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Larvicidal Activity of Aegle marmelos, Coleus aromaticus and Vitex negundo Leaf Extract Against Filarial Vector Culex quinquefasciatus

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Abstract

Plant products are considered to be potential alternatives for chemical pesticides. These to test the larvicidal activity *of Aegle marmelos, Coleus aromaticus* and *Vitex negundo* leaf extract are tested against II, III, IV instars and pupa of *Culex quinquefasciatus*. The LC_{50} values of *V. negundo* for II, III, IV instars and pupa is 66.31 ppm, 74.08 ppm, 84.36 ppm and 133.37 ppm respectively. LC_{50} value obtained for *A. marmelos* for II, III, IV and pupa is 91.52 ppm, 105.16 ppm, 151.43 ppm and pupa 203.78 ppm respectively. Similarly LC_{50} value obtained for *C. aromaticus* is 137.77 ppm for II instar, 175 ppm for III instar, 188.36 ppm for IV instar and 221.04 ppm for pupa. Among these three plants studied *V. negundo* is more effective than other two plant extract. The adult emergence recorded in this study indicates that there a reduction in adult emergence as a function of concentration irrespective of plant extracts studied.

Keywords: Larvicidal activity, *Culex quinquefasciatus,* plant extracts.

Introduction

Mosquitoes are major public vector throughout the world and about more than 3000 species are recorded throughout the world. Of these around hundred species are capable of transmitting various diseases to human (Reuda, 2008). Mosquitoes transmit many medically important pathogen and parasites such as viruses, bacteria, protozoan (Sathishkumar and Maneemegalai, 2008)

Several methods are used to control the mosquito menace and one such approach is by killing mosquitoes at its larval stages and this is achieved mainly based on synthetic insecticides. Though insecticides are most effective in controlling mosquitoes, indiscriminate use of insecticides leads to development of insecticide resistance (Govintharajan, 2001; Sarwar et al., 2009). This has necessitated developing an environmentally safe, bio-degradable indigenous method. Here herbal products have been recommended and used as natural insecticides (ICMR, 2003). Use of plant products against insects are learned from the co-evolution of plants with insect (Arivoli and Samuel, 2012).

In this context a number of plants derivatives are used against various species of mosquitoes and these studies are reviewed time to time (Sukumar et al., 1999; Gosh et al., 2012). In this time the present study was carried out to study the larvicidal activity of *V. negundo, A. marmelos* and *C. aromaticus* plant leaf extract tested against *Cx. quinquefasciatus* larvae and pupa.

A. marmelos is commonly known as Bael and belong to the family Rutaceae. Medicinal value of the leaf, root, bark, seeds and fruits (Dhankhar *et al.*2011).The leaves of Bael are astringent, a laxative, and an expectorant and are useful in the treatment of ophthalmia, deafness, inflammations, cataract, diabetes, diarrhoea, dysentery, heart palpitation and asthmatic complications (Arul et al., 2005) .

C.aromaticus a member of family Lamiaceae is an Indian traditional herb with several medicinal properties. The plant is traditionally used externally for burns and insect bites, while internally it is used as a carminative and to control asthma. *C.*

aromaticus is reported to also possess few other medicinal properties as antiepileptic, antimutagenic, antitumourogenic and antigenotoxic effects, anti-inflammatory and antitumor, diuretic, antioxidant and antimicrobial activities (Chatterjee and Parkrashi, 2001).

V. negundo is one of the common plants used in traditional medicine and reported to have Varity of pharmologycal activities (Baral and Kurmi, 2006). Though all parts of *V. negundo* have medicinal value, the leaves are much used. The decoction of leaves is used for treatment of inflammation, eye-disease, toothache, enlargement of the spleen, ulcer, cancer, catarrhal fever, rheumatoid arthritis, gonorrhea, sinuses, antibacterial, insecticidal, ovocidal, feeding deterrence, growth inhibition ect., (Gupata et al., 1999; Dharmasiri et al., 2003) .

Table 1. The LC_{50} and LC_{90} values of *Aegle marmelos, Coleus aromaticus* and *Vitex negundo,* leaf extract against the II, III, IV instar and pupa of *Cx. quinquefasciatus* under 24 hr exposure.

Plant species	Larval stages	LC_{50} (ppm) (UCL-LCL)	LC_{90} (ppm) (UCL-LCL)	$\chi 2$	Regression equation
Aegle marmelos	II instar	91.52 (93.48-89.56)	215.61 (217.94-213.28)	1.284	Y= 0.898 * X= 0.038
	III instar	105.16 (107.18-103.14)	203.46 (205.77-201.15)	2.975	Y= -0.702 * X= 0.046
	IV instar	151.43 (153.61-149.25)	203.60 (205.91-201.29)	.706	Y= -3.767 * X= 0.056
	Pupa	203.77 (206.08-201.46)	304.91 (307.39-302.4)	.217	Y= -2.998 * X= 0.038
Coleus aromaticus	II instar	137.77 (139.91-135.63)	243.98 (246.36-241.59)	1.594	Y= -1.357 * X= 0.0407
	III instar	175.00 (177.26-172.77)	306.31 (308.80-303.83)	5.651	Y= -2.581 * X= 0.040
	IV instar	188.36 (190.63-186.08)	337.17 (339.69-334.64)	6.359	Y= -2.604 * X= 0.038
	Pupa	221.04 (223.38-218.69)	355.35 (357.90-352.80)	.920	Y= -2.890 * X= 0.035
Vitex negundo	II instar	66.31 (68.13-64.49)	176.28 (178.52- 174.03)	3.682	Y= 1.042 * X= 0.048
	III instar	74.08 (75.94-72.20)	162.00 (164.21-159.79)	2.409	Y= 0.0105 * X= 0.054
	IV instar	84.36 (86.28-82.43)	200.75 (203.04-198.44)	2.450	Y= -0.234 * X= 0.052
	Pupa	133.371 (135.49-131.24)	238.069 (240.44-235.69)	2.561	Y= -2.372 * X= 0.053

Materials and Methods

Collection of the plants material

The leaves of *A.marmelos* (in Tamil *velvam*) (Rutaceae), *C.aromaticus* (in Tamil *Karpuravalli*) (Lamiaceae) and *V.negundo* (in Tamil *Nochchi*) (Verbenaceae) were collected from Karambayam, Thanjavur district, Tamilnadu, India.

Preparation of plant extract

The leaves of the these plants were washed with running tap water and dried in a shady place for 7-14 days at a day time temperature around 27°C to 37°C. The dried leaves (800g) were powdered mechanically using commercially available electrical stainless steel blender. The plant extract was derived from the powdered with the help of a Soxhlet apparatus using methanol as solvent (500 ml) (Boiling temperature ranges in between 45°C-50°C for 8 hours). The extract thus obtained was filter through a Buchner funnel with Whatman number 1 filter paper. The extract was concentrated under a reduced pressure of 22-26 mm Hg and the residue obtained was stored at 4°C. The residues were made in to a 1% stock solution with acetone (Stock solution) (Bagavan et al., 2009).

Culture of test animal

Filarial vector *Cx.quinquefasciatus* egg rafts were collected from stagnant sewage water of Thanjavur. The hatched larvae were culture and maintained in the laboratory at (27± 2°C room temperature and 75-85% relative humidity). The larvae were fed with ad libitum dog biscuit and yeast powder in the ratio 3:1. Adults emerged from these larvae were reared in mosquito cage and the females were allowed fed avian (from pigeon) blood and the males provide 10% glucose solution socked in cotton. Sample from this parent population is used to confirm the species by the district entomologist of malarial control program, Thanjavur. Eggs laid by these adults were cultured in a separate container and larvae developed from these eggs were used for bioassay studies (Dass and Mariappan, 2014).

Larvicidal bioassay

The larvicidal bioassay was carried out by using standard WHO protocols (WHO 2005). 200 ml of tap water was taken in a series of 250 ml beakers. The test concentration was made from 50 ppm to 300 ppm with methanol extract of *Aegle marmelos, Coleus aromaticus* and *Vitex negundo*. A control was also maintained separately by adding 2 ml of acetone to 200 ml of water. Since acetone was used as solvent to dissolve the extract. 10 larvae per concentration were used for all the experiments. A 24 hours larval mortality data was obtained for different larval stages and pupa for the three plant extracts. There were no mortality recorded in control and the mortality data was analyzed by using Abbott's formula (Abbott's, 1925).

Statistical Analysis

The mortality data calculated following Abbott were analyzed by log-probit method of Finney (Finney 1971) using SPSS.16 (SPSS 2010). Adult emergence in relation to concentration of the plant extracts is analyzed through regression and the slope and the elevation of the regression lines were tested through ANCOVA (Snedor and Cochran).

Results

Susceptibility of filarial vector *Cx. quinquefasciatus* against the methanol leaf extract of *A. marmelos, C. aromaticus* and *V. negundo* were studied. The LC_{50} and LC_{90} value obtained in the study is presented in Table 1. The 24 hrs LC_{50} value for II, III, IV instars and pupa in the plant *A. marmelos,* it is 91.52ppm, 105.16 ppm, 151.43 ppm and 203.78 ppm for II, III, IV and pupa respectively. In the case of *C. aromaticus* plant extract used, the LC_{50} value is 137.77 ppm for II instar, 175 ppm for III instar, 188.36 ppm for IV instar and 221.04 ppm for pupa. Likewise for *V. negundo* are 66.31 ppm, 74.08 ppm, 84.36 ppm and 133.37 ppm respectively. Similarly 24 hrs LC_{90} value for II, III, IV instars and pupa in the plant *A. marmelos* is 215.61ppm, 203.47 ppm, 203.6ppm and 304.91ppm respectively. Likewise *C.aromaticus* it is 243.99 ppm, 306.31ppm, 337.18 ppm, 355.35ppm. *V. negundo* is 168ppm, 176.28 ppm, 200.74 ppm, 238.06 ppm.

From this study, it is know that II instar larvae is more susceptible for plant extracts than other and the pupa is most tolerant to plant extract. Of these three plants tested *V. negundo* is more toxic to *Cx. quinquefasciatus* larvae. The relationship between dose dependent adult emergences is studied and the results indicate that irrespective of the plant extract the adult emergence is reduced while the concentration of plant extracts studied. ANCOVA indicates there is no difference ($P>0.05$) ($F = 2.93$) in the dose dependent adult emergence among the three plant extracts used (Figure.1).

Discussion

Naturally occurring insecticides of plant origin play a critical role in controlling mosquitoes

(Wandscheer et al., 2004). A study made Kaushik and Saini (2008) indicate that *Millingtonia hortensis* leaf extract is effective against *An. stepensi, Ae. aegypti* and *Cx. quinquefasciatus* larva. *Balanites aegyptica L.* (Simaroubaceae), *Nyctanthes arbortristis L.* (Oleaceae), *Plumbago zeylanica L.*(Plumbaginaceae) extracts were tested against IV instar larva of *Ae. aegypti* and *An. stepensi* where dichloromethane solvent extract was most effective than other solvents used (Patil et al., 2010). *Andographis paniculata* (Acanthaceae) leaf extract was also tested and promoting was obtained against *An. stepensi* the all the larvae stages and pupa (Kuppusamy and Murugan, 2009).

In the present study also *V. negundo* methanol extract shows as effective larvicide against *Cx. quinquefasciatus*. The finding of the present study reveals that the use of crude extract of *Vitex negundo* larvicide against *Cx. quinquefasciatus* as a potentential larvicide and isolation of active principle from these plants will help to control mosquitoes population.

Conclusion

Larvicidal activity of *A. marmelos, C. aromaticus* and *V. negundo,* against II, III, IV and pupa of *Cx. quinquefasciatus* was studied. It is observed that *V. negundo* is more toxic against *Cx. quinquefasciatus* than *A. marmelos* and *C. aromaticus*. The adult emergence is decreased when the concentration of plant extract is increased.

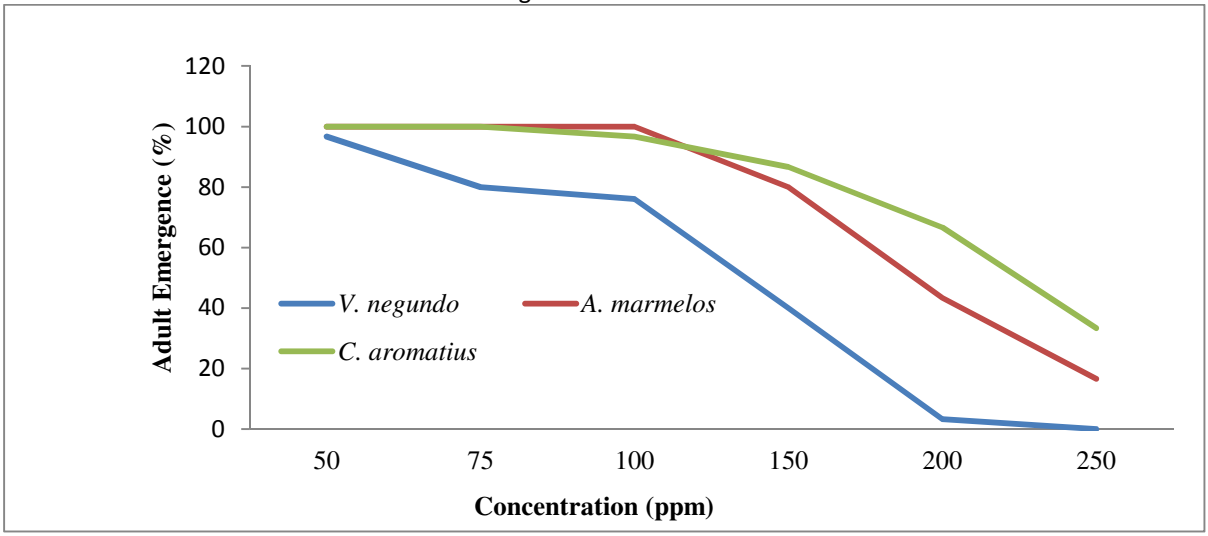

Figure 1. Relationship between concentration of the plant extracts and adult emergence of *Culex quinquefasciatus (Vitex negundo*: y =135.003 -0.44x; *Aegle marmelos* : y =132.66 - 0.3x ; *Coleus aromaticus* y=125.37- 0.59x).

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