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**Research Article**

# MOSQUITO LARVICIDAL EFFECT OF *CHROZOPHORA ROTTLERI* AGAINST *ANOPHELES STEPHENSI, AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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## ABSTRACT

In the present study, the larvicidal efficacy of the crude leaf extracts of *C. rottleri* with five different solvents like methanol, Chloroform, benzene, Ethyl acetate and Hexane was tested against the third instar larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The larval mortality was observed after 24 h of treatment. Among the five solvents the maximum efficacy was observed in methanol. The lethal concentration ($LC_{50}$) values of *C. rottleri* against third instar larvae of *Culex quinquefasciatus*, *Aedes Aegypti* and *Anopheles stephensi* were 142.90, 133.96, and 122.85 ppm respectively. No mortality was observed in controls. The chi-square value were significant at $p$ <0.05 level. The methanol extract of *C. rottleri* showed good larvicidal activity against three vector mosquitoes.

**Keywords:** *Chrozophora rottleri*, Larvicidal activity, *Culex quinquefasciatus, Aedes aegypti, Anopheles stephensi.*

## INTRODUCTION

Mosquitoes are the most important single group of insects well- known for their public importance, since they act as vector for many tropical and subtropical disease such as dengue fever, yellow fever, chikungunya, malaria, filariasis and encephalitis of different types including, Japanese encephalitis (Youdeowei, 1983). Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. Larviciding largely depends on the use of synthetic chemical insecticides – organophosphates (e.g. temephos and fenthion), insect growth regulators (e.g. diflubenzuron and methoprene), etc. Although effective, their repeated use has disrupted natural biological control systems and sometimes resulting in the widespread development of resistance. These problems have warranted the need for developing alternative strategies using eco-friendly products (Tiwary *et al.*, 2007). These steadily growing problems demand an intensive search for new products that are environmentally safe, target specific and degradable. The above facts prompted us to undertake investigations of some plant species traditionally used as insecticidal agents, as well as other endangered plant species, with the aim of identifying lead compounds for the development of new plant based insecticidal agents (Govindarajan *et al.*, 2012).

*Chrozophora rottleri* belongs to Euphorbiaceae family commonly known as Suryavarti. The plant occurs naturally throughout India, Myanmar, Thailand, Andaman Islands, and Central Java: Malesia. *C. rottleri*, an erect hairy annual common waste lands, blossoms profusely from January to April. It is an erect herb with silvery hairs; lower part of stem is naked, upper part hairy and has slender tap-root. The three-lobe leaves are alternative, thick and rugose. The plants are monoecious, the flowers borne in sessile axillary racemes with staminate flowers in upper and pistillate flowers in the lower part of raceme (Srivastava and Agarwal, 1953). *C. rottleri* is traditionally used by the tribes and native medical practitioners for the treatment of various diseases. In Sudan, powdered stems or whole plants are applied to wounds to improve healing. In Ethiopia, an infusion of the seeds and leaves is taken as a laxative. The plant is also used medicinally in Saudi Arabia, Pakistan and India (e.g. against jaundice and purifying blood). In

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Senegal, the plant is not browsed by most stock, except occasionally by sheep and goats, as it causes vomiting and diarrhoea, where as in Kenya, camels graze it. The fruits yield a purplish blue dye, which is used in East Africa to dye mats (Prota11 (1): Medicinal plants, cited 2010). In Nepal, juice of the fruit is given in cases of cough and colds (Manandhar and Manandhar, 2002), purifying agent (leaf) and laxative (seed), having bioactive components (Singh, 2010). The leaves are very much beneficial in treating skin diseases and also used as a depurative agent (Khare, 2007). The seeds are used as cathartic like Ghodtapde (Sasinath, 2007). Priyanka *et al.* (2010), reported that the aqueous extract of the leaves of this plant have significant anti-helmintic property against Pheritima posthuma (Indian Earth worm). Aqueous extract of this plant possessed phytotoxic activity on rice, wheat and mustard. In an experimental study by Suparna and Tapaswi (1999), the leaf extracts exhibited higher inhibition of shoot, root and radial elongation than the stem and root. The aim of the present study was to evaluate the larvicidal activity of different solvent extracts of *Chrozophora rottleri* against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi.*

## MATERIALS AND METHODS

### Collection of Plants

Fully developed leaves of the *C. rottleri* were collected from Nilgiris, Western Ghats (11° 10'N to 11° 45' N latitude and 76° 14'E to 77° 2' E longitude), Tamil Nadu State, India. The identity was confirmed at the Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu. Voucher specimens were numbered and kept in our laboratory and are available upon request.

### Extraction

The leaves were washed with tap water, shade dried and finely ground. The finely ground plant material (3.0 kg/solvent) was loaded in Soxhlet apparatus and was extracted with five different solvents namely benzene, hexane, ethyl acetate, chloroform and methanol individually. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of this plant varies with the solvents used. The *C. rottleri* with five different solvents yielded 84.60, 107.38, 99.37, 114.96 and 136.28 gm of crude residue respectively. Standard stock solutions were prepared at 1% by dissolving the residues with ethanol. From this stock solution, different concentrations were prepared and these solutions were used for ovicidal and repellent activity.

### Test Organisms

Laboratory-bred pathogen-free strains of mosquitoes were reared in the vector control laboratory, Department of Zoology, Annamalai University. At the time of adult feeding, these mosquitoes were 3–4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with Parafilm as membrane for 4 h. *Ae. aegypti* feeding was done from 12:00 p.m. to 4:00 p.m. and *An. stephensi* and *Cx. quinquefasciatus* were fed during 6:00 p.m. to 10:00 p.m. A membrane feeder with the bottom end fitted with Parafilm was placed with 2.0 ml of the blood sample (obtained from a slaughter house by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C were maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28±2 °C, 70–85 % relative humidity, with a photo period of 12-h light and 12-h dark.

### Larvicidal Bioassay

The larvicidal activity of the leaf extract of *C. rottleri* was evaluated as per the method recommended by WHO (1996). Different concentration of the test samples were used. 500 ml glass beaker containing 250 ml of tap water. Early third instar larvae of *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* were introduced to each of the test solution as well as control. Control consists of acetone only. For each experiment six replicates were maintained at a time. The lethal concentration ($LC_{50}$) values were calculated after 24 h by probit analysis (Finney, 1971).

### Statistical Analysis

Statistical evaluation was done using Statistical Package of Social Sciences (SPSS) 13.0 for windows, Significance level was set at $p<0.05$.

## RESULTS

The toxicity of *C. rottleri* was tested against the early third instar larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The data were recorded and statistical data regarding the $LC_{50}$, $LC_{90}$, regression equation, Chi-square and 95% confidence limits were calculated (Tables I, II, III.). The methanolic extract of *C. rottleri* showed highest larvicidal activity against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The $LC_{50}$ values of *C. rottleri* against early third instar larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* were 142.90, 133.96 and 22.85 ppm, respectively. No mortality was observed in control. The chisquare value were significant at $p$ <0.05 level. In the present study third instar was highly sensitive when compared with larvae of the three vector mosquitoes.

**Table 1.** Larvicidal activity of different solvent extracts of *Chrozophora rottleri* against *Anopheles stephensi.*

| Solvents | Concentration (ppm) | % of mortality±SD | $LC_{50}$ (ppm) (LCL-UCL) | $LC_{90}$ (ppm) (LCL-UCL) | $\chi^2$ |
|---|---|---|---|---|---|
| Hexane | 70 | 19.3±1.2 | 173.01 (157.90-187.13) | 315.54 (293.68-344.16) | 2.978 n.s. |
| | 140 | 38.6±0.6 | | | |
| | 210 | 61.2±0.4 | | | |
| | 280 | 79.5±1.2 | | | |
| | 350 | 97.4±0.8 | | | |
| Ethyl acetate | 70 | 22.9±0.6 | 163.28 (147.23-177.92) | 310.48 (288.29-339.69) | 1.105 n.s. |
| | 140 | 40.2±0.8 | | | |
| | 210 | 64.8±1.2 | | | |
| | 280 | 83.1±0.4 | | | |
| | 350 | 96.4±1.2 | | | |
| Benzene | 70 | 25.4±0.4 | 154.07 (137.52-168.89) | 300.68 (278.87-329.39) | 1.350 n.s. |
| | 140 | 43.9±0.6 | | | |
| | 210 | 66.2±1.2 | | | |
| | 280 | 85.7±0.8 | | | |
| | 350 | 97.3±1.2 | | | |
| Chloroform | 70 | 28.3±0.8 | 143.77 (127.37-158.28) | 282.74 (262..31-309.54) | 2.840 n.s. |
| | 140 | 46.2±1.2 | | | |
| | 210 | 69.7±0.6 | | | |
| | 280 | 88.9±0.4 | | | |
| | 350 | 99.1±1.2 | | | |
| Methanol | 60 | 29.6±1.2 | 122.85 (108.24-135.68) | 246.67 (228.44-270.73) | 4.404 n.s. |
| | 120 | 47.8±0.8 | | | |
| | 180 | 66.3±0.4 | | | |
| | 240 | 88.6±1.2 | | | |
| | 300 | 99.1±0.6 | | | |

[a] Values are mean ± SD of five replicates.

No mortality was observed in the control.

SD = standard deviation.

$LC_{50}$= lethal concentration that kills 50% of the exposed organisms.

$LC_{90}$= lethal concentration that kills 90% of the exposed organisms.

UCL= 95% upper confidence limit.

LCL= 95% lower confidence limit.

$\chi^2$= chi square.

n.s. = not significant (α=0.05).

**Table 2.** Larvicidal activity of different solvent extracts of *Chrozophora rottleri* against *Aedes aegypti.*

| Solvents | Concentration (ppm) | % of mortality±SD | $LC_{50}$ (ppm) (LCL-UCL) | $LC_{90}$ (ppm) (LCL-UCL) | $\chi^2$ |
|---|---|---|---|---|---|
| Hexane | 70 | 19.7±0.8 | 176.33 (159.95-191.56) | 333.92 (309.28-366.83) | 1.512 n.s. |
| | 140 | 40.5±0.4 | | | |
| | 210 | 58.2±1.2 | | | |
| | 280 | 77.6±0.6 | | | |
| | 350 | 94.3±1.2 | | | |
| Ethyl acetate | 70 | 21.6±0.6 | 172.49 (156.39-187.41) | 325.37 (301.78-356.66) | 1.425 n.s. |
| | 140 | 38.2±1.2 | | | |
| | 210 | 60.4±0.4 | | | |
| | 280 | 79.8±0.8 | | | |
| | 350 | 95.3±1.2 | | | |
| Benzene | 70 | 24.8±0.4 | 160.78 (143.95-175.98) | 314.29 (291.11-345.08) | 1.410 n.s. |
| | 140 | 41.6±1.2 | | | |
| | 210 | 63.9±0.6 | | | |
| | 280 | 82.7±0.8 | | | |
| | 350 | 96.2±1.2 | | | |
| Chloroform | 70 | 26.7±1.2 | 151.77 (134.91-166.78) | 299.95 (277.96-329.02) | 1.889 n.s. |
| | 140 | 44.5±0.6 | | | |
| | 210 | 66.2±0.8 | | | |
| | 280 | 85.8±0.4 | | | |
| | 350 | 97.6±1.2 | | | |
| Methanol | 60 | 26.8±0.8 | 133.96 (119.39-147.00) | 264.70 (245.05-290.81) | 3.067 n.s. |
| | 120 | 43.2±0.6 | | | |
| | 180 | 62.9±1.2 | | | |
| | 240 | 84.1±0.4 | | | |
| | 300 | 97.4±0.8 | | | |

[a] Values are mean±SD of five replicates.

No mortality was observed in the control.

SD = standard deviation.

$LC_{50}$= lethal concentration that kills 50% of the exposed organisms.

$LC_{90}$= lethal concentration that kills 90% of the exposed organisms.

UCL= 95% upper confidence limit.

LCL= 95% lower confidence limit.

$\chi^2$= chi square.

n.s. = not significant (α=0.05).

Table 3. Larvicidal activity of different solvent extracts of *Chrozophora rottleri* against *Culex quinquefasciatus.*

| Solvents | Concentration (ppm) | % of mortality±SD | $LC_{50}$ (ppm) (LCL-UCL) | $LC_{90}$ (ppm) (LCL-UCL) | $\chi^2$ |
|---|---|---|---|---|---|
| Hexane | 70<br>140<br>210<br>280<br>350 | 21.8±1.2<br>36.3±0.8<br>57.5±0.6<br>74.9±0.4<br>96.2±1.2 | 178.91 (162.79-194.03) | 335.63 (310.89-368.68) | 4.803 n.s. |
| Ethyl acetate | 70<br>140<br>210<br>280<br>350 | 20.4±0.8<br>37.6±0.4<br>59.2±1.2<br>78.9±0.6<br>97.3±1.2 | 174.58 (159.29-188.91) | 320.17 (297.68-349.74) | 3.690 n.s. |
| Benzene | 70<br>140<br>210<br>280<br>350 | 23.6±1.2<br>39.8±0.8<br>61.4±0.6<br>85.1±0.4<br>96.5±1.2 | 164.01 (148.16-178.54) | 309.98 (287.90-339.01) | 2.125 n.s. |
| Chloroform | 70<br>140<br>210<br>280<br>350 | 24.9±0.6<br>42.5±0.4<br>65.3±1.2<br>82.7±0.8<br>98.2±1.2 | 157.94 (141.61-172.68) | 304.97 (282.91-334.08) | 3.302 n.s. |
| Methanol | 60<br>120<br>180<br>240<br>300 | 24.6±0.4<br>40.4±0.8<br>59.2±1.2<br>81.7±0.6<br>95.3±0.4 | 142.90 (128.39-156.13) | 278.57 (257.71-306.47) | 2.179 n.s. |

[a] Values are mean±SD of five replicates.

No mortality was observed in the control.

SD = standard deviation.

$LC_{50}$= lethal concentration that kills 50% of the exposed organisms.

$LC_{90}$= lethal concentration that kills 90% of the exposed organisms.

UCL= 95% upper confidence limit.

LCL= 95% lower confidence limit.

$\chi^2$= chi square.

n.s. = not significant (α=0.05).

## DISCUSSION

The present findings corporate earlier findings of Macedo *et al.* (1997) who showed that ethanol extract of *Tagetes patula* was less active and only 50% larvae were killed at higher concentration (100 ppm). Methanolic leaf extract of *Cassia fistula* was tested for larvicidal activity against *Culex quinquefasciatus* and *Anopheles stephensi* with $LC_{50}$ values of 17.97 and 20.57 mg/l, respectively (Govindarajan *et al.*, 2008a). The petroleum ether fraction of *Acacia nolotica* and *Citrullus colocynthis* showed 100 per cent mortality in 100, 250 and 500 ppm and 60 and 50 percent mortality at 125 and 62.5 ppm respectively against *Culex quinquefasciatus*.

The leaf extract of *Acalypha in dica* with different solvents viz, benzene, chloroform, ethyl acetate and methanol were tested for larvicidal, ovicidal activity and oviposition attractancy against *Anopheles stephensi*. The larval mortality was observed after 24 h exposure. The $LC_{50}$ values are 19.25, 27.76, 23.26 and 15.03 ppm, respectively (Govindarajan *et al.*, 2008b). The leaf extract of *Cassia fistula* with different solvents viz, methanol, benzene and acetone were studied for the larvicidal, ovicidal and repellent activity against *Aedes aegypti*. The 24 h $LC_{50}$ concentration of the extract against *Aedes aegypti* were observed at 10.69, 18.27 and 23.95 mg/l respectively (Govindarajan, 2009).

Cheng *et al.* (2003) reported that the leaf and bark essential oil of *Cryptomeria japonica* showed larvicidal activity against *Aedes aegypti*. Methanolic fraction of leaves of *Menta piperita,Phyllanthus niruri* and *Letiota aspera* exhibited the $LC_{50}$ values of 43.65, 1819.70 and 2818.38 respectively against the larvae of *Culex* quinquefasciatus (Pandian *et al.*, 1994). Singh *et al*. (2003) reported the mosquito larvicidal properties of the leaf extract of a herbaceous plant *Ocinum canum* against *Aedes aegypti*. The $LC_{50}$ values for 2nd, 3rd and 4th larvae were 177.82, 229.08 and 331.13 ppm respectively. Gusmao *et al*. (2002) reported that the extract of *Derris urucu* showed larvicidal activity against *Aedes aegypti* with $LC_{50}$ values of 17.6 µg/ml. Muthukrishnan *et al*. (1997) reported that ethyl acetate fractions of *Solanum trilobatum* and *Letiota aspera* showed the $LC_{50}$ values of 23.5 and 138.6 ppm against 2nd and 3rd larvae of *Culex quinquefasciatus.*

Yang *et al*. (2002) reported that *Piper longum* fruit-isolated pipernonaline had strong larvicidal effects against the 4th stage larvae of *Aedes aegypti*. The $LC_{50}$ value of pipernonaline was 0.25 mg/L against *Aedes aegypti*. Kabaru and Gichia, (2001) reported that the mangrove plant *Rhizophora mucronata* bark and pith extract showed high toxicity with $LC_{50}$ values of 157.4 and 168.3 ppm respectively against *Aedes aegypti* larvae.

## CONCLUSION

In conclusion, the present study showed that extracts from *C. rottleri* can be effectively used in the control of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi mosquito larvae*. However, further studies on the identification of the active principles involved and their mode of action and field trials need presently other investigations.

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