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Mosquito larvicidal activity of *Solanum nigrum* berry extracts

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Phytochemicals are widely used as biocontrol agent against vector mosquitoes. The present study was undertaken to isolate and evaluate the mosquitocidal activity of various extracts of berries of *S. nigrum* against *Culex quinquefasciatus*. Crude and chloroform: methanol (1:1, v/v) extracts of fresh, mature, green berries of *S. nigrum* were tested against *Cx. quinquefasciatus*. The lethal concentration was determined and the chemical nature of the active substance was evaluated. A qualitative phytochemical analysis of chloroform: methanol (1:1, v/v) extract was performed in search of the active ingredient. The appropriate lethal concentrations at 24 h for chloroform: methanol (1:1, v/v) extract was also studied on non-target organisms. In a 72 h bioassay experiment with crude extract, the highest mortality was recorded in 3 per cent extract. In the chloroform: methanol (1:1, v/v) solvent extract, the maximum mortality was recorded at a concentration of 120 µg/ml. The log probit analysis (95% confidence level) recorded lowest LC_{50} value at 72 h of exposure. Both crude and chloroform: methanol (1:1, v/v) extracts showed good larvicidal activity against *Cx. quinquefasciatus.* The isolated active ingredient may be tested as a potential larvicide after determination of its structure.

Key words *Culex quinquefasciatus* - IR analysis - larvicidal activity - *Solanum nigrum*

Mosquitoes are vectors of many dreadful human diseases. *Culex quinquefasciatus* is the principal vector of filariasis and it is reported to infect more than hundred million people every year in more than 110 countries in the tropics[1]. New control methodologies aim at reducing mosquito breeding sites and biting activity by using a combination of chemical- biological methods to reduce the population of mosquito and to reduce the man-vector contact[2]. Plant products are given importance due to their biodegradable nature, easy availability and no effect on non-target organisms that share the same environmental guild with mosquito larvae. *Solanum nigrum* L. (*Solanaceae*: Solanales) is an Ayurvedic herb with multiple medicinal properties[3,4]. The objective of the present study was to examine the larvicidal activity of extracts of berries of *S. nigrum* against the larvae of *Cx. quinquefasciatus* and other non target organisms.

The present study was conducted at Burdwan, West Bengal, India, during June-August 2010. Fresh, mature, green berries of *S. nigrum* were randomly harvested from plants growing on the outskirts of Burdwan. Berries were initially rinsed with distilled water and dried on a paper towel. Crude extract was

prepared by grinding the plant material in a mortar and pestle and passing the ground material through Whatman No. 1 filter paper. Required concentrations of crude extract were prepared by mixing the crude extract with suitable amount of sterilized distilled water. In the laboratory bioassay, ten each of 1st to 4th instar larvae of *Cx. quinquefasciatus* were separately introduced into different petri-dishes containing graded concentrations (1, 1.5, 2, 2.5 and 3%) and the mortalities were recorded after 24, 48 and 72 h of exposure periods following Abbott's correction[5]. The mortality rate at 3 per cent concentration was higher (P<0.05) than all other concentrations of the crude extract tested for larval mortality (Table I). For the preparation of solvent extract, 50 g of fresh, mature berries were rinsed with distilled water and dried in a shed. The dried berries were put in a Soxhlet apparatus and the plant extract was prepared using chloroform: methanol (1:1, v/v), (extraction period 72 h and the temperature was <40°C). Total yield of chloroform: methanol (1:1, v/v) solvent extract was 4.53 g. Similar types of bioassay were conducted with chloroform: methanol (1:1, v/v) solvent extract with concentrations of 30, 50, 80, 100 and 120 µg/ml on 1st to 4th instars larvae. Higher mortality was recorded at 120 µg/ml for all instars larvae and it was significantly (P<0.05) higher than the mortality rates at 30, 50, 80 and 100 µg/ml concentrations at 24, 48 and 72 h of exposures (Table II). Results of regression analysis revealed that the mortality rate (Y) was positively correlated with the period of exposure (X) having a regression coefficient close to one in each case (Table III). The results of Finney's log probit analysis[6] (95% confidence level) revealed that LC_{50} values gradually decreased with the exposure periods having the lowest value at 72 h of exposure to 3rd instars larvae, followed by 1st, 2nd and 4th instars larvae (Table III). From thin layer chromatography (TLC) plates of chloroform: methanol (1:1, v/v) solvent extract, an unsaturated spot developed in the iodine chamber having R_f = 0.924 and larvicidal bioassay with this compound resulted more than 45 per cent mortality to all the instars at a concentration of 15 µg/ml. The results of preliminary phytochemical analysis of the chloroform:

Table I. Mean larval mortality of mosquito larvae of different instars of *Cx. quinquefaciatus* exposed to different concentrations of crude extracts of berries of *S. nigrum* (mean of three experiments)

Instars	Concentrations (%)	Mean mortality (%) ± Standard Error		
		24 h	48 h	72 h
1st	1.00	33.30 ± 0.33	43.30 ± 0.33	53.30 ± 0.33
	1.50	40.00 ± 0.58	53.30 ± 0.33	63.30 ± 0.33
	2.00	50.00 ± 0.58	56.70 ± 0.33	66.70 ± 0.33
	2.50	66.70 ± 0.33	70.00 ± 0.00	83.30 ± 0.33
	3.00	70.00 ± 0.58*	73.30 ± 0.33*	86.70 ± 0.33*
2nd	1.00	43.30 ± 0.33	46.70 ± 0.33	56.70 ± 0.33
	1.50	46.70 ± 0.33	60.00 ± 0.00	66.70 ± 0.33
	2.00	53.30 ± 0.33	56.70 ± 0.33	70.00 ± 0.00
	2.50	56.70 ± 0.33	66.70 ± 0.33	76.70 ± 0.33
	3.00	63.30 ± 0.33*	70.00 ± 0.58*	80.00 ± 0.00*
3rd	1.00	33.30 ± 0.33	40.00 ± 0.00	50.00 ± 0.58
	1.50	50.00 ± 0.58	53.30 ± 0.33	63.30 ± 0.33
	2.00	53.30 ± 0.33	66.70 ± 0.33	70.00 ± 0.00
	2.50	63.30 ± 0.33	70.00 ± 0.58	76.70 ± 0.33
	3.00	66.70 ± 0.33*	73.30 ± 0.33*	83.30 ± 0.33*
4th	1.00	26.70 ± 0.67	33.30 ± 0.33	43.30 ± 0.33
	1.50	40.00 ± 0.58	43.30 ± 0.88	53.30 ± 0.33
	2.00	43.30 ± 0.88	46.70 ± 0.67	56.70 ± 0.33
	2.50	46.70 ± 0.67	50.00 ± 0.58	60.00 ± 0.58
	3.00	50.00 ± 0.58*	56.70 ± 0.33*	66.70 ± 0.33*

*P<0.05 compared to other concentrations in the same group

Table II. Result of larval mortality of different concentrations of chloroform: methanol (1:1, v/v) solvent extract of berries of *S. nigrum* on all instar of *Cx. quinquefasciatus* (mean of three experiments)

Instars	Concentrations (µg/ml)	Mean Mortality (%) ± Standard Errors		
		24 h	48 h	72 h
1st	30	23.30 ± 0.33	33.30 ± 0.33	43.30 ± 0.33
	50	26.70 ± 0.33	36.70 ± 0.33	50.00 ± 0.58
	80	33.30 ± 0.33	43.30 ± 0.33	53.30 ± 0.33
	100	43.30 ± 0.33	50.00 ± 0.5	60.00 ± 0.58
	120	63.30 ± 0.33*	66.70 ± 0.33*	76.70 ± 0.33*
2nd	30	16.70 ± 0.33	23.30 ± 0.33	36.70 ± 0.33
	50	26.70 ± 0.33	40.00 ± 0.00	46.70 ± 0.33
	80	43.30 ± 0.33	46.70 ± 0.33	60.00 ± 0.00
	100	46.70±0.33	56.70 ± 0.33	63.30 ± 0.33
	120	53.30 ± 0.33*	63.30 ± 0.33*	70.00 ± 0.00*
3rd	30	30.00 ± 0.00	43.30 ± 0.33	56.70 ± 0.33
	50	43.30 ± 0.33	56.70 ± 0.33	60.00 ± 0.00
	80	46.70 ± 0.67	60.00 ± 0.58	66.70 ± 0.33
	100	53.30 ± 0.33	63.30 ± 0.33	70.00 ± 0.58
	120	63.30 ± 0.33*	70.00 ± 0.00*	80.00 ± 0.58*
4th	30	20.00 ± 0.58	20.00 ± 0.00	26.70 ± 0.33
	50	33.30 ± 0.33	36.70 ± 0.33	46.70 ± 0.33
	80	46.70 ± 0.33	53.30 ± 0.33	56.70 ± 0.33
	100	56.70 ± 0.33	60.00 ± 0.58	66.70 ± 0.67
	120	60.00 ± 0.58*	66.70 ± 0.67*	70.00 ± 0.58*

*$P<0.05$ compared to other concentrations in the same group

methanol (1:1, v/v) extract of the mature berries was carried out using standard methods of Harbone and Stahl[7,8]. The phytochemicals tested in the study were saponins, steroid, terpenoids, flavonoids, alkaloid, essential oils, phenolics and amino acids, of which alkaloids, steroids, flavonoids and steroidal glycosides were present in berries. The effect of the chloroform: methanol (1:1, v/v) extract was tested against two non-target invertebrates *Diplonychus annulatum* (5th instar larval forms) and *Chironomus circumdatus*. There was no change in the survival rate and swimming activity when these were exposed to the LC_{50} values at 24 h of chloroform: methanol (1:1, v/v) extract. From functional group analysis, it seemed that the bioactive compound was aromatic amide in nature.

Phytochemicals are basically the secondary metabolites from plants that serve as a means of defense mechanism of the plants against herbivore predators and other environmental factors. Applications of phytochemicals in mosquito control were in use from 1900[9]. Roark[10] described approximately 1,200 plant species having potential insecticidal value, while Sukumar *et al*[11] listed and discussed 344 plant species that only exhibited mosquitocidal activity. Shallan *et al*[12] reviewed the current state of knowledge on larvicidal plant species. Kishore et al[13] reviewed the chemical nature of several plant derived secondary materials, having larvicidal potentiality. Changes in the mosquitocidal potentiality of plant parts according to the choice of solvent used for extraction of bioactive substances have also been described[14]. But, most of the studies were based on laboratory evaluation of the plant parts with no effort to determine the structure and commercial production of active ingredient for wide field application.

In the present study, at a very low concentration of 120 µg/ml, the chloroform: methanol (1:1, v/v) solvent extract of green berry of *S. nigrum* resulted in 80 per cent mortality of 3rd instar larvae after 72 h of exposure which indicates its biocontrol potentiaity

Table III. Log-probit analysis and regression analysis of larvicidal activity of chloroform: methanol (1:1, v/v) solvent extracts of berries of S. *nigrum* against different instar larval forms of *Cx. quinquefasciatus*

Instars	Period of bioassay	LC_{50} (µg/ml)	LC_{90} (µg/ml)	Regression equation	R value	Lower and upper fiducidal limits (µg/ml)
1st	24	109.13	710.40	Y= 0.41x + 6.67	0.93	79.82 - 263.32
	48	82.59	910.68	Y= 0.34x + 19.97	0.95	53.93 - 214.39
	72	48.41	593.68	Y= 0.33x + 31.86	0.94	10.41 - 72.48
2nd	24	107.27	569.52	Y= 0.41x + 6.24	0.99	80.95 - 210.37
	48	79.13	458.77	Y= 0.42x + 14.27	0.98	58.51 - 121.95
	72	54.20	436.03	Y= 0.37x + 27.67	0.97	27.08 - 76.41
3rd	24	77.28	805.15	Y= 0.33x + 22.39	0.95	49.36 - 163.59
	48	40.91	746.75	Y= 0.26x + 39.04	0.98	0.12 - 65.02
	72	23.54	515.45	Y= 0.25x + 48.13	0.98	0.01 - 44.51
4th	24	85.64	421.48	Y= 0.46x + 9.02	0.98	65.99 - 129.66
	48	74.29	304.79	Y= 0.51x + 8.57	0.99	58.09 - 98.34
	72	61.15	296.97	Y= 0.46x + 1.14	0.97	43.23 - 79.98

R, regression coefficient; LC, lethal concentration

against *Cx. quinquefaciatus*. The qualitative and chromatographic study of green berries of *S. nigrum* revealed the presence of several secondary compounds. Infra Red (IR) spectra of the bioactive compounds indicated that any aromatic amide compound was responsible for the larval mortality. Leaves of *S. nigram* and *S. villosum* have been previously reported to have mosquito larvicidal properties[15,16]. As no mortality occurred in the non-target organisms it can be assumed that the plant extracts are safe to use in the aquatic ecosystem. Considering its high target specific toxicity, biodegradable nature and no toxicity to non target organisms, the isolated bioactive ingredient may be a potential larvicide in the control of mosquito larvae. However, further research needs to be carried out to determine the mode of action and elucidate the actual structure of active ingredients present in the plant.

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