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Yield, chemical composition, and bioactivity of essential oils from 12 species of *Eucalyptus* on *Aedes aegypti* larvae

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Abstract

The insecticidal activity of essential oils from 12 species of *Eucalyptus* (Myrtaceae) was evaluated on larvae of *Aedes aegypti* (L.) (Diptera: Culicidae), the most important vector of dengue and yellow fever in the Americas. Oils were obtained by hydrodistillation and their chemical composition was determined by gas chromatography coupled to mass spectrometry; yields ranged from 0.2 to 2.5%. Essential oils were mainly composed of 1,8-cineole, α-pinene, α-phellandrene, β-phellandrene, γ-terpinene, 4-terpineol, α-terpineol, *p*-cymene, and spathulenol. Larvicidal effects were tested on susceptible third or fourth stage *Ae. aegypti* larvae, determining median lethal concentration (LC_{50}) and median effective concentration (EC_{50}). Essential oils from *Eucalyptus dunnii* (Maiden), *Eucalyptus gunnii* (Hook), *Eucalyptus tereticornis* (Smith), *Eucalyptus camaldulensis* (Dehn), and *Eucalyptus saligna* (Smith) showed the best larvicidal activities with LC_{50} values of 25.2, 21.1, 22.1, 26.8, and 22.2, respectively. No significant differences were observed between LC_{50} and EC_{50} values of the same oil. Regression analysis revealed a significant relationship between total essential oil yields and 1,8-cineole concentration. Significant relationships were also revealed between larval mortality and the concentration of 1,8-cineole and *p*-cymene. This indicated that *Eucalyptus* species with high oil yields have higher 1,8-cineole concentrations and lower *p*-cymene concentrations and have less effect on *Ae. aegypti*. Our results suggest the potential of controlled crossing methods to obtain *Eucalyptus* trees with chemical profiles having enhanced activity against this mosquito.

Introduction

Several mosquito species attract attention due to their medical importance. In this sense, *Aedes aegypti* (L.) (Diptera: Culicidae) is one of the most important species in the world. In addition to its role as the main urban vector of the yellow fever virus, it is also the primary vector of dengue viruses (Eldridge, 2005). Both dengue fever (DF) and dengue hemorragic fever (DHF) are the most important and serious mosquito-borne diseases in Argentina (Carbajo et al., 2001).

During the 1950s and 1960s, more than 20 countries in Latin America were able to eradicate *Ae. aegypti*, but in the last decade almost all these countries, including Argentina, have become re-infested (Boffi, 2002). In northern Argentina, this re-infestation began in 1997 and, as a result, there have been quite a few sizable epidemics of DF (Aviles et al., 1999). With no vaccines available, controlling the vector becomes essential for ending epidemics in these tropical areas.

Temephos, an organophosphate insecticide, has been used in the form of sand granules (Abate® 1% SG) at a concentration of 1 g/10 l water (1 p.p.m. active ingredient) for controlling *Ae. aegypti* larvae in large-scale treatments. Although this product has a good larvicidal effect lasting several weeks or months in treated water tanks, and is of very low cost, it is frequently rejected by the general population for use in their water tanks due to its strong smell and slight turbidity. Additionally, a disadvantage is its non-target effects on beneficial aquatic arthropods and vertebrates, as

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well as the development of resistance in *Aedes* larvae (Mulla et al., 1986). *Aedes aegypti* already exhibits resistance to various insecticides and this resistance has spread widely in America (WHO, 1992; Pereira et al., 2005). In Brazil, acquired resistance to temephos has also been reported (Lima et al., 2003; Macoris et al., 2003; Braga et al., 2004).

Biological larvicides, such as *Bacillus thuringiensis* serovar *israelensis* (Bti), and insect growth regulators, such as methoprene or novaluron, could be useful alternatives for controlling mosquito larvae (Chavasse & Yap, 1997; Mulla et al., 2003), but large-scale treatments are expensive. Another alternative to conventional insecticides is the use of botanical products. It has been shown that plants produce chemical defense compounds against micro-orgasnisms and predators. These chemicals are natural candidates for the development of new products to control *Ae. aegypti* (Chantraine, 1998; Ciccia et al., 2000; Yang et al., 2002; Cheng, 2003; Barreira et al., 2004). Essential oils are special plant products composed of volatile substances found in a wide variety of species (Weinzieri, 1994, 2000). Their active components are isoprenoid compounds, mainly mono- and sesquiterpenes, which are responsible for the odor of aromatic plants (Franzios et al., 1997).

Some phytochemicals act as general toxicants to all life stages of mosquitoes, whereas others interfere with growth and reproduction, or act on the olfactory receptors, eliciting responses of attractancy or repellency (Sukumar et al., 1991). The mechanism of the toxic action of terpenoids is still obscure; however, the onset of toxic signs is usually rapid (Enam, 2001), indicating a neurotoxic mode of action in some pests (Isman, 2006). For example, Grundy & Still (1985) and Ryan & Byrne (1988) reported that these oils were competitive inhibitors of acetylcholinesterase, while others suggested that another possible target for essential oil activity is the octopaminergic system of insects (Enam, 2001; Kostyukovsky et al., 2002). On the other hand, Priestley et al. (2003) showed interference with γ-amino-butyric acid-gated chloride channels.

Some essential oils have been reported to have certain insect growth regulator properties, inhibiting adult emergence in addition to their larvicidal effects (Shaalan et al., 2005). Thus, it was interesting to evaluate the lethal concentration (LC) needed to obtain 50% mortality of larvae, and the effective concentration (EC) for a 50% inhibition of adult emergence (Shaalan et al., 2005), and to observe whether any of the essential oils studied interfere with the growth of *Ae. aegypti* larvae following a 24-h exposure.

Eucalyptus oils are obtained by hydrodistillation of the leaves and have a distinct species-characteristic aroma. The most important species, in terms of volume of oil production and trade, is the medicinal type, characterized by a high 1,8-cineole content in the oil (Coppen, 1995).

Taking into account the need to develop new mosquito larvicides with more favorable environmental properties and new modes of action to avoid the development of resistance, we studied the potential effect of some botanical products derived from *Eucalyptus* species on *Ae. aegypti* larvae. In this study, we present the chemical composition of 12 *Eucalyptus* essential oils and their corresponding bioactivity on *Ae. aegypti*. We also investigated the relationship between the essential oil yield of each *Eucalyptus* species, their percentage of 1,8-cineole and *p*-cymene, and their larvicidal effect on *Ae. aegypti* (expressed as larval mortality at 40 p.p.m.).

Materials and methods

Plant material

We used seeds that were 1 year old and 15 cm long. Those of *Eucalyptus saligna* (Smith), *Eucalyptus dunnii* (Maiden), *Eucalyptus globulus* ssp. *maidenii* (F. v. Muell), *Eucalyptus globulus* ssp. *globulus* (Labill), *Eucalyptus viminalis* (Labill), *Eucalyptus tereticornis* (Smith), *Eucalyptus camaldulensis* (Dehn), and two clonal hybrids (*Eucalyptus grandis* × *tereticornis* and *Eucalyptus grandis* × *camaldulensis*) were purchased from a forest tree nursery (Paul Forestal; INASE Register Number J/5188), San Isidro, Buenos Aires, Argentina. Seeds of *Eucalyptus cinerea* (F. v. Muell. ex Benth), *Eucalyptus sideroxylon* (Cunn), and *Eucalyptus gunnii* (Hook) were purchased from another forest tree nursery, Ferrari Hnos (INASE Register Number JL/2122), La Plata, Buenos Aires, Argentina. They were planted in an experimental plot at the Centro de Investigaciones de Plagas e Insecticidas (CITEFA-CONICET) (34°33′S, 58°30′W) located in Villa Martelli, Buenos Aires, Argentina. We collected fresh leaves from the plants in March 2006 after 18 months.

Extraction and essential oil yield

Samples from at least 800 g of fresh plant material of each *Eucalyptus* species were extracted during 70 min, using the hydrodistillation method in a modified Clevenger-type apparatus (Bandoni, 2002). The essential oil extract was then cooled, separated from the cohobated water, dehydrated with anhydrous sodium sulfate, and stored at −4 °C until use.

Essential oil yields were expressed as essential oil weight/100 g fresh plant material (% wt/wt).

Essential oil analysis

The chemical composition of *Eucalyptus* essential oils was determined by gas chromatography coupled to mass spectrometry using a GCMS-QM 5050A instrument (Shimadzu, Kyoto, Japan). Gas chromatography conditions were as follows: injection of 0.4 μl of a hexane solution of *Eucalyptus* essential oil (1 mg/ml); capillary column HP-1

(cross-linked methyl silicone gum) (50 m × 0.32 mm × 0.52 μm); and helium as carrier gas (1.3 ml/min). The analytical conditions were: injector and interface temperatures of 250 and 280 °C, respectively, split ratio of 13:1, initial isothermic temperature of 50 °C during 10 min, programed temperature of 50 to 68 °C (1 °C/min), programed temperature of 68 to 75 °C (0.5 °C/min), programed temperature of 75 to 250 °C (20 °C/min), final isothermic temperature of 280 °C during 10 min; and electron impact 70 eV.

Compounds identified in the samples were confirmed by comparing their GC retention times with standards through a comparison of the mass spectra with available NIST or Wiley mass spectral library resident in the system. Quantification of essential oil components (expressed in relative percentage on total area of chromatogram) was carried out by peak area normalization measurements.

Chemicals and biological material

α-Terpineol (90%), *p*-cymene (99%), 4-terpineol (96%), γ-terpinene (97%), β-pinene (97%), and α-pinene (97%) were purchased from Sigma-Aldrich (Buenos Aires, Argentina) and 1,8-cineole (99%) from Fritzsche (Buenos Aires, Argentina).

A susceptible CIPEIN strain of *Ae. aegypti*, originating from the Rockefeller strain of Venezuela, was used in all bioassays. The laboratory colony has been kept in the laboratory since 1996, free of exposure to pathogens, insecticides, or repellents, at 25–30 °C, 80–90% r.h., and L12:D12 photoperiod. Under these conditions, full development from egg to adult takes about 1 week. Mosquitoes were fed on pigeons. Eggs were collected on a wet filter paper and kept under the same conditions for 48 h. They were then dehydrated at room temperature and stored for at least 30 days. Eggs were re-hydrated in dechlorinated water (500 eggs/2 l water) at 25 ± 2 °C, and 24 h later the first instars were observed. Larvae were fed on rabbit food pellets and yeast, and used as third or early fourth instar for bioassays.

Larvicidal bioassay

The larvicidal bioassay was performed according to a protocol established during a meeting of the Latin American Network for Vector Control held in Iguazú (Misiones, Argentina) in December 2004 (Bisset et al., 2005), with some minor modifications. A 1-ml sample of an acetone solution of the experimental essential oil was added to 224 ml of distilled water in a 500-ml plastic jar, and shaken lightly to ensure a homogeneous test solution. Then, 20 late third or early fourth instars held in 25 ml distilled water were added to the experimental jar. A control solution was prepared using 1 ml acetone in 249 ml distilled water, while the untreated solution contained just 250 ml distilled water. All bioassays were conducted in a 27 ± 2 °C regulated chamber with L12:D12 photoperiod.

Larval mortality and survival were recorded after 24 and 48 h of exposure, during which no larval food was added. After 48 h of exposure, 5 mg of larval food was added to each 500-ml plastic jar, and maintained until more than 90% of the control mosquitoes either emerged or died. The amount of adults that did not emerge was recorded. Larvae were considered dead when they failed to move. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time), or those exhibiting the characteristic diving reaction when the water was disturbed. After 48 h, dead larvae were eliminated and not replaced.

Essential oils were tested at a final concentration of 10, 20, 30, 40, 50, 60, and 100 p.p.m. in 250 ml water. Three replicates were made for each treatment. A final concentration of 40 p.p.m. was eventually used, as it proved to be a limit for larvicidal activity for all the essential oils. To evaluate the presence of toxic effects due to the hydrophobic properties of water-insoluble essential oils, a positive control using an acetone solution of vegetable oil with a final concentration of 100 p.p.m. was performed.

Median lethal concentration (LC_{50}) values were calculated after 24 and 48 h of exposure. The effective concentration to reduce adult emergence by 50% (EC_{50}) was assessed twice: on the day that 100% of the control mosquitoes emerged as adults, and when the last specimen of the experimental group emerged or died. EC_{50} values were subsequently calculated.

Statistical analysis

Dose–mortality data from each *Ae. aegypti* pool were subjected to Probit analysis (Lichfield & Wilcoxon, 1949). LC_{50} and EC_{50} values of the essential oils were obtained using Micro Probit 3.0 software (T.C. Sparks & A. Sparks, Lily Research Labs, Greenfield, IN, USA) and expressed as p.p.m. final concentration. Regression analysis was performed using Statistical Graphics SGWIN® software (Statgraphics Plus 4.0; Statistical Graphics Corporation, 1994–1999, Herndon, VA, USA). The values of LC_{50} and EC_{50} were considered to be significantly different, if the 95% confidence limits did not overlap.

Results and discussion

The main essential oil component of *E. cinerea*, *E. globulus* ssp. *maidenii*, *E. globulus* ssp. *globulus*, *E. sideroxylon*, and *E. viminalis* was 1,8-cineole, whereas the main components of *E. grandis* × *E. tereticornis* and *E. grandis* × *E. camaldulensis* were 1,8-cineole and α-pinene (Table 1). *Eucalyptus tereticornis* and *E. camaldulensis* had α-phellandrene,

Table 1 Chemical composition of essential oils from 12 species of *Eucalyptus*, expressed as relative percentage of the total area of the chromatogram

	Plant species[2]											
Oil constituents[1]	1	2	3	4	5	6	7	8	9	10	11	12
α-Thujene	–	–	1.99	1.70	–	–	–	<1	–	–	–	–
α-Pinene	22.80	30.65	1.35	<1	12.95	4.94	5.45	4.04	5.84	11.09	1.95	1.16
Camphene	–	<1	–	–	–	–	–	–	–	–	–	–
β-Pinene	3.29	1.10	–	–	–	–	–	–	–	–	–	–
Sabinene	–	–	1.26	1.05	–	–	–	–	–	–	–	–
Myrcene	–	–	1.54	1.21	–	<1	–	<1	<1	<1	–	<1
α-Phellandrene	–	–	9.44	6.45	–	–	1.37	7.05	–	–	–	<1
Linalool acetate	–	1.43	–	–	–	–	–	–	–	–	–	–
α-Terpinene	–	–	<1	<1	–	–	–	–	–	–	–	–
p-Cymene	1.92	2.77	14.51	17.93	21.25	1.63	4.43	12.28	2.31	–	1.77	1.93
β-Phellandrene	–	–	22.64	16.34	–	–	–	–	–	–	–	–
1,8-Cineole	63.04	49.65	18.59	19.13	34.02	79.84	48.48	17.95	77.91	76.66	91.27	85.03
Cis-ocimene	–	–	–	–	–	–	<1	1.14	–	–	–	–
γ-Terpinene	–	–	2.25	1.87	20.13	–	12.96	3.35	4.82	<1	–	–
α-Terpinolene	–	–	–	<1	–	–	<1	–	–	–	–	–
Linalool	–	–	<1	3.08	–	–	–	–	–	–	–	–
p-Menthane-2-en-1-ol	–	–	<1	<1	–	–	–	–	–	–	–	–
p-Menthane-3,8-diol	–	–	–	–	–	–	–	–	–	–	–	–
Fenchol	<1	<1	–	–	–	–	–	–	–	–	–	–
Cryptone	–	–	4.14	5.71		–	–	–	–	–	–	–
α-Campholene aldehyde	<1	<1	–	–	1.15	–	–	–	–	–	–	–
Trans-pinocarveol	1.22	2.75	–	–	<1	–	–	–	–	–	–	–
Borneol	1	1.42	–	–	<1	–	–	–	–	–	–	–
4-Terpineol	<1	<1	5.83	6.73	2.63	<1	3.56	1.86	1.58	<1	<1	<1
α-Terpineol	2.55	4.05	1.59	–	3.45	3.79	3.59	1.38	6.04	1.45	2.61	1.75
α-Terpineol acetate	–	–	–	–		8.20	5.14		–	3.98	–	–
Bicycloelemene	–	–	–	–	–	–	–	1.71	–	–	–	–
β-Elemene	–	–	–	–	–	–	–	<1	–	–	–	–
Trans-caryophyllene	–	–	–	–	–	–	–	2.12	–	–	–	–
α-Gurjunene	–	–	–	–	–	–	<1		–	–	–	
Aromadendrene	–	–	–	–	–	–	3.40	1.21	–	1.11	–	1.98
α-Humulene	–	–	–	–	–	–		<1	–	–	–	–
Alloaromadendrene	–	–	–	–	–	–	< 1	1.67	–	–	–	–
Trans-carveol	–	–	–	–	<1	–	–	–	–	–	–	–
Carvacrol	–	–	–	–	<1	–	–	–	–	–	–	–
4-Isopropilbenzaldehyde	–	–	1.32	2.60		–	–	–	–	–	–	–
Phellandral	–	–	<1	1.45	–	–	–	–	–	–	–	–
γ-Elemene	–	–	<1	–	–	–	–	–	–	–	–	–
Caryophyllene oxide	–	–	1.69	–	–	–	–	–	–	–	–	–
Epiglobulol	–	–	–	–	–	–	<1	<1	–	–	–	1.24
Spathulenol	<1	<1	6.83	7.32	–	–		12.28	–	–	–	
Globulol	<1	–	–	–	–	–	4.15	<1	–	–	–	2.49
Unidentified compounds	1.56	2.66	2.01	2.96	2.43	1.22	3.29	16.20	0.70	1.70	1.64	1.85

[1]Main constituents of essential oils, determined with gas chromatography-mass spectrometry.

[2]Numbers represent the *Eucalyptus* species; 1, *E. grandis* × *E. tereticornis*; 2, *E. grandis* × *E. camaldulensis*; 3, *E. tereticornis*; 4, *E. camaldulensis*; 5, *E. saligna*; 6, *E. cinerea*; 7, *E. dunnii*; 8, *E. gunnii*; 9, *E. globulus* ssp. *maidenii*; 10, *E. globulus* ssp. *globulus*; 11, *E. sideroxylon*; and 12, *E. viminalis*. Unidentified compounds: less than 90% similarity index.

Table 2 Essential oil yields (% wt/wt) of 12 *Eucalyptus* species, expressed as the mean (SD) of four replicates

Species	Yield ± SD
E. cinerea	2.48 ± 0.09
E. globulus ssp. *maidenii*	2.25 ± 0.05
E. globulus ssp. *globulus*	1.66 ± 0.05
E. sideroxylon	1.65 ± 0.06
E. viminalis	1.46 ± 0.05
E. grandis × *E. tereticornis*	0.88 ± 0.02
E. dunnii	0.62 ± 0.02
E. tereticornis	0.59 ± 0.03
E. grandis × *E. camaldulensis*	0.54 ± 0.04
E. camaldulensis	0.38 ± 0.01
E. saligna	0.36 ± 0.01
E. gunnii	0.21 ± 0.01

β-phellandrene, and *p*-cymene as main essential oils in addition to 1,8-cineole. The essential oils of *E. dunnii* and *E. gunnii* had a complex composition, with 1,8-cineole, γ-terpinene, *p*-cymene, and spathulenol as main components (Table 1).

Essential oil yields, expressed as distillated oil weight/100 g fresh plant material, ranged from 0.2 to 2.5% (Table 2). Regression analysis showed a statistically significant relationship ($P<0.01$) between essential oil yield and the content of 1,8-cineole. The resulting fitted regression equation was: essential oil yield = exp (–1.619 + 0.026 × % 1,8-cineole). The coefficient of determination indicated that the model explains 81.9% of the variability in essential oil yields after transforming the data to a logarithmic scale in order to linearize the model. The standard deviation of the residuals was 0.356. The total essential oil yields were different for each species and the results showed that yields are higher when the main component of the essential oils was 1,8-cineole (Figure 1).

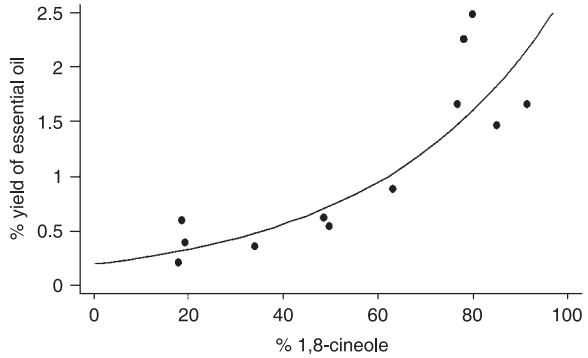

Figure 1 Relationship between essential oil yield and percentage of 1,8-cineole. Each point represents the mean of four values of essential oil yield from the leaves of each of 12 *Eucalyptus* species with their corresponding 1,8-cineole concentration.

Previous results obtained on the larvicidal activity of *Eucalyptus* essential oil components on *Ae. aegypti* showed that α- and β-pinene had the lowest LC_{50} values (15.4 and 12.1 p.p.m., respectively), while pure 1,8-cineole (99%, Fritzsche) had an LC_{50} of 57.2 p.p.m. (Lucia et al., 2007). The larvicidal effect of pure essential oil components after 24-h exposure to a final concentration of 40 p.p.m. was assessed from four replicates. *p*-Cymene exhibited a mortality rate of 100%, whereas pure 1,8-cineole had a mortality rate of 36% (±8.5). No larval mortality was observed in the control solution or in the positive control. *Eucalyptus* species that have essential oils with a high content of 1,8-cineole and a low concentration of *p*-cymene (*E. cinerea*, *E. globulus* ssp. *maidenii*, *E. globulus* ssp. *globulus*, *E. sideroxylon*, *E. viminalis*, *E. grandis* × *E. tereticornis*, and *E. grandis* × *E. camaldulensis*) (Table 1) had a lower larvicidal effect on *Ae. aegypti* (larval mortality <50% after 24 h at 40 p.p.m. final concentration) (Table 3).

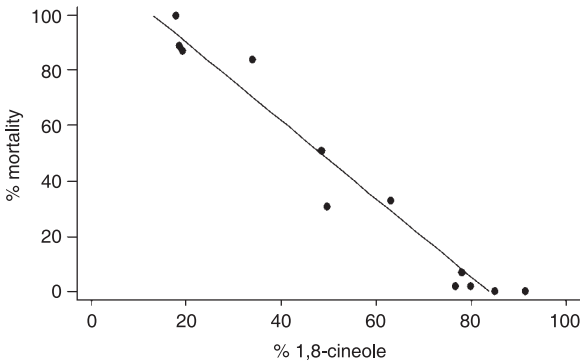

Figure 2 Relationship between *Aedes aegypti* larval mortality and the percentage of 1,8-cineole in essential oils from 12 species of *Eucalyptus*. Each point represents the mean of three values of larval mortality after 24 h of exposure to essential oil samples (40 p.p.m. final concentration) from each species of *Eucalyptus*, and their corresponding concentration of 1,8-cineole.

Regression analysis showed that there was a statistically significant relationship ($P<0.01$) between larval mortality and the concentration of 1,8-cineole. The fitted regression model was: larval mortality = 118.518 – 1.415 × % 1,8-cineole. The coefficient of determination indicated that the model explains 95.6% of the variability of larval mortality. The standard deviation of the residuals was 8.780 (Figure 2). The observed inverse correlation could be due to a dilution effect produced by the increase of the concentration of the less toxic compound 1,8-cineole.

Regression analysis also showed a statistically significant relationship ($P<0.01$) between larval mortality and the concentration of *p*-cymene. The fitted regression model

Table 3 Larvicidal activity of essential oils from different species of *Eucalyptus* on *Aedes aegypti*

Species	Larval mortality ± SD (%)[1]		LC_{50} (mg/l) (95% confidence interval)[2]				Adult emergence ± SD (%)[3]	EC_{50} (mg/l) (95% confidence interval)[4]			
			After 24-h exposure		After 48-h exposure			At 100% emergence in control		At end of assay	
	24 h	48 h	LC_{50}	Slope	LC_{50}	Slope		EC_{50}	Slope	EC_{50}	Slope
E. cinerea	2.0 ± 3.8	4.0 ± 3.8	–	–	–	–	96.7 ± 5.8	–	–	–	–
E. globulus ssp. *maidenii*	7.0 ± 11.5	13.0 ± 23.1	–	–	–	–	93.3 ± 5.8	–	–	–	–
E. globulus ssp. *globulus*	2.0 ± 3.8	4.0 ± 7.7	–	–	–	–	100.0 ± 0.0	–	–	–	–
E. sideroxylon	0.0 ± 0.0	0.0 ± 0.0	–	–	–	–	96.7 ± 5.8	–	–	–	–
E. viminalis	0.0 ± 0.0	0.0 ± 0.0	–	–	–	–	96.7 ± 5.8	–	–	–	–
E. grandis × *E. tereticornis*	33.0 ± 6.7	44.0 ± 20.4	–	–	–	–	96.7 ± 5.8	–	–	–	–
E. grandis × *E. camaldulensis*	31.0 ± 10.2	53.0 ± 17.6	–	–	–	–	93.3 ± 5.8	–	–	–	–
E. dunnii	51.0 ± 10.2	69.0 ± 21.4	25.23 (23.15–27.15)	8.22	23.87 (22.12–25.53)	9.72		20.54 (18.81–22.05)	10.58	20.02 (9.89–24.81)	10.28
E. tereticornis	89.0 ± 7.7	98.0 ± 3.8	22.14 (19.98–23.93)	8.18	21.06 (19.40–22.53)	10.91		20.02 (18.17–21.55)	10.28	19.74 (17.80–21.28)	10.19
E. camaldulensis	87.0 ± 6.7	96.0 ± 3.8	26.75 (24.93–28.58)	9.28	25.93 (24.15–27.72)	9.38		21.83 (19.89–23.46)	9.29	21.67 (19.92–23.20)	10.31
E. saligna	84.0 ± 7.7	89.0 ± 10.2	22.16 (19.87–24.08)	7.62	20.92 (19.49–22.26)	12.87		19.89 (18.01–21.28)	11.41	19.66 (17.65–21.08)	11.13
E. gunnii	100.0 ± 0.0	100.0 ± 0.0	21.13 (18.70–23.00)	7.74	20.63 (18.97–22.05)	11.24		20.11 (18.34–21.48)	11.68	20.11 (18.34–21.48)	11.68

[1]Larval mortality was determined after 24- and 48-h exposure to a final concentration of 40 p.p.m. Values are means of three replicates ± standard deviation (SD) (n = 15).

[2]LC_{50} values were determined after 24- and 48-h exposure.

[3]% adult emergence (40 mg/l).Values are means of three replicates ± SD (n = 15).

[4]EC_{50} values were evaluated when 100% of the control specimens emerged as adults and at the end of each assay.

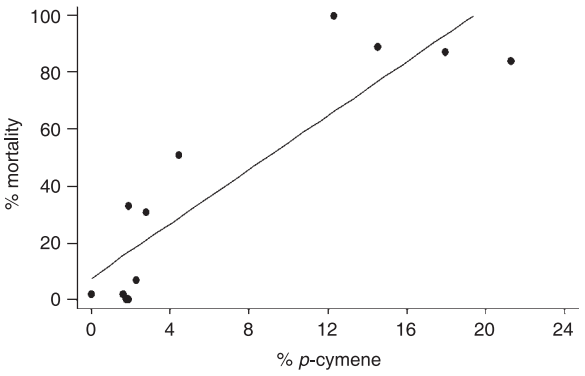

Figure 3 Relationship between larval mortality of *Aedes aegypti* and concentration of *p*-cymene in the essential oils from 12 species of *Eucalyptus*. Each point represents the mean of three values of larval mortality after 24 h of exposure to essential oil samples (40 p.p.m. final concentration) of each species of *Eucalyptus*, and their corresponding percentage of *p*-cymene.

was: larval mortality = 7.623 + 4.768 × % *p*-cymene. The R^2-value (0.789) indicated that the fitted model explains 78.9% of the variability of larval mortality. The standard deviation of the residuals was 19.240 (Figure 3).

Additionally, multiple regression analysis showed a statistically significant relationship ($P<0.01$) between larval mortality and two independent variables (concentration of 1,8-cineole and *p*-cymene). The fitted regression model was: larval mortality = 94.463 + 1.242 × % *p*-cymene − 1.134 × % 1,8-cineole. There was also a statistically significant relationship between the variables with a 99% confidence level. The coefficient of determination indicated that the model explains 97.2% of the variability of larval mortality. The standard deviation of the residuals was 7.383. The mean absolute error was 5.480. The Durbin–Watson (DW = 1.243) statistic was smaller than 1.4, which means there may be some indication of serial correlation. We determined whether the model could be simplified, but as the highest P-value of the independent variables, belonging to *p*-cymene, was 0.0495 ($P<0.05$), no variables could be removed from the model.

Values of LC_{50} and EC_{50} are shown in Table 3, and were obtained for essential oils that revealed a larval mortality >50% after 24 h of exposure to a 40 p.p.m. final concentration (*E. dunnii*, *E. gunnii*, *E. tereticornis*, *E. camaldulensis*, and *E. saligna*). Unlike the essential oils with reduced larvicidal effect on *Ae. aegypti*, these four species of *Eucalyptus* that exhibit a high larvicidal effect contain low concentrations of 1,8-cineole and high concentrations of *p*-cymene. No significant differences were observed between the values of LC_{50} after 24 and 48 h of exposure to each essential oil. Similarly, no significant differences were observed between the EC_{50} values on the day that 100% of the control mosquitoes had emerged as adults and the values on the day that the last specimen from the experimental group emerged or died (Table 3).

The most active compound was the essential oil of *E. gunni*, with LC_{50} = 21.13 p.p.m.; however, it was not significantly different from effects of the essential oils of *E. saligna* and *E. tereticornis* (LC_{50} = 22.16 and 22.14 p.p.m., respectively; Table 3). *Eucalyptus camaldulensis* had the highest LC_{50} value (26.75 p.p.m.), significantly higher than that of the group of *E. saligna*, *E. tereticornis*, and *E. gunni*, but not significantly different from the essential oils of *E. dunnii* with LC_{50} = 25.23 p.p.m. (Table 3). No significant differences were observed among the EC_{50} values for essential oils with a larval mortality >50% after 24 h of exposure to a concentration of 40 p.p.m. (Table 3).

Only the LC_{50} and EC_{50} values for essential oils from *E. dunnii* and *E. camaldulensis* had non-overlapping confidence intervals. However, no visible effects were observed during the bioassay on the subsequent growth of the remaining *Ae. aegypti* larvae, and there was no inhibition of adult emergence in addition to larvicide mortality after 24 h of exposure. These results suggested that at the concentrations studied, the essential oils of *Eucalyptus* had no visible effect on the subsequent growth of the remaining *Ae. aegypti* larvae following 24 h of exposure.

The bioassay results pointed out that an increase in the content of 1,8-cineole in the *Eucalyptus* essential oils studied reduces the larvicidal effect in *Ae. aegypti*. Conversely, when more *p*-cymene is present in the *Eucalyptus* oil, the larvicidal activity is higher. In conclusion, the results of this article represent a first step in the development of guidelines to predict the larvicidal activity of *Eucalyptus* essential oils on *Ae. aegypti* on the basis of their respective terpene composition. These results could be of interest in view of the fact that resistance to organophosphates is on the increase.

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