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Efficacy of Azadirachtin Treated Nets on Adults of *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract

Mosquitoes are vectors of a variety of diseases like malaria, dengue, chikungunya and filariasis causing several thousand deaths throughout the tropical and subtropical regions of the world. Vector control measures involving the use of insecticide treated net have become popular and has been recommended by world health organization for management of mosquitoes. However the effectiveness of the treated net is facing a threat due to the problem of emergence of insecticide resistance among mosquitoes. Alternatives to the conventional insecticides are being explored with emphasis on mammalian safety and prevention of rapid resistance development in insects. Botanical derivatives have proven to be an effective option for the same purpose. One such chemical is Azadirachtin. It is extracted from neem tree and is known to have potent toxicity against several insects. In the present study we tested the efficacy of Azadiracthin treated nets as a potential mosquito adulticide. Mosquito nets were treated with varying concentration of Azadiracthin in order to determine the optimum dose of the chemical required for a mortality of adult mosquitoes. Two species of mosquitoes which are abundant in Central Gujarat region were tested viz. *Aedes aegypti* and *Culex quinquefasciatus*. Both the insects were found to be susceptible to the Azadiracthin however LC_{50} and LC_{99} values for *Ae.aegypti* were lower suggesting its higher susceptibility to Azadirachtin compared to *Cx.quinqefasciatus*. The present study shows that Azadiracthin is effective in managing the population of adult mosquitoes and can be used as an alternative for conventional insecticides used in the insecticide nets.

1. Introduction

Malaria and other vector-borne diseases such as dengue, filariasis[1], and Japanese encephalitis[2] are major contributors to the communicable disease burden in the South-East Asia Region. The risk of different communities to vector-borne diseases varies according to their socioeconomic status (e.g. tribes, urban slum dwellers), physiological conditions (e.g. pregnant women, young children), geographical location (e.g. those living in areas with poor access to interventions, and in cross-border areas), occupational exposures (e.g. migrant workers, miners), natural hazards (e.g. disease exacerbated by earthquakes, floods, cyclones etc) and socio-political disturbances (e.g. refugees).

Two generations ago hopes were high that malaria could be eradicated. But from 1980s till date, malarial vectors have gained resistance to several classes of insecticides and pathogen has developed resistance to antimalarial drugs. Malaria has re-emerged in several Central Asian and Eastern European countries and in South-East Asia[3]. Pregnant women are high susceptibility to malaria and other diseases[4-6]. Mother and foetus are faced with adverse consequences which may even lead to the death of foetus[7-10] and in certain cases leading to maternal anemia[11] and of low birth weight[6].

Measures to fight malaria epidemic are two pronged, one by regulating the vector population and other by antimalarial drugs. Among the two, the former approach is cheaper and most efficient in protecting large population throughout the world. Several vector control measures like larvicidal control measures, indoor residual spraying have been undertaken, which though effective for a short time has its drawbacks. The spraying of pesticides especially pyrethroids and organochlorines expose the human population to the risk of pesticide toxicity and its residual effects. These vector control measures are costly when a large area has to be brought under vector control program.

The alternative method which is much more efficient, cheaper and long lasting is the use of insecticide treated nets (ITNs)[3]. ITNs when used by all the population of a community can bring down vector borne diseases by as much as 50% [1-2]. ITNs treated with conventional insecticides like pyrethroids have started becoming less effective due to the development of insecticide resistance among mosquitoes. In a few people using ITNs treated with pyrethroids has led to dermal irritation. Hence there is a need for a much more ecofriendly alternative which could solve the problem of insecticide resistance and at the same time is safer to humans.

One such alternative is botanical Azadiracthin extracted from Neem tree. Azadiracthin has been recognized as a potential insecticide having several properties like antifeedant activity, insect growth regulator, and steriliant activity [12-14]. In the present study we analyzed the efficacy of mosquito nets treated with Azadiracthin on the survival of vector population of *Culex quinquefasciatus* and *Aedes aegypti*. We also calculated the optimised dose required for the total management of adults of *Cx.quinquefasciatus* and *Ae.aegypti*.

2. Materials and Methods

Mosquito larvae were collected from urban areas of the city from overhead tanks, ponds, stagnant water bodies and brought to Insectary. The larvae were isolated based on their morphological characteristics and were reared till adulthood. Larvae were maintained in plastic trays having tap water and a diet comprising of glucose biscuit and brewer's yeast was provided. Water in beaker was changed every day. The larval culture was maintained at a temperature of 29±2° C, relatively humidity: 60% and 14:10h L: D photoperiod. The pupae developing from the larvae were separated from the larval stock and transferred to 500 ml glass beakers. Beakers containing pupae were kept in wire gauze rearing cages (45cm (L) × 45cm (B) × 45cm (H)); the adults emerging from pupae were then kept in the rearing cage. The newly emerged adults were provided with honey solution and blood meal on alternative days using rat. Plastic container having tap water was kept in adult rearing cages for oviposition. Eggs were harvested at regular intervals and transferred to glass beaker having water for hatching.

Bioassay Protocol: Polyester multifilament net having mesh size of 156 holes\inch, light pink in colour was used in the present study. The net (50cm x 50cm) was treated with the Azadiracthin and placed vertically in the center of Bioassay cage (45 x 45 x 45cms). A set of 9 holes (each 1 cm diameter) were made horizontally in the centre of the net. 15 sugar fed mosquitoes one day old were released in the cage in every test. The net was placed in such a way that the mosquitoes released from one side can pass to the other side only through holes by touching the net. This ensured the maximum likelihood of mosquitoes coming in contact with the net while moving from one end of the cage to other. Observations regarding mortality of the mosquitoes were taken after 12 hours after the release of mosquitoes. The test was repeated 20 times and appropriate control tests having water soaked polyester net were performed.

The insecticide used in the study was procured from Nicoorgo Manures Pvt ltd. The chemical is neem based product having Azadiracthin as active ingredient. Four concentrations of 50,100,200 and 300 ppm respectively were prepared from the stock solution. Polyester net was soaked in the insecticide solution overnight and then was allowed to dry in shade. This was done in accordance to WHOPES (2002) regulation regarding the use of insecticide treated net.

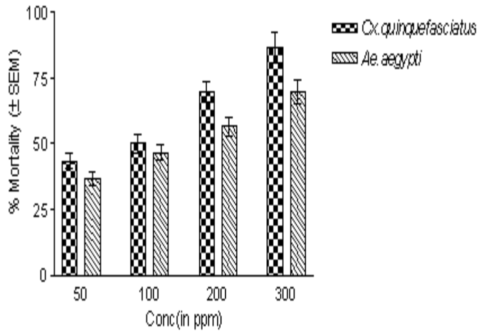
Data Analysis: Percentage mortality was corrected relative to the controls using Abbott's formula [15]. The data was subjected to probit analysis in accordance to Finney[16] to determine the optimum concentration of LC_{50} and LC_{99} for *Cx. quinquefasciatus* and *Ae.aegypti* using statistical software SPSS (ver 7.65).

3. Results and Discussion

The results of the study show that *Ae.aegypti* were susceptible at lower concentration of azadiracthin whereas *Cx.quinquefasciatus* mosquitoes showed susceptibility at comparatively higher doses. LC_{50} and LC_{99} values calculated using Probit table gave us the diagnostic concentration at which 50% and 99% of test populations are killed (Table 1). The data after 12 hour post exposure to Azadiracthin shows that *Ae.aegypti* (LC_{50} – 46.606 ppm; LC_{99} – 323.815 ppm) is more susceptible than *Cx.quinquefasciatus* (LC_{50} – 77.774 ppm; LC_{99} – 462.363 ppm) (Figure I).

The global strategy adopted by WHO in managing vector borne diseases using integrated vector control, lays emphasis on selective vector control methods. Among these methods, insecticide treated nets have been very effective comparable to indoor residual spray (IRS) and are also cheaper[17]. ITNs have been recommended for malaria preventions by WHO[18-19]. Presently only pyrethriod treated polyester nets have been used for community vector control programs. These net have been popular because they produce knockdown and lead to mortality among mosquitoes[20].

Figure 1: Percent Mortality (± SEM) of *Cx.quinquefasciatus* and *Ae.aegypti* adults exposed to different doses of Azadirachtin treated net



In recent years mosquitoes have started gaining resistance to pyrethroids[21-22] due to their intensive use as household insecticides. This has led to decreased effectiveness of ITNs as a vector control tool[23]. Washing of ITN has led to decreased bioefficacy of these nets[24] and permethrin treated nets have been known to cause dermal irritation in a few people. Alternatives to pyrethroids are botanicals most of which are relatively safer to human and other non target organisms and at the same time show selective toxicity towards insect pests.

In this study the effectiveness of Azadirachtin shows variation in LC_{50} and LC_{99} values in both the species of mosquitoes (Table 1) varies in different species of insects[25]. This emphasizes the need for a proper insecticide resistance monitoring program which should look into the resistance levels among mosquitoes at least on a yearly basis. This would help in the dose optimization for widespread use in the community health programs. The present study shows that Azadiracthin treated nets can be effective in the control of *Ae.aegypti* and *Cx.quinquefasciatus*. Higher diagnostic dose for *Culex* mosquitoes gives us indication about the development of cross resistance as a result of exposure to conventional insecticides used earlier in the study area.

Table 1: Probit regression response of *Cx.quinquefasciatus* and *Ae.aegypti* to Azadiracthin treated net

Species	LC_{50}		LC_{99}	
	LC_{50} (in ppm)	95% CI	LC_{99} (in ppm)	95% CI
Cx. quinquefasciatus	77.463	-77.774 to 135.229	462.363	325.892 to 1106.450
Ae aegypti	46.606	-100.298 to 94.700	323.815	232.979 to 726.196

In the study shows that nets treated with Azadiracthin can form an alternative for pyrethroids which can be effective in reducing the problem of insecticide resistance among mosquitoes. They can act as a cheaper and effective alternative in community vector control programs. Further studies needs to be undertaken regarding the efficacy of these net after washing and their efficacy against other species of mosquitoes also needs to be explored. However, the present studies are encouraging and suggest that the use of ITN treated with Azadirachtin can be a safe, cheap and effective alternative in community vector control programs.

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