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Comparative evaluation of *Bacillus thuringiensis* and *Bacillus sphaericus* against Dengue fever vector *Aedes albopictus* larvae, pupae emergence and adult inhibition in laboratory in Lahore, Pakistan

Wajeeha Hameed*[1], Dr. Nusrat Jahan[2], Ferhat Mehmood[3]

[1]*Department of Zoology, Student of Zoology Govt. College University, Lahore, Pakistan*

[2]*Department of Zoology, Faculty at Zoology Department, GC University, Lahore, Pakistan*

[3]*Department of Botany, Faculty at Botany Department, Govt. M.A.O. College, Lahore, Pakistan*

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Abstract

Dengue is a growing public health problem in many tropical and subtropical countries worldwide. At present the only method of controlling or preventing the disease is to eliminate its vectors viz *Aedes aegypti* and *Aedes albopictus*. In current study, susceptibility of wild caught laboratory reared *Aedes albopictus* early 4th instar larvae against, *Bti* TP vectobac® 5000 ITU/mg, *Bti* WDG vectobac® 3000 ITU/mg and *Bacillus sphaericus (Bsph)* Vectolex® (TP) 1380 ITU/mg was evaluated. Lethal concentrations LC_{50}-LC_{95} ranged between 0.26-1.21 and 0.047-0.28 respectively, against *Bti* TP, while 0.052 - 0.14 and 0.025-0.091 against *Bti* WDG and 0.44 -1.65 and 0.37-1.5 against *Bsph* at 24 and 48 hours post treatment. It indicated that early 4th instar larvae of *Aedes albopictus* were most susceptible against *Bti* WDG and least susceptible against *Bsp* in laboratory. Comparative probit regression analysis indicated that *Bti* WDG was highly toxic at 48 and 72 hours exposure at the same mortalities. Pupae emergence was completely inhibited at 1 ppm and > 50% at 0.01 ppm against *Bti* TP in laboratory. Mean percent mortalities were 81±3.53, 53±3.53, 37±2.31, 25±3.53 and 16±2.31 against 1, 0.1, 0.01, 0.001 and 0.0001 ppm at 24-72 hours post exposure respectively, with significant difference between *Bti* TP and *Bsph* at $P < 0.05$.

***Corresponding Author:** Wajeeha Hameed ✉ jiya.ravian@gmail.com

Introduction

Mosquitoes well known to human civilization since prehistoric time due to their biting nuisance, belong to phylum Arhropoda, class Insecta, order Diptera; the family Culicidae (Pratt and Moore, 1993). The three genera of mosquitoes; *Anopheles, Aedes* and *Culex*, are the primary vectors for various pathogens such as human malarial parasites (Plasmodium), the filarial parasites, (*Waucheri abancrofti* and *Brugia malayi*), and a number of arboviruses (Yellow fever, Dengue, Encephalitis) are the major cause of human mortality and morbidity in the world, owing to their obligate haematophagy. Blood feeding is an important feature of the mosquitoes in order to obtain protein needed to mature their eggs (Eritga *et al.*, 2005).

Aedes aegypti and *A. albopictus* are found in all habitats with later prevailing mainly in rainy seasons possibly acting as secondary vector (Tewari, *et al.*, 2004). Humans are the principle source of blood while utensils that can hold water like water jars, broken cans and plastic containers are the primary sites for oviposition and larval development for *A. albopictus* (Chareonviriyaphap *et al.*, 2003).

Aedes albopictus and *A. aegypti*are opportunistic and aggressive biters with feeding peaks in the early morning and late afternoon. *A. albopictus* with a wide host range including man, domestic and wild animals and birds, while *Ae. aegypti* prefer to feed exclusively on human blood. Multiple blood feeding and micro-movement to obtain blood sources have been confirmed for *A. aegypti* using PCR-based identification of human blood meals (Chow-Shaffer *et al.*, 2000). Dengue fever, dengue hemorrhagic fever and dengue shock syndrome are major cause of morbidity and mortality in children in many endemic Asian and South American countries (Guha-Sapir *et al.*, 2005).

Based on data from 112 national vital registration systems, 1200 deaths in Southeast Asia, 4000 deaths in western pacific and 2000 deaths in America for the year 2002 have been estimated due to dengue. Globally the incidence of dengue has increased at least four fold over the last three decades.

It is one of the most rapidly increasing mosquito-born diseases in the world and has been identified as a re-emerging disease in Southeast Asia. Efficacy of *Bti* and Copepods was evaluated for controlling *Aedes* larvae in water containers in Thailand (Kosiyachinda *et al.*, 2003). Microbial larvicides are specially processed bacterial compounds that are registered as pesticides for control of mosquito larvae in outdoor area. The microbial larvicides used for mosquito control are *Bacillus thuringiensis* var *israelensis (Bti)* and *Bacillus sphaericus (Bsph*).

Biological control with *Bacillus thuringiensis israelensis* (de Bac) and *Bacillus sphaericus larvicides* proved highly effective yet selective in action (Charles *et al.*, 2002). Therefore, environmentally safe to non-target organisms as well as for human exposure (W.H.O. 1999). In Pakistan first outbreak of Dengue hemorrhagic fever was reported in 1994 from Karachi (Chan *et al.*,1995). While recent outbreak of DF and DHF occurred in 2006. About 5,522 cases were reported. Among which 2025 were declared positive for dengue virus. Total of 50 deaths occurred comprising 43 in Karachi and 7 from Lahore.

The current study was designed for laboratory evaluation of *Bti* and *Bsph* as biological larvicide against laboratory-reared *Aedes albopictus* of *Bti*TP (Vectobac 5000ITU/mg) and WDG (water Dispersible Granules 3000 ITU/mg) as biological larvicides in artificial plastic containers against field collected 4th instars of *Aedes albopictus* from Lahore.

Material and method

All materials and glass wares were sterilized before use. Sterilization was done in the oven for 15 minutes at 200^{o}C. Deionized water was sterilized (for making any solution) by passing water through 0.2µm filter.

Bacillus formulations

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 laboratory and field assays. Test strain (2363) *Bacillus sphaericus* (*Bsph*) Vectolex® Technical powder (TP) 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 laboratory. The test strains consists of 1380 ITU (International Toxic Unit)/mg.

Mosquito rearing and maintenance

Wild caught *Aedes albopictus* were reared in the laboratory under standardized conditions at 27°C±3°C and 80%±3% Relative Humidity (RH) and a photoperiod of 16:8 (L:D) hours. Larvae for colony and experiments were maintained in batches of 200, each in 1200ml of deionized water in round plastic pans (21cm). Adults emerging within 24 hours period were maintained in cages 30cm³ and fed on 10% glucose solution and water.

Method

For laboratory bioassays, tests were performed with 7 different concentrations of *Bacillus thuringiensis israelensis (WDG and TP) and Bacillus sphaericus* (100, 10, 1, 0.1, 0.01, 0.001, 0.0001 ppm) in distilled water. Each concentration was replicated three times and three untreated cups were used as control in order to determine their active range and to find the minimum effective dose.

Test concentrations were obtained through sequential dilution of 100 ppm stock solution. For larval bioassays 25 larvae of early 4th instar of *A. albopictus* were placed in plastic cups (7.8cm diameter) consisting of 150ml of distilled water. Mortality in each concentration was recorded after every 24 hours. Mortality was counted by separating dead larvae from live with the help of camel hair brush. No food material was added during whole experiment.

Water was daily added to compensate the water loss by evaporation. Larval mortality in treated cups was corrected for any larval mortality in corresponding controls and percentage reduction in each group was calculated using the following formula Percentage reduction (%RD) = NC-NT\NC ×100.

Percentage of pupae/adult emergence inhibition was also recorded in each of the above group against all *Bacillus* strains/formulations.

Results

Susceptibility of wild caught laboratory reared *Aedes albopictus* early 4th instar larvae against, *Bti* TP vectobac® 5000 ITU/mg, *Bti* WDG vectobac® 3000 ITU/mg and *Bacillus sphaericus* (Bsph) Vectolex® (TP) 1380ITU/mg was evaluated.

Lethal concentrations LC_{50}-LC_{95} ranged between 0.26-1.21 and 0.047-0.28 against *Bti* TP, 0.052-0.14 and 0.025-0.091 against *Bti* WDG and 0.44-1.65 and 0.37-1.5 against *Bsph* at 24 and 48 hours post treatment indicated that early 4th instar larvae of *Aedes albopictus* were most susceptible against *Bti* WDG and least susceptible against *Bsp* in laboratory. Comparative Probit regression analysis (Table 1) indicated that *Bti* WDG was highly toxic at 48 and 72 hours exposure at the same mortalities. Hundred percent larval mortality occurred against 100 and 10ppm within 1-2 hours respectively.

Concerning percent larval mortalities 88, 64, 36, 28 and 12% occurred against 1, 0.1, 0.01, 0.001, 0.0001 ppm *Bti* TP at 24 hours post exposure (Table 2). Whereas 100, 72, 44, 36 and 28% and 100, 76, 52, 44, and 28% mortality was recorded at 48 and 72 hours post exposure with the same concentrations respectively.

Mean percent larval mortality was 96±4, 71±3.53, 44±4.62, 36±4.62 and 23±5.23 against 1, 0.1, 0.01, 0.001 and 0.0001 ppm at 24-72 hour post exposure. (Table 3, Fig. 1). Pupae emergence inhibition against Bti TP was recorded as 100, 67, 58, 43 and 17% and adult emergence inhibition was 100, 50, 40, 25 and 13% against 1, 0.1, 0.01, 0.001 and 0.0001 ppm respectively.

These results indicated that pupae emergence was completely inhibited at 1 ppm and > 50% at 0.01ppm against *Bti* TP in laboratory (Table 3).

Table 1. Susceptibility of laboratory reared *Aedes albopictus against Bacillus thuringiensis* (TP), (WDG) and *Bacillus sphaericus*.

Formulations	Time (Hours)	LC_{50}	95 %C.L	LC_{95}	95 %C.L
Bacillus thuringiensis (TP)	24	0.26	-0.075-1.10	1.21	0.68-6.73
	48	0.047	0.006-0.20	0.28	0.16-1.93
	72	0.028	0.050-0.12	0.27	0.151-2.61
Bacillus thuringiensis (WDG)	24	0.051	0.356-0.075	0.14	0.11-0.210
	48	0.025	0.013-0.041	0.09	0.066-0.149
	72	0.013	0.066-0.15	0.07	0.046-0.18
Bacillus sphaericus	24	0.44	0.095-2.33	1.65	0.93-11.89
	48	0.37	0.202-0.62	1.52	1.08-2.629
	72	0.23	0.076-0.41	1.24	0.89-2.12

Table 2. Probit- regression analysis of *Aedes albopictus* (early 4th instar) larvae against *Bacillus thuringiensisis raelensis* TP at 24 hours Post exposure.

Concentrations in ppm	Total no. of Larvae	No. of Dying	Expected Dying	Probit	LC_{50}	LC_{95}
10	25	25	25	1.00		
1	25	22	23	0.90		
0.1	25	16	10	0.38	0.26101	1.20581
0.01	25	9	8	0.33		
0.001	25	7	8	0.325		
0.0001	25	3	7	0.324		

Table 3. Mean Percent Mortality for wild caught Laboratory reared *Aedes albopictus* (early 4th instar) larvae with Pupae/Adult emergence inhibition against *Bacillus thuringiensis* TP at 24-72 hours post exposure.

Time in hours	Concentrations in ppm				
	1	0.1	0.01	0.001	0.0001
24	88	64	36	28	12
48	100	72	44	36	28
72	100	76	52	44	28
Mean Percent Larval Mortality ±	96 ± 4	71 ± 3.53	44 ± 4.62	36 ± 4.62	23 ± 5.23
Percent Reduction of Pupae	100	67	58	43	17
Percent Reduction of Adult	100	50	40	25	13

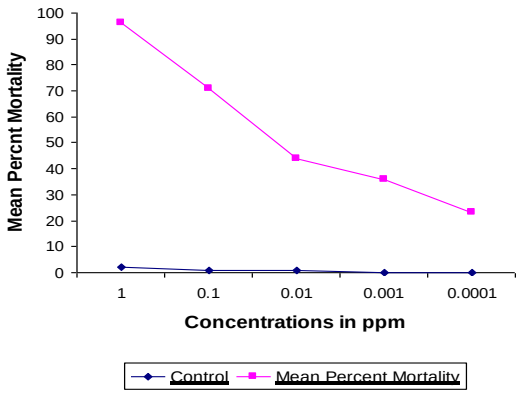

Fig. 1. Mean Percent Mortality for wild caught Laboratory reared *Aedes albopictus* (early 4th instar) larvae against *Bacillus thuringiensis* TP at 24-72 hours post exposure.

Percent larval mortalities recorded against *Bsph* were 76, 48, 36, 20 and 12% at 1, 0.1, 0.01, 0.001, and 0.0001 ppm at 24 hours post exposure (Table 4, Fig. 2) while 80, 52, 36, 24 and 16% at 48 hours post exposure and 88, 60, 40, 32 and 20% at 72 hours post exposure (Table 5, 6) with the same concentrations respectively. Percent inhibition of pupae emergence against *Bsph* was 67, 50, 33, 18 and 10% while adult emergence inhibition was 100, 40, 20, 7 and 5% against 1, 0.1, 0.01, 0.001 and 0.0001ppm respectively (Table 7). Mean percent mortalities were 81±3.53, 53±3.53, 37±2.31, 25±3.53 and 16±2.31 against 1, 0.1, 0.01, 0.001 and 0.0001 ppm at 24-72 hours post exposure respectively (Table 7).

ANOVA (Analysis of variance) indicated that mean percent larval mortalities are significantly higher against 1 and 0.1ppm *Bti* TP as compared to *Bsph* i-e P =0.051 and P < 0.05 = 0.025 respectively. In general the effect is dose dependent, higher doses up to 1ppm caused 100% larval/pupae mortalities at 48 hours exposure.

While 0.1ppm caused > 50% larval – pupae mortalities. However *Bti* TP and WDG are more toxic as compared to *Bsph* for laboratory reared *Aedes albopictus* larvae-pupae emergence. On the base of these laboratory results *Bsph* was not recommended to use in the field for *Aedes abopictus* control.

Table 4. Probit-regression analysis of *Aedes albopictus* (early 4th instar) larvae against *Bacillus sphaericus* at 24 hours Post exposure.

Concentrations in ppm	Total No. of Larvae	No. of Dying	Expected Dying	Probit	LC_{50}	LC_{95}
10	25	25	25	1.0000		
1	25	19	19	0.7761		
0.1	25	12	8	0.3192	0.44423	1.64857
0.01	25	9	7	0.2766		
0.001	25	5	6	0.2721		
0.0001	25	3	6	0.2721		

Table 5. Probit-regression analysis of *Aedes albopictus* (early 4th instar) larvae against *Bacillus sphaericus* at 48 hours Post exposure.

Concentrations in ppm	Total no. of Larvae	No. of Dying	Expected Dying	Probit	LC_{50}	LC_{95}
10	25	25	25	1.000		
1	25	20	20	0.815		
0.1	25	13	9	0.352	0.36697	1.524
0.01	25	9	8	0.305		06
0.001	25	6	7	0.300		

Table 6. Probit-regression analysis of *Aedes albopictus* (early 4th instar) larvae against *Bacillus sphaericus* at 72 hours Post exposure.

Concentrations in ppm	Total no. of Larvae	No. of Dying	Expected Dying	Probit	LC_{50}	LC_{95}
10	25	25	25	1.000		
1	25	22	22	0.895		
0.1	25	15	10	0.419		
0.01	25	10	9	0.363	0.22493	1.24093
0.001	25	8	9	0.357		
0.0001	25	5	9	0.357		

Table 7. Mean percent mortality for wild caught Laboratory reared *Aedes albopictus* (early 4th instar) larvae with Pupae/Adults emergence inhibition against *Bacillus sphaericus* at 24-72 hours post exposure.

Time in hours	Concentrations in ppm				
	1	0.1	0.01	0.001	0.0001
24	76	48	36	20	12
48	80	52	36	24	16
72	88	60	40	32	20
Mean Percent Larval Mortality	81 ± 3.53	53 ± 3.53	37 ± 2.31	25 ± 3.53	16 ± 2.31
Percent Reduction of Pupae	67	50	33	18	10
Percent Reduction of Adults	100	40	20	7	5

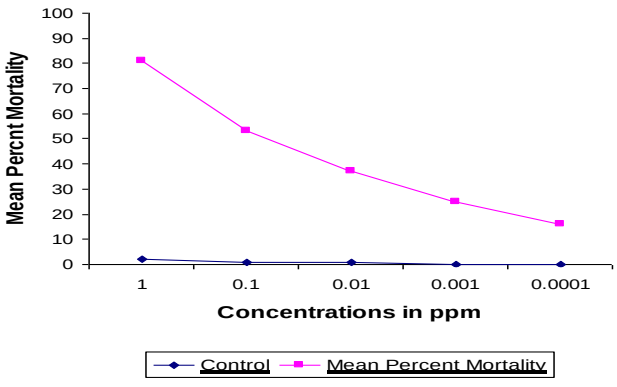

Fig. 2. Mean Percent Mortality for wild caught Laboratory reared *Aedes albopictus* (early 4th instar) larvae against *Bacillus sphaericus* at 24-72 hours post exposure.

Discussion

The only way to prevent dengue transmission, therefore is to reduce the population of its principal vectors, *A. aegypti* and *Aedes albopictus*, particularly in South East Asia including Pakistan. There has been no promising solution for sustainable control of dengue vector to present date. The trend for dengue vector has shifted from insecticides to biological control using biological control agents such as *Bacillus thuringiensis* var *israelensis* (*Bti*). Long-term vector control approaches include source reduction, environmental management and application of chemical and microbial larvicides.

Microbial larvicides have several advantages over other mosquito control agents, not only high efficacy but also environmentally safe for human. Based upon their properties, these larvicides have become powerful vector control tools that are ground for disease control in Africa and other parts of the tropics. These larvicides are being used effectively against different mosquito species in different regions of the world (Becker 1992; Barbazan *et al* 1997; Fillenger *et al* 2003). Although their safety to the environment and their efficacy against a variety of mosquito species have been demonstrated by several authors, both in laboratory and field conditions, their use in vector control program is less studied.

Laboratory studies with different strains and formulations of *B. sphaericus* revealed that *B. sphaericus* preparations are more effective against larvae of *Culex* sp than *Anopheles* sp. However, *B. sphaericus* was not effective against *A. aegypti*. Though, *Bti* formulations are very effective in the control of Aedes species, and have low potentiality for the development of resistance.

Recently the work in our laboratory indicated *Bti* WDG was highly effective against wild caught *Aedes albopictus* larvae in laboratory and field assays (MSc. Thesis Saima Hanif 2007). Current study evaluated efficacy and residual activity of *Bti* TP, WDG and *Bsph* in laboratory and field assays against dengue fever vector *Aedes albopictus* larvae-pupae/adults emergence in Lahore, Pakistan. According to our results are *Bsph* is ineffective for the control of *Aedes* mosquito larvae in present study LC_{50} – LC_{95} ranged from 0.37-1.52ppm for *Bsph* as compared to 0.047-0.28ppm for *Bti* TP 48 hours post exposure. Mean percent larval mortality and reduction in pupae/adults emergence was also significantly more against *Bti* TP and *Bti* WDG as compared to *Bsph* ($P < 0.05$).

However existing *Bti* formulations are found highly effective against *Aedes* mosquitoes. Although more improvement particularly to increase its long term effect and to enhance control will improve the process. Tablet and granule formulations of *Bti* have been developed which can be used by individuals and community particularly to control container breeding *A. aegypti* and *B. sphaericus* has no activity against Aedes spp. and *B. sphaericus*, however can also be used in rotation with *Bti* to delay the development of resistance in target mosquitoes.

In present study due to less toxicity in laboratory *Bsph* was not recommended to use in the field. Among the two *Bti* formulations and *Bti* WDG although, *Bti* TP has more potency (5000 ITU/mg) as compared to WDG (3000 IT/mg), the above mentioned LC_{50} – LC_{95} indicated that the later is more toxic for *Aedes albopictus* larvae- pupae/adults emergence.

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Refrences

Barbazan P, Baldet T, Darriet F, Escaffre H, Haman D, Hougard JM. 1997. Control of *Culex quinquefasciatus* (Diptera: Culicidae) with *Bacillus sphaericus* in Maroua, Cameroon. J Am Mosq Control Assoc **13,** 263-269.

Baumann G, Geisse S, Sullivan M. 1991. Cyclosporin A and FK-506 both affect DNA binding of regulatory nuclear proteins to the human interleukin-2 promoter. New Biol. **3(3),** 270-8.

Becker N.1992. Community participation in the operational use of microbial control agents in mosquito control programmes. Bull Soc Vector Ecol **17,** 114-118.

Chan SY, Kautner IM, Lam SK. 1995. Detection and serotyping of dengue viruses by PCR: a simple rapid method for the isolation of viral RNA infected mosquito's larvae. J. Trop. Med. Public Health **25,** 258-261.

Chareonviriyaphap T, Akratanakul P, Nettanomsak S. 2003. Larval habitats and distribution patterns of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (skuse) in Thailand. Southeast Asian Journal of Tropical Medicine and Public Health **34,** 529-535.

Charles JF, Nielsen-Le-Roux C. 2002. Mosquitocidal bacterial toxins: diversity, mode of action and resistance phenomena. Memories Do Institu to Oswaldo Cruz **95,** 201-206.

Chow-Shaffer E, Sina B, Hawley WA. 2000. Laboratory and field evaluation of polymerase chain reaction based forensic DNA profiling for use in identification of human blood meal sources of *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology **37(4),** 492-502.

Eritga R, Escosa R, Lucientes J, Marques E, Roiz D, Ruiz S. 2005. Worldwide invasion of mosquito's present European distribution and challenges for Spain. Biological Invasion **7(1),** 87-97.

Fillinger U, Knols BGJ, Becker N. 2003. Efficacy and efficiency of new *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* formulations against Afrotropicalanophelines in Western Kenya. Trop Med Int Health **8,** 37-47.

Guha-Sapir DM, Schimmert B. 2005. Dengue fever: new paradigms for a changing epidemiology. Emerging themes in epidemiology. Open access journal.

Kosiyachinda P, Bhumiratana A, Kittayapong P. 2003. Enhancement of the efficacy of a combination of *Mesocyclops aspericornis* and *Bacillus thuringiensis* var. *israelensis* by community-based products in controlling *Aedes aegypti* larvae in Thailand. The American journal of tropical medicine and hygiene **69(2),** 206-12.

Mittal CJ, Adak T, Batra CP. 2001. Comparative toxicity of selected larvicidal formulations against *Anopheles stephensi* Liston and *Aedes aegypti*. Linn. J. Commun **33(2),** 116-120.

Pratt HD, Moore CG. 1993. Mosquitoes of public health importance. Eco Access Publications 58-71.

Tewari SC, Munirathinam A, Ganjanana A. 2004. Dengue vector prevalence and viral infection in a rural area in South India. Trop. Med. Int. Health **4,** 499-507.

World Health Organization. 1999. International program on chemical safety (IPCS): microbial pest control agent *Bacillus thuringiensis*. Environmental Criteria **217,** 1-105.