Larvicidal efficacy of leaf extract of two botanicals against the mosquito vector *Aedes aegypti* (Diptera: Culicidae)

KM Remia and S Logaswamy*

PG and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India-641 029.

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Mosquitoes are the most important single group of insects in terms of public health. They transmit a number of diseases, such as malaria, filariasis, dengue fever, chikungunya, Japanese encephalitis, etc. causing millions of deaths every year. The application of easily degradable plant compounds is considered to be one of the safest methods to control insect pests and vectors as an alternative source for the synthetic pesticides. A study was made to monitor the effect of plant extracts on different instars of larvae and pupae of mosquito vector *Aedes aegypti*. Bio-assay was made using the solvent acetone to find out the median lethal concentration (LC$_{50}$). Plants, like *Lantana camara* Linn., *Catharanthus roseus* G. Don which possess insecticidal properties, are seemed to be better vector control agents than the synthetic xenobiotics.

**Keywords:** *Lantana camara*, *Catharanthus roseus*, Botanicals, Mosquito vector, *Aedes aegypti*, Insects, Larvicides.

**IPC code; Int. cl.**—A61K 36/00, A01N 25/00, A01N 65/00, A01N 65/22.

**Introduction**

There are currently more than 300 mosquito species in the world grouped in 39 genera and 135 subgenera. *Aedes aegypti* is a very important disease vector, transmitting the arbovirus causing dengue haemorrhagic fever (DHF) and chikungunya in human$^1$. At present, no effective vaccine is available for dengue; therefore, the only way of reducing the incidence of this disease is by mosquito control, which is frequently dependent on applications of conventional synthetic insecticides$^2$.

Insect pests have been controlled with synthetic insecticide over 50 years but problems of pesticide resistance and negative effects on non-target organisms, including human and environment have been reported$^1$. The synthetic insecticides are more hazardous to handle, leave toxic residues in food products and are not easily biodegradable. The world flora has a variety of plant species and in order to increase the number of plants used for pest control, more studies are being carried out to identify variety of effective substances found in different plant species. Consequently, substances alternative of chemical pesticides, which pollute our natural sources and threaten our future, can be discovered. More than 2000 plant species have been known to produce chemical factors and metabolites of values in pest control programs and among these plants, products of some 344 species have been reported to have a variety of activity against mosquitoes$^3$.

More important fact is that the plant extracts are sometimes more effective than the synthetic pesticide and phytochemicals have major role in mosquito control programme$^5-7$. Though several plants from different families have been reported for mosquitocidal activity, only a very few botanicals have moved from laboratory to the field use, like neem based insecticides, which may be due to the light and heat instability of phytochemicals compared to synthetic insecticides$^8$. An excellent review of the activity of neem, *Azadirachta indica* A. Juss. and other plants on mosquito control potential has been published for further studies$^9$.

*Lantana camara* Linn. (Family-Verbenaceae) is hairy shrub, native to tropical America, found wild and cultivated as an ornamental or hedge plant. About 8 species of Lantana occur in India and different parts of these plants are reported to be used in traditional medicine for the treatment of various human ailments such as ulcers, eczema eruptions, malaria and rheumatism$^{10}$. *Catharanthus roseus* G. Don belonging to Family- Apocynaceae, grows throughout India and found in wet and sandy land, possesses high medicinal value (Plate 1).

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*Correspondent author
E-mail: remiabalan@gmail.com, slogaswamy@yahoo.co.in
Phone: 09952833209, 09443006007
The present study was carried out to determine the biological activities of these two plants *Lantana camara* Linn. and *Catharanthus roseus* G. Don against mosquito species *Aedes aegypti*. The result of the present study would be useful in promoting research aiming at the development of new agents for mosquito control based on bioactive chemical compounds from indigenous plant sources.

**Materials and Methods**

**Collection of plants and preservation of acetone extract of leaves**

The leaves of *L. camara* and *C. roseus* were collected locally in and around Coimbatore identified by experienced botanist and they were washed with water, dried in shade at room temperature and powdered with the help of mechanical device. The dried powder (100g) was extracted with 300ml of acetone solvent with a minimum of 8h up to 20h in a Soxhlet apparatus. After extraction the solvent was evaporated using vacuum evaporator to obtain the extract in dried form. Dried residues obtained were 12g (*L. camara*) and 10.5g (*C. roseus*) of acetone extract; they were stored in airtight desiccators and used for experiment.

**Collection and storage of experimental animals**

The eggs of *Aedes aegypti* were collected from National Institute for Communicable Disease Center (NICDC), Coimbatore. The larvae were cultured and maintained in the laboratory at 27 ± 1°C and 85% of relative humidity. Larval forms were maintained in tray by providing dog biscuit and yeast powder in the 3:1 ratio.

**Test for larvicidal activity**

The laboratory colonies of *Aedes aegypti* were used for the larvicidal activity. II and IV instar larvae and pupae of the selected mosquito species were kept in 500ml glass beaker and different concentration of selected plant extract was added to find out LC$_{50}$.

**Bioassay experiment**

Different concentrations of extract (between 50ppm to 500ppm) were prepared using distilled water. The mosquito larvae were treated with extract by using the method of WHO. Ten larvae of *Aedes aegypti* were introduced in different test concentration of both plant extracts along with a set of control containing distilled water without any test solution. After adding the larvae, the glass dishes were kept in laboratory at room temperature. By counting the number of dead larvae at 24hrs of exposure, the mortality rate and the median lethal concentration were obtained. Five replications were maintained for each concentration. Dead larvae were removed as soon as possible in order to prevent decomposition, which may cause rapid death of the remaining larvae. The water used for the study was analyzed by using the method of APHA.

The LC$_{50}$ values and upper, lower confidence limit and chi-square test were calculated according to Probit methods of Finney.

**Results and Discussion**

Physical and chemical characteristics of water used for the study, like temperature 26.5±0.6°C, pH 7.5±0.5, dissolved oxygen 4.5±0.4mg/l, dissolved carbon dioxide 1.5±0.2mg/l, salinity 1.0±0.1ppt and
alkalinity 129±0.6 mg/l were within the permissible limits throughout the study periods.

The 24h bioassay is a major tool for evaluating the toxicity of phytotoxins, and a number of researchers have been applying this method to assess the toxic effect of different plant extraction against mosquitoes\(^{16,17}\). The mosquito larvae exposed under plant extracts showed significant behavioral changes. The changes were observed within 30 minutes of exposure. The most obvious sign of behavioral changes observed in *Aedes aegypti* was inability to come on the surface. The larvae also showed restlessness, loss of equilibrium and finally led to death. These behavioral effects were more pronounced in case of *C. roseus* than *L. camara* extracts after exposures. These effects may be due the presence of neurotoxic compounds in both the plants. No such behavioral changes were obtained in control groups.

Results of the experiment conducted for evaluating the larvicidal efficacy of both plants showed that they are toxic to the *Aedes aegypti* larvae. Lethal concentration of leaf extracts were 203.49ppm (*L. camara*) and 230.76ppm (*C. roseus*) for the IV instar larvae and it was 281.35ppm for pupae at 24hrs of exposure periods. The same concentration of plant extract gave 100% mortality after 96hrs of test period. The leaf extracts of *C. roseus* showed more effect than the *L. camara* toxicity (Table 2).

Neem and Karanja oil are employed extensively in Ayurvedic formulations for various purposes for

<table>
<thead>
<tr>
<th>Plants used</th>
<th>Stages of exposure</th>
<th>95% Fiducial LC(_{50}) (ppm)</th>
<th>Chi-square test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. camara</em></td>
<td>II instar</td>
<td>203.49</td>
<td>197.23 209.60</td>
</tr>
<tr>
<td></td>
<td>IV instar</td>
<td>230.76</td>
<td>235.64 225.17</td>
</tr>
<tr>
<td></td>
<td>Pupae</td>
<td>281.35</td>
<td>287.52 275.10</td>
</tr>
<tr>
<td><em>C. roseus</em></td>
<td>II instar</td>
<td>75.31</td>
<td>79.64 71.48</td>
</tr>
<tr>
<td></td>
<td>IV instar</td>
<td>156.85</td>
<td>161.79 150.64</td>
</tr>
<tr>
<td></td>
<td>Pupae</td>
<td>207.83</td>
<td>212.28 203.19</td>
</tr>
</tbody>
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<tr>
<th>Plants used</th>
<th>Parameters</th>
<th>Effective concentration in ppm</th>
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<tbody>
<tr>
<td><em>L. camara</em></td>
<td>II instar Larval Mortality (%)</td>
<td>Control 50 100 150 200 250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 4 22 36 48 65</td>
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<tr>
<td></td>
<td>IV instar Larval Mortality (%)</td>
<td>Control 200 210 220 230 240</td>
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<tr>
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<td>0 19 27 33 53 60</td>
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<tr>
<td></td>
<td>Pupae Pupal Mortality (%)</td>
<td>Control 220 240 260 280 300</td>
</tr>
<tr>
<td></td>
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<td>0 11 23 29 50 65</td>
</tr>
<tr>
<td><em>C. roseus</em></td>
<td>II instar Larval Mortality (%)</td>
<td>Control 20 40 60 80 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 24 30 39 50 69</td>
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<td>IV instar Larval Mortality (%)</td>
<td>Control 50 100 150 200 250</td>
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<td>Pupae Pupal Mortality (%)</td>
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<td>0 33 39 50 64 79</td>
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years. The use of vegetable oil presents a better option in comparison to chemical pesticides for the larval mosquito control, as chemicals may cause environmental hazards and proved troublesome in the long run. Extensive research has been carried out on the effect of botanical derivatives of the neem tree and its derivatives.

Methanolic extract of the leaves of *Atalantia monophylla* Corr. (Rutaceae) were evaluated for mosquitocidal activity against the immature stages of mosquitoes, *Culex quinquefasciatus*, *Anopheles stephensis* and *Aedes aegypti* in the laboratory. A survey of literature on control of different species of mosquito revealed that assessment of the efficacy of different phytochemicals obtained from various plants has been carried out by a number of researches on the field of vector control *Ageratina adenophora* (Spreng.) R. M. King & H. Rob. showed toxic effects on the mosquito species of *Aedes aegypti* and *Culex quinquefasciatus*. *Albizia amara* Boiv. and *Ocimum sanctum* Linn. showed larvicidal and repellent properties against *Aedes aegypti* and neem seed kernel extracts showed higher larvicidal activity. The authors determined the LC₅₀ and observed the behavioural changes and mortality in the larvae. Similar observations were noticed in the present study and support the potential applications of these herbs in mosquito control measures.

Molluscicidal and mosquito larvicidal efficacy of *Lantana indica* Roxb. and *Morinda officinalis* Linn. have already been reported and observed them safe for human health. These investigations support the present work.

**Conclusion**

As the leaf extract of *L. camara* and *C. roseus* are highly toxic even at low doses these plants may eventually prove to be useful larvicide. Further analysis is required to isolate the active principles and optimum dosages, responsible for larvicidal and adult emergence inhibition activity in *Aedes aegypti*. The product of these plants can be well utilized for preparing biocides or phytochemicals from which all the non-target organisms can be rescued from harmful vectors. These plants would be eco-friendly and may serve as suitable alternative to synthetic insecticides as they are relatively safe, inexpensive and are readily available in many areas of the world.

**References**