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Laboratory Evaluation of *Nyctanthes arbo-rtristis* Linn. Flower Extract and its Isolated Compound Against Common Filarial Vector, *Culex quinquefasciatus* Say (Diptera:culicidae) Larvae

Naznin Ara Khatune, Md. Ekramul Haque and [1]Md. Ashiik Mosaddik
Department of Pharmacy, University of Rajshahi- 6205 Bangladesh, Bangladesh

Abstract: The chloroform extract and its isolated pure compound NCS-2 from the flowers of *Nyctanthes arbor-tristis L.* were evaluated for larvicidal activity against common filaria vector, *Culex quinquefasciatus* Say at different instar under laboratory conditions. The LC_{50} values for the chloroform extract were 25.67, 22.19; 38.60, 28.95; 53.14, 42.14 and 72.60, 61.82 ppm and for the isolated compound NCS-2 were 73.31, 65.48; 83.02, 67.02; 97.26, 81.84 and 114.68, 99.02 ppm for 1st, 2nd, 3rd and 4th instar larvae respectively at 24 and 48 hours post exposure. The results also showed that the late instar larvae were more resistant to the extract than those of early instars.

Key words: NCS-2, larvicidal activity, *Nyctanthes arbortrists*

Introduction

Mosquito has been regarded as an important vector for transmission of several diseases such as malaria, encephalitidae, yellow fever, dengue fever and filariases. Recently, it is reported that mosquitoe can transmit hepatitis B virus (El-Alamy *et al.*, 1979; Siemens, 1987). Mosquito control is one of the major problems of the world today in view of its vector nature. Various control measures are adopted to control disease–transmitting mosquitoes. Particularly, chemical insecticides are commonly used for controlling mosquitoes in most parts of the world (Schofield, 1993; Pal, 1994). But in recent years, one of the main reasons for the failure of the mosquito control programs is that the insects develop resistance to these insecticides due to repeated use of same insecticides for a long time (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 no sophisticated technology but give excellent results (Minjas and Sarda, 1986). In view of this, the study of biologically active materials with antilarval properties has attracted considerable interest (Kalyanasundaram and Das, 1985; Kumar and Dutta, 1987). It is estimated that over 4,000,00 bioactive compounds exist but only about 10,000 of them have been characterized chemically (Swain, 1977). Many plant extracts of terrestrial origin have been reported to supress mosquitoe larval populations (Charan and Nikam,1982; Saxena and Vadav,1993) and suggested to be advantageous for field use in mosquito control programmes (Kalyanasundaram and Das, 1985).

Nyctanthes arbor-tristis L. (Family: Nyctantheceae) is a small tree of about 9m length and found to grow in the tropical Asia, including Bangladesh (Bruschia, 1957). The flowers of the plant are used as carminative, antibilious, expectorant, and hair tonic. It is also reported to be used in treatment of piles and various skin diseases (Kirtikar and Basu, 1993; Drury, 1973). 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

Extraction, isolation and characterization: Fresh flowers (500 gm) of the plant *Nyctanthes arbor-tristis L.* were collected from a garden of Prof. Md. Ekramul Haque, Rajshahi town and were extracted with rectified spirit at room temperature for 15 days with continuous changing fresh flowers with the old ones every three days interval. The solvent was then filtered and evaporated under reduced pressure in a rotary evaporator to afford crude extract (43.93 gm), as reddish orange mass. It was then successively extracted with pet. ether, chloroform, ethyl acetate and finally with methanol. Among these, chloroform extract was used for the evaluation of larvicidal effect. The compound NCS-2 was isolated from the chloroform extract by column chromatography (Backett and Stenlake, 1986) on silica gel of 60-120 mesh size. The column was first eluted with n-hexane: ethyl acetate (1:5) and then polarity of the solvent system was gradually increased and 50 ml were collected in each fraction. The fractions 3 to 6 showed almost identical spots on TLC. These fractions were combined and subjected to Preparative Thin Layer Chromatography (Egon and Stahl, 1969) using solvent system chloroform: methanol (7:1) to afford the compound NCS-2 as yellowish syrup. The compound NCS-2 was characterized on the basis of its ^{1}H-NMR, ^{1}H-^{1}H COSY 90^{0} spectrum, ^{13}C complete decoupling, ^{13}C DEPT 135^{0} spectra and long range correlation spectra. This compound was also used for the study of its larvicidal effects.

Test insects: To ensure a constant supply of the test insects *C. quinquefasciatus* larvae at different instars used in bioassay were reared in laboratory at room temperature (25 ± 5^{0}) and a relative humidity of 70 ± 10^{0}. 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 females in Gerberg mosquito cages (Gerberg, 1970) were also fed with chick blood twice a week, regularly.

Larvicidal effects: The chloroform extract and the compound, NCS-2 were tested for the larvicidal action at different concentrations, *viz.* 0 (control), 10, 20, 40 and 80 ppm after diluting the stock solution with dimethyl sulfoxide. The prepared doses were used in 3 replications, 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 hr 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 by using Abbott's formula (Abbott, 1925) and the results were subjected to probit analysis following the method of Busvine (1971).

Results and Discussion

The results of the larval susceptibility of *C. quinquefasciatus* to the chloroform extract of *N. arbor-tristis* L. flowers and its isolated pure compound NCS-2 are presented in Table 1

Table 1: Mortality data of *C. quinquefasciatus* larvae at different instars treated with chloroform extract and its isolated compound of *N. arbor-tristis* flowers.

Samples	Larval instar	Duration of treatment(hr)	LC_{50} value (ppm)	95% conf. Limits lower	–	upper	X^2 value
Chloroform extract	1st	24	25.67	20.94	-	31.48	1.88
		48	22.19	18.66	-	26.33	4.70
	2nd	24	38.60	30.30	-	49.16	4.20
		48	28.95	23.69	-	35.39	3.28
	3rd	24	53.14	40.55	-	69.63	0.11
		48	42.80	32.52	-	56.31	1.90
	4th	24	72.60	50.12	-	105.17	3.28
		48	61.82	42.21	-	90.54	0.18
NCS-2	1st	24	73.31	47.40	-	113.39	1.00
		48	65.48	44.15	-	97.12	2.11
	2nd	24	83.02	66.10	-	104.27	3.18
		48	67.02	55.66	-	80.70	4.14
	3rd	24	97.26	69.61	-	135.89	1.65
		48	81.84	65.26	-	102.63	3.86
	4th	24	114.68	79.54	-	165.35	1.39
		48	99.02	74.19	-	132.15	2.06

*P<0.05; ***P<0.001

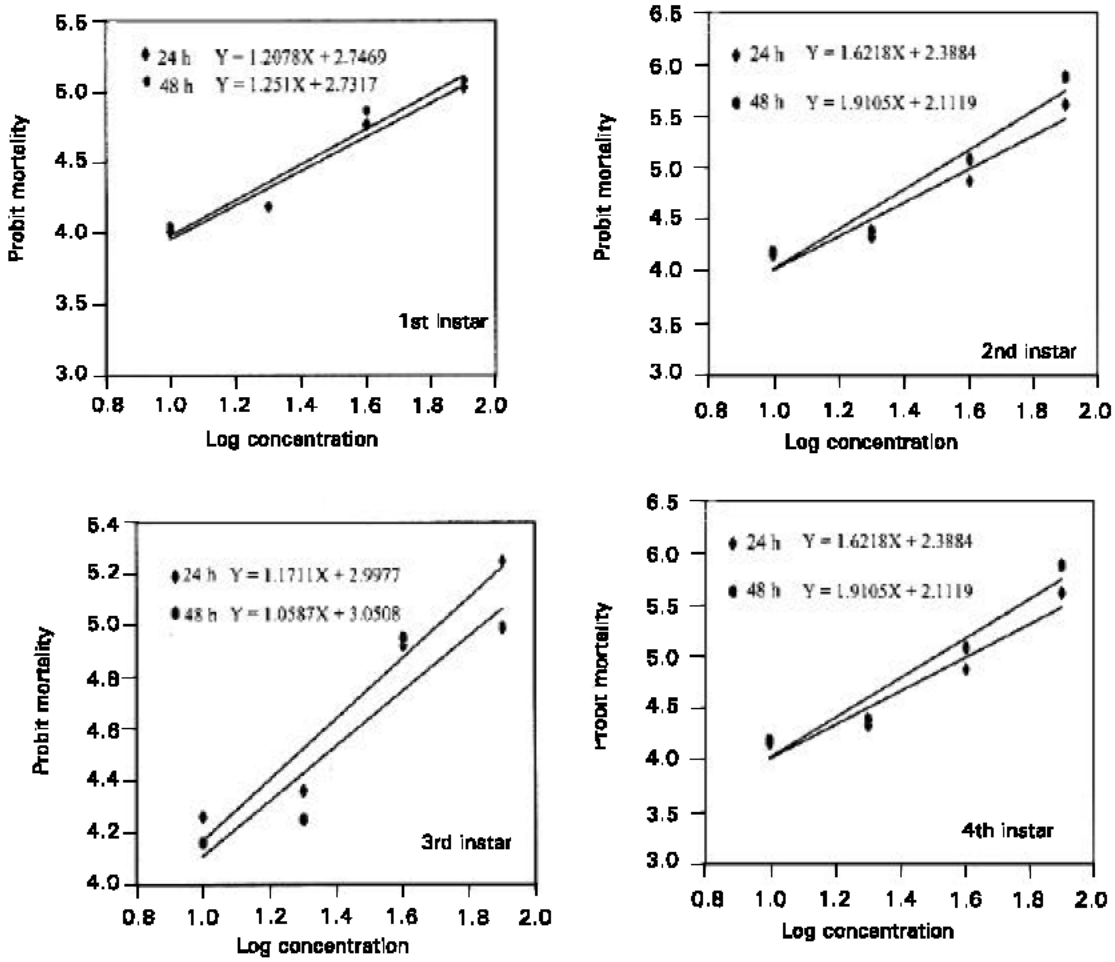

Fig. 1: Probit regression lines for the mortality of different instar larvae of *C. quinquefasciatus* treated with chloroform extract of flowers of *N. arbor-tristis* L. and its isolated pure compound, NCS-2, respectively.

and Fig 1. The chloroform extract and NCS-2 were effective against the larvae of different instars. The results showed that the mortality of larvae increase 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 lived. The 4th instar larvae of *C. quinquefasciatus* were more resistant to the samples than 1st instar larvae. The LC_{50} values of the samples were age dependent. This may clearly support the ideas of others that insect age play an important role in influencing the susceptibility (Kumar and Dutta, 1987; Mwangi and Mukiama, 1988). The sluggish movement and peculiar coiling of treated larvae seem to suggest some neutral or muscular disturbance by some active principle; which might cause acute lethal effect. This observation is more or less similar to Kiamuddein *et al.*(1979). The delayed lethal effects of the extract and the compound however, are more likely to be caused by a disturbance of the endocrine mechanisms that regulate the moulting and metamorphosis. This mechanism of action has been postulated previously for neem seed kernel extracts (Zebitz, 1986).

It is concluded that the chloroform extract and its isolated compound NCS-2 offer a significant potential as new control agents against *C. 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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