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Evaluation of Larval Toxicity of *Lantana Camara* L. and *Catharanthus Roseus* L. against *Culex Quinquefasciatus* say and *Aedes Aegypti* L

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Abstract

The present study aimed to evaluate the aqueous extracts from the leaves of *Catharanthus roseus* L. and *Lantana camara* L. against filarial vector mosquito *Culex quinquefasciatus* Say and dengue vector *Aedes aegypti* L. The plant material was macerated and extracted with distilled water. The aqueous extract was tested at different concentrations of 1000, 500, 250, 125 and 62.5 ppm concentrations against I, II, III and IV instar larvae of *C. quinquefasciatus* and *A. aegypti*. The LC_{50} values of *C. roseus* against I, II, III and IV instar larvae of *C. quinquefasciatus* were 30.28, 38.01, 59.12 and 71.81 and against *A. aegypti* 26.64, 34.64, 53.10 and 72.89 ppm. The LC_{50} values of *L. camara* against I, II, III and IV instar larvae of *C. quinquefasciatus* were 35.48, 46.74, 67.64 and 95.51 and against *A. aegypti* 35.19, 38.26, 65.98 and 91.90 ppm. No mortality of was observed in control. Our results suggest that the aqueous extract of *C. roseus* have the potential to be used as an ecofriendly approach for the control larvae of vector mosquito *C. quinquefasciatus* and *A. aegypti*.

Introduction

Mosquitoes are the most important hematophagous insect in terms of public health importance, which transmits a number of dreadful diseases, such as malaria, filariasis, Japanese encephalitis, dengue and chikungunya etc., causing huge number of deaths around the world. Among these diseases lymphatic filariasis infects 80 million people annually of which 30 million cases exist in chronic infection by *Culex quinquefasciatus*. *C. quinquefasciatus* is a worldwide vector of bancroftian filariasis in the tropical and subtropical countries. Filariasis is caused by *Wucheria bancrofti*, a helminth that lives in the lymph glands and vessels that provoke edemas by lymph obstruction. In India alone 25 million people harbour microfilaria (mf) and 19 million people suffer from filarial disease manifestations [1]. Lymphatic filariasis is a major vector–borne disease problem in many developing countries. About 119 million people in 73 countries are infected; 40% in India alone [2]. In India 419.85 million people (National Institute of Communicable Diseases/National Anti-malaria Programme, 1996) and nearly half of the country's population are at risk, while 27.64 million people are microfilaria (mf) carriers and 20.81 million people have one or other form of disease manifestation [3]. *Aedes aegypti* is an important vector for dengue, dengue hemorrhagic fever and chikungunya etc. In 2010–2012, outbreaks of dengue/ chikungunya-like illnesses with severe clinical manifestations were reported from several districts of Tamil Nadu, India. Although the exact number of fever cases or number of fatalities is not available, approximately few hundred thousand people were affected [4,5]; Tamil Nadu recorded second highest dengue cases [6].

The control of vector mosquitoes is an important general public health concern. Mosquito abatement is primarily dependent on continued applications of synthetic chemicals such as temephos, malathion and fenthion, insect growth regulators like diflubenzuron and methoprene, and selected bacterial larvicidal organisms like *Bacillus thuringiensis* H14 and *Bacillus sphaericus,* which are still the most effective larvicides [7]. Their repeated use has disrupted natural biological control systems and led to outbreaks of vector mosquitoes [8], often resulting in the widespread development of resistance, undesirable effects on non-target organisms, and eliciting environmental and human health concerns [9]. These problems have highlighted the need for the development of new strategies for selective mosquito control. The number of plant species showed potential insecticidal activity against various insects pests.

Lantana camara L. is a small perennial shrub which can grow 2 m tall and form dense thickets in a variety of environments [10]. Due to extensive selective breeding throughout the 17th and 18th Centuries for use as an ornamental plant there are now many different *L. camara* cultivars [11]. *L. camara* plant parts were reported for anticancer and antiproliferative activity. Leaves of *L. camara* were reported for antiproliferative activity against HEp-2 (laryngeal cancer) and NCIH292 (lung cancer) cell lines [12,13]. Anti-bacterial effect [14-16], Hemolytic activity [17], Oleanonic acid from *L. camara* exhibited promising cytotoxicity against A375 cells [18]. Antifungal activity [19,20], Anti mutagenic activity [21], antioxidant activity [22], mosquitocidal [23,24] and anti fertility activity [25].

Catharanthus roseus L. is an evergreen subshrub or herbaceous plant growing 1 m tall. The leaves are oval to oblong, 2.5-9 cm long and 1–3.5 cm broad, glossy green, hairless, with a pale midrib and a short petiole 1–1.8 cm long; they are arranged in opposite pairs. The flowers are white to dark pink with a darker red center, with a basal tube 2.5-3 cm long and a corolla 2-5 cm diameter with five petal-like lobes. The fruit is a pair of follicles 2-4 cm long and 3 mm broad [26]. *C. roseus* was found to be used from the traditional period as an anthelminthic agent [27]. Antioxidant enzyme activities [28], Anti hyperglycemic effect [29], Antineoplastic and antidiabetic effect [30-34], *In-vivo* antidiarrheal activity [35], Antimicrobial activity [36], Transcriptome

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analysis [37], Antifungal activity against *Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Candida albicans* and *Penicillium* species [38-40]. Antihelmintic activity [41]. In India this plant was used to treat depression, muscle pain, bleeding gums, mouth, ulcers and sore throats [42]. It is also used internally for loss of memory, hypertension, cystitis, gastritis and enteritis, diarrhoea and raised blood sugar levels [43]. Its application ranges from prevention of cancer, cancer treatment, anti-diabetic, stomachic, reduces high blood pressure, externally against nose bleeding, sore throat and mouth ulcer [44], Insecticidal activity [45]. The objective of this study was to investigate the larvicidal effect of aqueous extract from the leaves of *L. camara* and *C. roseus* against the larvae of *C. quinquefasciatus* and *A. aegypti.*

Materials and Methods

Plant collection and extraction

Fresh and diseased free leaves of *Lantana Camara* (Figure 1) and *Catharanthus roseus* (Figure 2) were collected in and around Pachaiyappa's College for Men, Kanchipuram, Tamil Nadu, and India. The species was identified by Dr. P. Paramasivan, Plant Biologist, Department of Botany, Pachaiyappa's College for Men, Kanchipuram, India and the voucher specimen (PCMH 106) was deposited at the departmental herbarium, Pachaiyappa's College for Men, Kanchipuram, India. The leaves were thoroughly washed with distilled water and shade dried under room temperature. The dried leaves were macerated with electric blender. The 1 kg of powdered plant material was soaked with 3 litre of distilled water for 72 hr and filtered using Whatman No. 1 filter paper. The filtrate was kept in a hot air oven at 50°C for evaporation of distilled water to get the crude aqueous extract. The aqueous extracts were stored at 4°C for further analysis.

Mosquito culture

C. quinquefasciatus and *A. aegypti* larvae were collected from stagnant water bodies in various places within Pachaiyappa's College for Men, Kanchipuram, Tamil Nadu, and India. They were colonized and maintained continuously for generations in the laboratory free of exposure to pathogens, insecticides or repellents. They were maintained at 27 ± 2°C, 75-85% RH under a photoperiod of 14:10 hr (light/dark) in the insectary. Larvae were fed on finely ground dog biscuit and yeast extract in the ratio of 3:1. Water was changed every day to avoid scum formation which might create toxicity. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages (30 × 30 × 30 cm dimension) where the adults emerged.

Figure 1: *Lantana camara* L.

Figure 2: *Catheranthus roseus* L.

The adult mosquitoes were reared in the screened cages of 30 × 30 × 30 cm dimension. The adult colony was provided with 10% sucrose solution and was periodically blood-fed on restrained rats. After three days, ovitrap was kept inside the cages and the eggs were collected and transferred to the enamel trays. Two developmental stages, larvae and adult females, were continuously available for the experiments and were maintained at the same condition as above.

Larvicidal activity

Larvicidal bioassays were performed with first, second, third and fourth instar larvae of *C. quinquefasciatus* and *A. aegypti.* The aqueous leaf extract of *L. camara and C. roseus* were tested at 1000, 500, 250, 125 and 62.5 ppm concentrations, respectively. A minimum of twenty-five larvae per concentration was used for all the experiments. Plant extract was dissolved in water with emulsifier (0.1% Tween 80). Tween 80 was used as negative control. The experiment was replicated five times. Mortality and survival rates were recorded after 24 hr of the exposure period. No food was offered during experimental period. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. Larvae were also observed for discoloration, unnatural positions, incoordination, or rigor. Considering the mortality of the larvae at the experimental concentrations were recorded.

Statistical analysis

The larval mortality rate was recorded and statistical data analysis regarding LC_{50}, LC_{90}, 95% confidence limit and slope were calculated using EPA Probit analysis software.

Results

In the present investigation the toxic effect of *L. camara* and *C. roseus* were tested at five different concentrations against *C. quinquefasciatus* and *A. aegypti*. Cent percent mortality was observed at 1000 ppm concentration of *C. roseus* followed by *L. camara* against I, II, III and IV instar larvae of *C. quinquefasciatus* and *A. aegypti* (Table 1 and 2). The LC_{50} values of *C. roseus* against I, II, III and IV instar larvae of *C. quinquefasciatus* were 30.28, 38.01, 59.12 and 71.81 and against *A. aegypti* 26.64, 34.64, 53.10 and 72.89 ppm. The LC_{90} values of *C. roseus* against I, II, III and IV instar larvae of *C. quinquefasciatus* were 96.37, 125.97, 365.97and 433.89 ppm and against *A. aegypti* 85.00, 118.92, 347.09 and 435.53 ppm, respectively. Similarly, the LC_{50} values

Plant	instars	Concentrations (ppm)					LC_{50}	95% Confidence Limit		LC_{90}	95% Confidence Limit		Slope
		1000	500	250	125	62.5		LFL	UFL		LFL	UFL	
Lantana camera	I	25 ± 0	25 ± 0	25 ± 0	22.4 ± 1.14	19.2 ± 1.09	35.48	22.12	46.15	106.01	90.59	128.92	2.69
	II	25 ± 0	25 ± 0	21.6 ± 0.89	18.6 ± 0.54	16.2 ± 0.44	46.74	3.847	85.73	227.50	134.48	1015.88	1.86
	III	25 ± 0	22.2 ± 0.83	19.4 ± 0.89	15.8 ± 0.83	13.2 ± 1.30	67.64	19.45	113.11	450.46	267.41	1625.95	1.55
	IV	24.8 ± 0.44	20.4 ± 0.54	17 ± 0.70	14 ± 0.70	11 ± 0.70	95.51	24.52	166.09	675.32	350.11	5592.93	1.50
Catheranthus roseus	I	25 ± 0	25 ± 0	25 ± 0	22.4 ± 1.14	19.2 ± 1.09	30.28	16.28	41.62	96.37	80.73	117.63	2.54
	II	25 ± 0	25 ± 0	24.4 ± 0.89	22.2 ± 0.44	17.8 ± 0.44	38.01	25.14	48.86	125.97	107.34	154.41	2.46
	III	25 ± 0	22.8 ± 1.09	20.8 ± 0.44	16.8 ± 1.30	13.8 ± 1.09	59.12	42.86	74.72	365.97	295.32	487.50	1.61
	IV	25 ± 0	22.6 ± 0.54	19.2 ± 1.09	15.6 ± 0.54	12.8 ± 0.44	71.81	54.60	88.41	433.89	348.87	580.68	1.64

Values are mean ± SD of five replicates, LFL - Lower Fiducial Limit, UFL - Upper Fiducial Limit.

Table 1: Larvicidal activity of *Lantana camara* L. and *Catheranthus roseus* L. against the immatures of *Culex quinquefasciatus* Say.

Plant	instars	Concentrations (ppm)					LC_{50}	95% Confidence Limit		LC_{90}	95% Confidence Limit		Slope
		1000	500	250	125	62.5		LFL	UFL		LFL	UFL	
Lantana camera	I	25 ± 0	25 ± 0	24.6 ± 0.54	22 ± 0.70	19.2 ± 0.86	35.19	21.91	46.01	110.11	93.79	134.34	2.58
	II	25 ± 0	24.2 ± 1.09	20.8 ± 0.83	19.4 ± 1.40	16.6 ± 2.07	38.26	3.20	75.49	266.58	157.42	968.82	1.70
	III	25 ± 0	22.4 ± 0.54	19.6 ± 0.54	16.2 ± 0.44	13.2 ± 1.30	65.98	48.73	82.62	427.93	341.98	578.82	1.57
	IV	24.4 ± 0.54	20.8 ± 0.44	17.8 ± 0.83	14.6 ± 0.54	10.6 ± 0.89	91.90	71.39	112.05	649.24	504.31	916.54	1.50
Catheranthus roseus	I	25 ± 0	25 ± 0	25 ± 0	23.4 ± 0.54	21 ± 0.70	26.64	11.95	38.51	85.00	69.20	103.88	2.54
	II	25 ± 0	25 ± 0	24.4 ± 0.54	22.6 ± 0.54	18.4 ± 0.54	34.64	21.49	45.77	118.92	100.66	146.05	2.39
	III	25 ± 0	23 ± 1.00	20.8 ± 0.44	17.6 ± 0.89	14.4 ± 0.54	53.10	37.06	68.53	347.09	279.23	464.75	1.57
	IV	25 ± 0	22.2 ± 0.44	19.8 ± 0.44	15.6 ± 0.54	12.4 ± 0.54	72.89	55.682	89.50	435.53	350.51	581.94	1.65

Values are mean ± SD of five replicates, LFL - Lower Fiducial Limit, UFL - Upper Fiducial Limit.

Table 2: Larvicidal activity of *Lantana camara* L. and *Catheranthus roseus* L. against the immatures of *Aedes aegypti* L.

of *L. camara* against I, II, III and IV instar larvae of *C. quinquefasciatus* were 35.48, 46.74, 67.64 and 95.51 and against *A. aegypti* 35.19, 38.26, 65.98 and 91.90 ppm. The LC_{90} values of *C. roseus* against I, II, III and IV instar larvae of *C. quinquefasciatus* were 106.01, 227.50, 450.46 and 675.32 and against *A. aegypti* 110.11, 266.58, 427.93 and 649.24 ppm, respectively. No mortality was observed in control group. When the experimental concentration is increased the larval mortality also increased. The highest potential larval mortality was observed in *C. roseus* against both vector mosquitoes.

Discussion

The results obtained in the present investigation clearly showed that the aqueous extract of *C. roseus* followed by *L. camara* showed prominent mosquitocidal activity against *C. quinquefasciatus* and *A. aegypti*. The highest larvicidal activity was noticed in *C. roseus* due to the presence of Catharanthine, serpentine, tabersonine, ajmalicine, secologanine and tryptamine (Alkaloids) [46]. The larval and pupal mortality were observed after 24 and 48 hr of exposure of aqueous, ethyl acetate and methanol extracts of *C. roseus*; no mortality was observed in the control group. The LC_{50} values against the fourth-instar larvae of *A. stephensi* were 68.62 and 72.04 mg/ml for the aqueous extract, 82.47 mg/ml for the ethyl acetate extract, and 78.80 and 86.64 mg/ml for the methanol extract, while the aqueous, ethyl acetate and methanol extracts had LC_{50} values of 85.21, 76.84 and 94.20 mg/ml against the fourth-instar larvae of *C. quinquefasciatus* [47]. The highest larval mortality was observed to be found in the flower extract of *C. roseus* plant at 100 mg/l concentration. The LC_{50} value was observed in the *C. roseus* flowers followed by leaves i.e. 37.15 mg/l and 67.61 mg/l, respectively after 24 hr of exposure time and 26.92 mg/l, and 35.48 mg/l, respectively at 48 hr of exposure time [48].

Our results agree with those obtained from some earlier reports. The lethal concentration of acetone extracts leaves were 203.49 ppm on *L. camara* and 230.76 ppm on *C. roseus* for the IV instar larvae of *A. aegypti* at 24 hr of exposure period. The same concentration of plant extract gave 100% mortality after 96 hr of test period. The leaf extracts of *C. roseus* showed more effect than the *L. camara*. LC_{50} of *C roseus* was 75.31 ppm for the II instar larvae. The II and IV instar larval mortality was higher when exposed under *C. roseus* compared to *L. camara* [49]. The leaf methanol extract of *Cassia fistula* was tested for larvicidal activity against *C. quinquefasciatus* and *A. stephensi*, respectively with the LC_{50} values of 17.97 and 20.57 mg/l [50]. The aqueous leaf extract of *Spathodea campanulata* exhibited larvicidal activity with LC_{50} value of 16.12 mg/l against fourth instar larvae of *A. aegypti* [51]. The larvicidal potential of different solvent crude (hexane, chloroform, ethyl acetate, acetone and methanol) leaf extracts of *Blepharis maderaspatensis, Elaeagnus indica, Maesa indica, Phyllanthus wightianus* and *Memecylon edule*) was tested against the fourth-instar larvae of *A. aegypti* and the maximum larval mortality was detected in acetone extract of *E. indica* and *M. indica* with LC_{50} value of 90.89 and 173.21 mg/l, respectively [52]. A significant larvicidal activity of *Cestrum nocturnum* leaf extract/fraction was reported against *A. aegypti* with the LC_{50} value of 6 mg/l for the active fraction (Hexane-Ethyl-acetate 1:1) compared to LC_{50} value of 14 mg/l of methanol extract [53]. Larvicidal potential of the crude benzene, chloroform, ethyl acetate and methanol solvent extracts of the medicinal plant *Impatiens balsamina* were assessed against *A. stephensi, A. aegypti* and *C. quinquefasciatus* and the highest activity

was observed in leaf methanol extract with LC_{50} values of 98.04, 119.68 and 125.06 mg/l, respectively [54]. The larvicidal action of acetone leaf extracts of 11 plants were evaluated against *A. aegypti* larvae and out of the plants tested, leaf extracts of *Millingtonia hortensis* was found to possess the most effective larvicidal activity (LC_{50} of 123 mg/l) followed by *Annona squamosa* (LC_{50} of 190.5 mg/l), *Bauhinia variegata* (LC_{50} of 204.2 mg/l), *Plumeria alba* (LC_{50} of 218.8 mg/l), *Psidium guajava* (LC_{50} of 223.9 mg/l), *Syzygium cumini* (LC_{50} of 223.9 mg/l) and *Alstonia scholaris* (LC_{50} of 239.9 mg/l) [55].

Methanol and ethanol extracts of flower and leaf of *L. camara* on the mortality percentage of 3rd and 4th instar larvae of *A. aegypti* 0.75 and 1.00 mg/ml concentration of extracts showed maximum effect on 3rd as well as 4th instar larvae. Comparing the effects of other extracts methanol leaf extract showed lesser activity Ethanol flower extract at a concentration of 0.75 mg/ml showed a 100% mortality of 3rd and 4th instar larvae [56]. Larvicidal activity of ethanol extracts from leaves of *Ricinus communis, Vinca rosea* and *Lantana camara* at different concentrations of 250- 3000 ppm against the 3rd instar larvae of *Anopheles arabiensis* [57]. They reported that the leaf extract of *R. communis* had greatest larvicidal effect against *A. arabiensis* larvae with lowest LC_{50} (282.7060 ppm) followed by *C. roseus* (471.6689 ppm) and *L. camara* (477.5257 ppm) extracts, respectively. Briefly, our findings suggested that the aqueous extract of leaves of *C. roseus* and its effective constituents may be explored as a potential natural mosquito larvicide. Further investigations for the mode action of the phytoconstituents, effects on non-target organisms and field evaluation are necessary. The aqueous extract from *C. roseus* may be a good source to develop newer bio pesticides.

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