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Larvicidal, ovicidal and repellent activities of the leaf extract of two cucurbitacious plants against filarial vector *Culex quinquefasciatus* (Say) (Diptera : Culicidae)

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Abstract. The leaf extract of two cucurbitacious plants *Citrullus colocynthis* and *Cucurbita maxima* with different solvents viz., benzene, ethylacetate, petroleum ether and methanol were tested for larvicidal, ovicidal and repellent activities against the mosquito *Culex quinquefasciatus*. Larval mortality was observed and recorded after 24 h exposure period. The LC_{50} values of *C. colocynthis* were 61.72, 47.58, 66.92 and 118.74 ppm respectively. *C. maxima* shows the LC_{50} values of 123.02, 75.91, 117.73 and 171.64 ppm respectively. The mean percent hatchability of the egg rafts were observed after 48 h treatment. 100% mortality was observed at 450 ppm for *C. colocynthis* and 600 ppm for *C. maxima*. Skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration of *C. colocynthis* gives the complete protection time ranges from 107 to 271 minutes. *C. maxima* exerted the complete protection time of 78 to 215 minutes. The leaf extract of these two plants shows the larvicidal and ovicidal properties and they can also be applied as an effective personal protection measure against mosquito bites.

INTRODUCTION

Mosquitoes are the vectors for the dreadful diseases of mankind. Of all the insects that transmit diseases, mosquitoes represent, the greatest menace. WHO has declared the mosquito "public enemy number one" because mosquitoes are responsible for the transmission of various dreadful diseases (WHO, 1996). One of the methods available for the control of mosquitoes is the use of insecticides. Chemical control using synthetic insecticides had been favourable so far, because of their speedy action and easy application. The relative toxicity of insecticides to various mosquito species has been studied by entomologists in detail (Rajavel *et al*., 1987; Saxena & Kaushik, 1988). Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air. There is a need to find alternatives to these synthetic pesticides. Botanical pesticides are promising in that they are effective, environment – friendly, easily biodegradable and also inexpensive. Botanical pesticides have been used traditionally by human communities in many parts of the world against pest species of insects (Jacobson, 1958).

The present study was an attempt to find new larvicide, ovicide and repellent products from the extracts of cucurbitacious plants to control the filarial vector *Culex quinquefasciatus*.

MATERIALS AND METHODS

Collection of plants and extraction

Fully developed leaves of the two cucurbitacious plants *Citrullus*

colocynthis Schrad and *Cucurbita maxima* were collected from Chavadi and Kothattai, Cuddalore district, Tamil Nadu, India. Voucher specimens have been deposited in the Botanical survey of India, Coimbatore, India. The leaves were washed with tap water, shade dried and finely ground. The finely ground plant material (125 gm/solvent) was loaded in soxhlet apparatus and was extracted with four different solvents namely benzene, petroleum ether, ethyl acetate and methanol individually (Vogel, 1978). The solvent from the extract was removed using a vacuum evaporator to collect the crude extract. The crude residue of these two plants vary with the solvents used. The *C. colocynthis* with four different solvents yielded 5.34, 10.13, 24.63 and 16.62 gm of crude residue respectively and the *C. maxima* extracts yielded 6.77, 8.32, 15.63 and 12.54 gm of crude residue respectively. Standard stock solutions were prepared at 1.0% by dissolving the residues the universal solvent DMSO (dimethyl sulphoxide). From this stock solution, different concentrations were prepared and these solutions were used for larvicidal and ovicidal bioassays. For the repellent activity the various range of stock solutions (1.0, 2.5, & 5.0 mg/cm^2) were prepared by dissolving the residues in ethanol.

Culture of test organism

The colonies of *Cx.quinquefasciatus* were cultured and maintained in the laboratory at 27 ± 1°C and 85% relative humidity. The larvae were fed with dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at 28 ± 2°C, 70 ± 5% RH, and a photoregime of 16:8 (L:D) hr.

Larvicidal bioassay

Five different test concentrations were prepared by adding different range of stock solution to 250 ml of water. Twenty five third instar larvae were exposed to the prepared 250ml of test concentrations. Each experiment was replicated six times. The control experiments were also run parallel with each replicate (WHO, 1996). The larval mortality was calculated after 24 hours of the exposure period. The corrected percent mortality was calculated by applying Abbott's formula (Abbott, 1925). The data were subjected to Probit analysis (Finney, 1971).

Ovicidal bioassay

The method of Su & Mulla (1998) was followed to test the ovicidal activity. The leaf extract was diluted in the respective solvent to achieve different concentrations. The freshly laid egg raft containing 100 eggs of *Cx. quinquefasciatus* were exposed to each dose of leaf extract until they hatched or died. Each concentration was replicated six times. Eggs exposed to respective solvents in water served as control. The hatch rate was assessed 48 h post treatment by the following formula.

$$\frac{\text{Number of hatched larvae}}{\text{Total number of eggs in egg rafts}} \times 100$$

Repellency test

The percentage of protection in relation to dose method was used (WHO, 1996) and 3-4 days old blood – starved female *Cx. quinquefasciatus* mosquito (100) were kept in a net cage (45 x 30 x 45 cm^2). The arms of the volunteer was washed and cleaned with ethanol and ethanol served as control. After air drying the arms of the volunteer, only 25 cm^2 dorsal side of the skin on the each arm was exposed and the remaining area being covered by rubber gloves. The extract of *C. colocynthis* and *C. maxima* were applied at 1.0, 2.5 and 5.0 mg/cm^2 separately (Venkatachalam & Jebanesan, 2001). The control and treated arm were introduced simultaneously into the cage. The number of bites were counted over 5 minutes every 30 minutes from 18:00h to 06:00h. In the event of no bites, in the initial 5 min the test arm was exposed after every 30 min for a duration of 5 min until a confirmed bite was received. The test was over after the

confirmation of mosquito bite in extract to be tested. The mosquito repellency of different extract was measured on the basis of the protection time (min) i.e., the time until the first confirmed bite after application (Schreck, 1977). The experiment was replicated five times in each concentration. It was observed that no skin irritation occurred from the leaf extract tested.

RESULTS

The experiments conducted for evaluating larvicidal efficacy of leaf extracts of *C. colocynthis* and *C. maxima* revealed that *C. colocynthis* exerted effective larvicidal properties than *C. maxima* against *Cx.quinquefasciatus*. The ethylacetate extract of *C. colocynthis* was more effective and the methanolic extract was least effective and the LC_{50} values ranging from 47.58 to 118.74 ppm. The leaf extract of *C.maxima* revealed the same results like *C. colocynthis*, ethyl acetate extract exerted effective larvicidal efficacy and the methanolic extract was least effective with the LC_{50} values ranging from 75.91 to 171.64 ppm (Table 1).

The mean percent hatchability of *Cx. quinquefasciatus* with *C.colocynthis* and *C. maxima* was shown in Table 2 and 3. The toxicity of leaf extracts was dependent on its concentration. Zero hatchability (100% mortality) was attained at the concentration of 450 ppm for *C. colocynthis* and 600 ppm for *C. maxima*. Control eggs (water with respective solvents) shows the hatchability ranged from 97.4 to 100% with *C. colocynthis* and *C. maxima* exhibited 100% hatchability for all the extracts (Table 2 and 3).

The leaf extract of *C. colocynthis* and *C. maxima* revealed the repellency activity against the adult mosquito *Cx. quinquefasciatus*. The results of complete protection time are presented in Table 4 and 5. The maximum protection time was observed in methanolic extract (271 min) and ethyl acetate extract exerted a least protection time but more than 3 hours at 5 mg/cm^2 (214 min) for *C. colocynthis*. The *C.maxima* leaf extract also shows the repellent activity against *Cx. quinquefasciatus* (Table 5).

DISCUSSION

The results of present study are comparable with earlier reports. The toxicity to the late third instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of *Memordica charantia*, *Trichosanthus anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* showed the LC_{50} values of 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm respectively (Prabakar & Jebanesan, 2004). Mullai & Jebanesan (2006) reported the larvicidal efficacy of the leaf extract of

Table 1. Larvicidal activity of leaf extract of certain Cucurbitacious plants against *Culex quinquefasciatus*

Plant species	Solvent	LC_{50} (ppm)	LC_{90} (ppm)	95% confidence limit (ppm)		Regression equation	Chi-square value (x^2)
				LCL	UCL		
Citrullus colocynthis	Benzene	61.72 ± 0.48	109.98 ± 1.01	59.65 ± 2.04	68.68 ± 1.69	Y = 0.76 + 0.80 X	1.27*
	Petroleum ether	66.92 ± 0.59	131.37 ± 0.60	48.75 ± 0.58	83.89 ± 0.47	Y = 0.60 + 0.78 X	14.67*
	Ethyl acetate	47.58 ± 1.43	88.79 ± 1.32	38.89 ± 1.10	56.03 ± 1.26	Y = 5.40 + 0.93 X	8.96*
	Methanol	118.74 ± 1.06	243.02 ± 0.29	75.26 ± 0.36	159.55 ± 1.10	Y = 10.47 + 0.33 X	21.88*
Cucurbita maxima	Benzene	123.02 ± 0.61	218.07 ± 1.06	99.07 ± 0.55	146.95 ± 0.82	Y = 1.84 + 0.39 X	12.18*
	Petroleum ether	117.73 ± 0.82	226.25 ± 1.18	91.58 ± 1.62	143.01 ± 1.33	Y = 7.14 + 0.36 X	11.63*
	Ethyl acetate	75.91 ± 0.67	166.29 ± 1.04	36.96 ± 1.56	106.09 ± 2.04	Y = 16.57 + 0.43 X	24.06*
	Methanol	171.64 ± 0.95	315.99 ± 1.26	141.59 ± 1.84	200.54 ± 0.27	Y = 5.23 + 0.26 X	8.30*

* Significant at P<0.05 level
Each value (X ± SD) represents mean of six values

Table 2. Ovicidal activity of *Citrullus colocynthis* leaf extract against egg of *Culex quinquefasciatus*

Solvent	Percentage of egg hatching						
	Concentration (ppm)						
	Control	75	150	225	300	375	450
Ethyl acetate	97.4 ± 0.63	94.8 ± 1.01	74.4 ± 0.85	65.3 ± 0.83	47.8 ± 0.68	24.3 ± 1.71	NH
Benzene	100 ± 0	95.7 ± 0.83	82.2 ± 0.40	68.2 ± 1.27	48.4 ± 0.81	27.8 ± 0.94	NH
Petroleum ether	99.0 ± 1.01	98.4 ± 0.85	86.8 ± 0.83	74.7 ± 0.40	62.6 ± 1.27	32.7 ± 1.00	NH
Methanol	100 ± 0	97.6 ± 0.63	77.4 ± 0.66	59.8 ± 1.28	46.7 ± 0.83	23.4 ± 0.79	NH

NH – No hatchability (100% mortality)
Each value (X ± SD) represents mean of six values

Table 3. Ovicidal activity of *Cucurbita maxima* leaf extract against egg of *Culex quinquefasciatus*

Solvent	Percentage of egg hatching						
	Concentration (ppm)						
	Control	100	200	300	400	500	600
Ethyl acetate	100 ± 0	97.3 ± 1.02	70.8 ± 0.93	40.0 ± 0.66	22.3 ± 0.81	11.6 ± 0.94	NH
Benzene	100 ± 0	94.0 ± 1.01	68.7 ± 1.46	38.9 ± 1.00	17.3 ± 0.94	7.4 ± 0.32	NH
Petroleum ether	100 ± 0	99.7 ± 0.45	78.6 ± 0.28	34.6 ± 0.31	19.7 ± 0.66	10.7 ± 0.85	NH
Methanol	100 ± 0	98.6 ± 0.55	74.3 ± 0.40	43.3 ± 0.85	28.6 ± 0.81	6.7 ± 0.94	NH

NH – No hatchability (100% mortality)
Each value (X ± SD) represents mean of six values.

Table 4. Repellent activity of *Citrullus colocynthis* leaf extract against *Culex quinquefasciatus*

Extract	Concentration mg/cm^2	Complete protection time (min)	
		Control	Treated
Methanol	1.0	6.2 ± 1.0	144 ± 3.18
	2.5	5.0 ± 0.7	200 ± 3.14
	5.0	5.5 ± 0.5	271 ± 2.79
Benzene	1.0	4.0 ± 0.8	115 ± 3.11
	2.5	5.0 ± 1.0	157 ± 3.44
	5.0	4.5 ± 1.1	240 ± 2.54
Petroleum ether	1.0	6.5 ± 0.7	120 ± 1.34
	2.5	4.3 ± 0.5	134 ± 2.16
	5.0	5.0 ± 0.7	230 ± 2.16
Ethyl acetate	1.0	6.0 ± 1.0	107 ± 1.34
	2.5	5.5 ± 0.7	125 ± 1.80
	5.0	4.0 ± 0.7	214 ± 2.94

Values of mean of six replication ± SD

Table 5. Repellent activity of *Cucurbita maxima* leaf extract against *Culex quinquefasciatus*

Extract	Concentration mg/cm^2	Complete protection time (min)	
		Control	Treated
Methanol	1.0	5.4 ± 0.6	100 ± 1.77
	2.5	4.0 ± 0.8	177 ± 2.99
	5.0	4.4 ± 1.0	200 ± 2.03
Benzene	1.0	4.4 ± 1.1	94 ± 2.61
	2.5	4.0 ± 0.7	115 ± 1.77
	5.0	3.5 ± 0.6	215 ± 2.65
Petroleum ether	1.0	4.0 ± 0.5	95 ± 2.87
	2.5	3.5 ± 1.2	108 ± 1.97
	5.0	2.2 ± 1.0	200 ± 3.30
Ethyl acetate	1.0	4.4 ± 0.7	78 ± 1.34
	2.5	3.0 ± 0.5	100 ± 2.48
	5.0	2.5 ± 0.7	192 ± 2.14

Values of mean of six replication ± SD

Cucumis pubescens with four different solvents against late third instar larvae of *Anopheles stephensi*, *Cx. quinquefasciatus* and *Aedes aegypti*.

The bioactive compound Azadirachtin (*Azadirachta indica*) showed complete ovicidal activity in the eggs of *Cx. tarsalis* and *Cx.quinquefasciatus* exposed to 10 ppm concentration (Su & Mulla, 1998). The ovicidal activity of *Moschosma polystachyum* leaf extract against the egg rafts of *Cx. quinquefasciatus* showed 100% mortality at 0-3 h and 3-6 h with concentrations of 125, 150, 175 and 200 mg/l (Rajkumar & Jebanesan, 2004).

The volatile oil of *M. polystachyum* and *Solanum xanthocarpum* possess effective skin repellent activity against *Cx. quinquefasciatus* (Rajkumar & Jebanesan, 2005). The mean protection time and total percentage protection in relation to dose of *Ferronia elephantum* leaf extract showed the percentage protection in relation to dose and time (h) (Venkatachalam & Jebanesan, 2001).

From these results it was concluded that the plants *C.colocynthis* and *C. maxima* exhibits larvicidal, ovicidal and repellent activities against *Cx. quinquefasciatus*. Further analysis to isolate the active compound for larval control is under way in our laboratory. More studies are needed to elucidate the ovicidal activity against a wide range of mosquito species and the active compound responsible for repellent activity should be identified which could be used to control different mosquito species in the future.

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