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Larvicidal effect of *Melia dubia* seed extract against the malarial fever mosquito, *Culex quinquefasciatus*

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The most important vector species belong to the genera *Anopheles*, *Culex* and *Aedes*. *Culex* species transmit *Wuchereria bancorfti* and arbovirus and *Aedes* species transmit important vector of yellow fever, Dengue, encephalitis viruses and recently Chigunkunya virus. Among the viruses' species, malaria alone kills 3 million each year, including 1 child every 30 seconds (Shell, 1997). Although mosquito-borne diseases currently represent a greater health problem in tropical and subtropical climate, no part of the world is immune to this risk (Fradin, 2002). Control of such disease is becoming increasingly difficult because of increasing resistance of mosquitoes towards pesticides, health hazard to human beings and also it affect non target organism (Ranson et al., 2001). An alternative approach for mosquito control is the use of natural products of plant origin. The botanical insecticides are generally pest specific, readily biodegradable and usually lack toxicity to higher animals (Bowers, 1992) Hence the present study was taken up to test the efficacy of *Melia dubia* extracts against the different developmental stages of the mosquito *Culex quinquefasciatus*.

Preparation of phytochemical extract

The study was carried out at Kongunadu Arts and Science College, Coimbatore during 2009. The seeds of *Melia dubia* were collected from forest college, Coimbatore. The seeds were shade dried and ground separately to fine powder. The dried and powdered seeds of *M. dubia* were extracted with 500ml of methanol, ethanol, ethyl acetate and petroleum ether each using soxhlet apparatus for 8 hours. The extract was concentrated in a vaccum evaporator to yield a dark brownish, gummy extract. The residue was then made into 1% stock solution with acetone and taken for further bioassay test (Vogal., 1978).

Maintenance of Larvae

The mosquito, *Culex quinquefasciatus* was maintained in laboratory at 27+2^{o}C, 75- 85% RH under 14 L: 10 D photoperiod cycles. The larva was fed with fish food. The feeding was continuing till the larvae transformed into the pupal stage.

Larvicidal Bioassay

Bioassay were performed using the different solvent extracts of *Melia dubia* seed on the larval (IIIrd & IVth instars) of the mosquito *Culex quinquefasciatus*. From 1% stock solution the following concentrations were prepared (3.0% to 5.5%)by using Acetone.To obtain different concentrations of test medium the crude extract,1 to 10 ml of the stock solution were dissolved in water and mixed thoroughly with the dry ingredients of the diet as suggested by Miller and chamberlain(1989).Each treatment had five replications and a separate control was maintained. The treatments were taken in 250ml distilled water in 500ml glass beakers. The different larval stages (IIIrd & IVth instars) with each replication of 25. A pinch of fish food was provided in all replications.

The larva were allowed to remain in the flasks for 24hrs and the rate of mortality of a larvae were calculated after the experimental period and corrected by Abbott's formula (Abbott, 1925) LC_{50} and LC_{90} were determined by probit analysis(Finney,1971) and the data were tabulated.

The results of *Culex quinquefasciatus* larval susceptibility to the four extracts of *Melia dubia* is shown in (Table 1). Much research has been conducted on plant derived chemicals which are non-toxic to man and domestic animals and serve as useful basis for the development of safer and more selective mosquito insecticides(Sukumar *et al.*,1991).The percent mortality values for IIIrd and IVth instar larvae of *Culex quinquefasciatus* treated with various concentrations ranging from 3.0 to 5.5 % of the seed extract of *Melia dubia* and LC_{50} and LC_{90} values and their 95 % lower and upper limits of the seed extract for 24 hour exposure of *Culex quinquefaciatus* are given in the table 2 and 3.

Highest mortality of 93 %and 81 % was recorded for third and fourth instar larvae of *Culex quiquefaciatus* respectively at 4.5% concentration.The data were recorded and statistic data regarding LC_{50} ,95% confidence limit,LC_{90} and chisquare values were calculated.The higest sensitivity of third instar larvae was evident by their lowest LC values (LC_{50} 3.240 and LC_{90} 4.786 ppm).Least susceptibility was shown by fourth instar larvae (LC_{50} 4.073 and LC_{90} 4.942 ppm).No mortality was observed in control. The present findings collaborate with earlier findings of Muthukrishnan *et al* (1997). They observed that the LC_{50} values of ethyl acetate extract of Leucas aspera were 75.40,93.09,132.20 and 138.60 ppm against first, second, third and fourth larvae of *C. quinquefasciatus,* respectively.

Sakthi vadivel and Daniel(2008) reported that the petroleum ether extract of *Leucas aspara* showed the LC_{50} value between 100 to 200 ppm against the larvae of *Culex quinquefasciatus,Anophelous stephensi and Anophelous aegypti.*The biological activity of these plant extracts might be due to the various compounds including alkaloids and terpenoides existing in this plant. These compounds may jointly or independently contribute to produce larvicidal activity against *Culex quinquefasciatus*.

From this it may concluded that plants could be an alternative source for Mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects.

CONCLUSION

The synthetic insecticides for mosquito control have resulted in environmental hazards and the botanicals form an important alternative for larval and pupal control as they constitute a rich source of bioactive chemicals. Hence, the larvicididal effect of various extracts of *Melia dubia* seed on *Culex quinquefasciatus*. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution.

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Table 1: Mortality of III Instar larvae of *Culex quinquefasciatus* using different percentage of mortality in different solvents.

Solvent extracts	Mortality (%)					
Concentration(%)	3.0	3.5	4.0	4.5	5.0	5.5
Methanolic	47	57	71	93	-	-
Ethanol	27	39	51	40	72	-
Petroleum ether	-	42	55	66	77	86
Ethyl acetate	-	-	40	47	78	91

Table 2: Larvicidal activity of different solvents seed extract against III instar larvae of *C. quinquefasciatus*

Plant	Solvents	Mosquito species	LC_{50} ± S.E. (ppm) LCL – UCL	LC_{90} ± S.E. (ppm) LCL – UCL	Chi-square (df = 4)
Melia dubia	Methanol	*C. quinquefasciatus*	3.24072 (2.88577-3.52012)	4.55528 (4.14244-5.46279)	1.162
	Ethanol		4.00966 (3.45724-4.57229)	6.20538 (5.28845-9.98957)	0.036
	Petroleum ether		3.82060 (2.72027-4.24557)	5.84336 (5.20528-8.14919)	0.009
	Ethyl acetate		4.58725 (4.33647-4.72382)	5.24124 (5.04149-5.77886)	0.468

Table 3: Larvicidal activity of different solvents seed extract against IV instar larvae of *C. quinquefasciatus*

Plant	Solvents	Mosquito species	LC_{50} ± S.E. (ppm) LCL – UCL	LC_{90} ± S.E. (ppm) LCL – UCL	Chi-square (df = 4)
Melia dubia	Methanol	*C. quinquefasciatus*	3.60766 (3.19501-4.09386)	5.46918 (4.71770-7.86958)	0.373
	Ethanol		4.16444 (3.69992-4.79610)	6.28457 (5.36469-9.88343)	0.131
	Petroleum ether		4.02445 (3.17862-4.43035)	6.03705 (5.35502-8.46011)	0.211
	Ethyl acetate		4.73439 (4.45044-4.93664)	5.64401 (5.28161-7.20834)	5.098