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Eco-friendly larvicides from Indian plants: Effectiveness of lavandulyl acetate and bicyclogermacrene on malaria, dengue and Japanese encephalitis mosquito vectors

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A B S T R A C T

Mosquitoes (Diptera: Culicidae) are a key threat for millions of people and animals worldwide, since they act as vectors for devastating pathogens and parasites, including malaria, dengue, Japanese encephalitis, filiariasis and Zika virus. Mosquito young instars are usually targeted using organophosphates, insect growth regulators and microbial agents. Indoor residual spraying and insecticide-treated bed nets are also employed. However, these chemicals have negative effects on human health and the environment and induce resistance in a number of vectors. In this scenario, newer and safer tools have been recently implemented to enhance mosquito control. The concrete potential of screening plant species as sources of metabolites for entomological and parasitological purposes is worthy of attention, as recently elucidated by the Y. Tu's example. Here we investigated the toxicity of *Heracleum sprengelianum* (Apiaceae) leaf essential oil and its major compounds toward third instar larvae of the malaria vector *Anopheles subpictus*, the arbovirus vector *Aedes albopictus* and the Japanese encephalitis vector *Culex tritaeniorhynchus*. GC–MS analysis showed that EO major components were lavandulyl acetate (17.8%) and bicyclogermacrene (12.9%). The EO was toxic to *A. subpictus*, *A. albopictus*, and *C. tritaeniorhynchus*, with LC_{50} of 33.4, 37.5 and 40.9 μg/ml, respectively. Lavandulyl acetate was more toxic to mosquito larvae if compared to bicyclogermacrene. Their LC_{50} were 4.17 and 10.3 μg/ml for *A. subpictus*, 4.60 and 11.1 μg/ml for *A. albopictus*, 5.11 and 12.5 μg/ml for *C. tritaeniorhynchus*. Notably, the EO and its major compounds were safer to three non-target mosquito predators, *Anisops bouvieri*, *Diplonychus indicus* and *Gambusia affinis*, with LC_{50} ranging from 206 to 4219 μg/ml. Overall, this study highlights that *H. sprengelianum* EO is a promising source of eco-friendly larvicides against three important mosquito vectors with moderate toxicity against non-target aquatic organisms.

1. Introduction

Arthropods are dangerous vectors of deadly pathogens and parasites. Among them, mosquitoes (Diptera: Culicidae) represent a key threat for millions of humans and animals worldwide (Reiter, 2001; Mehlhorn et al., 2012; Benelli, 2015a). Their medical and veterinary importance is mainly due to the fact that their act as vectors for a number of pathogens and parasites of public health relevance, including malaria, avian malaria, yellow fever, dengue, Japanese encephalitis, Zika virus, Rift Valley fever, Western equine encephalomyelitis, bancroftian and brugian filariae, canine heartworm disease (*Dirofilaria immitis*), and setariosis (*Setaria* spp.) (Benelli and Mehlhorn, 2016; Benelli et al., 2016).

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 (WHO, 2014). Furthermore, dengue ranks 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 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, 2012). In the past decade, West Nile virus has emerged in the Americas, becoming endemic throughout the region, while chikungunya, a formerly obscure arbovirus endemic to East Africa, also emerged causing millions of cases in the Indian Ocean basin and mainland

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South and Southeast Asia. Moreover, the Japanese encephalitis virus has expanded its range in the Indian subcontinent and Australasia, where it chiefly affects children (Tolle, 2009).

In this scenario, vector control is crucial. Mosquito young instars are usually targeted using organophosphates, insect growth regulators, and microbial agents. Indoor residual spraying and insecticide-treated bed nets are employed (Benelli, 2015a; Benelli and Mehlhorn, 2016). However, these chemicals have negative effects on human health and the environment and induce resistance in a number of vectors (Naqqash et al., 2016). In this scenario, newer and eco-friendly tools have been recently implemented to enhance control of mosquitoes (Amer and Mehlhorn, 2006; Benelli, 2015a, 2015b; Pavela and Benelli, 2016). The concrete potential of screening plant species as sources of metabolites for entomological and parasitological purposes is worthy of attention, as recently elucidated by the Y. Tu's example (Pavela, 2015; Benelli and Mehlhorn, 2016).

Particularly, in latest years a growing number of plant-borne molecules have been tested for their mosquitocidal, repellent and ovideterrent properties. Notably, plant extracts, essential oils and related compounds, including nanoformulated green pesticides (Govindarajan and Benelli, 2016a, 2016b, 2016b), often represent eco-friendly alternatives to synthetic pesticides, since they are biodegradable and have minimal side-effects on non-target organisms, as well as on the environment (Govindarajan et al., 2012, 2013; Pavela, 2015; Benelli, 2015b, Benelli, 2016a, 2016b, 2016b). Plant essential oils (EOs) show ovicidal, larvicidal, pupicidal and adulticidal activities, as well as oviposition deterrence, antifeedant activity and repellent actions (Ebadollahi, 2011; Zoubiri and Baaliouamer, 2011, 2014).

Some plant families are widely recognized as excellent sources of EOs with insecticidal properties. Among them, the Apiaceae family comprises 300–450 genus and 3000–3700 species. The genus *Heracleum* L. (Apiaceae) includes more than 70 species distributed worldwide. There are 15 species in India, five of which are endemic to peninsular India (Nayar, 1996; Pimenov and Leonov, 2004). *Heracleum sprengelianum* grows wildly in grasslands of Western Ghats at a height of 1500 m. Rhizomes and seeds are widely used in folk medicines. They are reported as effective in curing indigestion, sunburn, skin diseases and external tumors (Heywood, 1971). Many kinds of bioactive metabolites (i.e. anthraquinones, coumarins, furanocoumarins, furanocoumarin dimers, flavonoids and stilbenes) have been identified from various species of this genus (Doi et al., 2004; Niu et al., 2004; Taniguchi et al., 2005; Sayyah et al., 2005). Plants belonging to the genus *Heracleum* are highly aromatic, thus excellent sources of EOs (Karuppusamy and Muthuraja, 2011). The EO composition of different *Heracleum* species have been investigated, including *Heracleum dissectum* (Papageorgiou et al., 1985), *Heracleum candolleanum* (George et al., 2001a, 2001b), *Heracleum sphondylium* subsp. *ternatum* (Işcan et al., 2003), *Heracleum persicum* (Sefidkon et al., 2004), *H. candolleanum* (John et al., 2007) and *Heracleum crenatifolium* (Tosun et al., 2008). Mainly, they are sources of monoterpene hydrocarbons (e.g. *p*-cymene; γ-terpene; α- and β-pinene; limonene), oxygenated monoterpenes (e.g. isobornyl acetate, linalool, *n*-octanol, terpinene-1-ol-4.), and sesquiterpenes (e.g. caryophyllene oxide). To the best of our knowledge, no information was available on the mosquitocidal activity of *H. sprengelianum* EO.

In this study, the chemical composition of *H. sprengelianum* leaf EO was analyzed using gas chromatography–mass spectroscopy (GC–MS). The acute toxicity of the EO from *H. sprengelianum* and its major constituents was evaluated against larvae of the malaria vector *Anopheles subpictus*, the dengue and chikungunya vector *Aedes albopictus* and the Japanese encephalitis vector *Culex tritaeniorhynchus*. Furthermore, we studied the impact of EO on three non-target aquatic organisms, *Anisops bouvieri*, *Diplonychus indicus* and *Gambusia affinis*, sharing the same ecological niche of mosquito larvae in South India.

2. Materials and methods

2.1. Plant material and extraction of the essential oil

Fresh leaves of *H. sprengelianum* (Supplementary Online Material Fig. S1) was collected from Nilgiris, Western Ghats (11° 10′N to 11° 45′ N latitude and 76° 14′E to 77° 2′ E longitude), Tamil Nadu, India. The plant species was authenticated at the Department of Botany, Annamalai University. Voucher specimens (ID: AUDZ724) were deposited at the Department of Zoology, Annamalai University. The shade-dried leaves were hydrodistilled in a Clevenger-type apparatus (Govindarajan et al., 2016). The EO was dried over anhydrous sodium sulfate and stored at 4 °C in a vial covered with an aluminum foil to prevent the light-induced degradation of EO constituents.

2.2. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) was carried out using an Agilent 6890 GC that was also equipped with a 5973 N mass selective detector and an HP-5 (5% phenyl methylpolysiloxane) capillary column. The temperature of the oven was programmed from 50 to 280 °C at a rate of 4 °C/min and held at this temperature for 5 min. The temperatures of the inlet and interface were 250 and 280 °C, respectively. Helium, at a flow rate of 1.0 ml/min (constant flow), was used as the carrier gas. The sample (0.2 μL) was injected using a split of 20:1. Electron impact mass spectrometry was performed at 70 eV. The temperatures for the ion source and quadrupole were maintained at 230 and 150 °C, respectively. The compounds were identified by comparing their retention indices and mass spectra with those found in the NIST 98.1 and Mass Finder 3.1 commercial libraries. The integration area of the chromatographer was used to calculate the concentration of each component of the EO.

2.3. Mosquito rearing

Following the method by Govindarajan and Sivakumar (2015), laboratory-bred pathogen-free strains of mosquitoes were reared at the Department of Zoology, Annamalai University. At the time of adult feeding, these mosquitoes were 3–4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with Parafilm as membrane for 4 h. *A. albopictus* feeding was done from 12 noon to 4.00 p.m. and *A. subpictus* and *C. tritaeniorhynchus* were fed during 6.00 p.m. to 10.00 p.m. A membrane feeder with the bottom end fitted with Parafilm was placed with 2.0 ml of the blood sample (obtained from a slaughterhouse by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C were maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28 ± 2 °C, 70–85% relative humidity, with a photoperiod of 12-h light and 12-h dark.

2.4. Larvicidal toxicity on mosquito vectors

The larvicidal activity of the *H. sprengelianum* EO and its major compounds, lavandulyl acetate and bicyclogermacrene

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