Research Article

Larvicidal Efficacy of *Catharanthus roseus* Leaf Extracts against the Filarial Vector *Culex quinquefasciatus* (Diptera: Culicidae)

S. Vairavan, S. Thangapandiyan*, A.S. Alif Alisha
Department of Zoology, PSG College of Arts and Science, Peelamedu, Coimbatore, Tamil Nadu, India, 641014.
***Corresponding author's E-mail:** stp.nano@gmail.com

Received: 21-05-2018; **Revised:** 26-06-2018; **Accepted:** 08-07-2018.

ABSTRACT

Culex quinquefasciatus is an obligatory ectoparasitic vector since it plays a major role in the transmission of bancroftian filariasis all over the world. Finding an environment friendly bioinsecticides for the control of vector *Culex quinquefasciatus* is considered to be of paramount importance to reduce the negative impacts caused by chemical insecticides to the environment. The present investigation was aimed to find the phytochemical constituents of *Catharanthus roseus* leaf extracts in different solvent extracts such as ethanol, acetone and distilled water and to investigate the mosquito larvicidal activities against the third instar larvae of *Culex quinquefasciatus.* To identify the functional groups present in the compounds, Fourier Transform Infrared Spectroscopy analysis was performed. The mortality rates were recorded after 6^{th}, 12^{th}, 24^{th}, 36^{th}, 48^{th}, 72^{nd}, 96^{th} and 120^{th} hours of exposure. Among the three different *Catharanthus roseus* leaf extracts acetone leaf extract exhibited 100% mortality in the time period of 24 hours and the ethanol extract achieved 100% mortality at 120 hours at the concentration of 400 ppm. Similarly the distilled water shows 92.50 % at 120 hours of exposure. The Lethal $Concentration_{50}$ and Lethal $Concentration_{90}$ were determined followed by Probit Analysis. Acetone leaf extract of *Catharanthus roseus* exhibited maximum larvicidal activity than ethanol and distilled water extracts in 400 ppm at the time period of 24 hour against *Culex quinquefasciatus*. The result of the current work revealed that the leaf extract of *Catharanthus roseus* have the potential to be act as an alternative for the controlling of the mosquitoes.

Keywords: *Culex quinquefasciatus, Catharanthus roseus,* Fourier Transform Infrared Spectroscopy, Larvicidal activity.

INTRODUCTION

Mosquitoes are represented as the world's most dangerous vector which spreads diseases to human and domestic animals. Comprising approximately 3500 species, mosquitoes are found beyond the tropical and subtropical regions of the world.[1] They involved in spreading of more diseases which affects human health due to their ability to vector various pathogens that afflict millions of people worldwide.[2] There are about 3500 species of mosquitoes have been reported worldwide. Several species are belongs to the genera *Aedes*, *Anopheles* and *Culex*. The diseases like filariasis, dengue, yellow fever, malaria, Japanese encephalitis, and chikungunya are some of the deadly diseases which are spread by them.[3] The mosquito *Culex quinquefasciatus* having cosmopolitan habitat acts as a vector for *Wuchereria bancrofti* responsible for Filariasis in India.[4]

In the past years, the plant kingdom has been of great interest potential source of insecticidal products. Many number of plant species are playing an important role in insecticidal activities. Plants synthesize a variety of secondary metabolites which play a vital role in defense against mosquitoes.[5] Phytochemicals are mostly secondary metabolites extracted from various plant species have been tested for their larvicidal activity against mosquitoes.[6] The chemicals present in plants that protect its cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals.[7] The phytochemicals are found to be present in all parts of plants including leaves, fruits, seeds, flowers, stems and roots.[8] According to Koche *et al* [9] the phytochemicals are compounds are utilized as food and medicine, top reserve against illness and to ensure human health. Phytochemicals like terpenoids, tannins, flavonoids, saponins are present in the leaves and stem of most of the wild plants. According to Yadav *et al* [10] the use of plant products for mosquito control have several appealing features as they are easily degradable, less hazardous, house of chemicals of diverse biological activity and also economical as practical in application. *Catharanthus roseus* L. is an evergreen, perennial sub shrub, woody at the base, 30 cm to 1 m height, found in warm regions around the world, including India. The *Catharanthus roseus* has been historically used as a traditional remedy, as well as an insecticide around the world.[11] The present investigation was carried out to determine the mosquito larvicidal activity of *Catharanthus roseus* leaf extract against the filarial vector mosquito *Culex quinquefasciatus* using different solvents.

MATERIALS AND METHODS

Collection and identification of plant

Fresh, healthy and mature leaves of *Catharanthus roseus* were collected from the region of Thirumalaigiri, Salem District, Tamil Nadu, India. The collected plant material was identified and authenticated with the help of Dr. C. Murugan, Botanical Survey of India (BSI), Tamil Nadu Agricultural University (TNAU), Tamil Nadu.

Preparation of leaf extract

Fresh, mature and healthy leaves of *Catharanthus roseus* were collected and washed thoroughly using running tap water and again washed using distilled water. Then the leaves were allowed to dry under shade condition and powdered using electric blender.

1 gram of plant leaf powder was taken in 250 ml conical flasks. To this, 100 ml of different solvents such as acetone, ethanol and distilled water was added separately. After 6 days, the content were stirred thoroughly and filtered through Whatman's No.1 filter paper. The filtrate was collected and stored in sterile containers at 4°C for further use.

Phytochemical analysis of *Catharanthus roseus* leaves [12]

Freshly prepared leaf extracts were subjected to standard phytochemical analyses using standard procedure [13] in order to find out the presence of various phytoconstituents such as alkaloids, terpenoids, flavonoids, tannins, steroids, anthroquinones, saponins, resins, glycosides and phenols.

FT-IR Analysis

To identify the functional groups present in the compounds, IR- spectral analysis was performed using Shimadzu FTIR 8300 instrument. Potassium bromide pellet was prepared by mixing 1 mg of the fraction of the plant leaf powder with 100 mg of anhydrous potassium bromide. The spectra were recorded from 400 to 4000 cm^{-1}.

Preparation of stock solution

Dried and powdered leaf samples (1g) was mixed with 100 ml of acetone, ethanol and distilled water in separate conical flasks for a period of 96 hours and then filtered using Whatman's No.1.Filter paper. The filtered crude plant leaf extracts were kept in airtight containers separately at room temperature. It was said to be 10,000 ppm of stock solution.

Mosquito culture

The 3rd instar larvae of mosquito *Culex quinquefasciatus* were collected from National Center for Disease Control (NCDC), Mettupalayam, Coimbatore, Tamil Nadu, India. The collected larvae are kept without exposure to any insecticides and pathogens. The larvae were kept in plastic container containing tap water. The larvae were maintained at 25-29°C and 75-85% relative humidity under 14:10 light and dark cycles. The larvae were fed on powdered dog biscuit and yeast in the ratio of (3:1).

Larvicidal Bioassay

The larvicidal bioassay was carried out against laboratory reared 3rd instar larvae of *Culex quinquefasciatus*. In order to evaluate the larvicidal bioassay, the standard WHO [2] protocol was followed with minor modifications. The experiment was conducted in 150 ml paper cup. From the stock solutions, concentrations of 50, 100, 150, 200, 250, 300 and 400 ppm were prepared. From the culture 20 healthy 3rd instar larvae were introduced in 150 ml paper cups containing 100ml of distilled water with each test concentration. Larval mortality was observed at the time interval of 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 96 hr and 120 hr after introducing larvae. Larvae were considered dead when they showed no signs of movement when provoked on their respiratory siphon by using a needle. A total of three trials with three replicates per trial for each concentrations were carried out against 3rd instars larvae of mosquito species *Culex quinquefasciatus*. The larval percent mortality was calculated when the control mortality ranged from 5-20%.

The control mortality was corrected using Abbot's formula.[14]

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Statistical analysis:

The determination of LC_{50} and LC_{90} (Lethal concentration causing 50% and 90% mortality) were done using SPSS 20 version package. The experiment data of larval mortality and effect of concentration were subjected to analysis of variance (ANOVA) followed by probit analysis to determine the difference in larval mortality between various concentrations

RESULTS AND DISCUSSION

Qualitative phytochemical screening of the leaf extracts of *Catharanthus roseus*

Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive and are readily available throughout the world. The screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health.[15]

In the present study, results of the phytochemical composition of the *Catharanthus roseus* leaf extracts are given in the Table 1. The results of the phytochemical studies showed that all tested extracts (acetone, ethanol and distilled water) contains alkaloids, terpenoids, flavonoids, tannins, steroids, saponins, glycosides and phenols whereas, anthraquinones and resins were absent. *Catharanthus roseus* possesses immense medicinal properties. It exhibits high *in vitro* antiplasmodial activity, which may be due to the presence of compounds such as alkaloids, terpenoids, flavonoids and sesquiterpenes.[16]

Among the target organs, tannic acid mainly produces maximum damages to the midgut epithelium of some dipterian larvae.[17] Tannins are also having various physiological effects like antiparasitic anti-irritant, antiscretolytic and antimicrobial activities. The alkaloids

possess antioxidant activity. Terpenoids and essential oils have membrane disruption characteristics. Quinines and polyphenols inactivate the enzymes, bind to adhesions and forms complex with cell wall. Flavonoids inhibit gastro intestinal tract releasing acetylcholine. Saponin possesses membrane permeabilizing properties, leads to vacuolization and disintegration of integuments. Some of the characteristics of saponins include formation of foams in distilled water solutions, cholesterol binding properties and hemolytic activity which affects mosquito larvae.[18,19] Alkaloids inhibits the metabolic processes in mosquito larvae, interfere with growth hormones, and digest the protein in the larval body and turn it into peptone derivatives that will host larvae as food shortages and eventually leads to the death of larvae.[20]

Table 1: Qualitative phytochemical screening of Leaf Extracts of *Catharanthus roseus*

Phytoconstituents	Test Adopted	Acetone	Ethanol	Distilled
Alkaloids	Mayer's Test	+	+	+
Terpenoids	Knollar's Test	+	+	+
Flavonoids	Sodium hydroxide Test	+	+	+
Tannins	Lead acetate Test	+	+	+
Steroids	Chloroform Test	+	+	+
Anthraquinones	Free Anthraquinones Test	-	-	-
Saponins	Foam Test	+	+	+
Resins	Sodium hydroxide Test	-	-	-
Glycosides	Kellerkillian's Test	+	+	+
Phenols	Ferric chloride Test	+	+	+

+ = Present; - = Absent

FT-IR Analysis

The FT-IR analysis of *Catharanthus roseus* plant leaves is depicted in Fig 1. The phytochemical involvement of different functional groups in the plant extract is monitored by Fourier transformed infrared spectroscopy. The FTIR spectrum was recorded for the leaf powder of *Catharanthus roseus*. The resultant functional groups are listed in Table. 2. The predominant peak at 3579.88 cm^{-1}, 3630.03 cm^{-1} and 3691.75 cm^{-1} were attributed to the OH stretching of alcohol group. The peak at 2927.94 cm^{-1} and 2854.65 cm^{-1} are attributed to the C-H stretching vibration of alkanes. The observed peak at 1589.34 cm^{-1} is due to the N-O stretching vibration of nitro compound. The peak of 1026.13 cm^{-1} is specified to C-N stretching of aliphatic amines and the peak at 702.09 cm^{-1} and 732.95 cm^{-1} are specified to C=C stretching vibration of alkenes. The FTIR analysis confirmed the presence of phytochemicals belongs to the functional groups such as alkyl halides, aliphatic amines, alcohols, esters, alkanes, nitro group, aromatic hydrocarbons, Carbonyl group, imine group and phenols in *Catharanthus roseus* accounts for its usefulness as a powerful insecticidal agent.

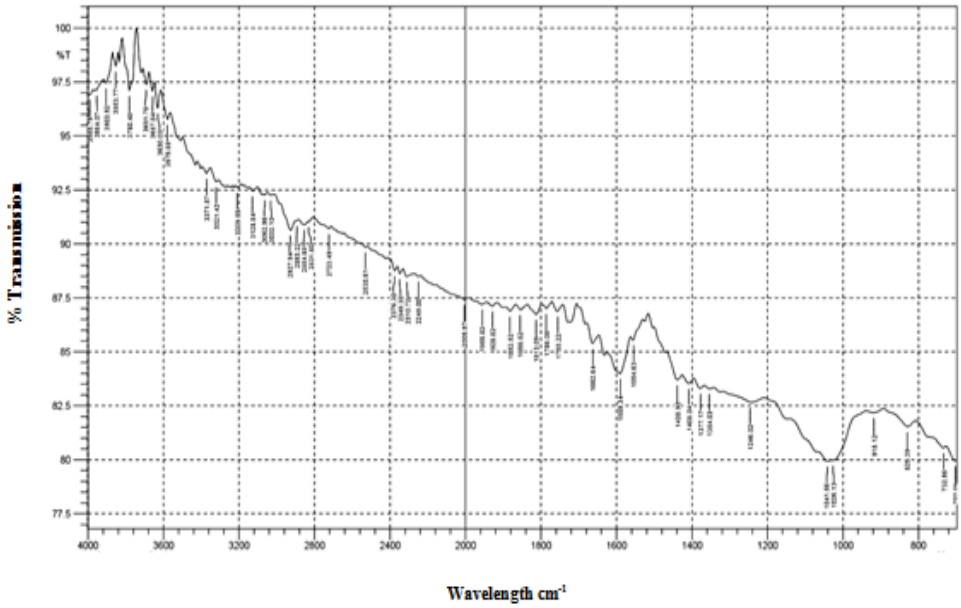

Figure 1: FT-IR spectrum of *Catharanthus roseus* leaf powder

Table 2: FT-IR Analysis of the powdered leaf sample of C*atharanthus roseus*

Bands cm^{-1}	Functional groups	Name of the Functional groups
702.09, 732.95	C-Cl	alkyl halides
1026.13, 3371.57	-NH2	aliphatic amines
1041.56	C-O	esters & alcohols
1354.03, 3209.55, 2854.65, 2927.94	C-H	alkanes
1408.04	C-C	aromatic ring
1377.17, 1589.34	N=O	nitro group
1662.64, 1813.09	C=O	Carbonyl group
2310.72, 2349.30	C=N	imine group
3321.42, 3579.88, 3630.03, 3691.75	-OH	alcohol & phenols

Larvicidal activity of *Catharanthus roseus* leaf extracts

Larvicidal activity of acetone, ethanol and distilled water extracts of *Catharanthus roseus* leaves at various concentrations were tested against filarial vector *Culex quinquefasciatus*. The results of the current study revealed that the acetone extract of *Catharanthus roseus* leaves exhibited potential larvicidal activity at varying concentrations followed by ethanol and distilled water extracts whereas, no larval mortality was observed in controls.

Among the three different *Catharanthus roseus* leaf extracts acetone leaf extract exhibited 100% mortality in the time period of 24 hours and the ethanol extract achieved 100% mortality at 120 hours at the concentration of 400 ppm. Similarly the distilled water shows 92.50 % at 120 hours after exposure. From this result it is cleared that the acetone leaf extract shows maximum mortality rate in short period of exposure. The larvicidal effect of acetone leaf extract of *Catharanthus roseus* exhibited in dose dependent as well as the duration of exposure (Fig 2).

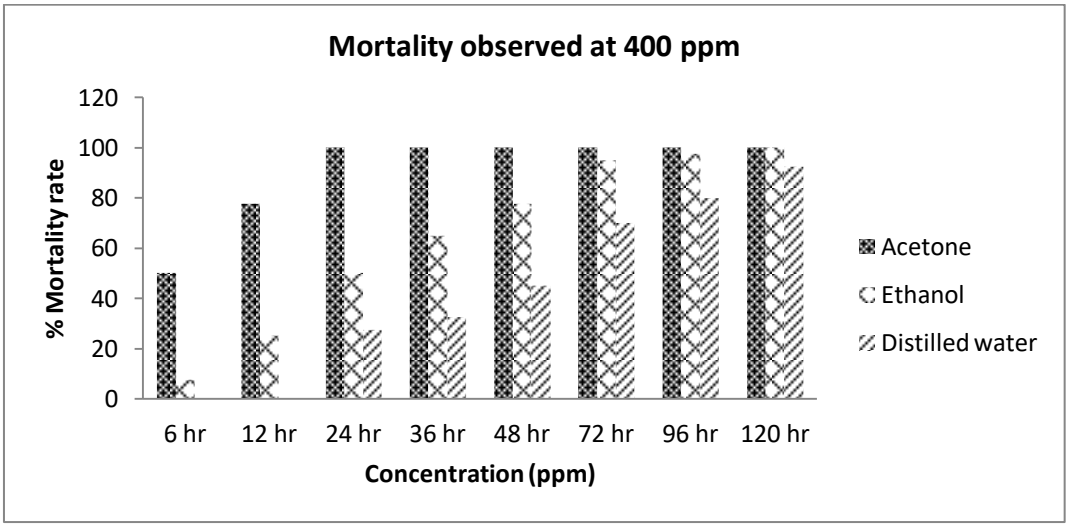

Figure 2: Comparative larvicidal activity of acetone, ethanol and aqueous leaf extracts of *Catharanthus roseus* against *Culex quinquefasciatus* at 400 ppm

The LC_{50} values of acetone leaf extract of *Catharanthus roseus* against *Culex quinquefasciatus* were found to be 403.57 ppm, 309.39 ppm, 240.88 ppm, 223.82 ppm, 193.18 ppm, 140.76 ppm, 94.46 ppm and 82.56 ppm for the time intervals of 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 96 hr and 120 hr respectively (Table 3). Similarly the LC_{50} values of ethanol (Table 4) and distilled water (Table 5) leaf extracts were found to be 471.84 ppm, 473.16 ppm, 397.99 ppm, 354.55 ppm, 301.81 ppm, 229.26 ppm, 161.99 ppm, 90.38 ppm and 00.00 ppm, 00.00 ppm, 478.05 ppm, 464.23 ppm, 426.78 ppm, 287.41 ppm, 223.65 ppm, 159.35 ppm for the time intervals of 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 96 hr and 120 hr respectively.

The LC_{90} values were also calculated. The LC_{90} values of acetone leaf extract of *Catharanthus roseus* against *Culex quinquefasciatus* were found to be 542.10 ppm, 480.40 ppm, 374.00 ppm, 360.54 ppm, 359.92 ppm, 377.17 ppm, 285.58 ppm and 264.95 ppm for the time intervals of 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 96 hr and 120 hr

respectively. Likewise LC_{90} values of ethanol and distilled water leaf extracts of *Catharanthus roseus* against larvae of *Culex quinquefasciatus* were found to be 535.72 ppm, 628.62 ppm, 605.46 ppm, 619.13 ppm, 579.15 ppm, 467.19 ppm, 377.11 ppm, 271.72 ppm and 00.00 ppm, 00.00 ppm, 715.03 ppm, 757.32 ppm, 818.73 ppm, 569.04 ppm, 466.04 ppm, 372.47 ppm for the time intervals of 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 96 hr and 120 hr respectively.

According to Parthasarathy *et al* [21] the LC_{50} values of *Catharanthus roseus* against 1st, 2nd, 3rd and 4th instar larvae of *Culex quinquefasciatus* were 30.28, 38.01, 59.12 and 71.81ppm respectively. The LC_{90} values of against 1st, 2nd, 3rd and 4th instar larvae were 106.01, 227.50, 450.46 and 675.32ppm respectively. Phytochemical derived from *Catharanthus roseus* flower extracts are effective mosquito vector control agent and the plant extracts may be useful for the further integrated pest management program.[22] The larvicidal activity shown by *Catharanthus roseus* is probably due to the presence of the alkaloid which are toxic substances. Phenolic and non-phenolic alkaloids isolated from *Catharanthus roseus* leaves are known to have toxic effects. In addition, the compounds of *Catharanthus roseus* extracts are neurotoxic in nature.[23] The mechanism of action of plant secondary metabolites on insect body documented several physiological disruptions, such as inhibition of acetylecholinestrase, sodium and potassium ion exchange disruption and inhibition of cellular respiration. The blockage of calcium channels, inhibition of nerve cell membrane action, octopamine receptors, disruption of hormonal balance, mitotic poisoning, disruption of the molecular events of morphogenesis and alteration in the behavior and memory of cholinergic system.[24]

Table 3: Probit analysis for the mortality rate of *Culex quinquefasciatus* using acetone leaf extract

Hours	Mean % larval mortality							95% Confidence Limit		x^2
	Concentration (ppm)							LC_{50}	LC_{90}	
	50	100	150	200	250	300	400			
6	00.00	00.00	5.00	00.00	2.50	20.00	50.00	403.57	542.10	25.45*
12	5.00	7.50	7.50	17.50	32.50	47.50	77.50	309.39	480.40	5.34*
24	7.50	15.00	12.50	27.50	45.00	72.50	100	240.88	374.00	25.13*
36	7.50	20.00	17.50	37.50	50.50	77.50	100	223.82	360.54	18.58*
48	17.00	32.50	25.00	52.50	60.00	77.50	100	193.18	359.92	19.77*
72	30.00	47.50	45.00	67.50	62.50	87.50	100	140.76	377.17	20.97*
96	42.00	50.00	64.50	76.00	79.50	92.50	100	94.461	285.58	5.37*
120	45.00	53.50	66.50	79.00	84.50	95.00	100	82.568	264.95	3.69*

x^2 : Chi square value; *= significant (P<0.05); LC: lethal concentration.

Table 4: Probit analysis for the mortality rate of *Culex quinquefasciatus* using ethanol leaf extract

Hours	Mean % larval mortality							95% Confidence Limit		x^2
	Concentration (ppm)							LC_{50}	LC_{90}	
	50	100	150	200	250	300	400			
6	00.00	00.00	00.00	00.00	00.00	00.00	7.50	471.84	535.72	0.02*
12	00.00	00.00	00.00	00.00	5.00	10.00	25.00	473.16	628.62	3.70*
24	00.00	5.00	7.50	10.00	17.50	28.00	50.00	397.99	605.46	2.96*
36	7.50	12.50	20.00	20.00	25.00	35.00	65.00	354.55	619.13	5.85*
48	15.00	20.00	25.00	30.00	35.00	40.00	77.50	301.81	579.15	10.90*
72	22.50	27.50	32.50	40.00	47.50	52.50	95.00	229.26	467.19	23.50*
96	30.00	35.00	45.00	57.50	70.00	72.50	97.50	161.99	377.11	8.37*
120	46.50	51.50	60.00	75.00	84.00	87.50	100	90.388	271.72	9.01*

x^2 : Chi square value; *= significant (P<0.05); LC: lethal concentration.

Table 5: Probit analysis for the mortality rate of *Culex quinquefasciatus* using distilled water leaf extract

Hours	Mean % larval mortality							95% Confidence Limit		x^2
	Concentration (ppm)							LC_{50}	LC_{90}	
	50	100	150	200	250	300	400			
6	00.00	00.00	00.00	00.00	00.00	00.00	7.50	471.84	535.72	0.02*
12	00.00	00.00	00.00	00.00	5.00	10.00	25.00	473.16	628.62	3.70*
24	00.00	5.00	7.50	10.00	17.50	28.00	50.00	397.99	605.46	2.96*
36	7.50	12.50	20.00	20.00	25.00	35.00	65.00	354.55	619.13	5.85*
48	15.00	20.00	25.00	30.00	35.00	40.00	77.50	301.81	579.15	10.90*
72	22.50	27.50	32.50	40.00	47.50	52.50	95.00	229.26	467.19	23.50*
96	30.00	35.00	45.00	57.50	70.00	72.50	97.50	161.99	377.11	8.37*
120	46.50	51.50	60.00	75.00	84.00	87.50	100	90.388	271.72	9.01*

x^2 : Chi square value; *= significant (P<0.05); LC: lethal concentration.

Results on the regression analysis revealed that the mortality rate (Y) was positively correlated with the concentration of exposure (X) having a regression coefficient (R) closer to 1 (Table 6). The chi-square values were significant at 5% level. Among the different time intervals and concentration, the acetone extract showed higher mortality on the vector mosquito *Culex quinquefasciatus*.

Table 6: Regression analysis of various samples in relation to the mortality rate at periods of exposure

Hours	Acetone		Ethanol		Distilled Water	
	Regression equation	R value	Regression equation	R value	Regression equation	R value
6	Y=12.99x+15.84	0.70	Y=01.65x+2.3	0.49	Y=00.00x+0.00	1
12	Y=21.35x+16.37	0.91	Y=06.88x+8.4	0.78	Y=00.00x+0.00	1
24	Y=27.82x+17.64	0.92	Y=13.72x+11.5	0.92	Y=08.70x+7.32	0.91
36	Y=27.60x+12.82	0.95	Y=14.99x+4.6	0.88	Y=09.73x+4.80	0.96
48	Y=24.20x+1.924	0.95	Y=16.08x+1.3	0.87	Y=10.18x+3.90	0.96
72	Y=19.77x+21.89	0.93	Y=19.05x+5.8	0.89	Y=16.08x+3.82	0.99
96	Y=17.32x+36.18	0.96	Y=19.61x+17.8	0.98	Y=18.97x+7.47	0.98
120	Y=16.70x+40.17	0.94	Y=16.31x+38.2	0.97	Y=19.85x+17.7	0.97

R value: Regression coefficient

The two way ANOVA of larvicidal effect exhibited by various concentration of acetone, ethanol and distilled water extracts of *Catharanthus roseus* on *Culex quinquefasciatus*. The larval mortality were statistically significant (P<0.05) at 24, 36, 48, 72, 96 and 120 hrs (F=151.72). Statistical significance were also observed between three different solvents (P<0.05) at various time intervals (F=54.79). Thus the plant extracts can be used as effective larvicidal agent instead of synthetic insecticides.

CONCLUSION

Mosquitoes play a predominant role in transmission of several life threatening diseases to the human population all around the world. They are the principle vector of many vector-borne diseases affecting human beings and animals, in addition to nuisance. In present study, the result clearly reveals that the acetone leaf extract of *Catharanthus roseus* could serve as a potential larvicidal agent against the filarial vector *Culex quinquefasciatus*. The mode of action and larvicidal efficacy of this plant leaf extract under the field conditions should be scrutinized and determined. Besides, further investigation regarding the effect on non-target organism and synergism with biocides are extremely important. As the plant of the present study is widely distributed, the commercial exploitation could provide an important step in the development of new plant based insecticide as one of the alternatives to expensive and environmentally harmful chemical insecticides.

Acknowledgements: The authors are grateful to the management of PSG College of Arts and Science, Coimbatore for their encouragement and providing facilities for this work.

REFERENCES

1. Balu S, Gokulakrishnan J, Elanchezhiyan K, Deepa J, Mosquito larvicidal, ovicidal and pupicidal activities of *Annona reticulata* linn (annonaceae) against *Aedes aegypti* (linn.), *Anopheles stephensi* liston and *Culex quinquefasciatus* (say) (diptera: culicidae), International Journal of Recent Scientific Research, 6, 2015, 2690-2696.
2. World Health Organization (WHO). Malaria fact sheets No. 94. WHO Report. 2010 Apr. Available from:http://www.who.int/mediacentre/factsheets/fs094/en/index.html.
3. Kumar D, Rakesh C, Dhamodaram P, Balakrishnan N, Larvicidal activity of *Cassia occidentalis* (linn.) against the larvae of bancroftian filariasis vector mosquito *Culex quinquefasciatus*, Journal of parasitology Research, 2014. DOI:http://dx.doi.org/10.1155/2014/236838.
4. Rajkumar S, Jebanesan A, Larvicidal and adult emergence inhibition effect of *Centella asiatica* brahmi (Umbelliferae) against mosquito *Culex quinquefasciatus* say (Diptera : Culicidae), African Journal of Biomedical Research, 8, 2005, 31-33.
5. Sakthivadivel M, Gunasekaran P, Sivakumar S, Samraj A, Arivoli S, Samuel T, Evaluation of *Solanum trilobatum* L (Solanaceae) aerial extracts for mosquito larvicidal activity against the filarial vector *Culex quinquefasciatus* Say (Diptera:Culicidae), Journal of Entomology and Zoology Studies. 2, 2014, 102-106.
6. Pavela R, Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* say larvae (Diptera: Culicidae), Parasitology Research, 3, 2008, 555-559.
7. Gibson EL, Wardle J, Watts CJ, Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children, Appetite, 31, 1998, 205-228.
8. Shaikh M, Revansiddha D, Mamtaram K, Phytochemical analysis of stem bark of some medicinal plants, International Journal of Research in Pharmacy and Pharmaceutical Sciences. 3, 2018, 86-89.
9. Koche D, Shirsat R, Imran S, Bhadange DG, Phytochemical screening of eight traditionally used ethanomedicinal plants from akola district (MS) India, International Journal of Pharma and Bio Sciences. 1, 2010, 253-256.
10. Yadav R, Tyagi V, Tikar SN, Sharma AK, Sukumaran D, Differential larval toxicity and oviposition altering activity of some indigenous plant extracts dengu chikungunya vector *Aedes albopictus*, Journal of Arthropod Borne Disease, 8, 2013, 174-185.
11. Singh D, Metha SS, Neoliya NK, Shukla YN, Mishra M, New possible insect growth regulators from *Catharanthus roseus*, Current Science, 84, 2003, 1184–1186.
12. Gowri sankar S, Thangapandiyan S, Alif Alisha AS, Saravana kumar P, Phytochemical Screening and Potential Bactericidal Efficacy of Annona Squamosa Leaf Extracts. World Journal of Pharmacy and Life Science. 4, 2018, 144-149
13. Trease GE, Evans WC, Pharmacognosy, W.B. Saunders Company Ltd, 1989;14:269-300.
14. Abbot WS, A method of computing the effectiveness of an insecticide, Journal of Economic Entomology, 18, 1925, 265-267.
15. Bowers WS, Sener B, Evans PH, Bingol F, Erdogani I, Activity of Turkish medicinal plants against mosquitoes *Aedes aegypti* and *Anopheles gambiae*, Insect Science and Its Application, 16, 1995, 339-341.
16. Jaleel CA, Panneerselvam R, Variations in antioxidant and indole alkaloid status in different parts of two varieties of *Catharanthus roseus*, Chinese Journal of Pharmacology and Toxicology, 6, 2007, 487-94.
17. Rey D, Pautou MP, Meyran JC, Histopathological effects of tannic acid on the midgut epithelium of some aquatic diptera larvae, Journal of Invertebrate Pathology, 73, 1999, 173-181.
18. Tiwari P, Kumar B, Kaur M, Gurpreet K, Harleen K, Phytochemical screening and extraction: A review, Internationale Pharmaceutica sciencia, 1, 2011, 98-106.
19. Okwu DE, Okwu ME, Chemical composition of *Spondias mombin* linn plant parts, Journal for Sustaining Agricultural Environment, 6, 2004, 140-147.
20. Utomo, Margo, Own power plant-based ingredients papaya seed powder against death Aedes aegypti larvae isolates SALATIGAB2P2VRP Laboratory (Proceedings of the National Seminar Unimus). 2013. DOI: http //: jurnal.unimus.ac.id.
21. Parthasarathi A, Chettiar K, Rajan M, Savarimuthu I, Evaluation of larval toxicity of *Lantana camara* L. and *Catharanthus roseus* L. against *Culex quinquefasciatus* say and *Aedes aegypti* L. Entomology, Ornithology and Herpetology, 5, 2016, 1-5.
22. Prasad A, Mathur P, Shrivastava M, Kumar D, Sharma E, Larvicidal efficacy of *Catharanthus roseus* linn leaves and flowers against the malaria vector *Anopheles stephensi* liston (insecta:diptera: culicidae), International Journal of Recent Scientific Research. 5, 2014, 1620-1623.
23. Waskito T, Isolasi dan uji aktivitas alkaloid dari daun *Tapak dara* (*Catharanthus roseus*) var albus, Tesis, Universitas Diponegoro, Semarang.
24. Rattan RS, Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Protection**,** 29, 2010, 913-922.

Source of Support: Nil, **Conflict of Interest:** None.