Larvicidal potentiality, longevity and fecundity inhibitory activities of *Bacillus sphaericus* (Bs G3-IV) on vector mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*

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Abstract

Intervention measures to control the transmission of vector-borne diseases include control of the vector population. In mosquito control, synthetic insecticides used against both the larvae (larvicides) and adults (adulticides) create numerous problems, such as environmental pollution, insecticide resistance and toxic hazards to humans. In the present study, a bacterial pesticide, *Bacillus sphaericus* (Bs G3-IV), was used to control the dengue and filarial vectors, *Aedes aegypti* and *Culex quinquefasciatus*. *Bacillus sphaericus* (Bs G3-IV) was very effective against *Aedes aegypti* and *Culex quinquefasciatus*, showing significant larval mortality. Evaluated lethal concentrations (LC_{50} and LC_{90}) were age-dependent, with early instars requiring a lower concentration compared with later stages of mosquitoes. *Culex quinquefasciatus* was more susceptible to *Bacillus sphaericus* (Bs G3-IV) than was *Aedes aegypti.* Fecundity rate was highly reduced after treatment with different concentrations of *Bacillus sphaericus* (Bs G3-IV). Larval and pupal longevity both decreased after treatment with *Bacillus sphaericus* (Bs G3-IV), total number of days was lower in the *B. sphaericus* treatments compared with the control. Our results show the bacterial pesticide *Bacillus sphaericus* (Bs G3-IV) to be an effective mosquito control agent that can be used for more integrated pest management programs.

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Introduction

Mosquitoes are insect vectors responsible for the transmission of many diseases. Mosquito-borne diseases include yellow fever, dengue fever and Chikungunya, transmitted mostly by *Aedes aegypti*; malaria, carried by the genus *Anopheles,* and *Culex* serves as a vector of important diseases such as West Nile virus, filariasis, Japanese encephalitis, St. Louis encephalitis, and avian malaria. Insect-transmitted disease remains a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million people annually and are responsible for several million deaths every year (WHO, 2012; Taubes, 2000; Kessler & Guerin, 2008).

Management of these vectors is a serious concern in a developing country like India, due to development of pesticide resistance and for socio-economic reasons. Every year, a large part of the population is affected by one or more vector-borne diseases. Vector control, which includes both anti-larval and anti-adult measures, constitutes an important aspect of any mosquito control program. Mosquito control using synthetic insecticides is an effective vector control strategy used extensively in daily life. Synthetic insecticides are still at the forefront of mosquito-controlling efforts. However, the environmental threat that these chemicals pose affects on non-target organisms, and resistance of mosquitoes to insecticides have all increased during the last five decades (Wattanachai & Tintanon, 1999; Amer & Mehlhorn, 2006a, 2006b).

In recognition of these facts, it is necessary to develop new insecticides for controlling mosquitoes that are environmentally safer, biodegradable, and more target-specific against the mosquitoes. Recent negative consumer perceptions concerning the use of chemicals as larvicides have shifted research efforts towards the development of alternatives that the public perceives as natural products, such as bacterial pesticides, predators, and plant extracts. Consequently, the present work deals with the insecticidal activities of natural products, such as bacterial pesticides.

Bacillus sphaericus is an aerobic, mesophilic, spore-forming bacterium with terminal swollen sporangia and spherical spores. As a consequence of the specific toxicity to mosquito larvae of binary toxin (Bin) and mosquitocidal toxins (Mtxs) produced during the sporulation and vegetative stages, respectively, some toxic strains have been widely used for many years as biopesticides in the field in mosquito control programs (Bei *et al*., 2007).

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. Depending on the formulation and environmental conditions, *B. sphaericus* is generally effective 1-4 weeks after application (Charles *et al*., 1996). The larvicidal binary toxin produced by *B. sphaericus* is composed of two chains, BinA (42 kDa) and BinB (51 kDa) that are deposited as parasporal crystals during sporulation (Baumann *et al*., 1991). These crystals bind to a specific receptor present on midgut brush-border membranes, resulting in damage to the midgut cells, which further leads to the mosquito's death (Neilsen-Leroux & Charles, 1992).

Bacillus sphaericus is specific and toxic against the genera *Culex* and *Anopheles*. It is harmless to humans, animals and the environment, and its use is recommended by the World Health Organization in public health programs worldwide (WHO, 1987). Many strains of *B. sphaericus* have been used as a toxicant in vector control programs all over the world (Regis *et al.,* 2001). In a recent study in Brazil, da Silva Pinto *et al.* (2012) cloned the BinA and BinB genes of *Bacillus sphaericus,* produced recombinant BinAB protein in three strains of *Escherichia coli,* and used these recombinant strains in toxicity assays against *Culex quinquefasciatus* larvae.

Research on *Bacillus sphaericus* in South Asian countries and India includes a number of strains, such as *Bacillus sphaericus* strain SI-1 (Hossain *et al.,* 2007), *Bacillus sphaericus strain* B-101 (serotype H5a, 5b) (Yadav *et al*., 1997), *Bacillus sphaericus* H5a5b (VCRC B42) (Prabhakaran *et al.,* 2007), *Bacillus sphaericus* H-5a5b (Manonmani & Hoti, 1999), *Bacillus sphaericus* (*Bs*) 2362 SPH-88 (serotype: H5a5b) (Poopathi *et al.,* 2009), *Bacillus sphaericus*, C3-41, 2362, and IAB59 (Pei *et al.,* 2002) for mosquito control. Mosquito larvicidal activity of *B. sphaericus* was assessed by isolating it from ecologically different soil habitats in South India (Surendran & Vennison, 2011).

The present study was conducted to test the larvicidal and pupacidal activities of the microbial insecticide *Bacillus sphaericus* (Bs G3-IV) on *Aedes aegypti* and *Culex quinquefasciatus* in the laboratory as well as in direct breeding sites. We also report the effects of *Bacillus sphaericus* (Bs G3-IV) on longevity and fecundity of *Aedes aegypti* and *Culex quinquefasciatus*.

Materials and methods

Collection of eggs and mosquitoes

Eggs of *Aedes aegypti* were collected using oviposition traps placed in shaded areas at a height of less than 1.2 m. Traps were filled with water plus a few dried leaves placed at the bottom of the container, with a muslin strip placed vertically inside the container and half-submerged in the water. *Culex quinquefasciatus* egg rafts were collected from sewage water bodies in Coimbatore district, Tamil Nadu, India, using CDC gravid traps (Reiter 1983, 1987). These eggs were brought to the laboratory and transferred to 18×13×4 cm enamel trays (with separate trays for each species) containing 500 mL of water, and held for larval hatch.

Maintenance of larvae

The mosquito larval culture was maintained in our laboratory at 27+2°C, 75-85% RH. The mosquito larvae were fed with dog biscuits and yeast (Scottlabs Pvt. Ltd., Hyderabad, India) at a 3:1 ratio. The feeding was continued until the larvae transformed into pupae.

Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred into plastic jar containers (12×12 cm) containing 500 mL of water. These plastic jars were kept in a 90×90×90 cm mosquito cage for adult emergence. The emerged adults were maintained at 27±2°C, 75-85% RH, under 14 light (L):10 dark (D) photoperiod cycles. Adults were fed with 10% sugar solution for a period of three days before they were given an animal for blood feeding.

Blood feeding of adult mosquitoes

The female mosquitoes were allowed to feed on the blood of a rabbit (exposed on the dorsal side) for two days. The males were provided with 10% glucose solution on cotton wicks. The cotton was kept moist with the solution and changed every day. An egg trap (cup) lined with filter paper containing water was placed in a corner of the egg collection cage.

Collection and preparation of *Bacillus sphaericus*

Bacillus sphaericus (Bs G3-IV) with improved toxicity was collected from Defence Research Laboratory, Tezpur, Assam, India. To assure good suspensions for selection and bioassay procedures, stock suspensions (1 ppm) of the primary powders were prepared in distilled water by vigorously shaking 1 g of the powder in 1000 mL of water in a screw-cap glass vial. Required concentrations (0.001 ppm, 0.01 ppm, 0.1 ppm, 1.0 ppm, and 10 ppm) were prepared by serial dilution of the stock solution in distilled water. All stocks and dilutions were kept refrigerated at -4°C for no more than four months.

Larval/pupal assays

Laboratory colonies of mosquito larvae/pupae (F1 generation) were used for the larvicidal/pupacidal activity. Twenty-five individual I-IV-instar larvae and pupae were introduced into a 500 mL glass beaker containing 250 mL of dechlorinated water with the desired concentrations of biopesticide. Larval food was provided for the test larvae. At each tested concentration, 2-5 trials were run and each trial consisted of 3 replicates. The larvae/pupae exposed to dechlorinated water without biopesticide served as a control. The control mortalities were corrected using Abbott's formula (Abbott, 1925) where:

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

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

Fecundity studies

The fecundity experiments were conducted by taking an equal number of male and female mosquito larvae that had emerged from the control and treated sets. These were placed in individual 30×30 cm cages for each concentration. Three days after the blood meal, eggs were collected daily from small plastic bowls containing water kept in an ovitrap in the cages. Fecundity was calculated from the number of eggs laid in ovitraps divided by the number of mated females. Death of adults in these experiments was taken into account.

Longevity test

The adult longevity of male and female mosquitoes (F1 generation) was also recorded. This was calculated as the number of days lived by the adult. Total number of days from adult emergence to death was recorded and the means were calculated to give the mean longevity in days.

Field trial

Field applications of plant extracts were made uniformly with a knapsack sprayer on the surface of the water in each habitat. Sampling of larvae was undertaken before treatment and 24, 48, 72 and 96 h after treatment by dipper sampling and counting. A separate sample was taken to determine the species composition of each larval habitat. Six trials were conducted for each area with similar temperature and alti-

tude. The required quantity of biopesticide was determined by calculating the total surface area, and the required concentration was prepared by multiplying ten times the observed laboratory LC_{50} values. Percent reduction of the larval density was calculated using the formula:

$$\text{Percent reduction} = \frac{C-T}{T} \times 100$$

where

C - total number of mosquitoes in control

T - total number of mosquitoes in treatment

Statistical analysis

The data obtained from the bioassay were subjected to statistical analysis using SPSS (Version 14.0) software (IBM Corp., Armonk, NY, USA). Lethal concentrations (LC), LC50 and LC90, DMRT (Duncan Multiple Range Test) and χ^2 tests were used.

Results

Table 1 illustrates the larval (I-IV) and pupal mortality data of the dengue vector, *Aedes aegypti*, after treatment with *Bacillus sphaericus* (Bs G3-IV) at different concentrations. The maximum mortality observed was 100% at 10 ppm concentration in I and II instar larvae. The observed mortality rate was greatly reduced in late instar larvae and pupae. Pupae showed high resistance to *Bacillus sphaericus* (Bs G3-IV), with low mortality rates of 2%, 2%, 3%, 12% and 30% at 0.001 ppm, 0.01 ppm, 0.1 ppm, 1.0 ppm and 10 ppm, respectively. Duncan's Multiple Range Test proved that the observed mortality rates were significant at $P<0.05$. LC_{50} values for I, II, III and IV instar larvae were 0.60 ppm, 0.72 ppm, 2.45 ppm and 3.76 ppm, respectively, and, for pupae, 14.08 ppm.

Percentages of larval and pupal mortality of the filarial vector, *Culex quinquefasciatus*, after treatment with *Bacillus sphaericus* (Bs G3-IV) at different concentrations (0.001 ppm, 0.01 ppm, 0.1 ppm, 1.0 ppm and 10 ppm) are shown in Table 2. Mortality values ranged from 22% to 100% for the larval stages, but were greatly reduced for the pupal stage. At the highest concentration (10 ppm), no larvae were found alive and 100% mortality was recorded. Duncan's Multiple Range Test proved that the observed mortality rates were significant at $P<0.05$. The calculated LC_{50} and LC_{90} values are 0.07 ppm and 0.56 ppm, 0.15 ppm and 0.87 ppm, 0.29 ppm and 1.14 ppm, 0.41 ppm and 1.32 ppm, and 10.84 ppm and 25.14 ppm for I, II, III, IV instar larvae and pupae, respectively.

Adult longevity and fecundity of the dengue vector, *Aedes aegypti*, after treatment with *Bacillus sphaericus* (Bs G3-IV) is shown in Table 3. A significant reduction in adult longevity and fecundity was recorded in this experiment when compared with the control. Longevity and fecundity after treatment with different concentrations of *Bacillus*

Table 1. Larvicidal and pupacidal activity of *Bacillus sphaericus* on the dengue vector, *Aedes aegypti*.

Larval and pupal stages	% Larval and pupal mortality (mean±SD) Concentration (ppm) 0.001	0.01	0.1	1	10	Standard error	LC_{50} (LC_{90}) (ppm)	95% Confidence limit LC_{50} LCL-UCL (ppm)	LC_{90} LCL-UCL (ppm)	χ^2 value
I	22±1.6^{d}	26±0.7^{cd}	38±0.4^{c}	65±1.1^{b}	100^{a}	0.16	0.60 (1.86)	0.46-0.80	1.48-2.56	4.07*
II	20±1.2^{d}	23±0.8^{cd}	34±1.5^{c}	60±0.7^{b}	100^{a}	0.16	0.72 (2.04)	0.56-0.97	1.61-2.85	3.35*
III	15±1.1^{d}	17±1.6^{cd}	26±2.1^{c}	47±1.1^{b}	98±0.5^{a}	0.04	2.45 (6.69)	0.85-12.13	3.71-37.20	15.35*
IV	12±1.6^{d}	15±0.4^{cd}	22±2.2^{c}	43±0.8^{b}	90±0.4^{a}	0.02	3.76 (9.64)	1.36-10.58	5.82-30.13	19.06*
Pupae	2±1.2^{c}	2±0.7^{c}	3±1.1^{c}	12±1.5^{b}	30±1.5^{a}	0.02	14.08 (24.52)	8.46-93.08	14.51-186.15	11.30*

Means±standard deviation (SD) followed by same letter within rows indicate no significant difference (Duncan's multiple range test, $P<0.05$). LC_{50}, LC_{90}, lethal concentration; LCL, lower confidence limits; UCL, upper confidence limits. *Significant at $P<0.001$ (heterogeneity factor used in calculation of confidence limits).

Table 2. Larvicidal and pupacidal activity of *Bacillus sphaericus* on the filarial vector *Culex quinquefasciatus*.

Larval and pupal stages	% Larval and pupal mortality (mean±SD) Concentration (ppm) 0.001	0.01	0.1	1	10	Standard error	LC_{50} (LC_{90}) (ppm)	95% Confidence limit LC_{50} LCL-UCL (ppm)	LC_{90} LCL-UCL (ppm)	χ^2 value
I	38±0.4^{cd}	43±1.1^{c}	58±0.7^{b}	99±0.4^{a}	100^{a}	0.42	0.07 (0.56)	0.02-0.13	0.43-0.79	2.00*
II	35±0.9^{cd}	39±1.6^{c}	53±2.1^{b}	93±0.5^{ab}	100^{a}	0.21	0.15 (0.87)	0.07-0.23	0.72-1.10	2.68*
III	27±1.2^{de}	32±0.7^{d}	48±1.2^{c}	85±1.2^{b}	100^{a}	0.18	0.29 (1.14)	0.08-0.54	0.79-2.13	5.49*
IV	22±0.9^{de}	28±1.1^{d}	42±2.2^{c}	79±0.9^{b}	100^{a}	0.17	0.41 (1.32)	0.19-0.72	0.92-2.48	5.48*
Pupae	11±1.5^{d}	13±0.5^{cd}	18±0.6^{c}	28±1.6^{b}	46±1.5^{a}	0.02	10.84 (25.14)	6.01-90.25	14.15-243.62	8.72*

Means±standard deviation (SD) followed by same letter within rows indicate no significant difference (Duncan's multiple range test, $P<0.05$). LC_{50}, LC_{90}, lethal concentration; LCL, lower confidence limits; UCL, upper confidence limits. *Significant at $P<0.001$ (heterogeneity factor used in calculation of confidence limits).

sphaericus (Bs G3-IV) were 26.4 days (d) in males and 37.9 d in females at 0.001 ppm concentration, 21.8 d in males and 35.7 d in females at 0.01 ppm concentration, 18.9 d in males and 31.2 d in females at 0.1 ppm concentration, 15.8 d in males and 27.3 d in females at 1.0 ppm concentration, and 11.6 d in males and 21.1 d in females at 10 ppm concentration. Fecundity was also reduced after treatment with *Bacillus sphaericus* (Bs G3-IV). A total of 178 eggs were recorded in the control, and the number of eggs recorded in the *B. sphaericus* treatments were 170, 155, 137, 116 and 82 at 0.001 ppm, 0.01 ppm, 0.1 ppm, 1.0 ppm and 10 ppm, respectively.

Adult longevity and fecundity of the filarial vector, *Culex quinquefasciatus*, after treatment with *Bacillus sphaericus* (Bs G3-IV) is shown in Table 4. Significant reduction in adult longevity and fecundity was recorded compared with the control. Longevity and fecundity recorded after treatment with different concentrations of *Bacillus sphaericus* (Bs G3-IV) were 30.9 d in males and 44.1 d in females at 0.001 ppm concentration, 25.8 d in males and 38.4 d in females at 0.01 ppm concentration, 18.2 d in males and 25.9 d in females at 0.1 ppm concentration, 11.8 d in males and 14.6 d in females at 1.0 ppm concentration, and 4.7 d in males and 7.1 d in females at 10 ppm concentration. The fecundity was also highly reduced after treatment with *Bacillus sphaericus* (Bs G3-IV). A total of 270 eggs were recorded in the control, compared with 249, 215, 178, 119 and 63 at 0.001 ppm, 0.01 ppm, 0.1 ppm, 1.0 ppm and 10 ppm, respectively, of *B. sphaericus*.

Table 5 shows the effect of *Bacillus sphaericus* (Bs G3-IV) on the dengue vector, *Aedes aegypti*, in their breeding sites. The field trial was conducted in stagnant water bodies at Vadavalli. The surface areas of the selected breeding sites were 0.6×0.7×0.5 m. A total of 481 larvae were found. After treatment with *Bacillus sphaericus* (Bs G3-IV), the percentage of reduction in larval density was 39.70%, 59.87%, 82.74% and 96.25% at 24 h, 48 h, 72 h and 96 h, respectively.

Field application of *Bacillus sphaericus* (Bs G3-IV) in the sewage water systems in Vadavalli (breeding sites of the filarial vector, *Culex quinquefasciatus*), is given in Table 6. The surface areas of the selected breeding sites were 0.4×1.7×0.28 m. Required quantity and concentration of biopesticide were calculated as 0.19 L and 2.88 ppm, respectively. A total of 788 larvae were found. After treatment with *Bacillus sphaericus* (Bs G3-IV), the percentage of reduction in larval density was 65.1%, 87.6%, 97.5% and 100% at 24 h, 48 h, 72 h and 96 h, respectively.

Discussion

Mosquitoes breed in varied habitats, such as ponds, marshes, ditches, pools, drains, water containers and other similar collections of water (Rozendaal, 1997). Mosquitoes such as *Anopheles, Culex* and *Aedes* are vectors responsible for diseases such as malaria, filariasis, Japanese encephalitis, dengue, dengue hemorrhagic fever, yellow fever and chikungunya. The increase in mosquito vectors and incidence of mosquito-borne diseases such as malaria, dengue, and chikungunya is rising in India due to climate change and water contamination. Unclean water bodies act as temporary and permanent breeding sites of mosquitoes, which tend to spread mosquito-borne diseases, along with cholera, dysentery, typhoid, etc.

Understanding the ecology of mosquitoes and the mechanism of disease management is a prerequisite to adopting any type of control. In general, the population of vector species must be of sufficient size so as to promote the transmission of vector-borne diseases. If the vector population falls below a critical density, the transmission of these diseases will not be very effective.

Effective mosquito control is often a complex, expensive task, frequently requiring the cooperative efforts of communities as well as industry, agriculture, and state and local governments. We must be concerned with the harmful effects of synthetic pesticides on the environment and living organisms, and reports have emerged on the resurgence of several mosquito-borne diseases in the world as a consequence of increasing resistance of mosquitoes to commercial insecticides (Becker *et al.,* 2003). This has necessitated the need for research and development of environmentally safe, biodegradable, indigenous methods for vector control.

We found *Bacillus sphaericus* (Bs G3-IV) to be significantly effective against *Aedes aegypti* and *Culex quinquefasciatus*. This may have been due to the presence of binary toxin (Bin) and mosquitocidal toxins (Mtxs). The Bin toxin produced by *Bacillus sphaericus* targets mosquito larval midgut epithelial cells, where it binds to Cpm1 (*Culex pipiens* maltase 1), a digestive enzyme, and causes severe intracellular damage, including dramatic cytoplasmic vacuolation (Opota *et al.,* 2011). *Culex quinquefasciatus* was much more susceptible to *Bacillus sphaericus* (Bs G3-IV), showing 100% mortality at a 10 ppm concentration against I-IV instars. Median lethal concentrations (LC50) observed were relatively low (0.07 ppm, 0.15 ppm, 0.29 ppm, 0.41 ppm, and 10.84 ppm for I, II, III, IV instar larvae and pupae, respectively) when compared with *Aedes aegypti*. Earlier, Yousten and Davidson (1982) and Davidson (1983) reported that *Bacillus sphaericus*, a spore-forming, entamopathogenic bacterium, possess potent larvicidal activity against several species of mosquito larvae. As a consequence of the specific toxicity to mosquito larvae of binary toxin (Bin) and mosquitocidal toxins (Mtxs) produced during the sporulation and vegetative stages, respectively, some toxic strains have been widely used for many years as biopesticides in the field in mosquito control programs (Bei *et al.,* 2007).

Table 3. Effect of *Bacillus sphaericus* on fecundity and longevity of dengue vector *Aedes aegypti* in the laboratory.

Treatment (ppm)	Adult longevity (Ins)		Fecundity
	Male	Female	
Control	29±0.2^{a}	39±0.5^{a}	178±0.4^{a}
0.001	26.4±0.9^{b}	37.9±0.6^{ab}	170±1.6^{ab}
0.01	21.8±0.9^{c}	35.7±1.1^{b}	155±1.1^{b}
0.1	18.9±0.6^{d}	31.2±0.6^{c}	137±0.9^{c}
1	15.8±0.2^{e}	27.3±0.9^{d}	116±0.6^{d}
10	11.6±0.9^{f}	21.1.±0.2^{e}	82±1.1^{e}

Means±standard deviation (SD) followed by same letter within rows indicate no significant difference (Duncan's multiple range test, P<0.05).

Table 4. Effect of *Bacillus sphaericus* on fecundity and longevity of the filarial vector *Culex quinquefasciatus* in the laboratory.

Treatment (ppm)	Adult longevity (Ins)		Fecundity
	Male	Female	
Control	34±0.2^{a}	47±0.2^{a}	270±0.6^{a}
0.001	30.9±1.5^{ab}	44.1±1.9^{ab}	249±2.8^{b}
0.01	25.8±1.5^{b}	38.4±1.6^{b}	215±3.2^{c}
0.1	18.2±2.1^{c}	25.9±1.1^{c}	178±1.9^{d}
1	11.8±0.9^{d}	14.6±1.5^{d}	119±1.6^{e}
10	4.7±1.0^{e}	7.1±0.6^{e}	63±0.9^{f}

Means±standard deviation (SD) followed by same letter within rows indicate no significant difference (Duncan's multiple range test, P<0.05).

In the present study, *Aedes aegypti* showed a slight reduction in mortality when compared with *Culex quinquefasciatus* after treatment with *Bacillus sphaericus* (Bs G3-IV). This may be due to its breeding habitats, characterized by high organic matter and oxygen content, which promotes the growth and development of *Bacillus sphaericus.* A previous study showed *Bacillus sphaericus* exhibited only a low level of toxicity against fourth instar larvae of *Aedes aegypti* (Mulla *et al.,* 1984), but the present isolate was more toxic when compared with those in earlier studies. Usually *B. sphaericus* is minimally toxic to *A. aegypti* because this species either lacks a specific receptor for the binary toxin of *B. sphaericus* or has an extremely low concentration of such receptors (Nielsen-Le Roux & Charles, 1992), whereas the isolate *B. sphaericus* (Bs G3-IV) used in the present study was improved with necessary qualities for targeting *A. aegypti*. Production of enhanced mosquitocidal toxin by *B. sphaericus* 2362 and *B. sphaericus* 14N1 using whey permeate (WP) under submerged fermentation conditions has resulted in high mosquitocidal activity (El-Bendary *et al.,* 2008). No significant differences have been found in other factors that could affect the activity of *B. sphaericus*, such as the rate of ingestion of the toxins or differences in proteolytic activation, between *C. quinquefasciatus* and *A. aegypti* (Aly *et al.,* 1989). The present findings also agree with those from earlier studies pointing out the high susceptibility of *Culex* spp. to *B. sphaericus* (Singer, 1980; Yousten, 1984; Mulla *et al.,* 1986). *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 (Poopathi & Abidha, 2010).

In the present study, *Bacillus sphaericus* (Bs G3-IV) reduced larval and pupal longevity and inhibited adult emergence in both species. The life span of emerged adults was also very low when mosquito larvae were treated with *Bacillus sphaericus* (Bs G3-IV), the reduction being more pronounced in *Culex quinquefasciatus* and less so in *Aedes aegypti*. *Aedes aegypti* was generally less susceptible to *Bacillus sphaericus* (Bs G3-IV), but effects on its longevity and fecundity were comparable with those seen in *Culex quinquefasciatus*. The affected pupae also resulted in a great reduction in fecundity. Adults emerging from treated larvae were morphologically normal but laid fewer eggs. The number of eggs laid by *Culex quinquefasciatus* was very low compared with *Aedes aegypti*. Murugan *et al.* (2002) reported changes in fecundity after treatment with Bti. Combined treatment of Bti with neem and pongamia showed 76% adult mortality and a reduction in fecundity (Senthil Nathan *et al.,* 2004). Poopathi & Tyagi (2002) reported a reduction in adult longevity (17% in males and 27% in females) in *Culex quinquefasciatus* after treatment with *Bacillus sphaericus* (GR strain), which supports the present findings.

This study showed that *Bacillus sphaericus* (Bs G3-IV) was also effective in a field environment. The percentage of reduction in larval density was observed every 24 h, and was seen to increase as the time after treatment increased. This supports the observation that the bacterial) pesticide was not negatively affected by the external environment and exhibits a persistent effect. This pesticide has also been found to be eco-friendly and non-toxic to non-target organisms. Mittal (2003) reported that the mosquitocidal toxins of certain strains of *Bacillus sphaericus* and *Bacillus thuringiensis* var *israelensis* H-14 (*Bti*) are highly effective against mosquito larvae in their direct breeding sites, even at very low doses, and are also safe to other non-target organisms. He also stated that the biolarvicide formulations of *B. sphaericus* are useful in the control of *Culex* and certain *Anopheles* spp., such as *An. stephensi* and *An. subpictus,* but are not very effective against *An. culicifacies*. Because *Bacillus sphaericus* distinctly affects the developmental stages of insects, it also has distinct advantages over synthetic pesticides. The specificity of the *B. sphaericus* toxin is in part due to differences in the number of target sites to which it binds (Baumann *et al.,* 1991). The binding of the protein toxin to the gastric caecum and posterior midgut has been observed in *Culex pipiens* (a susceptible species) but not in resistant *Aedes aegypti* (Mittal, 2003).

Table 5. Effect of *Bacillus sphaericus* against larval density of mosquito vectors in breeding sites (stagnant water) of *Aedes aegypti*.

Site no.	Larval density (%)				
	Before treatment	After treatment			
		24 h	48 h	72 h	96 h
1	66	39	26	9	0
2	90	56	38	16	4
3	69	40	28	12	1
4	78	45	30	14	4
5	96	59	38	16	3
6	82	51	33	16	6
Total	481	290	193	83	18
Average	80.16	48.33	32.16	13.83	3
% Reduction		39.70	59.87	82.74	96.25

Location, Vadavalli; habitat, stagnant water bodies; species, *Aedes aegypti*; stage, larvae; size, 0.6×0.7 m; depth, 0.5 m; required quantity, 0.6×0.7×0.5=0.42×0.5=0.21 L; required concentration, 2.450×10=24.50 ppm.

Table 6. Effect of *Bacillus sphaericus* against larval density of mosquito vectors at the breeding sites (sewage water) of *Culex quinquefasciatus*.

Site no.	Larval density (%)				
	Before treatment	After treatment			
		24 h	48 h	72 h	96 h
1	140	56	22	6	0
2	126	42	14	2	0
3	138	51	18	5	0
4	120	35	12	1	0
5	134	48	17	4	0
6	130	43	14	1	0
Total	788	275	97	19	0
Average	131.33	45.83	16.16	13.16	0
% Reduction	-	65.1	87.6	97.5	10

Location, Vadavalli; habitat, sewage water bodies; species, *Culex quinquefasciatus*; stage, larvae; size, 0.4×1.7 m; depth, 0.28 m; required quantity, 0.4×1.7×0.28=0.68×0.28=0.19 L; required concentration, 0.288×10=2.88 ppm.

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