https://doi.org/10.17221/49/2017-PPS

In Vitro Evaluation of Eight Plant Essential Oils for Controlling Colletotrichum, Botryosphaeria, Fusarium and Phytophthora Fruit Rots of Avocado, Mango and Papaya

Ali SARKHOSH[1,2], Bruce SCHAFFER[2], Ana I. VARGAS[2], Aaron J. PALMATEER[2], Patricia LOPEZ[2] and Asiye SOLEYMANI[3]*

[1]Katherine Research Station, Department of Primary Industry and Resources, Northern Territory Government, South Stuart Highway, Katherine, Australia;
[2]Tropical Research and Education Center, University of Florida, Homestead, USA;
[3]Department of Chemistry and Biochemistry, Florida International University, Miami, USA
**Corresponding author: sarkhosha@gmail.com*

Abstract

Sarkhosh A., Schaffer B., Vargas A.I., Palmateer A.J., Lopez P., Soleymani A. (2018): *In vitro* evaluation of eight plant essential oils for controlling Colletotrichum, Botryosphaeria, Fusarium and Phytophthora fruit rots of avocado, mango and papaya. Plant Protect. Sci., 54: 153–162.

In vitro efficacy of the essential oils extracted from eight plant species was tested at application rates of 100, 250, 500, 1000, or 2000 µl/l for controlling fruit rots. Results showed a 100% reduction of mycelium growth of *Colletotrichum*, *Fusarium*, *Phytophthora*, *Botryosphaeria* after applying thyme or savory oils at all concentrations tested. Mint and cinnamon oils inhibited mycelium growth of the five isolates at application rates of either 1000 or 2000 µl/l. Tea tree, lavender, myrtle, and eucalyptus oils were slightly effective at controlling mycelium growth of each fungus species tested. Savory oil with major constituent of carvacrol 71.2% and thyme oil with major constituent of thymol 73.3% showed the greatest potential of the essential oils tested for use as natural fungicides.

Keywords: fruit rots; mycelium growth; natural fungicides; *Satureja khuzistanica*

Avocado, mango, and papaya fruits are susceptible to several postharvest diseases, and some of the most common pathogens affecting tropical fruit in Florida include species of *Botryosphaeria*, *Colletotrichum*, *Fusarium*, and *Phytophthora* (Florida Extension Plant Diagnostic Clinic Database). All these pathogens infect immature fruit still attached to the plant, and are capable to damage fruit postharvest in storage at the packing house, in transit, or while sitting on the produce shelf (Ploetz 1994; Ploetz *et al.* 2003). Tropical fruit producers and packers strive to reduce factors leading to postharvest disease losses such as minimising fruit wetness periods and providing proper fruit storage conditions, but these cultural management strategies alone are not often enough for adequate disease control.

Most often commercial grade disinfectant products containing peroxyacetic acid, quaternary ammonium compounds or various fungicides are used to control postharvest fruit diseases. However, there are a limited number of fungicides legally labelled for use on tropical fruit crops for both commercial production and dooryard use in Florida (Sonke *et al.* 2005). Among fungicides that are currently labelled, azoxystrobin (often sold as Abound or Heritage) and copper-based fungicides (copper hydroxide and copper sulphate) are the most widely used for disease

Supported by the Australian Government (Department of Education), by the Department of Primary Industry and Resources of the Northern Territory, and partly by Endeavour Fellowship from the Australian Government.

https://doi.org/10.17221/49/2017-PPS

control. Azoxystrobin is often used repeatedly and not in a rotation with other fungicides allowing for the buildup of resistant pathogen populations. Pathogens are less likely to develop resistance to copper based fungicides, but their long-term use in tropical fruit production has raised concerns about potential adverse impacts on the health of terrestrial and aquatic ecosystems (Wightwick *et al.* 2010). The accumulation of copper can have a negative impact on soil organisms from earthworms to microbes and poses a threat to the long-term soil fertility (Wightwick *et al.* 2008; Komárek *et al.* 2010).

With increasing concerns about environmental pollution, consumer demand for organic produce has never been higher creating a need to find alternatives to synthetic pesticides. Genetic engineering, products containing biological control agents and pesticides derived from natural substances have been a primary focus for researchers to identify alternative options (Finckh *et al.* 2015). The objective of the current study was to assess the efficacy of eight plant essential oils *in vitro* for controlling fruit rot of avocado, mango, and papaya caused by *Colletotrichum gloeosporioides*, *Fusarium solani*, *Phytophthora palmivora*, and *Botryosphaeria* sp.

MATERIAL AND METHODS

Plant materials and essential oil preparation. The shoots (leaves and stems) of eight medicinal plants: mint (*Mentha piperita* Willd.), savory (*Satureja khuzistanica* Jamzad.), thyme (*Thymus daenensis* Celak.), cinnamon (*Cinnamomum zeylanicum* Blume.), lavender (*Lavandula angustifolia* Mill.), eucalyptus (*Eucalyptus globulus* Labill.), myrtle (*Mytrus communis* Linn.), and tea tree (*Melaleuca altenifolia* Cheel.) were used for oil extraction. The samples were dried at room temperature and ground in a grinder. The essential oil in each 2000-g sample from each plant species was extracted by hydrodistillation of air-dried leaves using a Clevenger set and hydro-distillation for three hours (Anonymus 1993). The extracted essential oils were dried over anhydrous sodium sulphate and stored at 4°C in dark vials until use.

GC-mass spectroscopy analysis. The components of extracted oils were separated with a gas chromatograph (ThermoQuest Corp., San Jose, USA) with a flame ionisation detector (FID). A fused silica capillary DB-5 column (30 m × 0.25 mm i.d., film thickness 0.25 μm) was used for analyses. Temperatures were set at 250°C for the injector and 300°C for the detector. Nitrogen was used as the carrier gas at a linear flow rate of 1.1 ml/minute. The initial column temperature was 60°C and then increased at 4°C/min to 250°C, and finally held isothermally for 10 minutes. Samples were then analysed by gas chromatography-mass spectrometry using a Thermoquest-Finnigan gas chromatograph equipped with fused silica capillary DB-1 column (60 m × 0.25 mm i.d., film thickness 0.25 μm) coupled with a TRACE mass spectrometer (Scientific Analysis Instrument – SAI, Manchester, UK). Helium was used as the carrier gas with an ionisation voltage of 70 eV. The ion source and interface temperatures were set at 200 and 250°C, respectively. The mass range was from *m/z* 43 to 456.

The retention indices were calculated using temperature-programmed conditions for *n*-alkanes (C6–C24) and the oil on a DB-5 column under the same chromatographic conditions. Single compounds were identified by comparison of their mass spectra with those of the internal reference mass spectra library (Adams and Wiley 7.0), or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature (Adams 2004). For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors.

Identity of the fruit rot pathogens. All pathogens used in this study were isolates obtained from the University of Florida's Extension Plant Diagnostic Clinic located at the Tropical Research and Education Center in Homestead, Florida. The single spore isolate of *Colletotrichum gloeosporioides* was obtained from samples of diseased mango fruit with anthracnose. Colonies produce abundant conidia that are hyaline, one-celled, straight, cylindrical, and average 14.7 × 5.0 μm with ranges of 12.5–17.5 × 3.8–7.5 μm. Cultural and morphological characteristics of our isolate matched those for *C. gloeosporioides* producing appressoria and characteristic lobed hyphopodia that average 10.5 (7.9–11.5) × 8.9 (7.0–10.1) μm (Bailey & Jeger 1992). A sequence from Internal Transcribed Spacer (ITS) regions 1 and 4 for the mango isolate (GenBank Accession No. KY447324) was nearly identical (99% homology) to *C. gloeosporioides* isolated from *Cymbidium sinensis* in China (Accession No. KC010549).

The single sporangium isolate of *Phytophthora palmivora* was obtained from mature papaya fruit

https://doi.org/10.17221/49/2017-PPS

covered with off-white mycelia of the pathogen with abundant sporangia that were present. The pathogen was identified as *P. palmivora* by the presence of numerous papillate, deciduous, ellipsoidal to ovoid sporangia with short pedicels. The sporangia averaged 51 × 33 µm with ranges of 46–61 × 22–34.5 µm (Erwin & Ribeiro 1996). *Phytophthora* species-specific primers (pal1s and pal2a) targeting part of the 18S rRNA gene, the ITS 1, the 5.8S rRNA gene, and the ITS 2 resulted in a PCR product of 879 bp, testing positive for *P. palmivora* (Tsai *et al.* 2006). The PCR product was cleaned (Qiagen Purification Kit; Qiagen, Hilden, Germany) and sequenced (Accession No. KY447326). The sequence from our isolate was nearly identical (exhibited 99% nucleotide identity) to an isolate of *P. palmivora* (Accession No. GQ398157) collected from bud rot of an African oil palm in Colombia.

The single spore isolate of *Fusarium solani* was obtained from diseased papaya fruit in a commercial storage facility with sunken water-soaked lesions that were up to 15 mm in diameter and covered with mycelia and conidia of the fungus. Colonies of the *Fusarium* isolate on PDA at 25 ± 2°C for 7 days were white to light purple with abundant cottony mycelia. Three- to five-septate macroconidia were 24.5–35.3 × 4.1–5.4 µm, and zero- to one-septate microconidia were 9.1–13.9 × 2.6–5.2 µm. The fungus was identified as *F. solani* based on both morphological and molecular characteristics (Booth 1977). ITS sequence (Accession No. KY447325) for the papaya isolate was nearly identical (99% homology) to *F. solani* isolated from cassava with root rot symptoms (Accession No. KT211516).

An isolate of *Botryosphaeria* was obtained from diseased avocado fruit acquired from a local packing house where the fruit had sunken black lesions covered with greyish-white mycelium of the fungus. Conidia were hyaline, aseptate, and fusoid. According to morphological criteria, the fungus was identified as a *Fusicoccum* sp., which is an anamorph of *Botryosphaeria* (Slippers *et al.* 2004). The ITS region of rDNA was amplified with primers ITS1/ITS4 and sequenced. BLAST analysis of the 390-bp fragment (Accession No. KY457243) showed 99% homology with a sequence of a *Botryosphaeria* sp. isolated from mangrove in China.

In vitro screening of the essential oil effects on mycelium growth. Effects of essential oils extracted from the different plant species and different concentrations of each oil on mycelia growth were tested in Petri dishes (polystyrene size 100 × 15 mm; Sigma-Aldrich, USA) containing 25 ml PDA. The essential oils were disseminated individually as an emulsion in sterilised water containing the surfactant Tween 80 (0.05%) which was added to PDA media immediately before it was poured into the Petri dishes at a temperature of 45–50°C. The concentrations of the essential oils were 0 (control), 100, 250, 500, 1000, and 1500 µl/l. A 5-mm diameter circular disk of each isolate was cut with a sterile cork borer from the margin of the actively growing cultures on the PDA and placed in the centre of each Petri dish containing the essential oil treatment. Petri dishes were then incubated at 25°C, and mycelial growth was measured every 24 h after initially placing the mycelium on the dish. The experiment was terminated when the Petri dishes containing the control treatment were fully covered with mycelium. The efficacy of each treatment was evaluated by measuring mycelial growth (mm) from the centre of the colony to the edge of the colony. The means of two radiuses at 90° angle from each other were combined for each measurement dish.

Statistical analyses. The experiment was arranged as a completely randomised design with a factorial combination of treatments and five replications of each treatment combination. Treatments were essential oil from each plant and application rates of each oil. Data were analysed by two-way analysis of variance (ANOVA) to test for interactions between essential oil and concentration of each oil. Differences between essential oils were tested by one-way ANOVA and Tukey's HSD test. Effects of application rates on mycelial growth were determined by linear and quadratic analysis of variance. All statistical analyses were done with SAS v9.3 statistical software (SAS Institute, Cary, USA). For each fungus species tested, there were significant statistical interactions ($P \leq 0.05$) between fungus species, essential oil, and application rate. Therefore, the effects of essential oils and application rates on mycelial growth were evaluated separately for each pathogen. The effects of the different essential oils on mycelial growth of each fungus were compared within each application rate and the effect of application rates on mycelial growth were compared separately for each oil.

RESULTS

Chemical composition of essential oils. The major chemical composition of the essential oil extracted

https://doi.org/10.17221/49/2017-PPS

Table 1. Chemical composition (relative area percentage) of essential oils extracted from five different plant species

Compound	RI*	*Mentha piperita*	*Satureja khuzistanica*	*Thymus daenensis*	*Cinnamomum zeylanicum*	*Lavandula angustifolia*	*Eucalyptus globulus*	*Mytrus communis*	*Melaleuca alteni-folia*
Isobutyl isobutyrate	910	–	–	–	–	–	–	1.5	0.9
α-Thujene	931	–	2.0	2.1	–	–	0.5	0.3	0.96
α-Pinene	940	–	1.5	0.5	1.6	0.5	12.1	23.1	4.45
Camphene	955	–	0.1	0.7	0.9	0.5	1.2	–	–
Benzaldehyde	961	–	–	–	1.3	–	–	–	–
β-Pinene	984	–	1.8	0.4	1.0	0.6	4.1	0.4	0.7
Myrcene	991	–	1.9	1.5	–	0.3	1.5	1.0	0.55
α-Phellandrene	1008	0.1	0.5	0.1	–	–	1.5	0.5	0.45
α-Terpinene	1018	–	–	1.7	0.2	–	–	–	5.6
p-Cymene	1024	–	7.6	5.8	0.3	0.2	–	0.5	11.5
Limonene	1030	–	–	0.5	1.1	1.8	–	17.6	1.6
β-Phellandrene	1032	–	–	0.1	–	–	–	–	0.3
1.8-Cineole	1035	11.4	1.1	–	6.7	4.3	39.1	24.0	5.2
γ-Terpinene	1059	–	8.1	4.6	0.2	0.5	0.8	–	13.5
cis-Sabinene hydrate	1070	–	0.2	0.5	–	–	–	–	–
Linalool	1098	0.9	0.2	–	1.2	26.3	0.8	11.4	0.6
Fenchol	1112	–	–	–	–	–	1.3	–	0.8
trans-Pinocarveol	1131	–	–	–	–	–	3.2	–	1.6
Menthone	1152	33.8	–	–	–	–	–	–	–
Borneol	1155	1.1	–	–	–	2.3	0.6		0.5
Pinocarvone	1156	–	–	–	–	–	1.7	–	1.2
Isomenthone	1158	4.2	–	–	–	–	–	–	1.5
Menthofuran	1162	3.6	–	–	–	–	–	–	–
4-Terpinol	1171	–	0.4	–	3.1	3.5	3.5	0.2	32.8
Menthol	1180	36.1	–	–	–	–	–	–	–
Myrtenol	1191	–	–	–	–	–	1.1	–	2.3
α-Terpineol	1193	–	–	–	3.5	2.5	2.6	4.4	4.8
p-Arill arisol	1202	–	–	–	–	–	–	1.3	0.3
Pulegone	1226	0.1	–	–	–	–	–	–	–
Linalyl acetate	1257	–	–	–	–	43.1	–	4.8	–
Lavandulyl acetate	1272	0.2	–	–	–	5.7	–	–	–
Thymol	1276	–	0.3	73.3	–	–	–	–	–
Menthyl acetate	1282	1.9	–	–	–	–	–	–	–
trans-Cinnamaldehyde	1284	–	–	–	2.6	–	–	–	–
cis-Cinnamaldehyde	1295	–	–	–	59.1	–	–	–	–
Carvacrol	1302	–	71.2	6.6	–	0.4	1.4	–	–
α-Terpinenyl acetate	1345	–	–	–	–	–	1.7	2.2	1.2
Carvacryl acetate	1355	–	1.4	–	–	–	–	–	–
Eugenol	1367	–	–	–	8.5	–	–	2.2	0.6
Greanyl acetate	1381	–	–	–	–	–	–	1.2	0.5
β-Caryophyllene	1414	0.1	0.7	0.2	–	2.6	–	–	–
trans-Caryophyllene	1417	2.5	–	–	0.6	–	–	0.8	–

https://doi.org/10.17221/49/2017-PPS

Table 1 to be continued

Compound	RI*	*Mentha piperita*	*Satureja khuzistanica*	*Thymus daenensis*	*Cinnamomum zeylanicum*	*Lavandula angustifolia*	*Eucalyptus globulus*	*Mytrus communis*	*Melaleuca alternifolia*
Cinnamyl acetate	1439	–	–	–	6.4	–	–	–	–
Germacrene D	1447	2.7	–	–	–	1.6	–	–	–
Aromadendrene	1455	–	–	–	–	–	10.3	–	1.6
γ-Muurolene	1474	–	–	–	–	–	3.1	–	–
α-Humulene	1476	–	–	–	–	–	–	1.2	0.15
γ-Elemene	1497	–	–	–	–	–	4.2	–	–
Caryophyllene oxide	1558	–	0.3	0.2	0.3	0.6	–	–	0.5
α-Cadinol	1583	–	–	–	0.4	0.3	–	–	–
Spathulenol	1578	–	–	–	–	–	1.4	–	–
Globulol	1581	–	–	–	–	–	1.0	–	0.7
Total	–	98.7	99.3	98.9	99.0	97.6	98.7	98.6	97.4

*RI – retention indices relative to C_6–C_{24} *n*-alkanes on the DB-1 column

from each plant species and the relative percentage of each compound as determined by GC-Mass spectroscopy analysis are shown in Table 1. The major compounds in mint oil were menthol (36.1%), menthone (33.8%), and 1,8-cineole (11.4%). Of the chemical compounds found in savory oil, carvacrol (71.2%) was by far the major constituent followed by γ-terpinene (8.1%) and *p*-cymene (7.6%). The essential oil extracted from thyme was primarily composed of thymol (73.3%) followed by much lesser quantities of carvacrol (6.6%), *p*-cymene (5.8%) and γ-terpinene (4.6%). The primary chemical component of cinnamon oil was cis-cinnamaldehyde (59.1%) followed by lesser concentrations of eugenol (8.5%), *cis*-cinnamaldehyde (59.1%), eugenol (8.5%), 1,8-cineole (6.7%), and cinnamyl acetate (6.4%). Linalyl acetate (43.1%), linalool (26.3%), lavandulyl acetate (5.7%), and 1,8-cineole (4.3%) were determined to be the main components of lavender oil. The major constituents of eucalyptus oil were 1,8-cineole (39.1%), α-pinene (12.1%), and aromadendrene (10.3%). Myrtle oil was primarily composed of α-pinene (23.1%), limonene (17.6%) and linalool (11.4%). The major constituents of tea tree oil were 4-terpinol (32.8%), γ-terpinene (13.5%), and *p*-cymene (11.5%).

Suppression of mycelial growth. The mycelial growth rate was different for each pathogen as can be seen by the final growth rate of each of the control treatments (Tables 2–5). Mycelial growth of *C. gloeosporioides* was completely inhibited by application

Table 2. Effect of essential oils on mycelial growth (mm/day) of *Colletotrichum gloeosporioides in vitro*

Essential oils	Application rate (µl/l)						Sig.	R^2
	0	100	250	500	1000	1500		
Savory	6.76^a	0.00^a	0.00^a	0.00^a	0.00^a	0.00^a	–	–
Thyme	6.76^a	0.00^a	0.00^a	0.00^a	0.00^a	0.00^a	–	–
Cinnamon	6.76^a	5.98^c	2.17^b	0.88^b	0.00^a	0.00^a	Q*	0.93
Mint	6.76^a	6.11^c	5.24^c	3.80^c	1.61^b	0.00^a	L*	0.98
Tea tree	6.76^a	5.15^b	4.80^c	3.97^c	2.87^c	1.04^b	L*	0.93
Lavender	6.76^a	5.88^c	5.71^e	5.10^e	4.08^e	2.13^c	L*	0.97
Myrtle	6.76^a	5.81^c	5.71^e	4.23^d	3.53^d	2.96^d	Q*	0.97
Eucalyptus	6.76^a	5.80^c	5.70^e	5.43^f	5.06^f	4.92^e	Q*	0.84

Different letters within columns indicate significant differences between essential oils within each application rate according to Tukey's HSD test ($P \leq 0.05$); asterisk indicates that the linear (L) or quadratic (Q) regression model was significant at $P \leq 0.01$ for the application rate of each essential oil

https://doi.org/10.17221/49/2017-PPS

Table 3. Effect of essential oils on mycelial growth (mm/day) caused of *Botryosphaeria* sp. *in vitro*

Essential oils	Application rate (µl/l)						Sig.	R^2
	0	100	250	500	1000	1500		
Thyme	10.8[a]	1.01[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	–	–
Savory	10.8[a]	1.11[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	–	–
Mint	10.8[a]	8.74[b]	7.10[b]	4.19[b]	0.94[b]	0.00[a]	L*	0.90
Cinnamon	10.8[a]	9.80[c]	9.62[c]	7.76[c]	4.37[c]	0.71[b]	L*	0.98
Tea tree	10.8[a]	9.70[bc]	9.41[c]	8.96[d]	5.75[d]	1.46[c]	L*	0.96
Lavender	10.8[a]	9.63[bc]	9.25[c]	8.46[d]	6.78[e]	1.39[c]	L*	0.92
Myrtle	10.8[a]	9.61[bc]	9.54[c]	8.96[d]	7.46[f]	4.89[d]	L*	0.95
Eucalyptus	10.8[a]	9.75[c]	9.53[c]	9.05[d]	8.40[g]	6.93[e]	L*	0.90

Different letters within columns indicate significant differences between essential oils within each application rate according to Tukey's HSD test ($P \leq 0.05$); asterisk indicates that the linear (L) regression model was significant at $P \leq 0.01$ for the application rate of each essential oil

of either savory or thyme oil at 100 µl/l, the lowest application rate tested (Table 2). Cinnamon oil was the next most effective essential oil, completely inhibiting mycelial growth of this fungus at an application rate of 1000 µl/l (Table 2). However, there was a significant quadratic relationship between the application rate of cinnamon oil and mycelial growth (Table 2). Thus, at even the lowest concentration tested, there was some inhibitory effect of cinnamon oil on mycelial growth of *C. gloeosporioides*. Mint completely inhibited mycelial growth of this fungus at a concentration of 1500 µl/l (Table 2). The application rate of mint oil was linearly related to mycelial growth (Table 2). Malaleuca, lavender, myrtle or eucalyptus did not inhibit mycelial growth of *C. gloeosporiodes* at any of the application rates tested (Table 2). However, each of these compounds did suppress mycelial growth to some extent with the relationship between application rate and mycelial growth linear for malaleuca and lavender oils and quadratic for myrtle and eucalyptus oils.

At the lowest application rate tested (100 µl/l), none of the essential oils completely inhibited mycelial growth of the *Botryosphaeria* sp. (Table 3). However, at 250 µl/l, thyme and savory oil completely inhibited mycelial growth of this fungus. At an application rate of 1500 µl/l, mint oil completely inhibited mycelial growth of the *Botryosphaeria* sp. and there was a strong positive linear relationship (R^2 = 0.90) between mint oil application rate and mycelial growth (Table 3). None of the other oils completely inhibited mycelial growth of the *Botryosphaeria* sp. at any of the concentrations tested. However, cinnamon, lavender, malaleuca, myrtle, and eucalyptus oil each had some inhibitory effect on mycelial growth of this fungus and there was a strong positive linear

Table 4. Effect of essential oils on *in vitro* mycelial growth (mm/day) of *Phytophthora palmivora*

Essential oils	Application rate (µl/l)						Sig.	R^2
	0	100	250	500	1000	1500		
Savory	4.40[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00 [a]	–	–
Thyme	4.40[a]	0.59[b]	0.00[a]	0.00[a]	0.00[a]	0.00 [a]	–	–
Cinnamon	4.42[a]	1.51[c]	0.76[b]	0.38[b]	0.00[a]	0.00 [a]	Q*	0.75
Mint	4.40[a]	3.43[d]	2.96[d]	1.56[d]	0.31[ab]	0.00 [a]	Q*	0.99
Lavender	4.43[a]	3.72[e]	2.29[c]	1.24[c]	0.65[abc]	0.00 [a]	Q*	0.95
Myrtle	4.40[a]	3.85[ef]	2.88[c]	1.99[d]	1.86[bc]	0.27[b]	L*	0.64
Tea tree	4.40[a]	4.04[fg]	3.32[e]	2.16[e]	0.62[abc]	0.00 [a]	L*	0.95
Eucalyptus	4.40[a]	4.10[g]	3.80[f]	3.24[f]	2.20[c]	0.75[c]	L*	0.97

Different letters within columns indicate significant differences between essential oils within each application rate according to Tukey's HSD Test ($P \leq 0.05$); asterisk indicates that the linear (L) or quadratic (Q) regression model was significant at $P \leq 0.01$ for the application rate of each essential oil

https://doi.org/10.17221/49/2017-PPS

Table 5. Effect of essential oils on *in vitro* mycelium growth (mm/day) caused by of *Fusarium solani*

Essential oils	Application rate (µl/l)						Sig.	R^2
	0	100	250	500	1000	1500		
Savory	6.11^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	–	–
Thyme	6.11^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	–	–
Mint	6.11^{a}	3.71^{b}	2.86^{b}	1.82^{b}	0.31^{b}	0.00^{a}	Q*	0.94
Cinnamon	6.11^{a}	4.57^{cd}	4.35^{ef}	3.53^{d}	0.00^{a}	0.00^{a}	L*	0.91
Lavender	6.11^{a}	4.41^{c}	3.72^{c}	3.42^{d}	1.39^{d}	0.00^{a}	L*	0.94
Tea tree	6.11^{a}	4.61^{d}	4.45^{f}	2.85^{c}	0.61^{c}	0.00^{a}	L*	0.93
Myrtle	6.11^{a}	4.58^{cd}	4.09^{d}	3.05^{c}	2.52^{f}	1.39^{b}	Q*	0.93
Eucalyptus	6.11^{a}	4.49^{cd}	4.21^{de}	3.89^{e}	2.41^{e}	1.34^{b}	L*	0.91

Different letters within columns indicate significant differences between essential oils within each application rate according to Tukey's HSD Test ($P \leq 0.05$); asterisk indicates that the linear (L) or quadratic (Q) regression model was significant at $P \leq 0.01$ for the application rate of each essential oil

relationship (R^2 = 0.92–0.98) between application rate and mycelial growth for essential oil from each of the plant species tested (Table 3).

For *Phytophthora palmivora*, the essential oil extracted from savory was the most effective at inhibiting mycelial growth, with a complete inhibition of growth at an application rate of 100 µl/l (Table 4). The next most effective oil tested was that extracted from thyme, which completely inhibited mycelial growth at an application rate of 200 µl/l (Table 4). Cinnamon oil completely inhibited mycelial growth of *P. palmivora* at 1000 µl/l and mint, lavender and malaleuca oils completely inhibited growth at 1500 µl/l (Table 4). There was a positive quadratic relationship between the application rate of essential oil extracted from each of these three species and mycelial growth of *P. palmivora*, with very high coefficients of determination for mint (R^2 = 0.99) and lavender (R^2 = 0.95) (Table 4). There was a strong linear relationship (R^2 = 0.95) between the application rate of malaleuca oil and mycelial growth of this pathogen (Table 4). Myrtle oil or eucalyptus oil did not completely inhibit mycelial growth at any of the application rates tested. However, for each of these oils, there was a significant linear relationship between application rate and mycelial growth (Table 4).

At the lowest application rate tested (100 µl/l), savory and thyme oil completely inhibited growth of *Fusarium solani* (Table 5). Cinnamon oil completely inhibited mycelial growth of this fungus at 1000 µl/l and there was a strong positive linear correlation (R^2 = 0.91) between the application rate of cinnamon oil and mycelial growth of *F. solani* (Table 5). Mint, lavender and tea tree oils completely inhibited mycelial growth at 1500 µl/l. For mint, there was as strong positive quadratic relationship (R^2 = 0.94) between essential oil concentration and mycelial growth, whereas for lavender and tea tree the relationship was linear (Table 5). Myrtle or eucalyptus oils at any concentration tested did not totally inhibit mycelial growth of *F. solani* (Table 5). However, there was a strong positive quadratic relationship (R^2 = 0.91) for myrtle oil and a strong positive linear relationship (R^2 = 0.93) for eucalyptus oil between application rate and *Fusarium* sp. mycelial growth (Table 5).

DISCUSSION

Each of the essential oils tested had some effect on reducing the mycelial growth of each pathogen tested compared to the control treatment with no oil added, although the effective concentration differed among oils. Tables 2–5 show a comparison of the different essential oils, each at the most effective concentration, on mycelial growth of the four pathogens. Savory, thyme, cinnamon, mint, lavender, and tea tree oils all inhibited mycelial growth of *F. solani* and *P. palmivora*, but savory oil was effective at the lowest concentration (100 µl/l) for inhibiting the growth of both species, whereas thyme oil was effective at this low concentration only for *F. solani* (Tables 2–5). Savory and thyme oils were also effective in inhibiting mycelial growth of *Botryosphaeria* sp. and *C. gloeosporioides* at relatively low concentrations (Tables 2–5). In the present study, carvacrol was the primary chemical constituent of savory oil (71.2%). Farsam *et al.* (2004) also found carvacrol to be the

https://doi.org/10.17221/49/2017-PPS

main chemical component of savory oil but at a significantly higher concentration (93.9%) than found in the present study. Hong *et al.* (2015) observed that carvacrol vapours inhibited *in vitro* conidial germination and mycelium growth of *C. gloeosporioides*. In the same study, carvacrol vapours reduced the lesion diameter on green pepper fruit inoculated with *C. gloeosporioides*. Zambonelli *et al.* (1996) observed that essential oil from a species of thyme, *Thymus vulgaris*, different from the one tested in the present study, was very effective in controlling mycelial growth of four different phytopathogenic fungi including *F. solani*. In that study, the effectiveness of thyme oil was attributed to thymol, which comprised 50.1% of the chemical components of that thyme species. In the same study, scanning electron microscope sections revealed that application of the oil resulted in degeneration of the fungal hyphae. The species of thyme, *Thymus daenensi*, tested in the present study, contained a significantly greater proportion of thymol (73.3%; Table 1) than Zambonelli *et al.* (1996) observed in *T. vulvaris*, which presumably was the primary chemical constituent responsible for inhibition of mycelial growth. Sellamuthu *et al.* (2013) found that vapours of essential oil extracted from *T. vulgaris* inhibited *in-vitro* mycelial growth of *C. gloeosporioides* and stopped the development of anthracnose caused by this fungus in avocado fruit. These investigators observed that in avocado fruit, thyme oil increased the concentration of fungal defence enzymes such as β-1,3-glucanase, chitinase, and peroxidase as well as antioxidant enzymes such as superoxide dismutase and catalase. A subsequent study found that fumigation with thyme oil induced the expression of β-1,3-glucanase and chitinase genes (Bill *et al.* 2016) responsible for stimulating production of these substances.

In the present study, mint oil completely inhibited mycelial growth of all pathogens tested but at a much higher concentration of 1500 µl/l compared to 100–250 µl/l for savory or thyme oil. Similarly, Moghaddam *et al.* (2013) found that essential oil from the same mint species, tested on three different fungus species from those that we tested, was most effective for inhibiting *in vitro* mycelial growth at a concentration1600 µl/l. They identified the two major chemical components, menthone and menthol, to collectively comprise 55.8% of the chemical constituents of mint oil. In the present study, mint oil was primarily composed of these two chemical compounds, collectively representing 69.9% of the chemical constituents (Table 1), which was closer to the concentration (70.9%) found by Zambonelli *et al.* (1996). These last authors also found that mint oil was most effective at inhibiting *in vitro* mycelial growth of several fungi, including *F. solani* at a concentration of 1600 µl/l.

Cinnamon oil completely inhibited mycelial growth of *F. solani* at a concentration of 1000 µl/l, and at the highest concentration applied (1500 µl/l) cinnamon oil completely inhibited mycelial growth of *P. palmivora*. We also observed that cinnamon oil inhibited mycelial growth of *Botryosphaeria* sp. and *C. gloeosporioides* substantially, but not completely (Tables 2–5). In a study of broad-spectrum anti=fungal activity and control of late leaf spot and crown rot in peanut, cinnamon oil inhibited *in vitro* spore germination of *Cercospora arachidicola*, *Phaeoisariopsis personata*, and *Puccinia arachidis* and reduced the incidence of crown rot caused by *Aspergillus niger* (Kishore & Pande 2007). Cinnamon oil applied in its volatile form also inhibited *in vitro* conidial germination of *C. gloeosporioides* and reduced the lesion diameter on pepper fruit inoculated with the fungus (Hong *et al.* 2015).

Lavender and malaleuca oils at the highest concentration tested (1500 µl/l) inhibited mycelial growth of *F. solani* and *P. palmivora* completely and inhibited mycelial growth of *Botryosphaeria* sp. and *C. gloeosporioides* substantially but not completely (Tables 2–5). In a previous study, Zambonelli *et al.* (1996) found that lavender oil completely inhibited mycelial growth of *Pythium ultimum*, *Rhizoctonia solani*, and *Colletotrichum lindemuthianum* at concentrations ranging from 400 µl/l to 800 µl/l, but at a concentration of 1600 µl/l it only inhibited mycelial growth of *F. solani* by 58%. Terzi *et al.* (2007) found that essential oil from tea tree and some of the major chemical components of this oil tested individually inhibited *in vitro* mycelial growth of four fungi pathogenic to cereals. In that study, the major chemical components of tea tree oil were 4-terpinol, 1,8-cineole, and terpinen, all of which were more effective at inhibiting mycelial growth than the general oil extract. We also detected 4-terpenol (32.8%) and 1,8-cineole (5.1%) but not terpinin to be major chemical components of tea tree oil. Based on the results of Terzi *et al.* (2007), it is possible that these isolated compounds would have a stronger inhibitory effect on the mycelial growth of the fungal species tested in the present study compared to the general essential oil extract, and thus they should be tested.

https://doi.org/10.17221/49/2017-PPS

Eucalyptus and myrtle oil were the least effective of the essential oils tested in the present study. Both these oils were most effective at inhibiting mycelial growth of all pathogens tested at concentrations of 1500 µl/l, but even at this concentration neither of these oils completely inhibited mycelial growth of any fungal species tested (Tables 2–5). López-Meneses *et al.* (2015) observed that eucalyptus oil concentrations as high as 10 000 µl/l were required to completely inhibit the radial mycelial growth of *Fusarium moniliforme* and never completely inhibited *in vitro* mycelial growth of *Aspergillus parasiticus* after 6 days (the time required to achieve maximum mycelium growth of the control). However, concentrations as low as 100 µl/l delayed growth of *F. moniliforme* for one day. Curini *et al.* (2003) found that at a concentration of 1600 µl/l, myrtle oil inhibited mycelial growth of *Rhizoctonia solani* by 60%, but it inhibited mycelial growth of *Colletotrichum lindemuthianum* by 21.4% only and *F. solani* only by 15.6%. In that study, the two primary chemical components of myrtle oil were limonene (52.9%) and α-pinene (32.9%). In the present study, these two chemical components were also found to be the major chemical constituents of myrtle oil, but the percentages of these compounds, 23.1% for α-pinene and 17.6% for limonene, were lower than those observed by Curini *et al.* (2003). Linalool was the third most abundant chemical constituent of myrtle oil in the study by Curini *et al.* (2003) and in the present study, but there was a higher percentage of this chemical (11.4%) in the present study compared to the percentage (4.2%) observed by those other investigators.

Results obtained in this *in vitro* study indicate that all eight essential oils were able to reduce mycelial growth of the four pathogens evaluated when compared to control treatments where no oil was applied. It is very encouraging to see that these essential oils demonstrate a potential for broad-spectrum efficacy against a range of common postharvest fruit rot pathogens including both fungi and oomycetes. With limited options for effective control of postharvest fruit rot pathogens our initial findings are promising and warrant further investigation on the treatment of fruit and disease development.

In the current study, the most promising findings include those obtained with savory and thyme oils, which were able to completely inhibit mycelial growth of all four pathogens evaluated. Thyme oil is currently an active ingredient sold as a commercial product (Proud 3), but to our knowledge savory oil would be a novel active ingredient if it were to be pursued for commercial use. The demand for alternative disease management products and especially those that can be labelled for organic use remains a high priority and we hope these findings encourage others to pursue alternative compounds such as essential oils for disease control.

Acknowledgements. Thanks to the Tropical Research and Education Centre at the University of Florida for providing facilities for this study. Special thanks go to Dr. Jonathan H. Crane, Ms. Pamela Moon, Ms. Tina Dispenza, and Dr. Georgina Sanahuja for their assistance during laboratory and field experiments.

References

Adams R.P. (2004): Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. 3rd Ed. Carol Stream, Allured Pub. Corp.

Anonymous (1993): British Pharmacopoeia. 15th Ed. London, HMSO.

Bailey J.A., Jeger M.J. (1992): *Colletotrichum*: Biology, Pathology and Control. Wallingford, CAB International.

Bill M., Sivakumar D., Beukes M., Korsten L. (2016): Expression of pathogenesis-related (PR) genes in avocados fumigated with thyme oil vapours and control of anthracnose. Food Chemistry, 194: 938–943.

Booth C. (1977): *Fusarium*: Laboratory Guide to the Identification of the Major Species. Surrey, Commonwealth Mycological Institute.

Curini M., Bianchi A., Epifano F., Bruni R., Torta L., Zambonelli A. (2003): Composition and *in vitro* antifungal activity of essential oils of *Erigeron canadensis* and *Myrtus communis* from France. Chemistry of Natural Compounds, 39: 191–194.

Erwin D.C., Ribeiro O.K. (1996): Phytophthora Diseases Worldwide. St. Paul, American Phytopathological Society.

Farsam H., Amanlou M., Radpour M.R., Salehinia A.N., Shafiee A. (2004): Composition of the essential oils of wild and cultivated *Satureja khuzistanica* Jamzad from Iran. Flavour and Fragrance Journal, 19: 308–310.

Finckh M.R., van Bruggen A.H.C., Tamm L. (2015): Plant Diseases Management in Organic Farming Systems. St. Paul, APS Press.

Hong J.K. Yang H.J. Jung H. Yoon D.J. Jeun Y.C. (2015): Application of volatile antifungal plant essential oils for controlling pepper fruit anthracnose by *Colletotrichum gloeosporioides*. Plant Pathology Journal, 31: 269–277.

Kishore G.K., Pande S. (2007): Evaluation of essential oils and their components for broad-spectrum antifungal ac-

https://doi.org/10.17221/49/2017-PPS

tivity and control of late leaf spot and crown rot diseases in peanut. Plant Diseases, 91: 375–379.

Komárek M., Čadková E., Chrastný V., Bordas F., Bollinger J.C. (2010): Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. Environment International, 36: 138–151.

López-Meneses A.K., Plascencia-Jatomea M., Lizardi-Mendoza J., Rosas-Burgos E.C., Luque-Alcaraz A.G., Cortez-Rocha M.O. (2015): Antifungal and antimycotoxigenic activity of essential oils from *Eucalyptus globulus*, *Thymus capitatus* and *Schinus molle*. Food Science and Technology (Campinas), 35: 664–671.

Moghaddam M., Pourbaige M., Tabar H.K., Farhadi N., Hosseini S.M.A. (2013): Composition and antifungal activity of peppermint (*Mentha piperita*) essential oil from Iran. Journal of Essential Oil Bearing Plants, 16: 506–512.

Ploetz R.C. (2003): Diseases of Tropical Fruit Crops. Wallingford, CABI Publishing.

Ploetz R.C., Zentmeyer G.A., Nishijima W.T., Rohrbach K.G., Ohr H.D. (1994): Compendium of Tropical Fruit Diseases. St Paul, APS Press.

Sellamuthu P.S., Sivakumar D., Soundy P., Korsten L. (2013): Essential oil vapours suppress the development of anthracnose and enhance defense related and antioxidant enzyme activities in avocado fruit. Postharvest Biology and Technology, 81: 66–72.

Slippers B., Crous P.W., Denman S., Coutinho T.A., Wingfield B.D., Wingfield M.J. (2004): Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia, 96: 83–101.

Sonke D.J., Gillett J.L., Leppla N.C. (2005): Chemsearch: a searchable pesticide database for tropical fruit crops. Proceeding Florida State Horticultural Society, 118: 218–220.

Terzi V., Morcia C., Faccioli P., Valè G., Tacconi G., Malnati M. (2007): *In vitro* antifungal activity of the tea tree (*Melaleuca alternifolia*) essential oil and its major components against plant pathogens. Letters in Applied Microbiology, 44: 613–618.

Tsai H.L., Huang L.C., Ann P.J., Liou R.F. (2006): Detection of orchid Phytophthora disease by nested PCR. Botanical Studies, 47: 379–387.

Wightwick A., Mollah M., Partington D., Allinson G. (2008): Copper fungicide residues in Australian vineyard soils. Journal of Agricultural and Food Chemistry, 56: 2457–2464.

Wightwick A., Walters R., Allinson G., Reichman S., Menzies N. (2010): Environmental risks of fungicides used in horticultural production systems. In: Carisse O. (ed.): Fungicides. Croatia, In-Tech Publishers: 273–304.

Zambonelli A., Zechini D'Aulerio A., Bianchi A., Albasini A. (1996): Effects of essential oils on phytopathogenic fungi *in vitro*. Journal of Phytophathology, 144: 491–494.

Received: 2017–04–16
Accepted after corrections: 2017–06–21
Published online: 2017–09–13