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Research Article

LARVICIDAL AND REPELLENT ACTIVITIES OF SALVIA SCLAREA MEDICINAL PLANT ESSENTIAL OIL AGAINST AEDES AEGYPTI (LINN.), ANOPLELES STEPHENSI (LISTON) AND CULEX QUINQUEFASCIATUS (SAY.) (DIPTERA:CULICIDAE)

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Abstract

To investigate the Larvicidal and repellent activities of Salvia sclarea essential oil against Ae. aegypti, An. stephensi and Cx. 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 LC50 values of the essential oil was determined by probit analysis. Repellent activity was carried out in a net cage (45×30×45 cm2) 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 Salvia sclarea was applied at 2.0, 4.0 and 6.0 mg/cm2 separately in the exposed area of the fore arm. The LC50 and LC90 values of Salvia sclarea essential oil on Ae. aegypti, An. stephensi and Cx. quinquefasciatus larvae in 24 h were 66.13, 71.47, 76.06 and 130.19, 142.63 and 144.28 ppm, respectively. The data is statistically significant at $P < 0.05$. The repellent activity of Salvia sclarea 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/cm2 provide100% protection up to 280, 240 and 160 min against Ae. aegypti, An. stephensi and Cx. quinquefasciatus, respectively. From the results it can be concluded the essential oil of Salvia sclarea as an excellent potential agent for controlling selected mosquitoes species.

Key Words: larvicidal activity, Repellent activity, Salvia sclarea, Aedes aegypti, Anopleles stephensi and Culex quinquefasciatus

INTRODUCTION

Mosquitoes not only create a nuisance as biting insects but are etiologic agents for some of the devastating diseases of human history such as malaria, filariasis, chikunguniya, dengue etc., (Molyneux *et al*., 2009). Mosquitoes are well known group of harmful insects that belongs to the Order Diptera that are wide spread, causing serious health problems to human beings (Bernhard *et al*., 2003). One of the approaches, the systematic application of insecticides is a common and widely accepted approach to control mosquito population, as it will provide rapid solution. On the other hand, the chemical control measures although highly effective, vector control is still facing a threat, as selective pressure imposed by conventional insecticides is enhancing resistance in various mosquito species resulting in disease outbreak (Liu *et al*., 2006). The recent negative impact of chemical insecticides has shifted the research efforts towards development of new environmentally compatible vector control methods by using naturalistic agents. In search of new vector control strategy, science has intensified the probes to plants in recent decades, so the plant kingdom is receiving renewed attention as mosquitocides (Pavela, 2009). The secondary phytochemicals of plants are a vast repository of compounds with a wide range of biological activities. The larvicidal activity of many of the secondary compounds, such as saponins (Wiesman and Chapagain, 2005; Pelah *et al*., 2002). Phenolics (Marston *et al*., 1993), isoflavonoids (Joseph *et al*., 2004), essential oil (Cavalcanti *et al*., 2004; Sukumar *et al*., 1991), alkaloids (Lee, 2000; Francois *et al*., 1996) and tannin compounds (Khanna *et al*., 2007), were well documented. Therefore the present study was carried out to determine the larvicidal and repellent activities of *Salvia sclarea* 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

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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 *Salvia sclarea* 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 150 ppm) 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).

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 *Salvia sclarea* 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 plant extract.

Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% 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.

Table 1 Larvicidal activity of essential oil of Salvia sclarea against selected vector mosquitoes

Mosquitoes	Concentration (ppm)	24 h mortality (%)	LC_{50} (ppm)	95%Confidence Limits(ppm)		LC_{90} (ppm)	Slope	Chi-square
				LCL	UCL			
Ae. aegypti	30	25.82±1.82	66.13	56.506	75.769	130.19	4.2585311	15.10214
	60	49.56±2.65						
	90	67.34±2.89						
	120	83.80±3.24						
	150	99.28±3.87						
	Control	0.00±0.00						
An. stephensi	30	19.64±1.43	71.47	57.846	79.104	142.63	3.687027	12.64301
	60	39.92±1.95						
	90	58.95±2.47						
	120	75.76±2.68						
	150	98.52±3.72						
	Control	0.00±0.00						
Cx.quinquefasciatus	30	16.64±1.86	76.06	58.437	81.695	144.28	3.6365441	13.46510
	60	35.57±1.64						
	90	54.83±2.37						
	120	72.42±2.80						
	150	97.26±3.24						
	Control	0.00±0.00						

Each value mean ± S.D represents mean of six values.*Statistically significant at $P < 0.05$. LCL-Lower confidence limit; UCL-Upper confidence limit; Slope; Regression.

RESULTS

Mosquitoes are nuisance pests and a major vector for the transmission of several life threatening diseases. With the development of resistance to conventionally used synthetic insecticides, vector management has become acutely problematic. Hence more attention has been focused on botanicals. Therefore our present study was aimed to evaluate the efficacy of *Salvia sclarea* essential oil against *Cx. quinquefasciatus* larvae in 24 h were 66.13, 71.47, the selected vector mosquitoes. The LC_{50} and LC_{90} values of

Table 2 Repellent activity of essential oil of Salvia sclarea against selected vector mosquitoes

Mosquitoes	Concentration mg/cm^2)	% of repellency							
		Time post application of repellent (min)							
		40	80	120	160	200	240	280	320
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	88.3±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	92.4±2.9
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.6±2.6
An. stephensi	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	89.5±2.3	82.3±1.4
	4.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.2±3.7	88.2±2.8
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	97.2±3.8	91.3±3.2
Cx.quinquefasciatus	2.0	100±0.0	100±0.0	100±0.0	100±0.0	84.6±2.3	81.3±2.6	71.5±2.3	68.6±2.3
	4.0	100±0.0	100±0.0	100±0.0	100±0.0	92.2±3.6	87.2±2.9	81.6±3.2	72.4±2.4
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	96.6±3.5	91.3±2.7	88.5±3.2	81.3±3.6

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

Salvia sclarea essential oil on *Ae. aegypti*, *An. stephensi* and 76.06 and 130.19, 142.63 and 144.28ppm, respectively. The data is statistically significant at $P < 0.05$ (Table 1). The repellent activity of *Salvia sclarea* 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 280, 240 and 160 min against *Ae. aegypti*, *An.stephensi* and *Cx. quinquefasciatus*, respectively(table 2).From the results it can be concluded the essential oil of *Salvia sclarea* as an excellent potential agent for controlling selected mosquitoes species.

DISCUSSION

The results of present study are comparable with similar reports of earlier workers, Amer and Mehlhorn (2006) reported that the mosquitoes in the larval stage are attractive targets for pesticides because it breeds in water and thus it is easy to deal with them in this habitat. Elango *et al.*(2009) who reported that the maximum repellent activity was observed at 500 ppm in methanol extracts of *Aeglemarmelos* and *Acacia lineata* and ethyl acetate extract of *Cytisus hirsutus*, and the mean complete protection time ranged from 90 to 120 min with the different extracts tested; no egg hatchability was observed with ethyl acetate extract of *A. marmelos*; methanol extracts *A. marmelos, A. lineata*, and C. *hirsutus* were exerted at 1 000 ppm, and the percentage of effective oviposition repellency were 92.60, 93.04, 95.20, 88.26, 92.80,94.01, 95.77, 96.93, and 92.54 at 500 ppm, and the lowest repellency were 47.14, 58.00, 56.52, 64.93, 71.09, 66.42, 50.62,57.62, and 65.73 at 31.25 ppm inacetone,ethyl acetate, and methanol extracts of *A. marmelos, A. lineata*, and C. *Hirsutus,* respectively.

Wiesman and Chapagain (2005) revealed that saponin extracted from the fruit of *Balanites aegyptica* showed 100% mortality against larvae of *Stegomyia aegypti*. Rawani *et al.* (2010) who also reported that ethyl acetate solvent extract of *Solanum nigrum* shows highest mortality against *Cx. quinquefasciatus* at 50 ppm. Watanabe *et al.* (1993) isolated a new compound, eucamol, and 4-isopropylbenzyl alcohol from *E. camaldulensis*. This new compound was compared with diet and proved to be highly active against *Ae. aegypti* ; after 3 h 75% of its repellency remained. Kerosene oil containing 1% neem oil resulted in the deviation of *Aopheles culicifacies* Giles, from living rooms to cattle sheds, followed by a reduced malaria incidence (Ansari and Razdan, 1994). For personal protection neem oil (1-4%) mixed in coconut oil resulted in a protection of 81-91% from bites of *Anopheles* mosquitoes, when tested in a forested village in India (Mishra *et al.*, 1995).

The result of the present study were also comparable to theearlier reports on the larvicidal activities of the four major compounds,.-terpinene, linalool, borneol and germacrene D. The LC_{50} values of.-terpinene were 30.7 and 29.8.g/mL against the larvae of *Anopheles aegypti* and *Anopheles albopictus* (Cheng *et al.*, 2009; Prabhu *et al.*, 2011; Nikkon *et al.*, 2011; Aziz *et al.*, 2011; Ravikumar *et al.*, 2011). Karunamoorthi *et al.* (2008) have also reported that the leaves of *Echinops* sp. (92.47%), *Ostostegia integrifolia*

(90.10%), and *Olea europaea* (79.78%) were also effective and efficient to drive away mosquitoes and the roots of *Silene macroserene* (93.61%), leaves of *Echinops sp.* (92.47%), *Ostostegia integrifolia* (90.10%), and *Olea europaea* (79.78%) were exhibited the significant repellency by direct burning. Tawatsin *et al.* (2001) have reported repellent activity against *Ae. aegypti, An. dirus* and *Cx. quinquefasciatus* which is due to 5% vanillin which has been added to the essential oil of *Curcuma longa*. Singh *et al.* (2008) demonstrated that the seed acetone extract of *Tribulus terrestris* showed 100% repellency in 0, 4, and 6 h and in 1 and 6 h and in 0, 2, and 4 h at 10% concentration against *An. culicifacies, An. stephensi* and *Cx. quinquefasciatus*, respectively. Amer and Mehlhorn (2006) have reported that 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 8 h at the maximum and a 100% repellency against *Ae. aegypti, An. stephensi,* and *Cx. quinquefasciatus*. Pushpanathan *et al.* (2008) reported that the essential oil of *Zingiber officinalis* showed repellent activity at 4.0 mg/cm2, which provided 100% protection up to 120 min against *Cx. quinquefasciatus*. Komalasmira *et al*, (2005) reported that the ethanol extracts of *P. beetle* has successfully killed the larvae of 4 mosquito vectors *A. aegypti, C. quinquefasciatus, A. dirus* and *Monsonia uniformis*. Dua *et al.* (1995) tested neem cream, also a repellent to be applied to the skin, against *Aedes, Culex* and *Anopheles* mosquitoes; one application was about 70% effective for 4 h. Two other study reported the LC_{50} values of linalool at 24 h were 155.73.g/mL against fourth instar larvae of *Ochlerotatus caspius* (Knio *et al.*, 2008) and the LC_{50} values of germacrene D were 63.6 and 59.5.g/mL against the larvae of *An. aegypti* and *An. stephensi* (Kiran *et al.*, 2006). *Lantana camara* flower extract in coconut oil provides 94.5% protection from *Aedes albopictus* and *Ae. aegypti*, with no undesirable adverse effects on human volunteers for 3 months after the application (Dua *et al.*, 1996). The LC_{50} values of Borneol were 43.5 mg/L against the larvae of *An. aegypti* (Rajkumar S, Jebanesan 2010).

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