LARVICIDAL ACTIVITIY OF *Phyllanthus debilis* PLANT EXTRACTS AGAINST *Culex quinquefasciatus*

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Abstract

Mosquitoes transmit serious human diseases, causing millions of deaths every year. Controlling mosquitoes at the larval stage is easy as target specificity of the larvicide used can be ensured. Plants may be alternative source of mosquito control agents. The Present study assessed the role of larvicidal activities of methanol (25, 50, 75, 100 mg) extracts of *Phyllanthus debilis* plant against early 4th instar larva of *Culex quinquefasciatus*. The larval mortality was observed after 24 h of exposure. The larvicidal effect of methanol extracts at lower concentrations the mortality was less, whereas at higher concentration the mortality rate increases. This study indicates that *Phyllanthus debilis* plant possesses larvicidal activity against *Culex quinquefasciatus* and may be a possible source of mosquito larvicides.

Keywords: *Culex quinquefasciatus* larvae, *Phyllanthus debilis* extracts, larvicidal activity, phytochemical analysis.

INTRODCUTION

Mosquitoes act as a vector for most of the life-threatening diseases like malaria, yellow fever, chikungunya fever, filariasis, encephalitis and many more. In both rural and urban areas mosquito menace are observed throughout the year. Mosquito borne diseases are still a problem, though personal prophylactic measures like repellent are widely used to escape from mosquito biting and thereby, prevent the mosquito borne diseases which impede national economic and social development (Komala Misra *et al.,* 2005). *Culex quinquefasciatus,* is a vector of lymphatic filariasis which is a widely distributed tropical disease, and there are nearly 1100 million people living in areas endemic for lymphatic filariasis and exposed to the risk of infection; there are 102 million cases of filariasis, either having patent micro filariae or chronic filarial disease. *C. quinquefasciatus* is an obligatory ectoparasitic vector since it plays a major role in the transmission of the nocturnal periodic form of *Bancroftian filariasis* all over the world (WHO, 1972).

Plants are rich source of alternative agents for control of mosquitoes, because they possess bio active chemicals, which act against limited number of species including specific target–insects and are ecofriendly. Traditionally plant-based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as a defense mechanism against insect attacks. Search for eco-safe, low cost and a highly potential insecticide for the control of mosquitoes needs the preliminary screening of plants to evaluate their insecticidal activities. (Shivakumar *et al.,* 2013). *Phyllanthus debilis* was found to have the higher larvicidal activity. The effect of the plant extract was reported to be dose dependent as evident by an increase in percent mortality with increasing concentrations and the dose dependency of the plant against mosquito larvae (Ranaweera, 1995). The present investigation was carried out to validate the larvicidal potential of different solvent extracts of four medicinal plants namely *Phyllanthus debilis* (Keelanelli) against mosquito larvae. This investigation is conducted to study the larvicidal effect. The assessment of plant for mosquito larval toxicity may help in the effective strategies for reduction of its population.

MATERIALS AND METHODS

Plant Material:

Healthy leaves of *Phyllanthus debilis* were selected on the basis of their easy availability, uncomplicated cultivation and possibility of commercialization. The plants were thoroughly washed with tap water in order to clean dust or any particles stuck to them. The plant leaves where observed carefully to find any kind of disease or infection and if found any were discarded. The selected leaves where kept for drying under shade at room temperature (27□2□c) for about 20 days till they dried completely. The dried leaves where mechanically grinded and sieved to get fine powder.

Preparation of extracts:

The 200g of each dried and powdered part was extracted with 1000 ml of alcohol using Soxhlet extraction apparatus for 24h at a temperature not exceeding the boiling point of the solvent.

The extracts were concentrated using a vacuum evaporator at 45□c under low pressure. After complete evaporation of the solvent the concentrated extract was collected and stored in a refrigerator at 4□c as the stock solution of 1000 ppm for further use.

Rearing of mosquitoes:

The eggs were collected in a bowl from Dept of Entomology, Loyola college, Chennai-34 and stagnant water surface around in Chengalpattu. The eggs were lined with Whatman filter paper and were allowed to hatch in trays filled with de-chlorined water. The mosquito larvae were collected from stagnant surface water of pools with the help of jar and stored in enamel trays containing tap water. They were maintained at 27□2□c temperature, 70□5□c relative humidity under 12:12 light and dark photo period cycle. The larvae were fed with the fresh food containing finely grounded dog biscuits and yeast extract in ratio of 3:2. If the pupae emerged were transferred to new trays containing tap water.

Larvicidal Assay:

In the larvicidal assay, fourth instar larvae of *Culex quinquefasciatus* were exposed to test concentrations of 25, 50,75,100 ppm of the extracts of the test plant namely *Phyllanthus debilis* in 100 ml of water. 100ml of tap water was taken in a series of 250 ml glass beakers. The measured amount of extracts was dissolved in 1ml of the solvent. A control was also maintained by adding 1ml of solvent to 100 ml water. 25 larvae per concentration were used for all the experiments. The number of dead larvae at the end of 24 h was recorded and the mortality percentage was calculated. This experiment was repeated three times.

RESULTS

The larvicidal activity of four plant namely, *Phyllanthus debilis* were tested against the larva of *Culex quinquefasciatus*. The 4th instar larvae of *Culex quinquefasciatus* were collected from Department of Zoology, Loyola college, Nungambakkam, Chennai, India. The plants were collected from in and around Chennai and shade dried. The dried plant samples were ground and sieved. The powdered samples were stored in polythene bags or plastic containers. The powdered sample (100 g) were extracted with methanol and water separately.

In the larvicidal assay, 4th instar larvae of *Culex quinquefasciatus* were exposed to test concentrations of 25, 50, 75 and 100 µg/mL of methanol extract and water extract of the four plants. Aliquots of 100 mL tap water were taken in plastic trays. The measured amounts of the plant extracts were added into water and dissolved, control with the solvents were maintained. Larval numbers of 25 per concentration was used for all the experiments. The number of dead larvae at the end of 24 h was recorded and the mortality percentage values were calculated. The experiment was repeated thrice.

The effect of plant extract on the development of the mosquito larvae was studied in two different extracts namely methanol and water. The table no 1 shows the dose dependent effect of extracts of *Phyllanthus debilis* on the mortality percentage of 4th instar larvae of *Culex quinquefasciatus*. After the 24 h time period the percentage mortality was compared with the control. In control the mortality was nil. At lower concentrations

the mortality was less, whereas at higher concentration the mortality rate increases. At 25 µg/mL concentration the mortality rate was 11 for methanol and 15 for water whereas at 100 µg/mL concentration the death rate was 87 and 98 for methanol and water respectively.

Table 1 - Effect of *Phyllanthus debilis* on *Culex quinquefasciatus* larvae.

Larvae	Extract	Concentration (µg/mL)				Control	IC^{50}
		25	50	75	100		
4th instar larva	Methanol	11	25	89	87	-	56
	Water	15	38	93	98		82

DISCUSSION

Mosquito borne diseases, such as malaria, filariasis and dengue fever are still major public health problems. Although chemical vector control programs have been carried on for long time, these mosquito vector diseases remain because of the development of resistance by the mosquito. And many of the house hold avoid spraying the chemicals due to allergic reaction among the inhabitants. Searching of new control agents from natural products such as plant secondary metabolites has gained popularity among researchers in countries with a strong herbal tradition and large numbers of plants have been reported to possess insecticidal activity (Komala Misra *et al*., 2005).

In the present study, the plant extracts expressed the presence of larvicidal activity in all the four plants tested namely *Phyllanthus debilis* was found to have the higher larvicidal activity. The effect of the plant extract was reported to be dose dependent as evident by an increase in percent mortality with increasing concentrations. Ranaweera (1995) reported the dose dependency of the plant against mosquito larvae.

A general behavioral change in the larvae of the mosquito was observed and it was seen that larvae slowly become inactive within few hours of treatment. Identification of various plant extract that have larvicidal potential activity against mosquito can be of advantage in reducing the problem of resistance and concern for the environmental safety. Previous studies of Ranaweera (1995) and Kumar and Venkatesulu (2012) showed that the essential oil of *Acorus calamus* rhizome can be used as a natural larvicidal agent against the larvae of filarial vector mosquito,

C. quinquefasciatus. Larval control of mosquito can reduce the population of the insect which could be transformed into reducing the burden of the disease.

This result is also comparable to earlier reports of Singh *et al.* (2003) who observed the larvicidal activity of *Ocimum canum* oil and *Culex quinquefasciatus* and *Anopheles stephensi*. The methanol extract of *Citrus sinensis* peel and the leaf and flower ethyl acetate extracts of *Ocimum canum* were tested against the larvae of *Anopheles stephensi* respectively (Kamaraj *et al*., 2008a) Karunamoorthi *et al*. (2008) reported the petroleum ether extracts of the leaves of *Vitex negundo* were evaluated for larvicidal activity against larval stage of *Culex tritaeniorhynchus*.

CONCLUSION

We can conclude from this study that the results of this experiment indicate that the extract of the plant *Phyllanthus debilis* can be used as potential larvicides in vector control programmes as field application of these extracts can be done. After further research into the potential of these plants they could be used to control the mosquito menace at larval stage by spraying in the breeding sites. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals for control of insect vectors.

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