

Mosquito Larvicidal Activity of Leaf Extract of *Azadirachta Indica* Against Dengue Vector, *Aedes aegypti*

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Abstract: The present study was conducted to find out the larvicidal effect of *Azadirachta indica* against 3rd and 4th instar larvae of *Aedes aegypti*. The larvicidal activity of crude methanol extract of the bark was assayed for the toxicity against mosquito larvae. Different concentration 25, 50, 100 and 300 ppm was prepared from the stock solution. The larvicidal mortality was observed after every 12 hours of exposures up to 48 hours. The lower mortality rate was found in lower concentrations. The highest larval mortality was found in methanol extract at 200 and 300 ppm. The result of this study shows that crude methanol extract of the bark be considered as a potent of mosquito larvicidal agent for *Aedes aegypti*,

Keywords: Mosquitoes, *Azadirachta indica*, mortality, larvicidal, *Aedes aegypti*

1. Introduction

Mosquitoes are the most important insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, yellow fever, and Japanese encephalitis, etc. these tiny assassins have the potential and lethal capacity to kill more than a million victims a year around the world (Vatandoost and Vaziri, 2000). Malaria alone kills three million people annually; including one child every 30 sec (Malaria fact sheet no 94). Pesticide exposure among humans has been linked to immune dysfunction, various forms of cancer and birth defects (Bounias,2003). Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Due to environmental concern on use of synthetic insecticides for vector control and due to existing and further risk of development of widespread insecticides resistance in disease vectors, interest on possible use of environment friendly natural products such as extracts of plant/plant parts increased for vector control. Sukumar *et al.* (1991) listed 346 species from 276 genera and 99 families which have been tested against mosquitoes for various effects such as toxicity, growth inhibition, ovipositional deterrence and repellency. Neem has been used as insecticides even before the advent of synthetic organic insecticides (Casida and Quistad, 1998). Azadirachtin, the active ingredient of neem has long been recognized for its mosquito larvicidal capability. Plant products have been used traditionally by human communities (Jacobson,1958). The plant world comprises a rich store house of phyto-chemicals, which are widely used in the place of synthetic insecticides since continuous use of synthetic insecticides cause side effects to non-target organisms and insecticide resistance against mosquitoes (Kelm *et al.*, 1997).

2. Materials and Methods

The healthy and matured bark of *Azadirachta indica* was collected from adjoining area of D.A.V.(P.G.) College

Dehradun, shade dried at room temperature and ground, sieved to get fine powder from which the extracts were prepared.

For the extraction, methanol was added and kept for 72 hours with periodic shaking, then filtered and the filtrate was collected. The pooled methanol extract was concentrated separately by rotary vacuum evaporator at 40^o C and evaporated to dryness and stored at 4^o C in an air tight bottle (Jang *et al.*, 2002).

For preparation of stock solution, one gram of the concentrated extract was dissolved in 100 ml methanol and kept as stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae.

Collection of Mosquitoes

The important Dengue vector species *Aedes aegypti* (say) was selected for this study. As *Aedes aegypti* is the vector of Dengue Virus which causes Dengue and Dengue Haemorrhagic Fever (DHF) and affect the blood and lymph vessels, resulting in platelet depletion, bleeding, pain behind the eyes, severe headache, joint pain, pain in muscles etc. and finally death

Aedes aegypti larvae were collected from stagnant sewage water. With the help of dipper. The collected larvae were reared from egg to larval stage and then to adults in the laboratory, to avoid the species mixture. From these adults, next F1 generation larvae were used in this study. This procedure facilitates to maintain the uniform age of larval instar (fourth instar). The larvae were fed with dry yeast powder on the water surface (50mg/l) (Senthilnathan, 2007)

BIO-ASSAY

The larvicidal activity was assessed by the standard guidelines of WHO (2005), with some modification and as per the method of Rahuman *et al.*, (2000), Prabakar and Jebanesan (2004). A laboratory colony of *Aedes aegypti* was used for the larvicidal activity studies. For bioassay test,

twenty numbers of fourth instars larvae of *Aedes aegypti* were taken in six batches of twenty each for the treatment. Bowls of 100 ml capacity were kept in series, and tested for the desired plant extract concentrations 25, 50, 100, 200 and 300 ppm. The control was set up with methanol and distilled water. The experimental media, in which 100% mortality rate of larvae occurred were selected for a dose response bioassay. The number of dead larvae was counted after every 12 hrs of treatment up to 48 hours. The percentage mortality has been calculated for all the results obtained by this study.

Table 1: Mortality rates of *Aedes aegypti* mosquito larvae at different concentrations of *Azadirachta indica* bark

Concentration (ppm)	Time (Hours)				N=20	
	12	24	36	48	Total	Percentage
Control	0	0	0	0	0	0
25	2	5	10	12	12	60
50	4	10	13	16	16	80
100	5	13	16	18	18	90
200	7	15	18	20	20	100
300	10	20	-	-	20	100

3. Results and Discussion

The Larvicidal activity of *Azadirachta indica* bark extract at different concentrations presented in Table: 1. the data shows that, maximum mortality rate 100 % was observed at 300 ppm after 24 hours, at 200 ppm after 48 hrs of the treatment. The efficacy of extract was reduced with decreasing concentrations of 100, 50 and 25 ppm by 90, 80 and 60 % respectively after 48 hrs of the treatment.

The most commonly studied plant for control of mosquito is *Azadirachta indica* which is commonly known as Neem in India. Neem contains at least 35 biologically active principles of which azadirachtin, a tritetraploid is the predominant active insecticidal ingredient in the seeds, leaves and other parts of the tree (Mulla and Su, 1999), Alouani et al., (2009) and Bhagavan and Rahuman, (2011) obtain similar result. Umar et al. (2006) showed the ability of some neem extracts to kill *Aedes papae* 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.

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