



## Larvicidal and pupicidal efficacy of *Momordica charantia* leaf extract and bacterial insecticide, *Bacillus thuringiensis* against malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae)

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### ABSTRACT

The efficacy of leaf extracts of *Momordica charantia* and *Bacillus thuringiensis* has been proven against larvicidal and pupicidal activities of the malarial vector, *Anopheles stephensi*. The present study investigated the larvicidal and pupicidal activity against the first to fourth instar larvae and pupae of the laboratory-reared mosquitoes, *An. stephensi*. The plant extract showed larvicidal and pupicidal effects after 24 h of exposure. All larval instars and pupae have considerably moderate mortality; however, the highest larval and pupal mortality was the methanol extract of leaf *M. charantia* against the first- to fourth instars larvae and pupae values of  $LC_{50}$  = I instar was 93.45 ppm, II instar was 123.74 ppm, III instar was 167.17 ppm, and IV instar was 216.15 ppm, and pupae was 256.66 ppm, respectively and bacterial insecticide, *B. thuringiensis* for the first-to fourth instars larvae and pupae recorded the  $LC_{50}$  values: 53.47 ppm, 62.09 ppm, 79.15 ppm, 95.39 ppm, and 105.76 ppm for the I-IV instar larvae and pupae, respectively. The combined treatment recorded the values of  $LC_{50}$  85.09 ppm, 90.51 ppm, 111.91 ppm, and 137.61 ppm for I to IV instars and 154.40 ppm for pupae respectively. The results of the present investigation revealed effect of methanolic extract of *M. charantia* and *B. thuringiensis* for controlling of larvicidal and pupicidal properties of against malarial vector, *A. stephensi*.

**Key words:** *Anopheles stephensi*, *Bacillus thuringiensis*, larvicidal, *Momordica charantia*, malarial vector, pupicidal

### INTRODUCTION

Malaria and other vector-borne diseases contribute to the major disease burden in India. One of the methods to control these diseases is to control the vectors for the interruption of disease transmission. Malaria is the largest single component of disease burden, epidemic malaria in particular, remains a major public health concern in developing tropical countries. In many developing countries, and especially in Africa, malaria exacts an enormous toll in lives, in medical costs, and in days of labor lost (Lambert, 2005). In the past, synthetic organic chemical insecticides based intervention measures for the control of insect pests and disease vectors have resulted in development of insecticide resistance in some medically important vectors of malaria, filariasis and dengue fever (WHO, 1992; Kumari *et al.*, 1998).

However, more concerted efforts have been undertaken to make environment-friendly compounds viable for field use and for large-scale vector control operations (Sukumar *et al.*, 1991) reported 99 families, 276 genera and 346 species to have insecticidal properties. An earlier study with a common medicinal and vegetable plant of *Momordica charantia* Linn (Cucurbitaceae), has shown the insecticidal activity of this

plant against mustered saw fly (Kumar Arun *et al.*, 1979) but there is no report about its insecticidal activity against mosquitoes. The present communication reveals the mosquito larvicidal property of *M. charantia* against three mosquito species- *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *M. charantia* is known as Hindi- karela; Tamil-pakal and is widely distributed and cultivated in many parts of India. This species is reported to have anti-plasmodial properties (Gbeassor *et al.*, 1990; Sharma *et al.*, 1998 and ) used in vegetable, unani and ayurvedic medicines in the treatment of many diseases particularly the fruits and leaves are useful in piles, leprosy, jaundice, vermifuge, sugar problem in snake-bite, and other diseases and it is found to have anti-oxidant properties (Chopra *et al.*, 1990 and Singh *et al.*, 2006).

Botanical insecticides and microbial pesticides are highly effective, safe, and ecologically acceptable. Among the microbial pesticides, bacterial insecticides belonging to *Bacillus thuringiensis* constitute a dominant group. *B. thuringiensis* produces many parasporal crystal toxins during sporulation that on proteolysis bind on the specialized midgut receptors, thereby causing disruption of gut epithelium, gut

paralysis, toxemia, and eventual death of the host insect (English and Slatis, 1992). However, due to the rapid development of resistance of mosquitoes to the *B. thuringiensis* toxin, alternate mosquito control measures are needed. Therefore, integrated vector control, which combines microbial pesticides and botanicals, is becoming the preferred approach (Murugan *et al.*, 2002).

*B. 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 (alpha-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* var. *israelensis* de Barjac has been shown to be effective against mosquitoes (Goldberg and Margalit, 1977; de Barjac and Coz, 1979; Garcia and Desrochers, 1979) and blackflies (Undeen and Nagel, 1978) belonging to nematoceros dipterans selectively. *B. thuringiensis* var. *israelensis* has also been known to have a larger safety margin for other non-target aquatic organisms. Since the discovery of the agent and its lethal effects against species of *Anopheles*, *Aedes*, *Culex*, *Ochlerotatus*, and *Uranotaenia* larvae by Goldberg and Margalit (1977), many evaluation reports in laboratory bioassay have been made (Goettel *et al.*, 1982; Nugud and White 1982). *B. thuringiensis* var. *israelensis* has been receiving increasing interest throughout the world as a microbial insecticide that has a highly lethal effect on various species of mosquito larvae and has proved to have a far safer margin for other non-target aquatic organisms (Laird and Miles, 1985). Hence in the present study, it was planned to evaluate the larvicidal and pupicidal properties of (methanol) crude extract of *M. charantia* leaves and *B. thuringiensis* against a potent malaria vector, *An. stephensi*.

## MATERIALS AND METHODS

### Collection and maintenance of insect

The eggs of *An. stephensi*, 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 pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage.

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% relative humidity, under a photoperiod of 14:10 hrs light/dark. A 10% sugar solution was provided for a period of 3 days before blood feeding. 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.

### Preparation of plant extract

*M. charantia* were collected from the Maruthamalai Hills (Western Ghats), Somaiyampalayam, Coimbatore, India. The plants were identified at Botanical Survey of India, Coimbatore, Tamil Nadu, India. *Momordica charantia* plant was washed with tap water and shade-dried at room temperature. An electrical blender powdered the dried plant materials (leaves). The powder (500 g) of the leaf was extracted with 1.5 L of organic solvents of methanol using a Soxhlet apparatus at 60–80°C for 8 h (Vogel, 1978). The extract was concentrated under reduced pressure 22–26 mm Hg at 45°C and the residue obtained was stored at 4°C. 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 100mL of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 50 ppm to 450 ppm respectively.

### Microbial preparation

*B. thuringiensis* subsp. *israelensis* was obtained from Tuticorin Alkali Chemicals and Fertilizers Limited, Chennai, India. *B. thuringiensis*, 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* was thoroughly mixed with distilled water and prepares various concentrations, ranging from 50 to 450, ppm respectively.

### Larval/pupal toxicity test

Laboratory colonies of mosquito larvae/pupae were used for the larvicidal/pupicidal activity. Twenty-five numbers of I to

IV instars larvae and pupae were introduced into 500 mL glass beaker containing 249 mL of dechlorinated water and 1mL of desired concentrations of plant extract, and bacterial toxins (*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 three replicates. The control was set up by mixing 1mL of acetone with 249 mL of dechlorinated water. The larvae and pupae which were exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula (Abbott's 1925). The  $LC_{50}$  and  $LC_{90}$  were calculated from toxicity data by using probit analysis (Finney, 1971).

### Statistical analysis

All data were subjected to analysis of variance; the means were separated using Duncan's multiple range tests by Alder and Rossler (1977). SPSS (Statistical software package) 9.0 version was used. Results with  $P < 0.05$  were considered to be statistically significant.

### RESULTS

Larval and pupal mortality of *An. stephensi* after the treatment of methanol extract of *M. charantia* leaf was observed. Table 1 shows the larval and pupal mortality of *An. stephensi* (I to IV Instars) after the treatment of *An. stephensi* at different concentrations (50 - 450 ppm). Forty eight percent mortality

was noted at I instar larvae by the treatment of *M. charantia* at 50 ppm, whereas it has been increased to 94% at 450 ppm of *M. charantia* leaf extract treatment. Similar trend has been noted for all the instars of *An. stephensi* at different concentration of *M. charantia* treatment. The  $LC_{50}$  values recorded as follows: 93.45 ppm, 123.74 ppm, 167.17 ppm, 216.15 ppm for I to IV instars, respectively. The  $LC_{90}$  values of were 454.96 ppm, 573.31 ppm, 630.66 ppm, 722.25 ppm for I to IV instars, respectively. The  $LC_{50}$  was 256.66 ppm, and the  $LC_{90}$  was 788.56 ppm, respectively for the pupae.

Table 2 provides the larval and pupal mortality of *An. stephensi* (I to IV Instars) after the treatment of *An. stephensi* at different concentrations (30 to 50 ppm). Forty one percent mortality was noted at I instar larvae by the treatment of *B. thuringiensis* at 30 ppm, whereas it has been increased to 89% at 150 ppm of *B. thuringiensis* treatment and 28% mortality was noted at pupae by the treatment of *B. thuringiensis* at 30 ppm and it has been increased to 65% at 150 ppm. Similar trend has been noted for all the instars of *An. stephensi* at different concentrations of *B. thuringiensis* treatment. Table 3 shows the considerable larval and pupal mortality after the combined treatment of *B. thuringiensis* and *M. charantia* leaf of methanol extract for all the larval instars and pupae. The concentration at (60 - 300 ppm) combined treatment of *B. thuringiensis* and *M. charantia* for I' instar larval mortality

**Table 1.** Larval toxicity effect of *M. charantia* leaf extract against the malarial vector, *A. stephensi*

Mosquito larval instars and pupae	% Larval and pupal mortality					$LC_{50}$ ( $LC_{90}$ )	95% confidence limit		$\chi^2$ ( $df = 4$ )
	Concentration of MCLE (ppm)						LFL	UFL	
	50	150	250	350	450		$LC_{50}$ ( $LC_{90}$ )	$LC_{50}$ ( $LC_{90}$ )	
I	48 <sup>a</sup>	57 <sup>a</sup>	66 <sup>a</sup>	79 <sup>a</sup>	94 <sup>a</sup>	93.45 (454.96)	35.23 (398.46)	134.01 (543.83)	4.52*
II	43 <sup>b</sup>	54 <sup>a</sup>	61 <sup>b</sup>	72 <sup>b</sup>	85 <sup>b</sup>	123.74 (573.31)	57.60 (488.30)	168.72 (722.80)	1.21*
III	38 <sup>c</sup>	49 <sup>b</sup>	57 <sup>c</sup>	68 <sup>c</sup>	80 <sup>c</sup>	167.17 (630.66)	110.61 (534.01)	209.40 (803.35)	0.48*
IV	33 <sup>d</sup>	46 <sup>b</sup>	52 <sup>d</sup>	61 <sup>d</sup>	74 <sup>d</sup>	216.15 (722.25)	164.49 600.58	260.83 (952.50)	0.75*
Pupa	30 <sup>d</sup>	41 <sup>c</sup>	50 <sup>d</sup>	58 <sup>d</sup>	68 <sup>e</sup>	256.66 (788.56)	207.96 (647.82)	306.87 (1064.73)	0.14*

Control- Nil mortality, *LFL* = Lower Fiducial Limit, *UFL* = Upper Fiducial Limit,  $\chi^2$  - *Chi*-square value, *df* - degrees of freedom, Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. \*Significant at  $P < 0.05$  level.

**Table 2.** Larval toxicity effect of bacterial insecticide, *B. thuringiensis* against the malarial vector, *A. stephensi*

Mosquito larval instars and pupae	% Larval and pupal mortality					LC <sub>50</sub> (LC <sub>90</sub> )	95% confidence limit		$\chi^2$ (df = 4)
	Concentration of <i>B. thuringiensis</i> (ppm)						LFL	UFL	
	30	60	90	120	150		LC <sub>50</sub> (LC <sub>90</sub> )	LC <sub>50</sub> (LC <sub>90</sub> )	
I	41 <sup>a</sup>	53 <sup>a</sup>	64 <sup>a</sup>	76 <sup>a</sup>	89 <sup>a</sup>	53.47 (164.64)	38.11 (145.94)	64.75 (194.28)	1.00*
II	39 <sup>a</sup>	48 <sup>b</sup>	59 <sup>b</sup>	73 <sup>a</sup>	81 <sup>b</sup>	62.09 (191.48)	45.85 (166.28)	74.18 (234.60)	0.38*
III	35 <sup>b</sup>	42 <sup>c</sup>	50 <sup>c</sup>	67 <sup>b</sup>	74 <sup>c</sup>	79.15 (221.59)	64.64 (188.90)	91.74 (280.83)	1.17*
IV	30 <sup>c</sup>	37 <sup>d</sup>	44 <sup>d</sup>	61 <sup>c</sup>	69 <sup>d</sup>	95.39 (239.99)	82.44 (203.29)	109.36 (307.39)	1.10*
Pupa	28 <sup>c</sup>	34 <sup>e</sup>	41 <sup>e</sup>	57 <sup>d</sup>	65 <sup>e</sup>	105.76 (257.35)	92.42 (215.51)	122.39 (336.89)	0.98*

Control-Nil mortality, *LFL* = Lower Fiducial Limit, *UFL* = Upper Fiducial Limit,  $\chi^2$  – *Chi*-square value, *df* - degrees of freedom, Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. \*Significant at  $P < 0.05$  level.

**Table 3.** Combined treatment of larval toxicity effect of *M. charantia* leaf extract and bacterial insecticide, *B. thuringiensis* against malarial vector, *An. stephensi*

Mosquito larval instars and pupae	% Larval and pupal mortality					LC <sub>50</sub> (LC <sub>90</sub> )	95% confidence limit		$\chi^2$ (df = 4)
	Concentration of MCLE (ppm) + <i>B. thuringiensis</i> (ppm)						LFL	UFL	
	40 +	80 +	120 +	160 +	200 +		LC <sub>50</sub> (LC <sub>90</sub> )	LC <sub>50</sub> (LC <sub>90</sub> )	
I	47 <sup>a</sup>	58 <sup>a</sup>	71 <sup>a</sup>	83 <sup>a</sup>	97 <sup>a</sup>	85.092 (269.19)	55.90 (242.58)	106.34 (308.30)	4.34*
II	45 <sup>a</sup>	56 <sup>a</sup>	68 <sup>b</sup>	79 <sup>b</sup>	91 <sup>b</sup>	90.51 (311.03)	56.09 (275.94)	114.68 (366.45)	1.03*
III	41 <sup>b</sup>	52 <sup>b</sup>	61 <sup>c</sup>	72 <sup>c</sup>	83 <sup>c</sup>	111.91 (379.80)	74.79 (328.28)	137.93 (469.87)	0.35*
IV	38 <sup>c</sup>	48 <sup>c</sup>	56 <sup>d</sup>	64 <sup>d</sup>	76 <sup>d</sup>	137.61 (455.49)	99.61 (381.30)	165.80 (600.38)	0.37*
Pupa	37 <sup>c</sup>	46 <sup>c</sup>	53 <sup>e</sup>	61 <sup>e</sup>	70 <sup>e</sup>	154.40 (522.42)	113.93 (423.33)	186.65 (739.57)	0.07*

was 97%, respectively. The  $LC_{50}$  recorded, 85.092 ppm, 90.512 ppm for I to V instars, respectively.

## DISCUSSION

Mosquito-borne diseases, such as filariasis, malaria, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries (Jang *et al.*, 2002). The direct and indirect contributions of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for of *A. stephensi*. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future (Wandscheer *et al.*, 2004).

The recently increased interest in developing plant-based insecticides as an alternative to chemical insecticides, this study was undertaken to assess the larvicidal potential of the various fruit wall extracts of *M. charantia* against two species of mosquito vectors, *An. stephensi* and *Cx. quinquefasciatus*. Among the extracts tested, petroleum ether extract was found more effective than carbon tetrachloride and methanol extracts towards anopheline and culicine larvae after 24 and 48 hrs of exposure respectively. Thus, all fruit wall extracts of *M. charantia* are toxic to both the larval species. *M. charantia* may, therefore, act as an effective biolarvicide against mosquitoes in the future (Maurya *et al.*, 2009).

Rahuman and Venkatesan (2008) have reported that the Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants, *Citrullus colocynthis*, *Coccinia indica*, *Cucumis sativus*, *Momordica charantia*, and *Trichosanthes anguina*, were tested against the early fourth instar larvae of *Aedes aegypti* L. and *Cx. quinquefasciatus*. The larval mortality was observed after 24 h of exposure. The petroleum ether extract of *C. colocynthis* and methanol extract of *M. charantia* were more effective than the other extracts. 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.

Kamaraj *et al.* (2009) have reported that the highest mortality was found in leaf petroleum ether and flower methanol extracts of *Cassia auriculata* against the larvae of *An. subpictus* and *Cx. tritaeniorhynchus*. Elango *et al.* (2009) have reported that the maximum repellent activity was observed at 500 ppm in methanol extracts of *Aegle marmelos* and *A. lineata* and ethyl acetate extract of *Cocculus hirsutus*, and the mean complete

protection time ranged from 90 to 120 min with the different extracts tested against *A. subpictus*; no egg hatchability was observed with ethyl acetate extract of *A. marmelos*; methanol extracts *A. marmelos*, *A. lineata*, and *C. hirsutus* were exerted at 1,000 ppm, and the percentage of effective oviposition repellency were 92.60, 93.04, 95.20, 88.26, 92.80, 94.01, 95.77, 96.93, and 92.54 at 500 ppm, and the lowest repellency were 47.14, 58.00, 56.52, 64.93, 71.09, 66.42, 50.62, 57.62, and 65.73 at 31.25 ppm in acetone, ethyl acetate, and methanol extracts of *A. marmelos*, *A. lineata*, and *C. hirsutus*, respectively.

*M. charantia* exhibited encouraging larvicidal effects against *An. stephensi* and *Cx. quinquefasciatus*. Toxicological studies have shown that *M. charantia* is safe for human health, and there are no toxic effects (Chopra, 1933; Chopra *et al.*, 1956). Cucurbitaceous plants contain many compounds and are commonly used in traditional medicines and have been reported to possess various biological activities. *M. charantia* is widely used as a vegetable, an antidiabetic, and for other common oilments. Moreover, its insecticidal activity was confirmed by Kumar *et al.* (1979). The larvicidal effect of *A. indica* and *M. charantia* have significant larvicidal potential against *Cx. quinquefasciatus*. Further, the extracts are eco-friendly larvicides as well as safe for use, as evidenced by use of plant extracts as ingredients in oral medicines and ointments (Poonam and Sharma, 1998; Grover and Yadav, 2004).

The addition of *B. thuringiensis* var. *israelensis* with plant extracts caused a significant mortality due to the avoidance of treated diet and may be due to increased toxicity (Gould *et al.*, 1991). It can therefore be concluded that *B. thuringiensis* var. *israelensis* and plant compounds caused swelling of the gut epithelial cells (Nasiruddin and Mordue Luntz, 1993). At naturally occurring concentrations, allelochemicals produce midgut lesions, reduce feeding and growth and increase mortality (Lindroth *et al.*, 1988).

In the present results of *M. charantia* against the first-to fourth instars larvae and pupae was recorded to produce a considerable mortality and bacterial insecticide, *B. thuringiensis* against the first-to fourth instars larvae and pupae also evidenced a considerable mortality.

An attempt has been made to evaluate the role of medicinal plant to control mosquitoes. Natural products are generally preferred in vector control measures due to their less deleterious effect on non-target organisms and their innate biodegradability. In the context of resistance developed by the mosquito larvae against chemical insecticides, it is worthwhile to identify new active compounds from natural products against mosquitoes. The findings of the present investigation revealed that the leaf crude extract of *M.*

*charantia* and bacterial insecticide, *B. thuringiensis* has good larvicidal and pupicidal properties against potent malarial vector, *An. stephensi* and can be recommended as a potent bio-pesticide.

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