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Synergistic and individual efficacy of certain plant extracts against dengue vector mosquito, *Aedes aegypti.*

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ABSTRACT

Mosquitoes are responsible for transmitting pathogens of many diseases such as malaria, filariasis, dengue haemorrhagic fever and chikunguinya than any other group of arthropods. The present study was undertaken to evaluate larvicidal efficacy of *Vitex negundo*, *Clerodendrum inerme* and *Gliricidia sepium* individually and synergistic activities combined with *Pongamia glabra* seed extract against early fourth instar dengue vector mosquito, *Aedes aegypti*. In the acute toxicity tests against the early fourth instar *A. aegypti* larval mortality increased with increased concentrations of the extract. The results revealed that, *C. inerme* found to be more toxic plant when tested individually. The maximum synergistic activities were found in the combination extracts of *V. negundo* with *P. glabra* (50: 50). These results are significantly effective than the combination extract ratio of *C. inerme* with *P. glabra* (50:50) and *G. sepium* with *P. glabra* (50:50) followed by other combinations with concentrations. The results of study revealed that *Vitex negundo, Clerodendrum inerme* plant leaves individual and/or combinations with extracts of *P. glabra* can be used as potent source of natural mosquito larvicidal agent.

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INTRODUCTION

Mosquitoes are important arthropods, transmitting diseases like malaria, filariasis, Japanese encephalitis and dengue haemorrhagic fever and have potential to feed on more than one individual during a single gonotropic cycle (Mackenzie *et al*., 2004; Govindarajan and Sivakumar, 2014). There are currently more than 3000 mosquito species in the world grouped in 39 genera and 135 subgenera. Among all subgenera mosquito, *Aedes aegypti* is a very important diseases transmitting vector, causing dengue hemorrhagic fever (DHF) and chikungunya in human (Ghosh *et al.,* 2012). Recently the dengue fever virus is found in the patients of Maharashtra, Karnataka, Kerala, Tamil Nadu and Andra Pradesh states severely. Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti* and at present, there is no effective vaccines are available for dengue virus control. Therefore, the way of reducing the incidence of this disease is by mosquito control, which is frequently dependent on applications of conventional insecticides (Remia and Logaswamy, 2010; Tabanca *et. al*., 2013). Proper control of mosquitoes lies in personal protection and public awareness as the most economical method in eradicating breeding sites and controlling these through environment friendly larvicides (Vadeyar *et al.,* 2010; Arivoli *et al.,* 2011; Poopathi, 2012; Ghosh *et al.,* 2012; Govindarajan and Sivakumar, 2014; Patil *et al.,* 2014). To overcome these problems, major emphasis has been made on the use of natural products as larvicidal means, which can provide an alternate to synthetic insecticides (Junwei *et al*., 2006). Many plants have been found to contain chemicals which are helpful for the control of insects (Robert, 2001) and are useful for field applications in mosquito control programmes (Sethuraman *et al.,* 2010; Yankanchi and Patil, 2010; Arivoli *et al.,* 2011; Palanisamy *et al.,* 2012; Muthu *et al.,* 2012; Patil *et al.,* 2014).

The pharmacological and insecticidal properties of plants have been recognized in many parts of the world, especially in India, where plant materials are easily available and their use in health practices is a tradition. The *Vitex negundo* L. (Verbenaceae) grows gregariously in wastelands and is also as a hedge-plant. Phytochemical studies on *Vitex negundo* have afforded several types of compounds, such as volatile oils lignans, Flavonoids, iridoids, terpenes, (triterpenes, diterpenes, sesquiterpenes) and steroids (Meena *et al.,* 2010).

The genus *Clerodendrum* (Verbenaceae) contains many plant species that are being used in various health care systems for the treatment of various disorders including life threatening diseases (Yankanchi and Koli, 2010). The *Clerodendrum inerme* L. Gaertn is a biennial hardy plant and grown as a hedge plant along home gardens throughout the India. The leaf powder of *C. inerme* reported to be insecticidal and growth inhibitory activities of against *A. aegypti* larvae and the ether extract was found to be insecticidal properties against *H. armigera* and *P. xylostella* in field conditions (Patil *et al.,* 2006; Yankanchi and Patil, 2009). The literature revealed that, selected plants extracts were used individual for insect pests control including mosquitoes also. However, there was inadequate information on selected plants extract combined with *Pongamia glabra.* Therefore, present study was undertaken to evaluate larvicidal efficacy of *Vitex negundo, Clerodendrum inerme* and *Gliricidia sepium* individually and synergistic activities combined with *Pongamia glabra* seed extract against dengue vector mosquito, *Aedes aegypti*.

MATERIALS AND METHODS

Insects

The mosquito larvae, *Aedes aegypti* were collected from the in and around the Kolhapur city vicinity. Insects were maintained in the laboratory conditions at 75±5% relative humidity, 27± 2°C temperature and 14L:10D. The larvae were fed on a powdered mixture of dog biscuits (Pedigree Pet food, Hyderabad) and yeast (Beakers yeast) tablets and in the ratio of 3:1. The emerged adults were fed with rat blood and with 10% glucose solution (Patil *et al.,* 2014).

Collection of plant materials and extraction

The plant leaves of *V. negundo, C. inerme* and *G. sepium*, and seeds of *P. glabra* were collected from foothill of Western Ghats area of Kolhapur district. Plant materials were shade dried at room temperature and powdered coarsely and extracted with petroleum ether (BP 60-80°C) in the soxhlet apparatus for 8-10 hrs (500 mL of petroleum ether for 100 g) according to Karmegam *et al.* (1997) method. The weighed quantity of the plant material was reduced to a viscous dark green residue and the crude extracts were further concentrated to paste.

Preparation of stock solutions

Two hundred and fifty milligrams (250 mg) of the crude extract was placed in a standard measuring flask and dissolved in 4.9mL of acetone and 0.1 mL of Tween 80 was added as an emulsifier. This mixture was made up to 250 mL using distilled water to prepare the stock solution. One milligram of extract in 1 mL of water was considered to be the 1000 ppm solution (Karmegam *et al.,* 1997). A mixture of 4.9mL of acetone and 0.1 mL of Tween 80 was made up to 250 mL in a standard measuring flask by adding distilled water to serve as the control solution.

Bioassay

From the stock solution, the following test concentrations *viz.* 500, 400, 300, 200 and 100 ppm solutions were prepared. The laboratory colonized twenty five (0-12 h) fourth instar larvae of *A. aegypti* were introduced in to each plant test solutions with four replicates and control was also run simultaneously (WHO, 2005). The results were observed at 24 hrs and the percentage mortality was corrected using the Abbott's (1925) formula. The LC_{50} values were calculated using the computation program of probit analysis (Finney, 1971). Results with $P<0.05$ were considered to be statistically significant. Since *C. inerme* showed high toxicity to larvae (LC_{50} values 292 ppm) so this concentration was considered as 100% and used for study the synergistic activities of three plants along with *P. glabra* extracts. The combinations of *V. negundo* and *P. glabra* combined in the ratios of V 75%: P

25%, V 50%: P 50% and V 25%: P 75%, *C. inerme* and *P. glabra* combined in the ratios of C 75%: P 25%, C 50%: P 50% and C 25%: P 75%, and *G. sepium* and *P. glabra* combined in the ratios of G 75%: P 25%, G 50%: P 50% and G 25%: P 75% prepared respectively. The synergistic factor (SF) was calculated using the formula of Kalyanasundaram and Das (1985).

$$SF = \frac{LC_{50} \text{ value of the insecticide alone}}{LC_{50} \text{ value of the insecticide with the assumed synergist}}$$

(Values of SF > 1 indicate synergism and SF < 1 indicate antagonism).

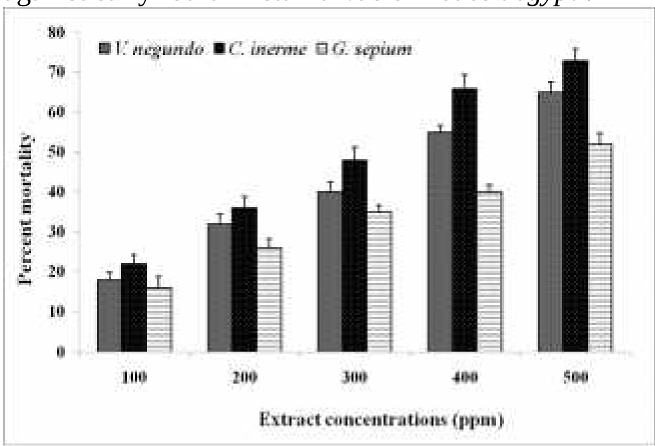
RESULTS AND DISCUSSION

The 24 h bioassay study of *V. negundo, C. inerme* and *G. sepium* at (500, 400, 300, 200 and 100 ppm) extract against early forth instar *A. aegypti.* The *C. inerme* extract showed highest toxicity having an LC_{50} value 292 ppm and *G. sepium* showed low toxicity having a highest LC_{50} value of 564 ppm. The plant crude extracts showed dose-dependent mortality of *A. aegypti* larvae (Fig.1). The LC_{50} value with other associated values such as 95% fiducial limit, regression equation and chi-squire were given in Table 1.The extract combinations of V 50% and P 50%, C 50% and P 50% found to be more toxic with less LC_{50} value 191 and 195 ppm respectively. The combinations of G 50% and P 50% produced less mortality with an LC_{50} value 328 ppm as compared to other combinations with all plant extracts. The extracts combination of V 50% and P 50%, C 50% and P 50% revealed maximum synergistic activity followed by other combinations, the *Pongamia* extract being considered as the synergist in the present study (Table 2).

The several plants possessing biological activities suggest that plants are of worth consideration with all the control strategies involved in vector control in terms of feasibility, cost effectiveness and easy availability of plant resources (Kumar *et al.,* 2012). On the other hand, selection of solvent system for extraction form plants material has impact on resultant efficacy and previous reports on the toxicity evaluation against vector mosquitoes showed that petroleum ether was effective than other solvents from plant extraction (Yankanchi, 2009; Sakthivadivel *et al.,* 2012). It is possible that the active components responsible for different bioactivities are extracted in greater measure only with certain specific solvents, and such specificity could be due to the polarity range of the solvents used for extraction. The 24 hrs bioassay is a major tool for evaluating the toxicity of phytotoxins, and a number of researchers have been applying this method to assess the toxic effect of different plant extracts on mosquitoes (Remia and Logaswamy, 2010; Sakthivadivel *et al.,* 2012; Patil *et al.,* 2014).

In the acute toxicity tests against the early fourth instar *A. aegypti* larval mortality increased with increased concentrations of the extracts (Fig. 1) and similar trend was observed by several workers with botanicals (Sakthivadivel *et al.,* 2012; Kumar *et al.,* 2012; Patil *et al.,* 2014). On the exposure to high concentrations, the larval body retraction accompanied with a sluggish behavior, loss of equilibrium and inability come on surface was observed. The mortality of larvae subjected to the extracts was attributed to the presence of feeding inhibition and/or neurotoxic compounds in the extract. The synergistic effect of various control agents have proved very effective in the control of various pests (Caraballo, 2000; Seyoum *et al.,* 2002). The *Pongamia* extract has been considered as a good synergist and so, it has been using in pest control agents for the control of various insect pests (Narasimhan *et al.,* 1998; Pathak and Shukla, 1998).

Fig 1. Percent mortality of selected plant extracts against early fourth instar larvae of *Aedes aegypti.*



It has been reported that, continuous use of synthetic insecticides caused insect pests to develop resistance in due course of time and it is often more economical to use synergists. The synergistic mixture can be incorporated into mosquito control programmes so as to avoid indiscriminate use of neem in blanket sprays. This type of approach will minimize the problem of induction of resistance in the mosquito population and will apparently continue to render the extracts effective for many years as insect pest control agents. The efficacy studies on mosquitoes susceptibility to different extracts, it was concluded that the various species of mosquitoes showed differential susceptibility to different extracts as reported earlier (Pathak *et al.,* 2000). Nevertheless, the synergistic mixture proved to be toxic to the larvae of *A. aegypti* and therefore the search for an alternative effective biopesticide is inevitable. The present results clearly indicated that better alternative and an effective substitute in the form of a synergistic mixture of *V. negundo* and *C. inerme* with *P. glabra* extracts, to the *A. indica* extract which has been widely acknowledged and currently available as a well known biopesticides.

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Table 1. Bioassay studies on the early fourth instar larvae of *Aedes aegypti* against petroleum ether extracts of selected plants.

Name of extract	Combination of extract	LC_{50} (ppm)	Fiducial limits		Regression equation	Chi-squire value (χ^2)
			Upper	Lower		
Vitex negundo	V 100%	364.04	308.03	455.42	Y = 0.9408 + 1.5850 X	2.46
Pongamia glabra	P 100%	306.35	260.56	369.16	Y = 1.0162 + 1.6017 X	1.91
V. negundo & *P. glabra*	V 75% & P 25%	286.40	198.67	440.80	Y = 0.3136 + 1.9099 X	6.21
V. negundo & *P. glabra*	V 50% & P 50%	191.73	95.50	271.62	Y = 0.7372 + 1.8861 X	5.39
V. negundo & *P. glabra*	V 25% & P 75%	259.08	173.28	378.59	Y = 0.5947 + 1.8267 X	5.70
Clerodendrum inerme	C 100%	292.36	258.83	332.60	Y = 0.3642 + 2.1772 X	2.16
Pongamia glabra	P 100%	306.35	260.56	369.16	Y = 1.0162 + 1.6017 X	1.91
C. inerme & *P. glabra*	C 75% & P 25%	249.85	218.38	283.74	Y = 0.0609 + 2.1113 X	0.92
C. inerme & *P. glabra*	C 50% & P 50%	195.02	163.91	224.32	Y = 0.4769 + 1.9756 X	1.69
C. inerme & *P. glabra*	C 25% & P 75%	225.37	192.49	258.81	Y = 0.4898 + 1.9198 X	1.99
Glericidia sepium	G 100%	564.28	441.74	875.93	Y = 1.1453 + 1.3968 X	2.60
Pongamia glabra	P 100%	306.35	260.56	369.16	Y = 1.0162+1.6017 X	1.91
G. sepium & *P. glabra*	G 75% & P 25%	488.19	402.65	660.91	Y = 0.6526 + 1.6123 X	4.60
G. sepium & *P. glabra*	G 50% & P 50%	328.72	237.99	541.33	Y = 0.2268 + 2.0781 X	6.98
G. sepium & *P. glabra*	G 25% & P 75%	377.47	269.44	788.40	Y = 0.3929 + 1.7861 X	6.17

Table 2. SF values of combined extracts of selected plants and *Pongamia* against early fourth instar larvae of *Aedes aegypti*.

Name of extract	Combination of extract	LC_{50} (ppm)	Synergistic factor (SF)	Effect
Vitex negundo	V 100%	364.04	---	---
Pongamia glabra	P 100%	306.35	---	---
V. negundo & *P. glabra*	V 75% & P 25%	286.40	1.27	Synergism
V. negundo & *P. glabra*	V 50% & P 50%	191.73	1.90	Synergism
V. negundo & *P. glabra*	V 25% & P 75%	259.08	1.40	Synergism
Clerodendrum inerme	C 100%	292.36	---	---
Pongamia glabra	P 100%	306.35	---	---
C. inerme & *P. glabra*	C 75% & P 25%	249.85	1.17	Synergism
C. inerme & *P. glabra*	C 50% & P 50%	195.02	1.50	Synergism
C. inerme & *P. glabra*	C 25% & P 75%	225.37	1.30	Synergism
Glericidia sepium	G 100%	564.28	---	---
Pongamia glabra	P 100%	306.35	---	---
G. sepium & *P. glabra*	G 75% & P 25%	488.19	1.16	Synergism
G. sepium & *P. glabra*	G 50% & P 50%	328.72	1.72	Synergism
G. sepium & *P. glabra*	G 25% & P 75%	377.47	1.50	Synergism

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