



MOSQUITOES LARVICIDAL ACTIVITY OF LEAF EXTRACT OF NEEM (*Azadirachta indica*)

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ABSTRACT

The larvicidal activity of Mosquito and phytochemical screening of Methanol extract of leaves belongs to the family Meliaceae have been evaluated in the present study. Larvicidal effect on 3rd and 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* to test concentrations of 50,100,150,200mg of Methanol and Ethanol extract of leaf of *Azadirachta indica* in 100ml of water.200mg concentration shows maximum mortality of the larvae when compared 150 (70%),100(50%) and 50(40%) concentration were observed. Presence of Caproic Acid,4-butoxy butanol, Oleic acid, Decanoic acid,8 methyl, methyl ester,N-methyl-N-N-di(2-(4-Pyridyl)ethyl)-(2-pyridyl)ethylamine,6(E), 9 (Z), 13(E),-Penedectriene, Phytol, Cis, Cis, Cis-7,10,13-Hexadecatrienal were found in GC/MS analysis of leaf extract.

KEYWORDS: *Azadirachta indica*, *Aedes aegypti*, *Culex quinquefasciatus*, Methanol extract.

INTRODUCTION

India encouraged scientific investigations of Neem tree as the part of his program to revitalize Indian tradition and also increase commercial interest on Neem (Stix, 1992) and presently some authors believe that no other plant or tree in the world has been so extensively researched or used, in all possible capacities so far, In Africa extracts from Neem leaves have provided various medicinal preparations.(Ekanem, 1971 and Udeinya, 1993). The medicinal properties of the plant *Azadirachta indica* were studied by several workers. They were Anti tumour effect (Fujiwara *et al.*, 1982), anti ulcer effect (Pillai and Santhakumari, 1984), Antidiabetic effect (Sonia Bajaj and Srinivasan, 1999). Anti microbial effect (Wafaa A., *et al.*, 2007). Anti fertility potential of Neem flower extract (Gbotolorun *et al.*, 2008). Mosquitoes transmit serious human diseases like malaria, filariasis, Japanese encephalitis, dengue haemorrhagic fever and yellow fever causing millions of deaths every year (Mittal, P.K., Subbarao, S.K., 2003).The widespread use of synthetic insecticides for the control of pests as well as human disease vectors has led to concerns about their toxicity and environmental impact (Bounias, 2003). Neem has been used as insecticides even before the advent of synthetic organic insecticides (Casida and Quistad, 1998). The purpose of the present study was to investigate the effects of alcoholic Neem leaf extracts adverse mosquitoes larvae of *Aedes aegypti* and *Culex quinquefasciatus*.

MATERIALS AND METHODS

Plant materials

The plant Neem (*Azadirachta indica*) was selected for study. Its leaves were collected from DKM college Garden in Vellore District, Tamil nadu. The collected leaves were identified and authenticated by

Prof. Dr. B. Annadurai, Research Co ordinator. Dept.of Plant biology and Biotechnology, CAH college, Melvisharam, Tamil Nadu, India.

Neem leaf extract

The leaves of the plant material were shade dried (28±2°C), ground and sieved to get fine powder from which the extracts were prepared. Methanol extracts of the plant was obtained by taking 50g of dried leaf and flower in a separate container. With this 250 ml of methanol was added and kept for 24hrs with periodic shaking, then filtered and the filtrate was collected. This procedure was repeated three times with fresh volume of methanol. The filtrates were pooled. Ethanol extract of the plant material was also prepared in a similar manner with that of methanol.

The pooled ethanol and methanol extract were concentrated separately by rotary vaccum evaporator at 40°C and evaporated to dryness and stored at 4°C in an air tight bottle. (Jang, Y.S., *et al.*, 2002)

Mosquito species

Eggs of *Aedes aegypti* and larvae of *Culex quinquefasciatus* were selected for the investigation. Eggs of *Aegypti* and larvae of *Culex quinquefasciatus* were collected in around Vellore.

Larvicidal Assay

In the larvidal assay, third and fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* were exposed to test concentrations of 50, 100,150,200 mg of methanol and ethanol extracts of leaf of *Azadirachta indica* in 100ml of water.

100ml of tap water was taken in series of 250ml glass beakers. The measured amount of extracts was dissolved in 1ml of the solvent which was used for preparing the extracts. The dissolved plant extract was added to the

Effect of Neem on Mosquito larvicidal activity

water in the beakers. A control was also maintained by adding 1ml of solvent to 100ml water. 25 larvae per concentration were used for all larvae experiment. The larvae were fed dry yeast powder on the surface (50mg/l) (Senthilnathan, S. 2007). The number of dead larvae at the end of 24hrs was recorded and the mortality percentage values were calculated. This experiment was repeated three times.

The Phytochemical screening of plant extract for Saponin, Flavonoids, Terpenoids, Tannin, cardiac glycosides and steroids were also carried out.

RESULTS

The results showed in the analysis presented in the graphs as shown in the graph-1, that Methanol extract of *A. indica* against *Aedes aegypti*. It showed maximum activity on 200 concentration when compared to 150, 100 and 50 percent

concentration in mgs. In this it is also observed that in 200 mg concentration 90 percent of Mortality rate of *Aedes aegypti* when compared to 150, 100 and 50% like 70, 50 and 40% respectively. In figure -2, extract of *A. indica* against *Aedes aegypti*. It showed maximum activity on 200 concentrations when compared to 150, 100 and 50 percent concentration in mgs. In this it is observed in 200 mg concentration 90 percent of Mortality rate of *Aedes aegypti* when compared to 150, 100 and 50% like 70, 50 and 40% respectively. Ethanol extracts of *A. indica* against *Culex quinquefasciatus*. Here it showed on 200 concentration maximum activity when compared to 150, 100 and 50 percent concentration in mgs. In this it is observed in 200 mg concentration 90 percent of Mortality rate of *Aedes aegypti* when compared to 150,100 and 50% like 70, 55 and 35% respectively

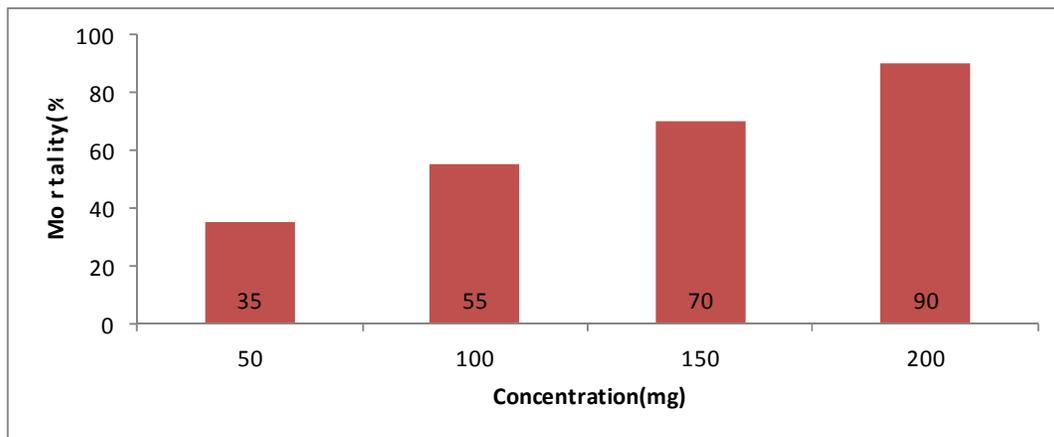


FIGURE 1. shows the Methanol extract of *A.indica* against *Aedes aegypti*. Here it shows 200 concentration shows maximum activity when compared to 150,100 and 50 percent concentration in mgs. In this it is observed in 200 mg concentration 90 percent of Mortality rate of *Aedes aegypti* when compared to 150,100 and 50% like 70,55 and 35% respectively.

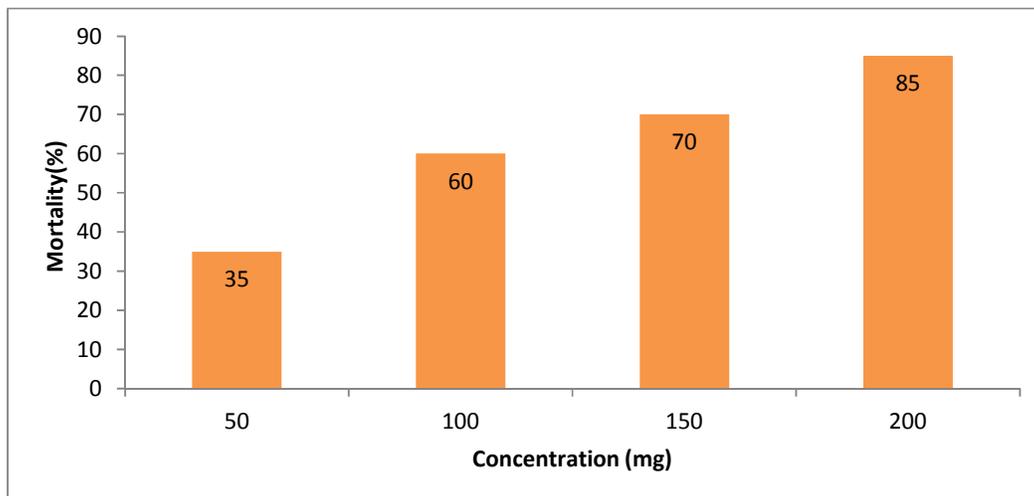


FIGURE 2. Portrays the Ethanol extract of *A.indica* against *Aedes aegypti*. Here it shows 200 concentration shows maximum activity when compared to 150,100 and 50 percent concentration in mgs. In this it is observed in 200 mg concentration 85 percent of Mortality rate of *Aedes aegypti* when compared to 150,100 and 50% like 70,60 and 35% respectively

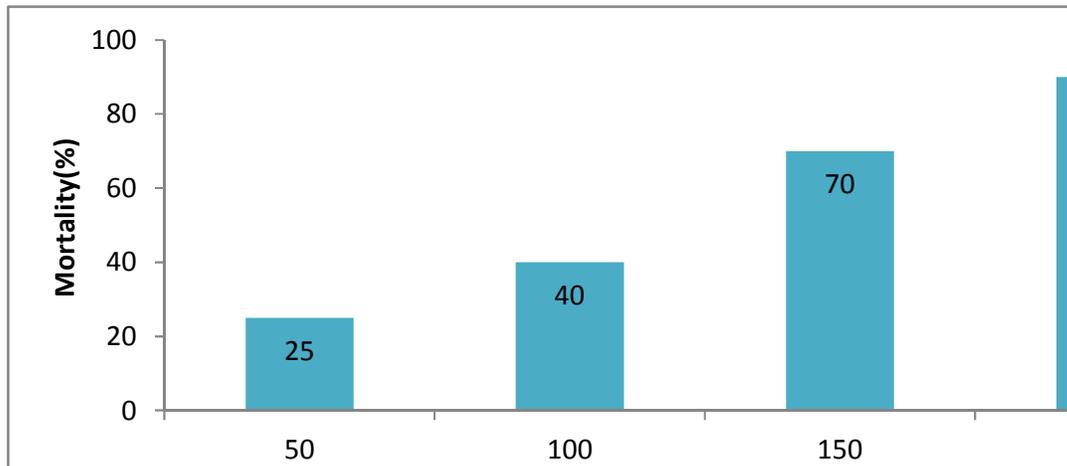


FIGURE 3. explains the Methanol extract of *A.indica* against *Culex quinque ficiatus*. Here it shows 200 concentration shows maximum activity when compared to 150,100 and 50 percent concentration in mgs.In this it is observed in 200 mg concentration 90 percent of Mortality rate of *Culex quinque ficiatus* when compared to 150,100 and 50% like 70,40 and 25% respectively

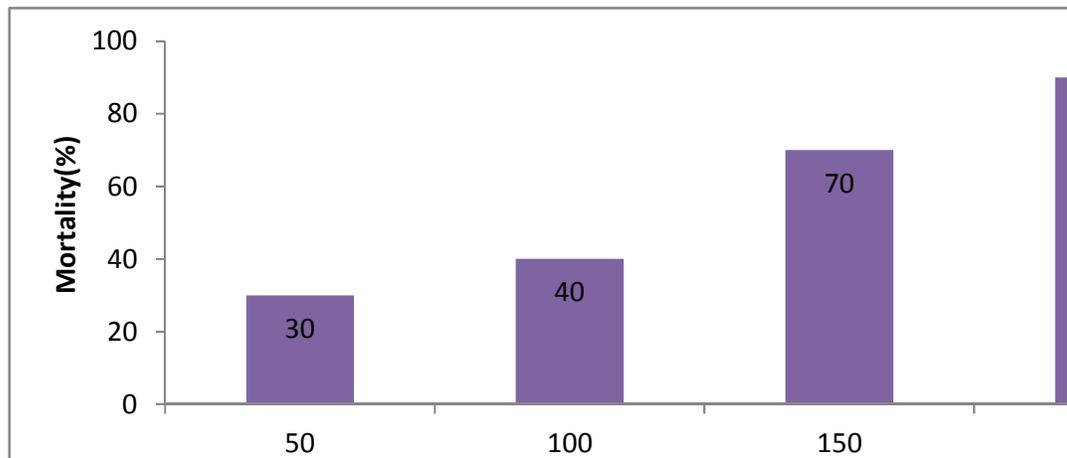


FIGURE 4. explains the Ethanol extract of *A.indica* against *Culex quine queficiatus*. Here it shows 200 concentration shows maximum activity when compared to 150,100 and 50 percent concentration in mgs. In this it is observed in 200 mg concentration 90 percent of Mortality rate of *Aedes aegypti* when compared to 150,100 and 50% like 70,40 and 30% respectively

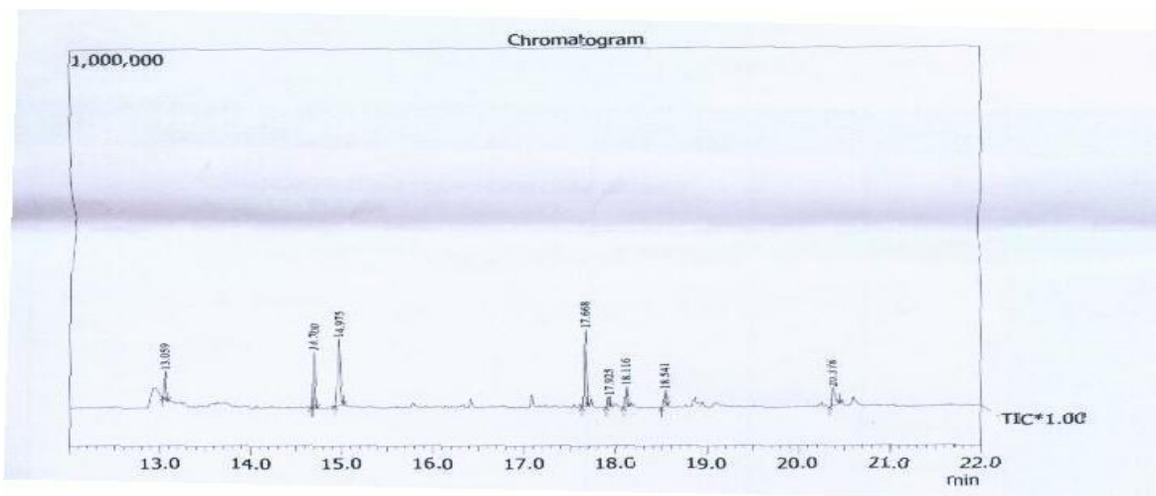


FIGURE 5. GC/MS Chromotogram of Metahanol leaf extract of *Azadirachta Indica*.

Gc/Ms Analysis

Gc/Ms Analysis of methanol leaf extract was determined by GC-MS. Shimadzu Model QP-2010 Mass spectrometer under the following conditions: DB-Polyethylene Glycol coated fused silica capillary column(30m length × 0.25mm ID×0.25µm film thickness): Helium Carrier Gas (1.34ml/minutes). 250°C injector temperature; 240°C interface temperature. 200°C Ion Source Temperature.

Column temperature programmed at 60°C with 10°C/minutes to rise to 230°C. For Gc/Ms detection ionization energy of 70ev was used. 50mg of Methanol leaf extract sample were taken and made up to 10 ml with methanol, from which 1µl of sample was injected (split mode) in the column. The components were identified based on NIST Library/Wiley Library. (M. Sathish kumar and S. Maneemagalai, 2008)

TABLE 1. Peak Report Total ion chromatogram of Methanol Leaf extract of *Azadirachta indica* by GC/MS

| Peak | Retention Time | Name |
|------|----------------|---|
| 01 | 13.059 | Caproic Acid |
| 02 | 14.700 | 4-butoxy butanol |
| 03 | 14.975 | Oleic Acid |
| 04 | 17.668 | Decanoic acid, 8 – methyl, methyl ester |
| 05 | 17.925 | N - methyl - N, N - di [2-(4-Pyridyl) ethyl] - (2-pyridyl) ethylamine |
| 06 | 18.116 | 6(E), 9(Z), 13(E), - Penedectriene |
| 07 | 18.541 | Phytol |
| 08 | 20.378 | Cis, Cis, Cis-7, 10, 13 - Hexadecatrienal |

the methanol extract of Leaf Presence of Caproic Acid,4-butoxy butanol, Oleic acid, Decanoic acid,8 methyl, methyl ester,N-methyl-N-N-di(2-(4-Pyridyl)ethyl)-(2-pyridyl) ethylamine, 6(E), 9(Z),13(E),-Penedectriene, Phytol, Cis, Cis, Cis-7,10,13-Hexadecatrienal were found in GC/MS analysis of leaf extract of *Azadirachta indica* were shown in Table.

DISCUSSION

The advent of Neem leaf extracts to mosquito larvae under laboratory conditions were studied. Raymond Demba Ndional *et al.* (2007) reported treatment and comparative analysis of the properties of aqueous extracts of seed kernel of *Azadirachta indica* A. Juss (Neem) was carried out on *Aedes aegypti* larvae.

Umar *et al* (2006) showed the ability of some neem extracts to kill *Aedes* pupae at relatively low concentrations present an alternative to the use of synthetic pesticides for control of mosquitoes. This technique is environmental friendly, biodegradable, less expensive, and locally available in mosquito endemic area. Potentials for adoption in mosquito management program cannot be overemphasized.

Leaves extract of *A. indica* and *D. melei* can be suggested as a natural larvicidal for controlling mosquitoes in India. Since, the both plants are economically safe and less expensive to control mosquito and the histological studies to be performed to recognize the mode of action between leaf extract and mosquito .(V.M. Chakkaravarthy *et al* 2011).

Gunasekaran *et al.*, (2009) suggested Neem Azal T/S 1.2 percent EC could be a promising candidate for the use in integrated vector management programme and replace chemical insecticides. The effect of Azadirachtin, a neem tree *Azadirachta indica* extract, against larvae and pupae of culex pipens mosquito in east of the Republic of Algeria.(Abdelouahab Alouani *et al.*, 2009). The Neem oil formulation was found effective in controlling mosquito larvae in different breeding sites under natural field conditions (Virendra K Dua *et al.*, 2009).

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