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Field evaluation of biolarvicides in Surat city, India

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Background & objectives : Two bacterial larvicide (bio-larvicide) formulations—Bacticide® and VectoBac® containing viable endospores and delta endotoxin of *Bacillus thuringiensis* var *israelensis* H-14 were evaluated in 2001 for their mosquito larvicidal efficacy under the operational conditions of urban malaria control programme in Surat city, India.

Methods : Larvicides were applied at the recommended dose in selected breeding habitats of *Anopheles* (*An. stephensi*), *Aedes* (*Ae. aegypti*) and *Culex* (*Cx. quinquefasciatus*) and reductions in the densities of III and IV instars were compared with that of untreated matched controls.

Results : At the construction sites in cemented tanks/chambers VectoBac produced reduction in the density of III and IV instar larvae of *An. stephensi* (98–100%) and *Ae. aegypti* (100%) in the first week of application whereas Bacticide produced 71–100% reduction in *An. stephensi* and 100% in *Ae. aegypti*. Re-application of VectoBac on Day 10 caused better control up to Day 20 when compared with Bacticide. In stagnant water pools, VectoBac produced 27.6–85.3% reduction in the larvae of *An. subpictus* and 18.5–83.8% in those of *Cx. quinquefasciatus* whereas Bacticide produced 23.3–30.3% and 39–97.2% reduction in *An. subpictus* and *Cx. quinquefasciatus* larval densities in the first week post application, respectively. Bacticide application gave better impact on *Cx. quinquefasciatus* larvae in the second week after re-application as compared to VectoBac. In storm water drains, VectoBac caused respectively 6.2–100% and 6.4–97.6% reduction in *An. subpictus* and *Cx. quinquefasciatus* larvae in the first week of application whereas Bacticide produced 100% and 13.3–98.8% reduction in *An. subpictus* and *Cx. quinquefasciatus* larval densities, respectively.

Interpretation & conclusion : Both the formulations were equally effective on *An. subpictus* and *Cx. quinquefasciatus* larvae after a second application. The results showed that application of these biolarvicides would be required at 7–10 day intervals. The health workers engaged in the application of biolarvicides reported a better ease of handling and application of the liquid formulation (VectoBac) than the wettable powder formulation (Bacticide).

Key words Biolarvicides – *Bti* – India – mosquito control – urban area

In urban settings, the vectors of malaria and dengue—*Anopheles stephensi* Liston and *Aedes aegypti* (Diptera : Culicidae) respectively co-breed in a wide variety of man-made habitats, water storage containers, ornamental tanks, construction-related water and wells. At present, there is no single and effective method of vector control available in most of these situations. Thus there is a need to incorporate new technologies for vector control to develop a comprehensive vector control programme for urban areas. Among the alternatives to larvicides, many strains of spore forming bacteria (*Bacillus*) have been proved useful against different mosquito species[1-5] and found environmentally safe[6,7]. One of the potent strains of bacterium—*Bacillus thuringiensis* var *israelensis* (*Bti*) H-14 has been found effective against all mosquito genera. Several commercial formulations of *B. thuringiensis* are now available in the market for use against mosquito vectors. The selection of these biological control agents is based on their host specificity that allows minimum disturbance to non-target organisms and the environment.

Surat city is endemic for malaria, dengue and filariasis. In 2001, Surat contributed more than 15% of all malaria cases and 27% of *Plasmodium falciparum* cases in Gujarat state (unpublished data, Deptt. of Health, Govt. of Gujarat, India). The health department of the Surat Municipal Corporation implements a mosquito-borne disease control programme. Although this programme heavily relies on the use of chemical larvicides and insecticides, since 2000 there has been a persistent effort in reducing reliance on the use of insecticides and incorporate eco-friendly methods such as environmental management and biological control. Towards this, in 2001 the city incorporated the use of two commercially available formulations of *Bti*—Bacticide? WP (wettable powder) and VectoBac? 12AS (liquid formulation) in vector control programme. This paper reports results of the operational effectiveness of these larvicides for control of *Anopheles, Culex* and *Aedes* mosquitoes in Surat city.

Material & Methods

For field evaluation Bacticide® was supplied by M/s. Biotech International Limited, Delhi and VectoBac® by M/s. Aventis CropSciences India Ltd., Mumbai to the Surat Municipal Corporation.

Preferable breeding habitats of *Anopheles* (*An. stephensi*), *Aedes* (*Ae. aegypti*) and *Culex* (*Cx. quinquefasciatus* Say) were surveyed and 46 potential habitats were selected. These included 18 cemented tanks/chambers at construction sites (50.9 m^2) supporting breeding of *An. stephensi* and *Ae. aegypti*, 17 stagnant water pools (1292.8 m^2) and 11 storm water drains (554.4 m^2) supporting breeding mainly of *Cx. quinquefasciatus* and *An. subpictus*. VectoBac was sprayed in 16 sites (504.1 m^2) and Bacticide in 15 sites (697 m^2). Fifteen unsprayed sites (696.9 m^2) were run as controls for comparison. Experimental and control sites were proportionally included. Further details of the breeding habitats are given in Table 1. Biolarvicides were applied with the help of knapsack sprayers with flat-fan nozzle after diluting the required quantity and applied as recommended by the manufacturers.

Table 1. Details of mosquito breeding habitats selected for the biolarvicide trial

Habitats	VectoBac®	Bacticide®	Untreated control
Cemented tanks and chambers at construction sites	5 (14.1)	5 (13.3)	8 (23.4)
Stagnant water pools	7 (379)	6 (558.8)	4 (355)
Storm water drains	4 (111)	4 (124.9)	3 (318.5)
Total	16 (504.1)	15 (697)	15 (696.9)

Figures in parentheses indicate area in square metre.

In clean waters (cemented tanks and chambers at construction sites), Bacticide was applied @ 5 kg/ha (0.5 g/m^2) and VectoBac was applied @1 l/ha (0.1–0.2 ml/m^2). In polluted waters (stagnant pools and storm water drains), Bacticide was applied @ 10 kg/ha and VectoBac was applied @ 2 l/ha.

To prepare the solution for spraying, 50 g (or 100 g in polluted water) of Bacticide powder was suspended in 10 litres of water to cover 100 m^2 of water surface— (100 ml/m^2). For application in clean water, 50 ml of VectoBac formulation (or 100 ml in polluted water) was diluted in 10 litres of water and applied @ 20 ml/m^2. Mosquito larval/pupal densities were measured on Day 0 taking 5 dips using a standard larval dipper of 300 ml capacity. Post-treatment densities were monitored on Day 1, 2, 3, 7 and 10 and thereafter on Day 17 and 20. On the basis of the re-appearance of III and IV instar larvae or pupae, a second round of biolarvicide application was carried out. Data were analysed to measure reduction in the average densities of III and IV instars in comparison with untreated controls using the following formula:

$$\text{Percent reduction} = 100 - [(C_1/T_1) \times (T_2/C_2)] \times 100$$

Where, C_1 and C_2 are densities of III and IV instars in untreated control on Day 0 and on subsequent days of monitoring; and T_1 and T_2 in treated habitats before and after treatment respectively[8].

Results

The overall results of the trial have been summarised and given in Tables 2–4. In cemented tanks/chambers at construction sites VectoBac produced 98–100% reduction in the density of III and IV instar *An. stephensi* and 100% reduction in larvae of *Ae. aegypti* during the first week of application. Bacticide caused 71–100% reduction in *An. stephensi* and 100% reduction in *Ae. aegypti* larvae. Percent reduction in larval density was higher (94.1%) in VectoBac treated habitats as compared to Bacticide (51.5%) on Day 10. VectoBac was more effective on *An. stephensi* larvae.

Table 2. Mean number of III and IV instars per dip and percent reduction in comparison to untreated control in cemented tanks and chambers at construction sites

Day	Untreated control	VectoBac®		Bacticide®	
		Treated	% reduction	Treated	% reduction
Target species: *An. stephensi*					
0	2.6	2.4	–	2	–
1	3.1	0	100	0.2	93.7
2	3.4	0	100	0	100
3	4.5	0	100	0	100
7	8	0.1	98.7	1.7	71.8
10*	6.5	0.4	94.1	2.4	51.5
17	5.7	0.8	84.3	4.4	0.4
20	3.9	1.6	57.3	5.2	**
Target species: *Ae. aegypti*					
0	3	1.6	–	0.8	–
1	3.2	0	100	0	100
2	1.2	0	100	0	100
3	2.1	0	100	0	100
7	0.6	0.7	**	0	100
10*	1.6	0.8	6.3	0	100
17	0.8	0.2	53.1	0	100
20	1	0.5	6.3	0	100

*Re-application of biolarvicides; **Not calculated since densities in treated sites were higher than those in the control.

In stagnant water pools, VectoBac produced 27.6–85.3 and 18.5–83.8% reduction in *An. subpictus* and *Cx. quinquefasciatus* larval densities respectively in the first week of post application. Bacticide produced 23.3–30.3 and 39–97.2% reduction in *An. subpictus* and *Cx. quinquefasciatus* larval densities respectively. Bacticide application showed better impact on *Cx.*

Table 3. Mean number of III and IV instar larvae per dip and percent reduction in comparison to untreated control in stagnant water pools

Day	Untreated control	VectoBac®		Bacticide®	
		Treated	% reduction	Treated	% reduction
Target species: *An. subpictus*					
0	20.8	14.9	–	5.8	–
1	14.4	1.5	85.3	2.8	30.3
2	13.4	7	27.6	2.1	43.8
3	18	5.1	60.7	3.9	22.3
7	14.8	5	52.9	16.6	**
10*	6.8	2.4	50.8	8.4	**
17	9.2	2.9	56.7	12.6	**
20	12.9	4.5	51.8	8.4	41.9
Target species: *Cx. quinquefasciatus*					
0	11.7	14	–	20.5	–
1	9.5	1.8	83.8	0.5	97.2
2	13.8	6.1	63.1	3.3	86.2
3	5.4	3.2	49.9	5.8	39
7	12.2	11.9	18.5	6.8	67.9
10*	8.8	8	24.2	3.8	75.6
17	19.6	5.6	76.1	6.8	80.2
20	23.4	8.1	70.9	9.4	76.9

*Re-application of biolarvicides; **Not calculated since densities in treated sites were higher than those in the control.

quinquefasciatus larvae in the second week after application on Day 10 as compared to VectoBac. However, higher larval densities of *An. subpictus* were recorded in Bacticide treated habitats as compared to untreated controls within one week of application. Whereas in VectoBac treated habitats, the larval densities remained low as compared to untreated controls throughout the trial period.

In storm water drains, VectoBac caused 6.2–100% and 6.4–97.6% reduction in *An. subpictus* and *Cx. quinquefasciatus* larvae respectively in the first week of post application. Bacticide produced 100% and 13.3–98.8% reduction in *An. subpictus* and *Cx. quinquefasciatus* larval densities respectively. Both the formulations were equally effective against *An. subpictus* larvae after second application on Day 10. Similar impact on *Cx. quinquefasciatus* larvae was also observed with both the formulations.

Based on the experience of field staff Bacticide powder was found to be cumbersome to measure in field,

Table 4. Mean number of III and IV instar larvae per dip and percent reduction in comparison to untreated control in storm water drains

Day	Untreated control	VectoBac®		Bacticide®	
		Treated	% reduction	Treated	% reduction
Target species: *An. subpictus*					
0	39.6	7.6	–	63	–
1	31.4	6.7	11.2	0	100
2	20.6	4.2	6.2	0	100
3	24.6	2.6	44.9	0	100
7	21	0	100	0	100
10*	35	0	100	0	100
17	15.8	0	100	0	100
20	5.8	0	100	0	100
Target species: *Cx. quinquefasciatus*					
0	33.3	24.3	–	34.4	–
1	85.5	1.5	97.6	1.1	98.8
2	71.3	2.2	95.8	3.2	95.7
3	33.9	5.2	79	7.5	78.6
7	32.5	22.2	6.4	29.1	13.3
10*	20.3	7	52.7	22.6	**
17	21.7	9.8	38.5	11.8	47.7
20	17.9	10	23.6	6.4	65.4

*Re-application of biolarvicides; **Not calculated since densities in treated sites were higher than those in the control.

mix in lukewarm water, while VectoBac was easy to use on these counts and the spray staff did not perceive any adverse reaction during its handling and use. Bacticide powder had a slightly unpleasant smell and caused slight skin irritation in case of three spray staff as reported in the questionnaire to mention their field perceptions.

Discussion

The renewal of interest in the integrated methods of vector control during the early 1980s has revived the use of environmental friendly approaches in vector control such as the biological control. In recent years, efficacy trials of various biolarvicides in India have shown their potential in the control of malaria[1], dengue[2], filariasis and Japanese encephalitis vectors[9,10]. Biolarvicides have been found cost-effective in comparison to the conventional larvicides—fenthion, temephos, paris green and malaria oil being used in vector control as the cost of larval control for four weeks with *B. thuringiensis* H-14 has been calculated to US $ 67.38 as compared to the cost for conventional larvicides (US $ 67–159)[11] . However, most of these trials were not done under the conditions of an operational vector control programme. This study, however, reports evaluation of the biolarvicides by the Surat Municipal Corporation in their vector control programme and amounts to a programmatic evaluation of the effectiveness of biolarvicide formulations.

The study showed that biolarvicides, VectoBac and Bacticide would be required to be used at an interval of about 7–10 days depending upon the quality of water in natural habitats. Between the two formulations, the liquid formulation (VectoBac) had a relative ease of operation.

Based on the results of this trial, it may be concluded that biolarvicides can be incorporated as a part of an integrated vector control programme in urban areas but have a potential for use in industrial, port and project areas where antilarval system is available or is feasible to organise. Biolarvicides may be selectively used in place of chemical larvicides or integrated with use of larvivorous fish.

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References

1. Ansari MA, Sharma VP, Mittal PK, Razdan RK, Batra CP. Evaluation of *B. sphaericus* to control breeding of malaria vectors. *Indian J Malariol* 1989; *26* : 25–31.
2. Ansari MA, Razdan RK. Laboratory and field evaluation of *Bacillus thuringiensis* H-14 (*Bti* H-14) granule formulation against *Aedes aegypti* in Delhi, India. *Dengue Bull* 1999; *23* : 94–8.
3. Dua VK, Sharma SK, Sharma VP. Application of Bactoculicide (*B. thuringiensis* H-14) for controlling mosquito breeding in industrial scrap at BHEL, Hardwar (U.P.). *Indian J Malariol* 1993; *30* : 17–21.
4. Kumar A, Sharma VP, Thavaselvam D, Sumodan PK. Control of *Anopheles stephensi* breeding in construction sites and abandoned overhead tanks with *Bacillus thuringiensis* var *israelensis*. *J Amer Mosq Contr Assoc* 1995; *11* : 86–9.
5. Mittal, PK, Pant CS, Basil A, Jayaraman K, Sharma VP. Evaluation of the formulations of the mosquito larvicidal agent Biocide-S from *Bacillus sphaericus* 1593 M. *Indian J Malariol* 1985; *22*: 71–6.
6. Mulla, MS. Activity, field efficacy and use of *Bacillus thuringiensis* var *israelensis* against mosquitoes. In : de Barjac H, Sutherland DJ, editors. *Bacterial control of mosquitoes and blackflies: biochemistry, genetics and application of Bacillus thuringiensis and Bacillus sphearicus*. New Brunswick NJ : Rutgers University Press 1990; p. 34–60.
7. Porter GA, Davidson EW, Liu J. Mosquitocidal toxins of bacilli and their genetic manipulation for effective biological control of mosquitoes. *Microbiol Rev* 1993; *57*: 838–61.

8. Mulla MS, Nodlard RL, Eanara DM, Darwazeh HA, Mckean DW. Control of chironomid midges in recreational lakes. *J Econ Entomol* 1971; *264* : 300–7.

9. Kumar A, Sharma VP, Thavaselvam D, Sumodan PK, Kamat RH, Audi SS, Surve BN. Control of *Culex quinquefaciatus* with *Bacillus sphaericus* in Vasco City, Goa. *J Amer Mosq Contr Assoc* 1996; *12* : 409–13.

10. Yadav RS, Sharma VP, Upadhayay AK . Field trial of *Bacillus sphaericus* strain B-101 (Serotype H5a, 5b) against filariasis and Japanese encephalitis vectors in India. *J Amer Mosq Contr Assoc* 1997; *13* : 158–63.

11. Balaraman K, Hoti SL. Comparative cost of mosquito control with larvicidal bacilli and insecticides. *Indian J Malariol* 1987; *24* : 131–34.

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