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Research Article

LARVICIDAL ACTIVITY OF *CLERODENDRON INERME* GAERTN. EXTRACTS AGAINST *AEDES AEGYPTI* L. AND *CULEX QUINQUEFASCIATUS* SAY. MOSQUITO SPECIES

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ABSTRACT

Objective: In the present investigation, larvicidal activity of organic solvents extracts of *C. inerme* plant were tested against third and fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.

Methods: Extracts of *C. inerme* sundried leaf powder were prepared by soxhlation using chloroform, petroleum ether and hexane and were tested for larvicidal activity against third and fourth instar larvae of *Ae. aegypti* and *Cx. quinquefaciatus*.

Results: The extract exhibiting the highest larvicidal activity against *Ae. aegypti* was found to be hexane extract with LC_{50} values of 87.2 (24 hour) and 81.5 ppm (48 hours) against third instar larvae and 121.1ppm (24 hours) and 100.3 ppm (48 hours) against fourth instar larvae. The hexane extract tested against third and fourth instar larvae of *Cx. quinquefasciatus* showed LC_{50} values of 127.1 ppm (48 hours) against third instar larvae and 240.7 ppm (24 hours) and 141.1 ppm (48 hours) concentrations against fourth instar larvae. Observations on the dead larvae in the treated groups revealed interference in the developmental period affecting the mid gut region and extrusion of peritropic membrane at the posterior end.

Conclusion: Our results suggest that the hexane extract of the *C. inerme* plant leaves possess promising larvicidal properties against *Ae. aegypti*, a fresh water breeding species, and against *Cx. quinquefasciatus* larvae, a species found breeding in polluted water. In conclusion the hexane extract can be a potential botanical insecticide for treating mosquito breeding sources of wide nature.

Keywords:

INTRODUCTION

Mosquitoes have been well known world wide not only for their nuisance caused by biting for blood meal but also due to their potentiality as vectors in disease transmission like malaria, dengue, chikungunya, elephantiasis etc [1]. Novel strategies in the current era for mosquito control are the most welcomed approach due to failure of conventional insecticides to curb the mosquito population without mitigating the risks associated with them like resistance development, environmental contamination, bioaccumulation etc. Resistance development in insects including the mosquitoes against several synthetic insecticides during the last decades has urged to explore possible alternative methods and resources for effective management of the pest insect and as well as vector insects [2, 3]. One of the alternative methods which have been under attention to certain extent are plant derived natural products. Plant based natural products have been well known for their usefulness to mankind in several aspects holding medicinal and insecticidal properties [4, 5, 6].

Plants have been explored and utilized to certain extent in controlling agricultural pest insects and medically important vector insects including mosquitoes [7, 8, 9]. One of such widely known plant, studied and reported for possessing broad spectrum of insecticidal properties is *Azadirachita indica* commonly known as neem tree [10]. Use of plant based products as insecticides in the present situation around the world holds a unique stance due to their ecofriendly nature in terms of non toxicity against non-target organisms, easy biodegradability and least chances of resistance development.

In the present study leaves of *C. inerme* plant was investigated for larvicidal activity against larval stages of *Ae. aegypti*, a primary vector dengue world wide. *C. inerme* plant is a commonly grown hedge plant and is commonly known as Kashmir bouquet [11]. There few reports suggesting that *C. inerme* plant have insecticidal as well as medicinal properties [12, 13]. Our earlier report have shown that *C. inerme* leaves possess insecticidal properties against *Ae. aegypti* larvae [14, 15]. Present study is in continuation of our recent report, on *C. inerme* extracts [16], to explore larvicidal effects of *C. inerme* against *Ae. aegypti*.

MATERIALS AND METHODS

Aedes aegypti L. colony

Ae. aegypti larvae used for the experiments were from the cyclic colony maintained in the Department of Zoology, Karnatak University, Dharwad, under laboratory conditions at 28±2°C temperature and RH 70 to 75%. Paper strips containing eggs from the cyclic colony were hatched by immersing under tap water in enamel trays. Eggs hatched were provided with food containing grounded dog's biscuit and yeast granules in 2:1 ratio in the form of pellets. Water was renewed alternate day so as to avoid formation of scum on the water surface due to yeast development. Pupae formed were collected and sex sorted, based on the size so as to release into the cage in 2: 1 male/female ratio. Emerged adults were provided with 5% honey, in addition female adults were allowed for accessing blood meal by introducing rat (Wester strain) in a small cage. Eggs were collected by introducing ovipositors every third day of blood meal in the cage which consisted of wet cotton pads covered with filter paper in a small container. Eggs laid on the paper strips were allowed for embryonic development under moist conditions for two days and later stored dry under laboratory conditions.

Cx. quinquefasciatus larval collection

The *Cx. quinquefasciatus* early stage larvae were collected from the septic tank around the Karnatak University campus and were reared in the source water, and stage specific instar were sorted for bioassay. All the experiments were conducted in the water collected from the source of larval collection.

Preparation of extracts

Fresh leaves of *C. inerme* plant collected around region of Dharwad, Karanataka state, India were washed and cleaned before drying under direct sunlight for six days and subsequent pulverization to

fine powder. Sun dried leaf powder was subjected for soxhlet extraction for 36 hours using organic solvents (50 grams for each) viz., chloroform, petroleum ether and hexane. The extracts obtained were evaporated in vacuum flash evaporator under reduced pressure and the concrete obtained was dissolved in acetone to prepare 10% (wt/vol) concentration of extract and stored under refrigerator for further experiments.

Larvicidal bioassay

Experiments were conducted against third / fourth instar larvae of *Ae. aegypti* obtained from the cyclic colony following WHO standard method [17]. Test concentrations of the extract were prepared in 100 ml of tap water in 250 ml capacity cups with four replicates along with control groups containing acetone added in tap water and a control group with water only. Twenty five larvae were released in each test concentrations. Observations were recorded every 24 hours and the results were subjected for probit analysis using SPSS software version 10.

RESULTS AND DISCUSSION

Use of commonly and naturally available resources like plant derived natural products is one of the economically feasible and ecofriendly method in the mosquito control program. Crude extracts derived from plants usually fetch several groups of compounds including bioactive compounds and their bioactivity is basically attributed to the combined action of several compounds, a phenomenon known as synergism [18]. In the present study *C. inerme* crude organic solvent extracts were evaluated for larvicidal activity against immature stages of *Ae. aegypti*. Our observations on the larvicidal activity for the three organic solvent extracts showed hexane extract to be highly effective against *Ae. aegypti* and *Cx. quinquefasciatus* larvae. Lethal concentrations (LC_{50}) for 24 hours and 48 hours were found to be 240.9 and 230.3 ppm, 142.2 and 121.4 ppm, 87.2 and 81.5 ppm for chloroform, petroleum ether and hexane extract respectively against third instar larvae of *Ae. aegypti* (Table 1 and 2). The hexane extract tested against fourth instar larval stage for larvicidal activity showed LC_{50} values of 121.1 and 100.3 ppm for 24 and 48 hours respectively (Table 3). Hexane extract tested against field collected *Cx. quinquefasciatus* third instar larvae showed LC_{50} values of 127.1 ppm (for 48 hours), and against fourth instar larvae the LC_{50} values were found to be 240.7 and 141.1 ppm for 24 hours and 48 hours respectively.

One of the significant observations made in the all the experiments was the prolongation of the larval developmental period for 4 to 7 days in the treated groups of third and fourth instar larvae compared to the control groups where the third instar larval period was found to be 2 days and fourth instar was for 3 days (Figure 1 to 6). This phenomenon of prolongation of the larval developmental period suggests that the extract interfered in the development process leading to survival of the larvae without moulting and eventually leading to death, further it was observed that the extract at higher concentration led to 100 percent mortality within 24 to 48 hours revealing toxic effect of the extract.

Table 1: Lethal concentrations of organic solvent extracts of *C. inerme* for 24 hours against third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.

Species	Extract	LC_{50} (ppm)	LC_{90} (ppm)	Intercept±SE	Pearson X^2 for goodness-of-fit (df)	Regression coefficient	*p* value
Ae. aegypti	Chloroform	240.9 (231.9 – 241.1)	281.1 (270.7 – 297.2)	-7.67±1.16	7.09 (10)	0.03187	NS
	Petroleum ether	142.2 (134.9 – 149.8)	198.3 (187.4 – 212.7)	-3.24±0.33	5.41 (10)	0.0228	NS
	Hexane	87.2 (80.6 – 93.5)	120.2 (110.7 – 137.5)	-3.38±0.005	5.00 (6)	0.03882	NS
Cx. quinquefasciatus	Hexane	--	--	--	--	--	--

Values indicated in the parentheses are lower and upper limit at 95% confidence interval; NS: Non-significant

Table 2: Lethal concentrations of organic solvent extracts of *C. inerme* for 48 hours against third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.

Species	Extract	LC_{50} (ppm)	LC_{90} (ppm)	Intercept±SE	Pearson X^2 for goodness-of-fit (df)	Regression coefficient	*p* value
Ae. aegypti	Chloroform	230.3 (214.0 – 242.1)	266.9 (253.5 – 293.7)	-8.07±1.43	13.38 (9)	0.03518	NS
	Petroleum ether	121.4 (111.3 – 131.7)	191.6 (176.9 – 211.9)	-2.21±0.22	7.91 (10)	0.1825	NS
	Hexane	81.5 (75.2 – 87.0)	113.0 (105.0 – 126.4)	-3.31±0.52	4.29 (6)	0.04069	NS
Cx. quinquefasciatus	Hexane	127.1 (109.1 – 143.5)	233.5 (207.9 – 275.0)	-1.53±0.21	9.4 (9)	0.01205	NS

Values indicated in the parentheses are lower and upper limit at 95% confidence interval; NS: Non-significant

Table 3: Lethal concentrations of hexane extract of *C. inerme* against fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.

Duration	Species	LC_{50} (ppm)	LC_{90} (ppm)	Intercept±SE	Pearson X^2 for goodness-of-fit (df)	Regression coefficient	*p* value
24 hours	*Ae. aegypti*	121.1 (114.3 – 127.6)	159.4 (150.5 – 172.5)	-4.04±0.58	2.19 (5)	0.03343	NS

48 hours	*Cx. quinquefasciatus*	240.7 (230.6 – 251.7)	360.3 (341.4 – 384.0)	-2.57±0.30	1.74 (7)	0.0107	NS
	Ae. aegypti	100.3 (94.3 – 106.4)	139.9 (131.4 – 151.3)	-3.24±0.40	2.48 (6)	0.0324	NS
	Cx. quinquefasciatus	141.1 (129.0 – 153.1)	216.4 (200.4 – 237.8)	-2.38±0.30	3.47 (7)	0.0168	NS

Values indicated in the parentheses are lower and upper limit at 95% confidence interval; NS: Non-significant

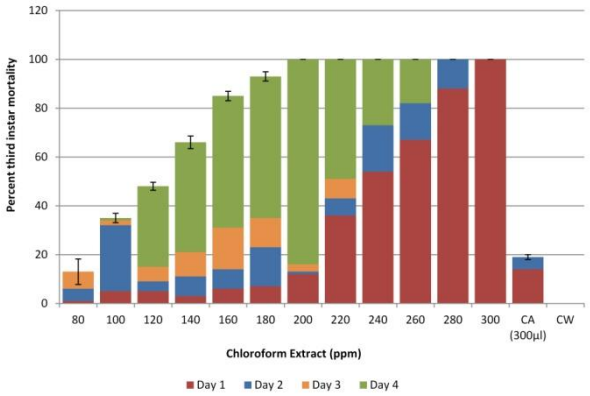

Fig.1: Dose-response relationship of chloroform extract treated against third instar larvae of *Ae. aegypti*.

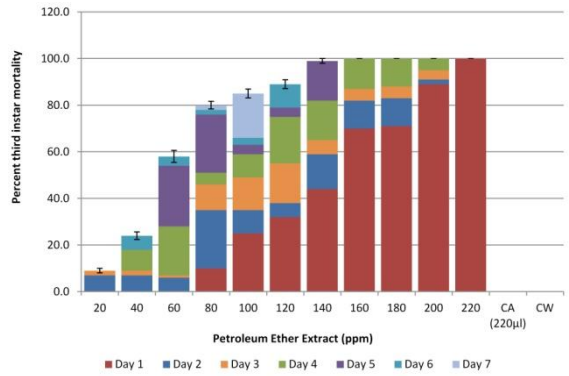

Fig.2: Dose-response relationship of petroleum ether extract treated against third instar larvae of *Ae. aegypti*.

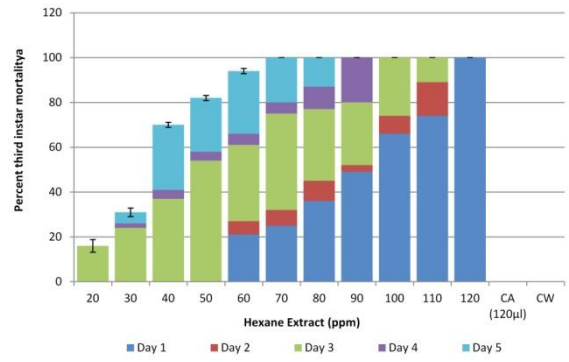

Fig.3: Dose-response relationship of hexane extract treated against third instar larvae of *Ae. aegypti*.

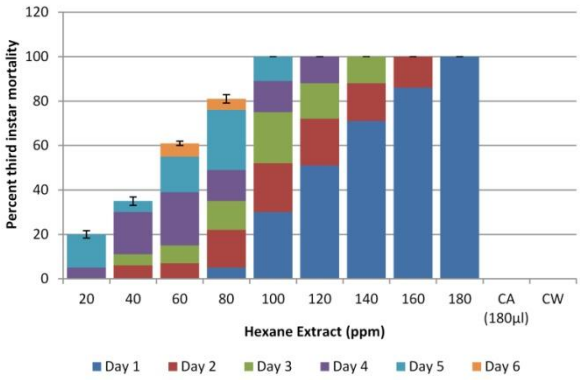

Fig.4: Dose-response relationship of hexane extract treated against fourth instar larvae of *Ae. aegypti*.

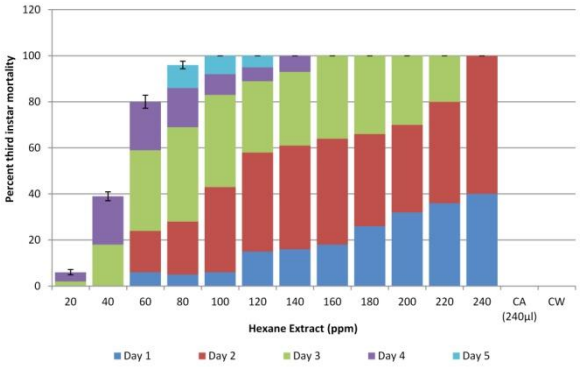

Fig.5: Dose-response relationship of hexane extract treated against third instar larvae of *Cx. quinquefasciatus*.

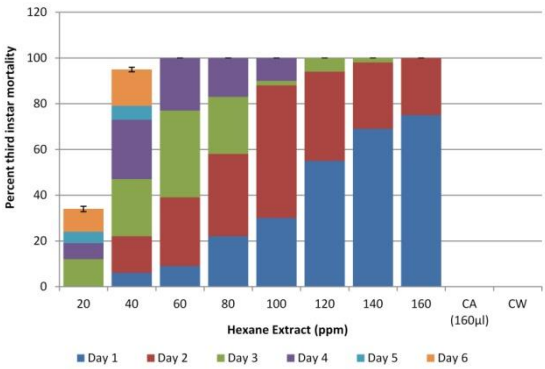

Fig.6: Dose-response relationship of hexane extract treated against fourth instar larvae of *Cx. quinquefasciatus*.

Earlier several researchers have shown that plants extracts treated against developmental stages of mosquitoes lead to prolongation of larval and pupal developmental period [19, 20, 21]. The larvae dead in the treated groups were found with peritropic membrane extruded at the posterior end between the anal palps (Figure 7). The peritrophic matrix (membrane) is an acellular chitin-containing sheath that separates the gut contents from the secretory/absorptive intestinal epithelium and also acts as a barrier for pathogens protecting the midgut region [22, 23, 24]. Observations in our study on extrusion of the peritropic membrane and prolongation of the larval period clearly indicate that the extracts affect the gut region, which could have led to substantial effect on the nutritional absorption in the larvae hindering the developmental process.

The present experimental results showed that the hexane extract possess effective larvicidal properties against *Ae. aegypti* and *Cx. quinquefasciatus* larvae. In conclusion, *C. inerme* is a commonly available plant and can be a cost effective and potential source of natural product in the integrated approach for mosquito control.

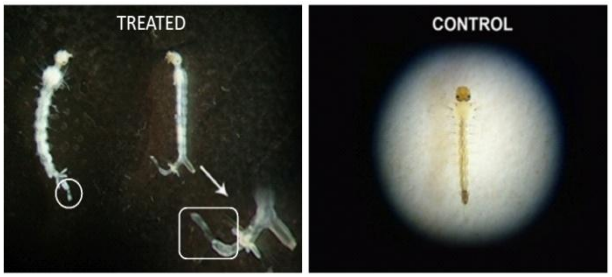

Fig.7: *Ae. aegypti* larvae with peritropic membrane extruded (arrow indicated) between the anal palps.

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