Toxicity effect of *Artemisia parviflora* against malarial vector *Anopheles stephensi* Liston

R. Uma Devi [1], D. Lakshmi[1] and N. Aarthi[2]

Abstract

The methanolic leaf extract of *Artemisia parviflora* (APLE) tested against *Anopheles stephensi* larvae and pupae and recorded the mortality rate, LC_{50} and LC_{90} values. This investigation revealed that this leaf extract possess higher toxicity against *Anopheles stephensi*. The biological activity of the plant extract might be due to the presence of active compounds â- Caryophyllene, germacrene D, Camphor, artemisia ketone, 1-8 Cineole, D-Copaene and Sabinyl acetae. These are all compounds are very toxic against the mosquito. The LC_{50} value for first instar larvae is 45.61 and it is increased in the IV instar larvae as 59.60. According our experimental view this plant can effectively play the biopesticide role and may contribute to an effective vector control tool. This new agent should preferentially to be applied in mosquito control strategies to reduce the mosquito populations and prevent the malaria.

Key words: *Artemisia parviflora, Anopheles stephensi*, biopesticide, mosquito control

INTRODUCTION

Insect-transmitted disease remains a major cause of illness and death worldwide (Pavela, 2009). Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year (Rahuman *et al.*, 2009, Borah *et al*., 2010). There are currently more than 3000 mosquito species in worldwide grouped in 39 genera and 135 subgenera (Clements, 1992; Reinert, 2000; Reinert, 2001). Among these 422 species of *Anopheles* found in worldwide, many of them sibling species that can only be identified using genetic techniques. Of these, about 70 are malaria vectors but only about 40 are important. Malaria is transmitted by different *Anopheles* species, depending on the region and environment. Malaria is from one person to another by the female anopheline mosquito. Before controlling the malarial first we have to control the vectors (Sivagnaname *et al*., 2004). The discovery and development of synthetic organic chemicals with persistent residual action not only over shadow the use of herbal products against mosquitoes, but also become the major weapon for mosquito control. Since the discovery and the development of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides. But the extensive uses of synthetic organic insecticides during the last five decades have resulted in environmental hazards and also in the development of physiological resistance in the major vector species (Hartzell, 1947 and Jacobson, 1971). This has necessitated the need for search and development of environmental safe, biodegradable, cheap, indigenous method for vector control, which can be used with minimum care by individual and communities in specific situation (Amer, 2006). One major drawback with the use of these chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. The toxicity problem, together with the growing incidence of insect resistance, underscores the need for development of effective insecticides, which are environmentally safe, target specific and biodegradable. Economically feasible plant secondary metabolites are considered to be a potential alternative approach against various stages and species of mosquitoes due to their excellent properties like cheap availability, environmental safety nature and the presence of rich source of bioactive compounds, such as larvicidal, repellent, insect growth regulators, antifeedants, ovicidal, oviposition deterrence and reduction of fecundity and fertility (Rajkumar and Jebanesan, 2005; Elango *et al.*, 2009; Kostic *et al.*, 2008; Pavela *et al.*, 2005; Borah *et al*.,2010) A large number of plant extracts have been reported to have mosquitocidal activity against mosquito vector, but very few plant product have shown practical utility for mosquito control. Plants belonging to the family *Asteraceae, Cledophoraceae, Labiatae, Meliaceae, Oocystaceae* and *Rutaceae* apper to have potential for providing future mosquito control agent (Sukumar *et al*.,1992). Recently

the discovery of insecticidal activity of phytotoxins present in the *Asteraceae* show very high toxic effect against the vector control. Because of these references toxicity studies was carried out the methanolic leaf extract of *Artemisia parviflora* test against the larvae and the pupae of *Anopheles stephensi*. This plant was cure malaria in the tribal medicine.

MATERIALS AND METHODS

Maintenance of mosquito

The larvae reared in plastic cups. They were daily provided with commercial fish food (Lyimo et al., 1992) *ad libitum*. Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed. The normal cultures as well as breeding cups used for any experimental purpose during the present study were kept closed with muslin cloth for preventing contamination through foreign mosquitoes. The pupae were collected from culture trays and were transferred to glass beakers containing 500 ml of water with help of a sucker. The glass beaker containing pupae was then kept in 90 x 90 x 90 cm size mosquito cage for adult emergence.

Collection and preparation of plant material

Artemisia parviflora plant leaves were collected from Kodaikannal hills, Tamil Nadu, India. The leaves were taken from this plant, this leaf washed with tap water to remove the soil and dust particles. The above plant materials were than spread over an absorbent paper to remove the water particles. The leaves were shade dried in enamel trays at laboratory temperature. Then the dried leaves were powdered with an electric blender. From the powder 200g of the plant material were extracted with 2.5 litres of organic solvent (Methanol) for 8 hrs in a soxhlet apparatus (Vogel, 1978). The crude plant extracts (APLE) were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1% stock solution. From this stock solution concentrations were prepared ranging from 0.5 to 100 ppm.

Bioassay

A laboratory colony of Anopheles stephensi larvae were used for the larvicidal activity. Twenty five numbers of first, second, third and fourth instar larvae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1ml of desired concentration of plant extracts. Larval food was given for the test larvae. At each tested concentration 2 to 5 trails were made and each trial consisted of three replicates. The control was set up by mixing 1ml of acetone with 249 ml of dechlorinated water. After the treatment of twenty four hours, the mortality was observed. The percentage of mortality was corrected by using Abbott's formula (Abbott's, 1925). Same procedure was followed in pupal toxicity. LC_{50}, LC_{90} were calculated from toxicity data by using probit analysis (Finney, 1971).

RESULTs and discussion

Data of the larvicidal activity of *A.parviflora* against the mosquito larvae and pupae of *A. stephensi*, Using probit analysis software regression lines were plotted for the dose response to APLE treatment of laboratory strain of A.stephensi. Table 1 shows the larval (I to IV instar) and pupal mortality after the treatment of APLE. There was considerable mortality was evident after the treatment of APLE. The LC_{50} of I instar was 45.61, II instar was 49.42, III instar was 55.20 and IV instar was 59.60, respectively. Similar trend has been noted for all the instars of A. stephensi at different concentration of APLE treatment. The LC_{90} of I instar was 45.61 ppm, 86.63 ppm and increased while the larvae grew older. Pupal mortality after the treatment of APLE also showed considerable toxicity. The LC_{50} value after the treatment of APLE was 52.33 ppm and LC_{90} value was 109.21 ppm, respectively.

Table 1. Larval and pupal toxicity effect of *Artemisia parviflora* (Methanolic extract) on of malarial vector, *Anopheles stephensi*

Larval stages and Pupa	LC_{30}	LC_{50}	LC_{90}	Regression Equation
I	40.38	45.61	93.36	Y = -1.22460 +0.02684 X
II	44.31	49.42	98.47	Y = —1.2967 +0.02612 X
III	50.51	55.20	102.99	Y = -1.48803 +0.02695 X
IV	55.36	59.60	102.90	Y = -1.76046 +0.02954 X
Pupa	32.73	52.33	109.21	Y = -0.17915 +0.02253 X

Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT.

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either countermeasures or development of newer insecticides (Cisneros, *et al.*, 2002). Botanical insecticides may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, degradable, and are readily available in many areas of the world. Though several plants from different families have been reported for mosquitocidal activity, only a very few botanicals have moved from the laboratory to field use, like neem based insecticides, which might be due to the light and heat instability of phytochemicals compared to synthetic insecticides (Green *et al.*, 1991).

Artemisia parviflora methanolic extracts have been brought out toxicity on different larval instars of *Anopheles stephensi* and moreover complete reduction of larval and pupal mortality was noticed after combination of *Artemisia parviflora*. Singh *et al.* (2006) worked on the bioassays with crude extract of *M. charantia* against larvae of *A. stephensi, C. quinquefasciatus* and *A.aegypti* revealed the LC_{50} values of 0.50, 1.29 and 1.45%, respectively. Hexane extract showed more potent larvicidal activity than the crude extract, indicating the non-polar characteristics of larvicidal components. The LC_{50} values of hexane extract against IV instar larvae of *An. stephensi, Cx. quinquefasciatus* and *Ae. Aegypti* were 66.05, 96.11 and 122.45 ppm, respectively. Eveline Solon Barreira Cavalcanti *et al.* (2004) concluded that the essential oils of *O. americanum* and *O. gratissimun* were shown to be as potent as *L. sidoides* and *C. citrates* in the larvicidal activity against *A. aegypti* and caused 100% mortality at a concentration of 100 ppm.

The secondary plants make up a vast repository of compounds with a wide range of biological activities (Chowdhury *et al.*, 2008). Most of the works clearly demonstrate that the plants secondary metabolites from the family Asteraceae, Cledophoraceae, Labiatae, Meliaceae, Oocystaceae and Rutaceae apper to have potential for providing mosquito control agent. In the present study toxicity activity of methanolic extract of *A. parviflora* against *A. stephensi*. This is also may be due to presence of active compounds caryophyllene, germacrene D, Camphor, aremisia ketone, 1, 8-cineole, copaene, aremisia alcohol, terpinen-4-ol, caryophyllene oxide, pinene, humulene, cadinene and sabinyl acetate, caryophyllene, germacrene D, Camphor and aremisia ketoneas from the *A. parviflora*. These active ingdients are the important neuro toxin. Of the toxic compounds identified, terpinen-4-ol was the most active and was as effective as dimethyl phthalate (Yih-Shen Hwang *et al.*, 1985). In this present study, biopesticides from plant origin may contribute to an effective vector control tools. These new agents should preferentially to be applied in mosquito control strategies to reduce the mosquito populations and prevent the malaria. This investigation clearly observes that botanical insecticides act as a good toxicant.

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R. Uma Devi [1], D. Lakshmi[1], N. Aarthi[2]

[1.]Department of Life Science, Karpagam University, Coimbatore, Tamil Nadu, India, Karpagam College of Education, Karpagam University, Coimbatore, Tamil Nadu, India, E-mail:umadevi.suba@gmail.com.

[2.]Department of Zoology, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India.