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ORIGINAL PAPER

Mosquito larvicidal and pupicidal efficacy of *Solanum xanthocarpum* (Family: Solanaceae) leaf extract and bacterial insecticide, *Bacillus thuringiensis*, against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract The bio-efficacy of *Solanum xanthocarpum* leaf extract and bacterial insecticide, *Bacillus thuringiensis*, were assessed against the first to fourth instar larvae and pupae of *Culex quinquefasciatus*, under the laboratory conditions. The medicinal plants were collected from the outskirt Bharathiar University, Coimbatore, Tamil Nadu, India. The shade dried plant materials were extracted by employing the Soxhlet apparatus with ethanol (organic solvent) for 8 h and filtered. The extracts were concentrated at reduced temperature on a rotary evaporator and stored at a temperature of 4°C. Both *S. xanthocarpum* and *B. thuringiensis* show varied degree of larvicidal and pupicidal activity against various stages of *C. quinquefasciatus*. The LC_{50} and LC_{90} of *S. xanthocarpum* against the first to fourth instar larvae and pupae were 155.29, 198.32, 271.12, 377.44, and 448.41 ppm and 687.14, 913.10, 1,011.89, 1,058.85, and 1,141.65 ppm, respectively. On the other hand, the LC_{50} values of *B. thuringiensis* against the first to fourth instar larvae and pupae were 133.88, 157.14, 179.44, 206.80, and 240.74 ppm; the LC_{90} values were 321.04, 346.89, 388.86, 430.95, and 492.70 ppm, respectively. However, the combined treatment of *S. xanthocarpum*+*B. thuringiensis* (1:2) material shows highest larvicidal and pupicidal activity of the LC_{50} values 126.81, 137.62, 169.14, 238.27, and 316.02 ppm and the LC_{90} values 476.36, 613.49, 705.29, 887.85, and 1,041.73 ppm against *C. quinquefasciatus* in all the tested concentrations than the individuals and clearly established that there is a substantial amount of synergist act. Therefore, the present investigation clearly exhibit that both *S. xanthocarpum* and *B. thuringiensis* materials could serve as a potential of highest mortality rate against the mosquito larvae laboratory as well as the field conditions. Since *C. quinquefasciatus* is a ditch breeder vector mosquito, this is a user and eco-friendly biopesticide for the control of mosquito vector management program.

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Introduction

Mosquitoes are responsible for the transmission of more diseases than any other group of arthropods and play an important role as etiologic agents of malaria, filariasis, dengue, yellow fever, Japanese encephalitis, and other viral diseases (James 1992). In 2001, resistance to insecticides concerned 540 species of arthropod, of which 198 were of medical and veterinary importance (Bills 2001).

Culex quinquefaciatus is a predominant house-resting mosquito in many tropical countries. It is important as a vector of filariasis in some countries as well as a nuisance mosquito. Mosquitoes breed in polluted waters such as blocked drains, damaged septic tanks, or soak age pools close to human habitations. Lymphatic filariasis is probably the fastest spreading insect-borne disease of man in the tropics, affecting about 146 million people (WHO, 1992). *C. quinquefasciatus* is the most widely distributed mosquito in India, mainly found in urban and suburban areas. The most efficient approach to control the vector is to target the immature stages of the life cycle. Lymphatic filariasis is a mosquito-borne disease caused by mosquito-transmitted filarial nematodes, including *Wuchereria bancrofti* and *Brugia malayi*.

The infected people carry the nocturnally periodic *W. bancrofti*, which has *C. quinquefasciatus* as the main mosquito vector. *C. quinquefasciatus* is a vector of lymphatic filariasis, which is a widely distributed tropical disease with around 120 million people infected worldwide, and 44 million people have common chronic manifestation (Bernhard et al. 2003). According to WHO, about 90 million people worldwide are infected with *W. bancrofti*, the lymphatic dwelling parasite, and ten times more people ate at the risk of being infected. In India alone, 25 million people harbor microfilaria (mf) and 19 million people suffer from filarial disease manifestations (NICD, 1990; Maheswaran et al. 2008).

Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans, and other advantages (Liu et al. 2000). Many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides (ICMR Bulletin, 2003). Natural products of plant origin with insecticidal properties have been tried in the recent past in order to control a variety of insect pests and vectors. Many approaches have been developed to control mosquito menace. One such approach to prevent mosquito-borne disease is by killing mosquito at larval stage. The current mosquito control approach is based on synthetic insecticides. Even though they are effective, they created many problems like insecticide resistance (Liu et al. 2005). This has necessitated the need for a research and development of environmentally safe, biodegradable indigenous method for vector control. Phytoextracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer, and risk-free properties as compared to isolated or synthesized biopesticides and can be used successfully in mosquito management (Rahuman and Venktesan 2008).

Plants may be a source of alternative agents for control of vectors because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are biodegradable. Phytochemical insecticides have received much attention, in this regard, as they are considered to be more environmentally biodegradable and considered safer than synthetic insecticides (Moretti et al. 2002; Cetin et al. 2004). Many researchers have reported on the effectiveness of plant extract against mosquito larvae (Kalyanasundaram and Das 1985; Govindarajan et al. 2008; Kovendan et al. 2011c, d).

The Solanaceae family comprises about 90 genera and 3,000 species which are widely distributed in the world. They are a rich source of active secondary metabolites (Coletto da Silva et al. 2004). Within this family, the genus *Solanum i*s the largest and most complex with more than 1,500 species (Chowdhury et al. 2007), which yield a great variety of steroidal saponins and glycoalkaloids of interest from ecological and human health viewpoints (Roddick et al. 2001). Numerous species of *Solanum* are known to possess a variety of biological activities including antimycotic (Singh et al. 2007), antiviral (Arthan et al. 2002), molluscicidal (Silva et al. 2006), teratogenic, and cytotoxic properties (Nakamura et al. 1996; Lu et al. 2009).

Solanum xanthocarpum (Family: Solanaceae) is an important medicinal herb in Ayurvedic medicine. Various studies indicated that *S. xanthocarpum* possesses antiasthmatic, hypoglycemic, hepatoprotective, antibacterial, and insect repellent properties. The fruits are reported to contain several steroidal alkaloids like solanacarpine (Gupta and Dutt 1938), and solamargine. Other constituents like caffeic acid coumarins like aesculetin and aesculin (Tupkari et al. 1972), steroids carpesterol, diosgenin, campesterol, daucosterol, and triterpenes like cycloartanol and cycloartenol were reported from the fruits (Sato and Latham 1953). Steroidal glycoalkaloids are naturally occurring, secondary plant metabolites that are formed in a number of foods including potatoes, tomatoes, and eggplants (Friedman and McDonald 1997).

Taxonomy

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Species	*xanthocarpum*
Botanical name	*Solanum xanthocarpum* (Schrad. & Wendl.)

Bacillus thuringiensis is an insecticide with unusual properties that make it useful for pest control in certain situations. *B. thuringiensis* is a naturally occurring bacterium common in soils throughout the world. Several strains can infect and kill insects. Because of this property, *B. thuringiensis* has been developed for insect control. At present, *B. thuringiensis* is the only "microbial insecticide" in widespread use. The gram-positive endospore-forming bacterium *B. thuringiensis* produces parasporal crystalline inclusions that contain polypeptides (δ-endotoxin) that are toxic to a variety of insect species. The toxin induces the formation of a lytic pore in the midgut epithelial membrane that results in cell lysis, cessation of feeding, and death of the larva (Charles and de Barjac 1983; Singh et al. 1996; Daniel et al. 1995).

B. thuringiensis strains, pathogenic to insects, produce two distinct types of toxin proteins, Cry and Cyt proteins (Crickmore et al. 1995). Generally, the genes encoding these proteins are located on large plasmids, and the proteins are

synthesized and form crystalline inclusions during sporulation. More than 100 different cry genes have been identified and sequenced, and significant homologies among the amino acid sequences of this group, in combination with experimental studies, suggest they have a common mode of action, colloid-osmotic lysis (Crickmore et al. 1998; Höfte and Whitely 1989).

The use of bacterial agents for mosquito control, especially *B. thuringiensis* is gaining widespread importance (de Barjac 1978; Abdel-Hameed et al 1980; Priest 1992; Porter 1996). The strategy of combining different vector control agents has proven to be advantageous in various pest management programs (Caraballo, 2000; Seyoum et al. 2002). Many biological control agents have been evaluated against larval stages of mosquitoes, of which the most successful ones comprise bacteria such as *B. thuringiensis* and *Bacillus sphaericus* (NICD 1990). Well-known bacterial agents which have been used successfully for mosquito control are *B. thuringiensis* and *B. sphaericus*. Two bacterial agents such as the *B. thuringiensis* and *B. sphaericus* are being widely used for control of mosquito breeding in a variety of habitats (Balaraman et al. 1983; 1987; Armengol et al. 2006; Medeiros et al. 2005; Geetha and Manonmani 2010; Kovendan et al. 2011a, b).

The purpose of the present investigation was to explore the mosquito control agent under laboratory as well as field conditions. The plant extracts and *B. thuringiensis* are reported to have mosquitocidal properties of the control, the lymphatic filarial vector, *C. quinquefasciatus*.

Materials and methods

Collection of eggs and maintenance of larvae

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

Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500 ml of water with the help of a dipper. The plastic jars were kept in a 90×90×90-cm mosquito cage for adult emergence. Mosquito larvae were maintained at 27+2°C, 75–85% RH under a photoperiod of 14L:10D. A 10% sugar solution was provided for a period of 3 days before blood feeding.

Blood feeding of adult *C. quinquefasciatus*

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 days to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Collection of plant and preparation of extract

The *S. xanthocarpum* plant was collected in and around Bharathiar University, Coimbatore, India. *S. xanthocarpum* plant was washed with tap water and shade dried at room temperature (27±2°C). An electrical blender powdered the dried plant materials (leaves). From the powder, 300 g of the plant materials was extracted with 1 L of organic solvents of ethanol for using a Soxhlet apparatus (Vogel 1978) boiling point range 60–80°C for 8 h. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 50 to 650 ppm, respectively.

Microbial bioassay

B. thuringiensis subsp. *israelensis* was obtained from Tuticorin Alkali Chemicals and Fertilizers Limited, Chennai, India. *B. thuringiensis* subsp. var *israelensis*, 630 ITU/mg (a.i.) 5% *w/w*; total proteins (including the active ingredient 5% *w/w*), 10% *w/w*; fermentation solids, 10% *w/w*; inert ingredient, 48% *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% *w/w* was active specifically against mosquito larvae. The required quantity of *B. thuringiensis* subsp. var *israelensis* was thoroughly mixed with distilled water and prepare to various concentrations, ranging from 50 to 250 ppm, respectively.

Larval/pupal toxicity test

Laboratory colonies of mosquito larvae/pupae were used for the larvicidal/pupicidal activity. Twenty-five numbers of first to fourth instar larvae and pupae were introduced into

500-ml glass beaker containing 249 ml of de-chlorinated water, and 1 ml of desired concentrations of plant extract and *B. thuringiensis* were added. Larval food was given for the test larvae. 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 and pupae were exposed to dechlorinated water without acetone which served as control. The control mortalities were corrected by using Abbott's formula (Abbott 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

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

The LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis (Finney 1971).

Field trail

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

$$\text{Percentage of Reduction} = \frac{C - T}{C} \times 100$$

Where *C* is the total number of mosquitoes in control, *T* is the total number of mosquitoes in treatment.

Statistical analysis

All data were subjected to analysis of variance; the means were separated using Duncan's multiple range tests (DMRT) by Alder and Rossler (1977). The average larval mortality data were subjected to probit analysis, for calculating LC_{50} and LC_{90}, values were calculated by using the (Finney 1971) method. SPSS (Statistical software package) 16.0 version was used. Results with $P<0.05$ were considered to be statistically significant.

Results

Larval and pupal mortality of *C. quinquefasicatus* after the treatment of ethanol *S. xanthocarpum* was observed. Table 1 provides the results of larval and pupal mortality of *C. quinquefasicatus* (first to fourth instar larvae) after the treatment at different concentrations (50 to 650 ppm). Forty three percent mortality was noted at first instar larvae by the treatment of *S. xanthocarpum* at 50 ppm, whereas it has been increased to 92% at 650 ppm; 21.2% mortality was noted at 50 ppm of *S. xanthocarpum* leaf extract treatment. Similar trend has been noted for all the instars of *C. quinquefasicatus* at different concentration of *S. xanthocarpum* treatment. The LC_{50} and LC_{90} values were represented as follows; LC_{50} value of first instar was 155.29 ppm, second instar was 198.32 ppm, third instar was 271.12 ppm, fourth instar was 377.44 ppm, and pupa was 448.41 ppm, respectively. The LC_{90} value of first instar was 687.14 ppm, second instar was 913.10 ppm, third instar was 1,011.89 ppm, fourth instar was 1,058.85 ppm, and pupa was 1,141.65 ppm, respectively.

Table 2 shows the results of larval and pupal mortality of *C. quinquefasicatus* (first to fourth instar larvae and pupae) after the treatment of *B. thuringiensis* at different concentrations (50 to 250 ppm). Mortality (30.8%) was noted at first instar larvae by the treatment of *B. thuringiensis* at 50 ppm, whereas it has been increased to 81.8% at 250 ppm of *B. thuringiensis* treatment, and 16.6% mortality was noted at pupae by the treatment of *B. thuringiensis* at 50 ppm, and it has been increased to 51.6% at 250 ppm, respectively. Similar trend has been noted for all the larval instars and pupae of *C. quinquefasciatus* at different concentrations of *B. thuringiensis* treatment. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of first instar was 133.88 ppm, second instar was 157.14 ppm, third instar was 179.44 ppm, fourth instar was 206.80 ppm, and pupa was 240.74 ppm, respectively. The LC_{90} value of first

Table 1 Larval and pupal toxicity effect of ethanolic extract of *S. xanthocarpum* against filarial vector, *C. quinquefasciatus*

Mosquito larval instars and pupae	% of Larval and pupal mortality±SD					LC_{50} (ppm) (LFL–UFL)	LC_{90} (ppm) (LFL–UFL)	x^2
	Concentration of *S. xanthocarpum* (ppm)							
	50	200	350	500	650			
First instar	43±1.74e	54±1.35f	64±1.67e	77±1.85ef	92±1.95ef	155.29 (79.75–210.66)	687.14 (600.93–821.27)	2.88*
Second instar	41.6±1.35de	51.4±1.85e	57±1.26 cd	66±1.78 cd	83.8±1.46de	198.32 (103.13–265.30)	913.10 (765.53–1,182.09)	3.17*
Third instar	35±1.67 d	45.6±1.95de	55.6±1.49c	64±1.09c	75.2±1.16 d	271.12 (190.32–336.41)	1,011.89 (842.19–327.44)	0.13*
Fourth instar	26.2±1.83b	35.2±1.72b	51.6±1.49b	60±1.26b	67.6±1.35b	377.44 (316.34–442.86)	1058.85 (891.97–1,354.47)	0.90*
Pupa	21.2±1.46a	33.2±1.93a	46±1.72a	52.6±1.35a	63.6±1.85a	448.41 (386.54–527.01)	1,141.65 (955.35–1,477.96)	0.75*

Control-Nil mortality, *LFL* lower fiducial limit, *UFL* upper fiducial limit, x^2 Chi-square value

Numbers within a column followed by the same letter(s) are not significantly different at 5% level by DMRT

Each value is the mean±SD of five replicates.

*$P<0.05$ significance level

instar was 321.04 ppm, second instar was 346.89 ppm, third instar was 388.86 ppm, fourth instar was 430.95 ppm, and pupa was 492.70 ppm, respectively.

Table 3 provides the considerable larval and pupal mortality after the combined effect of *B. thuringiensis* and *S. xanthocarpum* extract against all the larval instars and pupae. The concentration at 50+20 ppm combined treatment of *S. xanthocarpum* and *B. thuringiensis* for first instar larval mortality and pupal mortality was 32.4%, respectively. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of first instar was 126.81 ppm, second instar was 137.62 ppm, third instar was 169.14 ppm, fourth instar was 238.27 ppm, and pupa was 316.02 ppm, respectively. The LC_{90} value of first instar was 476.36 ppm, second instar was 613.49 ppm, third instar was 705.29 ppm, fourth instar was 887.85, and pupa was 1,041.73 ppm, respectively. The χ^2 values are significant at $P<0.05$ level. The 95% confidence limits LC_{50}, LC_{90} (LFL–UFL) values were also calculated. Larval and pupal mortality was observed after 24 h exposure. No mortality was observed in the control group.

A total number of 1,600 *C. quinquefasciatus* larvae were observed in the sewage water body systems. After treatment with *S. xanthocarpum* against *C. quinquefasciatus*, larval density was reduced by 25.3%, 70.3%, 94.1%, and 23.8% at 24, 48, and 72 h, respectively. Similarly, the reduction of *C. quinquefasciatus* larval densities after treatment with *B.*

Table 2 Larval and pupal toxicity effect of *B. thuringeinsis* against filarial vector, *C. quinquefasciatus*

Mosquito larval instars and pupae	Percent of larval and pupal mortality±SD					LC_{50} (ppm) (LFL–UFL)	LC_{90} (ppm) (LFL–UFL)	x^2
	Concentration of *B. thuringeinsis* (ppm)							
	50	100	150	200	250			
First instar	30.8±1.72de	37.8±1.46e	56±1.09f	63±1.67e	81.8±1.93e	133.88 (115.43–150.66)	321.04(283.62–380.67)	2.28*
Second instar	25.±1.96 d	33.6±1.74 d	48.4±1.49e	58.4±1.2 d	75.8±1.32 d	157.14 (139.98–175.12)	346.89(305.17–414.11)	0.87*
Third instar	23.2±1.46c	29±1.67c	43.4±1.35 cd	53.4±1.2c	68.2±1.72bc	179.44 (160.68–202.27)	388.86(336.11–478.75)	0.66*
Fourth instar	19.2±1.46b	26.4±1.01b	36.6±1.95b	48.8±1.6bc	60±1.75ab	206.80 (185.02–237.93)	430.95(366.51–546.38)	0.08*
Pupa	16.6±1.74a	23±1.41a	33.4±1.62a	41.6±1.85a	51.6±1.35a	240.74 (211.80–290.00)	492.70(407.16–660.70)	0.06*

Control-Nil mortality, *LFL* lower fiducial limit, *UFL* upper fiducial limit, x^2 *Chi*-square value

Numbers within a column followed by the same letter(s) are not significantly different at 5% level by DMRT

Each value is mean±SD of five replicates

*$P<0.05$ significance level

Table 3 Combined effect of larval and pupal activity of ethanolic extract of *S. xanthocarpum* and *B. thuringiensis* against filarial vector, *C. quinquefasciatus*

Mosquito larval instars and pupae	Percent of larval and pupal mortality±SD					LC_{50} (ppm) (LFL–UFL)	LC_{90} (ppm) (LFL–UFL)	x^2
	Concentration of *S. xanthocarpum* (ppm)+*B. thuringiensis* (ppm)							
	50+20	150+40	250+60	350+80	450+100			
First instar	44.8±1.72de	59.2±1.6e	71.4±1.01e	83.4±1.35ef	98.2±1.16ef	126.81 (73.09–166.77)	476.36 (426.23–548.75)	5.22*
Second instar	43.6±1.36cd	57.2±1.72d	66.6±1.35d	73.4±1.85cd	90.4±1.49e	137.62 (63.96–188.42)	613.49 (533.31–745.16)	2.92*
Third instar	42±1.67c	54.2±1.32bc	60.6±1.01c	66.8±1.16c	87.2±1.46cd	169.14 (93.44–221.64)	705.29 (602.43–885.78)	4.67*
Fourth instar	37.4±1.35b	46.2±1.63b	55.8±1.46b	62.8±1.72ab	74.4±1.85ab	238.27 (163.84–295.36)	887.85 (731.30–198.29)	0.26*
Pupa	32.4±1.01a	41±1.41a	52.6±1.62a	55.8±1.16a	66.2±1.32a	316.02 (248.62–385.23)	1,041.73 (834.35–491.52)	0.59*

Control-Nil mortality, *LFL* lower fiducial limit, *UFL* upper fiducial limit, x^2 Chi-square value *Significant at $P<0.05$ level.

Numbers within a column followed by the same letter(s) are not significantly different at 5% level by DMRT

Each value is the mean±SD of five replicates

thuringiensis were 23.87%, 67.5%, and 91.7%, respectively. Combined effect of *S. xanthocarpum* and *B. thuringiensis* were 15.34%, 68.12%, and 100% at 24, 48, and 72 h, respectively (Tables 4, 5).

Discussion

C. quinquefasciatus is one of the potential vectors of *W. bancrofti*, the causative agent of human lymphatic filariasis infecting over 120 million people all over the world (Terranella et al. 2006). Singh Karam and Bansal (2003) and Bansal et al. (2009) also observed that extracts from fresh green and yellow fruits of this plant were very much effective to the vectors of malaria and dengue. Mohan et al. (2005) also observed that the fruits of this plant were very effective against the larvae of *Anopheles stephensi* (24 h LC_{50} of CCl4 extract being 5.1 ppm) and *C. quinquefasciatus* (24 h LC_{50} of petroleum ether extract being 62.2 ppm), respectively.

The petroleum ether *S. xanthocarpum* extract exhibited maximum larvicidal activity against *A. stephensi* compared to the other extracts. Results regarding the larvicidal efficacy of this plant are supported by findings of Singh Karam and Bansal (2003), who studied the larvicidal activity of aqueous fruit (LC_{50}=0.058%) and root extract (LC_{50}=1.08%) of *S. xanthocarpum* against *A. stephensi*. Mohan et al. (2005) reported the larvicidal activity of carbon tetrachloride fruit extracts of the same plant (LC_{50}=5.11 ppm) against the same vector species. The individual bioefficacy of petroleum ether root extract of *S. xanthocarpum* and temephos was studied and noted their LC_{50} values 41.28 and 38.48 ppm; 0.0041 and 0.0029 ppm and LC_{90} 111.16 and 80.83 ppm; 0.0164 and

Table 4 Field trial by using plant extracts of *S. xanthocarpum* and bacterial insecticide *B. thuringiensis* sewage water bodies 2.0×1.8×1.4 against *C. quinquefasciatus*

Sample No.	Before treatment	Larval density					
		After treatment					
		S. xanthocarpum			*B. thuringiensis*		
		24 h	48 h	72 h	24 h	48 h	72 h
1	275	205	115	37	214	104	25
2	290	210	95	22	222	116	27
3	249	173	85	–	195	89	30
4	260	190	76	2	182	65	20
5	241	195	44	–	204	78	16
6	285	222	60	31	201	68	14
Total	1,600	1,195	475	94	1,218	520	132
Average	266.66	199.16	79.16	15.6	213.0	86.6	22.0
Reduction	–	25.31%	70.31%	94.12%	23.87%	67.5%	91.75%

Table 5 Field trial by using combined effect of sewage water bodies 2.0×1.8×1.4 against *C. quinquefasciatus*

Sample no.	Before treatment	Larval density		
		After treatment		
		S. xanthocarpum+*B. thuringiensis*		
1	275	220	98	–
2	290	241	97	–
3	249	236	72	–
4	260	190	81	–
5	241	214	79	–
6	285	253	83	–
Total	1,600	1354	510	0
Average	266.66	225.66	85	0
Reduction	–	15.34%	68.12%	100%

0.0116 ppm after 24 and 48 h of exposure, respectively (Mohan et al. 2006; 2008). In the present results, 43% mortality was noted at first instar larvae by the treatment of *S. xanthocarpum* at 50 ppm, whereas it has been increased to 92% at 650 ppm; 21.2% mortality was noted at 50 ppm of *S. xanthocarpum* leaf extract treatment at 24 h exposure; the LC_{50} values of first to fourth instars and pupae were 155.29, 198.32, 271.12, 377.44, and 448.41 ppm, respectively. The LC_{90} value of first instar was 687.14 ppm, second instar was 913.10 ppm, third instar was 1,011.89 ppm, fourth instar was 1,058.85 ppm, and pupa was 1,141.65 ppm, respectively.

Various compounds including phenolics, terpenoids, and alkaloids exit in plants (Wink 1993) which may jointly or independently contribute to the generation of larvicidal activities in mosquitoes (Hostettmann and Potterat 1997). Earlier authors reported that the effect of water extract of citrus-seed extract showed LC_{50} values of 135, 319.40, and 127,411.88 ppm against the larvae of *Aedes aegypti* and *C. quinquefasciatus* (Sumroiphon et al. 2006). Dua et al. (2006) have reported that the mean median lethal concentration values of the aqueous extract from the roots of *Hibiscus abelmoschus* against the larvae of *Anopheles culicifacies*, *A. stephensi*, and *C. quinquefasciatus* were 52.3, 52.6, and 43.8 ppm, respectively. The aqueous extract of *Rhinacanthus nasutus* showed LC_{50} values of 5,124 and 9,681 mg/l against *C. quinquefasciatus* and *A. aegypti*, respectively (Chansang et al. 2005).

In a previous study, the oils of 41 plants were evaluated for their effects against third instar larvae of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*. At first, the oils were surveyed against *A. aegypti* using a 50 ppm 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 third instar larvae of the three mosquito species in concentrations of 1, 10, 50, 100, and 500 ppm. The lethal concentration 50 values of three oils ranged between 1 and 101.3 ppm against *A. aegypti*, between 9.7 and 101.4 ppm for *A. stephensi* and between 1 and 50.2 ppm for *C. quinquefasciatus* (Amer and Mehlhorn 2006a).

Biological control with entomopathogenic bacteria has been increasingly used as a larvicide to control populations of various medically important dipterans of the genera *Culex* and *Aedes*. Like chemical larvicides, these agents can cause drastic density-dependent mortality, killing all larvae within 24–48 h, after breeding site treatment. Moreover, they are selective to insects and are consequently considered soft to non-target fauna commercial products. Based on this, *B. thuringiensis* subsp. *israelensis* is currently available (Thiery et al. 1996). The combined effect of neem and pongamia oil with *B. thuringiensis* var. *israelensis* showed higher larval toxicity on *C. quinquefasciatus* (Murugan et al. 2002). Kuppusamy and Ayyadurai (2011) reported that lyophilized powders of purified Cyt1A crystals of *B. thuringiensis* were much more toxic yielding a 50% LC_{50} of 11.332 mg/l, respectively.

Garcia and Desrochers (1979) observed appreciable mortality only with high concentrations (1×107cells/ml) of *B. thuringiensis* var. *israelensis*. The biocide at 1 to 10 kg/ha (0.25 to 2.5 ppm) caused 18% to 88% mortality of midges during a 4-week evaluation period. Younger instars are more susceptible than older ones as shown by *C. quinquefasciatus*. Exposure periods longer than 48 h in the laboratory may produce better activity results of the *B. thuringiensis* var. *israelensis* formulations against the midges' species (Ali, 1981). It was recently reported that *B. thuringiensis israelensis* against the first to fourth instar larvae were of values LC_{50}=9.332%, 9.832%, 10.212%, 10.622% and LC_{90}= 15.225%, 15.508%, 15.887%, and 15.986% larvae of *C. quinquefasciatus*, respectively (Kovendan et al. 2011a). In the present results, *B. thuringiensis* first to fourth instar larvae and pupae have LC_{50} values of 133.88, 157.14, 179.44, 206.80, and 240.74 ppm, respectively, against *C. quinquefasciatus*.

The persistency of larvicidal effects of 13 oils (camphor, thyme, amyris, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum, and sandalwood) was examined by storage of 50-ppm solutions under different conditions (open, closed, in the light, and in the dark) for 1 month after the preparation of the solutions. The stored solutions were tested against *A. aegypti* larvae for four times during the storage period. Some oils under some conditions stayed effective until the last test, while some solutions had lost their toxicity during a short time after preparation. Thus, the mode of storage is absolutely important for the larvicidal effects. The fresh preparations were always the best (Amer and Mehlhorn 2006b).

Rao et al. (1995) 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. According to Mustafa and Al Khazaraji (2008) *Azadirachta excels* Jack showed excellent larvicidal properties at low concentrations against *Culex pipiens molestus*. Its LC_{50} value after 1 day was 62.5 μg/mL. Dua et al. (2009) stated that emulsified concentration of neem oil formulation showed 95.5% reduction in larval population of *C. quinquefasciatus* in 1 day under field trials and thereafter 80% reduction was achieved up to the third week. In a recent study, the field trials were conducted by using *Clerodendron inerme* and *Acanthus ilicifolius* treatment in different habitats of three species of mosquito vectors namely malarial vector, *A. stephensi*, dengue vector, *A. aegypti*, and filarial vector, *C. quinquefasciatus* (Vadavalli, Mettupalayam, Navavoor privu, Pommanam palayam, Ooty, Mettupalayam (Kallaru) in Tamil Nadu, India. The percentage reduction of larval mortality also showed the 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 *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* at the breeding sites (Kovendan and Murugan, 2011). The maximum highest percentage of larval mortality *L. aspera* and followed by *Abutilon indicum*, *Hydnellum suaveolens*, and *Jatropha curcas* plant extracts of field trial 60.4%, 81.9%, and 99.7% and 51.7%, 77.6%, and 92%; and 50%, 73.5%, and 90.4%; 46.7%, 71.7%, and 89.9% at 24, 48 and 72 h, respectively, against *C. quinquefasciatus* (Kovendan et al. 2011e). In the present results, combined effect of *S. xanthocarpum* and *B. thuringiensis* in the field were 15.34%, 68.12%, and 100% at 24, 48, and 72 h, respectively.

In the present study, the larvicidal, pupicidal, and filed evaluation of plant extracts and Bti against *C. quinquefasciatus* were evaluated. These plant extracts and bacterial insecticide showed that they have good effective mosquito control and this work shows promising results. The natural products of biopesticdes are eco-friendly for the vector control management programs.

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