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RESEARCH ARTICLE

LARVICIDAL ACTIVITY OF *MELIA DUBIA* L. LEAF EXTRACTS AGAINST FOURTH INSTAR LARVA OF *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Inflammatory present study is aimed to derive natural products of plant origin with insecticidal properties to control insect vectors. The aqueous, ethanol and methanol leaf extracts of the traditional mosquito repellant species, *Melia dubia* were evaluated against the fourth instar larvae of two mosquito species *viz., Aedes aegypti* and *Culex quinquefasciatus.* The percentage mortality of these two different mosquito species was tested after 24 hrs of exposure to different concentrations of the leaf extract. The methanolic leaf extracts showed 100% mortality at 150 ppm and the aqueous and ethanol extracts showed 65% and 75% mortality respectively. Therefore it is known that the extracts of *M. dubia* have prominent mosquito control property.

INTRODUCTION

Mosquitoes are the major public health problem throughout the world. Among the 3492 species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases in human and other vertebrates (Rueda, 2008). Mosquitoes transmit malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis and chikungunya to humans (Nour *et al.,* 2009). Mosquito-borne diseases contribute significantly to disease burden, death, poverty and social debility all over the world, particularly in tropical countries. Among these diseases, malaria remains the most serious vector-borne disease affecting some 300-500 million people and 1.4 to 2.6 million deaths annually throughout the world. More than 40% of the world population lives in areas prone to malaria (Ghai and Gupta, 2000). Dengue fever can manifest as the classic form of the diseases, which debilitates the patient for a week or more, or as the haemorrhagic form which, in many cases leads to death (Neves-Filho *et al.,* 2009). Chikungunya virus, a member of alpha virus genus is of considerable public health concern in South-east Asian and African countries (Pastorino *et al.,* 2005). Mosquitoes in the larval stage are attractive target for control operation due to their low mobility in the breeding habitats and ease to control in these habitats (Howard *et al.,* 2007). Measures to control the mosquito form an essential component of diseases prevention programs in developing countries.

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Due to increasing resistance of mosquitoes to the insecticides, Lima *et al.* (2006) has focused interest on alternative compounds for mosquito control. Plants, being a natural source of various compounds are known to contain larvicidal agents which may act in combination or independently. According to Ghayal *et al.* (2010) phytochemicals act as general toxicants both against the adult as well as larval stages of mosquitoes, while others interfere with the growth and development and reproduction, produce olfactory stimuli action as a repellent or attractant. Natural products are best option because they are less harmful to environment and non-target organisms. Several extracts and compounds from different plant families have been evaluated for new and promising larvicides (Ester *et al.,* 2008). Researchers have proved the effectiveness of plant derived secondary compounds, such as saponin (Wiseman *et al.,* 2005), steroids (Chowdhurry *et al.,* 2008), isoflavonoids (Joseph *et al.,* 2004), essential oil (Cavalcanti *et al.,* 2004), alkaloids and tannins (Khana *et al.,* 2007) as mosquito larvicides. *Melia dubia* of Meliaceae family an important traditional mosquito repellant plant used by Irula tribals of Nilgiris, the Western Ghats, India (Venkatachalapathi et al., 2015). To validate the species scientifically its extracts were tested for larvicidal activity against the fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus.*

MATERIALS AND METHODS

Plant collection and extraction

The *Melia dubia* plant was collected in Maruthamalai hills, of Western Ghats, Tamil Nadu. The dried plant was powdered

and sieved to get fine powder using an electric blender. Seventy gram of the powder was filled in the thimble and extracted successively with water, ethanol and methanol using soxhlet extractor for 10 hrs. All the extracts were concentrated using rotary flash evaporator and preserved at 5°C in an airtight bottle until further use.

Larvicidal bioassay

Larvicidal activity was evaluated in accordance to WHO for the evaluation of new larvicidal agents (WHO, 2005). The larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were obtained and reared from the neonates in National Institute of Communicable Diseases, Southern India branch field station located at Mettupalayam, (Coimbatore), at 28 ± 2˚ C with a photoperiod of 12 hours light and dark and 80 ± 10% RH. A brewer's yeast powder mixed with an equal quantity (W/W) of ground dog biscuit is used in laboratory as a food for larvae. The fourth instar larvae were collected according to larval size and degree of chitinization of respiratory siphon (Cheng *et al.,* 2003). Different concentrations ranging from 25 to 150 ppm were prepared in 200 ml beaker and 25 fourth instar larvae were released in it and mortality as scored after 24 h. The beakers were kept in a temperature control room at 28°C±2°C. Each treatment was replicated five times (Tonk *et al.,* 2006). The corrected mortality was determined using Abbott's formula whenever required (1925). The dose mortality data was analyzed by log Probit – method of Finney (1971) and lethal concentration for 50 % and 90 % mortality were calculated (LC_{50} and LC_{90}).

Statistical analysis

The average larval mortality data was subjected to probit analysis for calculating LC_{50} and LC_{90} values and their statistics at 95% confidence limits of upper confidence limit, lower confidence limit and chi-square values were calculated using the SPSS 11.5 (Statistical Package of Social Sciences) software. Results with $P< 0.05$ were considered to be statistically significant.

RESULTS

The data based on the probit analysis between the concentrations of plant extract against fourth instar larvae of *Ae. Aegypti* and *Cx. quinquefasciatus* after 24 hrs exposure are represented in Table 1and 2. The results clearly indicate that the methanolic leaf extract of this species was found to be more potent against *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} and LC_{90} value of 39.56 and 118.68 ppm, and 35.39 ppm and 107.52 ppm respectively. Methanolic extract of *Melia dubia* showed 100% mortality at 150 ppm against the fourth instar larvae of the two tested mosquitoes, *Ae. aegypti* and *Cx. quinquefasciatus.* The ethanolic extracts showed 75% mortality and aqueous extracts showed 65% at 150 ppm against the fourth instar larvae of these two mosquito species. LC_{50} and LC_{90} values were 50.19 ppm and 155.68 ppm against *Cx. quinquefasciatus* and 60.98 ppm and 181.92 ppm against *Ae. Aegypti.* The aqueous extracts also showed mosquito larvicidal activity relatively at high concentration in comparison to that of methanol and ethanol extracts.

Table 1. Larvicidal activity of plant extracts of *Melia dubia* against fourth instar larvae of *Aedes aegypti*

Extract	Concentration (ppm)	Mortality (%) after 24hr	LC50 (UCL–LCL) (ppm)	LC90 (UCL–LCL) (ppm)
Aqueous	25	20	98.21	294.25
	50	25		
	75	50		
	100	55		
	150	65		
Ethanol	25	25	60.98	181.92
	50	40		
	75	55		
	100	65		
	150	75		
Methanol	25	40	39.56	118.68
	50	55		
	75	65		
	100	85		
	150	100		

Table 2. Larvicidal activity of plant extracts of *Melia dubia* against fourth instar larvae of *Culex quinquefasciatus*

Extract	Concentration (ppm)	Mortality (%) after 24hr	LC50 (UCL–LCL) (ppm)	LC90 (UCL–LCL) (ppm)
Aqueous	25	20	95.58	244.21
	50	25		
	75	50		
	100	55		
	150	65		
Ethanol	25	25	50.19	155.68
	50	40		
	75	55		
	100	65		
	150	75		
Methanol	25	40	35.39	107.52
	50	55		
	75	65		
	100	85		
	150	100		

DISCUSSION

Mosquito larval control using larvicidal agents is a major component in the control of vector borne diseases. Plant as potential larvicides is considered as viable and alternative source in the control of the mosquito species at community level. A large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vectors, but few plant products have shown practical utility for mosquito control (Sun *et al.,* 2006). In the present study methanolic leaf extract of *M. dubia* showed 100% larvicidal activity against the fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus.* Terpinoids and triterpenoids are generally credited with mosquito larvicidal activities (Gbolade, 2000, Venkatachalam, 2011, Ester *et al.,* 2008). As these two groups of secondary metabolites were largely in *M. dubia,* they may play major role in larvicidal activity against the two mosquitoes studied (Nagalakshmi et al., 2001). The terpenic compounds, mainly precocenes, with their antijuvenile hormonal activity are probably responsible for the insecticidal properties.

Conclusion

It is evident that the plant products are emerging as a potential source of mosquito control. Crude extract or isolated bioactive compounds from the plant *M. dubia* could be used in stagnant water bodies which are known to be the breeding grounds for the mosquitoes. The *M. dubia* extracts showed promising activity in mosquito control and its commercial utilization is very much feasible.

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