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Larvicidal efficacy of *Catharanthus roseus* Linn. (Family: Apocynaceae) leaf extract and bacterial insecticide *Bacillus thuringiensis* against *Anopheles stephensi* Liston.

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ABSTRACT

Objective: To explore the larvicidal activity of *Catharanthus roseus* (*C. roseus*) leaf extract and *Bacillus thuringiensis* (*B. thuringiensis*) against the malarial vector *Anopheles stephensi* (*An. stephensi*), when being used alone or together. **Methods:** The larvicidal activity was assayed at various concentrations under the laboratory and field conditions. The LC_{50} and LC_{90} values of the *C. roseus* leaf extract were determined by probit analysis. **Results:** The plant extract showed larvicidal effects after 24 h of exposure; however, the highest larval mortality was found in the petroleum ether extract of *C. roseus* against the first to fourth instars larvae with LC_{50}=3.34, 4.48, 5.90 and 8.17 g/L, respectively; *B. thuringiensis* against the first to fourth instars larvae with LC_{50}=1.72, 1.93, 2.17 and 2.42 g/L, respectively; and the combined treatment with LC_{50}=2.18, 2.41, 2.76 and 3.22 g/L, respectively. No mortality was observed in the control. **Conclusions:** The petroleum ether extract of *C. roseus* extract and *B. thuringiensis* have potential to be used as ideal eco-friendly agents for the control of *An. stephensi* in vector control programs. The combined treatment with this plant crude extract and bacterial toxin has better larvicidal efficacy against *An. stephensi*.

1. Introduction

Mosquitoes are the principal vector of many vector-borne diseases affecting human beings and animals, in addition to nuisance. Vector-borne diseases in India, *e.g.*, malaria, dengue, chikungunya, filariasis, Japanese encephalitis, and leishmaniasis, cause thousands of deaths per year. India reports 1.48 million malarial cases and about 1 173 deaths, 1.4 million suspected and 1 985 confirmed chikungunya cases, 5 000 Japanese encephalitis cases and approximately 1 000 deaths, and 383 dengue cases and 6 deaths during 2006 and 2007[1-3].

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In India, malaria is transmitted by six vector species, in which *Anopheles stephensi* (*An. stephensi*) is responsible in urban areas[4]. Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water; thus, it is easy to deal with them in this habitat. Management of disease vector using synthetic chemicals has failed because of resistance, effect on non-target organisms and environmental pollution. On the other hand, the recent public perception against the vector control using synthetic chemicals has shifted the research effort towards the development of environmentally sound and biodegradable agents. In that way, plant extracts including essential oils have attracted much attention to control the vector transmitted diseases[5].

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest-

managing agents. A number of plants and microbes have been reported as selectable subjects with little or no harmful effect on non-target organisms and the environment[6,7]. Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal, and adulticidal properties. Many researchers have reported the effectiveness of plant extract against mosquito larvae[8-10].

Madagascar Periwinkle [*Catharanthus roseus* (*C. roseus*)] belonging to the Apocynaceae family is formerly known as *Vinca rosea*. It is one of the important medicinal plants, due to the presence of the indispensable anti-cancer drugs, vincristine and vinblastine. It can erect bushy perennial herb and evergreen shrub grows to a height of 90 cm with a spread of 1 m. Its leaves are simple, opposite, exstipulate and petiolate. It contains more than 70 alkaloids mostly of the indole type. It has medicinal importance owing to the presence of alkaloids like ajamalicine, serpentine and reserpine, which are well known for their hypotensive and antispasmodic properties. The root bark contains alkaloid alstonine which has been used traditionally for its calming effect and its ability to reduce blood pressure. *C. roseus* exhibited high *in vitro* anti-plasmodial activity, which may be due to the presence of compounds such as alkaloids, terpenoids[11], flavonoids[12] and esquiterpenes[13] that were previously separated from the plant.

Bacillus thuringiensis (*B. thuringiensis*) subsp. var *israelensis* (Bti) is a Gram positive bacterium able to synthesize endotoxin protein crystals during sporulation. Bti produces four major insecticidal cryptochrome proteins and three cytolytic proteins[14]. The ingestion of these crystals by mosquito larvae rapidly leads to the formation of pores, cell lysis, septicemia and finally larva death[15,16]. One of the main advantages of Bti toxins is their capacity to act synergistically, improving the toxicity of the mixture[17,18] and reducing resistance to cryptochrome toxins in mosquitoes[19]. Biological control is an important component of the integrated vector control strategy and is being practiced in many countries for the control of mosquitoes[20,21].

Bacillus sp. produces large, spreading, gray-white colonies with irregular margins. A unique characteristic of this bacterium is its ability to produce endospores when environmental conditions are stressful. *B. thuringiensis* is a plant growth promoting bacterium which produces bacteriocin compounds of insecticidal properties and is marketed worldwide for control of many important plant pests, mainly caterpillars of Lepidoptera, mosquito larvae and black flies[22]. Well-known bacterial agents which have been used successfully for mosquito control are *B. thuringiensis* and *Bacillus sphaericus* (*B. sphaericus*)[23,24]. Two bacterial agents, *B. thuringiensis* and *B. sphaericus*, are being widely used for control of mosquito breeding in a variety of habitats[25,26]. In this context, the present study was designed to evaluate the mosquito larvicidal effects of *C. roseus* and *B. thuringiensis* against mosquito larvae *An. stephensi* under laboratory as well as field conditions.

2. Materials and methods

2.1. Collection of eggs and maintenance of larvae

The eggs of *An. stephensi* were collected from National Centre for Disease Control field station of Mettupalayam, Tamil Nadu, India, using an "O"-type brush. These eggs were brought to the laboratory and transferred to 18 cm × 13 cm × 4 cm enamel trays containing 500 mL of water for hatching. The mosquito larvae were fed on pedigree dog biscuits and yeast at a mass ratio of 3:1. The feeding was continued until the larvae transformed into the pupal stage.

2.2. Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred to plastic containers (12 cm × 12 cm) containing 500 mL of water with the help of a dipper. The plastic jars were kept in a 90 cm × 90 cm × 90 cm mosquito cage for adult emergence. Mosquito larvae were reared at (27±2) ℃ with 75%-85% relative humidity under a photoperiod of 14 h/10 h (light/dark). A 100 g/L sugar solution was provided for a period of 3 d before blood feeding.

2.3. Blood feeding of adult An. stephensi

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 d to ensure adequate blood feeding for 5 d. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

2.4. Collection of plant and preparation of extract

C. roseus plants were collected in and around Maruthmalai hills, Coimbatore, India. The plants were identified at Botanical Survey of India, Coimbatore, India. *C. roseus* leaves were washed with tap water and dried in shade at room temperature. The dried plant materials (leaves) were powdered by an electrical blender. From the powder, 500 g of the plant material were extracted with 1.5 L of organic solvents of petroleum ether using a Soxhlet apparatus at a boiling point of 60-80 ℃ for 8 h[27]. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in a rotary vacuum evaporator. Twenty gram of the plant residue was dissolved in 100 mL of acetone (stock solution)

considered as 200 g/L stock solution. From this stock solution, concentrations of 20, 50, 80, 110 and 140 g/L were prepared, respectively.

2.5. Microbial bioassay

B. thuringiensis "subsp" was obtained from Tuticorin Alkali Chemicals and Fertilizers Limited, Chennai, India. *B. thuringiensis* 630 ITU/mg (a.i.), 5.0% (w/w); total proteins [including the active ingredient 5.0% (w/w)], 10.0% (w/w); fermentation solids, 10.0% (w/w); inert ingredient, 48.0% (w/w); non-ionic surfactant, 0.2 (w/w); food grade preservative, 0.3%; UV protectant, 0.1%; and water, 71.4% were used. Total 100.0% (w/w) was active specifically against mosquito larvae. The required quantity of *B. thuringiensis* was thoroughly mixed with distilled water and prepared to various concentrations, ranging from 10 to 30 g/L, respectively.

2.6. Larval toxicity test

Laboratory colonies of mosquito larvae were used for the larvicidal activity. The first to fourth instar larvae and pupae, 25 each, were respectively introduced into a 500-mL glass beaker containing 249 mL of de-chlorinated water and 1 mL of desired concentrations of plant extract and *B. thuringiensis*. In fact, 0.5 mg larval food was provided for each test concentration. At each tested concentration, two to five trials were made and each trial consisted of five replicates. The control was set up by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae exposed to de-chlorinated water without acetone served as control. The mortalities (%) were corrected by using the following Abbott's formula[28], and lethal concentrations LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis[29].

Corrected mortality=(Observed mortality in treatment-Observed mortality in control)/(100%-control mortality)×100%. (1)

2.7. Field trial

For the field trial, the quantity of plant extract residues and required quantity of Bti (based on laboratory LC_{50} and LC_{90} values) for each treatment were determined by calculating the total surface area of sewage water bodies in each habitat. The required quantities of *C. roseus* and Bti were mixed thoroughly with water in a bucket with constant agitation. Teepol was used as emulsifying agent (0.05%, w/w). Field applications of the *C. roseus* leaf extracts and Bti were done with the help of a knapsack sprayer (Sujatha Products, India, 2010) and uniformly sprayed on the surface of the sewage water bodies in each habitat. Dipper sampling and counting of larvae were done to monitor the larval density after 24, 48, and 72 h post the treatment. A separate sample was taken to determine the composition of each larval habitat. Six trials were conducted for the *C. roseus* extract and *B. thuringiensis* alone and for the combined treatment, respectively. The percentage of reduction was calculated using the following formula:

Percentage of reduction=$(C-T)/C\times100\%$. (2)

where, C is the total number of mosquitoes in control, and T is the total number of mosquitoes in treatment.

2.8. Statistical analysis

All data were subjected to analysis of variance, and means were separated using Duncan's multiple range test[30]. The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper fiducidal limit (UFL) and lower fiducidal limit (LFL), and chi-square values were calculated using the SPSS 16.0 version (software package). The values are expressed as mean±SD of five replicates. Results with $P<0.05$ were considered to be statistically significant.

3. Results

3.1. Larval toxicity of the C. roseus extract against An. stephensi under laboratory conditions

The larval mortality of *An. stephensi* after the treatment with the petroleum ether extract of *C. roseus* leaves was observed. Table 1 provides the larval mortality of *An. stephensi* (the first to fourth instars) after the treatment with the *C. roseus* extract at various concentrations. A mortality of 47% was noted in the first instar larvae with the treatment of *C. roseus* at 20 g/L, and it gradually increased to 94% when the *C. roseus* leaf extract was used at 14 g/L.

Similar increasing trend was noted for all the instars of *An. stephensi* when treated with the *C. roseus* extract at different concentrations. The LC_{50} and LC_{90} values of the *C. roseus* extract alone against the *An. stephensi* larvae are also represented in Table 1.

3.2. Larval toxicity of B. thuringiensis against An. stephensi under laboratory conditions

Table 2 illustrates the larval mortality of *An. stephensi* (the first to fourth instars) after the treatment with *B. thuringiensis* at different concentrations. A mortality of 33% was noted in the first instar larvae after the treatment with *B. thuringiensis* at 10 g/L, and it increased to 84% at 30 g/L. Similar increasing trend was noted for all the instars of *An. stephensi* when treated with *B. thuringiensis* at different concentrations. The LC_{50} and LC_{90} values of *B. thuringiensis* alone against the *An. stephensi* larvae are also represented in Table 2.

3.3. Larval toxicity of the combined C. roseus leaf extract and B. thuringiensis against An. stephensi under laboratory conditions

Table 3 shows the considerable larval mortality of all the larval instars after the combined treatment with *C. roseus* leaf extract and *B. thuringiensis*. After treatment with the *C. roseus* extract at 60 g/L and *B. thuringiensis* at 2.5 g/L, the mortality of the *An. stephensi* in each larval stage was highest. The LC_{50} and LC_{90} values of the combined *C. roseus* leaf extract and *B. thuringiensis* against the *An. stephensi* larvae are also represented in Table 3.

3.4. Larval toxicity of the combined C. roseus leaf extract and B. thuringiensis against An. stephensi under field conditions

A total number of 375 *An. stephensi* larvae were observed in the overhead tanks of water body systems. In the field trial, after the treatment with the *C. roseus* extract alone, the larval density of *An. stephensi* was reduced by 12.26%, 28.80% and 79.46% at 24, 48, and 72 h, respectively. Similarly, the larval density was reduced by 10.93%, 25.86%, and 75.73% after 24, 48, and 72 h post the treatment with *B. thuringiensis* alone, respectively. The combined application of the *C. roseus* extract and *B. thuringiensis* caused 20.53%, 77.33%, and 100.00% reduction of larval density after 24, 48, and 72 h, respectively. The laval density after treatment is shown in Table 4.

4. Discussion

Malaria is the largest single component of disease burden; epidemic malaria, in particular, remains a major public health concern in tropical countries. In many developing countries, especially in Africa, malaria exacts an enormous toll in lives, medical costs, and days of labor lost[31].

Table 1
Larval toxicity of *C. roseus* leaf extract against *An. stephensi*.

Larval instar	Larval mortality (%) at different concentrations of extract					LC_{50} (LFL–UFL) (g/L)	LC_{90} (LFL–UFL) (g/L)	χ^2 (*df*=4)
	20 g/L	50 g/L	80 g/L	110 g/L	140 g/L			
First instar	47.00±1.78^{e}	58.00±1.41^{e}	66.00±1.26^{d}	79.00±0.89^{f}	94.00±1.49^{d}	3.34 (1.62–4.54)	14.08 (12.40–16.70)	4.21*
Second instar	42.00±1.63^{d}	54.00±1.09^{d}	60.00±1.41^{c}	68.00±1.32^{cd}	86.00±1.16^{c}	4.48 (2.56–5.81)	18.07 (15.44–22.72)	3.24*
Third instar	38.00±1.35^{bc}	48.00±1.41^{bc}	54.00±1.85^{b}	66.00±1.01^{c}	77.00±1.93^{b}	5.90 (4.11–7.24)	21.06 (17.63–27.54)	0.62*
Fourth instar	29.00±1.41^{a}	37.00±1.85^{a}	49.00±1.72^{a}	62.00±1.62^{ab}	70.00±1.16^{ab}	8.17 (6.92–9.45)	21.90 (18.60–27.79)	0.24*

The larval mortalities are expressed as mean±SD of five replicates. Nil mortality was observed in the control. Within a column, means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range test. LFL, lower fiducidal limit; UFL, upper fiducidal limit; *df*, degrees of freedom; χ^2, chi-square value. *Significant at $P<0.05$ level.

Table 2
Larval toxicity of *B. thuringiensis* against *An. stephensi*.

Larval instar	Larval mortality (%) at different concentrations of *B. thuringiensis*					LC_{50} (LFL–UFL) (g/L)	LC_{90} (LFL–UFL) (g/L)	χ^2 (*df*=4)
	10 g/L	15 g/L	20 g/L	25 g/L	30 g/L			
First instar	33.00±1.32^{d}	41.00±1.72^{d}	59.00±1.41^{d}	67.00±1.16^{d}	84.00±1.85^{e}	1.72 (1.52–1.88)	3.55 (3.21–4.10)	1.73*
Second instar	29.00±1.85^{c}	36.00±1.41^{c}	52.00±1.93^{c}	62.00±1.72^{bc}	78.00±1.16^{d}	1.93 (1.75–2.11)	3.87 (3.46–4.54)	1.06*
Third instar	26.00±1.72^{b}	32.00±1.16^{b}	47.00±1.41^{b}	55.00±0.80^{b}	71.00±1.32^{c}	2.17 (1.98–2.38)	4.31 (3.79–5.21)	0.99*
Fourth instar	22.00±1.32^{ab}	29.00±1.6^{a}	40.00±1.72^{a}	51.00±1.85^{a}	64.00±1.41^{ab}	2.42 (2.21–2.69)	4.67 (4.05–5.76)	0.20*

The larval mortalities are expressed as mean±SD of five replicates. Nil mortality was observed in the control. Within a column, means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range test. LFL, lower fiducidal limit; UFL, upper fiducidal limit; *df*, degrees of freedom; χ^2, chi-square value. *Significant at $P<0.05$ level.

Table 3
Larval and pupal toxicity of combined *C. roseus* leaf extract and *B. thuringiensis* against *An. stephensi*.

Larval instar	Larval mortality (%)					LC_{50} (LFL–UFL) (g/L)	LC_{90} (LFL–UFL) (g/L)	χ^2 (*df*=4)
	20/0.5†	30/1.0†	40/1.5†	50/2.0†	60/2.5†			
First instar	52.00±1.85^{d}	71.00±1.41^{d}	80.00±1.32^{d}	89.00±1.16^{d}	97.00±1.72^{d}	2.18 (1.56–2.59)	5.09 (4.71–5.67)	2.30*
Second instar	47.00±1.41^{c}	66.00±1.72^{c}	72.00±0.74^{c}	81.00±1.60^{c}	92.00±1.16^{c}	2.41 (1.70–2.87)	6.08 (5.52–6.99)	3.07*
Third instar	41.00±1.72^{b}	62.00±1.41^{b}	68.00±1.85^{b}	75.00±1.35^{bc}	88.00±1.93^{b}	2.76 (2.10–3.19)	6.73 (6.05–7.87)	4.44*
Fourth instar	35.00±1.85^{a}	56.00±1.62^{a}	61.00±1.41^{a}	74.00±1.72^{a}	82.00±1.16^{a}	3.22 (2.68–3.61)	7.26 (6.50–8.55)	3.86*

† indicates the concentrations of the extract (g/L) followed by that of *B. thuringiensis* (g/L), and the two concentrations are separated by a slash. The larval mortalities are expressed as mean±SD of five replicates. Nil mortality was observed in the control. Within a column, means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range test. LFL, lower fiducidal limit; UFL, upper fiducidal limit; *df*, degrees of freedom; χ^2, chi-square value. *Significant at $P<0.05$ level.

Table 4

Larval density in field trial by using leaf extracts of *C. roseus* and bacterial insecticide *B. thuringiensis* against malarial vector, *An. stephensi* (drinking water tanks with size of 0.10 m × 0.10 m × 2.00 m).

Sample no.	Before treatment	After treatment with *C. roseus* extract			After treatment with *B. thuringiensis*			After the combined treatment		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
1	44	39	28	12	41	31	14	31	18	0
2	55	48	39	18	49	44	17	39	12	0
3	62	51	43	10	53	49	11	51	8	0
4	71	66	54	12	64	51	18	62	19	0
5	89	76	62	17	78	54	21	73	16	0
6	54	49	41	8	49	49	10	42	12	0
Total	375	329	267	77	334	278	91	298	85	0
Average	62.5	54.8	44.5	12.8	55.6	46.3	15.6	49.6	14.6	0
Reduction percentage (%)	–	12.26	28.80	79.46	10.93	25.86	75.73	20.53	77.33	100.00

The larvicidal activity of many plant extracts against malarial vectors has been investigated. The petroleum ether (60-80 ℃) extracts of *Vitex negundo* leaves were evaluated for larvicidal activity with LC_{50} of 2.488 3 mg/L and LC_{90} of 5.188 3 mg/L against *Culex tritaeniorhynchus*[32]; the benzene, petroleum ether, ethyl acetate and methanol extracts of *Citrullus vulgaris* leaves were tested for larvicidal activity with LC_{50} values of 18.56, 48.51, 49.57 and 50.32 mg/L against *An. stephensi*, respectively[33]; and the compound beta-sitosterol isolated from petroleum ether extract of *Abutilon indicum* showed LC_{50} values of 11.49, 3.58 and 26.67 mg/L against *Aedes aegypti (Ae. aegypti)*, *An. stephensi* and *Culex quinquefasciatus (Cx. quinquefasciatus)*, respectively[34].

In previous study, the oils of 41 plants were evaluated for their effects against the third-instar larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. At first, the oils were surveyed against *Ae. aegypti* using 50 mg/L solution. Thirteen oils from 41 plants (camphor, thyme, amyris, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum and sandalwood) induced 100% mortality after 24 h or even after shorter periods. The pest oils were tested against the third-instar larvae of the three mosquito species at concentrations of 1, 10, 50, 100 and 500 mg/L.The LC_{50} values of three oils ranged between 1.0 and 101.3 mg/L against *Ae. aegypti*, between 9.7 and 101.4 mg/L for *An. stephensi*, and between 1.0 and 50.2 mg/L for *Cx. quinquefasciatus*[5].

Larvicidal activity of *Leucas aspera* leaf extract against *An. stephensi* was demonstrated with LC_{50} values of 96.95 g/L for the first instar, 102.72 g/L for the second instar, 08.23 g/L for the third instar, 113.03 g/L for the fourth instar and 127.32 g/L for pupae[35]. In the present results, the *C. roseus* leaf extract showed considerable mortality against *An. stephensi* with the LC_{50} values of 33.4, 44.8, 59.0, and 81.7 g/L against the first to fourth instars larvae, respectively, and the corresponding LC_{90} values were 140.8, 180.7, 210.6, and 219.0 g/L, respectively.

Nathan *et al* reported that combination of *B. thuringiensis* kurstaki and botanical insecticides caused a 2-fold decrease in the gut enzyme activity of larvae of rice leaf folder (*Cnaphalocrocis medinalis*) even at reduced concentrations. A synergistic effect was however found when botanical insecticides and bacterial toxins were combined at low doses. These effects were most obvious in early instars[36]. In another laboratory study, Nathan *et al* reported that the ingestion of bacterial toxins, *B. thuringiensis* (Berliner) subsp. kurstaki, neem seed kernel extract and *Vitex negundo* L. (Lamiales: Verbenaceae) leaf extract by rice leaf folder resulted in an altered leaf-folding behavior and biology. With the combination of Bti and botanicals, the average leaf consumption was decreased by a factor of 2 even at reduced concentrations when compared with the controls. During larval and pupal stages, adult longevity and fecundity were more affected by the treatments with the combination of both bacterial toxins and botanicals than by the treatment with the bacterial toxins or botanicals individually[37]. Well-known bacterial agents which have been used successfully for mosquito control are Bti and *B. sphaericus*[23,24], and *Bacillus subtilis* produce mosquitocidal toxins. However, they have not been fully studied for the nature of their toxins or their biocontrol potential[38]. The lyophilized powder of purified Cyt1A crystals of *B. thuringiensis* was much more toxic and yielded a LC_{50} of 11.332 mg/L[39]. In the present results, the LC_{50} values of *B. thuringiensis* against the first to fourth instars larvae were 17.2, 19.3, 21.7, and 24.2 g/L, respectively, and the corresponding LC_{90} values were 35.5, 38.7, 43.1, and 46.7 g/L, respectively.

Singh and Prakash have reported that six different concentrations of *B. sphaericus* (5, 10, 20, 30, 40 and 50 mg/L) were used in laboratory bioassays for *An. stephensi*[40]. Similarly, in the case of *Cx. quinquefasciatus*, six statistically significant different concentrations of *B. sphaericus* were used (0.01, 0.04, 0.05, 0.10, 5.00 and 10.00 mg/L). It was recorded that the mortalities were different for the different instars of *Cx. quinquefasciatus* and

An. stephensi after exposure of 24 h. Mahesh Kumar *et al* have reported the larvicidal and pupicidal efficacy of *Solanum xanthocarpum* against *Cx. quinquefasciatus* with the LC_{50} value of 155.29, 198.32, 271.12, 377.44 and 448.41 g/L against the first to fourth instars larvae and pupae, respectively. The LC_{90} values against the first to fourth instars larvae and pupae were 687.14, 913.10, 1011.89, 1 058.85, and 1 141.65g/L, respectively[41]. In the present results, the LC_{50} values of combined *C. roseus* and *B. thuringiensis* against the first to fourth instars larvae were 21.8, 24.0, 27.5, and 32.1 g/L, respectively, and the corresponding LC_{90} values were 50.9, 60.8, 67.3, and 72.6 g/L, respectively.

Rao *et al* reported that the field-tested relatively stable lipid-rich fractions of neem products were as effective as good quality crude neem products in the control of culicine vectors of Japanese encephalitis and produced a slight but significant reduction in population of anopheline pupae[42]. Dua *et al* stated that emulsified neem oil formulation showed 95.5% reduction in larval population of *Cx. quinquefasciatus* on the first day under field trials and thereafter 80% reduction were achieved up to the third week[43]. In a recent study, the field trials were conducted by using *Clerodendrum inerme* and *Acanthus ilicifolius* in different habitats to treat three species of mosquito vectors, namely, malarial vector *An. stephensi*, dengue vector *Ae. aegypti*, and filarial vector *Cx. quinquefasciatus*, in Vadavalli, Mettupalayam, Navavoor privu, Pommanam palayam, Ooty, Mettupalayam (Kallaru) in Tamil Nadu, India. The percentage reduction of larval mortality also showed variations among the different breeding habitats of mosquito vectors at 24, 48, and 72 h. This may be due to the impact of geographical distribution of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* at the breeding sites[44]. In field trial, *Leucas aspera* extract had the highest percentage of larval mortality against *Cx. quinquefasciatus*, *Abutilon indicum*, *Hyptis suaveolens* and *Jatropha curcas* extracts (60.4%, 51.7%, 50.0% and 46.7% at 24 h; 81.9%, 77.6%, 73.5% and 71.7% at 48 h; 99.7%, 92.0%, 90.4% and 89.9% at 72 h)[45].

In conclusion, the larvicidal potentiality of the crude extracts of *C. roseus* and *B. thuringiensis* was studied in the laboratory as well as field conditions. The *C. roseus* leaf extract and *B. thuringiensis* have been shown to be effective mosquito control agents. These results show that these two biological agents could reduce the malarial incidence. It also divulges the presence of active metabolites which are causes of larval mortality. Therefore, the botanicals are one of the best alternatives for chemical insecticides and are also eco-friendly bio-pesticides which create a healthy environment.

Conflict of interest statement

We declare that we have no conflict of interest.

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