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## Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*

M.V. Maciel<sup>a,\*</sup>, S.M. Morais<sup>b</sup>, C.M.L. Bevilaqua<sup>a</sup>, R.A. Silva<sup>c</sup>, R.S. Barros<sup>a</sup>, R.N. Sousa<sup>c</sup>, L.C. Sousa<sup>c</sup>, E.S. Brito<sup>d</sup>, M.A. Souza-Neto<sup>d</sup>

<sup>a</sup> Ceará State University, Postgraduate Program in Veterinary Science, Laboratory of Parasitic Diseases, Av. Paranjana 1700, CEP 60740-903 Fortaleza, Ceará, Brazil

<sup>b</sup> Ceará State University, Laboratory of Natural Products Chemistry, Ceará, Brazil

<sup>c</sup> Ceará State Health Secretariat, Entomology Laboratory of the Center for Vector Control, Ceará, Brazil

<sup>d</sup> Embrapa Tropical Agroindustry Research Unit, Ceará, Brazil

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### ABSTRACT

The chemical composition of essential oils from three species of plants belonging to the *Eucalyptus* genus was determined and, their insecticidal effects on egg, larva and adult phases of *Lutzomyia longipalpis* were assessed. The insects were collected in the municipality of Sobral in the State of Ceará, Brazil. Five treatments with different concentrations were performed along with two negative controls, distilled water and Tween 80 (3%), and a positive control, cypermethrin (0.196 mg/ml). The tests were carried out in plastic pots internally coated with sterile plaster and filled with a substrate made of rabbit feces and crushed cassava leaves. The eggs, larvae and adults were sprayed with the oils. The hatched larvae were counted for 10 consecutive days and observed until pupation. Insect mortality was observed after 24, 48 and 72 h. *E. staigeriana* oil was the most effective on all three phases of the insect, followed by *E. citriodora* and *E. globulus* oils, respectively. The major constituents of the oils were Z-citral and alpha-citral (*E. staigeriana*), citronellal (*E. citriodora*) and 1,8-cineole (*E. globulus*). The *Eucalyptus* essential oils constitute alternative natural products for the control of *L. longipalpis* since the median effective concentration (EC<sub>50</sub>) values revealed relevant action as compared with other natural products, some of their chemical constituents are already known for their insecticidal activity and these oils are produced in commercial scale in Brazil.

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### 1. Introduction

Visceral leishmaniasis (VL) is a serious chronic disease caused by protozoa of the *Leishmania* genus belonging to the *Leishmania* (*Leishmania*) *donovani* complex. In Brazil, the causative agent is *L. chagasi* (Gontijo and Melo, 2004). Sandflies, which are vectors for several species of *Leishmania*, comprise more than 40 species of *Phlebotomus* in the Old World and 30 *Lutzomyia* species in the Americas (Alexander and Maroli, 2003). *Lutzomyia long-*

*ipalpis* Lutz and Neiva 1912, is the main vector of this disease in Latin America, and, in Brazil alone it is responsible for 90% of disease cases (Lainson and Rangel, 2005).

Vector control using insecticides has been recommended by the World Health Organization (Gontijo and Melo, 2004). Mathematical models of the three methods of control (Dye, 1996) suggest that insecticide spraying and dog vaccination are better solutions to the problem than the euthanasia of serologically positive dogs (Tesh, 1995). In addition, acquired resistance and environmental pollution due to the repeated application of persistent synthetic insecticides have led to increased interest in new natural chemicals (Viegas-Júnior, 2003).

\* Corresponding author. Tel.: +55 3101 9853; fax: +55 3101 9840.  
E-mail address: [michellinevet@gmail.com](mailto:michellinevet@gmail.com) (M.V. Maciel).

In this context, screening of natural products has received the attention of researchers around the world, but seems to be particularly important for public health in developing countries. Since many diseases transmitted by insects (e.g., malaria, dengue, yellow fever, leishmaniasis and Chagas disease) are endemic in developing countries, the search for insecticides and repellents of botanical origin has been driven by the need to find new products that are effective, but also safer and cheaper than current products (De Paula et al., 2004). Additionally, people in our country like and sometimes prefer natural products than synthetics (Yaghoobi-Ershadi et al., 2006).

Many secondary plant metabolites are known for their insecticidal properties, and in many cases plants have a history of use as home remedies to kill or repel insects (Broussalis et al., 1999). In recent decades, research on the interactions between plants and insects has revealed the potential use of plant metabolites or allelochemicals for this purpose (Pavela, 2004). It is known that some chemical constituents of essential oils have insecticidal properties (Spitzer, 2004). In some studies, essential oils obtained from commercial sources were used. Specific compounds isolated from plant extracts or essential oils were tested for fumigation purposes (Rajendran and Sriranjini, 2008).

The Myrtaceae family comprises about 100 genera with approximately 3000 species of plants (Denardi and Marchiori, 2005). *Eucalyptus* is one of the most cultivated genera in the world, including more than 700 species belonging to this family. Various biological properties have already been attributed to the genus *Eucalyptus*, among them larvicidal activity on culicids (Cheng et al., 2009), insecticidal activity against beetles (Brito et al., 2006), and repellent action against *Phlebotomus papatasi* (Yaghoobi-Ershadi et al., 2006). In Brazil, the main species of *Eucalyptus* used to produce commercial essential oils are *E. staigeriana*, *E. citriodora* and *E. globulus* (Vitti and Brito, 2003). Since there have not been any reports about the use of these *Eucalyptus* oils against *L. longipalpis*, the objectives of this study were to determine the chemical composition of commercial essential oils of *E. staigeriana*, *E. citriodora* and *E. globules*, and to evaluate their insecticidal activity on the different developmental stages of *L. longipalpis* in the laboratory.

## 2. Materials and methods

### 2.1. Laboratory rearing of sandflies

The establishment and maintenance of the sandfly colony was based on the methods of Sherlock and Sherlock (1972), with some modifications. The vectors were captured in the municipality of Sobral, an endemic visceral leishmaniasis area in the State of Ceará, Brazil, using CDC traps and then brought to the entomology laboratory of the Vector Control Center (Health Secretariat of Ceará State). The insects were kept in nylon tulle cages inside a BOD incubator at a temperature of at 27 °C and 80% relative humidity (RH). The adult females were fed with anesthetized hamsters (10 mg/kg of Ketamine and 2 mg/kg of Xylazine by intramuscular injection). After 4 days, 25 females were placed for egg-laying in plastic pots that were internally coated with sterile plaster to maintain

moisture and filled with a substrate prepared from rabbit feces and crushed cassava leaves. Afterwards, several eggs were transferred to similar pots for hatching, and the formed larvae were maintained in this substrate until pupation. When the adults emerged, they were transferred to cages to continue the cycle.

Five treatments with three replicates were performed using different plant oil concentrations in all tests, as well as two negative controls, distilled water and Tween 80 (3%) and a positive control, cypermethrin (0.196 mg/ml).

### 2.2. Experimental design to assess the insecticide activity

In the *in vitro* tests on *L. longipalpis* eggs, aqueous solutions of plant oils were used at concentrations of 20, 10, 5, 2.5 and 1.2 mg/ml for *E. staigeriana* and 40, 20, 10, 5 and 2.5 mg/ml for both *E. citriodora* and *E. globulus*. Tests were performed at  $\pm 27$  °C and 80% RH using 30 eggs sprayed with 1 ml of each oil solution. The hatched larvae were counted for 10 days. In this experiment, 5 oil concentrations with 3 replicates/concentration per pot were used. Each pot contained 30 eggs of the vector, totaling 450 eggs. Thus, 1350 eggs were used for each oil in the experiment.

In the *in vitro* tests with larvae, forty eggs were transferred to plastic pots internally coated with sterile plaster containing a thin layer of food substrate for hatching. Six days after hatching, the larvae were counted and sprayed with oil solutions in the following concentrations: 5, 4, 3, 2 and 1 mg/ml for *E. staigeriana*; 6.5, 3.5, 1.6, 0.8 and 0.4 mg/ml for *E. citriodora* and 40, 35, 30, 25 and 20 mg/ml for *E. globulus*. The larvae were observed until pupation. In this experiment, the number of larvae used was the same as described for the eggs of the vector.

In the *in vitro* tests against *L. longipalpis* adults, each oil concentration (1 ml), cypermethrin or Tween 80 was applied to the inner surface and bottom of each pot using a pipette. Thirty adult *L. longipalpis* specimens (15 males and 15 females) were placed inside of the pots after the application of the oils, and the concentrations used were 5, 2.5, 1.2, 0.6 and 0.3 mg/ml for *E. staigeriana* oil and 10, 8, 6, 4 and 2 mg/ml for *E. citriodora* and *E. globulus* oils. In this experiment, the parameters observed were insect mortality after 24, 48 and 72 h, mortality rate differences between female and male insects and the number of eggs obtained from females subjected to the oils, the number of adults used was the same as described for the eggs and larvae of the vector.

### 2.3. GC and GC/MS analysis of essential oils

The *Eucalyptus* oils were purchased from Dierberger Óleos Essenciais Química Ltda. The essential oil was obtained by steam distillation using the plant leaves. The oils were analyzed by gas chromatography (GC) using a Varian CP-3800 gas chromatograph coupled to a computer equipped with a STAR WORKSTATION. The instrument was equipped with a 30-m fused silica capillary column (CP-Sil 8CB, Varian) with an internal diameter of 0.25 mm and a film thickness of 0.25  $\mu$ m. The hydrogen carrier gas had a delivery rate of 1.5 ml/min (controlled constant flow). The capillary injector operated

**Table 1**  
Relative percentage composition of leaf essential oils of *Eucalyptus* species.

Constituents	RI	<i>E. staigeriana</i>	<i>E. citriodora</i>	<i>E. globulus</i>
α-Pinene	928	3.27	1.1	4.15
o-Cymene	1021	1.76	–	2.93
(+) Limonene	1025	<b>28.82</b>	–	8.16
1,8-cineole	1029	5.39	0.8	<b>83.89</b>
α-Terpinolene	1081	9.4	–	–
(–) Isopulegol	1145	–	7.3	–
Beta-citronellal	1149	0.8	<b>71.77</b>	–
Isopulegol	1155	–	4.3	–
β-Citronellol	1223	–	2.9	–
Z-Citral	1235	<b>10.77</b>	–	–
Trans-geraniol	1247	4.2	–	–
E-Citral	1265	<b>14.16</b>	–	–
Methyl geranate	1317	3.66	–	–
Geraniol acetate	1374	3.86	–	–
Total		86.09	88.17	99.13

(–) means not detected.

The values in bold are to highlight the chemical constituents found in higher percentage in the essential oil.

at 250 °C in the split mode (1:100) and the flame ionization detector (FID) ran at 250 °C. The oven temperature program was 35 °C during injection, then increased from 35 to 180 °C at the rate of 4 °C/min, increased again to a final temperature of 280 °C at a rate of 17 °C/min and remained at 280 °C for 10 min.

Gas chromatography/mass spectrometry (GC/MS) was performed on a Hewlett-Packard 5971 instrument with a dimethylpolysiloxane DB-1 coated fused silica capillary column (30 m × 0.25 mm) and He as the carrier gas (1 ml/min). The injector temperature was 250 °C and the detector temperature was 200 °C. The column temperature program was 35–180 °C at 4 °C/min, then 180–250 °C at 10 °C/min. For MS, the electron impact was 70 eV. Compounds were identified by their GC retention time, expressed by Kovat's index, which was calculated by the Van den Dool and Kratz equation using a hydrocarbon homologous series and by comparison of test compound mass spectra with those present in the National Institute for Standard Technology computer data bank (NIST; 62,235 compounds) and published spectra (Adams, 2001).

#### 2.4. Statistical analysis

The data were processed with the formula  $\log(x + 1)$ , subjected to one-way variance analysis and compared by the Tukey test with 5% probability, using the Prism 3.0

program. The median effective concentration (EC<sub>50</sub>) values were calculated by using Probit SPSS 8.0 for Windows.

### 3. Results

The constituents of the commercial *Eucalyptus* oils are shown in Table 1. The major constituents of *E. staigeriana* oil were (+) limonene (28.82%), Z-citral (10.77%) and E-citral (14.16%); *E. citriodora* contained β-citronellal (71.77%) and *E. globulus* contained 1,8-cineole (83.89%). All the *Eucalyptus* oils studied were effective against the egg, larval and adult phases of *L. longipalpis*. However, *E. staigeriana* oil was the most effective on all three phases of the insect, followed by *E. citriodora* and *E. globulus* oils, respectively.

All tested oils inhibited egg hatching (Table 2). *E. staigeriana* oil was the most effective, since *E. citriodora* and *E. globulus* oils at a concentration of 40 mg/ml presented effectiveness of 94.68% and 92.73%, respectively, but *E. staigeriana* oil was 90.29% effective with 20 mg/ml ( $P > 0.05$ ). The EC<sub>50</sub> values of *E. staigeriana*, *E. citriodora* and *E. globulus* oils were 3.6 mg/ml (2.28–5.23), 9.44 mg/ml (2.47–35.25) and 9.23 mg/ml (6.73–12.59), respectively.

The results of the assays with insect larvae are shown in Table 3. *E. globulus* oil showed 100% effectiveness at 40 mg/ml, while *E. citriodora* and *E. staigeriana* oils showed this result at lower concentrations, 6.5 and 5 mg/ml, respectively ( $P > 0.05$ ). *E. citriodora* oil was superior to *E. globulus*

**Table 2**  
Efficacy (mean percentage ± S.D.) of essential oils of three species of *Eucalyptus* on the eggs of *Lutzomyia longipalpis*.

	<i>Eucalyptus staigeriana</i>		<i>Eucalyptus citriodora</i>		<i>Eucalyptus globulus</i>	
	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy
	20	90.29 ± 2.95 <sup>aA</sup>	40	94.68 ± 0.64 <sup>aA</sup>	40	92.73 ± 1.56 <sup>aA</sup>
	10	47.31 ± 0.38 <sup>bA</sup>	20	46.47 ± 2.97 <sup>bA</sup>	20	57.17 ± 1.70 <sup>bB</sup>
	5	26.19 ± 1.78 <sup>cA</sup>	10	20.46 ± 2.36 <sup>cB</sup>	10	22.55 ± 2.36 <sup>cAB</sup>
	2.5	15.03 ± 0.50 <sup>dA</sup>	5	11.95 ± 0.34 <sup>dA</sup>	5	13.00 ± 1.71 <sup>dA</sup>
	1.2	2.85 ± 1.38 <sup>eA</sup>	2.5	0.64 ± 1.12 <sup>eA</sup>	2.5	0.32 ± 0.56 <sup>eA</sup>
Cypermethrin	0.196	100.00 ± 0.00 <sup>f</sup>	0.196	100.00 ± 0.00 <sup>f</sup>	0.196	100.00 ± 0.00 <sup>f</sup>
Tween 80	3%	2.10 ± 1.85 <sup>g</sup>	3%	2.90 ± 0.43 <sup>f</sup>	3%	1.80 ± 0.75 <sup>g</sup>
Water		2.29 ± 0.37 <sup>g</sup>		1.14 ± 0.26 <sup>f</sup>		0.80 ± 0.73 <sup>g</sup>

Small letters compare means in the lines and capital letters in the columns.

**Table 3**Efficacy (mean percentage  $\pm$  S.D.) of essential oils of three species of *Eucalyptus* on the larvae of *Lutzomyia longipalpis*.

	<i>Eucalyptus staigeriana</i>		<i>Eucalyptus citriodora</i>		<i>Eucalyptus globulus</i>	
	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy
	5	100 $\pm$ 0.00 <sup>aA</sup>	6.5	100 $\pm$ 0.00 <sup>aA</sup>	40	100 $\pm$ 0.00 <sup>aA</sup>
	4	73.44 $\pm$ 1.64 <sup>bA</sup>	3.2	63.21 $\pm$ 1.35 <sup>bB</sup>	35	78.47 $\pm$ 3.20 <sup>bA</sup>
	3	51.43 $\pm$ 4.34 <sup>cA</sup>	1.6	37.54 $\pm$ 3.04 <sup>cB</sup>	30	59.22 $\pm$ 2.36 <sup>cC</sup>
	2	26.07 $\pm$ 3.47 <sup>dA</sup>	0.8	17.21 $\pm$ 1.66 <sup>dB</sup>	25	47.02 $\pm$ 2.23 <sup>dC</sup>
	1	5.32 $\pm$ 1.45 <sup>eA</sup>	0.4	5.72 $\pm$ 1.05 <sup>eA</sup>	20	30.39 $\pm$ 5.41 <sup>eB</sup>
Cypermethrin	0.196	100 $\pm$ 0.00 <sup>a</sup>	0.196	100 $\pm$ 0.00 <sup>a</sup>	0.196	100 $\pm$ 0.00 <sup>a</sup>
Tween 80	3%	2.71 $\pm$ 1.14 <sup>f</sup>	3%	2.29 $\pm$ 0.92 <sup>f</sup>	3%	1.60 $\pm$ 0.78 <sup>f</sup>
Water		2.84 $\pm$ 0.92 <sup>f</sup>		1.13 $\pm$ 0.64 <sup>f</sup>		1.47 $\pm$ 0.65 <sup>f</sup>

Small letters compare means in the lines and capital letters in the columns.

**Table 4**Efficacy (mean percentage  $\pm$  S.D.) of essential oils of three species of *Eucalyptus* in the first 24 h on adults of *Lutzomyia longipalpis*.

	<i>Eucalyptus staigeriana</i>		<i>Eucalyptus citriodora</i>		<i>Eucalyptus globulus</i>	
	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy
	5	99.62 $\pm$ 0.66 <sup>aA</sup>	10	88.13 $\pm$ 20.56 <sup>aA</sup>	10	95.50 $\pm$ 1.10 <sup>aA</sup>
	2.5	72.34 $\pm$ 1.85 <sup>bA</sup>	8	68.14 $\pm$ 2.60 <sup>bA</sup>	8	51.39 $\pm$ 5.73 <sup>bBA</sup>
	1.2	40.50 $\pm$ 2.87 <sup>cA</sup>	6	48.41 $\pm$ 4.04 <sup>cA</sup>	6	33.70 $\pm$ 0.37 <sup>cA</sup>
	0.6	12.48 $\pm$ 0.98 <sup>dA</sup>	4	31.01 $\pm$ 2.98 <sup>dB</sup>	4	18.70 $\pm$ 2.43 <sup>dAB</sup>
	0.3	4.90 $\pm$ 0.66 <sup>eA</sup>	2	10.60 $\pm$ 0.87 <sup>eA</sup>	2	4.10 $\pm$ 0.61 <sup>eA</sup>
Cypermethrin	0.196	100 $\pm$ 0.00 <sup>a</sup>	0.196	100 $\pm$ 0.00 <sup>a</sup>	0.196	100 $\pm$ 0.00 <sup>a</sup>
Tween 80	3%	0.61 $\pm$ 0.76 <sup>f</sup>	3%	1.11 $\pm$ 1.11 <sup>f</sup>	3%	1.38 $\pm$ 0.23 <sup>f</sup>
Water		0.49 $\pm$ 0.42 <sup>f</sup>		0.50 $\pm$ 0.43 <sup>f</sup>		0.48 $\pm$ 0.45 <sup>f</sup>

Small letters compare means in the lines and capital letters in the columns.

oil at all tested concentrations ( $P < 0.001$ ). The EC<sub>50</sub> values of *E. staigeriana*, *E. citriodora* and *E. globulus* oils were 2.63 mg/ml (1.69–3.67), 1.78 mg/ml (1.41–2.26) and 25.29 mg/ml (17.12–30.14), respectively.

Insecticide effects on adult sandflies after 24, 48 and 72 h of observation are shown in Tables 4–6. Of all compounds tested, *E. staigeriana* was the best. After 24 h, the *E. citriodora* and *E. globulus* oils at a concentration of 10 mg/ml were 88.13% and 95.50% effective, respectively, while *E. staigeriana* oil was 99.62% effective at a concentration of 5 mg/ml ( $P > 0.05$ ). After 48 h, *E. citriodora* oil was more effective than *E. globulus* oil at a concentration of 8 mg/ml ( $P < 0.01$ ). This difference persisted at 72 h of observation.

There were no statistical differences in the mortality rates of male and female insects ( $P > 0.05$ ). The EC<sub>50</sub> values of the *E. staigeriana*, *E. citriodora* and *E. globulus* oils were 0.59 mg/ml (0.37–0.82), 5.04 mg/ml (2.26–8.78) and 7.78 mg/ml (6.54–13.28), respectively. The effectiveness of the oils persisted through the whole observation period,

but had a tendency to diminish. However, this difference was only statistically significant at the 2.5 mg/ml ( $P < 0.01$ ) and 1.2 mg/ml ( $P < 0.001$ ) concentrations for *E. staigeriana*. There were no statistically significant differences in the number of eggs obtained from females or in larvae hatched from the eggs of the females subjected to the *Eucalyptus* spp. oils ( $P > 0.05$ ).

#### 4. Discussion

Visceral leishmaniasis is a zoonosis of great importance for public health and veterinary medicine. *L. longipalpis* is the main vector of this disease in Brazil. With the expansion of endemic areas and the significant increase in the number of cases, VL is considered by the World Health Organization to be a priority among tropical diseases (Gontijo and Melo, 2004).

In highly endemic areas, such as the states of North-eastern Brazil, attempts have been made to control VL on

**Table 5**Efficacy (mean percentage  $\pm$  S.D.) of essential oils of three species of *Eucalyptus* between 24 and 48 h on adults of *Lutzomyia longipalpis*.

	<i>Eucalyptus staigeriana</i>		<i>Eucalyptus citriodora</i>		<i>Eucalyptus globulus</i>	
	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy
	5	100.0 $\pm$ 0.0 <sup>aA</sup>	10	100 $\pm$ 0.0 <sup>aA</sup>	10	96.63 $\pm$ 0.72 <sup>aA</sup>
	2.5	72.24 $\pm$ 2.54 <sup>bA</sup>	8	70.14 $\pm$ 2.27 <sup>bA</sup>	8	52.53 $\pm$ 4.41 <sup>bB</sup>
	1.2	39.39 $\pm$ 4.32 <sup>cA</sup>	6	48.70 $\pm$ 3.35 <sup>cB</sup>	6	34.01 $\pm$ 2.30 <sup>cA</sup>
	0.6	12.76 $\pm$ 1.61 <sup>dA</sup>	4	29.79 $\pm$ 3.52 <sup>dB</sup>	4	15.51 $\pm$ 3.76 <sup>dA</sup>
	0.3	4.26 $\pm$ 1.26 <sup>eA</sup>	2	6.84 $\pm$ 1.70 <sup>eA</sup>	2	2.22 $\pm$ 0.86 <sup>eA</sup>
Cypermethrin	0.196	100 $\pm$ 0.00 <sup>a</sup>	0.196	100 $\pm$ 0.00 <sup>a</sup>	0.196	100 $\pm$ 0.00 <sup>a</sup>
Tween 80	3%	1.22 $\pm$ 1.08 <sup>f</sup>	3%	0.0 $\pm$ 0.0 <sup>f</sup>	3%	2.22 $\pm$ 1.28 <sup>f</sup>
Water		0.81 $\pm$ 0.02 <sup>f</sup>		1.39 $\pm$ 0.63 <sup>f</sup>		1.38 $\pm$ 0.48 <sup>f</sup>

Small letters compare means in the lines and capital letters in the columns.

**Table 6**  
Efficacy (mean percentage  $\pm$  S.D.) of essential oils of three species of *Eucalyptus* between 48 and 72 h on adults of *Lutzomyia longipalpis*.

	<i>Eucalyptus staigeriana</i>		<i>Eucalyptus citriodora</i>		<i>Eucalyptus globulus</i>	
	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy	Concentration (mg/ml)	% Efficacy
	5	100.00 $\pm$ 0.0 <sup>aA</sup>	10	100.00 $\pm$ 0.0 <sup>aA</sup>	10	96.47 $\pm$ 0.09 <sup>aA</sup>
	2.5	65.81 $\pm$ 0.38 <sup>bA</sup>	8	70.07 $\pm$ 2.58 <sup>bA</sup>	8	47.64 $\pm$ 1.33 <sup>bB</sup>
	1.2	32.34 $\pm$ 3.64 <sup>cA</sup>	6	45.25 $\pm$ 3.53 <sup>cB</sup>	6	25.80 $\pm$ 3.77 <sup>cA</sup>
	0.6	11.75 $\pm$ 0.62 <sup>dA</sup>	4	23.76 $\pm$ 0.61 <sup>dB</sup>	4	10.56 $\pm$ 1.61 <sup>dA</sup>
	0.3	1.66 $\pm$ 2.36 <sup>eFA</sup>	2	7.13 $\pm$ 2.90 <sup>eFA</sup>	2	3.13 $\pm$ 0.33 <sup>eFA</sup>
Cypermethrin	0.196	100 $\pm$ 0.00 <sup>a</sup>	0.196	100 $\pm$ 0.00 <sup>a</sup>	0.196	100 $\pm$ 0.00 <sup>a</sup>
Tween 80	3%	0.21 $\pm$ 0.36 <sup>f</sup>	3%	0.37 $\pm$ 0.32 <sup>f</sup>	3%	2.16 $\pm$ 1.9 <sup>f</sup>
Water		1.65 $\pm$ 0.77 <sup>f</sup>		3.00 $\pm$ 1.7 <sup>f</sup>		1.65 $\pm$ 0.77 <sup>f</sup>

Small letters compare means in the lines and capital letters in the columns.

three fronts: patient treatment, sacrifice of infected dogs and spraying of insecticides (Lainson and Rangel, 2005). Currently, residual insecticides are sprayed on house walls as well as inside chicken coops, pigsties and stables (Cabrera, 1999). In Brazil, these actions have always been sporadic and have failed to eradicate this disease, with reinfestation and resurgence of human and canine cases of VL (Gontijo and Melo, 2004). The first case of insecticide resistance was reported in Bihar, India, where *Phlebotomus papatasi* survived exposure to DDT at doses of 4% and 8% (Khaul et al., 1994).

Acquired resistance and environmental pollution due to repeated applications of persistent synthetic insecticides have created interest in discovering new natural insecticide products (Viegas-Júnior, 2003). The use of plants with insecticidal activity has several advantages over the use of synthetic products: natural insecticides are obtained from renewable resources and quickly degrade, the development of insect resistance to these substances is slow, the substances do not leave residues in the environment, they are easily obtained by farmers and they cost less to produce (Roel, 2001). The effects of essential oils on insects have been the subject of several studies. These oils are formed by a complex mixture of volatile constituents originating from the secondary metabolism of plants and are characterized by a strong scent (Bakkali et al., 2008).

The components in essential oils vary not only with plant species but also in relation to climate, soil composition, part of the plant and age of the plant. Many essential oils are composed of a variety of terpenoid compounds (De Paula et al., 2004). These substances are usually volatile and can be detected by the antennae or tarsi of insects. The major terpenoids contained in essential oils are monoterpenoids (citronellal, linalol, menthol, pinene, mentone, carvone and limonene), sesquiterpenoids (farnesol, nerolidol) and phenylpropanoids (safrol, eugenol), among other compounds (Spitzer, 2004). The great majority of the literature on the effects of terpenoids on insects has reported growth inhibition, impaired maturation, reduced reproductive capacity, appetite suppression and death of predator insects by starvation or direct toxicity (Viegas-Júnior, 2003). The monoterpene limonene demonstrated insecticidal activity by penetrating the cuticle of the insect (contact effect), by respiration (fumigant effect) and through the digestive system (ingestion effect) (Prates et al., 1998).

The essential oils of *Cymbopogon citratus*, *Lippia sidoides*, *Ocimum americanum* and *Ocimum gratissimum* showed good larvicidal activity against *Aedes aegypti*. The main constituents of these oils are the monoterpenoids geraniol and citral for *C. citratus*, thymol for *L. sidoides*, E-methylcinnamate for *O. americanum* and eugenol and 1,8-cineole for *O. gratissimum* (Cavalcante et al., 2006). The essential oil of *Myroxylum balsamum* presented good larvicidal activity against *A. aegypti* larvae, and the monoterpenes beta- and alpha-pinene were the main constituents (Simas et al., 2004).

The egg phase of *L. longipalpis* was the most resistant to the *Eucalyptus* oils, needing higher doses to obtain a better effect. Similar results were obtained with the compound 1,8-cineole on eggs, larvae and adults of *Tribolium confusum*. One hypothesis to explain the greater tolerance of the eggs is that the neurotoxic action of this compound acts only after nervous system of the embryo begins to grow. Another possible explanation is the lesser permeability of the egg surface at the beginning of embryogenesis (Stamopoulos et al., 2007). The constituents of *Eucalyptus* spp. oils were also tested on lice. 1,8-Cineole acted on eggs of the louse *Pediculus humanus capitis*, obtaining 67% effectiveness at a concentration of 1 mg/cm<sup>2</sup> (Yang et al., 2004).

The essential oil of *E. staigeriana* was the most effective on *L. longipalpis* larvae, followed by *E. citriodora* and *E. globulus*. These essential oils and the emulsified concentrate were tested against larvae and engorged females of the tick *Boophilus microplus*, in search of an acaricide less damaging to the environment. Citronellal, the main component of *E. citriodora* oil, and 1,8-cineole in *E. globulus* were considered responsible for their acaricidal action. *E. staigeriana* essential oil showed the best acaricidal action, and several substances acted in synergy against *B. microplus* (Chagas et al., 2002).

Studies performed with sandflies and plant insecticides are few. The compound azadirachtin added to the *L. longipalpis* larvae diet at 0.1, 1.0 and 10.0  $\mu$ g concentrations increased larval mortality compared to a control group (Coelho et al., 2006). Concentrations of 0.1 and 1.0  $\mu$ g/mg azadirachtin in the diet prevented the larvae from hatching. The bioactivity of phytochemicals against mosquito larvae can vary significantly in relation to plant species, part and age, as well as the solvent used in the extraction and the mosquito species involved (Shalan et al., 2005).



*E. staigeriana* oil was the most effective on adult insects, promoting mortality of  $99.62 \pm 0.66\%$  at a concentration of 5 mg/ml. *E. citriodora* and *E. globulus* oils were similarly effective at all tested concentrations, except for 8 mg/ml after 48 and 72 h. Lower efficacy was shown by aqueous extracts of *Antonia ovata* and *Derris amazonica* on *L. longipalpis* adults after 48 h of observation (Luitgards-Moura et al., 2002). The  $EC_{50}$  values were 233 and 212 mg/ml, respectively. However, when testing 1,8-cineole on males and females of *Musca domestica* and *Chrysomya megacephala*,  $EC_{50}$  values obtained of 118  $\mu\text{g}/\text{fly}$  (males, *Musca*) 177  $\mu\text{g}/\text{fly}$  (females, *Musca*), 197  $\mu\text{g}/\text{fly}$  (males, *Chrysomya*) and 221  $\mu\text{g}/\text{fly}$  (females, *Chrysomya*). With a topical application of 1,8-cineole on *M. domestica* adults, males were more susceptible than females (Sukontason et al., 2004).

Essential oils from *Citrus sinensis* was the most effective of five oils tested on adult *Culex pipiens quiquefasciatus* (Yang et al., 2005). Chemical analysis of this oil showed that *E*-citral and *Z*-citral were the major constituents (69.27%); however, citral tested separately was more effective against the insect in a short period of time. The  $EC_{50}$  values for *C. sinensis* oil and citral were, respectively, 0.0133% and 0.0012% (Yang et al., 2005). 1,8-Cineole on 10-day-old adult males and females of the coleopteron *Tribolium confusum* obtained a  $EC_{50}$  of 7.0  $\mu\text{l}/\text{l}$  for both sexes. The same experiment performed with 40-day-old males and females showed a lower  $EC_{50}$  (Stamopoulos et al., 2007). These data can explain the effectiveness of *Eucalyptus* oils on the three phases of *L. longipalpis* development. However, little information exists about the mechanism of action of the essential oils. One of the hypotheses suggested is that oil inhalation can kill the insect (Yang et al., 2005). Besides this, it is known that some terpenoids inhibit acetylcholinesterase activity. Another hypothesis is that the monoterpenes act on other vulnerable sites, such as cytochrome P450 (Lee et al., 2001), but understanding the real mechanism of action of these oils will require further investigation (Tsukamoto et al., 2005).

In this study, three essential oils of *Eucalyptus* were tested and shown to be effective on the developmental phases of *L. longipalpis* in the laboratory. The chemical compositions were different for each oil, in accordance with literature data, and the main constituents were tested in other insects and showed good activities. *E. staigeriana* oil was the most effective on all insect phases, and this oil constitutes a viable alternative for control of the vector of visceral leishmaniasis.

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