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Larvicidal Activity of a New Glycoside, Phenyl Ethyl β-D Glucopyranoside from the Stem of the Plant *Sida rhombifolia*

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Abstract: The isolated pure compound phenyl ethyl β-D glucopyranoside from the stem of the plant *Sida rhombifolia* was evaluated for larvicidal activity against common filaria vector, *Culex quinquefasciatus* at different instar under laboratory conditions. The LC_{50} values for the isolated compound was 36.22, 43.94 and 44.92, 58.34 and 60.40, 63.32 and 70.72, 82.52 ppm for 1st, 2nd, 3rd and 4th instar larvae *Culex quinquefasciatus* at 24 and 48 h post exposure, respectively.

Key words: Phenyl ethyl β-D glucopyranoside, larvicidal activity, *Sida rhombifolia*

Introduction

Mosquito control is one of the major problem especially in the developing countries today due to its vector nature. Since mosquito has been regarded as important vector for several diseases like malaria, encephalitidae, yellow fever and filariases. Recently, dengue, a dangerous disease also spread by a mosquito *Aedes aegypti*. Chemical insecticides are commonly used for controlling mosquitoes in most parts of the world (Schofield, 1993; Pal, 1994). But the problem is that after repeated use of the same insecticide the mosquito develop resistance to these insecticides (Hossain *et al.*, 1995). Apart from the development of insecticides and increased public concern over environmental pollution necessitate a continued search for alternatives, cheaper vector control methods which require little or not sophisticated technology but gives excellent results (Minjas and Sarda, 1986). In view of this, the study of biologically active materials with antilarval properties has attracted considerable interest (Kalyyanasundaram and Das, 1985; Kumar and Dutta, 1987). It is estimated that over 4,000.00 bioactive compounds so far been isolated but only about 10,000 of them have been characterized chemically (Swain, 1977). Many plant extracts of terrestrial origin have been reported to suppress mosquitoe larva populations (Chavan and Nikam, 1982; Saxena and Yadav, 1993) and suggested to be advantageous for field use in mosquito control programs (Kalyanasundaram and Das, 1985).

Sida rhombifolia Linn., locally known as Berela is an erect annual or perennial under shrub, widely distributed in Bangladesh, Asam, Madagascar and the topics of both hemispheres and belongs to the family Malvaceae (Khan, 1984). It has considerable reputation for its medicinal value in traditional medicine. The roots and leaves are used in fever, heart diseases, piles and all kinds of inflammation. The stems and roots are considered as aphrodisiac, diuretic and depurative; they are also given in the treatment of gonorrhoea and ring warm (Kirtikar, 1993). The preliminary screening of methanolic extract of leaves of *Sida rhombifolia* showed the anti-tumor and anti-HIV activities carried out against the human tumor cell lines in National Cancer Research Institute (Muanza *et al.*, 1995). There are many reports of plant species which have potent phytochemical effects on many species of dipteran insects (Chavan *et al.*, 1982; Kalyanasundaram and Das, 1985).

Materials and Methods

The powdered stem of *Sida rhombifolia* L. (3 Kg) were exhaustively extracted with methanol in a Soxhlet apparatus for 4 days at reflux temperature. The solvent was then filtered and evaporated off under reduced pressure in a rotary evaporator to afford crude extract as brick red mass. It was then successively extracted with pet ether, chloroform and ethylaceteate. The compound, phenyl ethyl β-D glucopyranoside was isolated from the chloroform extract by column chromatography (Backett and Stenlake, 1986) on silica gel followed by PTLC and isolated as oily syrup. The column was first eluted with n-hexane/chloroform and then polarity of the solvent system was gradually increased and 50 ml were collected in each fraction. The fractions 16-21 showed two distinct spots on thin layer chromatography (TLC). The compound was characterized on the basis of its ^{1}H-NMR, ^{1}H-^{1}H COSY 90^{0} spectrum and ^{13}C-NMR. This compound was used for the study of its larvicidal effect.

Table 1: Mortality data of *C. quinquefasciatus* larvae at different instar treated with the isolated compound phenyl ethyl β-D-glucopyranoside of *Sida rhombifolia* stems

Samples	Larvae instar	Duration of treatment	LC_{50} value	95% conf. Limits lower - upper	χ^2 value
Phenyl ethyl β-D-glucopyranoside	1st	24	36.92	30.00-43.67	4.01
		48	43.94	35.36-54.60	1.31
	2nd	24	44.92	35.71-56.50	1.23
		48	58.34	40.21-84.66	1.10
	3rd	24	60.40	43.15-84.55	1.96
		48	63.32	43.88-91.39	1.21
	4th	24	70.72	47.57-105.15	1.61
		48	82.52	52.55-129.58	1.72

*P<0.05; ***P<0.001

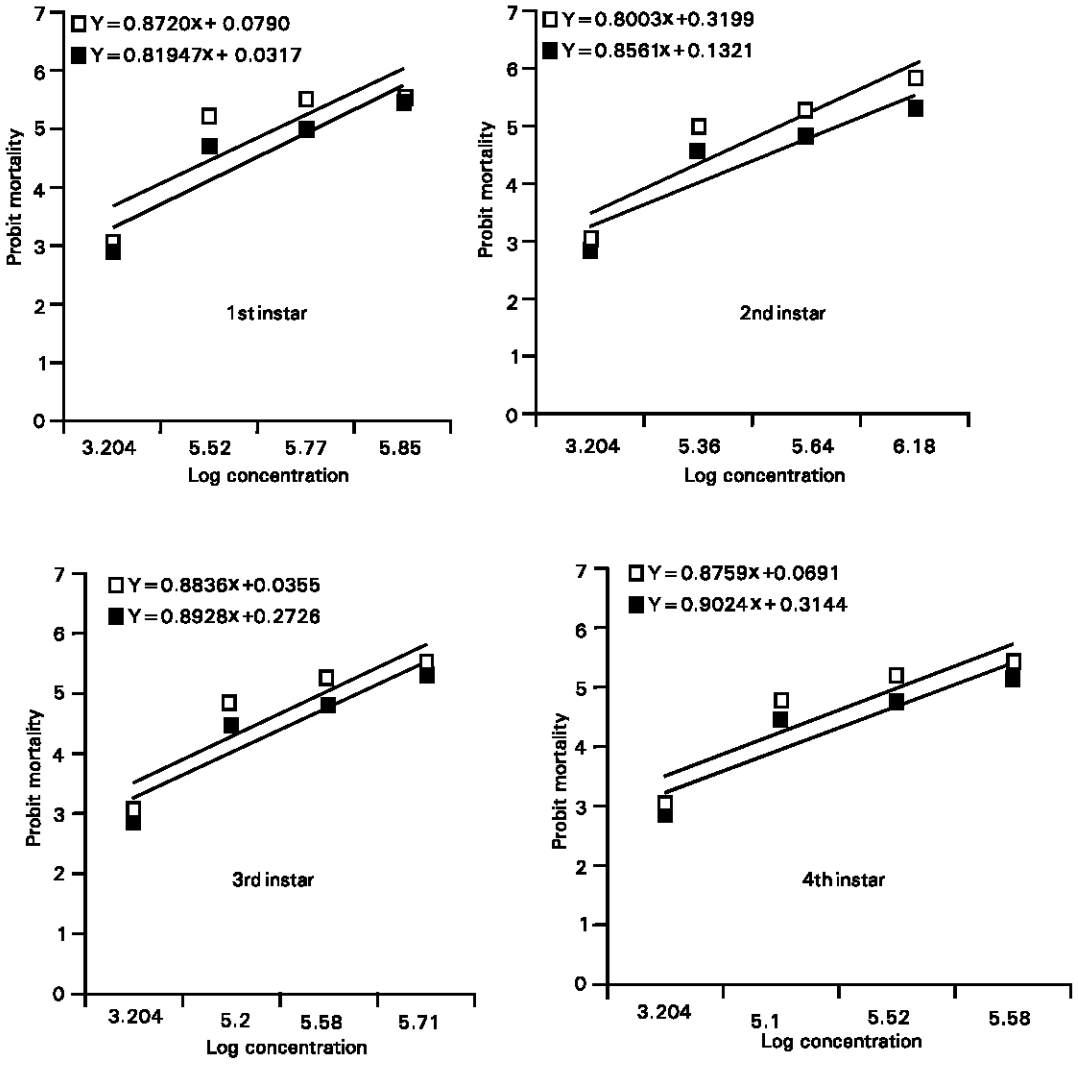

Fig. 1: Probit regression line for the mortality of different instar larvae of *C. quinquefasciatus* treated with phenyl ethyl β-D-glucopyranoside from the stems of *Sida rhombifolia.*

Test insects: To ensure a constant supply of the insects *Culex quinquefasciatus* larvae at different instars used in bioassay were reared in laboratory at room temperature of 25±5°C and a relative humidity of 70 ±10. Larvae were fed with powdered dry yeast: glucose granules (1:3) dissolved in distilled water. Adults were fed with 10% glucose solution soaked in cotton on petridishes. In addition to glucose feeding, the female in Gerberg mosquito cages were also feed with chick blood twice a week, regularly.

Larvicidal effects: The compound phenyl ethyl β-D-glucopyranoside was tested for the larvicidal action at different concentration, viz. 0 (control) 10, 20, 40 and 80 ppm after diluting with stock solution with dimethyl sulfoxide. The prepared doses used in 3 replication, each having 25 early 1st, 2nd, 3rd and 4th, instar mosquito larvae in test tubes with food medium. The mortality of the larvae at different instar was assessed after 24 and 48 h of treatment. The number of larvae was kept on untreated medium as controls. Drowning malformed larvae were recorded as being dead. The mortality was determined by counting survivors at the exposure period and the control mortality was adjusted using Abbotts formula (Abbott, 1925) and the results were subjected to probit analysis following the methods of Busvine (1971). The experiments were conducted at 26±2^{0}C.

Results and Discussion

The results of the larval susceptibility of *C. quinquefasciatus* to the isolated pure compound phenyl ethyl β-D-glucopyranoside from $CHCl_3$ extracts of *Sida rhombifolia* L. stems are presented in Table 1 and Fig. 1. The phenyl ethyl β-D-glucopyranoside was effective against the larvae of different instars. The results showed that the mortality of larvae increased as the doses of the samples were increased. The same trends were also observed in case of time elapse mortality. It was observed that many larvae were failed to ecdyse to perfect pupae, producing larvae-pupal intermediates which were short live. The 4th instar larvae of *Culex quinquefasciatus* were more resistant to the samples than 1st larvae. The LC_{50} values of the sample were age dependent. This may clearly support the ideas of others that insects age play an important role in influencing susceptibility (Kumar and Dutta, 1987; Mwangi and Mukiama, 1988). It would appear that 4th instar larvae are much more resistant to the sample as compared to the other. The sluggish movement and peculiar coiling of treated larvae seem to suggest some neutral or muscular disturbance by some active principle; which might be cause acute lethal effect. This observation more or less similar to Kiamuddein *et al.* (1979). The delayed lethal effect of the compound however, are more likely to be caused by a disturbance of the endocrine mechanisms that regulate moulting and metamorphosis. This mechanism of action has been postulated previously for neem seed kernel extracts (Zebitz, 1986).

It was concluded that the isolated compound offer a significant potential as new control agent against *Culex quinquefasciatus* larvae. However, more works are to be directed towards this line with varying concentration, extracts of different part of this plant on various mosquito species.

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