

Acute Toxicity of Two Tropical Plant Extracts on the Fecundity and Fertility of *Culex Quinquefasciatus* Say

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Abstract Acute toxicity tests were conducted to determine the biological effects of partially purified extract of two tropical plants *Croton hirtus* L'Her., *Pogostemon quadrifolius* (Benth.) F.Muell on the juveniles of *Culex quinquefasciatus* Say. Rearing of *C. quinquefasciatus* larvae in water at concentration less than the critical concentrations for inhibition of adult emergence in 50% of treated larvae (EC₅₀) of the ethyl acetate fraction of *Croton hirtus* and *Pogostemon quadrifolius* leaf extracts from hatching to emergence significantly decreased the fecundity of the *C. quinquefasciatus* and the hatchability of their eggs. At highest concentration of 50% EC₅₀ of the extracts the decrease in the fecundity over the control ranged between 72.4 and 85.4 %.

Keywords Plant extracts, *Croton Hirtus*, *Pogostemon Quadrifolius*, *Culex Quinquefasciatus*

1. Introduction

In view of the fact that mosquitoes develop genetic resistance to synthetic insecticides [1] and even to biopesticides such as *Bacillus sphaericus* [2,3] and the application of easily degradable botanicals for the control of mosquitoes is recommended [4]. Alkaloids, nicotine, anabasine, methyl anabasine and lupinine extracted from the Russian weed *Anabasis* were reported to have high larvicidal activity against *Culex* spp. [5]. Thangam and Kathiresan [6 and 7] have reported that acetone extracts of several species of marine angiosperm and algae possess very high mosquito larvicidal activity with their critical lethal concentrations (LC₅₀) ranging from 17.0 to 95.5 ppm. In an effort to increase the activity of plant extracts Pushpalatha and Muthukrishnan [8] purified the crude extracts in silica gel columns using solvents with a sharp polarity gradient and reported that 8.21 ppm of 3:1 petroleum ether (PE): ethyl acetate (EA) fraction of *Vitex negundo* leaf extract killed 50% of the treated II instar larvae of *Culex quinquefasciatus*. As a result of mosquitoes transmitting diseases, the critical

concentrations of botanicals which inhibit 50% of the treated larval population from emerging as adults (EC₅₀) are more meaningful than the LC₅₀ [9,10]. The larvicidal activity of extracts from two tropical plants and the effect on fecundity and fertility against *C. quinquefasciatus* are reported in this paper.

2. Materials and Methods

i) Preparation of Plant extracts

Mature leaves of *Croton hirtus* and *Pogostemon quadrifolius* were collected from the Calicut University Campus, dried under shade, powdered and extracted with analytical grade methanol (MeOH). The MeOH extract was concentrated and defatted with equal volume of MeOH and Petroleum ether (PE). The defatted MeOH fraction was then fractionated into ethyl acetate (EA) and water soluble fractions (Alkofahi et al [4]; modified). Preliminary bioassay tests revealed that the EA fractions of *Croton hirtus* and *Pogostemon quadrifolius* leaf extract were more toxic than the other fractions hence only these fractions were selected to study their effect on the fertility of the mosquitoes. The most active fractions of the selected plants were prepared and stored in refrigerator at 15°C.

ii) Bioassay (Estimation of LC₅₀ and EC₅₀)

Larvae of *C. quinquefasciatus* used in the present study were obtained from the laboratory culture maintained as described in Pushpalatha and Muthukrishnan [8]. Freshly hatched or moulted larvae were used for the bioassays. Different concentrations of the various fractions of the selected plant extracts were prepared in glass bowls. 20 freshly hatched /moulted larvae (I-IV instar) were exposed to each concentration in triplicates. Two different controls (water without methanol and water with the maximum volume of methanol used for making up the desired concentrations of the test medium) were maintained for each treatment. Mortality of the treated and control larvae over a period of 24hr was observed and percent mortality was calculated for the estimation of LC₅₀ using a modified probit

programme by Finney [11].

Freshly hatched first instar larvae were reared until their emergence as adults, in water with different concentrations less than the effective critical concentrations of the most active fractions of the extracts for inhibition of emergence (EC₅₀) of 50% of the treated larvae. To 100ml of water taken in sterilized glass bowls the appropriate volume of 1% stock solution of the most active fractions of the selected plant extracts were added to obtain 5, 10, 25 and 50% of the EC₅₀ of the extracts in the medium. The control medium (0% EC₅₀) contained the maximum volume of MeOH in the test medium. Fifty freshly hatched *C. quinquefasciatus* larvae were introduced into the different bowls. Three replicates were maintained for each tested concentration. The larvae provided with powdered dry yeast and dog biscuits in the ratio of 3:1. The level of water in the bowls was maintained by adding the required volume of dechlorinated water. After the larvae metamorphosed into pupae they were transferred to emergence cages and allowed to emerge. With the help of an aspirator 10 males and 10 females that emerged from each treatment were introduced into oviposition cages. The males were provided with 10% sugar solution through cotton buds the females with a blood meal from an immobilized chicken kept overnight inside the cage. The oviposition cages were covered with a wet cloth to maintain constant humidity (85±5%). One bowl containing dechlorinated water was also kept inside the cage to facilitate oviposition by females. The egg rafts oviposited by the females were removed from the cage next morning, counted under the low power of a binocular microscope and allowed to hatch. Fecundity (number of eggs/female) was monitored until the females died. The number of eggs that hatched into first instar larvae in each concentration was counted and hatchability was calculated as percentage of eggs deposited. Following Saxena et al [12], the sterility index (SI) was calculated as follows $SI = 100 - \{Fecundity\ of\ the\ treated\ larvae \times hatchability(\%) / fecundity\ of\ the\ control \times hatchability(\%)\}$

X 100.

3. Results

Fig 1 and 2 presents 24hr LC₅₀ (ppm) of the different fractions of the selected plant extracts for the different instars of *C. quinquefasciatus* larvae. The result show that EA fraction of the *Croton hirtus* and *Pogostemon quadrifolius* leaf extracts were the most active of the different fractions obtained. Table 1 provides data on EC₅₀ (ppm) and EC₉₀ (ppm) of the EA fractions of the selected plant extracts. The EA fraction of *Croton hirtus* and *Pogostemon quadrifolius* leaf extract was very highly active with regard to its efficacy to inhibit adult emergence. The lowest EC₅₀ (ppm) of the EA fraction is ranged between 8.79 ppm and 19.1 ppm against II instar larvae for *Croton hirtus* and *Pogostemon quadrifolius* respectively.

From the data provided in table 2 a dose dependent decrease in the fecundity of the selected species of mosquito obtained from the larvae subjected to treatment with less than EC₅₀ of the different extracts from hatching to emergence is evident. *C. quinquefasciatus* females in the control series deposited 136.4-140.9 eggs. Treatment of *C. quinquefasciatus* with 50% of the EC₅₀ of I instar *Culex* of *C. hirtus* leaf extract decreased the fecundity by 85.4%. On the other hand treatment of *Cx. quinquefasciatus* with 50% of EC₅₀ I instar *Culex* of *P. quadrifolius* leaf extract decreased the fecundity by 72.39% over control. Treatment of larvae, especially with 50% of the EC₅₀ of the active fractions of the extracts significantly decreased the hatchability of the eggs oviposited by the adults obtained from them (table 2). Treatment of the larvae with 50% of the EC₅₀ of the extracts induced 86 to 91 % sterility in the progeny of *C. quinquefasciatus*. Briefly, the active fraction of the two selected plant extracts decreased the fertility of the *C. quinquefasciatus* significantly.

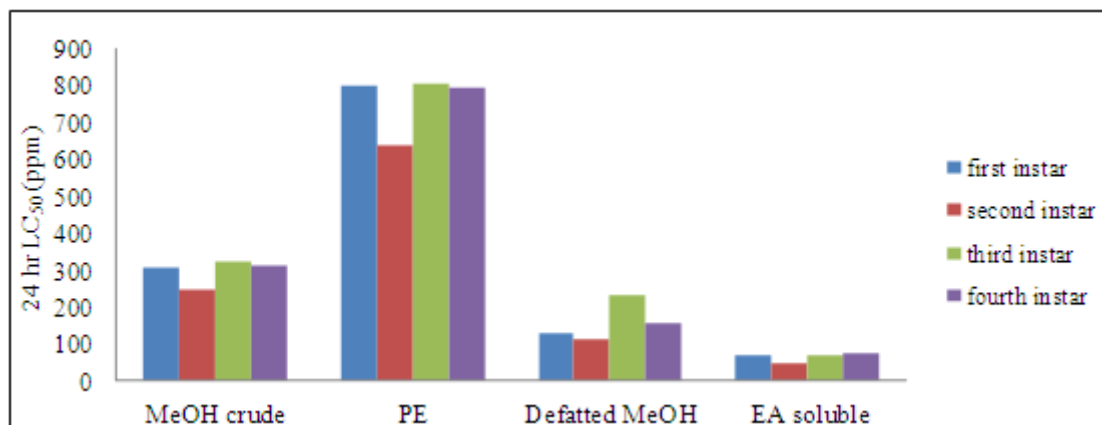


Figure 1. 24 hr LC₅₀ (ppm) of the different fractions of *C. hirtus* leaf extracts on the different instars of *Cx. quinquefasciatus*.

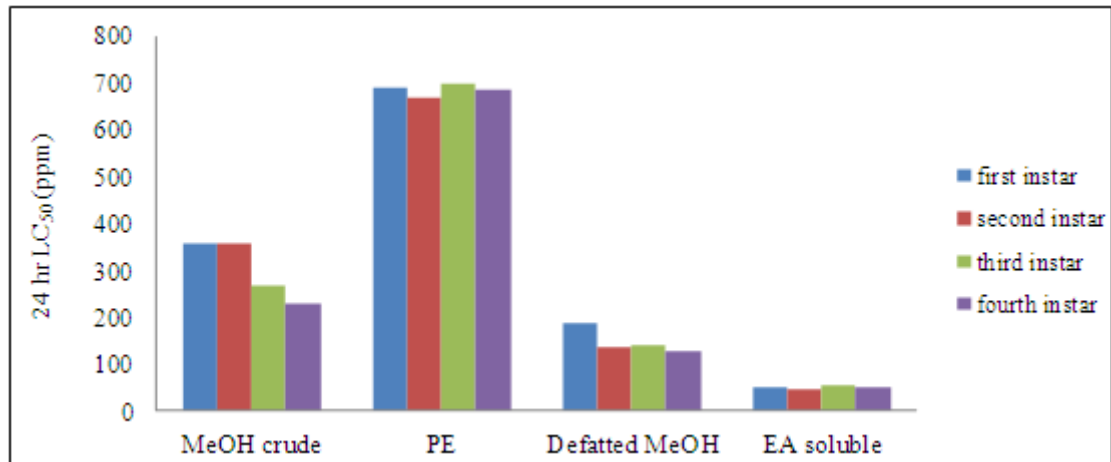


Figure 2. 24 hr LC₅₀ (ppm) of the different fractions of *P. quadrifolius* leaf extracts on the different instars of *Cx. quinquefasciatus*.

Table 1. EC₅₀ (Mean±SI) ppm of the EA fractions of the selected plant extracts tested against different instars of *Cx. quinquefasciatus*

Plant	Instar	EC50 (ppm)	EC90 (ppm)
<i>C. hirtus</i>	I	10.85 ± 0.63	93.7 ± 1.12
	II	8.79 ± 0.37	65.0 ± 1.48
	III	9.46 ± 0.44	74.3 ± 1.28
	IV	12.93 ± 0.72	82.8 ± 1.07
<i>P. quadrifolius</i>	I	23.9 ± 1.34	98.3 ± 12.7
	II	19.1 ± 1.24	84.9 ± 6.37
	III	28.9 ± 1.49	129.7 ± 9.21
	IV	25.8 ± 1.83	124.1 ± 9.36

Table 2. Effect of EA fraction of the selected plant extracts on fecundity and hatchability of the egg of *Cx. quinquefasciatus*. Each value represents the mean ± SD of three replicates of ten females raised from the treatment of sub lethal concentrations, subjected to *ad libitum* mating.

Plant	Conc, (ppm)	Fecundity (egg/female)	Hatchability (%)	Decrease over control		Sterility Index
				Fecundity	Hatchability	
<i>C. hirtus</i>	Control	136.4±0.80	83.0±0.5	0	0	--
	5	98.8±0.68	82.2±0.5	27.4	1.0	29.45
	10	63.6±0.87	69.3±1.2	53.4	16.5	61.15
	25	44.3±2.21	64.6±3.1	67.5	22.5	74.37
	50	19.97±8.33	49.8±2.78	85.4	40.0	91.49
<i>P. quadrifolius</i>	Control	140.9±0.81	97.9±0.2	0	0	--
	5	136.2±0.44	74.1±1.98	3.34	24.3	26.9
	10	96.1±0.70	73.0±0.62	31.8	25.4	49.2
	25	68.6±0.21	60.1±0.81	51.32	38.6	70.2
	50	34.9±1.01	49.8±0.12	72.39	49.1	86.0

4. Discussion

The plants tested in the present study are reported to have medicinal value and are not toxic to vertebrates [13]. Observations on critical concentrations have shown that the critical lethal concentration (LC₅₀) and the EC₅₀ of these extracts are far less than other partially purified extracts and that these extracts are highly efficacious for the control of mosquitoes. Results on the sterilizing effects of the different concentrations less than EC₅₀ of the active fraction of the

selected plant extracts on *C. quinquefasciatus* reported in the present study confirm their potential for control of mosquito population. The larvae which survive the treatment with sub lethal concentrations manage to emerge as adult ultimately oviposit a very few eggs, most of which fail to hatch successfully. The negative effects of these extracts on the fecundity and hatchability of eggs of the mosquitoes are remarkably greater than those reported for other plant extracts in the literature [10,12). The effect of the extracts on the fecundity and hatchability reported in the present study

prompt to speculate that the extracts produce these effects through their influence on the endocrine system. However further studies on the mechanism of action of these extracts and their efficacy for control is required for the development of a more potent bio control agent for the control of mosquito vectors.

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