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Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*

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Mosquitoes are the principal vectors of malaria and other vector borne diseases and contribute to major disease burden in India. Disease transmission can be interrupted by controlling the vectors using various methods. However, the extensive and unbalanced use of chemical insecticides have created problems like enhancing resistance of mosquito population to synthetic insecticides[1,2], pollution of environment and adverse effects on the non-target flora and fauna inhabiting the same aquatic habitat. These steadily growing problems, demand an intensive search for new products that are environmentally safe, target-specific and degradable.

The co-evolution of plants with insects have equipped them with a plethora of chemical defences which can be used against insects. Since botanicals are less likely to cause ecological damage, a large number of plants have been screened for their insecticidal activities against mosquitoes and some of these have been found to be promising[3-8].

A growing need to search more and more indigenous plants with insecticidal activities has led us to undertake the present investigation. In the present study, *Millingtonia hortensis* L. (Family: Bignoniaceae) a plant commonly known as 'Akas neem' and also as the "Indian cork tree" that is widely distributed and cultivated in many parts of India including semi-arid regions of Rajasthan has been screened against three species of mosquito vectors. Although some medicinal properties of this plant are known but so far there is no report of its biological activity against insects. The present communication is the first report which reveals the mosquito larvicidal property of *M. hortensis*.

Fresh leaves of *M. hortensis* were obtained from the plants growing in Forest Research Institute at Jaipur. Leaves were washed, air-dried and ground in a mixer to form a fine powder. The leaf powder was then extracted in acetone as per the soxhlet extraction method[9]. For extraction 30 g of the 40 mesh powder of leaves was extracted with 300 ml of acetone for 8 h over a mantle heater at 50°C.The extract was filtered and concentrated on water bath to evaporate the acetone. The filtrate was considered as pure material and redissolved in acetone to form 10% (w/v) standard formulation. By further dilutions with required amount of water, different ppm doses were prepared.

Tween-80 was used as an emulsifier at concentration of 0.02% (v/v) in final test solution.

A laboratory culture of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae) was maintained at controlled conditions of 28°C temperature and 70–80% relative humidity. Early II, III and IV instar larvae were selected for the experiments.

Standard methods for testing the susceptibility of mosquito larvae to insecticides[10] were followed in all the experiments with slight modifications. The acetone extract of plant leaves was used at 25, 50, 100, 200, 300 and 500 ppm dilutions in bioassays against different larval instars of the three mosquito species. Twenty larvae were exposed to the leaf extract at each concentration in a final volume of 100 ml formulation taken in 250 ml of glass beaker. Three replicates for each concentration and the control (with acetone and emulsifier) were tested for larval bioefficacy. The larval mortality at different concentrations and in control was recorded after 24h continuous exposure. The corrected mortality was analysed using Abbott's formula[11] wherever required. The mortality data were analysed by log-probit method[12] and lethal concentration (LC) values (50 and 90) were calculated. ANOVA test was applied to the data to analyse the significance of the results.

The percent mortality values for II, III and IV instar larvae of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* treated with various concentrations (ranging from 25 to 500) of the leaf extract of *M. hortensis* are presented in Table 1. LC_{50} and LC_{90} values and their 95% lower and upper limits of the leaf extract for 24 h exposure of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* are given in Table 2. From the results it is evident that the acetone extract of *M. hortensis* leaves showed efficacy against

Table 1. Toxicity of leaf extract of *Millingtonia hortensis* against mosquito larvae of different species

Dose (ppm)	*Anopheles stephensi* % mortality ± SEM			*Aedes aegypti* % mortality ± SEM			*Culex quinquefasciatus* % mortality ± SEM		
	II	III	IV	II	III	IV	II	III	IV
500	98.33± 0.333	96.67± 0.333	95±0.0	ND	ND	ND	ND	ND	ND
300	86.67± 0.333	80± 0.0	76.67± 0.333	95.01± 0.0	93.33± 0.333	91.70± 0.333	98.33± 0.333	96.67± 0.333	95± 0.0
200	81.67± 1.76	70± 0.0	68.33± 0.333	73.33± 0.333	71.67± 0.94	70± 1.0	85± 0.0	78.33± 0.333	76.67± 0.333
100	71.64± 1.666	30± 0.0	26.67± 0.333	15± 0.0	8.33± 0.333	6.70± 0.333	50± 0.0	30± 0.0	26.67± 0.333
50	36.67± 0.881	8.33± 0.333	6.67± 0.333	8.33± 0.333	3.33± 0.333	5± 0.0	28.33± 0.333	13.33± 0.333	10± 0.0
25	8.33± 0.333	1.67± 0.333	1.67± 0.333	1.67± 0.333	0.0± 0.0	0.0± 0.0	8.33± 0.333	1.67± 0.333	0.0± 0.0
Control	5±0.0	5± 0.0	5± 0.0	5± 0.0	5± 0.0	5± 0.0	5± 0.0	5± 0.0	3.33± 0.333
CD at 5%	0.929	0.203	0.26	0.22	0.34	0.42	0.22	0.25	0.22

Sixty larvae (3 replicates of 20 each) were treated at each dose level; ND– Not done.

Table 2. Larval susceptibility of three mosquito species to the leaf extract of *Millingtonia hortensis*

Mosquito species	Lethal concentrations*					
	II instar		III instar		IV instar	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
An. stephensi	104.70	281.8	162.2	363.1	223.9	426.6
	(102.7–106.7)	(279.4–284.2)	(160–164.4)	(360.6–365.6)	(221.6–226.2)	(424–429.2)
Ae. aegypti	138	269.2	195	302	208.9	316
	(135.9–140.1)	(266.8–271.6)	(192.8–197.2)	(299.6–304.4)	(206.7–211.3)	(314–318.9)
Cx. quinquefasciatus	83.18	190.5	147.9	257	138	275.4
	(81.27–85.10)	(188.2–192.8)	(145.8–150.1)	(254.6–259.4)	(135.9–140)	(273–277.8)

*All values are in ppm; Figures in parentheses are 95% fiducial limits.

all the larval stages of the three mosquito species tested. Highest mortality of 98.33% was recorded for II instar larvae of *Cx. quinquefasciatus* at 300 ppm. *An. stephensi* larvae were found to be relatively less susceptibile as they showed 98% mortality at a comparatively higher concentration of 500 ppm. *Ae. aegypti* larvae exhibited very little susceptibility at lower doses but a mortality of 95% was reported at the concentration of 300 ppm.

The highest sensitivity of II instar larvae of *Cx. quinquefasciatus* to the leaf extract of *M. hortensis* was also evident by their lowest LC values (LC_{50} 83.18 and LC_{90} 190.5 ppm). Least susceptibility was shown by IV instar larvae of *An. stephensi* (LC_{50} 223.9 and LC_{90} 426.6 ppm). Thus the susceptibility order of three mosquito species to the leaf extract was observed to be *Cx. quinquefasciatus* >*Ae. aegypti* >*An. stephensi*. Similar trend of differential susceptibility of mosquito species to plant extracts was reported earlier[13].

In the present study, a difference in the sensitivity of different larval stages of all the three mosquito species was reported. Earlier instars were more susceptible to the extract and showed higher mortality when compared to the later instars. Similar differences in the mortalities in various larval instars of *Cx. pipiens molestus* exposed to crude extract of *Melia volkensii* and *M. azaderach* (Family: Meliaceae) were also recorded by other workers[14]. Toxic effect of leaf extract against larvae was evident at higher concentrations particularly against *Ae. aegypti*.

The effect of the leaf extract was reported to be dose-dependent as evident by an increase in percent mortality with increasing concentrations. These results get substantial confirmation from the findings of other workers[15-17], who also reported the dose-dependency of the plant extract against mosquito larvae.

A general behavioural change in the larvae of all the three mosquito species was observed and it was seen that larvae slowly became inactive within few hours of treatment. The microscopic examination of dead larvae showed disintegration of integument probably due to removal of chitin and abnormal stretching of body specially the neck region was also observed. These symptoms suggest growth regulating and probably neurotoxic action of the leaf extract of *M. hortensis* but further research is needed to substantiate this view.

This study revealed that the plant *M. hortensis* has a potent mosquito larvicidal property and could be selected for further studies particularly those pertaining to its effect on growth and development of mosquitoes.

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