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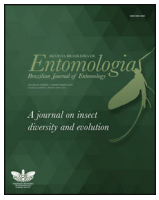

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Evaluation of the insecticidal activity of essential oils and their mixtures against *Aedes aegypti* (Diptera: Culicidae)

Natalia Ríos[a], Elena E. Stashenko[b], Jonny E. Duque[c,*]

[a] *Universidad Industrial de Santander, Escuela de Química, Centro de Investigaciones en Enfermedades Tropicales (CINTROP), Bucaramanga, Colombia*
[b] *Universidad Industrial de Santander, Escuela de Química, Centro de Investigación en Biomoléculas – CIBIMOL y Centro Nacional de Investigación para la Agroindustrialización de Plantas Aromáticas y Medicinales Tropicales – CENIVAM, Bucaramanga, Colombia*
[c] *Universidad Industrial de Santander, Escuela de Medicina, Departamento de Ciencias Básicas, Centro de Investigaciones en Enfermedades Tropicales (CINTROP), Bucaramanga, Colombia*

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ABSTRACT

The search for new insecticides to control dengue fever, chikungunya, and Zika vectors has gained relevance in the past decades. The aim of the present study was to evaluate the larvicidal action of essential oils (EOs) from *Thymus vulgaris*, *Salvia officinalis*, *Lippia origanoides*, *Eucalyptus globulus*, *Cymbopogon nardus*, *Cymbopogon martinii*, *Lippia alba*, *Pelargonium graveolens, Turnera diffusa*, and *Swinglea glutinosa* on *Aedes (Stegomyia) aegypti.* The EOs were extracted by microwave-assisted hydrodistillation and characterized by gas chromatography/mass spectrometry (GC/MS). The chemical components of the EOs were identified by linear retention indices and mass spectra. Lethal concentrations (LC_{50} and LC_{95}) were determined by probit analysis using larvae of *Ae. aegypti* between the third and the fourth instars. All EOs achieved larvicidal activity at LC_{50} values lower than 115 mg/L. The lowest LC_{50} value (45.73 mg/L) corresponded to *T. vulgaris* EO, whereas *C. martinii* EO showed the highest LC_{50} (LC_{50} = 114.65 mg/L). Some EO mixtures showed lower LC_{50} than oils used individually, such as the mixtures of *L. origanoides* + *S. glutinosa* (LC_{50} = 38.40 mg/L), *T. diffusa* + *S. glutinosa* (LC_{50} = 63.71 mg/L), and *L. alba* + *S. glutinosa* (LC_{50} = 48.87 mg/L). The main compounds of the EOs with highest larvicidal activity were thymol (42%) and p-cymene (26.4%).

Introduction

Several diseases such as yellow fever, dengue fever, chikungunya, and Zika fever, among several others, can be transmitted by *Aedes aegypti* (L., 1762) to human beings. Diseases are symptomatic manifestations of infections. Based on its morbidity and rates of mortality, dengue fever is considered the most serious disease from an epidemiological point of view. Approximately 60 million people around the world are estimated to acquire the virus each year resulting in about 10,000 deaths (Bhatt et al., 2013; Stanaway et al., 2016). In the case of Zika fever, global alarms have been activated due to the association of the virus with cases of microcephaly in newborns and Guillain-Barré syndrome reported by health institutions in Brazil and French Polynesian (Abushouk et al., 2016; Plourde and Bloch, 2016).

Due to the lack vaccines against these diseases, prevention strategies are focused on the control of larvae and adult *Ae. aegypti* populations. The application of synthetic insecticides (organophosphates-OP and pyrethroids-PI) is the most common approach used worldwide (Brandler et al., 2013). On the other hand, *Bacillus thuringiensis* var *israelensis* (Bti) is a bacteria widely evaluated in programs for Culicidae control. This mosquito control method is environmentally safe, commercially available and cheaper than synthetic insecticides (OP and PI). However, the principal disadvantage of using Bti in control programs is the low persistence in field conditions (Ritchie et al., 2010; Boyce et al., 2013; Moshi and Matoju, 2017).

Several studies have been conducted to identify new insecticides obtained from secondary metabolites of aromatic and medicinal plants, seeking effective alternatives to combat vector mosquitoes. The aim of such studies is to discover options to replace traditional chemical insecticides and determine natural ingredients to make formulations that can be used in the design of new insecticides (Carreño et al., 2014).

Compared to synthetic products, natural pesticides are less harmful to human health and ecosystems, and so they are widely accepted by the general population. Despite these benefits, commercial insecticides still have more effective lethal concentrations

* Corresponding author.
E-mail: jonedulu@uis.edu.co (J.E. Duque).

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(LC), lethal doses (LD) and lethal times (LT) than natural products (Shaalan et al., 2005; Koul et al., 2008). Therefore, it is important to characterize the insect-killing effectiveness of essential oils (EO) or plant extracts (PE) in their first screening phase in order to determine their promise as insecticides. One of the criteria to guide new larvicide research is that the candidate substances have an $LC_{50} < 100$ mg/L (Cheng et al., 2003; Dias and Moraes, 2014). However, this criterion does not include important aspects of the control and protection against mosquito bites, such as repellency, deterrence, and attraction (Castillo et al., 2017).

More studies are needed to compare the insecticidal action of EOs and PEs obtained at different geographical locations (Amer and Mehlhorn, 2006a; Pavela, 2008; Caballero-Gallardo et al., 2012; Manimaran et al., 2012). It is also important to understand that the chemical composition of an EO or PE can determine its insecticidal effect, and that this may vary intra- and interspecifically, according to soil, plant anatomy, edaphic factors, and environmental conditions (Bakkali et al., 2008; Dias and Moraes, 2014). Based on these premises, the aim of the present study was to evaluate the insecticidal activity of essential oils isolated from different aromatic plants, as follows: *Salvia officinalis* (Lamiaceae), *Thymus vulgaris* (Labiatae), *Eucalyptus globulus* (Myrtaceae), *Lippia alba* (Verbenaceae), *Turnera diffusa* (Turneraceae), *Pelargonium graveolens* (Geraniaceae), *Cymbopogon nardus*, and *Cymbopogon martinii* (Poaceae), *Swinglea glutinosa* (Rutaceae), as well as two different chemotypes of *Lippia origanoides* (phellandrene and thymol).

Material and methods

The experiments were developed using *Ae. aegypti* insects from the Rockefeller colony. Mosquitoes were kept in $40 \times 40 \times 40$ cm breeding cages under special conditions of humidity ($70 \pm 5\%$), photoperiod (12:12), and temperature ($25 \pm 5\,^{\circ}C$). Female mosquitoes were fed with Wistar rat blood (the UIS ethics committee was previously informed, as stated in CEINCI-UIS Minute No. 3, 2013; male mosquitoes were fed with 10% sucrose solution.

Essential oil isolation

The plants were collected from fields located in Santander, Colombia (Table 1). The EOs were extracted by microwave-assisted hydrodistillation (MWHD) as described by Stashenko et al. (2004). In the case of MWHD, plant material and the water were heated using a domestic microwave oven (2.45 GHz, 800 W), modified with a lateral orifice to connect the flask and the condenser. The microwave oven worked at full power (800 W) for 30 min (10 min × 3). The EO was collected in a Dean-Stark, and finally, the condensate was decanted and dried with anhydrous sodium sulfate.

The EOs were characterized by gas chromatography/mass spectrometry (GC/MS), using an Agilent Technologies 6890 (AT, Palo Alto, CA, USA) gas chromatograph with a DB-5MS capillary column ($60\,m \times 0.25\,mm$ id $\times 0.25\,mm$ d_f) using helium (99.995% purity) as carrier gas at a flow rate of 1 mL/min and an Agilent Technologies 5973 mass selective detector. Ionization was used electron energy achieved at 70 eV. The temperatures of the injector and the transfer line were set at 285 and 250 °C, respectively. The initial column temperature was 50 °C, which was increased by 3 °C/min up to 150 °C, and the 250 °C temperature was finally reached at 10 °C/min. The major components of the EOs were identified using the linear retention indeces and mass spectra, which were compared with those from the NIST, Wiley, and ADAMS databases (Stashenko et al., 2004).

Insecticidal activity

Experiments were initially conducted at exploratory concentrations (EC) of EO with larvae of *Ae. aegypti* between the third and the fourth instars. Larvae were placed in 100 mL plastic cups containing a solution of EO and mineral water. Mortality rates between 2 and 98% have been previously found after exposing larvae to EC of essential oils (Aciole et al., 2011; Vera et al., 2014). The concentrations being tested were initially 30, 300, and 1000 mg/L. Each treatment was repeated four times ($N = 120$ larvae), and experiments were replicated three times on different days. The control test used dimethyl sulfoxide (DMSO, 0.5%) and mineral water. Larvae counts were performed at 24 and 48 h after initial exposure to each EO concentration. The criteria to consider larvae as dead were that the individuals lacked all movement and failed to reach the water surface (WHO, 1996). Values of LC_{50}, LC_{95}, and mortality rates were determined for five selected EOs. The results of mortality and survival bioassays were subjected to Probit analysis (Finney, 1971).

Results

The EOs obtained by MWHD presented different extraction yields. *E. globulus* was the plant from which the highest amount of EO was obtained (2.0%, w/w). The major components in the oil were thymol (*T. vulgaris*), 1,8-cineole (*S. officinalis*), limonene (*L. origanoides* chemotype-phellandrene), thymol (*L. origanoides*, thymol chemotype), 1,8-cineol (*E. globulus*), citronellal (*C. nardus*), geraniol (*C. martinii*), carvone (*L. alba*), drima-7,9(11)-diene (*T. diffusa*), and citronellol in the EO of *P. graveolens* (Table 2).

All EOs displayed insecticidal action against *Ae. aegypti* larvae at 24 and 48 h (Table 3). The relationship between concentration and mortality was most effective with the oil mixture composed of *L. origanoides* and *S. glutinosa* (38.40 mg/L). *T. vulgaris* EO showed the

Table 1
Essential oil yields collection sites and registration numbers (voucher) of plants studied in this work.

Scientific name	Family	Common name	Voucher No.	Site of collection	EO yield, % (p/p)
Thymus vulgaris	Labiatae	Thyme	555843	Sucre, Santander	0.3
Salvia officinalis	Lamiaceae	Garden sage	555844	Sucre, Santander	0.4
Lippia origanoides (Phellandrene)	Verbenaceae	Wild oregano	519798	Cenivam, Bucaramanga	0.4
Lippia origanoides (thymol)	Verbenaceae	Wild oregano	519799	Cenivam, Bucaramanga	1.6
Eucalyptus globulus	Myrtaceae	Blue gum	C-470	Cenivam, Bucaramanga	2.0
Cymbopogon nardus	Poaceae	Citronella grass	578357	Cenivam, Bucaramanga	0.4
Cymbopogon martinii	Poaceae	Gingergrass	587116	Cenivam, Bucaramanga	0.4
Lippia alba	Verbenaceae	Quick relief	480750	Cenivam, Bucaramanga	0.5
Turnera diffusa	Turneraceae	Damiana	516293	Los Santos, Santander	0.7
Pelargonium graveolens	Geraniaceae	Wildemalva	51718	Cenivam, Bucaramanga	0.2
Swinglea glutinosa	Rutaceae	African lemon	521530	Cenivam, Bucaramanga	0.2
Mixture of *L. origanoides* and *S. glutinosa*		–	–	–	–
Mixture of *T. diffusa* and *S. glutinosa*		–	–	–	–
Mixture of *S. glutinosa* and *L. alba*		–	–	–	–

Table 2
Percentages of major components in the essential oils studied.

Plant	Major components (%)	Reference
T. vulgaris	Thymol (42.0), *p*-cymene (26.4), γ-terpinene (6.3), linalool (2.9), *trans*-β-caryophyllene (2.6)	Unpublished data
S. officinalis	1,8-Cineol (26.6), α-thujone (18.1), *trans*-β-caryophyllene (7.3), α-humulene (5.4)	Unpublished data
L. origanoides (phellandrene)	Limonene (15.0), *p*-cymene (14.6), α-phellandrene (10.3), *trans*-β-caryophyllene (5.8), α-humulene (2.9), α-pinene (2.5), γ-terpinene (2.1)	(Stashenko et al., 2010)
L. origanoides (thymol)	Thymol (66.1), *p*-cymene (7.2), γ-terpinene (4.4), *trans*-β-caryophyllene (3.6), α-humulene (2.4), methyl thymyl ether (2.3), thymyl acetate (2.0), α-thujone (1.0)	(Stashenko et al., 2010)
E. globulus	1,8-Cineol (69.4), α-pinene (4.6), viridiflorol (4.1), α-terpenyl acetate (3.3), limonene (3.0)	Unpublished data
C. nardus	Citronellal (21.8), citronellol (18.1), geraniol (11.3), germacrene D (4.6), limonene (3.5)	Unpublished data
C. martini	Geraniol (83.9), geranyl acetate (9.2), linalool (2.3), *trans*-β-caryophyllene (1.0)	(Rodríguez et al., 2012)
L. alba (carvone)	Carvone (35.3), limonene (35.0), bicyclosesquiphellandrene (9.6), piperitenone (3.4), piperitone (1.0)	(Agudelo-Gomez et al., 2010)
T. diffusa	Drima-7,9(11)-diene (22.9), β-viridiflorene (6.6), α-silinene (5.9), valencene (5.5), *trans*-β-caryophyllene (5.2), *trans*-muurola-4(14),5-diene (5.2), *p*-cymene (2.1)	Unpublished data
P. graveolens	Citronellol (14.9), geranial (8.4), geraniol (7.5), guainene (7.4) germacrene D (3.7), *iso*-menthone (3.7), geranyl formate (3.2)	Unpublished data
S. glutinosa	*trans*-Nerolidol (28.4), germacrene D (20.5), α-pinene (9.1), *trans*-β-caryophyllene (7.5), δ-elemene (3.6), α-cadinol (2.1), γ-elemene (2.6), δ-Cadinene (2.0), espatulenol (1.7), α-humulene (1.5), β-elemene (1.3), geranyl acetate (1.0)	(Stashenko et al., 2015)

Table 4
Larvicidal activity (in mg/mL) of the different EOs against *Ae. aegypti* larvae at 24 and 48 h.

Essential oil or mixture	24 h			48 h		
	LC_{50}	LC_{95}	X^2	LC_{50}	LC_{95}	X^2
(L. origanoides + S. glutinosa)	38.40 (35.52–42.37)	94.91 (77.38–128.56)	1.12	34.86 (32.54–37.81)	83.01 (69.64–106.87)	0.39
T. vulgaris	45.73 (41.29–53.80)	96.25 (75.01–149.01)	5.35	42.33 (40.13–44.73)	76.53 (68.64–89.18)	2.64
(S. glutinosa + L. alba)	48.87 (46.17–51.50)	101.76 (92.02–116.32)	0.22	45.93 (42.84–48.84)	109.41 (96.66–129.84)	1.73
L. origanoides (Phellandrene)	53.79 (50.90–56.69)	116.60 (102.56–140.31)	4.68	53.79 (50.90–56.69)	116.60 (102.56–140.31)	4.68
L. origanoides (Thymol)	56.18 (53.20–59.89)	124.55 (105.55–160.30)	5.71	38.73 (35.17–41.73)	102.75 (89.36–126.24)	3.46
(T. diffusa + S. glutinosa)	63.71 (60.75–67.71)	117.70 (103.39–141.81)	0.31	34.86 (32.54–37.81)	83.01 (69.64–106.87)	0.39
L. alba (Carvone)	72.34 (69.87–75.05)	110.84 (102.69–123.28)	0.12	63.61 (66.34–66.46)	98.91 (93.58–106.25)	5.38
S. officinalis	76.43 (71.84–83.79)	123.92 (106.98–136.75)	5.46	77.53 (69.71–91.14)	198.20 (149.17–322.5)	1.37
C. nardus	75.85 (69.15–86.82)	219.68 (165.93–345.92)	4.22	71.26 (65.60–80.11)	255.42 (182.79–465.99)	5.81
E. globulus	92.55 (89.37–97.00)	136.82 (124.67–157.14)	2.21	91.29 (88.35–95.30)	133.72 (122.54–152.00)	2.44
P. graveolens	108.96 (103.62–115.74)	176.61 (157.84–208.81)	2.80	113.16 (106.65–122.15)	198.54 (172.01–248.57)	0.73
C. martinii	114.65 (107.26–124.94)	251.26 (211.65–321.05)	1.96	114.82 (103.14–141.23)	290.06 (207.20–577.64)	0.99

LC_{50} is the lethal concentration causing mortality of 50% of organisms exposed to treatment. LC_{95} is the lethal concentration causing mortality of 95% of organisms exposed to treatment. The confidence interval is given in parentheses. The statistical analysis was well adjusted to the probit model (Finney, 1947).

lowest LC_{50} (45.73 mg/L). The EOs with highest LC_{50} were *C. martinii* and *P. graveolens*, with 114.65 and 108.96 mg/L, respectively, at 24 h (Table 4).

Discussion

All of the studied EOs, both individually and as mixtures, presented insecticidal activity against *Ae. aegypti* larvae. Only *C. martinii* and *P. graveolens* presented an $LC_{50} > 100$ mg/L, indicating that all EOs evaluated in this study can be utilized as good candidates for the design of new mosquito insecticides against mosquito control (Dias and Moraes, 2014). The mixture of *L. origanoides* and *S. glutinosa* was proven to cause the highest insect mortality ($LC_{50} = 38.40$ mg/L). As shown by Vera et al. (2014), the mixtures of EOs, in this case *L. origanoides* (53.37 mg/L) and *S. glutinosa* (65.71 mg/L), may enhance the toxic effect of individual oils on *Ae. aegypti* larvae.

Our results showed *T. vulgaris* to have the best larvicidal action (LC_{50} 45.73 mg/L). This bioactivity reflects a study by Massebo et al. (2009), who studied EO extracted from leaves and seeds of plants from Ethiopia, yet the LC_{50} was lower (17.3 mg/L). Also, *T. vulgaris* extracts from plants grown in the Czech Republic ($LC_{50} = 48$ mg/L) with *Culex quinquefasciatus* (Pavela, 2008) confirm our present results. Thymol and *p*-cymene were the major compounds identified in this plant, and the toxicity against mosquitoes was consistent with other reports on these metabolites (Dias and Moraes, 2014).

The *L. origanoides* EOs of phellandrene and thymol chemotypes, presented similar insecticidal effects ($LC_{50} = 53.79$ mg/L and $LC_{50} = 56.18$ mg/L, respectively), which matches the LC_{50} of *L. origanoides*, obtained by Vera et al. (2014) with *Ae. aegypti*.

Despite their different major compounds, the larvicidal effect of EOs from *L. alba, C. nardus*, and *S. officinalis* showed similar LC_{50} values (Table 3). In the case of *L. alba*, the LC_{50} value (72.34 mg/L) was higher than that found by Vera et al. (2014) with *Ae. aegypti* ($LC_{50} = 44.26$ mg/L). This lower activity could be related to a lower amount of carvone in the EO (35.3%) as compared to a previous report (38.3%) by Vera et al. (2014), who found an amount of 38.3%. Besides *L. alba* insecticidal action, the plant has a record as a repellent with other insects, such as *Tribolium castaneum* (Olivero-Verbel et al., 2013).

In the present study, the EO of *C. nardus* presented a much more effective larvicidal activity against *Ae. aegypti* than that reported by Tennyson et al. (2013) in India (1374.05 mg/L). On the other hand, we obtained lower LC_{50} values than those obtained by Manimaran et al. (2012) for *Ae. aegypti* ($LC_{50} = 47.21$) and *Anopheles stephensi* (47.61 mg/L); the EOs in that study were obtained from plants cultivated in India.

Pavela (2008) reported a LC_{50} of 159 mg/L with *Cx. quinquefasciatus* larvae in a study on *S. officinalis*, a plant of Eurasian origin; when we compared those results with our results on *Ae. aegypti*, we observed that the LC_{50} was lower (76.43 mg/L), indicating that the EO of this plant had higher insecticidal activity than *S. officinalis* extract.

Table 3
Ae. aegypti larvae mortality rate of each EO concentration tested at 24 and 48 h.

Essential oil	Concentration, mg/mL	Mortality rate (% ± SD)	
		24 h	48 h
T. vulgaris	0	0	0
	12	3 ± 1.7	0.0 ± 0.0
	20	4 ± 2.9	8 ± 3.5
	30	16 ± 7.2	18 ± 7.3
	45	37 ± 9.0	43 ± 10
	58	77 ± 4.0	80 ± 4.6
S. officinalis	0	0	0
	20	4 ± 2.1	1.2 ± 0.7
	30	3 ± 2.3	5 ± 1.7
	47	6 ± 4.0	16 ± 8.4
	63	30 ± 17.7	40 ± 20.5
	76	50 ± 24	50 ± 24.7
L. origanoides (phellandrene)	0	0	0
	32	14 ± 7.7	18 ± 9.9
	56	60 ± 18.5	50 ± 11.9
	67	60 ± 13.2	63 ± 7.6
	79	70 ± 18.8	84 ± 10
	93	100 ± 0.0	100 ± 0.0
L. origanoides (Thymol)	0	0	0
	27	3 ± 0.7	58 ± 3.5
	45	27 ± 4.0	59 ± 7.2
	52	40 ± 10.4	64 ± 5.4
	67	60 ± 11.7	76 ± 6.1
	79	50 ± 20.2	92 ± 5.8
E. globulus	0	0	0
	54	11 ± 5.9	13 ± 7.6
	61	4 ± 2.1	4 ± 1.9
	72	11 ± 4.2	13 ± 4.6
	81	20 ± 11.5	20 ± 12.4
	93	30 ± 10.8	30 ± 10.2
C. nardus	0	0	0
	37	16 ± 6.4	19 ± 5.3
	42	13 ± 5.7	14 ± 4.9
	56	34 ± 7.7	43 ± 8.1
	69	31 ± 9.8	39 ± 2.6
	78	50 ± 16.6	60 ± 15.3
C. martinii	0	0	0
	56	7 ± 5.7	18 ± 6.6
	78	16 ± 7.6	31 ± 6.3
	83	19 ± 9.6	30 ± 10.2
	97	20 ± 9.1	41 ± 6.1
	141	70 ± 10.9	70 ± 11.4
L. alba (Carvone)	0	0	0
	30	3 ± 0.6	4 ± 0.6
	59	21 ± 5.5	32 ± 5 .7
	73	50 ± 10.0	75 ± 2.6
	89	80 ± 11.6	90 ± 5.5
	96	95 ± 0.0	96 ± 5.3
P. graveolens	0	0	0
	30	3 ± 0.0	3 ± 0.0
	69	6 ± 1.6	6 ± 1.5
	76	13 ± 2.0	13 ± 2.5
	98	28 ± 3.5	28 ± 1.4
	130	76 ± 6.4	80 ± 4.2
Mixtures *L. origanoides* + *S. glutinosa*	0	0	0
	22	9 ± 4.9	9 ± 4.9
	30	12 ± 0.0	12 ± 0.0
	45	17 ± 7.0	21 ± 8.0
	53	40 ± 2.1	44 ± 9.2
	69	60 ± 9.6	60 ± 12.2
Mixtures *T. diffusa* + *S. glutinosa*	0	0	0
	22	3 ± 1.1	3 ± 1.53
	30	3 ± 1.4	4 ± 2.36
	45	12 ± 3.7	19 ± 3.3
	49	30 ± 12.7	34 ± 13.4
	53	31 ± 10.2	42 ± 9.8
	69	60 ± 12.8	66 ± 8.4
Mixtures *S. glutinosa* + *L. alba*	0	0	0
	30	13 ± 2.3	23 ± 5.0
	42	40 ± 15.3	40 ± 15.6
	57	60 ± 14.7	70 ± 14.8
	69	80 ± 10.1	80 ± 10.4
	78	86 ± 5.7	86 ± 5.7

SD: Standard deviation.

The EOs of *E. globulus* (LC_{50} = 92.55 mg/L), *P. graveolens* (LC_{50} = 108.96 mg/L), and *C. martinii* (CL_{50} 114.65 mg/L) showed less effectiveness. Based on the criterion of plants with CL_{50} < 100, only *E. globulus* EO would be promising as an insecticide (Dias and Moraes, 2014). The EO of this plant had the greatest yield (2.0%, w/w), and mortality rates of 32.92% at 24 h and 34.17% at 48 h were achieved with a concentration of 93 mg/L (Table 3). These results are consistent with those presented by Amer and Mehlhorn (2006b), who reported *Aedes* larval mortality rates from 16.7% with EO solutions (50 mg/L) at 24 h of treatment.

L. origanoides and *S. glutinosa* mixture showed the highest larvicidal activity (LC_{50} = 38.40 mg/L) of the three mixtures analyzed in this study. It should be highlighted that these EOs, separately, had higher LCs_{50} than when evaluated as part of mixtures, as has been observed with EOs of *L. origanoides* (LC_{50} = 53.79 mg/L) and *S. glutinosa* (LC_{50} = 65.71 mg/L) (Vera et al., 2014). These data indicate that the insecticidal effect of EOs can be potentiated by using mixtures of EO, probably due to a synergistic effect (Mansour et al., 2015).

Although there is extensive information on botanical products such as essential oils and plant extracts for mosquito control (larval and adults), it is unusual to find them in formulations of commercial insecticides. Plants such as *Azadirachta indica* and *Melia azedarach* (Meliaceae) are among the few that are part of commercial biopesticides. These two species of plants have been studied on at least 103 species of insects and have eco-friendly effects (Mazid, 2011; Thangavel and Sridevi, 2015; Moshi and Matoju, 2017). However, the insecticidal effect on mosquito larvae of these plants is not so effective (LC50 > 1×10^{-4} mg/L) as that of essential oils (LC50 < 50 mg/L) (Howard et al., 2009; Kishore et al., 2011; Dias and Moraes, 2014; Vera et al., 2014). This is a good reason to use the plants presented here as source of ingredients for design new insecticides.

Conclusion

All of the EOs evaluated in the present study showed insecticidal activity. The EO of *T. vulgaris* and the mixture of *L. origanoides* and *S. glutinosa* showed highest larvicidal action on *Ae. aegypti*. The main compounds of the EOs with higher larvicidal activity were thymol (42%) and p-cymene (26.4%).

Conflicts of interest

The authors declare no conflicts of interest.

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