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Preliminary evaluation of *Clerodendron phlomides* against dengue vector mosquito *Aedes aegypti* L. (diptera: culicidae)

R. Maheswaran[1,2,*], P. Pandikumar[1], N. Prakash Babu[1], S. Kingsley[1] and S. Ignacimuthu[1]

[1]Entomology Research Institute, Loyola College, Chennai – 600 034, India.

[2]Department of Zoology, Periyar University, Salem 636 011, Tamil Nadu, India.

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ABSTRACT

The objective of the present study was to evaluate the ovicidal and larvicidal activity of *Clerodendron phlomides* on *Aedes aegypti*. Maximum ovicidal and larvicidal activities were recorded in chloroform extract, followed by hexane and ethanol extracts of *C. phlomides*. The chloroform extract of *C. phlomides* at 1000 ppm showed an ovicidal effect of 15.6±1.30, 33.2±3.64 and 45.6±5.49 % on 0-6, 6-12 and 12-18 hr old eggs. The LC_{50} values for this extract were 10.21, 45.30, 235.06 and 335.51 ppm against the first, second, third and forth instar larvae of *Ae. aegypti* in 24hr. An increase in the concentration of the test extracts resulted in an increase in the ovicidal and larvicidal effects.

Introduction

Mosquitoes are not only the most important vectors for the transmission of malaria, filariasis and viral diseases (James, 1992) but are also an important pest to humans, causing allergic responses that include local skin reaction and systemic reactions such as angioedema, and urticaria (Peng *et al.*, 1999). *Aedes aegypti* is on focus worldwide because of its role as a vector of arboviruses, responsible for major diseases like dengue and dengue haemorrhagic fever and chikungunya. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue haemorrhagic fever and dengue shock syndrome or with unusual manifestion such as central nervous system involvement. About two-fifths of the world populations are now at risk of catching dengue (WHO 2003).

This consideration was behind this study in evaluating the ovicidal, larvicidal and repellent, oviposition deterrent and adulticidal effect of *C. phlomides* on *Aedes aegypti* Organophosphates such as temephos and fenthion and insect growth regulators such as diflunenzuron and methoprene are generally used for the control of mosquito larvae. Although they are effective, their repeated use has disrupted natural biological control systems and has led to outbreaks of insect species. Sometimes this has resulted in the widespread development of resistance, undesired effects on nontarget organisms and fostered environmental and human health concerns (Yang *et al.*, 2002).

Plant natural products are preferred because of their innate biodegradability and a rich source of bioactive organic chemicals. Plants synthesize a number of secondary metabolites to serve as defense chemicals against insect attack. These chemicals may serve as insecticides, antifeedants, oviposition deterrents, repellents, growth inhibitors, juvenile hormone mimics, moulting hormones, antimoulting hormones as well as attractants. They offer an advantage over synthetic pesticides as they are less toxic, less prone to the development of resistance, and easily biodegradable. Long before the advent of synthetic insecticides, plant and their derivates were used to kill pests of agriculture, veterinary and public health (Ignacimuthu, 2000). Insecticidal activity of plant derived compounds such as nicotine, rotenoids and pyrethroids have been evaluated and a few of these compounds have been exploited commercially (Jacobson and Crosby, 1971).

Several studies have focused on natural products for controlling *Aedes* mosquitoes, but with varied results (Tsao *et al.*, 2002). Besides their use as agricultural insect pest control agents, their use in mosquito larvae control is an interesting perspective. Considerable attention has been focused on the control of mosquito through natural products of plant origin. A number of bitter diterpenoid compounds, having a clerodane skeleton isolated from various *Clerodendron* sp. have been shown to inhibit the feeding of polyphagous insect pests (Kato *et al.*, 1972). Many plant extracts have been studied for their larvicidal efficacy of different species of mosquitoes (Zarroug *et al.*, 1988). The present work was undertaken with a view to evaluate the ovicidal and larvicidal activity of *C. phlomides* leaf extract against *Ae. aegypti*

Materials and Methods

Plant Collection and Extraction

Fresh mature leaves of *C. phlomoides* were collected from Alanthurai in Coimbatore district, Tamil Nadu, India. Leaves were shade dried and finally powdered using an electric blender. Fifty grams of leaf powder was soaked in 500 ml of hexane, chloroform and ethanol solvent, sequentially for a period of 72 hr each and filtered. The extracts were concentrated at reduced temperature on a rotary evaporator and stored at 4°C.

Mosquito Culture

Larvae of *Aedes aegypti* collected from clean stagnant water bodies from Chennai, India, were colonized and maintained continuously for generations since 2005, in a laboratory, free of exposure to pathogens, insecticides or repellents. They were maintained at 27±2°C, 75-85% RH under a photoperiod of 14:10

Tele:

E-mail addresses: mahes1380@gmail.com

hr (light/dark) in the insectary. Under these conditions, development from eggs to adults was about 3-4 weeks. Larvae were fed on finely ground dog biscuit and yeast extract in the ratio of 3:1. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages (23x23x32 cm dimension), where the adults emerged. The adults of *Ae. aegypti* were reared in the glass cages of 30x30x30 cm dimension. The adult colony was provided with 10% sucrose solution and 10% multivitamin syrup, and it was periodically blood-fed on restrained rats. Two developmental stages, larvae and adult females, were continuously available for the experiments. After three days, ovitrap was kept into the cages and the eggs were collected and transferred to the enamel trays. They were maintained at the same condition.

Ovicidal activity

Ovicidal activity was calculated as described by Su and Mulla, (1998). Fifty freshly laid eggs of 0-6, 6-12 and 12-18 hr old were treated with test extracts at 1000, 500, 250 and 125 ppm concentrations. Each treatment was replicated five times. Each crude extract was dissolved in water with an emulsifier (Tween 80) to get the experimental concentrations. Tween 80 was used as negative control. Newly hatched larvae were counted under the microscope. The hatch rate was calculated 120 hr post treatment using the following formula:

$$\frac{\text{Number of hatched larva}}{\text{Total number of eggs in treated water}} \times 100$$

Larvicidal Bioassay

Larvicidal activity was evaluated following WHO Method (1996) with slight modifications. Twenty five early third instar larvae were released in a 500 ml glass beaker containing 249 ml of dechlorinated water and 1.0 ml of desired plant extract concentration. Four replicates for each concentration were run at a time. Each crude extract was dissolved in water with 0.1% emulsifier (Tween 80) to get the experimental concentrations of 1000, 500, 250 and 125 ppm. Tween 80 was used as negative control. Mortality and survival were recorded after 24 hours of the exposure period. The moribund and dead larvae in four replicates were combined and expressed as a percentage of larval mortality of each concentration. Dead larvae were identified when they failed to more after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. They also showed discolouration, unnatural positions, tremors, uncoordination or rigor.

Results And Discussion

At 1000ppm concentration of chloroform extract of C. *phlomides*, the hatchability of eggs was 15.6±1.30, 33.2±3.64 and 45.6±5.49 % on 0-6, 6-12 and 12-18 hr old eggs (Table-1). Alka Prakash (1992) observed that *Ae. aegypti* eggs were highly susceptible to penfluron when they were 0-6 hr old. Pusphanathan *et al.* (2006) observed 100% ovicidal activity at 300 ppm with essential oil of *Cymbopogan citrates* against *Culex quinquefasciatus.* In the present evaluation, the LC_{50} values were observed at 10.21, 45.30, 235.06 and 335.51 ppm in chloroform extract of *C. phlomides* against first, second, third and fourth instar larvae of *Ae. aegypti* respectively in 24 hr (Table-2). Earlier, Maheswaran *et al.*, (2008) reported 72% of mortality with chloroform extract of *C. phlomides* against *Cx. quinquefasciatus*. Pereira and Gurudutt (1990) observed growth inhibition of *Cx. quinquefasciatus* on (-)-3- epicaryoptin isolated from *C. inerme*. Leaf powder of *C. inerme* at 200mg exhibited 100% larval mortality in *Ae. aegypti* (Patil *et al*., 2006). Maximum larvicidal activity was recorded in chloroform extract followed by hexane and ethanol extracts of *C. phlomides*. In the present study, it was found that the larval mortality increaseed with an increase in the concentration of the test extracts. Similar larvicidal effect of botanicals was also observed earlier on *Aedes aegypti* by Singh *et al.*, (2006). The results from the present study clearly demonstrate that the extracts of *C. phlomides* act as potential larvicides against *Ae. aegypti*. These results are very promising to develop new, effective and affordable biopesticide to control *Aedes* mosquito.

Table 1. Ovicidal activity of *Clerodendron phlomides* against *Aedes aegypti*

Solvent	Age of egg	1000ppm	500ppm	250ppm	125ppm
Hexane	0-6	44.0±3.00	75.6±4.02	87.6±4.38	89.6±3.34
	6-12	60.4±2.16	68.0±2.34	88.0±4.63	90.0±4.00
	12-18	64.8±3.28	81.2±4.56	96.0±1.41	99.2±0.54
Chloroform	0-6	15.6±1.30	39.6±2.86	41.2±3.36	48.4±4.08
	6-12	33.2±3.64	42.8±5.59	52.8±3.43	54.0±3.08
	12-18	45.6±5.49	60.4±1.92	65.6±3.03	67.2±2.50
Ethanol	0-6	78.8±5.17	81.2±3.36	82.2±2.30	84.4±1.92
	6-12	81.6±3.03	88.0±3.24	86.8±2.07	93.2±4.27
	12-18	92.0±2.23	94.8±1.34	92.8±4.61	98.0±1.41
Control	99.2±0.89				

Values are mean of five replicates ± S.D.

Table 2. Larvicidal activity of *Clerodendron phlomides* against *Aedes aegypti*

Instar	Solvent	LC_{50}	95% Confidence limit		LC_{95}	Slope	Chi-square
			LFL	UFL			
4th instar	Hexane	788.42	479.34	2310.73	16368.30	1.24	0.83*
	Chloroform	335.51	240.44	496.39	3153.05	1.69	1.87*
	Ethanol	1205.04	681.57	5067.85	21347.84	1.31	1.61*
3rd instar	Hexane	542.60	372.63	983.16	6411.40	1.53	1.63*
	Chloroform	235.06	173.54	315.96	1526.93	2.02	2.33*
	Ethanol	848.59	515.25	2523.03	16010.90	1.28	0.83*
2nd instar	Hexane	101.82	37.96	165.92	2679.31	1.15	0.39*
	Chloroform	45.30	12.68	75.62	424.28	1.69	1.08*
	Ethanol	177.28	68.08	325.05	11934.89	0.89	0.22*
1st instar	Hexane	20.28	0.36	50.22	370.82	1.30	0.61*
	Chloroform	10.21	0.00	37.00	207.60	1.25	0.82*
	Ethanol	61.75	1.48	136.27	8911.64	0.76	1.32*

Values were based on four concentrations and four replications with 25 larvae in each.

*Significant at $p<0.05$ level.

References

Alka Prakash. Ovicidal action of certain chitin synthesis inhibitors in mosquitoes. Entomon. 1992 17(1&2): 15 – 19.

Ignacimuthu S. The role of Botanicals in Combating mosquitoes. *In*: Proceedings of Recent Trends in Combating Mosquitoes. 2000.

Jacobson M, Crosby BG. Naturally occurring insecticides. 1971. NY Marcel Dekker Inc. USA, 210.

James AA. Mosquito molecular genetics: the hands that feed bite back. Science. 1992 257 (5066): 37 – 38.

Perira J, Gurudutt KN. Growth inhibition of *Musca domestica* L. and *Culex quinquefasciatus* (Say) by (-)-3-epicaryoptin isolated from leaves of *Clerodendron inerme* (Gaertn.) (Verbenaeae). J. Chem. Ecol., 1990, 16(7): 2297-2306.

Kato N, Takahashi M, Shinayama M, Munakata K. Antifeeding activity substances for insects in *Clerodendron tricotomum* Thumb. Agric. Biol. Chem., 1972, 36: 2579-2582.

Maheswaran R, Kingsley S, Ignacimuthu S. Larvicidal and repellent activity of Clerodendron phlomides *against* Culex quinquefasciatus *Say. (Diptera: Culicidae). In: Proceedings of Recent Trends in Insect Pest Management. 2008, Pp. 240-243.*

Patil PB, Holihosur SN, Kallapur VL. Efficacy of natural product, *Clerodendron inerme* against dengue mosquito vector

Aedes aegypti. Curr. Sci., 2006, 90(8):1064-1066.
Peng Z, Yang J, Wang H, Simons FER. Production and characterization of monoclonal antibodies to two new mosquitoes *Aedes aegypti* salivary proteins. Insect Biochem. Mol. Biol., 1999, 29(10): 909 – 914.
Pushpanathan T, Jebanesan A, Govindarajan M. Larvicidal, Ovicidal and repellent activities of *Cymbopogan citrates* Stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera : Culicidae). Tropical Biomed. 2006, 23(2): 208 – 212.
Singh RK, Dhiman RC, Mittal PK. Mosquito larvicidal properties of *Momordica charantia* Linn (Family: Cucurbitaceae). J. Vect. Borne Dis., 2006, 43: 88 – 91.
Su T, Mulla MS. Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera : Cilicidae). J. Am. Mosq. Control. Assoc., 1998, 14: 201 – 209.
Tsao R, Romanchuk FE, Peterson CJ, Coats JR. Plant growth regulatory effect and insecticidal activity of extracts of tree of Heaven (*Ailanthus altissima* L.). BMC Ecol., 2002, 2: 1 – 8.
World Health Organisation, 1996. Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides. CTD/WHOPES/IC/96.1, p.69.
World Health Organisation, 2003. Dengue. Available at http:// w.w.w.who.int/inf-fs/en/fact117.html.
Yang YC, Lee HK, Kim MK, Lee HS. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. J. Agric. Food Chem., 2002, 50(13): 3976 – 3767.
Zarroug IA, Nugud AD, Bashir AK, Mageed AA. Evaluation of Sudanese plant extracts as mosquito larvicides. Int. J. Crude Drug Res., 1988, 26: 77-80.