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Larvicidal Activity of Medicinal Plant against *Aedes aegypti* Mosquito Larvae to Control the Outbreak of Dengue

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Abstract: ***Phytochemical origin may have the potential to be used successfully as larvicides. The larvicidal activity of crude methanol extract of the bark of Azadirachta indica was assayed for the toxicity against the early fourth instar larvae of Aedes aegypti. Different concentrations 25, 50, 100, 200, 300 ppm was prepared from the stock solution. The larvicidal mortality was observed after every 12 hours of exposure, up to 48 hours. The extract showed larvicidal effects, and lower mortality rate was found in lower concentrations. However, the highest larval mortality was found in Azadirachta indica bark methanol extract at 200 and 300 ppm. The result of this study shows that crude methanol extract of the bark of Azadirachta indicamay be considered as a potent of mosquito larvicidal agent for Aedes aegypti, the carrier of DHV. The medicinal plant was easilybiodegradable than the synthetic insecticides, the products are less hazardous.***

Keywords: *Azadirachta indica,* Bark, Methanol, Larvicidal Activity, *Aedes aegypti*, Dengue Haemorrhagic Fever

1. Introduction

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, yellow fever, and Japanese encephalitis, etc. While most people consider mosquitoes as annoyance, these tiny assassins have the potential and lethal capacity to kill more than a million victims a year around the world [1]. Malaria alone kills three million people annually; including one child every 30 sec [2, 3]. The radical approach to combat mosquitoes today has been identified as most effective way of controlling mosquito borne diseases. Most of the insecticides available in the markets are synthetic chemical products which apart from their prohibitively high costs, their persistent applications have unintended implication including the production of resistant strains of mosquitoes, ecological imbalance and elimination of non-target organisms in the environment [4]. Pesticide exposure among humans has been linked to immune dysfunction, various forms of cancer and birth defects [5, 6]. 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.* [7] listed 346 species from 276 genera and 99 families which have been tested against mosquitoes for various effects such as toxicity, growth inhibition, ovipositional deterrency and repellency. Neem has been used as insecticides even before the advent of synthetic organic insecticides [8]. Azadiractin, the active ingredient of neem has long been recognized for its mosquito larvicidal capability. Plant products have been used traditionally by human communities [9]. The plant world comprises arich store house of phytochemicals, 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 [10].

2. Materials and Methods

2.1 Plant collection

The healthy and matured bark of *Azadirachta indica* was collected, shade dried at room temperature and ground, sieved to get fine powder from which the extracts were prepared.

2.2 Extraction

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 were concentrated separately by rotary vaccum evaporator at 40^0 C and evaporated to dryness and stored at 4^0 C in an air tight bottle [11].

2.3 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.

2.4 Mosquito species

The important vector species of mosquitoes is *Aedes aegypti* (say) is selected for this study. *Aedes aegypti* is the vector of Dengue Virus which causes Dengue Haemorrhagic Fever (DHF) which is known to 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 [12].

Aedes aegypti larvae were collected from stagnant sewage water of West Bengal. The collected larvae were reared from egg to larval stage and then to adults in the laboratory itself, to avoid the species mixture. From these adults, next F1 generation larvae were used for the present 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/1) [13].

2.5 BIO-ASSAY

The larvicidal activity was assessed by the standard guidelines of WHO (2005) [14] , with some modification and as per the method of Rahuman *et al*., (2000) [15] , Prabakar and Jebanesan (2004) [16] . A laboratory colony of *Aedes aegypti* was used for the larvicidal activity studies. For bioassay test, twenty numbers of fourth instars larvae 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	6	9	12	12	60
50	4	10	13	16	16	80
100	5	13	16	18	18	90
200	6	15	18	20	20	100
300	10	20	-	-	20	100

Figure 1: Azadirachta indica bark.

3. Results and Discussion

The Larvicidal activity of *Azadirachta indica* bark extract at different concentrations is 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 triterpenoid is the predominant active insecticidal ingredient in the seeds, leaves and other parts of the tree [17] . (Mulla and Su, 1999). Umar *et al.* (2006) [18] 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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