EFFICACY OF BACILLUS SPHAERICUS STRAIN ISOLATED FROM NORTH EAST REGION OF INDIA AS POTENTIAL MOSQUITO LARVICIDE

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Abstract: Efficacy of Bacillus sphaericus strain GC subgroup IV (Bs) isolated from Assam, India as water dispersible powder formulation has been evaluated in laboratory and field conditions. Laboratory evaluation revealed that Bs, Spherix, ABG 6262 and Bti 164 (serotype H-14) produced 90% mortality in Cx. quinquefasciatus @ 0.5, 0.6, 0.75, and 0.7 ppm respectively. In field trial Bs dosage @ 0.1 g/m² in fresh water ditches resulted in 92.7% reduction in An. crowfordi and An. annularis larvae whereas, Spherix and ABG 6262 resulted in 82.2 and 78.67% reduction respectively at same concentration. The application of 0.2 g/m² dosage in fresh water small ponds Bs, Spherix, ABG 6262 produced 97.43, 85.57, 81.33% reduction respectively in An. vagus and Cx. vishnui larvae. In polluted drains @ 0.5 g/m² dosage Bs, Spherix, ABG 6262 resulted in 95.57, 86.93, 87.25% reduction respectively in Cx. quinquefasciatus larvae. The shelf life of Bs was found more and its larvicidal efficacy persisted up to 30 months at room temperature. The study showed that Bs is an effective biolarvicide that can be used in different types of mosquito breeding habitats against different mosquito larvae.

Key words: Bacillus sphaericus, Biolarvicide, Mosquito larvae

INTRODUCTION

Despite advances in medical science and new drugs, mosquito-borne diseases such as malaria, filaria, dengue and viral encephalitis, remain the most important diseases of human with an estimated two billion people worldwide living in areas where these are endemic [1]. Since the World War II, disease control methods have relied heavily on broad-spectrum synthetic chemical insecticides to reduce vector populations. These insecticides are being discarded in many countries due to insecticide resistance in mosquito populations. In many countries the government has restricted the use of many insecticides owing to their environmental effects on non-target beneficial fauna and flora through contamination of food and water supplies. Therefore, the need of alternate, effective and environment-friendly control strategies, which could remain effective even in the face of growing insecticide, is an urgent need.

During last decade a number of biocontrol agents were screened for their efficacy, mammalian safety and environmental impact. Many organisms like viruses, fungi, bacteria, protozoa, nematodes, invertebrate predators and fishes have been investigated as potential agents for vector mosquito control [2]. However, most of these agents were shown to be of little operational use, largely because of the difficulty in multiplying them in large quantities. Only, a few spore forming bacteria, copepods and fish have reached operational use and are undergoing extensive field trials.

Biological means to control vectors, based on entomopathogenic bacteria Bacillus thuringiensis [3] and Bacillus sphaericus [4] has been studied
for more than 20 years. The larvicidal material of these bacterial formulations is the endotoxin proteins produced by bacteria during the sporulation. These biological formulations have the advantages of high specificity resulting in low negative impact to the environment.

The present paper describes the mosquito larvicidal activity of Bacillus sphaericus GC subgroup IV strain isolated from Sonitpur district of Assam and its comparison with some commercial bio-larvicides against common mosquito larvae.

MATERIALS AND METHODS

Materials: 217 soil samples were collected from different mosquito breeding habitats of Sonitpur district of Assam. Only seven soil samples were found positive for entomopathogenic bacteria [5]. The identification of bacterial species was done in the Institute of Microbial Technology (IMTECH) Chandigarh, India. Out of the seven colonies, five were identified as Bacillus sphaericus GC subgroup IV (Bs), one each as B. brevis and B. circulans. The Bacillus sphaericus strain isolates were inoculated in nutrient yeast and salt medium (NYSM) broth and incubated at 37 °C for 48 hrs for complete sporulation. The biomass was harvested by centrifugation at 6000 rpm for 10 minutes. The supernatant was discarded and the cell pellet was lyophilized. The lyophilized biomass was thoroughly mixed with filler (chalk powder) at the ratio of 1:10 and used as water dispersible powder formulation (WDP). The same sample was used for the toxicity test. The commercial Bs (Spherix and ABG 6262) WDP formulations were provided by Malaria Research Centre, Delhi whereas, Bti formulation (strain 164 serotype H-14) was provided by Biotech International Ltd.

Laboratory trial: A stock solution of 100 ppm was prepared and desired concentrations were made from the stock solution. Bioassays were conducted in 500 ml glass beakers and required concentration of each formulation was added in 250 ml of water. 25 healthy third instar larvae of Culex quinquefasciatus were introduced in each test concentration. The mortality was scored 24 h post treatment and corrected to control mortality, if any, using Abbott’s formula [6]. Larvae exposed to water served as control. The LC₅₀ and LC₉₀ values were calculated by probit regression analysis [7]. The bioassay of Bs was also carried out against Anopheles stephensi and Aedes albopictus.

Field trial: Field evaluation of Bs, Spherix and ABG 6262 was carried out in different mosquito breeding habitats. The breeding habitats chosen for application were small ponds (area 100 m²) harboring Cx. vishnui and An. vagus, fresh water ditches with An. crowfordi and An. annularis and kutcha & cemented polluted drains harbouring high density of Cx. quinquefasciatus larvae. One breeding habitat of each type was left untreated to serve as control. The quantity of biolarvicide required for different doses in each treatment was determined by calculating the total surface area of the water in each plot. Selected breeding habitats were treated with each bacterial formulation at the dosage ranging 0.1 g/m² to 0.5 g/ m² based on the presence and absence of visible organic pollutants in the water to estimate the minimum dosage which could provide long term control of mosquito population.

The required quantity of biolarvicide was mixed thoroughly with water in a bucket with constant agitation. Field application of the formulations were done with the help of a Knapsack sprayer uniformly on the surface of the water of each plot. The density of immature mosquitoes (3rd and 4th instar) was monitored from both treated and untreated habitats before and after the treatment at regular intervals up to one week with the help of a standard larval dipper (350ml) as per standard procedure [8]. The number of dips used to sample a population varied in relation to the size of test field (5-20 dips). The mean number of larvae per dip was calculated in each treatment from the total number of larvae dipped for all replicates. The percentage reduction in the density of larvae in the treated habitats was calculated using Mullà’s formula [9] (% Reduction = 100-(C/T1) X (T2/C2) X100. Where, C₁ = Density of larvae in control during pre-treatment, C₂ = Density of larvae in control during post-treatment, T₁ = Density of larvae in treated during pre-treatment, T₂ = Density of larvae in treated during post-treatment).

Shelf life of B. sphaericus GC subgroup IV formulation: The bacterial strain was grown in NYSM broth for 48 h and biomass was harvested and lyophilized. One half of the lyophilized biomass was formulated in water dispersible powder (WDP), and dispensed in to sterilized screw capped vials which were stored at -10 °C, 4 °C and 30 °C. Other half of the lyophilized biomass was dispensed equally into
Bacterial Formulation (10% WDP) | Mosquito Sp. | LC50 (mg/l) | LC90 (mg/l) |
--- | --- | --- | --- |
*B. sphaericus* GC sub group IV (Bs) | *Cx. quinquefasciatus* | 0.2 | 0.5 |
| *An. stephensi* | 0.3 | 0.6 |
| *Ae. albopictus* | 10 | 18 |
Spherix | *Cx. quinquefasciatus* | 0.25 | 0.6 |
ABG-6262 (Vectolex) | *Cx. quinquefasciatus* | 0.3 | 0.75 |
*Bti* (Strain 164, Serotype H-14) | *Cx. quinquefasciatus* | 0.1 | 0.7 |

Table 1: Comparative laboratory evaluation of *Bs* and *Bti* formulations

### Laboratory trial:

Results of laboratory evaluation of indigenous *Bs*, Spherix, ABG 6262 and *Bti* 164 (serotype H-14) against third instar larvae of *Cx. quinquefasciatus*, showed LC50 and LC90 values as 0.2 and 0.5 ppm, 0.25 and 0.6 ppm, 0.3 and 0.75 ppm, 0.1 and 0.7 ppm respectively (Table 1). The LC50 and LC90 values of *Bs* against larvae of *An. stephensi* were recorded at 0.3 and 0.6 ppm while that of *Ae. albopictus* were 10 and 18 ppm (Table 1). No control mortality was observed during the test.

### Field trial:

#### 1. Fresh water ditches: larval density of *An. annularis* and *An. crowfordi* was monitored up to one

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### Results

Laboratory trial:

#### 1. Fresh water ditches: larval density of *An. annularis* and *An. crowfordi* was monitored up to one

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week after single application @ 0.1g/m² (Table 2).

a). B. sphaericus subgroup IV: Reduction of 86.2% in larval population was achieved on day 1 and it was increased up to 94.0% on day 3 after treatment. However, no difference was observed in larval population from 3 and 7 day after treatment i.e., 93.7 and 92.7% reduction on 5 and 7 day.

b). Spherix: A day after post treatment 70.92% reduction was observed in larval population. On day 2 and 3 mortality was recorded as 79.02 and 83.7%. However, on day 5 larval density was increased while on day 7 it was again decreased to 81.6 and 82.2%.

c). ABG 6262: On day 2, 3, 5 and 7 after treatment larval reduction was recorded as 73.07, 70.94, 78.37 and 78.67% respectively.

2. Fresh water small pond: Mix larval density of An. vagus and Cx. vishnui larvae was monitored after application @ 0.2g/m² (Table 3).

a). B. sphaericus subgroup IV: After 1-day observation 79.28% reduction in larval population was achieved and highest mortality was recorded on day 7 (97.43%).

b). Spherix: On day one, post treatment reduction of larval population was recorded as 73.91% and it was increased up to 87.47% on day 3. On day 5 and 7, larval density was reduced to 86.6 and 85.57%.

c). ABG 6262: After application of the formulation 72.21% reduction was achieved on day 1 and 80.91% on day 2. The reduction in larval densities was increases on day 3 and 5 with 87.89 and 85.78% while it is decreased to 81.33% on day 7.

3. Kutcha polluted drain: Larval density of Cx. quinquefasciatus was recorded after application of the strain @ 0.2g/m² (Table 4)

a). B. sphaericus subgroup IV: 62.02% reduction of mosquito larvae on day 1 was observed after that it increases up to 87.65% on day 7.

b). Spherix: After application larval density was reduced to 66.93% on day 1 and 81.75% on day 3. However, 79.3 and 81.04% reduction of larvae was observed on 5 and 7 day post treatment during the study.

c). ABG 6262: Larval reduction was observed as 58.07% on day 1 and 72.31% on day 2. However, reduction in density was increased gradually from day 3 onwards and achieved highest 80.77% on day 7.

4. Cemented polluted drain: Larval density of Cx. quinquefasciatus was recorded after application of the strain @ 0.5 g/m² (Table 5).

a). B. sphaericus subgroup IV: One day after application, reduction of larval population was 86.31%. Reduction of larval density gradually increased from day 2 (95.04%) and reached 98.0% on day 5. However, on 7 day post treatment larval density started increasing and forms 95.57% reduction.

b). Spherix: The larval density decreased by 73.07% on day 1 and by 83.24% on day 2 and highest larval reduction was achieved on day 7 as 86.93%.

c). ABG 6262: After application larval population reduced to 74.03% on day 1 and 81.69% on day 2. Similarly, larval mortality increased gradually on day 3 from 86.42% to 87.76% on day 5 while no significant difference was found in larval reduction on day 7.

Shelf life: Studies revealed that LC₅₀ values of the lyophilized cells and WDP of the Bacillus sphaericus subgroup IV prior to storage was 0.02 and 0.2 mg/l respectively. When stored as lyophilized cells under hermetically sealed condition, the strain lost its 50% activity after 42 months at -10 °C, 33 months at 4 °C and 18 months at 30 °C.

When lyophilized cells stored in unsealed tubes the sample lost 50% of its activity after 12 months at -10 °C, 9 months at 4 °C and 6 months at 30 °C. When stored as WDP it lost only 20% of its activity after 48 months at -10 °C, 9 months at 4 °C and 6 months at 30 °C respectively.

DISCUSSION

It is well documented that conventional and broad acting pesticides are hazardous not only to non-target organisms but also to environment. Therefore, urgent need is there for microbial biolarvicides which could be safe to environment and humans and alternate to chemical pesticides. Several larvicide formulations
of *B. sphaericus* have been produced and tested extensively against culicine and anopheline mosquito species [10-13].

The entomotoxicity test of *B. sphaericus* GC subgroup IV (*Bs*) against laboratory reared third instar larvae showed that *Cx. quinquefasciatus* larvae were highly susceptible to this strain followed by *An. stephensi* and *Ae. albopictus*. The efficacy of the *Bs* against *Cx. quinquefasciatus* larvae was found to be more when compared with that of commercial larvicides Spherix and ABG 6262 (*B. sphaericus* strains) and more or less similar to *Bti* strain 164 serotype H-14, which highlights the potentiality of this strain. Spherimos and Vectolex produced 97 and 100 % mortality in *Cx. quinquefasciatus* larvae at a dose as low as 0.008 ml/m², while 1ml/m² was required for *An. stephensi* [14].

The susceptibility of Spherix against wild and mutant strains of *An. stephensi* was evaluated and LC_{50} and LC_{90} values varied from 0.088 to 1.42 mg/l and 0.314 to 10.98 mg/l respectively [15] which proved the potentiality of our strain against *An. stephensi*. The poor susceptibility of our *Bs* strain was found against the *Aedes* sp. In contrast more efficacy of *Bs* strains was reported in *Aedes* and *Culex* than *Anopheles* larvae [16,17] but high sensitivity was reported against *An. gambiense* [18] and poor sensitivity against *Aedes* sp. [19]. Two strains of *B. sphaericus* S2 and 2362 isolated from soil samples of Brazil were found highly toxic against *Cx. quinquefasciatus* followed by *An. albimanus* & *An. quadrimaculatus* and low toxic against *Ae. aegypti* [20]. The differential susceptibility among different mosquito species may be due to several factors such as gut pH [21], feeding behaviour, larval intrinsic factors [22] and presence of specific toxin binding sites [20,23].

In the present study effective control was achieved with *Bs* in the field conditions up to one week at the dosage of 0.1g/m² in fresh water ditches which resulted in 92.7% reduction in *An. crowfordi* and *An. annularis* breeding. On the other hand with ABG 6262 and Spherix 78.67 and 82.2 % reduction was recorded. In the small fresh water pond with our *B. sphaericus* strain (0.2 g/m²), the larval density of *An. vagus* and *Cx. quinquefasciatus* were reduced significantly 89.7-97.4% from day 2 to 7 whereas, 85.6 & 81.3% reduction was achieved with Spherix and ABG 6262 in the same habitat. This indicates that in both the habitats *Bs* is much more effective than other two commercial formulations.

The polluted water of kutcha drains treated with *Bs* formulation of our strain at 0.2 g/m² showed less effectiveness as compare to other habitats. Furthermore, when the dose was increased to 0.5 g/m², 95.6% reduction was observed in *Cx. quinquefasciatus* larvae. However, there was no significant reduction observed in other two strains with increased dose in the same habitat. This indicates that in polluted breeding habitats efficacy of *B. sphaericus* formulation decline considerably and require more than double dose in comparison to fresh water breeding habitats.

Single application of Spherimos (2ml/m²) resulted in 100 % reduction in *Cx. quinquefasciatus* larvae for 1 week in pools and 99 % reduction for 3 weeks in the wells [14]. The activity was enhanced for three weeks in pools when the dose was increased to 10 ml/m². These findings support our observation that in polluted breeding sites more dosage is required than in fresh water sites. The difference in efficacy can be due to the presence of pollutants in water which may reduce the pathogenicity of bacterial strains. In *Cx. quinquefasciatus* larvae 50-66 % reduction was observed in cesspools, unlined and cement lined drains and 28-31 % reduction in disused wells, cement tanks and cesspits when treated bimonthly with Spherifix at the dose of 15kg ai/ha [24]. The reduced activity in these habitats has been attributed to high degree of pollution and rapid setting of the active ingredient to the bottom mud. Variability in larvicidal activity of *B. sphaericus* mainly depends upon the breeding habitats, mosquito species and general ecology of the area [25].

In the present study 97% reduction in both anopheline and culicine larvae was recorded within 7 days of application with *B. sphaericus* formulation @ 0.2g/m² whereas, the same dosage of Spherix and ABG 6262 resulted in 86% and 81% reduction in anopheline and culicine larvae within 7 days. The finding indicates that same dose of *B. sphaericus* strains are equally effective for both the mosquito larvae. 95-100% reduction in the larval density of *An. stephensi* and *Cx. quinquefasciatus* within 48 hr of spraying Spherix @ 0.25-2 g/m² in different habitats and larvicidal activity persisted for 2-4 weeks [26]. In rural areas of Farrukhabad district, Uttar Pradesh more than 95% mortality was observed against both anopheline and culicine larvae within 48 hr of spray with Spherix @ 2 g/m² [27].
The studies on the shelf life are of paramount importance to sustain the efficacy of any product during storage. *B. sphaericus* (WDP) [28] lost 20, 24 and 40% efficacy after 10 weeks when stored at -40, 8 and 30°C and after 20 weeks 72, 86 and 100% efficacy was lost. Storage efficacy of our WDP formulation was found more as it lost only 20% activity after 48 months at -10°C, 40% activity at 4°C and 30°C after 33 and 30 months. When lyophilized powder of *B. sphaericus* [29] stored at 5°C retained complete activity after 10 weeks and lost 75% activity after 2 years. In another study two standard formulation of *B. sphaericus* (RB 80 and SPH 84), lost considerable efficacy within 5 weeks at 50°C [30]. In the present investigation storage efficacy of indigenous strain was found more than the other reported strains and considerable level of activity (70%) persists beyond 30 months at room temperature.

**CONCLUSION**

The *B. sphaericus* GC subgroup IV produced significant reduction of both culicine and anopheline mosquito larvae in different breeding habitats. When compared *B. sphaericus* GC subgroup IV formulations found to be more entomopathogenic than Spherix and ABG 6262. Furthermore, the minimum effective dosage to kill 100% larval population in different habitats was found to be extremely low. The present strain may therefore have a great potential for inclusion in integrated vector management operations.

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**REFERENCES**