EFFICACY OF *BACILLUS SPHAERICUS* IN DIFFERENT BREEDING HABITATS OF *CULEX QUINQUEFASCIATUS*

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Abstract. 'Spherifix', an alginate based slow release formulation of *Bacillus sphaericus* was field tested in different types of breeding habitats of *Culex quinquefasciatus* at the dose of 15 kg ai/ha at bimonthly interval. The efficacy of the formulation was higher in most of the months except in rainy and post-rainy months. The mean percentage reduction ± SD during the treatment phase of one year was 31.2 ± 17.9, 50 ± 29.4, 28.3 ± 17.6, 30.3 ± 21.1, 66 ± 22.5 and 53 ± 20.4 in larval density and 49 ± 20.8, 65.1 ± 26.1, 30.3 ± 21.9, 59.8 ± 22.6, 63.1 ± 21.9 and 47.7 ± 24.2 in pupal density respectively in cement tanks, cesspools, cesspits, disused wells, unlined drains and cement lined drains. The reduction in immature density was relatively higher in undisturbed, debris free and shallow habitats such as cesspools, unlined drains and cement lined drains. After withdrawal of treatment, the effect of the formulation could be seen for a period of four months.

INTRODUCTION

Control of mosquito breeding, especially that of *Culex quinquefasciatus* using various formulations of *Bacillus sphaericus* had been attempted in the past (Balaraman, 1995). Following the application of such formulations, significant level of reduction in the breeding was observed and the efficacy varied according to the nature of the formulation tested, dosage used and the type of breeding habitat. Most of these studies have been carried out on a small scale with limited number and types of habitats, and for a short period covering only a few weeks. In the present study, the efficacy of a *B. sphaericus* formulation was tested by treating all types of breeding habitats of *Cx. quinquefasciatus* present in two villages for a period of one year and by extending monitoring of immature density for another 12 months.

MATERIALS AND METHODS

The breeding habitats (with surface area in parenthesis) chosen for the application of Spherifix include unlined drains (74m^2), cement lined drains (104 m^2), cesspools (63 m^2), disused wells (208 m^2), cement tanks (31 m^2) and cesspits (132 m^2) which were spread over in 2 villages near Mayiladuturai, Tamilnadu, India. For comparison purpose, similar types of habitats *viz* unlined drains (244 m^2), cement lined drains (238 m^2), cesspools (271 m^2), disused wells (78 m^2), cement tanks (55 m^2) and cesspits (125 m^2) were selected in two other villages and kept untreated. All these habitats, except disused wells, serve as receptacles of sullage water. The wells, which were once the main drinking water source are now not used as the people have started using water from hand pumps and/or overhead tanks. As a result, the wells are not maintained and get polluted due to dumping of garbage.

'Spherifix', a *B. sphaericus* formulation developed at Vector Control Research Center was used in this study. It is an alginate based floating slow release formulation (in the form of granules packed in plastic floats having slits). An earlier study (Arunachalam *et al*, 1991) has shown that the formulation was highly effective in killing *Cx. quinquefasciatus* larvae present in disused wells at the dose of 10 kg active ingredient (ai)/hectare (ha). Since the present study involved six types of habitats, the dosage of 'spherifix' applied to the breeding sites of *Cx. quinquefasciatus* was increased to 15 kg ai/ha. The habitats were treated with the formulation for a period of one year at bimonthly interval. To prevent drifting of the floats (packed with the formulation) to different points of the habitats, they were anchored to the bottom of the habitats with a stone through a nylon thread.

Breeding of *Cx. quinquefasciatus* was monitored in three phases of 12 months each, *viz* pretreatment phase (July 1992 to June 1993), treatment phase (July 1993 to June 1994) and post-treatment phase (July 1994 to June 1995). Both treated and untreated habitats were sampled at weekly intervals using dipper as per standard procedures (Service, 1970). The larvae and pupae present in the samples were released back into the habitats after counting. The data were pooled to arrive at monthly means and expressed as density of larvae and pupae (number/dip). The efficacy of 'spherifix' was determined by calculating the percentage reduction in the density of larvae and pupae in the treated habitats using Mulla's formula (Mulla *et al,* 1971) as follows:

% Reduction = 100 - ((C1*T2) / (T1*C2))*100

Where,

C1 = density in untreated during pre-treatment phase
C2 = density in untreated during treatment phase
T1 = density in treated during pre-treatment phase
T2 = density in treated during treatment phase

The differences in the mean density of immatures and the comparative efficacy of 'spherifix' in different types of habitats were assessed using ANOVA test followed by Students Newman Keuls (SNK) test (Sokal and Rohlf, 1969).

RESULTS

The mean larval and pupal density of *Cx. quinquefasciatus* recorded in different types of habitats during pre-treatment phase in both experimental (treated) and check villages (untreated) are given in Table 1. The mean density of immatures differed significantly between habitats in experimental (larvae : $F = 12.86$, $df = 5$, $p < 0.05$; pupae: $F = 13.93$, $df = 5$, $p < 0.05$) as well as in check villages (larvae: $F = 20.18$, $df = 5$, $p < 0.05$; pupae : $F = 39.05$, $df = 5$, $p < 0.05$). In experimental villages, the highest larval density was recorded in disused wells followed by cement tanks, cesspools, cesspits and cement lined drains. In check villages, the cement tanks recorded the highest density followed by disused wells, cement lined drains, cesspits and cesspools. In both the zones, the larval density was the lowest in unlined drains. The pupal density was higher in cement tanks, cesspools, cesspits and cement lined drains and lower in disused wells and unlined drains.

The percentage reduction in the density of immatures (larvae and pupae) observed in different types of habitats in experimental villages during treatment phase is shown in Fig 1a-f. The reduction in the density of immatures was higher in most of the months (July 93 to October 93 and March 94 to June 94) except during the rainy and post-rainy months (November 93 to January 94). For the decade 1983-1993, the rainfall was the highest in 1993 and November and December alone received 75.6% of the total rainfall (1,548.6 mm) of the year. The heavy rainfall and subsequent flooding reduced the activity of the formulation due to

Table 1

Mean density (No./dip) of immatures of *Cx. quinquefasciatus* in different types of habitats in experimental and check villages during pre-treatment phase.

Habitat	Experimental villages				Check villages			
	Larvae		Pupae		Larvae		Pupae	
	Density*	SD	Density*	SD	Density*	SD	Density*	SD
CMT	157.9[a,b]	54.5	19.5[b,c]	9.5	301.2[a]	63.5	62.4[a]	20.1
CPL	144.3[b]	44.3	32.0[a]	11.0	122.8[b]	67.6	23.8[b]	10.1
CPT	137.0[b]	60.8	23.2[b]	10.6	146.0[b]	67.6	27.3[b]	13.2
DWL	199.6[a]	74.3	8.5[d]	7.6	181.7[b]	103.4	4.2[c]	2.7
ULD	34.8[d]	16.2	4.3[d]	2.8	18.5[c]	17.7	3.7[c]	5.5
CLD	82.6[c]	55.9	12.1[c,d]	10.6	169.1[b]	57.5	25.7[b]	7.6

CMT-cement tanks, CPL-cesspools, CPT-cesspits, DWL-disused wells, ULD-unlined drains, CLD-cement lined drains, SD-standard deviation.

* Values followed by the same letter do not differ significantly from each other (SNK test).

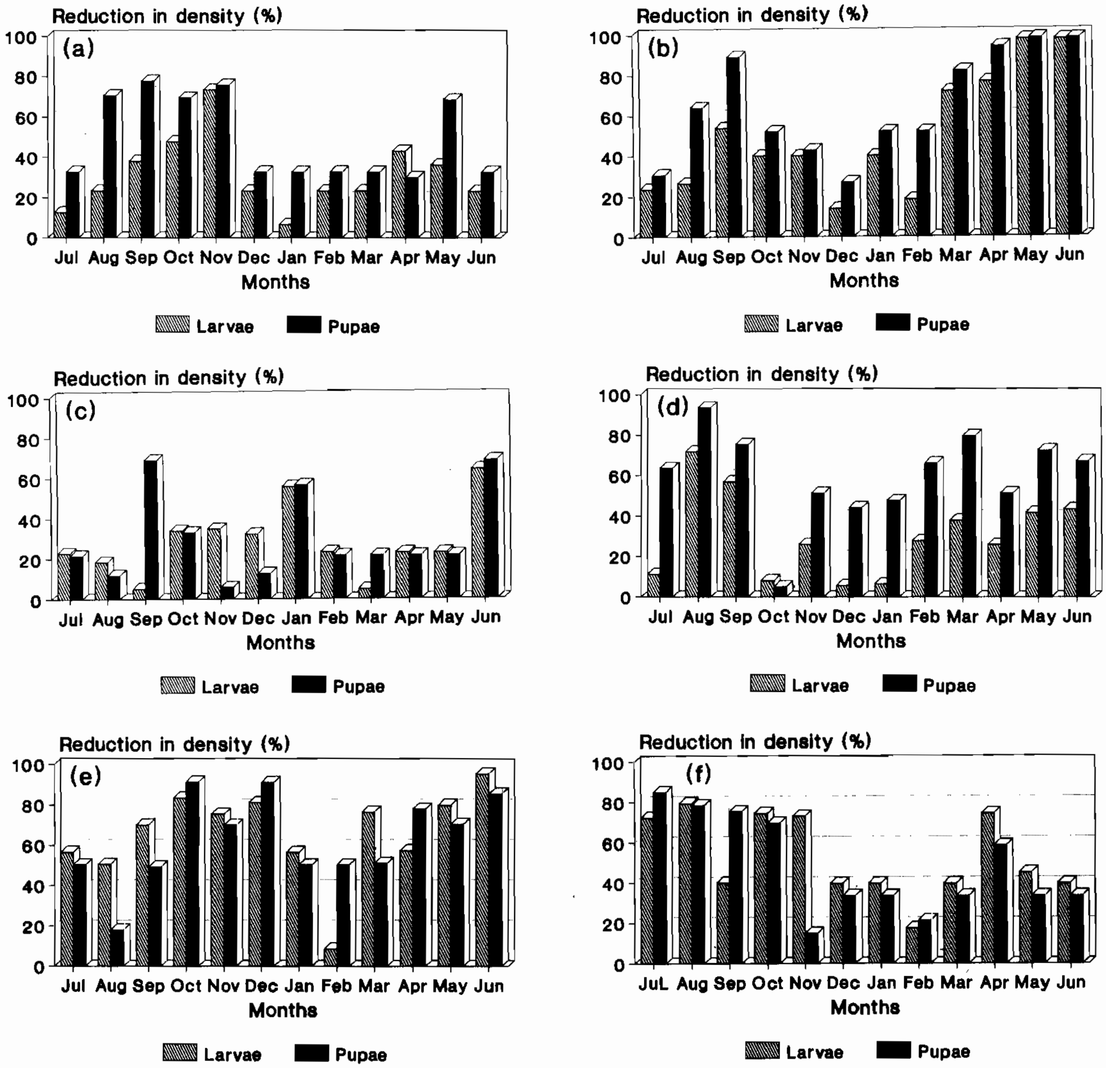

Fig 1–Reduction in immature density of *Cx. quinquefasciatus* in (a) cement tanks, (b) cesspools, (c) cesspits, (d) disused wells, (e) unlined drains and (f) cement lined drains during treatment phase.

accelerated release of active ingredient and its loss from the habitat. The reduction in the cement tanks, cesspools, cesspits, disused wells, unlined drains and cement lined drains was respectively in the range of 12.5 - 47.8%, 23.8 - 97.5%, 5 - 64%, 8 - 71.9%, 50.7 - 95.4% and 39.8 - 79.4% for larvae and 30 - 77.8%, 30.7 - 97.9%, 11.7 - 68.8%, 5 - 93.6%, 18.1 - 91.4% and 33.6 - 85.1% for pupae during July to October 1993 and March to June 1994. During the rainy and post-rainy months (November 1993 to February 1994), the reduction ranged from 6.6 to 73.4%, 14 to 40%, 23.1 to 55.7%, 5.7 to 27.8%, 8.7 to 81.3%, and 17.6 to 73.1%, in larval density and 32.7 to 75.9%, 27 to 52.1%, 5.8 to 56.4%, 44.2 to 66.3%, 50.5 to 91.2%, and 14.9 to 33.6% in pupal density respectively.

For the entire treatment period of 12 months, the mean percentage reduction ± SD was 31.2 ± 17.9, 50 ± 29.4, 28.3 ± 17.6, 30.3 ± 21.1, 66 ± 22.5 and 53 ± 20.4 in larval density and 49 ± 20.8, 65.1 ± 26.1, 30.3 ± 21.9, 59.8 ± 22.6, 63.1 ± 21.9 and 47.7 ± 24.2 in pupal density respectively in cement tanks, cesspools, cesspits, disused wells, unlined drains and cement lined drains. The ANOVA test indicated that the efficacy of 'spherifix' differed significantly between habitats (larvae : $F = 5.67$, $df = 5$, $p < 0.05$; pupae : $F = 4.0$, $df = 5$, $p < 0.05$). The SNK test further showed that the reduction in the larval density noticed in unlined drains, cement lined drains and cesspools were on par with each other and significantly higher when compared to that observed in the other three habitats *viz*, cement tanks, disused wells and cesspits. In the latter habitats, the reduction observed was on par with each other. In the case of pupae, same level of reduction was observed in all the habitats except cesspits in which the reduction was significantly lower.

During the post-treatment period *ie* after withdrawal of 'spherifix' application, the effect of the formulation could be seen for four months (July - October 1994) and the reduction in the density of larvae and pupae respectively was 12% and 43%, 16% and 34%, 7% and 0.7%, 26% and 52%, 46% and 47% and 64% and 60% in cement tanks, cesspools, cesspits, disused wells, unlined drains and cement lined drains. Thereafter, the immature density started increasing and the mean density ± SD of larvae and pupae was respectively 290.9 ± 71.5 and 27.3 ± 5.8, 343.9 ± 127.5 and 61 ± 26.6, 339.2 ± 59.5 and 55.3 ± 14.3, 150 ± 60.7 and 5 ± 2.5, 113.6 ± 54.1 and 15.1 ± 7.8 and 135 ± 43.5 and 18.6 ± 10.7 which were more or less equal to the pretreatment density for the corresponding period.

DISCUSSION

Several larvicide formulations prepared from *Bacillus sphaericus* have been introduced commercially and tested extensively against *Culex* species. Mulla *et al* (1988) tested BSP-2 FC (5.6 l/ha) in dairy waste water lagoons and observed complete initial and persistent control of *Cx. quinquefasciatus* for 14-21 days. Matanmi *et al* (1990) applied BSP-2 (4.8 l/ha) and ABG-6184 (FC : 2.24 l/ha) to a similar habitat and obtained 90% control of *Cx. stigmatosoma* for four weeks. A dust formulation, BIOCID-S (2.7 kg/ha) has been reported to yield 58-89% control of *Culex* spp in polluted ponds (Mittal *et al,* 1985). Bhalwar *et al* (1995) reported 93 - 100% control of culicines for 28 days with another dust formulation (22.6 kg/ha). A liquid formulation (10 l/ha) has been found to produce 100% mortality of *Cx. quinquefasciatus* and residual activity was seen upto five months (Lago *et al,* 1991). A sewage treatment plant was treated with Vectolex G (5.6 kg/ha) and 77 - 100% control of *Culex* spp was obtained (Sutherland *et al,* 1989). In rice fields, a microgel droplet formulation (2.2 and 4.3 kg/ha) yielded 83-100% reduction in pupal density of *Cx. vishnui* gr and *An. subpictus* for five weeks (Sundararaj and Reuben, 1991). Arunachalam *et al* (1991) tested three formulations, *viz,* vectolex (45 l/ha), spherimos (15 l/ha), and 'spherifix' (10 kg/ha), in polluted wells and observed respectively, 85%, 96-100% and 59-87% control of *Cx. quinquefasciatus* for a period of 63-67 days.

In the present study, it was observed that the application of 'spherifix' to *Cx. quinquefasciatus* breeding habitats resulted in 50%-66% reduction in larval density in cesspools, unlined and cement lined drains, and 28%-31% reduction in other three habitats. The reduction in pupal density ranged from 48%-65% in all types of habitats except cesspits in which the reduction was 30%. With regard to the efficacy in cesspits, the present observation is contrary to the report of Hougard (1990) who obtained good control of *Cx. quinquefasciatus* for 5-6 weeks by using a FC formulation (10 kg/ha). When compared to the other habitats, cesspits are subjected to constant disturbance by frequent and intermittent inflow of sullage water. Such inflows might have either adversely affected the release of the active ingredient by pushing the floats (packed with the formulation) deep into the slurry or accelerated the setting of the active ingredient released from the formulation on the bottom mud, thus, as reported earlier (Davidson *et al,* 1984; Balaraman *et al,* 1987), made the active ingredient unavailable for ingestion by larvae. It has also been reported that a powder preparation from M/s Stauffer and co when applied to highly polluted dairy discharge water-holding pond (Mulligan *et al,* 1978), street catch basins (Mulligan *et al,* 1980) and soakpits (Anonymous, 1977) caused lesser level of reduction in the density of *Cx. quinquefasciatus* larvae. 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. The presence of suspended particles and pollutants in water have also been observed to reduce the efficacy (Mulla, 1985).

The lower level of reduction in larval density noticed in the present study in disused wells and cement tanks with higher density of larvae compared to other habitats corraborate Mulla's observation (Mulla, 1985) that there was an inverse relationship between larval density and efficacy of *B. sphaericus*. Apart from this factor, the presence of large amount of debris might also have contributed for the reduced efficacy in these habitats by preventing an uniform distribution of the active ingredient over the water surface. However, the efficacy of 'spherifix' in cesspools was markedly higher in spite of the presence of high density of larvae. The higher efficacy of *B. sphaericus* in cesspools, unlined drains and cement lined drains was probably due to the fact that these habitats are shallow, facilitating the ingestion of spores from bottom water and mud by larvae (Matanmi *et al*, 1990).

In conclusion, it is inferred that the *B. sphaericus* formulation used in the present study had considerably reduced the breeding of *Cx. quinquefasciatus* in least disturbed shallow habitats and in habitats with less amount of debris. The efficacy of the formulation was lower in the habitats with relatively higher density of larvae.

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