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Field efficacy of *Bacillus Thuringiensis* compared with water Dispensible granules against *Aedes Albopictus* larvae

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Abstract

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are acute febrile diseases and transmitted to humans by the mosquito *Aedes aegypti* and *Aedes albopictus*. Dengue Fever, Dengue Hemorrhagic Fever and Dengue Shock Syndrome are among the major cause of morbidity and mortality in children in many endemic Asian and South American countries. It is regarded as major public health problem globally. In current study *Bacillus thuringiensis* var. *israelensis* Technical Powder (Vectobac 5000 ITU /mg) and WDG (Water dispersible granules) 3000 ITU mg were evaluated for the potential control of dengue vector, wild caught laboratory reared *Aedes albopictus* (early 4th instar) larvae in field conditions. Maximum residual activity of *Bti* TP was 35 days against 2.4ppm and minimum 14 days against 0.3ppm as compared to *Bti* WDG observed for 35 days against 0.26 ppm and minimum residual effect of 14 days with 0.05ppm.

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Introduction

Mosquitoes are important vector of human and animal diseases. In southeast asia, *Aedes aegypti* and *Aedes albopictus* are important vectors of Dengue fever (DF) and Dengue hemorrhagic fever (DHF). *A. albopictus* has a wide host range including man, domestic and wild animals and birds, whereas *A. aegypti* prefer to feed exclusively on human blood. *A. albopictus and A. aegypti* are opportunistic and aggressive biters with feeding peaks in the early morning and late afternoon (Hawley, 1988). DF and DHF have long been reported as the most common urban disease in the Southeast-Asian region since the 1950s, before spreading worldwide. Dengue virus is a Flavivirus (West away and Blok, 1997) having four serotypes; DEN-1, DEN-2, DEN-3 and DEN-4 (Gubler and Kuno, 1997).

As there is still no specific treatment and efficient vaccine available, vector control against *Aedes aegypti* remains the most effective solution to prevent dengue transmission. Unfortunately, vector control programs are facing operational challenges with the emergence and development of insecticide resistance in dengue vectors, especially *A. aegypti* (Ranson *et al.*, 2010). Resistance to organophosphates (OPs) and pyrethroids (PYRs) has been reported since the 1980s and 1990s respectively (Rosine, 1999;André-Yébakima,1995), and this resistance has recently been shown to be negatively impacting on the efficacy of vector control interventions (Marcombe *et al.*, 2009). The massive use of different insecticide families for vector control since the 1950s has probably contributed to select for insecticide resistance in mosquitoes (Brown, 1986).

Alternative methods of insect management offer adequate levels of pest control and pose fewer hazards. One such alternative is the use of microbial insecticides that contain microorganisms or their by-products. Microbial insecticides are especially valuable because their toxicity to non-target animals and humans is extremely low. Compared to other commonly used insecticides, they are safe for both, the pesticide user and consumers of treated crops. Microbial insecticides also are known as biological pathogens, and biological control agents. Chemical insecticides are far more commonly used in the world than microbial control however some microbial control agents, at least in part, can be used to replace some hazardous chemical pest control agents. A number of biological control agents formulated with bacteria, fungi, virus, pheromones, and plant extracts have been in use mainly for the control of insects responsible for the destruction of forests and agriculture crops (McDonald and Linde, 2002).

Keeping this in view, present study was carried out on *Bacillus thuringiensis israelensis* known as *Bti*. It has high toxicity to Dipterans especially mosquitoes and Black flies. The *Bti* formulation provides larvicidal activity with good residual effect. It has been also found safe in drinking water (WHO, 1999). Althogh simulated field evaluation of *Bti* against vector mosquitoes is relatively important to ensure the applicability of the *Bt*i formulations in the actual use, but It has been proven by researchers that *Bti* is also effectively used in the field. Proper dose of *Bti* on small scale (Rampal *et al.*, 1983) or large scale (Lacey and Inman, 1985) in field trials showed effective control against mosquitoes. *Bti* with different formulations have shown different levels of toxicity to host species. Culex and *Aedes* are highly susceptible while *Anopheles* is less susceptible, but can still be killed with *Bti* (Balaraman and Manomani, 1993). Even within one genus, some species are more susceptible than others (Chui *et al.*, 1995).

Vectobac (WDG) manufactured by Valent Bioscience Corp. IL, U.S.A. is the available mosquito biolarvicide containing the active ingredient *Bacillus thuringiensis israelensis*. It is a clean, dust free granular and can be applied with either aerial or ground equipment. In view of recent outbreak of Dengue fever in Pakistan, especially Punjab, and recently introduced use of biopesticide, there was need to carry out a study to evaluate the efficacy of *Bti* TP (Vectobac 5000ITU/mg) and WDG (water Dispersible Granules 3000 ITU/mg) as biological larvicide's. 4^{th} instars of *Aedes albopictus* were collected from artificial plastic containers and used tyres in field from Lahore for experimentation.

Material and method

Field collection of Larvae

Aedes albopictus overwinter in the egg stage in temperate climates (Lyon and Berry, 1991). Eggs are laid singly on the sides of water-holding containers such as tires, animal watering dishes, birdbaths, flowerpots and natural holes in vegetation (Hawley, 1988). There are 1-4 ovipositions per female (ISSG 2004). There are 30-50 to 250 eggs per oviposition. Egg laying occurs over a period of 2-3 days at 27C° depending upon temperature. 4 larval stages (instars) take 5-10 days for development.

Larval emergence occurs after rainfall raises the water level in the containers. The eggs may require several submersions before hatching (Hawley, 1988). The larvae develop through four instars prior to pupation. Transformation from the pupal stage to the adult stage generally takes 2-3 days. Field collected early 4th instar larvae *of A. Albopictus* were used in a field assay. Wild caught larvae were periodically collected from (July-Sep 2006 and July-August 2007) from natural and artificial containers at GC University, Lahore. These larvae were reared in insectory for laboratory experiment. Each female within 48-72 hours post blood feeding lays the eggs. In order to obtain eggs polystyrene/plastic cups (7.8cm diameter) containing clean water placed in the mosquito cage. After about 3-4 days of oviposition the eggs hatch into the 1st instar (larvae).

Larvae were plated in the plastic pans in the standardized condition. Pupae were separated daily with the help of mini vacuum pump in the plastic cups containing distilled water. Each cup with pupae was placed directly in cage for the emergence of adult. Field evaluations were conducted in 23cm diameter plastic tubs containing 2 liters of clear rainy water (obtained from a natural container, plastic drum of *Aedes* mosquito at GCU). In field evaluation, treatment concentration was evaluated on the basis of LC_{50} and LC_{95} from laboratory assays (Schnetter *et al.,* 1981).

Test strains used

Test strains of *Bacillus thuringiensis israelensis* WDG (Water dipersible granules) vectobac® 3000 ITU/mg and Technical powder (TP) 5000 ITU/mg by Valent Bioscience Corporation: IL, U.S.A. was used to evaluate efficacy of wild caught laboratory-reared early 4th instar larvae of *A. albopictus* in field assays.

Test formulations

Because of the difference in potencies of test formulations and based on LC_{50} and LC_{95} values in the laboratory different concentrations of the two test formulations were applied to evaluate effectiveness and residual activity in the field. Three replicates of each concentration were used. After 2 to 3 hours of larval acclimation four concentrations of *Bti* (TP) 0.3, 0.6, 1.2, 2.4 ppm was applied in four plastic containers. At the same time in another set of experiment three concentrations of *Bti* (WDG) 0.05, 0.13, 0.26 ppm was used in three plastic containers. Water temperature was 26°C to 35°C and relative humidity from 58% to 67% during the experiment.

Statistical analyses

Mean percent larval mortalities were analyzed by one way analysis of variance (ANOVA) with Tukeys test at 0.05 % confidence level using Minitab computer Software.

Results

Field evaluation of *Bti* TP and *Bti* WDG in plastic containers were conducted during August to September when air temperature ranged 29°C to 37°C (33°C), water temperature 26°C to 35°C (31°C) and relative humidity from 58 to 67% (63%). Based upon LC_{50} and LC_{95} values of *Bti* TP (0.3 – 1.2 mg/lit)and *Bti* WDG (0.05 – 0.13mg/lit) from laboratory with fixed volume of water (2 liter) various concentrations. *Bti* TP applied in plastic containers (tubs) showed LT_{100} (Lethal time for 100% death) for 5, 3, 2 and 1 days against 2.4, 1.2, 0.6 and 0.3 ppm.

Maximum residual activity of *Bti* TP was 35 days recorded against 2.4ppm in plastic containers (Table 1). In plastic containers residual effect of *Bti* TP was 35, 28, 21 and 21 days against 2.4, 1.2, 0.6 and 0.3 ppm (Table 1, Fig. 1). *Bti* WDG at 0.26, 0.13 and 0.05 ppm induced 100% larval mortality for 5, 2 and 1 day. LT_{50} in plastic containers was 15, 10 and 9 days respectively. Residual effect in plastic container with WDG, 0.26, 0.13 and 0.05ppm was 35, 28 and 21 days) respectively. Maximum residual activity of *Bti* WDG was observed in plastic containers for 35 days at 0.26 ppm. (Table 2, Fig. 2).

Table 1. Field Evaluation as maximum residual activity and percent larval mortality of *Aedes albopictus* against *Bacillus thuringiensis* TP in Plastic containers.

Concentration in ppm	Max. days of Residual activity	Percent mortality in no. of weeks				
		1st week	2nd week	3rd week	4th week	5th week
2.4	35	94	65	40	17	3
1.2	28	89	53	21	6	0
0.6	21	76	41	12	0	0
0.3	21	69	32	4	0	0

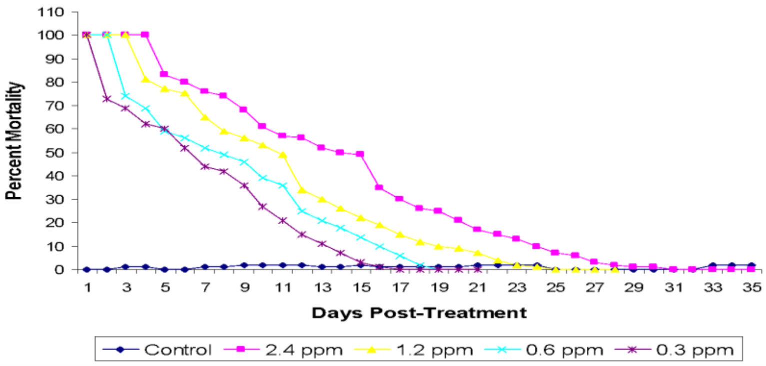

Fig. 1. Field Evaluation as maximum residual activity and percent larval mortality of *Aedes albopictus* against *Bacillus thuringiensis* TP in Plastic containers.

Table 2. Field Evaluation as maximum residual activity and percent larval mortality of *Aedes albopictus* against *Bacillus thuringiensis* WDG in Plastic containers.

Concentration in ppm	Max. days of Residual activity	Percent mortality in no. of weeks				
		1st	2nd	3rd	4th	5th
0.26	35	89	65	42	10	2
0.13	28	78	42	13	1	0
0.05	21	64	22	1	0	0

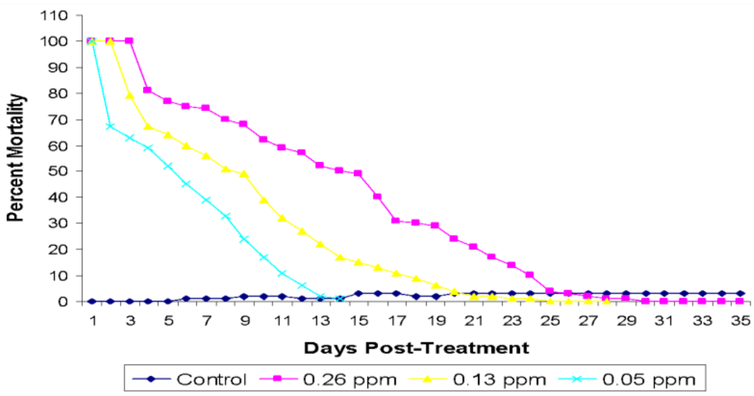

Fig. 2. Field Evaluation as maximum residual activity and percent larval mortality of *Aedes albopictus* against *Bacillus thuringiensis* WDG in Plastic containers.

Discussion

Microbial larvicides have several advantages over other mosquito control agents, not only due to high efficacy but also because it is environmentally safe for humans. These larvicides are being used effectively against different mosquito species in different regions of the world (Fillinger *et al.*, 2003).

Among the two *Bti* formulations, *Bti* TP has more potency (5000 ITU/mg) as compared to WDG (3000 ITU/mg). The LC_{50} – LC_{95} indicated that the latter is more toxic for *Aedes albopictus* larvae. These results are in accordance with the work of (Fansiri *et al.*, 2006), who showed that formulation technology can enhance the activity of products and that the *Bti* WDG formulations are promising for control of disease vectors. Similarly, LC_{50} – LC_{95} ranging from 0.39 to 0.84ppm for *Aedes albopictus* larvae using mosquito dunks (7000 ITU/mg *Bti*) investigated by (Fansiri *et al.*, 2006), are higher than our findings, for *Bti* TP and *Bti* WDG at 48 hours post exposure, indicating *Bti* TP and *Bti* WDG was 10X and 12X more toxic.

Residual activity using 0.3 – 2.4 ppm of the *Bti* TP formulation varied from 3 – 5 weeks (21 – 35 days) in plastic containers whereas, much lower concentrations of *Bti* WDG 0.05 – 0.26 ppm caused the same persistent effect i-e maximum 35 days at higher rate of 0.26 ppm and minimum effect for 14 – 21 days at lowest rate of 0.05 ppm. In general among the formulations *Bti* WDG has 10X more residual activity as compared to *Bti* TP. All these studies indicated LT_{100} and residual activity was dose dependant. Higher doses of *Bti* enhanced the residual effects against various species of mosquitoes.

In current study 100 % larval mortality was observed up to 5 days against *Bti* TP at 2.4 ppm *Bti* WDG at 0.26ppm. In conclusion, *Aedes albopictus* (Asian dengue vector) is found highly susceptible to *Bti* WDG at the rate of 0.26–0.05 ppm as studied in Pakistan. Furthermore, the minimum effective dosages to kill 100% of the larval population in a habitat have shown to be extremely low and product may have great potencies for inclusion in integrated vector management operations.

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