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Larvicidal Activity of Some Plant Extracts against Mosquitoes

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Abstract-- Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis. Malaria is one of the most important causes of direct or indirect infant, child and adult mortality with approximately two to three million new cases arising every year, in the tropics, in general and India, in particular. There is a need for more effective antimalaria drugs with broad host specificity. Medicinal plants have been known to have antimalarial activity; however there is lack of data as well as research of the same. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques. Although several plants have been reported for mosquitocidal activity, only a few plants are in actual use. The present work was aimed at finding the antimalarial activity of some medicinal plants like Momoradica charantia, Glyricidia sepium, Vitex neegundo, Gardenia jasminoides, Carica papaya, Annona reticulata L. and Chrysanthemum indicum L. The medicinal properties of these plants are known but the larvicidal activity has not been reported. The extracts of these plants were checked for larvicidal activity and the percentage mortality was calculated.

I. INTRODUCTION

Vector borne diseases are among the major causes of illness and death in many developing countries. Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. WHO has declared the mosquitoes as "public enemy number one" (WHO, 1996). Mosquito born diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population. They are nuisance to human beings and are the major vector for the transmission of life threatening diseases like malaria, dengue fever, yellow fever, chikunguniya fever, lymphatic filariasis, and Japaneses encephalitis ((Anupam Ghosh et al., 2012). In India malaria is one of the most important cause of direct and indirect infant, child and adult mortality with approximately two to three million new cases arising every year. Malaria is transmitted through bites of parasite -infected Anopheles mosquitoes. Anopheles is the major malarial vector in India. To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But mosquitoes have developed resistance against them. Though larvicides play a vital role in controlling mosquitoes in their breeding sites, these also show a negative impact in areas of beneficial and non-target organisms. Thus, synthetic insecticides have created several problems including the development of resistant insect strains, ecological imbalance and harm to mammals. Due to these drawbacks researchers are working hard to find biodegradable, target-specific and environmentally safe alternatives (Sritabutra et al., 2011; Luz Stella Nerio et al., 2010).

Plant products have been used in many parts of the world against the vectors and species of insects. Recent studies

stimulated the investigation of insecticidal properties of plantderived extracts such as Ocimum santum, Azadirachta indica, Lantana camera, Vitex negunda and Cleome viscose, Dalbergia sissoo and Mentha piperita etc and concluded that they are environmentally safe, degradable and target specific. (Senthil Nathan and Kalaivani, 2005).

Though larvicides play a vital role in controlling mosquitoes in their breeding sites, these also show a negative impact in areas of beneficial and non-target organisms. In view of an increasing interest in developing plant origin insecticides as an alternative to chemical insecticides, this study was undertaken to assess the larvicidal potential of the extracts of some medicinal plants against malaria vector.

II. MATERIALS AND METHODS

A. Collection of plant materials

The leaves of six locally available plant species such as Momoradica charanti (Cucurbitaceae), Vitex neegundo (Lamiaceae), Gardenia jasminoides (Rubiaceae), Carica papaya (Caricaceae), Annona reticulate (Annonaceae) Chrysanthemum indicum (Asteraceae)) and flowers of only one species i.e. Glyricidia sepium (Fabaceae) were collected from different localities in Ratnagiri region and kept separately in the plastic bags and brought to the laboratory for the extraction and authenticated.

B. Preparation of plant extracts

The leaves and flowers of above mentioned plants were dried for 24 hrs in an electric oven at 60 °C. The dried leaves and flowers were powdered mechanically (10g) using electrical stainless steel blender and extracted with ethanol and methanol (30 ml) for 24 hrs. The extracts were filtered with Whatman number 1 filter paper. All these plants were extracted in a separate container in 80% ethanol and placed on a orbital shaker by maceration or cold extraction method. After leaving the methanolic solution to rest for an overnight, it was filtered through Whatman No. 1 filter paper and the filtrate was collected. This procedure was repeated three times with fresh volume of ethanol and methanol. The filtrates were pooled and the pooled extracts were concentrated using a vacuum evaporator below 450 C under low pressure and over water bath until the solvent completely evaporated. The extract of each plant thus obtained was lyophilized and then place in the freezer at -20OC until testing of mosquito larvicidal activity. Standard stock solutions were prepared at 1% and from the stock solution various concentrations were prepared.

C. Test mosquitoes

The larvae of Anopheles spp. were collected from the sewage water of Ratnagiri city and were kept in plastic and enamel trays containing tap water. They were maintained and all the experiments were carried out at $27\pm 2^{\circ}$ C and 75-85 % relative humidity.

D. Larvicidal bioassay

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The larval bioassay tests for the larvicidal effect of ethanolic and methanolic extracts derived from seven different plants were carried out in accordance with the WHO standard method (WHO, 1981, 2005). For the bioassay test, 1 ml of each plant extracts were taken and 3 dilutions were made in distilled water (1:25, 1:50 and 1:75). Then 10 larvae were taken in different test tubes containing 10 ml of above plant concentrations. The control was set up with methanol and ethanol. The numbers of dead larvae were counted after 24 h of exposure and the percentage of mortality was reported from the average of five replicates. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Dead larvae were removed as soon as they were discovered to prevent decomposition, which may cause rapid death of the remaining larvae.

The experimental setup is given bellow,

Table 1:

No.	Concentrations Control	
1	1ml plant extract + 25 ml Distilled water	1ml ethanol or Methanol + 25 ml Distilled water
2	1ml plant extract + 50 ml Distilled water	1ml ethanol or Methanol + 50 ml Distilled water
3	1ml plant extract + 75 ml Distilled water	1ml ethanol or Methanol +75 ml Distilled water

III. RESULTS

Table 2: Mortality of Anopheles sp. Larvae in methanol extracts.

Plant extract used	Common names	% Mortality in different Concentrations		
		1:25	1:50	1:75
Control (Methanol)		0	0	0
Gardenia jasminoides	Ananta	80	70	20
Glyricidia sepium	Undir mari	90	80	10
Chrysanthemum indicum	Shevanti	80	50	20
Annona reticulata	Ram-fal	100	100	70
Momoradica charantia	Karale	90	60	20
Vitex neegundo	Nirgundi	100	90	00
Carica papaya	Papaya	90	60	20

Table 3: Mortality of Anopheles sp. Larvae in ethanol extracts.

Plant extract used	Common names	% Mortality in different Concentrations		
		1:25	1:50	1:75
Control	Ethanol	0	0	0
Gardenia jasminoides	Ananta	80	50	10
Glyricidia sepium	Undir mari	100	10	10
Chrysanthemum indicum	Shevanti	90	20	0
Annona reticulata	Ram-fal	100	70	30
Momoradica charantia	Karale	90	20	0
Vitex neegundo	Nirgundi	100	30	10
Carica papaya,	Papaya	10	10	0

DISCUSSION

Nowadays, mosquito control is mostly directed against larvae and only against adults when necessary. This is because the flight against adult is temporary, unsatisfactory and polluting for the environment, while larval treatment is more localized in time and space resulting in less-dangerous outcomes. The vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either counter measures or development of newer insecticides (Chandre et al., 1998). The preliminary screening is a good mean of evaluation of the potential larvicidal activity of plants popularly used for this purpose.

The application of botanicals for the control of mosquitoes is recommended (Alkofahi et al., 1989). Because they minimize the accumulation of harmful residues in the environment. The crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors(Mayura et al., 2007). The activity of crude plant extracts is often attributed to the complex mixture of active compounds. In the preliminary screening, potential larvicidal activity of seven plants as crude extracts in different solvents was noted. All plant extracts showed moderate toxic effect on Anopheles spp. after 24 hrs. of exposure at different concentrations. 100% mortality was found in methanol extract of Annona reticulate leaf in 1:25 (0.0132ml) and 1:50 (0.0066ml) concentrations. 100% mortality was observed in methanol extract of Vitex neegundo leaf in 1:25 (0.0132ml) concentration.

In ethanol extract of Glyricidia sepium, Annona reticulate and Vitex neegundo leaves 100% mortality was observed in 1:25(0.0132ml) concentration. 50% mortality was observed in methanol extract of Chrysanthenum indicum leaves in 1:50 (0.0066ml) concentration. In ethanol extract of Gardenia jasminoides leaves, 50% mortality was seen in 1:50(0.0066ml) concentration.

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CONCLUSION

Our findings showed, that leaf extract of Annona reticulata, Vitex neguundo and Glyricidia sepium can be developed as ecofriendly larvicides. Also our results open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts. The present findings have important implications in the practical control of mosquito larvae and adults by using botanical extracts. These plant extracts are easy to prepare, inexpensive and safe for mosquito control which possess enough potential in larvicide and adulticide can be used directly as larvicidal and adulticidal agents in small volume aquatic habitats or in /around human dwellings. The results suggest for a possible utilization of the cheap and readily available botanicals for possible control of mosquitoes as part of an integrated vector management program.

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