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ORIGINAL PAPER

Artemisia absinthium-borne compounds as novel larvicides: effectiveness against six mosquito vectors and acute toxicity on non-target aquatic organisms

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Abstract The eco-friendly control of mosquito vectors is a crucial challenge of public health importance. Here we evaluated the larvicidal potential of *Artemisia absinthium* essential oil (EO) and its three major chemical constituents against six mosquito vectors: *Anopheles stephensi*, *Anopheles subpictus*, *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, and *Culex tritaeniorhynchus*. The EO was obtained by leaf hydrodistillation. Its chemical composition was analyzed using gas chromatography-mass spectrometry. Major components were (*E*)-β-farnesene (31.6 %), (*Z*)-en-yn-dicycloether (11.12 %), and (*Z*)-β-ocimene (27.8 %). The EO was toxic effect against larval populations of *An. stephensi*, *An. subpictus*, *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus*, with LC_{50} values of 41.85, 52.02, 46.33, 57.57, 50.57, and 62.16 μg/ml. (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene were highly effective on *An. stephensi* (LC_{50} = 8.13, 16.24 and 25.84 μg/ml) followed by *An. subpictus* (LC_{50} = 10.18, 20.99, and 30.86 μg/ml), *Ae. aegypti* (LC_{50} = 8.83,17.66, and 28.35 μg/ml), *Ae. albopictus* (LC_{50} = 11.38,23.47, and 33.72 μg/ml), *Cx. quinquefasciatus* (LC_{50} = 9.66, 19.76, and 31.52 μg/ml), and *Cx. tritaeniorhynchus* (LC_{50} = 12.51,25.88, and 37.13 μg/ml). Notably, the EO and its major compounds were safer to the non-target organisms *Chironomous circumdatus*, *Anisops bouvieri* and *Gambusia affinis*, with LC_{50} values ranging from 207.22 to 4385 μg/ml. Overall, our results highlight that (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene from the *A. absinthium* EO represent promising eco-friendly larvicides against six key mosquito vectors with moderate toxicity against non-target organisms.

Keywords Biosafety · Culcidae · (*E*)-β-farnesene · (*Z*)-en-yn-dicycloether · (*Z*)-β-ocimene · Non-target arthropods · *Gambusia affinis*

Introduction

Arthropods are important vectors of a great number of pathogens and parasites, which may hit as epidemics or pandemics in the increasing world populations of humans and animals (Mehlhorn 2015; Benelli and Mehlhorn 2016; Benelli et al. 2016a). Mosquitoes (Diptera: Culicidae) represent a key threat for millions of organisms worldwide, since they act as vectors of the agents of malaria, dengue, yellow fever, West Nile virus fever, Japanese encephalitis, filariasis and, more recently, Zika virus (Mehlhorn et al. 2012; Benelli 2015a; Benelli et al. 2016b, c).

According to the latest estimates, there were at least 198 million cases of malaria in 2013 and an estimated 584,000 deaths. Malaria mortality rates have fallen by 47 % globally since 2000 and by 54 % in the African region, but are still high. Most deaths occur among children living in Africa, where a child dies every minute from malaria (Jensen and Mehlhorn 2009; WHO 2014). Dengue is ranked among the most important mosquito-borne viral diseases in the world. In the last 50 years, the incidence has increased 30-fold. An estimated 2.5 billion people live in over 100 endemic

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countries and areas where dengue viruses can be transmitted. Up to 50 million infections occur annually with 500,000 cases of dengue hemorrhagic fever and 22,000 deaths, mainly among children (WHO 2012a). In the past decade, West Nile virus has emerged in the Americas, becoming endemic throughout the region. Chikungunya, a formerly obscure arbovirus endemic to East Africa, has also emerged, causing millions of cases in the Indian Ocean basin and mainland South and Southeast Asia. The Japanese encephalitis virus has expanded its range in the Indian subcontinent and Australasia, where it chiefly affects children (Tolle 2009; Benelli and Mehlhorn 2016).

Currently, the use of mosquito larvicides faces several serious problems. Besides the negative effects of synthetic insecticides on the environment and non-target organisms, including man (Hodgson and Levi 1996; WHO 2012b), the development of resistant mosquito populations in particular is one of the most serious problems (Hemingway and Ranson 2000; Naqqash et al. 2016). Insecticide resistance is viewed as an extremely serious threat to crop protection and vector control, and is considered by many parties, including industry, the WHO, regulatory bodies, and the public, to be an issue that needs a proactive approach (Hemingway and Ranson 2000; McCaffery and Nauen 2006; Nauen 2007; WHO 2012b).

These problems highlighted the needing of new pest control alternatives, acceptable for the environment and human health (Benelli 2016a, b; Pavela and Benelli 2016). Among the existing alternative tools aimed at decreasing pest populations, the use of pesticides based on plant extracts is currently one of the most promising (Amer and Mehlhorn 2006a, b, c, d; Dubey 2011; Benelli 2016c; Govindarajan et al. 2016a, b, c, d). Essential oils and related main compounds are also an environmentally interesting tool because they are biodegradable and have minimal side effects on non-target organisms, as well as on the environment (Govindarajan 2010; Govindarajan et al. 2012, 2013; Pavela 2014, 2015; Benelli 2015b, c Govindarajan and Benelli 2016a, b).

Essential oils can be used as an alternative to synthetic larvicides for vector control programs (Pavela 2015). It is well known that plant-derived natural products are extensively used as biologically active compounds (Zebitz 1984). Among them, essential oils were the first preservatives used by the man (Bakkali et al. 2008). Essential oils are mainly composed by isoprenoid compounds, mainly mono- and sesquiterpenes, which are mainly responsible of the smell of many aromatic plants (Franzios et al. 1997). Commercially, essential oils are used in four primary ways: as pharmaceuticals, as flavor enhancers in many food products, as odorants in fragrances, and as insecticides (Zhu et al. 2001; Pavela 2015; Benelli 2015a).

Artemisia, the largest and most widely distributed genus of family Asteraceae, comprises over 500 species geographically spread in the temperate zones of Europe, North America, Asia, and South Africa (He et al. 2009). Many *Artemisia* species are popular traditional Chinese medicinal plants and have been used for the treatment of a variety of diseases, such as malaria, hepatitis, inflammation, bruising, diuresis, hypertension, allergy, jaundice, cancer, and infections caused by bacteria, fungi, and viruses (Rustaiyan and Masoudi 2011). *Artemisia* species possess medicinal, insecticidal, repellent, or antifeedent properties (Grainge and Ahmed 1988; Negahban et al. 2006a, b). *Artemisia abrotanum*, *Artemisia absinthium*, *Artemisia vulgaris*, and *Artemisia dracunculus* are used medicinally (Evans 2001). *Artemisia herba-alba* inhibited the asexual reproduction of *Aspergillus niger*, *Penicillium italicum*, and *Zygorrhychus* sp. (Tantaoui-Elaraki et al. 1993). *A. vulgaris* has been reported as repellent and toxic to *Tribolium castaneum* (Wang et al. 2006). *Artemisia scoparia* is used as choleretic, antiinflammatory, and diuretic agents in the treatment of hepatitis (Hikino 1985). Lastly, *A. vulgaris* has been recently extensively studied for the present of the antiplasmodial drug artemisinin (Tu 2011; Benelli and Mehlhorn 2016).

A. absinthium is an herbaceous, perennial plant with fibrous roots. The stems are straight, growing to 0.8–1.2 m tall, grooved, branched, and silvery-green. The leaves are spirally arranged, greenish-gray above and white below, covered with silky silvery-white trichomes, and bearing minute oil-producing glands; the basal leaves are up to 25 cm long, bipinnate to tripinnate with long petioles, with the cauline leaves smaller, 5–10 cm long, less divided, and with short petioles; the uppermost leaves can be both simple and sessile. Its flowers are pale yellow, tubular, and clustered in spherical bent-down heads, which are in turn clustered in leafy and branched panicles. Flowering is from early summer to early autumn; pollination is anemophilous (Flora of North America 2016). To the best of our knowledge, no information was available on the larvicidal activity of *A. absinthium* essential oil against mosquito vectors of economic importance.

In this study, the chemical composition of *A. absinthium* essential oil was analyzed using gas chromatography–mass spectroscopy (GC-MS). Major components were (*E*)-β-farnesene (31.6 %), (*Z*)-en-yn-dicycloether (11.12 %), and (*Z*)-β-ocimene (27.8 %). Furthermore, the acute toxicity of essential oil from *A. absinthium* essential oil (EO), (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene was evaluated against larvae of the malaria vectors *Anopheles stephensi* and *Anopheles subpictus*, the dengue and Zika virus vector *Aedes aegypti* and *Aedes albopictus*, the filariasis vector *Culex quinquefasciatus*, and the Japanese encephalitis vector *Culex tritaeniorhynchus*. We also studied the acute toxicity of (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene on non-target aquatic organisms *Chironomous circumdatus*, *Anisops bouvieri*, and *Gambusia affinis*. This study would be

useful for the development of newer and safer larvicides against malaria, filariasis, and arbovirus vectors.

Materials and methods

Plant material and extraction of essential oil

A. absinthium 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. It 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. EO was obtained by the hydro-distillation of 3 kg of fresh leaves in a Clevenger apparatus for 8 h. The oil layer was separated from the aqueous phase using a separating funnel. The resulting essential oil was dried over anhydrous sodium sulfate. The essential oil was stored in 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 % dimethylpolysiloxane) 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

GC-MS was performed using an Agilent 6890 GC equipped with 5973N mass selective detector and an HP-5(5 % phynyl 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 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 essential oil component was calculated from the integration area of the chromatographer (Govindarajan and Benelli 2016a, b).

Mosquito rearing

Laboratory-bred pathogen-free strains of mosquitoes were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. 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* and *Ae. albopictus* feeding was done from 12 noon to 4:00 p.m. and *An. stephensi*, *An. subpictus*, *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus* were fed during 6:00 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 slaughter house by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes (Govindarajan and Sivakumar 2014). The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C was 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 % relative humidity, with a photoperiod of 12-h light and 12-h dark.

Larvicidal activity

Larvicidal activity of the *A. absinthium* EO and its major compounds, (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene, were evaluated following World Health Organization (2005) method. EO was tested at concentrations ranging from 20 to 125 μ g/ml. Furthermore, each compound was tested at various concentrations (ranging from 10 to 100 μg/ml). EO or/and individual compounds were dissolved in 1 ml DMSO, 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, for a total of 100 tested larvae. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae. The lethal concentrations (LC_{50} and LC_{90}) were calculated by probit analysis (Finney 1971). The Statistical Package of Social Sciences 12.0 software was used for all the analyses.

Biotoxicity on non-target aquatic organisms

Here, the effect of non-target organisms was assessed following the method by Sivagnaname and Kalyanasundaram

Table 1 Chemical composition of the *A. absinthium* essential oil

Peak	Components	Retention time (Kovats Index)	Composition (%)	Mode of identification
1	Hexanal	802	1.2	RI, MS
2	Santolina triene	907	4.9	RI, MS
3	α-Pinene	939	0.9	RI, MS
4	Benzaldehyde	962	1.3	RI, MS
5	*para*-Cymene	1029	1.8	RI, MS
6	(*Z*)-β-ocimene	1043	27.8	RI, MS
7	(*E*)-β-ocimene	1052	2.1	RI, MS
8	γ-Terpinene	1060	2.6	RI, MS
9	*trans*-Sabinene hydrate	1097	1.5	RI, MS
10	Allo-ocimene	1130	1.4	RI, MS
11	Terpinen-4-ol	1181	1.9	RI, MS
12	α-Terpineol	1193	1.6	RI, MS
13	*cis*-Verbenyl acetate	1289	0.8	RI, MS
14	β-Caryophyllene	1419	1.2	RI, MS
15	(*E*)-β-farnesene	1463	31.6	RI, MS
16	(2*Z*,6*E*)-farnesyl acetate	1818	1.2	RI, MS
17	(*Z*)-en-yn-dicycloether	1882	11.2	RI, MS
18	(*E*)-en-yn-dicycloether	1892	2.3	RI, MS
–	Total	–	97.3	–

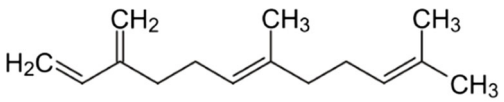
RI retention index, *MS* mass spectra

(2004). The effect of EO and its major compounds, (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene of the potential plant was tested against non-target organism, *viz.*, *C. circumdatus*, *G. affinis*, and *Anisops bouvieri*. The species were field collected and separately maintained in cement tanks (85 cm diameter and 30 cm depth) containing water at 27 ± 3 °C; the external relative humidity was 85 %.

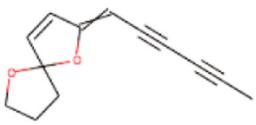
The major compounds, (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene as well as EO of *A. absinthium* were evaluated at a concentration of 50 times higher the LC_{50} dose for mosquito larvae. Ten replicates will be performed for each concentration along with four replicates of untreated 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 the tested botanicals on survival and swimming activity.

Data analysis

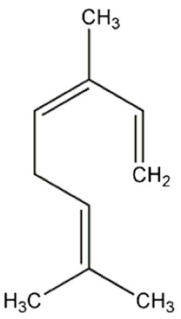
Mortality data were subjected to probit analysis. LC_{50} and LC_{90} were calculated using the method by Finney (1971). In experiments evaluating the biotoxicity on non-target



(*E*)-β-farnesene



(*Z*)-en-yn-dicycloether



(*Z*)-β-ocimene

Fig. 1 Major chemical constituents of the *A. absinthium* essential oil

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

Yield and GC-MS analysis

The yield of *A. absinthium* leaf EO was 12.4 ml/kg fresh weight. Table 1 shows the constituents of the EO, their percentage composition, and their Kovats Index (KI) values listed in order of elution. A total of 18 compounds representing 97.3 % of the EO were identified. The major constituents of this oil were (*E*)-beta-farnesene (31.6 %), (*Z*)-en-yn-dicycloether (11.12 %), and (*Z*)-beta-ocimene (27.8 %). Chemical structures of three major compounds were shown in Fig. 1. The percentage compositions of remaining 15 compounds ranged from 0.8 to 4.9 %.

Larvicidal potential against mosquito vectors

Results of acute toxicity experiments conducted against the larvae of mosquito vectors *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus* are presented in Tables 2, 3, 4, and 5. The EO from the leaves of *A. absinthium* exhibited significant larvicidal activity, with the LC_{50} values of 41.85, 46.33.

Table 2 Larvicidal activity of the essential oil from *A. absinthium* against *An. stephensi*, *An. subpictus, Ae. aegypti*, *Ae. albopictus*, *Cx. 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	20	29.3 ± 0.2	41.85 (37.01–46.13)	83.56 (77.38–91.75)	3.47	$y = 11.22 + 0.903x$	6.959 (4) n.s.
	40	47.6 ± 1.5					
	60	62.5 ± 2.0					
	80	88.4 ± 1.7					
	100	99.2 ± 0.1					
Ae. aegypti	20	25.8 ± 1.2	46.33 (41.64–50.59)	89.41 (82.85–98.10)	2.89	$y = 5.74 + 0.929x$	5.854 (4) n.s.
	40	42.1 ± 1.8					
	60	57.6 ± 0.2					
	80	84.3 ± 2.1					
	100	97.6 ± 1.7					
Cx. quinquefasciatus	25	22.4 ± 0.2	50.57 (46.05–54.81)	94.22 (87.38–103.30)	2.50	$y = 0.81 + 0.951x$	5.186 (4) n.s.
	50	37.2 ± 1.5					
	75	53.8 ± 0.9					
	100	79.5 ± 1.8					
	125	96.3 ± 0.3					
An. subpictus	25	28.6 ± 1.0	52.02 (46.21–57.18)	101.76 (94.40–111.42)	3.08	$y = 10.48 + 0.739x$	5.499 (4) n.s.
	50	45.3 ± 2.1					
	75	68.4 ± 1.5					
	100	87.3 ± 1.7					
	125	100.0 ± 0.0					
Ae. albopictus	25	24.2 ± 1.8	57.57 (52.04–62.63)	107.86 (100.29–117.78)	2.55	$y = 4.08 + 0.774x$	3.319 (4) n.s.
	50	39.6 ± 1.7					
	75	63.4 ± 2.0					
	100	85.2 ± 0.7					
	125	98.1 ± 0.8					
Cx. tritaeniorhynchus	25	21.6 ± 1.0	62.16 (56.76–67.22)	113.46 (105.54–123.84)	2.33	$y = -0.21 + 0.787x$	4.362 (4) n.s.
	50	36.2 ± 0.7					
	75	57.5 ± 1.2					
	100	81.3 ± 2.1					
	125	97.4 ± 0.4					

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

Table 3 Larvicidal activity of (*E*)-β-farnesene against *An. stephensi*, *An. subpictus*, *Ae. aegypti*, *Ae. albopictus*, *Cx. 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	4	28.2 ± 0.3	8.13	16.20	3.42	$y = 12.27 + 4.533x$	5.052 (4)
	8	49.5 ± 2.0	(7.18–8.98)	(15.01–17.76)			n.s.
	12	68.4 ± 1.5					
	16	87.2 ± 0.7					
	20	100.0 ± 0.0					
Ae. aegypti	4	24.3 ± 1.2	8.83	17.28	3.1	$y = 8.26 + 4.605x$	3.796 (4)
	8	46.8 ± 0.3	(7.88–9.68)	(16.02–18.95)			n.s.
	12	65.4 ± 1.8					
	16	82.6 ± 2.0					
	20	98.5 ± 1.0					
Cx. quinquefasciatus	4	21.2 ± 2.0	9.66	18.49	2.78	$y = 4.07 + 4.648x$	3.093 (4)
	8	42.8 ± 1.5	(8.72–10.52)	(17.13–20.30)			n.s.
	12	60.5 ± 1.8					
	16	78.3 ± 0.7					
	20	96.4 ± 0.5					
An. subpictus	5	29.6 ± 1.8	10.18	20.17	3.30	$y = 11.97 + 3.642x$	6.800 (4)
	10	48.2 ± 2.1	(9.00–11.22)	(18.69–22.11)			n.s.
	15	65.7 ± 1.5					
	20	89.5 ± 0.7					
	25	100.0 ± 0.0					
Ae. albopictus	5	24.5 ± 1.8	11.38	21.80	2.78	$y = 5.9 + 3.764x$	4.756 (4)
	10	44.2 ± 1.5	(10.23–12.42)	(20.23–23.86)			n.s.
	15	59.8 ± 0.2					
	20	85.2 ± 2.0					
	25	98.1 ± 1.7					
Cx. tritaeniorhynchus	5	20.8 ± 0.9	12.51	23.04	2.40	$y = 0.39 + 3.87x$	5.390 (4)
	10	39.2 ± 1.6	(11.41–13.54)	(21.40–25.20)			n.s.
	15	55.4 ± 0.2					
	20	79.3 ± 2.1					
	25	97.5 ± 0.7					

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

50.57, 52.02, 57.57, and 62.16 μg/ml, respectively. The three major pure constituents extracted from the *A. absinthium* EO were tested individually against the six mosquito vector larval populations. (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene appeared to be most effective against *An. stephensi* (LC_{50} = 8.13, 16.24, and 25.84 μg/ml) followed by *An. subpictus* (LC_{50} = 10.18, 20.99, and 30.86 μg/ml), *Ae. aegypti* (LC_{50} = 8.83,17.66, and 28.35 μg/ml), *Ae. albopictus* (LC_{50} = 11.38,23.47, and 33.72 μg/ml), *Cx. quinquefasciatus* (LC_{50} = 9.66, 19.76, and 31.52 μg/ml), and *Cx. tritaeniorhynchus* (LC_{50} = 12.51,25.88, and 37.13 μg/ml).

Biotoxicity on non-target aquatic organisms

Toxicity of the *A. absinthium* EO and its three major compounds against non-target organisms *C. circumdatus*, *G. affinis*, and *Anisops bouvieri* is presented in Table 6. *C. circumdatus*, *G. affinis*, and *Anisops bouvieri* were the least susceptible, with LC_{50} values ranging from 207.22 to 4385 μg/ml. *G. affinis* was less susceptible to the EO and its three major compounds than *Anisops bouvieri* and *C. circumdatus*. SI/PSF indicated that this EO and its three major compounds is less harmful to the non-target organism tested (Tables 7 and 8). Survival and swimming activity of the test species were not altered during the exposure at mosquito LC_{50} and LC_{90} of the plant EO and related chemicals.

Discussion

EOs are volatile, natural, complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites and generally lower density than that of water

Table 4 Larvicidal activity of (*Z*)-en-yn-dicycloether against *An. stephensi*, *An. subpictus*, *Ae. aegypti*, *Ae. albopictus*, *Cx. 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	8	28.5 ± 1.5	16.24 (14.27–17.96)	32.79 (30.36–35.99)	3.75	$y = 12.85 + 2.239x$	3.247 (4)n.s.
	16	49.6 ± 0.3					
	24	68.2 ± 1.7					
	32	87.5 ± 2.0					
	40	99.1 ± 1.1					
Ae. aegypti	8	25.4 ± 2.0	17.66 (15.74–19.38)	34.73 (32.18–38.10)	3.17	$y = 8.45 + 2.291x$	3.318 (4) n.s.
	16	45.8 ± 0.8					
	24	64.3 ± 1.6					
	32	83.5 ± 0.7					
	40	98.2 ± 0.2					
Cx. quinquefasciatus	8	21.0 ± 2.1	19.76 (17.96–21.43)	36.83 (34.19–40.30)	2.50	$y = 6.66 + 1.763x$	2.996 (4) n.s.
	16	38.2 ± 0.6					
	24	60.8 ± 1.5					
	32	78.4 ± 0.3					
	40	96.4 ± 1.2					
An. subpictus	10	27.5 ± 1.9	20.99 (18.61–23.10)	41.48 (38.46–45.47)	3.26	$y = 10.58 + 1.83x$	3.678 (4) n.s.
	20	46.8 ± 2.1					
	30	67.5 ± 0.7					
	40	86.4 ± 0.5					
	50	99.2 ± 0.3					
Ae. albopictus	10	23.4 ± 1.6	23.47 (21.17–25.57)	44.66 (41.44–48.92)	2.71	$y = 4.54 + 1.886x$	4.258 (4) n.s.
	20	41.2 ± 2.0					
	30	62.5 ± 0.8					
	40	80.4 ± 1.7					
	50	98.1 ± 0.2					
Cx. tritaeniorhynchus	10	20.6 ± 1.6	25.88 (23.56–28.06)	48.59 (44.97–53.43)	2.57	$y = 0.92 + 1.862x$	4.017 (4) n.s.
	20	37.5 ± 2.0					
	30	56.3 ± 1.7					
	40	74.1 ± 0.6					
	50	95.4 ± 0.4					

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

(Bakkali et al. 2008). The chemicals derived from plants have been projected as weapons in future mosquito control programs as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents, and oviposition-deterrent (Sukumar et al. 1991; Pavela 2015; Benelli 2015b). EOs are mostly composed of complex volatile mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes (Isman 2008), and the insecticidal constituents of EOs were mainly monoterpenoids, as also found for most of these mixtures. Lee et al. (2003) explained that monoterpenoids are volatile and lipophilic compounds, which are able to penetrate quickly inside insects and interfere with their physiological functions and therefore is very complicated to elucidate their mode of action. Monoterpenoid compounds have been considered as potential pest control agents because they are acutely toxic to insects and possess repellent (Watanabe et al. 1993) and antifeedant properties (Hough-Goldstein 1990). Many studies have evaluated of the monoterpenoids on various insect pests have established their biological activity as larvicides, ovicides, fumigants, and contact toxicants (Karr and Coats 1988; Rice and Coats 1994; Pavela 2015; Benelli 2015b).

Our results shed light on the promising potential of *A. absinthium* essential EO and its major constituents (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene as larvicidal agents against mosquito vectors *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus*. In particular, the EO pure constituents (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)- β -ocimene were more than threefold more active than the whole *A. absinthium* EO in larvicidal assays against early third instar larvae of *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus*. To the best of our knowledge, the larvicidal activity of *A. absinthium*

Table 5 Larvicidal activity of (*Z*)-β-ocimene against *An. stephensi*, *An. subpictus*, *Ae. aegypti*, *Ae. albopictus*, *Cx. 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	12	26.3 ± 0.3	25.84 (22.98–28.39)	50.82 (47.12–55.71)	3.18	$y = 9.36 + 1.532x$	3.971 (4) n.s.
	24	45.2 ± 1.5					
	36	68.4 ± 2.1					
	48	83.6 ± 1.9					
	60	99.0 ± 0.5					
Ae. aegypti	12	23.2 ± 1.8	28.35 (25.51–30.94)	54.72 (50.69–60.07)	2.89	$y = 5.22 + 1.542x$	3.912 (4) n.s.
	24	41.6 ± 0.3					
	36	63.1 ± 2.0					
	48	78.4 ± 1.4					
	60	97.3 ± 0.3					
Cx. quinquefasciatus	12	19.6 ± 1.9	31.52 (28.82–34.08)	58.03 (53.82–63.64)	2.40	$y = -0.93 + 1.588x$	3.699 (4) n.s.
	24	35.2 ± 0.5					
	36	56.8 ± 0.7					
	48	74.1 ± 1.2					
	60	95.4 ± 2.0					
An. subpictus	15	28.2 ± 1.8	30.86 (27.21–34.06)	61.79 (57.25–67.78)	3.54	$y = 11.92 + 1.205x$	2.874 (4) n.s.
	30	47.5 ± 2.0					
	45	69.4 ± 0.7					
	60	86.7 ± 1.3					
	75	99.0 ± 0.1					
Ae. albopictus	15	25.3 ± 1.0	33.72 (30.10–36.97)	66.25 (61.37–72.73)	3.16	$y = 7.71 + 1.222x$	2.087 (4) n.s.
	30	43.2 ± 0.5					
	45	65.1 ± 1.8					
	60	82.7 ± 1.6					
	75	97.2 ± 0.3					
Cx. tritaeniorhynchus	15	22.1 ± 2.0	37.13 (33.62–40.37)	70.57 (65.38–77.48)	2.69	$y = 2.77 + 1.245x$	2.141 (4) n.s.
	30	37.5 ± 1.6					
	45	61.2 ± 1.8					
	60	77.8 ± 0.7					
	75	95.3 ± 0.2					

No mortality was observed in the control

[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 ($\alpha = 0.05$)

EO and related constituents against these mosquito species has been not evaluated previously.

Several studies investigated the composition of the EOs derived from *Artemisia* species. Eucalyptol, camphor, and caryophyllene oxide are the major compounds of EOs from *Artemisia annua* (Soylu et al. 2005) and *Artemisia argyi* (Guan et al. 2006). Camphor, eucalyptol, and terpinen-4-ol are major components of EOs from *Artemisia feddei* (Cha et al. 2007), *A. herba-alba* (Mighri et al. 2009), *Artemisia haussknechtii* (Jalali and Sereshti 2007), and *Artemisia austriaca* (Guvenalp et al. 1998). Eucalyptol, germacrene D, camphor, and caryophyllene are the major compounds of EOs from *A. annua* (Goel et al., 2008; Padalia et al., 2011), *A. vulgaris* (Govindaraj et al. 2008; Williams et al. 2012), *Artemisia verlotiorum* (Chericoni et al. 2004), and *Artemisia parviflora* (Rana et al. 2003).

To the best of our knowledge, there are no previous reports on the bioactivity of *A. absinthium* EO against mosquito vector larvae. Zhu and Tian (2013), testing leaf EOs of *Artemisia gilvescens* on *Anopheles anthropophagus* larvae, observed that 1,8-cineole, camphor, and germacrene D were the most potent compounds with LC_{50} and LC_{90} values of 49 and 97 ppm, respectively. In addition, the fumigant activity of some EOs from *Artemisia* species has been evaluated against a number of stored product insects. Fumigant toxicity of the essential oils has been reported for *A. annua* against *Sitophilus oryzae* (Aggarwal et al. 2001), for *Artemisia tridentata* against some stored grain insects (Dunkel and Sears 1998). *Artemisia aucheri* Boiss oil also had fumigant toxicity to stored product pests (Shakarami et al. 2004), and *Artemisia sieberi* oil to *S. oryzae* and *T. castaneum* (Negahban et al. 2006a, b).

More generally, a growing number of plant-borne compounds related to the family Asteraceae have been screened for larvicidal potential against mosquito vectors. Borneol, germacrene D, and caryophyllene from *Blumea densiflora*

Table 6 Effect of *A. absinthium* essential oil, (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene of against non-target organisms sharing the same ecological niche of *Aedes*, *Anopheles*, and *Culex* mosquito vectors

Treatment	Non-target organism	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Essential oil	*Chironomus circumdatus*	1040.25 (926.20–1141.81)	2014.65 (1870.48–2203.38)	2.93	$y = 10.07 + 0.037x$	4.813 (9) n.s.
	Anisops bouvieri	3132.70 (2743.48–3471.83)	6472.18 (5977.39–7134.17)	4.55	$y = 12.81 + 0.012x$	4.145 (9) n.s.
	Gambusia affinis	4385.00 (3888.81–4825.48)	8776.51 (8121.52–9647.46)	3.53	$y = 9.5 + 0.009x$	1.783 (9) n.s.
(*E*)-β-farnesene	*Chironomus circumdatus*	207.22 (183.56–228.15)	409.13 (379.33–448.34)	3.24	$y = 11.1 + 0.183x$	5.391 (9) n.s.
	Anisops bouvieri	637.40 (566.84–700.11)	1247.33 (1157.24–1365.75)	3.09	$y = 9.69 + 0.062x$	3.332 (9) n.s.
	Gambusia affinis	905.51 (811.59–990.23)	1751.52 (1624.75–1918.81)	2.92	$y = 6.67 + 0.047x$	1.957 (9) n.s.
(*Z*)-en-yn-dicycloether	*Chironomus circumdatus*	507.89 (447.13–561.15)	1019.45 (944.35–1118.48)	3.60	$y = 12.54 + 0.072x$	3.678 (9) n.s.
	Anisops bouvieri	1719.50 (1524.52–1892.37)	3422.86 (3170.41–3756.84)	3.41	$y = 9.93 + 0.023x$	2.675 (9) n.s.
	Gambusia affinis	2037.75 (1812.96–2238.66)	4070.98 (3763.88–4481.54)	3.48	$y = 8.37 + 0.02x$	1.705 (9) n.s.
(*Z*)-β-ocimene	*Chironomus circumdatus*	635.15 (565.57–697.12)	1235.47 (1146.74–1351.84)	3.00	$y = 9.53 + 0.062x$	3.356 (9) n.s.
	Anisops bouvieri	2010.43 (1803.91–2196.91)	3854.72 (3579.21–4216.65)	2.80	$y = 6.68 + 0.021x$	2.711 (9) n.s.
	Gambusia affinis	2401.29 (2173.63–2610.86)	4525.85 (4201.82–4953.50)	2.61	$y = 3.17 + 0.019x$	2.050 (9) n.s.

No mortality was observed in the control

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, *d.f.* degrees of freedom, *n.s.* not significant ($\alpha = 0.05$)

Table 7 Suitability index of different non-target organisms over young instars of *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*, exposed to *A. absinthium* essential oil, (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene

Treatment	Non-target organism	*Culex quinquefasciatus*	*Aedes aegypti*	*Anopheles stephensi*
Essential oil	*Chironomus circumdatus*	20.57	22.45	24.85
	Anisops bouvieri	61.94	67.61	74.85
	Gambusia affinis	86.71	94.64	104.77
(*E*)-β-farnesene	*Chironomus circumdatus*	21.45	23.46	25.48
	Anisops bouvieri	65.98	72.18	78.40
	Gambusia affinis	93.73	102.54	111.37
(*Z*)-en-yn-dicycloether	*Chironomus circumdatus*	25.70	28.75	30.96
	Anisops bouvieri	87.01	97.36	105.88
	Gambusia affinis	103.12	115.38	125.47
(*Z*)-β-ocimene	*Chironomus circumdatus*	20.15	22.40	24.58
	Anisops bouvieri	63.78	70.91	77.80
	Gambusia affinis	76.18	84.70	92.93

Table 8 Suitability index of different non-target organisms over young instars of *Cx. tritaeniorhynchus*, *Ae. albopictus*, and *An. subpictus*, exposed to *A. absinthium* essential oil, (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene

Treatment	Non-target organism	*Culex tritaeniorhynchus*	*Aedes albopictus*	*Anopheles subpictus*
Essential oil	*Chironomus circumdatus*	16.73	18.06	19.99
	Anisops bouvieri	50.39	54.41	60.22
	Gambusia affinis	70.54	76.16	84.29
(*E*)-β-farnesene	*Chironomus circumdatus*	16.56	18.20	20.35
	Anisops bouvieri	50.95	56.01	62.61
	Gambusia affinis	72.38	79.57	88.94
(*Z*)-en-yn-dicycloether	*Chironomus circumdatus*	19.62	21.63	24.19
	Anisops bouvieri	66.44	73.26	81.91
	Gambusia affinis	78.73	86.82	97.08
(*Z*)-β-ocimene	*Chironomus circumdatus*	17.10	18.83	20.58
	Anisops bouvieri	54.14	59.62	65.14
	Gambusia affinis	64.67	71.21	77.81

were effective against the larvae of *An. anthropophagus* with the LC_{50} and LC_{90} values of 71 and 143 ppm, respectively (Zhu and Tian 2011). Marques et al. (2011) noted that piperitone from *Tagetes erecta* was effective against *Ae. aegypti* with the LC_{50} and LC_{90} values of 79 and 100 ppm, respectively. In another investigation, Ruiz et al. (2011) found that *trans*-ocimenone from *Tagetes minuta* had LC_{50} values of 52 ppm, when tested against *Ae. aegypti*. Comparisons of our results with these data revealed that (*E*)-β-farnesene, (*Z*)-en-yn-dicycloether, and (*Z*)-β-ocimene examined in this study exhibited great larvicidal activity.

Concerning acute toxicity on non-target aquatic organisms, such as other arthropods and fishes, our research highlighted low susceptibility to the *A. absinthium* EO, at variance with previous research conducted with other phytochemicals (Conti et al. 2014). On the other hand, recent research showed little acute toxicity of *Pinus kesiya* EO on mosquito predators *Anisops bouvieri*, *D. indicus* and *G. affinis*, with LC_{50} values ranging from 4135 to 8390 mg/ml (Govindarajan et al. 2016c). In addition, the *Syzygium zeylanicum* EO and its major components α-humulene and β-elemene tested against the mosquito natural enemy *G. affinis*, showed a LC_{50} of 20,374 μg/ml (Govindarajan and Benelli 2016a); the biotoxicity of the *Heracleum sprengelianum* EO and its two major compounds lavandulyl acetate and bicyclogermacrene on *Anisops bouvieri*, *D. indicus*, and *G. affinis* was also negligible, with LC_{50} values ranging from 414 to 4219 μg/ml (Govindarajan and Benelli 2016b).

Notably, even if it has been stated that the acute toxicity of monoterpenes against arthropods is relatively low compared to conventional insecticides (Lee et al. 2003; Silva et al. 2015), we believe that the toxic activity exerted by *A. absinthium* EO towards the studied species could be due to the anticholinesterase activity of this EO and its components (Mills et al. 2004). On the other hand, several researches have indicated that plant EOs can act also as growth and/or reproduction inhibitors (Pushpanathan et al. 2006). A dedicated research is required to understand the main physiological pathway(s) through which the *A. absinthium* EO exert its toxic activity.

Lastly, it is worthy to note that our focal observations on the non-target organisms exposed to the EO and its major constituents showed no post-treatment impact of these botanicals on survival and swimming activity of the aquatic organisms. It is worthy to note that most of the studies on non-target effects of green pesticides usually focus on the acute toxicity on non-target organisms, while detailed analysis of sub-lethal effects, including genotoxicity and behavioral modifications, is quite rare (reviewed by Isman 2000, see also Desneux et al. 2007). This is particularly true for studies focused on mosquito vectors. However, recent research showed that in some cases ultra-low doses of green-synthesized nanocomposites may boost the predation efficiency of natural enemies (e.g., copepods, tadpoles, and mosquito fishes) against mosquito young instars (e.g., Murugan et al. 2015a,b,c, 2016a,b; Subramaniam et al. 2015, 2016). As regards to genotoxicity, Murugan et al. (2016c) recently reported no significant damages of *Carassius auratus* erythrocytes post-treatment with graphene quantum dots at doses lower than 25 ppm.

Conclusions

Overall, our study reveals that the EO of *A. absinthium* has remarkable larvicidal properties against six mosquito vectors of medical and veterinary importance. The flora of India has

rich aromatic plant diversity, which acts as an outstanding reservoir of natural products active against arthropod pests. Our findings suggested that the EO from *A. absinthium* could be candidated as a novel and safer source of effective mosquito larvicides that can be employed in malaria and arbovirus control programs.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest. Giovanni 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.

Compliance with ethical standards All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

Aggarwal KK, Veena Prajapati AK, Kumar S (2001) Toxicity of 1,8-cineol towards three species of stored product coleopterans. Insect Sci 21:155–160

Amer A, Mehlhorn H (2006a) Repellency effect of forty-one essential oils against *Aedes*, *Anopheles* and *Culex* mosquitoes. Parasitol Res 99: 478–490

Amer A, Mehlhorn H (2006b) The sensilla of *Aedes* and *Anopheles* mosquitoes and their importance in repellency. Parasitol Res 99:491–499

Amer A, Mehlhorn H (2006c) Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). Parasitol Res 99:466–472

Amer A, Mehlhorn H (2006d) Persistency of larvicidal effects of plant oil extracts under different storage conditions. Parasitol Res 99:473–477

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils—a review. Food Chem Toxicol 46:446–475

Benelli G (2015a) Research in mosquito control: current challenges for a brighter future. Parasitol Res 114:2801–2805

Benelli G (2015b) Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. Parasitol Res 114:3201–3212

Benelli G (2016a) Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol Res 115:23–34

Benelli G (2016b) Plant-mediated synthesis of nanoparticles: a newer and safer tool against mosquito-borne diseases? Asia Pacif J Trop Biomed 6:353–354

Benelli G (2016c) Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer—a brief review. Enzyme Microbial Technol doi. doi:10.1016/j.enzmictec.2016.08.022

Benelli G, Mehlhorn H (2016) Declining malaria, rising dengue and Zika virus: insights for mosquito vector control. Parasitol Res 115:1747–1754

Benelli G, Pavela R, Canale A, Mehlhorn H (2016a) Tick repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? Parasitol Res doi. doi:10.1007/s00436-016-5095-1

Benelli G, Lo Iacono A, Canale A, Mehlhorn H (2016b) Mosquito vectors and the spread of cancer: an overlooked connection? Parasitol Res 115:2131–2137

Benelli G, Canale A, Higuchi A, Murugan K, Pavela R, Nicoletti M (2016c) The outbreaks of Zika virus: mosquito control faces a further challenge. Asia Pacif J Trop Dis 6:253–258

Cha JD, Jung EK, Kil BS, Lee KY (2007) Chemical composition and antibacterial activity of essential oil from *Artemisia feddei*. J Microbiol Biotechnol 17:2061–2065

Chericoni S, Flamini G, Campeol E, Cioni PL, Morelli I (2004) GCMS analyses of the essential oil from the aerial parts of *Artemisia verlotiorum*: variability during the year. Biochem Syst Ecol 32: 423–429

Conti B, Flamini G, Cioni PL, Ceccarini L, Macchia M, Benelli G (2014) Mosquitocidal essential oils: are they safe against non-target aquatic organisms? Parasitol Res 113:251–259

Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81–106

Deo PG, Hasan SB, Majumdar SK (1988) Toxicity and suitability of some insecticides for household use. Int Pest Control 30:118–129

Dubey NK (2011) Natural products in pest management. CAB International, London

Dunkel FV, Sears LJ (1998) Fumigant properties of physical preparations from mountain big sagebrush, *Artemisia tridentata* Nutt. ssp. vaseyana (Rydb.) beetle for stored grain insects. J Stored Prod Res 34:307–321

Evans WE (2001) Trease and Evans phamacognosy, 15th edn. Saunders Company Ltd, London, p 600

Flora of North America (2016) Common wormwood, armoise absinthe, *Artemisia absinthium* Linnaeus, Sp. Pl. 2: 848. 1753, vol 19, 20 and 21., p 519, http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=200023158

Finney DJ (1971) Probit analysis. Cambridge University Press, London, pp 68–72

Franzios G, Mirotsou M, Hatziapostolou E, Kral J, Scouras ZG, Mavragani-Tsipidou P (1997) Insecticidal and genotoxic activities of mint essential oils. J Agric Food Chem 45:2690–2694

Goel D, Mallavarupu GR, Kumar S, Singh V, Ali M (2008) Volatile metabolite compositions of the essential oil from aerial parts of ornamental and artemisinin rich cultivars of *Artemisia annua*. J Essent Oil Res 20:147–152

Govindaraj S, Kumari BD, Cioni PL, Flamini G (2008) Mass propagation and essential oil analysis of *Artemisia vulgaris*. J Biosci Bioeng 105(3):176–183

Govindarajan M (2010) Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (willd.) Hook. F. Benth (Rutaceae) against three mosquito species. Asian Pacific J Trop Med 3:874–877

Govindarajan M, Benelli G (2016a) α-Humulene and β-elemene from *Syzygium zeylanicum* (Myrtaceae) essential oil: highly effective and eco-friendly larvicides against *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus* (Diptera: Culicidae). Parasitol Res 115:2771–2778

Govindarajan M, Benelli G (2016b) Eco-friendly larvicides from Indian plants: effectiveness of lavandulyl acetate and bicyclogermacrene on malaria, dengue and Japanese encephalitis mosquito vectors. Ecotoxicol Environ Saf 133:395–402

Govindarajan M, Sivakumar R, Rajeswari M, Yogalakshmi K (2012) Chemical composition and larvicidal activity of essential oil from *Mentha spicata* (Linn.) against three mosquito species. Parasitol Res 110:2023–2032

Govindarajan M, Sivakumar R, Rajeswary M, Yogalakshmi K (2013) Chemical composition and larvicidal activity of essential oil from *Ocimum basilicum* (L.) against *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus* (Diptera: Culicidae). Experiment Parasitol 134:7–11

Govindarajan M, Sivakumar R (2014) Larvicidal, ovicidal, and adulticidal efficacy of *Erythrina indica* (Lam.) (Family: Fabaceae) against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res 113(2):777–791

Govindarajan M, Rajeswary M, Hoti SL, Bhattacharyya A, Benelli G (2016a) Eugenol, α-pinene and β-caryophyllene from *Plectranthus barbatus* essential oil as eco-friendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors. Parasitol Res 115:807–815

Govindarajan M, Rajeswary M, Hoti SL, Benelli G (2016b) Larvicidal potential of carvacrol and terpinen-4-ol from the essential oil of *Origanum vulgare* (Lamiaceae) against *Anopheles stephensi*, *Anopheles subpictus*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* (Diptera: Culicidae). Res Vet Sci 104:77–82

Govindarajan M, Rajeswary M, Benelli G (2016c) Chemical composition, toxicity and effects on non-target organisms of *Pinus kesiya* essential oil: an eco-friendly larvicide against mosquito vectors. Ecotoxicol Environ Saf 129:85–90

Govindarajan M., Rajeswary M., Benelli G (2016d) δ-Cadinene, calarene and δ-4-carene from *Kadsura heteroclita* essential oil as novel larvicides against malaria, dengue and filariasis mosquitoes. Combinatorial Chemistry & High Throughput Screening. doi: 10.2174/1386207319666160506123520

Grainge M, Ahmed S (1988) Handbook of plants with pest-control properties. Wiley, New York, 470 pp

Guan WQ, Li SF, Yan RX, Huang YY (2006) Comparison of composition and antifungal activity of *Artemisia argyi* Levl. et Vant inflorescence essential oil extracted by hydrodistillation and supercritical carbon dioxide. Nat Prod Res 20:996–998

Guvenalp Z, Cakir A, Harmandar M, Gleispach H (1998) The essential oils of *Artemisia austriaca* Jacq. and *Artemisia spicigera* C. Koch. from Turkey. Flavour Frag J 13:26–28

He ZZ, Yan JF, Song ZJ, Ye F, Liao X, Peng SL, Ding LS (2009) Chemical constituents from the aerial parts of *Artemisia minor*. J Nat Prod 72:1198–1201

Hemingway J, Ranson H (2000) Insecticide resistance in insect vectors of human disease. Ann Rev Entomol 45:371–391

Hikino H (1985) Antihepatoxic constituents of Chinese drugs. China Pharmac Bull 20:415–417

Hodgson E, Levi PE (1996) Pesticides: an important but underused model for the environmental health sciences. Environ Health Perspect 104: 97–106

Hough-Goldstein JA (1990) Antifeedant effects of common herbs on the Colorado potato beetle (Coleoptera: Chrysomelidae). Environ Entomol 19:234–238

Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608

Isman MB (2008) Botanical insecticides: for richer, for poorer. Pest Manag Sci 64:8–11

Jalali HM, Sereshti H (2007) Determination of essential oil components of *Artemisia haussknechtii* Boiss. using simultaneous hydrodistillation-static headspace liquid phase microextraction-gas chromatography mass spectrometry. J Chromatogr A 1160:81–89

Jensen M, Mehlhorn H (2009) Seventy-five years of Resochin® in the fight against malaria. Parasitol Res 105:609–627

Karr LL, Coats JR (1988) Insecticidal properties of d-limonene. J Pest Sci 13:287–290

Lee S, Peterson CJ, Coats JR (2003) Fumigation toxicity of monoterpenoids to several stored product insects. J Stored Prod Res 39:77–85

Marques MMM, Morais SM, Vieira ÍGP, Vieira MGS, Silva ARA, Almeida RR, Guedes MIF (2011) Larvicidal a activity of *Tagetes erecta* against *Aedes aegypti*. J Am Mosq Control Assoc 27:156–158

McCaffery A, Nauen R (2006) The Insecticide Resistance Action Committee (IRAC): public responsibility and enlightened industrial self interest. Outlook Pest Manage 17:11–14

Mehlhorn H, Al-Rasheid KA, Al-Quraishy S, Abdel-Ghaffar F (2012) Research and increase of expertise in arachno-entomology are urgently needed. Parasitol Res 110:259–265

Mehlhorn H (ed) (2015) Encyclopedia of parasitology, 4th edn. Springer, New York, p 893

Mighri H, Akrout A, Casanova J, Tomi F, Neffati M (2009) Influence of drying time and process on *Artemisia herba-alba* Asso essential oil yield and composition. J Essent Oil Bea Plants 12:358–364

Mills C, Cleary BV, John JJW, Gilmer F (2004) Inhibition of acetylcholinesterase by tea tree oil. J Pharm Pharmacol 56(3):375–379

Murugan K, Dinesh D, Paulpandi M, Althbyani AD, Subramaniam J, Madhiyazhagan P, Wang L, Suresh U, Kumar PM, Mohan J, Rajaganesh R, Wei H, Kalimuthu K, Parajulee MN, Mehlhorn H, Benelli G (2015a) Nanoparticles in the fight against mosquito-borne diseases: bioactivity of *Bruguiera cylindrica*-synthesized nanoparticles against dengue virus DEN-2 (in vitro) and its mosquito vector *Aedes aegypti* (Diptera: Culicidae). Parasitol Res 114:4349–4361

Murugan K, Benelli G, Panneerselvam C, Subramaniam J, Jeyalalitha T, Dinesh D, Nicoletti M, Hwang JS, Suresh U, Madhiyazhagan P (2015b) *Cymbopogon citratus*-synthesized gold nanoparticles boost the predation efficiency of copepod *Mesocyclops aspericornis* against malaria and dengue mosquitoes. Exp Parasitol 153:129–138

Murugan K, Priyanka V, Dinesh D, Madhiyazhagan P, Panneerselvam C, Subramaniam J, Suresh U, Chandramohan B, Roni M, Nicoletti M, Alarfaj AA, Higuchi A, Munusamy MA, Khater HF, Messing RH, Benelli G (2015c) Enhanced predation by Asian bullfrog tadpoles, *Hoplobatrachus tigerinus*, against the dengue vector *Aedes aegypti* in an aquatic environment treated with mosquitocidal nanoparticles. Parasitol Res 114:3601–3610

Murugan K, Anitha J, Dinesh D, Suresh U, Rajaganesh R, Chandramohan B, Subramaniam J, Paulpandi M, Vadivalagan C, Amuthavalli P, Wang L, Hwang JS, Wei H, Alsalhi MS, Devanesan S, Kumar S, Pugazhendy K, Higuchi A, Nicoletti M, Benelli G (2016a) Fabrication of nano-mosquitocides using chitosan from crab shells: impact on non-target organisms in the aquatic environment. Ecotoxicol Environ Saf 132:318–328

Murugan K, Panneerselvam C, Aziz AT, Subramaniam J, Madhiyazhagan P, Hwang JS, Wang L, Dinesh D, Suresh U, Roni M, Higuchi A, Nicoletti M, Saleh Alsalhi M, Benelli G (2016b) Eco-friendly drugs from the marine environment: spongeweed-synthesized silver nanoparticles are highly effective on *Plasmodium falciparum* and its vector *Anopheles stephensi*, with little non-target effects on predatory copepods. Environ Sci Poll Res doi. doi:10.1007/s11356-016-6832-9

Murugan K, Nataraj D, Madhiyazhagan P, Sujitha V, Chandramohan B, Panneerselvam C, Dinesh D, Chandirasekar R, Kovendan K, Suresh U, Subramaniam J, Paulpandi M, Vadivalagan C, Rajaganesh R, Wei H, Syuhei B, Aziz AT, Saleh Alsalhi M, Devanesan S, Nicoletti M, Canale A, Benelli G (2016c) Carbon and silver nanoparticles in the fight against the filariasis vector *Culex quinquefasciatus*: genotoxicity and impact on behavioral traits of non-target aquatic organisms. Parasitol Res 115:1071–1083

Naqqash MN, Gökçe A, Bakhsh A, Salim M (2016) Insecticide resistance and its molecular basis in urban insect pests. Parasitol Res 115: 1363–1373

Nauen R (2007) Insecticide resistance in disease vectors of public health importance. Pest Manage Sci 63:628–633

Negahban M, Moharramipour S, Sefidkon F (2006a) Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored product insects. J Stored Prod Res 43(2):123–128

Negahban M, Moharramipour S, Sefidkon F (2006b) Insecticidal activity and chemical composition of *Artemisia sieberi* Besser oil from Karaj, Iran. J Asia- Pacif Entomol 9:61–66

Padalia RC, Verma RS, Chauhan A, Chanotiya CS, Yadav A (2011) Variation in the volatile constituents of *Artemisia annua* var. CIM-Arogya during plant ontogeny. Nat Prod Commun 6:239–242

Pavela R (2014) Insecticidal properties of *Pimpinella anisum* essential oils against the *Culex quinquefasciatus* and the non-target organism *Daphnia magna*. J Asia-Pacif Entomol 17:287–293

Pavela R (2015) Essential oils for the development of eco-friendly mosquito larvicides: a review. Ind Crops Prod 76:174–187

Pavela R, Benelli G (2016) Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors—a review. Exp Parasitol 167C:103–108

Pushpanathan T, Jebanesan A, Govindarajan M (2006) Larvicidal, ovicidal and repellent activities of *Cymbopogan citratus* Stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Trop Biomed 23:208–212

Rana VS, Juyal JP, Blazquez MA, Bodakhe SH (2003) Essential oil composition of *Artemisia parviflora* aerial parts. Flavour Frag J 18:342–344

Rice PJ, Coats JR (1994) Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J Econ Entomo l 87:1172–1179

Ruiz C, Cachay M, Domínguez M, Velásquez C, Espinoza G, Ventosilla P, Rojas R (2011) Chemical composition, antioxidant and mosquito larvicidal activities of essential oils from *Tagetes filifolia*, *Tagetes minuta* and *Tagetes elliptica* from Perú. Planta Med 77:PE30

Rustaiyan A, Masoudi S (2011) Chemical constituents and biological activities of Iranian *Artemisia* species. Phytochem Lett 4:440–447

Shakarami I, Kamali K, Moharramipour S (2004) Application of *Artemisia aueheri* Boiss as a botanical insecticide. Proceeding of the 1st Iranian National Seminar on Development of Agrochemical Industries, Tehran, Iran. p. 31

Silva LL, Garlet QI, Koakoski G, Oliveira TA, Barcellos LJG, Baldisseroto B, Pereira AMS, Heinzmann BM (2015) Effects of anesthesia with the essential oil of *Ocimum gratissimum* L. in parameters of fish stress. Rev Bras Pl Med 17:215–223

Sivagnaname N, Kalyanasundaram M (2004) Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutaceae) against immature stages of mosquitoes and non-target Organisms. Mem Inst Oswaldo Cruz Rio de Janeiro 99(1):115–118

Soylu EM, Yigitbas H, Tok FM, Soylu S, Kurt S, Baysal O, Kaya AD (2005) Chemical composition and antifungal activity of the essential oil of *Artemisia annua* L. against foliar and soil-borne fungal pathogens. J Plant Dis Protect 112:229–239

Subramaniam J, Murugan K, Panneerselvam C, Kovendan K, Madhi yazhagan P, Mahesh Kumar P, Dinesh D, Chandramohan B, Suresh U, Nicoletti M, Higuchi A, Hwang JS, Kumar S, Alarfaj AA, Munusamy MA, Messing RH, Benelli G (2015) Eco-friendly control of malaria and arbovirus vectors using the mosquitofish *Gambusia affinis* and ultra-low dosages of *Mimusops elengi*-synthesized silver nanoparticles: towards an integrative approach? Environ Sci Poll Res. doi:10.1007/s11356-015-5253-5

Subramaniam J, Murugan K, Panneerselvam C, Kovendan K, Madhiyazhagan P, Dinesh D, Mahesh Kumar P, Chandramohan B, Suresh U, Rajaganesh R, Alsalhi MS, Devanesan S, Nicoletti M, Canale A, Benelli G (2016) Multipurpose effectiveness of *Couroupita guianensis*-synthesized gold nanoparticles: high antiplasmodial potential, field efficacy against malaria vectors and synergy with *Aplocheilus lineatus* predators. Environ Sci Pollut Res. doi:10.1007/s11356-015-6007-0

Sukumar K, Perich MJ, Boobar LR (1991) Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc 7:210–237

Tantaoui-Elaraki A, Ferhout F, Errifi A (1993) Inhibition of the fungal asexual reproduction stages by three Moroccan essential oils. J Essen Oil Res 5:535–545

Tolle MA (2009) Mosquito-borne diseases. Curr Prob Pediatr Adolesc Health Care 39:97–140

Tu Y (2011) The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nat Med 17:1217–1220

Wang J, Zhu F, Zhou XM, Niu CY, Lei CI (2006) Repellent and fumigant activity of essential oil from *Artemisia vulgaris* to *Tribolium eastaneum* (Herbst). J Stored Prod Res 42:339–347

Watanabe K, Shono Y, Kakimizu A, Okada A, Matsuo N, Satoh A, Nishimura H (1993) New mosquito repellent from *Euculyptus camuldulensis*. J Agric Food Chem 4(1):2164–2166

WHO (2012a) Global Plan for Insecticide Resistance Management in Malaria Vectors (GPIRM)., accessed 15.04.2

WHO (2012b) Impact of dengue. Global Alert and Response (GAR) (accessed 14.04.12.)

WHO (2014) Malaria. Fact sheet N 94. WHO, Geneva

Williams JD, Saleh AM, Acharya DN (2012) Composition of the essential oil of wild growing *Artemisia vulgaris* from Erie, Pennsylvania. Nat Prod Commun 7:637–640

World Health Organization (2005) Guidelines for laboratory and field-testing of mosquito larvicides. Communicable disease control, prevention and eradication, WHO pesticide evaluation scheme. WHO, Geneva, 2005; WHO/CDS/WHOPES/GCDPP/1.3

Zebitz CPW (1984) Effect of some crude and azadirachtin-enriched neem (*Azadirachta indica*) seed kernel extracts on larvae of *Aedes aegypti*. Entomol Exp Appl 35:11–16

Zhu BCR, Henderson G, Chen F, Fei H, Laine RA (2001) Evaluation of vetiver oil and seven insect-active essential oils against the *Formosan subterranean* termite. J Chem Ecol 27:1617–1625

Zhu L, Tian Y (2011) Chemical composition and larvicidal activity of *Blumea densiflora* essential oils against *Anopheles anthropophagus*: a malarial vector mosquito. Parasitol Res 109:1417–1422

Zhu L, Tian Y (2013) Chemical composition and larvicidal activity of essential oil of *Artemisia gilvescens* against *Anopheles anthropophagus*. Parasitol Res 112:1137–1142