academicJournals

Vol. 6(1), pp. 11-15, January, 2014
DOI: 10.5897/JPVB10.032
ISSN 2141-2510 ©2014 Academic Journals
http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Short Communication

Evaluation of larvicidal activity of the different extracts against important species of mosquito: *Anopheles stephensi*

Babita R. Malik[1]*, Mukesh K. Malik[1], N. Balakrishnan[2] and B.suresh[1]

[1]Department of Pharmacognosy, J.S.S. College of Pharmacy Rockland, Ooty-643 001, T.N., India.
[2]National Institute of Communicable Diseases, Southern India branch Conoor-643101, T.N., India.

Accepted April 17, 2013

The aim of this work was to compare larvicidal activity of insecticidal plants against malaria vector mosquito *Anopheles stephensi.* The larvicidal activity of *Lantana camara* Linn. and *Bauhinia racemosa* Lam., extracted in petroleum ether, chloroform and ethyl acetate were tested against mosquito larvae of *A. stephensi*. Late third or early fourth instar larvae were used for the screening. These extracts were used for determining the larvicidal activity using World Health Organization (WHO) method for evaluation of new larvicidal agents. The petroleum ether extract of *L. camara* showed highest larvicidal activity in comparison to petroleum ether extract of *B. racemosa*, and ethyl acetate extract of *B. racemosa* showed highest larvicidal activity in comparison to chloroform extract of *L. camara* against the mosquito vector *A. stephensi*. No mortality was observed in control. The result suggests the use of the plants in insect control as an alternative method for minimizing the noxious effect of some pesticide compounds on the environment. Thus, the extracts of whole plant of *L. camara* Linn. and leaf extracts of *B. racemosa* Lam. may deliver promising, more selective and biodegradable agents.

Key words: *Lantana camara*, *Bauhinia racemosa, Anopheles stephensi*.

INTRODUCTION

Mosquitoes are one of the most medicinally significant vectors and they transmit parasites and pathogens which continue to have devastating impact on human beings (Maheswaran et al., 2008). Several numbers of species belong to genera *Anopheles, Culex, Aedes* and vectors for the pathogens of various diseases like malaria, filaria, Japanese encephalitis, dengue, and yellow fever. Thus, one of the approaches for control of these vector-borne diseases is the interruption of disease transmission by killing mosquitoes or preventing mosquito bites (Das, 1989). Herbal products which have proven potential as insecticides or replicants can play an important role in the interruption of the transmission of mosquito-borne diseases both at the individual and community level. However the discovery, development and use of synthetic organic insecticidal chemicals with persistent residual action not only over shadowed the use of herbal products as insect-cides of choice against mosquitoes but also become the major weapon for mosquito control (Sakthivadivel and Daniel, 2008). The extensive use of synthetic organic insecticides during the last decade has resulted in environmental hazards and also in the development of physiological resistance in most vector species. This has necessitated the need for research and development of environmentally

*Corresponding author. E-mail: tinaindia771@yahho.co.in. Tel: 09305730760, 919634401029. Fax: 2582330.

safe, biodegradable, low cost indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situations (Singh et al., 2006). The plant *Lantana camara* Linn. (Verbenaceae) and *Bauhinia racemosa* Lam. (Caesalpiniaceae) are described in Ayurveda and Siddha, as a potent drug against a variety of ailments. These plants are widely distributed and cultivated in various parts of India (Yoganarasimhan, 2000; Varier, 2006; Prajapati et al., 2007; Ivan, 1999; Singh and Himadri, 2005; Raveendra and Martin, 2006).

MATERIALS AND METHODS

Collection of plant material

The plants were collected during flowering stage in the month of July to August from Nilgiris. Then, their identification was established with the aid of an expertise botanist and by making comparison with herbarium sheets of the authentic sample. Many of the defensive components are biodegradable, with non-residual effect on the biological environment hence; an attempt has been made in the present investigation to identify plants with the potential to control vector mosquitoes.

Extraction

The plant *L. camara* Linn. and *B. racemosa* Lam. were powdered and extracted in soxhlet, with petroleum ether, chloroform and ethyl acetate. The extracts were concentrated under reduced pressure to a semisolid mass. These extracts were used for determining the larvicidal activity against mosquito larvae.

Biological assay

Larvicidal activity was evaluated in accordance with World Health Organization (WHO) for the evaluation of new larvicidal agents (WHO, 1985). The larvae of *Anopheles stephensi* was obtained and reared from the neonates in National Institute of Communicable Diseases, Southern India branch field station located at Mettupalayam (District Coimbatore of Tamil Nadu State), at 28 ± 2°C with a photoperiod of 12 h light and dark, and 80 ± 10% relative humidity. A brewer's yeast powder mixed with an equal quantity (w/w) of ground dog biscuit was used in the laboratory as food for the larvae. The late third or early fourth instar larvae were collected according to larval size and the degree of chitinization of respiratory siphon (Cheng et al., 2003). Different concentrations of the extracts were prepared in 1 ml of acetone for each experiment. All experimental exposure was done in 500 ml glass beaker in triplicate. Twenty-five (25) larvae were collected with a pasture pipette, placed on a filter paper for removal of excess of water and placed in 250 ml dechlorinated tap water containing various concentration of crude extracts. Three controls in triplicate were setup, one with acetone (1 ml), the other with distilled water (250 ml).

The beakers were covered with muslin cloth to avoid entry of any foreign material. Sufficient control was also kept for each extracts. The observed mortality (crude mortality) was recorded at 24 h of exposure to test solution. From this crude mortality, percentage crude mortality was obtained. Subsequently, controlled mortality, if any, was recorded and percentage crude mortality was obtained. The percentage crude mortality was corrected by using Abbot's formula. The corrected probit mortality and expected mortality was also obtained but no control mortality was recorded during the experiment, so Abbot's formula was not used.

Statistical analysis

Lethal concentration (LC_{50} and LC_{90}) values and their 95% confidence limits were estimated by fitting a probit regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. Separate probit models were fitted for each extract (Finnely, 1971). All analysis was carried out using the statistical package for social sciences (SPSS) software, version 13.0.

RESULTS AND DISCUSSION

Seven different concentrations of test solution ranging from 50 to 350 ppm for petroleum ether extract and six concentrations of test solution ranging from 50 to 300 ppm for chloroform extract for *L. camara* Linn. and six different concentrations of test solution ranging from 50 to 300 ppm for petroleum ether extract and five different concentrations of test solution ranging from 40 to 200 ppm for ethyl acetate extract were subjected to 24 h bioassay, using late 3rd or early 4th instar larvae of *A. stephensi.* Based on observations made in the 24 h bioassay studies among the different plant extracts, the petroleum ether extract of *L. camara* Linn. was more potent than petroleum ether extract of *B. racemosa* Lam. and ethyl acetate extract of *B. racemosa* was more potent than chloroform extract of *L. camara* Linn. for vector mosquito and were identified as efficient against them. The results from the *A. stephensi* larvicidal bioassay using different extracts of two different plants, the most active extract against late third or early fourth instar larvae of *A. stephensi*, were the petroleum ether extract of *L. camara* and Ethyl acetate extract of *B. racemosa*. The results of susceptibility of larvae for the extracts were given in Tables 1 and 2; and Figures 1 and 2.

Conclusion

The use of the plants in insect control offers a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. Moreover, these results could be useful in the search for newer, more selective and biodegradable larvicidal natural compounds.

ACKNOWLEDGEMENTS

The authors are thankful to the J.S.S. College of Pharmacy, Ooty, Tamil Nadu and National Institute of

Table 1. Observed and expected mortality of *Anopheles stephensi* larvae exposed to *Lantana camara* with petroleum ether and chloroform extracts. Expected mortality is based on probit regression analysis.

Concentration (µg/ml)	No. of Larvae		Mortality (%)		Expected mortality			Probit (mortality) = a + b × concentration	χ^2, DF, P-value	LC_{50} (95% CI)	LC_{90} (95% CI)
	Exposed	Dead	Crude	Corrected	Probit	Dead	%				
Petroleum extract											
50	75	17	22.7	22.7	-0.80	15.8	21.1	-1.3270+0.0105×conc.	χ^2=1.56, DF=5, P=0.9	126.7 (112.0-139.7)	248.9 (231.2-271.8)
100	75	30	40.0	40.0	-0.28	29.3	39.1				
150	75	44	58.7	58.7	0.25	44.8	59.8				
200	75	56	74.7	74.7	0.77	58.5	78.0				
250	75	67	89.3	89.3	1.30	67.7	90.3				
300	75	73	97.3	97.3	1.82	72.4	96.6				
350	75	75	100.0	100.0	2.35	74.3	99.1				
Chloroform extract											
50	75	8	10.7	10.7	-1.95	1.9	2.6	-2.7408+0.0158×conc.	χ^2=39.9, DF=4, P<0.005	173.8 (120.2-235.3)	255.0 (205.5-435.0)
100	75	9	12.0	12.0	-1.16	9.2	12.3				
150	75	13	17.3	17.3	-0.37	26.7	35.5				
200	75	44	58.7	58.7	0.42	49.7	66.2				
250	75	73	97.3	97.3	1.21	66.5	88.7				
300	75	75	100.0	100.0	2.00	73.3	97.7				

DF = degrees of freedom, conc. = concentration, CI = confidence interval.

Table 2. Observed and expected mortality of *Anopheles stephensi* larvae exposed to *Bauhinia racemosa* with petroleum ether and ethyl acetate extracts. Expected mortality is based on probit regression analysis.

Concentration (µg/ml)	No. of Larvae		Mortality (%)		Expected Mortality			Probit (mortality) = a + b x concentration	χ^2, DF, P-value	LC_{50} (95% CI)	LC_{90} (95% CI)
	Exposed	Dead	Crude	Corrected	Probit	Dead	%				
Petroleum ether extract											
50	75	11	14.7	14.7	-1.21	8.4	11.2	-1.8238+0.0122×conc.	χ^2 = 9.7, DF=4, P=0.046	149.3 (121.2-175.3)	254.3 (220.5-316.0)
100	75	24	32.0	32.0	-0.60	20.5	27.3				
150	75	31	41.3	41.3	0.01	37.7	50.2				
200	75	49	65.3	65.3	0.62	54.8	73.1				
250	75	69	92.0	92.0	1.23	66.7	89.0				

Table 2. Contd

300	75	75	100.0	100.0	1.84	72.5	96.7				
						Ethyl acetate extract					
40	75	12	16.0	16.0	-1.14	9.5	12.7				
80	75	25	33.3	33.3	-0.43	25.1	33.5				
120	75	42	56.0	56.0	0.28	45.9	61.2	-1.8513+0.0178×conc.	χ^2=5.9, DF=3, P=0.11	104.0 (95.2-112.5)	176.1 (163.3-193.1)
160	75	60	80.0	80.0	1.00	63.0	84.1				
200	75	75	100.0	100.0	1.71	71.7	95.6				

DF = degrees of freedom, conc. = concentration, CI = confidence interval.

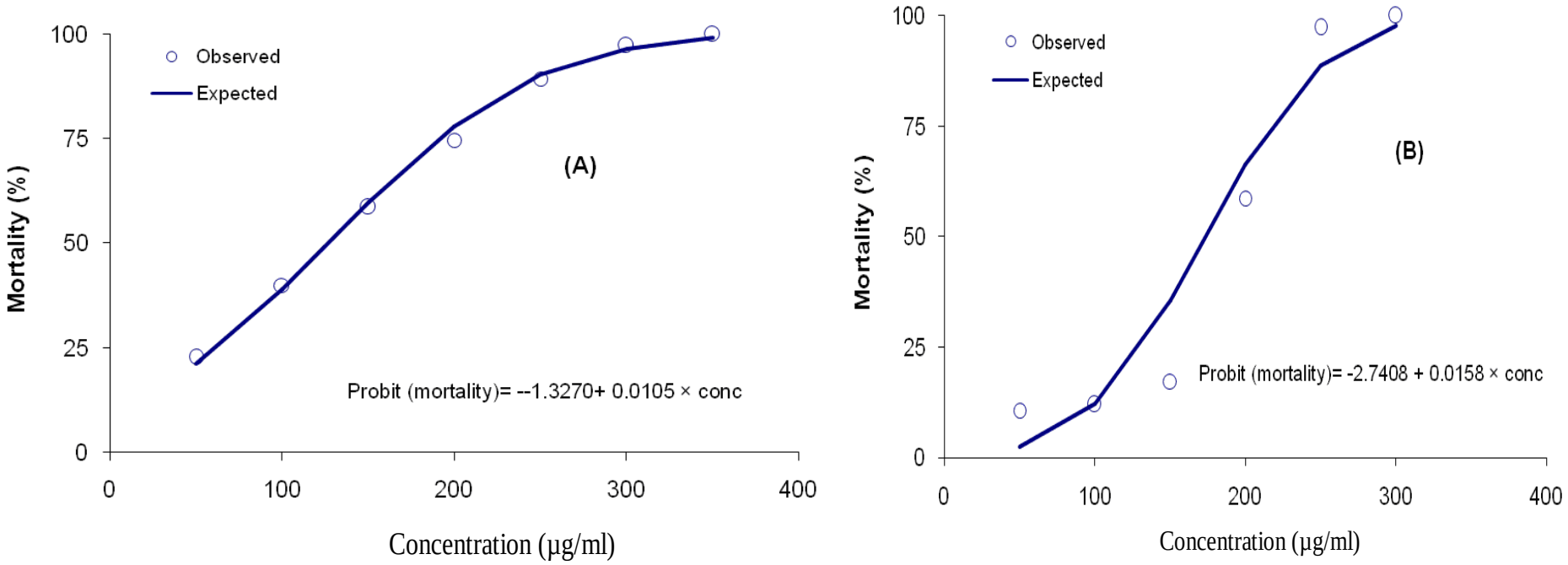

Figure 1. Relationship between *Anopheles stephensi* larval mortality and concentration of *Lantana camara* with (A) petroleum ether and (B) chloroform extracts. Expected values are based on probit regression analysis. Conc = concentration.

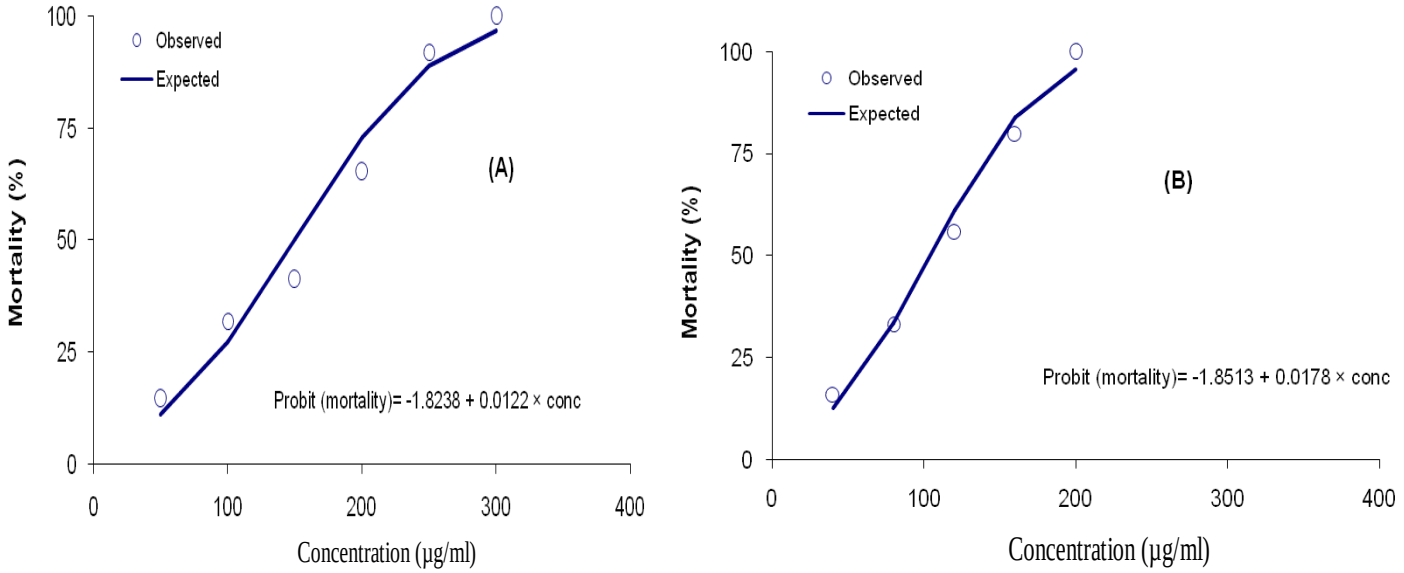

Figure 2. Relationship between *Anopheles stephensi* larval mortality and concentration of *Bauhinia racemosa* with (A) petroleum ether and (B) ethyl acetate extracts. Expected values are based on probit regression analysis. Conc = concentration.

Communicable Diseases, (Coonoor), Southern India branch field station located at Mettupalayam (District Coimbatore of Tamil Nadu State) for providing all necessary facilities required FOR completing this research work.

REFERENCES

Cheng SS, Chang HT, ST Chang, Tsai KH, Wei JC (2003). Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. Bio. Resour. Tech. 89:99-102.

Das PKKM (1989). A module for chemical control of mosquito vectors, V.C.R.C Pondicherry.

Finnely DJ (1971). Probit Analysis, 3rd ed. Cambridge: Cambridge Univ.press; p 333.

Ivan AR (1999). Medicinal Plants of the World Chemical Constituents Traditional and Modern medicinal Uses. NJ: Published by Humana Press Inc. Totowa. pp. 179-180.

Maheswaran R, Kingsley S, Ignacimuthu S (2008). Larvicidal and Repellent activity of Clerodendron phlomides against Culex quinquefasciatus Say (Diptera: Culicidae). proceed. Recent Trends Insect Pest Manage. pp.240-243.

Prajapati ND, Purohit SS, Sharma AK, Kumar T (2007). Handbook of Medicinal Plants: A complete source book. India: Published by Agrobis. pp. 306-307.

Raveendra KR, Martin P (2006). Ethnomedicinal Plants. Published by Agrobis (India), Jodhpur, p 80.

Sakthivadivel M, Daniel T (2008). Evaluation of certain insecticidal plants for the control of vector mosquitoes viz. *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. Appl. Entomol. Zool. 43(1):57-63.

Singh MP, Himadri P (2005). Medicinal herbs with their formulations. Published by Daya Publishing House, Delhi, Volume I, p. 158.

Singh RK, Dhiman RC, Mittal P K (2006). Mosquito larvicidal properties of *Momordica charantia* Linn. J. Vect. Borne Dis. 43:88-91.

Varier PS (2006). Indian Medicinal Plant: A Compendium of 500 Species, Orient Longman Arya Vaidya Sala. Chennai: Orient Longman Private Limited; pp. 300-301.

World Health Organisation (WHO) (1985). Bioassay method for the titration of *Bacillus sphaericus* preparations with RB standard. Informal consultations on the development of *Bacillus sphaericus* as a microbial larvicide. World Health Organization, Geneva. (TDR/BVC/SPHAERICUS/ 85.3).

Yoganarasimhan SN (2000). Medicinal Plants of India. Tamil Nadu: 2:315-373.