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

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Abstract: In the present study larvicidal activity of methanolic extract of leaf of *Dysoxylum binectariferum* Hook (Meliaceae) was assessed against 3rd & 4th instar larvae 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, LC_{50} and LC_{90} after 24-hour exposure. The highest mortality was observed in *Cx. quinquefasciatus* [LC_{50} = 340 ppm, (95% CI: 240 - 460 ppm); LC_{90} = 600 ppm (CI: 390- 1083 ppm)] followed by *Ae. aegypti* [LC_{50} = 3236 ppm, (CI: 2500-4180 ppm); LC_{90} = 55410 ppm (CI: 34720-103910 ppm)] and *An. stephensi* [LC_{50} = 13460 ppm (CI: 12840-14180 ppm); LC_{90} = 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[2]. To curb these diseases, vector control is as important as diagnosis and treatment of the cases. So far, chemical insecticides have been the main stay 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 [4, 18, 23, 10, 7, 24] necessitating search for new products that are novel, environmentally safe, target specific and readily degradable. 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[8] many of which are selective and have little or no harmful effect on non-target organisms and the environment[12,3]. In the past, plants of family Meliaceae have been tested for their insecticidal contents[3,11,33]. Several investigators have demonstrated biological effects of limonoids & triterpenoids on insects isolated from different species of *Dysoxylum,* which is also a member of Meliaceae [23, 9, 22, 14, 13]. Using 4% leaf extract of *D. malabaricum* 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 [26] 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 Ambegao 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.

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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 temperature of 50° C and a dark green residue from leaves 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 extract for complete solubility in the solution. The stock solution was then diluted to required concentration with water 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 [32] 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[20]. Larvae of the three mosquito species were reared in the mosquito colony maintained at 26 ± 2°C, 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 larvae per concentration were used for

all the experiments and control. Mortality was recorded after 24 hours of exposure based on counts of dead larvae.

Statistical analysis

The percent mortalities were corrected using Abbott's formula [1] and the average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90}, 95 per cent confidence limits and Chi-square values were calculated using the SPSS (Statistical package for Social sciences) software. P<0.05 was considered to be statistically significant.

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.

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

Species	LC_{50} (ppm)	95% Confidence limit (lower-upper)	LC_{90} (ppm)	95% Confidence limit (lower -upper)	SE	Z	p	S/ NS
Culex quinquefasciatus	340	240-460	600	390-1083	0.100	10.309	.000	S
Aedes aegypti	3236	2500-4180	55410	34720-103910	0.088	11.766	.000	S
Anopheles stephensi	13460	12840-14180	18010	16650-20360	1.013	10.021	.000	S

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

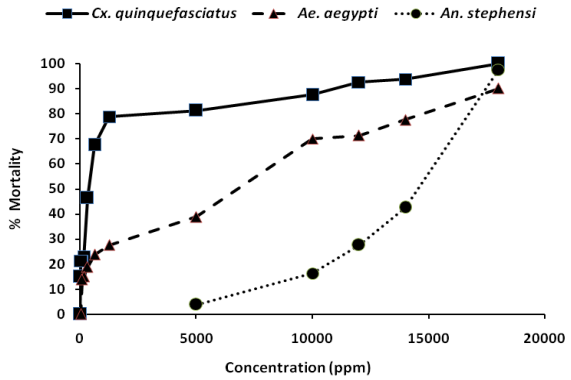

Figure 1: Bioassay of leaf extract of *D. binectariferum* against larvae of different vector species

The mortality in *Cx. quinquefasciatus* larvae in the range of 15-100% was observed at dose range of 20-18000 ppm with LC_{50} and Lc_{90} estimated as 340 (95% CI: 240-460 ppm) and 600 ppm (95% CI: 390-1083 ppm). On the other hand, in *Ae. aegypti*, LC_{50} was 3236 ppm (95% CI: 2500-4180) and LC_{90} of 55410 (95% CI: 34720-103910 ppm). In case of *An. stephensi* LC_{50} and LC_{90} were 13460 ppm (95% CI: 12840-14180 ppm) and 18010 ppm (95% CI: 16650-20360 ppm). These results imply that although effective, yet there was varied dose mortality response in the 3 test species. When LC_{50} 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.

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[23, 19,31]. Preliminary screening is considered a good approach to evaluate potential larvicidal activity of plants and their parts[27]. Approximately 2,000 plant derivatives have been screened for insecticidal properties [2] and good deal of work is being done in India to search for alternative eco-friendly and effective larvicides of plant origin[21]. This study here has shown larvicidal potentiality of methanolic leaf extract of *Dysoxylum binectariferum* against the three mosquito species with varied activities, *Cx. quinquefasciatus* being the most sensitive mosquito species followed by *Ae. Aegypti and An. stephensi*. In an earlier study, by Senthil *et al.,* 2006, 4% methanolic leaf extract of *D. malabaricum* resulted in 92% mortality of *An. stephensi* 4th instar larvae. Tiwary *et al.,* 2007 found similar activity when they exposed larvae of *An. stephensi* to the essential oils.

Though in our experiments larvicidal activity of *D. binectariferum* against III & IV instar larvae of all 3 test species was observed at comparatively high doses, much scope exists to explore effects of this extract on lower instars of larvae and the overall effect of exposure at sub lethal doses on pupae as well as fecundity and longevity of adults emerged from such pupae. It will be also interesting to see whether the effects of extracts in various other solvent systems differ, as there are certain bioactive principles the activity of which gets enhanced in certain solvent systems. Raghvendra *et al.,* 2009 reported that hexane extract was comprehensively more effective against mosquitoes when compared with aqueous extract. There are also reports wherein the extracts when tested individually were found effective at much higher doses or ineffective totally [5, 30, 29, 16, 6, 17]. On the contrary, when suitably blended, they might show enhanced activity due to blending effects. Recent finding of promising larvicidal activities of many essential oils against mosquito vectors have reemphasized

the need to explore the beneficial effects of essential oil-based products as supplementary and complimentary measures for the control of mosquito borne diseases[23]. 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 *An. stephensi, Ae. aegypti* and *Cx. 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.

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