Academic Journal of Entomology 2 (2): 62-66, 2009
ISSN 1995-8994

Larvicidal, Ovicidal and Repellent Activities of *Pemphis acidula* Forst. (Lythraceae) Against Filarial and Dengue Vector Mosquitoes

[1]K. Samidurai, [1]A. Jebanesan, [2]A. Saravanakumar, [1]M. Govindarajan and [1]T. Pushpanathan

[1]Department of zoology, Annamalai University, Annamalai Nagar, 608002, Tamil Nadu, India
[2]CAS in Marine biology, Annamalai University, Parangipettai, 608502, Tamil Nadu, India

Abstract: Crude leaf extracts of *Pemphis acidula* were evaluated for larvicidal, ovicidal and repellent activities against *Culex quinquefasciatus* and *Aedes aegypti*. The larval mortality was observed after 24 h exposure. The LC_{50} values of methanol, benzene, acetone were 10.81, 41.07, 53.22ppm and 22.10, 43.99, 57.66ppm respectively. Hundred percent ovicidal activities were observed at 350 ppm and 450 ppm. Skin repellent test at 1.0, 2.5 and 5.0 mg/cm2 concentration of *P.acidula* gave 100% protection up to 2.30, 4.00 and 6.45 hrs and 2.45, 4.30 and 7.0 hrs respectively. From the present study it was revealed that extracts from *P. acidula* can be effectively used in the control of *Cx. quinquefasciatus* and *Ae. aegypti*.

Key words: *Pemphis acidula* · different extract · *Culex quinquefasciatus* · *Aedes aegypti*

INTRODUCTION

Mosquito spread various vector-borne diseases such as malaria, filariasis, Japanese encephalitis and dengue fever, which are transmitted by the four genera of mosquitoes namely *Anopheles, Culex, Aedes* and *Monsonoids*. 40 million people in India suffer from mosquito borne diseases annually. They are over 3000 mosquito species belonging to 34 genera in the world. Of these, only about 300 transmit human and animal diseases. These diseases devastate Indian economy every year [1]. Filariasis, a disease affecting the arms, legs and genitals, is much prevalent in India. Filariasis caused by *Wuchereria bancrofti* is transmitted by *Cx. quinquefasciatus* mosquitoes which are widespread in the country now and lymphatic filariasis infects 80 million people annually of which 30 million cases exist in chronic infection. There are 45 million cases of lymphatic filariasis in India alone [2]. Dengue and dengue haemorrhagic fever are transmitted by *Aedes aegypti*. Dengue fever continues in recurrent epidemic afflicting millions and causing thousands of deaths annually which is transmitted by *Ae. aegypti*. Mosquito are a serious threat to public health through which several dangerous diseases are transmitted in both animals and human beings [3].Vector control is a global problem. It may be directed against the immature or adult stages of mosquitoes. The problems of vector control differ from country to country and may not be similar even in different areas of the same country [4]. The residual spraying of insecticides is the most common method of vector control, but usefulness of insecticides in the control of vector-borne diseases is limited. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects [5]. Recent studies stimulated the investigation of insecticidal properties of plant derived from materials or botanicals and concluded that they are environmentally safe, degradable and target specific [6]. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programmes. The plant product of phytochemical, which is used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. Several indigenous plants viz, *Ocimum basilicum, O. santum, Azadirachta indica, Lantana camera, Vitex negundo* and *Cleome viscosa* were studied for their larvicidal action on the field which collected fourth instar of *Cx. Quinquefasciatus* [7]. *Leucas aspera, O. santum, A. indica, Allium sativum* and *Curcuma longa* had a strong larvicidal, antiemergence, adult repellency and antireproductive activity against *A. stephensi* [8]. Other essential oils from plants like *Myrtus comunis, Origanum syriacum, Laventula stoechos* and pure compounds like thymol, carvacrol and α-pinene

Corresponding Author: K. Samidurai, Centre of Advanced Study in Marine Biology, Annamali University, Parangipettai, Tamil Nadu, India - 608 502

have been documented for larvicidal activities towards *Cx. pipens molestus* [9]. The toxicity of the plant *Moschosma polystachyum* was evaluated against mosquito *Cx. Quinquefasciatus* [10]. The larvicidal activity of extracts from *Calophyllum inophyllum* (Clusiaceae), *Rhinacanthus nasutus* (Acanthaceae), *Solanum suratense* (Solanaceae) and *Samadera indica* (Simaroubaceae), *Myriophyllum spicatum* (Haloragaceae) against *A. stephensi* [11]. In addition, *Pelargonium citrosa* [12], *Cymbopogan citrates* [13] and *Mentha piperita* [14] were shown to contain larvicidal and growth inhibitory activity against *A. stephensi*. The present study deals with larvicidal, ovicidal and repellent effects of *Pemphis acidula* against *Cx. quinquefasciatus* and *Ae. aegypti.*

MATERIALS AND METHODS

Preparation of Plant Extract: The plant *Pe.acidula* (Lythraceae) was collected from Gulf of Mannar Biosphere Reserve, (9º14'47.2N lat. and 79º12'38.6E long.) Tamilnadu, India. The fresh leaves of *Pe. acidula* were washed with tap water and shade dried at room temperature (28 ± 2°C). The dried leaves (1.0 kg) were powdered by electrical blender. Three litre methanol, acetone and benzene separately were used for the extraction of 1.0 kg in the Soxhlet apparatus followed by the standard procedure [16]. The plant material was loaded in the inner tube of the Soxhlet apparatus and then fitted into a round bottomed flask containing methanol. The solvent was boiled gently (40°C) over a heating mantle using the adjustable rheostat. The extraction was continued until complete extraction was effected (8 hrs.) and the solvent was removed at the reduced pressure with the help of rotary vaccum evaporator to yield a viscous dark green residue (12.5 g) of each solvent of methanol, acetone and benzene leaf extracts.

Test Organisms: To satisfy the enormous number of mosquitoes need for the day to day bioassays, a colony was essential. The eggs and egg rafts of *Cx. quinquefasciatus* and *Ae. aegypti* were procured from Vector Control Research Centre (VCRC) at Puducherry, India. The mosquito colony maintained at 70-85% RH, 28±2°C temperature and 14:10 light and dark photoperiod cycle. The larvae were fed on powdered mixture of dog biscuits and yeast tablets in 3:1 ratio. The blood meal was given to the female adult mosquitoes and 5.0% glucose solution and honey were given to the male adult mosquitoes.

Larvicidal Activity: Testing of the plant extract for larvicidal activity was carried out at different concentration by preparing the required stock solutions by following the standard procedure [17]. The desired concentrations of the test solution were achieved by adding 1.0 ml of an appropriate stock solution to 249 ml of dechlorinated water. Six replicates for each concentration were maintained. Twenty five number of late third larvae were introduced into the beaker, were obtained from the laboratory colony. Acetone was used as control. The larval mortality in both treated and control was recorded after 24 hrs and the percentage of mortality was calculated using Abbott's formula [18].

Ovicidal Activity: Ovicidal activity of *Cx. quinquefasciatus* and *Ae. aegypti* was assessed using the standard method [19]. The leaf extract was diluted in methanol to achieve different concentrations of 100 to 350 ppm for *Cx. quinquefasciatus* and 100 to 450 ppm for *Ae. aegypti*. Each egg raft containing 100 eggs of *Cx. quinquefasciatus* and the eggs of *Ae. aegypti* (100 numbers) were exposed to each dose of leaf extract until they hatched or died. Eggs exposed to methanol and water served as control. After treatment the egg rafts and eggs from each concentration were transferred to distilled water cups for hatching assessment after counting the eggs under microscope. The test was replicated six times. The hatch rate was calculated 48 h post treatment by the following formula.

$$\frac{No.of\ hatched\ larvae}{Total\ number\ of\ eggs}\times 100$$

Repellent Activity: The repellent activity was determined by the percentage protection time in relation to dose method [17]. Three to four days old blood-starved 100 adult female of *Cx. quinquefasciatus* and *Ae. aegypti* mosquito were kept in a different net cages (45 × 30 × 45 cm3). The arms of the test person were cleaned with isopropanol. After air drying the arm of the test person, only 25 cm2 dorsal side of the skin on each arm was exposed and the remaining area being covered by rubber gloves. The plant extract was dissolved in isopropanol and this alcohol served as control. The leaves of *Pe. acidula* plant extract of 1.0, 2.5, 5.0 mg/cm2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The first bite was noted to 5 minutes for every 30 minutes, from 20:00 to 06:00 h for *Cx. quinquefasciatus* and 08:00 to 18:00 h for *Ae. aegypti*. The experiment was conducted for

five times. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula.

$$\frac{No.\ of\ bites\ received\ by\ control - No.of\ bites\ received\ by\ treated}{No.of\ bites\ received\ by\ control} \times 100$$

RESULTS

The LC50 value of *P. acidula* ranged from 10.81 to 57.66 ppm with three different solvents. The methanol extract of *P. acidula* found to be more effective than the other extracts (Table 1). The chi-square values were significant at P<0.05 level. The statistical data LC90, regression equation and 95% confidence limits were also calculated. The fresh eggs *of Cx. quinquefasciatus* and *Ae. aegypti* treated with different concentrations of leaf extracts caused ovicidal activity resulting in failure to hatch the eggs. Hundred percent ovicidal activity was observed at higher concentration of 350 ppm for *Cx. quinquefasciatus* and 450ppm for *Ae. aegypti*. (Table 2). The ovicidal effects were generally dose dependent. This study revealed that *P. acidula* had repellency activity against the adult mosquito *Cx. quinquefasciatus* and *Ae. aegypti*. The results of mean protection in relation to dose of *P. acidula* extracts are presented in (Table 3). Maximum of 100% protection time was obtained at the concentration of 5.0 mg/ cm2. This is statistically significant (1.0 mg/cm^2, t = 32.229 and 0.922; 2.5 mg/cm^2, t = 31.486 and 2.891; 5 mg/ cm^2, t =63.807 and 18.984, p<0.001) between treated and control groups.

Table 1: Larvicidal activity of *Pemphis acidula* against *Culex quinquefasciatus* and *Aedes aegypti*

					95% confidence limit (ppm)		
Name of the species	Name of the solvents	LC50	LC90	Regression equation	UCL	LCL	Chi-square value
Culex quinquefasciatus	Methanol	10.81	20.64	Y = 8.41 + 3.77x	8.10	13.33	9.21*
	Benzene	41.07	81.89	Y = 11.14 +0.91x	31.46	49.93	10.11*
	Acetone	53.22	104.55	Y = 8.28 + 0.75x	41.17	64.60	8.42*
Aedes aegypti	Methanol	22.10	43.71	Y = 9.61 + 1.80x	16.52	27.29	10.54*
	Benzene	43.99	84.87	Y = 8.47 + 0.93x	33.47	53.80	10.17*
	Acetone	57.66	106.51	Y = 5.48 + 0.76x	45.10	69.80	10.39*

Values were based of five concentration and six replication ± SE

*P<0.05 level

Table 2: Ovicidal activity of *Pemphis acidula* leaf extract against eggs of *Culex quinquefasciatus* and *Aedes aegypti*

		Percentage of egg hatchability Concentration of extract (ppm)									
Name of the species	Extraction	Control	150	200	250	300	350	400	450	500	F-value
Cx quinquefasciatus	Methanol	100.0	72.3±4.3	55.8±4.3	36.6±2.8	18.8±2.7	NH	NH	NH	NH	273.585
	Benzene	100.0	92.1±3.1	69.1±3.0	51.6±4.6	35.5±2.0	11.5±1.8	NH	NH	NH	624.811
	Acetone	100.0	100.0	90.1±4.0	71.6±3.0	49.6±4.5	33.5±2.0	15.5±1.8	NH	NH	483.874
Ae. aegypti	Methanol	100.0	100.0	92.1±3.1	69.1±3.0	51.6±4.5	31.5±2.0	11.5±1.8	NH	NH	626.288
	Benzene	100.0	100.0	100.0	100.0	83.6±4.1	71.0±3.3	55.8±4.3	36.6±3.5	18.8±2.7	299.972
	Acetone	100.0	100.0	100.0	88.1±4.0	69.6±3.0	49.6±4.5	35.5±2.0	11.8±2.0	NH	390.535

NH – No hatchability (100% mortality)

Values are mean of six replicates ± SE

Table 3: Laboratory repellent activity of *P. acidula* leaf extract against *Culex quinquefasciatus* and *Aedes aegypti*

Species	Concentration of leaf extracts (mg/cm^2)	Mean no. of bites received in control	Mean no. of bites received in treated	Total % of protection for 10 hrs	t- value (df)
Cx.quinquefasciatus	1.0	71±1.4	41±1.1	2.30	32.229*(5)
	2.5	69±1.2	35±1.4	4.00	31.486*(5)
	5.0	67±1.4	25±1.2	6.45	63.807*(5)
Ae. aegypti	1.0	49.0±1.0	33.0±1.6	2.45	0.922*(5)
	2.5	50.0±1.4	29.0±1.2	4.30	2.891*(5)
	5.0	50.0±1.8	21.0±0.8	7.0	18.984*(5)

Values are mean of six replicates ± SE

*P <0.001 level

DISCUSSION

Acalypha indica leaf extract exerted larval mortality against larvae of *A. stephensi*. The LC_{50} values was observed at 19.25, 27.76, 23.26 and 15.03ppm respectively [20]. *Piper longum* fruit-isolated pipernonaline had strong larvicidal effects against the 4th stage larvae of *Ae. Aegypti* [20]. The LC_{50} values of pipernonaline were 0.25 mg/l against *Ae. aegypti*. The mangrove plant *Rhizophora mucronata* bark and pith extract showed high toxicity with LC_{50} values of 157.4 and 168.3 ppm respectively against *Ae. aegypti* larvae [22]. Exposure of A. stephensi larvae to sub-lethal doses of neem extracts in the laboratory prolonged larval development, reduced pupal weight, high oviposition deterrence and high mortality [23]. The direct and indirect contributions of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for management of *A. stephensi*. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future [24]. The repellent activity of turmeric, kaffir lime, citronella grass and hairy basil with the addition of 5% vannillin provided protection up to six hours [25]. Bioinsecticide IR 3535 in the USA tested against laboratory reared *Ae. Aegypti* [26] female mosquitoes and provided protection for an average of 22.9 minutes and also reported that the repellent activity of DEET (N,Ndiethyl- 3 methylbenzamide) 23.8% ingredient provided protection for an average of 301 min. Although DEET has been claimed to be safe for use against biting insects for over 40 year it may still pose a risk to human health. All toxins used in pest control pose some hazards to the user and also to the aquatic environment [27]. In the present study it was concluded that the extracts of *Pe. acidula* exhibited effective larvicidal, ovicidal and repellent properties against *Cx. quinquefasciatus* and *Ae. aegypti*. Further studies on identification of active compounds for larval control and commercial preparation of repellent products and field trials are needed to recommend the development of ecofriendly chemicals from this plant based extract for mosquito control and protection against the bites of haematophagous insects.

ACKNOWLEDGEMENT

The authors are grateful to Professor and Head, Department of Zoology, Annamalai University for the facilities provided and encouragement.

REFERENCES

1. Jaswanth, A., P. Ramanathan and K. Ruckmani, 2001. Evaluation of mosquitocidal activity of *Annona squamosa* leaves against filarial vector mosquito, *Culex quinquefasciatus*. Indian J. Exp. Biol., 40: 363-365.
2. Bowers, W.S., B. Sener, P. Evans, F. Bingol and I. Eradogon, 1995. Activity of Turkish medicinal plant against mosquitoes *Aedes aegypti* and *Anopheles gambiae*. Insect Sci Appl., 16: 339-342.
3. Service, M., 2004. Medical entomology for students. Cambridge University Press.
4. Rao, R.D., 1992. Evaluation of four azadirachtin rich fractions from neem *Azadirachta indica* A. Juss (Family: Meliaceae) as mosquito larvicides. Ind J Entomol., 25: 67-72.
5. Ghosh, G.K., 1991. Biopesticide and integrated pest management. A.P.H. Publishing Corporation, New Delhi, 145-146.
6. Govindarajan, M., A. Jebanesan and T. Pushpanathan, 2008a. Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. Parasitol Res., 102: 289-292.
7. Kalyanasundaram, M. and P.K. Das, 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. Indian J. Med. Res., 82: 19-23.
8. Murugan, K. and D. Jeyabalan, 1999. Mosquitocidal effect of certain plants extracts on *Anophels stephensi*. Curr Sci., 76: 631-633.
9. Traboulsi, A.F., K. Taoubi, S. El-Haj, J.M. Bessiere and Salma Ramal, 2002. Insecticidal properties of essential plant oils against the mosquito *Culex pipiens*.
10. Molestus (Diptera: Culicidae). Pest Management Science., 58: 491-495.
11. Rajkumar, S. and A. Jebanesan, 2004. Mosquitocidal activity of octacosane from *Moschosma polystachyum* Linn. (Lamiaceae). J Ethnopharmacol., 90: 87-89.
12. Muthukrishnan, J. and E. Puspalatha, 2001. Effects of plant extracts on fecundity and fertility of mosquitoes. J. Appl. Entomol., 125: 31-35.
13. Jeyabalan, D., N. Arul and P. Thangamathi, 2003. Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, Anopheles stephensi Liston. Biores Tech., 89(2): 185-189.

14. Pushpanathan, T., A. Jebanesan and M. Govindarajan, 2006. Larvicidal, ovicidal and repellent activities of *Cymbopogan citrates* stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera:Culicidae). Trop Biomed., 23: 208-212.
15. Ansari, M.A., P. Vasudevan, M. Tandon and R.K. Razdan, 2000. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. Biores Technol., 71: 267-271.
16. Vogel, A.I., 1978. Text Book of Practical Organic Chemistry. The English Language Book Society and Longman, London. pp: 1368.
17. WHO, 1996. World Health Organisation, Instruction for determining the susceptibility and resistance of mosquito larvae to insecticides. WHO/VBC/75.583, Monographed document.
18. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
19. Su, T. and S. Mulla, 1999. Ovicidal activity of neem products (*Azadirachtin*) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J. Am. Mos. Control. Assoc., 14(2): 204-209.
20. Govindarajan, M., A. Jebanesan, T. Pushpanathan and K. Samidurai, 2008b. Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera:Culicidae). Parasitol. Res., 103: 691-695.
21. Yang, Y.C., S.G. Lee, H.K. Lee, M.K. Kim and H.S. Lee, 2002. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. J. Agric. Food Chem., 50(3): 3765-3767.
22. Kabaru, J.M. and L. Gichia, 2001. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against the arthropods. Afr. J. Sci. Technol., 2(2): 44-49.
23. Wandscheer, C.B., J.E. Duque, Y. Fukuyama, J.L. Wohlke and J.D. Fontana, 2004. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. Toxicon., 44: 829-835.
24. Tawatsin, A., S.T. Wratten, R.R. Scott, U. Thavara and T. Techadamrongsin, 2001. Repellency of volatile oils from plants against three mosquitoes. J. Vec. Ecol., 6: 76-82.
25. Fradin, M.S. and J.F. Day, 2002. Comparative efficacy of insect repellent against mosquito bites. The New England Journal of Medicine., 13: 347-418.
26. Osimitz, T.G. and R.H. Grothaus, 1995. The present safety assessment of DEET. J. Am. Mos. Con. Ass., 7: 210-237.
27. Kreutzweiser, D.P., 1997. Non-target effects of neem-based insecticides on aquatic invertebrates. Ecotoxicol Environ Safety., 36: 109-117.