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ADULTICIDAL AND OVICIDAL ACTIVITIES OF *RHODOMYRTUS TOMENTOSA* LEAF EXTRACTS AGAINST DENGUE VECTOR *AEDES AEGYPTI*

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ABSTRACT

Mosquitoes has developed the resistance to synthetic insecticides, unfavorable effects to human and animal health, beneficial organisms and the environment, there is an urgent need to develop new insecticides of botanical based, which are suitable alternative bio control techniques with effective, safe, biodegrable and target-specific in the future. The adulticidal activities of crude hexane, acetone, and methanol extracts of the leaf of *Rhodomyrtus tomentosa* were assayed for their toxicity against dengue, chikungunya and zika virus vector, *Aedes aegypti*, the adult mortality was observed after 24 h of exposure. All extracts showed moderate adulticidal effects; however, the highest adult mortality observed was found in methanol extract. The LC_{50} and LC_{90} values of *R. tomentosa* leaf extracts against adulticidal activity of (acetone methanol and aqueous) *A. aegypti,* were the following: LC_{50} values were 113.09 ppm for (RTAE), 106.38 ppm for (RTME) and 125.25 ppm for (RTAQE); LC_{90} values were 251.18 for (RTAE), 229.94 for (RTME) ppm and 273.38 ppm for (RTAQE), respectively. The results of the ovicidal activity of acetone and methanol extracts of *R. tomentosa* plant at six different concentrations of 60-420 ppm were applied on eggs of *A. aegypti*. The toxicity of leaf extracts was dependent on its concentration against *A. aegypti*. There was zero hatchability (100% mortality) was attained at the concentration of 240 ppm for methanol extract and 300 ppm acetone extract and 360 ppm for aqueous extract. In this observation, this plant crude extracts gave the potential to be used as an ideal eco-friendly approach for the control of mosquitoes. This is the first report on mosquito adulticidal and ovicidal activities of the tribal medicinal plant, *R. tomentosa* against mosquito vectors from Southern India.

Keywords: Insecticide, Vector borne diseases, Eco friendly, Public health, Crude extracts.

INTRODUCTION

Mosquito-borne diseases, such as malaria, Japanese encephalitis, filariasis, yellow fever, dengue and zika remain a major source of illness and death worldwide, particularly in tropical and subtropical countries (Becker *et al*., 2003). Dengue is a mosquito-borne flavivirus found in tropical and sub-tropical regions of the world, mostly in urban and semi-urban regions. Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and, to a lesser extent, *Ae. albopictus*. This mosquito's also can transmit chikungunya, yellow fever and Zika infection (WHO, 2015). *Aedes aegypti*, the primary carrier for viruses that cause dengue fever, dengue haemorrhagic fever, chikungunya fever, and yellow fever is widespread over large areas of the tropics and subtropics (Kasinathan *et al*., 2018; Service & Youdeowei, 1983; Yang *et al*.,2004). It is the fastest spreading vector borne viral disease and is now endemic in over 100 countries, resulting in 40% of the world's population living in an area at risk for dengue. Dengue fever / dengue hemorrhagic fever (DHF) continues to be of major public health importance in countries of the Western Pacific and Southeast Asia. It is caused by one of four distinct serotypes (DEN -1, DEN - 2, DEN - 3 and DEN - 4) reported by WHO, (2016). The chemical based insecticides are play a very important role in controlling mosquito vector in their breeding sites, but this kind of control methods confirmed a negative impact in areas of beneficial and non target organisms and still mosquito vectors resistance to synthetic insecticides. In the view of the

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recently increased interest in developing plant origin insecticides as an alternative to synthetic insecticides (Amer & Mehlhorn, 2006; Wattanachai & Tintanon, 1999a). The environmental safety and low cost insecticide is considered to be of paramount importance and should not cause mortality on non-target organism in order to be acceptable (Kabaru & Gichia, 2001). However, the environmental threat that these chemicals pose, effects on non–target organisms, and the resistance of mosquitoes to insecticides have all increased during the last five decades (Amer & Mehlhorn, 2006; Wattanachai & Tintanon, 1999).

Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans, and other advantages (Liu *et al*., 2000). Many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides. Plants may be an alternative source of mosquito control agents because they constitute a rich source of bioactive chemicals (Kamaraj *et al*., 2010; Tiwary *et al*., 2007).

Rhodomyrtus tomentosa (Aiton) Hassk is a small shrub belonging to the Myrtaceae family and this herbal plants that are commonly used in traditional medicine in Southeast Asia (Winotai *et al*., 2005). *R. tomentosa* have been recognized by several researchers. All parts of this plant (leaves, roots, buds, and fruits) have been often used in traditional medicine to treat colic diarrhoea (Ong & Nordiana, 1999), dysentery, abscesses, haemorrhage, and gynecopathy. In addition, it is used to formulate skin-whitening, antiaging, and skin beautifying agents (Miyake & Nojima, 2006). The extract from this plant possess strong inhibitory activity against gram-positive bacteria (Limsuwan *et al*., 2009; Saising *et al*., 2008), antimicrobial properties (Limsuwan & Voravuthikunchai, 2008; Sianglum *et al*., 2011; Voravuthikunchai *et al*., 2010) the Larvicidal and Pupicidal (Kasinathan *et al*.,2018). The *R. tomentosa* plant part has been employed in traditional medicine to treat colic diarrhoea, haemorrhage, dysentery, abscesses, and gynaecopathy (Kanner *et al*., 1994; Lavanya *et al*., 2012; Sanchez moreno, 2002; Vinson & Hontz, 1995). No data regarding its adulticidal properties are available. Hence, the present investigation was aimed to explore the adulticidal and ovicidal activity of the extracts (ethanol, methanol and aqueous) from *Rhodomyrtus tomentosa* leaves against dengue and zika virus vector, *Aedes aegypti* under the laboratory condition.

MATERIAL AND METHODS

Collection of eggs Maintenance of larvae *Aedes aegypti*

The eggs of *Aedes aegypti* were collected from Vector Control Research Centre (VCRC - ICMR, Pondicherry), India without expose to any insecticide and in and around Coimbatore district, India at different breeding habitats with the help of 'O' type brush. These eggs were brought to the laboratory and were transferred to 18 X 13 X 4 cm size enamel trays containing 500 ml of water and kept for larval hatching. The mosquito larval culture was maintained in our laboratory at 27 ± 2°C, 75 - 85% RH, under 14L: 10D photoperiod cycles. The mosquito larvae were fed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till the larvae were transformed into pupae.

Maintenance of pupae and adult *Aedes aegypti*

The pupae were collected from the culture trays and were transferred to plastic containers (12 X 12 cm) containing 500 ml of water. The plastic jars were kept in 90 X 90 X 90 cm sized mosquito cage for adult emergence. The freshly emerged adults were maintained 27 ± 2°C, 75 - 85% RH, less than 14L: 10D photoperiod cycles. The adults were fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding.

Blood feeding of adult Mosquitoes

The female mosquitoes were allowed to feed on the blood of a rabbit (exposed on the dorsal side) for two days. The males were provided with 10% glucose solution on cotton wicks. The cotton was always kept moist with the solution and changed every day. An egg trap (cup) lined with filter paper containing water was placed at a corner of the cage egg collection.

Collection of plant materials

The tribal medicinal plant, *Rhodomyrtus tomentosa* were collected from Nilgiri hills, Western Ghats, Tamil Nadu, India. The plants were identified (Taxonomy) at BSI (Botanical Survey of India, Coimbatore, Tamil Nadu, India.) and the specimens were deposited at Zoology Department, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India. This plant is a small tree growing up to 5 to 10 feet. It is an edible plant of higher altitude. It's used by the native tribal for stomach pain.

Preparation of plant extracts

R. tomentosa plant was washed with tap water and shade dried at room temperature (27 ± 2°C). An electrical blender powdered the dried plant materials (leaves). From the powder, 300 g of the plant materials was extracted with 1 L of organic solvents of Acetone and Methanol for using a Soxhlet apparatus boiling point range 60 - 80°C for 8 h. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) and considered as 1% stock

solution. From this stock solution, different concentrations were prepared ranging from 120 to 600 ppm, respectively.

Adulticidal bioassay

Sugar - fed adult female mosquitoes (5 to 6 days old) were used. The *R. tomentosa* leaf extract were diluted with acetone to make different concentrations. The diluted plant extracts were impregnated on filter papers (140 × 120 mm). A blank paper consisting of only ethanol was used as control. The papers were left to dry at room temperature to let the ethanol evaporate overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring 125 × 44 mm following the method of (WHO, 1981). Mortality of the mosquitoes was recorded after 24 h. The above procedure was carried out in triplicate for plant extract of each concentration.

Ovicidal Activity

For ovicidal activity, slightly modified method of (Su & Mulla, 1998) was performed. The egg raft / eggs of *A. aegypti* were collected from vector control Laboratory, Annamalai University. The *R. tomentosa* leaves extracts diluted in the appropriate solvent to achieve various concentrations ranging from 60 to 420 ppm. Eggs of these mosquito species (100 nos.) were exposed to each concentrations of leaf extract until they hatched or died. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula.

$$\%\ \text{of egg mortality (Ovicidal activity)} = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100$$

Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95 % fiducidal limits of upper fiducidal limit and lower fiducidal limit, and Chi-square values were calculated using the SPSS Statistical software package 16.0 version was used. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The results of the adulticidal activity of acetone, methanol and aqueous extracts of *R. tomentosa* against the adult of dengue and chikungunya vector *A. aegypti*, are Tables 1 presented in (Figure 1 and 2). Among the three vectors tested, the highest adulticidal activity was observed in the highest mortality followed by methanol, acetone and aqueous extract. At higher concentrations, the adult showed restless movement for some time with abnormal wagging and died. The rates of mortality were directly proportional to concentration. The LC_{50} and LC_{90} values of *R. tomentosa* leaf extracts against adulticidal activity of (acetone, methanol and aqueous) *A. aegypti*, were the following: Acetone extract LC_{50} and LC_{90} values were 113.09 and 251.18 ppm; Methanol extract LC_{50} (LC_{90}) values were 106.38 (229.94) ppm; Aqueous extract LC_{50} values were 125.25 ppm and LC_{90} values were 273.38 ppm, respectively. Similarly, highest adulticidal effect was established from *Piper sarmentosum*, followed by *Piper ribesoides*, and *Piper longum*, with LD_{50} values of 0.14, 0.15, and 0.26 µg / female, respectively. Govindarajan *et al*. (2013) have reported that the moderate adulticidal properties were found in methanol extract of *A. paniculata* against the adults of *C. quinquefasciatus* and *A. aegypti* with the LC_{50} and LC_{90} values were 149.81 and 172.37 ppm, and 288.12 and 321.01 ppm, respectively. Plant borne extracts and essential oils can be tested for their adulticidal properties against a number of mosquitoes of economic importance (Amerasan *et al.* (2012); Govindarajan *et al*., 2013; Panneerselvam & Murugan, 2013). For example, Subramaniam *et al.* (2015) reported that the adulticidal activity of methanol extracts of seaweeds *D. dichotoma*, *P. pavonica* and *V. pachynema* on the costal malarial vector *Anopheles sundaicus*, LC_{50} values were 147.18 ppm, 161.9 pm and 133.79 ppm, respectively. In particular, 88 % adult mortality was observed from *Pelargonium citrosa* leaf extracts at 2 % concentration against *A. stephensi* (Jeyabalan *et al*., 2003).

The ethanol extract of *Citrus sinensis* showed LC_{50} and LC_{90} values 320.38 and 524.57 ppm against *A. aegypti* adults (Murugan et al., 2012). Recently, (Subramaniam *et al*., 2016) have reported that the adulticidal properties of *C. guianensis* flower extract and nanoparticles showed $LC_{50}$and LC_{90} of 133.96 and 11.23 ppm and 287.65 and 24.61 ppm, respectively. For instance, (Subramaniam *et al*., 2015) were highlighted the adulticidal properties of *Mimusops elengi* extract, which are highly toxic against *A. stephensi* and *A. albopictus*, respectively. The percentage of egg hatchability of dengue and zika vector, *A. aegypti* was tested with three different solvents (acetone, methanol and aqueous) at various concentrations of R. *tomentosa* leaf extracts and the results are listed in (Table 2). The toxicity of leaf extracts was dependent on its concentration against *A. aegypti*. There was zero hatchability (100% mortality) was attained at the concentration of 240 ppm for methanol extract and 300 ppm acetone extract and 360 ppm for aqueous extract. At 360 ppm all the three extracts exerted the zero hatchability. Control eggs exerted the hatchability rate ranged from 100%. The hatchability rate of control group with acetone, methanol and aqueous were 97.6%, 96.2% and 98.2%, respectively. The ovicidal activity of *R. tomentosa* against *A. aegypti* revealed that the hatchability rate of control egg rafts was ranged from 96.2% to 100%. The bio-active compound *Azadiracta indica* showed highest ovicidal activity in the eggs of *Culex tarsalis* and *Culex quinquefasciatus* exposed to 10 ppm concentration (Su & Mulla, 1998). Plant extracts have been screened and studied for their ovicidal repellency activity against mosquitoes (Tennyson *et al*., 2011; Thavara, *et al*., 2002).

For examples, some researchers have been reported the essential oil and plant extracts, which have highest ovicidal potential against major mosquito vectors, *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* (Prajapati *et al*., 2005), *Cinnamomum zeylanicum*, *Zingiber officinale* and *Rosmarinus officinalis* oils (Miyake & Nojima, 2006) *Citrullus vulgaris* (Samidurai *et al*., 2009). Recently, *Amerasan et al.* (*2015*) *Morinda tinctoria and Pongamia glabra*, Subramaniam & Murugan (2013) *Myristica fragrans* seed and Subramaniam *et al.* (2017) *Chenopodium ambrosioides* and Kasinathan *et al.* (2018) have reported the larvicidal and pupicidal activity of methanolic leaf extract of *R. tomentosa* (RTME) at different concentrations (120 to 600 ppm). The LC_{50} values were 263.82, 299.43, 331.64, 386.16 and 419.56 ppm (I - IV instar and pupae), respectively.

Table 1. Adulticidal toxicity of *R. tomentosa* leaf extracts against the Dengue and Zika virus vector, *Ae. aegypti.*

Treatment	Dosage (ppm)	Mortality (%)	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	$\chi 2$
R. tomentosa-acetone extract	Control	0.0 ± 0.0	113.09 (97.85 - 126.27)	251.18 (229.10 - 282.37)	3.29 n.s.
	50	31.6 ± 2.0^{e}			
	100	43.8 ± 1.8^{d}			
	150	59.0 ± 2.0^{bc}			
	200	76.8 ± 2.2^{ab}			
	250	93.4 ± 2.8^{a}			
R. tomentosa-methanolic extract	Control	0.0 ± 0.0	106.38 (54.09 - 139.27)	229.94 (187.06 - 342.47)	8.60 n.s.
	50	33.2 ± 2.6^{e}			
	100	45.8 ± 2.2^{d}			
	150	61.0 ± 2.6^{bc}			
	200	79.8 ± 2.0^{ab}			
	250	98.4 ± 1.8^{a}			
R. tomentosa-aqueous extract	Control	0.0 ± 0.0	125.25 (110.04 -138.91)	273.38 (247.92 - 310.24)	3.92 n.s.
	50	29.4 ± 1.2^{e}			
	100	39.8 ± 1.8^{cd}			
	150	55.4 ± 2.6^{c}			
	200	69.8 ± 2.2^{b}			
	250	90.6 ± 1.6^{a}			

Mortality rates are means ± SD of five replicates. No mortality was observed in the control. Within each column means followed by the same letter(s) are not significantly different (P<0.05). Chi-square value followed by an asterisk is significant (heterogeneity factor used in calculation of confidence limits) (P<0.05).

LC_{50} lethal concentration that kills 50 % of the exposed organisms, LC_{90} lethal concentration that kills 90% of the exposed organisms, LCL lower confidence limit, UCL upper confidence limit, $\chi 2$ Chi-square test, NS not significant.

Table 2. Ovicidal activity of *R. tomentosa* leaf extracts against the eggs of Dengue and Zika virus vector, Ae. *aegypti*

Treatment	Total	Control	Egg hatchability (%) Concentration (ppm)						
			60	120	180	240	300	360	420
R. tomentosa-acetone extract	100	*97.6* ± 2.05^{a}	*67.5* ± 1.80^{b}	*48.9* ± 1.50^{c}	*25.7* ± 1.55^{d}	*11.2* ± 1.24^{e}	*NH*	*NH*	*NH*
R. tomentosa-methanolic extract	100	*96.2* ± 1.82^{a}	*62. 3*± 1.56^{c}	*44.5* ± 1.32^{d}	*22.2* ± 1.78^{e}	*NH*	*NH*	*NH*	*NH*
R. tomentosa-aqueous extract	100	*98.2* ± 1.82^{a}	*69.3* ± 1.16^{b}	*54.5* ± 1.72^{c}	*36.2* ± 1.18^{d}	*20.4* ± 0.82^{e}	*08* ± 1.82^{f}	*NH*	*NH*

Values in a row with a different superscript are significantly different at p < 0.05% level (DMRT test). Each value (X± SD) represents the mean of six values. NH - No hatchability (100% mortality).

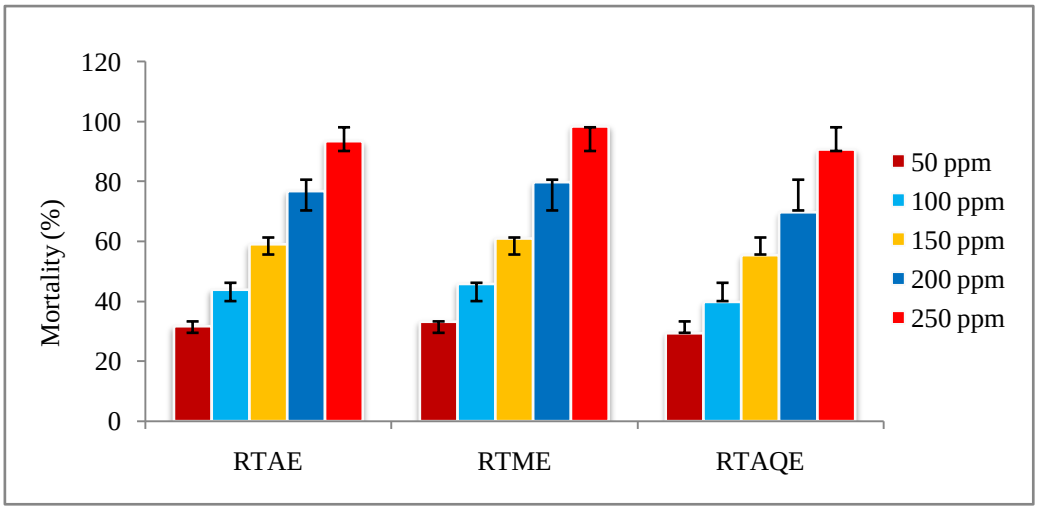

Figure 1. Adulticidal toxicity of *R. tomentosa* leaf extracts against the Dengue and Zika virus vector, *Ae. aegypti.*

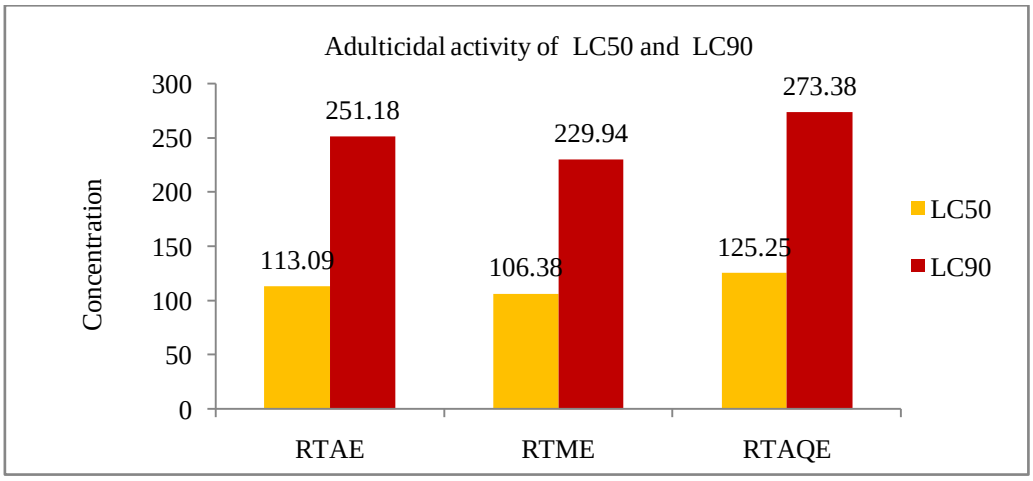

Figure 2. Adulticidal activity of *R. tomentosa* leaf extracts against the eggs of Dengue and Zika virus vector, *Ae. Aegypt.*

CONCLUSION

The conclude the results showed that the adulticidal and ovicidal activities of acetone methanol and aqueous extract of *R. tomentosa* leaves has potential to be developed as an insecticide against dengue, zika and chikungunya vector, *A. aegypti*. However, further studies to evaluate its toxicity and effects on non-target organisms and the environment need to be conducted. These are environmentally safe and eco-friendly approaches for the vector control programs. The results of the present study could be useful in promoting research aimed at the development of new agents for mosquito control based on bioactive chemical compounds from indigenous plant sources.

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