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RESEARCH ARTICLE

LARVICIDALACTIVITY OF *CALOTROPIS GIGANTEA* LEAF EXTRACT AGAINST MOSQUITOE *AEDES AEGYPTI*

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ABSTRACT

Mosquitoes act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile Virus infection, etc. Under the Integrated Mosquito Management (IMM), emphasis was given on the application of alternative strategies in mosquito control. The continuous application of synthetic insecticides causes development of resistance in vector species, biological magnification of toxic substances through the food chain and adverse effects on environmental quality and non target organisms including human health. Application of active toxic agents from plant extracts as an alternative mosquito control strategy was available from ancient times. In this article, the larvicidal activity of *Calotropis gigantea* leaf extract were studied in laboratory on the concentration of 1.0, 2.5 and 5.0 mg/cm² against 3 to 4 days 1d unfed *Aedes aegypti* in a dial cycle from 7 am to 5 pm. This study reveals that the extract of *C.gigantea* has remarkable larvicidal as well as repellent properties. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

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INTRODUCTION

Mosquitoes and other biting arthropods are pests because of their biting activity and their ability to carry and transmit arthropod borne diseases (Olkowski, 2001). Mosquitoes can often interfere with daily outdoor activities, curtailing or strictly limiting activities to indoors. In the past, removal of mosquito breeding areas, chemical treatment using pesticides, repellents, or avoidance when mosquitoes are active were the only options a homeowner relied upon. Although the use of pesticides can eliminate mosquitoes from a treated area, it was often a temporary measure and not always environmentally safe (Peterson, 2003). *Aedes aegypti* (L.) is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa.

Dengue fever has become an important public health problem as the number of reported cases continue to increase, especially with more severe forms of the disease, dengue haemorrhagic fever and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement (Hendarto and Hadinegoro, 1992; Pancharoen et al., 2002). About two-fifths of the world's population is now at risk of catching dengue according to the World Health Organization (WHO 2003).

MATERIALS AND METHODS

The *Aedes aegypti* mosquito is the primary carrier for viruses that cause dengue fever and yellow fever. It is often called as the "yellow fever mosquito". Worldwide, this species has a cosmopolitan range extending from 40 degree N to 40 degree S latitude.

This species is found throughout most tropical to subtropical world regions. Survival is poor in hot, dry climates. It is abundant in towns and cities.

Collection of plant materials: The plant *Calotropis gigantea* (L.) T. Aiton, (Asclepiadaceae) was collected from Annamalai University Campus, Annamalai Nagar, Chidambaram (11:24 N lat. and 79°: 5E long; 5.79 above MSL) Tamilnadu, India. The vernacular name of the plant is Eruku. The plant was taxonomically identified at the Department of Botany, Annamalai University and voucher specimen was deposited at the Department of Zoology, Annamalai University.

Preparation of plant extract: The leaves of *Calotropis gigantea* were carefully examined and old, insect-damaged, fungus infected leaves, twigs and flowers were removed. Healthy leaves were washed with tap water and shade dried at room temperature (28 + 2°C) for 5-8 days or until they broke easily by hand. Once completely dry, leaf material (1.0 kg) was ground to a fine powder using electrical blender. Three liter ethanol, methanol, acetone and petroleum ether separately was used for the extraction of 1.0 kg in the Soxhlet apparatus followed by the standard procedure (Vogel, 1978). The plant material was loaded in the inner tube of the Soxhlet apparatus and then fitted into a round bottomed flask containing ethanol. The solvent was boiled gently (40°C) over a heating mantle using the adjustable rheostat. The extraction was continued until complete extraction was effected (8 hrs.) and the solvent was removed at the reduced pressure with the help of rotary vacuum evaporator to yield a viscous dark green residue (12.5 g) of each solvent of ethanol, methanol and acetone leaf extracts.

Laboratory colonization of *Aedes aegypti*: The eggs of *Ae. aegypti* procured from Vector Control Research Centre (VCRC) at Puducherry, India. The mosquito colony maintained at 70-85% RH, 28 + 2°C temperature and 14:10 light and dark photoperiod cycle. The larvae were fed on powdered mixture of dog biscuits and yeast tablets in 3:1 ratio. The blood meal was given to the female adult mosquitoes and 5.0% glucose solution and honey were given to the male adult mosquitoes.

Test for larvicidal activity: Testing of the plant extract for larvicidal activity was carried out at different concentration by preparing the required stock solutions by following the standard procedure (WHO, 1996). The desired concentrations of the test solution was achieved by adding 1.0 ml of an appropriate stock solution to 249 ml of dechlorinated water. Six replicates for each concentrations were maintained. Twenty five number of late third larvae were introduced into the beaker, were obtained from the laboratory colony (Plate 1). Acetone was used as control. The larval mortality in both treated and control were recorded after 24 hrs and the percentage of mortality was calculated using Abbott's formula (Abbott, 1925).

$$\% \text{ Mortality} = \frac{\text{Mortality at treatment} - \text{Mortality at control}}{100 - \text{Mortality at control}} \times 100$$

The statistical evaluation of LC₅₀, LC₉₀, regression equation and 95 percent confidence limit LCL and UCL were calculated from the data, which was carried out by Probit analysis (Finney, 1971).

RESULTS

Larvicidal activity of extract of *C. gigantea* against dengue vector *Ae. Aegypti*: The result of the larvicidal activity of methanol, ethanol and acetone extract of *C. gigantea* against II, III and IV larval mosquito of *Ae. aegypti*.

Methanolic extract

a) II Instar: The LC₅₀ value of the methanolic of *C. gigantea* was 44.69 ppm for the second instar of *Ae. aegypti*. The LC₉₀ and regression equation were 83.27 ppm, Y=12.825 + 0.785x. The 95% lower and upper confidence limit of LC₅₀, LC₉₀ (LCL-UCL) were (30.71-57.25) and (68.66-11.78) ppm respectively. The chi-square value 17.363 was significant at P<0.05 level.

b) III Instar: The LC₅₀ value of the methanolic extract of *C. gigantea* was 51.31 ppm for the third instar of *Ae. Aegypti*. The LC₉₀ and regression equation were 94.79 pp, Y=7.904 + 0.790x. The 95% lower and upper confidence limit of LC₅₀, LC₉₀ (LCL-UCL) were (30.59-62.35). and (80.91-118.60) ppm respectively. The chi-square value 12.090 was significant at P<0.05 level.

c) IV Instar: The LC₅₀ value of the methanolic extract of *C. gigantea* was 60.72 ppm for the fourth instar of *Ae. Aegypti*. The LC₉₀ and regression equation were 111.32 pp, Y=4.238 + 0.750x. The 95% lower and upper confidence limit of LC₅₀, LC₉₀ (LCL-UCL) were (45.99-75.19). and (93.16-146.72) ppm respectively. The chi-square value 15.939 was significant at P<0.05 level.

Ethanolic extract

a) III Instar : The LC₅₀ value of the ethanolic extract of *C. gigantea* was 63.49 ppm for the second instar of *Ae. aegypti*. The LC₉₀ and regression equation were 125.56ppm, Y=12.825 + 0.785x. The 95% lower and upper confidence limit of LC₅₀, LC₉₀ (LCL-UCL) were (30.71-57.25) and (68.66-11.78) ppm respectively. The chi-square value 17.363 was significant at P<0.05 level.

b) III Instar: The LC₅₀ value of the ethanolic extract of *C. gigantea* was 69.51 ppm for the third instar of *Ae. aegypti*. The LC₉₀ and regression equation were 127.18 pp, Y=2.571 + 0.694x. The 95% lower and upper confidence limit of LC₅₀, LC₉₀ (LCL-UCL) were (56.86-82.79). and (108.72-160.50) ppm respectively. The chi-square value 10.794 was significant at P<0.05 level.

c) IV Instar: The LC₅₀ values of the Methanolic extract of *C. gigantea* was 72.83 ppm for the fourth instar of *Ae. aegypti*. The LC₉₀ and regression equation were 127.80 ppm, Y=-0.476 + 0.706x. The 95% lower and upper confidence limit of LC₅₀, LC₉₀ (LCL - UCL) were (59.54-82.79) and (108.49-164.63) ppm respectively. The chi-square value 12.875 was significant at P<0.05 level.

Acetonic extract

a) II Instar: The LC₅₀ values of the acetonic extract of *C. gigantea* was 55.67 ppm for the second instar of *Ae. aegypti*. The LC₉₀ and regression equation were 103.96 ppm, Y=7+0.76x. The 95% lower and upper confidence limit of LC₅₀, LC₉₀ (LCL - UCL) were (36.42-73.41) and (83.72-

151.01) ppm respectively. The chi-square value 24.476 was significant at $P < 0.05$ level.

b) III Instar larvae : The LC_{50} values of the acetonetic extract of *C. gigantea* was 62.81 ppm for the third larvae of *Ae. aegypti*. The LC_{90} and regression equation were 116.00 ppm, $Y = 4.047 + 0.732x$. The 95% lower and upper confidence limit of LC_{50} , LC_{90} (LCL - UCL) were (50.72- 74.92) and (99.57-144.54) ppm respectively. The chi-square value 10.763 was significant at $P < 0.05$ level.

c) IV Instar : The LC_{50} values of the acetonetic extract of *C. gigantea* was 74.93 ppm for the fourth instar of *Ae. aegypti*. The LC_{90} and regression equation were 133.79 ppm, $Y = 0.428 + 0.673x$. The 95% lower and upper confidence limit of LC_{50} , LC_{90} (LCL - UCL) were (58.86-93.71) and (110.32-185.75) ppm respectively. The chi-square value 16.782 was significant at $P < 0.05$ level.

DISCUSSION

There is a renewed interest in the use of natural products to control destructive insects and vectors of diseases due to the prevalent occurrence of vector resistance to synthetic insecticides and the problem of toxic nonbiodegradable residues contaminating the environment and adversely affecting nontarget organisms. More than 2000 plant species are already known to have insecticide properties (Sukumar *et al.*, 1991). Humans have used plant parts, products, and metabolites in pest control since early historical times. Plants are the chemical factories of nature, producing many chemicals, some of which have medicinal and pesticidal properties. By using plant parts in early historical times and plant extracts and concentrated components in more recent times, man has been able to control certain pests with these remedies quite successfully. *C. gigantea* leaf extracted were extracted with three different solvent namely methanol, ethanol and acetone. Stock solution was prepared using acetone, because basic toxicological investigation and screening the flora for insecticidal activity, acetone is commonly used as a solvent. It has good solvency for synthetic insecticides (Singh and Jain, 1987) and easy to evaporate (Chauhan *et al.*, 1987). The *Calotropis gigantea* leaf extract tested for the mosquito larvicidal activity (II, III and IV instar) at different concentration for 24 hour exposure period against *Ae. aegypti* larvae. All the extract having significant toxicity to *Ae. aegypti*. High mortality rate was observed for II instar in all three extract tested than III and IV instar larvae. The LC_{50} values methanolic extract for II, III, IV instar were 44.69, 51.31 and 60.72 ppm respectively. The LC_{50} values ethanolic extract for II, III, IV instar were 63.48, 69.51 and 72.83 ppm respectively. The LC_{50} values acetone extract for II, III, IV instar were 55.67, 62.81 and 74.93 ppm respectively. It was also revealed that higher concentration was needed for the toxicity of III and IV instar, which was evident from LC_{50} values. The larvicidal results are also comparable with earlier reports, similar results was observed by Cheng *et al.* (2003) and reported that the leaf and bark essential oil of *Cryptomeria japonica* showed larvicidal activity against *Aedes aegypti*. The LC_{50} values are 37.6 $\mu\text{g/l}$ and 48.1 $\mu\text{g/l}$. Silva *et al.* (2003) reported that the *Copaifera reticulata* Duke oil resin produced larval mortality against *Culex quinquefasciatus*. Sing *et al.* (2003) reported that the mosquito larvicidal properties of the leaf extract of a herbaceous plant, *Ocimum canum* against *Aedes aegypti*.

The LC_{50} values for 2nd, 3rd and 4th instar were 177.82, 229.08 and 331.13 ppm respectively. Kalyanasundaram and Das (1985) reported that the plant extracts of *Vinca rosea*, *Calatropis* sp. and *Adathoda* sp. possess larvicidal activity against *Ae. aegypti*.

CONCLUSION

Increased during recent years because of the drawbacks of synthetic chemical pesticides, among which are impact on environment, toxicity to mammals and non-targets, resistance development in insect populations and so forth. In view of these facts, in the present study, deals the larvicidal and repellent activity of extracts of *Calotropis gigantea* against the dengue vector, *Aedes aegypti*. This study reveals that the extract of *C. gigantea* has remarkable larvicidal as well as repellent properties. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

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