Laboratory and Field Evaluation of *Bacillus thuringiensis H-14 (*Bt.H-14) Granule Formulation* Against *Aedes aegypti* in Delhi, India‡

By

M.A. Ansari and R.K. Razdan

Malaria Research Centre Indian Council of Medical Research, 20, Madhuban, Delhi-110 092 (India)

Abstract

Laboratory and field evaluations of Bt.H-14 granule formulation were carried out against *Aedes aegypti,* the vector of dengue and dengue haemmorhagic fever in Delhi, India. The results of laboratory evaluation revealed 100% mortality of *Aedes aegypti* larvae @ 0.5 gm/m^2 within 24 hours of exposure in enamel trays. The field evaluation revealed that biolarvicide @ 0.5 gm/m^2 provided an effective control of this species for more than four weeks in evaporation coolers and disused tyres.

Key words: Vector control, *Aedes aegypti*, Bt.H-14, Evaporation coolers, disused tyres

Introduction

Aedes aegypti is the vector of dengue, dengue haemmorhagic fever (DHF) and dengue shock syndrome (DSS). The control of this species by space spraying of chemical insecticides has not been successful because of deeper and secure resting habits of the species. Sustainable and cost-effective control can only be achieved by eliminating the breeding places using eco-friendly technologies with the help of civic organizations and involvement of the community. Earlier surveys carried out in the Delhi National Capital Region revealed that the maximum amount of breeding of *Aedes*

* Bt.H-14 granule formulation was received through the courtesy of Hoechst AgrEvo Ltd., Hoechst Centre, 54A, Andheri Kurla Road, Post Box No. 9473, Andheri (East), Mumbai-400 093 (India)

‡ *Aedes aegypti* larvae are known to feed at sides and bottom of the containers. The containers although are of various sizes/shapes, but the volume of water can be calculated. Bti formulation under this study is generally for surface feeders, however the authur has evaluated it against *Aedes aegypti* larvae - Editor.

aegypti takes place in evaporation coolers in domestic and disused tyres and other rain-filled receptacles in peri-domestic situations during the monsoon and post-monsoon periods[1,2]. Bt.H-14 formulations have been found to be effective against mosquitoes in general and against *Aedes aegypti* in particular[3,4]. The Bt.H-14 has been reported to be safe for humans when the biolarvicide is used in potable water in normal dosages[5]. However, normal formulations of Bt.H-14 tend to settle rapidly at the bottom of water bodies and may require frequent applications. Recently new slow-release formulations have been developed to prolong the larvicidal activity, particularly for those breeding places which are not easily and frequently accessible. In view of this, studies were initiated to evaluate Bt.H-14 granule formulation under laboratory and field conditions against *Aedes aegypti.* Results of this study are presented in this paper.

Materials and methods

Laboratory-reared larvae of *Aedes aegypti*[6] were used for bioassay tests. Twenty-five late IInd and IIIrd instar larvae were introduced in enamel trays (15x20 cm) containing about 500 ml water and 100 mg of larval food. Bt.H-14 @ 250, 500 and 1000 mg/m^2 was applied on the water surface. The experiment was repeated 10 times when each dosage and larval mortality was recorded at 24, 48 and 72 hours post-exposed period. Corrected mortality was calculated by using the Abbott formula.

The Mehrauli rural circle No. 141, South zone, was selected for field trials of the biolarvicide in evaporation coolers, and Mayapuri (Motia Khan) circle no.8, West zone, was selected for discarded tyres. Selection of the localities was decided on the basis of the breeding potential and operational convenience. The trials were conducted during August-October 1997 in 20 experimental and 4 control coolers and 36 experimental and 6 control tyres. The mean larval density was calculated on the basis of 5 dips/cooler and 6 pipette dips per tyre. Prior to the experiment the surface areas of coolers and tyres were measured along with the pre-spray density of larvae. The biolarvicide was applied @ 250, 500 and 1000 mg/m^2 in both coolers and unused tyres and the post-spray density of I-II and III-IV instar larvae was recorded after 24 hours. Successive observations were made at an interval of one week. The percentage reduction was calculated by the following formula earlier described by Mulla[7]:

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

where C_1 and T_1 are the pre-treatment density and T_2 and C_2 are the post-treatment density of III+IV instar larvae per dip in the control and treated habitats, respectively.

Results

Laboratory evaluation

The results of laboratory evaluation revealed that the formulation had shown a high larvicidal activity against immature mosquitoes. Of the three species tested, *Aedes aegypti* was the most susceptible followed by *Cx. quinquefasciatus* and *An. stephensi*. Bt.H-14 granule formulation @ 0.25 gm/m^2 produced 100% mortality

among all the three species of mosquitoes within 24 hours of post exposure. A lower dosage, i.e. 0.125 gm/m^2, also produced a 100% mortality in *Aedes aegypti* within 24 hours as against 94.0% and 98.0% mortality after 72 hours of post-exposure period for *An. stephensi* and *Cx. quinquefasciatus* respectively (Table 1).

Table 1. *Bio-efficacy of Bt.H-14 granule formulation against larvae of Aedes aegypti under field conditions*

Dose in Mg/m^2	% reduction after				
	24 hrs	I week	II week	III week	IV week
1000	100.0	100.0	100.0	100.0	98.5
500	100.0	100.0	100.0	100.0	97.0
250	100.0	100.0	100.0	92.0	82.0
Control	0.0	0.0	0.5	0.0	0.0

Note: Five replicates of each dose were made in enamel trays consisting of 200 IInd and IIIrd instar larvae.

Table 2.*Bio-efficacy of vectobac granules formulation against Aedes aegypti* in evaporation coolers under field conditions

Day/ Week	I + II (Instar)		III + IV (Instar)		% Reduction
	E	C	E	C	
@ 1.0 gm/m^2					
Pre-0 day	2.8	2.0	2.0	1.5	-
I day	0.08	1.5	0.07	1.7	97.0
I week	0.0	2.4	0.0	1.7	100.0
II week	0.0	3.3	0.0	2.09	100.0
III week	0.0	3.2	0.0	2.5	100.0
IV week	0.0	2.1	0.0	2.9	100.0
@ 0.5 gm/m^2					
Pre-0 day	1.3	2.0	0.8	1.5	-
Post-I day	0.1	1.5	0.13	1.7	86.3
I week	0.0	2.4	0.0	1.7	100.0
II week	0.0	3.3	0.0	2.09	100.0
III week	0.0	3.2	0.0	2.5	100.0
IV week	0.0	2.1	0.0	2.9	100.0
@ 0.25 gm/m^2					
Pre-0 day	1.5	2.0	1.2	1.5	-
Post-I day	0.2	1.5	0.3	1.7	78.9
I week	0.0	2.4	0.4	1.7	71.8
II week	0.0	3.3	0.6	2.09	65.6
III week	0.5	3.2	0.9	2.5	56.8
IV week	0.1	2.1	1.3	2.9	46.3

Note : 1. Percent reduction was calculated on the basis of IIIrd and IVth instar density.
2. E = Experimental coolers, C = Control coolers.

Table 3. *Bio-efficacy of Bt.H-14 granule formulation against Aedes aegypti larvae in disused tyres under field conditions*

Day/ Week	I + II (Instar)		III + IV (Instar)		% Reduction
	E	C	E	C	
@ 1.0 gm/m^2					
Pre-0 day	11.8	10.6	8.6	6.7	---
I day	0.3	8.6	0.3	7.2	96.8
I week	0.0	8.9	0.0	7.4	100.0
II week	0.0	11.2	0.0	7.8	100.0
III week	0.0	10.8	0.0	8.2	100.0
IV week	0.0	8.2	0.0	8.6	100.0
@ 0.5 gm/m^2					
Pre-0 day	8.6	10.6	6.2	6.7	-
Post-I day	0.3	8.6	0.4	7.2	94.5
I week	0.0	8.9	0.0	7.4	100.0
II week	0.0	11.2	0.0	7.8	100.0
III week	0.0	10.8	0.0	8.2	100.0
Iv week	0.0	8.2	0.0	8.6	100.0
@ 0.25 gm/m^2					
Pre-0 day	6.6	10.6	4.2	6.7	-
Post-I day	0.6	8.6	0.8	7.2	83.4
I week	0.0	8.9	1.2	7.4	75.7
II week	0.0	11.2	1.8	7.8	65.4
III week	0.4	10.8	2.5	8.2	54.3
IV week	0.7	8.2	3.0	8.6	47.7

Note: 1. Percent reduction was calculated on the basis of IIIrd and IVth instar density.
2. E = Experimental tyres, C = Control tyres.

Field evaluation

The application of Bt.H-14 in evaporation coolers resulted in a high degree of reduction in density of the immature of *Aedes aegypti;* however, the persistence of biolarvicide was directly proportional to its dosage. Higher dosages, i.e. 1 and 0.5 gm/m^2, produced 100% reduction in the larval density of these species for about four weeks as compared to the untreated control. Lower concentration, i.e. 0.125 gm/m^2, also

showed a high larvicidal activity against this species, but the percentage reduction was not as pronounced as with higher dosages. The percentage reduction @ 0.25 gm/m^2 was 71.8, 65.6, 56.8 and 46.8, respectively, during 1^{st}-4^{th} week as against 100 with 0.5 gm/m^2 (Table 2). The application of Bt.H-14 formulation in disused tyres @ 1, 0.5 and 0.125 gm/m^2 produced variable degrees of percentage reduction (83-100%) in the larval density of *Aedes aegypti* (Table 3). As observed earlier, the formulation @ 1 and 0.5 gm/m^2 also resulted in 100% reduction in the larval density up to four weeks in tyres and the reduction was consistent in successive weeks of post-exposure periods. The formulation @ 0.25 gm/m^2 also showed considerable larvicidal activity (47.7%-83.4%) in this habitat but a hundred per cent reduction in immature density was not evident.

Discussion

Laboratory and field trials clearly indicate that the Bt.H-14 granule formulation has broad spectrum activities against larvae of mosquitoes in general and against *Aedes aegypti,* the principal vector of dengue and dengue haemmorhagic fever in India, in particular. *B. sphaericus,* which has recycling properties, has not been found effective against the larvae of *Aedes aegypti* in comparison to *Culex quinquefasciatus* and *An. stephensi*[1,8,9]. However, this formulation was most effective against *Aedes aegypti* @ 0.5gm/m^2 and the larvicidal activity persisted up to four weeks. Therefore, the formulation can be effectively used for the composite control of dengue, DHF, malaria and filaria vector species of mosquitoes in urban areas. As the formulation produced larvicidal activity for longer duration in domestic and peri-domestic habitats, it will be operationally feasible to treat specific breeding places at the onset of the monsoon, particularly in abandoned coolers and tyres, to prevent the rapid build-up of vector density and thereby of dengue and DHF epidemic. Nevertheless, before incorporating this method into the strategy for urban malaria control, pilot studies are required to be undertaken with conventional larvicides and oil to evaluate the relative efficacy, operational feasibility and cost-effectiveness of each method of intervention.

Acknowledgements

Technical assistance rendered by the staff is gratefully acknowledged. Thanks are also due to Mr N.L. Kalra, Consultant, Malaria Research Centre, Delhi for critically examining the manuscript.

References

1. Ansari MA. Domestic mosquito breeding places and their management. Bull. Environ. Science (1993), 11:56-62.
2. Ansari MA and Razdan RK. Seasonal prevalence of *Aedes aegypti* in five localities of Delhi, Dengue Bulletin (1998), 22:38-44.
3. Tyrell DJ, Davidson LI, Bulla LA, and Ramoska WA. Toxicity of parasporal crystals of *Bacillus thuringiensis* sub sp. *israelensis* to mosquitoes. Appl. and Environ. Microbiol. (1979)38:656-658.
4. Progress report of biolarvicides in vector control (1993). MRC Publication pp 1-79.
5. WHO SEARO guidelines on DF/DHF prevention and control (1999). Publication No. 29. pp 1-133.
6. Ansari MA, Singh KRP, Brooks GD, Malhotra PR and Vaidyanathan V. The development of procedure and techniques for mass survey of *Aedes aegypti.* Ind. J. Med. Res. (1977). 65 (Supple) 91-99.
7. Mulla MS. Control of chironomide midges in recreational lake. J. Econ. Entomol (1971), 64: 300-307.
8. Ansari MA, Sharma VP, Mittal PK, Razdan RK and Batra CP. Evaluation of *Bacillus sphaericus* to control breeding of malaria vectors. (1989) 26:25-31.
9. Ansari MA, Sharma VP, Mittal PK and Razdan RK. Efficacy of two flowable formulations of *Bacillus sphaericus* against larvae of mosquitoes. (1995) 32:76-84.