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ORIGINAL PAPER

Larvicidal and repellent potential of *Zingiber nimmonii* (J. Graham) Dalzell (Zingiberaceae) essential oil: an eco-friendly tool against malaria, dengue, and lymphatic filariasis mosquito vectors?

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Abstract Mosquitoes (Diptera: Culicidae) are important vectors of terms of public health relevance, especially in tropical and sub-tropical regions. The continuous and indiscriminate use of conventional pesticides for the control of mosquito vectors has resulted in the development of resistance and negative impacts on non-target organisms and the environment. Therefore, there is a need for development of effective mosquito control tools. In this study, the larvicidal and repellent activity of *Zingiber nimmonii* rhizome essential oil (EO) was evaluated against the malaria vector *Anopheles stephensi*, the dengue vector *Aedes aegypti*, and the lymphatic filariasis vector *Culex quinquefasciatus*. The chemical composition of the EO was analyzed by gas chromatography–mass spectroscopy (GC-MS). GC-MS revealed that the *Z. nimmonii* EO contained at least 33 compounds. Major constituents were myrcene, β-caryophyllene, α-humulene, and α-cadinol. In acute toxicity assays, the EO showed significant toxicity against early third-stage larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, with LC_{50} values of 41.19, 44.46, and 48.26 μg/ml, respectively. Repellency bioassays at 1.0, 2.0, and 5.0 mg/cm^2 of *Z. nimmonii* EO gave 100 % protection up to 120, 150, and 180 min. against *An. stephensi*, followed by *Ae. aegypti* (90, 120, and 150 min) and *Cx. quinquefasciatus* (60, 90, and 120 min). Furthermore, the EO was safer towards two non-target aquatic organisms, *Diplonychus indicus* and *Gambusia affinis*, with LC_{50} values of 3241.53 and 9250.12 μg/ml, respectively. Overall, this research adds basic knowledge to develop newer and safer natural larvicides and repellent from Zingiberaceae plants against malaria, dengue, and filariasis mosquito vectors.

Keywords Malaria · Arbovirus · Biosafety · GC-MS · Mosquito vectors · Zingiberaceae

Introduction

Mosquitoes (Diptera: Culicidae) are vectors of important pathogens and parasites, such as malaria, lymphatic filariasis, Japanese encephalitis and yellow and dengue fevers which cause morbidity, mortality, economic loss, and social disruption (Mehlhorn et al. 2012; Benelli 2015a). The repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It also resulted in the development of resistance (Brown 1986), undesirable effects on non-target organisms, and fostered environmental and human health concern (Thomas et al. 2004). Culicidae eggs, larvae, and pupae are usually targeted using organophosphates, insect growth regulators, and microbial control agents. Indoor residual spraying and insecticide-treated bed nets are also employed

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to reduce transmission of malaria in tropical countries (Benelli 2015a). However, synthetic chemicals have strong negative effects on human health and the environment and induce resistance in a number of mosquito species (Wattanachai and Tintanon 1999; Hemingway and Ranson 2000).

Eco-friendly control tools are urgently needed. In the latest years, extensive research has been carried out to investigate the efficacy of botanical products against mosquito vectors (Benelli 2015b; Pavela 2015a, 2015b). People entering into regions where dengue, malaria, or yellow fever risks exist may protect themselves using plant-derived repellents (Mehlhorn et al. 2005, 2011, 2012; Amer and Mehlhorn 2006a, 2006b). On the other hand, people living in endemic regions have to protect themselves using several strategies at the same time, since infection rates of mosquitoes may be extremely high (Pushpanathan et al. 2006; Amer and Mehlhorn 2006c, 2006d; Semmler et al. 2009; Benelli et al. 2015a, 2015b, 2015c; Govindarajan and Benelli 2015; Pavela 2015a; Benelli 2015b). In this framework, recent research tested essential oils obtained from various plants from India, including *Origanum vulgare* (Govindarajan et al. 2016), *Plectranthus barbatus* (Govindarajan et al. 2015), *Coleus aromaticus* (Govindarajan et al. 2013a), *Ocimum basilicum* (Govindarajan et al. 2013b), *Clausena anisata* (Govindarajan 2010), and *Mentha spicata* (Govindarajan et al. 2012), against larvae of *Aedes aegypti, Anopheles stephensi,* and *Culex quinquefasciatus*.

Furthermore, repellency plays an important role in preventing the vector-borne diseases by reducing man–vector contact. However, some repellents of synthetic origin may cause skin irritation and affect the dermis (Das et al. 2000). The majority of commercial repellents are prepared by using chemicals like allethrin, N,N-diethyl-meta-toluamide (DEET), dimethyl phthalate (DMP), and N,N-diethyl mendelic acid amide (DEM). It has been reported that these chemical repellents are not safe for public use (Ronald et al. 1985). Because of unpleasant smell, oily feeling to some users, and potential toxicity, some prefer to use natural insect repellent products (Robbins and Cherniack 1986). Repellents of plant origin do not pose hazards of toxicity to human and domestic animals and are easily biodegradable. Natural products are safe for humans when compared to that of synthetic compounds (Sharma and Ansari 1994).

The genus *Zingiber* has about 85 species of aromatic herbs mostly distributed in East Asia and tropical Australia (Mabberley 1990). Plants belonging to Zingiberaceae are known for a number of medicinal properties (Basu 2002; Prajapathi et al. 2005). The term "Zingiber" is derived from the Sanskrit word "shringavera," owing to their "horn shaped" rhizomes. *Zingiber* species are rich in volatile oils and are commonly used in traditional medicine and as spices. Zingiberaceae plants have significant medicinal properties (Kumar et al. 2006). They are having insecticidal, repellant (Millar 1998; Chane-Ming et al. 2003), anti-inflammatory, and chemopreventive activities (Kirana et al. 2003; Nakamura et al. 2004).

Zingiber nimmonii (J. Graham) Dalzell is an endemic species from the Western Ghats in South India, which grows both at low and high altitudes, in moist areas under the shades of trees (Sabu 2003). *Z. nimmonii* rhizome oil is a unique natural product with 69.9 % of isomeric caryophyllenes, viz. β-caryophyllene (42.2 %) and α-caryophyllene (27.7 %), along with traces of isocaryophyllene (0.03 %) in it. The major constituents of the rhizome oil of *Z. nimmonii* varied from the rhizome oils of *Zingiber zerumbet* and *Zingiber officinale*. The oil showed significant activities against the human pathogenic fungi, *Candida glabrata*, *Candida albicans*, and *Aspergillus niger* (Baby et al. 2006). To the best of our knowledge, the biotoxicity of *Z. nimmonii* essential oil (EO) against mosquito vectors is unknown.

In the present study, we investigated the larvicidal and repellent activity of the essential oil extracted from the rhizome of *Z. nimmonii* against the malaria vector *An. stephensi*, the dengue vector *Ae. aegypti* and the filariasis vector *Cx. quinquefasciatus*. The EO obtained from hydro-distillation was analyzed by gas chromatography–mass spectrometry (GC-MS), in order to identify its major constituents. Furthermore, the toxicity of this EO was assessed against two non-target species sharing the same ecological niche of mosquito larvae, *Diplonychus indicus* and *Gambusia affinis*.

Materials and methods

Plant material and extraction of essential oil

Z. nimmonii was collected from Nilgiris, Western Ghats (11° 10 N to 11° 45 N latitude and 76° 14 E to 77° 2 E longitude), Tamil Nadu, India. The plant was authenticated at the Department of Botany, Annamalai University. Vouchers specimens are deposited at the herbarium of Plant Phytochemistry Division, Department of Zoology, Annamalai University. The EO was obtained by the hydro-distillation of 3 kg of rhizomes in a Clevenger apparatus for 8 h. The oil layer was separated from the aqueous phase using a separating funnel. The resulting EO was dried over anhydrous sodium sulfate. The essential oil was stored in the dark at 4 °C until the testing phase.

Gas chromatography

Gas chromatography (GC) was carried on a Varian gas chromatograph equipped with a flame ionization detector and a BPI (100 % dimethyl polysiloxane) capillary column. Helium at a flow rate of 1.0 ml min^{-1} and

8 psi inlet pressure was employed as a carrier gas. Temperature was programmed from 60 to 220 °C at 5 °C min^{-1} with a final hold time of 6 min. The injector and detector temperatures were maintained at 250 and 300 °C, respectively. The sample (0.2 μl) was injected with 1:20 split ratio.

Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC-MS) was performed using an Agilent 6890 GC equipped with 5973 N mass selective detector and an HP-5(5 % phenyl methyl polysiloxane) capillary column. The oven temperature was programmed from 50 to 280 °C at the rate of 4 °C min^{-1} and held at this temperature for 5 min. The inlet and interface temperatures were 250 and 280 °C, respectively. The carrier gas was helium at a flow rate of 1.0 ml min^{-1} (constant flow). The sample (0.2 μl) was injected with a split of 20:1. Electron impact mass spectrometry was carried out at 70 eV. Ion source and quadrupole temperatures ware maintained at 230 and 150 °C, respectively. The identification of EO compounds was based on the comparison of their retention indices and mass spectra with those in commercial libraries NIST 98.1 and Mass Finder 3.1. The concentration of each EO component was calculated from the integration area of the chromatographer.

Mosquito rearing

Laboratory-bred pathogen-free strains of the three mosquito vectors tested in this study were reared in the Vector Control Laboratory, Department of Zoology, Annamalai University. At the time of adult feeding, these mosquitoes were 3–4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with Parafilm as membrane for 4 h. *Ae. aegypti* feeding was done from 12 noon to 4.00 p.m. and *An. stephensi* and *Cx. quinquefasciatus* were fed during 6.00 p.m. to 10.00 p.m. A membrane feeder with the bottom end fitted with Parafilm was placed with 2.0 ml of the blood sample (obtained from a slaughterhouse by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C were maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28 ± 2 °C, 70–85 % R.H., with a photoperiod of 12-h light and 12-h dark.

Larvicidal activity

Larvicidal activity of the *Z. nimmonii* EO was evaluated following World Health Organization (2005). EO was tested at 20, 40, 60, 80, and 100 μg/ml. EO was dissolved in 1 ml DMSO, and then diluted in 249 ml of filtered tap water to obtain each of the desired concentrations. The control was prepared using 1 ml of DMSO in 249 ml of water. Twenty early third instar larvae were introduced into each solution. For each concentration, five replicates were performed. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae.

Repellent activity

The EO was applied on a membrane used for membrane feeding of unfed mosquitoes in a 1-ft cage. About 50 unfed 3–4-day-old laboratory-reared pathogen-free strains of *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* was introduced in a 1-ft cage fitted with a membrane with blood for feeding with temperature maintained at 37 °C through circulating water bath maintained at 40–45 °C. The time taken for the first feeding in the cage containing the membrane treated with repellent needs to be observed at 30-min intervals, and each observation was made for 60 s. The experiment was repeated at this application rate for five times to confirm reproducible results. The time taken for feeding is considered as the protection time (in hours). Each test included one membrane feeding unit as control without applying any repellent. The testing period was 6:00 a.m. to 2:00 p.m. for *Ae. aegypti* and 6:00 p.m. to 2:00 a.m. for *An. stephensi* and *Cx. quinquefasciatus*.

If more than 4 h are taken at 2 mg/cm^2 application, the EO was considered to exhibit promising repellency. If the protection time is <1–2 h at 2 mg/cm^2 application rate, the EO may be discarded for repellence testing. 'The percentage of repellency was calculated by the following formula:

$$\%\ \text{Repellency} = [(T_a - T_b)/T_a] \times 100$$

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

Acute toxicity on non-target organisms

The acute toxicity of *Z. nimmonii* EO to non-target organisms was assessed following the method by Sivagnaname and Kalyanasundaram (2004). The effect of EO was tested against non-target organisms *D. indicus* and *G. affinis*. The species were field collected and separately maintained in cement tanks (85-cm

diameter and 30-cm depth) containing water at 27 ± 3 °C and relative humidity 85 %. The *Z. nimmonii* EO was also tested at a concentration of even 50 times higher than the lethal concentration $(LC)_{50}$ dose for mosquito larvae. Ten replicates were performed for each concentration along with four replicates of untreated controls. The non-target organisms were observed for mortality and other abnormalities such as sluggishness and reduced swimming activity after 48-h exposure. The exposed non-target organisms were also observed continuously for 10 days to understand the post treatment effect of this extract on survival and swimming activity.

Data analysis

Mortality data were subjected to probit analysis. LC_{50} and LC_{90} were calculated using the method by Finney (1971). Repellency data were analyzed using two-way ANOVA followed by Tukey's honest significant difference (HSD) test ($P < 0.05$). In experiments evaluating toxicity against non-target organisms, the Suitability Index (SI) was calculated for each non-target species using the following formula (Deo et al. 1988)

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

All data were analyzed using the SPSS Statistical Software Package version 16.0. A probability level of $P < 0.05$ was used for the significance of differences between values.

Results

Chemical composition of the essential oil

The yield of *Z. nimmonii* EO was 16.9 ml/kg fresh weight. Table 1 showed the constituents of the EO, their percentage composition, and their Kovats Index (KI) values listed in order of elution. A total of 33 compounds were identified, representing 97.3 % of the EO. The major constituents were myrcene (5.1 %), β-caryophyllene (26.9 %), α-humulene (19.6), and α-cadinol (5.2). Chemical structures of four major constituents were reported in Fig. 1. The percentage compositions of the remaining 29 compounds ranged from 0.7 to 2.8 %.

Mosquito larvicidal and repellent activity

The toxicity of EO from *Z. nimmonii* against early third larvae of mosquito vectors *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, were presented in Table 2.

Table 1 Chemical composition of *Zingiber nimmonii* essential oil

Peak	Components	Retention time (Kovats index)	Composition (%)	Mode of identification
1	Camphene	948	2.2	RI, MS
2	Sabinene	972	1.4	RI, MS
3	β-Pinene	976	0.8	RI, MS
4	Myrcene	992	5.1	RI, MS
5	α-Phellandrene	1011	1.3	RI, MS
6	α-Terpinene	1015	1.4	RI, MS
7	*p*-Cymene	1023	1.2	RI, MS
8	*o*-Cymene	1027	0.9	RI, MS
9	Limonene	1030	1.8	RI, MS
10	(*E*)-β-ocimene	1047	1.4	RI, MS
11	γ-Terpinene	1056	1.3	RI, MS
12	Terpinolene	1089	0.9	RI, MS
13	Camphor	1148	1.2	RI, MS
14	Camphene hydrate	1151	0.9	RI, MS
15	α-Phellandren-8-ol	1165	0.7	RI, MS
16	Borneol	1171	2.1	RI, MS
17	Terpinen-4-ol	1180	1.9	RI, MS
18	α-Terpineol	1193	1.8	RI, MS
19	*n*-Decanal	1206	0.9	RI, MS
20	β-Elemene	1397	1.3	RI, MS
21	β-Caryophyllene	1440	26.9	RI, MS
22	α-Humulene	1472	19.6	RI, MS
23	β-Bisabolene	1512	1.1	RI, MS
24	γ-Cadinene	1523	0.9	RI, MS
25	δ-Cadinene	1535	0.9	RI, MS
26	Germacrene B	1556	1.2	RI, MS
27	Caryophyllene oxide	1602	2.1	RI, MS
28	3-Octadecyne	1627	1.8	RI, MS
29	τ-Muurolol	1658	2.8	RI, MS
30	Cubenol	1662	0.8	RI, MS
31	α-Cadinol	1671	5.2	RI, MS
32	β-Bisabolol	1679	2.2	RI, MS
33	(2E,6Z)-Farnesol	1752	1.3	RI, MS
	Total		97.3	

RI retention index, *MS* mass spectra

The EO from the rhizome of *Z. nimmonii* exhibited significant larvicidal activity, with the LC_{50} and LC_{90} values of 41, 44, and 48 and 80, 85, 90 ppm, respectively. The EO of *Z. nimmonii* shows significant repellency against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (Table 3). Repellency depended on the strength of the EO concentration. A higher concentration of 5.0 mg/cm^2 provided 100 % protection up to 180, 150, and 120 min, respectively.

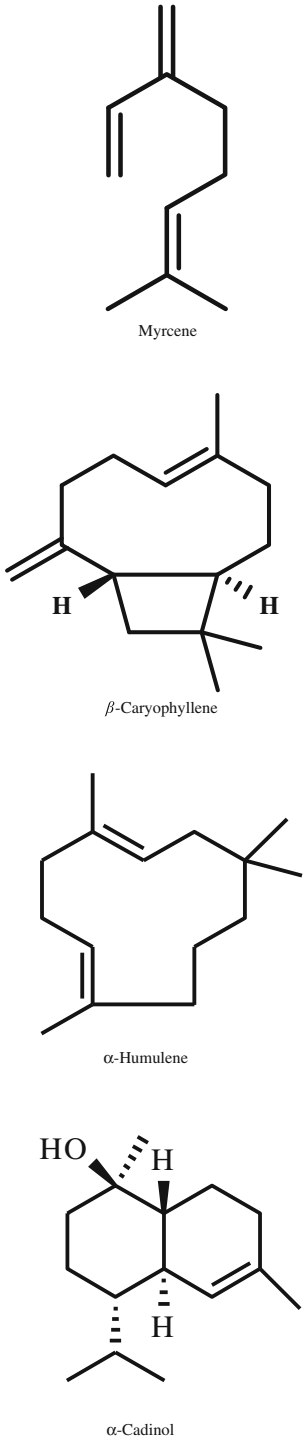

Fig. 1 Chemical structure of the four major constituents of *Zingiber nimmonii* essential oil

Effect on non-target aquatic organisms

The acute toxicity of the *Z. nimmonii* EO towards non-target organisms *D. indicus* and *G. affinis* were presented in Table 4. Interestingly, LC_{50} values were 3241.53 and 16,670.30 μg/ml. *G. affinis* was less susceptible to the *Z. nimmonii* EO when compared to *D. indicus*. Overall, SI/PSF indicated that the *Z. nimmonii* EO was less harmful to the non-target organism if compared to the targeted mosquito species (Table 5). Focal observations conducted during the testing phase also showed that the survival and swimming activity of the non-target species were not altered during the exposure concentrations of target species.

Discussion

Different parts of plants contain a complex of chemicals with unique biological activity (Farnsworth and Bingel 1977; Benelli, 2015b; Pavela 2015a) which is thought to be due to toxins and secondary metabolites, which act as attractants or deterrents (Fisher 1991). Our result showed that EO from the rhizome of *Z. nimmonii* has significant larvicidal as well as repellent activity against several mosquito vectors of economic importance. This result is also comparable to earlier research by Singh et al. (2003) who observed the larvicidal activity of *Ocimum canum* oil against vector mosquitoes *Ae. aegypti* and *Cx. quinquefasciatus* (LC_{50} 301 ppm) and *An. stephensi* (234 ppm). Traboulsi et al. (2005) reported that the larvicidal activity of EO of *Citrus sinensis*, *Eucalyptus* spp., *Ferula hermonis*, *Laurus nobilis*, and *Pinus pinea* against *Cx. pipiens*. LC_{50} values were 60.0, 120.0, 44.0, 117.0, and 75.0 ppm, respectively. The EO of *Z. nimmonii* exhibited higher toxic action, if compared to the reported plants. Also, the EO of *Tagetes minuta*, providing a repellency of 90 % protection for 2 h was observed by Tyagi et al. (1994). EO obtained from *Vitex negundo* leaves shows repellency ranged from 1 to 3 h (Hebbalkar et al. 1992).

In the present study, *Z. nimmonii* rhizome extracted essential oil exhibited larvicidal activity LC_{50}=41.19, 44.46, and 48.26 ppm against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, respectively. These values are higher or comparable to other recent reports. For instance, Pushpanathan et al. (2008) have reported larvicidal activity of *Z. officinalis* oil as LC_{50}=50.78 ppm against *Cx. quinquefasciatus*, while in the present work, it was 48.26 ppm. This variation may be also due to the difference in strains of *Cx. quinquefasciatus*, since it has been shown that there is a vast difference between two strains of same species in respect of bioactivity (Tare et al. 2004). Pushpanathan et al. (2008) also reported that *Z. officinalis* oil offers 100 % protection for 2 h against *Cx. quinquefasciatus* at 4 mg/cm^2, while we have obtained 2-h protection at 0.5 mg/cm^2.

Monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor,

Table 2 Larvicidal activity of essential oil from *Zingiber nimmonii* against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*

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	20	28.3 ± 0.4	41.19	80.31	3.03	$y = 10.75 + 0.928x$	4.820 (4)
	40	46.1 ± 0.8	(36.59–45.28)	(74.53–87.87)			n.s.
	60	69.4 ± 1.2					
	80	88.2 ± 0.6					
	100	100.0 ± 0.0					
Ae. aegypti	20	25.4 ± 0.8	44.46	85.88	2.90	$y = 7.17 + 0.937x$	2.586 (4)
	40	42.6 ± 1.2	(39.82–48.64)	(79.70–94.02)			n.s.
	60	66.2 ± 0.6					
	80	84.5 ± 0.4					
	100	98.1 ± 0.8					
Cx. quinquefasciatus	20	21.6 ± 1.2	48.26	90.95	2.61	$y = 2.95 + 0.952x$	1.621 (4)
	40	39.3 ± 0.6	(43.69–52.46)	(84.43–99.56)			n.s.
	60	62.7 ± 0.8					
	80	80.4 ± 0.4					
	100	96.2 ± 0.6					

No mortality was observed in the control

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 ($\alpha = 0.05$)

[a] Values are mean ± SD of five replicates

and thymol are common constituents of a number of EOs described in the literature as mosquito repellents (Yang et al. 2004; Park et al. 2005; Jaenson et al. 2006). Among sesquiterpenes, β-caryophyllene is highly cited as a strong repellent against *Ae. aegypti* (Gillij et al. 2008). Although repellent properties of several EOs regularly appear to be associated with the presence of monoterpenoids and sesquiterpenes (Jaenson et al. 2006), Odalo et al. (2005) also showed that phytol, a linear diterpene alcohol, has high repellent activity against *Anopheles gambiae*. Notably, the oxygenated compounds phenylethyl alcohol, β-citronellol, cinnamyl alcohol, geraniol, and α-pinene, isolated from the essential oil of *Dianthus caryophyllum*, also showed strong repellent activities against ticks (*Ixodes ricinus*) (Tunón et al. 2006).

Toloza et al. (2008) evaluated the repellent activity of 16 essential oils from native and exotic Argentine plants and 21 isolated metabolites; three alcohols (benzyl alcohol, menthol and thymol) were found as the most effective towards *Pediculus humanus capitis*. Omolo et al. (2004) and Odalo et al. (2005) evaluated the repellent

Table 3 Repellent activity of essential oil of *Zingiber nimmonii* against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*

Mosquito species	Concentration (mg/cm^2)	Repellency% ± SD						
		Time of post application (minutes)						
		30	60	90	120	150	180	210
An. stephensi	1.0	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	90.4 ± 0.8 b	76.2 ± 0.8 c	64.1 ± 1.2 d
	2.0	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	82.2 ± 1.2 b	72.2 ± 0.8 c
	5.0	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	90.2 ± 1.0 b
Ae. aegypti	1.0	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	89.6 ± 0.8 b	76.8 ± 1.2 c	68.2 ± 0.8 d	57.6 ± 1.2 e
	2.0	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	91.7 ± 1.2 b	81.4 ± 0.6 c	68.5 ± 0.6 d
	5.0	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	90.5 ± 1.2 b	83.7 ± 0.8 c
Cx. quinquefasciatus	1.0	100 ± 0.0 a	100 ± 0.0 a	86.8 ± 0.8 b	75.9 ± 0.6 c	64.3 ± 1.2 d	56.7 ± 0.6 e	44.5 ± 0.8 f
	2.0	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	87.2 ± 0.8 b	76.2 ± 0.6 c	68.4 ± 1.2 d	57.4 ± 1.2 e
	5.0	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	89.6 ± 0.6 b	77.8 ± 1.2 c	71.1 ± 0.8 d

Within each row, different letters indicate significant differences (Tukey's HSD, $P < 0.05$)

Table 4 Biotoxicity of *Zingiber nimmonii* essential oil against two non-target organisms sharing the same ecological niche of *Anopheles*, *Aedes* and *Culex* mosquito vectors

Non-target organism	Concentration (μg/ml)	Mortality (%) ± SD[a]	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Diplonychus indicus	1500	22.4 ± 0.8	3241.53	6040.30	2.50	$y = 6.54 + 0.013x$	2.932 (4)
	3000	46.7 ± 1.2	(2919.39–3533.31)	(5624.31–6579.91)			n.s.
	4500	69.2 ± 0.6					
	6000	87.3 ± 0.4					
	7500	99.6 ± 0.8					
Gambusia affinis	4000	19.8 ± 1.2	9250.12	16,670.30	2.24	$y = 1.79 + 0.005x$	4.591 (4)
	8000	42.3 ± 0.6	(8428.01–	(15,551.22–			n.s.
	12,000	65.7 ± 1.2	10,011.49)	18,118.14)			
	16,000	84.2 ± 0.4					
	20,000	99.8 ± 0.8					

No mortality was observed in the control

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 ($\alpha = 0.05$)

[a] Values are mean ± SD of five replicates

activities of 12 Kenyan plants from different genus against *An. gambiae*, and some pure metabolites extracted from them. The most effective repelling chemicals were perillyl alcohol, cisverbenol, cis-carveol, geraniol, citronellal, perillaldehyde, caryophyllene oxide, carvacrol, 4-isopropyl benzene methanol, thymol, 3-carene, and myrcene. These belong to different structural types such as sesquiterpenoid, diterpenoid and acyclic, monocyclic, and bicyclic monoterpenoids.

Panneerselvam and Murugan (2013) observed the repellent activity of *An. stephensi* the hexane, ethyl acetate, benzene, aqueous, and methanol extract of *Andrographis paniculata*, *Cassia occidentalis*, and *Euphorbia hirta* plants at three different concentrations of 1.0, 3.0, and 6.0 mg/cm^2. Five different concentrations, 5, 10, 15, 20, and 25 % (*v*/*v*), were prepared from each extract stock. Topical application of the extract concentrations on human volunteers revealed that 20 and 25 % repelled mosquitoes for at least 2 and 5 h, respectively. The methanol extract of *Ervatamia coronaria* was found to be more repellent than *Caesalpinia pulcherrima* extract. A higher concentration of 5.0 mg/cm^2 provided 100 % protection up to 150, 180, and 210 min against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*, respectively (Govindarajan et al. 2011). Karunamoorthi et al. (2008) have reported that the leaves of *Echinops* sp. (92.47 %), *Ostostegia integrifolia* (90.10 %), and *Olea europaea* (79.78 %) were effective and efficient to drive away mosquitoes, and the roots of *Silene macroserene* (93.61 %), leaves of *Echinops* sp. (92.47 %), *Os. integrifolia* (90.10 %), and *Ol. europaea* (79.78 %) exhibited a significant repellency by direct burning.

Table 5 Suitability index of non-target organisms over young instars of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* exposed to *Zingiber nimmonii* essential oil

Non-target organism	*Anopheles stephensi*	*Aedes aegypti*	*Culex quinquefasciatus*
Diplonychus indicus	78.69	72.90	67.16
Gambusia affinis	224.57	208.05	191.67

Choi et al. (2002) tested the repellent activity of *Lavandula officinalis* and *Rosmarinus officinalis* EOs against *Culex pipiens pallens*, showing an effective repellent effect mainly due to adult mosquitoes due to α-terpinene, carvacrol, and thymol. Tawatsin et al. (2001) have reported repellent activity against *Ae. aegypti*, *Anopheles dirus*, and *Cx. quinquefasciatus*, which is due to 5 % vanillin, which has been added to the EO of *Curcuma longa*. Neem products are good mosquito repellents, showing from 90 to 100 % protection against malaria vectors and about 70 % against *Cx. quinquefasciatus* (Ansari and Razdan 1994). Autran et al. (2009) have reported that the EO from leaves and stems of *Piper marginatum* exhibited an oviposition deterrent effect against *Ae. aegypti* at 50 and 100 ppm, since significantly lower numbers of eggs (<50 %) were laid in glass vessels containing the test solutions compared with the control. The oviposition deterrent properties against *An. stephensi* have been observed for various plant extracts including the methanol extract of *Pelargonium citrosa*, which exhibited 56 and 92 % inhibition of oviposition at 1 and 4 ppm, respectively (Jeyabalan et al. 2003).

Conclusions

Overall, this research adds knowledge to develop newer and safer natural larvicides and repellent against malaria, dengue, and lymphatic filariasis mosquito vectors. The plant tested in the study, *Z. nimmonii*, is available in large quantities in India and other Asian countries. The cost involved in the preparation of the *Z. nimmonii* EO is minimal. In addition, natural products are generally preferred in vector control measures due to their less deleterious effect on non-target organisms and their innate biodegradability (Pavela 2015a). In the context of resistance developed by the mosquito larvae against chemical insecticides, it is worthwhile to identify new larvicidal compounds from natural products against mosquitoes (Benelli 2016a, b). The results reported here open the possibility of further investigations of efficacy on their larvicidal properties of natural product extracts. In particular, *Z. nimmonii* EO may be utilized by local people for controlling mosquito larvae in small breeding places like water coolers, tree holes, abandoned wells, drums, and containers in and around the rural/suburban dwellings. Such practice would not only reduce environmental pollution but also promote sustainable utilization of locally available bio-resources by marginalized rural communities.

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Conflicts of interest The authors declare that they have no competing interests. G. Benelli is an Editorial Board Member of *Parasitology Research*. This does not alter the authors' adherence to all the *Parasitology Research* policies on sharing data and materials.

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