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Larvicidal Efficacy of Extracts of Selected Plants against *Aedes aegypti* (Linnaeus)

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Abstract

Background: *Aedes aegypti* (Diptera: Culicidae) is considered to be a primary vector of viral diseases such as dengue fever, chikungunya and yellow fever. Plant compounds having insecticidal properties are a preferable option for the control of these vectors because they are less harmful to non-target organism and are easily biodegradable. The present study is designed to test the larvicidal efficacy of six plants found locally in NE India against *Aedes aegypti*.

Methods: The larvicidal efficacy of ethanol extracts of whole plant of *Alternanthera philoxeroides* and *Ipomoea aquatica*, fronds of *Amphineuron opulentum* and *Sphaerostephanos unitus*; leaves of *Ziziphus jujuba* and the stem bark of *Zanthoxylum nitidum* were tested against the fourth instar larvae of *Aedes aegypti*. The mortality was observed after 24 and 48 hours of treatment. Data were subjected to probit analysis to determine the lethal concentrations.

Results: Most of the plants showed moderate to high level of toxicity. The ethanol extract of *Zanthoxylum nitidum* stem bark was found to be the most effective with an LC_{50} value of 6.10 ppm after 24 hours of exposure followed by that of *Ziziphus jujuba* with an LC_{50} value of 79.98 ppm. *Alternanthera philoxeroides*, *Amphineuron opulentum* and *Sphaerostephanos unitus* showed moderate efficacy whereas *Ipomoea aquatic* showed very low larval toxicity against the mosquito.

Conclusion: The results obtained suggest *Zanthoxylum nitidum*, *Ziziphus jujuba* and *Amphineuron opulentum* as potential candidate for the control of *Aedes aegypti* mosquitoes.

Keywords: *Aedes aegypti*, plant extract, larvicide, lethal concentration

Introduction

The mosquito *Aedes aegypti* (Linnaeus, 1854) is the primary vector for diseases like dengue fever, chikungunya and yellow fever. These mosquitoes are peridomestic and well adapted to urban areas and the domestic environment (Bara *et al*., 2013). The key breeding sites of *Aedes aegypti* include water containers both inside and outside human dwellings (Preechaporn *et al*., 2006, 2007; Saleeza *et al*., 2011). It is most important to check the proliferation of these vectors in order to reduce the occurrence of diseases spread by them. Pyrethroids have long been used as most favoured household insecticides. Other insecticides commonly used are DDT, organophosphates, etc (WHO, 2009). Continuous and prolonged human exposure to these insecticides cause serious

neurotoxic and immunotoxic hazards. Some common health problems associated with the use of insecticides include breathing problem, eye irritation, cough, cold and sneezing, headache, asthma, bronchial irritation, itching, ear, nose and throat pain, giddiness, vomiting, nausea, allergy, etc. (Sharma, 2001; Kamble, 2012).

Phytochemicals are nowadays considered as ideal alternatives to hazardous and non-biodegradable synthetic pesticides. It is reported that plant derivatives are target specific and not toxic to non-target organisms of the environment in which they are applied (Sharma *et al*., 2011). Need for the application of new environment friendly pesticide for insect pest management has led to search for biologically active natural products with low mammalian toxicity in order to avoid some of the deleterious effects of synthetic pesticides on the environment (Ce´spedes *et al*., 2005). Furthermore, unlike commercial insecticides that are based on single active component, phytoderivatives comprise hundreds of secondary metabolites, which can act synergistically on both behavioral and physiological processes. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants are in use for their insecticidal properties. They can act as larvicide, adulticide, antifeedent, insect growth regulators, repellents and ovipositor attractant and have different other activities (Adeniyi et al., 2010 ; Sharma *et al*., 2011; Ghosh *et al*., 2012). Development of resistance against repeatedly used compounds demand search for new compound from new sources. The present study is designed to test the larvicidal efficacy of six plants that are available locally in NE India.

Materials and methods

Plant collection: The plant materials selected for the study are whole plant of *Alternanthera philoxeroides* and *Ipomoea aquatica*, fronds of *Amphineuron opulentum* and *Sphaerostephanos unitus*; leaves of *Ziziphus jujuba* and the stem bark of *Zanthoxylum nitidum*. Plants samples were collected from different locality of Dibrugarh district of Assam, India. Samples were identified by comparing their morphological characters, habit and habitats with those described in the taxonomic literature and were authenticated by a plant taxonomist from Department of Life Sciences, Dibrugarh University.

Mosquito collection: Eggs of *Aedes aegypti* were kindly provided by the Regional Medical Research Centre, Dibrugarh. Eggs were hatched in tap water and larvae were fed on a diet composed of yeast powder and dog biscuit in a ratio of 3:1. Feeding was continued until larvae obtained fourth instar and was ready for assay.

Preparation of extract: Extracts were prepared by following Harborne (1998) and Gogoi and Bora (2012). The collected plants were shade dried for several days. The dried leaves were then ground into a homogenous coarse powder. 100g of the powder was then extracted with 200ml ethanol at room temperature for 72 hours. The solvent was then evaporated in rotaevaporator and the residue obtained was used for larvicidal assay.

Larvicidal assay: Extracts were initially dissolved in ethanol and diluted with distilled water to obtain 1000ppm, 500ppm, 250ppm, 125ppm and 62.5ppm concentrations. Tween 20 was added as an emulsifier. For the larvicidal assay, larvae were taken in three replicates of 10 in 10ml of extract of each concentration. The control was set up with ethanol, distilled water and Tween 20. The numbers of dead larvae were counted after 24 and 48 hours of exposure and the percentage mortality was recorded from the average of three replicates.

Statistical analysis: The data of average larval mortality was subjected to Probit analysis to calculate the lethal concentration (LC_{50} and LC_{90}) causing 50% and 90% of

larval mortality after 24 hours and 48 hours of exposure.

Results and discussions

The effects of the six plants against the larvae of *Aedes aegypti* after 24 hours and 48 hours of exposure is presented in Table 1. 100% mortality was seen in larvae exposed to all the five concentrations (1000ppm, 500ppm, 250ppm, 125ppm and 62.5ppm) of *Zanthoxylum* extract. The extract of *Ziziphus jujuba* and *Amphineuron opulentum* showed similar toxicity against the larvae. In case of these two extracts, 100% mortality was seen at 250ppm concentration after 24 hours of exposure and at 125 ppm concentration after 48 hours of exposure. Extracts of *Alternanthera philoxeroides* showed moderate level of toxicity against the larvae. 100% mortality was seen in larvae exposed to concentrations upto 250ppm but mortality was significantly low in the lower concentrations. Extracts of *Sphaerostephanos unitus* and *Ipomoea aquatica* showed very low level of toxicity and caused no mortality in larvae exposed to lower concentrations.

Table 1: Percentage Mortality of *Aedes aegypti* mosquito larvae treated with various concentrations of plant extracts.

Plants	Concentration (ppm)	Mortality (%)	
		24 hours of exposure	48 hours of exposure
Alternanthera philoxeroides	1000	100	100
	500	100	100
	250	100	100
	125	16.67	26.67
	62.5	6.67	16.67
Amphineuron opulentum	1000	100	100
	500	100	100
	250	100	100
	125	83.33	96.67
	62.5	0	26.67
Ipomoea aquatica	1000	66.67	100
	500	53.33	73.34
	250	16.67	33.34
	125	0	6.67
	62.5	0	0
Sphaerostephanos unitus	1000	100	100
	500	100	100
	250	13.33	20
	125	0	6.67
	62.5	0	0
Zanthoxylum nitidum	1000	100	100
	500	100	100
	250	100	100
	125	100	100
	62.5	100	100
	31.25	100	100
	15.62	100	100
	7.81	93.33	96.67
Ziziphus jujuba	1000	100	100
	500	100	100
	250	100	100
	125	86.67	100
	62.5	33.33	56.67
	31.25	13.33	26.67

Table 2: Lethal Concentrations of the plant extracts after 24 hours and 48 hours of exposure.

Plants	***Family***	***Lethal Concentration***			
		24 hours of exposure		**48 hours of exposure**	
		LC_{50}	**LC_{90}**	**LC_{50}**	**LC_{90}**
Alternanthera philoxeroides	Amaranthaceae	155.37	213.09	140.13	212.73
Amphineuron opulentum	Thelypteridaceae	104.00	130.05	78.22	110.41
Ipomoea aquatica	Convolvulaceae	585.10	1663.50	319.85	663.99
Sphaerostephanos unitus	Thelypteridaceae	292.15	350.06	283.59	457.05
Zanthoxylum nitidum	Rutaceae	6.10	7.79	5.78	7.43
Ziziphus jujuba	Rhamnaceae	79.98	129.63	54.28	88.50

Table 2 represents the Lethal Concentrations (LC_{50} and LC_{90}) of the tested plants after 24 hours and 48 hours of exposure. The LC_{50} value of *Zanthoxylum* was 6.10 ppm and 5.78 ppm after 24 hours and 48 hours of exposure respectively. Extracts of *Ziziphus jujuba* also had a low LC_{50} value of 79.98ppm and 54.28ppm after 24 and 48 hours. *Amphineuron* extract had moderate lethal concentration of 104.00ppm and 78.22ppm while the LC_{50} values of the other three plants were comparatively higher.

In the present study, the extracts of the six plants studied showed different larvicidal activities against *Aedes aegypti.* The differential biological activity of the experimental plants is due to the presence of different classes of secondary metabolites which may independently or collectively produce the toxic effect through different mechanism of action viz. inhibition of acetylecholinestrase by essential oils, GABA-gated chloride channel by thymol , sodium and potassium ion exchange disruption by pyrethrin and inhibition of cellular respiration by rotenone, mitotic poisioning by azadirachtin disruption of the molecular events of morphogenesis and alteration in the behaviour and memory of cholinergic system by essential oil etc.(Rattan, 2010). The effect varies not only with the chemistry of the compound(s), but also with species variation (Arivoli and Tennyson, 2011).

The present study revealed that the stem bark extract of *Zanthoxylum nitidum* is highly toxic to the larvae at a very low lethal concentration. This toxic effect may be attributed to the presence of Alkaloids and Flavonoids in the ethanol extracts of stem bark of *Zanthoxylum nitidum* .The present study is the first report on the mosquito larvicidal activity of *Zanthoxylum nitidum.* Different species of *Zanthoxylum* belonging to family Rutaceae is reported to have medicinal and insecticidal properties by other authors too (Bhattacharya *et al.,* 2009; Gogoi and Bora, 2012). *Zanthoxylum limonella* pericarp has been found effective against *Aedes albopictus* larvae at concentration as low as 0.01 ppm (Nath *et al.,* 2006).

The leaf extract of *Ziziphus jujuba* (Rhamnaceae) showed potential larvicidal effect against *Aedes aegypti* in the present study. In earlier study it was reported to be effective against *Culex pipiens* larvae in which the petroleum ether extract and oil were shown to cause pathological effect on pupa and adult (El Husseiny *et al.* 2014 and Iman *et al.,* 2015). However the susceptibility of different species of mosquitoes towards the same plant extract varies and hence for every species studies are required to be conducted separately (Singh *et al.,* 2006; Arivoli and Tennyson, 2011).

In the present study, among the six plants studied, based on LC_{50} values

Amphineuron opulentum (Thelypteridaceae) was ranked third. Literature search has not revealed any information regarding prior use of this plant as mosquitocide.

Conclusion

The results obtained suggest *Zanthoxylum nitidum, Ziziphus jujuba* and *Amphineuron opulentum* as potential candidate for the control of *Aedes aegypti* mosquitoes.

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