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ORIGINAL PAPER

Larvicidal activity of *Blumea eriantha* essential oil and its components against six mosquito species, including Zika virus vectors: the promising potential of (4*E*,6*Z*)-allo-ocimene, carvotanacetone and dodecyl acetate

Giovanni Benelli[1] · Marimuthu Govindarajan[2] · Mohan Rajeswary[2] · Sengamalai Senthilmurugan[2] · Periasamy Vijayan[2] · Naiyf S. Alharbi[3] · Shine Kadaikunnan[3] · Jamal M. Khaled[3]

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Abstract The effective and environmentally sustainable control of mosquitoes is a challenge of essential importance. This is due to the fact that some invasive mosquitoes, with special reference to the *Aedes* genus, are particularly difficult to control, due to their high ecological plasticity. Moreover, the indiscriminate overuse of synthetic insecticides resulted in undesirable effects on human health and non-target organisms, as well as resistance development in targeted vectors. Here, the leaf essential oil (EO) extracted from a scarcely studied plant of ethnomedicinal interest, *Blumea eriantha* (Asteraceae), was tested on the larvae of six mosquitoes, including Zika virus vectors. The *B. eriantha* EO was analyzed by GC and GC-MS. The *B. eriantha* EO showed high toxicity against 3rd instar larvae of six important mosquito species: *Anopheles stephensi* (LC_{50}=41.61 μg/ml), *Aedes aegypti* (LC_{50}=44.82 μg/ml), *Culex quinquefasciatus* (LC_{50} =48.92 μg/ml), *Anopheles subpictus* (LC_{50}=51.21 μg/ml), *Ae. albopictus* (LC_{50}=56.33 μg/ml) and *Culex tritaeniorhynchus* (LC_{50}=61.33 μg/ml). The major components found in *B. eriantha* EO were (4*E*,6*Z*)-allo-ocimene (12.8%), carvotanacetone (10.6%), and dodecyl acetate (8.9%). Interestingly, two of the main EO components, (4*E*,6*Z*)-allo-ocimene and carvotanacetone, achieved LC_{50} lower than 10 μg/ml on all tested mosquito species. The acute toxicity of *B. eriantha* EO and its major constituents on four aquatic predators of mosquito larval instars was limited, with LC_{50} ranging from 519 to 11.431 μg/ml. Overall, the larvicidal activity of (4*E*,6*Z*)-allo-ocimene and carvotanacetone far exceed most of the LC_{50} calculated in current literature on mosquito botanical larvicides, allowing us to propose both of them as potentially alternatives for developing eco-friendly mosquito control tools.

Keywords biosafety · biopesticide · essential oil · larvicidal activity · GC-MS · non-target toxicity · WHO

✉ Giovanni Benelli
benelli.giovanni@gmail.com

✉ Marimuthu Govindarajan
drgovind1979@gmail.com

[1] Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

[2] Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar, Tamil Nadu 608 002, India

[3] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

Introduction

Mosquitoes constitute a major public health problem as vectors of serious human and animal diseases, such as malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya, yellow fever, and – more recently – Zika virus. These diseases cause high mortality and morbidity among people living in tropical and sub-tropical zones (Benelli 2015a; Mehlhorn 2015; Benelli et al. 2016; Yakob and Walker 2016). Nowadays, the effective and environmentally sustainable control of mosquitoes is a challenge of essential importance. This is due to the fact that some mosquitoes, with special reference to the *Aedes* genus, are particularly difficult to control, due to their high ecological plasticity. A good example is the Asian tiger mosquito, *Aedes albopictus*, which is now ranked among the one hundred most invasive organisms worldwide (Benedict et al. 2007; Becker

2008; Becker et al. 2013; Benelli and Mehlhorn 2016). In addition, the indiscriminate overuse of synthetic insecticides resulted in undesirable effects on human health and non-target organisms, as well as resistance development in targeted vectors, with consequent loss of efficacy (see Hemingway and Ranson 2000, as well as Naqqash et al. 2016 for dedicated reviews). This current scenario is worsened by the lack of access to modern and costly mosquito control tools by rural populations of developing countries, which are the most afflicted by mosquito-borne diseases (Benelli 2015a).

Therefore, the use of plant derivatives with multiple mechanisms of action and eco-friendly features has been proposed as alternative tools against Culicidae, ticks, and other vectors (Benelli 2015b; Pavela and Benelli 2016a, b). In latest years, plant essential oils (EOs), plant extracts, plant extraction byproducts (e.g. neem cake), as well as botanical-fabricated capped mosquitocidal nanoparticles have been evaluated as mosquito control agents (Amer and Mehlhorn 2006a, b, c, d; Dinesh et al. 2015; Subramaniam et al. 2015, 2016; Murugan et al. 2016a, b; Panneerselvam et al. 2016; Benelli 2016a, b, 2017; Benelli and Govindarajan 2017).

In particular, a wide number of EOs extracted from aromatic plants have been tested as mosquitocides, ovideterrents and/or repellents, against different mosquito species (Benelli 2015a; Pavela 2015; Pavela and Govindarajan 2016; et al. 2016a, b, c). Good examples include *Origanum majorana* (El-Akhal et al. 2014), *Ocimum gratissimum* (Pratheeba et al. 2015), *Lavandula stoechas* (El Ouali et al. 2016), *Corymbia citriodora* (Santi and Simone 2014), *Myristica fragrans* (Carolina and Maman 2016), *Crataeva magna* (Veni et al. 2016), *Laurus nobilis* (Verdian-Rizi 2009), *Apium graveolens* (Kumar et al. 2014), *Clausena anisata* (Jayaraman et al. 2015), *Melissa officinalis* (Baranitharan et al. 2016) and *Coleus aromaticus* (Govindarajan et al. 2013b; Baranitharan et al. 2017). Results underlined that the plant EOs may be an alternative source of mosquito larval control agents, since are rich in bioactive compounds that show multiple mechanisms of action, are biodegradable into non-toxic products, and potentially suitable for use in IPM programs (Pavela and Benelli 2016a, b).

However, most of the studies focused on routine testing of EOs without digging deep in their chemical composition (i.e. no GC-MS, HPLC-MS, HPTLC and NMR analyses have been performed) and evaluating the bioactivity of selected pure constituents, formulated alone or in synergistic blends (Benelli et al. 2017a, b, c). Most importantly, as recently reviewed by Pavela (2015), the majority of tested EOs achieved LC_{50} higher than 50 ppm on mosquitoes, highlighting the value of systematic screening endemic flora of tropical and sub-tropical countries searching for effective mosquitocidal and antiplasmodial products (Benelli and Mehlhorn 2016).

Blumea is a genus of shrubs and small trees, comprising about 80 species distributed in tropical and subtropical Asia, Africa, and Oceania (Liang et al. 2011). This genus includes some key medicinal plants largely used in traditional medicine. For example, *Blumea membranacea* EO led to blood pressure reduction (Mehta et al. 1986). Besides the ethno-pharmacological potential of *Blumea* species, the EOs from *B. mollis* (Senthilkumar et al. 2008), *Blumea perrottetiana* (Owolabi et al. 2010) and *B. densiflora* (Zhu and Tian 2011) have been reported for their insecticidal activity, while *B. membranacea* EO shows antifungal activity (Mehta et al. 1986).

Blumea eriantha is an annual aromatic herb, which grows abundantly along roadsides and degraded forestlands. Common names are "Nimurdi" in Marathi and "Kukronda" in Hindi. *B. eriantha* is distributed in Bihar, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Uttar Pradesh and Southern India (Singh et al. 2011). The juice extracted from this herb has been reported as a carminative, while the warm leaf infusion is used as sudorific, and the cold infusion is considered as a diuretic and herbal emmenagogue. The EO extracted from *B. eriantha* is traditionally recognized for its antibacterial and antifungal uses in folk medicine (Khare 2007). A recent study focused on the antimicrobial efficacy of *B. eriantha* EO (Pednekar et al. 2012).

However, the toxicity of *B. eriantha* EO against insect vectors of medical and veterinary relevance is unknown. In the present research, we investigated the environmentally sustainable use of *B. eriantha* EO and its main chemical components for the development of new products to combat the spread of the mosquito-borne diseases, with special reference to dengue and Zika virus. We tested the *B. eriantha* EO larvicidal activity on six key mosquito vectors, i.e. *Anopheles stephensi*, *An. subpictus*, *Ae. aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, and *Cx. tritaeniorhynchus*. Furthermore, *B. eriantha* EO was analyzed using gas chromatography-mass spectrometry (GC-MS). The major components of *B. eriantha* EO, i.e. (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate, were also tested against the six mosquito vectors. Lastly, to shed light on non-target effects of the *B. eriantha* EO as well as (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate, we evaluated them in acute toxicity tests on four non-target predators of mosquito larvae.

Materials and methods

Extraction, GC and GC-MS of the *B. eriantha* essential oil

Fresh leaves of *B. eriantha* were collected in the Munnar mountains, India (10°05'21"N 77°03'35"E, 1700 m a.s.l.) in May 2016. *Blumea eriantha* EO was hydro-distilled using 3 kg of fresh leaves, then analyzed by GC and GC-MS as described by Govindarajan and Benelli (2016a, b). Compound identification was carried out comparing retention indices and mass spectra with those available in NIST 98.1, Mass Finder 3.1 and Adams (2007).

Larvicidal activity of (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate

The *B. eriantha* EO as well as (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate, were tested against 3rd instar larvae of *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus* following the protocol by WHO (2005), slightly modified by Govindarajan and Benelli (2016c). For each tested product, 5 replicates of each dose were prepared. 10 3rd larvae were transferred into each beaker. Mortality was assessed after 24 h of exposure.

Toxicity on non-target predators

The effect of *B. eriantha* EO as well as (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate, on non-target aquatic predators was assessed following the method by Sivagnaname and Kalyanasundaram (2004) modified Govindarajan et al. (2016d, e). The toxicity of the

Table 1 Chemical composition of *Blumea eriantha* essential oil

Peak	Constituent	Retention time (Kovats index)	Composition (%)	Mode of identification
1	*n*-Hexanol	871	1.2	RI, MS
2	α-Thujene	928	0.9	RI, MS
3	α-Pinene	935	2.9	RI, MS
4	Sabinene	972	1.5	RI, MS
5	β-Pinene	975	0.9	RI, MS
6	Vinyl amyl carbinol	981	0.8	RI, MS
7	Myrcene	993	1.8	RI, MS
8	α-Terpinene	1015	0.8	RI, MS
9	Limonene	1025	0.7	RI, MS
10	γ-Terpinene	1056	0.7	RI, MS
11	Phenethyl alcohol	1114	3.2	RI, MS
12	Isophorone	1121	0.8	RI, MS
13	*(4E,6Z)-Allo-ocimene*	1133	12.8	RI, MS
14	3-Terpinen-1-ol	1137	0.9	RI, MS
15	*trans*-Verbenol	1147	1.8	RI, MS
16	Borneol	1173	0.7	RI, MS
17	Terpinen-4-ol	1180	3.5	RI, MS
18	Myrtenal	1193	1.2	RI, MS
19	(4*Z*)-Decenal	1197	2.9	RI, MS
20	Nerol	1235	0.7	RI, MS
21	*Carvotanacetone*	1253	10.6	RI, MS
22	Neryl formate	1275	2.2	RI, MS
23	Carvacrol	1304	0.9	RI, MS
24	α-Gurjunene	1402	3.6	RI, MS
25	(*E*)-Caryophyllene	1424	3.9	RI, MS
26	Nopyl acetate	1427	2.7	RI, MS
27	α-Humulene	1455	2.2	RI, MS
28	γ-Muurolene	1483	3.1	RI, MS
29	α-Cedrene epoxide	1577	3.3	RI, MS
30	Caryophyllene oxide	1585	4.2	RI, MS
31	*Dodecyl acetate*	1614	8.9	RI, MS
32	α-Muurolol	1648	2.6	RI, MS
33	7-*epi*-alpha-Eudesmol	1661	3.4	RI, MS
34	α-Santalol	1677	1.9	RI, MS
	Total		94.2	

Compounds given in italics represent the main constituents

RI retention index, *MS* mass spectra

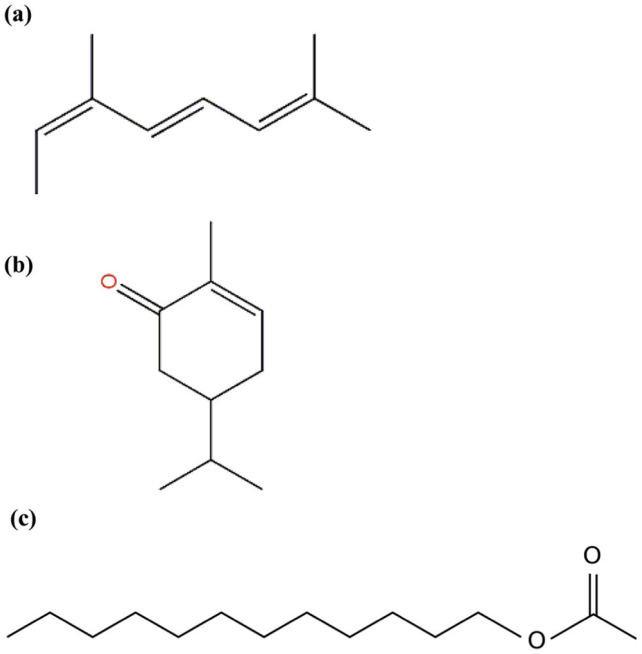

Fig. 1 Chemical structures of the three major constituents of the *Blumea eriantha* essential oil. **a** (4*E*,6*Z*)-Allo-ocimene. **b** Carvotanacetone. **c** Dodecyl acetate

B. eriantha EO, (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate was tested against adults of the non-target biological control agents of mosquito young instars, including adult backswimmers and water bugs i.e. *Anisops bouvieri* and *Diplonychus indicus,* and larvivorous fish *Gambusia affinis* and *Poecilia reticulata*. The non-target species were reared as reported by Govindarajan and Benelli (2016d). The *B. eriantha* EO, (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate were evaluated at doses higher than 50xLC_{50} calculated on the six mosquito species. 10 replicates were performed for each dose. 4 control replicates were also done (where no *B. eriantha* EO, (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate were added to the water). Mortality of each non-target predator was assessed after 48 h of exposure (Govindarajan and Benelli 2016b; Benelli et al. 2017c).

Data analysis

Mortality data were analyzed by probit analysis (Benelli 2017). LC_{50} and LC_{90} were calculated following Finney (1971). Concerning non-target predators, the Predator Safety

Table 2 Larvicidal activity of *Blumea eriantha* essential oil on six mosquito vectors

Mosquito species	Concentration (μg/ml)	24-h mortality (%) ± SD	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anopheles stephensi	20	27.5 ± 1.2	41.61 (36.94–45.74)	81.46 (75.56–89.20)	3.1	$y = 10.62 + 0.922x$	5.634 (4) n.s.
	40	48.2 ± 0.8					
	60	66.4 ± 0.6					
	80	87.6 ± 1.2					
	100	100.0 ± 0.0					
Aedes aegypti	20	24.9 ± 0.6	44.82 (40.08–49.08)	87.34 (80.95–95.79)	3.02	$y = 7.49 + 0.924x$	3.787 (4) n.s.
	40	45.3 ± 1.2					
	60	62.7 ± 0.8					
	80	83.4 ± 0.4					
	100	98.2 ± 1.2					
Culex quinquefasciatus	20	20.6 ± 0.8	48.92 (44.38–53.13)	97.83 (85.22–100.56)	2.56	$y = 2.39 + 0.951x$	3.132 (4) n.s.
	40	41.5 ± 0.6					
	60	58.9 ± 1.2					
	80	79.4 ± 0.4					
	100	96.7 ± 1.2					
Anopheles subpictus	25	29.4 ± 0.4	51.21 (45.34–56.40)	101.02 (93.67–110.70)	3.23	$y = 11.54 + 0.732x$	5.899 (4) n.s.
	50	46.9 ± 1.2					
	75	67.2 ± 0.8					
	100	88.7 ± 0.6					
	125	100.0 ± 0.0					
Aedes albopictus	25	25.6 ± 1.2	56.33 (50.42–61.66)	109.64 (101.64–120.21)	3.01	$y = 7.06 + 0.742x$	2.228 (4) n.s.
	50	42.3 ± 0.8					
	75	63.8 ± 0.6					
	100	84.5 ± 1.2					
	125	97.2 ± 0.4					
Culex tritaeniorhynchus	25	21.9 ± 0.6	61.33 (55.69–66.58)	114.81 (106.58–125.69)	2.54	$y = 1.98 + 0.764x$	2.452 (4) n.s.
	50	38.4 ± 1.2					
	75	59.2 ± 0.8					
	100	80.6 ± 0.4					
	125	96.3 ± 1.2					

Factor (PSF) was calculated as described by Deo et al. (1988). Data were analyzed by SPSS version 16.0.

Results

Yield and composition of *B. eriantha* essential oil

The yield of *B. eriantha* leaf EO was 2.5 ml/kg of leaf fresh weight. Table 1 showed a total of 34 chemical constituents, representing 94.2% of the *B. eriantha* leaf EO. The major constituents of *B. eriantha* EO were (4*E*,6*Z*)-allo-ocimene (12.8%), carvotanacetone (10.6%) and dodecyl acetate (8.9%). The chemical structures of (4*E*,6*Z*)-allo-ocimene, carvotanacetone and dodecyl acetate are reported in Figure 1. The amount of remaining 31 molecules ranged from 0.7 % to 4.2 % (Table 1).

Larvicidal activity of (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate

The *B. eriantha* EO showed acute toxicity against third instar larvae of *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus*, with LC_{50} of 41.61, 44.82, 48.92, 51.21, 56.33 and 61.33 μg/ml, respectively (Table 2).

Furthermore, the three major pure compounds extracted from the *B. eriantha* EO, (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate, were tested individually against six mosquito vector larval populations. We observed that (4*E*,6*Z*)-allo-ocimene, carvotanacetone and dodecyl acetate

Table 3 Larvicidal activity of (4*E*,6*Z*)-allo-ocimene from *Blumea eriantha* essential oil on six mosquito vectors

Mosquito species	Concentration (μg/ml)	24-h mortality (%) ± SD	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anopheles stephensi	2	28.5 ± 0.8	4.06 (3.58–4.47)	8.04 (7.45–8.81)	3.31	$y = 12.14 + 9.11x$	5.134 (4) n.s.
	4	49.2 ± 1.2					
	6	67.9 ± 0.6					
	8	88.4 ± 0.4					
	10	100.0 ± 0.0					
Aedes aegypti	2	22.7 ± 1.2	4.52 (4.05–4.94)	8.69 (8.07–9.52)	2.85	$y = 6.4 + 9.39x$	1.764 (4) n.s.
	4	45.2 ± 0.8					
	6	64.8 ± 0.6					
	8	83.6 ± 1.2					
	10	97.4 ± 0.4					
Culex quinquefasciatus	2	20.3 ± 0.4	4.92 (4.45–5.35)	9.36 (8.67–10.28)	2.70	$y = 3.03 + 9.335x$	1.840 (4) n.s.
	4	41.7 ± 1.2					
	6	59.4 ± 0.8					
	8	78.6 ± 0.6					
	10	95.2 ± 1.2					
Anopheles subpictus	3	27.4 ± 0.8	6.14 (5.45–6.76)	12.02 (11.16–13.17)	3.10	$y = 11.25 + 6.15x$	4.876 (4) n.s.
	6	49.2 ± 1.2					
	9	67.9 ± 0.6					
	12	88.5 ± 0.4					
	15	100.0 ± 0.0					
Aedes albopictus	3	23.8 ± 0.6	6.70 (6.01–7.33)	12.90 (11.97–14.12)	1.13	$y = 6.9 + 6.253x$	3.671 (4) n.s.
	6	46.2 ± 1.2					
	9	62.9 ± 0.8					
	12	84.6 ± 0.4					
	15	98.4 ± 0.6					
Culex tritaeniorhynchus	3	20.5 ± 1.2	7.26 (6.59–7.88)	13.55 (12.59–14.83)	2.52	$y = 2.35 + 6.397x$	4.020 (4) n.s.
	6	42.9 ± 0.6					
	9	57.6 ± 0.8					
	12	81.4 ± 0.4					
	15	97.2 ± 1.2					

Table 4 Larvicidal activity of carvotanacetone from *Blumea eriantha* essential oil on six mosquito vectors

Mosquito species	Concentration (μg/ml)	24-h mortality (%) ± SD	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anopheles stephensi	3	28.5 ± 1.2	6.20 (5.50–6.82)	12.15 (11.27–13.31)	3.13	$y = 10.9 + 6.14x$	5.780 (4) n.s.
	6	47.2 ± 0.8					
	9	66.7 ± 0.6					
	12	88.4 ± 1.2					
	15	100.0 ± 0.0					
Aedes aegypti	3	24.7 ± 0.8	6.77 (6.07–7.39)	13.03 (12.09–14.27)	2.86	$y = 6.5 + 6.247x$	3.577 (4) n.s.
	6	43.9 ± 0.4					
	9	62.5 ± 1.2					
	12	84.3 ± 0.6					
	15	98.2 ± 1.2					
Culex quinquefasciatus	3	20.6 ± 0.6	7.38 (6.72–8.00)	13.66 (12.69–14.92)	2.43	$y = 1.07 + 6.463x$	2.172 (4) n.s.
	6	39.2 ± 0.8					
	9	58.4 ± 1.2					
	12	81.7 ± 0.4					
	15	96.3 ± 1.2					
Anopheles subpictus	4	27.5 ± 0.6	8.43 (7.51–9.26)	16.42 (15.23–17.97)	3.00	$y = 9.77 + 4.638x$	6.188 (4) n.s.
	8	46.9 ± 1.2					
	12	65.3 ± 0.8					
	16	87.4 ± 0.6					
	20	100.0 ± 0.0					
Aedes albopictus	4	23.8 ± 0.4	9.21 (8.29–10.05)	17.59 (16.32–19.25)	2.75	$y = 5.25 + 4.723x$	2.649 (4) n.s.
	8	42.5 ± 1.2					
	12	61.7 ± 0.8					
	16	84.2 ± 0.4					
	20	97.4 ± 1.2					
Culex tritaeniorhynchus	4	20.4 ± 0.6	10.02 (9.13–10.85)	18.55 (17.23–20.30)	2.44	$y = 0.65 + 4.813x$	2.743 (4) n.s.
	8	38.6 ± 1.2					
	12	57.3 ± 0.8					
	16	79.5 ± 0.4					
	20	96.2 ± 1.2					

were extremely toxic to the six mosquito species. (4*E*,6*Z*)-allo-ocimene LC_{50} values estimated on *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. subpictus*, *Ae. albopictus*, and *Cx. tritaeniorhynchus,* were 4.06, 4.52, 4.92, 6.14, 6.70 and 7.26 μg/ml, respectively (Table 3). Carvotanacetone LC_{50} values were 6.20, 6.77, 7.38, 8.43, 9.21 and 10.02 μg/ml, respectively (Table 4). Dodecyl acetate LC_{50} values were 10.22, 11.18, 12.16, 12.31, 13.45 and 14.68 μg/ml, respectively (Table 5). No mortality was recorded in controls.

Toxicity on non-target predators

The acute toxicity of *B. eriantha* EO was tested on the four non-target predators *A. bouvieri*, *D. indicus*, *P. reticulata* and *G. affinis*. Results are reported in Table 6. *B. eriantha* EO LC_{50} values were 4139.79, 6285.59, 10251.51 and 11431.04 μg/ml, respectively.

Furthermore, the three major pure compounds extracted from the *B. eriantha* EO, (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate, were tested individually against four important non-target predators of mosquito larvae. We observed that (4*E*,6*Z*)-allo-ocimene, carvotanacetone and dodecyl acetate were scarcely toxic to the non-target predators. (*4*E,6*Z*)-allo-ocimene LC_{50} estimated on *A. bouvieri*, *D. indicus*, *P. reticulata* and *G. affinis* were 519.97, 845.65, 1656.78 and 1854.25 μg/ml, respectively (Table 7). Carvotanacetone LC_{50} were 631.59, 1051.39, 1863.86 and 2075.07 μg/ml, respectively (Table 8). Dodecyl acetate LC_{50} were 823.94, 1483.11, 2065.56 and 2369.78 μg/ml, respectively (Table 9). No mortality was recorded in control treatments.

Table 5 Larvicidal activity of dodecyl acetate from *Blumea eriantha* essential oil on six mosquito vectors

Mosquito species	Concentration (μg/ml)	24-h mortality (%) ± SD	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anopheles stephensi	5	29.4 ± 1.2	10.22 (9.01–11.28)	20.45 (18.94–22.43)	3.52	$y = 12.21 + 3.61x$	5.524 (4) n.s.
	10	47.2 ± 0.8					
	15	68.7 ± 0.6					
	20	86.5 ± 0.4					
	25	100.0 ± 0.0					
Aedes aegypti	5	25.9 ± 0.8	11.18 (9.99–12.26)	21.92 (20.30–24.05)	3.11	$y = 7.79 + 3.674x$	3.716 (4) n.s.
	10	43.5 ± 0.6					
	15	64.3 ± 1.2					
	20	82.6 ± 0.4					
	25	98.2 ± 1.2					
Culex quinquefasciatus	5	21.6 ± 0.6	12.16 (11.03–13.20)	22.76 (21.13–24.91)	2.54	$y = 2.35 + 3.822x$	3.518 (4) n.s.
	10	39.8 ± 0.8					
	15	60.2 ± 1.2					
	20	79.5 ± 0.4					
	25	97.3 ± 1.2					
Anopheles subpictus	6	28.4 ± 0.8	12.31 (10.89–13.57)	24.37 (22.59–26.70)	3.28	$y = 11.61 + 3.042x$	5.143 (4) n.s.
	12	47.9 ± 1.2					
	18	68.3 ± 0.4					
	24	87.2 ± 0.6					
	30	100.0 ± 0.0					
Aedes albopictus	6	24.7 ± 1.2	13.45 (12.04–14.72)	26.12 (24.22–28.62)	2.98	$y = 7.16 + 3.1x$	2.074 (4) n.s.
	12	43.2 ± 0.8					
	18	65.9 ± 0.6					
	24	83.4 ± 1.2					
	30	97.6 ± 0.4					
Culex tritaeniorhynchus	6	20.4 ± 0.6	14.68 (13.33–15.94)	27.46 (25.50–30.04)	2.53	$y = 2.04 + 3.19x$	1.818 (4) n.s.
	12	39.8 ± 1.2					
	18	61.3 ± 0.4					
	24	79.6 ± 0.8					
	30	96.2 ± 1.2					

The estimated PSF indicated that the *B. eriantha* EO and its main constituents showed little toxicity on *A. bouvieri*, *D. indicus*, *P. reticulata* and *G. affinis* (Table 10).

Lastly, focal observations conducted daily until 10 days from the exposure to *B. eriantha* EO and its main constituents, formulated at the LC_{50} and LC_{90} calculated on the targeted six mosquito vectors, indicated that the survival and swimming activity of the non-target predators were not damaged.

Discussion

Wide chemical diversity of *Blumea* essential oils

Results from GC and GC-MS analyses showed that 34 compounds were identified in the *B. eriantha* EO, with (4*E*,6*Z*)-allo-ocimene, carvotanacetone and dodecyl acetate as main components. This highlighted a quite different chemical composition, if compared to other EOs extracted from close-related *Blumea* species. Currently, the EOs of several species of the genus *Blumea* have been examined. Good examples are *B. balsamifera* (Sakee et al. 2011), *Blumea lacera* (Khair et al. 2014), *B. balsamifera* (Norikura et al. 2008), and *B. lacera* (Jahan et al. 2014). At variance with our results on *B. eriantha*, it is worthy to note that *B. perrottetiana* aerial part EO was mostly composed by 2,5-dimethoxy-*p*-cymene (30.0 %), 1,8-cineole (11.0 %) and sabinene (8.1 %) (Owolabi et al. 2010). *Blumea balsamifera* leaf EO was mostly composed by borneol (33.22 %), caryophyllene (8.24 %) and ledol (7.12 %) (Bhuiyan et al. 2009). *Blumea mollis* leaf EO was mainly composed by linalool (19.43 %) and γ-elemene (12.19 %) (Senthilkumar et al. 2008). *Blumea brevipes* EO main

Table 6 Toxicity of *Blumea eriantha* essential oil on four non-target predators of mosquitoes

Non-target predator	Concentration (μg/ml)	48-h mortality (%) ± SD	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anisops bouvieri	2000	28.3 ± 0.8	4139.79 (3674.55–4552.34)	8108.45 (7521.30–8879.82)	3.11	$y = 10.83 + 0.009x$	5.952 (9) n.s.
	4000	47.6 ± 1.2					
	6000	66.2 ± 0.6					
	8000	88.5 ± 0.4					
	10,000	100.0 ± 0.0					
Diplonychus indicus	3000	25.6 ± 1.2	6285.59 (5604.95–6892.80)	12,114.04 (11,252.75–13,239.59)	2.87	$y = 9.53 + 0.006x$	3.390 (9) n.s.
	6000	48.2 ± 0.8					
	9000	67.9 ± 0.4					
	12,000	88.3 ± 1.2					
	15,000	99.5 ± 0.6					
Poecilia reticulata	5000	27.4 ± 0.4	10,251.51 (9027.02–11,324.66)	20,598.26 (19,081.66–22,598.63)	3.62	$y = 12.22 + 0.004x$	2.377 (9) n.s.
	10,000	49.6 ± 1.2					
	15,000	68.5 ± 0.8					
	20,000	87.2 ± 0.6					
	25,000	98.7 ± 1.2					
Gambusia affinis	5000	22.6 ± 1.2	11,431.04 (10,227.59–12,514.36)	22,337.98 (20,698.00–24,513.17)	3.07	$y = 6.89 + 0.004x$	1.221 (9) n.s.
	10,000	44.8 ± 0.8					
	15,000	65.3 ± 0.6					
	20,000	81.7 ± 0.4					
	25,000	96.2 ± 1.2					

chemicals were terpinen-4-ol (27.6 %) and germacrene-D (15.4 %) (Mwangi et al. 1994), while *B. lanceolaria* EO mainly contained methyl thymol (Dung et al. 1991). Lastly, *B. lacera* leaf EO was composed by thymoquinol di-mether, β-caryophyllene, α-humulene, and (*E*)-β-farnesene (Laakso et al. 1989). Main compounds in *B. densifora* EO were borneol (11.43%), germacrene D (8.66%) and β-caryophyllene (6.68%) (Zhu and Tian 2011). Based on the presence of (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate in our GC-MS analysis of *B. eriantha* EO, we selected these molecules for further toxicity screenings on mosquito vectors.

Blumea essential oils really work against mosquitoes!

The use of plant EOs in vector control may represent a cheap alternative method to minimize the side effects of chemical

Table 7 Toxicity of (4*E*,6*Z*)-allo-ocimene from *Blumea eriantha* essential oil on four non-target predators of mosquitoes

Non-target predator	Concentration (μg/ml)	48-h mortality (%) ± SD	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anisops bouvieri	250	26.4 ± 1.2	519.97 (463.51–570.32)	1001.87 (930.48–1095.24)	2.86	$y = 9.91 + 0.075x$	5.451 (9) n.s.
	500	48.9 ± 0.8					
	750	66.3 ± 0.6					
	1000	89.2 ± 1.2					
	1250	100.0 ± 0.0					
Diplonychus indicus	400	25.4 ± 0.6	845.65 (751.97–928.92)	1652.52 (1533.68–1808.33)	3.07	$y = 9.69 + 0.046x$	1.521 (9) n.s.
	800	47.6 ± 1.2					
	1200	68.2 ± 0.8					
	1600	87.5 ± 0.4					
	2000	98.3 ± 1.2					
Poecilia reticulata	800	28.6 ± 0.8	1656.78 (1468.86–1823.20)	3260.19 (3023.59–3570.98)	3.19	$y = 10.89 + 0.023x$	4.068 (9) n.s.
	1600	46.2 ± 1.2					
	2400	67.5 ± 0.6					
	3200	88.7 ± 0.4					
	4000	99.3 ± 1.2					
Gambusia affinis	800	23.7 ± 0.4	1854.25 (1670.79–2021.56)	3533.93 (3280.17–3868.34)	2.73	$y = 4.95 + 0.024x$	2.474 (9) n.s.
	1600	41.5 ± 0.8					
	2400	62.6 ± 1.2					
	3200	83.2 ± 0.6					
	4000	97.4 ± 1.2					

Table 8 Toxicity of carvotanacetone from *Blumea eriantha* essential oil on four non-target predators of mosquitoes

Non-target predator	Concentration (μg/ml)	48-h mortality (%) ± SD	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anisops bouvieri	300	27.4 ± 0.8	631.59 (562.56–693.12)	1226.24 (1138.18–1341.70)	2.97	$y = 9.67 + 0.062x$	5.232 (9) n.s.
	600	45.2 ± 0.6					
	900	68.9 ± 1.2					
	1200	86.3 ± 0.4					
	1500	100.0 ± 0.0					
Diplonychus indicus	500	26.4 ± 0.4	1051.39 (935.15–1154.77)	2051.12 (1903.37–2245.00)	3.04	$y = 9.93 + 0.037x$	3.728 (9) n.s.
	1000	48.2 ± 0.6					
	1500	65.9 ± 1.2					
	2000	88.3 ± 0.8					
	2500	99.1 ± 1.2					
Poecilia reticulata	900	28.4 ± 0.6	1863.86 (1643.60–2057.55)	3741.48 (3465.80–4105.05)	3.60	$y = 11.62 + 0.02x$	1.934 (9) n.s.
	1800	46.8 ± 0.8					
	2700	67.3 ± 1.2					
	3600	89.2 ± 0.4					
	4500	97.6 ± 1.2					
Gambusia affinis	900	22.7 ± 0.4	2075.07 (1877.25–2256.42)	3873.90 (3603.06–4227.90)	2.52	$y = 3.92 + 0.022x$	2.896 (9) n.s.
	1800	41.8 ± 1.2					
	2700	63.2 ± 0.8					
	3600	84.6 ± 0.6					
	4500	98.3 ± 1.2					

pesticides on human health and the environment (Benelli 2015b; Govindarajan et al. 2013a, b, 2016a, b, c; Pavela 2015). Although a number of compounds of botanical origin have been currently reported (Wang et al. 2006; Cheng et al. 2009; Pavela and Benelli 2016b), the discovery of more effective plant products is of paramount importance to improve insecticide formulation and develop environmentally acceptable insecticides (Alkofahi et al. 1989).

In our experiments, the EO extracted from the leaves of *B. eriantha* showed high toxicity against 3rd instar larvae of *Anopheles*, *Aedes* and *Culex* species, including Zika virus vectors, with LC_{50} ranging from 41.61 to 61.33 μg/ml. Our results fit the criteria of EO larvicidal efficacy outlined by Pavela (2015). Earlier, it has been reported that two other EOs from the *Blumea* genus are highly toxic to anopheline vectors. Indeed, the *B. densiflora* EO tested on *Anopheles anthropophagus* larvae showed a LC_{50} of 10.55 ppm after 24 h, and 22.32 ppm after 12 h (Zhu and Tian 2011), and Senthilkumar et al. (2008) showed the larvicidal

Table 9 Toxicity of dodecyl acetate from *Blumea eriantha* essential oil on four non-target predators of mosquitoes

Non-target predator	Concentration (μg/ml)	48-h mortality (%) ± SD	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anisops bouvieri	400	27.9 ± 0.8	823.94 (732.06–905.51)	1604.57 (1489.10–1755.94)	3.01	$y = 10.78 + 0.046x$	5.723 (9) n.s.
	800	48.2 ± 1.2					
	1200	66.5 ± 0.6					
	1600	89.4 ± 0.4					
	2000	100.0 ± 0.0					
Diplonychus indicus	700	25.9 ± 0.6	1483.11 (1320.62–1627.88)	2885.67 (2678.76–3156.73)	3.00	$y = 9.39 + 0.027x$	1.891 (9) n.s.
	1400	46.2 ± 0.8					
	2100	68.7 ± 1.2					
	2800	87.3 ± 0.4					
	3500	98.6 ± 0.6					
Poecilia reticulata	1000	28.9 ± 1.2	2065.56 (1824.53–2277.91)	4119.13 (3816.45–4518.31)	3.45	$y = 11.61 + 0.018x$	4.112 (9) n.s.
	2000	47.3 ± 0.8					
	3000	66.5 ± 0.6					
	4000	88.2 ± 1.2					
	5000	99.1 ± 0.4					
Gambusia affinis	1000	21.7 ± 0.8	2369.78 (2146.45–2575.41)	4437.85 (4123.57–4851.01)	2.54	$y = 3.2 + 0.019x$	3.421 (9) n.s.
	2000	42.6 ± 1.2					
	3000	59.3 ± 0.4					
	4000	83.2 ± 0.6					
	5000	97.5 ± 1.2					

Table 10 Predator safety factors calculated on four non-target predators over six key mosquito species exposed to *Blumea eriantha* essential oil, (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate

Treatment	Non-target predator	*Culex quinquefasciatus*	*Aedes aegypti*	*Anopheles stephensi*	*Culex tritaeniorhynchus*	*Aedes albopictus*	*Anopheles subpictus*
Blumea eriantha essential oil	*Anisops bouvieri*	84.62	92.36	99.49	67.50	73.49	80.83
	Diplonychus indicus	128.48	140.24	151.05	102.48	111.58	122.74
	Poecilia reticulata	209.55	228.72	246.37	167.15	181.99	200.18
	Gambusia affinis	233.66	255.04	274.71	186.38	202.92	223.21
(4*E*,6*Z*)-Allo-ocimene	*A. bouvieri*	105.68	115.03	128.07	71.62	77.60	84.68
	D. indicus	171.88	187.09	208.28	116.48	126.21	137.72
	P. reticulata	336.74	366.54	408.07	228.20	247.28	269.83
	G. affinis	376.88	410.23	456.71	255.40	276.75	301.99
Carvotanacetone	*A. bouvieri*	85.58	93.29	101.86	63.03	68.57	74.92
	D. indicus	142.46	155.30	169.57	104.92	114.15	124.72
	P. reticulata	252.55	275.31	300.62	186.01	202.37	221.09
	G. affinis	281.17	306.50	334.68	207.09	225.30	246.15
Dodecyl acetate	*A. bouvieri*	67.75	73.69	80.62	56.12	61.25	66.93
	D. indicus	121.96	132.65	145.11	101.02	110.26	120.48
	P. reticulata	169.86	184.75	202.10	140.70	153.57	167.79
	G. affinis	194.88	211.96	231.87	161.42	176.19	192.50

effectiveness of *B. mollis* EO on *Cx. quinquefasciatus*, with LC_{50} of 52.2 ppm.

Concerning the larvicidal activity of other EOs and extracts from the Asteraceae family, Macêdo et al. (1997) evaluated the toxicity of *Tagetes minuta* extract on *Ae. fluviatilis* (1.0 mg/l). The ethyl acetate leaf extract of *Eclipta prostrata* achieved a LC_{50} of 119.89 ppm on *Cx. tritaeniorhynchus* (Elango et al., 2009), while *Achilea millefolium* methanolic stem extract led to LC_{50} of 120.0 ppm on *Cx. quinquefasciatus* (Pavela 2008), *Tanacetum vulgare* methanolic flower extract (LC_{50} = 178.0 ppm) and methanolic stem extract of *Otanthus maritimus* (LC_{50} = 195.0 ppm) were also toxic to *Cx. quinquefasciatus* (Pavela et al. 2009).

Larvicidal activity of (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate

Interestingly, two of the main *B. eriantha* EO components, (4*E*,6*Z*)-allo-ocimene and carvotanacetone, achieved LC_{50} lower than 10 μg/ml on all tested mosquito species. As mentioned above, a wide number of plant EOs have been tested against mosquitoes. Indeed, screening the abundance of research products using "essential oil mosquito" as keywords on Scopus database (accessed: January 2017) we found more than 900 studies on this topic.

Unfortunately, only a very limited number of them considered testing single molecules identified in the EO (Pavela 2015; Benelli et al. 2017a). Some recent and noteworthy exceptions with larvicidal LC_{50} lower than 50 ppm are reviewed here. For example, the leaf EO from *Clausena anisata* contained β-pinene, sabinene, germacrene-D, estragole and linalool, which achieved LC_{50} of 23.17, 19.67, 16.95, 11.01 and 35.17 ppm on *An. stephensi*, LC_{50} of 27.69, 21.20, 18.76, 12.70 and 38.64 ppm on *Ae. aegypti,* and LC_{50} of 32.23, 25.01, 21.28, 14.01 and 42.28 ppm on *Cx. quinquefasciatus* (Govindarajan 2010). The LC_{50} of germacrene D-4-ol from *Zanthoxylum monophyllum* EO ranged from 6.12 to 7.26 μg/mL, while the LC_{50} for α-cadinol ranged from 10.27 to 12.28 μg/mL (Pavela and Govindarajan 2016). *ar*-curcumene and *epi*-β-bisabolol from *Hedychium larsenii* EO were toxic to *An. stephensi* (LC_{50} = 10.45 and 14.68 μg/ml), *Ae. aegypti* (LC_{50} = 11.24 and 15.83 μg/ml) and *Cx. quinquefasciatus* (LC_{50} = 12.24 and 17.27 μg/ml) (AlShebly et al. 2017). *Artemisia absinthium* EO-isolated (E)-β-farnesene, (Z)-en-yndicycloether, and (Z)-β-ocimene were toxic to *An. stephensi* (LC_{50} = 8.13, 16.24 and 25.84 μg/ml), *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) (Govindarajan and Benelli 2016a). Lavandulyl acetate and bicyclogermacrene from *Heracleum sprengelianum* EO were toxic to *An. subpictus* (LC_{50} = 4.17 and 10.3 μg/ml), *Ae. albopictus* (LC_{50} = 4.60 and 11.1 μg/ml) and *Cx. tritaeniorhynchus* (LC_{50} = 5.11 and 12.5 μg/ml) (Govindarajan and Benelli 2016b). from *Syzygium zeylanicum*

EO was a source of α-humulene and β-elemene, which were toxic to *An. subpictus* (LC_{50} = 6.19 and 10.26 μg/ml), *Ae. albopictus* (LC_{50} = 6.86 and 11.15 μg/ml), and *Cx. tritaeniorhynchus* (LC_{50}= 7.39 and 12.05 μg/ml) (Govindarajan and Benelli 2016c). Eugenol, α-pinene and β-caryophyllene were identified in the *Plectranthus barbatus* EO, and they showed toxicity to *An. subpictus* (LC_{50} = 25.45, 32.09 and 41.66 μg/ml, respectively), *Ae. albopictus* (LC_{50} = 28.14, 34.09 and 44.77 μg/ml) and *Cx. tritaeniorhynchus* (LC_{50} = 30.80, 36.75 and 48.17 μg/ml) (Govindarajan et al. 2016a). Carvacrol and terpinen-4-ol isolated from the *Origanum vulgare* EO showed toxicity to *An. stephensi* (LC_{50} = 21.15 and 43.27 μg/ml), *An. subpictus* (LC_{50} = 24.06 and 47.73 μg/ml), *Cx. quinquefasciatus* (LC_{50} = 26.08 and 52.19 μg/ml) and *Cx. tritaeniorhynchus* (LC_{50} = 27.95 and 54.87 μg/ml) (Govindarajan et al. 2016d). δ-cadinene, calarene and δ-4-carene from the *Kadsura heteroclita* EO acted as larvicides on *An. stephensi* (LC_{50} = 8.23, 12.34 and 16.37 μg/ml), *Ae. aegypti* (LC_{50} = 9.03, 13.33 and 17.91 μg/ml) and *Cx. quinquefasciatus* (LC_{50} = 9.86, 14.49 and 19.50 μg/ml) (Govindarajan et al. 2016e).

Testing selected chemicals from plant essential oils is really important, since in a number of instances the single molecules are more effective if compared to the raw oil. In addition, testing single products for which the mechanism (s) of action is well known may help to shed light on the precise alterations induced on insect biochemical pathways (Pavela and Benelli 2016b). Lastly, the most effective molecules could be formulated in dedicated blends to shed light on possible synergistic and antagonistic toxicity effects (Benelli et al. 2017b, d). Further research on potential synergic as well as antagonistic effects occurring among the *B. eriantha*-borne molecules tested in blend is ongoing.

Toxicity of selected *Blumea*-borne molecules on non-target aquatic predators

The acute toxicity of *B. eriantha* EO, as well as (4*E*,6*Z*)-allo-ocimene, carvotanacetone, and dodecyl acetate on four aquatic predators was limited, with a LC_{50} range of 519-11.431 μg/ml (Tables 6, 7, 8, and 9). Plant EOs have been recognized as important sources of biopesticides, with limited toxic effects on human health and non-target organisms (Pavela and Benelli 2016b). For instance, recent research showed very limited acute toxicity of *Pinus kesiya* EO on *A. bouvieri*, *D. indicus* and *G. affinis*, with LC_{50} ranging from 4135 to 8390 mg/ml. Also in the above-cited research, in agreement with the present results, *G. affinis* has been found less susceptible to EO-based treatments, if compared to *A. bouvieri*, *D. indicus* and *P. reticulata* (Govindarajan et al. 2016c). Besides size differences, enzymatic assays to shed light on the reasons at the basis of this differential susceptibility are required. Furthermore, Govindarajan et al. (2016b) reported that the *Zingiber nimmonii* EO was safer towards *D. indicus* and *G. affinis*, with LC_{50} of 3241.53 and 9250.12 μg/ml, respectively. Pavela and Govindarajan (2016) showed that *Z. monophyllum* EO and its main constituents germacrene D-4-ol and a-cadinol tested on *G. affinis* had LC_{50} of 4234, 414 and 635 μg/ml, respectively. *H. sprengelianum* EO and its two major compounds lavandulyl acetate and bicyclogermacrene tested on *A. bouvieri*, *D. indicus* and *G. affinis* led to LC_{50} ranging from 414 to 4219 μg/ml. The *S. zeylanicum* EO (LC_{50} = 20,374 μg/ml), β-elemene (LC_{50} = 2073 μg/ml), and α-humulene (LC_{50} = 1024 μg/ml) from *Syzygium zeylanicum* are scarcely toxic towards *G. affinis* (Govindarajan and Benelli 2016c). Taken together, the data reported above underline the eco-friendly features of EO-borne molecules used as mosquito larvicides, allowing us to claim their potential employ as larvicides in urban and rural areas, with special reference to developing countries where mosquito-borne diseases are endemic and people should synergize different control tools in the fight against mosquitoes (see also Benelli 2015a).

Conclusions

Overall, the present research showed the toxicity of *B. eriantha* EO on six important mosquito vectors. Besides the effective larvicidal potential of the *B. eriantha* EO, which led to LC_{50} values lower than 50 ppm for most of the tested mosquitoes (Pavela 2015), is extremely noteworthy the toxicity of two main EO components, (4*E*,6*Z*)-allo-ocimene and carvotanacetone, which achieved LC_{50} lower than 10 μg/ml on all tested mosquito species, including two aedine vectors of Zika virus. Therefore, the extremely high larvicidal activity of (4*E*,6*Z*)-allo-ocimene and carvotanacetone far exceed most of the LC_{50} calculated in current literature on botanical mosquito larvicides, coupled with their eco-friendly features on non-target aquatic predators of mosquito larval instars, allowing us to propose both of them as potentially alternatives for developing eco-friendly mosquito control tools.

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