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ORIGINAL PAPER

Identification of chemical constituents and larvicidal activity of essential oil from *Murraya exotica* L. (Rutaceae) against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae)

Shanmugam Krishnamoorthy · Manivachagam Chandrasekaran · Gnanaprakasam Adaikala Raj · Mahalingam Jayaraman · Venugopalan Venkatesalu

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Abstract This study was conducted to evaluate the phytochemical composition and larvicidal effect of leaf essential oil from *Murraya exotica* against early fourth-instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS) analyses revealed that the essential oil contained 27 components. The major chemical components identified were β-humulene (40.62 %), benzyl benzoate (23.96 %), β-caryophyllene (7.05 %) and α-terpinene (5.66 %). The larval mortality was observed after 12 and 24 h of exposure period. The results revealed that essential oil showed varied levels of larvicidal activity against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus.* After 12 h of exposure period, the larvicidal activities were LC_{50}=74.7 and LC_{90}=152.7 ppm (*A. aegypti*), LC_{50}=56.3 and LC_{90}=107.8 ppm (*A. stephensi*), and LC_{50}=74.4 and LC_{90}=136.9 ppm (*C. quinquefasciatus*) and the larvicidal activities after 24 h of exposure period were LC_{50}=35.8 and LC_{90}=85.4 ppm (*A. aegypti*), LC_{50}=31.3 and LC_{90}=75.1 ppm *(A. stephensi)*, and LC_{50}=43.2 and LC_{90}= 103.2 ppm (*C. quinquefasciatus*). These results suggest that leaf essential oil from *M. exotica* is a promising and eco-friendly source of natural larvicidal agent against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*.

S. Krishnamoorthy · M. Chandrasekaran (✉) · G. A. Raj · M. Jayaraman
Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu 608 002, India
e-mail: chandruphd@yahoo.co.in

V. Venkatesalu
Botany Wing-DDE, Annamalai University, Annamalainagar, Tamil Nadu 608 002, India

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Introduction

Vector-borne diseases are illnesses caused by pathogens and parasites in human populations. Every year, more than one billion people are infected and more than one million people die from vector-borne diseases including malaria, dengue, schistosomiasis, leishmaniasis, Chagas disease, yellow fever, lymphatic filariasis and onchocerciasis (WHO 2014). Mosquitoes represent a significant threat because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide (WHO 2010). Several species belonging to genera *Aedes*, *Anopheles* and *Culex* are vectors for the pathogens of various diseases like dengue fever, dengue hemorrhagic fever, malaria, Japanese encephalitis and filariasis (Borah et al. 2010; Rahuman et al. 2009). Dengue is prevalent in more than 100 countries and threatens the health of approximately 2.5 billion people. Around 50 million people are infected annually, characterizing a pandemia (WHO 2002).

In recent years, the emphasis to control the mosquito populations has steadily shifted from the use of conventional chemicals towards more specific and environmentally friendly materials which are generally of botanical origin. For this purpose, many phytochemicals from various plant species have been tested for their larvicidal and repellent actions against mosquitoes (Ciccia et al. 2000; Ansari and Razdan 2000).

Aedes aegypti is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to southeast Asia, the pacific island area, Africa and America. This mosquito is also the vector of yellow fever in central South America and West Africa. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement (Pancharoen et al. 2002). *Culex quinquefasciatus*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people being infected worldwide and 44 million people having a common chronic manifestation (Bernhard et al. 2003). *A. stephensi* Liston is the primary vector of malaria in India and other west Asian countries. Malaria remains one of the most prevalent diseases in the tropical world with 200 million to 450 million infections annually worldwide; it causes up to 2.7 million deaths (WHO 2010).

Essential oils from plants may be an alternative source of mosquito larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in integrated management programmes. In fact, many researchers have reported on the effectiveness of plant essential oils against mosquito larvae, and the recent examples are studied by (Jantan et al. 2005; Thomas et al. 2004). Among them, essential oils were the first preservatives used by man, originally in their natural state within plant tissues and then as oils obtained by water distillation (Bakkali et al. 2008). The essential oil extracted from fresh leaves of *Hyptis suaveolens* and its main constituents were evaluated for larvicidal and repellent activity against the Asian tiger mosquito, *Aedes albopictus* (Conti et al. 2012).

Murraya exotica L. (family Rutaceae) is a handsome evergreen shrub or small tree 3–4 m in height with a spreading crown and short, often crooked, trunk found almost throughout India and in the Andaman Islands. The plant is commonly grown in gardens for its glossy green foliage and large clusters of fragrant flowers. It is a popular hedge plant and is well adapted for topiary work. Propagation may be done by seeds, cuttings or layering. The leaves are stimulant and astringent. The leaves and bark are reported to be used for diarrhoea and dysentery in the Philippines and China (Chopra 1956; Li et al. 1988; Anonymous 1964). The acetone extract of *M. exotica* leaves showed an antifeedant activity against the early third-stage larvae of *Spodoptera litura* (Wang et al. 2009).

The purpose of the present investigation was made to explore the larvicidal activity of leaf essential oil from *M. exotica* against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* larvae in search for effective and affordable natural products to be used in the control of vectors.

Materials and methods

Plant material

The leaves of *M. exotica* were collected from Annamalainagar (Lat. 11.39° N; Long. 79.71° E), Cuddalore District, Tamil Nadu, India, during April, 2012. The plant was identified by former professor Dr. R. Selvaraj, Department of Botany, Annamalai University. Herbarium specimen was deposited in the Department of Botany, Annamalai University.

Extraction of the essential oil

Healthy and well-grown fresh leaves of *M. exotica* were collected and immediately brought to the laboratory using polythene bags. The fresh leaves were cut into small pieces and subjected to hydro-distillation using the Clevenger X77 type of apparatus for 4 h. The obtained essential oil was dried over anhydrous sodium sulphate, and the purified essential oil was stored in an amber colour vial (sealed with parafilm) at 4 °C for further analysis and larvicidal assay.

Chemical analysis of essential oil

Gas chromatography (GC) analysis was carried out using Thermo GC-Trace Ultra Ver: 5.0, Thermo MS DSQ II. The chromatograph was fitted with DB 5-MS capillary non-polar column. The injector temperature was set at 300 °C and the oven temperature initially at 80 °C then programmed to 200 °C at the rate of 5 °C/min and held at 200 °C for 10 min. Then the temperature was increased to 300 °C at the rate of 20 °C/min, finally held at 300 °C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 ml/min. The sample was injected in the split mode in the ratio of 1:100. The percentage of composition of the essential oil was calculated by the GC peak areas.

GC-MS analysis

GC and gas chromatography-mass spectrometry (GC-MS) analyses of essential oils were performed by using Varian 3800 Gas Chromatography equipped with Varian 1200 C single quadrupole mass spectrometer. GC conditions were the same as reported for GC analysis, and the same column was used. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV. Ion source and transfer line temperature was kept at 300 °C. The mass spectrum was obtained by centroid scan of the mass range from 40 to 800 amu. Identification of components of the essential oil was matching their recorded spectra with the data bank mass spectra of NIST and WILEY library provided by the instrument software, and the components were confirmed by comparing with previous literature.

Mosquito larvicidal bioassay

The eggs of *A. aegypti* and *A. stephensi* were received from the Field Station, Centre for Research in Medical Entomology (ICMR-Government of India), Viruthachalam, and the egg rafts of *C. quinquefasciatus* were collected from drainage of local residential area of Annamalai Nagar (11° 23′ 17 N, 79° 42′ 57 E) and reared in the laboratory (29±3 °C, 75 to 85 % RH). The larvae were fed with Brewer's yeast/dog biscuit (1:3). The larvae at the early fourth instar stage were used for larvicidal assay.

The larvicidal effect of leaf essential oil from *M. exotica* against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* was studied with the standard procedures recommended by the WHO (1981). The essential oil was dissolved in 1 ml of acetone and prepared into different concentrations *viz.* 12.5, 25, 50, 75 and 100 ppm with distilled water. Twenty larvae (in a 100-ml beaker) of the early fourth instar stage were used for larvicidal assay, and five replicates were maintained for each concentration. During this experiment, no food was offered to the larvae. The larval mortality was calculated after 12 and 24 h of exposure period. The lethal concentrations, LC_{50} and LC_{90}, and their 95 % confidence limit of upper and lower confidence levels were calculated by probit analysis (SPSS, Version 16.0).

Results

Yield and chemical constituents of essential oil

The hydro-distillation of leaf of *M. exotica* yielded 0.1 % (*v*/*w*) of essential oil with pale yellow in colour. Table 1 shows the chemical constituents of the essential oil analysed by GC (Fig. 1) and GC-MS. In total, 27 compounds were identified and account about 99.26 % of the total oil. The major chemical constituents were β-humulene (40.62 %), benzyl benzoate (23.96 %), β-caryophyllene (7.05 %) and α-terpinene (5.66 %).

Table 1 Chemical composition of *Murraya exotica* leaf essential oil

Peak no.	Retention time (min)	Chemical constituents[a,b]	Composition (%)
1	12.62	α-Terpinene	5.66
2	13.86	β-Caryophyllene	7.05
3	14.74	Benzyl benzoate	23.96
4	15.04	α-Muurolene	1.62
5	15.40	β-Elemene	7.56
6	15.84	γ-Muurolene	1.80
7	16.60	β-Humulene	40.62
8	16.91	α-Gurjunene	0.71
9	17.14	α-Selinene	0.17
10	17.44	Germacrene B	0.87
11	17.90	α-Thujenal	1.03
12	18.39	α-Amorphene	0.10
13	18.70	Thujopsene	0.96
14	19.04	α-Bisabolol	3.61
15	19.33	t-Muurolol	1.91
16	19.88	Methyl linoleate	0.04
17	20.41	Pentadecanal	0.25
18	20.81	Beta-bourbonene	0.02
19	21.23	Spathulenol	0.11
20	22.68	Caryophyllene oxide	0.02
21	23.25	Elemol	0.11
22	23.94	3-octen-1-ol, (E)-	0.02
23	24.53	Methyl linolenate	0.07
24	26.06	Ethyl palmitate	0.07
25	28.19	α-Humulene	0.03
26	29.06	Manool	0.86
27	43.60	Docosane	0.03
Total			99.26

[a] Compounds listed in order of elution from DB 35-MS Capillary standard non-polar column

[b] Components identified based on computer matching of the mass peaks with WILEY and NIST Library

Larvicidal activity of essential oil

The larvicidal activity of leaf essential oil from *M. exotica* was investigated. The essential oil had promising larvicidal activities (Table 2) against the early fourth instar larvae activity after 12 h of exposure period which were LC_{50}=74.7 and LC_{90}=152.7 ppm (*A. aegypti*), LC_{50}=56.3 and LC_{90}=107.8 ppm *(A. stephensi*), and LC_{50}=74.4 and LC_{90}=136.9 ppm *(C. quinquefasciatus*), and the larvicidal activities after 24 h of exposure period were LC_{50}=35.8 and LC_{90}=85.4 ppm (*A. aegypti*), LC_{50}=31.3 and LC_{90}=75.1 ppm (*A. stephensi*), and LC_{50}=43.2 and LC_{90}=103.2 ppm *(C. quinquefasciatus*). The essential oil showed 98 % larval mortality against *A. stephensi* at 100 ppm (after 24 h).

Discussion

Botanical insecticides may serve as suitable alternatives to synthetic insecticides as they are relatively safe, degradable and are readily available in many areas of the world. Although several plants form different families have been reported for mosquitocidal activity, only a few botanicals have moved from the laboratory to field use like neem-based insecticides, which might be due to the light and heat instability of phytochemicals compared to synthetic insecticides (Green and Singer 1981). In the present study, the leaf essential oil

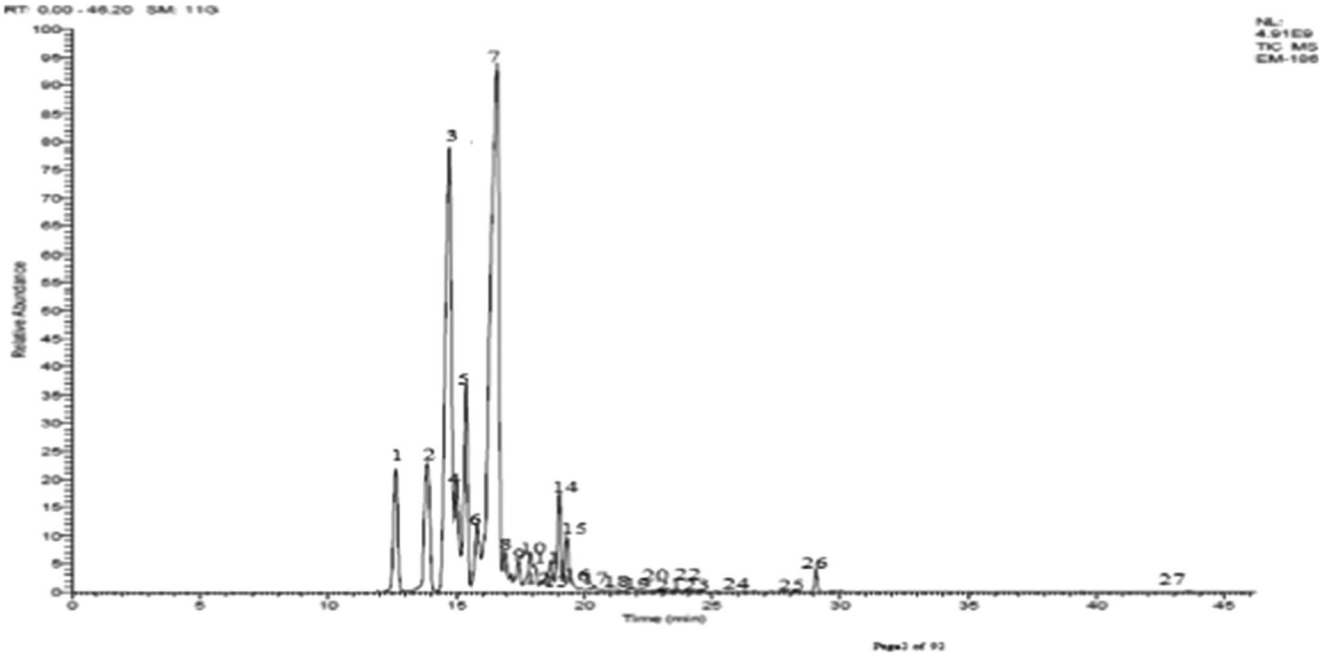

Fig. 1 Gas chromatogram of *Murraya exotica* leaf essential oil

from *M. exotica* showed larvicidal activity against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. Similarly, these results are supported by previous reports in the same species of *M. exotica* leaf extracts having larvicidal activity against *C. quinquefasciatus*. The LC_{50} values of *M. exotica* for III and IV instar larvae and pupae are 135.539 and 154.361 ppm, respectively (Dass and Mariappan 2014), although several studies have reported the larvicidal activity of essential oils against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. Govindarajan et al. (2012) evaluated that larvicidal activity of essential oil from *Mentha spicata* (Linn.) against three mosquito species, *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. The oil of *M. spicata* exhibited a significant toxic effect against the larvae of three species under study, with LC_{50} values of 49.71 ppm for *A. stephensi*, 56.08 ppm for *A. aegypti* and 62.62 ppm for *C. quinquefasciatus*. Senthilkumar and Venkatesalu (2012) reported that larvicidal potential against *C. quinquefasciatus* in five compounds was derived from essential oils from *Acorus calamus* leaf including Beta asarone, *cis*-beta-terpineol, Limonene, Carvone and Amyl isovalerate with an LC_{50} value of 63.43 ml/L and LC_{90} value of 145.95 ml/L. Govindarajan (2011) reported the larvicidal and repellent effect against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* in *Zingiber officinale* with LC_{50} and LC_{90} values of 98.83 and 57.98 ppm, and 186.55 and 104.23 ppm. Ravi Kiran et al. (2006) reported the larvicidal effect against *A. aegypti* and *A. stephensi* in *Chloroxylon swietenia* leaves and stem essential oil with LC_{50} values of 16.5 and 14.9 μg/ml and 20.2 and 19 μg/ml. Senthilkumar et al. (2013) reported the larvicidal activity of essential oil of *Feronia limonia* leaf against *A. stephensi* with LC_{50} 38.93 and LC_{90} 108.64 ppm (after 12 h), LC_{50} 15.03 and LC_{90} 36.69 ppm (after 24 h); *A. aegypti* with LC_{50} 37.60 and LC_{90} 104.69 ppm (after 12 h), LC_{50} 11.59 and LC_{90} 42.95 ppm (after 24 h); and *C. quinquefasciatus* with LC_{50} 52.08 and LC_{90} 124.33 ppm (after 12 h), LC_{50} 22.49 and LC_{90} 60.90 ppm (after 24 h), respectively. The essential oil of *Plectranthus amboinicus* leaves possessed larvicidal activity against *A. stephensi* with LC_{50} values of 33.54 (after 12 h) and 28.37 ppm (after 24 h) and LC_{90} values of 70.27 (after 12 h) and 59.38 ppm (after 24 h), respectively (Senthilkumar and Venkatesalu 2010).

In the present study, chemical components were identified from essential oil of *M. exotica* leaf, and major chemical components were β-humulene (40.62 %), benzyl benzoate (23.96 %), β-caryophyllene (7.05 %) and α-terpinene (5.66 %). Li et al. (2010) reported that *M. exotica* aerial parts of essential oils are composed of spathulenol, α-pinene, caryophyllene oxide, bicyclogermacrene, 1,2,3,5,6,7,8,8a-octahydro-1-methyl-6-methylene-4-(1-methylethyl)-naphthalene and γ-selinene. This observed differences in the chemical composition may be attributed to occurrence of chemotypes, geographical locations, season at the time of collection, stage of development, culture climate and other culture conditions, which may affect biological activities (Runyoro et al. 2010).

The result of the present study showed that the significant larvicidal activity of the essential oil of *M. exotica* leaf may be due to the presence of major chemical constituents like β-humulene, benzyl benzoate and β-caryophyllene. Vera et al. (2014) reported that benzyl benzoate is one of the major chemical components from the essential oil of *Cananga odorata* with an LC_{50} value of 52.9 ppm. This essential oil

Table 2 Larvicidal properties of the leaf of essential oil *Murraya exotica* against the larvae of *A. aegypti, A. stephensi* and *C. quinquefasciatus* after 12 and 24 h of exposure period

Name of the mosquito species	Time	Concentration (ppm)	% Mortality±SD	LC_{50} (LCL-UCL)[a]	LC_{90} (LCL-UCL)[a]	$X^2(d=4)$[b]
A. aegypti	After 12 h	12.5	12±0.83	74.7 (67.1–84.3)	152.7 (133.6–182.6)	5.22
		25	19±0.54			
		50	43±1.50			
		75	51±0.90			
		100	62±0.83			
	After 24 h	12.5	21±0.50	35.8 (30.4–40.7)	85.4 (77.8–95.5)	5.38
		25	43±1.00			
		50	71±0.84			
		75	82±1.14			
		100	94±0.05			
A. stephensi	After 12 h	12.5	14±0.83	56.3 (51.3–61.4)	107.8 (98.7–120.1)	1.87
		25	23±1.14			
		50	44±0.84			
		75	63±0.89			
		100	89±0.83			
	After 24 h	12.5	23±0.44	31.3 (26.2–35.8)	75.1 (68.5–83.7)	6.02
		25	48±0.94			
		50	76±1.14			
		75	87±0.76			
		100	98±0.89			
C. quinquefasciatus	After 12 h	12.5	9±0.70	74.4 (68.2–82.0)	136.9 (123.0–156.9)	0.62
		25	16±0.49			
		50	31±0.89			
		75	53±0.89			
		100	68±0.83			
	After 24 h	12.5	18±0.54	43.2 (37.2–48.8)	103.2 (93.2–117.1)	8.07
		25	38±0.83			
		50	66±0.70			
		75	73±1.09			
		100	84±0.89			

Control-Nil activity

SD standard deviation, *LCL* lower confidence level, *UCL* upper confidence level

[a] 95 % confidence interval

[b] Degrees of freedom X^2 -Chi-square value

had a larvicidal activity against *A. aegypti*. β-caryophyllene is a major component from the essential oil of *Alpinia purpurata* and showed larvicidal activity against fourth instar larvae of *A. aegypti* with IC_{50} values of 80.7 and 71.5 ppm, respectively (Santos et al. 2012). Senthilkumar and Venkatesalu (2010) reported that α-humulene and caryophyllene oxide are major chemical components from the essential oil of *P. amboinicus*. This essential oil had strong larvicidal activity against *A. stephensi*. The essential oil from *Blumea densiflora* had larvicidal activity against *A. anthropophagus*. β-caryophyllene is one of the major chemical components of this essential oil (Zhu and Tian 2011).

The vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either counter measures or development of newer insecticides (Chandre et al. 1998). The isolation and identification of the larvicidal components from the leaf essential oil of *M. exotica* as described here could lead to the development of natural mosquitocidal products to replace the synthetic

insecticides. The use of natural plant-based products by individuals and communities would generate local employment, reduce dependence on expensive imported synthetic products and stimulate local efforts to enhance public health (Bowers et al. 1995). For example, essential oil from citronella, lemon and eucalyptus provides the active ingredients of some commercial repellents. Such substances are sold under several brand names (Trongtokit et al. 2005).

The mode of action and site of effect for larvicidal essential oils and isolated compounds has received little attention. In this study, the mode of action of tested materials was not studied. The larvicidal mode of action of essential oils was previously investigated by Corbet et al. (1995); WHO observed that essential oils increase the tendency of tracheal flooding and chemical toxicity in mosquito larvae. Usta et al. (2002) demonstrated that isolated pure compounds interfered with proton transfer in the mitochondria leading to larval mortality.

Mosquito control is vital for many countries and is still in a state of evolution. During the last decades, it depended upon synthetic organic insecticides, many of which have been removed from the arsenal of weapons (Floore 2006) and botanicals are the new weapons of mosquito control under exploration. Natural pesticides derived from plants are a promising tool especially for targeting mosquitoes in the larval stage (Amer and Mehlhorn 2006). Cheng et al. (2004) showed the data and revealed that a-phellandrene, limonene, p-cymene, c-terpinene, terpinolene and -terpinene examined in this study exhibited great larvicidal performance. Vector control is one of the most powerful weapons in the process of managing vector populations to reduce/interrupt the transmission of disease. As a result, vector control remains considered to be a cornerstone in the vector-borne disease control programme due to a lack of reliable vaccine, drug resistance parasites and insecticide resistance of insect vector disease (Karunamoorthi 2011). Some of our reported LC_{50} values differ from reported data (Perumalsamy et al. 2009) which may be the results of different methodologies and analysis. Additionally, different species, from different ecological niches, appear to be more susceptible or resistant to specific compounds (Waliwitiya et al. 2009). The use of natural products may be considered as an important alternative insecticide for the control of vector-borne diseases since they constitute a rich source of bioactive compounds that are biodegradable, nontoxic and potentially suitable for use in integrated larvae management programmes. In conclusion, the present study has shown that the leaf essential oil from *M. exotica* may have a potential in the control of larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. The results could be useful in search of newer, safer and more effective natural compounds as larvicides.

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