**ABSTRACT**

**Objective:** The prime objective of this study is to evaluate the larvicidal and enzyme inhibitory effects of *Acalypha fruticosa* and *Catharanthus roseus* leaves 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 (LC50) value of 253.08 ppm and (LC90) value of 455.40 ppm followed by hexane extract of *C.* roseus with LC50 value of 645.33 ppm and LC90 value of 1452.88 ppm against the larvae of *C. quinquefasciatus*. In control, there were five different fractions of α-carboxylesterases resolved in the gel with the mobilities ranging between 0.14 and 0.66. β-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 LC50 value of 48 hrs treatments. *A.* fruticosa ethyl acetate extract, and *C.* roseus hexane extract exposed larvae indicated increased quantities of β-Est4/5 isozymes in the concentration of 1/4 and 1/10 of LC50 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, β-carboxylesterase, *Culex quinquefasciatus*, Lethal concentration

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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 larvical 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]. Prench-Constant et al. [17] described an electrophoretic technique, based on patterns of esterase bands on polyacrylamide gel (PAGE) for
distinguishing large samples of *Myzus antirrhinic* from susceptible or resistant populations of the closely related species of *Myzus persicae*. In addition to the insecticidal activity, most of the 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 inductances 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 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 aceton 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 aceton with 2.49 ml of dechlorinated water. The larvae exposed to dechlorinated water without aceton 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 (LC₅₀) and LC₆₀; 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 appendage 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.

\[
\text{Protein concentration in the sample (mg/ml) =} \frac{\text{OD of sample} - \text{OD of standard}}{\text{Concentration of standard (40 µg)}} \times \text{mg/ml}
\]

**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/min/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. 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/min/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-
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 been observed that the ethyl acetate extracts of A. fruticosa possess maximum larvicidal activity against 4th instar larvae of C. quinquefasciatus. The percentage mortality of larvae treated with A. fruticosa recorded with 87.25% at 5% concentration followed by hexane (57.50%) and methanol (52.50%) extracts compared with untreated control. The results of 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 LC50 and LC90 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 LC50 value of 253.08 ppm and LC90 value of 455.40 ppm followed by extracts of hexane with LC50 value of 429.99 ppm and LC90 value of 774.45 ppm and methanol with LC50 value of 574.82 ppm and LC90 value of 1035.00 ppm against 4th instar larvae of C. quinquefasciatus. The efficacy of hexane extract of C. roseus was most active with LC50 value of 645.33 ppm and LC90 value of 1452.88 ppm followed by extracts of methanol with LC50 value of 715.39 ppm and LC90 value of 1287.54 ppm and ethyl acetate with LC50 value of 1370.06 ppm and LC90 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 α- and β-naphthyl acetates as staining substrates for all the plant extracts treated mosquito larvae of C. quinquefasciatus. In control, there were five different fractions of α-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 α-carboxylesterase enzyme fractions (Fig. 1). On the contrary the staining intensity of one slow moving fraction α-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 β-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 LC50 value at 24 hrs treatment of 1/10 of LC90 value at 24 hrs treatment. Likewise, β-carboxylesterase isozyme

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

<table>
<thead>
<tr>
<th>Name of the plants</th>
<th>Solvent</th>
<th>Control</th>
<th>Concentration in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>A. fruticosa</td>
<td>Hexane</td>
<td>0±0</td>
<td>13.75±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>0±0</td>
<td>25.00±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0±0</td>
<td>10.00±1.41&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. roseus</td>
<td>Hexane</td>
<td>0±0</td>
<td>12.25±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>0±0</td>
<td>11.25±1.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0±0</td>
<td>10.75±1.41&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>Values carrying same alphabets in a column are not statistically significant by Turkey’s Test at p=0.05% level</sup>
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 LC₅₀ value of 48 hrs treatments (Fig. 2).

In the case of relative mobilities of β-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 LC₅₀ value (Fig. 2).

Individual band intensities of α- or β-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 α- 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 LC₅₀ value of 24 hrs treatment except hexane extract of *A. fruticosa*. Likewise, α- Est5/6 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 LC₅₀ 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 LC₅₀ value of 48 hrs treatment were intensely stained in β- 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 β- Est4/5 isozymes in the concentration of 1/4 and 1/10 of LC₅₀ 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 α-carboxylesterase and β-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 α-carboxylesterase was 0.89 µM.minute⁻¹.mg protein⁻¹. It was observed to be much lower against all the treatments except 1/4 of LC₅₀ value of hexane extracts of *C. roseus* and in which it was recorded as 0.92 µM.minute⁻¹.mg protein⁻¹ (Table 3).

The quantity of β-carboxylesterases in control was observed to be 1.87 µM.minute⁻¹.mg protein⁻¹. Among various treatments, the results revealed that except a few, all the other solvent extracts of plants yielded higher quantities of β-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

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

<table>
<thead>
<tr>
<th>Name of the plants</th>
<th>Solvent extract</th>
<th>LC₅₀ (µM)</th>
<th>LC₉₀ (µM)</th>
<th>Chi-square Value</th>
<th>Regression value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. fruticosa</em></td>
<td>Hexane</td>
<td>429.99</td>
<td>744.45</td>
<td>0.82*</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>253.08</td>
<td>455.40</td>
<td>1.52*</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>574.82</td>
<td>1035.00</td>
<td>8.19</td>
<td>2.88</td>
</tr>
<tr>
<td><em>C. roseus</em></td>
<td>Hexane</td>
<td>645.33</td>
<td>1452.88</td>
<td>2.40*</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>1370.06</td>
<td>2089.43</td>
<td>0.96*</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>715.39</td>
<td>1287.54</td>
<td>5.82*</td>
<td>2.96</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Name of the plants</th>
<th>Solvent extract</th>
<th>Esterase activity (µM.minute⁻¹.mg protein⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/4th dilution</td>
<td>1/10th dilution</td>
</tr>
<tr>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
</tr>
<tr>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
</tr>
<tr>
<td><em>A. fruticosa</em></td>
<td>Ethyl acetate</td>
<td>3.92</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>5.70</td>
</tr>
<tr>
<td><em>C. roseus</em></td>
<td>Hexane</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Control - 0.89 µM.minute⁻¹.mg protein⁻¹, *C. roseus*: Catharanthus roseus, *A. fruticosa*: Acalypha fruticosa

**Fig. 2: β-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**

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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
The present study clearly showed 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, flavonoids, 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 LC50 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 *Trichosanthes spiralis* with promising larvicidal activity against *C. gelidus* and *C. quinquefasciatus* [38]. Zahir et al. [39] studied the inhibition of adult emergence and adulicial 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 *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 LC50 values of 17.97 and 20.57 mg/L respectively [41]. The petroleum ether fraction of *Acacia nilotica* and *Citrus colonycath* 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 *Alphyla 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 LC50 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 LC50 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 LC50 values for 2nd, 3rd and 4th 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 LC25 value of 17.6 µg/ml which supports the presents results were comparably good. Singh et al. [45] demonstrated that the larvicidal activity of *O. canum* oil tested against *A. aegypti* and *C. quinquefasciatus* (LC50 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 α-carboxylesterases and β-carboxylesterases are commonly found in the organophosphate resistant strains [49]. In some cases, overproduction is seen only in one type of esterase, that is, α - esterase or β-esterase, in others both the types are overproduced simultaneously [50]. In the present study, we have also observed an increased α - and β-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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37. Pavunraj et al. 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.


