# EVALUATION OF A MICROGEL DROPLET FORMULATION OF *BACILLUS SPHAERICUS* 1593 M (BIOCIDE-S) FOR CONTROL OF MOSQUITO LARVAE IN RICE FIELDS IN SOUTHERN INDIA

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ABSTRACT. A new microgel droplet formulation of *Bacillus sphaericus* 1593 M (Biocide-S) was tested at 2 rates against culicine vectors of Japanese encephalitis and *Anopheles subpictus*. A single application just after transplantation of rice seedlings prevented the buildup of anopheline as well as culicine populations, and gave 83–100% reduction of pupal density at the lower rate (2.2 kg/ha) and 87–100% reduction at the higher dosage (4.3 kg/ha) for at least 5 weeks. During the last 3 wk before harvest, density was naturally very low and the efficacy of the treatment could not be assessed. This formulation has a potential for control of riceland mosquitoes, but a suitable delivery system is required before it can be considered for practical control.

## INTRODUCTION

There have been relatively few attempts to evaluate the bacterial agent *Bacillus sphaericus* against mosquito larvae in rice fields. Kramer (1984), working in India, obtained 94–99% mortality of *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi at rates of 1.5–2.0 kg/ha, but suppression of breeding was limited to 3–4 days. Lacey et al. (1986, 1988) obtained up to 82% suppression of *An. quadrimaculatus* Say by aerial spraying, while a granular formulation penetrated dense stands of rice satisfactorily. However, the level of control was considered inferior to that obtained by the use of *Bacillus thuringiensis* H-14 in the same habitat (Lacey and Lacey 1990). Control of *Psorophora* sp. in ricefields was as good or better than that obtained with *B. thuringiensis* H-14 (Lacey and Lacey 1990). As pointed out by these authors, there remains considerable scope for improvement of efficiency and spectrum of activity by improved formulation and delivery systems. A single application of a new formulation of *B. sphaericus* almost completely eliminated pupae of culicine and anopheline mosquitoes for the whole duration of the rice crop, as reported in this paper.

## MATERIALS AND METHODS

A microgel droplet formulation of *B. sphaericus* 1593 M (Biocide-S) was received from Professor Kunthala Jayaraman, Anna University, Madras, India. The formulation consisted of a highly viscous water soluble bio-polymer in which bacilli were entrapped. The average particle size ± SD was 8.6 ± 4.8 micrometers. The formulation was stable and had been kept for up to 6 months at room temperature without losing potency. Prior to application it was appropriately diluted with water. There were no particular problems in handling, but workers were advised to handle it with the care usually employed when spraying any pesticide.

The study was carried out close to Madurai, in Tamil Nadu, during the first crop season, beginning in July 1990. Treatment was carried out immediately after transplantation of rice seedlings, using a knapsack power sprayer. Three 440 $m^2$ plots were treated at each of 2 rates, 2.2 kg/ha and 4.3 kg/ha (20 mg/$ft^2$ and 40 mg/$ft^2$), and there were 3 similar sized control plots. All the plots had strong separating bunds and separate inlets and outlets for irrigation water. Normal agricultural practices were followed.

Measurement of immature abundance was made by taking 10 dipper samples per plot. Observations were carried out 24 h after treatment, and 3 times a week thereafter until harvest. Larval instar and genus were recorded in the field, and the immatures were returned to the plot from which they were collected. Pupae were, however, taken to the laboratory for identification of emerging adults. Nontarget organisms were also identified, counted and recorded.

The percent reduction was calculated using the formula $(1 - n/N) \times 100$, where n = mean no. of immatures in treated plots and N = mean no. of immatures in control plots during the same period. The formula of Mulla et al. (1971) could not be used to correct for pretreatment abundance levels because treatment was carried out immediately after transplantation.

## RESULTS

The culicine species identified from rice fields in this study were *Culex vishnui* Theobald

(79%), *Cx. tritaeniorhynchus* Giles, *Cx. pseudovishnui* Colless and *Cx.* (*Lutzia*) *fuscanus* Wied. (21%). The first 3 of these species are recognized vectors of Japanese encephalitis in southern India. The anopheline species identified were *An. subpictus* (99%) and *An. vagus* Doenitz (1%).

In the Madurai area, intense breeding of both anopheline and culicine species takes place during the first 2–3 wk after transplantation, after which there is a natural decline (Rajendran and Reuben 1991). This pattern was repeated in the control plots with a clear peak in culicine breeding during the 2nd week. No immatures could be collected during the 6th wk, but there was a low level of breeding in weeks 7 and 8, just before harvesting. During the first 5 wk after transplantation, 54–69% reduction of early instar and 68–100% reduction of late instar larvae were obtained at 2.2 kg/ha, while the higher rate gave 44–79% and 82–100% reduction, respectively (Table 1). No pupae were collected in any of the treated plots during the first 3 weeks. Small numbers were collected during weeks 4 and 5 in plots treated at the lower rate, and in week 5 only at the higher rate. The data were subjected, after log transformation, to Factorial Randomized Block Design (FRBD) analysis. Reduction of all instars was significant during the first 5 wk, after which abundance was so low in all plots that significance could not be demonstrated. Overall, the higher rate gave significantly better control of early as well as late instar larvae, but there was no significant difference in numbers of pupae between plots treated at different rates, although both were significantly lower than the control.

Anopheline abundance in the control plots also followed the normal pattern, with peak larval and pupal production in the 2nd week. Reduction in abundance of early instar larvae ranged from 40 to 100% at the lower rate and between 18 and 100% at the higher rate. Except during the 2nd wk the differences were significant (Table 2). At the higher rate reduction of late instars was 73–100% and 79–100% at the lower rate. The differences were significant except during the 8th week. Pupal reduction ranged from 94 to 100% during the first 5 wk, and the differences were significant. Overall both rates were at par and gave significant reduction over control.

Numbers of nontarget organisms captured in treated plots did not differ significantly from those in control plots (Table 3), except for chi-

Table 1. Bioefficacy of *Bacillus sphaericus* 1593 M against culicine species in rice fields in southern India. Values presented are mean number/10 dips (% reduction).

| Weeks after treatment | Control | | | 2.2 kg/ha | | | 4.3 kg/ha | | |
|---|---|---|---|---|---|---|---|---|---|
| | I/II | III/IV | Pupae | I/II | III/IV | Pupae | I/II | III/IV | Pupae |
| 1 | 57.9 | 60.4 | 2.1 | 26.5* (54.2) | 0.0* (100.0) | 0.0* (100.0) | 15.5* (73.2) | 0.0* (100.0) | 0.0* (100.0) |
| 2 | 109.6 | 70.8 | 16.2 | 44.6* (59.3) | 2.3* (96.7) | 0.0* (100.0) | 29.8* (72.8) | 1.1** (98.7) | 0.0* (100.0) |
| 3 | 73.7 | 21.7 | 0.0 | 23.0* (68.8) | 6.2* (71.5) | 0.0* (100.0) | 15.2* (79.4) | 0.8** (96.2) | 0.0* (100.0) |
| 4 | 69.2 | 37.3 | 3.2 | 44.0* (63.6) | 11.9* (68.2) | 0.6* (82.9) | 27.2* (60.7) | 3.4** (90.8) | 0.0** (100.0) |
| 5 | 37.6 | 18.8 | 1.3 | 13.7* (63.6) | 0.0* (100.0) | 0.2* (88.0) | 16.5* (43.9) | 3.3** (82.3) | 0.2* (87.2) |
| 6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | 5.6 | 2.2 | 0.0 | 7.3 (−23.3) | 1.0 (55.4) | 0.0 — | 1.5 (73.3) | 0.0 (100.0) | 0.0 — |
| 8 | 16.5 | 3.2 | 0.0 | 10.1 (38.8) | 2.3 (26.3) | 0.0 — | 7.9 (52.1) | 2.6 (19.0) | 0.0 — |

* Significantly different ($P < 0.05$) from same instar in control in the same week.
** Significantly different from both control and lower rate in the same week.

FRBDA analysis between treatments.

| Instar | Av. log(n + 1) | | |
|---|---|---|---|
| | Control | 2.2 kg/ha | 4.3 kg/ha |
| I/II | 1.74a | 1.46b | 1. 30c |
| III/IV | 1.42a | 0.62b | 0.41c |
| Pupae | 0.52a | 0.06b | 0.01b |

CD value for treatments vs. instars = 0.1. Values followed by different letters are significantly different.

Table 2. Bioefficacy of *Bacillus sphaericus* 1593 M against anopheline species in rice fields in southern India. Values presented are mean number/10 dips (% reduction).

| Weeks after treatment | Control | | | 2.2 kg/ha | | | 4.3 kg/ha | | |
|---|---|---|---|---|---|---|---|---|---|
| | I/II | III/IV | Pupae | I/II | III/IV | Pupae | I/II | III/IV | Pupae |
| 1 | 160.1 | 163.3 | 7.0 | 86.2* (46.2) | 1.5* (99.1) | 0.0* (100.0) | 44.1* (72.5) | 0.2** (99.9) | 0.0* (100.0) |
| 2 | 244.0 | 156.3 | 25.8 | 145.9 (40.2) | 32.3* (79.3) | 0.1* (99.6) | 200.5 (18.0) | 16.8* (89.3) | 0.0* (100.0) |
| 3 | 129.7 | 52.2 | 2.5 | 31.3* (75.9) | 7.2* (86.3) | 0.0* (100.0) | 40.0* (69.2) | 7.8* (85.0) | 0.0* (100.0) |
| 4 | 210.3 | 103.7 | 7.2 | 50.1* (76.2) | 13.9* (86.6) | 0.4* (93.9) | 31.1* (85.2) | 8.0* (92.3) | 0.0* (100.0) |
| 5 | 141.8 | 58.8 | 8.3 | 10.7* (92.5) | 3.7* (93.8) | 0.0* (100.0) | 14.3* (89.9) | 8.3* (85.8) | 0.3* (96.0) |
| 6 | 31.3 | 0.3 | 0.0 | 0.0* (100.0) | 0.0* (100.0) | 0.0 — | 0.0* (100.0) | 0.0* (100.0) | 0.0 — |
| 7 | 25.2 | 1.2 | 0.0 | 4.2* (83.5) | 0.2* (83.3) | 0.0 — | 0.8** (96.7) | 0.3* (73.0) | 0.0 — |
| 8 | 16.3 | 6.0 | 0.0 | 2.2* (86.4) | 0.2 (96.3) | 0.0 — | 3.1* (81.0) | 0.3 (94.5) | 0.0 — |

* Significantly different ($P < 0.05$) from same instar in control in same week.
** Significantly different from both control and lower rate in the same week.

FRBD analysis between treatments.

| Instar | Av. log(n + 1) Control | 2.2 kg/ha | 4.3 kg/ha |
|---|---|---|---|
| I/II | 2.08a | 1.47b | 1.40b |
| III/IV | 1.64a | 0.81b | 0.72b |
| Pupae | 0.84a | 0.05b | 0.01b |

CD value for treatments vs. instars = 0.11. Values followed by the same letter are not significantly different.

Table 3. Total number of nontarget organisms captured in the experimental rice fields.

| Nontarget organisms | Total number recorded Control | 2.2 kg/ha | 4.3 kg/ha |
|---|---|---|---|
| *Corixa* sp. | 208 | 227 (0.56) | 213 (0.12) |
| *Notonecta* sp. | 58 | 58 (0.00) | 56 (−0.09) |
| *Chironomus* larvae | 253 | 103 (−4.87)* | 236 (−0.39) |
| Ephemeropteran naiads | 181 | 230 (0.83) | 231 (1.16) |
| Zygopteran naiads | 3 | 7 (0.83) | 7 (0.88) |

Figures in parenthesis are values of *t*.
* Significant at $P = 0.01$, df 7.

ronomid larvae, which were significantly fewer in plots treated at 2.2 kg/ha than in the control plots. This is not, however, likely to be due to the treatment, because there was no reduction in plots treated at 4.3 kg/ha.

Water temperatures recorded during the study ranged from 25.9 to 32.5°C, and pH from 6.9 to 8.9.

## DISCUSSION

These trials have shown that a single treatment with the microgel droplet formulation of Biocide-S significantly reduces pupation of culicines as well as anophelines for at least 5 wk after transplantation in rice fields. An earlier liquid suspension concentrate of the same bacterial agent was effective against culicines in ricefields but had no impact on anopheline density (CRME, unpublished data). The microgel formulation disperses in the water, but a substantial portion remains at the surface, thus making bacterial toxin available in the feeding zones of both anopheline and culicine larvae. Floating formulations of *B. thuringiensis* H-14 have been shown to have enhanced efficiency against anophelines, which feed most actively at the air-water interface (Cheung and Hammock 1985, Aly et al. 1987).

Biocide-S therefore shows considerable promise for control of ricefield breeding vectors of Japanese encephalitis and may also be effective against malaria vectors breeding in this habitat. It has not been possible to test its efficacy against *Anopheles culicifacies* Giles since this species has been absent (Chandrahas and Rajagopalan 1979, Kramer 1984) or present at low densities (Chandrahas et al. 1979, Tewari et al. 1984, Rajendran and Reuben 1991) in rice fields in southern India in recent years. However, trials in casuarina pits in coastal Tamil Nadu have shown that Biocide-S is effective against the species in that habitat (CRME, unpublished data).

Before this agent can be considered for practical control in the vast rice-growing tracts in developing countries, cost will have to be taken into account. It took a man 2.5 h to spray 0.5 ha; a less labor intensive delivery system must be developed, perhaps by multiple point source introduction into irrigation water entering fields (McLaughlin and Vidrine 1984).

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