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CONTROL OF *CULEX QUINQUEFASCIATUS* WITH *BACILLUS SPHAERICUS* IN VASCO CITY, GOA

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ABSTRACT. In a locality (Sada) of Vasco City, Goa, India, that was highly infested with mosquitoes, weekly spraying of *Bacillus sphaericus* (strain 101, serotype H 5a 5b) at the rate of 1 g/m^2 in polluted water habitats, *viz.*, surface drains, cess pits, cess pools, and septic tanks, resulted in a sharp decline in the immature and adult populations of *Culex quinquefasciatus*. The per man-hour adult densities, percent habitat positivity, and immature densities were significantly lower ($P < 0.001$) in the treated area compared to the control area through out the study period.

INTRODUCTION

Mosquito-borne diseases such as malaria, filariasis, Japanese encephalitis, dengue/dengue hemorrhagic fever, and yellow fever continue to afflict mankind heavily around the world, particularly in tropical and subtropical regions, despite the conserted efforts being made internationally to control these diseases. The failure to control these diseases is often attributed to technical, administrative, and political reasons. The development of resistance in mosquito vectors, clear appreciation of the long-term detrimental effects of insecticides on nontarget organisms, as well as environmental pollution caused by insecticides leading to contamination of food chains have necessitated the search for safer and viable alternatives (Davidson 1990). Biological control methods, particularly the exploitation of indigenous larvivorous fishes and microbial or "biorational" insecticides provide an attractive alternative. The distinct advantages with microbial-based control are their ability to kill target insects at low doses, safety to nontarget species, easy application in the field, inexpensive production, lack of infectivity and pathogenicity towards mammals including humans, and little evidence of development of resistance in target mosquito species (Davidson et al. 1990). The entomopathogenic bacteria that exhibit the above qualities are *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus*. *B. sphaericus* strains in the H serotype, H 5a 5b including 1593–94, 1691, 1881, and 2362, exhibit high mosquitocidal activity particularly against *Culex quinquefasciatus* Say, *Cx. pipiens* Linn., *An. stephensi* Liston, *Toxorhynchites rutilis rutilis* (Coq.), and *Aedes aegypti* (Linn.). Recently, Bhalwar et al. (1993) and Kumar et al. (1994) have found *B. sphaericus* strain B 101, serotype H 5a 5b, to be highly toxic against *Cx. quinquefasciatus* and *An. stephensi*. *Bacillus sphaericus* H6 (strain 1A 59) and H 25 (strain 2297) are also very toxic to *Cx. pipiens, Cx. quinquefasciatus,* and *An. stephensi,* but less toxic to *Ae. aegypti, Mansonia uniformis* (Theobald), and *Tx. r. rutilis* (Lacey et al. 1988, Yap et al. 1988, Thiery and de Barjac 1989).

The current longitudinal study was undertaken in 1993 and 1994 to evaluate the efficacy of *B. sphaericus* B 101 (Spherix formulation) against *Cx. quinquefasciatus* in a locality of Vasco City, Goa, India, that was highly infested with mosquitoes. The target mosquito *Cx. quinquefasciatus* develops in surface drains, cess pits, cess pools, and septic tanks. Vasco City is endemic to Bancroftian filariasis, which is transmitted by *Cx. quinquefasciatus*. The conventional means of mosquito control undertaken by the State Health Services in the city included weekly application of fenthion (Baytex®) at 1 ppm and mosquito larvicidal oil (MLO) at 20 ml/m^2.

MATERIALS AND METHODS

The field trials were conducted in the Sada locality of Vasco City in Goa state of India in an area of approximately 4 km^2 to evaluate the efficacy of *B. sphaericus* against *Cx. quinquefasciatus*. A similar area at a distance of 4 km from the experimental area was held as a control for comparison. The experimental area is located on a plateau surrounded on 3 sides (north, east and west) by the Arabian Sea. In this area, the workers of Vasco Harbour and Marmagao Port Trust mainly reside in small concrete houses. Both study areas have no discernible sewage system and the storm water drains are used to carry human waste water from houses. In the beginning of April 1993, large and small surface drains, cess pits, cess pools, and septic tanks, the major larval mosquito habitats, were treated

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with Spherix[4] at the rate of 1 g/m^2 surface area using knapsack sprayers with a flat fan nozzle.

The physical and chemical properties of the Spherix formulation used in the experiment were: particle size 90–500 μm, bulk density 0.49–0.58 g/cm^2, pH 6.05–6.40, and both endotoxins and spores constitute 14–20% of the dry weight of the formulation. The LD_{50} values of the product varied from 0.008 to 0.012 mg/liter against *Cx. pipiens* (autogenous strain) and had a potency of 450 ITU/mg against the SPH 8 standard reference powder.

Prior to the commencement of *B. sphaericus* spraying operations, the routine chemical control measures such as use of Baytex® and mosquito larvicidal oil (MLO) were withdrawn by the National Filaria Control Programme of the State Health Services. In the initial phase of the experiment, blanket spraying of Spherix was done in all the polluted water habitats in the experimental area irrespective of their breeding status. Subsequently, however, weekly application was done only in the positive habitats detected during larval surveillance. The densities of immatures were estimated weekly in all the habitats using 300-ml ladles in the surface drains and septic tanks and with 300-ml bowls in the cess pits and cess pools both in the experimental and control areas. A definite sampling plan was followed for different breeding habitats. The number of samples varied with the surface area of the breeding habitat; five samples for an area up to 5 m^2, 10 samples for an area from 5 to 10 m^2, and 20 samples for an area greater than 10 m^2. In surface drains, samples were taken at distances of 1 m along the linear length of the drain. In septic tanks and cess pits, which were mostly less than 5 m^2 in area, 4 samples were taken in the corners and one in the center. In cess pools, the sampling for immatures was done along the periphery where the maximum larval density was encountered.

The data collected on densities of immatures for different weeks of a particular month was pooled separately for instars I + II, instars III + IV, and pupae and averaged. Similarly, habitat positivity was calculated separately for the experimental and control areas. Furthermore, to evaluate the impact of the Spherix spraying program on adult populations of *Cx. quinquefasciatus,* mosquito collections were conducted in both experimental and control areas every 2 wk between 0500 and 0730 h using aspirators. Each of the 4 insect collectors employed collected adult mosquitoes for 15 min nonstop in 8 structures in a given locality. Thus, the 4 insect collectors spent a total of 8 h collecting mosquitoes in 32 structures per day. The mosquitoes collected from each structure were kept in a separate test tube that was suitably labelled. The mosquitoes were etherized in the laboratory and identified using keys of Barraud (1934). The per man-hour (pmh) density of adult mosquitoes was calculated using the following formula:

$$\text{pmh density} = \frac{\text{No. of adult } Cx.\ quinquefasciatus \text{ females collected}}{\text{Total collecting time in minutes}} \times 60.$$

For the statistical analysis, Student's *t*-test for differences between paired means was applied.

RESULTS AND DISCUSSION

Impact of B. sphaericus *on densities of immatures*: The estimation of the habitat positivity and densities of immatures was done from 11,879 habitats in the experimental area and 4,495 habitats in the control area (Table 1). During the pretreatment phase, the percentage of habitats found to be positive was 66.4% in the experimental area; this declined to 28.0% during the first month (April 1993) of Spherix spraying. In the subsequent months, from May 1993 to March 1994, the Spherix had a much greater impact with the percentage of positive habitats ranging from 2.7 to 28.0% with a mean of 5.9%. In contrast, in the control area, the percentage of habitats found to be positive was 23.9%, corresponding to the pretreatment phase of the experimental area; this increased to 40.7% by May 1993 and up to March 1994, ranged from 10.5 to 40.7%, with a mean habitat positivity of 20.3%. The difference in the percentage of positive habitats between the control and the experimental area was highly significant ($t = 5.8$, $P < 0.001$) over the entire study. Similarly, the densities of the total immatures in the positive habitats declined from 38.7 per dip in the pretreatment phase and ranged from 0.4 to 13.0 per dip from April 1993 to March 1994. In comparison, the densities of immatures increased in the control area from 115.1 per dip during the pretreatment phase to 141.4 per dip in April 1993 and ranged from 28.3 to 141.4 per dip in the corresponding period. These densities were also significantly higher than those observed in the experimental area ($t = 5.09$, $P < 0.001$) over the entire study. As is apparent from Table 1, the spraying of Spherix produced a noticable impact on the densities of higher instars ($t = 5.22$,

[4] The biolarvicide was manufactured by the Berdsk Plant of Biological Preparation, Russia, and supplied under the trade name of Spherix through the courtesy of the Ministry of Health and Family Welfare, Delhi, India.

Table 1. Comparison of percent habitat positivity and per dip densities of culicines (in positive as well as total habitats) between the experimental area where *Bacillus sphaericus* (Spherix formulation) was sprayed at 1 g/m² and the control area.

Month	% habitats positive: Experimental (*n*)	% habitats positive: Control (*n*)		Density per dip: Spherix experimental area: Total dips	Spherix experimental area: No. immatures	Control area: Total dips	Control area: No. immatures
Pretreatment (March and up to April 18, 1993)	66.4 (301)	23.9 (197)	1[1]	1,380	38.7	125	115.1
			2[2]	6,070	8.8	555	25.9
Post-treatment (April 19 onwards)	28.0 (327)	32.9 (310)	1	625	13.0	150	141.4
			2	2,253	3.6	620	34.2
May	6.6 (515)	40.7 (455)	1	255	1.0	150	132.4
			2	4,705	0.1	565	35.1
June	3.7 (488)	28.4 (423)	1	145	0.4	220	85.9
			2	7,760	0.01	930	20.3
July	2.7 (1,080)	25.7 (588)	1	290	2.4	350	49.9
			2	8,506	0.1	1,760	9.9
August	2.9 (761)	15.2 (335)	1	130	9.4	355	41.8
			2	8,715	0.1	1,885	7.9
September	4.8 (874)	16.6 (328)	1	335	7.8	210	28.3
			2	10,316	0.3	1,690	3.5
October	4.2 (1,190)	18.3 (284)	1	625	6.7	210	38.9
			2	14,855	0.3	1,660	4.9
November	4.5 (1,190)	14.5 (276)	1	390	5.3	115	34.0
			2	14,397	0.1	1,295	3.0
December	3.6 (1,421)	16.0 (388)	1	420	9.2	80	37.7
			2	17,340	0.2	1,325	2.3
January 1994	4.3 (984)	13.8 (291)	1	585	9.6	150	54.1
			2	11,715	0.5	995	8.2
February	2.9 (1,327)	10.5 (304)	1	415	13.0	195	80.0
			2	15,950	0.3	1,825	8.6
March	2.9 (1,421)	10.8 (316)	1	370	11.4	185	46.7
			2	15,611	0.3	1,660	5.2

[1] 1 = Positive habitats alone.
[2] 2 = All habitats.

$P < 0.001$) when the figures of the experimental area were compared with those of the control area.

Similar trends were witnessed when the data were analyzed using all the habitats within each experimental area, both negative as well as positive, for the estimation of densities of immatures (Table 1). The density of total immatures, which was 8.8 per dip during the pretreatment period in the experimental area, declined substantially and the overall mean ranged from 0.05 to 3.6 per dip during the posttreatment phase from April 1993 to March 1994. This mean density was significantly less than that of the control area ($t = 3.56$, $P < 0.01$). Concurrently, numbers of instar I + II larvae ($t = 3.49$, $P < 0.01$), III + IV larvae ($t = 3.5$, $P < 0.01$), and pupal production ($t = 4.0$, $P < 0.001$) declined significantly during the posttreatment phase in the experimental area when compared with the control area (Table 1).

Impact on densities of adult Cx. quinquefasciatus: The results of collections of adults are shown in Fig. 1. In the pretreatment phase during February and March 1993, the pmh densities of *Cx. quinquefasciatus* were 76.7 and 55.2, respectively, in the experimental area and 46.5 and 32.3 in the control area. During the posttreatment phase, however, there was a sudden impact starting in the month of May as the densities of adult *Cx. quinquefasciatus* declined from 44.1 pmh in April to 7.3 pmh in June and ranged from 7.3 to 44.1 pmh during the posttreatment phase. In comparison, the control area had an

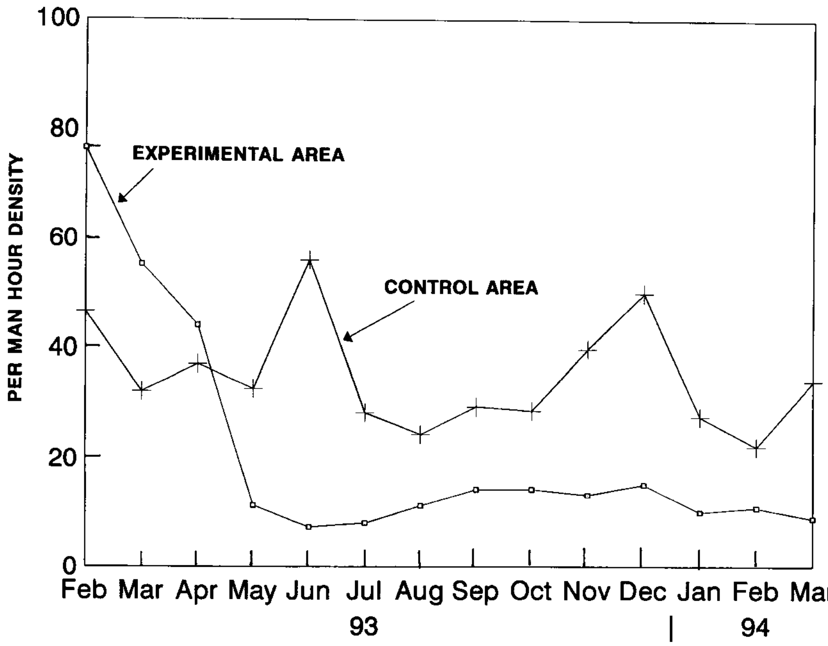

Fig. 1. Comparison of per man-hour densities of *Culex quinquefasciatus* in the preapplication and postapplication phases of the experimental (Spherix-sprayed) and the control area.

increase in densities of adults to 56.0 pmh in June and the density ranged from 22.2 to 56.0 pmh in the posttreatment months from April 1993 to March 1994. The difference in the densities of adult *Cx. quinquefasciatus* between the experimental and control area was highly significant ($t = 5.16$, $P < 0.001$).

It is evident that the weekly spraying of Spherix in major breeding sites of *Cx. quinquefasciatus* resulted in a significant drop in the habitat positivity as well as densities of immatures in the experimental area. The impact of the *B. sphaericus* spraying program on the *Cx. quinquefasciatus* population became apparent within a month after the commencement of Spherix spraying, that is, from May 1993 onwards. Moreover, the effective control of *Cx. quinquefasciatus* could be sustained even with selective spraying of the positive habitats, which could greatly reduce cost of application and material. In the control area, on the other hand, the trend was reversed as both percent habitat positivity and densities of immatures were significantly higher throughout the study period. The density of adult *Cx. quinquefasciatus,* which was much higher in the experimental area during the pretreatment phase, also dropped significantly by May 1993 and remained more or less static throughout the study period.

In a recent study malaria control has been demonstrated utilizing the Spherix formulation against *An. stephensi* (Kumar et al. 1994). Earlier, Bhalwar et al. (1993) had found Spherix to be highly effective against *Cx. quinquefasciatus.* Thus, Spherix formulations can be utilized in the major action program aimed at control of Bancroftian filariasis. The possibility of development of resistance in mosquitoes against this biolarvicide appears to be remote (Davidson 1988). Moreover, the bacterial toxins are considered to be environmentally safe (Mulla 1990, Porter et al. 1993) and also they have been found to be cost-effective as compared to MLO, temephos, and Paris green (Balaraman and Hoti 1987).

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