

## STUDIES ON TOXICITY OF CERTAIN PLANT EXTRACTS TO LARVAE OF THE YELLOW FEVER MOSQUITO, *Aedes aegypti*, WITH A NOTE ON THEIR EFFECT ON DEVELOPMENT OF THE MOSQUITO LARVAE

Midhun, P. and B. Dhanakkodi

Department of Zoology, Kongunadu Arts and Science College, Coimbatore.

\*E.mail: [midhun.p2010@gmail.com](mailto:midhun.p2010@gmail.com)

### ABSTRACT

A study was carried out to reveal the toxicity of crude extracts obtained from leaves of herbs to the developmental stages of mosquito, *Aedes aegypti*. Further, experiments were conducted to find out the effect of the plant leaf extracts on certain indices of development of mosquito larvae viz., development period, rate of population, rate of emergence, survival rate and growth index. LC<sub>50</sub>/24 hours values of four plants to I, II, III, IV instars and pupae of *Aedes aegypti* were also calculated. The data indicated that there was a considerable increase in the LC<sub>50</sub>/24 values with the age of larvae. Compared to control period of development, form I instar to pupa, extended in the larvae reared in medium of plant extracts. Survival rate and growth index for mosquito larvae were remarkably reduced when treated with plant extracts. To conclude, the leaf extracts of the plant could be used for the control of mosquito larvae. *Kirganelia reticulata* leaf extract found to possess relatively higher toxicity.

**Keywords:** *Aedes aegypti*, Mosquito larvae, LC<sub>50</sub>

### 1. INTRODUCTION

Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to human (Kovendan and Murugan, 2011). The effectiveness of vector control has declined because of the reduced effectiveness of insecticides caused by the emergence of resistance in mosquitoes against the currently used insecticides (Chandre *et al.*, 1998). The application of easily degradable plant compounds is considered to be one of the safest methods to control insect pests and vectors (Alkofahi *et al.*, 1989).

In recent years there has been much interest in natural insecticides derived from plants which are biodegradable, easily available at low cost, and safe for human health. Various studies on the natural plant products as larvicides against mosquito vectors have been reported [Pizarro *et al.*, 1999; Singh *et al.*, 2001; Singh *et al.*, 2005, 2006, 2007, 2010]. However, more concerted efforts would be needed to make these environment friendly compounds viable for field use and for large scale vector control operations.

### 2. MATERIALS AND METHODS

#### 2.1. Test Animal

The larvae of mosquitoes were collected from nearby water bodies and reared in the laboratory until they emerge into adults. They were then identified in to their species and

maintained in separate cages. The adult mosquitoes were maintained in a cage of size 1 cu.ft. A total number of 20 mosquitoes with a sex ratio of 1:1 were regularly maintained in the cage for continued supply of eggs. The adult female mosquitoes were fed with blood of chick in every alternative day. Both male and female were provided with 10% glucose solution. The cotton was always kept moist with the solution and changed everyday. An egg trap (cup) lined with filter paper containing filtered water was always placed at a corner of the cage. This arrangement made collection of eggs easier. The larvae were reared in plastic cups. They were daily provided with commercial fish food (Lyimo *et al.*, 1992) *ad libitum*. Water was changed alternate days. The normal cultures as well as those kept for experimental purpose were covered with muslin cloth which will prevent contamination through foreign mosquitoes.

#### 2.2. Test compounds

Crude water leaf extracts of herbal plants, *Kirganelia reticulata*, *Pavetta indica*, *Cleome viscosa*, Vedathi (Plate I-IV) were used as toxicants in the present study. The leaves were picked out, cleaned and air dry under the shade till they become fit to be powdered. A non quantity of finally powdered leaves of any type of plant was taken in a container with 200 ml of filtered tap water (Unchlorinated) and stirred for 1 hour with

magnetic stirrer and kept for 24 hours. The mixture was then filtered through whatman no:1 filterpaper. The filtrate was used as the experimental medium. The amount of the leaf powder taken at a time was in proportion to the concentration of the medium required. For example, 1% medium of any of the sample was prepared by dissolving 2 gm of leaf powder in 20ml of filtered tap water.

### 2.3. Bioassay Test

To test the efficacy of crude water extracts of the on *Aedes aegypti* at different developmental stages viz., I, II, III and IV instar and pupa were subjected to bioassay experiments. Different concentrations (0.2 - 5%) of any of the test compounds were prepared using filtered tap water as described earlier. Clean plastic cups were used as test containers. 20 larvae at a particular stage of development were exposed to 200ml of a particular concentration of test solution (plate 7-10). Mortality rates of larvae were recorded after 24 hours. Five or more concentrations of attest compound giving between 0 and 100% mortality for larvae at different instar stages were recorded. Parallel control was maintained. Three replicate were done at each concentration. In recording the percentage mortality for each concentration, the moribund and dead larvae were combined. For computing  $LC_{50}/24$  hrs the data were subjected to Finney's method of probit analysis (Finney, 1971).

### 2.4. Toxicity of the plant leaf extracts to the larvae of *Aedes aegypti*

The larvae of *Aedes aegypti* at different instar stages I, II, III, IV and pupae were exposed to medium contain serious of concentrations of extracts obtained from leaves of the test toxicant plants and doses giving mortality of larvae ranging from 0 to 100% was recorded. The data were objected to finney's method of probit analysis to derived median lethal concentration which was expressed in terms of  $LC_{50}/24$  hours.

## 3. RESULTS AND DISCUSSION

$LC_{50}/24$  hours values of *Pavetta indica* to I, II, III and IV instar larvae and pupae of *Aedes aegypti* were 1.6099, 1.704, 1.8092, 1.8776 and 2.3371%, respectively. *Kirganelia reticulata* showed higher toxicity compared to that any other plants used in the present study. This was exhibited from the low  $LC_{50}/24$  hr values recorded: 0.6684, 0.6968, 0.7061, 0.7462, 0.893% for I, II, III and IV instar larvae and pupae, respectively. Median lethal concentration of *Cleome viscosa* to the mosquito

larvae were 1.5764, 1.828, 2.0231, 2.2099 and 2.4391 %, respectively. And those of *Vedathi* were 1.6095, 1.7825, 1.9237, 2.3755 and 2.532% for I, II, III and IV instar larvae and pupae respectively (Table.1 and Fig.1).

A notable observation of the present study was that susceptibility of different larval stages to the leaf extracts of the plants varied according to their age. Susceptibility of first instar larvae to the test compound was always higher compared to that of the larvae at other stages of development which were in the order of II III IV Pupae in their response as the  $LC_{50}/24$  hours values to the mosquito larvae were in the order of I II III IV Pupae.

### 3.1. Effect of plant leaf extracts on pupation and emergence of *Aedes aegypti*

Medium contain Sublethal dose of leaf extracts of any of the plants was individually introduced with 30 I instar larvae of the mosquito. They were provided with fish food *ad libitum* till emergence. The medium was checked every 24 hours to record number of larvae moulted to the next stage and dead if any, were removed. From the data obtained percentage of pupation and adult emergence was calculated. The experiment was continued till the last larvae emerged into adult. The experiment was repeated thrice of every plant extract and a parallel control was maintained.

Mean percentage of larvae pupated in Sublethal medium of *Pavetta indica*, *Vedathi* and *Cleome viscosa* was 85, 86.66 and 92.2 were those reared in control showed 95.53% among the plant extracts used, *Kirganelia reticulata* showed highest effect of pupation by allowing only 80% of larvae to pupate and 43.33% emerged into adult. The percentage of emergence was also considerably low among the larvae treated with extracts of *Pavetta indica*, *Vedathi* and *Cleome viscosa* as 46.66%, 46.83% and 53.33% respectively (Table 2 and Fig 2).

### 3.2. Effect of the plant extracts on indices of development of *Aedes aegypti*

The mosquito larvae were reared in the Sublethal medium of extracts obtained from plants *Pavetta indica*, *Kirganelia reticulata*, *Cleome viscosa*, and *Vedathi*. Daily record of their transmission from one stage to another was maintained till the larvae emerged into adult.

The larvae reared in control medium took 6.5 days for pupation, whereas those grown in leaf extract of *Kirganelia reticulata* pupated on day 10. Extracts of *Pavetta indica* Vedathi and *Cleome viscosa* also considerably prolonged the development period to 7.65, 7.36 and 7.38 respectively compared to that of the control. This value was obtained by dividing percent survival of larvae at pupation by development period. Growth Index for the mosquito larvae at control medium was 15.40. This was considerably less for those *Aedes aegypti* larvae grown in medium made of leaf extracts of *Kirganelia reticulata*, *Pavetta indica*, *Cleome viscosa* and *Vedathi*, 10, 11.11, 11.77 and 12.49, respectively (Table 3 and Fig.3).

Larvicidal property of *Kirganelia reticulata*, *Pavetta indica*, *Cleome viscosa* and *Vedathi* to different instar stages and pupae *Aedes aegypti*, was evidently proved from the median lethal concentration obtained in the present study. LC50/24 hour values of *Kirganelia reticulata* to I, II, III, IV instar stages and pupae were 0.6684, 0.6968, 0.7061, 0.7461 and 0.893%, respectively and that of *Pavetta indica* 1.6099, 1.704, 1.746, 1.8776 and 2.3371%; *Cleome viscosa* 1.5764, 1.8328, 2.0231, 2.2099 and 2.4391% and *Vedathi* 1.6095, 1.7825, 1.9237, 2.3755 and 2.532%, respectively. Though direct report on the toxicity of the plant selected for this study are not available, the result could be compared to those of investigations conducted using products obtained from other plant species.

#### 4. CONCLUSION

In current study, a considerable increase in the LC<sub>50</sub> values with the age of larvae was observed i.e early instar larvae were more susceptible to the plant extracts than late instars. It was found that pupae and fourth instar larvae, in particular, were more tolerant to the toxicants than early instars. The decrease in susceptibility may be attributed to increased size and weight of the older larvae (Gbolade, 2001).

*Aedes aegypti* larvae reared in sublethal concentration of the herbs *Kirganelia reticulata*, *Pavetta indica*, *Cleome viscosa*, *Vedathi* used in the present study showed prolonged pre-adult development time in comparison to control. Larvae in control medium pupated in 6.5 days, whereas those exposed to extracts of the plants took 10, 9, 8.5 and 8 days, among the treated compared to that of control (95.53%). The lowest percentage of pupation (80%) was found at

medium contained leaf extracts of *Kirganelia reticulata*. Computation of Growth Index also confirmed the delayed effect of plant leaf extracts on the development of the mosquito larvae.

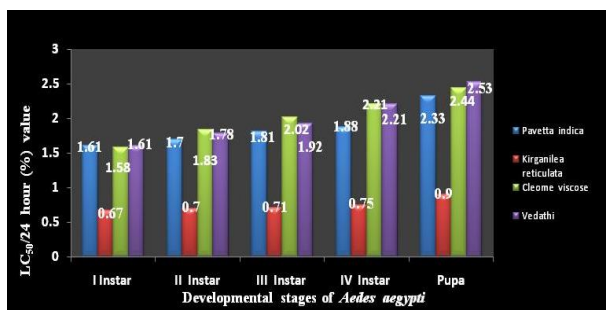
#### REFERENCES

- Alkofahi, A., J.K. Rupprecht, J.E. Anderson, J.L. McLaughlin, K.L. Mikolajczak and B.A. Scott, (1989). Search for new pesticides from higher plants. In: Morand P, ed. Insecticides of plant origin. Washington, DC: American Chemical Society 25-43.
- Chandre, E., E. Damet and M. Darder, (1998). Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Med Vet Entomol.* **12**: 359-66.
- Cheng Chang, S.S. H.T. Chang, S.T. Tsai and K.H. Chen, (2003). Bioactivity of Selected Plant Essential Oils Against the Yellow Fever Mosquito *Aedes aegypti* larvae, *Bioresource Technol* **89**: 99-102.
- Finney, D.J. (1971). Probit analysis (3<sup>rd</sup> edition) Cambridge University Press, Cambridge, UK.
- Gbolade, A. A. (2001). Plant-derived insecticides in the control of malaria vector. *J. Trop. Med. Plants*, **2**: 91-97.
- Kovendan, K. and K. Murugan, (2011). Effective of Medicinal Plants on the Mosquito Vectors from the Different Agroclimatic Regions of Tamil Nadu, India, *Advances in Environmental Biology* **5**(2): 335-344.
- Pizarro, A.P.B., F. Oliveira, J.P. Parente, M.T.V. Melo, C.E. Santos and P.R. Lima, (1999). Utilization of the waste of sisal industry in the control of mosquito larvae. *Rev Soc Bras Med Trop.* **32**: 23-29.
- Singh, R.K., R.C. Dhiman, P.K. Mittal and M.K. Das, (2010). Susceptibility of malaria vectors to insecticides in Gumla district, Jharkhand state, India. *J Vect Born Dis.* **47**: 116-118.
- Singh, R.K., R.C. Dhiman and P.K. Mittal, (2006). Mosquito larvicidal properties of *Momordica charantia* Linn (Family- Cucurbitaceae). *J Vect Born Dis.* **43**: 88-91.
- Singh, R.K., R.C. Dhiman and P.K. Mittal, (2007). Studies on Mosquito larvicidal Properties of *Eucalyptus citriodora* Hook (Family- Myrtaceae). *J. Commun Dis.* **39**: 233-236.
- Singh, R.K., P.K. Mittal and R.C. Dhiman, (2005). Laboratory study on larvicidal properties of leaf extract of *Calotropis procera* (Family- Asclepiadaceae) against mosquito larvae. *J Commun Dis* **37**: 109-13.
- Singh, S.P., K. Raghavendra, R.K. Singh and S.K. Subbarao, (2001). Laboratory Studies on the larvicidal properties of leaf extract of *Solanum nigrum* Linn (Family- Solanaceae). *Current Science.* **81**(12): 1529-30.

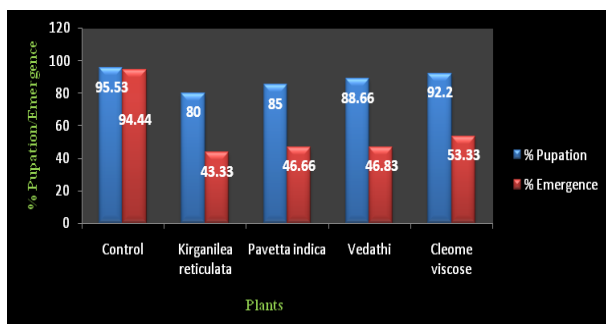
**Table. 1. LC<sub>50</sub>/24 hour (%) value of Plants (1,2,3 and 4) on the developmental stages ( I - IV instars and Pupae) of *Aedes aegypti*.**

S.No	Plants	I Instar larva	II Instar larva	III Instar larva	IV Instar larva	Pupa
1	<i>Pavetta indica</i>	1.01 (1.33-1.9299)	1.70 (1.40-2.07)	1.81 (1.53-2.14)	1.88 (1.59-2.24)	2.33 (2.08-2.60)
2	<i>Kirganilea reticulata</i>	0.67 (0.56-0.81)	0.70 (0.58-0.86)	0.71 (0.60-0.81)	0.75 (0.60-0.92)	0.90 (0.80-1.004)
3	<i>Cleome viscosa</i>	1.50 (1.29-1.90)	1.83 (1.53-2.17)	2.02 (1.70-2.40)	2.21 (1.93-2.50)	2.44 (2.12-2.80)
4	<i>Vedathi</i>	1.01 (1.32-1.94)	1.78 (1.45-2.23)	1.92 (1.61-2.28)	2.21 (2.07-2.71)	2.53 (2.21-2.91)

**Fig.1. LC<sub>50</sub>/24 hour (%) value of Plants (1,2,3 and 4) on the developmental stages ( I - IV instars and Pupae) of *Aedes aegypti*.**



**Fig.2. Change in per cent pupation/emergence of *Aedes aegypti* larvae reared in medium contained Sublethal concentration of plant leaf extracts.**



**Table.3. Change in the development period of *Aedes aegypti* larvae reared in Sublethal medium of plant extracts and control.**

Plants	Days taken	Developmental Period	Growth index
Control	6.5	6.20	15.40
<i>Kirganilea reticulata</i>	10.0	8.0	10
<i>Pavetta indica</i>	9.0	7.65	11.11
<i>Vedathi</i>	8.5	7.36	11.77
<i>Cleome viscosa</i>	8.0	7.38	12.49

**Fig.3. Change in the development period of *Aedes aegypti* larvae reared in Sublethal medium of plant extracts and control.**

