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ORIGINAL ARTICLE

Mosquito larvicidal activity of *Aloe vera* (Family: Liliaceae) leaf extract and *Bacillus sphaericus*, against Chikungunya vector, *Aedes aegypti*

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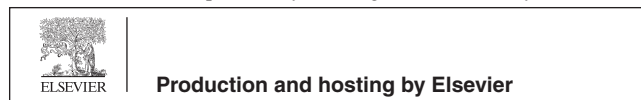
Abstract The bio-efficacy of *Aloe vera* leaf extract and bacterial insecticide, *Bacillus sphaericus* larvicidal activity was assessed against the first to fourth instars larvae of *Aedes aegypti*, under the laboratory conditions. The plant material was shade dried at room temperature and powdered coarsely. *A. vera* and *B. sphaericus* show varied degrees of larvicidal activity against various instars larvae of *A. aegypti*. The LC₅₀ of *A. vera* against the first to fourth instars larvae were 162.74, 201.43, 253.30 and 300.05 ppm and the LC₉₀ 442.98, 518.86, 563.18 and 612.96 ppm, respectively. *B. sphaericus* against the first to fourth instars larvae the LC₅₀ values were 68.21, 79.13, 93.48, and 107.05 ppm and the LC₉₀ values 149.15, 164.67, 183.84, and 201.09 ppm, respectively. However, the combined treatment of *A. vera* + *B. sphaericus* (1:2) material shows highest larvicidal activity of the LC₅₀ values 54.80, 63.11, 74.66 and 95.10 ppm; The LC₉₀ values of 145.29, 160.14, 179.74 and 209.98 ppm, against *A. aegypti* in all the tested concentrations than the individuals and clearly established that there is a substantial amount of synergist act. The present investigation clearly exhibits that both *A. vera* and *B. sphaericus* materials could serve as a potential larvicidal agent. Since, *A. aegypti* is a container breeder vector mosquito this user and eco-friendly and low-cost vector control strategy could be a viable solution to the existing dengue disease burden. Therefore, this study provides first report on the mosquito larvicidal activity the combined effect of *A. vera* leaf extract and *B. sphaericus* against as target species of *A. aegypti*.

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1. Introduction

A recent estimate shows that more than 50 million people are at risk of dengue virus exposure worldwide. Annually, there are 2 million infections, 500,000 cases of dengue hemorrhagic fever, and 12,000 deaths (Guha-Sapir and Schimme, 2005).

Aedes aegypti is generally known as a vector for an arbo-virus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito also acts as a vector of yellow fever in Central and South America and West Africa. However, Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever, and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement (Pancharoen et al., 2002).

A. aegypti is a cosmopolitan species that proliferates in water containers in and around houses. Secondary vectors include *Aedes albopictus*, an important vector in Southeast Asia and that has spread to the Americas, western Africa and the Mediterranean rim, *Aedes mediiovittatus* in the Caribbean, and *Aedes polynesiensis* and *Aedes scutellaris* in the western Pacific region. *A. aegypti* breeds in many types of household containers, such as water storage jars, drums, tanks, and plant or flower containers (Muir and Kay, 1998; Honorio et al., 2003; Harrington et al., 2005; Murugan et al., 2011).

Mosquito control, in view of their medical importance, assumes global importance. In the context of ever increasing trend to use more powerful synthetic insecticides to achieve immediate results in the control of mosquitoes, an alarming increase of physiological resistance in the vectors, its increased toxicity to non-target organism and high costs are noteworthy (WHO, 1975). Most of synthetic chemicals are expensive and destructive to the environment and also toxic to humans, animals and other non-target organisms. Besides, they are non-selective and harmful to other beneficial organisms. Some of the insecticides act as carcinogenic agents and are even carried through food chain which in turn affects the non-target organism. Therefore alternative vector control strategies, especially effective and low cost are extremely imperative (Piyarat et al., 1974; Kalyanasundaram and Das, 1985).

The use of different parts of locally available plants and their various products in the control of mosquitoes has been well established globally by numerous researchers. The larvicidal properties of indigenous plants have also been documented in many parts of India along with the repellent and anti-juvenile hormones activities (Singh and Bansal, 2003). Almost all tropical regions of the world are experiencing the resurgence and reoccurrence of one of the world's most deadly diseases, i.e., malaria, filariasis, dengue, and Chikungunya in world and India is no exception. Traditionally, plants and their derivatives were used to kill mosquitoes and other household and agricultural pests. In all probability, these plants used to control insects contained insecticidal phytochemicals that were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects (Shalan et al., 2005; Preeti Sharma et al., 2009).

Aloe vera is a perennial plant belonging to the family of Liliaceae, of which there are about 360 species (Klein and Penneys, 1988). Taxonomists now refer to *Aloe barbadensis* as *A. vera* (Coats and Ahola, 1979). Aloe is one of the few medicinal plants that maintain its popularity for a long period of time. The plant has stiff, gray-green lance-shaped leaves containing clear gel in a central mucilaginous pulp. *A. vera* gel has hypoglycemic (Rajasekaran et al., 2004), wound healing (Pandarinathan et al., 1998) and anti-inflammatory effects (Davis et al., 1991). The *A. vera* (L.) Burm. f., plant (synonym = *Aloe*

barbadensis Miller) is commonly referred to as *A. vera* and belongs to the lily family (family: Liliaceae, tribe Aloinae). This species is one of the approximately 420 species of aloe (Burdock, 1997).

Since, 1986 *A. vera* has been used as a traditional medicine and as an ingredient in many cosmetic products; it has gained high importance for its diverse therapeutic properties. The plant, being succulent, contains 99.5% water and the remaining solid material contains over 75 different ingredients including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, tannic acids, polysaccharide, glycoproteins, saponins, sterols, amino acids, and salicylic (Reynolds and Dweck, 1999). *A. vera* provides nutrition, shows anti-inflammatory action and has a wide range of antimicrobial activity (Reynolds and Dweck, 1999).

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. *B. sphaericus* is effective against *Culex* spp. but is less effective against some other mosquito species. Commercially available formulations of *B. sphaericus* are sold under the trade name Vectolex. 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 (Meisch, 1990).

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 (Kalfon et al., 1984). 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 (Wirth et al., 2001).

Indeed, source reduction is one of the key components in the malaria vector control program since the target is exceptionally specific unlike adult control. Innovative vector control strategy like use of phytochemicals as alternative sources of insecticidal/larvicidal agents in the fight against the vector-borne diseases has become inevitable. Above and beyond, in recent epoch, around the globe phytochemicals have gained massive attention by various researchers because of their bio-degradable and eco-friendly values (Karunamoorthi and Ilango, 2010). In this context, the purpose of the present investigation is to explore the larvicidal properties of *A. vera* leaf extract and bacterial insecticide, *B. sphaericus* against Chikungunya vector, *A. aegypti*, under the laboratory conditions. Therefore, this study provides first report on the mosquito larvicidal activity combined effect of *A. vera* leaf extract and *B. sphaericus* against *A. aegypti* as target species.

2. Materials and methods

2.1. Collection of eggs and maintenance of larvae

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

2.2. Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred to plastic containers (12 × 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 (light/dark). A 10% sugar solution was provided for a period of 3 days before blood feeding.

2.3. Blood feeding of adult *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.

2.4. Collection of plant and preparation of extract

A. vera was collected in and around Bharathiar University Campus, Coimbatore, India. The voucher specimen has been deposited and kept in our research laboratory for further reference. *A. vera* 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 for 8 h using a Soxhlet apparatus (Vogel, 1978). The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in a rotary vacuum evaporator. 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 and 400 ppm, respectively.

2.5. Microbial bioassay

B. sphaericus was obtained from T. Stanes & 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): FeSO₄·7H₂O,

0.01; MnSO₄, 0.1; MgSO₄·7H₂O, 0.2; CaCl₂, 0.08; K₂HPO₄, 0.025; yeast extract, 2; peptone, 4; and D-glucose, 1 and casein, 5. Solutions of yeast extract, peptone casein, D-glucose, K₂HPO₄ and CaCl₂ 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 25, 50, 75, 100 and 125 ppm, respectively.

2.6. 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 *A. vera* leaf 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 dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula (Abbott, 1925).

Corrected mortality

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

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

The LC₅₀ and LC₉₀ were calculated from toxicity data by using probit analysis (Finney, 1971).

2.7. 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). The average larval mortality data were subjected to probit analysis, for calculating LC₅₀ and LC₉₀, values were calculated by using the Finney (1971) method. SPSS (Statistical software package) 9.0 version was used. Results with *P* < 0.05 were considered to be statistically significant.

3. Results

Larval mortality of *A. aegypti* after the treatment of petroleum ether *A. vera* was observed. Table 1 provides the results of larval mortality of *A. aegypti* (I–IV instars) after the treatment of

Table 1 Larvicidal activity of *A. vera* leaf extract against *A. aegypti*.

Mosquito larval instars	% of larval mortality					LC ₅₀ (LC ₉₀)	95% confidence limit		χ^2 (df = 4)
	Concentration of AVLE (ppm)						LFL	UFL	
	80	160	240	320	400		LC ₅₀ (LC ₉₀)	LC ₅₀ (LC ₉₀)	
I	34 ^a	53 ^a	64 ^a	71 ^a	89 ^a	162.74 (442.98)	128.19 (395.17)	189.79 (516.81)	2.87*
II	29 ^a ^b	48 ^b	56 ^b	64 ^b	81 ^b	201.43 (518.86)	168.33 (455.12)	229.81 (623.39)	2.27*
III	24 ^b ^c	36 ^c	47 ^c	58 ^c	75 ^c	253.30 (563.18)	225.36 (493.94)	282.81 (675.91)	0.66*
IV	19 ^c	28 ^d	41 ^d	50 ^d	68 ^d	300.059 (612.96)	271.32 (534.46)	336.01 (742.76)	0.67*

Control – nil mortality, LFL – lower fiducial limit, UFL – upper fiducial limit, χ^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. Each value is five replicates.

* Significant at *P* < 0.05 level.

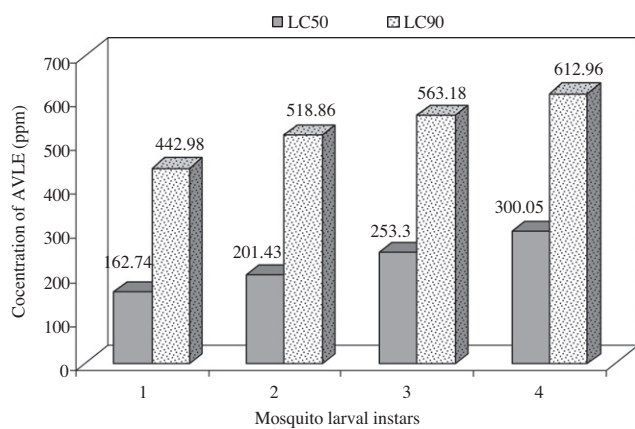


Figure 1 Larvicidal activity of *A. vera* leaf extract against *A. aegypti* expressed as LC₅₀ and LC₉₀.

A. aegypti at different concentrations (80–400 ppm). Thirty-four percent mortality was noted at I instar larvae by the treatment of *A. vera* at 80 ppm, whereas it has been increased to 89% at 400 ppm of *A. vera* leaf extract treatment. Similar trend has been noted for all the instars of *A. aegypti* at different concentrations of *A. vera* treatment. The LC₅₀ and LC₉₀ values were represented as follows; LC₅₀ value of I instar was 162.74 ppm, II instar was 201.43 ppm, III instar was 253.30 ppm, and IV instar was 300.05 ppm, respectively. The LC₉₀ value of I instar was 442.98 ppm, II instar was 518.86 ppm, III instar was 563.18 ppm and IV instar was 612.96 ppm, respectively (Fig. 1).

Table 2 shows the results of larval mortality of *A. aegypti* (I–IV instars) after the treatment of *B. sphaericus* at different concentrations (25–125 ppm). Twenty-seven percent mortality was noted at I instar larvae by the treatment of *B. sphaericus* at 25 ppm, whereas it has been increased to 85% at 125 ppm of *B. sphaericus* treatment and 12% mortality was noted at pupae by the treatment of *B. sphaericus* at 25 ppm and it has been increased to 60% at 125 ppm. Similar trend has been noted for all the instars of *A. aegypti* at different concentrations of *B. sphaericus* treatment. The LC₅₀ and LC₉₀ values were represented as follows: LC₅₀ value of I instar was 68.21 ppm, II instar was 79.13 ppm, III instar was 93.48 ppm, and IV instar

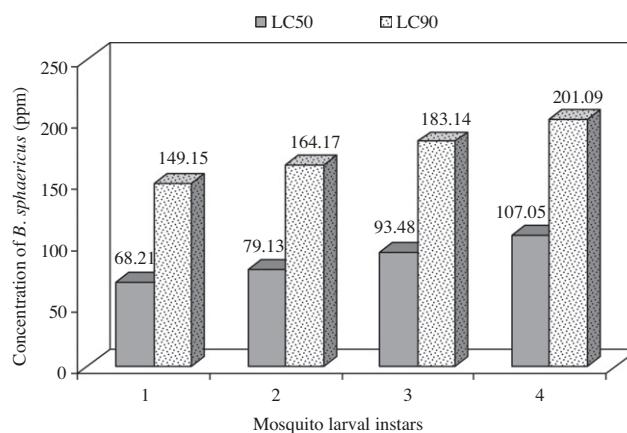


Figure 2 Larvicidal activity of *B. sphaericus* against *A. aegypti* expressed as LC₅₀ and LC₉₀.

was 107.05812 ppm, respectively. The LC₉₀ value of I instar was 149.15 ppm, II instar was 164.67 ppm, III instar was 183.84 ppm, and IV instar was 201.09 ppm, respectively (Fig. 2).

The considerable larval mortality after the combined effect of *B. sphaericus* and *A. vera* extract against all the larval instars in *A. aegypti* is provided in (Table 3 and Fig. 3). The concentration at 20 + 10 ppm combined treatment of *B. sphaericus* and *A. vera* for I instar larval mortality was 41%. The LC₅₀ and LC₉₀ values were represented as follows: LC₅₀ value of I instar was 54.80 ppm, II instar was 63.11 ppm, III instar was 74.66 ppm and IV instar was 95.10 ppm. The LC₉₀ value of I instar was 145.29 ppm, II instar was 160.14, III instar was 179.74 ppm and IV instar was 209.98 ppm, respectively.

4. Discussion

Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, which makes it easy to deal with them in this habitat. The use of conventional pesticides in the water sources, however, introduces many risks to people and the environment. Natural pesticides, especially those derived from plants, are more promising in this aspect. Aromatic plants and their essential oils are very important

Table 2 Larvicidal activity of bacterial insecticide, *B. sphaericus* against *A. aegypti*.

Mosquito larval instars	% of larval mortality					LC ₅₀ (LC ₉₀)	95% confidence limit		χ^2 (df = 4)
	Concentration of <i>B. sphaericus</i> (ppm)						LFL	UFL	
	25	50	75	100	125		LC ₅₀ (LC ₉₀)	LC ₅₀ (LC ₉₀)	
I	27 ^a	39 ^a	50 ^a	67 ^a	85 ^a	68.21 (149.2)	60.35 (134)	75.58 (171.8)	2.05*
II	21 ^{ab}	35 ^b	46 ^a	59 ^b	78 ^b	79.13 (164.7)	71.36 (146.8)	87.28 (192.2)	1.07*
III	16 ^{bc}	28 ^c	41 ^{ab}	50 ^c	69 ^c	93.48 (183.8)	85.12 (162.1)	103.71 (218.6)	0.84*
IV	12 ^c	23 ^d	35 ^b	44 ^d	60 ^d	107.05 (201.1)	97.36 (175.3)	120.48 (243.7)	0.55*

Control-Nil mortality, LFL – lower fiducial limit, UFL – upper fiducial limit, χ^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. Each value is five replicates.

* Significant at $P < 0.05$ level.

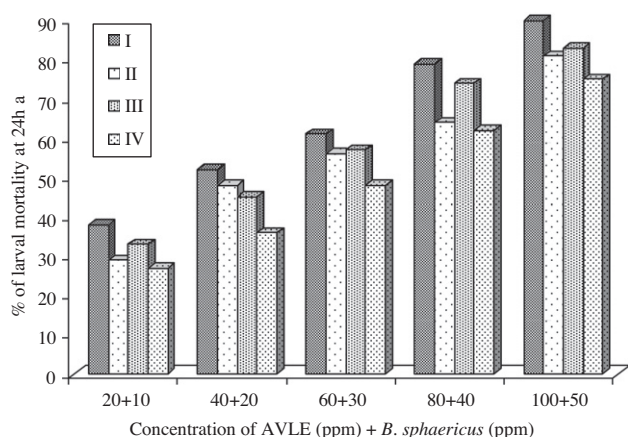
Table 3 Combined treatment of larvicidal activity of *A. vera* leaf extract and bacterial insecticide, *B. sphaericus* against *A. aegypti*.

Mosquito larval instars	% of larval mortality					LC ₅₀ (LC ₉₀)	95% confidence limit		χ^2 (df = 4)
	Concentration of AVLE (ppm) + <i>B. sphaericus</i> (ppm)						LFL	UFL	
	20 + 10	40 + 20	60 + 30	80 + 40	100 + 50		LC ₅₀ (LC ₉₀)	LC ₅₀ (LC ₉₀)	
I	41 ^a	56 ^a	69 ^a	82 ^a	94 ^a	54.80 (145.29)	41.63 (131.78)	64.65 (165.23)	4.73*
II	38 ^a	52 ^a	61 ^b	79 ^{ab}	90 ^a	63.11 (160.14)	50.52 (144.29)	72.79 (184.28)	4.11*
III	33 ^b	45 ^b	57 ^b	74 ^b	83 ^b	74.66 (179.74)	62.93 (160.35)	84.30 (210.42)	3.48*
IV	27 ^c	36 ^c	48 ^c	62 ^c	75 ^c	95.10 (209.98)	84.57 (184.25)	105.73 (252.87)	3.16*

Control – nil mortality, LFL – lower fiducial limit, UFL – upper fiducial limit, χ^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. Each value is five replicates.

* Significant at $P < 0.05$ level.

**Figure 3** Combined treatment of larval mortality of *A. vera* leaf extract and *B. sphaericus* against *A. aegypti* at 24 h.

sources of many compounds that are used in different respects (Amer and Mehlhorn, 2006a).

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 (Kovendan and Murugan, 2011).

The isolated compound saponin from ethyl acetate extract of *Achyranthes aspera* was effective against the larvae of *A. aegypti* and *Culex quinquefasciatus* with LC₅₀ value of 18.20 and 27.24 ppm, respectively (Bagavan et al., 2008). The neem formulation, Neem Azal, produced an overall mortality or inhibition of emergence of 90% (EI₉₀, when third-instar larvae were treated) at 0.046, 0.208, and 0.866 ppm in *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti*, respectively (Gunasekaran et al., 2009). Fraction A1 of ethanol from *Sterculia guttata* seed extract was found to be most promising; its LC₅₀ was 21.552 and 35.520 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively (Katade et al., 2006a,b). With *A. barbadensis* the

larvicidal activity increases with increase in the exposure period from 24 to 48 h with decrease in LC₅₀ values from 15.31 to 11.01 ppm (carbon tetrachloride extract), 25.97 to 16.60 ppm (petroleum ether extract) and 144.44 to 108.38 ppm (methanol extract). Similar trend was also observed in case of *Cannabis sativa* with LC₅₀ values 88.51 to 68.69 ppm (carbon tetrachloride extract), 294.42 to 73.32 ppm (petroleum ether extract) and 160.78 to 71.71 ppm (methanol extract) on increase in the exposure period. Further, Barnard and Rui De (2004) observed the repellent activity of *A. vera* against *A. albopictus* and *Culex nigripalpus*.

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 (Kovendan et al., 2012b,c). In the present results, the LC₅₀ and LC₉₀ values of *A. vera* against first to fourth instars larvae were 162.74, 201.43, 253.30 and 300.05 ppm; the LC₉₀ values of 442.98, 518.86, 563.18 and 612.96 ppm, respectively.

However, in our case, early detection of resistance against *B. sphaericus* could be better for the management of resistance development. Experts of resistance management have been involved in resistance detection with generations of mosquitoes. Moreover, the detection of resistance in an early stage could be a better approach to control mosquitoes. Laboratory- and field-collected *C. quinquefasciatus* exposed to *B. sphaericus* strain 2362 for 35 generations in the laboratory showed a level of resistance 43- and 12-fold than that of potential generation, respectively (Rodeharoen and Mulla, 1991). *B. sphaericus*, a spore-forming, entamopathogenic bacterium, has been shown to possess potent larvicidal activity against several species of mosquito larvae (Davidson, 1983; Yousten and Wallis, 1987). A flowable concentrate of *B. sphaericus* (Neide) strain 2362 was applied against *Anopheles gambiae* Giles s.l. mosquito larvae in small plot field trials in Bobo-Dioulasso area, Burkina-Faso. Third and fourth instar larvae were controlled for 10–15 days with a dosage of 10 g/m², 3–10 days with 1 or 0.1 mg/m², and 2 days with 0.01 g/m² (Nicolas et al., 1987).

B. sphaericus showed a good control over *A. stephensi* which may be due to the presence of Bin and mosquitocidal

toxins (Mtxs). As a consequence of the specific toxicity to mosquito larvae of Bin and Mtxs produced during the sporulation and vegetative stages, respectively, some toxic strains have been widely used for many years as bio-pesticides in the field of mosquito control programs (Bei et al., 2006). The soil bacterium showed varied mortality rate related to the larval stages and concentrations. The younger larval stages were much susceptible than the later ones. Active strains of *B. sphaericus* are known to produce considerable quantities of at least two sets of proteinaceous mosquito larvicidal factors at the onset of stationary phase (Souza et al., 1988; Baumann et al., 1991; Porter et al., 1993). Larvicidal activity was observed in all strains of *B. sphaericus* from Amazonia in differentiated toxicity levels (Eleiza de et al., 2008). In the present study, *B. sphaericus* treatment reduced the larvicidal properties of microbial insecticides development of growth control.

Singh and Prakash (2009) have reported that six different concentrations were used in laboratory bioassays (0.5, 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 were used in laboratory bioassays (0.5, 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 (Rodrigues et al. 1998). *B. sphaericus* against the first to fourth instar larvae and pupae had the following values: I instar was 0.051%, II instar was 0.057%, III instar was 0.062%, IV instar was 0.066%, and for the pupae was 0.073%, respectively. *B. sphaericus*, an obligate aerobe bacterium, showed that it has good and effective mosquito control properties and also can act as an eco-friendly, biopesticide for further vector control programs. In a previous study, *B. sphaericus*, the bacterial pesticide was isolated from the soil samples and used to control the malarial vector, *A. stephensi* (Kovendan et al., 2012a). In the present results, the LC₅₀ and LC₉₀ values of *B. sphaericus* were 68.21, 79.13, 93.48, and 107.05 ppm; The LC₉₀ values of 149.15, 164.67, 183.84 and 201.09 ppm, respectively.

Vector control is one of the most powerful weapons in the process of managing vector populations to reduce/interrupt the transmission of disease. As a result, vector control remains considered to be a cornerstone in the vector-borne disease control program due to lack of reliable vaccine, drug resistance parasites and insecticide resistance of insect vectors disease (Karunamoorthi, 2011). In previous study, *B. sphaericus* and *Leucas aspera* first to fourth instars larvae and pupae against *A. stephensi* the LC₅₀ and LC₉₀ values were represented as follows: LC₅₀ values of 2.03%, 2.04%, 2.05%, 2.05% and 2.07%; the LC₉₀ values of 2.10%, 2.11%, 2.12%, 2.13% and 2.16%, respectively (Kovendan et al., 2012a). In the present results, the LC₅₀ and LC₉₀ values of *A. vera* leaf extract and *B. sphaericus* against first to fourth instars larvae were 54.80, 63.11, 74.66 and 95.10 ppm; the LC₉₀ values of 145.29, 160.14, 179.74 and 209.98 ppm, respectively.

5. Conclusion

This result clearly reveals that both the leaf extract of *A. vera* and bio-control agent *B. sphaericus* could serve as a potential larvicidal agents against the dengue vector *A. aegypti* and they have demonstrated a synergist act too. This approach could not only improve the bio-efficacy of *B. sphaericus* but also substantially reduce the possibilities of physiological resistance development in mosquito population. Therefore, the present strategy should be promoted in the dengue vector control program. The mode of action and larvicidal efficiency of the *A. vera* extract under the field conditions should be scrutinized and determined. Besides, further investigation regarding the effect on non-target organism is extremely important and imperative in the near future.

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References

- Abbott, W.S., 1925. A method of computing the effectiveness of insecticides. *J. Ecol. Entomol.* 18, 265–267.
- Alder, H.L., Rossler, E.B., 1977. *Introduction to Probability and Statistics*. Freeman, San Francisco, pp. 246.
- Amer, A., Mehlhorn, H., 2006. Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera: Culicidae). *Parasitol. Res.* 99, 466–472.
- Bagavan, A., Rahuman, A.A., Kamaraj, C., Geetha, K., 2008. Larvicidal activity of saponin from *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol. Res.* 103, 223–229.
- Barnard, D.R., Rui De, Xue, 2004. Laboratory evaluation of mosquito repellent against *Aedes albopictus*, *Culex nigripalpus* and *Ochlerotatus triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* 41, 726–730.
- Baumann, P., Clark, M.A., Baumann, L., Broadwell, A.H., 1991. *Bacillus sphaericus* as a mosquito pathogen: properties of the organism and its toxins. *Microbiol. Rev.* 55, 425–436.
- Bei, H., Haizhou, L., Xiaomin, H., Zhiming, Y., 2006. Preliminary characterization of a thermostable DNA polymerase I from a mesophilic *Bacillus sphaericus* strain C3–41. *Arch. Microbiol.* 186, 203–209.
- Burdock, G.A., 1997. Aloe, extract (*Aloe* spp.). In: *Encyclopedia of Food and Color Additives*. vol. I. CRC Press, Boca Raton, FL, pp. 105–106.
- Coats, Ahola, R., 1979. *A Modern Study of Aloe vera*. Garland, Dallas.
- Davidson, E.W., 1983. Alkaline extraction of toxin from spores of the mosquito pathogen *Bacillus sphaericus* strain 1593. *Can. J. Microbiol.* 29, 271–275.
- Davis, H., Parker, L., Samson, T., 1991. The isolation of an active inhibitory system from an extract of *Aloe vera*. *J. Am. Podiatr. Med. Assoc.* 81, 258–261.

- Eleiza de, C., Litaiff, E., Wanderli, P.T., Jorge, I., Porto Ila, R., De, M., Aguiar, O., 2008. Analysis of toxicity on *Bacillus sphaericus* from amazonian soils to *Anopheles darlingi* and *Culex quinquefasciatus* larvae. *Acta Amaz.* 38 (2), 255–262.
- Finney, D.J., 1971. *Probit Analysis*. Cambridge University Press, London, pp. 68–78.
- Guha-Sapir, D., Schimme, B., 2005. Dengue fever: new paradigms for a changing epidemiology. *Emerg. Themes Epidemiol.* 12, 13.
- Gunasekaran, K., Vijayakumar, T., Kalyanasundaram, M., 2009. Larvicidal and emergence inhibitory activities of NeemAzal T/S 1.2 per cent EC against vectors of malaria, filariasis and dengue. *Indian J. Med. Res.* 130, 138–145.
- Harrington, L.C., Scott, T.W., Lerdthusnee, K., Coleman, R.C., Costero, A., Clark, G.G., Jones, J.J., Kitthawee, S., Kittayapong, P., Sithiprasasna, R., Edman, J.D., 2005. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities, part 1. *Am. J. Trop. Med. Hyg.* 72, 209–220.
- Honorio, N.A., Silva, W.C., Leite, P.J., Gonc alves, J.M., Lounibosn, L.P., Lourenço-de-Oliveira, R., 2003. Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic dengue area in the state of Rio de Janeiro, Brazil. *Mem. Inst. Oswaldo Cruz* 98, 191–198.
- Kalfon, A., Charles, J.F., Bourgouin, C., de Barjac, H., 1984. Sporulation of *Bacillus sphaericus* 2297: an electron microscope study of crystal like inclusion, biogenesis and toxicity to mosquito larvae. *J. Gen. Microbiol.* 130, 893–900.
- Kalyanasundaram, M., Das, P.K., 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. *Indian J. Med. Res.* 82, 19–23.
- Karunamoorthi, K., 2011. Vector control: a cornerstone in the malaria elimination campaign. *Clin. Microbiol. Infect.* 17, 1608–1616.
- Karunamoorthi, K., Ilango, K., 2010. Larvicidal activity of *Cymbopogon citratus* (DC) Stapf. and *Croton macrostachyus* Del. against *Anopheles arabiensis* Patton (Diptera: Culicidae), the principal malaria vector. *Eur. Rev. Med. Pharmacol. Sci.* 14, 57–62.
- Katade, S.R., Pawar, P.V., Wakharkar, R.D., Deshpande, N.R., 2006a. *Sterculia guttata* seeds extractives—an effective mosquito larvicide. *Ind. J. Exp. Biol.* 44, 662–665.
- Katade, S.R., Pawar, P.V., Tungikar, V.B., Tambe, A.S., Kalal, K.M., Wakharkar, R.D., Deshpande, N.R., 2006b. Larvicidal activity of bis(2-ethyl-hexyl) benzene-1,2-dicarboxylate from *Sterculia guttata* seeds against two mosquito species. *Chem. Biodiversity* 3, 49–53.
- Klein, D., Penneys, N., 1988. *Aloe vera*. *J. Am. Acad. Dermatol.* 18, 714–720.
- Kovendan, K., Murugan, K., 2011. Effect of medicinal plants on the mosquito vectors from the different agro-climatic regions of Tamil Nadu, India. *Adv. Environ. Biol.* 5, 335–344.
- Kovendan, K., Murugan, K., Vincent, S., Barnard, D.R., 2012a. Studies on larvicidal and pupicidal activity of *Leucas aspera* Willd. (Lamiaceae) and bacterial insecticide, *Bacillus sphaericus* against malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). *Parasitol. Res.* 110, 195–203.
- Kovendan, K., Murugan, K., Vincent, S., 2012b. Evaluation of larvicidal activity of *Acalypha alnifolia* Klein ex Willd. (Euphorbiaceae) leaf extract against the malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and *Bancroftian filariasis* vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol. Res.* 110, 571–581.
- Kovendan, K., Murugan, K., Naresh Kumar, A., Vincent, S., Hwang, J.S., 2012c. Bio-efficacy of larvicidal and pupicidal properties of *Carica papaya* (Caricaceae) leaf extract and bacterial insecticide, spinosad against Chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). *Parasitol. Res.* 110, 669–678.
- Meisch, M.V., 1990. Evaluation of *Bacillus sphaericus* against *Culex quinquefasciatus* in septic ditches. *J. Am. Mosq. Control Assoc.* 6, 496–499.
- Muir, L.E., Kay, B.H., 1998. *Aedes aegypti* survival and dispersal estimated by mark-release-recapture in northern Australia. *Am. J. Trop. Med. Hyg.* 58, 277–282.
- Murugan, K., Hwang, J.S., Kovendan, K., Prasanna Kumar, K., Vasugi, C., Naresh Kumar, A., 2011. Use of plant products and copepods for control of the dengue vector, *Aedes aegypti*. *Hydrobiologia* 666, 331–338.
- Nicolas, L., Darriet, F., Hougard, J.M., 1987. Efficacy of *Bacillus sphaericus* 2362 against larvae of *Anopheles gambiae* under laboratory and field conditions in West Africa. *Med. Vet. Entomol.* 1 (2), 157–162.
- Pancharoen, C., Kulwichit, W., Tantawichien, T., Thisyakorn, U., Thisyakorn, C., 2002. Dengue infection: a global concern. *J. Med. Assoc. Thai.* 85, 25–33.
- Pandinathan, C.B., Sajithlal, G.B., Gowri, C., 1998. Influence of *Aloe vera* on collagen turn over in healing of dermal wounds in rats. *Ind. J. Exp. Biol.* 36, 896–901.
- Piyarat, S.W.K., Freed, M., Roy, S., 1974. Biologically active plant extract for the control of mosquito larvae. *Mosq. News* 34, 398.
- Porter, A.G., Davidson, E.W., Liu, J.W., 1993. Mosquitocidal toxins of bacilli and their genetic manipulation for effective biological control of mosquitoes. *Microbiol. Rev.* 57, 838–861.
- Rajasekaran, S., Sivagnanam, K., Ravi, K., Subramanian, S., 2004. Hypoglycemic effect of *Aloe vera* gel on streptozotocin-induced diabetes in experimental rats. *J. Med. Food.* 7, 61–66.
- Reynolds, T., Dweck, A.C., 1999. *Aloe vera* leaf gel: a review update. *J. Ethnopharmacol.* 68, 3–37.
- Rodeharen, J., Mulla, M.S., 1991. Resistance in *Culex quinquefasciatus* to *Bacillus sphaericus*: An Annual Report. University of California Riverside.
- Rodrigues, I.B., Tadei, W.P., Dias, J.M.C.S., 1998. Studies on the *Bacillus sphaericus* larvicidal activity against malaria vector species in Amazonia. *Mem. Inst. Oswaldo Cruz* 93, 441–444.
- Shalan, E., Canyon, D., Faried, M.W., Abdel-Wahab, H., Mansour, A., 2005. A review of botanical phytochemicals with mosquitocidal potential. *Environ. Int.* 31, 1149–1166.
- Sharma, P., Mohan, L., Srivastava, C.N., 2009. *Amaranthus oleracea* and *Euphorbia hirta*: natural potential larvicidal agents against the urban Indian malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol. Res.* 106, 171–176.
- Singh, G., Prakash, S., 2009. Efficacy of *Bacillus sphaericus* against larvae of malaria and filarial vectors: an analysis of early resistance detection. *Parasitol. Res.* 104, 763–766.
- Singh, K.V., Bansal, S.K., 2003. Larvicidal properties of a perennial herb *Solanum xanthocarpum* against vectors of malaria and dengue/DHF. *Curr. Sci.* 84, 749–751.
- Souza, A.E., Rajan, V., Jayaraman, K., 1988. Cloning and expression in *Escherichia coli* of two DNA fragments from *Bacillus sphaericus* encoding mosquito-larvicidal activity. *J. Biotechnol.* 7, 71–82.
- Vogel, A.I., 1978. *Textbook of Practical Organic Chemistry*. The English Language Book Society and Longman, London, pp. 1368.
- Wirth, M.C., Yang, Y., Walton, W.E., Federici, B.A., 2001. Evaluation of alternative resistance management strategies for *Bacillus sphaericus*. Mosquito Control Research, Annual Report. Division of Agriculture and Natural Resources, University of California.
- World Health Organization, 1975. *Manual on Practical Entomology in Malaria Part I*. Who division of malaria and other parasitic diseases, pp. 160.
- Yousten, A.A., Wallis, D.A., 1987. Batch and continuous culture production of the mosquito larval toxin of *Bacillus sphaericus* 2362. *J. Ind. Microbiol. Biotechnol.* 2, 277–283.