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Research Article

Efficiency of *Nerium oliender* L. and *Gliricidia sepium* (Jacq.) Kunth ex Walp. as a larvicidal agent against aquatic stages of *Aedes aegypti* (L)

D. Banupriya, S. Umavathi, S. K. Bhasheer, M. Thangavel and M. Cinthamani

PG and Research Department of Zoology, J. K. K. Nattraja College of Arts and Science, Kumaraplayam, Namakkal District, Tamilnadu.
*Corresponding author: *uma_kasc@yahoo.co.in*

Abstract

In the current study the ethyl acetate leaf extract of *Nerium oliender* and *Gliricidia sepium* were tested for larvicidal activity against the mosquito *Aedes aegypti.* Larval (I-IV) and pupal mortality was observed and recorded after 24 hours exposure period. The crude ethyl acetate extracts showed different degrees of phytotoxicity. It was also observed that the toxicity of the extracts varies with concentration. In addition preliminary phytochemical analysis showed the presence of alkaloid, flavonoids, steroid, and tannin, chlorogenic acid and phenolic compounds in *N. oliender* and alkaloids, glycosides, steroids and carbohydrate in *G. sepium* ethyl acetate leaf extract.

Keywords: Phytochemicals, *Nerium oliender, Gliricidia sepium*, *Aedes aegypti* and LC_{50}

Introduction

Mosquitoes are responsible for the spread of more diseases, than any other group of arthropods. Mosquito borne diseases such as malaria, filariasis, dengue fever, yellow fever and Japanese encephalitis contribute significantly to disease burden, death, poverty and social debility in tropical countries (Hafeez *et al.,* 2011). Vector control is an essential and effective means for controlling the transmission of these mosquito borne diseases. The control of mosquitoes at the immature stage is necessary and efficient in integrated vector control management (Shaalan *et al.,* 2012). The major tool in mosquito control operation is the application of synthetic insecticides such as organo chlorine, organophosporous, carbamates, pyrethrins and pyrethroids (Ali *et al.,* 2012). Chemical pesticides have been used for several decades in controlling pests and vectors of various human diseases as they have a quick knock down effect.

In recent years use of synthetic insecticides in mosquito control programme has been limited. It's due to lack of novel insecticides, resurgence of pests, elimination of natural enemies, non biodegradable nature, high cost of synthetic insecticides, concern of environmental sustainability, harm full effect on human health and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem and increasing insecticide resistance on a global scale (Russell *et al*.,2009). When they applied carelessly, they may also cause undesirable, acute and long-term side effects. Hence it is an important to search for easily degradable alternative insecticides to control vector mosquitoes.

The pharmacological and insecticidal properties of plants have been recognized in many parts of the world especially India, where plant materials are easily available and their use in health practices is a tradition (Shakthivadivel and Daniel, 2008).

Biologically active compounds of plants are biodegradable with non-residual effects in the environment. Hence in the present study an attempt has been made to assess the larvicidal potential of ethyl acetate leaf extract of *Nerium oleander L* and *Gliricidia sepium.*

Materials and Methods

The leaves of *N. oleander* and *G. sepium* were thoroughly washed with tap water and were dried under shade at room temperature (29 ± 2 ^{0} C) for about 20 days. The completely dried leaves were powdered and sieved to get fine powder. The powdered leaves 100 gms were extracted separately with 300ml ethyl acetate by using the Soxhlet apparatus for 8 hours. The extracts were concentrated using a vacuum evaporator at 45^{0}C under low pressure. After complete evaporation of the solvent the concentrated extract was collected and stored in a refrigerator for further experiments. One gram of concentrated extract was dissolved in 100 ml of the respective solvent, kept as a stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae. The larvicidal bioassay was done using standard WHO Protocols (WHO, 2005). Twenty freshly moulted I-IV instar larvae and pupae of *A. aegypti* were exposed to different desired concentrations of plant extracts. Controls were maintained using respective solvents along with the experiment. Mortality of different developmental stages of the treated and control over a period of 24
hours was observed. The percentage of larval mortality was corrected by Abbot's formula (1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortalityin treatment - Observed mortality in control}}{\text{100- Control mortality}} x100$$

$$\text{Percentagemortality} = \frac{\text{Numberof deadlarvae}}{\text{Numberof larvae introduced}} \times 100$$

from toxicity data by using probit analysis (Finney, 1971). The ethyl acetate extract of *N.oleander* and *G.sepium* were subjected to preliminary phytochemical tests to determine the groups of secondary metabolites present in the plant materials (Harborne, 1998).

Results and Discussion

Bioassay test were conducted to find out the effect of *N. oleander* and *G. sepium* ethyl acetate leaf extract on the developmental stages of *A. aegypti*. The larvae and pupae of *A. aegypti* were exposed to 50 to 250ppm of *N. oleander* and 100 to 300ppm of *G. sepium* (Fig.1&2). Phytochemical extract of *N. oleander* and *G. sepium* showed the concentration based effect on the developmental stage of *A. aegypti*. In higher concentration (250&300ppm) the mortality percentage ranges from 100, 86.0, 74.0, 66.0, 64.0 in *N.oleander*; 86.0, 76.0, 66.0, 60.0, 52.0 in *G.sepium* against I - IV instar larvae and pupae respectively. In *N.oleander* extract the lower concentrations (50ppm) mortality percentages ranging from 48.0, 46.0, 44.0, 28.0 and 22.0 on I- IV larvae and pupae of *A.aegypti*. In *G.sepium* ethyl acetate extract, lower concentrations (100ppm) mortality percentage ranging from 36.0, 34.0, 22.0, 16.0, and 20.0 on I-IV larvae and pupae *A. aegypti*.

The LC_{50} and LC_{90} values of ethyl acetate extract of *N. oleander* 59.117, 204.662ppm for I instar larvae; 62.512, 306.924 ppm for II instar larvae; 81.066, 357.203 ppm for III instar larvae 128.989, 417.197ppm for IV instar larvae and 165.423, 426.738 ppm for pupae (Table 1). The ethyl acetate extract of *G. sepium* showed LC_{50} values were 115.719, 172.919, 222.588, 246.357 and 276.824 ppm; the LC_{90} values were 335.324, 401.464, 442.260, 450.086, and 579.560 ppm for the I-IV instars and pupae of *A. aegypti* respectively. Among the developmental stages the first instar larvae were more susceptible than the other aquatic stages of *A. aegypti* (Table. 2).

The LC_{50} and LC_{90} values were age depended in the present study. IV Instar larvae of *A. aegypti* showed least susceptibility than pupae and larval stages against the ethyl acetate extract of *N. oleander* and *G. sepium*. This may clearly support the insect age place an important role in influencing the susceptibility to pesticide and plant extracts (Umavathi and Manimagalai, 2010). The 100%mortality might be due to the chemical constituent present in the ethyl acetate extract of *N. oleander* and *G. sepium* that arrest the metabolic activity of larvae which cause high percentage of mortality. Studies involving the plant constituents indicate that much of their effects are due to their growth regulating properties rather than their direct toxicity (Moore *et al*., 2003). Botanicals have

widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases.

Fig. 1. Larvicidal and pupicidal effect of ethyl acetate extract *of N. oleander* against the dengue vector *A.aegypti*.

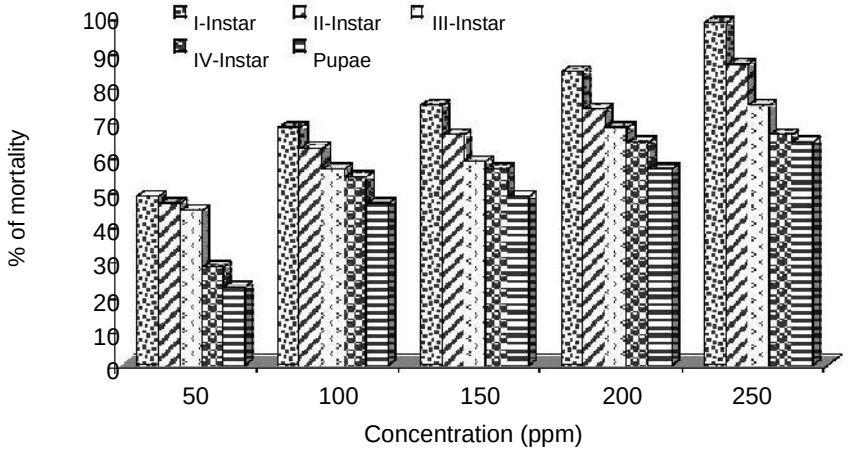

Fig. 2. Larvicidal and pupicidal effect of ethyl acetate extract *of G. sepium* against the dengue vector *A.aegypti*.

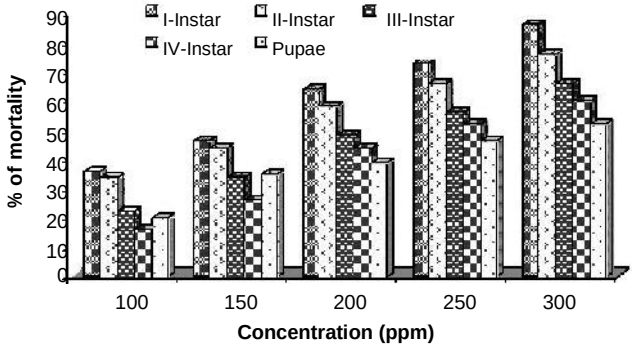

Table. 1. The LC_{50} values and their 95% Fiducial (upper and lower) limits regression equation and chi-square(x^2) value of the leaf extract of *N. oleander* for different stages of *A. aegypti*

Larval and pupal stages	LC_{50}(PPM) (LCL-UCL)	LC_{90}(PPM) (LCL-UCL)	Regression equation	Chi square
I-Instar	59.117 (40.662-104.529)	204.662 (156.731-389.131)	Y=-0.521+0.009X	9.879
II-Instar	62.512 (16.433-90.219)	306.924 (262.035-390.403)	Y=-0.328+0.005X	1.974
III-Instar	81.066 (19.963-113.717)	357.203 (286.866-409.555)	Y=-0.309+0.004X	0.704
IV-Instar	128.989 (112.311-225.609)	417.197 (279.332-481.614)	Y=-0.574+0.004X	7.609
Pupa	165.423 (104.287-257.183)	426.738 (304.660-507.633)	Y=-0.081+0.005X	5.518

Table.2. The LC_{50} values and their 95% Fiducial (upper and lower) limits regression equation and chi-square(x^2) value of the leaf extract of *Gliricidia sepium* for different stages of *Aedes aegypti*

Larval and pupal stages	LC_{50}(PPM) (LCL-UCL)	LC_{90}(PPM) (LCL-UCL)	Regression equation	Chi square
I-Instar	115.719 (134.258 -172.723)	335.324 (303.340-384.411)	Y=1.111+0.007X	0.734
II-Instar	172.919 (148.157-193.179)	401.464 (351.911-489.157)	Y=0.970+0.006X	0.215
III-Instar	222.588 (203.120-245.442)	442.260 (336.878-538.964)	Y=1.299+0.006X	0.694
IV-Instar	246.357 (227.229-271.565)	450.086 (396.223-541.442)	Y=1.550+0.007X	2.066
Pupa	276.824 (245.738-332.987)	579.560 (472.833-817.317)	Y=1.172+0.004X	0.990

One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides to botanical origin as a simple and sustainable method of mosquito control. In the present investigation the ethyl acetate extract of *N. oleander* showed the presence of secondary metabolites such as alkaloid, flavonoids, steroid, tannin, chlorogenic acid and phenolic compounds. *G.sepium* ethyl acetate leaf extract showed the presence of alkaloids, glycosider, steroids and carbohydrate (Table.3). The biological activity of this *N. oleander* and *G.sepium* extracts might be due to the presence of compounds existing in the plant. These compounds may jointly or independently contribute to produce larvicidal activity against the developmental stages of *A.aegypti*.

Table. 3. Qualitative analysis of the phytochemicals in acetone extract *N. oleander* and *G.sepium.*

S. No	Phytochemical constituents	Name of the Test	*N. oleander*	*G. sepium*
1	**Alkaloid**	Mayer's test	+	+
		Dragendroff's test	+	+
		Wagner Test	+	+
2	**Carbohydrate**	Molish Test	+	-
		Fehling Test	-	-
		Benedicts Test	-	-
3	**Flavonoids**	Ammonia test	+	+
4	**Saponin**	Foam Test	-	-
5	**Coumarin**	Sodium chloride test	-	-
6	**Steroids**	Libermann's test	+	+
		Salkowaski test	+	+
7	**Tannin**	Ferric chloride test	+	-
8	**Chlorogenic acid**	Ammonia test	-	-
9	**Anthocyanin**	H_2So_4 test	-	-
10	**Phenol**	Phenol reagent	+	-
11	**Flavones**	Shinoda's Test	+	+
12	**Anthracene Glycoside**	Borntrager's test	-	+

+ Presence of compounds - Absence of compound

Rawani *et al*.,(2013) reported the larvicidal activity due to the presence of saponins, steroid, terpenoid, flavonoids, alkanoid, essential oils, phenolics compounds and amino acids, and steroidal glycosides were present in the chloroform:methonal (v/v) extracts of fresh, mature and green berries of *Solanum nigrum*. Shaalen *et al.,* (2005) reviewed the current state of knowledge on larvicidal plant species extraction processes, growth and reproduction inhibiting phytochemicals, botanical ovicides, synergistic, additive and antagonistic joint action effects of mixtures, residual capacity, effect on non-target organisms, resistance and screening methodologies and discussed some promising advances made in phytochemical research. They are also proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential.

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