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LARVICIDAL AND PUPICIDAL EFFICACY OF *ALOCASIA MACRORRHIZA* (L.) SCHOTT (ARACEAE) LEAF EXTRACT AND BACTERIAL INSECTICIDE, *BACILLUS THURINGIENSIS ISRAELENSIS* AGAINST THE MALARIAL VECTOR, *ANOPHELES STEPHENSI* LISTON (DIPTERA: CULICIDAE)

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A B S T R A C T

Objective: To evaluate the larvicidal and pupicidal potential of the methanolic extracts from *Alocasia macrorrhiza* (*A. macrorrhiza*) plant leaf against malarial vector *Anopheles stephensi* (*A. stephensi*) mosquitoes at different concentrations (60, 120,180,240 and 300 ppm).

Methods: *Alocasia macrorrhiza* was collected from the area of around Bharathiar University, Coimbatore. The dried plant materials were powdered by an electrical blender. From each sample, 50 g of the plant material were extracted with 300 mL of methanol for 8 h in a Soxhlet apparatus. The extracts were evaporated to dryness in rotary vacuum evaporator to yield 122 mg and 110 mg of dark greenish material (residue) from *Alocasia macrorrhiza*. One gram of the each plant residue was dissolved separately in 100 mL of acetone (stock solution) from which different concentrations, i.e., 60, 120,180,240 and 300 ppm were prepared.

Results: Larvicidal activity of *Alocasia macrorrhiza* exhibited in the first to fourth instar larvae of the A. stephensi, and the LC_{50} and LC_{90} values were larval and pupal mortality of *A. stephensi* (first to fourth instars and pupae) after the treatment of *Alocasia macrorrhiza* at different(60 to 300 ppm). 32.7 % mortality was noted at I-instar larvae by the treatment of *Alocasia macrorrhiza* at 60 ppm, whereas it has been increased to 98.4 % at 300 ppm of *Alocasia macrorrhiza* leaf extract treatment. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 126.55 ppm, II instar was 143.19 ppm, III instar was 165.10 ppm, and IV instar was 186.13 ppm. The LC_{90} value of I instar was 278.81 ppm, II instar was 327.47 ppm, III instar was 380.01 ppm, and IV instar was 421.04 ppm. The LC_{50} value of pupae was 205.68 ppm, and the LC_{90} value of pupae was 456.92 ppm. **Conclusions:** The present study indicates that the phytochemicals derived from *Alocasia macrorrhiza* plant leaf extracts are effective mosquito vector control agents and the plant extracts may be used for further integrated pest management programs

INTRODUCTION

Mosquitoes are insects that have been around for more than 30 million years. And it seems that, during those millions of years, mosquitoes have been honing their skills so that they are now experts at finding people to bite. Mosquitoes are common flying insects in the family that are found around the world. There are about 3,500 species. Disease organisms transmitted by mosquitoes include West Nile virus, Saint Louis encephalitis virus, and Eastern equine Encephalomyelitis virus, Everglades virus, Highlands J virus, La Crosse Encephalitis virus in the United states; Dengue fever, yellow fever, Ilheus virus and malaria in the American tropics; Rift Valley fever, *Wuchereria bancrofti*, Japanese Encephalitis, dengue fever, yellow fever, Chikungunya and malaria in Africa and Asia; There are three main mosquito vectors namely *Anopheles, Culex* and *Aedes* which are responsible for millions of death worldwide.

Malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes up to 2.7 million deaths [1]. In India, malaria is transmitted by six vector species, in which *A. stephensi* is responsible in urban areas. It is endemic in all parts of India, and periodic epidemics of

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malaria occur every 5 to 7 years [2]. Malaria alone kills 3 million each year, including 1 child every 30 Malaria continues to be a major public health problem in the tropical world. Of the total world population of about 5.4 billion people, 2,200 million are exposed to malarial infections in some 90 countries or areas. The most recent estimates indicate that there may be 300–500 million clinical cases each year, with countries in tropical Africa accounting more than 90 % of these. Malaria is also the cause of an estimated 1–4 to 2–6 million deaths worldwide every year, with more than 90 % in Africa alone [3] [4]

Anopheles is a genus of mosquito (Culicidae). *Anopheles stephensi* is the primary vector of malaria in India and other West Asian countries, and improved methods of control are urgently needed [5];[6]. There are approximately 460 recognized species; while over 100 can transmit human malaria, only 30-40 commonly transmit parasites of the genus *Plasmodium* that cause malaria which affects humans in endemic areas. The known vectors of *Anophles* species, which are common in India include *Anopheles stephensi, Anopheles Culicifacies, Anopheles fluviattis, Anopheles minimums, Anopheles sudanicus* and *Anopheles philippinensis malari*a is caused by *Plasmodium*,viz: *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale* and *Plasmodium vivax*. Malaria is one of the most common infectious diseases and an enormous public health problem. The disease is caused by protozoan parasites of the genus *Plasmodium*. Usually, people get malaria by being bitten by an infective female *Anopheles* mosquito. Only *Anopheles* mosquitoes can transmit malaria, and they must have been infected through a previous blood meal taken on an infected person.[7]Botanical and microbial insecticides have been increasingly used for mosquito control because of their efficacy and documented non-toxic effects on non-target organisms [8]. The highest number of malaria, *Plasmodium falciparum*, cases, and malaria-related deaths are recorded from the state of Orissa located in the eastern part of India [7].

Alocasia macrorrhiza is a member of the *Araceae* family. It is a tall succulent herbaceous plant that can reach up to 4.5m. It has a large elongated stem. The leaves are huge about 0.9m long and generally arrow shaped, with shallow and rounded lobes. The leaves point upwards forming a straight line with the main axis of the petiole. They have a conspicuous mid-rib and are green in colour. The spathe has a glaucous, yellowish-green blade. The ovules are one to a few in each ovary cell in sub basal placenta. The leaves nearly peltate. The caudex well developed. The tuber of *A. macrorrhiza* is used to treat influenza, high fever and malaria; diarrhoea and typhoid fever, rheumatic; pulmonary tuberculosis and tuberculous lyphadenopathy; headache; abscesses and ringworm; venomous bites of snakes, dogs and insects; leucorrhoea acute abdominal pains [9]. It can be eaten after being put through a detoxifying process where the oxalate content is eliminated [10]. It has used in the treatment of scorpion sting amongst the Indian traditional practitioners [11][12]. The leaf of *A. macrorrhiza* is traditionally used as astringent, styptic and antitumour. The root and leaf is used as rubefacient. A decoction of the leaf and stem is used in a bath for treatment of skin conditions like itching and burns. A poultice of the fresh leaves helps in improving circulation, prevent bursting and reduce pain attributed to varicose veins. The steamed oiled leaves can help relieve rheumatic pains by applied around painful joints overnight. Toated, powdered leave speed up wound healing [13]. Antioxidant, Antinociceptive, Anti-inflammatory, Antimicrobial, Antitumour, Lymphcyte stimulation, Haemagglutinating and Hepatoprotective activity.

Trypsin/Chymotrypsin Inhibitor

The tuber of *A. macrorrhiza* was found to contain a trypsin/chymotrypsin inhibitor which specifically inhibits human trypsin but not human chymotrypsin [14]. The inhibitor was found to be a protein with 184 amino-acid sequence and exist in two dimmers [15].

Toxicities

The plant is considered toxic. All parts (leaves, stems and tubers) can be injurious. The toxins include raphides of water-insoluble calcium oxalate and sapotoxin (a neurological poison).Toxicity due to the presence of Calcium oxalate and a neurological toxin called sapotoxin [16].

Bacillus thuringiensis israelensis is a subspecies of the common insecticidal bacterium; it was discovered in 1978 and has high toxicity to *Diptera. Bacillus thuringiensis* (*Bt*) strains and varieties are pathogenic to a number of pests, including *Lepidoptera* and *Diptera*. The discovery of *Bacillus thuringiensis israelensis* (*Bti*), a variety specific to *Diptera* (especially mosquitoes and blackflies) in Israel in 1978, has led to the development of many products based on this bacterium. These products have been used extensively in mosquito and biting flies control programmes, especially in Africa, USA and Asia.

The use of bacterial agents for mosquito control, especially *B. thuringiensis* is gaining widespread importance [17]; [18]. The strategy of combining different vector control agents has proven to be advantageous in various pest management programs [19]. 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*. 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 mosquitoes breeding in a variety of habitats [20];[21];[22].

The present study would be useful and new way for mosquito control based on plant source. In view of the recent increased interest in developing plant-based insecticides as an alternative to synthetic insecticides, in this research was undertaken to assess the larvicidal and pupicidal activities of *Alocasia macrorrhiza* leaf extract and *B. thuringiensis* against the medically important malarial vector *Anopheles stephensi* L.

MATERIALS AND METHODS

Collection of eggs and maintenance of larvae

The eggs of malarial vector, *Anopheles stephensi* were collected from the 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 18×13×4-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% relative humidity, under a photoperiod of 14:10 L/D. A 10% sugar solution was provided for a period of 3 days before blood feeding.

Blood feeding of adult mosquito vectors

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 plant extract

The *Alocasia macrorrhiza* plant was collected in and around Coimbatore, India. *Alocasia macrorrhiza* 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, 500 g of the plant materials was extracted with 1.5 L of organic solvents of ethanol for using a Soxhlet apparatus boiling point ranging 60–80°C for 8 h [23]. 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 80, 160, 240, 320 to 400 ppm, respectively.

Microbial bioassay

B. thuringiensis subsp. var. *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 prepared to various concentrations, ranging from 25, 50, 75, 100 and 125 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-instar larvae and pupae were introduced into 500-mL glass beaker containing 249 mL of dechlorinated water and 1 mL of desired concentrations of plant extract and *B. thuringiensis* was 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 served as control. The control mortalities were corrected by using [24] formula.

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

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

The LC50 and LC90 were calculated from toxicity data by using probit analysis [25].

Statistical analysis

The average larval, pupal and adult mortality data were subjected to probit analysis for calculating LC50, LC90 and other statistics at 95 % fiducial limits of upper fiducidal limit and lower fiducidal limit, and Chi-square values were calculated using the 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 *A. stephensi* after the treatment of methanolic extract of *Alocasia macrorrhiza* leaf was observed. Table 1 shows the larval and pupal mortality of *A. stephensi* (I to IV instars and pupae) after the treatment of *A. stephensi* at different concentrations (60 to 300 ppm). 32.7 % mortality was noted at I-instar larvae by the treatment of *Alocasia macrorrhiza* at 60 ppm, whereas it has been increased to 98.4 % at 300 ppm of *Alocasia macrorrhiza* leaf extract treatment. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 126.55 ppm, II instar was 143.19 ppm, III instar was 165.10 ppm, and IV instar was 186.13 ppm. The LC_{90} value of I instar was 278.81 ppm, II instar was 327.47 ppm, III instar was 380.01 ppm, and IV instar was 421.04 ppm. The LC_{50} value of pupae was 205.68 ppm, and the LC_{90} value of pupae was 456.92 ppm.

Table 2 provides the larval and pupal mortality of *A. stephensi* after the treatment of B*. thuringiensis* at different concentrations (25 to 125 ppm). 29.2 % mortality was noted at I instar larvae by the treatment of *B. thuringiensis* at 20 ppm, whereas it has been increased to 91.4 % at 100 ppm of *B. thuringiensis* treatment. Similar trend has been noted for all the instars of *A. stephensi* at different concentrations. The LC_{50} and LC_{90} values were represented as follows: The LC_{50} values of I instar to IV instar and pupae were 58.68, 64.38, 74.12, 85.19 and 92.27 ppm respectively. The LC_{90} values were 129.95, 142.14, 162.57, 183.80 and 195.62 ppm, respectively.

Table 1 Larval and pupal toxicity effect of *Alocasia macrorrhiza* leaf

Larva and Pupal stage	% larval and pupal mortality Concentration of of *Alocasia macrorrhiza* (ppm)					LC_{50} and (LC_{90})
	60	120	180	240	300	
I instar	32.7±1.2	47.4±1.7	63.2±1.2	77.1±1.3	98.4±27	126.55 (278.

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

Larva and Pupal stage	% larval and pupal mortality					LC_{50} and (LC_{90})	Regression equation	95% confidence Limit		Chi-square value
	Concentration of *B. thuringiensis* (ppm)							LCL LC_{50} (LC_{30})	UCL LC_{50} (LC_{90})	
	25	50	75	100	125					
I instar	29.2±1.8	43.7±1.9	59.2±1.8	74.1±1.9	91.4±27	58.68 (129.95)	Y=-1.055 +0.018=X	51.01 (65.39)	118.27 (146.62)	1.85
II instar	27.5±1.7	40.5±1.4	54.8±2.0	69.8±1.2	86.7±1.1	64.38 (142.14)	Y=-1.061 +0.016=X	56.53 (71.49)	128.34 (162.48)	1.11
III instar	25.8±1.9	34.7±1.6	49.7±2.1	62.8±1.5	79.0±1.2	74.12 (162.57)	Y=-1.074 +0.014=X	65.92 (82.26)	144.48 (190.70)	0.73
IV instar	21.7±2.7	31.6±2.0	47.0±1.3	55.5±1.8	70.4±1.2	85.19 (183.80)	Y=-1.107 +0.013=X	76.38 (95.21)	160.54 (222.14)	0.44
Pupa	19.4±2.1	30.0±2.9	43.1±1.3	54.2±1.0	64.7±2.9	92.27 195.62	Y=-1.144 +0.0012=X	82.91 (103.93)	169.28 (240.32)	0.19

Table 3 combined larval and pupal toxicity effect *Alocasia macrorrhiza* leaf extract and Bacterial insecticide, *B. thuriengiensis* against the malarial vector, *A. stephensi*

Larva and Pupal stage	% larval and pupal mortality					LC_{50} and (LC_{90})	Regression equation	95% confidence Limit		Chi-square value
	Concentration of *Alocasia macrorrhiza* + *B. thuringiensis* (ppm)							LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{30})	
	30+15	60+30	90+45	120+60	150+75					
I instar	36.5±1.2	51.4±1.7	68.2±1.2	79.8±1.3	100±0.0	85.02 197.43	Y=0.969 +0.011=X	18.09 (118.33)	156.71 (323.26)2	10.45
II instar	33.2±1.4	47.1±2.4	62.4±1.3	73.5±2.5	93.4±2.1	97.12 (230.38)	Y=-0.142 +0.001=X	81.79 (109.93)	08.88 (261.50)	3.97
III instar	30.1±1.0	45.3±1.1	57.2±2.1	71.0±0.9	82.2±2.4	109.22 (271.48)	Y=-0.60048 +0.00553=X	91.93 (123.94)	241.27 (318.70)	0.12
IV instar	27.4±1.2	43.0±2.0	53.1±1.3	68.5±1.8	76.0±1.0	121.32 (305.55)	Y=-0.67398 +0.00506=X	103.95 (137.06)	267.90 (366.96)	0.61
Pupa	24.2±2.2	38.7±1.8	50.1±1.8	63.0±1.0	70.1±1.9	138.28 326.36	Y=-0.139 +0.001=X	121.15 (155.81)	283.35 (399.02)	0.73

Table 3 shows the considerable larval and pupal mortality after the combined treatment of *B. thuringiensis* and *Alocasia macrorrhiza* leaf of extract for all the larval instars and pupae. The concentration at (45 - 225 ppm) combined treatment of *B. thuringiensis* and *Alocasia macrorrhiza* for It instar larval mortality was 100% respectively. The LC_{50} and LC_{90} values were represented as follows: The LC_{50} values of I - IV instar and pupae were 85.02, 97.12, 109.22, 121.32 and 138.28 ppm respectively. The LC_{90} values were 197.43, 230.38, 271.48, 305.55 and 326.36 ppm, respectively.

DISCUSSION

Recent studies on the larval and pupal mortality of *Anopheles stephensi* after the treatment of methanol extract of *Clerodendron inerme* leaf extract showed 22% mortality at I instar larvae as a result of treatment at 20 ppm; in contrast, it was increased to 81% at 100 ppm of *C. inerme* leaf extract of larval and pupal mortality of *A. stephensi* (I–IV instars) after the treatment of methanol extract of *Acanthus ilicifolius* at different concentrations (20–100 ppm). A 23% mortality was noted at I instar larvae by the treatment of *A. ilicifolius* at 20 ppm, whereas it was increased to 89% at 100 ppm of *A. ilicifolius* leaf extract treatment [26].

Many authors have reported a number of plants have been investigated for their larvicidal and pupicidal properties against mosquito vector control programs. Recently, studies reported that the leaf extract of methanol *J. curcas* against the first to fourth instar larvae showed values of LC_{50}=1.200%, 1.290%, 1.358%, and 1.448% and LC_{90}=2.094%, 2.323%, 2.444%, and 2.544% larvae of *C. quinquefasciatus*, respectively. [27]. have reported the larval and pupal mortality of *An. stephensi* (I to IV Instars) after the treatment of *An. stephensi* at different concentrations (50 - 450 ppm) [28]. 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, was 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.

The leaf extract of *Acalypha alnifolia* with different solvents hexane, chloroform, ethyl acetate, acetone, and methanol were tested for larvicidal activity against three important mosquitoes such as malarial vector, *A. stephensi*, dengue vector, *A. aegypti* and *Bancroftian filariasis* vector, *C. quinquefasciatus* and highest larval and pupal mortality were found in the leaf extract of methanol *Carica papaya* against the first to fourth instar larvae and pupae of values LC50 =51.76, 61.87, 74.07, 82.18 and 440.65 ppm, respectively [29].

In our present results of research larval and pupal mortality of *A. stephensi* (I to IV instars and pupae) after the treatment of *A. stephensi* at different concentrations (60 to 300 ppm). 32.7 % mortality was noted at I-instar larvae by the treatment of *Alocasia macrorrhiza* at 60 ppm, whereas it has been increased to 98.4 % at 300 ppm of *Alocasia macrorrhiza* leaf extract treatment. The LC_{50} and LC_{90} values were represented as follows: LC_{50} values were 126.55, 143.19, 165.10, 186.13 and 205.68 ppm. The LC_{90} values were 278.81, 327.47, 380.01, 421.04 and 456.92 ppm, respectively [30] reported the *B. thuringiensis* against the first- to fourth-instar larvae of values LC500 9.332%, 9.832%, 10.212%, and 10.622% and LC90015.225, 15.508, 15.887, and 15.986%values of *Culex quinquefasciatus*, respectively. LC50 values of I to IV instars and pupae were 155.29, 198.32, 271.12, 377.44, and 448.41 ppm, respectively. have reported that the LC_{90} value of I instar was 687.14 ppm, II instar was 913.10 ppm, III instar was 1011.89 ppm, IV instar was 1058.85 ppm, and pupa was 1141.65 ppm of *Culex quinquefasciatus*, respectively[31]. In the present results, the LC_{50} and LC_{90} values I- to IV-instar larvae and pupae. The LC_{50} values were 58.68, 64.38, 74.12, 85.19 and 92.27 ppm respectively. The LC_{90} values were 129.95, 142.14, 162.57, 183.80 and 195.62 ppm, respectively.

The combined effect of neem and pongamia with *B. thuringiensis* var. *israelensis* showed higher larval toxicity on *C. quenquefasciatus* [32]. 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 was 97%, respectively. The LC_{50} recorded, 85.092 ppm, 90.512 ppm, respectively. 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. It can therefore be concluded that *B. thuringiensis* var. *israelensis* and plant compounds caused swelling of the gut epithelial cells [33]. At naturally occurring concentrations, allelochemicals produce midgut lesions, reduce feeding and growth and increase mortality [34].

In this present study the considerable larval and pupal mortality after the combined treatment of *B. thuringiensis* and *Alocasia macrorrhiza* leaf of extract for all the larval instars and pupae. The concentration at (45 - 225 ppm) combined treatment of *B. thuringiensis* and *Alocasia macrorrhiza* for 1st instar larval mortality was 100% respectively. The LC_{50} and LC_{90} values were represented as follows: The LC_{50} values of I - IV instar and pupae were 85.02, 97.12, 109.22, 121.32 and 138.28 ppm respectively. The LC_{90} values were 197.43, 230.38, 271.48, 305.55 and 326.36 ppm, respectively.

Conflict of interest statement

We declare that we have no conflict of interest.

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