

Zingiber cernuum (Zingiberaceae) essential oil as effective larvicide and oviposition deterrent on six mosquito vectors, with little non-target toxicity on four aquatic mosquito predators

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Abstract Mosquitoes are responsible for the transmission of many pathogens and parasites, which cause serious diseases in humans and animals. Currently, botanical products have been suggested as alternative tools in the fight against arthropod vectors. In this study, the essential oil (EO) extracted from *Zingiber cernuum* was tested as larvicide and oviposition deterrent on six mosquito species of public health relevance, including malaria and Zika virus vectors. The EO showed high toxicity on third instar larvae of *Anopheles stephensi* (LC₅₀ = 41.34 µg/ml), *Aedes aegypti* (LC₅₀ = 44.88 µg/ml), *Culex quinquefasciatus* (LC₅₀ = 48.44 µg/ml), *Anopheles subpictus* (LC₅₀ = 51.42 µg/ml), *Aedes albopictus* (LC₅₀ = 55.84 µg/ml), and *Culex tritaeniorhynchus* (LC₅₀ = 60.20 µg/ml). In addition, low doses of *Z. cernuum* EO reduced oviposition rates in six mosquito species. The acute toxicity of *Z. cernuum* EO on four mosquito predators was scarce; LC₅₀ ranged from 3119 to 11,233 µg/ml. Overall, our results revealed that the *Z. cernuum* EO can be considered

for the development of effective and environmental-friendly mosquito larvicides and oviposition deterrents.

Keywords Aquatic toxicology · Biosafety · Dengue · Japanese encephalitis · Malaria · Neglected tropical disease · Zika virus

Introduction

Arthropods are dangerous vectors of important life-threatening and debilitating diseases. Among them, mosquitoes (Diptera: Culicidae) belonging to the genera *Anopheles*, *Aedes*, and *Culex* act as vectors of pathogens and parasites causing malaria, filariasis, Japanese encephalitis, dengue and dengue hemorrhagic fever, yellow fever, chikungunya, and, very recently, Zika virus (Benelli and Mehlhorn 2016; Ward and Benelli 2017). Several efforts have been made to improve the control of mosquito vectors. Besides the efficacy at low doses, the use of synthetic insecticides can lead to high costs, concerns for environmental sustainability, harmful effects on human health and other non-target populations, and development of insecticide resistance in the targeted pests (Benelli 2015a; Naqqash et al. 2016).

While chemical insecticides are usually based on a single active ingredient, plant-derived pesticides consist of a combination of molecules which can act concertedly on both behavioral and physiological processes (Jain et al. 2001; Pavela 2015). Thus, there is very little chance of resistance development in the treated arthropods. In this scenario, the identification of effective and eco-friendly biopesticides is crucial for the successful management of arthropod vectors (Lucia et al.

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2007; Cheng et al. 2003, 2004, 2008, 2009a; Govindarajan and Benelli 2016a, b).

Essential oils (EOs) are complex mixtures of volatile organic compounds produced as secondary metabolites in plants. They include blends of terpenes, sesquiterpenes, and oxygenated compounds, such as alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, and phenol ethers (Chericoni et al. 2004; Astani et al. 2010; Almeida et al. 2011). Plant EOs and their constituents have been proposed as effective pesticides, since they are able to evoke acute toxicity and oviposition and feeding deterrence, as well as repellency (Barnard 1999). The insecticidal properties of EO are widely documented (e.g., Pathak and Dixit 1988; Araujo et al. 2003; Traboulsi et al. 2002; Ansari et al. 2000a, b, 2005; Koul et al. 2008; Rattan 2010; Pavela 2008a, b; Urzúa et al. 2010; Benelli 2015b). To the best of our knowledge, any development of resistance to EOs and their constituents has been reported (Sharma et al. 1992; Cavalcanti et al. 2004; Pavela and Benelli 2016).

A growing number of researchers focused on the effectiveness of plant EOs against young mosquito instars (Pavela 2015). Good examples include *Lippia sidoides* (Carvalho et al. 2003), *Tagetes patula* (Dharmagadda et al. 2005), *Pinus kesiya* (Govindarajan et al. 2016a), *Cordia leucomalloides* and *C. curassavica* (Santos et al. 2006), *Blumea mollis* (Senthilkumar et al. 2008), *Piper klotzschianum* (Do Nascimento et al. 2013), *Tetradium glabrifolium* (Liu et al. 2015), *Chloroxylon swietenia* (Kiran et al. 2006), *Origanum majorana* (El-Akhal et al. 2014), *Thymus vulgaris* (El-Akhal et al. 2015), *T. magnus* (Park et al. 2012), *T. transcaspicus* (Dargahi et al. 2014), *Cinnamomum osmophloeum* (Cheng et al. 2009b), *Clausena excavata* (Cheng et al. 2009c), *Toddalia asiatica* (Liu et al. 2013), *Saussurea lappa* (Liu et al. 2012), *Ipomoea carica* (Thomas et al. 2004), and *Zingiber officinalis* (Pushpanathan et al. 2008). Pitasawata et al. (2007) and Champakaew et al. (2007) reported that *Curcuma zedoaria* EO showed larvicidal toxicity on *Aedes aegypti*, while the *Zingiber zerumbet* rhizome EO had larvicidal and pupicidal activity on anopheline mosquitoes (Tewtrakul et al. 1998). Sutthanont et al. (2010) recommended the use of this EO as mosquito larvicide. Kamaraj et al. (2010) reported that the hexane extract of *Z. zerumbet* had larval toxicity against *Culex quinquefasciatus*.

The family of Zingiberaceae represents a key source of herbal preparations and phytoconstituents of interest for current pharmacology, parasitology, and entomology (Burkill 1966; Negi et al. 1999; Scartezzini and Speroni 2000; Youko et al. 2000; Patricia et al. 2003; Jirovetz et al. 2003; Bendjeddou et al. 2003; Nguefack et al. 2004; Govindarajan et al. 2016b). The *Zingiber officinale* rhizome EO is one of the most studied Zingiberaceae EOs. It contains monoterpenoids and sesquiterpenoids. Main molecules are α -zingiberene, α -curcumene, β -bisabolene, and β -sesquiphellandrene (Menon 2007; Rana et al. 2008; Padmakumari et al. 2009), while *Z. zerumbet* EO shows a high

content of the monocyclic sesquiterpene ketone zerumbone, as well as an oxygenated humulene derivative (Srivastava et al. 2000; Bhuiyan et al. 2009).

Zingiber cernuum Dalzell (Zingiberaceae), commonly known as curved-stem ginger, is widely found in the evergreen forests of Western Ghats, India. It is a large perennial herb, 1–2 m tall, with curved stem. The leaves are 15–30 cm in length, narrow-elliptic, and long-pointed. The flowers are borne in spikes 5–10 cm long, directly from the rootstock, rising just above the ground. Bracts are 2–3 cm long, greenish-yellow. The sepal cup is shortly three-lobed. Stamen is single, with a short filament. The style is threadlike. The capsules are 1 cm long, smooth with red, channeled seeds. The flowers are yellow colored, variegated with red, with the lab broad and three-lobed (Sanjay 2015). From a phytochemical point of view, *Z. cernuum* is extremely scarcely studied. Kasarkar and Kulkarni (2011) recently identified flavonoids and tannins in *Z. cernuum* extracts, which showed antioxidant activity (Sanjay 2015). This species is rich in iron and manganese and showed low amounts of molybdenum, sulfur, and nitrate in rhizome and leaves (Kasarkar and Kulkarni 2011). To the best of our knowledge, the composition and mosquitocidal bioactivity of *Z. cernuum* EO have not been explored.

In this research, we analyzed the chemical composition of *Z. cernuum* EO using gas chromatography-mass spectroscopy analysis. Furthermore, we studied the larvicidal and oviposition deterrent activity of the *Z. cernuum* EO on six mosquito species, the malaria vectors *Anopheles stephensi* and *Anopheles subpictus*; the dengue and Zika virus vectors *Aedes aegypti* and *Aedes albopictus*; the filariasis, West Nile virus, and St. Louis encephalitis vector *Culex quinquefasciatus*; and the Japanese encephalitis vector *C. tritaeniorhynchus*. In addition, to assess the biosafety of *Z. cernuum* EO-based treatments in the aquatic environment, we investigated the toxicity of *Z. cernuum* EO on four non-target enemies of mosquito young instars, the insects *Anisops bouvieri* and *Diplonychus indicus* and the fishes *Poecilia reticulata* and *Gambusia affinis*.

Materials and methods

Extraction and GC-MS analysis of the *Z. cernuum* essential oil

Fresh rhizomes of *Z. cernuum* were collected during May 2016 in the Munnar mountains (India 10° 05' 21" N 77° 03' 35" E, 1700 m a.s.l.). Four hundred grams of fresh rhizomes of *Z. cernuum* was hydrodistilled for 3 h using a modified Clevenger-type apparatus; then, the *Z. cernuum* EO was dried over anhydrous sodium sulfate and stored in the dark at 5 °C. GC and GC-MS analyses were carried out as recently described by Govindarajan and Benelli (2016a). The

constituents of the *Z. cernuum* EO were identified by comparison of their mass spectra and retention indices (Table 1) with the ones indexed in the Wiley library, as well as those available in the literature (Adams 2007).

Larvicidal and oviposition deterrence assays

The six mosquito species were reared as described by Govindarajan and Benelli (2016a). Early third instar larvae and adults were used to evaluate the larvicidal potential and oviposition deterrence, respectively. The larvicidal activity of the *Z. cernuum* EO was studied following the method by WHO (2005). Various doses of the *Z. cernuum* EO were dissolved in 1 ml dimethyl sulfoxide (DMSO) and then diluted in 249 ml of filtered tap water. Control was 1 ml of DMSO diluted in 249 ml of water. Within each replicate, 20 early

third instar larvae were tested (WHO 2005); *n* = 5 per each dose.

In oviposition deterrent experiments, *Z. cernuum* EO was evaluated at a dose range of 40–250 µg/ml in DMSO. DMSO diluted in water served as a control. We followed the method by Xue et al. (2001). Twenty gravid females (5–7 days old) of each mosquito species were released in the bioassay cage (60 × 60 × 45 cm). After 24 h, the number of eggs laid in treated and control bowls was counted using a stereomicroscope (Olympus, Japan).

Toxicity on non-target organisms

Toxicity on the four predators was assessed following Sivagnaname and Kalyanasundaram (2004) with minor modifications by Govindarajan and Benelli (2016a). The

Table 1 Chemical composition of *Zingiber cernuum* essential oil

Peak	Component	Retention time (Kovats index)	Composition (%)	Mode of identification
1	α-Thujene	925	0.9	RI, MS
2	α-Pinene	927	1.6	RI, MS
3	Camphene	942	0.8	RI, MS
4	Sabinene	971	4.6	RI, MS
5	β-Pinene	974	1.2	RI, MS
6	Myrcene	987	1.7	RI, MS
7	α-Phellandrene	1002	1.3	RI, MS
8	δ-3-Carene	1005	13.2	RI, MS
9	α-Terpinene	1015	1.2	RI, MS
10	<i>p</i> -Cymene	1021	1.9	RI, MS
11	β-Phellandrene	1026	2.6	RI, MS
12	γ-Terpinene	1055	2.3	RI, MS
13	Terpinolene	1081	1.7	RI, MS
14	Terpinen-4-ol	1178	5.8	RI, MS
15	α-Terpineol	1192	1.2	RI, MS
16	β-Elemene	1383	0.9	RI, MS
17	<i>cis</i> -Caryophyllene	1397	0.8	RI, MS
18	α-Gurjunene	1402	0.9	RI, MS
19	<i>trans</i> -Caryophyllene	1416	31.8	RI, MS
20	α- <i>trans</i> -Bergamotene	1431	0.9	RI, MS
21	α-Humulene	1452	10.4	RI, MS
22	BicycloGermacrene	1497	1.2	RI, MS
23	δ-Amorphene	1515	1.6	RI, MS
24	<i>trans</i> -Nerolidol	1561	0.9	RI, MS
25	Spathulenol	1573	0.8	RI, MS
26	Caryophyllene oxide	1575	3.6	RI, MS
27	Humulene epoxide II	1601	1.2	RI, MS
28	<i>epi</i> -α-Cadinol	1635	0.9	RI, MS
	Total		96.2	

RI retention index, MS mass spectra

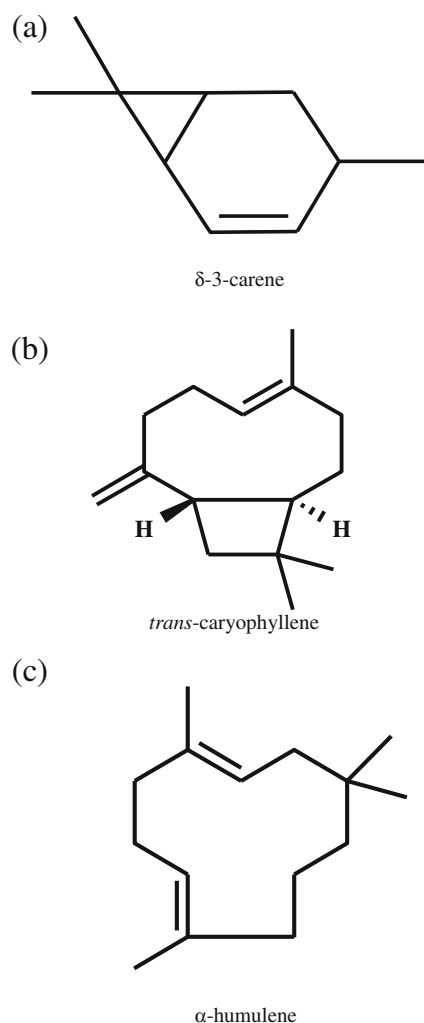


Fig. 1 Chemical structures of the three major constituents of *Zingiber cernuum* essential oil. **a** δ -3-Carene. **b** *trans*-Caryophyllene. **c** α -Humulene

Z. cernuum EO was evaluated at doses of even $50 \times LC_{50}$ values calculated for mosquito larvae studied in the paragraph above, 10 replicates per each dose, plus 4 control replicates (where no EO was added to the water). The mortality of non-target species was assessed 48 h post-treatment.

Data analysis

All data were analyzed using the SPSS Statistical Software Package version 16.0. LC_{50} and LC_{90} were estimated following the method by Finney (1971). The oviposition activity index (OAI) was calculated as indicated by Kramer and Mulla (1979):

$$OAI = (NT - NC) / (NT + NC)$$

Effective repellency (ER %) due to *Z. cernuum* EO was estimated following Xue et al. (2001). In non-target assays,

the suitability index (SI) was calculated as described by Deo et al. (1988).

Results

GC and GC-MS of *Z. cernuum* essential oil

The yield of the *Z. cernuum* rhizome EO was 1.8 ml/kg of rhizome fresh weight. Table 1 showed a total of 28 compounds representing 96.2% of the *Z. cernuum* EO. The major constituents of *Z. cernuum* EO were δ -3-carene, *trans*-caryophyllene, and α -humulene (Fig. 1). The other 25 compounds ranged from 0.8 to 5.8%.

Larvicidal and oviposition deterrent activity

The *Z. cernuum* EO showed acute toxicity on third instar larvae of *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*, with LC_{50} of 41.34, 44.88, 48.44, 51.42, 55.84, and 60.20 $\mu\text{g/ml}$, respectively (Table 2). No mortality was detected in the control.

The results obtained from the oviposition deterrence experiments testing *Z. cernuum* EO on the six mosquito species are reported in Table 3. The mean number of eggs laid in sites treated with the *Z. cernuum* EO tested at the highest doses (i.e., 200–250 $\mu\text{g/ml}$) was 44.5, 41.5, and 36.7 eggs per bowl for *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*, respectively, and 56.2, 52.8, and 47.5 eggs per bowl for *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*, respectively. Significant differences ($P < 0.05$; $P < 0.01$) were detected comparing these values to the respective controls (Table 3). The range of OAI achieved by *Z. cernuum* EO tested against the six mosquito vectors at 200 and 250 $\mu\text{g/ml}$ ranged from -0.79 to -0.84 (Table 3).

Toxicity on non-target predators

Z. cernuum EO toxicity on *A. bouvieri*, *D. indicus*, *P. reticulata*, and *G. affinis* was reported in Table 4. LC_{50} values were 3119, 5273, 10,363, and 11,233 $\mu\text{g/ml}$, respectively. PSF indicated that the *Z. cernuum* EO showed scarce toxicity on *A. bouvieri*, *D. indicus*, *P. reticulata*, and *G. affinis* (Table 5). Survival and swimming activity of the non-target water bugs and fishes were not affected by the exposure to *Z. cernuum* EO LC_{50} and LC_{90} estimated on the six mosquito species.

Table 2 Larvicidal activity of the essential oil from *Zingiber cernuum* on six mosquito vectors

Mosquito	Concentration (µg/ml)	Mortality (%) ± SD	LC ₅₀ (µg/ml) (LCL-UCL)	LC ₉₀ (µg/ml) (LCL-UCL)	Slope	Regression equation	χ ² (d.f.)
<i>Anopheles stephensi</i>	20	28.5 ± 1.2	41.34	81.00	3.11	y = 10.89 + 0.922x	5.528 (4) n.s.
	40	46.9 ± 0.8	(36.68–45.46)	(75.13–88.71)			
	60	67.3 ± 0.6					
	80	88.2 ± 0.4					
	100	100.0 ± 0.0					
<i>Anopheles subpictus</i>	25	29.2 ± 1.2	51.42	101.86	3.31	y = 11.55 + 0.729x	5.533 (4) n.s.
	50	46.5 ± 0.8	(45.49–56.66)	(94.42–111.66)			
	75	68.3 ± 0.6					
	100	87.2 ± 0.4					
	125	100.0 ± 0.0					
<i>Aedes aegypti</i>	20	25.2 ± 0.6	44.88	86.49	2.86	y = 6.75 + 0.937x	3.684 (4) n.s.
	40	43.8 ± 0.8	(40.25–49.07)	(80.24–94.71)			
	60	62.4 ± 1.2					
	80	85.1 ± 0.4					
	100	98.2 ± 0.8					
<i>Aedes albopictus</i>	25	26.7 ± 0.6	55.84	110.28	3.25	y = 8.16 + 0.73x	2.467 (4) n.s.
	50	42.6 ± 0.8	(49.77–61.27)	(102.11–121.13)			
	75	63.8 ± 1.2					
	100	84.2 ± 0.4					
	125	97.1 ± 0.8					
<i>Culex quinquefasciatus</i>	20	22.3 ± 0.4	48.44	90.15	2.49	y = 2.01 + 0.965x	3.790 (4) n.s.
	40	39.2 ± 1.2	(44.00–52.56)	(83.76–98.56)			
	60	58.6 ± 0.8					
	80	81.9 ± 0.6					
	100	97.4 ± 1.2					
<i>Culex tritaeniorhynchus</i>	25	23.4 ± 0.4	60.20	114.22	2.68	y = 3.39 + 0.755x	2.512 (4) n.s.
	50	38.6 ± 1.2	(54.45–65.51)	(105.94–125.18)			
	75	60.2 ± 0.6					
	100	81.5 ± 0.8					
	125	96.3 ± 0.4					

Discussion

GC and GC-MS of *Z. cernuum* essential oil

Our results showed that 28 compounds were identified in the *Z. cernuum* EO, with δ-3-carene, trans-caryophyllene, and α-humulene as main components. This highlighted a quite surprising composition, if compared to EOs extracted from other *Zingiber* species. Indeed, several studies have been conducted on the EOs from other *Zingiber* species, such as *Z. officinale* (Foko et al. 2011), *Z. cassumunar* (Jantan et al. 2003), *Z. zerumbet* (Tewtrakul et al. 1998), *Z. piperitum* (Kamsuk et al. 2006), *Z. limonella* (Somanabandhu et al. 1992), *Z. armatum* (Tiwary et al. 2007), and *Z. monophyllum* (Pavela and Govindarajan 2017). Campbell et al. (2011) pointed out that several mono- and sesquiterpenes, including trans-caryophyllene, α-terpineol, β-pinene, germacrene-D, limonene, and α-zingiberene, present in the EOs evoke responses in *Aedes aegypti* antennae. Sesquiterpenes α-curcumene, β-sesquiphellandrene, zingiberene, and β-bisabolene from *Z. officinale* EO (Campbell 2009), as well as

trans-caryophyllene and *Ocimum forskolei* (Dekker et al. 2011), also induced antennal responses by the antennae of *A. aegypti* females.

Larvicidal and oviposition deterrent potential

Essential oils from plants can represent an alternative source of eco-friendly and biodegradable mosquito ovicides (Benelli 2015b), larvicides (Pavela 2015) and adult repellents (Barnard 1999). A growing number of researches concentrated on the effectiveness of plant EOs against mosquito young instars, with special reference to larvae (Sukumar et al. 1991; Benelli 2015b; Pavela 2015). According to Tawatsin et al. (2001), the bioactivity of EOs depends on various factors, including the plant species and cultivar, the growing conditions, the harvesting time, the storage conditions, and the extraction method (see also Pavela and Benelli 2016).

In our assays, the EO extracted from the rhizome of *Z. cernuum* showed high toxicity against third instar larvae of *Anopheles stephensi* (LC₅₀ = 41.34 µg/ml), *Aedes aegypti* (LC₅₀ = 44.88 µg/ml), *Culex quinquefasciatus* (LC₅₀ = 48.44 µg/ml), *Anopheles subpictus* (LC₅₀ = 51.42 µg/ml), *Aedes albopictus*

Table 3 Oviposition deterrent activity of the *Zingiber cernuum* essential oil on six mosquito vectors

Mosquito	Concentration (µg/ml)	Eggs laid in bowl (n)		Effective repellency (%)	OAI
		Treated	Control		
<i>Anopheles stephensi</i>	40	95.2 ± 1.4**	368.9 ± 2.2	74.10	-0.58
	80	82.6 ± 0.8**	389.6 ± 1.8	78.79	-0.65
	120	65.4 ± 1.2**	433.8 ± 2.4	84.92	-0.73
	160	52.9 ± 1.2*	474.2 ± 2.8	88.84	-0.79
	200	44.5 ± 1.4**	512.7 ± 1.6	91.32	-0.84
<i>Anopheles subpictus</i>	50	94.2 ± 0.8**	375.3 ± 3.2	74.90	-0.59
	100	82.6 ± 1.2**	402.8 ± 2.4	79.49	-0.65
	150	70.7 ± 1.2*	438.9 ± 2.8	83.89	-0.72
	200	64.9 ± 0.8*	465.3 ± 2.2	86.05	-0.75
	250	56.2 ± 1.4**	501.2 ± 3.2	88.78	-0.79
<i>Aedes aegypti</i>	40	91.4 ± 1.2**	375.5 ± 1.8	75.65	-0.60
	80	78.6 ± 0.8**	399.2 ± 2.2	80.31	-0.67
	120	62.7 ± 1.2*	452.7 ± 2.8	86.14	-0.75
	160	46.8 ± 1.4*	488.3 ± 3.2	90.41	-0.82
	200	41.5 ± 1.4**	523.1 ± 1.8	92.06	-0.85
<i>Aedes albopictus</i>	50	92.7 ± 1.2**	388.5 ± 2.8	76.13	-0.61
	100	78.3 ± 1.2**	412.8 ± 2.6	81.03	-0.68
	150	68.9 ± 0.8*	445.7 ± 2.4	84.54	-0.73
	200	61.4 ± 1.4**	476.9 ± 2.8	87.12	-0.77
	250	52.8 ± 1.2**	522.5 ± 2.6	89.89	-0.81
<i>Culex quinquefasciatus</i>	40	87.6 ± 0.8**	388.4 ± 2.4	77.44	-0.63
	80	71.4 ± 1.2**	425.3 ± 1.8	83.21	-0.71
	120	58.6 ± 1.2*	469.4 ± 2.6	87.51	-0.77
	160	41.8 ± 0.8**	494.2 ± 2.2	91.54	-0.84
	200	36.7 ± 1.4**	544.5 ± 2.8	93.25	-0.87
<i>Culex tritaeniorhynchus</i>	50	87.4 ± 1.6**	396.3 ± 2.2	77.94	-0.63
	100	75.9 ± 1.4*	433.7 ± 2.6	82.49	-0.70
	150	61.3 ± 0.8**	462.9 ± 3.2	86.75	-0.76
	200	55.1 ± 1.2**	497.1 ± 2.6	88.91	-0.80
	250	47.5 ± 0.8**	538.9 ± 2.2	91.18	-0.83

OAI oviposition activity index

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

($LC_{50} = 55.84 \mu\text{g/ml}$), and *Culex tritaeniorhynchus* ($LC_{50} = 60.20 \mu\text{g/ml}$). Concerning the bioactivity of other EOs and extracts from the Zingiberaceae family, Rahuman et al. (2008) evaluated the larvicidal activity of 4-gingerol from *Z. officinale*, against *A. aegypti* (4.25 ppm) and *C. quinquefasciatus* (5.52 ppm). Sutthanont et al. (2010) reported that *Z. zerumbet* and *Kaempferia galanga* EOs are effective on *A. aegypti*, with LC_{50} of 48.88 and 53.64 ppm, respectively. Tewtrakul et al. (1998) showed the toxicity of *Z. zerumbet* ethanol extract on anopheline larvae, with LD_{50} of 18.9 µg/ml. The *Z. cassumunar* EO is effective against *A. aegypti* larvae ($LT_{50} = 1.4$ min) (Jantan et al. 2003). Pitasawata et al. (2007) and Champakaew et al. (2007) noted that the *C. zedoaria* EO showed larvicidal activity on *A. aegypti*, with LC_{50} of 33.45 ppm. *Z. zerumbet* EO also

showed larvicidal toxicity on *A. aegypti* and *A. nuneztovari*, with LC_{50} of 89.8 and 62.8 µg/ml, respectively (Tewtrakul et al. 1998). Other studies reported that *Z. officinale* EO tested at 20 mg/ml and 700 µl/ml effectively repelled stored product pests, such as *Sitophilus zeamais* and *Prostephanus truncatus* adults (Ogbonna et al. 2014).

The *Z. cernuum* EO tested in this study was mainly composed of δ -3-carene, *trans*-caryophyllene, and α -humulene. Recently, several effective mosquitocidal molecules have been identified in the EOs of other Indian plants. For example, Govindarajan and Benelli (2016a) investigated the toxicity of α -humulene and β -elemene from *Syzygium zeylanicum* EO on *A. albopictus* ($LC_{50} = 6.86$ and $11.15 \mu\text{g/ml}$), *C. tritaeniorhynchus* ($LC_{50} = 7.39$ and $12.05 \mu\text{g/ml}$), and *A. subpictus* (LC_{50} values were 6.19 and $10.26 \mu\text{g/ml}$).

Table 4 Toxicity of the *Zingiber cernuum* essential oil on non-target mosquito predators

Non-target species	Concentration (µg/ml)	Mortality (%) ± SD	LC ₅₀ (µg/ml) (LCL-UCL)	LC ₉₀ (µg/ml) (LCL-UCL)	Slope	Regression equation	χ ² (d.f.)
<i>Anisops bouvieri</i>	1500	26.4 ± 1.2	3119.35 (2769.42–3429.59)	6104.44 (5664.60–6681.22)	3.09	y = 10.52 + 0.012x	3.146 (4) n.s.
	3000	48.9 ± 0.8					
	4500	67.2 ± 0.6					
	6000	88.5 ± 0.4					
	7500	99.1 ± 1.2					
<i>Diplonychus indicus</i>	2500	24.8 ± 0.8	5273.84 (4707.74–5779.89)	10,135.89 (9419.71–11,069.24)	2.83	y = 8.91 + 0.008x	1.146 (4) n.s.
	5000	47.2 ± 0.6					
	7500	68.5 ± 1.2					
	10,000	89.9 ± 0.4					
	12,500	98.1 ± 1.2					
<i>Gambusia affinis</i>	5000	25.7 ± 0.4	11,233.25 (10,033.35–12,311.09)	22,026.60 (20,406.96–24,172.77)	3.12	y = 7.60 + 0.004x	2.128 (4) n.s.
	10,000	42.6 ± 0.6					
	15,000	64.8 ± 1.2					
	20,000	83.4 ± 0.8					
	25,000	97.2 ± 1.2					
<i>Poecilia reticulata</i>	5000	28.5 ± 0.6	10,363.75 (9177.37–11,412.67)	20,489.41 (18,996.93–22,452.17)	3.27	y = 11.05 + 0.004x	3.533 (4) n.s.
	10,000	46.3 ± 1.2					
	15,000	67.8 ± 0.4					
	20,000	88.2 ± 0.8					
	25,000	99.1 ± 1.2					

Further research focusing on potential synergic larvicidal effects occurring among the abovementioned molecules is ongoing.

Concerning the oviposition deterrent potential, we observed that the range of OAI achieved by the *Z. cernuum* EO tested at 200 and 250 µg/ml compared with controls ranged from -0.7 to -0.8. Recently, a growing number of studies focused on the oviposition deterrent activity of plant extracts and EOs against mosquito vectors of economic importance (Elango et al. 2009). However, few of them investigated the oviposition deterrent potential of *Zingiber* species. Coria et al. (2008) reported 100% oviposition deterrent effect obtained with *Melia azedarach* leaf extract tested at 1 g/l concentration against *Aedes aegypti*. Prajapati et al. (2005) noted that the bark EO of *Cinnamomum zeylanicum* reduced the oviposition rates of *A. aegypti* to 50% when tested at 33.5 ppm. Autran et al. (2009) recorded the oviposition deterrent effect of EO obtained from leaves, inflorescences, and stems of *Piper marginatum*; the EOs from leaves and stems of *P. marginatum* exhibited oviposition deterrent effect on

A. aegypti females at 50 and 100 ppm concentrations and the number of eggs laid was significantly lower (<50%), if compared to control.

Biotoxicity on mosquito predators

It is worthy to note that the toxicity of *Z. cernuum* EO on the mosquito predators *A. bouvieri*, *D. indicus*, *G. affinis*, and *P. reticulata* was very low, with LC₅₀ values always higher than 3000 µg/ml. EOs have been recently recognized as novel and reliable biopesticides, which do not induce resistance and have few toxic effects on human health and non-target species. For example, scarce toxicity of *P. kesiya* EO on *A. bouvieri*, *D. indicus*, and *G. affinis* was noted, with LC₅₀ from 4135 to 8390 mg/ml, and in agreement with the present results, *G. affinis* has been found less susceptible to EO, if compared to *A. bouvieri* and *D. indicus* (Govindarajan et al. 2016a). *S. zeylanicum* EO tested on *G. affinis* showed LC₅₀ = 20,374.26 µg/ml (Govindarajan and Benelli 2016a). Moreover, *Heracleum sprengeianum* EO, lavandulyl acetate

Table 5 Predator safety factor calculated on four mosquito predators and mosquito young instars post-treatment with *Zingiber cernuum* essential oil

Non-target species	<i>Anopheles stephensi</i>	<i>Anopheles subpictus</i>	<i>Aedes aegypti</i>	<i>Aedes albopictus</i>	<i>Culex quinquefasciatus</i>	<i>Culex tritaeniorhynchus</i>
<i>Anisops bouvieri</i>	75.45	60.66	69.50	55.86	64.39	51.81
<i>Diplonychus indicus</i>	127.57	102.56	117.50	94.44	108.87	87.60
<i>Poecilia reticulata</i>	250.69	201.55	230.92	185.59	213.95	172.15
<i>Gambusia affinis</i>	271.72	218.46	250.29	201.16	231.90	186.59

and bicyclogermacrene, tested on *A. bouvieri*, *D. indicus*, and *G. affinis*, led to LC₅₀ ranging from 414 to 4219 µg/ml (Govindarajan and Benelli 2016b). Taken together, the data reported above underline the environmental-friendly nature of botanicals from selected Asian plant species, which can be further considered for as larvicides and oviposition deterrents in urban and peri-urban areas.

Conclusions

Overall, the present research sheds light on the chemical composition of the EO of *Z. cernuum*, as well as on its larvicidal and oviposition deterrent activity on six important mosquito species. Notably, really limited non-target effects of *Z. cernuum* EO were found on four important mosquito predators. Therefore, the results from this study supported our hypothesis to consider the *Z. cernuum* EO for the development of effective and eco-friendly larvicides and oviposition deterrents effective against a broad range of mosquito vector species.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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