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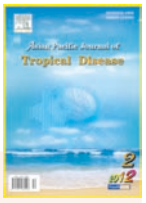

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Mosquito larvicidal and ovicidal activity of *Delonix elata* (L.) Gamble against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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ABSTRACT

Objective: To determine the larvicidal and ovicidal efficacy of different solvent leaf and seed extract of *Delonix elata* (*D. elata*) against filariasis vector *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** Twenty five early third instar larvae of *Cx. quinquefasciatus* were exposed to various concentrations and were assayed in the laboratory by using the protocol of WHO 2005. The larval mortality was observed after 24 h of treatment. The ovicidal activity was determined against *Cx. quinquefasciatus* mosquito to various concentrations ranging from 75-600 mg/L under the laboratory conditions. **Results:** Among five solvent extracts tested the maximum efficacy was observed in the leaf and seed methanol extracts. The LC_{50} and LC_{90} values of *D. elata* against early third instar of *Cx. quinquefasciatus* were 124.84, 147.86 mg/L and 213.88, 289.43 mg/L , respectively. No mortality was observed in controls. The Chi-square values were significant at $P<0.05$ level. Among five tested solvent, methanol extract was found to be most effective for ovicidal activity against *Cx. quinquefasciatus* mosquito. 100% mortality was observed at 375 mg/L . **Conclusions:** From the results it can be concluded the crude extract of *D. elata* was an excellent potential for controlling *Cx. quinquefasciatus* mosquito larvae and eggs.

1. Introduction

Mosquitoes are important vectors of etiological agents of diseases to humans and domestic animals. A primary element in the current global strategy for the control of vector-borne diseases is vector control, with chemical control remaining a main component of integrated vector management. The factors including the development of resistance of mosquito vectors to insecticides are leading to a rise in morbidity and mortality from malaria and other vector-borne infections. Resistance has been reported to every chemical class of insecticide used in vector control programs, including microbial drugs and insect growth regulators[1]. Thus, the search for alternative pesticides and control measures posing minimal risk to human health and the environment is of great interest from the preventive medicine point of view. Several phytochemicals extracted from various botanical sources have been reported to have detrimental effects on mosquitoes[2-5]. Govindarajan et al [6] reported that methanolic leaf extract of *Cassia fistula* was tested for larvicidal and ovicidal activity against *Culex quinquefasciatus* (*Cx. quinquefasciatus*) and *Anopheles stephensi* (*An. stephensi*). Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants, *Citrullus colocynthis*, *Coccinia indica*, *Cucumis sativus*, *Momordica charantia*, and *Trichosanthes anguina*, were tested against the early fourth instar larvae of *Aedes aegypti* (*Ae. aegypti*) and *Cx. quinquefasciatus*[7]. 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*[8]. 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*[9]. The larvicidal and ovicidal efficacy of different extracts of *C. halicacabum* against *Cx. quinquefasciatus* and *Ae. aegypti*[10].

As far as our literature survey could ascertain, no information was available on the ovicidal and larvicidal activities of the experimental plant species given here against *Cx. quinquefasciatus*. Therefore, the aim of this study was to investigate the mosquito ovicidal and larvicidal activities of the different solvent extracts of *D. elata* plant species from Tamil Nadu, India. This is an ideal eco-friendly approach for the control of the filariasis vector, *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Collection of plants

Fully developed leaves and seeds of the *D. elata* were collected

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from Thanjavur District (Between 9° 50′ and 11° 25′ of 78° 45′ N and 70° 25′ E), Tamil Nadu, 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.

2.2. Extraction

The leaves and seeds were washed with tap water, shade-dried, and finely ground. The finely ground plant leaf powder (1.0 kg/solvent) was loaded in Soxhlet apparatus and was extracted with five different solvents, namely, hexane, benzene, chloroform, ethyl acetate and methanol, individually. The solvents from the extracts were removed using a rotary vacuum evaporator to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in ethanol. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal and ovicidal bioassays.

2.3. Test organisms

The mosquito, *Cx. quinquefasciatus* was 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 1-week-old chick for blood meal. Mosquitoes were held at (28±2) ℃, 70%-85% relative humidity, with a photo period of 14-h light and 10-h dark.

2.4. Larvicidal bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by World Health Organization[11]. Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 mL of water. The appropriate volume of dilution was added to 200 mL 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 ethanol was added. The LC_{50} value was calculated after 24 h by probit analysis[12].

2.5. Ovicidal activity

For ovicidal activity, slightly modified method of Su et al[13] was performed. The egg rafts of *Cx. quinquefasciatus* were collected from vector control laboratory, Annamalai University. The plant crude extracts diluted in the ethanol to achieve various concentrations ranging from 75 to 600 mg/L. Eggs of these mosquito species (100) were exposed to each concentration of crude extracts. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula.

$$\% \text{ of mortality} = \frac{\text{No of hatched larvae}}{\text{Total no. of eggs}} \times 100$$

2.6. 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 (UCL) and lower confidence limit (LCL), and Chi-square values were calculated using the SPSS12.0 software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The results of the larvicidal activity of crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts of leaf and seed of *D. elata* against the early third instar larvae of *Cx. quinquefasciatus* are presented in Table 1. Among five solvent extracts tested the maximum efficacy was observed in the methanol extracts. The LC_{50} and LC_{90} values of leaf and seed of *D. elata* against *Cx. quinquefasciatus* were 124.84, 147.86 mg/L and 213.88, 289.43 mg/L , respectively. The Chi-square values are significant at $P<0.05$ level. The Chi-square values in the bioassays indicated probably the heterogeneity of the test population. The 95% confidence limits LC_{50} and LC_{90} were also calculated. The mean percent of egg hatchability of *Cx. quinquefasciatus* were tested with five different solvents at different concentrations of *D. elata* leaf and seed extracts, and the results are presented in Table 2. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. Among the five solvent extracts tested, methanol extracts of leaf and seed of *D. elata* exerted 100% mortality at (zero hatchability) 375 and 600 mg/L, respectively. Control eggs showed the 100% hatchability. The leaf extract of *D. elata* was found to be most effective than seed against larvae and eggs of *Cx. quinquefasciatus*.

Table 2

Ovicidal activity of *D. elata* plant leaf and seed extracts against *Cx. quinquefasciatus*.

Parts used	Solvents	Percentage of egg hatch ability					
		Concentration (mg/L)					
		75	150	225	300	375	450
Leaf	Hexane	76.1±1.7	66.7±1.5	52.5±1.4	39.4±1.9	28.6±1.3	17.6±1.8
	Benzene	70.7±1.6	61.8±1.9	49.3±1.8	36.2±1.0	24.9±1.6	NH
	Chloroform	66.2±1.1	56.9±1.2	44.1±1.5	29.6±1.4	18.2±1.3	NH
	Ethyl acetate	60.1±1.3	51.2±1.4	39.4±1.6	22.7±1.5	NH	NH
	Methanol	54.1±1.5	45.6±2.0	30.2±1.0	19.8±1.1	NH	NH
Seed		100	200	300	400	500	600
	Hexane	92.2±1.7	80.9±1.4	66.8±1.3	52.9±1.0	35.6±1.5	27.9±1.4
	Benzene	84.3±1.3	72.5±1.6	57.6±1.4	45.7±1.6	30.1±1.4	22.1±1.6
	Chloroform	79.4±1.6	66.6±1.3	51.3±1.8	38.7±1.4	24.4±1.7	NH
	Ethyl acetate	72.8±1.5	60.2±1.1	47.7±0.9	32.6±1.3	19.6±1.9	NH
	Methanol	67.9±1.1	56.3±1.9	40.3±2.1	26.8±1.0	17.3±1.0	NH

NH- No hatch ability. Control-100 mg/L.

Table 1.

Larvicidal activity of different solvent leaf and seed extracts of *D. elata* against *Cx. quinquefasciatus*.

Solvents	LC_{50} (LCL-UCL) (mg/L)		LC_{90} (LCL-UCL) (mg/L)		χ^2	
	L	S	L	S	L	S
Methanol	124.84 (99.58-150.06)	147.86 (83.72-202.22)	213.88 (182.59-271.07)	289.43 (228.81-436.59)	14.440*	26.854*
Ethyl acetate	130.55 (94.13-164.55)	178.79 (120.67-233.96)	238.43 (197.88-319.01)	343.71 (277.51-497.16)	18.223*	21.806*
Chloroform	135.93 (102.11-168.25)	201.89 (143.43-263.62)	243.16 (204.01-318.15)	390.27 (313.69-577.42)	16.620*	19.936*
Benzene	144.58 (113.32-175.30)	220.99 (171.00-278.38)	251.39 (213.55-321.38)	416.93 (341.48-585.56)	15.020*	15.280*
Hexane	160.91 (117.40-202.47)	235.23 (192.31-286.44)	290.22 (240.67-390.86)	429.06 (358.81-571.77)	18.896*	11.988*

* $P<0.05$. L - Leaf; S - Seed; LCL - Lower Confidence Limits ; UCL - Upper Confidence Limits; χ^2 - Chi square;

4. Discussion

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents. Phytoextracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer, and risk-free properties. Our results showed that crude extract of *D. elata* have significant larvicidal and ovicidal activity against *Cx. quinquefasciatus*. This result is also comparable to earlier reports of Chowdhury *et al.*[14] have reported that the chloroform and methanol extracts of mature leaves of *S. villosum* showed that the LC_{50} value for all instars were between 24.20 and 33.73 mg/L after 24 h and between 23.47 and 30.63 mg/L after 48 h of exposure period against *An. subpictus*. The n-hexane, ethyl acetate and methanol extracts of *Cassia nigricans*, *Jatropha curcas* (skin and seeds) and *Datura innoxia*, *Strophantus hispidus*, *Securidaca longepedunculata* and *Sapium grahamii* exhibited 100% mortality at 250 μg/mL concentration against fourth instar larvae of *Ochlerotatus triseriatus*[15]. Govindarajan[16] 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). Mathivanan *et al.*[17] 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. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants. It is apparent from the results of this research, as well as that of other authors, that plant leaves and seeds have strong potential in the new larvicides and ovicides safe for the environment and for human health. *D. elata* extracts may thus contribute greatly to the reduction of environmental chemicalization and to an overall reduction in the population density of *Cx. quinquefasciatus*.

Conflict of interest statement

We declare that we have no conflict of interest.

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