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LARVICIDAL ACTIVITY OF *CASSIA FISTULA* FLOWER AGAINST *CULEX TRITAENIORHYNCHUS* GILES, *AEDES ALBOPICTUS* SKUSE AND *ANOPHELES SUBPICTUS* GRASSI (DIPTERA: CULICIDAE)

M. GOVINDARAJAN*

Unit of Vector Biology and phytochemistry, Department of Zoology, Annamalai University, Annamalainagar-608 002, Tamilnadu, India

*Corresponding Author Email: drgovind1979@gmail.com, Tel: +91 9080220148

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ABSTRACT

Mosquitoes transmit serious human diseases, causing millions of deaths every year and the development of resistance to chemical insecticides resulting in rebounding vectorial capacity. Plants may be alternative sources of mosquito control agents. The present study assessed the role of larvicidal efficacy of different solvent flower extract of *Cassia fistula (C. fistula)* against *Culex tritaeniorhynchus* (*Cx. tritaeniorhynchus*) *Aedes albopictus* (*Ae. albopictus*) and *Anopheles subpictus* (*An. subpictus*). Twenty five early third instar larvae of *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus* were exposed to various concentrations and were assayed in the laboratory. The larval mortality was observed after 24 h of treatment. Among three solvent extracts tested the maximum efficacy was observed in the methanol extract and the LC_{50} and LC_{90}values of *C. fistula* flower against early third instar of *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus* were 136.59, 118.64, 96.51 ppm and 243.67, 231.79, 174.39 ppm, respectively. No mortality was observed in controls. The chi-square values were significant at $p < 0.05$ level. From the results it can be concluded the crude extract of *C. fistula* flower was an excellent potential for controlling *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus* mosquito larvae.

Keywords: Larvicidal activity, *Culex tritaeniorhynchus, Aedes albopictus, Anopheles subpictus, Cassia fistula* flower.

INTRODUCTION

Mosquitoes act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, *etc.*, in almost all tropical and subtropical countries and many other parts of the world. To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological

magnification through ecosystem, and increasing insecticide resistance on a global scale (Russell I *et al*., 2009) It has prompted researchers to look for alternative approaches ranging from provision of or promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species (Govindarajan *et al*., 2011*a &b*).The larvicidal and ovicidal activity of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of the leaf of three plants, *Eclipta alba*, *Cardiospermum halicacabum* and *Andrographis paniculata*, were tested against the early third-instar larvae of *An. stephensi* (Govindarajan, 2011*a*).

The larvicidal and ovicidal efficacy of different extracts of *C. halicacabum* against *Cx. quinquefasciatus* and *Ae. aegypti* (Govindarajan, 2011*b*). Samidurai *et al*. (2009) observed that the leaf extracts of *Pemphis acidula* were evaluated for larvicidal, ovicidal and repellent activities against *Cx. quinquefasciatus* and *Ae. aegypti*. Govindarajan (2010*a*) investigated the larvicidal efficacy of different extracts of *Ficus benghalensis* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. The larvicidal activity of *Sida acuta* was evaluated against 3rd instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus* (Niraimathi *et al*., 2010). The larvicidal activity of methanol extracts of *Cassia obtusifolia, C. tora* and *Vicia tetrasperma* were tested against early fourth stage larvae of *Ae. aegypti* and *Cx. pipiens* (Jang *et al*., 2002). The acetone, chloroform, ethyl acetate, hexane, and methanol leaf extracts of *Azardirachta indica*, *Achyranthes aspera*, *Lucus aspera, Morinda tinctoria*, and *Ocimum sanctum* were studied against the early fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* (Bagavan *et al*., 2008). Laboratory evaluation of a phytosteroid compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of *Cx. quinquefasciatus* (Diptera: Culicidae) and nontarget organisms (Ghosh *et al*., 2008). Ovicidal and repellent activities of methanol leaf extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were evaluated against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* (Govindarajan *et al*., 2011*a*). In view of this, in this study, we examine the mosquito larvicidal activity of different solvent extracts from the plant flowers of *C. fistula* against the larvae of *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus*.

MATERIALS AND METHODS

Plant collection

Fully developed flowers of the *C. fistula* were collected from different regions of Cuddalore District, Tamilnadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of plant phytochemistry division, Department of Zoology, Annamalai University.

Preparation of the extract

The flowers were washed with tap water, shade dried and finely ground. The finely ground plant material (1.0 kg/solvent) was loaded in soxhlet apparatus and was extracted with three different solvents namely ethyl acetate, chloroform and methanol individually. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of this plant varies with the solvents used. The *C. fistula* with three different solvents yielded 102.34, 96.21 and 121.25 gm of crude residue respectively. Standard stock solutions were prepared at 1% by dissolving the residues in appropriate solvent. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassay.

Test organisms

Cx. tritaeniorhynchus, Ae. albopictus and *An. subpictus* were reared in the vector control laboratory, Department of zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at 28 ± 2°, 70 - 85 % relative humidity (RH), with a photo period of 14 h light, 10 h dark.

Larvicidal bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by World Health Organization (2005). Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200ml of water. The appropriate

volume of dilution was added to 200ml water in the cups to obtain the desired target dosage starting with the lowest concentration. Four replicates were set up for each concentration and an equal number of controls were set up simultaneously using tap water. To this 1 ml of appropriate solvent was added. The LC_{50} value was calculated after 24 h by probit analysis (Finney, 1979).

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit and chi-square values were calculated using the SPSS12.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ were considered to be statistically significant.

RESULTS

The efficacy of *C. fistula* flower was tested against the early third larvae of *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus.* The data were recorded and statistical data regarding the LC_{50}, LC_{90}, Chi-square and 95% confidence limits were calculated (Table 1). The methanolic extract of *C. fistula* flower showed highest larvicidal activity against *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus* and the LC_{50} and LC_{90} values of *C. fistula* flower against early third larvae of *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus* were 136.59, 118.64, 96.51 ppm and 243.67, 231.79, 174.39 ppm, respectively. No mortality was observed in control. The chi-square value were significant at $p < 0.05$ level.

Table 1. Larvicidal activity of different solvent crude extracts of *C. fistula* flower against *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus.*

Mosquitoes	Solvents	LC_{50} (ppm)	LCL-UCL	LC_{90} (ppm)	LCL-UCL	χ^2
Culex tritaeniorhynchus	Ethyl acetate	167.29	132.11-180.64	286.26	271.62-299.39	13.576*
	Chloroform	148.36	131.02-162.79	264.92	252.57-279.33	13.010*
	Methanol	136.59	121.76-156.64	243.67	232.07-269.64	13.653*
Aedes albopictus	Ethyl acetate	153.67	139.79-174.61	274.02	259.32-286.28	15.176*
	Chloroform	126.56	110.82-139.47	247.62	231.68-259.57	14.126*
	Methanol	118.64	109.24-131.73	231.79	219.14-247.33	15.749*
Anopheles subpictus	Ethyl acetate	132.14	124.06-149.76	239.64	221.26-256.37	14.636*
	Chloroform	107.67	92.94-126.71	192.76	178.15-209.64	14.197*
	Methanol	96.51	82.57-112.69	174.39	162.76-195.19	13.166*

*Significant at $P<0.05$ level. LC_{50}: Lethal Concentration; LCL: Lower Confidence Limit;

UCL: Upper Confidence Limit.

DISCUSSION

The results showed that crude extract of *C. fistula* flower have significant larvicidal activity against *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus*. This result is also comparable to earlier reports of the petroleum ether (60–80°C) extracts of the leaves of *V. negundo* were evaluated with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/L against larval stages of *Cx. tritaeniorhynchus* in the laboratory (Karunamoorthi *et al*., 2008). Mullai and Jebanesan (2007) have reported that the ethyl acetate, petroleum ether and methanol leaf extracts of *Citrullus colocynthis* and *Cucurbita maxima* showed that the LC_{50} values were 47.58, 66.92 and 118.74 ppm and 75.91, 117.73 and 171.64 ppm, respectively, against *Cx. quinquefasciatus* larvae. (Mathivanan *et al*., 2010) to determine the LC_{50} and LC_{90} values of crude methanol extract of leaves of *E. coronaria* on *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* larvae in 24 h were 72.41, 65.67, 62.08 and 136.55, 127.24 and 120.86 mg/l, respectively. The effect of water extract of citrus-seed extract showed LC_{50} values of 135, 319.40 and 127, 411.88 ppm against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus* (Sumroiphon

et al., 2006). The benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *A. paniculata* was found to be more effective against *C. quinquefasciatus* than *A. aegypti*. The LC_{50} values were 112.19, 137.48, 118.67, 102.05, 91.20 ppm and 119.58, 146.34, 124.24, 110.12, 99.54 ppm respectively (Govindarajan, 2011*c*).

The benzene extracts of *C. vulgaris* exerted 100% mortality (zero hatchability) at 250 ppm, a very low hatchability (11.8%) at 200 ppm, and complete ovicidal activity at 300 ppm. The fraction I at 80 ppm exerted a very low hatchability rate of 3.2% followed by fraction II (6.9%), and fractions III and IV afforded 4.9% and 5.3% hatchability recorded against *An. stephensi* and *Ae. aegypti*, respectively (Mullai *et al*., 2008). Govindarajan (2010*b*) to evaluate the larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging between 38 to 48 mg/L. The crude extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *An. stephensi* for 180 min followed by *Ae. aegypti* (150 min) and *Cx. quinquefasciatus* (120 min). The essential oil from the leaves of *C. anisata* exhibited significant larvicidal activity, with 24 h LC_{50} values of 140.96, 130.19 and 119.59 ppm, respectively (Govindarajan, 2010*c*).

Compared with earlier authors reports, our results revealed that the experimental plant extracts were effective to control *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus.* From these results it was concluded that the plant *C. fistula* flower exhibits larvicidal activity against two important vector mosquitoes. Further analysis to isolate the active compound for larval control is under way in our laboratory.

Conclusion

The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest associated with this article.

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***Corresponding author address:** Dr. M. Govindarajan, (Principal Investigator, DST-Fast Track Young Scientist Project, UGC- Major Research Project and ICMR *Ad-hoc* Research Project), Unit of Vector Biology and Phytochemistry, Department of Zoology, Annamalai University, Annamalainagar-608 002, Tamilnadu, India.