

Short Communication

Larvicidal Activity of *Elytraria acaulis* against *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Background: Mosquitoes are blood sucking arthropods and serve as vectors of many diseases causing serious health problems to human beings. *Culex quinquefasciatus* and *Aedes aegypti* were responsible for Filariasis and Dengue. Synthetic pesticides were effective against mosquitoes as well as main sources of environmental pollution and most of them are immunosuppressant. Botanicals were widely used as insecticides, growth disruptors, repellents, etc. The aim of this research was to determine larvicidal properties of powdered leaf, *Elytraria acaulis* against late third or early fourth instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti*.

Methods: Larvae of *Cx. quinquefasciatus* and *Ae. aegypti* were tested at various concentrations of 100, 120, 140, 160, 180 and 200mg/100ml and mortality was recorded after 24h. The LC₅₀ values of the *E. acaulis* leaf powder were calculated by Probit analysis.

Results: The plant powder exhibited strong larvicidal activity against *Cx. quinquefasciatus* with LC₅₀ value of 116.07mg/100ml against *Ae. aegypti* 124.25mg/100ml respectively. The result indicated that the plant powder of *E. acaulis* showed potential larvicidal activity against *Cx. quinquefasciatus* and *Ae. aegypti*.

Conclusion: The overall findings of the present investigation suggested that the *E. acaulis* highly effective against *Cx. quinquefasciatus* and *Ae. aegypti* larvae. *Elytraria acaulis* may be used as an alternative to synthetic chemical pesticides for control of vectors to reduce vector borne diseases and did not harm to total environment.

Keywords: *Elytraria acaulis*; Larvicidal activity; *Culex quinquefasciatus*; *Aedes aegypti*

Introduction

Man suffers extensively due to the nuisance of vector mosquito population in public health manner. Mosquitoes directly transmit diseases such as filarial, malaria and dengue fever. Mosquitoes are blood sucking insects and serve as vectors for spreading human diseases and therefore, they continue to pose a serious health problem throughout the world. These are not only the most important vector for the transmission of diseases (1) but also an important pest to humans, causing allergic responses that include local skin reaction and systemic reaction such as angio-edema and urticaria (2). *Culex quinquefasciatus*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic mani-

festation (3). Despite its debilitating effects, lymphatic filariasis is given a very low control priority (4). In most of its range the females intensely anthropophilic, fed actively only at night and it causes nuisance (5) and are vectors of Japanese encephalitis, West Nile virus St. Louis encephalitis and avian malaria. *Aedes aegypti*, the principal vector of dengue, chikungunya, Zika and yellow fever viruses, is an anthropophilic species adapted to urban environments, particularly to housing (6). Dengue Hemorrhagic Fever (DHF) and Chikungunya are the major mosquito-borne diseases in India. The first dengue hemorrhagic fever was reported in Thailand and Philippines in 1950s. Dengue infections are reported throughout the world including India, where the first dengue outbreak was re-

ported in Delhi in 1988 (7). Now the dengue infection has been reported from all over the country (8-13) with major outbreaks reported from Tamil Nadu (14-17). Chikungunya which is endemic to South Asia, the Pacific island area, Africa, and the Americas and has infected millions of people mainly in developing countries (18). The lack of a commercial vaccine and the failure of vector control strategies to limit the expansion of chikungunya have prompted the need for further options to prevent the spread of this disease. Nowadays synthetic insecticides are at the fore front of mosquito-controlling agents. The continual usage of the synthetic chemical insecticides possess various environmental hazards such as development of resistance in vectors mosquitoes to these chemicals, disruption of natural biological control systems in mosquito populations (19). Hence, the necessity of plant derived insecticides especially target specific, eco-friendly, readily biodegradable and cost-effective (20). In general, plant essential oil has been recognized as important natural resources of insecticides (21). Many researchers have reported the control of mosquito larvae using the plant extract and the essential oils obtained from the different parts of the plants (22-24). Natural insecticides meet the needs for alternatives to controlling resistant populations of different species of mosquitoes. They can affect different stages of development through a variety of mechanisms. In this study, we have chosen *Elytraria acaulis* belongs to the family Acanthaceae is a small shrub, which grows in shady dry places and it is commonly known as Asian Scalystem. It is a stem less perennial herb with one to several unbranched flowering stems; up to 30cm. Stems are covered with overlapping bracts. Leaves occur in a rosette at the base. They are obovate, 4–10 centimeter long. Flowers are white, lower lip and lateral lobes spreading, 2-lobed. This plant is frequently found on rocky or sandy soils. The whole shrub is used for medicinal purposes (25). The decoction of *E. acaulis* leaves prescribed for fever, venereal diseases and root is used in

mammary tumor, abscesses, pneumonia, anti-diabetic effects, antibacterial activity, treating wounds infected with worms and infantile diarrhea as well as traditional medicine for long days (26-27). The sub-acute toxicity of methanolic extract of *E. acaulis* was tested against female Wistar rats with the concentrations of 50 to 2000mg/kg by oral administration (28). They observed that no significant alteration on any of the biological parameters. The present study was aimed to investigate the larvicidal properties of powdered leaf of *E. acaulis* against late third or early fourth instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti*.

Materials and Methods

Selected medicinal plant

Fresh and matured leaves of *E. acaulis* was used for the research work. The selected plant was collected from Kattukollai, Kanchipuram District, Tamil Nadu based on their abundance, availability, medicinal and insecticidal properties. The plant specimen was identified by Dr P Paramasivam, Department of Botany, Pachaiyappa's College for Men, Kanchipuram, India. The specimen plant was preserved at herbarium of Department of Botany, Pachaiyappa's College for Men, Kanchipuram, India for further reference. The collected plant material were washed with tap water to remove all the unwanted impurities and shade dried at laboratory temperature (27 ± 2 °C) and macerated with electric blender and stored at 4 °C for larvicidal bioassay.

Maintenance of mosquito larvae

Culex quinquefasciatus and *Ae. aegypti* mosquito larvae were collected from stagnant water in and around of Kattukollai, Kanchipuram District, Tamil Nadu, India. All the larvae were kept in plastic trays containing tap water and were maintained in the laboratory. The larvae were fed with dog biscuits and yeast powder in the 3:1 ratio. All the experiments were carried out at 27 ± 2 °C and 75–85% relative hu-

midity under a photoperiod of 14:10h (light/dark) cycles. They were maintained until the larvicidal bioassay.

Larvicidal activity

Larvicidal activity was evaluated by using the standard method (29). Twenty five individuals of late third or early fourth instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* were released in a 250ml glass beaker containing 100 ml of dechlorinated tap water mixed with desired *E. acaulis* plant powder at different concentrations (mg), an equal number of controls were set up simultaneously using tap water. Five replicates of each concentration were run at a time. The experimental concentrations were 100, 120, 140, 160, 180 and 200mg/100ml respectively. Negative control (water) was run simultaneously. Mortality and survival rate were recorded after 24 hours. Based on the WHO protocol no food was offered to avoid the difference in mortality. The moribund and dead larvae in five replicates were combined and expressed as a percentage of larval mortality for each concentration. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within reasonable period of time) or the water was disturbed the characteristic diving reaction was not seen. The LC₅₀ value was calculated by EPA Probit analysis software.

Results

In the present investigation the toxic effect of powdered leaf of *E. acaulis* tested at six different concentrations such as 200, 180, 160, 140, 120 and 100mg/100ml to evaluate the larvicidal activity against the larvae of *Cx. quinquefasciatus* and *Ae. aegypti*. Besides, the control set up also compared with different concentrations of plant powder. The highest concentration (200mg/100ml) of powdered leaf of *E. acaulis* showed 100% mortality against the

larvae of *Cx. quinquefasciatus* and *Ae. aegypti*. However, 180 and 160 and 140mg/100ml of *E. acaulis* leaf powder inflicted moderate larval mortality. The least concentrations of 120 and 100mg/100ml exhibited least larvicidal activity. In comparison with the control, all the concentrations of *E. acaulis* leaf powder contributed potential larvicidal activity. The LC₅₀ and LC₉₀ value of *E. acaulis* leaf powder exhibited 116.07 and 190.38mg/100ml against *Cx. quinquefasciatus* and 124.25 and 198.21 mg/100ml against *Ae. aegypti* (Table 1).

No larval mortality of observed in control. After 96hrs 100% mortality was observed in all the tested concentrations *E. acaulis*. Symptomatological observations were carried out through the exposure period at laboratory temperature among the two species of mosquitoes revealed that immediately after exposure to *E. acaulis*. All larvae were active and exhibited a normal appearance with the siphon pointed up and head hung down. After 5 minutes of treatment, some of the larvae became restless and frequently sank down and floated up quickly at 200mg/100ml concentration. At 30th minute, the restlessness persisted; tremor and convulsion at the bottom of the container were observed approximately in 1 to 2 larvae. Similar evidences of restlessness, tremors, and convulsions followed by paralysis were clearly seen after an hour approximately in 4 to 5 larvae. At 12h, approximately 1 to 2 moribund and dead larvae were found. After 24h of treatment, approximately one-third of the larvae was paralyzed and sank to the bottom of the bowl. More and more larvae exhibited toxic symptoms during 12h. Subsequently, all of them died within 24 h in the 200mg/100ml treatment. The powdered leaf of *E. acaulis* caused rapid mortality, suggesting larvicidal property. The symptoms observed in treated larvae were similar to those caused by nerve poisons, such as excitation, convulsion, paralysis and death. Dead larvae were observed under the light microscope after 24h of exposure, where the body attained a dark brown color; length of the larvae was shrunk.

Table 1. Larvicidal activity of *Elytraria acaulis* against the larvae of *Culex quinquefasciatus* and *Aedes aegypti*

<i>Culex quinquefasciatus</i>													
Replication	Concentration (mg/100ml)/ Number of dead out of 25 tested							LC ₅₀	95% Confidence limit		LC ₉₀	95% Confidence limit	
	Control	200mg	180mg	160mg	140mg	120mg	100mg		LCL	UCL		LCL	UCL
1	0	25	20	19	15	13	10						
2	0	25	20	20	15	13	9						
3	0	25	21	20	16	13	9						
4	0	25	20	20	18	14	11						
5	0	25	20	19	16	12	11	116.07	90.05	131.08	190.38	164.26	279.40
Total dead	0/125	125/125	101/125	98/125	80/125	65/125	50/125						
S.D.	0	0	0.44	0.54	1.22	0.70	1						
% of mortality	0	100	80.8	78.4	64	52	40						
<i>Aedes aegypti</i>													
1	0	25	18	19	16	12	8						
2	0	25	17	18	15	11	7						
3	0	25	20	18	14	14	8						
4	0	24	22	17	15	12	8						
5	0	25	20	16	16	12	6	124.25	102.97	138.73	198.21	171.24	284.61
Total dead	0/125	124/125	97/125	88/125	76/125	61/125	37/125						
S.D.	0	0.44	1.94	1.14	0.83	1.09	0.89						
% of mortality	0	99.2	77.6	70.4	60.8	48.8	29.6						

Values are mean \pm SD of five replicates. In each concentration 25 larvae were used

Discussions

World Health Organization has estimated globally the contribution of commercial pesticides to health (30). Naturally occurring pesticides thus appear to have a prominent role in the development of future safety of the environment and public health (31). There are different methods for controlling mosquitoes. The plant crude formulation was responsible for larvicidal activity. The pool of plants processing insecticidal substance is enormous. The use of plant essential extract for the pest and diseases management has recently been reversed. The preliminary screening is a good means of evaluating the potential larvicidal activity of plants popularly used for this purpose. In the present investigation *E. acaulis* inflicted potential larvicidal activity with the LC₅₀ value of 116.07 mg/100ml against *Cx. quinquefasciatus* and 124.25mg/100ml against *Ae. aegypti*. Like ways, the sustained toxicity test of some medicinal plants such as *Nerium oleander*, *Calotropis procera* and *Ricinus communis* powders against *Anopheles arabiensis*, *Cx. quinquefasciatus* (32). They reported that the after 6 days 100% mortality was observed in *An. arabiensis*, Whereas, *Cx. quinquefasciatus* 60% mortality was observed. The larvicidal activity due to the presence of Alkaloids, Flavonoids, Protein, Amino Acid, Glycosides, Carbohydrates, Phenol, Steroids, Saponins and Tannins from *E. acaulis* (33).

The results were coincides with earlier findings in which the leaf powder of *Croton sparsiflorus* had LC₅₀ value of 122.73mg/100ml and LC₉₀ value of 180.04mg/100ml followed by *Bauhinia variegata* with LC₅₀ value of 142.47mg/100ml and LC₉₀ value of 210.16mg/100ml, respectively (34). In another study the solvent extracts of *E. acaulis* showed moderate effect on *Cx. quinquefasciatus* and *Ae. aegypti* (35). Similarly, 1mg/ml of ethanolic extracts of the leaves of *Lantana camara* exhibited 84% larval mortality while treated with methanolic extract caused 48% mortality on

fourth instar larvae of *Ae. aegypti* (36). The ethanolic extract from leaves of *Cassia occidentalis* caused larval mortality against malarial vector mosquito *An. stephensi* at a dose equivalent to LC₅₀ of 70.56% for fourth instar larvae (37). Ethanolic extract from bulbs of *Allium sativum* inflicted remarkable insecticidal activity against larvae of *Aedes albopictus* with LC₅₀ value of 4.48g/L (38). The toxic effect of *Ricinus communis* crude extract was tested against immatures of *Cx. Quinquefasciatus* and *An. arabiensis* (39). They recorded LC₅₀ values as 403.65, 445.66, and 498.88ppm against second, third, and fourth instar larvae of *An. arabiensis* and 1091.44, 1364.58, and 1445.44 ppm against second, third, and fourth instar larvae of *Cx. quinquefasciatus*, respectively. Several plants were evaluated against main malaria vector, *An. stephensi*, and *Cx. pipiens* including *Mentha spicata*, *Cymbopogon olivieri*, *Azadirachta indica*, *Melia azedarach*, *Lagetes minuta*, *Calotropis procera*, *Eucalyptus camaldulensis*, *Cupressus arizonica*, *Thymus vulgaris*, *Lawsonia inermis*, *Cedrus deodara*, *Cionura erecta*, *Bunium persicum*, *Carum carvi*, *Artemisia dracunculus*, *Rosmarinus officinalis*. *Mentha spicata* and *Eucalyptus camaldulensis*, had the lowest and highest LC₅₀ respectively (40-55). Our results clearly indicated the *E. acaulis* highly effective against *Cx. quinquefasciatus* and *Ae. aegypti*. Powdered leaves of *E. acaulis* may be a good source to develop newer mosquitocidal biopesticide.

Conclusion

The overall findings of the present investigation suggested that the *E. acaulis* highly effective against *Cx. quinquefasciatus* and *Ae. aegypti* larvae. However solvent extractions are time consuming and costlier technique. *Elytraria acaulis* may be used as an alternative for synthetic chemical pesticides to control vec-

tors mosquitoes and reduce vector borne diseases. According to our knowledge it seems not harmful to the natural environment and it needs more study to understand the level of toxicity.

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