EVALUATION OF *BACILLUS SPHAERICUS* FORMULATIONS AGAINST THE VECTOR OF BANCROFTIAN FILARIASIS

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Abstract. Three different formulations of *Bacillus sphaericus* viz, Spherimos, Vectobac and Spherifix, were evaluated for their efficacy and residual activity against *Culex quinquefasciatus* breeding in polluted disused wells. Spherimos, a flowable concentrate formulation, exerted 96-100% control when treated at the dosage of 10 l/ha for 17 days, whereas the effective residual activity lasted up to 67 days at 15 l/ha. In the case of Vectolex, a granular formulation, the residual activity lasted up to 56 days with the dosage of 30 l/ha and up to 66-77 days with higher dosages of 45 and 60 l/ha. The residual activity of Spherifix, a floating controlled release formulation, lasted up to 67 days with a dosage of 10 kg/ha.

INTRODUCTION

Bacillus sphaericus Neide has exhibited excellent larvicidal activity against mosquitos (Balaraman, 1980; Davidson *et al,* 1981; Mulla *et al,* 1986) and it has been introduced commercially too (Karch *et al,* 1990). When used in mosquito breeding habitats its efficacy was found to be influenced by several factors, such as the availability of toxin in the larval feeding zone (Aly, 1983), the feeding behavior of mosquito larvae (Shipitsina, 1930; Aly *et al,* 1987), quality of water (Mian and Mulla, 1983), etc. Although it is reported to have exerted extended control of culicines under certain situations (Lacey *et al,* 1984; Silapnuntakul *et al,* 1983), generally its activity is restricted to 24-28 hours after application (Mulligan *et al,* 1978; Davidson *et al,* 1981; Hoti and Balaraman, 1984; Kramer, 1984; Paily *et al,* 1987). So as to enhance its activity and also to reduce the cost, suitable formulations need to be developed. At the Vector Control Research Center a floating type of controlled release formulation of *B. sphaericus,* "Spherifix", was developed and evaluated for larval control and residual activity in comparison with commercial formulations. This paper presents the results of this evaluation.

MATERIALS AND METHODS

Test site and formulation: The target sites chosen for the study were 30 disused polluted wells situated in different parts of Bangalore, a cosmopolitan city. *Culex quinquefasciatus,* the vector of bancroftian filariasis, was breeding in very high densities in these wells. The wells were 1-2 m in diameter with a water column of 2-4 m. The water was rich in organic contents.

Three formulations were taken up for the study. They were i. Spherimos (BSP-2), a flowable concentrate formulation from Solvay and Co, Brussels, Belgium, ii. Vectolex (ABG 6262), a granular formulation from Abbott laboratories, North Chicago, Illinois, USA and iii. Spherifix a floating type controlled release formulation from VCRC, Pondicherry (Kuppusamy *et al,* 1987). The latter was based on an indigenous strain B42 of *B. sphaericus* H-5a5b (de Barjac *et al,* 1985).

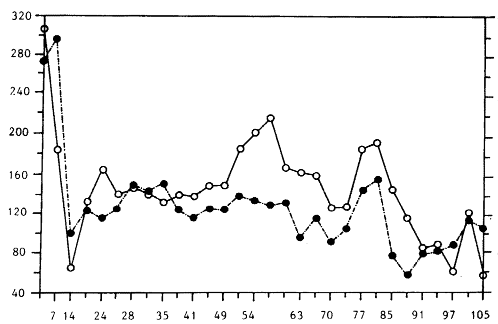
Orientational dosages of different formulations were determined by bioassay against field collected larvae as follows: larvae were collected from the wells, brought to the laboratory and III instars were isolated. They were exposed to different doses of the *B. sphaericus* formulations for 48 hours in the well water. The LC90 values were calculated by standard procedures (Balaraman *et al,* 1987) and were 1ml, 3ml and 1g/m^2 (equivalent to 10 l/ha, 30 l/ha and 10 kg/ha), for Spherimos, Vectolex and Spherifix, respectively.

Application and evaluation: Vectolex was applied at dosages of 30, 45 and 60 l/ha, Spherimos at 10, 15 and 20 l/ha and Spherifix at 10 and 15 kg/ha. Each dosage was replicated three times. The former two formulations were treated after suspending them in tap water and spraying with a knap-sac sprayer, ensuring uniform and complete coverage of water surface of the habitat. The latter formulation was applied by introducing appropriate numbers of vials into each well.

The density of immatures (III and IV instar larvae and pupae) was monitored for 18 days before and 87 days after application of formulations, by dipper sampling as follows: the wells were sampled five times, at random places every 3rd or 4th day by using a 3 l capacity bucket. The immatures in the water sample were counted at the site and released back into the wells. The data on the density of the immatures of treated and untreated wells were averaged and are presented as mean number of larvae or pupae per 5 dips

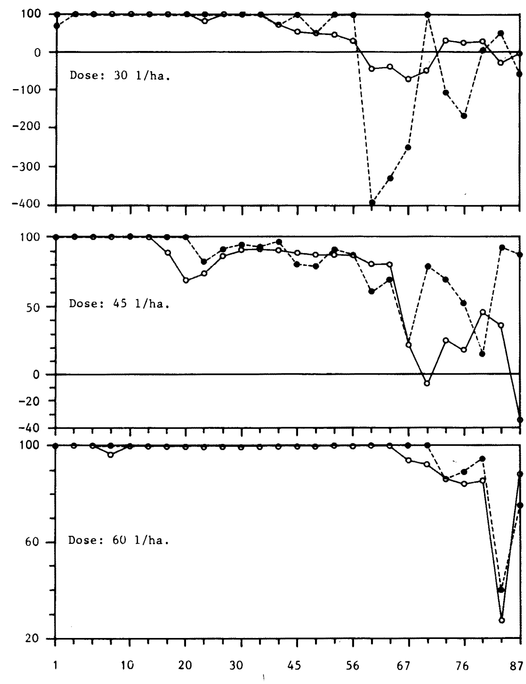
RESULTS

In the control wells the density of the immatures (larvae and pupae) fluctuated. The pretreatment density in these wells ranged from 64 to 307 larvae and 8 to 28 pupae and the post-treatment density from 132 to 216 larvae and 8 to 13 pupae per 5 dips (Fig 1).



X axis title: Days of observation
Y axis title (Left side): No. of pupae per 5 dips
Y axis title (Right side): No. of larvae per 5 dips

Fig 1—Density of the immatures of *Cx. quinquefasciatus* in untreated wells (o–o larvae, ●--● pupae).



X axis title: Days after treatment
Y axis title: % Reduction

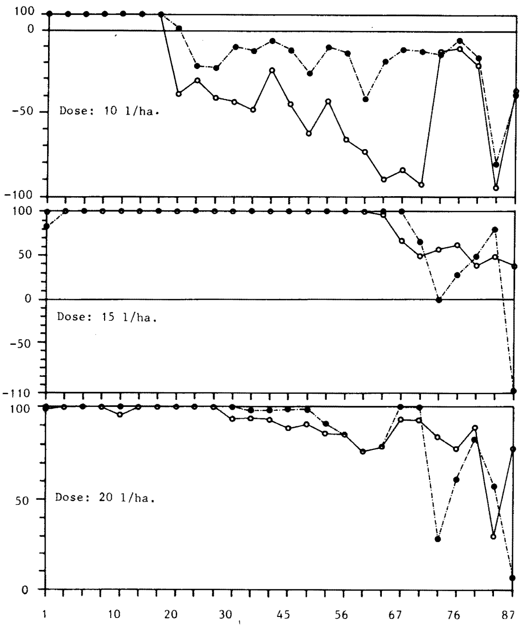
Fig 2—Effect of Vectolex on the breeding of *Cx. quinquefasciatus* in wells (o–o larvae, ●--● pupae).

Vectolex: In the wells treated with 30 l/ha of Vectolex the pre-treatment density of larvae and pupae was 103 and 3, respectively. Twenty four hours after application, the larval density was reduced by 100% (Fig 2). Thereafter and till 36th day the reduction was 98%. Then the breeding slowly picked up but still the reduction was near 50% till the 56th day. With pupae, 68% reduction was noticed in the density by the 24th hour after application and it increased to 100% by 48th hours. The reduction in the pupal density continued to be at the same level till the 36th day, after which the breeding slowly picked up and the pupal density returned to the pre-treatment level by the 60th day.

At the next higher dosage of 45 l/ha the density of larvae and pupae were reduced by 100% by the 24th and 48th hours respectively and then remained at the same level till the 14th and 21st days. Thereafter until the 63rd day the mean reduction was still substantial (85% and 84%, respectively). By the 86th day the reduction was 29% in the case of larvae and 60% in the case of pupae. On the 87th day the density of larvae increased to more than the pre-treatment level.

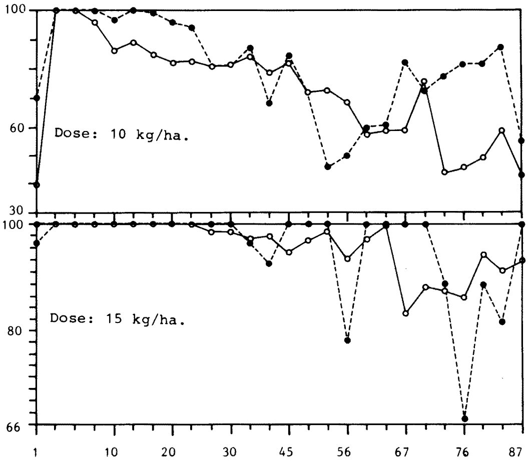
At the highest dosage of 60 l/ha, Vectolex extended the 100% reduction in the density of both larvae and pupae till 60 days after treatment. Thereafter the reduction in the density fluctuated between 80 and 100%. Even by the 87th day after treatment there were only 103 larvae and 30 pupae and which was still well below the pre-treatment density.

Spherimos: The densities of larvae and pupae were 67 and 9, respectively during the pre-treatment period in the wells treated with 10 l/ha of Spherimos (Fig 3). It was reduced by 96-100% 24 hours after treatment and continued to remain at the same level until the 17th day. On the 18th day the reduction was only 13%, whereupon the density of larvae and pupae increased to more than the



X axis title: Days after application
Y axis title: % Reduction

Fig 3—Effect of Spherimos on the breeding of *Cx. quinquefasciatus* in wells (o—o larvae, ●--● pupae).



X axis title: Days after application
Y axis title: % Reduction

Fig 4—Effect of Spherifix on the breeding of *Cx. quinquefasciatus* in wells (o—o larvae, ●--● pupae).

pre-treatment level.

Application at the next higher dosage of 15 l/ha, maintained the 100% reduction of immatures until the 63rd day. Thereafter and till the 87th day the reduction in the larval density was 52% and that of pupae 55%. However, in the wells treated with the next higher dosage of 20 l/ha the reduction in the density was 100% only till the 31st day after application. This curtailment in the length of the period of 100% reduction to just 31 days, compared to that observed with 15 l/ha (63 days) may be due to the initial excessive immature density observed during the pre-treatment period. After the 31st day and until the 79th day the reduction was 89%.

Spherifix: In the wells treated with 10 kg/ha of Spherifix the larval and pupal densities were reduced by 40% and 70%, respectively, 24 hours after application (Fig 4). By the 48th hour 100% reduction was noticed in both the cases which lasted up to 6 days. From the 7th day until the 45th day after application and from the 46th day until the 87th day the mean reduction in the larval density was 87 and 59% respectively. The reduction in the density of pupae for the corresponding periods was 92% and 69% respectively.

Upon treatment with the next higher dosage of 15 kg/ha, within 24 hours after application the density of larvae and pupae was reduced by 100% and 97%, respectively. Subsequently 100% reduction was noticed in both the cases till the 23rd and 31st days, respectively. Thereafter and till the 89th day the reduction in their density was 95% and 93%, respectively.

The results obtained in this study indicate that in the case of Spherimos, the effective residual activity (100% control) lasts for 17 days at the dosage of 10 l/ha and for 67 days at 15 l/ha. In the case of Spherifix, the residual activity lasts up to 67 days with the dosage of 15 kg/ha and that in the case of Vectolex, the residual activity lasts up to 56 days with the dosage of 30 l/ha, up to 66-77 days with higher dosages of 45 or 60 l/ha.

DISCUSSION

Treatment with Spherimos at 10 l/ha resulted in the complete control of immatures 14 days post-treatment and it gradually decreased over the next 58 days, upon which the breeding returned to pre-treatment level. Increasing the dosage to 15 l/ha extended the effective control (100% control) of immatures for as long as 58 days. However, at 20 l/ha effective control was observed for 27 days only although still a considerable but decreasing level of control was maintained for up to 78 days. In the latter case, even though the formulation was treated at a higher dose, effective control was observed for comparatively shorter duration. The reason for this could be the very high density of immature populations present in these wells during the pre-treatment period. Vectolex also exerted substantial to complete control of immatures for up to 35 days at 30 l/ha and during the next 23 days the activity of the formulation decreased gradually. Similar to Spherimos, increasing the application rate increased the duration of effective control.

Spherifix, unlike the other two formulations, has caused effective control at 10 kg/ha and showed a gradually diminishing trend at a very slow pace with time. Effective control of the immatures was observed for a short duration at this dose indicating the inadequacy of the dose to act upon the exceptionally high immature density (1600/5 dips) compared to that which existed in other wells. However, upon treatment with 15 kg/ha effective control was observed throughout the post-treatment period of 87 days.

The results lead us to conclude that all the three *B. sphaericus* formulations exerted considerable pressure on the immature density of *Cx. quinquefasciatus* breeding in highly polluted well water. Karch *et al* (1990) observed control of *Cx. pipiens* for 14 days with 4 treatments of Vectolex at 4 l/ha dose. Mulla *et al* (1988) obtained 99% control of *Cx. quinquefasciatus* for 49 days with 4.48 kg/ha of a primary powder. Although considerably higher dosages were used in the present investigation they were based on the bioassays conducted with field collected larvae. The extent and duration of the activity of the formulations was dependent upon the immature density present in the polluted wells. Application of the larvicide based on the density of the target mosquito has also been emphasized by Karch *et al* (1990). This suggests that an optimum dosage of the formulation, worked out taking into consideration the mosquito immature density in the habitat, needs to be applied to obtain high levels of control for extended period.

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