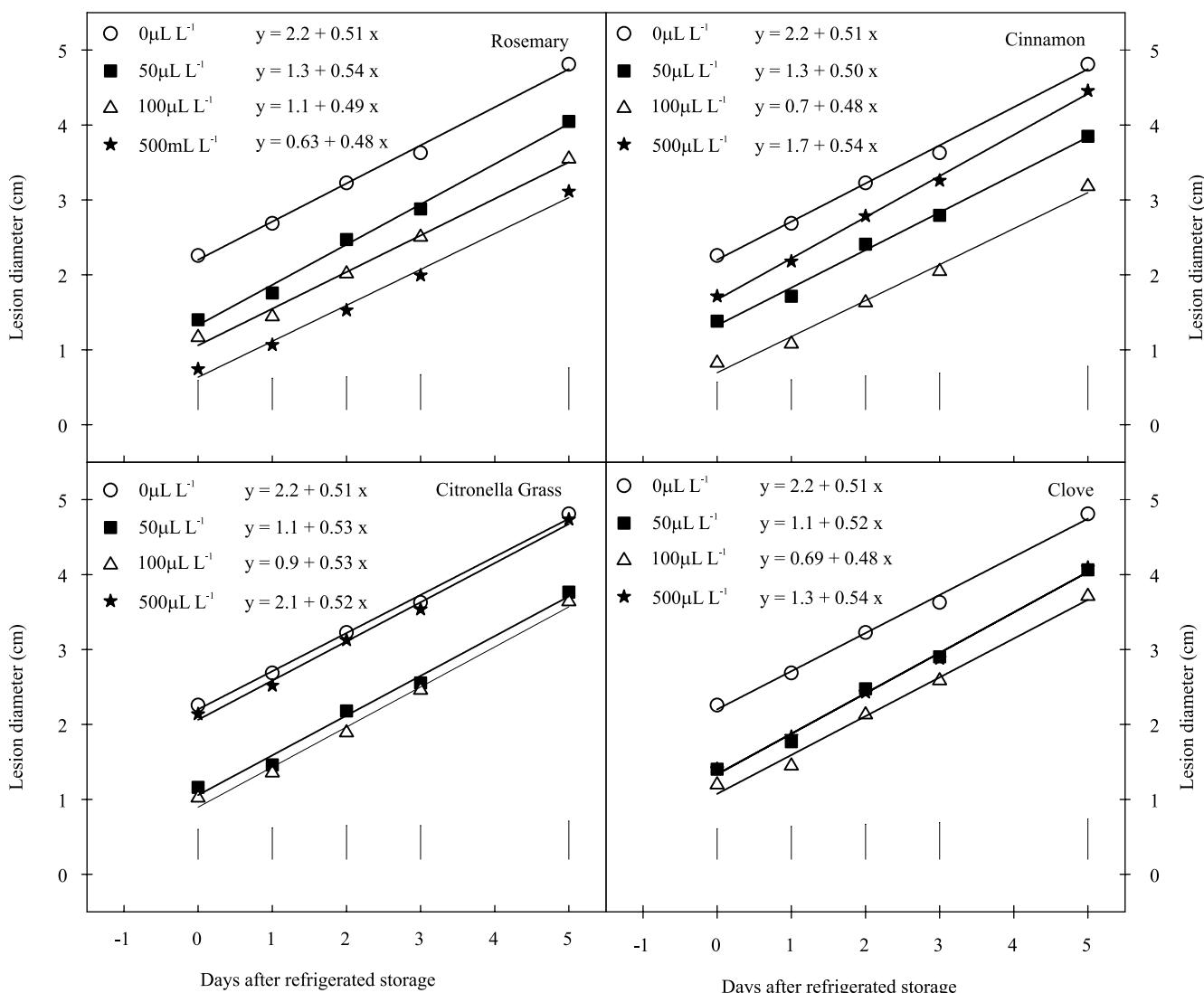
Oil essential	GI (%)		Spores $\text{mL}^{-1} (\times 10^6)$	Spore viability (%)	
	6 days	10 days		24 hours	48 hours
Concentration 100 $\mu\text{L L}^{-1}$					
Control	0b	0b	154.5a	97a	100a
Rosemary	11b	12b	57.9b	87ab	97a
Cinnamon	78a	76a	20.4c	63bc	78a
Citronella grass	57a	59a	47.9bc	62bc	92a
Clove	67a	63a	34.7bc	58c	83a
Mean	43	42	63.1	73	90
SD	34.8	33.7	53.0	17	9
Concentration 1,000 $\mu\text{L L}^{-1}$					
Control	0c	0c	153.2a	93a	100a
Rosemary	80b	67b	1.7b	0b	53ab
Cinnamon	100a	100a	3.1b	3b	67ab
Citronella grass	100a	100a	0.7b	2b	92a
Clove	100a	100a	2.4b	0b	23b
Mean	80	73	32.2	20	67
SD	43.3	43.4	67.7	41	31

<sup>(1)</sup>Means followed by equal letters, in the columns at each concentration, do not differ by Tukey's test, at 5% probability. Control, sterilized distilled water. SD, standard deviation.

control. Bomfim et al. (2015) reported that rosemary essential oil ( $150 \mu\text{g ml}^{-1}$ ) has a fungitoxic effect on the in vitro growth of *Fusarium verticillioides*, and inhibits the fungus mycotoxin production (fumonisins).

The concentration of  $1,000 \mu\text{L L}^{-1}$  of rosemary essential oil inhibited 80 and 67% of in vitro growth of *P. expansum* after 6 and 10 days of incubation, respectively, and differed from the 100% growth inhibition promoted by oils of the cinnamon, citronella grass, and clove (Table 2). The essential oils reduced

the number of fungal spores at both concentrations, in comparison to that of the control treatment. Lorenzetti et al. (2011) observed that cinnamon, lemon grass, clove, and eucalyptus essential oils at  $200 \mu\text{L L}^{-1}$  reduced the in vitro growth of the fungus *Colletotrichum gloeosporioides* by more than 30%; however, at  $1,000 \mu\text{L L}^{-1}$ , the effect of the oils on the fungus improved, and the growth reduction reached 100% for cinnamon, lemon grass, and clove oils, and 71% for eucalyptus oil. In the present study, cinnamon,



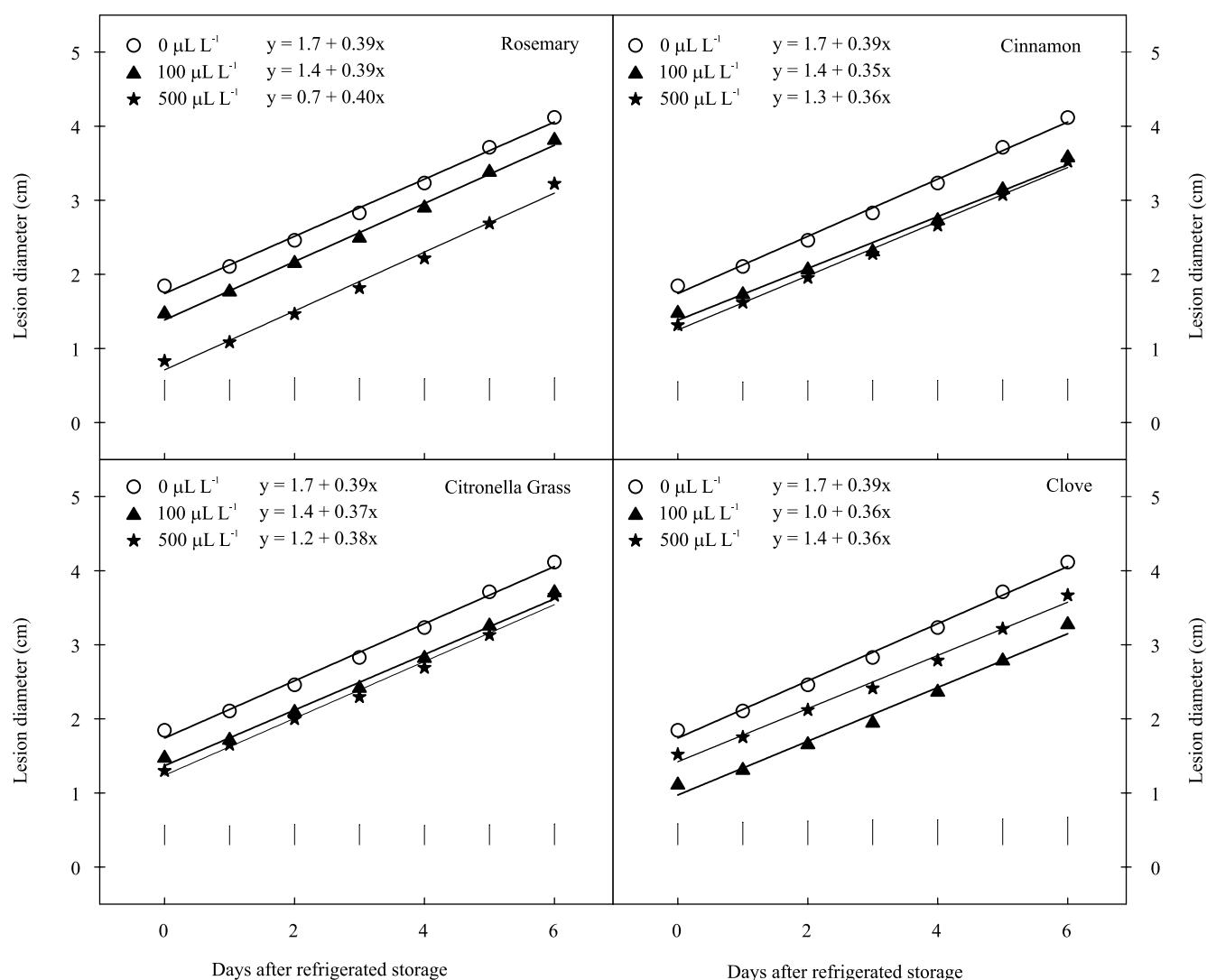
**Figure 1.** Diameter of blue mold (*Penicillium expansum*) lesions in 'Fuji' apples (*Malus domestica*) treated with volatilized rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum zeylalicum*), citronella grass (*Cymbopogon winterianus*), and clove (*Syzygium aromaticum*) essential oils at 0, 50, 100, and  $500 \mu\text{L L}^{-1}$  concentrations, after 30 days of cold storage ( $0 \pm 0.5^\circ\text{C}$  and  $90 \pm 2\%$  RH), followed by five days at room temperature ( $23 \pm 5^\circ\text{C}$  and  $70 \pm 5\%$  RH) without essential oils. Vertical bars indicate the least significant difference between the means, by the Tukey's test, at 5% probability. RH, relative humidity.

clove, and citronella grass oils inhibited in about 80% the growth of *P. expansum* at 100  $\mu\text{L L}^{-1}$  (Figure 1).

'Fuji' apples treated with different essential oils showed a smaller diameter of blue mold lesions than the nontreated fruit; nonetheless, the effect of the oils varied according to concentration used, days of treatment, and evaluation time. The concentration increase of the rosemary essential oil resulted in a

reduced lesion diameter in fruit treated both for 30 and 2 days (Figures 1 and 2).

In the performed evaluation, as soon as the fruit were removed from the chamber (day 0), the reductions of *P. expansum* growth in fruit treated for 30 days with rosemary essential oil at 50, 100, and 500  $\mu\text{L L}^{-1}$  were 38, 48, and 67%, respectively. After five days at room temperature, a greater inhibition of fungus growth was observed at the highest oil concentrations, with



**Figure 2.** Blue mold (*Penicillium expansum*) lesion diameter in 'Fuji' apples (*Malus domestica*) treated with volatilized rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum zeylalicum*), citronella grass (*Cymbopogon winterianus*), and clove (*Syzygium aromaticum*) essential oils at 0, 100, and 500  $\mu\text{L L}^{-1}$  concentrations, after 45 days of cold storage ( $0\pm0.5^\circ\text{C}$  and 95% RH), with two days in the presence of the oils, followed by six days at room temperature ( $23\pm4^\circ\text{C}$  and  $70\pm5\%$  RH). Vertical bars indicate the least significant difference between the means, by the Tukey's test, at 5% probability. RH, relative humidity.

a 26 and 35% reduction of blue mold growth, in the treatments at 100 and 500  $\mu\text{L L}^{-1}$ , respectively.

The treatment with the highest concentration (500  $\mu\text{L L}^{-1}$ ) of cinnamon and citronella grass essential oils, when applied for 30 days, did not reduce the development of blue mold. However, the cinnamon essential oil at 100  $\mu\text{L L}^{-1}$  provided 64% inhibition of fungus growth (Figure 1). The treatment with citronella grass oil at 500  $\mu\text{L L}^{-1}$  did not differ from the control in the evaluation times. The lesion diameters of the fruit treated with citronella grass oil at 50 and 100  $\mu\text{L L}^{-1}$  did not differ from each other; lesions were inhibited around 50% at both concentrations, when the fruit were removed from the chamber, and slightly more than 20% after five days at room temperature. The treatment with clove essential oil reduced the diameter of the blue mold lesions, in comparison to fruit without treatment with this essential oil; however, there was no significant difference between the tested concentrations of this oil (50, 100, and 500  $\mu\text{L L}^{-1}$ ).

In fruit treated with rosemary oil for two days, the inhibition of blue mold development was 20 and 55% for the concentrations of 100 and 500  $\mu\text{L L}^{-1}$ , respectively, by the time the fruit were removed from the chamber. After six days at room temperature, the inhibition of fungus growth reached 7 and 22% at the respective concentrations (Figure 2).

The clove oil at 500  $\mu\text{L L}^{-1}$  was less efficient in reducing the blue mold development than at 100  $\mu\text{L L}^{-1}$ ; however, at both concentrations, the fruit showed lower lesion diameters than that of the control (Figure 2).

The treatment with the cinnamon and citronella grass oils reduced the diameter of blue mold lesions, but no significant difference was observed between the concentrations of 100 and 500  $\mu\text{L L}^{-1}$  (Figure 2).

The growth of the lesions throughout the days, at room temperature, was linear with no observed difference in the daily growth rate of the rot lesions among all oils, concentrations, and treatment times (Figures 1 and 2). The fungus growth rate ranged from 0.36 to 0.54 cm per day, depending on the essential oil and treatment time.

The inhibitory effect of fungus growth was more evident in vitro than in vivo for most essential oils, which was also reported by other authors (Plotto et al., 2003; Sun et al., 2014; Shao et al., 2015). Shao et al. (2015) reported that the incorporation of clove essential oil into the culture medium significantly reduced the in vitro

growth of *P. digitatum*, and they observed extravasation of the cellular fluid and alteration of the hyphae morphology of the fungus. However, these authors were unable to reproduce the same results in citrus fruit artificially infected with the fungus, and immersed in clove essential oil at concentrations of 500, 1,000, or 2,000  $\mu\text{L L}^{-1}$ . Carvacrol, trans-cinnamaldehyde, and cinnamaldehyde are compounds mostly found in clove, cinnamon, oregano, and thyme essential oils. These oils showed antimicrobial activity on *Escherichia coli* and *Penicillium digitatum* in blueberry fruit, but there were more substantial effects on the inhibition of microbial growth in vitro (Sun et al., 2014).

The effects of the essential oils depend on their composition and concentration, the microbial species, and time and form (immersion or volatilization) of treatment (Droby et al., 2008; Wang et al., 2012). The mechanisms of antimicrobial action of essential oils are not fully understood, but some have been proposed. There is evidence that the lipophilic characteristic of the oils allows of the expansion of the plasma membrane of microbial cell, and the increase of membrane fluidity and permeability, inhibiting the cellular respiration, and altering the ion transport process and extravasation of cellular contents (Fadili et al., 2012). Alteration of cell morphology and cell growth block may also occur (Tao et al., 2014).

According to Jiménez-Del Rio & Vélez-Pardo (2004), the essential oils penetrate the cells because of the increased permeability of the membrane, causing damage mainly to the membrane of the mitochondria. Through the change in the flow of electrons in the transport chain, they produce free radicals, which oxidize and damage lipids, proteins, and DNA. Some phenolic or terpenoid compounds, present in essential oils, undergo oxidation by the contact with these free radicals; therefore, these compounds produce highly reactive phenoxy radicals, which can cause even greater damage to microbial cells (Jiménez-Del Rio & Vélez-Pardo, 2004). When antioxidant molecules present in essential oils – such as phenolic and terpenoid compounds – interact with reactive forms of oxygen, they are converted into pro-oxidants. Pro-oxidants have the ability to oxidize lipids, proteins, and DNA, causing a severe damage to various organelles of microorganism cells, compromising the entire antioxidant defense of the microbial cell (Bakkali et al., 2008).

Although the direct action of the oils on the microorganisms is known, they can also act as inducers of resistance. Pereira et al. (2012) observed an increased activity of the enzymes peroxidase and chitinase, after spraying coffee plants with 1,000 µL L<sup>-1</sup> of citronella grass essential oil, which promoted a reduced growth of *Hemileia vastatrix* and *Cercospora cafeeicola* in 47 and 29%, respectively. Shao et al. (2015) observed an increase of the defense enzymes chitinase and phenylalanine ammonia-lyase in citrus fruit after immersion in a solution with clove essential oil at 500 µL L<sup>-1</sup> concentration. These authors observed also a reduced diameter of green mold lesions (*P. digitatum*), and attributed this result to the synergistic effect of the antimicrobial and inducing resistance actions of the essential oil.

Irrespective of the concentrations employed, the evaluated essential oils had no substantial effect on the poststorage quality of 'Fuji' apples (pulp firmness, soluble solids content, titratable acidity, and background color of the epidermis), in fruit treated for both 30 and 2 days (Table 3).

**Table 3.** Color of the epidermis, soluble solids content (SS), titratable acidity (TA) and pulp firmness (PF) of 'Fuji' apple (*Malus domestica*) fruit treated with volatilized essential oils, for 2 or 30 days under refrigeration, and maintained for another five months under refrigeration (0±0.5°C and 90±2% RH), followed by 7 days at room temperature (23±4°C and 70±5% RH)<sup>(1)</sup>.

Oil essential	Color of the epidermis			SS (%)	TA (%)	PF (N)
	L	C	h°			
Essential oil treatment for 30 days						
Rosemary	70.4	42.6	97.3	13.8	0.122	59.4
Cinnamon	71.0	43.0	100.1	13.9	0.129	63.4
Citronella grass	70.5	42.8	98.5	13.8	0.109	59.7
Clove	69.3	42.6	99.4	13.5	0.125	-
Mean	70.4	42.9	99.1	13.7	0.118	60.8
SD	1.40	0.93	2.36	0.63	0.013	2.23
Essential oil treatment for 2 days						
Rosemary	69.6	41.3	96.0	13.8	0.135	63.8
Cinnamon	68.9	42.0	94.7	13.7	0.145	69.0
Citronella grass	70.0	41.7	95.5	13.5	0.146	68.5
Clove	69.9	41.7	96.5	13.3	0.138	64.3
Mean	69.6	41.7	95.7	13.6	0.141	66.4
SD	0.50	0.29	3.18	0.22	0.005	2.73

<sup>(1)</sup>Means in the columns, in each treatment time, do not differ by Tukey's test, at 5% probability. Values referring to the averages obtained with the essential oil concentrations of 0, 100 and 500 µL L<sup>-1</sup>, and 0, 50, 100 and 500 µL L<sup>-1</sup> for 2 and 30 days, respectively. RH, relative humidity; SD, standard deviation.

## Conclusions

1. Rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum zeylaciun*), citronella grass (*Cymbopogon winterianus*), and clove (*Syzygium aromaticum*) essential oils are composed mainly of eucalyptol, eugenol, citronellal, and eugenol, respectively.
2. The essential oils at 100, and 1,000 µL L<sup>-1</sup> reduce the growth, number, and viability of *Penicillium expansum* spores 24 hours after the induction to germination; they also reduce the diameter of blue mold lesions in apple after 30 days of cold storage, regardless of the evaluation time, except for the citronella oil, at 500 µL L<sup>-1</sup>.

3. There is no change in the soluble solids, titratable acidity, pulp firmness, or the background color of the epidermis of 'Fuji' apples subjected to treatments with essential oils after cold storage for five months, followed by seven days at room temperature.

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