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Screening of some semi-arid region plants for larvicidal activity against *Aedes aegypti* mosquitoes

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Mosquitoes pose a major threat to human health by transmitting serious diseases. Development of resistance, cross-resistance, rising cost and possible toxicity hazards associated with synthetic insecticides are some of the reasons for revival of interest in plant-based products in recent years[1–4]. Search for cost-effective, safe and highly potent plant-based insecticides for the control of mosquitoes requires the preliminary screening of plants to evaluate their effectiveness in mosquito control and selecting the plants with high potency for further study. In the present study, we have screened 11 plant species of local flora against the IV instar larvae of *Aedes aegypti* (Diptera: Culicidae). These selected plants are *Millingtonia hortensis*, *Annona squamosa*, *Bauhinia varigata*, *Plumeria alba*, *Psidium guajava*, *Syzygium cumini*, *Alstonia scholaris*, *Michelea champaca*, *Holoptelia integrifolia*, *Quisqualis indica* and *Nerium indicum*. Taxonomic identification was performed by botanists from the Department of Botany, University of Rajasthan, Jaipur, India where voucher specimens were deposited.

The leaves of the plants were dried in the shade, ground in a mixer and extracted with acetone (95%) at room temperature. The plant material (300 g) was soaked in acetone in an airtight wide mouthed bottle and kept for seven days. After that the cold extracts from the bottle along with acetone were filtered and kept in petri dishes for drying at room temperature. Dried extracts were used for larvicidal bioassay as per WHO standard method[5]. Stock solutions (10% w/v) were prepared by dissolving the plant extract in acetone. Tween-80 was also added as emulsifying agent. Different concentrations were prepared by adding required volume of stock solution in beakers containing 100 ml of water.

A laboratory culture of *Ae. aegypti* was maintained at 26 ± 2°C, 70 ± 10% relative humidity and a photoperiod of 12 : 12, Light : Dark at the Ecotoxicological Laboratory, Department of Zoology, University of Rajasthan, Jaipur, India.

Standard method for testing the susceptibility of mosquito larvae to insecticide, as suggested by WHO[6] was followed in all the experiments. In the present study, IV instar larvae of *Ae. aegypti* were treated separately with the leaf extracts of 11 plants at 100, 200 and 300 ppm concentrations. For each concentration 100 ml of tap water was kept in three glass beakers (250 ml capacity). Required amount of stock solution was added in each beaker to obtain a particular concentration of the extract. A control with acetone and emulsifier was run with each concentration. Twenty larvae of *Ae. aegypti* were introduced in control and in three replicates of a particular concentration. After 24 h of exposure, the number of larvae surviving in each concentration was recorded and the percent mortalities were calculated. The mortality data were analysed by log-probit method of Finney[7] as described by Busvine[8].

The results showing larvicidal action of leaf extracts of 11 plants against *Ae. aegypti* are presented in Table 1. Out of the 11 plants tested, leaf extracts of

Table 1. Lethal concentration of different plant extracts against larvae of *Ae. aegypti*

Plant species screened	Lethal concentration in ppm	
	LC_{50}	LC_{90}
Millingtonia hortensis	123 (120.9–125.09)	323.6 (321.1–326.1)
Annona squamosa	190.5 (188.2–192.8)	323.6 (321.1–326.1)
Bauhinia varigata	204.2 (201.9–206.5)	446.7 (444.1–449.4)
Plumeria alba	218.8 (216.5–221.1)	501.2 (498.5–503.9)
Psidium guajava	223.9 (221.6–226.3)	316.2 (313.7–318.7)
Syzygium cumini	223.9 (221.6–226.3)	524.8 (522.1–527.5)
Alstonia scholaris	239.9 (237.5–242.3)	501.2 (498.5–503.9)
Michelea champaca	263 (261.6–265.4)	562.3 (559.6–565.1)
Holoptelia integrifolia	281.6 (279.4–284.3	707.9 (705.1–710.8)
Quisqualis indica	281.8 (297.4–284.3)	794.3 (791.4–797.2)
Nerium indicum	316.2 (313.7–318.7)	812.8 (809.9–815.7)

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

Millingtonia hortensis was found to possess the most effective larvicidal activity (LC_{50} of 123 ppm) followed by *Annona squamosa* (LC_{50} 190.5 ppm), *Bauhinia varigata* (LC_{50} 204.2 ppm), *Plumeria alba* (LC_{50} 218.8 ppm), *Psidium guajava* (LC_{50} 223.9 ppm), *Syzygium cumini* (LC_{50} 223.9 ppm) and *Alstonia scholaris* (LC_{50} 239.9 ppm). Among the other plants, *Michelea champaca*, *Holoptelia integrifolia*, *Quisqualis indica* and *Nerium indicum* also showed some larvicidal activity against *Ae. aegypti* but at comparatively higher doses (LC_{50} >263 and LC_{90} >562.3).

The leaf extract of *Millingtonia hortensis* has been earlier reported to posses larvicidal activity against *Anopheles stephensi, Ae. aegypti* and *Cx. quinquefasciatus*[9]. However, as evident from the present results crude leaf extract of *Millingtonia hortensis* is significantly more effective (LC_{50} 123 ppm) when compared to its extracts prepared by soxhlet method (LC_{50} 208.9 ppm). This confirms the effect of extraction method on efficacy of the plant as reviewed by Shallan[10]. The larvicidal and growth regulating activities of *Annona squamosa* and *Syzygium cumini* have been reported against *An. stephensi* and other mosquitoes[11–14]. The high potency of *Annona squamosa* and *Syzygium cumini* as a larvicide against mosquito species is re-emphasized in the present study.

In the present study, after the preliminary screening with crude leaf extracts some plants with strong larvicidal activity against *Ae. aegypti* have been identified. However, further study is needed to conduct the fractionation of crude extracts which will help in identification of active toxic compound(s) responsible for larval mortality. It is suggested that a further detailed study of some of these plants against *Ae. aegypti* and other mosquito species should be undertaken to evaluate their insecticidal, growth regulating, repellent and antifeedant properties.

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