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Effect of some crude leaf extracts on larval development of culex *Quinque fasciatus*

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Abstract
The effects of crude extracts of leaves of *Ocimum sanctum* (OSL) *Catharanthus roseus* (CRL) *Acalypha indiaca* (AIL) and *Vitex negundo* (VNL) at four different concentrations were observed in the IV instar larvae of *Culex quinquefasciatus*, a vector of filariasis. The two higher concentrations *viz*. 750 and 1000 ppm of *Vitex negundo* leaf extracts resulted in 86.67% mortality in the IVth instar larvae of *C. quinquefasciatus*. The order of effect of leaf extracts is VNL< CRL < OSL< AIL. The order of percent mortality in VNL, CRL, OSL and AIL is 86.67, 80.00, 46.67 and 46.67% respectively in 1000 ppm. In lower concentrations (250 and 500 ppm), there is no mortality due to the leaf extracts of OSL and AIL, whereas the leaf extracts of *Vitex negundo* and *Catharanthus roseus* leaf extracts have more potential effect over *C.quinquefasciatus* than the leaf extracts of other two plants *viz*., plants *Ocimum sanctum and Acalypha indiaca.*

Keywords: crude leaf extracts, larval mortality, *C.quinquefasciatus*

Introduction
Long before the advent of chemical insecticides, plants and their by products were used to kill the pests of agriculture, veterinary and public health (Slama and Williams, 1996) [13] Plants may be an alternative source of insecticidal agents because they constitute a rich source of bioactive chemical (Wink 1993) [15]. Several workers have proved that the phyto chemicals derived from plants have been projected as weapons in mosquito control programmes (Arnason *et al*., 1989; Sugunar *et al* 1991; Latha *et al*. 1999; Opender Koul *et al*. 2000; Singaravelu *et al*. 2000; Prasad *et al*. 2001; Rajkumar and Jebanesan, 2002; Abdel-shafy and Zyed 2002 and Shah and Maheswari, 2002) [2, 5, 7, 9, 10, 11, 12]. All these studies have indicated the phytochemicals can be used as inhibitors on feeding growth and reproduction. The present paper reports the larvicidal potential of four indigenous plant species against the fourth instar larvae of a principal vector of filariasis, *Culex quiquefasciatus* in the laboratory bioassay.

Materials and methods
Selection of plants
Leaves of *Ocimum sanctum* (OSL), *Catharnathus roseus* (CRL), *Acalypha indica* (AIL) and *Vitex negundo* (VNL) were selected to investigate on the larval development of *C.quinquefasciatus.*

Preparation of phytochmical extracts
The chopped leaves of four plants *viz*., *Ocimum sanctum*, *Catharnathus roseus*, *Acalypha indica* and *Vitex negundo* were air dried at controlled temperature 40-60^{o} C for 5-12 days, powdered separately and stored at temperature ranging from 59-20^{o}C. Leaf powder materials were extracted individually in petroleum ether (40-60^{o}C for 24 h) using Soxhelt apparatus and further concentrated in a rotary vacuum evaporator. The residue was used to prepare stock solutions of 1% (w/v) in acetone. Test concentrations *viz*., 250,500,700 and 1000 ppm were prepared by dilution with 90 – 95 % acetone.

Test organism culture
The test mosquitoes *C.quinquefasciatus* were maintained in a 10% aqueous sucrose solution and blood from a live mouse, while larvae reared in plastic containers (24 cm) containing sterilized diet (40 mesh chick chow powder/ yeast 80:20) They were held at 28$\pm$2^{o} C and 70$\pm$ 5% relative humidity with a photoperiod 16 L: 8 D (L - light:D-dark cycle)
The eggs of *C.quinquefasciatus* were collected in the plastic container (100 ml capacity) and allowed to hatch in enamel trays containing 2 litres of water. The freshly emerged IV instar larvae were kept in the enamel trays for further rearing at 28$\pm$2^{o}C

Topical application
Direct contact toxicity of extracts in various concentrations was demonstrated by topical application using acetone solutions. For topical application the test materials in 0.03µl of acetone were applied to the dorsum of the insect larvae of *C.quinquefasciatus* using a fine 5 µl syringe (710S Sena Syringe Hamilton & Co. Reno. Neveda. USA). Four concentrations ranging from 250 to 1000 ppm were tested (acetone alone as control) with IV instar larvae (n=20) treated at each concentration individually in the plastic cup. Treated larvae were reared on artificial diet for 72 h. Mortality of the larvae was recorded at 24 hrs intervals for 5 days. Both the control and treated larvae were observed for mortality. The percent larval mortality was calculated as per equation given below:

$$\text{Percent Larval Mortality} = \frac{\text{No. of dead larvae}}{\text{No. of larvae treated (n=20)}} \text{X } 100$$

From the mortality date the percent corrected mortality was also completed as per the equation devised by Abbott (1925) [1]

$$\text{Percent Corrected Mortality} = \frac{\text{\% Killed in treated - \% killed in Control}}{\text{100 \% killed in Control}} \text{X } 100$$

Results

Effect of two lower concentrations of leaf extracts on IV instar larvae of *C.quinquefasciatus*

No larval mortality was recorded at 250 and 500 ppm concentration of OSL extract and AIL extract, while at the same concentration CRL extract has resulted in mortality ranging between as 25 and 46.67% and VNL extract caused mortality ranging between 40 and 53% in 250 and 550 ppm respectively.

Effect of two higher concentrations of leaf extracts on IV instar larvae of *C.quinquefasciatus*:

At two higher concentrations *viz*. 750 and 1000 ppm, CRL extract caused percent larval mortality ranging between 60 and 80 % respectively. Percent larval mortality obtained due to topical application of OSL extract was less effective than the other leaf extracts (AIL and VNL at concentrations 750 and 1000 ppm respectively). Percent mortalities due to the application AIL extract caused 26.67 at 750 ppm and 46.67 at 1000 ppm concentrations.

Discussion

The present study was performed to evaluate plant extracts for controlling mosquitoes. In the last decade, plant extracts were widely used against phytophagous pests and mosquitoes (Amason *et al.*, 1987; Chavan and Nikam, 1988) [6].

The results showed that the significant larval mortality were due to higher concentrations (750 and 1000 ppm of *Vitex negundo* and *Catharnathus roseus.*. Moreover, it reached a mortality rate of 86.67% at both higher concentrations *viz*., 750 and 1000 ppm of VNL extract (Table 1). Mortality in VNL extract treatment was significantly greater than the Ocimum and Acalypne plant leaf extract. These findings fall in line with the earlier observation made by Vimalraj *et al*., (2000) [14] in *Culex quinquefasciatus* and Shah and Maheswari (2002) [12] in *Culex faigans*.

It is concluded from the results of the present study that greater larval mortality with the treatment of *Vitex negundo* (VNL) and *Catharanthus roseus* (CRL) extracts suggest their potentiality of phytochemicals passing through delicate cuticle layer, thereby increasing mortality percentage at two higher concentrations, yet another possibility suggested is the obvious effect of *V.negundo* and *C.roseus* leaf phytotoxins preventing the synthesis of cuticle during the moulting period. Only those survived larvae from topical treatments were transformed into pupae as suggested by Mwangi and Rembold, (1988); Anupama Sharma, (1994) [4] in their studies.

Table 1. Studies on the percent larval mortality in IV instar larvae of *C.quinquefasciatus* after topical application from leaf extracts

Leaf extract Concentration (ppm)	OSL extract	CRL extract	VNL extract	AIL extract
250	0.00(0)	25.00 (6)	50.00 (6)	0.00 (0)
500	0.00(0)	46.67 (8)	53.34 (8)	0.00 (0)
750	40.00(6)	60.00 (13)	86.67 (13)	26.67 (6)
1000	46.67(7)	80.00 (13)	86.67 (13)	46.67 (7)
Control	ND	ND	ND	ND

Each Value is average of three observations. Figures in parentheses denote the number of dead larvae. ND = Not Detectable.

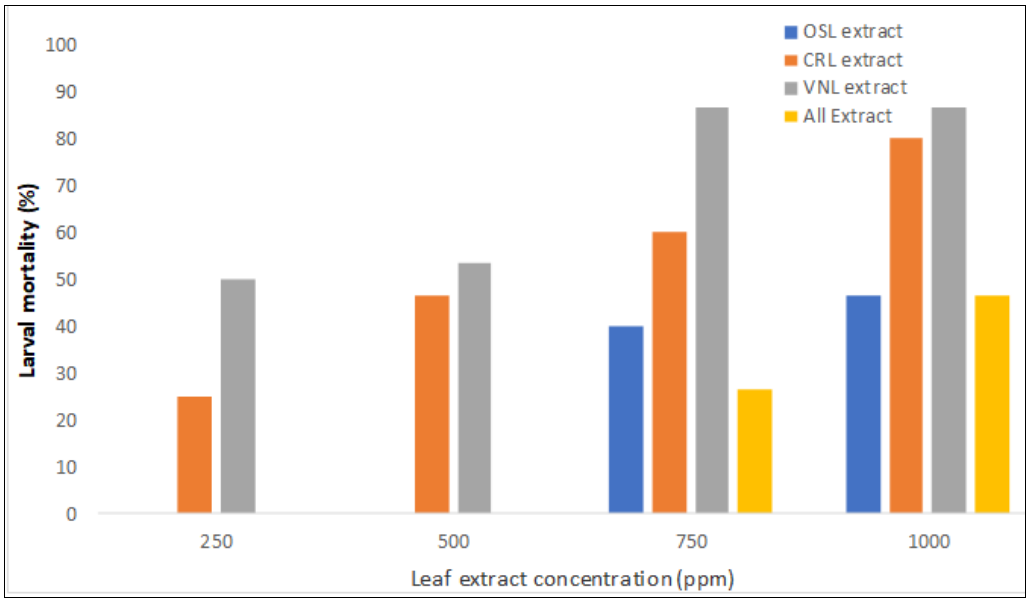

Fig 1: Bar diagram showing the percent larval mortality in IV instar larvae of *C.quinquefasciatus* after topical application by varied leaf extracts (Based on the data presented in the Table 1)

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