LARVICIDAL AND CHEMOSTERILANT ACTIVITY OF *ANNONA SQUAMOSA* ALKALOIDS AGAINST *ANOPHELES STEPHENSI*

R. C. SAXENA, V. HARSHAN, A. SAXENA, P. SUKUMARAN, M. C. SHARMA AND M. LAKSHAMANA KUMAR

Post Graduate Department of Zoology, S. S. L. Jain College, Vidisha, M. P. 464001, India

ABSTRACT. Alkaloids isolated from *Annona squamosa* have shown larvicidal growth-regulating and chemosterilant activities against *Anopheles stephensi* at concentrations of 50 to 200 ppm. Adults exposed as larvae to different treatments showed reduced fecundity and fertility in females. Mortality in the larvae, pupae and adults produced about a 52–92% decrease in the laboratory experiment. The total developmental period was slightly reduced from the control. Treatment with the alkaloids had a significant effect on the mortality, emergence and reproductive physiology of *An. stephensi*.

INTRODUCTION

Plant alkaloids have been found to affect physiological systems in higher animals as well as in insects (Saxena and Tikku 1990). Schneider et al. (1982) have mentioned that these compounds in general are very toxic to insects and can be used as insecticides.

Some plants known to contain toxic principles can play a useful role in the control of vectors. A variety of plant species of different families have been reported to exhibit insecticidal and other biological activities (Jotwani and Srivastava 1981, Banerji et al. 1985, Kalyanasundaram and Das 1985, Saxena and Sumithra 1985, Chavan and Nikam 1988, Saxena and Saxena 1992, Saxena et al. 1992).

Since, *Annona squamosa* (Family Annonaceae) extracts have shown insecticidal activity against stored grain insects (Dixit 1992[1]), we deemed it appropriate to study the larvicidal and chemosterilant activity of *A. squamosa* alkaloids against *Anopheles stephensi* Liston, an important urban vector of malaria in India.

MATERIALS AND METHODS

Collection and extraction of plant material: Green aerial parts of the plant, *A. squamosa*, were collected locally during the winter season. The collected plant material was washed thoroughly with water and air dried in the shade for about a month. For extraction of alkaloids the methods reported by Chopra et al. (1933) were employed as follows: A known quantity of powdered material was extracted with hot acidulated (HCl) water until the alkaloid was completely removed. The acidulated solution was cooled with ice and treated with petroleum ether for the removal of oily substances. The solution was then neutralized with caustic soda, filtered and then eluted in chloroform. The chloroform extract was then concentrated and acidulated with dilute hydrochloric acid (2N). From this solution the total alkaloids present were precipitated with caustic soda. The alkaloids thus obtained were used for experimental bioassay.

Experimental bioassay: Laboratory colonies of *An. stephensi* used in this study were maintained at 27 ± 2°C, 75–85% RH, under a 14L:10D photoperiod cycle. The larvae were fed with a diet of finely ground brewer's yeast and dog biscuits (3:1). Adults were fed *ad libitum* on 10% sucrose solution. Females were allowed to bloodfeed from a rabbit placed in a restraining cage 3 days after emergence.

For experimental treatment, 25 second or fourth instar larvae of *An. stephensi* were kept in 500 ml glass beakers containing 249 ml of distilled water and 1 ml alkaloids. Ethanol (50%) was used as solvent to dilute the alkaloids to an appropriate test concentration. The treatments were replicated 3 times. Each replicate set contained one control which received 1 ml of 50% ethanol and 249 ml of distilled water and one untreated which contained only 250 ml of distilled water. The number of dead larvae, pupae and adults were recorded. Mortality was corrected according to Abbott (1925). The fecundity and fertility experiments were conducted by taking equal number of male and female mosquitoes which had emerged from the treated and untreated sets and mated in cages (30×30×30 cm), and placed into the following groups: 1) treated females with treated males (T ♀×T ♂), 2) treated females with untreated males (T ♀×UT ♂), 3) untreated females with treated males (UT ♀×T ♂), and 4) untreated females with untreated males (UT ♀×UT ♂). Three days after the blood meal, eggs were collected daily from the small plastic bowls containing test concentrations from ovitraps kept in the cages. Just after hatching larvae were transferred to the enamel trays

[1] Dixit, O. P. 1992. Study on the effect of selected plant bioproducts on some pulse beetles (Coleoptera: Bruchidae) Ph.D. Thesis Faculty of Life Sciences, Barkatullah University, Bhopal, India.

Table 1. Effect of *Annona squamosa* alkaloid on development, molting and metamorphosis of *Anopheles stephensi*.

Concentration (ppm)	Average larval period (days)	Larval mortality (%)	Average pupal period (days)	Pupal mortality (%)	Adult emergence (%) (a)	Adult mortality (%)	Average development (b) (days)	Total mortality (%)	Growth index (a/b)
50	13.5	58	2.0	4	48	4	15.5	52	3.09*
100	13.5	60	1.5	6	34	4	15	76	1.71*
150	13	70	1.5	16	14	8	14.5	86	0.97*
200	13	74	1.5	18	8	8	14.5	92	0.53*
Control	14	4	2.5	4	92	0	16.5	8	5.57
Untreated	14	0	2.5	4	96	0	16.5	4	5.82

* Values are significantly different from control and untreated groups (Duncan's multiple range test, $P < 0.005$).

(30×25×5 cm) containing distilled water. Larval mortality, developmental period and growth index were recorded until adult emergence. Percentage of hatching and the effect upon metamorphosis were assessed (Saxena and Sumithra 1985) as follows:

Sterility Index (SI)

$$= 100 - \frac{\text{Treated number of eggs} \times \%\text{ of eggs hatched}}{\text{Control number of eggs} \times \%\text{ of eggs hatched}} \times 100$$

Growth Index (GI)

$$= \frac{\text{Adult emergence}}{\text{Average developmental period}}$$

Statistical evaluation of data were carried out by probit analysis (Finney 1971) and level of significance by Duncan's (1963) multiple range test.

RESULTS AND DISCUSSION

The plant alkaloids applied in the present study showed larvicidal activity against second and fourth instar larvae of *An. stephensi*. The 24-h LC_{50} and LC_{90} values were 178, 126 ppm and 751, 428 ppm, respectively, for second and fourth instar larvae. It was also observed that treated larvae exhibited abnormal behavior such as aggregation of larvae on the water surface, and movement in circles near the periphery of the beaker as opposed to normal zig-zag motion in the untreated sets. Mortality of larvae and pupae in different concentrations during the experiment ranged from 52 to 92% as compared with 8% in the control and untreated groups (Table 1). These results are quite comparable to our previous reports on indigenous plant extracts against *An. culicifacies* Giles and *An. stephensi* in which crude extracts of 2 plants, *Premina integrifolia* and *Ageratum conyzoides*, were found toxic causing 100% mortality to second instar larvae at 500 ppm concentration (Saxena and Saxena 1991, 1992). The larvicidal activity of various plant extract such as *Pedalium murax, Cleome icosandra* and *Dictyosa dichotoma* have been found to be promising against *Culex quinquefasciatus* Say showing 24-h LC_{50} values of about 23, 11 and 2 ppm, respectively (Kalyanasundaram and Das 1985). Similar results were also reported for *Cx. quinquefasciatus* exposed as larvae to 7 different concentrations of *Haplophyllum tuberculatum* extract (Mohsen et al. 1989).

In our study some morphological abnormalities such as formation of larval-pupal intermediates and half-ecdysed adults were observed. Spielman and Skaff (1967) reported that a butanol extract of the soapberry plant, *Phytolacca dodecandra*, induced morphogenetic aberrations in *Aedes aegypti* (Linn.), *Culex pipiens* and *Anopheles quadrimaculatus* Say. Similarly, Supavarn et al. (1974) using methanol extract of plant species from 17 families, reported that in addition to acute toxicity, compounds from these plants significantly lengthened the larval period in *Ae. aegypti* which was suggested to be due to the interference in normal hormonal activity. Sujatha et al. (1988) have also described some morphogenetic abnormalities in treated larvae of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* when 5 and 10 mg/liter concentrations of *M. longifolia, Acorus calamus* and *Ageratum conyzoides* were applied. They have observed the formation of larval-pupal intermediates, decolourized and extended pupae, and incompletely emerged adults. However in this study, only larval-pupal intermediates and half-ecdysed adults could be seen.

From the results, it is apparent that the plant alkaloid did not affect the length of the larval developmental period. The growth index was found to be significantly different ($P < 0.005$) from the control and untreated groups (Table 1). This is in agreement with the earlier reports

Table 2. Fecundity and fertility effect of *Annona squamosa* alkaloids adults of *Anopheles stephensi* from treated larvae.

Treated groups*	% of females dead	Oviposition day after blood meal	Average number of eggs obtained	% of hatching	% larval mortality	% adult emergence	% sterility index (SI)
T ♀ × T ♂	54.0	4	75*	68.0*	44	52	50.67
T ♀ × UT ♂	28.2	3	78*	72*	42	64	54.31
UT ♀ × T ♂	42.0	4	88*	79.5	38	78	66.80
UT ♀ × UT ♂	10.5	3	110	94	4	92	—

* 10 each treated females and males were taken in each replicate.
** Values are significantly different than the control (Duncan's multiple range test, $P < 0.01$).
T = treated; UT = untreated.

on the effects of *Ipomea cornea* extract on the growth and development of *An. stephensi* and *Cx. quinquefasciatus* in which the growth index of treated mosquitoes is shorter than the control and untreated groups (Saxena and Sumithra 1985).

Plant alkaloids resulted in a significant loss in fecundity and fertility in the adults from treated larvae as compared to the control and untreated larvae (Table 2). The sterility index was found to be significantly reduced when larvae treated adult females were mated with equal number of treated males ($P < 0.01$). Egg hatching was also affected in this group. These results were found to be consistent in our recent reports on some anti-juvenile hormone-like activity of five plant extracts against *Cx. quinquefasciatus* in which *Ageratum conyzoides, Cleome icosandra* and *Tridax procumbens* induced loss in biting behavior and fecundity and fertility at sublethal concentrations. Other investigators have also reported reduced egg production following larval exposure to sublethal concentration of insecticides in *Cx. quinquefasciatus* (Ferrari and Georghiou 1981, Robert and Olson 1989).

Alkaloids of *A. squamosa* at sublethal concentrations against larvae of *An. stephensi* caused developmental defects as well as larval mortality. Females obtained from the treated larvae showed loss in fecundity and fertility. The plant alkaloids noticed to have possess growth stimulating activities. Further studies of active principles involved and field trials are needed to recommend the alkaloids of *A. squamosa* for mosquito control programs.

REFERENCES CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265–267.

Banerji, R. G., G. Mishra and S. K. Nikam. 1985. Role of indigenous plant material in pest control. Pesticides 19:32–38.

Chavan, S. R. and S. T. Nikam. 1988. Investigation of alkanes from neem leaves and their mosquito larvicidal activity. Pesticides 12:32–33.

Chopra, R. N., J. C. Gupta and B. Mukherjee. 1933. The pharmacological action of an alkaloid obtained from *Rauwolfia serpentina*. Benth. Indian J. Med. Res. 21:261–271.

Duncan, J. 1963. Post-treatment effects of sublethal doses of dieldrin on the mosquito *Aedes aegypti* (L.). Ann. Appl. Biol. 52:1–6.

Ferrari, J. A. and G. P. Georghiou. 1981. Effects on insecticidal selection and treatment on reproductive potential of resistant, susceptible and heterozygous strains of the Southern house mosquito. J. Econ. Entomol. 74:323–327.

Finney, D. J. 1971. Probit analysis. Third edition. Cambridge Univ. Press, London.

Jotwani, M. G. and Srivastava, K. P. 1981. Neem insecticides of the future-II protection against field pests. Pesticides 15:40–47.

Kalyanasundaram, M. and P. K. Das. 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. Indian. J. Med. Res. 82:19–23.

Mohsen, Z. H., H. J. Jaffer, M. Al. Sadia and Z. S. Ali. 1989. Insecticidal effects of *Haplophyllum tuberculatum* against *Culex quinquefasciatus*. Int. J. Crude Drug Res. 27:17–21.

Robert, L. L. and J. K. Olson. 1989. Effects of sublethal dosages of insecticides on *Culex quinquefasciatus*. J. Am. Mosq. Control Assoc. 5:239–246.

Saxena, A. and R. C. Saxena. 1991. A new plant extract to suppress the population of malaria vector *Anopheles culicifacies*. Geobios New Reports 10:154–156.

Saxena, A. and R. C. Saxena. 1992. Effect of *Ageratum conyzoides* extract on the developmental stages of malaria vector *Anopheles stephensi* (Diptera: Culicidae). J. Environ. Biol. 13:207-209.

Saxena, B. P. and K. Tikku. 1990. Impact of natural products on the physiology of phytophagous insects. Proc. Indian Acad. Sci. (Animal Sci.) 99:185–198.

Saxena, S. C. and L. Sumithra. 1985. Laboratory evaluation of leaf extract of a new plant to suppress the population of malaria vector *Anopheles stephensi* Liston. Curr. Sci. 54:201–202.

Saxena, R. C., O. P. Dixit and P. Sukumaran. 1992. Laboratory assessment of indigenous plant extracts of anti-juvenile hormone activity in *Culex quinque-*

fasciatus. Indian J. Med. Res. 95:204–207.
Schneider, D., M. Boppre, J. Zweig, S. B. Horsley, T. W. Bell, J. Meinwald, K. Hansen and E. W. Diehl. 1982. Scent organ development in *Creatonotos* moths: regulation by pyrrolizidine alkaloids. Science 215:1264–1265.
Spielman, A. and V. Skaff. 1967. Pharmacology of insect juvenile hormones in comprehensive insect physiology, pp. 357–394. *In:* G. A. Kerkut and L. I. Gilbert (eds.), Biochemistry and pharmacology. vol. 2. Pergamon Press. Oxford.
Sujatha, C. H., V. Vasuki, T. Mariappan, M. Kalyanasundaram and P. K. Das. 1988. Evaluation of plant extract for biological activity against mosquitoes. International Pest Control 30:122–124.
Supavarn, P., W. K. Fred and S. Roy. 1974. Biologically active plant extracts for control of mosquito larvae. Mosq. News 34:398–402.