Evaluation of larvicidal effect of *Dysoxylum binectariferum* Hook against *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* L.

Uma Masur¹, Hemanth Kumar²* and Ashwani Kumar²

¹Department of Botany, Parvatibai Chowgule College, Gogol, Margao, Goa-403601, India.
²National Institute of Malaria Research, Field Station, Panaji, Goa-403001, India.

Received for publication: September 29, 2015; Revised: October 04, 2015; Accepted: November 13, 2015

**Abstract:** In the present study larvicidal activity of methanolic extract of leaf of *Dysoxylum binectariferum* Hook (Meliaceae) was assayed against 3rd & 4th instar larv of 3 mosquito vector species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. The bioassays revealed that *Cx. quinquefasciatus* was most sensitive species. The dose mortality data were subjected to log probit regression analysis to determine median lethal concentrations, LC₅₀ and LC₅₀ after 24-hour exposure. The highest mortality was observed in *Cx. quinquefasciatus* [LC₅₀ = 340 ppm, (95% CI: 240 - 460 ppm); LC₅₀ = 600 ppm (CI: 390-1083 ppm)] followed by *As. aegypti* [LC₅₀ = 3236 ppm, (CI: 2500-4180 ppm); LC₅₀ = 55410 ppm (CI: 34720-103910 ppm)] and *An. stephensi* [LC₅₀ = 13460 ppm (CI: 12840-14180 ppm); LC₅₀ = 18010 ppm (CI: 16650-20360 ppm)]. As a larvicide, leaf extracts of *Dysoxylum binectariferum* could be explored further.

**Key words:** *Dysoxylum binectariferum* Hook; larvicidal effect; leaf extract; *Anopheles stephensi*; *Culex quinquefasciatus*; *Aedes aegypti*.

**INTRODUCTION**

Mosquito borne diseases take a heavy toll of mankind in terms of morbidity and mortality. To curb these diseases, vector control is as important as diagnosis and treatment of the cases. So far, chemical insecticides have been the mainstay of the vector control programmes. The selection pressure imposed by continued use of limited number of conventional insecticides is responsible for development of resistance in mosquito populations. Plants have been the source of many drugs, repellents as well as insecticides. Plant sources have received renewed attention as agents for disease vector control. They offer an alternative source of insect-control agents as they contain a range of bioactive chemicals many of which are selective and have little or no harmful effect on non-target organisms and the environment.

In the past, plants of family Meliaceae have been tested for their insecticidal contents. Several investigators have demonstrated biological effects of limonoids & triterpenoids on insects isolated from different species of *Dysoxylum*, which is also a member of Meliaceae. Using 4% leaf extract of *D. malabaricus* Senthil, 2006 has reported 90% mortality in all the instars of *An. stephensi*. *Dysoxylum binectariferum* Hook (Meliaceae) is a large tree endemic to Western Ghats. Though limonoids and alkaloids from this tree showed pharmacological activity its bioactivity against mosquito larvae remained unexplored. Hence in the present study, methanolic extract of leaves of *D. binectariferum* was tested for larvicidal activity against 3rd & 4th instar larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*, the vectors of Malaria, Filaria and Dengue and Chikungunya respectively.

**MATERIALS AND METHODS**

**Plant materials**

Fresh, mature, healthy leaves were collected from the trees of *D. binectariferum* growing in Ambegaoo village near Kolhapur, Maharashtra, India. Leaves were brought to the laboratory washed thoroughly under the tap water and dried at room temperature for further processing.

**Extraction**

Methanol extracts of leaves were obtained according to the method of Warthen et al., 1994. First the plant leaves were crushed to a fine particle size and dried in shade at room temperature. 100 gm of dried crushed material were stirred in 1000 ml of methanol. Solution was left to rest overnight and then filtered through Whatman no. 40 filter paper. The procedure was repeated for solid filtration and two filtrates were combined. The solvent was evaporated at 50º C and a dark green residue was obtained. This crude extract was then used to prepare a stock solution. Crude extract was dissolved in methanol to 500 ml of volume. A drop of emulsifier (Tween-20 Merck Specialties Private Limited) was added to the stock solution to prepare test solutions.

**Bioassay**

Preliminary screening bioassays with the obtained leaf extracts of plant, *D. binectariferum* were carried out against the larvae of the mosquito species, *An. stephensi*. These mosquitoes were reared as per WHO methodology in the insectary of the National Institute of Malaria Research, Field Unit at Campal, Panaji, Goa, India. Larvae were fed on a diet of commercially available baby food of trusted brand (Nestle) mixed with powdered fish food in a ratio of 2:1. Late 3rd and early 4th instar larvae were used to screen the larvicidal activity of the methanolic extract of the leaves.

Main bioassay on 3rd - 4th instar larvae of three vectors, *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* were carried out. Methanolic leaf extracts of *D. binectariferum* of various concentrations were prepared as per the standard WHO Protocol. Larvae of the three mosquito species were reared in the mosquito colony maintained at 26 ± 2ºC, 70 ± 10% RH and a photoperiod of 12:12, L: D at the National Institute of Malaria Research, Goa. Methanol solution of respective concentrations was used as a control. Four replicates of 25 larva per concentration were used for...
Statistical analysis

The percent mortalities were corrected using Abbott’s formula and the average larval mortality data were subjected to probit analysis for calculating LC50 and LC90. 95% confidence limit and Chi-square values were calculated using the SPSS (Statistical Package for Social sciences) software. P<0.05 was considered to be statistically significant.

Table 1: Log Probit values for Cx. quinquefasciatus, Ae. aegypti and An. stephensi, against methanolic leaf extract of D. binectariferum based on bioassays in ppm

<table>
<thead>
<tr>
<th>Species</th>
<th>LC50 (ppm)</th>
<th>95% Confidence limit (lower-upper)</th>
<th>LC90 (ppm)</th>
<th>95% Confidence limit (lower-upper)</th>
<th>SE</th>
<th>Z</th>
<th>p</th>
<th>S/NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culex quinquefasciatus</td>
<td>340</td>
<td>240-460</td>
<td>660</td>
<td>390-1083</td>
<td>0.100</td>
<td>10.309</td>
<td>.000</td>
<td>S</td>
</tr>
<tr>
<td>Aedes aegypti</td>
<td>3236</td>
<td>2500-4180</td>
<td>55410</td>
<td>34720-103910</td>
<td>0.088</td>
<td>11.766</td>
<td>.000</td>
<td>S</td>
</tr>
<tr>
<td>Anopheles stephensi</td>
<td>13460</td>
<td>12840-14180</td>
<td>18010</td>
<td>16650-20360</td>
<td>1.013</td>
<td>10.021</td>
<td>.000</td>
<td>S</td>
</tr>
</tbody>
</table>

SE= Standard Error, Z= Z-test, P= Probability, S= Significant NS= Not Significant

![Graph showing larval mortality](image)

Results and Discussion

Results of the study to determine the effect of treatment of methanolic leaf extracts of D. binectariferum on the III and IV instar larvae of An. stephensi, Ae. aegypti and Cx. quinquefasciatus indicated deleterious effect resulting in larval mortality. There was no mortality in control. The results of screening are shown in Table 1 & Fig.1.

Many plant derived chemicals have larvicidal effects. The differential responses induced by their active ingredients on various species of mosquitoes are influenced by several factors such as plant species, parts of the plant, solvents used for extractions, geographical location where the plants grow and methods employed for extraction. Preliminary screening is considered a good approach to evaluate potential larvicidal activity of plants and their parts. Approximately 2,000 plant derivatives have been screened for insecticidal properties and good deal of work is being done in India to search for alternative eco-friendly and effective larvicides of plant origin.

The mortality in Cx. quinquefasciatus larvae in the range of 15-100% was observed at dose range of 20-18000 ppm with LC50 and LC90 estimated as 340 (95% CI: 240-460 ppm) and 600 ppm (95% CI: 390-1083 ppm). On the other hand, in Ae. aegypti, LC50 was 3236 ppm (95% CI: 2500-4180) and LC90 of 55410 (95% CI: 34720-103910 ppm). In case of An. stephensi LC50 and LC90 were 13460 ppm (95% CI: 12840-14180) and 18010 ppm (95% CI: 16650-20360 ppm). These results imply that although effective, there was varied dose mortality response in the 3 test species. When LC50 values obtained for the 3 species were compared, Cx. quinquefasciatus was 9.5 X & 39.5 X more sensitive than Ae. aegypti and An. stephensi respectively and Ae. aegypti was 4.15 X more sensitive than An. stephensi.

The pattern of larvae mortality in the three vectors also differed (Fig. 1). The mortality in Cx. quinquefasciatus increased steeply at lower doses but it stabilized with little incremental effect at higher doses. In contrast, mortality in An. stephensi increased gradually with the increasing doses. Ae. aegypti mortality curve was between these two extremes. The extent of toxicity however varied in these three species, Cx. quinquefasciatus being most sensitive while An. stephensi was the least sensitive among the three species.
the need to explore the beneficial effects of essential oil-based products as supplementary and complimentary measures for the control of mosquito borne diseases[3]. Since in the current study, bioassay with methanolic extracts of D. binectariferum have shown promising larvicidal activity, it may be worthwhile to consider enhancing the efficacy by blending it with the other agents as an effective and affordable approach to control vector mosquitoes responsible for various diseases. Also awareness can be brought about this tree as a suitable source for controlling mosquito larvae in small breeding places thus reducing the chemical burden on the environment and also promote sustainable utilization of this locally available bioresource by the rural communities. Thus D. binectariferum if used judiciously can also be a component in integrated vector management.

**CONCLUSION**

We evaluated the role of D. binectariferum methanolic leaf extract for its larvicidal activity against Aa. stephensi, Aa. aegypti and Cs. quinquefasciatus as target species. From the results, it can be concluded that the crude methanolic extracts of D. binectariferum possess excellent potential for controlling mosquito vectors. The results reported in this study open up the possibility for further investigations on larvicidal properties of natural product extracts. These results could encourage the search for more botanical sources of natural compounds offering an alternative to synthetic repellents and insecticides. These environmentally safer and eco-friendly approaches need to be encouraged and popularized among local communities to achieve integrated vector control.

**ACKNOWLEDGEMENTS**

Thanks are due to University Grants Commission, Western Zone, Pune for financial assistance vide letter No.: 47- 444/08 (WRO) dated 18th Dec. 2008. The authors are also thankful to Dr. S. R. Yadav, Professor and Head, Shivaji University, Kolhapur for identifying the plant. We acknowledge the cooperation of the Insectary staff of NIMR, Goa for their help and cooperation. Thanks to Sh. Ajeet Kumar Mohanty of NIMR for the statistical analysis of data. This manuscript bears publication screening committee approval no. ’056/2014’ of National Institute of Malaria Research, New Delhi.

**REFERENCES**


25. Sharma VP. Urbanization and Vector Borne Diseases Commentary. Malaria Research Center (ICMR), New Delhi; 2001


Source of support: University Grants Commission, Western Zone, Pune for financial assistance
Conflict of interest: None Declared