

# LARVAL SUSCEPTIBILITY OF *AJUGA REMOTA* AGAINST ANOPHELINE AND CULICINE MOSQUITOS

Preeti Sharma, Lalit Mohan and CN Srivastava

Applied Entomology and Vector Control Laboratory, Department of Zoology, Faculty of Science, Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra, India

**Abstract.** The objective of the present study is to determine the bioefficacy of different crude extracts of *Ajuga remota* against anopheline and culicine larvae. Larval susceptibility of crude carbon-tetrachloride, methanol and petroleum-ether extracts of *Ajuga remota* leaves was observed against the malaria vector, *Anopheles stephensi* and the filariasis vector, *Culex quinquefasciatus*. Among the extracts tested, petroleum-ether extract was the most effective with LC<sub>50</sub> values of 0.033% after 24 hours and 0.029% after 48 hours of treatment against the larvae of *Anopheles stephensi*. In the case of the larvae of *Culex quinquefasciatus*, the carbon-tetra-chloride extract exhibited maximum efficacy with LC<sub>50</sub> values of 0.043% after 24 hours and 0.026% after 48 hours of exposure, respectively. It is, therefore, concluded that *Ajuga remota* can be applied as an ideal larvicide against *An. stephensi* and *Cx. quinquefasciatus*.

## INTRODUCTION

Mosquitos are the carrier of a number of vector-borne diseases, such as malaria, filariasis, yellow fever, brain fever, dengue fever, etc (Jaswanth *et al*, 2002). Men are compelled to fight against them using available technical armaments. There was initial success in controlling vectors by using synthetic insecticides. The major drawbacks with these synthetic insecticides are that they are generally non-biodegradable, toxic to non-targets, and vectors develop resistance against them (Evans and Raj, 1988). Efforts are ongoing to seek insecticides of natural origin as a safer alternative to these synthetic insecticides. Many plant extracts have been studied for their efficacy in controlling larvae of different mosquito species the world over (Kumar and Dutta, 1987; Evans and Raj, 1988; Markouk *et al*, 2000; Jaswanth *et al*, 2002; Singh and Bansal, 2003). India has a lot of unexploited vegetative wealth for its insecticidal property. In this direction, we have evaluated the larvicidal nature of various extracts of *Ajuga remota* against *Anophales stephensi* and *Culex quinquefasciatus*.

## MATERIALS AND METHODS

The leaves of *Ajuga remota* were collected from an area adjacent to the institute. The collected leaves were washed with water, dried in the shade, and powdered. The leaf powder was subjected to soxhlation by using petroleum ether, carbon-tetrachloride and methanol as solvents so as to fractionate the constituents of *Ajuga remota* leaves depending upon their solubility in these solvents of increased polarity. After the soxhlation, the solvents *ie* petroleum ether, carbon-tetra-chloride and methanol were evaporated completely by distillation and the crude extracts were collected. These crude extracts were further used to prepare the stock solution in a suitable carrier solvent (acetone/alcohol). Different test concentrations were prepared by diluting these stocks in water.

The colonies of *An. stephensi* and *Cx. quinquefasciatus* were maintained in the laboratory at a temperature of 27° ± 1°C and 85% relative humidity. The larvae of both the species were maintained in separate containers and provided with yeast powder as food during experimentation.

The third instar larvae of anopheline and culicine, collected from the maintained-laboratory culture, were acclimatized for the experimental conditions of bioassay. The tests were carried out by placing 20 larvae in a 500-ml glass beaker containing 250 ml of test concentration of differ-

---

Correspondence: CN Srivastava, Applied Entomology and Vector Control, Laboratory, Department of Zoology, Faculty of Science, Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra 282 005, India. Tel: +91-562-3103817 (R); Fax: +91-562-2121226 E-mail: chandnarayan\_dei@rediffmail.com

ent extracts individually. Three replicants of each dosage were arranged for the six-test solutions. Control experiments were conducted in parallel with each replicant. All the experiments were performed according to a WHO (1971) standard technique. Mortality counts were made after 24 and 48 hours of exposure. Controls with more than 20% mortality were discarded. When the mortality ranged from 5-20%, the corrected mortality was calculated by Abbott's formula so as to remove the error, if any, on account of the mortality due to factors other than the toxic effect of the extract (Abbott, 1925). The  $LC_{50}$  was calculated using Probit analysis (Finney, 1971).

## RESULTS

The larvicidal potency of different extracts of *Ajuga remota* leaves against *An. stephensi* and *Cx. quinquefasciatus* are given in Tables 1 and 2, respectively. Among the three extracts tested, the extract obtained by soxhlation with petroleum-ether was found to be the most toxic and the extract obtained by soxhlation with methanol was the least effective against *An. stephensi*. In the case of culicine larvae, the extract collected after carbon-tetra-chloride soxhlation was the most effective and the extract collected after methanol soxhlation was the least toxic. As mentioned in Table 1, the  $LC_{50}$  values of the carbon-tetra-chloride extract were 0.045% and 0.031% after 24

and 48 hours of exposure, respectively, against anopheline larvae. The methanol soxhlated extract had  $LC_{50}$  values were 0.046% and 0.043% after 24 and 48 hours of treatment, respectively. The  $LC_{50}$  values of the petroleum-ether soxhlated extract were 0.033% and 0.029% after 24 and 48 hours of exposure. Among the extracts tested against culicine larvae as per Table 2, the carbon-tetra-chloride soxhlated extract had  $LC_{50}$  values of 0.043% and 0.026% after 24 and 48 hours of treatment, respectively. The  $LC_{50}$  values of the methanol soxhlated extract were 0.045% and 0.042% after the same exposure periods, respectively. The petroleum ether soxhlated extract had  $LC_{50}$  values of 0.442% and 0.359% after 24 and 48 hours of treatment, respectively.

## DISCUSSION

The efficacy of several plant extracts has been established against mosquito larvae (Kalyansundram and Das, 1985; Kumar and Dutta, 1987; Evans and Raj, 1988; Markouk *et al*, 2000; Jaswanth *et al*, 2002; Singh and Bansal, 2003). The aqueous extracts of different parts of *Solanum xanthocarpum* were tested against the larvae of certain mosquito species by Singh and Bansal (2003). Among the parts used, the fruit extract was the most effective with a  $LC_{50}$  value of 0.052% against *Aedes aegypti*. The root extract was the least effective with a  $LC_{50}$  value of

Table 1  
Efficacy of different extracts of *Ajuga remota* leaves against the larvae of *Anopheles stephensi*.

Phytoextract	Exposure period (hours)	Regression equation	Chi-square ( $\chi^2$ )	$LC_{50}$ (%)	Fiducial limits	Relative potency	Relative potency irrespective of time period
Fraction-I (obtained with petroleum-ether)	24	-10.5x+96.5	2.40	0.033	0.031 0.035	1.0	1.14
	48	-10x+104	0.48	0.029	0.027 0.032	1.0	1.0
Fraction-II (obtained with carbon-tetra-chloride)	24	-11.5x+60.5	1.51	0.045	0.033 0.063	1.36	1.55
	48	-13x+72	2.61	0.031	0.023 0.041	1.07	1.07
Fraction-III (obtained with methanol)	24	-19.66x+104.28	4.59	0.046	0.045 0.047	1.39	1.59
	48	-21.67x+117.65	14.90	0.043	0.042 0.045	1.48	1.48

Table 2

Efficacy of different extracts of *Ajuga remota* leaves against the larvae of *Culex quinquefasciatus*.

Phytoextract	Exposure period (hours)	Regression equation	Chi-square ( $\chi^2$ )	LC <sub>50</sub> (%)	Fiducial limits	Relative potency	Relative potency irrespective of time period
Fraction-I (obtained with petroleum-ether)	24	-11x+95	4.06	0.442	0.422 0.464	10.28	17.0
	48	-6.5x+92.5	0.19	0.359	0.312 0.414	13.81	13.81
Fraction-II (obtained with carbon-tetra chloride)	24	-12.5x+62.5	0.71	0.043	0.033 0.059	1.0	1.65
	48	-14x+77	0.53	0.026	0.021 0.033	1.0	1.0
Fraction-III (obtained with methanol)	24	-13.5x+87.5	18.51	0.045	0.044 0.047	1.05	1.73
	48	-13x+94	17.89	0.042	0.04 0.044	1.61	1.61

1.16% against *Anopheles culicifacies*. The results of the *Ajuga remota* extracts are, therefore, comparable to earlier observations made by Singh and Bansal (2003). Among the extracts of *A. remota* tested, the petroleum-ether soxhlated extract was the most effective with LC<sub>50</sub> values of 0.033% after 24 hours and 0.029% after 48 hours of exposure against *An. stephensi*. According to the results, the petroleum-ether extract of *A. remota* was 1.57 times more effective than the *S. xanthocarpum* aqueous-fruit-extract after 24 hours and 1.79 times more toxic after 48 hours of treatment against *An. stephensi*. In the case of the culicine larvae, the carbon-tetra-chloride extract was 1.15 times more toxic after 24 hours and 2.0 times more effective after 48 hours of exposure than in previous studies. The results show that in both cases the *Ajuga remota* was more effective than *S. xanthocarpum*. The studies suggest that the active ingredient of the extract responsible for larval mortality in mosquitos should be identified and utilized, if possible, in preparing a commercial product/formulation to be used as a mosquito larvicide.

## REFERENCES

- Abbott WS. A method of computing of the effectiveness of an insecticide. *J Econ Entomol* 1925; 18: 265-7.
- Evans DA, Raj PK. Extract of Indian plants as mosquito larvicides. *Indian J Med Res* 1988; 88: 38-41.
- Finney DJ. Probit analysis. 3<sup>rd</sup> ed. Cambridge: Cambridge University Press, 1971.
- Jaswanth A, Ramanathan P, Ruckmani K. Evaluation of the mosquitocidal activity of *Annona squamosa* leaves against filarial vector mosquito, *Culex quinquefasciatus* Say. *Indian J Exptl Biol* 2002; 40: 363-5.
- Kalyansundaram M, Das PK. Larvicidal and synergistic activity of plant extracts for mosquito control. *Indian J Med Res* 1985; 82: 19-21.
- Kumar A, Dutta GP. Indigenous plant oils as larvicidal agents against *Anopheles stephensi*. *Curr Sci* 1987; 56: 959-60.
- Markouk M, Bekkouche K, Larhsini M, Bousaid M, Lazrek H B, Jana M. Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. *J Ethnopharmacol* 2000; 73: 293-7.
- Singh KV, Bansal SK. Larvicidal properties of a perennial herb *Solanum xanthocarpum* against vectors of malaria and dengue/DHF. *Curr Sci* 2003; 84: 749-51.
- WHO. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. *WHO/VBC/75*, 1971: 583.