# DEVELOPMENT OF ALGINATE-BASED SLOW RELEASE FORMULATION OF *BACILLUS SPHAERICUS* FOR CONTROLLING *CULEX QUINQUEFASCIATUS*

K Gunasekaran, V Vijayan, AN Shriram, S Subramanian, and K Balaraman

Vector Control Research Centre, Indira Nagar, Pondicherry-605 006, India

**Abstract.** Seven types of formulations were prepared as granules using the larvicidal factor of *Bacillus sphaericus* and different concentrations of calcium alginate which was used as matrix to immobilize and entrap the active ingredient (ai). All formulations were tested in disused wells against *Culex quinquefasciatus* at the rate of 15 kg ai per hectare. Among the seven types tested, the type 2 which contained 5% calcium alginate as immobilizing agent, exhibited the maximum larvicidal activity. Persistent control in breeding was noticed for 8 weeks with this formulation type.

## INTRODUCTION

Several formulations of *Bacillus sphaericus* Neide have been found effective in eliminating mosquito larval populations in different breeding sites within 24 hours of application (Davidson *et al*, 1981; Mulla *et al*, 1986; Karch *et al*, 1990). However, vast differences have been noticed regarding residual activity of such formulations. Several reasons are attributed for the differences including continuous availability of the active ingredient in the feeding zone (Aly, 1983), feeding behavior of larvae (Aly *et al*, 1987) and pollution level of the larval habitat (Mian and Mulla, 1983). Therefore, choice of appropriate formulations for larval control in specific habitats depends on the above factors. In the present study, efficacy of seven types of alginate-based slow-release floating formulations of *Bacillus sphaericus* was evaluated for the control of *Culex quinquefasciatus* Say 1821 larvae and the results are presented in this communication.

## MATERIALS AND METHODS

The freeze-dried whole cellmass (spore-toxin complex) of a strain of *Bacillus sphaericus* serotype H5a5b (culture number VCRC B42) obtained from the stock of Vector Control Research Centre (VCRC) was the larvicidal factor/active ingredient (ai) used in this study. The ai had a Lc50 value of 10 microgram to third instar larvae of *Cx. quinquefasciatus* (obtained from the cycling colony being maintained at VCRC) placed in 250 ml of water. Calcium alginate (d-Mannuronic and l-guluronic acid joined by 1, 4 linkages), obtained from Robert Johnson, India was used as matrix to immobilize and entrap the active ingredient. Seven types of formulations were prepared in the form of granules using the active ingredient and different concentrations of calcium alginate. The seven types, with percentage of calcium alginate in parenthesis, are Type-1 (4%), Type-2 (5%), Type-3 (6%), Type-4 (8%), Type-5 (10%), Type-6 (12%), Type-7 (16%). The alginate granules (entrapping the ai) were dried under shade and packed in a floating aid (plastic vials with slits) (Fig 1) at the rate of 1 g/float.

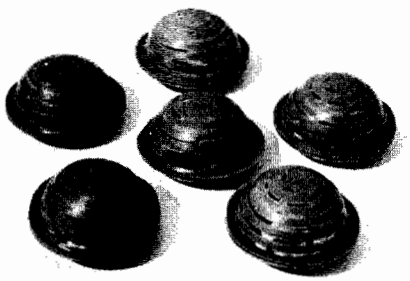

Fig 1–Plastic floats with slits, containing alginate-based slow release formulation of *B. sphaericus*. These floats were applied to the habitats at the required dose. The formulation inside the floats got soaked and released slowly through the slits.

Correspondence: K Gunasekaran, Tel: 91-0413-72784, 72397, 72422; Fax: 91-413-72041, 72422

The study was carried out in Porayar, a small town in Nagai Quaid-E-Milleth district of Tamil Nadu (India). All seven types of formulations were tested in disused wells (10 replicates for each type), one of the major breeding habitats of *Cx. quinquefasciatus* in this area, at the rate of 15 kg active ingredient per hectare. Ten wells were kept untreated for comparison. Breeding status of *Cx. quinquefasciatus* before (day '0') and after the application (day 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, 56 and 63) of the formulations was monitored by taking immature samples using an iron bucket (2,000 ml).

Percentage reduction in immature density (number/one bucket sample) in the treated wells was estimated using Mulla's formula (Mulla *et al*, 1971). These percentage values were transformed to arcsine values to normalize the data and analysis of variance (ANOVA) was performed to assess the efficacy of different types of the formulations by taking the percentage reduction (arcsine values) as dependent variable and formulation types (7) and days (12) as factors. Comparison of means of percentage reduction obtained with different formulation types was done using Student-Newman Keuls (SNK) test (Sokal and Rohlf, 1969).

## RESULTS

Density of immatures of *Cx. quinquefasciatus* recorded in the wells prior to the application of the formulations (day zero) is given in Table 1. Mean density of early and late instars and pupae varied from 233.76 ± 145.76 to 741.37 ± 369.01, 179.85 ± 141.31 to 571.12 ± 436.01 and 38.9 ± 49.3 to 180.29 ± 258.28 respectively on day zero.

After treatment, a reduction of 41.9%-87.9%, 61.8%-92.7%, 54.7%-79.1%, 47.5%-72.2%, 38.2%-85.7%, 50.8%-92.8% and 29.7%-84.4% was observed in the density of early instars with the formulation type 1, 2, 3, 4, 5, 6 and 7 respectively during the study period of 63 days. Initial reduction was higher with type 2 and 6. However, persistent control was achieved only with the type-2. The highest reduction in early instar density with these two formulation types was noticed on day 14 posttreatment. The type-1 and 7 caused the highest reduction on day 21 and 28 respectively. The other types exhibited delayed effect and the highest reduction was noticed only after day 49 posttreatment (Fig 2).

Percentage reduction in the density of late instar larvae was 53-94.9, 73.7-99.6, 56.6-90.7, 59.4-88.9, 33-97.4, 40-98.8 and 48-89.5 with the formulation type-1, 2, 3, 4, 5, 6 and 7, respectively (Fig 3). The corresponding values for pupal density were 55.6-90.9, 59.8-100, 62.4-99.8, 58.2-97.6, 48-97.2, 60.8-96.4 and 33-92.1 (Fig 4). Highest reduction in the density of late instar larvae and pupae was noticed on 14th and 35th day respectively with the formulation type 1, 7th and 21st day with type 2, 28th day (late instar as well as pupae) with type 3 and 5, 7th and 35th day with type 4, 21st and and 42nd day with type 6 and 35th and 49th day with

Table 1

Mean immature density of *Cx. quinquefasciatus* in habitats prior to treatment (day '0') with different types of *B. sphaericus* formulations.

| Formulation type | Early instars | | Late instars | | Pupae | |
|---|---|---|---|---|---|---|
| | Density | SD | Density | SD | Density | SD |
| 1 | 302.59 | 228.44 | 179.85 | 141.31 | 38.90 | 49.30 |
| 2 | 741.37 | 369.01 | 571.12 | 436.01 | 128.85 | 209.41 |
| 3 | 233.76 | 145.76 | 229.12 | 306.58 | 48.31 | 68.86 |
| 4 | 300.57 | 210.47 | 386.10 | 298.95 | 73.81 | 95.85 |
| 5 | 299.67 | 153.59 | 204.52 | 221.43 | 39.82 | 45.51 |
| 6 | 508.29 | 587.69 | 237.39 | 389.17 | 42.15 | 91.87 |
| 7 | 460.43 | 717.45 | 340.80 | 261.10 | 83.12 | 117.37 |
| Control | 461.65 | 393.25 | 376.01 | 382.00 | 180.29 | 258.28 |

SD - Standard deviation

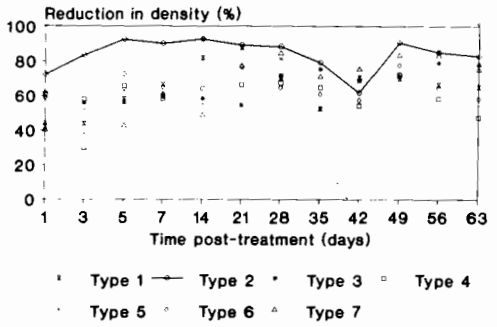


Fig 2–Reduction in density of early instars (stages I, II) after the treatment with different types of *B. sphaericus* formulations.

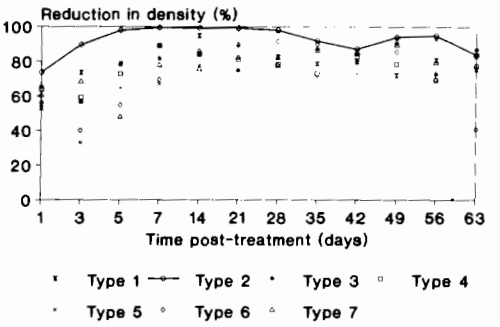


Fig 3–Reduction in density of late instars (stages III, IV) after the treatment with different types of *B. sphaericus* formulations.

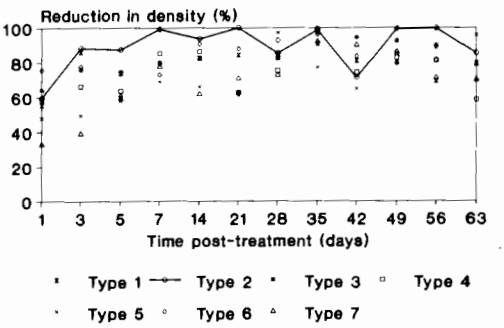


Fig 4–Reduction in density of pupae after the treatment with different types of *B. sphaericus* formulations.

type 7 (Figs 3, 4).

ANOVA test indicated that the larvicidal efficacy (in terms of percentage reduction in immature density) of different formulation types differed significantly (early instar density: $F = 4.04$, $df = 6$, $p = 0.001$; late instar density: $F = 4.65$, $df = 6$, $p < 0.01$; pupal density: $F = 4.48$, $df = 6$, $p < 0.01$). Their effect on different stages of immatures on different days post-treatment also differed significantly (early instar density: $F = 3.78$, $df = 11$, $p < 0.01$; late instar density: $F = 7.80$, $df = 11$, $p < 0.01$; pupal density: $F = 5.46$, $df = 11$, $p < 0.01$). However, the interaction effect between the formulation types and post-treatment days did not differ significantly (early instar density: $F = 0.85$, $df = 66$, $p = 0.793$; late instar density: $F = 0.98$, $df = 66$, $p = 0.531$; pupal density: $F = 0.80$, $df = 66$, $p = 0.867$). The SNK test confirmed that the reduction in early and late instar density obtained with the formulation type-2 was significantly higher than that observed with the other types. In the case of pupae, though reduction in density was relatively higher with the formulation type-2, the reduction was on par with that noticed with the type-3, 6 and 1 (Table 2).

## DISCUSSION

Efficacy of a formulation depends upon the availability of adequate concentration of larvicidal factor in larval feeding zone. In the case of slow release formulations, quality and strength of immobilizing agent play a vital role in deciding the efficacy. In the present study, the seven types of slow release formulations, prepared with larvicidal factor of *B. sphaericus*, differed only with respect to concentration of the immobilizing agent (calcium alginate). The efficacy of these formulation types was compared for (i) initial reduction (ii) the post-treatment day on which maximum larvicidal activity was noticed, (iii) residual activity and (iv) overall reduction in immature density.

Reduction in both late instar and pupal density was the highest with the formulation type-2 indicating that the immobilizing agent, calcium alginate, when used at 5%, released the required quantity of larvicidal factor from the beginning leading to higher reduction in immature density. With the increase in the strength of calcium alginate the

Table 2

Mean percentage reduction in immature density of *Cx. quinquefasciatus* in habitats treated with different types of *B. sphaericus* formulation.

| Early instars[a] | | Late instars[b] | | Pupae | |
|---|---|---|---|---|---|
| Formulation type | Reduction in density (%)[c] | Formulation type | Reduction in density (%)[c] | Formulation type | Reduction in density (%)[c] |
| 2 | 84.04 | 2 | 92.50 | 2 | 89.13 |
| 6 | 66.72 | 1 | 78.95 | 3 | 81.40 |
| 3 | 65.97 | 3 | 78.80 | 6 | 80.83 |
| 1 | 64.55 | 7 | 76.25 | 1 | 78.96 |
| 5 | 64.45 | 4 | 75.85 | 4 | 74.32 |
| 7 | 64.35 | 5 | 74.86 | 5 | 71.71 |
| 4 | 61.41 | 6 | 71.94 | 7 | 68.69 |

[a] Larval stages I and II
[b] Larval stages III and IV
[c] Similar means are indicated by continuous line on the left side of the values (SNK test)

amount of larvicidal factor released had decreased and thereby a reduction in the larvicidal activity.

The type-2 caused not only a higher level of initial reduction within 3-5 days after application but also persistent control upto 63 days. The activity of type 4 was inconsistent. In the case of types 3 and 5, and of 1, 6 and 7, the efficacy was low up to day 27 and 20 post-treatment respectively.

Moreover, a 100% reduction in pupal density was obtained by 3rd week after treatment with the formulation type-2, whereas with the other types, the maximum reduction (ranging from 91% to 99.8%) was noticed only on day 28-35 post-treatment. These observations indicate that larvicidal activity of the formulation type-2 was faster and consistent enough to cause 100% reduction in recruitment to pupal stage by 3rd week itself.

Overall, reduction in early and late instar larval density obtained with the formulation type-2 was the highest. Regarding reduction in pupal density, the efficacy of the type-2 was on par with that of types 3, 6 and 1. Though, the types 2, 3, 6 and 1 had similar efficacy, when rapidity and consistency in larvicidal action were considered, the formulation type 2, which contains 5% alginate as immobilizing agent, could be the best choice for controlling *Cx. quinquefasciatus* in disused wells consistently for a period of 8 weeks.

A granular preparation (at 2.8-22.4 kg/ha) and a flowable concentrate formulation (FC) (at 2.24-5.6 kg/ha) were reported to produce more than 80% control in breeding for 14-21 days (Mulla *et al*, 1988). A significant reduction in larval population was obtained for 14 days with a liquid formulation (Vectolex) at 4 l/ha (Karch *et al*, 1990). Hougard (1990) used a FC formulation at 10 kg/ha and reported residual effect for 5-6 weeks and Yebakima (1991) used Spherimos, another FC preparation and observed control for 28 days. Vectolex G (granules) was reported to control breeding for 11-14 days at 4.64-5.00 lb/ac (Sutherland *et al*, 1989; Sutherland and McNelly, 1990). A briquette formulation was found to keep larval populations of *Culex* spp at significantly low levels for 13-17 days (Schmidt, 1990). Effective residual activity was reported upto 67 days with spherimos (FC) at 15 l/ha and 56 days with vectolex (granular) at 30 l/ha against *Cx. quinquefasciatus* in polluted disused wells (Arunachalam *et al*, 1991). Though, direct comparison was not possible as these formulations were of different nature and the doses used were not uniform, the alginate-based formulation type-2 tested in the present study was more effective with reference to initial as well as persistent control in breeding and its efficacy was comparable to that of

spherimos (Arunachalam *et al*, 1991).

## ACKNOWLEDGEMENTS

The authors are very grateful to the technical staff, VCRC Field Station, Mayiladuturai for their help.

## REFERENCES

Aly C. Feeding behaviour of *Aedes vexans* larvae (Diptera: Culicidae) and its influence on the effectiveness of *Bacillus thuringiensis* var *israelensis*. *Bull Soc Vector Ecol* 1983; 8 : 94-100.

Aly C, Mulla MS, Schneter E, Xu BZ. Floating bait formulations increase effectiveness of *Bacillus thuringiensis* var *israelensis* against *Anopheles* larvae. *J Am Mosq Control Assoc* 1987; 3 : 583-8.

Arunachalam N, Reddy CMR, Hoti SL, Kuppusamy M, Balaraman K. Evaluation of *Bacillus sphaericus* formulations against the vector of bancroftian filariasis. *Southeast Asian J Trop Med Public Health* 1991; 22 : 160-164.

Davidson EW, Sweeny AW, Cooper R. Comparative field trial of *Bacillus sphaericus* strain 1593 and *Bacillus thuringiensis* var. *israelensis* commercial powders. *J Econ Entomol* 1981; 74 : 350-4.

Hougard JM. Formulations and persistence of *Bacillus sphaericus* in *Culex quinquefasciatus* larval sites in tropical Africa. In: de Barjac H, Gutherland DJ, eds Bacteriological control of Mosquitoes and Black flies: Biochemistry, genetics and applications of *Bacillus thuringiensis israelensis* and *Bacillus sphaericus*. New Brunswick, NJ: Rutgers Univ Press 1990; 295-306.

Karch S, Monteny N, Jullien JL, Sinegre G, Coz J. *Bacillus sphaericus*, a biological agent against mosquitoes and its effects on natural aquatic medium. *J Am Mosq Control Assoc* 1990; 6 : 47-54.

Mian LS, Mulla MS. Factors influencing activity of microbial agent *Bacillus sphaericus* against mosquito larvae. *Bull Soc Vector Ecol* 1983; 8 : 128-34.

Mulla MS, Norland RL, Fanara DM, Darwazeh HA, McKean DW. Control of chironomid midges in recreational lakes. *J Econ Entomol* 1971; 264 : 300-7.

Mulla MS, Darwazeh HA, Aly C. Laboratory and field studies on new formulations of two microbial agents against mosquitoes. *J Am Mosq Control Assoc* 1986; 11 : 255-63.

Mulla MS, Axelrod H, Darwazeh HA, Matanmi BA. Efficacy and longevity of *Bacillus sphaericus* 2362 formulations for control of mosquito larvae in dairy waste water lagoons. *J Am Mosq Control Assoc* 1988; 4 : 448-52.

Schmidt RF. Field tests of a dry formulation of *Bacillus sphaericus*. *Proc/Ann Meet New Jersey Mosq Control Assoc* 1990; 77 : 121-3.

Sokal RR, Rohlf FJ. *Biometry*. WH Freeman and company, San Francisco 1969; 776 pp.

Sutherland DD, McNelly JJ, Hansen JA. Evaluation of granular *Bacillus sphaericus* to control *Culex* in sewage treatment ponds in cape May. *Proc Ann Meet, New Jersey Mosq Cont Assoc* 1989; 76 : 84-90.

Sutherland DD, McNelly JJ. Evaluation of granular aerially applied *Bacillus sphaericus* in 1989. *Proc Ann Meet New Jersey Mosq Cont Assoc* 1990; 77 : 124-32.

Yebakima A. Larvicidal effect of *B. thuringiensis* and *B. sphaericus* on *Ae. aegypti* and *C. quinquefasciatus* in Martinique (FWI). In: Pavis C, Karmarrec A, ed. Recontres caraibes en lutta biologique. Caribbean Meetings on biological control, 5-7 Nov. 1990. *A Paris, France: Inst National de la Recharche Agronomique* 1991; 281-3.