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Efficacy of *Solanum villosum* Mill. (Solanaceae: Solanales) as a Biocontrol Agent against Fourth Instar Larvae of *Culex quinquefasciatus* Say

Nandita CHOWDHURY[1], Indranil BHATTACHARJEE[1], Subrata LASKAR[2], Goutam CHANDRA[1,*]

[1]Mosquito Research Unit. Department of Zoology. The University of Burdwan. Burdwan- 713104. INDIA

[2]Natural Products Laboratory, Department of Chemistry. The University of Burdwan. Burdwan- 713104. INDIA

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Abstract: Dried ground leaves of *Solanum villosum*, Mill. were extracted with 5 different solvents [petroleum ether, benzene, choloform:methanol (1:1 v/v), acetone, and absolute alcohol] to determine the best extractant for subsequent isolation and characterization of larviciding compounds. Each batch of larvae (10 per batch) was treated with 30 ppm, 50 ppm, and 100 ppm of each extract in 3 replicates. All eluted fractions were found to induce significant mortality in test mosquito species. The petroleum ether eluted fraction was the least toxic, whereas the chloroform:methanol (1:1 v/v) eluted fraction was the most toxic to the larvae. A very high mortality rate (86.67%) was observed at 100 ppm test solution after 24 h of exposure. LC_{50} values of the leaves with biologically active different solvent extracts like petroleum ether, absolute alcohol, benzene, acetone, and chloroform:methanol (1:1 v/v) were 645.745 ppm, 321.890 ppm, 204.302 ppm, 107.657 ppm, and 39.192 ppm, respectively, after 24 h of exposure period. Mortality rate with chloroform:methanol (1:1 v/v) extract was significantly higher ($P < 0.05$) in 100 ppm than in other extracts. The bioactive fraction of chloroform:methanol (1:1 v/v) was isolated by thin layered chromatography (TLC), and the LC_{50} value was determined as 3.179 mg/100 ml after 24 h of study period.

Key Words: *Solanum villosum*, *Culex quinquefasciatus*, biocontrol, bioassay, LC_{50}

Introduction

Culex quinquefasciatus Say is generally known as the vector of the pathogen responsible for bancroftian filariasis in warm and humid areas. Phytochemicals have a major role in mosquito control programs (Hag et al., 1999; Palsson and Janeson, 1999; Markouk et al., 2000; Saktivadivel and Thilagavathy, 2003; Ghosh and Chandra, 2006). Some herbal products such as pyrethrums from *Chrysanthemum cinerarifolium* flowers (Hartzell and Wilcoxon, 1941), and *Anabasis aphylla* (Campbell et al., 1993) have been used as natural insecticides before and after the discovery of synthetic organic insecticides (Jacobson and Crosby, 1971). Since the discovery of DDT, synthetic insecticide based method not only contributes to environmental hazards but also increases resistance to disease vectors (Wattal et al., 1981). In recent years, the search for new insecticides that are easily biodegradable and do not have any ill effect on nontarget organisms remains the top priority (Redwane et al., 2002). The plant product can be obtained from the whole plant or from a specific part by extraction with different types of solvents, such as water, petroleum ether, chloroform, and methanol, depending on the polarity of the phytochemicals. Hartzell and Wilcoxon (1941) evaluated extracts from 150 plant species for their toxicities to mosquito and found several to be very effective. Jantal et al. (2003) evaluated 17 methanol extracts and 9 essential oil preparations of Malaysian plants for their larvicidal activities against *Aedes aegypti.*

The Solanaceae, to which the genus *Solanum* belongs, is a cosmopolitan family, composed of approximately 90 genera and between 2000 and 3000 species (Schilling et al., 1989). The family is widely distributed throughout tropical and temperate regions of the world (Edmonds, 1978). Within this family, *Solanum* constitutes the largest and most complex genus, with more than 1500 species. *Solanum villosum* is commonly known as red-fruit nightshade. This plant is a perineal herb,

* E-mail: goutamchandra63@yahoo.co.in

subglabrous to villous annual, up to 50 cm high and widely distributed in many parts of India. Leaves rhombic to ovate-lanceolate, 2.0-7.0 cm long x 1.5-4.0 cm broad, margins entire to sinuate-dentate. Inflorescence simple, umbellate to slightly lax solitary cymes. Calyces 1.2-2.2 mm long, slightly accrescent, deflexed or adhering to base of mature berry. Berries usually longer than wide, orange, 6-10 mm broad, falling from calyces when ripe. Stems angled with dented ridges.

This species is an ayurvedic herb with multiple medicinal properties. For example, it is used for swelling and sore eye, and is easily available to the local people. Leaves of this plant are also eaten as boiled salad and its orange berries are consumed as fruit.

The objective of the present study was to determine the role of mature leaf extracts of *S. villosum* as a biocontrol agent against the fourth instar larval form of *Cx. quinquefasciatus*.

Materials and Methods

Collection of plant material

Fresh mature leaves of *S. villosum* Mill. were harvested randomly from rural areas of Burdwan (23°16′N, 87°54′E), West Bengal, India, from mid June to mid July.

Preparation of leaf extract

Fresh mature leaves were harvested, rinsed with distilled water and dried in the shade at room temperature (20 °C), and milled into a fine powder with a Jankel and Kunkel model A10 mill. To determine the efficacy of different extractants, 25 g of finely ground leaves were plunged in different 250 ml solvents of analytical grades (Merck) of varying polarity: petroleum ether, benzene, chloroform: methanol (1:1 v/v), acetone, and absolute alcohol with vigorous shaking (Kotze and Eloff, 2002). The extracted liquid was subjected to rotary evaporation in order to remove the chemicals. The semisolid extract produced was kept in a deep freeze at –80 °C (REVCO model No. ULT 790-3-V32) overnight and then subjected to freeze drying for 24 h at –60 °C. Then the extract was stored in an air-tight container at 4 °C in a refrigerator for further use. The dried residues were weighed and dissolved in suitable volumes of distilled water to make different concentrations.

Bioassay experiments

Different concentrations (30 ppm, 50 ppm, and 100 ppm) were made with distilled water. Different concentrations of each of the solution (WHO/VBC, 1981) were poured separately into sterile glass dishes (9 cm diameter/150 ml capacity). Ten fourth instar *Cx. quinquefasciatus* larvae were placed in each test solution following the standard WHO larval susceptibility test method (WHO/VBC, 1981) along with a set of controls containing distilled water without any test solution. After adding the larvae, the glass dishes containing the larvae were kept in the laboratory at room temperature (23-28 °C). By counting the number of dead larvae at 24-h intervals for up to 72 h of exposures, we monitored the effects of all graded solutions. The dead larvae were identified when they failed to move after being probed by a needle in the siphon or cervical region. They were also considered dead if they were unable to reach the water surface (Macedo et al., 1997). Pupations and adult emergence were recorded up to 72 h. The experiments were replicated 3 times. Percentage of mortality (%M) was corrected by Abbott's formula (WHO/VBC, 1981). The data were subjected to log probit analysis to calculate median lethal concentration (LC_{50}) values (Finney, 1971).

Data analysis

The statistical significance of differences between solvent extracts was determined by Student's *t*-test, calculating mean values for mortality rate. The significance level was set at 5%.

Preparation of samples for active part responsible for mortality

The dried residue of chloroform:methanol (1:1 v/v) (as it exhibited the highest mortality) extract was eluted with the same solvent, and then diethyl ether (AR) solvent was added using a separating funnel to make 3 fractions: basic, acidic and neutral. The precipitate formed after adding ether solvent was eluted with hot absolute alcohol and filtered through Whatman 40 filter paper. This dried sample was eluted with absolute alcohol (as it showed mortality) and chromatographed using precoated TLC plates (Sigma, USA, Silica gel; Unoplan-Shandon, London coating apparatus, thickness 0.5 mm) with chloroform:methanol (1:1 v/v) as a mobile phase.

Obtaining and Bioassay of Purified fraction

All 4 fractions were evaporated to dryness, and then dissolved in suitable volumes of distilled water to find out larval mortality. Out of the 4 fractions, the one that was fractioned with hot dehydrated alcohol showed mortality and was subjected to TLC. All the spots found on the TLC plate were scraped (from 12 plates) and dissolved in 20 ml of dehydrated alcohol and were heated in a water bath (60-65 °C) for 15 min. Clear solutions were taken in conical flasks. The solid mass present at the bottom of the conical flask was weighed. All were dissolved separately in distilled water to make concentrations and were tested against larvae. Then purified fractions were made in different concentrations (1.5 mg/100 ml, 2.0 mg/100 ml, and 2.5 mg/100 ml) and treated against fourth instar larvae of *Cx. quinquefasciatus* and larval death was recorded after 24, 48, and 72 h (Table 3). The R_f value of the positive spot was measured.

Table 1. Mortality of fourth instar larvae of *Culex quinquefasciatus* from different concentrations of nonpolar to polar solvent extracts (average of 3 experiments).

Solvent Extracts	Concentration (ppm)	Exposures								
		24 h			48 h			72 h		
		M	S	P	M	S	P	M	S	P
Petroleum	30	0	100	0	3.33	96.67	0	6.67	86.66	6.67
Ether	50	3.33	96.67	0	10.00	86.67	3.33	13.33	76.67	10.00
	100	10.00	90.00	0	20.00	80.00	0	33.33	60.00	6.67
	Control	0	100	0	3.33	90.00	6.67	3.33	83.33	13.33
Benzene	30	10.00	90.00	0	20.00	73.33	6.67	26.67	63.33	10.00
	50	13.33	86.67	0	26.67	70.00	3.33	36.67	53.33	10.00
	100	30.00	70.00	0	40.00	53.33	6.67	50.00	36.66	13.33
	Control	0	100.00	0	0	100.00	0	3.33	93.33	3.33
Chloroform:	30	33.33	66.67	0	43.33	50.00	6.67	46.66	39.99	13.33
Methanol	50	66.67	33.33	0	80.00	16.67	3.33	86.67	6.66	6.67
(1:1 v/v)	100	86.67	13.33	0	90.00	10.00	0	93.33	0	6.67
	Control	0	100.00	0	3.33	90.00	6.67	3.33	83.33	13.33
Acetone	30	13.33	86.67	0	20.00	76.67	3.33	30.00	60.00	10.00
	50	16.67	83.33	0	20.00	80.00	0	36.67	60.00	3.33
	100	46.67	53.33	0	53.33	40.00	6.67	60.00	26.67	13.33
	Control	3.33	96.67	0	6.67	86.66	6.67	6.67	80.00	13.33
Absolute	30	0	100.00	0	3.33	93.33	3.33	6.67	86.66	6.67
alcohol	50	10.00	90.00	0	16.67	83.33	0	16.67	76.66	6.67
	100	16.67	83.33	0	23.33	73.33	3.33	26.67	66.66	6.67
	Control	0	100.00	0	3.33	90.00	6.67	0	86.67	13.33

M = % of larval mortality
S = % of larvae survived
P = % of pupal development
Adult emergence was nil in all cases.

Table 2. Larvicidal activity in different nonpolar to polar solvent extracts of *Solanum villosum* leaves on the fourth instar *Culex quinquefasciatus* larvae.

Solvent extracts	LC_{50}a (ppm in water)
Petroleum ether	645.745
Benzene	204.302
Chloroform:Methanol (1:1 v/v)	39.192
Acetone	107.657
Absolute alcohol	321.890

a = lethal concentration to kill 50% larvae

Results

The results of the present study on the toxicity of polar and nonpolar solvent extract against fourth instar larvae of *Cx. quinquefasciatus* are presented in Table 1. In the case of petroleum ether, only 10% mortality was found after 24 h of exposure. Moreover, no toxicity was observed at the lowest concentration in the case of both petroleum ether and absolute alcohol extracts. The other 3 solvent extracts showed toxicity at their lowest concentration (30 ppm). Among all 5 solvent extracts, chloroform:methanol (1:1 v/v) exhibited the highest mortality (86.67%), which was significantly ($P < 0.05$) higher than that of petroleum ether (t = 10.941), benzene (t = 8.08), acetone (t = 4.25), and absolute alcohol (t = 7.439) (against tabulated value of 2.83). The present study also revealed that the mortality rate in the 100 ppm concentrations of chloroform:methanol (1:1 v/v) was higher than 50 ppm (t = 2.60) and 30 ppm (t = 8.336) concentrations in the 24-h study period.

The LC_{50} values of the leaves with biologically active different solvent extracts like petroleum ether, benzene, chloroform:methanol (1:1 v/v), acetone, and absolute alcohol were 645.745 ppm, 204.302 ppm, 39.192 ppm, 107.657 ppm and 321.890 ppm, respectively, after the 24-h study period (Table 2).

The fraction showing mortality gave an R_f value of 0.93. The results of the bioassay test with that fraction against fourth instar larvae of *Cx. quinquefasciatus* are presented in Table 3. The highest mortality was found at the 2.5 mg/100 ml concentration (40%), as expected, as a result of the concentration. Probit analysis with different concentrations of fractions isolated by TLC revealed the LC_{50} value of 3.197 mg/100 ml after the 24-h study period.

Discussion

Vector control is facing a threat of developing resistance in vector species to synthetic insecticides, which increases the interest in the development of newer insecticides. Insecticides of botanical origin are safe to use because of their target specificity, biodegradability, and availability. Although several plants showed toxicity to

Table 3. Mortality of fourth instar larvae of *Culex quinquefasciatus* due to the action of active part isolated by TLC of chloroform: methanol (1:1 v/v) extract.

Amount of extract (mg/100 ml)	Exposure								
	24 h			48 h			72 h		
	M	S	P	M	S	P	M	S	P
1.5	23.33	76.67	0	26.67	73.33	0	33.33	63.33	3.33
2	30.00	70.00	0	40.00	56.67	3.33	46.67	43.33	10.00
2.5	40.00	60.00	0	53.33	43.33	3.33	56.67	36.66	6.67
Control	0	100.00	0	3.33	93.33	3.33	3.33	93.33	3.33

M = % of larval mortality
S = % of larvae survived
P = % of pupal development
Adult emergence was nil in all cases.

mosquitoes (Saxena et al., 1992, 1993; Perich et al., 1994; Karam and Bansal, 2003; Bishnu and Zee, 2005; Patil et al., 2005), only a few have been used in field conditions like *Chrysanthemum cinerarifolium* (family Compositae) (Bruce and Leonard, 1985), which has also been used in indoor sprays (Sharma et al., 1996).

In the present study, among the polar and nonpolar solvent extracts of mature leaves, the highest efficacy as a larviciding agent against fourth instar larval form is found in chloroform:methanol (1:1 v/v) extract, which definitely suggests that any secondary metabolites of the plant are responsible for larval mortality.

In recent years, environmentally friendly and easily biodegradable insecticides have gained renewed importance. The results of the present study with solvent extracts of *S. villosum* mature leaves clearly indicate that this species can serve as a potent larvicide against *Cx. quinquefasciatus* larvae. The bioactive part responsible for mortality was isolated and the lethal concentration was determined. It will be cost effective as it works at a very low dose rate; it is indigenously available, easily biodegradable, and safe in comparison to synthetic insecticides. The study suggests that the active ingredients of the chloroform:methanol (1:1 v/v) extract, responsible for causing mortality to mosquito larvae, should be identified and utilized, if possible, in preparing commercial product formulations as a mosquito larvicide.

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