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Larvicidal activity of Selected Medicinal Plant extracts against A*edes aegypti, Anopheles stephensi* and *Culex quiquefasciatus* (Diptera: Culicidae)

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Abstract
The current study was aimed to evaluate the larvicidal activity of methanolic crude extracts of *Azadirachta indica* and *Melia azedarach* (40, 60, 80 and 100 ppm) against different instars of three mosquito species *Aedes aegypti, Anopheles stephensi* and *Culex quiquefasciatus*. The crude methanolic extracts of *A.indica* showed highest mortality against *A. aegypti, A. stephensi* and *C. quiquefasciatus* in the range of 90.6, 93.37 and 83.6 % respectively at 100 ppm, where as the *M. azedarach* showed 90.37, 96.37 and 88.3 % against *A. aegypti, A. stephensi* and *C. quiquefasciatus* at 100 ppm. The LC_{50} values of *A.indica and M. azedarach* were51.48, 48.26 ppm against *A.aegypti*, 41.98, 43.32 ppm against *A.stephensi* and 54.27, 52.82 ppm against *C. quiquefasciatus*. The results evidenced that the methanolic extracts of *A. indica* and *M. azedarach have* potential activity to control the larval instar of *A. aegypti, A.stephensi* and *C.quiquefasciatus*.

Keywords: Azadirachta indica, Melia azedarach, Aedes aegypti, Anopheles stephensi, Culex quinquefaciatus

1. Introduction
Vector mosquitos are capable of transmitting the pathogen to human beings which cause several infectious diseases like malaria, filariasis, yellow fever, dengue fever and chickungunya [8].
Mosquito borne diseases are endemic over 100 countries reported the mortality rate of nearly 2 million people every year and atleast 1 million children dies for the disease each year [4]. In India various species of *A. aegypti, A. stephensi* and *C. quinquefaciatus* mosquito are important vector of human diseases. The causative agent of chickengunya and dengue fever are generally known as arbovirus responsible for dengue fever which found endemic over Southeast Asia [9]. It has affected more than 1.25 million people in 151 districts within 8 states of tropical country [11]. In recent years lot of insecticide were used to control the mosquito population, unfortunately there was no promising effects were achieved to control mosquito population effectively.
Vector control is a crucial prevention against mosquito-borne diseases. Mosquito larvae are initially controlled by using organophosphates, insect growth regulators, indoors residual spraying and insecticide-treated bed nets etc., [1, 5].However, these synthetic chemical insecticide were showing negative effects on human health environment and also leads to resistance to mosquito populations [5]. Therefore, it is necessary to look for an alternative method to control mosquito population specially by using plant metabolites.

Plant based products do not have any hazardous effect on ecosystem. Recent research on the plant derived compounds such as saponins, steroids, flavonoids, essential oils, alkaloids and tannins were found to have potential mosquito larvicidal activity [14].
M .azedarach and *A. indica* belongs to the *Meliaceae* family in Indian tradition increase a commercial interest all over the world. *M. azedarach* has been reported as analgesic, anticancer, antimalaria and antimicrobial activity [15]. Many of the plant of meliaceae family posses insecticidal activity against facundity and fertility of mosquito vector at the same time it is much eco-friently [7]. In the present investigation an attempt were made to study the larvicidal efficacy of methanolic crude extracts of *A. indica* and *M .azedarach*, against the larval instar of *A. aegypti, A. stephensi* and *C. quinquefasciatus*.

2. Materials and methods

2.1 Plant collection

A. indica and *M. azedarach* plant leaves were collected in and around region of kanchipuram district, Tamil Nadu. The voucher specimen of both plant species was verified by Prof. Muthumery, CAS in Botany University of Madras, Guindy Campus, Chennai-25.

2.2 Preparation of extracts

The plant materials were washed with tap water and shade dried at room temperature [10]. The leaves were powdered in an electrical grinder, 15 g of plant powder was taken and dissolved in 50 ml of methanol and stirred for 24 h at room temperature. The extract was filtered, evaporated in rotary evaporator and stored in refrigerator (5° C) for further bioassay.

2.3 Preparation of stock solution

Stock solution of 100 ppm extract was prepared by using 200 mg of methanolic crude extract in 1 ml of acetone and made upto 100 with distilled water [13]. From this stock solution, working standard was prepared in the range of 40, 60, 80 and 100 ppm.

2.4 Collection of Mosquito larvae and rearing

The larvae of *A. aegypti* were collected from the ditch of Medavakkam and Oorapakkam areas, Chennai. The egg raft of *C. quinquefasciatus* and the larva of *A. stephensi* were collected from Adyar River near Saidapet, Chennai. The collected larvae were transferred to dechlorinated water under laboratory condition. The mosquito eggs were placed in plastic trays with 1L tap water and kept at room temperature (26± 2°C) with a photoperiod of 16:8 h (L:D) for larval hatching. The hatched larvae of each mosquito species were maintained at separate trays under the same condition and feed with a mixture of dog biscuit and baker's yeast (3:1ratio) in a powder form, after three days the fourth instar larvae to pupae of each mosquito species were maintained in separate mosquito cages (26± 2°C) at the photoperiod of 16:8 (L:D) for adult emergence. Cotton soaked in 10% aqueous glucose solution was used to feed adult male mosquitoes and blood meal was given to the female mosquitoes with young immobile chicken. The plastic tray was placed in each cage with filter paper immersed on water surface for egg laid. The eggs obtained from the laboratory- reared mosquitoes were used for further larvicidal assays.

2.5 Larvicidal bioassay

The larvicidal bioassy were performed according to Imran Khan *et al.*[3] with some modification. Different instars of each larval species were exposed to different concentrations (40, 60, 80 and 100 ppm) of methanolic extract of *A. indica* and *M. azedarach* in 50 mL of tap water at room temperature (26± 2°C) with naturally prevailing photoperiod (18:6 h / L:D) in the laboratory. The mortality was observed and recorded after 48 h of exposure.

3. Results

The larvicidal activity of methanolic extract of *A. indica* and *M.azedarach* were studied against three different vector mosquito species *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. During this investigation, highest mortality was occurred when increased the concentration (ppm level) of extracts against fourth instar of three mosquito species. The percentage of mortality for *A.indica* was 90.6 (Fig. 1A), 93.37 (Fig. 1B) and 83.6 % (Fig. 1C) against fourth instars *A.aegypti*, *A.stephensi* and *C. quiquefasciatus* at 100 ppm respectively. Similarly *M.azedarach* showed maximum mortality against three species in the range of 93.67 (Fig. 2A), 96.36 (Fig. 2B) and 88.37 % (Fig. 2C) against *A.aegypti*, *A. stephensi* and *C. quinquefasciatus* respectively at 100 ppm after 48 h exposure of the extracts.

The LC_{50} value of *A.indica* and *M. azedarach* showed 51.48, 48.26 ppm against *A.aegypti*, 43.32, 41.98 ppm against *A.stephensi* and 54.27, 52.82 ppm against *C. quinquefasciatus*. *M. azedarach* showed LC_{90} value of 81.62, 76.3 and 112.65 ppm against all three mosquito species respectively. Interestingly among these two plants the *M. azedarach* elicit highest mortality rate against all instars mosquito species than the *A. indica*. Both *A. indica* and *M. azedarach* leaves extracts showed maximum mortality against *A. aegypti*, *A. stephensi*, than *C. quinquefasciatus* at 100 ppm. Hence these two plants has maximum larvicidal activity against all three species, at one stage of increasing the concentration of test medium, also it is beneficial to aquatic systems and not toxic to other organisms.

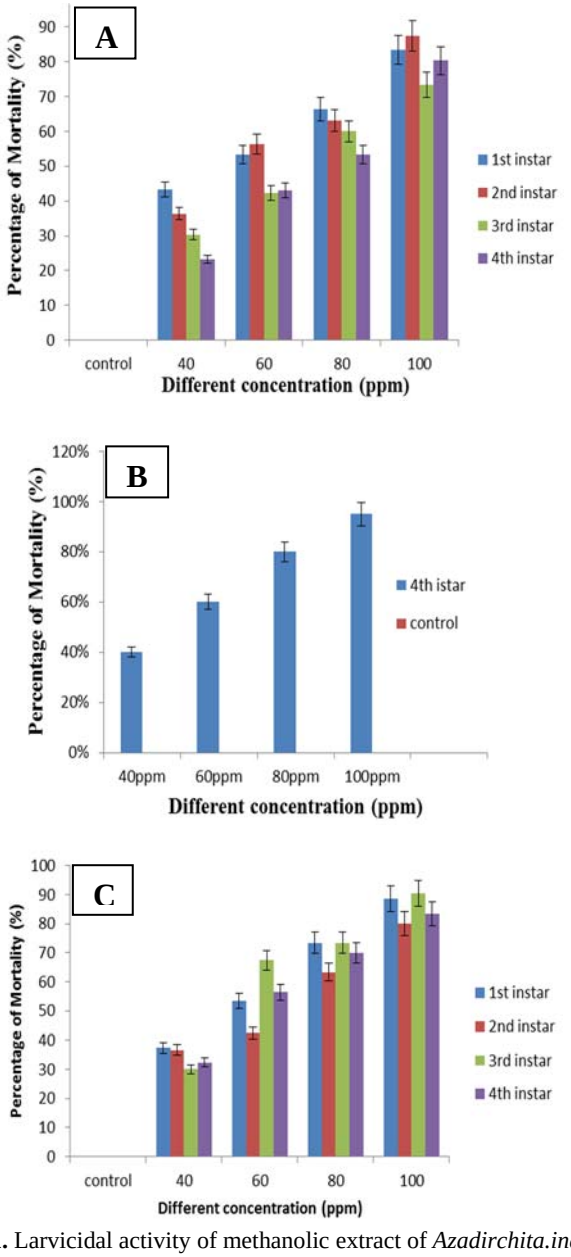
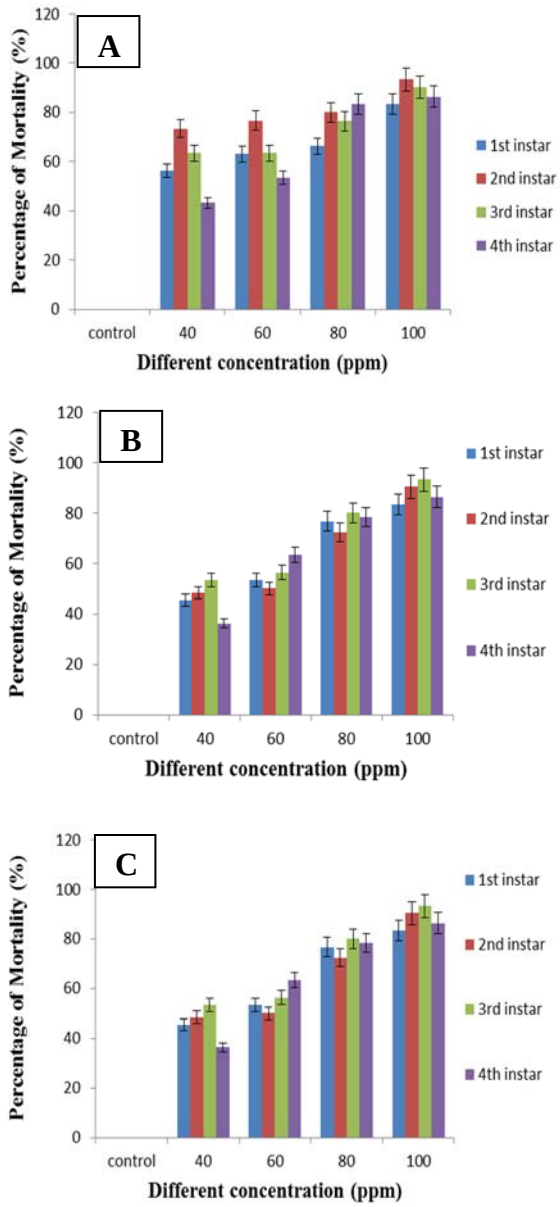


Fig 1. Larvicidal activity of methanolic extract of *Azadirchita.indica* against (A) *Aedes aegypti* (B) *Anopheles stephensi* and (C) *culex quinquefasicatus*



4. Discussion

Plant has major resources for primary and secondary metabolites with a wide variety of biological and insecticidal activity including antimosquito property. Compounds isolated from *M. azedarach* have received much attention from appliedentomologists because of their excellent insecticidalproperties [6]. The insecticidal effect of *M.azedarach* could be attributed due to the presence of limonoids, it posses poisonous effect on insects [2].The aqueous extracts of seed kernel of *A. indica* showed shows maximum 50% of the mortality at 4 mg/L (LC_{50}) after 24 h against *C. quinquefasciatus* and at 6 mg/L after 96 h against *Anopheles gambiae* was recorded [12].

In this study the crude methanolic extract of *A. indica* and *M. azedarach* showed potential larvicidal activity against three major vectors species with maximum mortality rate, interestingly the mortality rate was increased dose-dependently against these mosquito species. Moreover the larvicidal activity of these selected plants has been earlier reported against *C. quinquefasciatus* only [3]. However, the present study investigated the larvicidal activity against three major vector species of *A. aegypti*, *A.stephensi* and *C. quinquefasciatus* showed mortality rate of 90.3, 93.6 and 83.6 % at 100 ppm exposure with *A. indica*. Similarly the *M. azedarach* elicit has maximum mortality rate than *A. indica* in the range of 93.6, 96.3 and 88.3 % at 100 ppm. Hence the methanolic extracts of *A. indica* and *M. azedarach* showed maximum point to control the mosquitoes in their early stages of larval instars. This may use as mosquito repellents in near future.

5. Conclusion

The study concluded that the methanolic crude extracts of both *A. indica* and *M. azedarach* possessed potent larvicidal acitvity against three mosquito species of *A. aeqypti*, *A. stephensi* and *C. quinquefaciatus* with 90% mortality. Furthermore, the selected plants should may used as a potent ecofriently plant based insecticides to control mosquito populations.

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