

## LARVICIDAL AND ENZYME INHIBITORY EFFECTS OF *ACALYPHA FRUTICOSA* (F.) AND *CATHARANTHUS ROSEUS* L (G) DON. LEAF EXTRACTS AGAINST *CULEX QUINQUEFASCIATUS* (SAY.) (DIPTERA: CULICIDAE)

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### ABSTRACT

**Objective:** The prime objective of this study is to evaluate the larvicidal and enzyme inhibitory effects of *Acalypha fruticosa* and *Catharanthus roseus* leaf extracts against *Culex quinquefasciatus*.

**Methods:** Insecticidal susceptibility tests were carried out using the World Health Organization standard method, and the mortality was observed after 24 hrs exposure.

**Results:** The tested extracts showed a significant larvicidal activity depending on the dose of the plant extracts. However, maximum larval mortality was detected in ethyl acetate extract of *A. fruticosa* with a lethal concentration (LC<sub>50</sub>) value of 253.08 ppm and (LC<sub>90</sub>) value of 455.40 ppm followed by hexane extract of *C. roseus* with LC<sub>50</sub> value of 645.33 ppm and LC<sub>90</sub> value of 1452.88 ppm against the larvae of *C. quinquefasciatus*. In control, there were five different fractions of  $\alpha$ -carboxylesterases resolved in the gel with the mobilities ranging between 0.14 and 0.66.  $\beta$ -carboxylesterase isozyme profile of larvae treated with hexane extract of *A. fruticosa* showed higher enzyme activities by way of intense staining of fractions both at 1/4 and 1/10 LC<sub>50</sub> value of 48 hrs treatments. *A. fruticosa* ethyl acetate extract, and *C. roseus* hexane extract exposed larvae indicated increased quantities of  $\beta$ -Est4/5 isozymes in the concentration of 1/4 and 1/10 of LC<sub>50</sub> value of 24 hrs treatment when compared to control. The protein quantity in the majority of treatments decreased compared to control.

**Conclusion:** The results revealed that the organic leaf extract of *A. fruticosa* and *C. roseus* had significant larvicidal and enzyme inhibitory effects against *C. quinquefasciatus*.

**Keywords:** *Acalypha fruticosa*, *Catharanthus roseus* leaves extract,  $\beta$ -carboxylesterase, *Culex quinquefasciatus*, Lethal concentration<sub>50</sub>

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### INTRODUCTION

Mosquitoes are medically most important and responsible for transmitting the vector-borne diseases, parasites, and pathogens which continue to have a devastating impact on human beings, public hygiene and ecological perspectives [1]. Mosquitoes under the genus *Culex* are the vectors of encephalitis and filariasis [2]. Frequent outbreaks of these diseases result in restricting the socioeconomic status in developing countries in the tropical and subtropical belts. Considerable economic, ecological, and public health impacts of vector-borne diseases are expected to continue, given limited domestic and international capabilities for detecting, identifying, and addressing likely epidemics. It is estimated that every year at least 500 million people in the world suffer from one or the other tropical diseases that include malaria, lymphatic filariasis, schistosomiasis, dengue, trypanosomiasis, and leishmaniasis. 1-2 million deaths are reported annually due to malaria worldwide. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia, and Pacific Islands. Synthetic insecticides are effective in controlling mosquitoes, but their environmental consequences are unpredictable. In the context of ever-increasing trend to use more powerful synthetic insecticides to achieve immediate results in the control of mosquitoes, an alarming increase of physiological resistance in the vectors, its increased toxicity to non-target organism and high costs are noteworthy [3]. Most of the synthetic chemicals are expensive and destructive to the environment and also toxic to humans, animals, and other beneficial organisms. Besides, they are non-selective and harmful to other beneficial organisms. Therefore,

effective and low-cost alternate vector control strategies, especially are extremely imperative [4,5]. Use of different parts of locally available plants and their various products in the control of mosquitoes have been accepted globally by numerous researchers.

Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, and ovipositional attractants and also have deterrent actions as observed by many researchers [6-8]. The larvicidal properties of indigenous plants have also been documented in many parts of India along with the repellent and anti-juvenile hormones activities [9]. Traditionally, plants and their derivatives were used to kill mosquitoes and other household and agricultural pests. In all probability, these plants used to control insects contained insecticidal phytochemicals that were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects [10,11].

Esterase is an enzyme that played a significant role both physiologically and biochemically during any stress on insects. They are excellent key enzymes involved in the metabolism of hormones [12,13], digestion [14], and resistance to insecticides. Esterases are considered an important tool for analysis of genetic differentiation and evolutionary relationship of insects [12]. These enzymes are tissue specific in insects [15] and are closely linked with morphological, physiological, or biochemical ontogenetic alterations [16]. French-Constant *et al.* [17] described an electrophoretic technique, based on patterns of esterase bands on polyacrylamide gel (PAGE) for

distinguishing large samples of *Myzus antirrhini* from susceptible or resistant populations of the closely related species of *Myzus persicae*. In addition to the insecticidal activity, most of toxicological approaches on botanical insecticides have been focused on inhibition or induction of detoxifying enzymes such as carboxylesterase, glutathione-S-transferase and cytochrome P450. Rachokarn et al. [18] reported similar results on different enzymes, that is, carboxylesterase activity had a tendency to be induced after treated with *Amaranthus viridis* extracts. These inductions of detoxifying enzyme suggest a possibility on the development of resistance against the extracts as shown in many reports on increased induction in detoxifying enzyme activity and discussed on development of resistance of many insect pests [19-22]. In the present work, both quantitative and qualitative analyses of esterases were undertaken to assess the efficacy of hexane, ethyl acetate, and methanol extracts of *Acalypha fruticosa* and *Catharanthus roseus* leaves against esterase induction/activity in the fourth-instar larvae of *Culex quinquefasciatus*. With this background, the present investigation was undertaken to assess the efficacy of various solvent extracts of the leaves of very well-known Indian medicinal plants *A. fruticosa* and *C. roseus* against the fourth-instar larvae of *C. quinquefasciatus*.

## MATERIALS AND METHODS

### Materials

#### Plant collection and preparation of leaf extracts

The healthy leaves of the selected plant species of *A. fruticosa* (Euphorbiaceae) and *C. roseus* (Apocynaceae) were collected during 2012 from in and around Chennai, Tamil Nadu, India. Plant specimens were identified by plant taxonomists and standard flora. The leaves were shade dried at room temperature and coarsely powdered in a powdering machine. A total of 200 g powder of the plant was extracted sequentially with increasing polarity of hexane, ethyl acetate, and methanol at room temperature for 1 week with occasional shaking in an aspirator bottle. The extract was filtered through Watmann No.1 filter paper and evaporated in vacuum evaporator.

### Rearing of mosquitoes

The test was carried out against laboratory reared *C. quinquefasciatus* mosquitoes free of exposure to insecticides and pathogens. Cyclic generations of *C. quinquefasciatus* were maintained at 25-29°C and 80-90% R.H in the insectarium. Larvae were fed on larval food (powdered dog biscuits and yeast in the ratio of 3:1) and adult mosquitoes on ten percent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

### Larval toxicity test

A laboratory reared colony of *C. quinquefasciatus* larvae was used for the larvicidal activity. Twenty individuals of fourth-instar larvae were kept in a 500 ml glass beaker containing 249 ml of dechlorinated water and desired concentration of *C. roseus* and *A. fruticosa* leaf extracts in 1 ml of acetone with Tween-20 (1%). Larval food was given for the test larvae. At each tested concentration, two to five trials were made, and each trial includes five replicates. The negative control was setup by mixing 1 ml of acetone with 249 ml of dechlorinated water. The larvae exposed to dechlorinated water without acetone served as positive control. The control mortalities were corrected by using Abbott's formula [23].

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

### Statistical analysis

All data were subjected to analysis of variance. The means were separated using Duncan's multiple range tests by Alder and Rossler [24]. The average larval mortality data were subjected to probit analysis for calculating lethal concentration (LC<sub>50</sub>) and LC<sub>90</sub>. Values were calculated using the Finney [25] method. Statistical software package 9.0 version was used. Results with p<0.05 were considered statistically significant.

### Preparation of whole body homogenates of larvae and pupae for esterase analyses

The level of esterase enzymes (against both alpha and beta naphthyl acetates as substrates) were estimated in the fourth-instar larvae of *C. quinquefasciatus* subjected to hexane, ethyl acetate, and methanol extracts of *A. fruticosa* and *C. roseus* leaves. A suitable control was also maintained. After treatment, the larvae were removed from the treatment tray, washed with double distilled water, and the adhering water was completely removed from the body by blotting with tissue paper. The larvae (25 individuals) from each of the treatments were transferred separately to eppendorf tubes and homogenized using a teflon hand homogenizer in 150 µl of ice-cold phosphate buffer (20 mM, pH 7.0) for extraction of esterases. The whole body homogenates were centrifuged at 10000 rpm at 4°C for 20 minutes, and the clear supernatants were collected for further esterase assays.

### Quantitative analyses of biochemical constituents

The impact of exposure of plant extracts on fourth-instar larvae of *C. quinquefasciatus* was quantitatively analyzed in response to the level of protein and expressed as unit enzyme activity per mg protein.

### Determination of protein concentration

Protein concentrations in the larval homogenates were estimated following the method of Bradford [26]. To 5 µl of protein or blank solution, 195 µl of Bradford reagent was added and mixed well. The optical density was read between 5 and 20 minutes after addition of protein reagent at 595 nm. Protein concentration per ml of the test sample was calculated as follows.

$$\text{Protein concentration in the sample} \left( \frac{\text{mg}}{\text{ml}} \right) = \frac{\text{OD of sample}}{\text{OD of standard}} \times \text{Concentration of standard (40 } \mu\text{g)}$$

### Estimation of carboxylesterase activity

The carboxylesterase activity in the whole body homogenates was measured by the modified method of Van Asperen [27] as described by Argentine and James [28].

### Estimation of α-carboxylesterase activity

A volume of 10 µl of whole body homogenate of larvae was incubated with 198 µl of 20 mM sodium phosphate buffer (pH 7.0) containing 250 µM of α-naphthyl acetate for 10 minutes at RT. After incubation, 50 µl of freshly prepared 0.3% fast blue B salt in 3.3% SDS were added to stop the enzymatic reaction and allowed to develop color for 15 minutes at RT. The optical density of samples was read at 430 nm against the blank consisting of same reagents and buffer substituted for the homogenate. The level of α-carboxylesterase activity was calculated and expressed as µM/minute/mg protein.

### Estimation of β-carboxylesterase activity

The whole body homogenate of larvae was first diluted 3 times with sodium phosphate buffer (20 mM, pH 7.0), and 100 µl of diluted homogenate was incubated with 1 ml of sodium phosphate buffer (20 mM, pH 7.0) containing 250 µM of β-naphthyl acetate for 10 minutes at RT. After incubation, 400 µl of freshly prepared 0.3% fast blue B salt in 3.3% SDS were added to stop the enzymatic reaction and allowed to develop color for 15 minutes at RT. Blank consisted of same reagents and buffer instead of homogenate. The optical density of samples was read at 580 nm against the blank. The level of β-carboxylesterase activity was calculated and expressed as µM/minute/mg protein.

### Qualitative analyses of esterase enzymes using native PAGE

The effect of exposure of fourth-instar larvae to various solvent extracts of plants was qualitatively analyzed by native-PAGE with esterase enzymes.

### Native-PAGE

The profiles of esterase enzymes in the whole body homogenates of fourth-instar larvae were analyzed in discontinuous PAGE under non-

denaturing conditions following Maurer [29]. This was performed using 3% stacking gel (pH 6.7) and 7% separating gel (pH 8.9) in Tris-glycine buffer (pH 8.3). Samples of the whole body homogenates (each 80  $\mu$ l) of control and experimental larvae were electrophoresed at a constant current of 3 mA per sample at 10°C on a slab gel (170  $\times$  150  $\times$  1.5 mm). After electrophoresis, the gels were suitably stained for detection of esterase activity.

#### Detection of carboxylesterase activity

The electrophoretically separated bands with esterase activity were detected in the gel following the method of Argentine and James [28]. Accordingly, the gel was first incubated with phosphate buffer (20 mM, pH 7.0) for 15 minutes at RT. After decanting the buffer, the gel was then re-incubated for 30 minutes at RT with freshly prepared  $\alpha$ -naphthyl acetate + fast blue B solution for detection of  $\alpha$ -carboxylesterase or  $\beta$ -naphthyl acetate + fast blue B solution for detection of  $\beta$ -carboxylesterase. The gels were washed with distilled water and stored in 7% acetic acid. After the electrophoresis, the destained gel was measured for relative mobility of protein bands. Relative mobility is the distance migrated by a band divided by the distance migrated by the dye (i.e., dye front).

#### Densitometry analysis of esterase isozyme

All the native PAGE gels performed for  $\alpha$ -carboxylesterase or  $\beta$ -carboxylesterase banding pattern were subjected to densitometry analysis using Image J 1.46r software developed by National Institutes of Health (NIH), U.S. Department of Health and Human Service.

## RESULTS

The results of larvicidal activity of hexane, ethyl acetate and methanol extracts of *A. fruticosa* and *C. roseus* are summarized in Table 1. The effect on larval mortality was concentration dependent. It has observed that the ethyl acetate extracts of *A. fruticosa* possess maximum larvicidal activity against 4<sup>th</sup> instar larvae of *C. quinquefasciatus*. The percentage mortality of larvae treated with *A. fruticosa* recorded with 87.25% at 5% concentration followed by hexane (65.0%) and methanol (57.50) extracts compared with untreated control. The results of the larvicidal activities of different crude extracts of *C. roseus* in hexane against *C. quinquefasciatus* was 62.50% at 5% concentration followed by extracts of methanol (58.75%) and ethyl acetate (43.75%) when compared with untreated control. No mortality was recorded in various replicates of control.

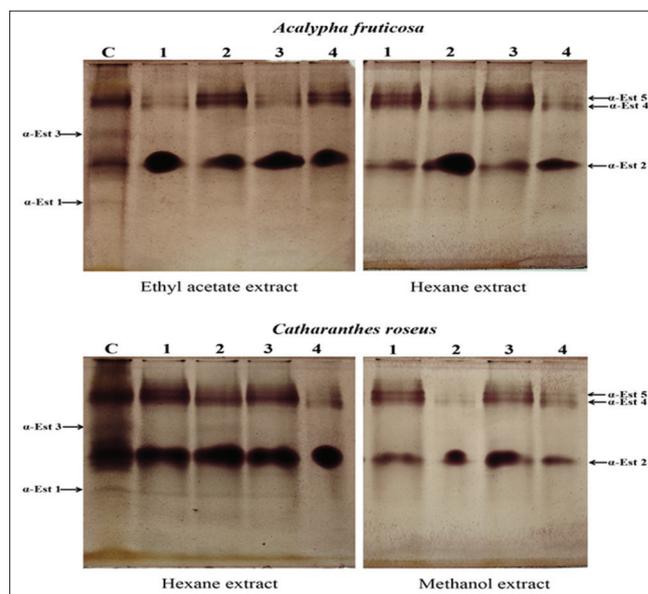
The results of the LC<sub>50</sub> and LC<sub>90</sub> values of the plant extracts after 24 hrs against the larvae of *C. quinquefasciatus* are shown in Table 2.

The ethyl acetate leaf extract of *A. fruticosa* was found to be effective with a LC<sub>50</sub> value of 253.08 ppm and LC<sub>90</sub> value of 455.40 ppm followed by extracts of hexane with LC<sub>50</sub> value of 429.99 ppm and LC<sub>90</sub> value of 774.45 ppm and methanol with LC<sub>50</sub> value of 574.82 ppm and LC<sub>90</sub> value of 1035.00 ppm against 4<sup>th</sup> instar larvae of *C. quinquefasciatus*. The efficacy of hexane extract of *C. roseus* was most active with LC<sub>50</sub> value of 645.33 ppm and LC<sub>90</sub> value of 1452.88 ppm followed by extracts of methanol with LC<sub>50</sub> value of 715.39 ppm and LC<sub>90</sub> value of 1287.54 ppm and ethyl acetate with LC<sub>50</sub> value of 1370.06 ppm and LC<sub>90</sub> value of

2089.43 ppm against the larvae of *C. quinquefasciatus*. This study showed the larvicidal potency of various solvent extracts of leaves of these two plants against *C. quinquefasciatus*. Thus *A. fruticosa* and *C. roseus* could be exploited further for identification of specific larvicidal agents.

Esterase enzyme gel electrophoresis was carried out using 7% PAGE (Native-PAGE) with  $\alpha$ - and  $\beta$ -naphthyl acetates as staining substrates for all the plant extracts treated mosquito larvae of *C. quinquefasciatus*. In control, there were five different fractions of  $\alpha$ -carboxylesterases resolved in the gel with the mobilities ranging between 0.14 and 0.66. On exposure of the larvae to different plant extracts, intensity of staining of various enzyme fractions was decreased with disappearance of at least two or three  $\alpha$ -carboxylesterase enzyme fractions (Fig. 1). On the contrary, the staining intensity of one slow moving fraction  $\alpha$ -Est1 with a mobility of 0.49 was increased significantly or maintains the same level in all experimental groups when compared to control. A similar trend was observed in the larvae of *C. quinquefasciatus* treated against ethyl acetate and hexane extracts of *A. fruticosa* and hexane and methanol extracts of *C. roseus* (Fig. 1). The relative mobility of  $\beta$ -carboxylesterase isozyme profile of larvae of *C. quinquefasciatus* treated against ethyl acetate extract of *A. fruticosa* revealed four different enzyme fractions in control and three fractions in 24 hrs treatment and four fractions in 48 hrs treatment (Fig. 2).

Among various treatments, the staining intensities of various enzyme fractions were significantly higher in 24 hrs and 48 hrs treatments of ethyl acetate at 1/4 of LC<sub>50</sub> value at 24 hrs treatment of 1/10 of LC<sub>50</sub> value at 24 hrs treatment. Likewise,  $\beta$ -carboxylesterase isozyme



**Fig. 1:**  $\alpha$ -Carboxylesterase isozyme pattern of *Culex quinquefasciatus* upon exposure to different medicinal plant extracts. C: Control, 1: 1/4 dilution 24 hrs, 2: 1/4 dilution 48 hrs, 3: 1/10 dilution 24 hrs, 4: 1/10 dilution 48 hrs

**Table 1:** Percent larvicidal activity of leaf extracts of *Acalypha fruticosa* and *Catharanthus roseus* against fourth instar larvae of *Culex quinquefasciatus*

Name of the plants	Solvent	Control	Concentration in ppm				
			100	125	250	500	1000
<i>A. fruticosa</i>	Hexane	0 $\pm$ 0 <sup>a</sup>	13.75 $\pm$ 0.95 <sup>bc</sup>	21.25 $\pm$ 1.01 <sup>bc</sup>	35.00 $\pm$ 1.91 <sup>c</sup>	48.11 $\pm$ 1.15 <sup>d</sup>	65.00 $\pm$ 0.22 <sup>b</sup>
	Ethyl acetate	0 $\pm$ 0 <sup>a</sup>	25.00 $\pm$ 0.12 <sup>c</sup>	33.75 $\pm$ 1.36 <sup>d</sup>	53.75 $\pm$ 1.20 <sup>c</sup>	65.75 $\pm$ 1.33 <sup>c</sup>	87.25 $\pm$ 1.52 <sup>c</sup>
	Methanol	0 $\pm$ 0 <sup>a</sup>	10.00 $\pm$ 1.41 <sup>b</sup>	20.00 $\pm$ 1.41 <sup>b</sup>	16.25 $\pm$ 0.83 <sup>a</sup>	46.00 $\pm$ 0.56 <sup>b</sup>	57.50 $\pm$ 0.51 <sup>ab</sup>
<i>C. roseus</i>	Hexane	0 $\pm$ 0 <sup>a</sup>	12.25 $\pm$ 0.77 <sup>b</sup>	18.50 $\pm$ 1.77 <sup>a</sup>	24.75 $\pm$ 1.65 <sup>b</sup>	44.50 $\pm$ 1.02 <sup>b</sup>	62.50 $\pm$ 0.44 <sup>a</sup>
	Ethyl acetate	0 $\pm$ 0 <sup>a</sup>	11.25 $\pm$ 1.59 <sup>b</sup>	16.25 $\pm$ 1.30 <sup>a</sup>	23.75 $\pm$ 0.36 <sup>b</sup>	28.75 $\pm$ 1.07 <sup>a</sup>	43.75 $\pm$ 1.20 <sup>a</sup>
	Methanol	0 $\pm$ 0 <sup>a</sup>	10.75 $\pm$ 1.41 <sup>b</sup>	27.5 $\pm$ 1.54 <sup>c</sup>	42.50 $\pm$ 0.96 <sup>b</sup>	52.50 $\pm$ 0.63 <sup>d</sup>	58.75 $\pm$ 1.38 <sup>a</sup>

Values carrying same alphabets in a column are not statistically significant by Turkey's Test at p= 0.05% level

**Table 2: Probit analysis on larvicidal efficacy of *Acalypha fruticosa* and *Atharanthus roseus* against fourth instar larvae of *Culex quinquefasciatus***

Name of the plants	Solvent extract	LC <sub>50</sub>	LC <sub>90</sub>	Chi-square Value	Regression value
<i>A. fruticosa</i>	Hexane	429.99	774.45	0.82*	2.72
	Ethyl acetate	253.08	455.40	1.52*	2.54
	Methanol	574.82	1035.00	8.19	2.88
<i>C. roseus</i>	Hexane	645.33	1452.88	2.40*	2.92
	Ethyl acetate	1370.06	2089.43	0.96*	3.22
	Methanol	715.39	1287.54	5.82*	2.96

\*Significant at P<0.05 level, *C. quinquefasciatus*: *Culex quinquefasciatus*, *A. fruticosa*: *Acalypha fruticosa*

profile of larvae treated with hexane extract of *A. fruticosa* showed higher enzyme activities by way of intense staining of fractions both at 1/4 and 1/10 LC<sub>50</sub> value of 48 hrs treatments (Fig. 2).

In the case of relative mobilities of  $\beta$ -carboxylesterase enzyme profile of larvae of *C. quinquefasciatus* treated against hexane and methanol extracts of *C. roseus* indicated higher enzyme activities in the gel, especially 24 hrs treatment in both the concentrations of 1/4 and 1/10 of LC<sub>50</sub> value (Fig. 2).

Individual band intensities of  $\alpha$ - or  $\beta$ -carboxylesterase isozyme from the profile obtained from native PAGE gel were quantified using ImageJ (NIH). Values in the linear range of enzyme fraction/band intensities were selected for quantifications. Samples of control and treatments (control and plant extract exposed larval samples) were run side by side on the same gel. The densitometric analysis revealed that the  $\alpha$ - Est2 isozyme expression level was increased in *A. fruticosa* ethyl acetate extract, *C. roseus* hexane and methanol extract exposed larval samples, especially in the concentrations of 1/4 and 1/10 of LC<sub>50</sub> value of 24 hrs treatment except hexane extract of *A. fruticosa*. Likewise,  $\alpha$ - Est4/5 isozymes were found to be intensely stained in *A. fruticosa* ethyl acetate extract *C. roseus* hexane and methanol extract exposed larval samples (especially 24 hrs treatment in the concentrations of 1/4 and 1/10 of LC<sub>50</sub> value) except *A. fruticosa* ethyl acetate extract exposed larvae (Fig. 3).

*A. fruticosa* ethyl acetate extract, and *C. roseus* hexane and methanol extract exposed larval samples, especially in the concentrations of 1/4 and 1/10 of LC<sub>50</sub> value of 48 hrs treatment were intensely stained in  $\beta$ -Est2 isozyme when compared to control (Fig. 4). Furthermore, *A. fruticosa* ethyl acetate extract and *C. roseus* hexane extract exposed larvae indicated increased quantities of  $\beta$ -Est4/5 isozymes in the concentration of 1/4 and 1/10 of LC<sub>50</sub> value of 24 hrs treatment when compared to control.

Total protein concentrations of larvae exposed to various plant extract treatments revealed that there were decreased levels of protein in the majority of treatments when compared to control. These values of protein were also used to express the quantity of enzymes (Fig. 5).

The  $\alpha$ -carboxylesterase and  $\beta$ -carboxylesterase in the fourth-instar larvae treated with various solvent extracts of *A. fruticosa* and *C. roseus* exhibited significantly high enzyme quantities in all the treatments when compared to control. In control, the quantity of  $\alpha$ -carboxylesterase was 0.89  $\mu\text{M}\cdot\text{minute}^{-1}\cdot\text{mg protein}^{-1}$ . It was observed to be much lower against all the treatments except 1/4 of LC<sub>50</sub> value of hexane extracts of *C. roseus* and in which it was recorded as 0.92  $\mu\text{M}\cdot\text{minute}^{-1}\cdot\text{mg protein}^{-1}$  (Table 3).

The quantity of  $\beta$ -carboxylesterases in control was observed to be 1.87  $\mu\text{M}\cdot\text{minute}^{-1}\cdot\text{mg protein}^{-1}$ . Among various treatments, the results revealed that except a few, all the other solvent extracts of plants yielded higher quantities of  $\beta$ -carboxylesterases (Table 4).

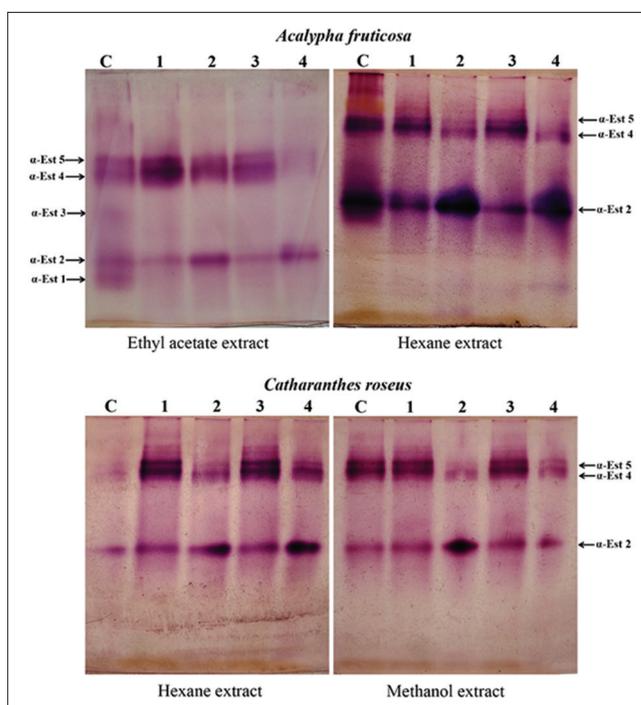
## DISCUSSION

Plant-based medicine, which uses medicinal plants as the first medicine is a general phenomenon. Every civilization on earth, through written

**Table 3: Quantity of  $\alpha$ -carboxylesterase in the fourth instar larvae treated with various solvent extracts of plants**

Name of the plants	Solvent	Esterase activity ( $\mu\text{M}\cdot\text{minute}^{-1}\cdot\text{mg protein}^{-1}$ )			
		1/4 <sup>th</sup> dilution		1/10 <sup>th</sup> dilution	
		24 hrs	48 hrs	24 hrs	48 hrs
<i>A. fruticosa</i>	Ethyl acetate	3.92	3.68	4.42	3.64
	Hexane	5.70	4.62	5.08	3.69
<i>C. roseus</i>	Hexane	1.17	0.92	1.21	1.27
	Methanol	2.96	1.48	2.24	2.11

Control - 0.89  $\mu\text{M}\cdot\text{minute}^{-1}\cdot\text{mg protein}^{-1}$ , *C. roseus*: *Catharanthus roseus*, *A. fruticosa*: *Acalypha fruticosa*



**Fig. 2:  $\beta$ -Carboxylesterase isozyme pattern of *Culex quinquefasciatus* upon exposure to different medicinal plant extracts C: Control, 1: 1/4 dilution 24 hrs, 2: 1/4 dilution 48 hrs, 3: 1/10 dilution 24 hrs, 4: 1/4 dilution 48 hrs**

or oral tradition, has relied on the vast variety of healing plants for their healing attributes. The majority of medicinal plant products available today, originated from the same traditional [30] traditional medicine has spread throughout the world and has gained popularity not only in the developing countries but also to industrialized countries which have access to alternative medicine [31]. The control of mosquitoes at larval stage is focused with plant extracts. The advantage of targeting mosquito at the larval stage is that they cannot escape from their breeding habitat

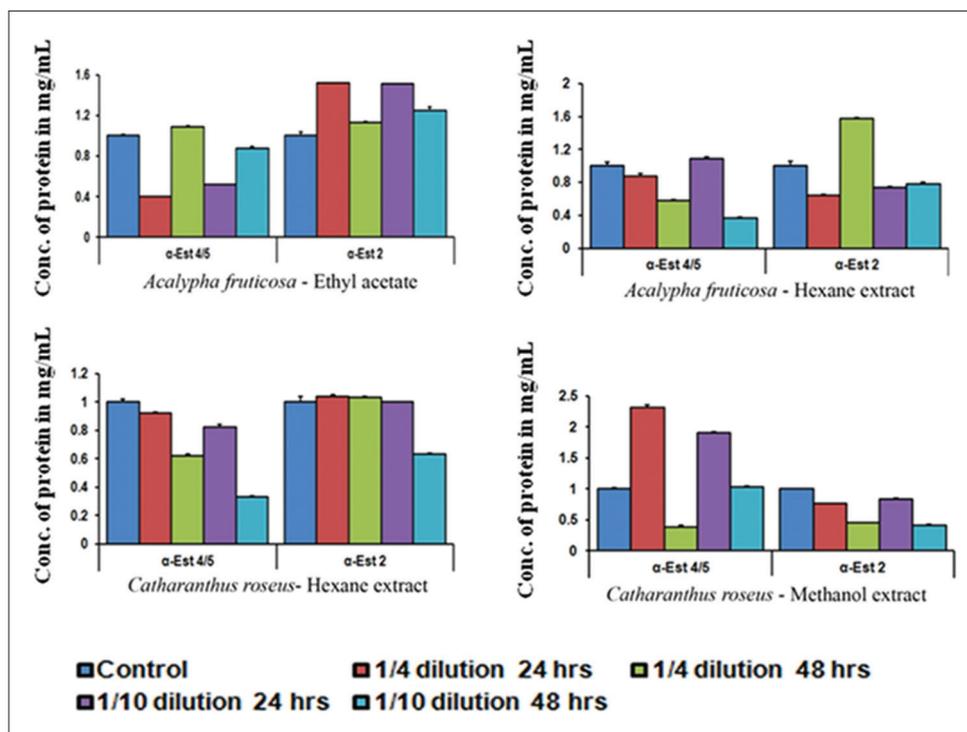


Fig. 3: Densitometry analysis of  $\alpha$ -carboxylesterase isozyme banding pattern by using image 1.46r software

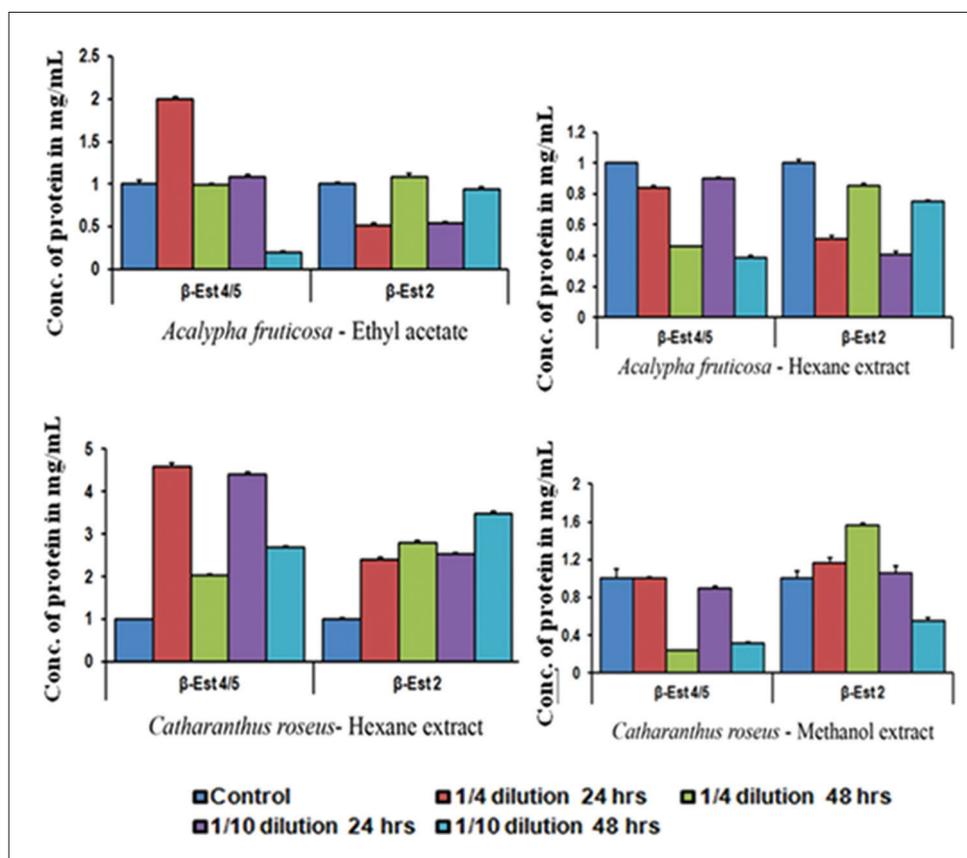


Fig. 4: Densitometry analysis of  $\beta$ -carboxylesterase isozyme banding pattern by using image 1.46r software

until the adult emergencies and also to reduce the overall pesticide use to control of adults by aerial application of adulticidal chemicals. The application of synthetic insecticides could be thus reduced by botanical pesticides through natural control mechanism. These botanicals are often active against a number of insect pest species, less expensive,

easily biodegradable to non-toxic products and potentially suitable for use in mosquito control program [32]. Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase plant resistance to insect attack [33]. Recently, bio-pesticides with plant

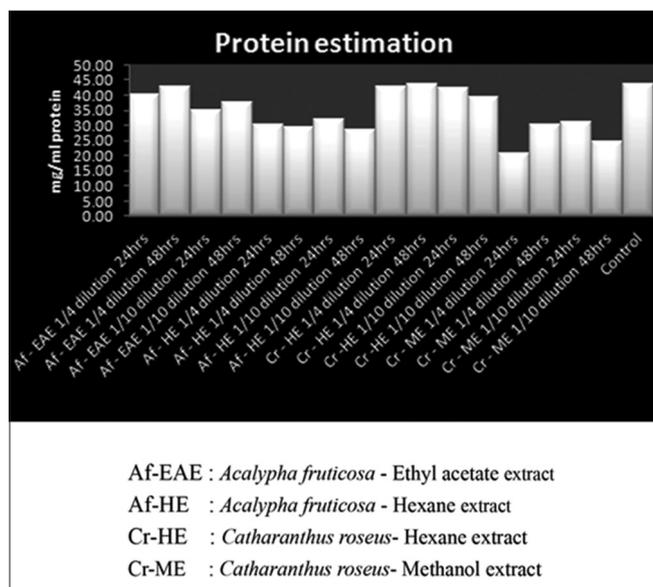


Fig. 5: Determination of protein concentration from whole larval homogenate

Table 4: Quantity of  $\beta$ -carboxylesterase in the fourth instar larvae treated with various solvent extracts of plants

Name of the plants	Solvent	Esterase activity ( $\mu\text{M}\cdot\text{minute}^{-1}\cdot\text{mg protein}^{-1}$ )			
		1/4 <sup>th</sup> dilution		1/10 <sup>th</sup> dilution	
		24 hrs	48 hrs	24 hrs	48 hrs
<i>A. fruticosa</i>	Ethyl acetate	1.37	1.90	3.02	3.13
	Hexane	3.50	3.35	4.14	1.55
<i>C. roseus</i>	Hexane	1.40	1.97	2.64	3.11
	Methanol	5.53	3.35	4.70	2.00

Control:  $1.87 \mu\text{M}\cdot\text{minute}^{-1}\cdot\text{mg protein}^{-1}$ , *C. roseus*: *Catharanthus roseus*, *A. fruticosa*: *Acalypha fruticosa*

origins are used against several insect species especially for the control of insect vectors [34,35]. Natural pesticides, derived from plants are more promising in this aspect. It is evident from our results that these two plants would be useful for further exploration of active compounds for the control of *C. quinquefasciatus* larvae.

The present study showed clearly that the ethyl acetate and hexane extracts of *C. roseus* and *A. fruticosa* had a prominent larvicidal effect on *C. quinquefasciatus*. As reported earlier in similar such studies it may be due to the presence of the phytochemicals such as alkaloids, carboxylic acids, flavanoids, phenols, proteins, quinones, resins, steroids, and saponins in the extracts of the plants [36]. In a related study conducted by Maheswaran et al. [37] on hexane extracts of leaves of *Leucas aspera* against the fourth-instar larvae of *C. quinquefasciatus* with  $\text{LC}_{50}$  values of 122.50 ppm was reported.

The findings of the present study are quite comparable with previous reports of Maheswaran et al. [1] who have reported that the maximum larvicidal activities of different solvent leaf extracts of *L. aspera* in which hexane, chloroform and ethanol extracts showed 230.71, 518.88, and 1059.13 ppm against *Aedes aegypti*. Likewise, there were reports of hexane, ethyl acetate, and methanol extracts of *Momordica charantia*, *Moringa oleifera*, *Ocimum gratissimum*, *Ocimum tenuiflorum*, *Punica granatum* and *Tribulus terrestris* with promising larvicidal activity against *C. gelidus* and *C. quinquefasciatus* [38]. Zahir et al. [39] studied the inhibition of adult emergence and adulticidal activities of hexane, chloroform, ethyl acetate, and acetone leaves extracts of *Anisomeles malabarica*, *Euphorbia hirta*, *Ocimum basilicum*, *Ricinus communis*,

*Solanum trilobatum*, *Tridax procumbens* and seeds of *Gloriosa superba* against *Anopheles stephensi*. These studies provided a clue that there are probabilities for the presence of active principles in these plants with several solvent extracts. The larvicidal and adult emergence inhibition activities of castor (*R. communis*) seed extract against three potential mosquito vectors *A. stephensi*, *C. quinquefasciatus*, *A. albopictus* in India studied by Mandal [8] (2010) suggest that the seed extract provided an excellent potential property for the control of mosquito vectors.

Many similar experiments on the efficacy of plant extracts on the larvicidal action on several species mosquitoes were conducted previously. The findings of the presence results, report of 50% larval mortality with some of the solvent extracts of these two plants also corroborate with earlier findings of Macedo et al. [40] who reported that the ethanol extract of *Tagetes patula* was able to cause only 50% larval mortality at 100 ppm concentration.

Similarly, methanolic leaf extract of *Cassia fistula* was tested for larvicidal activity against *C. quinquefasciatus* and *A. stephensi* with  $\text{LC}_{50}$  values of 17.97 and 20.57 mg/l, respectively [41]. The petroleum ether fraction of *Acacia nolotica* and *Citrullus colocynthis* showed 100% mortality at 100, 250, and 500 ppm and 60% and 50% mortality at 125 and 62.5 ppm, respectively, against *C. quinquefasciatus* [42]. The leaf extract of *Acalypha indica* with different solvents viz, benzene, chloroform, ethyl acetate and methanol were tested for larvicidal and ovicidal activity against *A. stephensi*. The larval mortality was observed after 24 hrs exposure. The  $\text{LC}_{50}$  values are 19.25, 27.76, 23.26, and 15.03 ppm, respectively [41]. The leaf extract of *C. fistula* with different solvents such as methanol, benzene, and acetone were studied for the larvicidal, ovicidal and repellent activity against *A. aegypti*. The 24 hrs  $\text{LC}_{50}$  concentration of the extract against *A. aegypti* were observed at 10.69, 18.27, and 23.95 mg/l, respectively [43]. Cheng et al. [44] reported that the leaf and bark essential oil of *Cryptomeria japonica* showed larvicidal activity against *A. aegypti*. Singh et al. [45] reported the mosquito larvicidal properties of the leaf extract of *Ocimum canum* against *A. aegypti*. The  $\text{LC}_{50}$  values for 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar larvae were 177.82, 229.08, and 331.13 ppm, respectively. Gusmao et al. [46] reported that the extract of *Derris urucu* (*Lonchocarpus*) showed larvicidal activity against *A. aegypti* with  $\text{LC}_{50}$  value of 17.6  $\mu\text{g}/\text{ml}$  which supports the present results were comparably good. Singh et al. [45] demonstrated that the larvicidal activity of *O. canum* oil tested against *A. aegypti* and *C. quinquefasciatus* ( $\text{LC}_{50}$  301 ppm) and *A. stephensi* (234 ppm).

The increased detoxification is a common mechanism of resistance to pesticides (Openoorth, 1985) [47]. In *Culex pipiens*, such a mechanism is often involved in resistance to organophosphates. However, the low levels of organophosphate and pyrethroid resistance could be conferred by either the elevated esterase enzymes [48]. Elevation in the  $\alpha$ -carboxylesterases and  $\beta$ -carboxylesterases are commonly found in the organophosphate resistant strains [49]. In some cases, overproduction is seen only in one type of esterase, that is,  $\alpha$ -esterase or  $\beta$ -esterase, in others both the types are overproduced simultaneously [50]. In the present study, we have also observed an increased  $\alpha$ - and  $\beta$ -carboxylesterase activities in *C. quinquefasciatus* to various solvent extracts of *A. fruticosa* and *C. roseus*. *C. quinquefasciatus* being an urban vector that breeds in the water stagnations might not have been exposed to various agricultural pesticides, before the introduction of plant products. This might have triggered the overproduction of both esterases through genetic selection/gene amplification. Therefore, increased tolerance to the extracts of these plants in *C. quinquefasciatus* might be a result of gene amplification of esterases through genetic selection, resulting in elevated levels of esterase activities.

## CONCLUSION

The present findings on various solvent extracts of leaves of *A. fruticosa* leaves are promising sources for the control of *C. quinquefasciatus* larvae. It is an indigenous medicinal plant of India and is easily available to local people. It may be a safe alternative to synthetic chemicals.

Further studies on isolation of bioactive fraction/constituent as well as application on larger scales in the field may provide promising lead products for effective mosquito control. The results of the present investigations are more or less in agreement with aforementioned findings.

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