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Correspondence

Mosquito larvicidal properties of *Mirabilis jalapa* (Nyctaginaceae) against *Anopheles stephensi*, *Aedes aegypti* & *Culex quinquefasciatus* (Diptera: Culicidae)

Sir,

Mosquitoes are the most important single group of insects known for their public importance, since they act as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, chikungunya, malaria, filariasis and encephalitis of different types including, Japanese encephalitis[1]. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. Larviciding largely depends on the use of synthetic chemical insecticides – organophosphates (*e.g*. temephos and fenthion), insect growth regulators (*e.g*. diflubenzuron and methoprene), *etc*. Although effective, their repeated use has disrupted natural biological control systems and sometimes resulting in the widespread development of resistance. These problems have warranted the need for developing alternative strategies using eco-friendly products[2]. We undertook investigations of certain plant species traditionally used as insecticidal agents, as well as other endangered plant species, with the aim of identifying lead compounds for the development of new plant based insecticidal agents[3].

Mirabilis jalapa Linn (Nyctaginaceae) is a perennial herb and is known as "Gulambasa" in Ayurveda. The presence of oxymethyl anthraquinone, trigonelline, arabinose, galactose, beta-sitosterol in leaves has been reported. It is used in the traditional system of medicine in the treatment of piles, abscess, boils and ulcers[4,5]. There is no information available on the larvicidal activity of *M. jalapa* against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*. Therefore, the present study was carried out to determine the larvicidal efficacy of *M.jalapa* leaves extract against malaria, dengue and filariasis vector mosquitoes.

The leaves of *M.jalapa* were collected from in and around Gingee, Tamil Nadu, India, and were authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen was deposited at the herbarium of Plant Phytochemistry Division, Department of Zoology, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

Cx. quinquefasciatus, Ae. aegypti and *An. stephensi* 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 per cent sucrose solution and membrane feeding on goat blood. Mosquitoes were held at 28 ± 2°C, 70-85 per cent relative humidity (RH), with a photoperiod of 12 h light : 12 h dark. The dried leaves (1 kg) were extracted with four different solvents, namely, benzene, chloroform, ethyl acetate and methanol (500ml), individually and the extract was evaporated in a rotary vacuum evaporator. Standard stock solutions were prepared at 1 per cent by dissolving the residues in ethanol, which was used for the larvicidal bioassay.

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by the World Health Organization[6]. Batches of 25 third instar larvae were transferred to a small disposable paper 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 (25-250 mg/l). 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} (lethal concentration that kills 50 per cent of the exposed larvae) and LC_{90} (lethal concentration that kills 90 per cent of the exposed larvae) values were calculated after 24 h by probit analysis[7]

The results of the larvicidal activity of crude benzene, chloroform, ethyl acetate, and methanol solvent leaf extracts of *M. jalapa* against the larvae of three important vector mosquitoes, viz. *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* are presented in the Table. Among extracts tested, the highest larvicidal activity was observed in leaf methanol extract of *M. jalapa* against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with the LC_{50} and LC_{90} values as 57.55, 64.58, 84.53 ppm and 104.20, 120.28, 159.25 ppm, respectively (Table). Regression analysis showed that the mortality rate (Y) was positively correlated with concentration of exposure (X). The result of log probit analysis (95% confidence level) showed that LC_{50} values gradually decreased (benzene < chloroform < ethyl acetate < methanol).

It is a well recognized fact that plant extracts and phytochemicals could be developed into products suitable for mosquito control, because many of these are selective, often biodegradable to non-toxic products, and may be applied to mosquito breeding sites in the same way as conventional insecticides. Our result showed that the crude benzene, chloroform, ethyl acetate and methanol solvent extracts of leaf of *M. jalapa* had significant larvicidal properties against three vector mosquitos viz. *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus.* This result was also comparable to our earlier reports of the LC_{50} values of benzene, hexane, ethyl acetate, methanol, and chloroform extract of *Eclipta alba* against early third-instar larvae of *Ae. aegypti* which were 151.38, 165.10, 154.88, 127.64, and 146.28 ppm, respectively[8], and for the larvicidal efficacy of benzene, hexane, ethyl acetate, methanol, and chloroform leaf extract of *Cardiospermum halicacabum* against *Cx. quinquefasciatus* and *Ae. aegypti*, the LC_{50} values were

Table. Larvicidal activity of different solvent leaf extract of *M. jalapa* against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*

Mosquito species	Solvent used	LC_{50} (mg/l) (LCL-UCL)	LC_{90} (mg/l) (LCL-UCL)	Slope	Regression equation	χ^2 (df)	Comparative toxicity[a] (fold)
An. stephensi	Benzene	79.67 (67.16-92.41)	137.36 (120.19-165.52)	2.000	Y=0.581+0.642x	9.495 (4)	1.38
	Chloroform	70.95 (54.89-86.55)	127.25 (107.75-163.30)	2.215	Y=3.633+0.649x	14.639 (4)	1.23
	Ethyl acetate	63.67 (52.61-74.77)	109.98 (95.48-134.46)	2.010	Y=0.162+0.786x	10.914(4)	1.11
	Methanol	57.55 (43.52-70.99)	104.20 (87.62-135.59)	2.270	Y=4.629+0.780x	16.118(4)	-----
Ae. aegypti	Benzene	97.03 (79.72-113.99)	172.15 (149.81-208.91)	2.150	Y=2.567+0.489x	10.101(4)	1.69
	Chloroform	88.20 (65.62-109.38)	162.16 (136.13-211.08)	2.395	Y=6.310+0.487x	15.691(4)	1.53
	Ethyl acetate	72.77 (59.66-85.66)	127.91 (111.09-155.88)	2.095	Y=2.019+0.658x	10.593(4)	1.26
	Methanol	64.58 (45.29-82.38)	120.28 (99.11-163.42)	2.500	Y=7.467+0.644.x	19.110(4)	1.12
Cx. quinquefasciatus	Benzene	116.05 (93.15-138.18)	207.78 (179.27-255.95)	2.125	Y=1.290+0.378x	44.553(4)	2.02
	Chloroform	105.11 (87.36-123.12)	184.89 (160.65-225.67)	2.100	Y=0.771+0.473x	10.126(4)	1.83
	Ethyl acetate	94.82 (75.46-113.60)	170.66 (146.45-212.66)	2.240	Y=3.752+0.485x	12.087(4)	1.65
	Methanol	84.53 (57.86-108.81)	159.25 (130.54-218.70)	2.610	Y=8.381+0.480x	19.841(4)	1.47

LC_{50}, lethal concentration that kills 50 per cent of the exposed larvae; LC_{90}, lethal concentration that kills 90 per cent of the exposed larvae; UCL, upper confidence limit; LCL, lower confidence limit; χ^2, Chi square; Chi-squared test, comparing experimental and control group, with a significance level established at $P<0.05$. df- degrees of freedom; [a]comparative toxicity of species with reference to LC_{50} of *An. stephensi* (methanol extract).

174.24, 193.31, 183.36, 150.44, and 154.95 and 182.51, 200.02, 192.31, 156.80, and 164.54 ppm, respectively[9]. The leaf oil extracts of *Eucalyptus tereticornis* showed 100 per cent mortality at 160 ppm against the larvae of *An. stephensi*[10]. The acetone extracts of *Nerium indicum* and *Thuja orientelis* have been studied with LC_{50} values of 200.87 and 127.53 ppm against III instar larvae of *An. stephensi*[11]. The isolated compound neemarin from *Azadirachta indica* exhibited LC_{50} and LC_{90} values of 0.35 and 1.81 mg/l for *An. stephensi* [12]. Compared with earlier reports, our results revealed that the experimental plant extracts were effective against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* and the plant *M. jalapa* exhibited larvicidal activity against three important vector mosquitoes. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

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