JBiopest 6(2):178-181

Mosquito larvicidal activity of 8, 11, 14-eicosatrienoic acid of *Gliricidia sepium* Jacq.

Jiji Thomas[1]*, M. Shonima Govindan[1] and G. Muraleedhara Kurup[2]

ABSTRACT

As part of an ongoing program to identify phytochemicals with mosquitolarvicidal activity, an ethno botanical survey was conducted and a very common plant *Gliricidia sepium* was selected. Petroleum ether extract of dried leaves after bio-assay guided purification procedures yielded 8, 11, 14-eicosatrienoic acid. Toxicity studies were conducted against larvae and pupae of *Culex quinquefasciatus* mosquito, and LC_{50} and LC_{90} were calculated using probit analysis. Effect of the phytochemical on non target organisms was also tested using guppy fish. Results proved the nontoxicity of 8, 11, 14-eicosatrienoic acid on non-target aquatic organisms. These results could boost up amelioration of mosquito menace studies to a great extent.

MS History:21.2.2013 (Received)-2.10.2013 (Revised)-4.10.2013 (Accepted)

Key words: *Gliricidia* plant*; Culex* mosquito, phytochemical

INTRODUCTION

Culex quinquefasciatus is one of the most important mosquito vectors of the pathogens that cause lymphatic filariasis and Japanese encephalitis in India and many developing countries (Kovendan *et al*., 2012; Arivoli *et al*., 2011). The prevalent practice of ignition of coils and other repellents are sugar coated poisons. They help repel mosquitoes, but invariably bring many uninvited hazards. The apprehended depth of these hazards for health and environment still remains incomprehensible. Many of the chemicals available commercially for controlling mosquitoes are of synthetic types. They induce tolerance and resistance in mosquitoes, besides environmental pollution (Nazar *et al*., 2009; Singh and Prakash, 2009). So before mosquitoes gain complete resistance against the key weapons now used among them, the mosquito control personnel should initiate extensive research to explore and launch safe, effective and eco-friendly biomaterials for their control. In recent years there is an increased tendency for the revival of plant based products because of the development of resistance and cross resistance associated with synthetic insecticides (Ansari *et al*., 2005).

Gliricidia sepium (*Leguminosae*) is a medium sized tree introduced into India from the American continent. This tree is used as shade for cocoa and coffee plantations and for this reason it is called 'Madre cacao' (mother of cocoa). Crude ethanol extracts of dried leaves, fresh leaves, dried petioles and stem bark of this plant showed toxic properties against late third instar larvae of *Anopheles stephansi, Aedes aegypti* and *Culex quinquefasciatus* (Sharma *et al.,* 1998) and also against other insects, nematodes and microbes (Nazli *et al.,* 2008).

But its detailed bioactive compound impacts were not recorded against any insects. The principal objectives of this investigation are to study the lethal dose of the phytochemical extracted from *Gliricidia sepium* against larvae and pupae of *C. quinquefasciatus*, and to test the toxicity of phytochemical on non-target organisms.

.

MATERIALS AND METHODS

Culturing and maintenance of mosquito

Mosquitoes collected from fields were used for raising the colony. After oviposition, eggs were collected in filter paper and kept separately at 27±2 °C. The adult mosquitoes were provided with water soaked raisins and cotton swabs dipped in 5 % glucose solution. The female mosquitoes were fed on blood meal. Plastic cups of 6 cm height and 8 cm diameter lined with filter paper and half filled with water were introduced into the cages for oviposition. After the eggs were laid, the ovitraps were taken out of the cages and fresh ones were placed in the cages for subsequent ovipositions.

After hatching, the first instar larvae were transferred to an enamel tray of 30×25×5 cm^3 containing well water. The larvae were fed on a diet of finely powdered biscuits and yeast in the ratio 3:1. The water in the tray was changed every day and dead larvae were removed (Gerber *et al*., 1994).

Plant materials

An ethno botanical survey revealed that leaves of *Gliricidia sepium* were commonly used as a mosquito repellent in many areas of Kerala state. The plant was selected for the study because of its high degree of desired activity and as it is ethnobotanically important, easily available, and ubiquitous in the flora of Kerala.

Collection and extraction

Fresh leaves were collected from Vagamon, located 1100 m above sea level at Idukki district, in Kerala state. Petroleum ether extract of dried powdered leaves were extracted again with acetonitrile and column chromatographic separation was done using acetone: methanol (9:1) after extraction with chloroform. The eluted compounds were tested for their larvicidal activity. The compound with desired quality was subjected to structural analysis by GCMS, FTIR, NMR and identified as 8, 11, 14-eicosatrienoic acid.

Bioassay

8,11,14-eicosatrienoic acid extracted from *G.sepium*, were prepared in concentrations ranging from 0.004 to 0.50 mg/ml using 0.1 % of Tween 20 as emulsifier. Sample in each concentration was in replicates of four and, a control containing only Tween 20 (0.1 %) was run for comparison. Twenty five numbers of larvae or pupae were used for all the experiments. The number of dead larvae or pupae at the end of 24 h was recorded and percentage of mortality was calculated. LC_{50} and LC_{90}, of the extracts with 95 % confidence limits, were found out by the mortality data analyzed by log probit method.

Studies on non target organisms

The test was conducted to determine the susceptibility of a selected nontarget organism to the phytochemical as described by Promsiri *et al*. (2006) and Singha *et al*. (2011). Non-target organisms *Poecilia reticulata* fish, were exposed to 0.01 mg/ml and 0.1 mg/ml concentration of 8, 11, 14-eicosatrienoic acid to observe the mortality and other behavioural abnormalities such as sluggishness and reduced swimming up to 48 h. Male and female guppies (*Poecilia reticulata*), reared in the laboratory were used for the toxicity experiments. Their age varied from two to three months. Thirty guppy fish were placed in a rectangular, glass aquarium containing 400 ml of plant extract water solution in three replicates. Each group of 30 fish was exposed to a test solution. A control, consisting of 30 fish in fresh water, was studied at the same time. The number of dead fish was checked first after 24 h and also after 48 h. All of these bioassay tests were conducted at a room temperature of approximately 27-28 °C, without aeration or changing the water.

RESULTS AND DISCUSSION

It has been well recognized that plant extracts and phytochemicals could be developed into products suitable for mosquito control because many of them are selective, often biodegradable to nontoxic products, and may be applied to mosquito breeding places in the same way as conventional insecticides (Sukumar *et al*., 1991; Hostettmann *et al*., 1997). The phytochemical 8,11,14-eicosatrienoic acid extracted from *G. sepium* demonstrated high toxicity against *C. quinquefasciatus*. The result indicated the LC_{50} values as 4, 6, 9, 11 and 49 ppm and LC_{90} values as 8, 23, 43, 60 and 121 ppm for 1^{st}, 2^{nd}, 3^{rd}, 4^{th} instars and pupae respectively. Singh *et al*. 2006 have studied the mosquito larvicidal properties of *Momordica charantia* against larvae of *An. stephensi, C. quinquefasciatus and A. aegypti* and revealed the LC_{50} values as 0.50, 1.29 and1.45 %, respectively. The himachalene sesquiterpenoid isolated from *Hugonia busseana* showed moderate activity against *Anopheles* mosquito after 24 h at a concentration of 237 ppm (Baraza *et al*., 2007). Gluanol acetate isolated from *Ficus racemosa* (Moraceae) was reported by Rahuman *et al*. (2008), to have shown a high potency against 4^{th} instar larvae of *Anopheles stephensi* with LC_{50} and LC_{90} values of 28.50 and 106.50 ppm, respectively LC_{50} values are 4, 6, 9, 11 and 49 ppm and LC_{90} values as 8, 23, 43, 60 and 121 ppm for 1^{st},2^{nd}, 3^{rd} 4^{th} instars and pupae. Tween 20 did not exert any

Table 1. LC_{50} and LC_{90} values of 8, 11, 14- eicosatrienoic acid of *G. sepium* against larvae and pupae of *C. quinquefasciatus*

Life stages	LC_{50}mg/ml	95% confidence limit		LC_{90}mg/ml	95% confidence limit	
		LL	UL		LL	UL
1st instar	0.004	0.002	0.005	0.008	0.006	0.014
2nd instar	0.006	0.005	0.007	0.023	0.019	0.030
3rd instar	0.009	0.008	0.010	0.043	0.033	0.063
4th instar	0.011	0.010	0.013	0.060	0.044	0.095
Pupae	0.049	0.032	0.107	0.121	0.327	0.017

toxic effect as the control showed no mortality. The effect of the phytochemical 8, 11, 14-eicosatrienoic acid on *C. quinquefasciatus* larvae with LC_{50} and LC_{90} values are given in Table 1. A positive correlation was observed between the concentration of phytochemical and the percentage of mortality. Bioassay studies showed that the 1st instars are more susceptible to the phytochemical. The present study clearly revealed the larvicidal activity of the biochemical 8, 11, 14-eicosatrienoic acid. It produced high toxicity against the larvae and pupae of *C. quinquefasciatus*.

In the toxicity studies on guppy fish, no change in the swimming behaviour and survivability was observed in control as well as in experimental groups clearly indicating the non-toxicity of the phytochemical on non target organisms. The experiments on fish also showed the safety of the phytochemical, 8,11,14-eicosatrienoic acid. According to Rukiye, *et al*. 2003, the 48 h LC_{50} value of deltamethrin, to *Poecilia reticulata* was 5.13 mg/l. Singha *et al*. 2011 have conducted larvicidal assay using *Mesua ferrea* plant extract against *C.quinquefasciatus* mosquito larvae and tested the toxicity in nontarget organisms *P. reticulata*. They found out that plant extracts were safe to those non target organisms that share the same habitat of larvae.

The experiments proved that 8,11,14-eicosatrienoic acid is an effective molecule which could be recommended for the control of the severe threat, acing by the present society from mosquitoes. This work will definitely help maintaining the well being of people, by preventing the diseases spread by mosquitoes. The experiments on fish also showed the safety of the phytochemical, 8,11,14-eicosatrienoic acid on non target organisms.

Acknowledgement

Authors are thankful to University Grants Commission, New Delhi (UGC Letter no:F.FIP/KLMG068TF01 dated on 21-01-2009) India for providing financial support

REFERENCES

Ansari, M. A., Mittal, P. K., Razdan, R. K., Sreehari, U. 2005. Larvicidal and mosquito repellent activities of Pine (*Pinus longifolia*, family: Pinaceae) oil. *Journal of Vector Borne Diseases,* **42**:95-99.

Arivoli, S., Samuel Tennyson and J. Jesudoss Martin. 2011 Larvicidal efficacy of *Vernonia cinerea* (L.) (Asteraceae) leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae) *Journal of Biopesticides,* **4** (1): 37 – 42.

Baraza, LD., Joseph C C., Nkunya M.H.H. 2007 A new cytotoxic and larvicidal himachalenoid, rosanoids and other constituents of *Hugonia busseana*. *Natural Product Research,* **21**: 1027-1031.

Gerber, FJ., Barnard, DR., Ward, RA. 1994 Manual for mosquito rearing and experimental techniques. *American Mosquito Centre Association Bulletin,* **5**: 1-98.

Hostettmann, K and Potterat, O. 1997 Strategy for the isolation andanalysis of antifungal, molluscicidal, and larvicidal agents from tropical plants. In: *Phytochemicals for Pest Control* (Hedin, P.A., Hollingworth, R. M., Masler, E. P., Miyamoto, J. and Thompson,D. G. eds.), ACS Symposium Series 658; American Chemical Society: Washington, DC, 14-26 **PP**.

Kovendan, K., Murugan, K., Vincent, S., Barnard, D.R. 2012. Efficacy of larvividal and pupicidal properties of *Acalypha alnifolia* Klen ex Willd. (Euphorbiaceae) leaf extract and *Metarhisium anisopilae* (Metsch.) against *Culex quinquefasciatus* Say. (Diptera: Cuclicidae). *Journal of Biopesticides*, **5** (sup):170-176.

Nazar, S., Ravikumar, S., Prakash Williams, G., Syed Ali, M., P. Suganthi, P. (2009). Screening of Indian coastal plant extracts for larvicidal activity of *Culex quinquefasciatus*. *Indian Journal of Science and Technology*, **2(**3): 24-27.

Nazli, R., Akhter, M., Ambreen, S., Solangi, A. H., & Sultana, N. (2008). Insecticidal, nematicidal and antibacterial activities of *Gliricidia sepium*. *Pakistan Journal of Botany*, **40(**6): 2625-2629.

Promsiri, S., Naksathit, A., Kruatrachue, M. and Thavara U.. 2006 Evaluations of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non target fish. *Insect Science*, **13**: 179-188.

Rahuman, AA., Venkatesan, P., Geetha, K., Gopalakrishnan, G., Bagavan, A. and Kamaraj, C. 2008 Mosquito larvicidal activity of gluanol acetate, a tetracyclic triterpenes derived from *Ficus racemosa. Linn. Parasitol Research,***102**: 333–339.

Rukiye, V., Figen, U. E., Hilal, P. and Oner, K. 2003 Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). *Ecotoxicology and Environmental Safety*, 55: 82–85.

Sharma, N., Qadry, J. S., Subramanium, B., Verghese, T., Rahman, S. J., Sharma, S. K. and Jalees, S. 1998. Larvicidal activity of Gliricidia sepium against mosquito larvae of Anopheles stephansi, Aedes aegypti and Culex quinquefasciatus. *Pharmaceutical Biology*, **36**(1): 3-7.

Singha, S., Utpal, A. and Goutam, C. 2011. Smoke repellency and mosquito larvicidal potentiality of *Mesua ferra* L. leaf extract against filarial vector *Culex quinquefasciatus* Say. *Asian Pacific Journal of Tropical Biomedicine* S119-S123.

Singh, G. and Prakash, S. 2009. Efficacy of *Bacillus sphaericus* against larvae of malaria and filarial vectors: an analysis of early resistance detection. *Parasitology Research*, **1-4** (4): 763-766.

Singh, R. K., Dhiman, R.C. and Mittal, PK. 2006 Mosquito larvicidal properties of *Momordica charantia* Linn (Family: Cucurbitaceae). *Journal of Vector Borne Diseases*, **43**: 88–91.

Sukumar, K., Perich, M.J. and Boobar, L .R. 1991 Botanical derivatives in mosquito control: a review. *Journal of American Mosquito Control Association*, **7**: 210-237.

Jiji Thomas[1]*, Shonima Govindan M[1], Muraleedhara Kurup G[2]

[1]School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India.

[2]Department of Biochemistry, Kariavattom, Kerala University, Trivandrum, Kerala, India

*Communication author Email: jijimoolayil@gmail.com