Effect of medicinal plant and microbial insecticides for the sustainable mosquito vector control

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ABSTRACT

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. Dengue is an acute viral infection with potential fatal complications. Dengue fever is spread by the bite of infected Aedes mosquitoes. To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages. Many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides, and also some of biological control agents have been evaluated against larval stages of mosquitoes, of which the most successful ones comprise bacteria such as *Bacillus sphaericus*. The aim of this present study to determine the larvicidal and pupicidal activity of *Ocimum sanctum* and Microbial insecticide, *Bacillus sphaericus* on dengue vector, *Aedes aegypti*. Lethal dose concentrations (LC_{50} and LC_{90}) were calculated for different larval instars and pupal stages. Field trials were conducted at the breeding sites of the *A. aegypti* and the mortality was observed after 72 hours of treatment.

Keywords: *Ocimum sanctum, Bacillus sphaericus, Aedes aegypti,* Larvicide, field trail

INTRODUCTION

Mosquitoes are known to perceive visual, thermal and olfactory stimuli which enable them to detect light source, odor and several other volatile chemicals emanating from the skin, breath and waste products of their hosts [1,2]. Dengue is a vector-borne disease of tropical and subtropical human populations, which occurs predominantly in urban areas. The global increase in urbanization, such that the world's urban population of 1.7 billion in 1980 is expected 6.9 billion by the United States Census Bureau (2010), is likely to lead to an increase in dengue in the future. Dengue is an arboviral disease mainly transmitted by the mosquito *Ae. aegypti* and in the last years has become a major international public health concern [3]. Vector control is facing a threat due to the emergence of resistance vector mosquitoes to conventional synthetic insecticides, warranting counter measures such as developmental of novel insecticides [4]. The use of conventional pesticides in the water sources, however, introduces many risks to people and the environment. Natural products of plant origin with insecticidal properties have been tried in the recent past control of variety of insect pests and vectors.

Many approaches have been developed to control the mosquito menace. One such approach to prevent mosquito-borne disease is by killing the mosquito at larval stage. The current mosquito

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control approach is based on synthetic insecticides. Even though they are effective, they created many problems like insecticide resistance [5], pollution, and toxic side effect on human beings [6]. This has necessitated the need for a research and development of environmentally safe, biodegradable indigenous method for vector control. One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioral and physiological processes. Thus there is very little chance of pests developing resistance to such substances. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases [7].

The plants of genus *Ocimum* belonging to family Labiatae are very important for their unique properties. *Ocimum sanctum* L. (Tulsi), *Ocimum gratissium* (Ram Tulsi), *Ocimum canum* (Dulal Tulsi), *Ocimum basilicum* (Ban Tulsi), *Ocimum kilimandscharicum, Ocimum ammericanum, Ocimum camphora* and *Ocimum micranthum* are examples of known important species of genus *Ocimum* which grow in different parts of the world and are known to have medicinal properties [8,9]. *Ocimum sanctum* is, a small herb seen throughout India, commonly cultivated in gardens. In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum*, have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite, etc. The *Ocimum sanctum* L. has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions. A variety of biologically active compounds have been isolated from the leaves, including ursolic acid, apigenin and luteolin. Some other main chemical constituents of Tulsi are Oleanolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, and β-caryophyllene [10].

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* [11]. 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 [12,13]. *Bacillus sphaericus* is a naturally occurring soil bacterium that can effectively kill mosquito larvae present in water. *B. sphaericus* has the unique property of being able to control mosquito larvae in water that is rich in organic matter. When community mosquito control is needed to reduce mosquito- borne disease, the Department of Health favors the use of larvicide applications targeted to the breeding source of mosquitoes [14]. Bacterial larvicides have been used for the control of nuisance and vector mosquitoes for more than two decades. The discovery of bacterium like *B. sphaericus*, which is highly toxic to dipteran larvae, has opened the possibility of its use as a potential biolarvicide in mosquito eradication program worldwide [15]. The mosquitocidal activity of the highly active strain of *B. sphaericus* resulted in their development as commercial larvicides. This is now used in many countries in various parts of the world to control vector and nuisance mosquito species [16]. In this context, the purpose of the present investigation is to explore the larvicidal properties of *Ocimum sanctum* leaf extract and bacterial insecticide, *B. sphaericus* against dengue vector, *Ae. aegypti,* under the laboratory conditions and as well as in field.

MATERIALS AND METHODS

Collection of eggs and maintenance of larvae

The eggs of *Ae. aegypti,* 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 × 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 14 L: 10D. A 10% sugar solution was provided for a period of 3 days before blood feeding.

Blood feeding of adult mosquito *A. aegypti*

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 *O. sanctum* plant was collected in and around Bharathiar University, Coimbatore, India. *O. sanctum* plant was washed with tap water and shade dried at room temperature. 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 petroleum ether 8 h using a Soxhlet apparatus [17]. 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 60 to 300 ppm, respectively.

Microbial bioassay

B. sphaericus was obtained from T. Stanes and Company Limited, Research and Development Centre, Coimbatore, Tamil Nadu, India. The organism was grown in a liquid medium containing (in grams per liter of distilled water): $FeSO_4.7H_2O$, 0.01; $MnSO_4$, 0.1; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.08; K_2HPO_4, 0.025; yeast extract, 2; peptone, 4; and D-glucose, 1 and casein, 5. Solutions of yeast extract, peptone casein, D-glucose, K_2HPO_4 and $CaCl_2$ were separately prepared, sterilized, and added before inoculation. The pH of the medium was adjusted to 7.1 before sterilization. The required quantity of *B. sphaericus* was thoroughly mixed with distilled water and prepared at various concentrations ranging from 10, 20, 30, 40 and 50 ppm, respectively.

Larval toxicity test

A laboratory reared colony of *A. aegypti* larvae was used for the larvicidal activity. Twenty-five individuals of first, second, third and fourth instars larvae were kept in a 500 mL glass beaker containing 249 mL of dechlorinated water and 1-mL of desired concentration of *O. sanctum* extracts and *B. sphaericus* were added. Larval food was given for the test larvae. At each tested concentration, two to five trials were made and each trial consists of five replicates. The control was setup by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae exposed to decholorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula [18].

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

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

The LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis [19].

Statistical analysis

All data were subjected to analysis of variance; the means were separated using Duncan's multiple range tests by [20]. The average larval and pupal mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% fiducidal limits of upper fiducidal limit and lower fiducidal limit, and Chi-square values were calculated using the SPSS Statistical software package 13.0 version was used. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The results of larvicidal and pupicidal toxicity effect of *O. sanctum* petroleum ether leaf extract was noted and presented in table 1. The graded concentrations (60, 120, 180, 240 and 300 ppm) of *O. sanctum* petroleum ether leaf extract were treated against first to fourth instars larvae and pupae of *A. aegypti*. A. *aegypti* had values of LC_{50} = 147.87, 169.31, 195.30, 228.06 and 257.14 ppm; the LC_{90} = 326.61, 373.41, 414.42, 459.38 and 491.69 ppm, respectively.

Table 2 provides the effect of *B. sphaericus* at different concentrations on the mortality and toxicity of different larval instars of *A. aegypti*. Considerable mortality was evident after the treatment of *B. sphaericus*. The lowest mortality recorded after treating with *B. sphaericus* was 15.8 % at 10 ppm on the IV instars, and the highest mortality was 84.6 % at 50 ppm on first instars. Similar trend has been noted for all the larval instars and pupae. The LC_{50} and LC_{90} values of first to fourth instars larvae and pupae against *A. aegypti* were represented as follows: *A. aegypti* had values of LC_{50} = 27.18, 30.51, 34.95, 40.58 and 46.81 ppm and the LC_{90}= 58.76, 65.52, 73.21, 78.97 and 87.05 ppm, respectively

Table 3 provides the combined larval mortality after the treatment of *O. sanctum* petroleum ether leaf extract and *B. sphaericus* for all the larval instars of *A. aegypti*. The concentration at 150+25 combined treatment *O. sanctum* petroleum ether leaf extract and *B. sphaericus* treatment for fourth instars larval mortality was 76.6 %. A similar trend has been noted for all the instars and pupae of *A. aegypti* at different concentration of treatment. Combining treatment against the first- to fourth-instars larvae and pupae against vector species of *A. aegypti*, the LC_{50} and LC_{90} values were

represented as follows: *A. aegypti* had values of LC_{50} = 69.44, 78.43, 93.05, 109.01 and 130.66 ppm and the LC_{90} = 156.63, 165.15, 188.94, 229.42 and 274.09 ppm.

A total of 624 *A. aegypti* larvae found were observed in the storage water systems. After being treated with *O. sanctum* petroleum ether leaf extract, the *A. aegypti* larval density was reduced by 19.2%, 41.9% and 83.1% at 24, 48, and 72 h, respectively; similarly, the reduction of *A. aegypti* larval densities after treatment with *B. sphaericus* was 16.8%, 39.4% and 78.5%; and combined treatment *O. sanctum* petroleum ether leaf extract and *B. sphaericus* treatment, the *A. aegypti* larval density was reduced by 52.8%, 80.7% and 100% at 24, 48, and 72 h, respectively (Table 4).

Table 1. Larval and pupal activity of *O. sanctum* petroleum ether leaf extract against dengue vector, *Ae. aegypti*.

Mosquito larval instar and pupa	% of Larval and pupal mortality ± SD Concentration of *O. sanctum* (ppm)					LC_{50} (ppm) (LFL–UFL)	LC_{90} (ppm) (LFL–UFL)	x^2
	60	120	180	240	300			
1st Instar	29.0±0.81	41.9±0.73	55.3±0.47	72.1±0.23	89.6±0.97	147.87 (129.29-164.40)	326.61 (296.09- 370.85)	2.21
2nd Instar	25.6±0.94	36.8±0.62	52.1±0.84	67.3±2.05	79.8±0.23	169.31 (149.85-187.86)	373.41 (333.74-434.00)	0.12
3rd Instar	21.3±1.24	33.0±0.81	45.6±0.47	62.5±0.70	71.8±1.07	195.30 (175.58-216.55)	414.42 (366.32-490.62)	0.30
4th Instar	18.1±0.62	26.8±0.23	38±0.75	55.5±0.40	64.3±0.94	228.06 (206.64-255.05)	459.38 (401.30-554.80)	0.52
Pupa	13.9±0.29	22.4±1.14	33.6±1.24	48.1±0.83	58.0±1.63	257.14 (233.07-290.88)	491.69 (426.47-601.25)	0.20

Control-Nil mortality, *LFL* - Lower Fiducidal Limit, *UFL* - Upper Fiducidal Limit, x^2 – *Chi*-square value, *Significant at $P < 0.05$ level. Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT; Each value (Mean ± SD) five replicate.

Table 2. Larval and pupal toxicity effect of *B. sphaericus* dengue vector, *Ae. Aegypti*.

Mosquito larval instar and pupa	% of Larval and pupal mortality ± SD Concentration of *O. sanctum* (ppm)					LC_{50} (ppm) (LFL–UFL)	LC_{90} (ppm) (LFL–UFL)	x^2
	10	20	30	40	50			
1st Instar	26.4±0.41	38.3±0.94	50.8±0.84	69.0±0.75	84.6±2.05	27.18 (24.11-30.06)	58.76 (52.95-67.36)	1.21
2nd Instar	23.8±0.86	34.0±1.63	47.3±1.24	65.5±1.42	76.0±2.16	30.51 (27.30-33.77)	65.52 (58.29-76.71)	0.43
3rd Instar	19.6±1.24	31.0±1.95	43.6±0.94	58.3±0.47	68.0±1.63	34.95 (31.51-38.93)	73.21 (64.19-87.89)	0.20
4th Instar	15.8±082	24.3±0.94	34.3±1.24	52.0±1.41	61.3±1.88	40.58 (36.85-45.56)	78.97 (68.85-95.69)	0.52
Pupa	12.1±0.23	19.3±1.24	29.0±1.34	43.6±1.95	52.8±1.57	46.81 (42.20-53.78)	87.05 (74.81-08.22)	0.28

Control-Nil mortality, *LFL* - Lower Fiducidal Limit, *UFL* - Upper Fiducidal Limit, x^2 – *Chi*-square value, *Significant at $P < 0.05$ level. Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. Each value (Mean ± SD) five replicate.

Vector control is a serious concern in developing countries; every year, a large part of the population in the world is affected by one or more vector-borne diseases. Mosquitoes alone transmit disease to more than 700 million people annually [21]. Mosquito nuisance is a worldwide problem. It is observed at elevations of 5,500 m and in mines situated at depths of 1,250 m below sea level. Dengue is a vector-borne disease of tropical and subtropical human populations, which occurs predominantly in urban areas. The global increase in urbanization, such that the world's urban population of 1.7 billion in 1980 is expected to reach in 2010, 6.9 billion according to the United States Census Bureau, is likely to lead to an increase in dengue in the future.

Table 3. Combined effect of larval and pupal activity of *O. sanctum* petroleum ether leaf extract and *B. sphaericus* against dengue vector, *A. aegypti.*

Mosquito larval instar and pupa	% of Larval and pupal mortality ± SD Concentration of *O. sanctum* + *B. sphaericus* (ppm)					LC_{50} (ppm) (LFL–UFL)	LC_{90} (ppm) (LFL –UFL)	x^2
	30+5	60+10	90+15	120+20	150+25			
1st Instar	37.0±0.94	48.3±1.52	62.5±0.40	80.8±0.62	100±0.00	69.44 (10.96-97.79)	156.63 (122.39-279.34)	12.52
2nd Instar	33.3±0.47	42.1±1.75	58.1±1.47	75.4±1.22	100±0.00	78.43 (12.39-112.54)	165.15 (126.30-346.32)	16.46
3rd Instar	27.0±1.63	35.0±0.81	52.6±1.24	68.0±2.16	92.3±2.05	93.05 (65.25-116.40)	188.94 (154.89-275.09)	7.01
4th Instar	23.0±1.72	32.3±1.88	47.6±0.47	62.9±2.13	76.6±1.24	109.01 (98.03-120.36)	229.42 (204.48-267.83)	0.28
Pupa	20.2±1.97	27.3±1.69	43.3±2.05	52.6±0.94	65.3±0.21	130.66 (117.59-147.10)	274.09 (237.41-336.08)	0.48

Control-Nil mortality, *LFL* - Lower Fiducidal Limit, *UFL* - Upper Fiducidal Limit, x^2 – *Chi*-square value, *Significant at $P < 0.05$ level. Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. Each value (Mean ± SD) five replicate.

Many researchers have reported on the effectiveness of plant extract against mosquito larva studies [22-24]. Tulsi is described as sacred and medicinal plant in ancient literature. The name Tulsi is derived from *'*Sanskrit*'*, which means "matchless one". This plant belongs to the family *Labiatae*, characterized by square stem and specific aroma. Botanical name of Tulsi is *Ocimum sanctum* (Linn). In India, the plant is grown throughout the country from Andaman and Nicobar islands to the Himalayas up to 1800 meters above the sea level. It is also abundantly found in Malaysia, Australia, West Africa and some of the Arab countries. *Ocimum sanctum* (Linn) is the most prominent species of the genera. The leaves of the plant are considered to be very holy and often form a consistent part of the Hindu spiritual rituals (Tirtha or Prasada). *Ocimum sanctum* has two varieties i.e. black (Krishna Tulsi) and green (Rama Tulsi), their chemical constituents are similar. Both the varieties also have common medicinal properties. *O. sanctum* is used as a mosquito repellent and has toxic properties [25,26], and the essential oil showed larvicidal activity *against C. quinquefasciatus, A. aegypti* and *A. stephensi* [27]. It has been [28] observed significant mortality after the treatment of *Ocimum sanctum* leaf extract (OSLE) at different concentrations against *A. stephensi* in laboratory. After the treatment of OSLE at different concentration levels (0.5-8%), 38% mortality was noted at I instar larvae by the treatment of OSLE at 0.5%, whereas this increased to 90% at 8% of OSLE treatment. Mortality increased with increasing concentration.

Table 4. Field trail by using plant extracts of *O. sanctum* with *B. sphaericus* against dengue vector, *Ae. aegypti*.

No.	Larval density						
	Before treatment	After treatment					
		O. sanctum			*B. sphaericus*		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1	90	80	62	12	85	76	20
2	139	114	74	34	124	82	26
3	98	80	52	11	78	64	19
4	88	60	48	9	56	54	12
5	94	72	59	19	76	40	26
6	115	98	67	22	102	62	31
Total	624	504	362	105	519	378	134
Average	104	84	60.3	17.5	86.5	63	22.3
Reduction	-	19.23	41.98	83.17	16.82	39.42	78.52

Kovendan et al. [29] have reported hexane, chloroform, ethyl acetate and methanol extract of *J. curcas* with LC_{50} values of 230.32, 212.85, 192.07 and 113.23 ppm; *H. suaveolens* with LC_{50} values

of 213.09, 217.64, 167.59 and 86.93 ppm; *A. indicum* with LC_{50} values of 204.18, 155.53, 166.32 and 111.58 ppm; and *L. aspera* with LC_{50} values of 152.18, 118.29, 111.43 and 107.73 ppm, respectively. In the present study, after treatment of Petroleum ether leaf extract of *O. sanctum* against the dengue vector, *Ae. aegypti,* the LC_{50} and LC_{90} values were represented as follows; LC_{50} value of first instar was 147.87 ppm, second instar was 169.31 ppm, third instar was 195.30 ppm, fourth instar was 228.06 ppm, and pupa was 257.14 ppm, respectively. The LC_{90} value of first instar was 326.61 ppm, second instar was 373.41 ppm, third instar was 414.42 ppm, fourth instar was 459.38 ppm, and pupa was 491.69 ppm, respectively, This result is similar to the [30]. He reported that, highest larval mortality was found in chloroform and methanol extract of *O. sanctum*. Among the crude extracts tested are the chloroform extracts of acetone leaf of *O. sanctum* (LC_{50}=425.76). Mahesh Kumar et al. [24] have reported that 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 instar larvae and pupae were 155.29, 198.32, 271.12, 377.44, and 448.41 ppm, respectively. The LC_{90} values of first to fourth instar larvae and pupae were 687.14, 913.10, 1,011.89, 1,058.85, and 1,141.65 ppm, respectively.

Table 5. Field trail by using combine effect of plant extracts of *O. sanctum* with *B. sphaericus* against dengue vector, *Ae. aegypti*.

No.	Larval density			
	Before treatment	After treatment		
		O. sanctum with *B. sphaericus*		
		24hrs	48hrs	72hrs
1	90	40	22	-
2	139	58	12	-
3	98	49	14	-
4	88	31	18	-
5	94	62	20	-
6	115	54	34	-
Total	624	294	120	-
Average	104	49	20	-
Reduction (-)	-	52.88	80.76	100

B. sphaericus, a spore-forming, entamopathogenic bacterium, has been shown to possess potent larvicidal activity against several species of mosquito larvae [31,32]. In previous study, *B. sphaericus* and *Leucas aspera* first to fourth instars larvae and pupae against *A. stephensi* the LC_{50} and LC_{90} values were represented as follows: LC_{50} values of 2.03%, 2.04%, 2.05%, 2.05% and 2.07%; the LC_{90} values of 2.10%, 2.11%, 2.12%, 2.13% and 2.16%, respectively [33]. In the present results, the LC_{50} and LC_{90} values of *O. sanctum* leaf extract and *B. sphaericus* against first to fourth instars larvae and pupae were 69.44, 78.43, 93.05, 109.01 and 130.66 ppm; the LC_{90} values of 156.63, 165.15, 188.94, 229.42 and 274.09 ppm, respectively. Singh and Prakash [34] have reported that six different concentrations were used in laboratory bioassays (05, 10, 20, 30, 40, and 50 mg/l) for *A. stephensi*. Similarly, in the case of *C. quinquefasciatus*, six statistically significant different concentrations were used (0.01, 0.04, 0.05, 0.10, 5.0, and 10.0 mg/l) of *B. sphaericus*. It was recorded after exposure of 24 h. The percentages of mortalities were different for the different instars of *C. quinquefasciatus* and *A. stephensi*. Bioassay studies of *B. sphaericus* have been carried out in different parts of the world, including India, on mosquitoes in laboratories and fields [35].

Rao et al. [36] 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. Table 4 and 5 shows field trail using *O. sanctum* and *C. trifoliata* individually and combine

treatment against dengue vector *Ae. Aegypti*. . In a recent study, field trials were conducted using Clerodendron inerme and *Acanthus ilicifolius* treatment in different habitats of three species of mosquito vectors, namely, malarial vector *An. stephensi*, dengue vector *Ae. aegypti* and filarial vector *C. quinquefasciatus*. After treated with *O. sanctum* extracts, the *Ae. aegypti* larval density was reduced by 19.2%, 41.97%, and 83.1% at 24, 48, and 72 h, respectively; similarly, the reduction of *Ae. aegypti* larval densities after treatment with *B. sphaericus* was 16.8%, 39.4% and 78.5% at 24, 48, and 72 h, respectively, A total of 624 *Ae aegypti* larvae were observed in the stored water systems. After being treated with combine effect of plant extracts of *O. sanctum* leaf extract and *B. sphaericus* extract, the *Ae. aegypti* larval density was reduced by 52.8%, 80.7%, and 100% at 24, 48, and 72 h, respectively. Earlier, [37] reported that field trail conducted in Vadavalli, Mettupalayam, Navavoor privu, Pommanam palayam, Ooty, and 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 due to the impact of geographical distribution of *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* at the breeding sites.

In the present study, the larvicidal, pupicidal, and filed evaluation of plant extracts and Bs against *Ae. aegypti* 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 and sustainable mosquito vector control programs.

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