Indian J Med Res 147, March 2018, pp 299-307 DOI: 10.4103/ijmr.IJMR\_1631\_16



# Field testing & evaluation of the efficacy & duration of effectiveness of a biolarvicide, Bactivec<sup>®</sup> SC (*Bacillus thuringiensis* var. *israelensis* SH-14) in Bengaluru, India

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Received October 5, 2016

*Background & objectives*: Different formulations of *Bacillus thuringiensis* var. *israelensis (Bti)* have been tested against different mosquito vectors and other insects for their residual activity. In the present study, the efficacy and residual activity of a new formulation of *Bti* (Bactivec Suspension Concentrate) were evaluated against immature stages of *Anopheles stephensi* Liston (Diptera: Culicidae), *Aedes aegypti* Linnaeus (Diptera: Culicidae) and *Culex quinquefasciatus* Say (Diptera: Culicidae), in natural habitats in Phase II and Phase III in Bengaluru, India.

*Methods*: Preferential breeding habitats of the mosquito species were selected and four dosages (0.25, 0.5, 1 and 2 ml/50 l) were tested in Phase II trial. Two most effective dosages, 0.5 and 1 ml/50 l were selected for Phase III trial. The evaluation was carried out essentially following the guidelines of the World Health Organization Pesticide Evaluation Scheme. Pre-treatment and post-treatment densities were recorded at regular intervals, and >80 per cent reduction in pupae was taken as the duration of effectiveness.

*Results*: Bactivec SC treated at the dosage of 1 ml/50 l could produce 10-17 days efficacy (>80% reduction in pupae) in clean water habitats tested, whereas 0.5 ml/50 l dosage showed residual activity from 7 to 14 days against *Ae. aegypti* and *An. stephensi* in Phase III studies. In polluted water habitats, 4-7 days efficacy could be recorded against *Cx. quinquefasciatus* in Phase III.

*Interpretation & conclusions*: The Bactivec SC formulation was operationally feasible and easy to handle. For the control of *Anopheles* and *Aedes* mosquitoes in freshwater habitats, 1 ml/50 l dosage was found effective, whereas in polluted water habitats against *Cx. quinquefasciatus* 5 ml/m<sup>2</sup> was found effective.

Key words Aedes aegypti - Anopheles stephensi - Bacillus thuringiensis var. israelensis - Bactivec - Culex quinquefasciatus - larvicidal efficacy

Mosquito-borne diseases are of major public health concern in the world. Among these dengue, malaria, chikungunya, filariasis, *etc.* are causing high morbidity and mortality in many countries of the world<sup>1</sup>. Adult mosquito control and larval control are being undertaken by many vector control programmes to contain these diseases in many countries. *Bacillus thuringiensis* var. *israelensis* (*Bti*) is a Gram-positive, spore-forming bacterium, and toxins secreted by it are being used as biolarvicide against caterpillars, beetles, and flies, including mosquitoes and black flies<sup>2</sup>. The spores enter into the gut of the mosquito and disrupt the midgut endothelium, thereby causing the death of the larvae. These toxins are only effective against the feeding aquatic stages of mosquitoes. Two *Bti* formulations, namely WG, water-dispersible granule; and DT, ready-to-use tablet have been evaluated using the World Health Organization Pesticide Evaluation Scheme (WHOPES) and recommended as mosquito larvicides<sup>3,4</sup>.

Much emphasis has been given by the pesticide manufacturers to produce formulations that are safe in storage, handling and spraying operations in the field and may substantially influence effectiveness and safety<sup>5</sup>. Controlled release formulations such as microencapsulation, wettable granules, capsule suspensions and suspension concentrates have been developed to minimize the exposure during spray preparations, handling, slow release to extend the bioavailability of the insecticide on the surface, extended efficacy and to minimize the environmental contamination, operational feasibility, storage, *etc*<sup>6</sup>.

Bactivec SC is recommended for the control of mosquitoes such as Anopheles stephensi and Aedes aegypti (Diptera: Culicidae). In a study conducted at Nova Igua, Brazil, Bactivec resulted in a reduction in ovitrap positivity and also the highest house index reduction in Ae. aegypti when compared to Vectobac Granular (G) and Vectobac water dispersible granules (WDGs) formulations7. In another study conducted by Harwood et al<sup>8</sup>, different formulations of Bt showed efficacy in controlling mosquito immatures of Aedes vector mosquitoes in simulated tree holes and other aquatic habitats. In another study<sup>9</sup>, Vectomax Water Soluble Pouch, a formulation containing Bti and Bacillus sphaericus was found to be effective in controlling third and fourth instar larvae of Culex pipiens (Diptera: Culicidae) in septic tanks. In a study carried out by Tamilselvan et al<sup>10</sup>, using Bti with fly ash based formulation for the control of Culex quinquefasciatus (Diptera: Culicidae) larvae in their natural breeding habitats reported high efficacy in causing mortality in immature stages. Terbot et al<sup>11</sup> emphasized the efficacy of Bti formulation in the control of the larval population of mosquitoes. A few other studies also reported the efficacy of different Bti formulations in different settings<sup>12-20</sup>. In the present study, the efficacy and residual activity of Bactivec

SC were tested in different breeding habitats in Phase II and Phase III manner against immature stages of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* in Bengaluru city, India.

# **Material & Methods**

Bengaluru, the capital of Karnataka State, India is divided into 198 municipal wards. The population of the city is approximately 8.5 million, with a 1-1.2 million floating population (*www.bbmp.gov.in*). The temperature in Bengaluru ranges from 21 to  $35^{\circ}$ C. The average annual rainfall is about 970 mm. The study was undertaken during the period of June-December 2015 in north and east Bengaluru, covering 40 wards where *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* were reported.

*Test product*: Bactivec<sup>®</sup> SC supplied by M/s Labiofam Enterprise Group, La Habana, Cuba in 1 l bottles, is a biological larvicide that targets immature stages of mosquitoes and kills larvae at all the stages. The test formulation Bactivec SC contains *Bti* serotype H-14, strain 266/2 as active ingredient (6 g/l insecticidal toxins and spores; and 994 g/l other ingredients). The biopotency of the compound is >1200 International toxic units/mg (ITU/mg). According to its material data safety sheet, it is classified as slightly hazardous Class III. The product is unstable at  $4 \le pH \ge 10$ . The compound is water soluble, non-toxic to persons, warm-blooded animals or hydrobionts. It is biodegradable and ecosystem friendly<sup>21</sup>.

Phase II field trial in natural breeding habitats small scale): The study was conducted according to the WHO standard guidelines for small scale field trials of biological larvicides<sup>22</sup>. Field surveys were undertaken in the northern and eastern parts of Bengaluru City for identifying potential natural breeding sources of mosquitoes. Larval and pupal samples were collected from different breeding habitats and placed in separate containers. These were maintained in insectary of National Institute of Malaria Research Field Unit, Bengaluru, for adult emergence for identification of species. The habitats which were supporting the breeding of An. stephensi, Ae. aegypti and Cx. quinquefasciatus were selected for the study. Plastic containers, plastic tanks used for storing water, flower pots (both earthen and cement make), and domestic cement tanks which are the most preferred breeding habitats of Aedes mosquitoes in Bengaluru were included. For Anopheles species cement tanks and flower pots were included. Polluted stagnant drains, cement tanks and pools (both polluted and clean water) were included for testing on *Cx. quinquefasciatus* species. Water temperature and *p*H of the habitats were noted and the habitats having *p*H <4 and >10 were excluded preferably. In all, 4-6 habitats were selected for each type of habitat in Phase II study.

*Larval sampling*: Larval densities of all Stages I + II and III + IV instars and pupal counts were monitored in the habitats by dipping method (300 ml enamel dipper). Where dipping was not possible in small containers, the contents were poured into a tray and developmental stages were counted and the entire contents were returned to the habitats. Five dips were taken in each habitat to assess the density per dip as described elsewhere<sup>22</sup>. After assessing the densities in all the habitats, these were allocated to five arms of four each. Four dosages were tested in Phase II trial, *i.e.* 0.25, 0.5, 1 and 2 ml/50 l in clean water habitats. Where possible, the volume of the water was calculated. Habitats from each type with comparable pre-treatment densities were assigned to either treatment or control groups.

For large habitats such as wastewater drains and pools, the drain at its entire length/entire pool was treated with one dosage and each segment of 10-12 m<sup>2</sup> was considered as a replicate. Separate drains were selected for each dosage as well as for control. Four to six replicates were tested per dose/control for each habitat. The dosages tested as per surface area were 2, 3, 5 and 7 ml/m<sup>2</sup>. The habitats where observations were made continuously for a minimum of 10 days were only considered for analysis. Habitats that lost for reasons such as emptying by owners, becoming dry and diluted due to rains were excluded.

*Treatment procedure*: Large water bodies (more than 500 l) were treated with hand atomizer sprayer (2 l capacity) with respective dosages according to the surface area. For containers containing <500 l of water manual application of the insecticide using graded pipette was done.

*Monitoring and evaluation of impact*: Mosquito larvae/pupae were sampled using enamel dippers, counted by stage and returned to their habitats. Post-treatment monitoring of the density of mosquitoes larvae/pupae was done on 1, 3, 7, 10, 14, 17, 20 and 24<sup>th</sup> day post-application until the pupal density in the treated habitats reached the level comparable to the untreated habitats. The criterion of 80 per cent reduction in larval/pupal counts as per the WHOPES larvicide

guidelines<sup>22</sup> was used to determine the performance of the test dose for each habitat. The percentage reductions in I-II instars, III-IV instar larvae and pupae were calculated as per Mulla's formula<sup>23</sup>.

*Phase III field trial in natural breeding habitats (large scale)*: This study was designed in accordance with the WHO standard guidelines for the large-scale field testing of biolarvicides<sup>22</sup>.

Habitat identification: The field trials were conducted with natural populations of anopheline and culicine larvae in native habitats. Concurrent control replicates were maintained for comparison. Care was taken to select the type of habitats generally preferred by these species in nature for breeding. The selected natural habitats were treated with the Bactivec SC formulation at two effective field application dosages determined in the Phase II field trial for clean and polluted water habitats, i.e. 0.5 and 1.0 ml/50 l dosages for clean water habitats and 5 and 7 ml/m<sup>2</sup> for larger water bodies and polluted habitats. The treatment procedure, larval and pupal assessments described in Phase II testing were followed as specified for respective breeding habitats. In all, about 25-40 habitats were selected for each type of habitat and dosage.

Statistical analysis: The per cent reduction was calculated as per Mulla's formula<sup>23</sup>. The percentage data were  $\text{Log}_{10}$  transformed in case of positive values and in the case of negative percentages additional Log Modulus transformation was used.  $\text{Log}_{10}$  transformed data were used for two-way analysis of variance keeping days of observation and dosages as independent variables. Student's *t* test was used for comparison between two dosages in Phase III trial. Only percentage data were used for presentation of results.

## Results

*Phase II: Small scale field trial*: Phase II studies were conducted with four dosages of Bactivec SC, *i.e.* 0.25, 0.5, 1.0 and 2 ml/50 l in clean water habitats and 2, 3, 5 and 7 ml/m<sup>2</sup> in polluted water habitats against three mosquito vector species. In most of the habitats tested, >80 per cent reduction in pupal density was observed up to 10 days with all the dosages against *Ae. aegypti* and *An. stephensi*, except 0.25 ml/50 l dose, where >80 per cent reduction was observed up to seven days in cement tanks and plastic containers (Table I). Based on the results, 0.5 ml/50 l and 1.0 ml/50 l dosages were

Type of habitat	Dosage (ml/50 l)	Duration of effectiveness in days (>80% reduction in pupal densities over control)						
		Aedes aegypti	Anopheles stephensi	Culex quinquefasciatus				
Flower pots	2	10	10	ND				
	1	10	10	ND				
	0.5	10	10	ND				
	0.25	10	7	ND				
Cement tanks	2	10	10	7				
	1	10	10	1				
	0.5	10	10	3				
	0.25	7	7	3				
Plastic drums	2	10	ND	ND				
	1	10	ND	ND				
	0.5	10	ND	ND				
	0.25	7	ND	ND				
Polluted drains	7 ml/m <sup>2</sup>	ND	ND	3				
	5 ml/m <sup>2</sup>	ND	ND	3				
	3 ml/m <sup>2</sup>	ND	ND	1				
	$2 \text{ ml/m}^2$	ND	ND	ND				

**Table I.** Duration of effectiveness of different dosages of Bactivec suspension concentrate (>80% reduction in pupal densities) against three mosquito species in different breeding habitats in Phase II trial

selected for large-scale Phase III trial in clean water habitats.

In case of *Cx. quinquefasciatus,* residual activity was observed up to a maximum of seven days only in cemented containers treated with 2 ml/50 l dosage. In cemented clean water habitats, all the tested dosages produced >80 per cent reduction up to three days only. In polluted habitats, *i.e.* drains >80 per cent reduction in pupal and 3-4 instar larval densities was observed up to three days in case of 5 and 7 ml/m<sup>2</sup> dosages and 0 to 1 day in case of 2 and 3 ml/m<sup>2</sup> dosages. No significant difference could be observed when per cent reductions were compared among the dosages. Although results of *Cx. quinquefasciatus* were not conclusive, 5 and 7 ml/m<sup>2</sup> dosages were chosen for Phase III trial.

# Phase III: Large scale trial

ND, not done

<u>Aedes aegypti:</u> Mean larval and pupal densities of *Ae. aegypti* and per cent reductions over control in habitats treated with 0.5 and 1 ml/50 l are shown in Table II. The results revealed that duration of effectiveness in causing >80 per cent reduction in pupal densities in treated habitats was 10-14 days for the 1 ml/50 l dosage and 7-10 days for 0.5 ml/50 l dosage in all the three habitats tested. There was no significant difference in per cent reductions when compared between the two dosages tested for early, late instars, and pupae also in all the three types of habitats tested. From the results, it was observed that 1 ml/50 l dosage was effective up to 10-14 days and 0.5 ml/50 l was effective up to 7-10 days.

Anopheles stephensi: Mean larval and pupal densities of An. stephensi and per cent reductions over control in habitats treated with 0.5 and 1 ml/50 l are shown in Table III. The results revealed that the residual activity (>80% reduction in pupal density) was 10-14 days in cement tanks and flower pots treated with 0.5 ml dosage, whereas 14-17 days in case of 1 ml/50 l dosage. There was no significant difference between the two dosages in reducing the density of larvae as well as pupae in both types of habitats tested, indicating the equal effectiveness of both the dosages in controlling the immatures.

<u>Culex quinquefasciatus</u>: Mean larval and pupal densities of Cx. quinquefasciatus and per cent reductions over control in habitats treated with different dosages are shown in Table IV. Cement tanks, drains, polluted, and clean water pools were selected for assessing the efficacy against Culex larvae. The results showed that in clean water habitats such as cemented containers and pools, >80 per cent reduction was seen up to 10 days

Table II. Mean larv	al and pupal	densities per	dip (% reduc	tion over cor	ntrol) of Aede	es aegypti in c	lifferent habita	ts in Phase III evaluation
Dosage in ml/50 l (number of habitats)	Pre -treatment	Day 1	Day 4	Day 7	Day 10	Day 14	Day 17	Duration of effectiveness (>80% reduction in densities over control)
Plastic containers								
Mean larval density								
Control (29)	5.47	2.90	2.51	2.46	1.98	2.54	3.08	
1 (41)	12.26	0.02 (100)	0.18 (97)	0.60 (89)	2.58 (42)	2.57 (55)	1.56 (77)	7
0.5 (31)	6.25	0.03 (99)	0.37 (87)	1.14 (59)	2.72 (-20)	2.76 (5)	2.17 (38)	4
Mean pupal density								
Control (29)	1.72	3.19	2.30	1.67	1.23	1.17	0.98	
1 (41)	1.87	0.05 (98)	0 (100)	0 (100)	0.04 (97)	1.81 (-42)	1.89 (-77)	10
0.5 (31)	1.72	0.11 (97)	0.01 (99)	0.04 (99)	0.67 (45)	1.74 (-50)	2.05 (-110)	7
Cement tanks								
Mean larval density								
Control (34)	12.45	9.50	3.64	2.41	2.43	3.36	3.68	
1 (31)	10.56	0.23 (97)	0.00 (100)	1.17 (43)	2.43 (-18)	3.37 (-18)	2.94 (6)	4
0.5 (31)	10.81	0.30 (96)	0.00 (100)	1.40 (33)	2.31 (-9)	3.01 (-3)	2.05 (36)	4
Mean pupal density								
Control (34)	3.51	4.36	5.20	3.79	1.37	1.74	2.36	
1 (31)	3.95	0.57 (88)	0.02 (100)	0 (100)	0.23 (85)	0.39 (80)	2.38 (10)	14
0.5 (31)	2.72	0.45 (87)	0.04 (99)	0 (100)	0.07 (93)	1.57 (-16)	2.5 (-37)	10
Flower pots								
Mean larval density								
Control (27)	7.18	5.46	2.68	2.48	5.23	4.10	2.29	
1 (23)	10.51	0.01 (100)	0.01 (100)	2.07 (43)	4.17 (46)	2.93 (51)	2 (40)	4
0.5 (32)	4.93	0.15 (96)	0.16 (92)	2.37 (-39)	3.03 (16)	2.17 (23)	2.67 (-70)	4
Mean pupal density								
Control (27)	1.39	1.73	2.70	2.11	1.58	1.94	1.94	
1 (23)	2.39	0.21 (93)	0.09 (98)	0.08 (98)	0.51 (81)	2.05 (39)	2.26 (32)	10
0.5 (32)	1.73	0.24 (89)	0.04 (99)	0.35 (87)	1.45 (27)	1.99 (17)	1.96 (19)	7

in 1 ml/50 l dosage, seven days in case of lower dosage 0.5 ml/50 l in cement tanks. In pools with clean water, seven days efficacy was observed with both the dosages. In polluted drains, only four days residual activity was observed with both dosages of 5 ml and 7 ml/m<sup>2</sup>. In contrast, in polluted pools, seven days residual activity was observed. There was no significant difference in between the dosages in reducing the densities of either

larvae or pupae, inferring that both the dosages are equally effective.

# Discussion

In the present study, suspension concentrate of Bti was tested against aquatic stages of mosquitoes in their natural breeding habitats. The duration of effectiveness ranged from 7 to 17 days in different

Dosage in ml/50 1 (number of habitats)	Pre -treatment	Day 1	Day 4	Day 7	Day 10	Day 14	Day 17	Day 20	Day 24	Duration of effectiveness (>80% reduction in densities over control)
Flower pots										
Mean larval density										
Control (23)	3.48	3.14	2.51	1.69	1.75	2.04	2.77	2.13	1.63	
1 (30)	3.31	0.13 (96)	0.15 (94)	0.32 (80)	0.70 (58)	1.54 (21)	1.89 (28)	2.36 (-17)	2.58 (-67)	7
0.5 (27)	3.01	0.04 (99)	0.09 (96)	0.54 (63)	1.35 (11)	1.73 (3)	2.02 (16)	2.14 (-16)	1.61 (-14)	4
Mean pupal density										
Control (23)	1.09	1.30	1.55	1.51	1.12	0.70	0.75	1.10	1.26	
1 (30)	1.21	0.20 (86)	0 (100)	0 (100)	0 (100)	0.15 (81)	0.83 (1)	1.41 (15)	1.35 (5)	14
0.5 (27)	1.16	0.52 (63)	0.01 (99)	0 (100)	0 (100)	0.32 (58)	0.86 (-6)	1.05 (11)	1.28 (5)	10
Cement tanks Mean larval densities										
Control (23)	5.00	4.01	2.68	1.57	2.30	1.99	2.39	3.45	2.62	
1 (30)	4.54		0.26 (89)		0.83 (61)		1.90 (12)	1.50 (52)	1.71 (28)	4
0.5 (27)	4.70	. ,	. ,				3.63 (-61)	2.33 (55)	2.01 (19)	7
Mean pupal density		(00)			(*/)			()		·
Control (23)	1.06	1.53	2.21	2.07	1.63	1.57	1.32	1.82	1.52	
1 (30)	1.66	0.25 (89)	0.03 (99)	0.01 (100)	0.08 (97)	0.14 (94)	0.39 (81)	1.01 (64)	1.56 (34)	17
0.5 (27)	1.76	0.49 (81)	0.09 (98)	0.01 (100)	0.07 (98)	0.09 (96)	0.49 (77)	1.52 (50)	1.64 (35)	14

clean water habitats and up to 4-7 days in polluted water habitats. The formulation was found effective in killing the immature stages with all the dosages tested. The results were in conformity with other studies that reported the efficacy of Bti in reducing the larval density in many habitats. Harwood et al8 in their study on different formulations of Bti reported the effectiveness in controlling mosquito larvae in tree holes and other habitats. Cetin et al9 reported 24 days efficacy of Bti + Bs combination in controlling the larvae of Cx. pipiens in septic tanks. Dambach et al<sup>24</sup> also reported the efficacy of Bti in controlling Anopheles mosquito larvae in natural conditions in sub-Saharan Africa. Li et al<sup>25</sup> reported the efficacy of Bti WP formulations in controlling the mosquito immatures of Aedes, Anopheles and Culex immatures. A study on two formulations of Bti, WDGs and an

extruded pellet against *Ae. albopictus* reported 100 per cent reduction in mosquito larvae and about three weeks residual efficacy<sup>26</sup>.

Vectobac The efficacy of GR (potency 200 ITU/mg), a new formulation of bacterial larvicide Bt var. israelensis Strain AM65-52, was tested against An. gambiae and Cx. quinquefasciatus in the simulated field and natural habitats in Benin and an efficacy of 2-3 days against larvae and up to 10 days against pupae in natural habitats was reported<sup>27</sup>. Guidi et al<sup>28</sup> in their evaluation of a commercial biolarvicide based on Bt var. israelensis and Lysinibacillus sphaericus to control mosquitoes breeding in catch basins in southern Switzerland reported >97 per cent of reduction of late instars (3<sup>rd</sup> and 4<sup>th</sup> instars) and pupae for four weeks. In contrast, Gezelbash et al16 reported the low efficacy of Bti MH-14 (Bioflash) in laboratory

Dosage (number of habitats)	Pre -treatment	Day 1	Day 4	Day 7	Day 10	Day 14	Duration of effectiveness (>80% reduction in densities over control)
Cement tanks (ml/50 l) Mean larval density							
Control (25)	8.14	6.89	5.14	4.62	4.91	3.70	
1 (26)	5.29	0.29 (93)	0.58 (83)	1.47 (51)	3.28 (-3)	2.62 (-9)	4
0.5 (26)	5.74	0.25 (95)	0.74 (80)	2.72 (16)	4.85 (-40)	4.04 (-42)	4
Mean pupal density							
Control (25)	2.48	3.13	3.85	3.21	2.58	2.36	
1 (26)	2.61	0.18 (95)	0 (100)	0.20 (94)	0.53 (81)	2.36 (5)	10
0.5 (26)	2.42	0.17 (94)	0 (100)	0.24 (92)	1.45 (42)	2.52 (-9)	7
Polluted pools (ml/m <sup>2</sup> )							
Mean larval density							
Control (4)	9.40	8.40	5.25	5.10	4.25	3.65	
7 (7)	8.46	0.57 (92)	0.09 (98)	2.86 (38)	3.34 (13)	3.03 (8)	4
5 (9)	7.56	0.51 (92)	1.69 (60)	2.93 (28)	4.18 (-22)	3.4 (-26)	1
Mean pupal density							
Control (4)	3.5	3.45	4.35	4.1	2	2.1	
7 (7)	3.11	0.51 (83)	0 (100)	0.06 (98)	1.00 (44)	2.43 (-30)	7
5 (9)	2.42	0.89 (63)	0.24 (92)	0.53 (81)	2.07 (-49)	2.24 (-54)	7
Clean water pools (ml/m <sup>2</sup> )							
Mean larval density							
Control (6)	7.20	5.03	3.40	3.77	3.00	4.60	
7 (9)	7.47	0.20 (96)	2.44 (31)	2.33 (40)	3.78 (-21)	4.04 (15)	1
5 (7)	6.66	0.31 (93)	2 (36)	2.6 (25)	4.8 (-73)	4.11 (-14)	1
Mean pupal density					( )		
Control (6)	4.00	4.00	2.53	2.27	2.47	1.70	
7 (9)	4.18	0.56 (87)	0.04 (98)	0.44 (81)	2.29 (11)	2 (-13)	7
5 (7)	2.34	0.29 (88)	0.09 (94)	0.26 (80)	2.86 (-98)	2.11 (-113)	7
Polluted drains (ml/m <sup>2</sup> )			( )		( )	( )	
Mean larval density							
Control (18)	11.96	10.44	9.70	6.70	4.84	-	
7 (20)	10.03	1.01 (88)	4.18 (49)	5.59(1)	6.11 (-50)	-	1
5 (19)	13.40	1.24 (89)	6.56 (40)	6.51 (13)	5.26 (3)	-	1
Mean pupal density		. (~~)		()			-
Control (18)	3.67	4.38	5.66	3.70	3.00	-	
7 (20)	3.9	1.91 (59)	0.91 (85)	2.55 (35)	3.18 (0)	-	4
5 (19)	4.07	1.59 (67)	0.89 (86)	3.60 (12)	3.21 (4)		4

Table IV Mean larged and pupel densities per dip (% reduction over control) of Culor avinguation in different babitats in Phase

and field trials against *Anopheles* larvae. In the present study, low residual activity was reported against *Cx. quinquefasciatus* probably due to the fast settling of the spores of the formulation, bacterial degradation and toxic effluents in the drainage pools.

In conclusion, in our study Bactivec SC, a biological larvicide showed promising efficacy in reducing the larval and pupal densities within 24 h post-treatment in the majority of the habitats and at low dosages. The product was easy to handle and operationally feasible for the application. For comprehensive control in clean water habitats 1 ml/501 dosage could be effective up to two weeks against *Anopheles* and *Aedes* larvae. For the control of *Cx. quinquefasciatus* in polluted habitats, 5 ml/m<sup>2</sup> dosage was found to be effective for a week.

### Acknowledgment

The authors thank the staff of the National Institute of Malaria Research Field Unit, Bengaluru for their assistance in Field work, and to Dr Mehul Chaurasia and Shrimati Mala Selvam for their suggestions in the statistical analysis of the data.

*Financial support & sponsorship*: This study was sponsored by World Health Organization Pesticide Evaluation Scheme (WHOPES).

# Conflicts of Interest: None.

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