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RESEARCH ARTICLE

MOSQUITOCIDAL ACTIVITY OF *NEPTA CATARIA* ESSENTIAL OIL AGAINST *AEDES AEGYPTI* (LINN.), *ANOPLELES STEPHENSI* (LISTON) AND *CULEX QUINQUEFASCIATUS* (SAY.) (DIPTERA:CULICIDAE)

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ABSTRACT

To investigate the mosquitocidal activities of *Nepta cataria* essential oil against *Aedes aegypti*, *Anopleles stephensi* and *Culex quinquefasciatus*. Twenty five early third instar larvae of selected mosquitoes were exposed to various concentrations (30- 150ppm) and were assayed in the laboratory by using the protocol of WHO 2005; the 24h LC_{50} values of the essential oil was determined by probit analysis. The ovicidal activity was determined against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* exposed to various concentrations were tested under laboratory conditions and the hatch rates were assessed 48hrs post treatment. Similarly, selected essential oil were tested against pupae of selected mosquitoes at various concentrations and mortality of each pupa was recorded after 2days post treatments of exposure. Repellent activity was carried out in a net cage (45×30×45 cm^2) containing 100 blood starved female selected mosquitoes and were assayed in the laboratory condition by using the protocol of WHO 1996; The essential oil of *Nepta cataria* was applied at 2.0, 4.0 and 6.0 mg/cm^2 separately in the exposed area of the fore arm. LC_{50} and LC_{90} values of *Nepta cataria* essential oil on *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus larvae* in 24 h were 67.53, 73.09, 78.62 and 130.61, 145.24 and 152.77 ppm respectively. The data is statistically significant at $P < 0.05$. It has been noticed that the higher concentrations of *Nepta cataria* essential oil possesses strong ovicidal activity at 300ppm concentration against *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus*, were 84.46%, 94.33% and 96.54%, respectively. In pupicidal activity, among the three mosquito tested against *Nepta cataria* essential oil at 300ppm higher concentrations, the *C. quinquefasciatus* was found to be most effective for pupicidal activity provided 96.25% and *Ae. aegypti* and *An. stephensi* were85.61% and 92.87%, respectively. The repellent activity of *Nepta cataria* essential oil was found to be most effective for repellent activity against *Ae. aegypti* followed by *An. stephensi* and *Cx. quinquefasciatus* and a higher concentration of 6mg/cm^2 provide100% protection up to 210, 180 and 150 min against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, respectively. From the results it can be concluded the essential oil of *Nepta cataria* as an excellent potential agent for controlling selected mosquitoes species.

INTRODUCTION

The mosquito is a common insect found around the world. There are about three thousand five hundred species of mosquitoes identified in our world. Mosquitoes are the major vector of diseases, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year (James, 1992; Morisson *et al*., 2008;WHO, 2005). Mosquitoes are the important single group of insects in terms of public health importance and causing millions of death every year (Das *et al*., 2004).These diseases not only cause high levels of morbidity and mortality, but also inflict great economic loss and social disruption on developing countries such as India, China, etc., India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 crore (Hotez*et al*., 2007; Rahuman *et al*., 2009; Kamaraj *et al*., 2011).

Aedes aegypti is the most important vector of dengue viruses world-wide, yellow fever virus in urban settings, and is a competent vector of chikungunya virus. Dengue causes more human morbidity and mortality than any other vector-borne viral infection. *Ae. aegypti* is uniquely adapted to a close association with humans, which facilitates efficient virus transmission (Morrison *et al*., 2008). This mosquito is more widely dispersed now than any time in the past, placing billions of humans at risk of infection. It enjoys greater geographical distribution and is established virtually in all tropical countries (Halstead *et al*., 2008). *Anopheles stephensi* is the primary vector of malaria in India and other Western Asian countries. Malaria remains one of the most prevalent diseases in the

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tropical world. With 200 million to 450 million infections annually worldwide, it causes up to 2.7 million deaths (WHO, 2010). *Culex quinquefasciatus*, a vector of lymphatic filariasis, is widely distributed in tropical and subtropical countries, with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard *et al*., 2003; Hotez *et al*., 2004).

Over and injudicious use of synthetic insecticides in vector control has resulted in environmental hazards through persistence and accumulation of non-biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effects on human health and non-target organisms (Bansal *et al*., 2011; Devine and Furlong 2007; Elumalai *et al*., 2012a,b; Krishnappa *et al*., 2012a,b). Thus new approaches are urgently needed. Interest on possible use of environment friendly natural products such as extracts of plants or plant parts increased for vector control. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticide properties (Pankaj and Anita 2010; Kamaraj *et al*., 2011; Nazar *et al*., 2009; Nataya *et al*., 2010; Krishnappa and Elumalai, 2012; Elumalai *et al*., 2013; Dhanasekaran *et al*., 2012; Balu Selvakumar *et al*., 2012; Elangovan *et al*., 2012; Gokulakrishnan *et al*., 2012). Plant derived materials are comparatively safer to humans and ecosystem and easily biodegradable (Kalu *et al*., 2010). Plant derived natural products have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic insecticides. Phytochemicals extracted from various plant species have been tested for their larvicidal activity against mosquitoes (Pavela, 2008; Pitasawat *et al*., 2007; Krishnappa and Elumalai, 2014). Therefore the present study was carried out to determine the larvicidal, ovicidal, pupicidal and repellent activities of *Nepta cataria* essential oil against important vector mosquitoes.

MATERIALS AND METHODS

Plant collection and extraction

The plant materials (leaves) were collected from various parts of Malappuram District, Kerala, located at the foothills of Western Ghats of Southern India. The leaves were collected during the January 2013- June 2013 and brought to the laboratory where, they were washed thoroughly with tap water and kept in sunlight for 45 minutes for the complete evaporation of water and then shade dried on blotting paper spread at room temperature (28 ± 2 °C). The dried plant material hydrodistilled in a clavenger apparatus for 4 h. the distilled oil was dried over anhydrous sodium sulphate and stored under nitrogen atmosphere until further use.

Test organisms

All tests were carried out against laboratory reared vector mosquitoes viz., *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* free of exposure to insecticides and pathogens. Cyclic generations of vector mosquitoes were maintained at 25-29 °C and 80-90 % relative humidity in the insectariums. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio of 3:1) and adult mosquitoes on 10 % glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

Larvicidal activity

The larvicidal activity of *Nepta cataria* essential oil was assessed by using the standard method as prescribed by World Health Organization (2005). From the stock solution, six different test concentrations (30, 60, 90, 120 and 150ppm) were prepared and they were tested against the freshly moulted (0 – 6 hrs) third instar larvae of selected mosquito. The larvae of test species (25) were introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water + 1ml of emulsifier; DMSO) and the required amount of essential oil was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The LC_{50} value was calculated by using probit analysis (Finney 1979).

Ovicidal activity

The method of Su and Mulla (1998) was slightly modified and used to test the ovicidal activity. The various concentrations as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs of selected mosquitoes were counted individually with the help of hand lens. Freshly laid eggs (100) were exposed to each concentration of essential oil until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment by the following formula.

Pupicidal assay

Batches of thirty number of pupae were introduced into 500 ml of the test medium containing particular concentration of the essential oil in a plastic cups in five replications. In control, the same number of pupae was maintained in 500 ml of dechlorinated water containing appropriate volume of DMSO. All containers were maintained at room temperature (28±2°C) with naturally prevailing photoperiod (12:12h /L:D) in the laboratory. Any pupa was considered to be dead if did not move when prodded repeatedly with a soft brush. Mortality of each pupa was recorded after 24 of exposure to the extract.

Repellent activity

The repellent study was following the methods of World Health Organization (2009). 3-4 days old blood-starved selected female mosquitoes (100) was kept in a net cage (45×45× 40cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of the test person were cleaned with isopropanol. After air drying the arm only 25 cm^2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves. The plant extract was dissolved in isopropanol and this alcohol served as control. The *Nepta cataria* essential oil at 2.0, 0.4 and 6.0mg/cm^2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min every

30 min and the experiment were conducted five times. It was observed that there was no skin irritation from the essential oil.

Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated using the SPSS 12.0 version software. Results with $p \leq 0.05$ were considered to be statistically significant.

RESULTS

Despite centuries of control efforts, mosquito-borne diseases are flourishing worldwide. With a disproportionate effect on children and adolescents, these conditions are responsible for substantial global morbidity and mortality. Efforts to limit the effect of mosquito borne diseases in endemic areas face the twin challenges of controlling mosquito populations and delivering effective public health interventions. LC_{50} and LC_{90} values of *Nepta cataria* essential oil on *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus larvae* in 24 h were 67.53, 73.09, 78.62 and 130.61, 145.24 and 152.77 ppm respectively.The data is statistically significant at $P < 0.05$ (table 1). It has been noticed that the higher concentrations of *Nepta cataria* essential oil possesses strong ovicidal activity at 300ppm concentration against *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus*, were 84.46%, 94.33% and 96.54%, respectively. In pupicidal activity, among the three mosquito tested against *Nepta cataria* essential oil at 300ppm higher concentrations, the *C. quinquefasciatus* was found to be most effective for pupicidal activity provided 96.25% and *Ae. aegypti* and *An. stephensi* were 85.61% and 92.87%, respectively (table 2). The repellent activity of *Nepta cataria* essential oil was found to be most effective for repellent activity against *Ae. aegypti* followed by *An. stephensi* and *Cx. quinquefasciatus* and a higher concentration of 6mg/cm^2 provide100% protection up to 210, 180 and 150 min against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, respectively (table 3). From the results it can be concluded the essential oil of *Nepta cataria* as an excellent potential agent for controlling selected mosquitoes species. From the results it can be concluded the essential oil of *Nepta cataria* essential oil as an excellent potential agent for controlling selected mosquitoes species.

DISCUSSION

The results of present study are comparable with similar reports of earlier workers, Shyamapada (2010) reported that the *Ricinus communis* seed extract exhibited larvicidal effects with 100% killing activities at concentrations 32-64 µg/mL, and with LC_{50} values 7.10, 11.64 and 16.84 µg/mL for *C. quinquefasciatus*, *An. stephensi* and *Ae. albopictus* larvae,

Table 1 Larvicidal activity of *Nepta cataria* essential oil against selected vector mosquitoes

Mosquitoes	Concentration (mg/l)	24 h mortality (%)	LC_{50} (ppm)	95%Confidence Limits (mg/l)		LC_{90} (ppm)	Slope	¡ ^{2} value
				LCL	UCL			
Ae. aegypti	30	23.4±1.6^b	67.53	64.907	83.167	130.61	3.9649388	12.34052
	60	47.6±1.4^c						
	90	64.8±1.9^d						
	120	81.2±1.6^e						
	150	99.4±1.2^f						
	Control	0.0±0.0^a						
An. stephensi	30	21.2±1.8^b	73.09	67.469	87.733	145.24	3.6678423	12.19064
	60	42.8±1.4^c						
	90	60.2±1.8^d						
	120	77.6±1.6^e						
	150	98.4±1.2^f						
	Control	0.0±0.0^a						
Cx. quinquefasciatus	30	18.8±1.4^b	78.62	69.000	89.254	152.77	3.747174	11.97823
	60	37.5±1.8^c						
	90	58.4±1.7^d						
	120	75.8±1.2^e						
	150	98.2±1.9^f						
	Control	0.0±0.0^a						

Each value mean ± S.D represents mean of five values. Different alphabets in the column are statistically significant at p<0.05. (MANOVA; LSD -Tukey's Test). LCL-Lower confidence limit; UCL-Upper confidence limit; Slope; Chi-square.

Table 2 Ovicidal and pupicidal activity of *Nepta cataria* essential oil against selected mosquitoes

Solvent tested	Concentrations tested (ppm), Ovicidal and Pupicidal activity %				
	60	120	180	240	300
	Ovicidal activity %				
Ae. aegypti	15.92 ± 1.64^b	27.61 ± 1.38^b	66.18 ± 2.28^b	78.62 ± 2.16^b	84.46 ± 2.41^b
An. stephensi	16.46 ± 1.76^c	33.62 ± 1.34^c	76.82 ± 2.82^c	86.72 ± 2.36^c	94.33 ± 2.68^c
Cx. quinquefasciatus	24.16 ± 1.59^d	37.73 ± 1.22^d	81.84 ± 2.46^d	89.68 ± 2.22^d	96.54 ± 2.56^d
Control	2.54 ± 1.36^a	2.54 ± 1.36^a	2.54 ± 1.36^a	2.54 ± 1.36^a	2.54 ± 1.36^a
	Pupicidal activity %				
Ae. aegypti	16.65 ± 1.37^b	26.61 ± 1.44^b	45.34 ± 2.27^b	68.15 ± 2.36^b	85.61 ± 4.32^b
An. stephensi	18.60 ± 1.22^c	29.64 ± 1.51^c	56.84 ± 2.12^c	76.72 ± 2.59^c	92.87 ± 2.94^c
Cx. quinquefasciatus	21.86 ± 1.74^d	35.62 ± 1.22^d	59.93 ± 2.53^d	76.84 ± 2.31^d	96.25 ± 4.76^d
Control	2.63 ± 1.99^a	2.63 ± 1.99^a	2.63 ± 1.99^a	2.63 ± 1.99^a	2.63 ± 1.99^a

Values represent mean ± S.D. of five replications. Different alphabets in the column are statistically significant at p<0.05. (MANOVA; LSD -Tukey's Test). Eggs and pupae in control groups were sprayed with no phytochemicals.

Table 3 Repellent activity of *Nepta cataria* essential oil against selected vector mosquitoes

Mosquitoes	Concentration (mg/cm^2)	% of repellency							
		Time post application of repellent (min)							
		30	60	90	120	150	180	210	240
Ae. aegypti	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	87.6±2.6
	4.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.4±3.8
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	95.3±3.2
An. stephensi	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	89.2±2.3	78.3±2.4
	4.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	94.8±3.8	80.2±3.6
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.5±3.4	91.3±3.4
Cx. quinquefasciatus	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	83.3±2.8	71.5±2.3	68.6±2.2
	4.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.2±3.3	88.6±2.9	82.4±2.6
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	98.3±3.4	92.5±3.2	89.3±2.4

Each value mean± S.D represents mean of six values.

respectively. Bagavan *et al*. (2009) who have been reported that ethyl acetate and methanol extracts of *P. emblica* showed highest larval mortality against *C. tritaeniorhynchus* with LC_{50} = 54.82 ppm; LC_{90} 199.89 ppm, respectively and adult mortality was found in leaf methanol extracts against *H. bispinosa* and *P. cervi* with LC_{50} = 256.08; 60.60 ppm; LC_{90} = 1025.60; 287.48 ppm respectively. Pushpalatha and Muthukrishnan (1995) reported that The methanol leaf extracts of *V. negundo*, *Vitex trifolia*, *Vitex peduncularis*, and *Vitex altissima* possessed varying levels of larvicidal activity on *C. quinquefasciatus* and *A. stephensi* and were found with LC_{50} value of 212.57, 41.41, 76.28, and 128.04 ppm, respectively. Sumroiphon *et al*. (2006) who have reported that the effect of citrus seed extract showed LC_{50} values of 135,319.40 and 127,411.88 ppm against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Sharma *et al*. (2009) reported that, petroleum ether extract of *Ageratum conyzoides* leaves exhibited larvicidal activity with LC_{50} value of 425.60 and 267.90 ppm after 24 and 48 h of exposure. The toxicity to the third instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of *Memordica charantia*, *Trichosanthus anguina* and *Luffa acutangula* showed the LC_{50} values of 465.85, 567.81 and 839.81 ppm respectively (Prabakar and Jebanesan, 2004). The five most effective oils were those of Litsea (*Litsea cubeba*), Cajeput (*Melaleuca leucadendron*), Niaouli (*Melaleuca quinquenervia*), Violet (*Viola odorata*), and Catnip (*Nepeta cataria*), which induced a protection time of 8h at the maximum and a 100% repellency against *Ae. aegypti*, *A. stephensi*, and *C. quinquefasciatus* (Amer and Mehlhorn, 2006).

Early, The repellent activity of methanol extract of *Ferronia elephantum* leaves against *Ae. aegypti* activity at 1.0 mg/cm2 and 2:5 mg/cm^2 concentrations gave 100% protection up to (2.14±0.16) h and (4.00±0.24) h, respectively, and the total percentage protection was 45.8% at 1.0 mg/ cm^2 and 59.0% at 2.5 mg/cm2 for 10 h (Venketachalam and Jebanesan2001). Vahitha *et al*., 2002 reported that the larvicidal efficacy of leaf methanol extracts of *Pavonia zeylanica* and *Acacia ferruginea* were tested against the late third instar larvae of *Culex quinquefasciatus* with LC_{50} values of 2,214.7 and 5,362.6 ppm, respectively. The petroleum ether (60–80°C) extracts of the leaves of *V. negundo* were evaluated for larvicidal activity against larval stages of *Culex tritaeniorhynchus* in the laboratory with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/l, respectively (Karunamoorthi *et al*. 2008).Tyagi *et al*. (1994) who reported that the essential oil of *T. minuta* providing a repellency of 90% protection for 2 h against *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. Saravanan *et al*. (2007) reported that the petroleum ether extract of *Solanum xanthocarpum* was observed to be the most toxic with LC_{50} of 1.41 and 0.93 ppm and LC_{90} of 16.94 and 8.48 ppm at 24 and 48 h after application, respectively against *An. Stephensi*. Singh *et al*. (2003) who observed the larvicidal activity of *Ocimum canum* oil against vector mosquitoes, namely, *Aedes aegypti* and *Culex quinquefasciatus* (LC_{50} 301 ppm) and *Anopheles stephensi* (234 ppm).

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