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RESEARCH ARTICLE

MOSQUITO LARVICIDAL, OVICIDAL AND PUPICIDAL ACTIVITIES OF *ANNONA RETICULATA* LINN (ANNONACEAE) AGAINST *AEDES AEGYPTI* (LINN.), *ANOPHELES STEPHENSI* LISTON AND *CULEX QUINQUEFASCIATUS* (SAY) (DIPTERA : CULICIDAE)

Balu Selvakumar, Gokulakrishnan J, Elanchezhiyan K and Deepa J

Department of Zoology, Poompuhar College (Autonomous), Poompuhar, Melaiyur- 609107, Tamilnadu, India

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ABSTRACT

The present investigation was aimed to investigate the mosquito larvicidal, ovicidal and pupicidal activities of different solvent extracts of *Annona reticulata* Linn. (Annonaceae) against selected mosquitoes *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). *A. reticulata* benzene, chloroform, ethyl acetate and methanol extract were tested against 3rd instar larvae of *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus* for 24 hr and mortality were recorded at various concentrations. The LC_{50} and LC_{90} values were determined following probit analysis. The ovicidal activity was determined against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* exposed to various concentrations were tested under laboratory conditions and the hatch rates were assessed 48hrs post treatment. Similarly, selected plant extracts were tested against pupae of selected mosquitoes at various concentrations and mortality of each pupa was recorded after 24hr post treatments of exposure. The LC_{50} and LC_{90} values of benzene, chloroform, ethyl acetate and methanol extract of *A. reticulata* against early third instar larvae of *Ae. Aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were 83.56, 96.24, 106.52, 79.56, 83.81, 95.27, 69.56, 76.28, 86.32, 62.82, 74.36, 80.44ppm and 115.28, 124.65, 132.67, 112.86, 116.35, 119.77, 99.56, 104.58, 107.89, 88.31, 93.85 and 96.28 ppm, respectively. The chi-square values are significant at $p \leq 0.05$ level. It has been noticed that the higher concentrations of *A. reticulata* extracts possesses strong ovicidal activity at 200ppm concentration against *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus*, no egg hatchability was recorded. In the same way, methanol extracts showed maximum ovicidal activity followed by benzene, chloroform and ethyl acetate against selected vector mosquitoes. In pupicidal activity, among the four solvent extracts tested against selected mosquitoes at 200ppm higher concentrations, the methanol was found to be most effective for pupicidal activity provided 29.67 (98.90%), 29.14 (97.13%) and 28.37 (94.56%) against *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* respectively. The present investigation lead the path of exploration of *Annona reticulata* for eradication of selected medically important human vector mosquitoes, thereby, gaining a real momentum to include this plant product for intense vector control programme.

INTRODUCTION

Mosquitoes are still representing the world's number-one vector of human and domestic animals.Comprising approximately 3500 species, mosquitoes are found beyond the tropical and subtropical regions of the world with which they are classically associated. Particularly true for the chief genera which vector human disease-causing pathogens *Anopheles* (malaria, filariasis), *Aedes* (yellow fever, dengue, chikungunya), and *Culex* (West Nile, Japanese encephalitis, filariasis) mosquitoes are distributed globally and most female mosquitoes take blood meals from vertebrates to obtain the necessary nutrition to produce their eggs, injecting saliva (which may contain pathogens) into the host animal. While many mosquitoes are distinctly selective feeders, restricted to one or a few closely related species, some feed in a less restrictive manner, varying between mammals, birds, and reptiles.1 Mosquitoes breed in water, occasionally depositing eggs directly on water, but generally using a variety of moist surfaces, tree holes, and containers (Reiter, 2001; White, 2004; WHO, 2009; 2010). *Aedes aegypti* (L.) is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever, and dengue shock syndrome, or with unusual manifestations such

Corresponding author:* **Balu Selvakumar
Department of Zoology, Poompuhar College (Autonomous), Poompuhar, Melaiyur, Tamilnadu, India

as central nervous system involvement (Pancharoen *et al.,* 2002). The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health. *Anopheles stephensi* Liston is the common vector of malaria in India and other West Asian countries. Malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes upto2.7 million deaths (WHO, 1999). The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health. Lymphatic filariasis is a major vector borne disease making about 120 million peoples in 83 countries physically disabled (WHO, 2006) which is transmitted by *C. quinquefasciatus* mosquito having cosmopolitan distribution. WHO (1992) have suggested various controlling strategies to control vector transmission at different levels. Among the available vector control methods, chemical control is decisively superior over environmental and biological control strategies that have limited applicability in mitigating sporadic unpredictable outbreaks of vector borne disease. However, *Cx.quniquefasciatus* has also shown resistance to different insecticides used in mosquito control such as organochlorines, organophosphorous, pyrethroids and microbial insecticides throughout the world (Tikar *et al.,* 2008).

The use of chemical insecticides in controlling mosquitoes has been encountered by many problems due to detrimental hazards of organic synthetic pesticides to human, domestic animals, wildlife and the environment (Matsumura, 1975). In addition to adverse environmental effects from conventional insecticides, most mosquitoes and other pest species has become physiologically resistant to many of these compounds (Brown, 1986). These problems have highlighted the need for the development of new strategies for selective mosquito control. Phytochemicals are advantageous due to their eco-safety, target-specificity, non development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas. A survey conducted on 344 plant species, revealed that certain phytochemicals act as general toxicants to all life stages of mosquitoes, whereas others interfere with growth and reproduction, or act on the olfactory receptors, eliciting responses of attractancy or repellency (Sukumar *et al.,* 1991). Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programs. The plant product of phytochemical, which is used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. Phytochemicals obtained from the whole plant or specific part of the plant by the extraction with different types of solvent such as aqueous, methanol, chloroform, benzene, ethyl acetate, acetone, etc., depending on the polarity of the phytochemical. Some phytochemicals act as toxicant (insecticide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and growth inhibitor or with reproduction or produce an olfactory stimulus, thus acting as repellent or attractant (Gosh, 1991; Elumalai *et al.,* 2012a,b; Krishnappa *et al.,* 2012a,b; Krishnappa and Elumalai, 2012; Dhanasekaran *et al.,* 2012; Balu Selvakumar *et al.,* 2012; Elangovan *et al.,* 2012; Gokulakrishnan *et al.,* 2012; Markouk *et al.,* 2001; Krishnappa and Elumalai, 2014). Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are biodegradable (Krishnappa and Elumalai, 2013; Elumalai *et al.,* 2013). In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal, ovicidal and pupicidal potential of the benzene, chloroform, ethy acetate and methanol extract of *Annona reticulata* against the medically important vector mosquitoes.

MATERIALS AND METHODS

Collection of plants

Matured leaves of *Annona reticulata* Linn (Annonaceae) were collected during the flowering season (January - April 2014) from different places of Poompuhar Village, Sirkali, Nagapattinam districts of the Tamilnadu, India. Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared per species and identified with the help of plant taxonomist, Department of Botany, Poompuhar College, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

Extraction procedure

The leaves of *Annona reticulata* linn (Annonaceae) were washed with tap water, shade-dried, and finely ground with the help of electrical blender and extracted sequentially with benzene, chloroform, ethy acetate and methanol in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22–26 mm Hg at 45°C by 'Rotavapour' and the residue obtained was stored at 4°C in an amber vial. Then the vials were named and covered with silver foil and transported to the laboratory. Until use those vials were kept in cool and dark place at 4^0C.

Test organisms

The larvae of mosquitoes, *Ae. aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* were collected from the agricultural gardens and field and continuously reared in the laboratory. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week-old chick for blood meal. Mosquitoes were held at 27 ± 2°C, 75±5% RH, with a photo period of 12L:12D.

Larvicidal activity

The larvicidal activity of the plants crude extracts was evaluated as per the method recommended by World Health Organization (2005). Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 ml of water. The appropriate volume of dilution was added to 200 ml water in the cups to obtain the desired target dosage, starting with the lowest concentration. Five replicates were set up for each concentration and an equal number of controls were set up simultaneously using tap water. To this, 1 ml of appropriate solvent was added. The larval mortality was calculated by using the formula of Abbott (1925) and LC_{50}

value was calculated after 24 hr by probit analysis (Finney, 1971).

Ovicidal activity

For ovicidal activity, slightly modified method of Su and Mulla (1998) was performed. The eggs/egg rafts of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* were reared in the Department of Zoology, Poompuhar College. The different leaf extracts diluted in the appropriate solvent to achieve various concentrations ranging from 50 to 200 ppm. Eggs of these mosquito species (100) were exposed to each concentration of leaf extracts. 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 five times along with appropriate control. The hatch rates were assessed 48 hr post treatment by following formula.

Pupicidal activity

Batches of thirty number of pupae were introduced into 500 ml of the test medium containing 100 and 200ppm concentration of the crude extract in a plastic cups in five replications. In control, the same number of pupae was maintained in 500 ml of dechlorinated water containing appropriate volume of DMSO. All containers were maintained at room temperature (28±2°C) with naturally prevailing photoperiod (12: 12h/L: D) in the laboratory. Any pupa was considered to be dead if did not move when prodded repeatedly with a soft brush. Mortality of each pupa was recorded after 24hr of exposure to the extract.

RESULTS

Results of the present study reflected spectrum of activity with *A. reticulata* different solvent crude extracts tested against the selected mosquitoes larvae, pupae and eggs. Generally, as the concentration increases the rate of larval mortality are also increases. The LC_{50} and LC_{90} values of benzene, chloroform, ethyl acetate and methanol extract of *A. bracteata* against early third instar larvae of *Ae. Aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were 83.56, 96.24, 106.52, 79.56, 83.81, 95.27, 69.56, 76.28, 86.32, 62.82, 74.36, 80.44ppm and 115.28, 124.65, 132.67, 112.86, 116.35, 119.77, 99.56, 104.58, 107.89, 88.31, 93.85 and 96.28 ppm, respectively. The chi-square values are significant at $p \leq 0.05$ level (table 1). It has been noticed that the higher concentrations of *A. reticulata* extracts possesses strong ovicidal activity at 200ppm concentration against *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus*, no egg hatchability was recorded. In the same way, methanol extracts showed maximum ovicidal activity followed by benzene, chloroform and ethyl acetate against selected vector mosquitoes (table 2). In pupicidal activity, among the four solvent extracts tested against selected mosquitoes at 200ppm higher concentrations, the methanol was found to be most effective for pupicidal activity provided 29.67 (98.90%), 29.14 (97.13%) and 28.37 (94.56%) against *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* respectively (table 3). Results of this study show that the *A. reticulata* selected extracts may be a potent source of natural larvicidal, ovicidal and pupicidal activities against selected important vector mosquitoes.

DISCUSSIONS

Due to indiscriminate use of synthetic chemicals to control the mosquitoes in the natural habitats, they have developed strong resistance to almost all the chemicals that are available today. Moreover, chemical pesticides gradually altered the behaviour of non-target organisms. Thus, in this context, the world scientific community intensively searching for the alternative mosquitocidal agent preferably from plants available in nature. Today, the environmental safety of an insecticide is considered to be of important milestone in the field of pest control in general and vector control programme in particular. An insecticide must not cause high mortality in target organisms in order to be acceptable (Kabaru and Gichia, 2001). The extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion. It is evident from the present study that exposure of *An. stephensi* eggs to the leaf extracts of various solvents not only elicited egg mortality but also delayed hatchability to larval stages. Similar kind of observation was also noted earlier by several workers (Rajkumar *et al.*, 2011; Aarthi and Murugan, 2011). The ovicidal activity indicated an important finding that the larvae which hatched out of the treated eggs were succumbed to death within an hour or two. In the present study, our aim was to determine whether *Annona reticulata* could be used for mosquito control. We observed a functional response of the ovicidal activity exhibited by the methanol extract. In the case of ovicidal activity, exposure to the freshly laid eggs was more effective than that to the older eggs. Similarly, ovicidal and gravid mortality effects of ethanolic extract of *Andrographis paniculata* was assessed by Kuppusamy *et al* (2008) against *An. stephensi.* Larvicidal and oviposition activity of *Cassia obtusifolia* leaf extract against *An. stephensi* was also evaluated by Rajkumar and Jebanesan (2009). Similarly, the aqueous and hydro-alcoholic extracts of *Melia azedarach* leaves and seeds were tested to explore the in vitro ovicidal and larvicidal activity against *Haemonchus contortus* (Kamaraj *et al.*, 2010) and the results were comparable with our results. Additionally, through screening several plants for their larvicidal activity, Sharma *et al* (2006) found that *Artimisia annua* was the most toxic against anopheles with an LC_{50} of 16.85 ppm and 11.45 ppm after 24 and 48 h of exposure, respectively.

In addition, the larvicidal effects of *Momordica charantia* fruit on *An. stephensi* (LC_{50} of 66.05 ppm) were also investigated by Singh *et al* (2006). Abdalla *et al.*, 2009 have also reported that the *A. arabiensis* extracts against *Cx. quinquefasciatus* that caused high, moderate and low larval mortality in the larvicidal experiment against 3rd instar larvae. It was found that, LC_{50}–LC_{90} values calculated were 273.53–783.43, 366.44–1018.59 and 454.99–1224.62 ppm for 2nd, 3rd and 4th larval instars, respectively, of *An. Arabiensis* and 187.93–433.51, 218.27–538.27 and 264.85–769.13 ppm for 2nd, 3rd and 4th larval instars, respectively, of *Cx. quinquefasciatus.* These and other naturally occurring insecticides may play a crucial role in vector control programs in the near future (Wandscheer *et al.*, 2004). Since *An. stephensi* breeds in drinking water tank, many of the plant extracts are subject to risk factors in mosquito control (Ahmed *et al.*, 2011). Siddiqui *et al.*, (2004) who reported spipnoohine and pipyahyine

Table 1 Larvicidal activity of *Annona reticulata* different extracts tested against the thired instar larvae of selected mosquitoes.

Extracts tested	Mosquitoes	LC_{50}	95% Confidence Limit		LC_{90}	95% Confidence Limit		¡ ^{2} value
			LCL	UCL		LCL	UCL	
Benzene	*Aedes aegypti*	83.56	52.79	96.35	115.28	82.94	122.45	11.264
	Anopheles stephensi	96.24	58.47	107.55	124.65	94.76	128.70	12.591
	Culex quinquefasciatus	106.52	61.34	115.36	132.67	98.56	137.89	12.587
Chloroform	*Aedes aegypti*	79.56	52.46	96.37	112.86	96.55	120.26	13.185
	Anopheles stephensi	83.81	59.95	98.74	116.35	98.53	122.50	13.832
	Culex quinquefasciatus	95.27	63.16	101.15	119.77	101.17	124.34	13.679
Ethyl acetate	*Aedes aegypti*	69.56	55.28	81.65	99.56	81.45	106.55	13.194
	Anopheles stephensi	76.28	57.22	86.26	104.58	84.97	111.16	11.460
	Culex quinquefasciatus	86.32	55.92	95.26	107.89	85.65	118.45	12.530
Methanol	*Aedes aegypti*	62.82	39.56	78.33	88.31	76.47	92.38	11.468
	Anopheles stephensi	74.36	46.97	82.20	93.85	76.49	98.52	12.970
	Culex quinquefasciatus	80.44	54.48	86.24	96.28	81.73	105.88	11.241

LC_{50}=Lethal Concentration 50%; LC_{90}=Lethal Concentration 90%; LCL=Lower Confidence Limit; UCL= Upper Confidence Limit; χ^2= Chi square.

Table 2 Ovicidal activity of *Annona reticulata* different extracts tested against the freshly laid eggs of selected mosquitoes.

Extracts tested	Mosquito species and their Percentage of egg hatch ability			
	Concentrations (ppm)	*Aedes aegypti*	*Anopheles stephensi*	*Culex quinquefasciatus*
Benzene	50	39.56±1.46	42.35±1.67	48.47±1.4
	100	22.38±1.17	31.72±1.93	35.33±1.8
	150	18.37±1.69	25.28±1.68	29.68±1.6
	200	NH	NH	NH
Chloroform	50	34.51±1.81	39.87±1.28	42.74±1.57
	100	23.64±1.57	29.64±1.27	32.23±1.93
	150	18.86±1.21	22.54±1.47	25.79±1.14
	200	NH	NH	NH
Ethyl acetate	50	24.62±1.68	25.71±1.62	27.66±1.17
	100	15.71±1.37	19.59±1.89	23.24±1.35
	150	NH	NH	NH
	200	NH	NH	NH
Methanol	50	22.30±1.45	26.50±1.46	28.67±1.48
	100	NH	NH	NH
	150	NH	NH	NH
	200	NH	NH	NH
Control	00	100.0±0.0	100.0±0.00	100.0±0.00

Values represent mean ± S.D of five replications.

compounds isolated from dried fruits of *Piper nigrum* and gluanol acetate, a tetracyclic triterpene isolated from *Ficus racemosa* (Rahuman *et al.,* 2008). David *et al.,* (2000) reported that the toxicity of the phenolic derivative, methyl-p-hydroxybenzoate control the mosquito larvae. Komalasmira *et al.,* (2005) who have been reported the ethanol extracts of *P. beetle* has successfully killed the larvae of 4 mosquito vectors *Ae. aegypti*, *C. quinquefasciatus*, *An. dirus* and *Monsonia uniformis*. Mosquito control is vital for many countries and is still in a state of evolution. During the last decades, it depended upon synthetic organic insecticides, many of which have been removed from the arsenal of weapons (Floore, 2006) and botanicals are the new weapons of mosquito control under exploration. The activity of crude plant extracts is often attributed to the complex mixture of active compounds. Natural pesticides derived from plants are a promising tool especially for targeting mosquitoes in the larval stage (Amer and Mehlhorn, 2006). Ansari *et al.,* (2000) suggested that the peppermint oil (*Mentha piperita*) showed strong repellent activity against adult mosquitoes when applied on the human skin. The protection obtained against *An. annularis*, *An. culicifacies*, and *C. quinquefasciatus* was 100.0%, 92.3%, and 84.5%, respectively. Nathan *et al.,* (2005) considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity against *An. stephensi* and the larval mortality was dose-dependent with the highest dose of 1 ppm azadirachtin, evoking almost 100% mortality, affecting pupicidal and adulticidal activity and significantly decreased fecundity and longevity of *An. stephensi*. The isolated compound saponin from ethyl acetate extract of (*Acanthus aspera*) was effective against the larvae of *Ae. aegypti* and *C. quinquefasciatus* with LC_{50} value of 18.20 and 27.24 ppm, respectively (Bagavan *et al.,* 2008). Kamaraj *et al.,* (2008) have reported that the peel methanol extract *C. sinensis*, leaf and flower ethyl acetate extracts of *O. canum* against larvae of *An. Stephensi* (LC_{50} 95.74, 101.53, 28.96; LC_{90} 303.20, 492.43 and 168.05 ppm) respectively. Similarly, the aqueous and hydro-alcoholic extracts of *Melia azedarach* leaves and seeds were tested to explore the in vitro ovicidal and larvicidal activity against *Haemonchus contortus* (Sharma *et al.,* 2006). Karunamoorthi and Ilango, (2010) have reported that the LC_{50} and LC_{90} values of methanol leaf extracts of *Croton macrostachyus* were 89.25 and 224.98 ppm, respectively against late third instar larvae of malaria vector, *An. arabiensis.*

The screening of *Artimisia annua* plants against larvicidal activity of *Anopheles* mosquito, it produced maximum activity and LC_{50} values were 16.85 ppm and 11.45

promising effect on selected mosquitoes and it has no deleterious effects against human beings since it has been used in Indian auyurvedhic medicine for several ailments.

Table 3 Pupicidal activity of different extracts of *Annona reticulata* tested against pupae of selected moaquitoes

Solvent tested	Concentration	Vector mosquito	Two days post treatment			
			Mortality*		Adult emergence	
			Pupal mortality	% Mortality	Adult	% Emergence
Benzene	100ppm	*A.aegypti*	23.22±1.26	77.40	6.78±1.71	22.60
		An. stephensi	22.38±1.38	74.60	7.62±1.35	25.40
		C.quinquefasciatus	24.66±1.49	82.20	5.34±1.54	17.80
	200ppm	*A.aegypti*	28.68±1.62	95.60	1.32±1.49	4.40
		An. stephensi	27.79±1.88	92.63	2.21±1.98	7.36
		C.quinquefasciatus	29.33±1.22	97.76	1.67±1.37	2.23
Chloroform	100ppm	*A.aegypti*	22.98±1.64	76.60	7.02±1.53	23.40
		An. stephensi	21.79±1.94	72.63	8.21±1.48	27.36
		C.quinquefasciatus	23.77±1.66	79.23	6.23±1.74	20.76
	200ppm	*A.aegypti*	27.33±1.72	91.10	2.67±1.55	8.90
		An. stephensi	25.92±1.42	86.40	4.08±1.82	13.60
		C.quinquefasciatus	28.68±1.59	95.60	1.32±1.48	4.40
Ethy acetate	100ppm	*A.aegypti*	21.67±1.68	72.23	8.33±1.22	27.76
		An. stephensi	20.55±1.97	68.50	9.45±1.45	31.50
		C.quinquefasciatus	22.43±1.38	74.76	7.57±1.67	25.23
	200ppm	*A.aegypti*	25.56±1.94	85.20	4.44±1.28	14.80
		An. stephensi	23.22±1.22	77.40	6.78±1.55	22.60
		C.quinquefasciatus	27.33±1.68	91.1	2.67±1.44	8.90
Methanol	100ppm	*A.aegypti*	23.81±1.22	79.36	6.19±1.38	20.63
		An. stephensi	22.64±1.36	75.46	7.54±1.22	25.13
		C.quinquefasciatus	24.79±1.29	82.63	5.21±1.64	17.36
	200ppm	*A.aegypti*	29.14±1.44	97.13	1.86±1.17	2.86
		An. stephensi	28.37±1.31	94.56	1.68±1.52	5.60
		C.quinquefasciatus	29.67±1.94	98.90	1.33±1.68	1.10
Control	0ppm	Selected mosquito	0.0±0.0	0.0	30.0±0.0	100

Value represents mean ± S.D. of five replications. *Mortality of the pupae observed two days post treatment.

ppm after 24 and 48 h of exposure, respectively (Singh *et al.,* 2006). The larvicidal and adulticidal activities of ethanolic and water mixture (50:50) of plant extracts *Eucalyptus globulus, Cymbopogan citratus, Artemisia annua, Justicia gendarussa, Myristica fragrans, Annona squamosa* and *Centella asiatica* were tested against *An. stephensi*, and the most effective between 80% and 100% was observed in all extracts (Senthilkumar *et al.,* 2009). The biological activity of the plant extract might be due to a variety of compounds in *V. zizanioides* roots, including phenolics, terpenoids and alkaloids. These compounds may jointly or independently contribute to cause oviposition deterrent and ovicidal activity against *A. stephensi* (Medhi *et al.,* 2010). Similarly, the oviposition deterrent activity, ovicidal and gravid mortality effects of ethanolic extract of *Andrographis paniculata* against the malarial vector *An. stephensi* was evaluated by (Kuppusamy and Murugan, 2008). The root extract of *Valeriana jatamansi* which exhibited adulticidal activity of 90% lethal concentration against adult *An. stephensi, An. culicifacies, Ae. aegypti, An. albopictus* and *Cx. quinquefasciatus* were 0.14, 0.16, 0.09, 0.08, and 0.17 and 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm^2, respectively (Dua *et al.*, 2008). In addition, the larvicidal effects of *Momordica charantia* fruit on *A. stephensi* (LC_{50} of 66.05 ppm) and *C. quinquefasciatus* (LC_{50} of 96.11 ppm) were also investigated Singh *et al.,* (2006). Sharma *et al.,* (2005) reported that the acetone extract of *Nerium indicum* and *Thuja orientelis* had LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against III instar larvae of *An. stephensi* and *Cx. quinquefasciatus*, respectively. The plant extracts which are highly toxic against *An. stephensi* are also toxic to human beings. In the present study, *Annona reticulata* extract showed

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