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FIELD TRIAL OF *BACILLUS SPHAERICUS* STRAIN B-101 (SEROTYPE H5a, 5b) AGAINST FILARIASIS AND JAPANESE ENCEPHALITIS VECTORS IN INDIA

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ABSTRACT. A large-scale operational field trial was conducted from June 1993 to October 1994 to evaluate the efficacy of *Bacillus sphaericus* (strain B-101, serotype H5a,5b) for control of the vectors of filariasis (*Culex quinquefasciatus*) and Japanese encephalitis (*Cx. tritaeniorhynchus* and *Cx. vishnui*) in Rourkela city. Application of *B. sphaericus,* when sprayed at 1 g/m^2 in storm drains, wastewater pools, abandoned masonry tanks, peripheral paddy fields, ditches, and other small water collections and at 4 g/m^2 in domestic septic tanks, significantly reduced larval and pupal counts ($P < 0.0001$) and significantly reduced the percentage of habitats containing larvae (3rd–4th instars) ($P < 0.0001$) as compared with routine antilarval measures. This in turn resulted in a reduction in the indoor density of disease vectors in particular and a reduction in mosquito nuisance in general. The trial demonstrated that *B. sphaericus* has good potential for use against disease vectors and mosquito breeding in polluted as well as clean waters.

INTRODUCTION

Since the first documentation of the isolation of an active *Bacillus sphaericus* isolate in California (Kellen and Meyers 1964), several *B. sphaericus* strains have been reported to have varying mosquito larvicidal activity (Singer 1990). Demonstration of the efficacy of *B. sphaericus* against malaria vectors and other mosquitoes in the field (Ansari et al. 1989, Karch et al. 1992), its recycling and long persistence of efficacy (Des Rochers and Garcia 1984), and its mammalian safety (Shadduck et al. 1980) has encouraged isolation of new strains as well as evaluation of different formulations (Mittal et al. 1985, Mulla et al. 1988) to achieve better efficacy. In 1991, we received a small quantity of *B. sphaericus* strain B-101, serotype H5a,5b, manufactured by the Berdsk Plant of Biological Preparations, Russia. Strains with serotype H5a,5b are reported to be among the most toxic (de Barjac 1990). A preliminary field test with *B. sphaericus* showed good larvicidal efficacy, which prompted us to undertake a larger, longitudinal field trial from June 1993 to October 1994 for control of mosquitoes and, in particular, the vectors of filariasis (*Culex quinquefasciatus* Say) and Japanese encephalitis (*Cx. tritaeniorhynchus* Giles and *Cx. vishnui* Theobald) in Rourkela city, which encompasses a major steel plant of India. An outbreak of Japanese encephalitis (JE) was recently reported in the city (Vajpayee et al. 1991), while bancroftian filariasis is endemic in the region (Ghosh and Yadav 1995).

MATERIALS AND METHODS

Bacillus sphaericus had the following specifications: homogeneous wettable powder (particle size >90 μm below 2% and >500 μm below 0.2%); spore content 7–10% w/w; endotoxin 7–10% w/w; bulk density 0.49–0.58 g/cm^3; pH 6.05–6.4 and LD_{50} for *Culex pipiens* Linn. (autogenous strain) 0.008–0.012 mg/liter.

The trial was conducted in 2 major colonies of Rourkela city, Orissa state in eastern India beginning June 1993, and a 3rd colony was taken for comparison. Most houses in the area were made of brick and concrete, but there were small slums interspersed in the area. Due to the lack of a sanitary sewer system, individual houses had septic tanks which mostly opened into the storm drains for discharge of excess water. Initially, permanent mosquito larval habitats were identified. These were domestic septic tanks (mostly with semicovered lids), storm drains, wastewater pools, abandoned masonry tanks at construction sites, rice/fallow fields on the periphery receiving drainage water, and a few wells. During rains, temporary collections such as ditches and borrow pits also provided larval habitats.

The existing antilarval operation of the Rourkela municipality was discontinued for 2 weeks from the area to be treated with *B. sphaericus* spray. In the area selected for comparison, the municipality continued routine antilarval measures, i.e., spraying with Abate®, Baytex®, or larvicidal oil and cleaning drains. An aqueous suspension of *B. sphaericus* was sprayed at 1 g/m^2 on the water surfaces of larval habitats using knapsack sprayers with flat fan nozzles. The larval habitats were checked weekly, and only those found with 3rd–4th instar larvae were resprayed at the dose stated above. The treatment dose for septic tanks had to be increased to 2 g/m^2 after 2 weeks of initial spraying and then to 4 g/m^2 after the 4th week. The existing municipal spray staff undertook *B. sphaericus* spray under our guidance. Almost all wells in the area were being used; hence, guppy (*Poecilia reticulata* Peters) fish were released in them.

Larval and pupal counts (density/dip) were made

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Table 1. Percentages of various mosquito species that emerged from the larval samples taken from major habitats in Rourkela city.

Species	Storm drains	Septic tanks	Waste water pools	Masonry tanks	Paddy fields	Wells
Aedes						
1. *albopictus* Skuse	0	0	0	2.1	0	0
2. *vittatus* (Bigot)	0.2	0	0	10.1	0	0
Anopheles						
3. *barbirostris* Van der Wulp	0	0	0.3	2.1	0	5.6
4. *culicifacies* Giles	0	0.2	0.03	0.7	0	5.6
5. *nigerrimus* Giles	0	0	0.2	2.6	6.7	3.7
6. *ramsayi* Covell	0	0	0.2	0	0	0
7. *subpictus* Grassi	3.7	0.2	26.6	1.6	2.2	3.7
8. *vagus* Donitz	2.4	0.4	4.7	3.2	0	29.6
Armigeres						
9. *obturbans* Walk	5.8	63.3	0.7	3.0	0	0
Culex						
10. *bitaeniorhynchus* Giles	0.1	0	2.7	0	0	0
11. *cornutus* Edwards	0.2	0	1.03	0.2	16.9	0
12. *fuscanus* Wiedemann	0.6	0	0.74	0.7	0	14.8
13. *gelidus* Theobald	1.5	0	0.3	0	0	0
14. *nigropunctatus* Edwards	0.9	0	2.1	0	12.4	0
15. *pallidothorax* Theobald	6.4	0	10.6	2.6	33.7	12.9
16. *quinquefasciatus* Say	76.2	35.7	32.8	65.4	0	24.1
17. *sinensis* Theobald	0	0	0.9	0.4	0	0
18. *tritaeniorhynchus* Giles	0.9	0.2	11.9	5.1	28.1	0
19. *vishnui* Theobald	0.9	0	3.8	0.2	0	0
20. *whitmorei* (Giles)	0.2	0	0.4	0	0	0
Total mosquitoes emerged	3,437	3,484	567	1,292	89	57

on a weekly basis from several index habitats using a 300-ml larval dipper. Larval/ pupal samples were brought to the laboratory from these sites in the routine treatment area during the trial. Samples were brought from the treatment area during the pretreatment period and from new habitats created by rains and new construction during the trial. Adult mosquito species were identified on emergence. To monitor densities of indoor resting mosquitoes, morning catches between 0500 h and 0700 h were made fortnightly from 12 fixed and 12 randomly selected rooms by employing 4 collectors in each of the sprayed and routine treatment areas. Every collector searched for mosquitoes for 15 minutes in each of the 6 assigned rooms using aspirators and flashlights. Mean monthly man-hour densities were calculated based on fortnightly catches. The pH of water in larval habitats was measured with a digital pH meter. Rainfall data were taken from a local meteorological station. Differences in the means of larval counts and habitat positivity rates in the *B. sphaericus*-sprayed and control areas were analyzed by a paired *t*-test.

RESULTS AND DISCUSSION

From the larval and pupal samples collected from the major habitats, 20 mosquito species emerged. Total mosquitoes emerged from these samples and proportions of various species emerged from different habitats are given in Table 1. Among the vector species, *Cx. quinquefasciatus* accounted for major emergence from storm drains, wastewater pools, and masonry tanks and was the second largest species breeding in septic tanks and wells. *Culex tritaeniorhynchus* emerged mainly from paddy fields, wastewater pools, and masonry tanks, while *Cx. vishnui* emerged mainly from the wastewater pools. *Anopheles culicifacies* Giles was rarely found in this study.

There was a very significant reduction ($t = 4.34$; df 64; $P < 0.0001$) in the larval counts in storm drains sprayed with *B. sphaericus* as compared to the control (Fig. 1). During weekly checks, anopheline pupae were present in only 2.2% (6/276) of the samples taken from the drains treated with *B. sphaericus,* compared to 20% (14/70) of the samples from the routine treatment area. Culicine pupae were found in 11.2% (31/276) and 72.8% (51/70) of the samples from the treated and untreated drains, respectively. Since the major species emerging from drains was *Cx. quinquefasciatus, B. sphaericus* application controlled its breeding considerably. During the monsoon period (0 to 20 weeks and week 51 onward), breeding in drains was minimal in both areas, apparently due to the

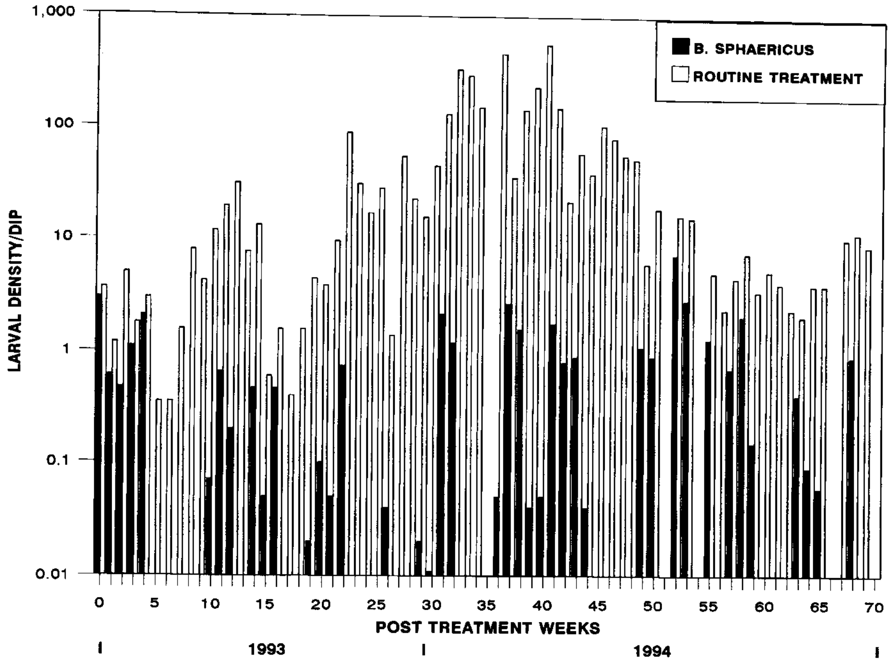

Fig. 1. Impact of spraying *B. sphaericus* @ 1 g/m² on larval counts (3rd and 4th instars) in urban storm drains. In the control routine, antilarval measures were taken by the municipality. Note that the *y* axis is on log scale.

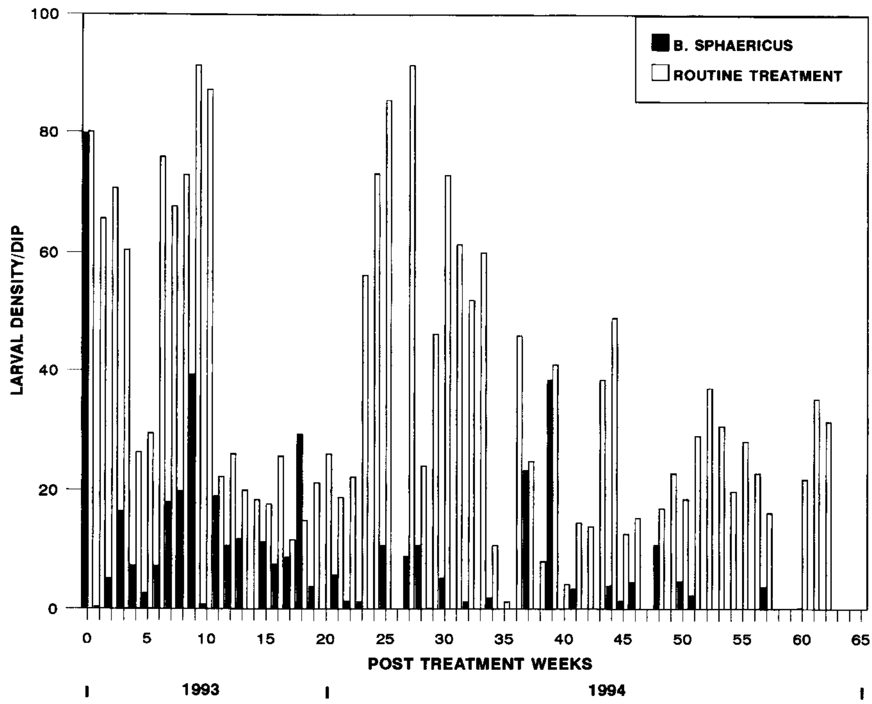

Fig. 2. Impact of spraying *B. sphaericus* @ 4 g/m² on the breeding of *Armigeres obturbans* (3rd and 4th instars) in domestic septic tanks.

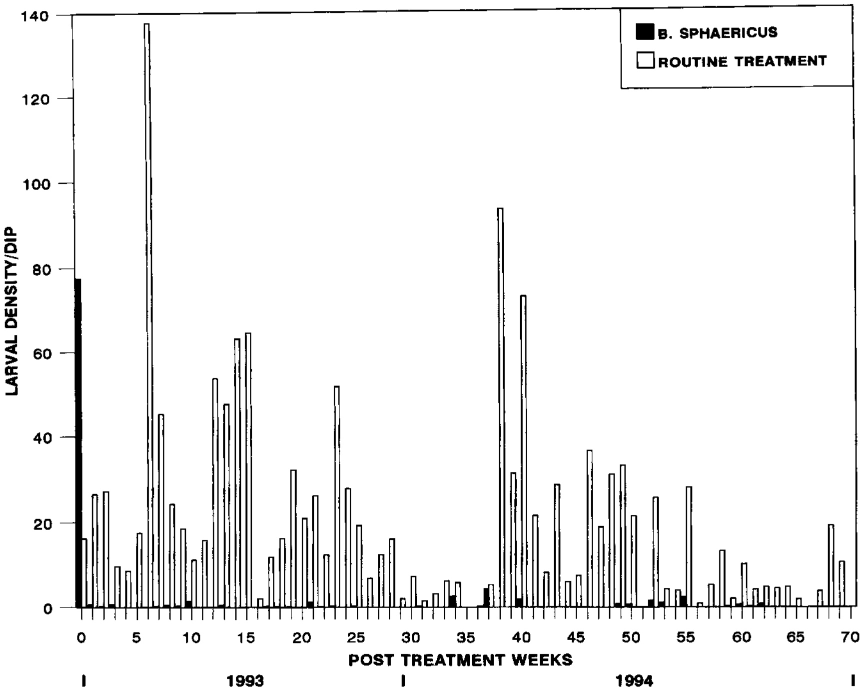

Fig. 3. Impact of spraying *B. sphaericus* @ 1 g/m² on larval counts (3rd and 4th instars) in wastewater pools.

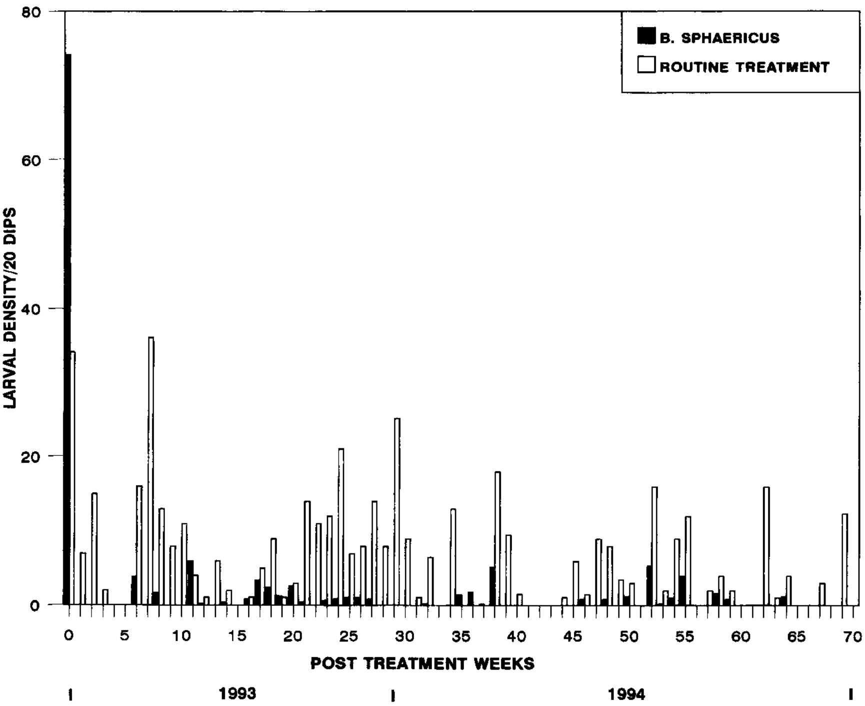

Fig. 4. Impact of spraying *B. sphaericus* @ 1 g/m² on larval counts (3rd and 4th instars) in paddy/fallow fields. Crop period was from week 10 to week 27 and beyond 62 weeks.

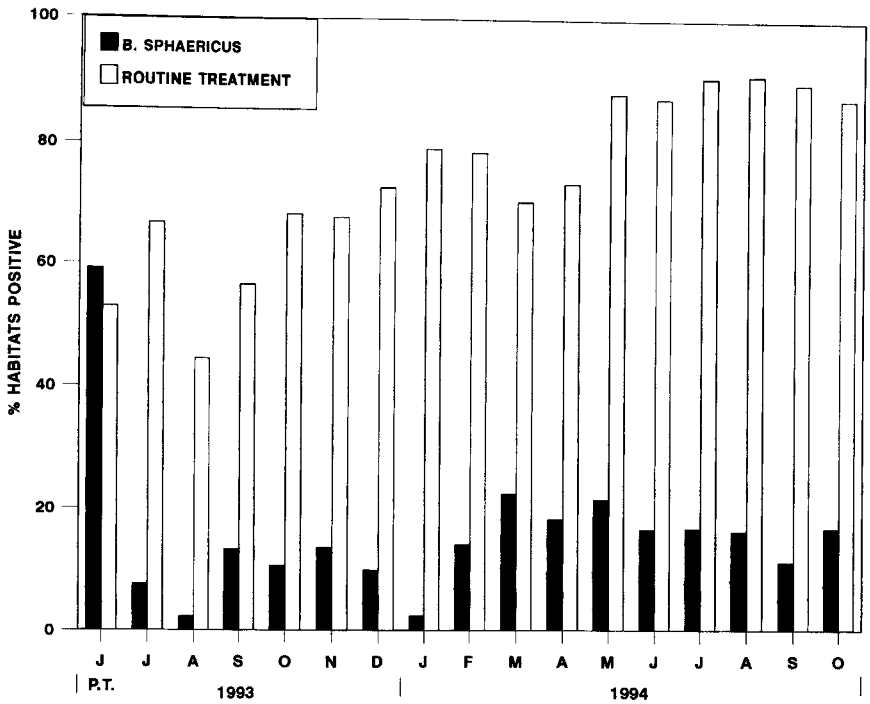

Fig. 5. Percentages of permanent larval habitats positive for 3rd/4th instar larvae in the area sprayed with *B. sphaericus* compared with those in the routine treatment area.

Table 2. Impact of the application of *Bacillus sphaericus* strain B-101 (*Bs*) on the indoor density of disease vectors (*Culex quinquefasciatus, Cx. tritaeniorhynchus,* and *Cx. vishnui*) and all mosquitoes compared with routine treatment.

		Man-hour density			
		Vectors		All mosquitoes	
Months	Rainfall (mm)	*Bs*	Routine treatment	*Bs*	Routine treatment
May 1993[1]	34.8	23.0	30.5	33.0	30.5
June[1]	212.6	58.9	64.1	68.2	84.1
July	256.4	92.1	118.4	95.6	149.4
August	548.6	55.7	74.7	74.5	162.5
September	282.4	52.0	84.8	58.0	148.3
October	94.0	42.4	71.1	44.4	99.2
November	0	45.4	67.9	48.0	184.7
December	0	33.0	140.9	34.9	146.7
January 1994	17.2	60.2	163.3	61.4	174.7
February	25.6	69.2	288.8	71.5	292.6
March	0	70.9	242.1	72.6	254.8
April	35.6	50.8	145.8	52.0	149.0
May	63.4	22.5	83.3	23.3	88.4
June	475.2	16.1	30.7	16.9	54.7
July	871.7	58.2	103.3	61.4	121.1
August	451.8	55.2	64.8	58.7	89.4
September	336.6	49.0	50.6	48.2	64.8
October	66.6	59.0	70.1	61.5	78.8
November	29.6	72.5	109.5	75.5	114.0

[1] Pretreatment densities.

flushing effect of rains. However, during the postmonsoon period, larval density increased considerably in the routine treatment area, although it remained largely nil in the *B. sphaericus* treated drains. The reapplication interval for drains was 3 weeks (median value).

Application of *B. sphaericus* reduced the breeding of *Cx. quinquefasciatus* in septic tanks very greatly, i.e., from the pretreatment density of 61/dip (600 ml cup) to 2.8/dip after a week of treatment. However, due to subsequent increase in the larval counts, the dose was increased to 2 g/m^2 during weeks 3 and 4, and thereafter to 4 g/m^2, which produced good results. The impact on the breeding of *Armigeres obturbans* Walk in septic tanks was relatively lower at the initial dose of 1 g/m^2 (Fig. 2); however, the enhanced dose of 4 g/m^2 caused a significant reduction in larval counts (t = 9.59; df 57; $P < 0.0001$). *Armigeres* pupae were found in 23.4% (29/124) of the samples taken from the sprayed tanks during weekly surveys, compared to 45.2% (28/62) of the samples from those treated routinely. However, reapplication of *B. sphaericus* to septic tanks was required after 7-(*Armigeres*) or 14-day (*Culex*) median intervals. The relatively poor activity of *B. sphaericus,* for which higher dosages were used, could be attributed to a large amount of suspended material (Mulligan et al. 1978), the depth of water in the tanks, and presumably, a high tolerance of *Armigeres* to *B. sphaericus.* Since frequent applications of *B. sphaericus* in domestic septic tanks may not be operationally economical, it is suggested that these tanks may be made mosquito-proof to obviate the need for biological or chemical control.

Application of *B. sphaericus* in pools receiving wastewater from houses or contiguous drains caused significant reduction (t = 7.18; df 65; P < 0.0001) in larval counts, thereby reducing the breeding of disease vectors and nuisance mosquitoes (Fig. 3). In 276 samples taken from the sprayed pools during weekly surveys, anopheline and culicine pupae were present in only 1.1 and 9.8% of the samples, respectively, compared to 58.5 (41/70) and 65.7% (46/70) of those from the routine treatment area, respectively. The median reapplication interval was 4 wk.

Paddy fields mostly received drainage water, which supported the breeding of *Cx. tritaeniorhynchus* besides nonvector anophelines and culicines. *Bacillus sphaericus* caused a significant reduction in larval counts (t = 6.99; df 60; P < 0.0001) in paddy/fallow fields (Fig. 4). Of the samples collected from sprayed paddy fields during weekly surveys, anopheline and culicine pupae were present in only 2 and 3.7%, respectively, compared to 14.4 and 20.5% of those from the routine treatment area, respectively. *Bacillus sphaericus* was sprayed only once at the beginning of each of the 2 monsoons, since these fields received wastewater from the drains treated with *B. sphaericus*.

Main species emerging from the larval samples from abandoned masonry tanks at construction sites were *Cx. quinquefasciatus, Cx. tritaeniorhynchus,* and *Aedes vittatus* (Bigot). Larval counts in these tanks declined very significantly after treatment with *B. sphaericus* compared to those in the routine treatment area (t = 3.76; df 60; P < 0.0001). Anopheline and culicine pupae were present in only 1.8 and 2.4% of samples from treated tanks, respectively, compared to 14.4 and 20.5% of those from the routine treatment area, respectively. The median reapplication interval was 4 wk.

During the pretreatment period in June 1993, the percentages of permanent habitats, i.e., wastewater pools, storm drains, and masonry tanks with mosquito breeding, in the routine treatment and proposed spray areas were almost comparable. However, *B. sphaericus* applications reduced the percentages of positive mosquito habitats (Fig. 5) very significantly (t = 21.04; df 15; P < 0.0001) compared to those in the routine treatment area. The pH of water in larval habitats ranged from 6.5 to 7.5, which was suitable for the application of the biolarvicide. There was a drop in the indoor density of the vectors of filariasis and JE in particular, and of all mosquitoes in general, in the sprayed area (Table 2).

By using the same formulation as was used in this trial, control of the malaria vector *An. stephensi* Liston breeding in construction waters was demonstrated earlier by Kumar et al., (1994), and this trial has demonstrated successful control of filariasis and JE vectors in particular, and of mosquito nuisance in general, in an urban area.

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