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LARVICIDAL ACTIVITY OF *ANNONA SQUAMOSA* LEAF AND SEED EXTRACT AGAINST IMMATURE STAGES OF *CULEX QUINQUEFASCIATUS*

K. MANIMEGALAI
DEPARTMENT OF ZOOLOGY,
AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND
HIGHER EDUCATION FOR WOMEN,
COIMBATORE-641 043, INDIA
(e-mail : manijeysu@gmail.com)

Petroleum ether, chloroform and ethanol extracts of the leaf and seed of *Annona squamosa* were evaluated for larvicidal activity against laboratory reared fourth instar larvae of *Culex quinquefasciatus*, a vector of the *Bancroftian filariasis* and urban nuisance mosquito. The larval mortality was observed after 24 hr exposure. The extract was found to suppress the population of the vector at higher dosage, while the lower dosages found to induce several developmental defects.

Key words : Larvicidal activity, *Annona squamosa, Culex quinquefasciatus*

INTRODUCTION

Phytochemicals can act as larvicides, insect growth regulators, repellents and ovipositional attractants.(Babu & Murugan, 1998;Venkatachalam & Jebanesan, 2001). More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes (Ahmed *et al.*, 1984) and plants products of some 344 species have been reported to have a variety of activity against mosquitoes.

MATERIALS AND METHODS

Plants of *Annona squamosa* leaf and seed were collected from in and around Avinashilingam University campus at Coimbatore. 10g of each of the leaf and seed powder was weighed using an electronic balance (Denver XS-210) and made into packets using Zerohaze filter paper. These powders were subjected to extraction with 500 ml of the solvents for 8 h using a Soxhelt apparatus (Harbourne, 1973; Vogel, 1978). Petroleum ether (60-80°C) extraction was followed by chloroform extraction and ethanol extraction, so that the powder were subjected to extraction with solvents of increasing polarity . The leaf and seed extract thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40°C.

Bioassay studies : A laboratory colony of *C. quinquefasciatus* was used for the larvicidal activity. The larvae were maintained at 70-85% relative humidity, 27 ± 2°C temperature and 14L : 10D photoperiod cycle. Larvicidal activity of *C. quinquefasciatus* was assessed by using the standard method (WHO, 1996).

Experimental design : The experiment was laid down in a Completely Randomized Design (CRD). The experimental set up consisted of six treatments, each with three replications, one set for leaf extract and another for seed extract. Twenty newly emerged IV-instar larvae of *C. quinquefasciatus* were introduced into the beakers for the bio-assay studies. Controls with three replications were also maintained simultaneously. Larval and pupal behaviour, morphological changes and incidences of malformations were observed.

Test for larvicidal activity : The larval mortality in both treatment and control was recorded at 24h of treatment and the percentage of mortality was calculated using Abbott's formula (Abbott, 1925).

$$\% \text{ Mortality} = \frac{\text{Mortality in treatment (\%) - Mortality in control (\%)}}{\text{100 - Mortality in control (\%)}} \times 100$$

RESULT

Among the three *A. squamosa* leaf extracts, petroleum ether extract was found to be more effective than others by inducing 50% and above larval mortality in 24 h. However, 100% mortality was observed in higher concentrations ranging from 80 to 120 ppm in all the three leaf treatments by 96 h. In all higher concentrations 100% mortality was recorded at 96 h and there was no pupation. In lower concentrations pupation ranged from 5 - 40% and pupal mortality ranged from 5 - 25%. There was no adult emergence in higher concentrations of all the three extracts. In lower concentrations 5 - 25% adult emergence was recorded. 24 h LC_{50} indicated petroleum ether extract as the most effective one with a LC_{50} value of 82.3 ppm and the regression equation of Y = 1.77 + 1.69X, 95% confidence limits UCL LC_{50} calculated was 116.9 ppm and LCL LC_{50} was 57.90 ppm. In comparison the LC_{50} value of chloroform extract was 2340.5 ppm and ethanol extract was 288.3 ppm (Table I).

Petroleum ether extract of *A. squamosa* seed was found to be more effective than chloroform and ethanol extracts. The larval mortality of 50% and above was obtained in petroleum ether extract by 24 h. However, 100% larval mortality was observed in higher concentrations of 100 to120 ppm of all the three extracts at 72 h. Pupation was minimum in 40 ppm chloroform seed extract (10%) and maximum was in 20 ppm ethanol extract (40%). Maximum pupal mortality (20%) was observed in 20 ppm ethanol extract. No adult emergence was recorded in all higher concentrations and it was minimum in 40 ppm chloroform extract (5%).

The 24 h LC_{50} value of petroleum ether extract was 75.7 ppm, with a regression equation, Y = 2.45+1.36x, 95% confidence limits UCL LC_{50} 90.93 ppm and LCL LC_{50} 62.94 ppm (Table II). Compared to this, the LC_{50} value of chloroform extract was 327.1 ppm and ethanol extract was 305.3 ppm. Abnormal behaviour of larvae such as restlessness, circular movement near the periphery of the beaker as compared to zigzag movement in the control set, sluggish movement, loss of equilibrium, muscular tetany and stay of larvae at the bottom of the beaker for comparatively longer periods were observed in the study.

DISCUSSION

Plants are rich sources of bioactive organic chemicals and synthesize a number of synthetic 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 (Ignacimuthu,

Table I : Lethal concentration of leaf extracts of *Annona squamos* against *Culex quinquefaseiatus*

Solvents used	Hours	LC_{50} (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression equation	95% confidence limits				X^2	SE
						UCL (ppm)		LCL (ppm)			
						LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	24	82.26	168.42	473.82	Y = 1.77 +1.69X	116.88	1539.80	57.90	145.80	10.07	0.60
	48	39.86	77.11	199.91	Y = 2.07 + 1.83X	46.69	272.77	34.03	146.51	7.00	0.41
	72	28.77	53.95	133.72	Y =2.80+ 1.92X	61.93	440.19	13.37	40.62	17.66	0.80
	96	7.22	64.32	1511.36	Y = 4.53 + 0.55X	67.10	234.28	0.78	9.75	5.84	0.74
Chloroform	24	2340.46	13523.09	170120.1	Y = 2.68 + 0.69X	34469.31	5.96E+07	158.92	485.94	2.95	0.51
	48	115.44	327.33	1473.67	Y = 2.61 + 1.16X	155.54	4634.24	85.68	468.62	4.29	0.41
	72	32.96	63.15	161.42	Y = 2.18 + 1.86X	60.46	416.27	17.97	62.60	22.45	0.60
	96	8.14	21.23	84.67	Y = 3.85 + 1.26X	21.50	165.08	3.08	43.43	0.84	0.82
Ethanol	24	288.26	795.15	3440.22	Y = 2.07 + 1.19X	540.33	17152.46	153.78	689.99	1.21	0.48
	48	73.11	156.71	471.08	Y = 2.05 +1.58X	85.44	817.59	62.56	271.43	4.97	0.42
	72	32.31	57.25	130.72	Y = 1.81 + 2.11X	57.13	333.71	18.27	51.20	13.99	0.80
	96	17.00	28.30	59.06	Y = 2.08 + 2.37X	2771.58	9550.32	0.10	0.37	8.02	5.55

X^2 : Chi-Square; SE : Standard Error; UCL : Upper Confidence Limit; LCL : Lower Confidence Limit.

Table II : Lethal concentration of seed extracts of *Annona squamosa* against *Culex quinquefasciatus.*

Solvents used	Hours	LC_{50} (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression equation	95% confidence limits				X^2	SE
						UCL (ppm)		LCL (ppm)			
						LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	24	75.65	184.01	663.92	Y = 2.45+ 1.36 X	90.93	1381.26	62.94	319.12	7.21	0.41
	48	34.33	65.80	168.29	Y = 2.15 + 1.86 X	53.24	340.49	22.14	83.18	12.69	0.59
	72	25.03	44.72	103.35	Y = 2.09 + 2.08 X	98.41	834.22	6.37	12.80	18.56	1.33
	96	15.56	38.31	140.60	Y = 3.40 + 1.34 X	26.94	88.82	0.28	29.26	9.23	4.94
Chloroform	24	327.13	1257.44	8783.09	Y = 2.74 + 0.90 X	768.66	103634.2	139.22	744.37	1.12	0.45
	48	50.38	106.07	310.70	Y = 2.24 + 1.62 X	58.77	481.40	43.20	200.53	5.55	0.41
	72	21.74	39.88	95.72	Y=2.34+1.99 X	27.75	129.77	17.04	70.61	2.48	0.60
Ethanol	24	305.33	872.27	3969.73	Y = 2.14 + 1.15 X	596.85	22006.54	156.19	716.09	1.16	0.48
	48	64.37	134.60	390.42	Y = 2.04 + 1.64 X	74.50	635.65	55.62	239.79	8.53	0.41
	72	28.25	51.65	123.43	Y = 2.10 + 2.00 X	34.17	174.54	23.36	87.28	0.91	0.58

X^2 : Chi-Square; SE : Standard Error; UCL : Upper Confidence Limit; LCL : Lower Confidence Limit

2000). Therefore, the search for insecticides of plant origin has gained great impetus in recent times.

In the present observation 100% larval mortality was observed in higher concentrations of petroleum ether, chloroform and ethanol leaf extracts *of A. squamosa* Petroleum ether extract of leaf and seed of *A. squamosa* was found to be the most effective one based on their 24 h LC_{50} values. Acute toxicity causing 100% larval mortality was recorded by Daniel *et al.* (1995) in their studies using *Acalypha indica,* Karmegam *et al.* (1997) using *Pergularia extensa, Argemone. mexicana* and *Withania somnifera* and Anuradha *et al.* (2000) using *Acacia nilotica* and *Citrullus colocynthus* against the larvae of *C. quinquefasciatus.*

Dose dependent larvicidal and pupicidal activity were seen both in leaf and seed extracts in the present study as previously reported on *C. quinquefasciatus* with *A squamosa* seed extract (Mehra & Hiradhar, 2000). Compared the individual combined efficacy of the extracts of *A. squamosa* and *Pongamia glabra* was also tested by George & Vincent (2005). In the present work also higher concentrations of both leaf and seed extracts produced 100% larval mortality.

In the present study several morphogenetic abnormalities such as formation of hypermelanized pupae, partially melanized pupae and non-melanized pupae were observed in treatment with all the leaf and seed extracts. Abnormal formations such as larval-pupal intermediates and pupal-adult intermediates were also seen. This corroborates with the findings of Saxena & Saxena (1992) who observed various defective stages in *C. quinquefasciatus* with *Thevetia neriifolia* leaf and seed extracts. The present observation is also comparable to the description of Zebitz (1984) in neem seed kernel extracts against *A. aegypti,* and that of Sujatha *et al.* (1988) who also found *A. calamus* extract inducing malformations to a greater extent in *A. stephensi* and to lesser extent in *C. quinquefasciatus.*

The prolongation of developmental period and induction of morphogenetic abnormalities of mosquito larvae treated with plant extracts may be ascribable to the interference of the bioactive compounds with the endocrine mechanism that regulate moulting and metamorphosis as suggested by Zebitz, (1986) or to the hormonal interference in the co-ordination of the metabolic processes of the developing stages as suggested by Supavarn *et al.* (1974). Abnormal behaviour of larvae observed in the present study has also been documented by several authors using plant extracts (Zebitz (1986); Saxena & Saxena (1992); Yadav *et al.* (2002) and Sakthivadivel & Thilagavathi (2003).

Conclusion : It is clear that both leaf and seed extracts of *Annona squamosa* plant have larvicidal and pupicidal potency and thereby arresting the adult emergence. Therefore, these plant extracts can be recommended for use in the management of mosquitoes.

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