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RESIDUAL ACTIVITY OF BRIQUETTE AND ALGINATE FORMULATIONS OF BACILLUS SPHAERICUS AGAINST MOSQUITO LARVAE

by

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ABSTRACT

Briquette and alginate formulations of Bacillus sphaericus were prepared and tested in the laboratory for residual activity against Culex quinquefasciatus larvae.

Ninety to one hundred per cent larval mortality was induced by briquettes, with a mean spore release of 0.71-1.57 x 10^3/ml/day for the first 15 days. During the next 13 days the mean number of spores released was 4.21-6.42 x 10^3/ml/day, which caused 100% mortality.

Wet alginate beads induced 50-100% larval mortality from the 6th to 78th day, whereas dried beads did not induce larval mortality unless pretreated with solubilizing agents, viz., KH_2PO_4, sodium citrate or EDTA. The KH_2PO_4-treated alginate beads caused 100% mortality from the 1st to 78th day, but the per cent mortality fluctuated widely on different days. On the other hand, Na-citrate- and EDTA-treated beads caused mortality after a lag period of 4-8 days for 58-60 days and the per cent mortality steadily increased from 14 to 100.

1. INTRODUCTION

Of the various microbial agents, bacteria continue to hold the key for further development as larvicides for vector control (1). In the past, both Bacillus thuringiensis H-14 and B. sphaericus were successfully produced, formulated as water-dispersible powders, emulsifiable concentrates, and granules, and tested in different mosquito breeding habitats (2,3). However, the larvicidal activity of these formulations lasted for only a few days after treatment and this has led to attempts to develop slow-release formulations (4,5). At the Vector Control Research Centre, Pondicherry, such formulations were developed using B. sphaericus; their larvicidal activity was evaluated in the laboratory and the results are presented in this paper.

2. MATERIALS AND METHODS

The freeze-dried biomass of a B. sphaericus strain (VCRC B42), with an LC_{50} value of 15 µg/250 ml for third-instar Culex quinquefasciatus larvae, was used in the study.

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2.1 Floating briquettes

The briquettes were prepared by mixing 1 g biomass of B. sphaericus per briquette and certain buoyancy and binding agents to enable the briquettes to float. One briquette was added to each plastic trough containing 8 litres of chlorine-free tap-water and different numbers of third-instar C. quinquefasciatus larvae, i.e., 1000, 500, 300 and 100. Two sets of troughs containing chlorine-free tap-water served as controls - one set had only larvae (500/trough) and the other only a briquette/trough. The experiment was run in duplicate. Observations were made of larval mortality and the number of spores released from the briquettes into the troughs on different days, until the briquettes disintegrated. After each day of observation, the briquettes were transferred to another set of troughs containing healthy larvae and water as stated earlier. The per cent larval mortality was worked out using Abbot's formula (6), after correcting for control mortality, and LC_{90} values were calculated according to standard methods. The number of spores released from briquettes on each day was assessed as follows: after removing the briquettes and larvae (both dead and alive) from the troughs, the water was thoroughly stirred with a glass rod, five 1-ml samples were taken randomly at different depths and different places of each trough, pooled and used for spore count. For the spore count water samples were heat-treated at 80°C for 10 min, serially diluted and spread on nutrient-glucose agar containing 0.01% streptomycin sulphate. The agar plates were incubated at 30°C for 48 hours and the developing B. sphaericus colonies were counted.

2.2 Alginate beads

The biomass of B. sphaericus was mixed with a 2% aqueous solution of sodium alginate to a final strength of 20% w/v (7). This mixture was added, drop by drop, through a No. 20 hypodermic needle, to a solution of 0.1 mol/l $CaCl_2$, at 30°C and with constant stirring. The resulting spherical alginate beads were hardened by being kept in the $CaCl_2$ solution for a further 1 hour. A portion of the beads was then removed and stored at 4°C (in wet form), whereas the remaining beads were dried at 35°C for two days and stored in sealed plastic containers.

Larvicidal activity of the alginate beads was evaluated in the laboratory. The experimental kit consisted of bowls containing 250 ml of chlorine-free tap-water and 50 third-instar C. quinquefasciatus larvae. The alginate beads were of five different kinds: (a) wet; (b) dried; and dried beads pretreated for 1 hour with a 0.1 mol/l solution of either (c) potassium dihydrogen orthophosphate (KH_2PO_4) or (d) sodium citrate ($C_6H_5O_7Na_3$) or (e) ethylene diamine tetra acetic acid (EDTA). Except for the wet alginate beads which were added in amounts of 200 mg (equivalent to 100 mg dried beads), the remaining kinds of beads were added in quantities of 100 mg per bowl. Appropriate controls were also maintained and the larvae were fed daily sterilized dog-biscuit and yeast powder. The experiment was run in triplicate. Observations were made for larval mortality every 24 hours and per cent mortality was calculated after correcting for control mortality (6). After each day of observation, the alginate beads were transferred to a fresh set of bowls containing healthy larvae and water as stated above, using stainers until the beads dissolved completely.

3. RESULTS AND DISCUSSION

3.1 Floating briquettes

The number of spores released from briquettes into the troughs containing different numbers of larvae varied from 0.08×10^3/ml - 14.07×10^3/ml, whereas in the control troughs which had only briquettes the spore count ranged from 0.07×10^3/ml - 5.03×10^3/ml (Fig. 1). The mean number of spores released per day during the first 15 days in troughs containing 1000, 500, 300 and 100 larvae, respectively, was 0.71×10^3, 1.57×10^3, 0.78×10^3 and 0.88×10^3; in the control troughs the mean spore count was only 0.28×10^3/ml. Thereafter, the number of spores released from the briquettes increased sharply for up to 28 days. During the latter 13 days, the

mean number of spores released was 4.21-6.42 x 10^3/ml and 2.21 x 10^3/ml per day, respectively, in the treated and control troughs. A maximum of 7.3-14.1 x 10^3 spores/ml were released in the treated troughs during the experimental period, compared to 5 x 10^3 spores/ml in the control troughs. The total number of spores released by briquettes for 28 days in troughs containing 1000, 500, 300 and 100 larvae was 6.7 x 10^4/ml, 7.3 x 10^4/ml, 8.1 x 10^4/ml and 6.8 x 10^4/ml, respectively, whereas in troughs containing briquettes but no larvae the number was 3.04 x 10^4 spores/ml.

On the first day after addition of B. sphaericus briquettes, larval mortality in treated troughs ranged from 44% to 69% (Fig. 1). The mortality increased to 97-98% thereafter until the 28th day. The LC_{90} values increased with increase in the number of larvae added to the treated troughs as follows: (a) 490 spores/ml for 1000 larvae (first 15 days); (b) 410 spores/ml for 500 larvae (first 15 days); (c) 410 spores/ml for 300 larvae (first 11 days); and (d) 217 spores/ml for 100 larvae (first 9 days). The trend of larvicidal activity observed in this study is similar to what has been reported earlier on B. thuringiensis H-14 briquette formulations (8).

A general pattern of disintegration of the briquettes was noticed during the study period. The briquettes were intact for 15 days after addition, with no apparent change on the surface. However, dissociation of their components was faster thereafter as indicated by the exposure of granules of floating agents as protrusions, imparting a rough appearance to the briquettes. After 22 days, small portions of the briquettes started withering off, ultimately resulting in total disintegration by the 28th day.

The results indicate that B. sphaericus spores are released from the formulation in a sustained way for the first 15 days after treatment, causing 95-100% mortality, and the mean number of spores released is 0.71-1.57 x 10^3/ml. During the next 13 days the number of spores released is several folds higher than the minimum required due to rapid disintegration of briquettes. In an earlier study pellet formulations of B. sphaericus were found to release 10^3-10^5 spores/ml for 30 days causing significant larval mortality (9). The LC_{90} values (in terms of number of spores released from the formulation) increased with the increase in the number of larvae added to the troughs, indicating the existence of a definite correlation between these two factors. The total number of B. sphaericus spores released from the formulation during the entire experimental period was much higher in the treated troughs, where both briquettes and larvae were added, than in the control troughs with only briquettes, suggesting that higher numbers of spores were released due to either direct or indirect disturbances caused by the presence of larvae.

3.2 Alginate beads

The wet alginate beads did not induce larval mortality during the first five days. But on the 6th day they caused 50% mortality which increased to 100% on the 8th day (Fig. 2(A)). From then on and until the 78th day larval mortality fluctuated between 60 and 100%.

The dried alginate beads which were not pretreated with any chemical did not exhibit larvicidal activity, whereas those pretreated with KH_2PO_4 or sodium citrate or EDTA induced larval mortality. In the bowls to which beads treated with KH_2PO_4 were added, 100% larval mortality was observed already on the first day which then fluctuated between 10 and 100% (Fig. 2(B)). Alginate beads treated with sodium citrate induced 40% larval mortality on the 9th day. From the 10th to 60th day, mortality ranged from 92% to 100% (Fig. 2(C)). The EDTA-treated alginate beads induced approximately 10% larval mortality on the 5th day which increased to 100% by the 16th day (Fig. 2(D)). Thereupon and until the 58th day larval mortality remained at 100%. Whereas the wet beads and those treated with KH_2PO_4 completely dissolved by 71-78 days, those treated with sodium citrate and EDTA lasted for 58-60 days.

Bacteria such as Azospirillum and Pseudomonas that promote plant growth have been entrapped in sodium alginate and the beads made from them were used to inoculate

plants (10). It has been reported that such beads released the bacteria at a slow and constant rate for prolonged periods. In the present study, it was observed that the alginate beads induced larval mortality for as long as 58-78 days, indirectly indicating that they released the entrapped B. sphaericus spores continuously for a prolonged period. However, marked variations were found in the trend of larvicidal activity caused by the various types of beads, i.e., wet beads, dried beads, and dried pretreated beads. The dried beads which were not pretreated with any of the solubilizing agents did not cause larval mortality throughout the experimental period, indicating that they did not release the entrapped B. sphaericus spores. Whereas the wet beads and the dried beads pretreated with sodium citrate or EDTA caused larval mortality after an initial lag period of 4-8 days, the dried beads treated with KH_2PO_4 caused larval mortality from the very first day onwards. Also, while the per cent mortality induced by the wet beads, the dried beads pretreated with sodium citrate, and the dried beads pretreated with EDTA was steady and increasing until the end of the experiment, mortality was erratic in the case of dried beads pretreated with KH_2PO_4. This indicates that in the latter case B. sphaericus spores were not released in a controlled way. The study also reveals that the dried alginate beads pretreated with sodium citrate or EDTA dissolved completely within 58-60 days while the wet beads and beads treated with KH_2PO_4 dissolved after 71-78 days. It must be noted that none of the alginate beads containing B. sphaericus floated in water.

The present study leads to conclude that B. sphaericus briquettes released adequate numbers of spores to cause the desired level of larval mortality, in a sustained manner, and remained intact for 15 days. But the rapid disintegration of the briquettes during the next 13 days resulted in wastage of spores (this could be avoided by improving the formulation). The trial also suggests that B. sphaericus or any other larvicidal bacillus can be incorporated into sodium alginate and released at a low and constant rate during larval treatments. These beads can be stored at ambient temperature over a long period and are biodegradable. However, since they do not float in water, they can be used only in shallow waters. Therefore, further research is necessary to develop alginate beads that float in water so that they could be used in other mosquito breeding habitats where depth of water is a limiting factor.

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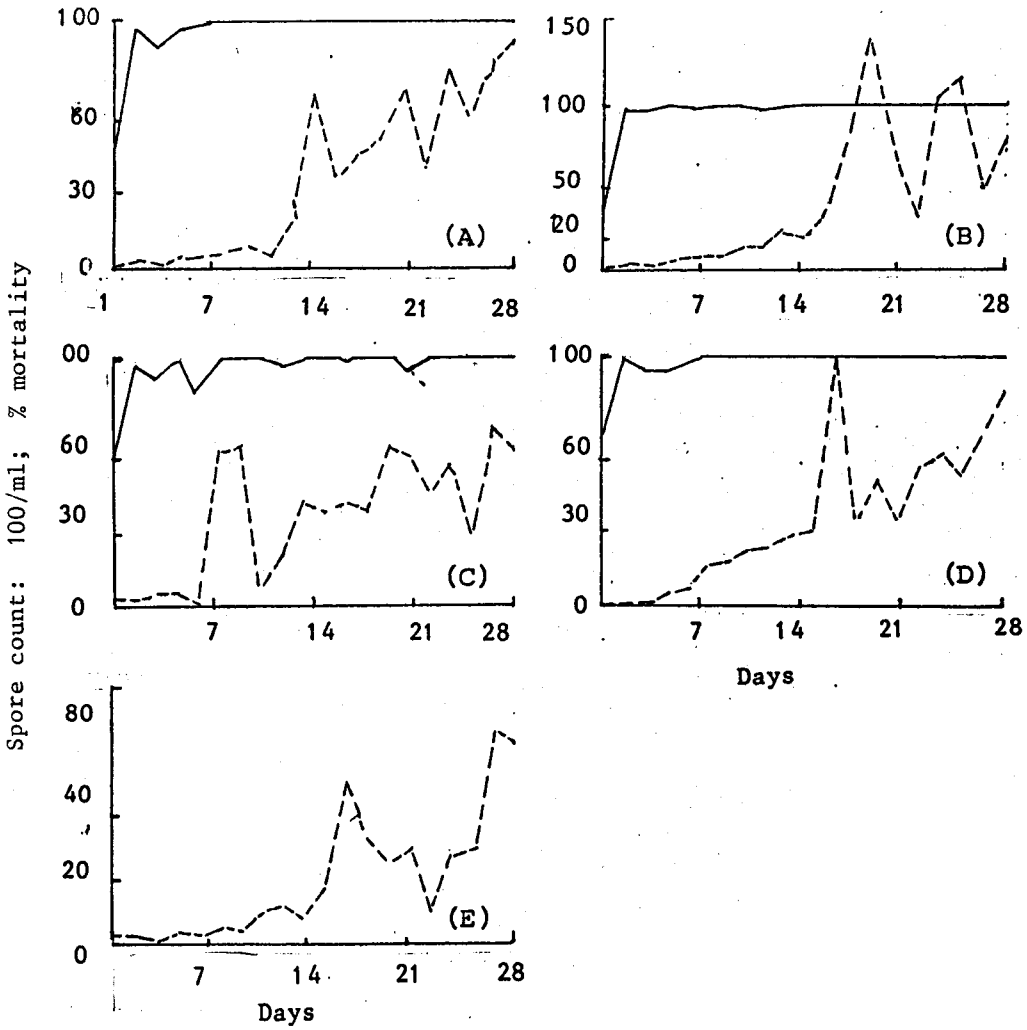
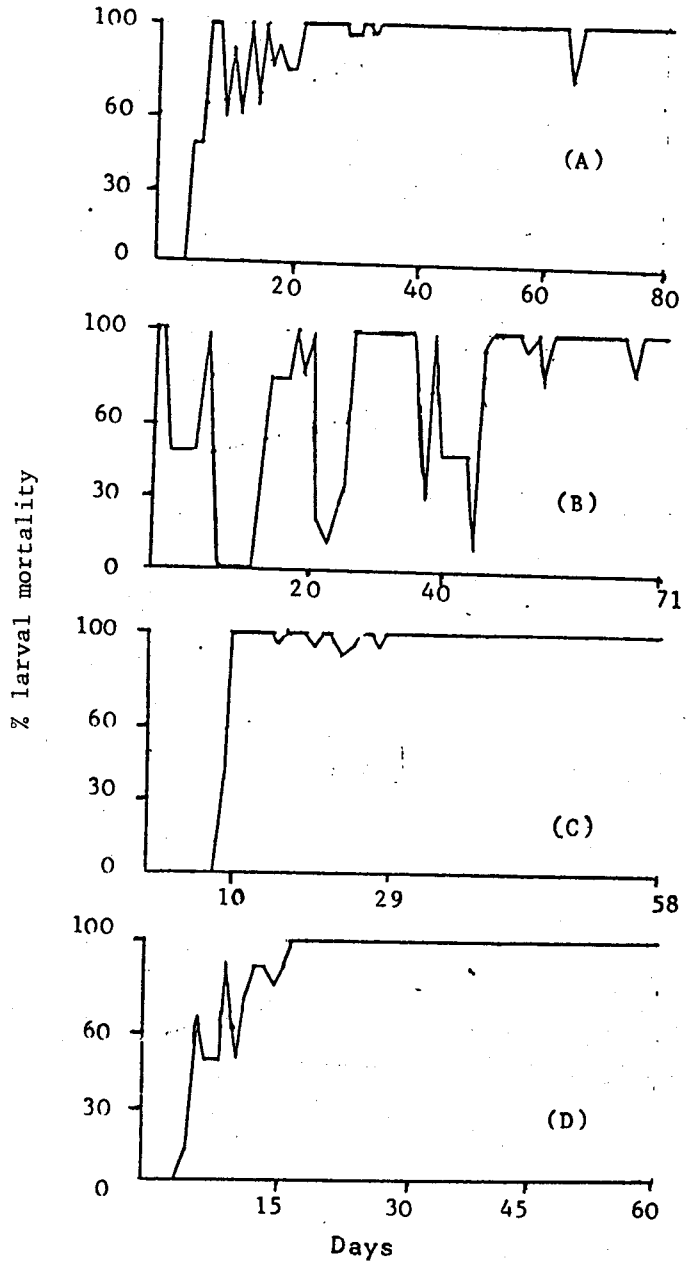
FIG. 1. SPORE RELEASE RATE AND LARVICIDAL ACTIVITY OF B. SPHAERICUS BRIQUETTES



Legend: (A) = briquette + 100 larvae;
(B) = briquette + 300 larvae;
(C) = briquette + 500 larvae;
(D) = briquette + 1000 larvae;
(E) = briquette + 0 larvae.
———— % larval mortality.
---------- spore count.

FIG. 2. LARVICIDAL ACTIVITY OF B. SPHAERICUS EMBEDDED IN ALGINATE BEADS



Legend: (A) = wet beads.
Dried beads:
(B) = KH_2PO_4-treated beads;
(C) = sodium-citrate-treated beads;
(D) = EDTA-treated beads.
———— % larval mortality.

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