CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjb.htm
2015 Vol. 4 (2) April-June, pp.13-19/Mathew et al.
Research Article

LARVICIDAL ACTIVITY OF *GLIRICIDIA SEPIUM* LEAF EXTRACTS ON MOSQUITO LARVAE AND ITS LETHAL EFFECT ON NONTARGETED ORGANISMS

Jiby John Mathew, *Prem Jose Vazhacharickal, Sajeshkumar N.K and Jesmi Sunil
Department of Biotechnology, Mar Augusthinose College, Ramapuram, Kerala, India
**Author for Correspondence*

ABSTRACT
Mosquitoes are known vectors for transmitting malarial parasites. To prevent proliferation of mosquito borne diseases and to improve public health, mosquito control has been employed using unfriendly synthetic insecticides which cause side effects and environmental pollution. Alternative to these synthetic agents are natural products derived from various plant sources. The present study was designed to investigate the larvicidal activity of the Petroleum ether crude extract of the leaves of *Gliricidia sepium* (Cheemakonna) against the fourth instar larvae of Anopheles mosquitoes and also check its effect on non targeted organisms; tadpole and guppy fry (*Poecilia reticulata*). A quantity of the powdered leaves was exhaustively extracted by the Soxhlet extraction method using petroleum ether (80%). Samples of larvae were transferred into the extract at the various concentrations (0, 25, 50, 100, 150, 200, and 250 ppm). The mortality was observed and the dead larvae counted after 15 min., 30 min, 1 hr, 3 hrs, 4 hrs, 5 hrs and 6 hrs. There were progressive increases in the lethal effect on the Anopheles larvae with respect to time and concentration. Lethality was observed from 1 hr, at a concentration of 200 ppm and by the 6th hour, all the concentrations were lethal. After probit analysis, the calculated LC50 (larvae) was 70.68 ppm/6 hrs. No lethal effects on non-targeted organisms were observed. *Gliricidia sepium* leaf extract has potent effect on controlling mosquito larvae which can be explored further.

Keywords: *Lethal Concentration; Larvicidal Activity; Gliricida Sepium; Probit Analysis*

INTRODUCTION
Mosquitoes are the major vectors of many diseases and it alone transmits disease to more than 700 million peoples annually (Cancrini *et al.*, 1997; Waterhouse *et al.*, 2007; Sardelis *et al.*, 2001; Nene *et al.*, 2007). Mosquitoes borne diseases currently represents a greater health problem in tropical and subtropical climates of the world (Taubes, 1997; Shell, 1997; Fradin and Day, 2002). Mosquitoes transmit many serious diseases like Dengue fever and Yellow fever which recently struck most part of Pakistan and turned up to an endemic proportion. Mosquito can thrive urban and suburban regions due to ideal conditions for breeding (Ramson *et al.*, 2001).

Mosquito control is essential for the proliferation of mosquito borne diseases, environmental quality and public health. Synthetic insecticides have been not very successful due to human, technical, operational, ecological and economic factors. This may resulted to the development of resistant strains of mosquitoes. One of the major drawbacks of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment (Lee and Kim, 2001). So there is an urge to look for an environment friendly, cost effective, biodegradable and target specific insecticides against mosquitoes (Gbolade, 2001).

Many of the organic insecticides are based on single ingredient, but plant products comprises of a number of chemicals which can act on the physiological process of mosquitoes. It was reported that more than two hundred plant species belonging to different families and genera have toxic components which are effective against insects (Govindarajan and Jebanesan, 2008). Extracts from various part of the plant such as leaves, roots, stem and fruits are shown mosquitocidal (larvicidal) activities (Kalyanasundaram and Dass, 1985; Gbolade, 2001).

In India many compounds (Phytochemicals) derived from plants has been recognized as an important source of insecticide (Gbolade and Oyedele, 2000) and many of them exhibit harmful effect on

mosquitoes (Kuo, 2007; Ghosh, 2008, Rahuman, 2009). *Gliricidia sepium* is a medium sized tree found in most part of India and it was observed that it is relatively free from insects and disease problems (Boa and Lenne, 1996). It was also found that the tinctures made from *Gliricidia sepium* inhibit the growth of various strains of *Neisseria gonorrhoea* invitro test (Caceres *et al.,* 1995). The presence of *Gliricidia sepium* in the field reduces incidence of some fungal and insect attack (Glover, 1989).

Given lacking qualitative and quantitative data on various biological larvicidal agents in Kerala, objective of this study were to assess the potential of the extracts of *Gliricidia sepium* leaves on mosquito larvae and its effect on non-targeted organisms.

MATERIALS AND METHODS

Sample Collection

Gliricidia sepium leaves were collected from different places of Wayanad district of Kerala state, India. The fresh leaves were collected in poly ethylene zipper bags, later washed two times with distilled water. The plant materials were thoroughly washed with distilled water and fresh weight were determined. The samples are then oven dried (KOA4, KEMI lab equipments, Ernakulam, India) at 60°C for 24 h. The dried samples were powdered using a waring blender (Magic V2, Preethi Kitchen Appliances Pvt Ltd, Chennai, India) and stored in air-tight polyethylene bottles until further analysis.

Mosquito larvae were collected from rubber plantations, from the natural habitat along with their immediate surrounding water. The collected larvae were carried to the lab for further experiments. Sufficient quantity of pond or stagnant water was drawn from the marsh environment and taken to the laboratory for investigation. Hand lens was used to observed stream of larvae moving in the water and for the counting. Only lively, highly motile larvae were used after allowing them to acclimatize to the laboratory conditions. The tadpole was collected from the pond of situated in the garden of Mar Augusthinose College Ramapuram, Kottayam district in Kerala state. Guppy fry were collected from a local aquarium.

Extraction Method

About 40 g weight quantity of the Gliricidia sepium leaf powder was extracted in 600 ml petroleum ether (80%) by Soxhlet (KHM3, KEMI lab equipments, Ernakulam, India) extraction technique for 48 hours (Figure 1). The final extract ants were filtered using a Whatman filter paper 42 (GE Healthcare UK Ltd, Buckinghamshire, UK) and transferred to an airtight polyethylene bottles which were stored on refrigerator for further inhibition studies.

Working Solution of Extract

Stock solution was prepared by dissolving 1 g of crude extract in 10 ml petroleum ether (Merck, Mumbai, India) and volume made up to 100 ml with distilled water. Seven different dilutions of 0 ppm 25 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm were prepared in 200 ml de-ionized water in 250 ml bottles and specified amount of larvae (25) were released into each. The number of death against time was taken at 15 min, 30 min, 1hr, 2hr, 3hr, 4 hr, 5 hr and 6 hrs respectively. The beakers were kept in room temperature at 30 °C ± 2 °C. The mortality data were subjected to probit analysis to determine the lethal concentration to kill 50% of the treated larvae (Randhawa 2009). For the control experiment, the same numbers of larvae used above were placed in 200 ml water containing 0.1 ml of petroleum ether in a 250 ml beaker and observed. Each treatment was replicated three times.

Larvicidal Activity Determination

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by, WHO, 2005. From the stock solution, seven different test concentrations (0, 25, 50, 100, 150, 200 and 250 ppm) were prepared and tested against the freshly moulted (0-6 h) third instar larvae of mosquito and Guppy Fry). A control also maintained. The larvae of test species (25) were introduced in bottles containing 200 ml of aqueous medium and the required amount of plant extract was added. The larval mortality was observed and recorded after 6 h of post treatment. For each experiment, three replicates were maintained at a time. The larval mortality was observed and recorded.

Statistical Analysis

The survey results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA).

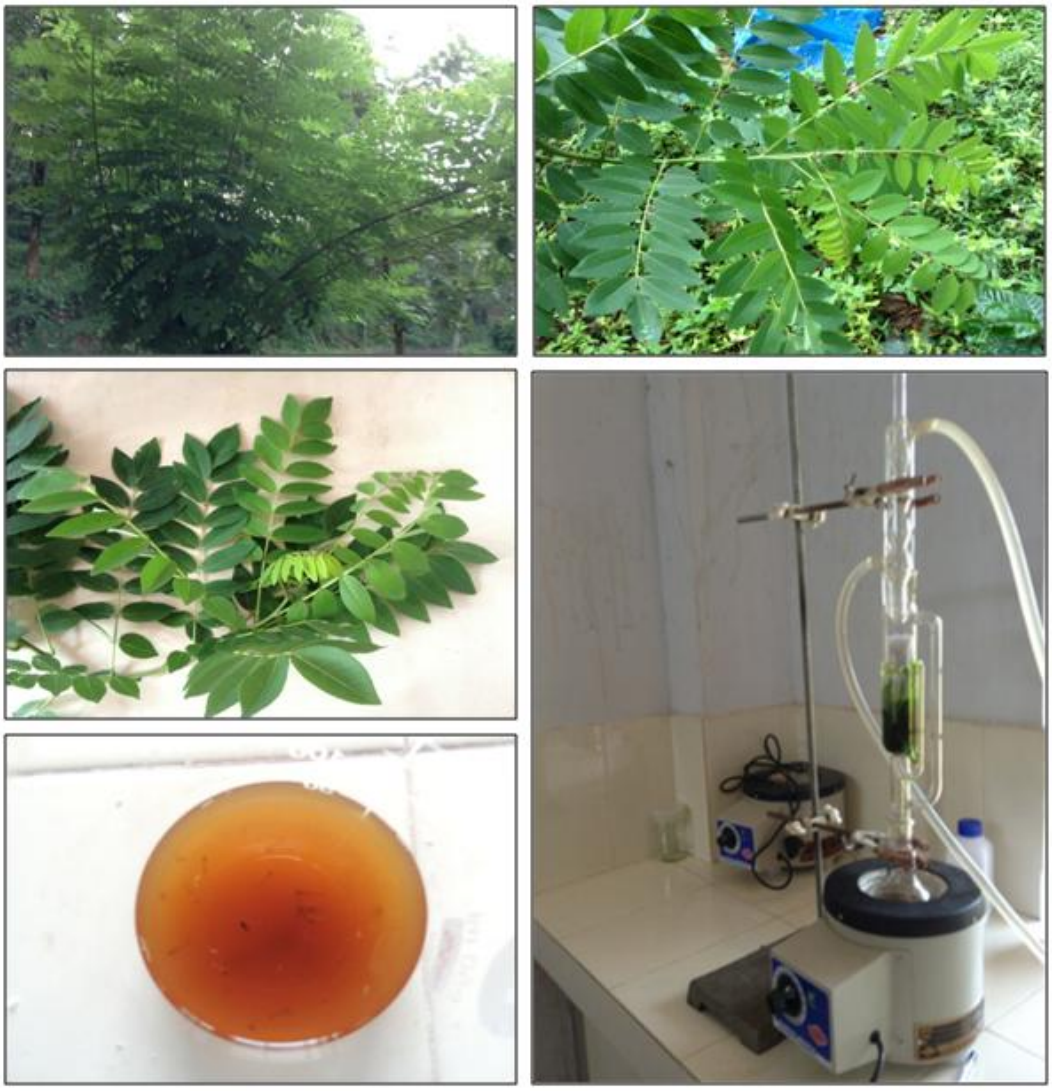

Figure 1: *Gliricidia sepium* plant in the field (top left); *Gliricidia sepium* leaves (top right); young leaves collected (middle left); Soxhlet apparatus extraction of dry leaves (bottom right); extracts action on mosquito larvae (bottom right)

RESULTS AND DISCUSSION

The result shows there was no lethal effect was observed after an hour in all the concentrations. There were progressive increases in the lethal effect on the larvae after about three hours of exposure to the extract. The percent mortality and lethal concentration were calculated (Table 1 to 6). At the concentration of 150, 200 and 250 ppm, mortality was 70.68, 84.00 and 93.00% respectively. The lethal concentration for 50% mortality (LC50), after probit analysis, was found to be 70.68 ppm at 6 hours.

It was also observed that the crude extract was no lethal effect on untargeted organisms that present in the water body. Table 3, 4, 5 and 6 shows respectively that there was no lethal effect of *Gliricidia sepium* extract on freshly moulted tadpole and guppy fry. The crude extract of *Gliricidia sepium* had no effect on

the life of guppy fry, the fish that commonly consumes mosquito larvae for their food. From the control it was clear that petroleum ether had no direct effect on these organisms. The result shows that the crude extract of the leaves of the *Gliricidia sepium* plant has activity against the larvae of mosquitoes. The toxic effects of the extract on the larvae depend on the concentration and duration of exposure. This indicated that the toxicity levels of the extract are dependent on time of exposure of the larvae and the applied concentration.

This is comparable to previous reports on larvicidal activities of plant extracts. Kamaraj *et al.*, (2011) reported LC50 values of 93.80 and 104.94 for the methanol extract of *Annona squamosa* leaves and methanol extract of the leaves of *Chrysanthemum indicum* L, respectively, against Anopheles subpicus. Other similar reports include the larvicidal study on the components of the leaves of *Azadirachta indica* and *Artemisia annua* Linn, where the LC50 of crude extracts were to be 19.9 and 69.0 ppm, while LC50 of 72 ppm and 136 ppm for crude extracts of *Vitex negundo* and *Nerium oleander* leaves. The crude extract of the leaves of Gliricidia sepium could be used as one of the source of finding chemical substances to design insecticidal agents, especially anti-mosquito agents.

Table 1: Larvicidal activity of Gliricidia sepium crude extract against freshly moulted larvae of mosquito

Treatment	Concentration	Larvae mortality (ml)	Mortality (%)
C	0	1.33 ± 0.46	5.3
S1	0	1.00 ± 0.46	4.0
S2	25	6.00 ± 0.69	24.0
S3	50	8.33 ± 0.05	33.3
S4	100	10.33 ± 0.05	41.3
S5	150	13.33 ± 0.05	53.3
S6	200	21.00 ± 0.28	84.0
S7	250	23.30 ± 0.05	93.2

Table 2: Bioassay testing of Gliricidia sepium crude extract at different concentrations against freshly moulted larvae of mosquito at various time intervals

Concentration (ppm)	15 min	30min	1hr	2hrs	3hrs	4hrs	5hrs	6hrs
25	-	-	-	-	-	-	-	+
50	-	-	-	-	-	+-	+	+
100	-	-	-	-	+	+	+	+
150	-	-	+	+	+	+	+	+
200	-	-	+	+	+	+	+	+
250	-	-	+	+	+	+	+	+

Key: - = no noticeable lethal effect + = lethal effect observed

Table 3: Larvicidal activity of Gliricidia sepium crude extract against freshly moulted tadpole

Treatment	Concentration	Larvae mortality (ml)	Mortality (%)
C	0	1.50 ± 0.46	6.0
S1	0	1.50 ± 0.46	6.0
S2	25	1.33 ± 0.69	5.3
S3	50	1.00 ± 0.05	4.0
S4	100	2.00 ± 0.05	8.0
S5	150	1.60 ± 0.05	6.4
S6	200	2.00 ± 0.28	8.0
S7	250	3.00 ± 0.05	12.0

Table 4: Bioassay testing of *Gliricidia sepium* crude extract at different concentrations against tadpole at various time intervals

Concentration (ppm)	15 min	30min	1hr	2hrs	3hrs	4hrs	5hrs	6hrs
25	-	-	-	-	-	-	-	+
50	-	-	-	-	-	+-	+	+
100	-	-	-	-	+	+	+	+
150	-	-	+	+	+	+	+	+
200	-	-	+	+	+	+	+	+
250	-	-	+	+	+	+	+	+

Key: - = no noticeable lethal effect + = lethal effect observed

Table 5: Larvicidal activity of Gliricidia sepium crude extract against freshly moulted guppy fry

Treatment	Concentration	Larvae mortality (ml)	Mortality (%)
C	0	0.67 ± 0.46	6.7
S1	0	0.67 ± 0.46	6.7
S2	25	1.00 ± 0.69	10.0
S3	50	1.00 ± 0.05	10.0
S4	100	1.00 ± 0.05	10.0
S5	150	2.00 ± 0.05	20.0
S6	200	3.33 ± 0.28	33.3
S7	250	4.67 ± 0.05	46.7

Table 6: Bioassay testing of Gliricidia sepium crude extract at different concentrations against guppy fry at various time intervals

Concentration (ppm)	15 min	30min	1hr	2hrs	3hrs	4hrs	5hrs	6hrs
25	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	+	+
250	-	-	-	-	-	-	+	+

Key: - = no noticeable lethal effect + = lethal effect observed

It can be concluded that the crude extracts of the leaves of the *Gliricidia sepium* plant have insecticidal activity, especially mosquitocidal activity, against the larvae of the mosquitoes. It was also proved that the extract has no lethal effect on non-targeted organisms. Therefore, it could be formulated and used as an ecological friendly natural product for anti-mosquito activity.

Conclusions

Plant derived mosquitocidal agents have promising results and eco-friendly. However the full potential of various plants are still underutilized or explored. Research should also focus on the enhancing the activity by different formulations. These agents are very economical for mosquito control in developing and under developing countries.

ACKNOWLEDGEMENT

The authors are grateful for the cooperation of the management of Mar Augsthinose college for necessary support. Technical assistance from Binoy A Mulanthra is also acknowledged. We also thank an anonymous farmer for proving majority of samples used in the study.

REFERENCES

Boa ER and Lenne JM (1996). *Diseases and Insect Pest* 73 -76.

Caceres A, Menendez H, Mendez E, Colionbon E, Samayoa BE, Jauregu E, Paralta E and Carrillo G (1995). Antigonorrhoea activity of plants used in Guatemala for the treatment of sexually transmitted diseases. *Journal of Ethnopharmacology* **48**(2) 85-88.

Cancrini G, Pietrobelli M, Di Regalbono AF and Tampieri MP (1997). Mosquitoes as vectors of *Setaria labiatopapillosa*. *International Journal for Parasitology* **27**(9) 1061-1064.

Fradin MS and Day JF (2002). Comparative efficacy of insect repellent against Mosquito bite. *New England Journal of Medicine* **347**(1) 13-18.

Gbolade AA (2001). Plant-derived insecticides in the control of malaria vector. *Journal of Tropical Medicinal Plants* **2**(1) 91-97.

Gbolade AA, Oyedele AO, Sosan MB, Adewayin FB and Soyela OL (2000). Mosquito repellent activities of essential oils from two Nigerian Ocimum species. *Journal of Tropical Medicinal Plants* **1**(1/2) 146-148.

Ghosh A, Chowdhury N and Chandra G (2008). Laboratory evaluation of a phytosteroid compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) and nontarget organisms. *Parasitology Research* **103**(2) 271-277.

Glover N (1989). *Gliricidia Production and Use* (Nitrogen fixing trees Association, Waimanalo, Hawaii, USA) 44.

Govindarajan M, Jebanesan A and Pushpanathan T (2008). Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. *Parasitology Research* **102**(2) 289-292.

Kalyanasundaram M and Dass PK (1985). Larvicidal and synergistic activity of plant extracts for mosquito control. *Indian Journal of Medical Research* **82**(1) 19-23.

Kamaraj C, Bagavan A, Elango G, Zahir A, Rajakumar G and Rahuman AA (2011). Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Indian Journal of Medical Research* **134**(1) 101 -106.

Kuo PM, Chu FH, Chang ST, Hsiao WF and Wang SY (2007). Insecticidal activity of essential oil from *Chamaecyparis formosensis*. *Holzforschung* **61**(5) 595-599.

Lee SE, Kim JE and Lee HS (2001). Insecticide resistance in increasing interest. *Journal of Applied Biological Chemistry* **44**(3) 105-112.

Nene V, Wortman JR, Lawson D, Haas B, Kodira C, Tu ZJ and Li S (2007). Genome sequence of *Aedes aegypti*, a major arbovirus vector. *Science* **316**(5832) 1718-1723.

Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA and Elango G (2009). Efficacy of larvicidal botanical extracts against *Culexquinque fasciatus* Say (Diptera: Culicidae). *Parasitology Research* **104**(6) 1365-1372.

Randhawa MA (2009). Calculation of LD50 values from the method of Miller and Tainter, 1944. *Journal of Ayub Medical College Abbottabad* **21**(3) 184-185.

Ranson H, Rossiter L, Ortelli F, Jensen B, Wang X, Roth C, Collins FH and Hemingway J (2001). Identification of a novel class of insect glutathione S- transferees in valued in resistance to DDT in malaria vector Anopheles gambiae. *Biochemical Journal* **2001**(359) 295-304.

Sardelis MR, Turell MJ, Dohm DJ and O'Guinn ML (2001). Vector competence of selected North American Culex and Coquillettidia mosquitoes for West Nile virus. *Emerging Infectious Diseases* **7**(6) 1018.

Shell ER (1997). Resurgence of a deadly diseases. *Aflantic Monthly* 45-60.

Taubes G (1997). A mosquito bites back. *New York Times Magazine* **24** 40-46.

Waterhouse RM, Kriventseva EV, Meister S, Xi Z, Alvarez KS, Bartholomay LC and Christophides GK (2007). Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. *Science* **316**(5832) 1738-1743.

WHO (2005). Pesticides and their application for the control of vectors and pests of public health importance. World Health Organization. Available: WHO/CDS/WHOPES/GCDPP/.Geneva.