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Full Length Research Paper

Toxicity effect of *Croton sparciflorus linn*. (Euphorbiaceae) leaf extract against *Culex quinquefasciatus say*

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The effect of leaf extract of *Croton sparciflorus* (Euphorbiaceae) on larvae and pupae of *Culex quinquefasciatus say* was studied under laboratory conditions. Preliminary screening of hexane, chloroform and ethyl acetate extracts at 1000ppm showed that hexane extract was the most effective treatment, which killed 100 and 89 percent larvae and pupae after 24hours. At various concentrations, viz. 25, 50, 100, 200 and 400ppm, the hexane extract caused concentration dependent larval and pupal mortality. The LC_{50} and LC_{90} of hexane extract were 145.3 and 466.9 ppm respectively for larvicidal and 335.2 and 714.7 ppm respectively for pupicidal activities. The results suggest that the leaf extract of *C. sparciflorus* is an effective larvicidal and pupicidal agent against *Cx. quinquefasciatus.*

Key words: *Croton sparciflorus, c. quinquefasciatus,* larvicidal, pupicidal, leaf extract.

INTRODUCTION

Mosquitoes not only cause nuisance by their bites but also transmit deadly diseases like malaria, filariasis, yellow fever, dengue, Japanese encephalitis and also contribute significantly to poverty and social debility in tropical countries (Jang et al., 2002). *Cx. quinquefasciatus Say*, 1822 acts as a vector for *Wuchereria bancrofti* responsible for lymphatic filariasis, which is a prevalent disease in India. L. filariasis infects 112 million people annually, of which 30 million cases exist in chronic infection. There are 45 million cases of L. filariasis in India alone (Bowers et al., 1995). Control of such diseases is becoming increasingly difficult because of the development of detoxifying mechanisms by *Culex* against chemical insecticides (Severrini *et al.,* 1993). Many plant extracts have been reported to suppress mosquito larval populations (Chavan and Nikam, 1982; Saxena and Yadav, 1983). The botanical insecticides are generally pest specific and are relatively harmless to non-target organisms including man (Prakash and Rao, 1997). They are also biodegradable and harmless to the environment. *Crotan sparsiflorus* (Euphorbiaceae) grows as a weed in most part of Asia. This plant was already tested against three mosquito species to find out its synergistic activity along with chemical pesticide (Kalyanasundram and Babu, 1982). In this study, the plant extract is tested for its larvicidal and pupicidal activities against *Cx. quinquefasciatus* under laboratory condition. Hence this study was undertaken with the intention of finding out the efficacy of this plant as anti-mosquito agents for commercial purposes.

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MATERIALS AND METHODS

Plant Material

The leaves of *Crotan sparciflorus* were collected during May 2006 in and around Chennai. It was taxonomically identified by Dr. D.Narasimhan, Department of Botany, Madras Christian College,Chennai. A voucher specimen is deposited in the herbarium of the Entomology Research Institute, Loyola College, Chennai.

Preparation of Plant Extract

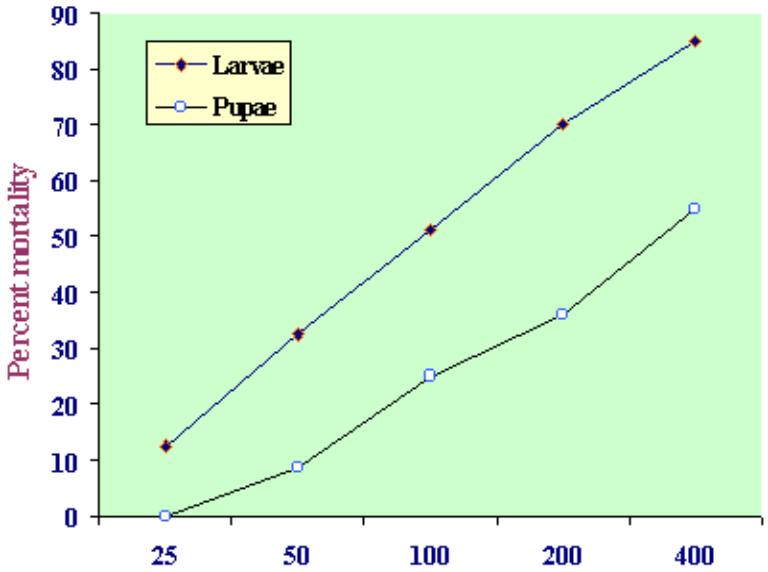
The leaves were washed with water and shade dried at

Table1. Preliminary screening of hexane, chloroform and ethyl acetate extracts of *Croton sparciflorus* at 1000ppm against fourth instar larva and pupa of *Culex quinquefasciatus* after 24 hours of exposure (Mean ± S.E).

S / No.	Treatment	Mortality (%) Larvae	Pupae
1	Hexane	100.00 ± 0.0[a]	89.00 ± 2.5 [a]
2	Chloroform	26.00 ± 1.3 [c]	08.00 ± 1.3 [c]
3	Ethyl Acetate	35.00 ± 2.7 [b]	22.00 ± 3.5 [b]
4	Control	00.00 ± 0.0 [d]	00.00 ± 0.0 [d]

Means followed by different alphabets are statistically different by LSD at 5% level.

Figure1. Effect of hexane leaf extract of *Croton sparciflorus* at different concentrations on larval and pupal mortality of *Culex quinquefasciatus Say*.



at room temperature and powdered using an electric blender. About 500g of the powder was soaked in hexane (1.5 lit) for 72hrs and the extract was filtered through Whatman No.1 filter paper. The solvent was removed using vacuum rotary evaporator at 40ºC. About 55g of a dark greenish crude extract was obtained and it was stored at 4ºC for further use. The remaining plant powder was extracted with chloroform and ethyl acetate sequentially.

Test Organism

The larval and pupa stages of *Cx. quinquefasciatus* were collected from nearby water bodies and a laboratory culture was raised at 28±2ºC, 65-70% RH with a photo period of 11± 0.5h. The larvae were reared on dog biscuits and yeast powder in the ratio of 3:1 in plastic tray (24x35x5cm). Adults were fed with 10% sucrose solution. In addition to sucrose feeding, the female mosquitoes were also fed with live rat blood every four days. The fourth instar larvae and pupae were used for the bioassays.

Bioassay Procedure

In a preliminary study, three solvent extracts (Hexane, chloroform and ethyl acetate) were screened at one concentration (1000ppm) against larvae and pupae and the most effective extract was studied at different concentrations. Hexane extract was found to be the most active treatment in the preliminary screening. For insecticidal and pupicidal experiments, different concentrations such as 25, 50, 100, 200 and 400ppm were screened. The extract was made into a 10% stock solution with acetone. Different concentrations were prepared from the stock solution by adding required qua-

Table2. Median lethal concentration (LC_{50}) and LC_{90} of *Croton sparciflorus* hexane extract against *Culex quinquefasciatus.*

Stage	LC_{50} (ppm)	95% Confidence limits		LC_{90} (ppm)	95% Confidence limits	
		Lower	Upper		Lower	Upper
Larvae	146.3	23.8	358.6	396.1	245.5	1814.0
Pupae	335.2	239.45	1845.44	714.9	432.5	5173.2

ntity of water.

Larvicidal and pupicidal activities were determined by following the standard procedure (WHO, 1975). Twenty early fourth instar larvae of *Cx. quinquefasciatus* were transferred to 500ml bowls, which contained 250ml of tap water mixed with required concentration solutions. In control, 1ml of acetone alone was added. The same method was followed for the treatment of pupae. Five replicates were maintained for each test concentration and control. Mortality in larvae/pupae was recorded 24hrs post treatment.

In the case of pupicidal activity, the mouth of each bowl containing pupae was covered with muslin cloth to prevent the escape of any emerged adult mosquitoes. The dose response relationship was elucidated by Probit analysis and the results are expressed as LC_{50} and LC_{90} (Finney, 1971).

Statistical Analysis

One way Analysis of Variance was performed to find out significance of treatments. The treatment means were separated by Least Significant Difference (LSD) at 5% level.

RESULTS

Preliminary screening of crude extracts

Table 1 shows the percentage of larval and pupal mortality of *Cx. quinquefasciatus* at 1000ppm concentration. Among the three solvent extracts, hexane extract caused 100 percent mortality at the larval stage and 89 percent mortality at the pupa stage at the end of 24hours. Next to hexane, ethyl acetate extract presented high larval (35%) and pupa (22%) mortality. Chloroform extract caused the least larval (26%) and pupa (8.0%) mortality. In general, larvae were found to be more susceptible than pupae.

Effect of hexane extract at different concentrations

The larvicidal and pupicidal effect of different concentrations of hexane extract is given in Figure 1. Concentration dependent mortality was recorded in both larvae and pupae. Larval form was found to be more susceptible than pupa. The larval mortality at 25, 50, 100, 200 and 400ppm concentration was recorded as 12.5, 32.5, 51.25, 70.0 and 85.0 percent respectively. Pupa mortality was 3, 8.75, 25.0, 36.25 and 55.0 percent at 25, 50, 100, 200 and 400ppm concentrations respectively (Figure 1).

Lethal concentration

The probit analysis data is given in Table 2. The LC_{50} and LC_{90} values for fourth instar *Cx. quinquefasciatus* larvae were calculated as 146.3 and 396.1ppm respectively. The LC_{50} and LC_{90} for pupal stage were recorded as 335.2 and 714.9ppm respectively. The 95% confidence limits for LC_{50} lie between 23.8 and 358.6 for larva and 239.45 and 1845.44 for pupa.

DISCUSSION

The Hexane extract of the leaves of the plant *C. sparsiflorus* has been found to possess both larvicidal and pupicidal properties against *Cx. quinquefasciatus*. Earlier works done by Deshmukh and Renapurkar (1987) and Thangam and Kathiresan (1988) showed that plant extracts including mangrove plants were found to exhibit either larvicidal or insect growth regulating activity against mosquito larvae. In the present study, 200ppm of hexane extract of *C. sparsiflorus* presented 70 percent larval mortality. Earlier study by Anuratha *et al.,* (2000) reported that petroleum ether fraction of *Hydrocotyle javanica* caused 50 percent mortality in *Cx. quinquefasciatus* at 282.7 ppm concentration. Larval stage was found to be more susceptible than pupa. The body cuticle of the larva is softer than pupa, which might have led to easy penetration of the compound through the cuticle or spiracle and caused more larval death.

Pandian *et al.,* (1994) have reported that methanolic extract of leaves of *Mentha piperita*, *Phyllanthus niruri, Leucas aspera* and *Vitex negundo* had the LC_{50} as 43.65, 1819.70, 2818.38 and 3019.95 ppm against the fourth instar larvae of *Cx. quinquefasciatus.* Minija and Sarda (1986) reported that crude extract of saponin from the fruit pods of *Swartzia madagascariensis* produced higher

mortality in *Cx. quinquefasciatus*. Muthukrishnan *et al.,* (1997) reported that ethyl acetate fractions of *Solanum trilobatum* and *Leucas aspera* showed LC_{50} values of 23.5 and 138.6ppm against second and fourth instar larvae of *Cx. quinquefasciatus.*

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either countermeasures or development of newer insecticides (Chandre *et al.,* 1998). Though several plants from different families have been reported for mosquitocidal activity, only a very few botanicals have moved from the laboratory to field use, like neem based insecticides, which might be due to the light and heat instability of phytochemicals compared to synthetic insecticides (Green *et al.,* 1991).

Since the plant *C. sparciflorus* hexane extract showed promising activity against *Cx. quinquefasciatus.* In the present study, this plant can be used for mosquito control programme. But before that, the active compound present in the hexane extract should be identified and field trials should also be undertaken. The present finding of the investigation revealed that *C. sparciflorus* has good larvicidal and pupicidal activity against *Cx. quinquefasciatus*. Their mode of actions is presently under investigations.

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