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Research Article

LARVICIDAL AND REPELLENT ACTIVITIES OF *TYLOPHORA INDICA* (BURM. F.) MERR. (ASCLEPIADACEAE) AGAINST *CULEX QUINQUEFASCIATUS* SAY AND *AEDES AEGYPTI* L. (DIPTERA: CULICIDAE)

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ABSTRACT

Mosquitoes transmit serious human diseases and cause millions of deaths every year. Natural products obtained from plants are good alternative sources for mosquito control. The aim of the present study was to assess the larvicidal and repellent activities of extracts from *Tylophora indica* against *Culex quinquefasciatus* and *Aedes aegypti*. Three solvents viz., hexane, ethyl acetate and methanol were used to prepare the extracts from shade-dried leaves of *T. indica*. The results of larvicidal activity clearly showed that the hexane extract was the most potent larvicide against both vector mosquitoes. The lethal concentrations varied; LC_{50} value of 324 ppm was recorded for *Cx. quinquefasciatus* and LC_{50} value of 619 ppm for recorded *Ae. aegypti*. At 5mg/cm^2 concentration, the hexane extract presented the highest repellent duration against *Cx. quinquefasciatus* up to 187 min. At this same concentration, the hexane extract gave a protection time of 122 min against *Ae. aegypti*. The hexane extract of *T. indica* can be probed further for isolation of active principles against *Cx. quinquefasciatus* and *Ae. aegypti*.

Keywords: Larvicidal activity, Repellency, *Tylophora indica, Culex quinquefasciatus, Aedes aegypti.*

INTRODUCTION

The incidence of dengue fever (DF), dengue hemorrhagic fever (DHF) and filariasis has increased tremendously in recent years. *Ae. aegypti* is the primary vector that carries the arbo-virus responsible for dengue fever. DHF/DF are considered as the major cause of childhood mortality and nearly 400,000 cases of DHF/DF are reported annually in many Asian countries (Halstead, 2000). *Cx. quinquefasciatus* is the main vector of filariasis in India. Filariasis is also a major mosquito borne disease.

Most widely used vector control method is larviciding with synthetic insecticides. These synthetic insecticides pollute the environment, affect the non-target organisms and cause pesticide resistance in vector mosquitoes (Wattal *et al*., 1981). It is clearly evident from the literature that botanical pesticides can offer ecofriendly vector mosquito control. *T. indica* has been traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma, bronchitis, rheumatism, dermatitis and the extracts are used as antiallergenic medication by Ayurvedic practitioners (The Wealth of India, 2008). The present study was aimed to evaluate the larvicidal and repellent activities of *T. indica* leaf extracts against *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes.

MATERIALS AND METHODS

Collection of Plant material

T. indica leaves were collected from Vengal, Thiruvallur District, India. Leaves were shade dried for 72 h and then powdered.

Solvent extracts

The shade dried powder (500 gm) was sequentially soaked in hexane, ethyl acetate and methanol for 96 h respectively with intermittent shaking. After 96 h of soaking, each solvent extract was filtered and concentrated under

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reduced pressure using rotary vacuum evaporator.

Test mosquitoes

Larval instars (third instars) of *Ae. aegypti* and *Cx. quinquefasciatus* were obtained from laboratory culture, that was maintained at 27±1°C, 75-85% RH and 14±10 h photoperiod at Entomology Research Institute, Loyola College, Chennai.

Larvicidal activity

The larvicidal activity of the crude extracts was assessed following the protocol of World Health Organization (WHO, 2005). The third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to the concentrations of 62.5, 125, 250, 500 ppm. Acetone was used to dissolve the extract. Five replicates were maintained for every concentration of each extracts. Solvent control (acetone in water) and water control were maintained separately. Larval mortality was determined after 24h. Larvae were considered dead when they did not respond to stimulus or did not rise to the surface of the solution. The larvicidal activity was corrected by Abbott's formula (Abbott, 1925) and the lethal concentration values LC_{50} and LC_{90} were calculated by probit analysis (SPSS 11.5 version).

Repellent activity

For repellent study, the hands of six volunteers from ERI, Loyola College were exposed to vector mosquitoes in cages sized 30 cm × 30 cm × 30 cm as per the protocol of WHO (1996) with slight modifications. The right hand of the volunteers was served as treated and left hand served as control. In each replicate 100 blood-starved female mosquitoes were used. DEET (12%) was used as positive control.

RESULTS

The results of larvicidal activity clearly showed that hexane extract was the most effective treatment against the two vector mosquitoes. The lethal concentrations varied; LC_{50} value of 324 ppm was recorded against *Cx. quinquefasciatus* and LC_{50} value of 619 ppm against *Ae. aegypti* (Table 1). The hexane extract gave a significantly high protection time of 187 min against *Cx. quinquefasciatus* and 122 min against *Ae. aegypti* at 5mg/cm^2 concentration.

The methanol extract and ethyl acetate extract were not effective and showed very low repellent activities (Tables 2 and 3).

Table 1. Lethal concentrations (in ppm) of *Tylophora indica* extracts against the larvae of *Culex quinquefasciatus* and *Aedes aegypti.*

Mosquito species	Treatment	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	χ^2
			LL	UL		LL	UL		
Culex quinquefasciatus	Hexane	324.08	273.06	393.52	679.68	569.80	866.93	-0.1 ± 0.5	9.5*
	Ethyl acetate	424.80	373.06	493.52	679.68	679.08	966.93	-0.2 ± 0.5	5.5*
	Methanol	478.64	368.87	372.74	1105.83	815.36	1881.16	-0.8 ± 0.5	2.6*
Aedes aegypti	Hexane	619.19	496.40	881.06	1095.00	846.37	1663.94	-1.6 ± 0.1	6.8*
	Ethyl acetate	719.19	596.40	981.06	1195.00	746.37	1763.94	-0.2 ± 0.5	2.8*
	Methanol	819.19	696.40	1081.06	1295.00	946.37	1863.94	-0.8 ± 0.5	6.8*

LC_{50} -lethal concentration that kills 50 % of the exposed larvae, LC_{90}-lethal concentration that kills 90 % of the exposed larvae, LL lower limit (95 % confidence limit), UL upper limit (95 % confidence limit).

*$p \leq 0.05$, level of significance of chi-square values.

Table 2. Complete protection time recorded by three solvent extracts of *Tylophora indica* leaves against *Culex quinquefasciatus.*

Extract	Concentration mg/cm^2	Complete protection time (min)	
		Control	Treated
Hexane	1.0	1.4 ± 0.54^{a}	105 ± 3.16^{g}
	2.5	2.2 ± 0.83^{a}	150 ± 2.91^{e}
	5.0	2.0 ± 1.22^{a}	273 ± 2.54^{b}
Ethyl acetate	1.0	1.6 ± 0.54^{a}	44 ± 2.73^{j}
	2.5	1.1 ± 0.19^{a}	99 ± 3.16^{h}
	5.0	1.4 ± 0.54^{a}	178 ± 1.58^{d}
Methanol	1.0	1.2 ± 0.44^{a}	44 ± 2.44^{j}
	2.5	1.6 ± 1.34^{a}	76 ± 2.73^{i}
	5.0	1.8 ± 0.44^{a}	120 ± 2.73^{f}
N-N Diethyl benzamide (12%)	1.0	2.4 ± 0.89^{a}	110 ± 1.0^{fg}
	2.5	1.8 ± 0.83^{a}	194 ± 1.58^{c}
	5.0	2.0 ± 0.70^{a}	323 ± 2.0^{a}

Each value represents mean of five replicates ± SD; values carrying different letters in a column are statistically different by Tukey's test at p=0.05.

Table 3. Complete protection time recorded by three solvent extracts of *Tylophora indica* leaves against *Aedes aegypti.*

Extract	Concentration mg/cm^2	Complete protection time (min)	
		Control	Treated
Hexane	1.0	0.33 ± 1.11^{a}	44 ± 1.58^{h}
	2.5	0.30 ± 0.08^{a}	87 ± 2.54^{d}
	5.0	0.52 ± 0.30^{a}	165 ± 1.0^{b}
Ethyl acetate	1.0	0.51 ± 0.30^{a}	28 ± 1.58^{i}
	2.5	1.16 ± 0.11^{a}	64 ± 3.16^{f}
	5.0	0.35 ± 0.10^{a}	118 ± 1.87^{c}
Methanol	1.0	1.2 ± 0.44^{a}	25 ± 2.12^{i}
	2.5	0.51 ± 0.05^{a}	51 ± 1.58^{g}
	5.0	0.40 ± 0.10^{a}	75 ± 2.91^{e}
N-N Diethyl benzamide (12%)	1.0	1.2 ± 0.70^{a}	55 ± 1.0^{g}
	2.5	1.4 ± 0.54^{a}	95 ± 1.41^{d}
	5.0	1.2 ± 0.44^{a}	182 ± 0.70^{a}

Each value represents mean of five replicates ± SD; values carrying different letters in a column are statistically different by Tukey's test at p=0.05.

DISCUSSION

Solvent extracts of many plants possess larvicidal and repellent effects against different mosquito species and are suggested for reducing the vector mosquito population and mosquito bites. Recent studies have pointed out that many plant extracts possess cost-effective and ecofriendly molecules that can be effectively used as mosquito repellents and pesticides without any side effects to non-target organisms. The active molecules are reported from different parts of plants namely fruit pulp, kernel, root, bark, and leaf (Ansari *et al.*, 2000; Ezeonu *et al*.,

2001; Sen-Sung *et al*., 2003; Chapagain *et al.*, 2007; Rawani *et al.*, 2013).

Results of the larval mortality experiments in the present study, clearly confirm the potential of hexane extract of *T. indica* in mosquito control. The larvicidal and repellent properties of this plant can be exploited for personal protection and mosquito population eradication in their breeding sites. Chockalingam *et al*. (1992) reported that the crude plant extracts containing compounds exhibiting synergistic or potentiating interactions have higher and longer-lasting effects on pests. The LC_{50} value of 324ppm was recorded against *Cx. quinquefasciatus* and LC_{50} value of 619ppm against *Ae. aegypti* was recorded with hexane extract of *T. indica* during the present study. These results of larvicidal activity was comparable with the earlier reports of Ansari *et al*. (2005) who observed larvicidal activity of *Pinus longifolia* oil against *Ae. aegypti* (LC_{50}-82.1 ppm), *Cx. quinquefasciatus* (LC_{50}-85.7 ppm).

The repellent results of present study indicated that hexane extract possessed good repellent activity and can give a protection time up to 182 min against *Cx. quinquefasciatus* and 122 min against *Ae. aegypti*. These results are comparable with the earlier report of Rajkumar and Jebanesan (2007) who reported a repellency of 140.0 and 119.0 minutes with *C. asiatica* and *P. guajava*, respectively at 6% concentration.

CONCLUSION

It is concluded that the hexane extract of *T. indica* possessed larvicidal and repellent activities against *Cx. quinquefasciatus* and *Ae. aegypti* and can be used in mosquito control programmes.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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