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Larvicidal Efficacy of *Strychnos nuxvomica* Linn. (Loganiaceae) Leaf Extracts Against the Filarial Vector Culex *quinquefasciatus* Say (Diptera: Culicidae)

[1]S. Arivoli and [2]Samuel Tennyson

[1]Department of Advanced Zoology and Biotechnology,
Loyola College, Chennai 600 034, Tamilnadu, India
[2]Department of Zoology, Madras Christian College, Chennai 600 059, Tamilnadu, India

Abstract: The larvicidal activity of hexane, chloroform and ethyl acetate leaf extracts of *Strychnos nuxvomica* against the third instar larvae of *Culex quinquefasciatus* was studied. The ethyl acetate leaf extract was found to be the effective larvicide with LC_{50} value of 222.28 and 146.99 ppm after 24 and 48 hours. The results indicate promising larvicidal activity against *Culex quinquefasciatus*. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed.

Key words: *Strychnos nuxvomica* · Leaf Extracts · Larvicidal Activity · *Culex quinquefasciatus*

INTRODUCTION

Mosquitoes are major public health pests throughout the world. Among the 3492 species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases to humans [1]. Mosquito menace is particularly high in South East Asian countries [2] and in recent years global warming has lead to the spread of mosquitoes into temperate countries and higher altitude regions and the people in these regions are severely affected [3]. Mosquito serve as crucial vectors for a number of arboviruses (arthropod-borne viruses) and parasites that are maintained in nature through biological transmission between susceptible vertebrate hosts by blood feeding arthropods responsible for inflammation, encephalitis, dengue, malaria, rift valley fever, yellow fever and others [4]. The mosquito *Culex quinquefasciatus* acts as a vector for *Wuchereria bancrofti* responsible for filariasis [5] which is an endemic, disabling, disfiguring disease. *Culex quinquefasciatus* Say (Diptera: Culicidae) is a pantropical pest and urban vector of *Wuchereria bancrofti* [6] and is probably the most abundant house mosquito in towns and cities of the tropical countries [7]. Interest in the control of *Culex quinquefasciatus* lies in the fact that it acts as a vector of filarial fever, a serious public health problem in India and many developing countries. One of the strategies of WHO in combating tropical diseases is to destroy their vectors or intermediate hosts. Since no effective vaccine is available for filarial fever, the only efficacious approach of minimizing the incidence of this disease is to eradicate and control mosquito vectors mainly by application of insecticides to larval habitats. Vector control is by far the most successful method for reducing incidences of mosquito-borne diseases, but the emergence of widespread insecticide resistance and the potential environmental issues associated with some synthetic insecticides (such as DDT) has indicated that additional approaches to control the proliferation of mosquito population would be an urgent priority research. Currently, numerous products of botanical origin, especially the secondary metabolites, have received considerable renewed attention as potentially bioactive agents used in insect vector management [8].

The use of herbal products is one of the best alternatives for mosquito control. The search for herbal preparations that do not produce any adverse effects in the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control [9]. *Strychnos nuxvomica* Linn. (Loganiaceae) is found predominantly distributed in India, Srilanka, Southeast Asia and Northern America [10]. It is cultivated throughout India and is popularly known as 'snake wood' in English [11], 'bailewa' in Hindi [12] and

Corresponding Author: Samuel Tennyson, Department of Zoology, Madras Christian College, Chennai 600 059, Tamilnadu, India.

'etti' in Tamil [13]. It has been known for its medicinal properties and Ayurveda recommends use of *Strychnos nuxvomica* in purified form since time immemorial in treatment of various diseases [14] and also in folk medicine for alleviating inflammation and joint pains [15].

Different formulations of the plant are used in treatment of metabolic ailments [16]. The seeds of *Strychnos nuxvomica* are used as febrifuge, emmenagogue, purgative, stimulant, stomachic, aphrodisiac, antihelmentic [17] and also in treating anaemia, asthma, bronchitis, constipation, diabetes, skin diseases, paralysis, nervous disorders [18], chicken pox fever [19], eczema [20] and rheumatism [21]. The dried seeds are used as herbal remedies in traditional Chinese medicine for treatment of nervous disorders, arthritis and vomiting [22]. The leaves are used for treating chronic wounds, ulcers and the root bark in treatment of cholera [11]. The whole plant is used for treating digestive disorders, epilepsy [23], migrane headaches and to treat problems related to menopause [24].

The plant possesses anti-inflammatory [25], analgesic [26], antidepressant [27], antitumour [28] and convulsant properties [29] and also used as an antidote for snake poison [30]. The phytochemical constituents present in *Strychnos nuxvomica* include strychnine, brucine, strychnicine, glycosides [31], indole alkaloids [32], iridoids [33], iridoid glycosides [22] and glucomannas [34]. Besides these, the acetone extract of *Strychnos nuxvomica* dried stem exhibited toxicity against polyphagous beetles [35]. Further, the plant is reported to possess antimalarial activity and antimalarial compounds and the seed extracts of *Strychnos nuxvomica* showed larvicidal activity against the fourth instar larvae of *Aedes aegypti* [36]. Therefore, the present study has been carried out to evaluate the larvicidal activity of *Strychnos nuxvomica* leaf extracts against the filarial vector, *Culex quinquefasciatus*.

MATERIALS AND METHODS

Plant Collection and Extraction: *Strychnos nuxvomica* leaves collected in and around Tamilnadu, India were brought to the laboratory, shade dried under room temperature and powdered using an electric blender. Dried and powdered leaves (1 kg) was subjected to sequential extraction using 3 L of hexane, chloroform and ethyl acetate for a period of 72 h to obtain the crude extracts using rotary vacuum evaporator. The hexane, chloroform and ethyl acetate crude extracts were thus obtained, lyophilized and a stock solution of 1,00,000 ppm prepared from each crude extract by adding adequate volume of acetone was refrigerated at 4°C until testing for bioassays.

Test Mosquitoes: Tests were carried out against laboratory reared *Culex quinquefasciatus* free of exposure to insecticides and pathogens. Cyclic generations of *Culex quinquefasciatus* were maintained at 25-29°C and 80-90 per cent R.H. in the insectarium. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on 10 per cent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

Larvicidal Activity: Standard WHO [37] protocol with slight modifications was adopted for the study. From the stock solution, concentrations of 250, 500, 750 and 1000 ppm were prepared. Twenty five early third instar larvae were introduced in 250 ml beaker containing 200 ml of water with each concentration. A control was prepared by the addition of acetone to water. Mortality was recorded after 24 and 48 hours. A total of three trials were carried out with five replicates per trial against vector mosquitoes. However, when the control mortality ranged from 5-20 per cent, the observed percentage mortality was corrected by Abbott's formula [38],

$$\text{Per cent mortality} = \frac{\text{\% Mortality in treated} - \text{\% Mortality in control}}{100 - \text{\% Mortality in control}} \times 100$$

Statistical Analysis: SPSS 11.5 version package [39] was used for determination of LC_{50} and LC_{90}. Data from mortality and effect of concentrations were subjected to Chi square analysis ($P < 0.05$).

RESULTS AND DISCUSSION

Results of the larvicidal effects of leaf extracts of *Strychnos nuxvomica* against *Culex quinquefasciatus* reported in the present study exhibit the presence of larvicidal properties in the plant suggesting their use in larval mosquito population control (Tables 1, 2). The leaf extracts of *Strychnos nuxvomica* showed larval mortality against the third instar larvae of *Culex quinquefasciatus*. *Strychnos nuxvomica* ethyl acetate leaf extract was found to be more effective than hexane and chloroform by producing 100 per cent mortality in 500 ppm at 48 hours. The LC_{50} value of ethyl acetate extract was 222.28 and 146.99 ppm followed by chloroform extracts with LC_{50} value of 265.41 and 241.96 ppm after 24 and 48 hours

Table 1: Per cent larvicidal activity of *Strychnos nuxvomica* leaf extracts against *Culex quinquefasciatus*

	Concentration (ppm)							
	250		500		750		1000	
Extracts	24h	48h	24h	48h	24h	48h	24h	48h
Hexane	35.2±0.76	48.0±0.63	74.4±1.82	84.8±0.95	84.8±1.37	95.0±0.71	100±0.0	100±0.0
Chloroform	49.6±0.72	55.2±0.71	76.8±0.95	90.4±1.15	88.8±0.65	97.6±0.35	100±0.0	100±0.0
Ethyl acetate	58.4±1.3	96.8±0.33	85.6±0.66	100±0	95.0±0.33	100±0.0	100±0.0	100±0.0
Control	0	0	0	0	0	0	0	0

Values are mean (%) of the five-replicates of three trials ±standard deviation.

Table 2: Probit analysis of larvicidal activity of *Strychnos nuxvomica* leaf extracts against *Culex quinquefasciatus*

	LC_{50} (ppm)		LC_{90} (ppm)		Slope		Chi square	
Extracts	24h	48h	24h	48h	24h	48h	24h	48h
Hexane	327.79	261.91	946.53	694.27	3.57±0.70	3.89±0.84	2.13*	0.43*
Chloroform	265.41	241.96	895.74	593.99	3.11±0.71	4.22±0.97	1.58*	0.16*
Ethyl acetate	222.28	146.99	692.84	459.26	3.33±0.82	3.32±1.19	0.59*	0.77*

*Significant at P <0.05 level

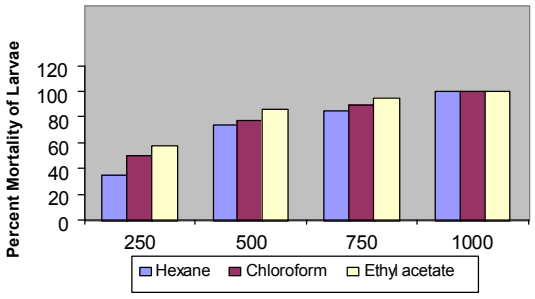

Fig. 1: Per cent larvicidal activity of Strychnos nuxvomica leaf extracts at 24h against Culex quinquefasciatus

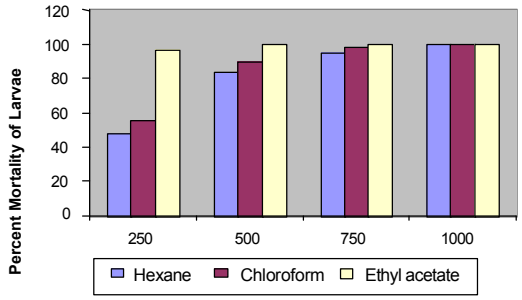

Fig. 2: Per cent larvicidal activity of Strychnos nuxvomica leaf extracts at 48h against Culex quinquefasciatus

respectively. Further, 100 per cent mortality was also observed in hexane and chloroform extracts in 1000 ppm after 24 hours (Figures 1, 2).

Mosquito control programmes largely target the larval stage at their breeding sites with larvicides [40]. Larviciding is a successful method of reducing mosquito population in their breeding places before they emerge as adults [41]. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices. Plant extracts are reported to be eco-friendly mosquito control agents [42-45]. The results of the present study are comparable with earlier reports. One hundred per cent larval mortality was recorded by Daniel *et al* [46] in their studies using *Acalypha indica* and Karmegam *et al* [47] using *Pergularia extensa, Argemone mexicana* and *Withania somnifera* against the larvae of *Culex quinquefasciatus*. In the present observation, hundred per cent larval mortality was observed and ethyl acetate leaf extract of *Strychnos nuxvomica* was found to be effective. Similarly,Arivoli *et al* [48] studied the effect of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts of shoot with leaves of *Leucas aspera* and leaves of *Vitex negundo* against the larvae of *Culex quinquefasciatus* and proved that ethyl acetate extract of both plants provided maximum mortality.

The toxicity to the third instar larvae of *Culex quinquefasciatus* by methanolic leaf extract of *Memordica charantia*, *Trichosanthus anguina* and *Luffa acutangula* showed LC_{50} values of 465.85, 567.81 and 839.81 ppm respectively [49]. The dichloromethane extract of *Citrullus colocythis* whole plant provided hundred per cent mortality with LC_{50} value of 240.36 ppm [50] and the diethyl ether extract of *Abutilon indicum* leaf exhibited a LC_{50} value of 395.90 ppm [51] and the hexane extract of *Hyptis suaveolens* aerial parts a LC_{50} value of 203.37 ppm [52] after 24 hours of exposure against the third instar larvae of *Culex quinquefasciatus*. The findings of the present investigation revealed that the leaf extracts of

Strychnos nuxvomica possess larvicidal activity against *Culex quinquefasciatus*. It may be concluded that natural products as extracts from parts of plants of insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal toxicity. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *Strychnos nuxvomica* leaf extracts to control the larval stages of *Culex quinquefasciatus*.

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