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Comparative efficacy of Azadirachtin on the larval population of *Culex quinquefasciatus, Anopheles stephensi, and Aedes aegypti* (Diptera: Culicidae) in Gujarat, India.

Corresponding Author

M.S.Shivakumar

[1]Department of Zoology, Faculty of Science, The M.S.University of Baroda, Vadodara - 390002, India.
[2]Molecular Entomology Lab, Department of Biotechnology, Periyar University, Salem -636011, Tamilnadu, India.

Co Authors

Ruchika Kataria

[1]Department of Zoology, Faculty of Science, The M.S.University of Baroda, Vadodara - 390002, India.

ABSTRACT

In the present study, the larvicidal action of Azadirachtin was tested on 4th Instar larvae of *Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti*. Graduated concentrations of Azadirachtin at 1, 3, 5, 10, 15 and 30 ppm were tested as per WHOPES protocol for larvicidal efficacy. Results indicate that *Culex* larvae require a higher concentration of 30ppm for 100% larval mortality whereas for *Aedes* it is 10ppm. In *Anopheles* 100% mortality was recorded at 3ppm. LC_{99} estimates suggest that *An.stephensi* is highly susceptible to the Azadirachtin as compared to *Cx.quinquefasciatus* and *Ae.aegypti*.

KEY WORDS

Botanical Insecticide; *Anopheles stephensi; Aedes aegypti; Culex qinquefasciatus;* Azadirachtin.

Running head: Efficacy of Azadirachtin formulation on larval mosquito population

INTRODUCTION

Vector Borne diseases remains the major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million people annually.[1] World Health Organization (WHO) has taken measures to counter malaria with the help of Insecticide Treated Nets and development of larvicidal compounds. With regular use of these conventional insecticides, mosquitoes have developed resistance.[2] In order to find more efficient and ecofriendly method of larval control, molecules from botanical origin are being tested for larvicidal activity. An alternative approach for mosquito control is the use of natural products of plant origin. Many plant based products is widely used for their insecticidal / repellent properties for control of mosquitoes or protection from mosquito bites. In recent years, interest in plant based products has been revived because of the development of cross resistance and possible toxicity hazards associated with synthetic insecticides and their rising cost.

A large number of plant based products have been reported to have mosquito larvicidal or repellant activity. Botanical insecticides offer a more natural and eco-friendly approach to pest control than do synthetic pyrethroids. Neem contains several active ingredients of which one is Azadirachtin. The neem plant and its derived products have shown a variety of insecticidal properties on a broad range of insect species. Neem products have been shown useful for malaria control and include antifeedant[3-4] , ovicidal activity, fecundity suppression, insect growth regulation and repellency.[5-6] Recent studies have shown that bioactivitiy of neem includes effects on vitellogenesis degeneration of follicle cells during oogenesis in mosquitoes.[7] Neem based products are relatively safe towards non target biota, with only minimal risk of direct adverse effects on aquatic macro invertebrate,resulting from contamination of water bodies with neem based insecticides. Neem products are less likely to induce resistance due to their multiple mode of action on insect.

Extensive work has been done on several aspects of the insecticidal action of neem based products on the mosquitoes. The study pertaining to the Vadodara city and its outer limits is not there and also the resistance levels of the mosquitoes are largely unknown. The current study is aimed at understanding the larvicidal efficacy of the emulsified formulation of Neem (Azadirachtin, EC 0.03%) on the fourth instar larvae of *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti*.

MATERIALS AND METHODS

Three different genera of mosquitoes (*Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus)* were used for the experiment. Stock culture of all the three mosquitoes was maintained in the insectary. For the bioassay the fourth instar larvae were taken.

Formulation of concentration: The present study is done by using botanical insecticide Niconeem (Azadirachtin, 0.03% EC), which is neem based product. Four concentrations of 3, 5, 10, 15 ppm and an untreated control were taken for the present study. For each concentration of the chemical three replicates were kept. For each replicate 10 larvae were taken.

Experimental setup: In the present study ten fourth instar mosquito larvae (*An.stephensi, Ae.aegypti or Cx.quinquefasciatus*) were released into the beaker have required concentration of chemical dissolved in water. The observations for larval mortality were taken at 1, 3 and 7th day after application of the chemical. Corrected mortality was calculated using Abbott's formula.[8] Data obtained from the study was subjected to Probit analysis for finding out LC_{99} estimates.

RESULTS

In *Cx.quinquefasciatus* 100% mortality was observed at 30ppm in 72hours after the treatment, whereas the lower concentrations of 10 and 15 ppm produced 70% mortality (Fig 1). A chronic exposure at 5ppm concentration delayed mortality in more than 60% is observed. In *Ae.aegypti* larvae at 10 and 15 ppm, 100% mortality was observed within 24 hours. At lower concentrations of 3 and 5 ppm more than 50% larval mortality was observed (Fig 2). In *An.stephensi* larvae, 10 and 15 ppm concentration produced 100% mortality within 72 hours after treatment in all the concentrations (Fig 3).

Figure 1
Percent mortality (± SEM) rates of 4th instar Culex quinquefasciatus larvae exposed to various concentrations of Azadirachtin (in ppm) after 1, 3 and 7 days of treatment.

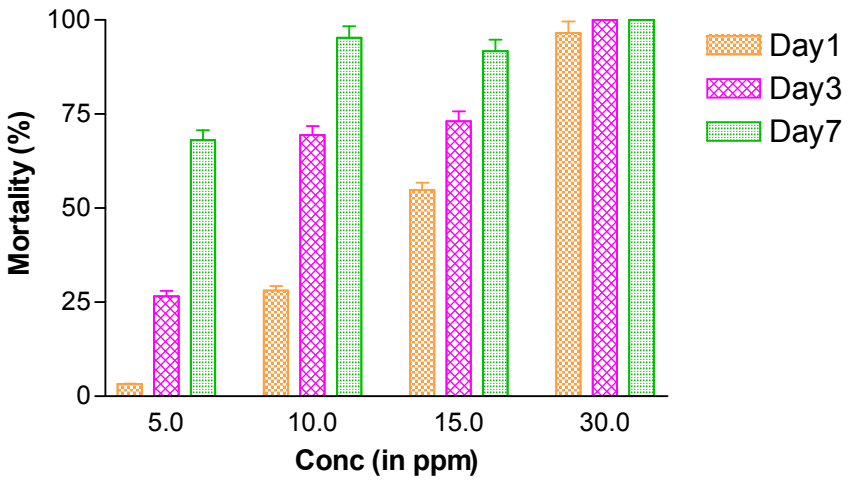


Figure 2
Percent mortality (± SEM) rates of 4th instar Aedes aegypti larvae exposed to various concentrations of Azadirachtin (in ppm) after 1, 3 and 7 days of treatment.

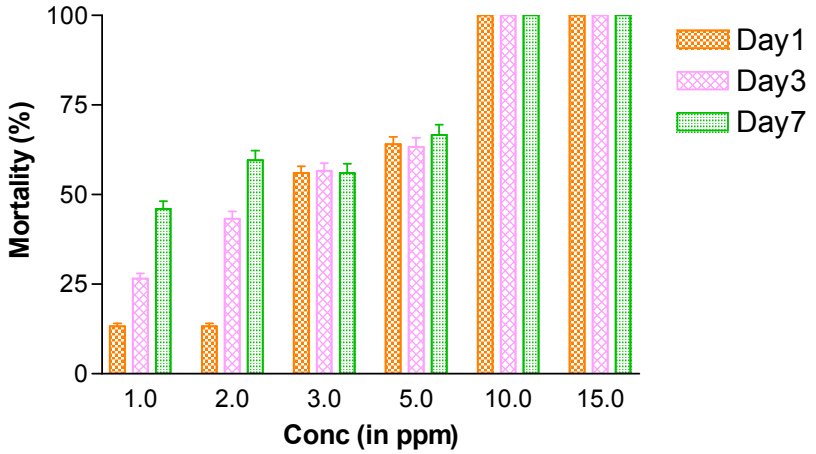
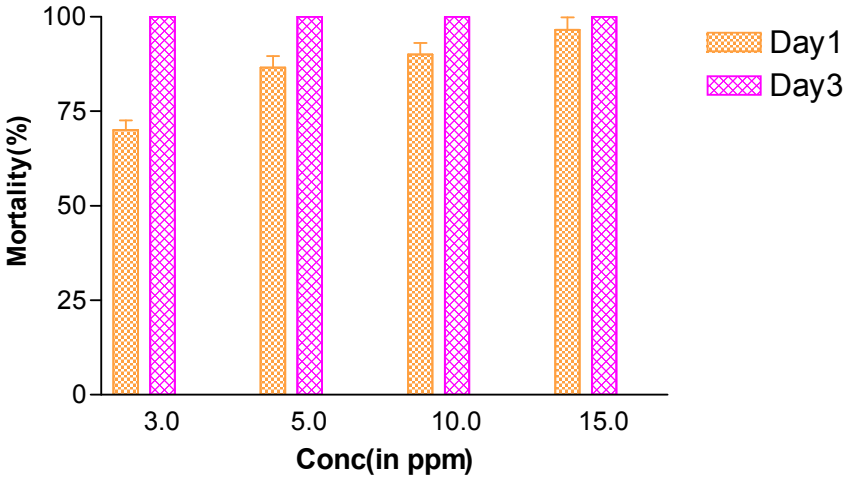


Figure 3
Percent mortality (± SEM) rates of 4th instar Anopheles stephensi larvae exposed to various concentrations of Azadirachtin (in ppm) after 1, 3 and 7 days of treatment.



Comparing the three species of mosquito larvae, *Cx.quinquefascitus* is able to resist a higher concentration of azadirachtin (LC_{99} – 28.33) as compared to *Ae.aegypti* (LC_{99} – 20.933) and *An.stephensi*. Among all the three species *An.stephensi* is highly susceptible (LC_{99} – 15ppm) to very low doses of azadirachtin (Table 1).

Table 1

Susceptibility of Mosquito larvae (An.stephensi, Ae.aegypti and Cx.quinquefasciatus to varying concentrations of Azadiracthin after 1, 3 and 7 DAT (days after treatment).

DAP	*Cx.quinquefasciatus*			*Ae.aegypti*			*An.stephensi*		
		Lower Limit	Upper Limit		Lower Limit	Upper Limit		Lower Limit	Upper Limit
	LC_{99}	(95% CI)		LC_{99}	(95% CI)		LC_{99}	(95% CI)	
1	28.883	24.478	36.907	20.900	14.828	50.747	15.942	10.464	49.441
3	23.716	19.479	32.963	20.900	14.828	50.747	12.104	8.433	27.802
7	17.740	13.560	34.757	20.900	14.828	50.747	11.922	7.332	18.556

DISCUSSION

Secondary metabolites from Neem tree have insecticidal, antifeedant activity [9] on a variety of pests of agricultural and medical importance.[10-11] The active principles in the neem are azadirachtin and nimbin which have insecticidal property. [12] Azadirachtin, a complex tetranotriterpenoid luminoid from the neem seeds, is the main component responsible for the toxic effects in insects.[13] considering the importance of botanicals in the management of insect vectors of diseases; neem formulation was tested for larvicidal activity. An advantage of using botanicals as mosquito larvicide is due to its photodegradative property[12,7] as compared to chemical insecticides which causes toxicity in non-target organisms and also causes environmental contamination.

Comparing the larvicidal efficacy, *Anopheles* shows a high susceptibility to very low concentrations of *Azadirachta indica* (1ppm - 5ppm), whereas in the case of *Aedes* the diagnostic concentration is slightly higher at 10ppm for larva. For *Culex* the diagnostic concentration for larvae is 30ppm. Of the three mosquito genera *Culex* larvae have a higher tolerance towards the Neem as compared to the other two genera. The possible explanation to the above phenomenon is that, *Culex* is the dominant mosquito fauna found in the urban habitats and peripheral limits of the city. Being found in high densities, Culex adults and larvae are exposed to a variety of mosquitocidal chemicals which can results in strong selection pressure leading to the development of resistance among them.

Studies on larvicidal action of Neem formulation conducted in *An. gambiae* in Africa has shown that 32 ppm is the diagnostic concentration for the larvae to be killed within 7days of treatment.[14] The LC_{50} value of 10.68ppm produces larval mortality in *An.gambiae*. However in our present study pertaining to the larvicidal action on *Anopheles stephensi*, at a concentration of 3ppm resulted in 100% mortality within 72 hours and a concentration of 15 ppm produces 90% mortality within 24 hours of the treatment. The possible explanation for this for this variation among anopheles species in two areas is due to the quantum of chemicals used in Africa is higher as compared to India which confers insecticidal cross resistance. Su and Mulla (1998)[9] reported that a

concentration of 5-10ppm of Neem based compound Neemix (EC 4.5 %) and Azadiracthin (EC 4.5%) produces ovicidal and antifeedant effect in *Culex tarsalis* and *Culex quinquefasciatus*.

From the present it is clear that the neem formulation of azadirchtin (Niconeem) is highly effective as larvicide. Our study provides baseline information needed for monitoring the developmental of resistance among the vector population in Vadodara. Further studies on resistance potential of the local mosquito population need to be done for getting a comprehensive picture regarding the magnitude of the problem which could be utilized for vector control program.

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