American Journal of Plant Sciences, 2014, 5, 1181-1190
Published Online April 2014 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2014.59131

Essential Oils from *Lippia origanoides* Kunth. and *Mentha spicata* L.: Chemical Composition, Insecticidal and Antioxidant Activities

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Received 16 January 2014; revised 3 March 2014; accepted 21 March 2014

Abstract

This work describes the chemical composition of the essential oils extracted from fresh leaves of *Lippia origanoides* Kunth. and *Mentha spicata* L. and their antioxidant and insecticidal activities. The essential oils were extracted by steam distillation using a modified Clevenger apparatus and subsequently analyzed by gas chromatography-flame ionization detector and gas chromatography-mass spectrometry. The antioxidant activities were determined by the β-carotene-linoleic acid method and by sequestration of the 2,2-diphenyl-1-picryl-hidrazila radical. The concentrations of the essential oils and the synthetic standard, butylated hydroxyltoluene, were 25, 50, 100, 150, 200, 250, 300 and 500 μg mL^{-1}. Insecticidal activity was analyzed by non-preference with choice and no choice against the aphid *Myzus persicae* Sulzer. Gas chromatography analysis of the essential oil from *Lippia origanoides* Kunth. revealed carvacrol (41.51%), *p*-cymene (18.36%), γ-terpinene (17.03%) and thymol (4.86%) as major constituents, and the essential oil from *Mentha spicata* L. contained piperitona (81.18%), piperitenone (14.57%) and limonene (1.47%) as the principal components. The essential oils and the standard exhibited dose-dependent antioxidant activities at the concentrations tested. The essential oils were shown to be potential agents in the integrated management of the aphid *Myzus persicae* Sulzer.

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How to cite this paper: Teixeira, M.L., *et al.* (2014) Essential Oils from *Lippia origanoides* Kunth. and *Mentha spicata* L.: Chemical Composition, Insecticidal and Antioxidant Activities. *American Journal of Plant Sciences*, **5**, 1181-1190.
http://dx.doi.org/10.4236/ajps.2014.59131

Keywords

Antioxidant Activity; Insecticidal Activity; Lamiaceae; Natural Products; Verbenaceae

1. Introduction

For many years, humankind has been using plants for biotechnological purposes because they contain natural compounds capable of acting against predators and insects and as preventive agents for cardiovascular and neurodegenerative diseases [1]. Considering the possible problems that might be affected by the consumption of synthetic antioxidants, research has been undertaken to encounter natural products with antioxidant activity that could replace the synthetic products or complement them.

For many years, a variety of synthetic compounds have been employed to control pests and plant diseases. However, the use of these substances has been limited by their carcinogenic and teratogenic activities, toxicity and environmental pollution [2]. Among the pests of economic importance in Brazil, the *Myzus persicae* Sulzer aphid, one of the most damaging species from the agricultural point of view, should be emphasized because, in addition to the direct damages resulting from the continuous ingestion of sap, it causes indirect losses because of the transmission of a virus [3].

Because of the harmful consequences resulting from the use of synthetic compounds as antioxidants and insecticides, the search for alternative methods to control pests has increased. Among these natural compounds, the essential oils are secondary metabolites produced by plants and characterized by being complex mixtures of organic compounds [4]. Their use is increasing and gaining a greater share of the market and consumer preference.

Brazil has a variety of flora, and this variety facilitates the study of the functional properties of the species that compose the flora. *L. origanoides* (Verbenaceae) is an aromatic bush that is native to Central America and northern South America. It is used in traditional medicine for the treatment of gastrointestinal and respiratory diseases [5]. *M. spicata* (Lamiaceae) is an aromatic, creeping, rhizomatous plant. It is popularly used as a flavoring agent in tea and as a calming agent [6]. The objective of the present study was the characterization of the essential oils from the fresh leaves of *Lippia origanoides* and *Mentha spicata* and the evaluation of the antioxidant and insecticidal activities of these essential oils.

2. Material and Methods

2.1. Collection of the Vegetal Material and Extraction of the Essential Oil

The *Lippia origanoides* Kunth. (Verbenaceae) and *Mentha spicata* L. (Lamiaceae) plants were collected in the morning in the Medicinal Plants Garden of the Federal University of Lavras (UFLA) in Lavras, MG, Brazil, on a mild day with no precipitation. Lavras is located in the southern region of the state of Minas Gerais at 21˚14'S, longitude 45˚00'W Gr and at an altitude of 918 m. The essential oils were extracted in the Laboratory of Organic Chemistry-Essential Oils of the Federal University of Lavras by hydrodistillation with a modified Clevenger apparatus [7]-[9]. For the extraction of the essential oil from *L. origanoides*, 250 g of fresh leaves was added to a round bottom flask (5 liters) with 2500 mL of distilled water. Fresh *M. spicata* leaves (225 g) were extracted in a similar manner. Three extractions were performed for each plant material by boiling for 2 hours. The essential oil was separated from the hydrolact by centrifugation at 965g for 5 minutes using a bench-top centrifuge with a horizontal crosspiece (Fanem Baby® Model 206 BL). The oil was removed with the aid of a Pasteur pipette, stored in a glass flask protected from moisture and light and stored at a low temperature (4˚C).

2.2. Chemical Identification and Quantification of the Essential Oils

The chemical analysis of the essential oils was performed in the Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

The GC-MS analyses were performed on a Perkin Elmer Autosystem XL gas chromatograph equipped with a DB-1 fused silica column (30 m × 0.25 mm ID, film thickness, 0.25 μm; J & W Scientific Inc.) and coupled to a Perkin Elmer Turbomass mass spectrometer (software version 4.1). The oven temperature was initially pro-

grammed from 45˚C to 175˚C, with a heating rate of 3˚C min^{-1}, followed by 15˚C min^{-1} to 300˚C, where the temperature was maintained for 10 minutes. The injector temperature was 280˚C and the helium carrier gas was adjusted to a linear velocity of 30 cm s^{-1}. The transfer line temperature was 280˚C, the temperature of the ion trap, 220˚C; the split ratio, 1:40, the ionization energy, 70 eV, the scan range, 40-300U, and the scan time, one second.

The identity of the compounds was determined by comparison of their retention indices relative to C_9-C_{21} n-alkanes and the mass spectra with those of standard commercial and reference compounds present in oils existing in the laboratory and with a library of mass spectra developed in the laboratory of the Centro de Biotecnologia Vegetal, Faculdade de Ciências, Universidade de Lisboa [10].

The volatile components were analyzed by gas-liquid chromatography on a Perkin Elmer 8700 gas chromatograph equipped with two flame ionization detectors (FID), a system for processing data and an automatic injector. Two columns of different polarities with the following characteristics were used: fused silica with a DB-1 immobilized methylsilicone phase (30 m × 0.25 mm ID, film thickness, 0.25 µm; J & W Scientific Inc.) and a DB-17HT immobilized phenylmethylsilicone phase (30 m x 0.25 mm id, film thickness, 0.25 µm; J & W Scientific Inc.).

The oven temperature was initially programmed from 45˚C to 175˚C, with a heating rate of 3˚C min^{-1}, followed by 15˚C min^{-1} to 300˚C, where the temperature was maintained for 10 minutes. The temperature of the injector and detector were 290˚C and 280˚C, respectively; the flow rate of the hydrogen carrier gas was 30 cm s^{-1}. The split ratio was 1:50, and the injected volume was 0.1 µL of oil in pentane. The composition of the essential oils was calculated by normalizing the peak areas without using correction factors. The values shown correspond to the average from two injections [10].

2.3. Antioxidant Activity

For the antioxidant activity of essential oils by the β-carotene-linoleic acid method, 60 mg of linoleic acid, 600 mg of Tween 20, 6 mg of β-carotene and 30 mL of chloroform were added to a round bottom flask. After homogenization, the chloroform was completely evaporated at 50˚C, and the residue was dissolved in 150 mL of distilled water saturated with oxygen (emulsion A). Aliquots of 2.5 mL of the emulsion were transferred to test tubes, and 0.2 mL of each of the dilutions of the essential oil in methanol at the following concentrations 25, 50, 100, 150, 200, 250, 300 and 500 µg mL^{-1} was added. The blank emulsion was prepared without the addition of β-carotene and the control, by the addition of 2.5 mL of the emulsion A and 0.2 mL of methanol. The analysis for each reaction mixture was performed with four replicates, and the absorbance was measured immediately on a Shimadzu UV-160 1 PC spectrophotometer at 470 nm. The tubes were incubated at 50˚C, and the absorbance was measured again after 60 minutes. BHT (Butylated hydroxytoluene) was used as a positive control in the same concentrations as the essential oils. The percentage of antioxidant activity was calculated according to Lopes-Lutz *et al*. (2008) [11] using the following equation:

$$AA\% = 100 \times \left[1 - \left(A_0 - A_t / A_{00} - A_{0t}\right)\right]$$

where A_0 is the absorbance at the beginning of incubation with the sample; A_t is the absorbance after incubating the sample for 60 minutes; A_{00}: is the absorbance at the start of incubation without the sample; A_{0t} is the absorbance after 60 minutes without sample.

For the sequestration of the DPPH (1,1-diphenyl-2-picryl-hidrazila) radical method, a stock solution contained DPPH at a concentration of 40 mg mL^{-1} in methanol was prepared. The essential oils were dissolved in methanol at concentrations of 25, 50, 100, 150, 200, 250, 300 and 500 µg mL^{-1}. The stock solution of DPPH (2.7 mL) was added to a test tube, followed by the addition of 0.3 mL of the dilutions of the essential oil. The blank contained 2.7 mL of methanol and 0.3 mL of the most concentrated essential oil, and the control contained 2.7 mL of the stock solution of DPPH and 0.3 mL of methanol. Analysis was performed for 60 minutes with four replicates (in the absence of light); readings were performed on a spectrophotometer (Shimadzu UV-160 1 PC) at 515 nm. BHT was used as a positive control in the same concentrations as the essential oils. The percentage of antioxidant activity was calculated according to Lopes-Lutz *et al*. (2008) [11] using the following equation:

$$\%\, AA = \left[1 - \left(A_{am} / hap\right)\right] \times 100$$

where A_{am} is the absorbance of the sample; hap is the absorbance of the control.

The experimental design was a completely randomized design (CRD) with a 3 × 8 factorial arrangement (es-

sential oils/standard x concentrations), with four replications. The statistical program used was SISVAR [12]. Data were subjected to analysis of variance, and the average was compared by the Tukey test at 5% probability.

2.4. Insecticide Activity

The experiments were performed in the Laboratory of Plant Resistance to Insects, Department of Entomology, Federal University of Lavras. The planting of tomato plants was accomplished using vessels with a capacity of 1 kg of soil, in which the tomato seedlings of the Santa Clara cultivar, provided in Styrofoam trays, were transplanted.

The collection of aphids was conducted in the field. The aphids were brought to the laboratory and reared in cages with "joá de capote" (*Nicandra physaloides*) plants in an environmental chamber at 25 ± 2˚C and a photoperiod of 12 hours. In biological tests, adult aphids of uniform size were chosen. The leaves of the tomato plants were detached, and sections were cropped with the aid of a circular plastic cutter six centimeters in diameter. These sections were sprayed on the abaxial surface with the sample solutions to the point of total runoff and fixed in Petri dishes on a layer of 1.0% agar covered with PVC film pierced with a pin.

The treatments used in the experiments with the essential oils were: T1—water with Tween 80; T2—0.1% essential oil; T3—0.5% essential oil [13].

For the test with a chance of choice, each Petri dish contained a leaf section of each of the three treatments, arranged in a circle, forming an arena (Silveira *et al*., 1998) [14]. In the center of each plate, 15 wingless adult *M. persicae* aphids were released.

In the no choice test, each Petri dish contained a leaf section of each treatment set in the center of the plate. Five wingless adult *M. persicae* aphids were released [14]. The plates were kept in a climatic chamber at 25 ± 2˚C with a photoperiod of 12 hours. The evaluations were made 24, 48 and 72 hours after the release of the aphids by counting the number of aphids and nymphs on each leaf section.

The experimental designs were a randomized block design (RBD) and a completely randomized design (CRD) with ten replicates for tests with and without choice, respectively. The statistical program used was SISVAR [12]. The data were transformed into $\sqrt{(x+0.5)}$ and subjected to analysis of variance with the parts subdivided in time, and the means were compared by the Tukey test at 5% probability.

3. Results and Discussion

3.1. Identification and Quantification of the Constituents of the Essential Oils

Thirty-two constituents were identified in the essential oil from *L. origanoides* described in **Table 1**. The major components were carvacrol (41.51%), *p*-cymene (18.36%), γ-terpinene (17.03%) and thymol (4.86%).

The results corroborate those found by Santos *et al*. (2004) [15], who analyzed three collections of *L. origanoides*. They found carvacrol (33.5 to 42.9%), followed by *p*-cymene (11.9 to 15.8%), γ-terpinene (8.0 to 10.5%) and thymol (5.1 to 8.4%) as the principal constituents. Escobar *et al*. (2010) [16] analyzed the essential oils from plants grown in different locations in Colombia and obtained carvacrol as the major component with a concentration of 20.5%, followed by *p*-cymene (14.8%), thymol (13.4%) and γ-terpinene (7.5%).

The essential oil from *M. spicata*, known as peppermint, consists mainly of oxygenated monoterpenes (25 constituents were identified, **Table 2**), with piperitona (81.18%), piperitenone (14.57%) and limonene (1.47%) as the principal constituents.

Sartoratto *et al*. (2004) [17] analyzed the essential oil from *M. spicata* and obtained piperitone oxide as the major constituent (94.8%). Chauhan *et al*. (2009) [18] studied the essential oils from plants of the same species in the flowering period, collected in different regions of the Himalayas (Northern India), and found carvone as the major constituent (49.62 to 76.65%), followed by limonene (9.57 to 22.31%), 1,8-cineole (1.32 to 2.62%) and *trans*-carveol (0.3 to 1.52%), results that differ from those found in this work.

Koliopoulos *et al*. (2010) [19], investigating plants of the Lamiaceae family in different regions of Greece, cited piperitenone oxide (35.7%) and 1,8-cineole (14.5%) as the major constituents of the essential oil from *M. spicata*.

According Gobbo-Neto and Lopes (2007) [20], the production of secondary metabolites can be influenced by various environmental factors such as seasonality, rainfall, circadian rhythm, altitude, temperature, vegetative cycle of the plant, and soil type, among others. In this context, quantitative and qualitative changes in the chemical composition of the essential oils can be influenced by these environmental factors because the plants

Table 1. Chemical composition of the essential oil from leaves of *L. origanoides*.

Compounds	$RI_{cal.}$	N. area (%)
α-Thujene	924	2.12
α-Pinene	930	0.62
Sabinene	958	v
1-Octen-3-ol	961	0.04
β-Pinene	963	0.12
β-Myrcene	975	3.32
α-Felandrene	995	1.03
α-Terpinene	1002	2.14
p-Cimene	1003	18.36
β-Felandrene	1005	0.11
Limonene	1009	0.68
trans-β-Ocimene	1027	v
γ-Terpinene	1035	17.03
trans-Sabinene hydrate	1037	0.28
2,5-Dimethylestirene	1059	v
Terpinolene	1064	v
cis-Sabinene hydrate	1066	0.03
Linalol	1074	v
Terpinen-4-ol	1148	0.53
Thymol	1275	4.86
Carvacrol	1286	41.51
Thymol acetate	1330	0.17
Carvacrol acetate	1348	2.12
β-Caryophyllene	1414	3.28
trans-α-Bergamotene	1434	0.05
α-Humulene	1447	0.17
Germacrene-D	1474	0.04
β-Bisabolene	1500	0.04
δ-Cadinene	1500	v
β-Caryophyllene oxide	1561	0.62

*RIcal. = Calculated retention index, N. area = Normalization of the area, v = vestigial.

were collected in very distinct environments.

3.2. Antioxidant Activity

The percentage of antioxidant activity of the essential oils and the BHT standard in relation to their concentrations, evaluated by the β-carotene-linoleic acid method, are presented in **Table 3**. It's possible to observe a significant effect of the factors essential oil/standard, concentration and the interaction of these factors on the percentage of antioxidant activity.

An increase in the antioxidant activity with concentration revealed a dose dependence of the antioxidant activity of the essential oils and the BHT standard. This dose dependence of the essential oil was reported in the work of Wang *et al*. (2008) [21], who evaluated the antioxidant activity of the essential oil from *Rosmarinus officinalis* L. by the β-carotene-linoleic acid method.

The BHT standard exhibited the highest antioxidant activity, followed by the essential oil from *L. origanoides* and *M. spicata*, respectively. Results obtained by Morais *et al*. (2006) [22] and by Duarte-Almeida *et al*. (2006) [23] corroborate those observed in this study regarding the high antioxidant activity of the synthetic BHT, which is used as an antioxidant in the food industry. The fact that the essential oil from *L. origanoides* exhibited a higher activity than that from *M. spicata* may be related to the presence of carvacrol as the principal compound

Table 2. Chemical composition of the essential oil from leaves of *M. spicata*.

Compounds	RIcal.	N. area (%)
α-Pinene	930	0.51
Canfene	938	v
Sabinene	958	0.50
1-Octen-3-ol	961	v
β-Pinene	963	0.50
3-Octanol	974	v
Myrcene	975	v
Isobutyl isovalerate	986	v
1,8-Cineole	1005	0.36
Limonene	1009	1.47
cis-β-Ocimene	1017	0.17
trans-β-Ocimene	1027	v
n-Octanol	1045	v
2-But acid methyl ester isoamyl	1072	v
Terpinen-4-ol	1148	v
α-Terpineol	1159	v
Piperitona	1211	81.18
Carvacrol	1286	v
Piperitenone	1289	14.57
Piperitenone oxide	1330	v
β-Bourbonene	1379	v
β-Elemene	1388	0.29
β-Caryophyllene	1414	0.28
Germacrene-D	1474	0.11
Bicyclogermacrene	1487	v

*RIcal. = Calculated retention index, N. area = Normalization of the area, v = vestigial.

Table 3. Percentages of antioxidant activity of essential oils and BHT observed using the β-carotene-linoleic acid method.

[ ] (μg mL^{-1})	Essential oils/Standard		
	LO	**MS**	**BHT**
25	9.22 dB	3.34 bB	53.00 cA
50	17.68 cB	4.76 bC	61.43 bA
100	23.71 Cb	4.95 bC	65.86 abA
150	36.88 bB	22.99 aC	65.29 abA
200	40.44 bB	24.61 aC	69.86 aA
250	43.49 bB	24.80 aC	70.57 aA
300	44.28 bB	24.89 aC	71.00 aA
500	52.27 aB	25.37 aC	72.14 aA

The means followed by the same lower case letter in the columns and the same upper case letter in the rows do not differ by the Tukey test at the 5% probability level. Legend: [ ] (μg mL^{-1}) = Concentrations (μg mL^{-1}); LO = *Lippia origanoides* Kunth.; MS = *Mentha spicata* L.

in the oil. According Ruberto and Barata (2000) [24], phenols and their derivatives are effective antioxidants; thus, molecules such as thymol and carvacrol are responsible for the high antioxidant activity of many essential oils.

As can be deduced from **Table 4**, the percentage of antioxidant activity was also dose dependent at the concentrations tested, and the BHT standard exhibited greater antioxidant activity than the essential oils from *L. origanoides* and *M. spicata*, employing the same concentrations, in the method that involved the sequestering of the DPPH radical. The oil from *L. origanoides* appeared to exhibit greater stabilization of the DPPH radical than

Table 4. Percentages of antioxidant activity of the essential oils and BHT in the method involving the sequestering of the DPPH radical.

[ ] (μg mL^{-1})	Essential oils/Standard		
	LO	MS	BHT
25	4.90 fB	1.81 cB	47.38 eA
50	9.86 eB	2.92 cC	50.51 dA
100	20.58 dB	3.41 bcC	59.67 cA
150	25.18 cdB	4.23 bcC	86.57 bA
200	29.41 cB	5.57 bcC	90.06 abA
250	38.38 bB	6.80 bcC	90.98 abA
300	39.06 bB	8.60 abC	91.43 abA
500	50.90 aB	13.81 aC	91.85 aA

The means followed by the same lower case letter in the columns and the same upper case letter in the rows do not differ by the Tukey test at the 5% probability level. Legend: [ ] (μg mL^{-1}) = Concentrations (μg mL^{-1}); LO = *Lippia origanoides* Kunth.; MS = *Mentha spicata* L.

the oil from *M. spicata*. However, the activity of the oils exhibited in the DPPH method was lower than that observed with the β-carotene-linoleic acid method. This lower activity may be explained by the low solubility of the constituents of the essential oil in the reaction medium of the test, usually conducted in a polar solvent such as methanol or ethanol.

Safaei-Ghomi *et al*. (2009) [25] reported that compounds containing hydrogen atoms on allylic, benzylic and phenolic carbons exhibit higher activity in the β-carotene-linoleic acid method because of the relative ease of abstraction of the hydrogen atoms by the peroxyl radicals formed in the test. Thus, the antioxidant capacity of phenolic compounds is due to their ability to donate hydrogen atoms, thereby confirming the high antioxidant capacity of the essential oil from *L. origanoides* compared with that of the oil from *M. spicata.*

The results obtained by the method of sequestration of the DPPH radical are based on the bleaching of a solution composed of the violet-colored DPPH radical when this is added to substances that can release a hydrogen atom (such as phenols). Thus, the greater antioxidant activity presented by the essential oil from *L. origanoides* is to be expected because carvacrol is present in a high concentration in this oil.

3.3. Insecticidal Activity

The test of preference with a choice assessed the preference of the aphids for leaf sections sprayed with different concentrations of the essential oils. From the data presented in **Table 5**, it can be observed that aphids had a greater preference for sections of the control (water + Tween 80) and for sections with a concentration of 0.1% essential oil of *L. origanoides* than for those treated with a 0.5% concentration. From the numbers of nymphs present in leaf sections treated with different concentrations of the essential oil of *L. origanoides* (**Table 5**), it is clear that the 0.1 and 0.5% concentrations of essential oil influenced the reproduction of aphids, as compared with the control, being less preferred for egg laying by adults. This fact proves that natural products such as essential oils can be considered as alternative sources for the management of insect pests.

When evaluating the influence of the time interval on the number of live adults in the data presented in **Table 6**, the interval did not alter the preference of aphids; the numbers of aphids on the leaf sections remained constant with the passage of time. Nor did the time interval influence the effect of the essential oil, and the number of nymphs on the leaf sections remained constant over time. The results of the test of preference with no choice evaluating the essential oil from *M. spicata* L. are summarized in **Table 7**. There was a significant interaction between the factors concentration and the time interval. Thus, the sections treated with the 0.1 and 0.5% concentrations of the oil were less preferred for food by adult aphids, regardless of the interval evaluated. With regard to the number of nymphs (**Table 7**), the leaf sections treated with the control and with the 0.1% concentration of oil were most preferred for the reproduction of aphids during for the first 24 hours and presented a greater number of nymphs than was observed with the 0.5% concentration. After 48 hours, the number of nymphs decreased proportionally to the concentrations tested, *i.e.*, the higher the concentration, the smaller the number of nymphs, and, at 72 hours, the treatments with 0.1 and 0.5% differed statistically from the control, being less preferred for egg laying.

Table 5. The numbers of live adults and nymphs in the preference with a choice test at different concentrations of the essential oil from *L. origanoides.*

Concentration (%)	Number of live adults	Number of nymphs
0	2.67 a	4.23 a
0.1	2.07 ab	3.12 b
0.5	1.70 b	2.13 b

The means followed by the same letter in the column do no differ statistically by the Tukey test at 5% of probability.

Table 6. Numbers of live adults and nymphs in the preference with a choice test according to the time interval for the essential oil from *L. origanoides.*

Time interval (hours)	Number of live adults	Number of nymphs
24	210 a	2.90 a
48	2.16 a	3.26 a
72	2.17 a	3.33 a

The means followed by the same letter in the column do no differ statistically by the Tukey test at 5% of probability.

Table 7. Numbers of live adults and nymphs in the preference with a choice test by concentration and time interval of the essential oil from *M. spicata.*

Concentration (%)	Number of live adults			Number of nymphs		
	Time interval			Time interval		
	24	48	72	24	48	72
0	2.36 a	2.74 a	2.56 a	2.95 a	4.16 a	4.16 a
0.1	2.05 b	1.91 b	1.87 b	2.74 a	2.69 b	2.99 b
0.5	1.97 b	1.67 b	1.85 b	2.07 b	1.95 c	2.58 b

The means followed by the same letter in the column do no differ statistically by the Tukey test at 5% of probability.

Nerio *et al*. (2009) [26] studied the repellent activity of the essential oils from the aromatic plants *Cymbopogon citratus*, *Eucalyptus citriodora*, *Lippia origanoides*, *Lippia alba*, *Cananga odorata*, *Tagetes lucida* and *Rosmarinus officinalis*, grown in Colombia, against *Sitophilus zeamais* Motschulsky using the method of preferred area. They used concentrations of 0.063, 0.126, 0.252, and 0.503 mL $(cm^2)^{-1}$ and observed that all the essential oils, except for that from *L. alba*, exhibited some repellent activity. The oils with the highest repellency were those from *L. origanoides*, *E. citriodora* and *T. lucida*. According to the authors, the essential oil from *L. origanoides* was the most active repellent, possibly because of the presence of thymol, which was the major component found and is considered to be a botanical insecticide. These results corroborate those found in the present work, because the essential oils also presented repellent activity. The essential oil from *L. origanoides* contained carvacrol, an isomer of thymol, as the principal compound. Thus, there is a similarity between the two studies with regard to the repellent activity.

The test of preference with no choice evaluated the toxicity of the essential oils to *Myzus persicae* Sulzer aphids. No toxicity was observed when the concentrations of the essential oils from *L. origanoides* and *M. spicata* L. were varied. Thus, the number of adult aphids was not significantly different when compared with the control (**Table 8**). For the essential oil from *L. origanoides*, there was no statistically significant difference between the control and the treatment with 0.1% essential oil with respect to the number of nymphs, although the numbers were higher than those observed in the treatment with 0.5% essential oil. Thus, a greater insecticidal effect against the adult aphids was observed for the higher oil concentration. With the essential oil from *M. spicata* L., there was a decrease in the number of nymphs deposited by aphid females as the concentration of the oil increased. That is, the control afforded the greatest number of nymphs, followed by the treatments with 0.1 and 0.5% essential oil, respectively.

In the analysis of the influence of the time interval on the insecticidal effect of essential oils (**Table 9**) in the test of preference with no choice, no significant variation in the number of live adults with time was observed with both the essential oils. Nor was there any variation in the number of nymphs with time when the oil from *L. origanoides* was tested. However, the essential oil from *M. spicata* L. did not behave like that from *L. origa-*

Table 8. Mean numbers of live adults and nymphs in the preference without a choice test with variation in the concentrations of the essential oils.

Concentration (%)	Number of live adults		Number of nymphs	
	Lippia origanoides Kunth.	*Mentha spicata* L.	*Lippia origanoides* Kunth.	*Mentha spicata* L.
0	2.34 a	2.34 a	3.98 a	3.90 a
0.1	2.33 a	2.28 a	3.54 a	2.90 b
0.5	2.27 a	2.26 a	2.66 b	2.30 c

The means followed by the same letter in the column do no differ statistically by the Tukey test at 5% of probability.

Table 9. Variation of the mean numbers of live adults and nymphs in the preference with no choice with time interval.

Time interval	Number of live adults		Number of nymphs	
	Lippia origanoides Kunth.	*Mentha spicata* L.	*Lippia origanoides* Kunth.	*Mentha spicata* L.
24	2.33 a	2.31 a	3.36 a	2.91 b
48	2.32 a	2.29 a	3.37 a	3.00 ab
72	2.30 a	2.28 a	3.45 a	3.18 a

The means followed by the same letter in the column do no differ statistically by the Tukey test at 5% of probability.

noides; there was an increase in the number of nymphs deposited with time. This fact implies that the efficacy against aphids decreased with time. The difference in the performance of the essential oils can probably be explained by the difference in their chemical compositions because the essential oil from *M. spicata* consisted mostly of terpenes, while that from *L. origanoides* is composed mainly of phenolic compounds.

Zapata and Smagghe (2010) [27] evaluated the repellent activity as well as the contact and fumigant toxicities against *Tribolium castaneum* of four essential oils extracted from the leaves and bark of *Laurelia sempervirens* and *Drimys winteri*. They observed that the four oils had a strong repellent activity against *T. castaneum* in the arena test with filter paper. After four hours of exposure, over 90% repellency was obtained for the oils from *L. sempervirens* at low concentrations [0.032 µL $(cm^2)^{-1}$]. Concentrations of the oils from *D. winteri* three-to-ten times higher were necessary to achieve this activity. Both oils were toxic to *T. castaneum* when applied topically or by spraying. The LD_{50} values for topical application of the *L. sempervirens* oils were 39 - 44 µg mg^{-1} insect, and those for the oils from *D. winteri* were 75 to 85 µg mg^{-1} insect. When sprayed, the LC_{50} values for the oils from *L. sempervirens* were 1.6 to 1.7 µL L^{-1} of air, while those for the oils from *D. winteri* were 9.0 a 10.5 µL L^{-1} of air.

Acknowledgements

The authors thank the support of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG), the Universidade Federal de Lavras and Pest OE/EQB/LA0023/2011. D.L. Nelson was the recipient of a PVNS fellowship from the Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES).

References

[1] Dixit, S. and Ali, H. (2010) Antioxidant Potential Some Medicinal Plants of Central India Antioxidant Potential Some Medicinal Plants of Central India. *Journal of Cancer Therapy*, **1**, 87-90. http://dx.doi.org/10.4236/jct.2010.12014

[2] Viegas Junior, C. (2003) Terpenos com Atividade Inseticida: Uma Alternativa para o Controle Químico de Insetos. *Química Nova*, **26**, 390-400. http://dx.doi.org/10.1590/S0100-40422003000300017

[3] Blackman, R.L. and Eastop, V.P. (1984) Aphids on the World's Crops: An Identification Guide. John Wiley & Sons Ltd., Chichester.

[4] Okunowo, W., Oyedeji, O., Afolabi, L. and Matanmi, E. (2013) Essential Oil of Grape Fruit (*Citrus paradisi*) Peels and Its Antimicrobial Activities. *American Journal of Plant Sciences*, **4**, 1-9.

[5] Vicuña, G.C., Stashenko, E.E. and Fuentes, J.L. (2010) Chemical Composition of the *Lippia origanoides* Essential Oils and Their Antigenotoxicity against Bleomycin-Induced DNA Damage. *Fitoterapia*, **81**, 343-349. http://dx.doi.org/10.1016/j.fitote.2009.10.008

[6] Lawrence, B.M. (2006) Mint: The Genus *Mentha*. CRC Press, Boca Raton.

[7] BRASIL (2000) Farmacopéia Brasileira. 4th Edition, Ateneu, São Paulo, Part I, 2-7.

[8] Gomes, M.S., *et al*. (2013) Multivariate Analysis of the Essential Oil Components of the Genus Citrus and Their Antifungal Activity. *Científica*, *Jaboticabal*, **41**, 111-121.

[9] Teixeira, M.L., *et al*. (2012) Citrumelo Swingle: Caracterização Química, Atividade Antioxidante e Antifúngica dos óleos Essenciais das Cascas Frescas e Secas. *Magistra*, **24**, 194-203.

[10] Mendes, M.D., *et al*. (2011) ISSR Molecular Characterization and Leaf Volatiles Analysis of *Pittosporum undulatum* Vent. Naturalized in the Azores archipelago (Portugal). *Industrial Crops and Products*, **33**, 710-719. http://dx.doi.org/10.1016/j.indcrop.2011.01.010

[11] Lopes-Lutz, D., Alviano, D.S., Alviano, C.S. and Kolodziejczyk, P.P. (2008) Screening of Chemical Composition, Antimicrobial and Antioxidant Activities of *Artemisia* Essential Oils. *Phytochemistry*, **69**, 1732-1738. http://dx.doi.org/10.1016/j.phytochem.2008.02.014

[12] Ferreira, D.F. (2011) Sisvar: A Computer Statistical Analysis System. *Ciência e Agrotecnologia*, **35**, 1039-1042.

[13] Lima, R.K., *et al*. (2008) Composição dos Óleos Essenciais de Anis-estrelado *Illicium verum* L. e de Capim-limão *Cymbopogon citratus* (DC.) Stapf: Avaliação do Efeito Repelente sobre *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). *BioAssay*, **3**, 1-6.

[14] Silveira, L.C.P., Vendramim, J.D. and Rosseto, C.J. (1998) Não-preferência para Alimentação da Lagarta-do-Cartucho em Milho. *Bragantia*, **57**, 105-111. http://dx.doi.org/10.1590/S0006-87051998000100012

[15] Santos, F.J.B., *et al*. (2004) Composition and Biological Activity of Essential Oils from *Lippia origanoides* HBK. *Journal of Essential oil Research*, **16**, 504-506.

[16] Escobar, P., *et al*. (2010) Chemical Composition and Antiprotozoal Activities of Colombian *Lippia* spp Essential Oils and Their Major Components. *Memórias do Instituto Oswaldo* Cruz, **105**, 184-190. http://dx.doi.org/10.1590/S0074-02762010000200013

[17] Sartoratto, A. (2004) Composition and Antimicrobial Activity of Essential Oils from Aromatic Plants Used in Brazil. *Brazilian Journal Microbiological*, **35**, 275-280. http://dx.doi.org/10.1590/S1517-83822004000300001

[18] Chauhan, R.S., *et al*. (2009) Chemical Composition of Essential Oils in *Mentha spicata* L. Accession [IIIM(J)26] from North-West Himalayan Region, India. *Industrial Crops and Products*, **29**, 654-656. http://dx.doi.org/10.1016/j.indcrop.2008.12.003

[19] Koliopoulos, G., *et al*. (2010) Chemical Composition and Larvicidal Evaluation of *Mentha*, *Salvia*, and *Melissa* Essential Oils against the West Nile virus Mosquito *Culex pipiens*. *Parasitological Research*, **107**, 327-335. http://dx.doi.org/10.1007/s00436-010-1865-3

[20] Gobbo-Neto, L. and Lopes, N.P. (2009) Plantas Medicinais: Fatores de Influência no Conteúdo de Metabólitos Secundários. *Química Nova*, **30**, 374-381. http://dx.doi.org/10.1590/S0100-40422007000200026

[21] Wang, W., *et al*. (2008) Antioxidative Activity of *Rosmarinus officinalis* L. Essential Oil Compared to Its Main Components. *Food Chemistry*, **108**, 1019-1022. http://dx.doi.org/10.1016/j.foodchem.2007.11.046

[22] Morais, S.M., *et al*. (2006) Atividade Antioxidante de Óleos Essenciais de Espécies de *Croton* do Nordeste do Brasil. *Química Nova*, **29**, 907-910. http://dx.doi.org/10.1590/S0100-40422006000500004

[23] Duarte-Almeida, J.M., Santos, R.J., Genovese, M.I. and Lajolo, F.M. (2006) Avaliação da Atividade Antioxidante Utilizando Sistema β-Caroteno/ácido Linoleico e Método de Sequestro de Radicais DPPH. *Ciência e Tecnologia Alimentos*, **26**, 446-452. http://dx.doi.org/10.1590/S0101-20612006000200031

[24] Ruberto, G. and Baratta, M.T. (2000) Antioxidant Activity of Selected Essential Oil Components in Two Lipid Model Systems. *Food Chemistry*, **69**, 167-174. http://dx.doi.org/10.1016/S0308-8146(99)00247-2

[25] Safaei-Ghomi, J., Ebrahimabadi, A.H., Djafari-Bidgoli, Z. and Batooli, H. (2009) GC/MS Analysis and in Vitro Antioxidant Activity of Essential Oil and Methanol Extracts of *Thymus caramancus* Jalas and Its Main Constituint Carvacrol. *Food Chemitry*, **115**, 1524-1528. http://dx.doi.org/10.1016/j.foodchem.2009.01.051

[26] Nerio, L.S., Olivero-Verbel, J. and Stashenko, E.E. (2009) Repellent Activity of Essential Oils from Seven Aromatic Plants Grown in Colombia against *Sitophilus zeamais* Motschulsky (Coleoptera). *Journal of Stored Products Research*, **45**, 212-214. http://dx.doi.org/10.1016/j.jspr.2009.01.002

[27] Zapata, N. and Smagghe, G. (2010) Repellency and Toxicity of Essential Oils from the Leaves and Bark of *Laurelia sempervirens* and *Drimys winteri* against *Tribolium castaneum*. *Industrial Crops and Products*, **32**, 405-410. http://dx.doi.org/10.1016/j.indcrop.2010.06.005