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BIOACTIVITY OF *CHENOPODIUM AMBROSIOIDES* L. (FAMILY: CHENOPODIACEAE) AGAINST THE FILARIASIS VECTOR *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

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ABSTRACT

Identification of novel effective secondary metabolites obtained from the plants can be used as an alternative to synthetic insecticides under the integrated vector control. The bioactivity of methanolic extract of the leaves of *Chenopodium ambrosioides* was studied under laboratory conditions for larvicidal, ovicidal and oviposition activity against *Culex quinquefasciatus*. The extract was the most potent in all activities and produced 50% larval mortality at 7.12 mg/l concentration. Clear dose - response relationships were established with the highest dose of 25 mg/l causing 100% mortality. In ovicidal activity, the egg rafts aged for 0-3, >3-6, >6-9, >9-12 and >12-15 h were individually exposed to different concentrations of extract ranging from 25 to 125 mg/l for 3 h. The results suggest that the ovicidal activity of the extract was influenced by concentration of the extract and age of the egg rafts. The effects of extract on the oviposition behaviour of *Cx. quinquefasciatus* was also evaluated in the present study. The concentrations of 100, 200, 300 and 400 mg/l reduced egg laying by gravid female mosquitoes from 27.7 to 90.2 %. The results of oviposition activity indicate that the extract exhibited oviposition deterrent activity against gravid female mosquitoes. The results obtained from this bioactivity study suggest that the leaf extract of *C.ambrosioides* is promising as mosquitoidal against Cx.quinquefasciaus and could be useful in the search for new natural mosquitocidal products in future

Keywords: *Chenopodium ambrosioides, Culex quinquefasciatus,* Larvicide, ovicide, oviposition activity.

INTRODUCTION

Culex quinquefasciatus is a major vector in India as well as in tropical regions of the world and has been shown to be directly responsible for 80 million annual lymphatic filariasis of which 30 million cases exist in chronic infection. There are 45 million cases of lymphatic filariasis in India alone (Bowers *et al*., 1995). The present proliferation of this disease is not only due to higher number of breeding places in urban area, but also due to increasing resistance of mosquitoes to current commercial insecticides such as organochlorides, organophosphates, pyrethroid, and carbamates (Das and Amalraj, 1997). Secondly, the 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 insecticides. Identification of botanical insecticides are alternative method in that they are effective, environment – friendly, easily biodegradable, inexpensive and also multiple effects in a variety of insect species (Su and Mulla, 1999). These effects include larvicidal, ovicidal, antifeedancy, growth regulation, fecundity suppression, male sterility and changes in oviposition activity mostly as repellency. *Chenopodium ambrosioides*, commonly known as worm seed, is originated in Central America, though it has been distributed to much of the world. The plant is annual perrenial herb that grows to a height of 40 cm. The leaves are oval and toothed. It has been used as an anthelmintic for many years (Kliks, 1985). The present study was undertaken to study the bioactivity of *Chenopodium ambrosioides* against *Cx. quinquefasciatus* in a search for effective natural products to be used in the control of filariasis vector.

MATERIALS AND METHODS

Plant collection and extraction

Fully developed leaves of *C. ambrosioides* were collected from natural forests of Sirumalai hills, Dindugal district, Tamil Nadu, India. Leaves were washed with dechlorinated water, shade dried and powdered using a mechanical grinder. Powdered leaves (2.0 kg) were extracted with methanol (6.0 l) in a soxhlet apparatus for 8 h and the extract was concentrated in a rotary vacuum evaporator to yield 183 g of a dark greenish material, which was used for the bioactivity.

Mosquito culture

Adult *Cx. quinquefasciatus* were obtained from a laboratory colony maintained at 27 ± 2°C, 70-80%

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relative humidity and 14:10 light and dark photo period cycle. Larvae were fed dog biscuits and yeast powder in the ratio of 3:1. The adults mosquitoes were reared in the glass cages and provided with 10% sucrose solution and were periodically blood fed on restrained 5-7 week-old chicks.

Larvicidal activity

The larval bioassay test was carried out by following the standard procedure (WHO, 1996). The leaf extract was volumetrically diluted in 250 ml filtered tap water to obtain the test concentrations of 5, 10, 15, 20 and 25 mg/l. The methanol was served as a control. Twenty five early third instar larvae were introduced to each of the test concentration as well as control. For each concentration five replicates were run at a time. The larval mortality was recorded after 24 h of exposure, during which no food was given to the larvae. The lethal concentration (LC_{50}) was calculated by probit analysis (Finney, 1971).

Ovicidal activity

Ovicidal activity of the extract was determined by using the following procedure (Su and Mulla, 1998). The extract was volumetrically diluted in 100 ml filtered tap water to obtain the test concentrations of 25, 50, 75, 100 and 125 mg/l. Control was set up with the 1 ml of methanol in 99 ml of water. The egg rafts of different age groups 0-3, >3-6, >6-9, >9-12 and >12-15 h old were used. Ten egg rafts of each age group was individually transferred to the different concentrations of extract for 3 h treatment. After treatment, 5 egg rafts were selected at random from each concentration and control and individually transferred to distilled water for hatching assessment after counting the eggs in each egg raft under microscope. Each treatment was done with five replicates. The percentage hatching was assessed 120 h after treatment by the following formula

$$\frac{\text{Number of hatched larvae}}{\text{Total no. of eggs in all 5 egg rafts}} \times 100$$

Oviposition activity

The oviposition behaviour test was determined by using the following protocol (Xue *et al*., 2001). Twenty gravid female *Cx. quinquefasciatus* (10 days old, 5 days after blood feeding) transferred to each mosquito cage (45 x 38 x 38 cm) covered with a plastic screen, with a glass top and a muslin sleeve for access. Concentrations of 100, 200, 300 and 400 mg/l were made in 100 ml of filtered tap water. Two enamel bowls holding 100 ml of water were placed in opposite corners of each cage, one treated with the test material and the other with a methanol control. The positions of the bowls were alternated between the different replicates so as to nullify any effect of position on oviposition. Five replicates for each concentration were run, with cages placed side by side for each bioassay. After 24 h, the number of egg rafts laid in treated and control bowls were recorded. The percent effective repellency for each concentration of the extract was calculated using the following formula.

$$ER\ (\%) = \frac{NC - NT}{NC} \times 100$$

Where, NC is the number of egg rafts laid in control and NT is the number of egg rafts laid in test solution.

RESULTS

The leaf extract of *C. ambrosioides* have been studied for use as natural insecticides instead of organic phosphorous materials or other synthetic agents. Results on the larvicidal, ovicidal and oviposition effects of leaf extract was reported in the present study, confirm their potential for control of the mosquito populations. Results on per cent mortality of larvae presented in Table 1. The extract was found to be highly lethal to larvae of *Cx. quinquefasciatus* with a LC_{50} of 7.12 mg/l. This low dose is comparable to some well established botanical insecticides. At highest concentrations (15, 20 and 25 mg/l), the larvae showed restless movement for some time and then settled at the bottom of the beakers with abnormal wagging and died slowly. However, at lowest concentrations of extract (5 and 10 mg/l) the larvae did not show such type of restless movement. The results of larvicidal activity clearly indicates that the percentage of mortality being directly proportional to concentration of the extract.

The data on the ovicidal activity presented in Table 2. Egg rafts with ages of 0-3 and >3-6 h were exposed to 75, 100 and 125 mg/l of extract exhibited complete ovicidal activity. In addition the egg rafts aged for >9-12 and >12-15 h did not show complete mortality even at higher concentrations. This fact indicates that the ovicidal activity of extract depended upon two key factors: concentration of the extract and age of the egg rafts. The oviposition activity of the extract shown in Table 3. In oviposition activity, the leaf extract greatly reduced the number of egg rafts laid by gravid *Cx. quinquefasciatus* at all the concentrations. The results obtained in the oviposition activity clearly revealed that the greatest effective repellency was noted at highest concentration so, the oviposition deterrent was concentration dependent. The oviposition deterrent may be due to volatile compounds from extract escape into the mosquito cage, which could be responsible for repelling the gravid females for oviposition.

DISCUSSION

Mosquito control is facing a thread due to the emergence of resistance mechanism in mosquito species to conventional synthetic insecticides. Undoubtedly, plant derived toxicants are a valuable source of potential

Table 1. Larvicidal activity of *Chenopodium ambrosioides* leaf extract against early 3rd instar larvae of *Culex quinquefasciatus*

Concentration (mg/l)	Mortality (%)	LC_{50} (mg/l)	95% confidence limit (mg/l)	X^2
5	42.2 ± 0.4a	7.12	5.86 – 8.51	0.22
10	61.4 ± 0.5b			
15	80.6 ± 0.5c			
20	92.4 ± 0.7d			
25	100.0 ± 0e			
Control	1.4 ± 0.2f			

Each value (mean ± S.E) represents mean of five values. Values with different letters in a column are significantly different at P<0.05 level (Tukey's test).

Table 2. Ovicidal activity of *Chenopodium ambrosioides* leaf extract against eggs of *Culex quinquefasciatus*

Age of the egg raft (h)	Percentage of egg hatching					
	Concentration of the extract (mg/l)					
	Control	25	50	75	100	125
0-3	94.2 ± 0.3a	6.4 ± 0.3a	3.4 ± 0.3a	-	-	-
>3-6	97.6 ± 0.2b	12.8 ± 0.4b	5.4 ± 0.3b	-	-	-
>6-9	99.2 ± 0.2c	27.8 ± 0.5c	18.6 ± 0.5c	11.4 ± 0.3a	6.4 ± 0.3a	2.6 ± 0.2a
>9-12	100.0 ± 0d	42.6 ± 0.5d	35.0 ± 0.5d	26.8 ± 0.5b	17.4 ± 0.4b	9.6 ± 0.4b
>12-15	100.0 ± 0d	67.6 ± 0.7e	52.4 ± 0.6e	39.0 ± 0.6c	28.6 ± 0.5c	19.6 ± 0.4c

'-' indicate zero hatchability (100% mortality). Each value (mean ± S.E) represents mean of five values. Values with different letters in a column are significantly different at P<0.05 level (Tukey's test).

Table 3. Oviposition responses of gravid *Culex quinquefasciatus* to different concentrations of the *Chenopodium ambrosioides* leaf extract

Concentration (mg/l)	Number of egg rafts in bowl		Effective repellency (%)
	Treatment	Control	
100	6.8 ± 0.3a	9.4 ± 0.2a	27.7
200	5.2 ± 0.2b	11.2 ± 0.2b	53.6
300	3.4 ± 0.2c	14.4 ± 0.3c	76.4
400	1.6 ± 0.1d	16.4 ± 0.4d	90.2

Each value (mean ± S.E) represents mean of five values. Values with different letters in a column are significantly different at P<0.05 level (Tukey's test)

insecticides. These and other naturally occurring insecticides may play a more prominent role in mosquito control programmes in the future (Mordue and Blackwell, 1993). Unlike synthetic insecticides, the literature on mosquitocidal botanical agents does not contain any evidence to suggest that resistance to these substances has emerged. This is most likely not due to any reason of consequence but because botanical agents are not often used in vector control and also they are mixtures of various related compounds with different modes of action and hence the development of resistance to such product is somewhat difficult (Shaalan *et al*., 2005).

A large number of different plant species representing different geographical areas around the world have been shown to possess phytochemicals that are capable of causing a range of acute and chronic toxic effects. Not only have many botanical extracts been shown to cause remarkable deleterious effects on the fecundity and hatchability of mosquito eggs, but they have been shown to have significant and promising larvicidal properties that include growth regulating effects. Any one of these effects taken alone is usually not impressive, but the combined ovicidal, larvicidal, oviposition and IGR effects possessed by many phytochemicals can produce impressive results. When joint – action is considered, the application possibilities for vector control increase significantly. In this study, the leaf extract of *C. ambrosioides* exhibited combined larvicidal, ovicidal and oviposition activity against *Cx. quinquefasciatus.*

The larvicidal results of this present study are similar to our earlier report of mosquitocidal compound octacosane which isolated from leaves of *Moschosma polystachyum* exhibited remarkable larvicidal activity against *Cx. quinquefasciatus* (Rajkumar and Jebanesan, 2004a). The bioactivity of phytochemicals against mosquito larvae vary widely from promising to very unpractical doses depending on plant species, plant part used, age of plant part, solvent used in extraction, method of extraction and mosquito species (Sukumar *et al.*, 1991). The results of ovicidal activity obtained in this present work indicate that egg rafts of 0-3 and >3-6 h old were more susceptible than older age of egg rafts (>6-9, >9-12 and >12-15 h) and also increasing concentration of the extract found to increase the ovicidal activity. The present ovicidal results are comparable with our previous study of *Solanum trilobatum* leaf extract against egg rafts of *Cx.quinquefasciatus* and *Cx.tritaeniorhynchus* (Rajkumar and Jebanesan, 2004b). The results of oviposition activity in the present study indicate that mosquitoes were acutely sensitive to chemical stimuli, with significant amounts of oviposition deterrent occurring in response to different concentrations of extract. Mosquitoes are known to select (or) reject their specific hosts and oviposition sites by sensing chemical signals, that are detected by sensory receptors on the antennae (Davis and Bowen, 1994). The present oviposition deterrent activity study is comparable to previously screened leaf extract of *Solanum trilobatum* against eggs of *Anopheles stephensi* in our laboratory (Rajkumar and Jebanesan, 2005) and also comparable to well-established synthetic insect repellent DEET which exhibit deterrent activity against *Aedes albopictus* (Xue *et al*., 2001).

The screening of locally available plants for mosquitocidal activity may eventually lead to their use in natural – product based mosquito abatement practices. Such practices would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health (Bowers *et al*., 1995). In this way, the results obtained in this study suggest that the leaf extract of *C.ambrosioides* is promising as natural insecticide against *Cx. quinquefasciatus*. Moreover, these results could be useful in the search for newer, more selective, and biodegradable mosquitocidal natural compounds. Further investigations are currently underway to isolate these active compounds.

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