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RESEARCH ARTICLE

LARVICIDAL ACTIVITY OF *VITEX NEGUNDO* LEAF EXTRACT AGAINST *CULEX QUINQUEFASCIATUS* MOSQUITO LARVAE

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ABSTRACT

The present study was carried out to establish the properties of leaf extract of *Vitex negundo* was tested for larvicidal activity against early 4th instar larvae of *Culex quinquefasciatus* mosquito using standard WHO technique. The mortality rate was observed at 5 to 200 ppm, after 12, 24, 36 and 48 hrs of the treatment. And no mortality rate was observed in control. The methanolic extract of *V. negundo* was found effective and 100% result was observed in this treatment. This plant extracts are easy to prepare, inexpensive, and safe for mosquito control which might be used directly as larvicidal agents in small volume aquatic habitats or breeding sites of around human dwellings.

INTRODUCTION

Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability. Many studies on plant extracts against mosquito larvae have been conducted around the world. Many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Sharma *et al*., 2006; Amer and Mehlhorn 2006). Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis, and Japanese encephalitis (James 1992; Gubler 1998). Mosquitoes also cause allergic responses that include local skin and systemic reactions such as angioedema in humans (Peng *et al.,* 1999). *Culex quinquefasciatus*, a vector of lymphatic filariasis, is a widely distributed tropical disease with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard *et al.,* 2003). The yellow fever mosquitoes, Aedes aegypti, are responsible for dengue fever in India where the number of dengue fever cases has increased significantly in recent years. Dengue viruses occur as four antigenically related but distinct serotypes, which cause a broad range of disease, including clinically asymptomatic forms, classic dengue fever, and the more severe forms such as dengue hemorrhagic fever–dengue shock syndrome (Fundacao Nacional de Sau de 2002). Dengue is present in more than 100 countries and threatens the health of approximately 2.5 billion people. Worldwide, around 80 million people are infected annually at an attack rate of 4% (Monath 1994).

Control of the mosquito larvae is frequently dependent on continued applications of organophosphates and insect growth regulators (Yang *et al*., 2002). An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. It has also provoked undesirable effects, including toxicity to nontarget organisms, and fostered environmental and human health concerns (Lee *et al*., 2001). The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides (Macedo *et al*., 1997) and for more detailed studies of naturally occurring insecticides (Ansari *et al*., 2000).

MATERIALS AND METHODS

Plant collection and extraction

Matured fresh leaves of *Vitex negundo* were collected from Adilabad district, Telangana state, India. The plant material was washed and shade dried at room temperature. The dried material was powdered and extracted with methanol for a period of 72 hrs and filtered with whatman 1 filter paper. The extracts were concentrated at reduced temperature on a rotary evaporator and stored at a temperature of 4 ^{o} C.

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Preparation of stock solution

One gram of crude extract was first dissolved in100 ml of methanol and stored as stock solution. The anthelmintic assay was carried as per the method of Tandon *et al*. (1997). This stock solution was used to prepare the desired concentrations of the extract for the larvicidal activity on the mosquito larvae. From the stock solution 5, 10, 25, 50, 100 and 200 ppm concentrations were prepared with dechlorinated tap water. The control was set up with 100 ml tap water by adding 2ml of methanol.

Mosquito culture

Culex quinquefasciatus larvae were collected from stagnant sewage water of the River Musi in Hyderabad. The identification of mosquito larvae was done by Dr. B. Redya Naik, Entomologist, Department of Zoology, Osmania University, Hyderabad, Telangana state. 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 stage (fourth instar).

Larvicidal Bioassay

Larvicidal activity was evaluated using WHO method (2005) with slight modification. For bioassay test, twenty numbers of early 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 each desired plant extract concentrations 5, 10, 25, 50, 100 and 200 ppm. The control was set up with 2 ml methanol and distilled water. The experimental media, in which 100% mortality rate of larvae occurred were selected for a dose response bioassay. Based on the screening results, crude methanol solvent extracts of leaf extracts of the plant is subjected to dose response bioassay for larvicidal activity against the larvae of *Cx. quinquefasciatus*. The numbers of dead larvae were counted after every 12 hrs of treatment up to 48 hours. The percentage mortality and standard error of mean have been calculated for all the results obtained by this study.

RESULTS AND DISCUSSION

Table 1. The larvicidal activity of *Vitex negundo* leaf crude extract at different concentration is represented in Table 1. The data shows that, 100 % mortality rate of mosquito larvae was observed at 25, 50, 100 and 200 ppm of concentrations. And at the concentration of 200 ppm 100 % mortality rate was recorded within 12 hours of the treatment, for 100 ppm it is around 24 hrs, for 50 ppm after 36 hours, and for 25 ppm it was recorded after 48 hrs of the treatment. The efficacy of crude extract on the mosquito larvae showed lesser activity when the concentrations of the same was decreased to 10 and 5 ppm which showed 75 and 45 % mortality rate respectively within 48 hours of the treatment. The control did not show any mortality the mean and standard error also showed in Table 1. Krishnan *et al*. (2007) reported 50 % mortality rate for the methanolic extract of *Vitex negundo* leaves at the concentration of 41 ppm and for *V. trifolia* leaves at the concentration of 212 ppm. As the earlier results are in support of the present study the *V. negundo* leaf extract may be considered as the potential control against mosquito larvae which is eco-friendly in nature.

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Table 1. Mortality rates of *Culex quinquefasciatus* mosquito larvae at different concentrations of crude extract of *Vitex negundo* leaves

Conce.	Time (hours)				*n = 20*			
	12	24	36	48	Total	%	Mean	SE (+/-)
5	3	5	7	9	9	45	2.25	1.291
10	7	10	12	15	15	75	3.75	1.683
25	10	14	18	20	20	100	5.00	2.217
50	14	17	20	-	20	100	6.66	1.436
100	18	20	-	-	20	100	10.00	0.500
200	20	-	-	-	20	100	20.00	0.000
Control	0	0	0	0	0	0	0.000	0.000

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