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Towards green oviposition deterrents? Effectiveness of *Syzygium lanceolatum* (Myrtaceae) essential oil against six mosquito vectors and impact on four aquatic biological control agents

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Abstract Essential oils (EOs) from plants may be alternative sources of molecules toxic against mosquito vectors of public health relevance. Most of researches in this field focused on EOs as larvicides or ovicides, while limited efforts focused on the exploitation of EOs as oviposition deterrents. In the present study, the larvicidal and oviposition deterrent activity of *Syzygium lanceolatum* leaf EO was evaluated against six mosquito species, *Anopheles stephensi*, *An. subpictus*, *Aedes aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, and *Cx. tritaeniorhynchus*. The chemical composition of the *S. lanceolatum* EO was analyzed by GC-MS analysis, showing the presence of phenyl propanal, β-caryophyllene, α-humulene, and caryophyllene oxide as major constituents. *S. lanceolatum* EO showed high acute toxicity on *An. stephensi* (LC_{50} = 51.20 μg/ml), *Ae. aegypti* (LC_{50} = 55.11 μg/ml), *Cx. quinquefasciatus* (LC_{50} = 60.01 μg/ml), *An. subpictus* (LC_{50} = 61.34 μg/ml), *Ae. albopictus* (LC_{50} = 66.71 μg/ml), and *Cx. tritaeniorhynchus* (LC_{50} = 72.24 μg/ml) larvae. Furthermore, the EO was effective as oviposition deterrent against the six tested mosquito species, with OAI on *An. stephensi*, *An. subpictus*, *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus* reaching −0.83, −0.81, −0.84, −0.83, −0.84, and −0.86, respectively. The toxicity of *S. lanceolatum* EO against several biological control agents of mosquitoes, including water bugs (*Anisops bouvieri* and *Diplonychus indicus*) and fishes (*Gambusia affinis* and *Poecilia reticulata*), was extremely low, with LC_{50} ranging between 4148 and 15,762 μg/ml. Overall, our results pointed out the promising potential of the *S. lanceolatum* leaf EO as a source of environmental-friendly oviposition deterrents and larvicides effective against a wide number of mosquito species of importance for parasitology.

Keywords Biosafety · Dengue · Filariasis · Japanese encephalitis · Larvicides · Malaria · Zika virus

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Introduction

Mosquitoes constitute an important group of arthropods for public health. *Anopheles*, *Aedes*, and *Culex* vector a wide range of human diseases such as malaria, dengue, yellow fever, filariasis, Japanese encephalitis, St. Louis encephalitis, and Zika virus, causing millions of deaths worldwide each year (Mehlhorn 2015; Benelli et al. 2016a). Global patterns of climate change and urbanization have increased the threat of humans contracting arthropod-borne viral infections (Benelli 2016a; Benelli and Mehlhorn 2016). However, high levels of pesticide resistance have been developed through chemical control of arthropod vectors, threatening the effectiveness of current control programs (Hemingway and Ranson 2000; Naqqash et al. 2016). To overcome these problems, it is necessary to search for alternative, more environmentally benign mosquito control methods (Benelli 2015a, b, c; Benelli et al. 2015; Pavela and Benelli 2016a). In this framework, botanical-borne pesticides may

provide a cheap and eco-friendly alternative to synthetic insecticides (e.g., Elango et al. 2010; Govindarajan and Benelli 2016a, b, c), due to their generally low toxicity to human health and the environment (Isman 2008; Benelli 2015b, 2016b,c; Pavela 2015).

Among botanical-based insecticides, plant essential oils (EOs) have a broad spectrum of bioactivity (Isman 2008; Govindarajan et al. 2016a, b, c, d, e) because of the presence of several active ingredients that exert toxicity through several mechanisms of action (Pavela and Benelli 2016b). Several plant species of the family Myrtaceae, which includes 4620 species and 140 genera distributed all over the world (Mabberly 1997), are being used in folk medicine due to their antidiarrheal, antimicrobial, antioxidant, antirheumatic, anti-inflammatory, and anti-cholesterol properties (Stasi and Hiruma-Lima 2002). Several species belonging to the genus *Syzygium* are employed to treat diabetes mellitus. The chemical composition of EOs from several *Syzygium* species has been previously reported, with special reference to *S. aqueum*, *S. samarangense*, *S. malaccense*, *S. aromaticum*, *S. guineense*, and *S. caryophyllatum* (Wong and Lai 1996; Raina et al. 2001; Lee et al. 2009; Noudogbessi et al. 2008; Nassar et al. 2007; Bhuiyan et al. 2010; Stalin and Swamy 2013; Ahmed et al. 2009). Sesquiterpenes (hydrocarbons and oxygenated derivatives) have been found the main class of volatile constituents responsible of the antibacterial, antifungal, anti-inflammatory, and cytotoxic activities. In addition, monoterpenes and phenylpropanoids have been also reported as important constituents of *Syzygium* EOs (Boulos 1983).

Syzygium lanceolatum (synonyms: *Eugenia lanceolata* Lam.; *Syzygium wightianum* Wall. ex Wt. and Arn.) belongs to the family Myrtaceae, which has 10 genera and 154 species in the Indian subcontinent. Of these, *Syzygium* Gaertn. is the largest genus, with 11 species that are endemic to Western Ghats of Tamil Nadu, India (Gamble and Fischer 1923). Plants of this family are known to be rich in volatile EOs, which are reported for their uses in Indian traditional medicine (Mahmoud et al. 2001; Reynertson et al. 2005). However, to the best of our knowledge, no information is available on the chemical composition and insecticidal activity of *S. lanceolatum* EO.

Notably, most of the researches in the field of mosquito control with plant-borne pesticides focused on plant EOs as larvicides or ovicides (see Benelli (2015b) and Pavela (2015) for recent reviews), while only limited efforts focused on the exploitation of EOs as oviposition deterrents (Prajapati et al. 2005; Autran et al. 2009; Khandagle et al. 2011; Rajaganesh et al. 2016. Therefore, in the present study, the larvicidal and oviposition deterrent activity of the *S. lanceolatum* leaf EO was evaluated against six mosquito species, the malaria vectors *Anopheles stephensi* and *An. subpictus*, the dengue and Zika virus vectors *Aedes aegypti* and *Ae. albopictus*, the filariasis and St. Louis encephalitis and West Nile vector *Culex quinquefasciatus*, and the Japanese encephalitis vector *Cx. tritaeniorhynchus.* Furthermore, the chemical composition of the *S. lanceolatum* EO was analyzed by gas chromatography-mass spectroscopy (GC–MS) analysis. Lastly, the non-target toxicity of the *S. lanceolatum* EO was evaluated against several biological control agents of mosquitoes, including water bugs (*Anisops bouvieri* and *Diplonychus indicus*) and fishes (*Gambusia affinis* and *Poecilia reticulata*).

Materials and methods

Extraction and GC–MS analysis of the *S. lanceolatum* essential oil

Fresh leaves of *S. lanceolatum* were collected during May 2016 in the Munnar mountains (India 10° 05′ 21″ N, 77°03′35″ E, 1700 m a.s.l.). *S. lanceolatum* samples were identified, and authenticated voucher specimens were deposited at the Herbarium of the Faculty of Science, Annamalai University (India). 400 grams of *S. lanceolatum* fresh leaves were hydrodistilled using a modified Clevenger-type apparatus for 3 h; then, *S. lanceolatum* EO was dried over anhydrous Na_2SO_4 and stored into amber-colored vials at 5 °C until the testing phase (Govindarajan and Benelli 2016a, b). *S. lanceolatum* EO was stored in an airtight container prior to analysis by gas chromatography (GC) and GC–MS.

GC and GC–MS analyses

Analytical gas chromatography was carried out using an HP gas chromatograph. The separation was achieved by use of a HP_1 (fused silica) capillary column (30 m × 0.25 mm; film thickness 0.25 μm), split ratio, 1:25, and using a flame ionization detector. GC settings were programmed as reported by Govindarajan and Benelli (2016c). He was employed as carrier gas; the flow rate was 1 ml/min. GC–MS was performed on Agilent Technology 5973 mass selective detector connected with a HP 6890 gas chromatograph. Separation was achieved relying to the HP_1 MS capillary column described above, with split ratio 1:25, equipped with a flame ionization detector (FID). MS was operated at 70 eV ionization energy. Quantitative data on *S. lanceolatum* EO composition were obtained from the electronic integration of FID peak areas.

The identification of *S. lanceolatum* EO components was based on retention indices, which were calculated by using retention times of *n*-alkanes *injected* after the *S. lanceolatum* EO at the same chromatographic conditions. The components of the *S. lanceolatum* EO were identified by comparison of their mass spectra and retention indices (Table 1) with the ones from Wiley library and Adams (2007).

Table 1 Chemical composition of *Syzygium lanceolatum* essential oil

Peak	Compound	Retention time (Kovats Index)	Composition (%)	Mode of identification
1	Phenyl ethyl alcohol	1101	1.7	RI, MS
2	Phenyl propanal	1153	18.3	RI, MS
3	δ-Elemene	1331	1.4	RI, MS
4	β-Elemene	1381	5.9	RI, MS
5	β-Caryophyllene	1412	12.8	RI, MS
6	Aromadendrene	1433	2.4	RI, MS
7	α-Humulene	1443	14.5	RI, MS
8	β-Selinene	1480	3.4	RI, MS
9	α-Selinene	1491	3.9	RI, MS
10	*trans*-calamenene	1511	2.6	RI, MS
11	Spathulenol	1567	5.1	RI, MS
12	Caryophyllene oxide	1575	10.7	RI, MS
13	Viridiflorol	1584	1.5	RI, MS
14	Humulene epoxide II	1602	6.5	RI, MS
15	Alloaromadendrene epoxide	1627	1.6	RI, MS
16	Caryophylla-4(12),8(13)-dien-5-ol	1631	1.2	RI, MS
17	Selina,3,11-dien-6α-ol	1632	1.7	RI, MS
18	Cubenol	1635	2.1	RI, MS
	Total		97.3	

RI retention index, *MS* mass spectra

Larvicidal and oviposition deterrence assays

Pathogen- and parasite-free strains of *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus* were reared as recently described by Govindarajan and Benelli (2016c), at 27 °C, 12:12 L/D photoperiod, 80 ± 10 % R.H. Early third instar larvae and adult females (5–7 days old) were used for larvicidal and oviposition deterrence experiments, respectively (Govindarajan et al. 2016d).

The larvicidal activity of the *S. lanceolatum* EO was evaluated following the method by WHO (2005) slightly modified by Govindarajan and Benelli (2016c). Various doses of the *S. lanceolatum* EO were dissolved in 1 ml DMSO, and then diluted in 249 ml of filtered tap water to obtain the tested concentrations (Table 2). Control was 1 ml of DMSO diluted in 249 ml of water. Within each replicate, 20 early third instar larvae were tested. No food was given to the larvae (WHO 2005). For each concentration, five replicates were performed. Mortality was recorded after 24 h of exposure.

In oviposition deterrent assays, the *S. lanceolatum* EO was evaluated at various concentrations (40–250 μg/ml) prepared in DMSO. As reported above for larvicidal experiments, DMSO diluted in water served as a control. The experiments were carried out as described by Xue et al. (2001) slightly modified by Benelli and Govindarajan (2016). As oviposition support, we used a filter paper strip placed on the internal surface of treated and control bowls of 500-ml capacity filled with 100 ml distilled water, and the filter paper was half submerged in water. Twenty gravid females (5–7 days old) of *An. stephensi, Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, or *Cx. tritaeniorhynchus* were released in the bioassay cage (60 × 60 × 45 cm). After 30 min, the treated and control bowls were placed in diagonal position inside bioassay cage. After 24 h, the number of eggs laid in treated and control bowls were counted under a stereomicroscope (Olympus, Japan).

Toxicity on biological control agents

The effect of *S. lanceolatum* EO on the four non-target organisms was assessed following the method by Sivagnaname and Kalyanasundaram (2004) with minor modifications by Govindarajan et al. (2016a, b). The toxicity of the *S. lanceolatum* EO was tested against adults of the water bugs *A. bouvieri* and *D. indicus* and the larvivorous fishes *G. affinis* and *P. reticulata*. The non-target species were reared as described by Govindarajan and Benelli (2016c), maintaining them in cement tanks (diam.: 85 cm; depth: 30 cm) filled with tap water, 27 ± 3 °C, and external R.H. 85 %. *S. lanceolatum* EO was evaluated at doses 50 × LC_{50} calculated for the tested mosquito larvae. Ten replicates were performed for each dose plus 4 control replicates (where no EO was added to the water). Mortality of each non-target species was assessed after 48 h of exposure (Govindarajan and Benelli 2016b).

Table 2 Larvicidal activity of the essential oil from *Syzygium lanceolatum* against *Anopheles stephensi*, *An. subpictus*, *Aedes aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, and *Cx. tritaeniorhynchus*

Mosquito species	Concentration (μg/ml)	24 h mortality (%)± SD[a]	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 *(d.f.)*
An. stephensi	25	29.2 ± 1.2	51.20 (45.29–56.42)	101.30 (93.90–111.04)	3.29	$y = 11.7 + 0.73\times$	5.882 (4) n.s.
	50	47.6 ± 0.8					
	75	66.9 ± 0.6					
	100	88.4 ± 0.4					
	125	100.0 ± 0.0					
An. subpictus	30	27.4 ± 0.6	61.34 (54.33–67.52)	120.41 (111.72–131.81)	3.14	$y = 11.43 + 0.614\times$	4.379 (4) n.s.
	60	48.6 ± 1.2					
	90	69.8 ± 0.4					
	120	87.5 ± 0.8					
	150	100.0 ± 0.0					
Ae. aegypti	25	26.8 ± 0.6	55.11 (49.18–60.42)	107.79 (99.92–118.22)	3.09	$y = 8.2 + 0.738\times$	4.180 (4) n.s.
	50	44.2 ± 0.8					
	75	62.7 ± 1.2					
	100	85.6 ± 0.4					
	125	98.3 ± 0.8					
Ae. albopictus	30	24.6 ± 1.2	66.71 (59.45–73.19)	131.43 (121.75–144.26)	3.21	$y = 8.29 + 0.61\times$	2.151 (4) n.s.
	60	45.9 ± 0.6					
	90	64.7 ± 0.8					
	120	83.2 ± 0.4					
	150	97.4 ± 1.2					
Cx. quinquefasciatus	25	22.6 ± 0.4	60.01 (54.22–65.32)	114.18 (105.88–125.17)	2.71	$y = 3.74 + 0.752\times$	2.287 n.s.
	50	40.9 ± 0.6					
	75	59.3 ± 1.2					
	100	81.7 ± 0.8					
	125	96.2 ± 1.2					
Cx. tritaeniorhynchus	30	21.9 ± 0.8	72.24 (65.01–78.83)	140.01 (129.57–153.97)	2.94	$y = 4.89 + 0.611\times$	1.643 (4) n.s.
	60	42.6 ± 0.6					
	90	60.4 ± 1.2					
	120	79.3 ± 0.4					
	150	95.2 ± 0.6					

[a] Values are mean ± SD of five replicates

SD standard deviation, LC_{50} lethal concentration that kills 50 % of the exposed organisms, LC_{90} lethal concentration that kills 90 % of the exposed organisms, *UCL* 95 % upper confidence limit, *LCL* 95 % lower confidence limit, χ^2 chi square, *d.f.* degrees of freedom, *n.s.* not significant ($\alpha = 0.05$)

Data analysis

All mortality data were analyzed by probit analysis. LC_{50} and LC_{90} were estimated following the method by Finney (1971). The oviposition activity index (OAI) was calculated as follows (Kramer and Mulla 1979):

$$OAI = (NT\text{-}NC)/(NT + NC)$$

Effective repellency (ER %) evoked by *S. lanceolatum* EO was calculated as indicated by Xue et al. (2001):

$$ER(\%) = [(NC\text{-}NT)/NC]*100$$

NT was the total number of eggs in the treated solution. NC was the total number of eggs in the control solution.

Concerning non-target organisms, the suitability index (SI) was calculated for each species as follows (Deo et al. 1988):

$$SI = \frac{LC_{50}\,of\ non\text{-}target\ organisms}{LC_{50}\,of\ target\ vector\ species}$$

All data were analyzed using the SPSS Statistical Software Package version 16.0. $P < 0.05$ was used to assess the significance of differences among values.

Results

Yield and chemical composition

Yield of *S. lanceolatum* leaf EO was 6.3 ml/kg of leaf fresh weight. Table 1 shows the constituents of *S. lanceolatum* EO, their percentage composition, and the Kovats Index (KI) values listed in order of elution. 18 compounds representing 96.3 % of the *S. lanceolatum* EO composition were identified. Major constituents of this oil were phenyl propanal (18.3 %), β-caryophyllene (12.8 %), α-humulene (14.5), and caryophyllene oxide (10.7 %). The chemical structures of the four major compounds were shown in

Fig. 1. The percentage compositions of remaining 14 compounds ranged from 1.2 to 6.5 %.

Larvicidal activity and oviposition deterrent activity

The acute toxicity of the *S. lanceolatum* EO on larvae of *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus* is presented in Table 2. The EO extracted from the leaves of *S. lanceolatum* exhibited effective larvicidal activity, with the LC_{50} values of 51.20, 55.11, 60.01, 61.34, 66.71, and 72.24 μg/ml, respectively. No mortality was recorded in controls.

Results obtained from oviposition deterrent assays testing the *S. lanceolatum* EO on *An. stephensi*, *A. aegypti*, *C. quinquefasciatus*, *A. subpictus*, *A. albopictus*, and *C. tritaeniorhynchus* are reported in Table 3. The mean number of eggs laid in sites treated with the EO tested at the highest concentration (i.e., 250–300 μg/ml) was 46.2, 44.6, and 37.5 eggs per bowl for *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* and 52.3, 47.1, and 39.7 eggs per bowl for *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus*. These data showed significant oviposition deterrent activity if compared to the relative controls ($P < 0.05$) (Table 3). Overall, *S. lanceolatum* EO was effective as oviposition deterrent against the six tested mosquito species, with OAI on *An. stephensi*, *An. subpictus*, *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus* reaching −0.83, −0.81, −0.84, −0.83, −0.84, and −0.86, respectively.

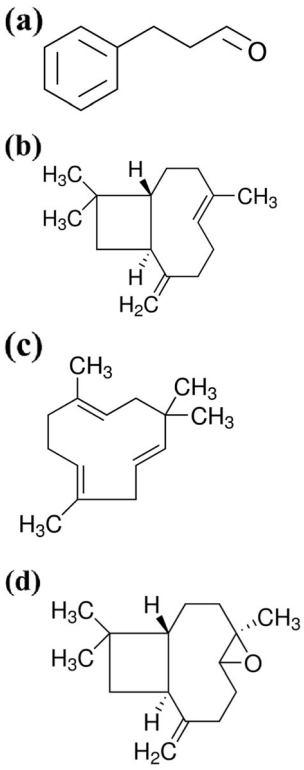

Fig. 1 Chemical structures of the three major constituents of the *Syzygium lanceolatum* essential oil: **a** phenyl propanal, **b** β-caryophyllene, **c** α-humulene, and **d** caryophyllene oxide

Toxicity on biological control agents

The acute toxicity of *S. lanceolatum* EO tested on four non-target mosquito natural enemies *A. bouvieri*, *D. indicus*, *P. reticulata*, and *G. affinis* is given in Table 4. LC_{50} values were 8133, 6189, 14,528, and 15,762 μg/ml, respectively. SI/PSF indicated that *S. lanceolatum* EO showed low toxicity on *A. bouvieri*, *D. indicus*, *P. reticulata*, and *G. affinis*, if compared to the targeted mosquito species (Table 5). As a final remark, our focal observations outlined that the survival and swimming activity of the non-target species were not altered during the exposure to LC_{50} and LC_{90} doses of the *S. lanceolatum* EO calculated on mosquito larvae.

Discussion

Chemical composition of the *S. lanceolatum* essential oil

Our GC and GC–MS results showed that at least 18 compounds were present in the *S. lanceolatum* EO, with phenyl propanal (18.3 %), β-caryophyllene (12.8 %), α-humulene (14.5), and caryophyllene oxide (10.7 %) as main constituents. This is a quite different chemical composition, if compared to the EOs extracted from other *Syzygium* species, such as *S. aromaticum* (Gurib-Fakim 2006), *S. zeylanicum* (Govindarajan and Benelli 2016b), and *S. cumini* (Ayyanar and Subash-Babu 2012). Indeed, in EO of close related species *S. zeylanicum*, the main components were α-humulene (37.8 %) and β-elemene (10.7 %), while only low amounts of phenyl propanal (4.2 %), β-caryophyllene (2.3 %), and caryophyllene oxide (4.9 %) were detected. Moreover, in *S. cumini*, a completely different composition of the EO was found, with high percentages of α-terpineol (16.67 %) and α-pinene (17.53 %) (Ayyanar and Subash-Babu 2012), which could be responsible of the high antioxidant activity of this EO (see Kim et al. 2004). Indeed, as a general trend, *Syzygium* plant parts, with special reference to seeds, are well documented as sources of natural antioxidants in traditional Thai medicine (Maisuthisakul et al. 2007), while information on their toxic activity against insect pests is extremely scarce (Govindarajan and Benelli 2016b).

Larvicidal and oviposition deterrent activity of the *S. lanceolatum* essential oil

In latest years, a wide array of plant EOs have been tested against arthropods pests, including mosquitoes, ticks, and other important vectors of medical and veterinary relevance, with promising results (see Benelli 2015b; Benelli et al. 2016b; Pavela et al. 2016 for reviews). EOs from plants may represent a valuable source of mosquitocidal products. Indeed, many studies focused on the effectiveness of EOs and related

Table 3 Oviposition deterrent activity of the *Syzygium lanceolatum* essential oil against mosquito vectors *Anopheles stephensi*, *An. subpictus*, *Aedes aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, and *Cx. tritaeniorhynchus*

Mosquitoes	Concentration (μg/ml)	Eggs laid in bowl (*n*)		Effective repellency (%)	OAI
		Treated	Control		
An. stephensi	50	97.2 ± 1.6**	355.4 ± 3.2	72.65	−0.57
	100	84.5 ± 0.8**	375.9 ± 2.8	77.52	−0.63
	150	67.6 ± 1.2**	428.3 ± 2.6	84.21	−0.72
	200	55.4 ± 0.8*	466.4 ± 3.2	88.12	−0.78
	250	46.2 ± 1.4**	518.7 ± 2.4	91.09	−0.83
An. subpictus	60	95.3 ± 0.8**	366.8 ± 2.8	74.01	−0.58
	120	81.9 ± 1.2*	412.4 ± 2.8	80.14	−0.66
	180	73.4 ± 1.6*	444.9 ± 2.6	83.50	−0.71
	240	66.2 ± 1.2**	476.2 ± 3.2	86.09	−0.75
	300	52.3 ± 1.4**	515.6 ± 2.8	89.85	−0.81
Ae. aegypti	50	94.3 ± 1.2**	372.5 ± 3.0	74.68	−0.59
	100	81.5 ± 1.6*	392.7 ± 2.2	79.24	−0.65
	150	65.9 ± 0.8**	465.4 ± 2.8	85.84	−0.75
	200	48.4 ± 1.2**	495.8 ± 3.2	90.23	−0.82
	250	44.6 ± 1.2**	533.9 ± 2.8	91.64	−0.84
Ae. albopictus	60	91.5 ± 0.8**	392.1 ± 2.4	76.66	−0.62
	120	75.9 ± 1.2**	432.5 ± 2.6	82.45	−0.70
	180	64.2 ± 1.4*	461.2 ± 3.2	86.07	−0.75
	240	54.3 ± 1.4**	499.7 ± 2.8	89.13	−0.80
	300	47.1 ± 1.6**	536.9 ± 2.6	91.22	−0.83
Cx. quinquefasciatus	50	89.5 ± 1.6**	392.4 ± 2.6	77.19	−0.62
	100	74.2 ± 0.8**	433.8 ± 2.4	82.89	−0.70
	150	59.1 ± 1.2**	472.5 ± 2.8	87.49	−0.77
	200	42.6 ± 0.8*	494.2 ± 3.0	91.38	−0.84
	250	37.5 ± 1.4*	554.4 ± 3.2	93.23	−0.87
Cx. tritaeniorhynchus	60	85.2 ± 0.8**	411.5 ± 2.4	79.29	−0.65
	120	71.8 ± 1.2**	455.9 ± 3.0	84.25	−0.72
	180	59.3 ± 1.2*	489.3 ± 2.8	87.88	−0.78
	240	48.1 ± 1.4*	512.4 ± 2.6	90.61	−0.82
	300	39.7 ± 1.2**	565.2 ± 3.2	92.97	−0.86

Each value (mean ± SE) represent mean of five replicates

t values are significant at $*P < 0.05$; $**P < 0.01$

OAI Oviposition Activity Index

constituents against mosquito young instars, with special reference to eggs (Benelli 2015b) and larvae (Pavela 2015).

Our results showed that the *S. lanceolatum* EO was effective as larvicide against *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus*, leading to LC_{50} values of 51.20, 55.11, 60.01, 61.34, 66.71, and 72.24 μg/ml, respectively. In a review of all the plant EOs tested as mosquito larvicides, Pavela (2015) recently pointed out that only 77 EOs showed LC_{50} values lower than 50 ppm, while only 7 of them showed LC_{50} lower than 10 ppm. On the other hand, Komalamisra et al. (2005) considered products showing $LC_{50} \leq 50$ mg/l as active, 50 mg/l < $LC_{50} \leq 100$ mg/l as moderately active, 100 mg/l < $LC_{50} \leq 750$ mg/l as effective, and $L_{50} > 750$ mg/l as inactive. In addition, Ravi Kiran et al. (2006) considered compounds with $LC_{50} < 100$ mg/l as significant mosquito larvicides. However, it should be stressed that these criteria must be directly correlated with the time of exposure and the origin of larvae, which are variables that can alter the LC_{50} values. In this framework, our results are promising, at variance with a wide number of EOs which led to LC_{50} values higher than 100 ppm. Good examples are *Achillea millefolium* EO (LC_{50} = 211.3 μg/ml), *Helichrysum italicum* EO (LC_{50} = 178.1 μg/ml), and *Foeniculum vulgare* EO (LC_{50} = 142.9 μg/ml) tested on *Ae. albopictus* (Conti et al. 2010), as well as the *A. conyzoides* EO evaluated against fourth instar larvae of *Ae. aegypti* (LC_{50} = 148 μg/ml) (Mendonca et al. 2005).

Table 4 Toxicity of *Syzygium lanceolatum* essential oil against biological control agents of *Aedes*, *Anopheles*, and *Culex* mosquito vectors

Non-target organism	Concentration (μg/ml)	24 h mortality (%)± SD[a]	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 *(d.f.)*
Anisops bouvieri	2000	26.8 ± 1.2	4148.34 (3680.50–4562.75)	8133.50 (7546.67–8903.12)	3.12	$y = 10.69 + 0.009\times$	2.896 (4) n.s
	4000	48.3 ± 0.8					
	6000	67.9 ± 0.6					
	8000	88.4 ± 1.2					
	10,000	99.1 ± 0.4					
Diplonychus indicus	3000	28.4 ± 0.6	6189.25 (5457.23–6832.62)	12,409.58 (11,497.27–13,611.65)	3.57	$y = 11.74 + 0.006\times$	1.655 (4) n.s
	6000	46.3 ± 0.8					
	9000	69.5 ± 1.2					
	12,000	87.9 ± 0.4					
	15,000	98.1 ± 1.2					
Gambusia affinis	7000	23.7 ± 0.4	15,762.82 (14,090.80–17,265.49)	30,788.72 (28,539.66–33,763.11)	3.06	$y = 7.3 + 0.003\times$	1.377 (4) n.s.
	14,000	45.6 ± 1.2					
	21,000	62.8 ± 0.8					
	28,000	85.4 ± 0.6					
	35,000	96.2 ± 1.2					
Poecilia reticulata	7000	27.4 ± 0.8	14,528.10 (12,851.53–16,008.01)	28,830.42 (26,727.43–31,596.58)	3.34	$y = 11.11 + 0.003\times$	2.646 (4) n.s.
	14,000	48.3 ± 1.2					
	21,000	66.2 ± 0.6					
	28,000	89.6 ± 0.4					
	35,000	98.1 ± 1.2					

[a] Values are mean ± SD of five replicates

SD standard deviation, LC_{50} lethal concentration that kills 50 % of the exposed organisms, LC_{90} lethal concentration that kills 90 % of the exposed organisms, *UCL* = 95 % upper confidence limit, *LCL* 95 % lower confidence limit, χ^2 chi square, *d.f.* degrees of freedom, *n.s.* not significant ($\alpha = 0.05$)

To our mind, the larvicidal action of *S. lanceolatum* EO on the six mosquito species tested in our research can be mainly due to the presence of α-humulene, *β*-caryophyllene, caryophyllene oxide, and phenyl propanal as the main compounds. Indeed, α-humulene, which is also one of the main components of *S. zeylanicum* EO, has been reported as highly toxic against *An. subpictus* (LC_{50} = 6.19 μg/ml), *Ae. albopictus* (LC_{50} = 6.86 μg/ml), and *Cx. tritaeniorhynchus* (LC_{50} = 7.39 μg/ml), while caryophyllene oxide exhibited larvicidal activity on *Ae. albopictus* larvae with a LC_{50} of 65.6 μg/ml (Cheng et al. 2009).

Furthermore, we recently showed that β-caryophyllene was toxic against third instar larvae of *An. subpictus* (LC_{50} = 41.66 μg/ml), *Ae. albopictus* (LC_{50} = 44.77 μg/ml), and *Cx. tritaeniorhynchus* (LC_{50} = 48.17 μg/ml) (Govindarajan et al. 2016a). β-caryophyllene has been also reported as a good larvicide against *Ae. aegypti* larvae with a 48-h LC_{50} value of 34 μg/ml (Cheng et al. 2004). Chaubey (2012) also noted that β-caryophyllene from the EO of *Zingiber officinale* (Zingiberaceae) and *Piper cubeba* (Piperaceae) was toxic to adults and larvae of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and adults of *Sitophilus oryzae* (Coleoptera: Curculionidae). After 24 h of exposure, β-caryophyllene showed LD_{50} of 0.173 μl/cm^{-2} on *T. castaneum* adults, 0.17 μl/cm^{-2} on *T. castaneum* larvae, and 0.159 μL/cm^{-2} on *S. oryzae* adults. Benelli et al. (2012) also highlighted that β-caryophyllene from the EO of *Hyptis suaveolens* (Lamiaceae) showed 65 % of repellence activity against *Sitophilus granarius* (Coleoptera: Curculionidae).

It can be argued that the use of pure compounds, such as in the case of α-humulene (Govindarajan and Benelli 2016b), should be preferred over the employ of whole EO. However, to our mind, the employ of the EOs is also an important alternative, for two main reasons. First, the EOs from local aromatic plants are easy to obtain, cheap, and still effective for a number of marginalized rural populations worldwide. Second,

Table 5 Suitability index/predator safety factor of different biological control agents of mosquitoes over young instars of *Anopheles stephensi*, *An. subpictus*, *Aedes aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, and *Cx. tritaeniorhynchus* exposed to the *Syzygium lanceolatum* essential oil

Non-target organism	*An. stephensi*	*An. subpictus*	*Ae. aegypti*	*Ae. albopictus*	*Cx. quinquefasciatus*	*Cx. tritaeniorhynchus*
Anisops bouvieri	81.02	67.62	75.27	62.18	69.12	57.42
Diplonychus indicus	120.88	100.90	112.30	92.77	103.13	85.67
Gambusia affinis	307.86	256.97	286.02	236.28	262.66	218.20
Poecilia reticulata	283.75	236.84	263.62	217.77	242.09	201.10

the substances contained in an EO, such as the one from *S. lanceolatum*, represent a rich blend with good larvicidal potential, which is exerted through multiple mechanism(s) of action, including inhibition of cytochrome P450 (CYPs), damage of GABA receptors, inhibition of cholinergic system, and modulation of octopaminergic system (Pavela and Benelli 2016b). This strongly reduced the chances of development of EO resistance in mosquito populations (Benelli 2015a).

As a further confirmation of the importance of screening close-related botanical as mosquitocidal products, we would like to point out the higher efficacy of the *S. lanceolatum* EO tested in this study, if compared to the EO extracted from *S. zeylanicum*, which achieved LC_{50} values of 83.11, 90.45, and 97.96 μg/ml on third instar larvae of *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus*, respectively (Govindarajan and Benelli 2016b).

Notably, most of the researches in mosquito control focused on plant EOs as larvicides or ovicides, while limited efforts focused on their exploitation as oviposition deterrents (Prajapati et al. 2005; Khandagle et al. 2011). Our findings showed that *S. lanceolatum* EO was effective as oviposition deterrent against the six tested mosquito species, with OAI reaching −0.83, −0.81, −0.84, −0.83, −0.84, and −0.86 on *An. stephensi*, *An. subpictus*, *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus*, respectively. In this framework, we hypothesize that EO component phenyl propanal may be one of the main molecules responsible of the larvicidal and ovideterrent action exerted by the *S. zeylanicum* EO on mosquitoes, since it has been reported that phenyl propyl compounds showed larvicidal activity in recent literature. (Nascimento et al. 2013; Silva et al. 2016). In particular, Bezerra-Silva et al. (2016) reported that the aldehyde dodecanal showed deterrent effect against *Ae. egypti* mosquitoes, allowing us to formulate that close-related aldehyde compounds, such as phenyl propanal, may evoke comparable deterrent effects. Further research on the activity of these compounds is ongoing.

More generally, in agreement with our oviposition deterrence assays, Autran et al. (2009) tested EOs from stems, leaves, and inflorescences of *Piper marginatum* showing that in presence of 50 and 100 ppm of the EOs, the *A. aegypti* females laid 40 % fewer eggs if compared to the controls. Oviposition deterrence was also observed testing the EO from inflorescences of *Alpinia purpurata* at a minimum concentration of 100 ppm, which lead to a reduction of at least 50 % in the number of eggs laid in test vessels in comparison with controls (Santos et al. 2012). Also, the *Commiphora leptophloeos* leaf EO at concentrations of 25, 50, and 100 ppm exerted a strong effect on the oviposition of *Ae. aegypti* females, resulting in a reduction ranging from 59 to 63 % in the number of eggs laid (Prajapati et al. 2005). Lastly, we noted that the oviposition deterrent action of *S. lanceolatum* EO can be partially due to the presence of α-humulene and β-caryophyllene, which have been recently reported as an effective oviposition deterrent against *Ae. aegypti* (da Silva et al. 2015).

Biotoxicity on biological control agents

In our experiments, acute toxicity of *S. lanceolatum* EO and its major compounds against mosquito biocontrol agent *A. bouvieri*, *D. indicus*, *G. affinis*, and *P. reticulata* was extremely low, with LC_{50} values higher than 4148.34 μg/ml. Recently, plant EOs are gaining increasing attention important sources of biopesticides for control of agricultural and urban arthropod pests. This is mostly due to the fact that they do not induce resistance and have limited toxic effects on non-target organisms (Benelli 2015a; Pavela 2015). In agreement with our work, recent research showed little acute toxicity of *S. zeylanicum* EO on *G. affinis*, with a LC_{50} value of 20,374.26 μg/ml (Govindarajan and Benelli 2016b). Similarly, the biotoxicity of *Heracleum sprengelianum* EO and its two major compounds lavandulyl acetate and bicyclogermacrene on *A. bouvieri*, *D.indicus*, and *G. affinis* was also moderate, with LC_{50} values ranging from 1840 to 4219 μg/ml (Govindarajan and Benelli 2016a). Taken together, all these findings highlight the eco-friendly nature of plant-borne molecules extracted from Asian plant species, supporting their potential employ as mosquito larvicides in aquatic breeding sites (Benelli 2015a).

Conclusions

Overall, our study highlights the promising larvicidal and oviposition deterrent activity of the *S. lanceolatum* leaf EO on six mosquito species of public health importance. Notably, the EO was effective as oviposition deterrent with maximum OAI ranging from −0.81 to −0.86. GC and GC–MS studies showed that the toxic action of the tested EO can be due to the presence of phenyl propanal, β-caryophyllene, α-humulene, and caryophyllene oxide as major constituents. In particular, the latter three molecules have been recently reported as effective larvicides against anopheline and culicine species. In addition, α-humulene and β-caryophyllene are able to effectively deter *Ae. aegypti* females from egg laying. Notably, the toxicity of *S. lanceolatum* EO against several biological control agents of mosquitoes, including water bugs (*A. bouvieri* and *D. indicus*) and fishes (*G. affinis* and *P. reticulata*) was extremely low. On this basis, we pointed out the potential of the *S. lanceolatum* leaf EO as a source of environmental-friendly oviposition deterrents and larvicides effective against a wide number of mosquito species of importance for parasitology.

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