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Comparative study of toxicity of *Bacillus thuringiensis* against *Aedes albopictus* larvae, pupae and adults in plastic container and used tyres

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Abstract

Bioefficacy and residual activities of *Bti* TP and WDG were evaluated, in outdoor conditions, with various concentrations in two types of water storage containers i-e plastic containers and used tyres, which constitute vast developmental/breeding sites for *Aedes* mosquitoes in urban/semi urban areas. Maximum residual activity of *Bti* TP was 35 days against 2.4ppm in plastic containers and minimum 14 days against 0.3ppm in used tyres as compared to *Bti* WDG observed in plastic containers for 35 days against 0.26 ppm and minimum residual effect of 14 days with 0.05ppm in used tyres. In general *Bti* WDG showed 10-12 X more residual effect as compared to *Bti* TP in both types of containers. In comparison of two types of containers, residual activity of *Bti* TP and WDG was low in used tyres as compared to plastic containers with respect to larval mortalities. However, there was no significant difference ($P > 0.05$) between two types of containers.

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Introduction

Mosquitoes are important vector of human and animal diseases. The three genera of mosquitoes; *Anopheles, Aedes* and *Culex*, are the primary vectors for pathogens owing to their obligate haematophagy. Pathogens causing the most important past and present human mortality and morbidity, are transmitted by mosquitoes. The female serve as intermediate host for numerous diseases. Such as human malarial parasites (Plasmodium), the filarial parasites, (*Waucheria bancrofti* and *Brugia malayi*), and a number of arboviruses (Yellow fever, Dengue, Encephalitis), which are the major cause of human mortality and morbidity in the world (Yogesh and Patole, 2000).

Life cycle of *Ae. albopictus* is closely associated with human habitats, and it is a container-inhabiting species which lays its eggs in water containing receptacles in urban, suburban, rural and forested areas. The primary immature habitats of this species are artificial containers such as tyres, flower pots, cemetery, vases, buckets, tin cans, rain gutters, ornamental ponds, drums etc have been reported. Larvae are also found in natural containers such as tree holes, bamboo pots and leaf axils. (Eritja *et al.,* 2005). The ability of *Ae. albopictus* to occupy a wide variety of habitats further enhances its chances of being a vector species. (O'Meara *et al.,* 1993).

Ae. albopictus was found prevalent mainly during rainy seasons preferring to breed outdoors in discarded containers (Hawley, 1988) possibly acting as secondary vector in rural areas of South India (Tewari *et al.,* 2004). Distribution of dengue among children in rural and sub-rural areas in Thailand has been studied. The results showed that most transmission occurs in residential environments and within a young age group (3-8 years old), which has a significantly higher risk of infection than older children (Strickman *et al.,* 2000). At present there is no vaccine for preventing dengue. Prevention and control of dengue and DHF currently depends on controlling the mosquito vector. Space sprays with insecticides to kill adult mosquitoes are not usually effective unless they are used indoors.

The most effective way to control the mosquitoes that transmit dengue is larval source reduction, i.e. elimination or cleaning of water-holding containers that serve as the larval habitats for *Aedes* mosquitoes in the domestic environment (Gubler, 1989; Reiter and Gubler, 1997). The removal of mosquito breeding habitat can be an effective method for mosquito control (Dame and Fasulo, 2003).

The use of *Bti* more toward *Aedes* control rather than other mosquito species may be due to the bottom feeding behavior of the *Aedes* species as most of the *Bti* toxins sediment to the base of container during treatment (Su and Mulla, 1999). Among mosquitoes, different preparations of *Bti* 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 *et al.,* 1993). However, even within one genus, some species are more susceptible than others. Mosquito's species, which are not filter feeders, do not seem susceptible. There are several types of *Bti* based products available, ranging from liquid to pellet and angular formulations. *Bti* products that are available commercially include Dipel, Javelin, Leptox, Novabac, Vectobac, Teknar, Bactimos, Skeetal and Mosquito attack.

Vectobac (WDG) manufactured by Valent Bioscience Crop. IL, U.S.A. is a mosquito biolarvicide containing the active ingredient *Bacillus thuringiensis israelensis*. WDG is a clean, dust free granular and can be applied with either aerial or ground equipment.

Vector control in tropics (Africa) can targ*et al*l stages of mosquito life cycle but larviciding and source reduction have a major advantage in that they control mosquitoes before they disperse and transmit diseases (Killeen *et al.,* 2002). Biological control with *Bacillus thuringiensis israelensis* (de Bac) and *Bacillus sphaericus* larvicides proved highly effective yet selective in action (Charles *et al.,* 2000) and therefore, environmentally safe to non-target organisms as well as for human exposure (WHO, 1999).

These Bacillus products are characterized by easy handling, cost effectiveness and capability of being produced locally. Furthermore, application of larvicides does not require expensive equipment; it can be organized locally and highly acceptable in the community (Becker, 1992). In view of this present study was designed to analyze toxicity of *Bacillus thuringiensis* against *Aedes albopictus* larvae, pupae and adults in plastic container and used tyres

Material and method

Mosquito Rearing

Field collected early 4th instar larvae *of Ae. albopictus* were used in the field assay. Wild caught larvae were periodically collected from (July-Sep 2006 and July-August 2007) from natural and artificial containers at various sites of GC University Lahore. These larvae were reared in insectory for laboratory experiment at Zoology Dep't GCU Lahore.

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 *Ae. albopictus* in laboratory and field assays. Wild caught *Aedes albopictus* were reared in the laboratory under standard conditions at 27°C±3°C temperature, 80%±3% Relative Humidity (R H) and a photoperiod of 16:8 (L:D) hours. Larvae for colony and experiments were maintained in batches of 200 each in 1200 ml of deionized water in round plastic pans (21cm). Each batch was fed with 2 drops of 10% sugar solution daily for the first 3 days then finely coursed fish food.

Method

Keeping in view that *Aedes* mosquitoes breed in clear water of house hold containers and used tyres, field evaluations were conducted in 23 cm diameter plastic tubs and medium sized automobile used tyres containing 2 liters of clear rainy water (obtained from a natural container, plastic drum of *Aedes* mosquito at GCU) (Fig: 18, Fig: 19).

The tubs and tyres were maintained outdoors in GCU open field with dense green habitat under a shade of hard board to protect from rain and direct sun light. One hundred larvae of *Aedes albopictus (*early 4th instar) were introduced into each tub/used tyres. In field evaluation, treatment concentration was evaluated on the basis of LC_{50} and LC_{95} from laboratory assays.

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 acclimatization four concentrations of *Bti* (TP) 0.3, 0.6, 1.2, 2.4 ppm were applied in four tubs/used tyres. In another set of experiment three concentrations of *Bti* (WDG) 0.05, 0.13, 0.26ppm were used in three tubs/used tyres at the same time.

Each tub was kept covered with fine-mesh plastic screen to protect from air-borne debris, wild insects and any oviposition by wild mosquitoes. An opaque plastic cover was used to cover the used tyres for the same purpose. All tubs/tyres were examined daily to record post-treatment larval and pupal mortality or survivorship and adult emergence. A large asteur pipette was used (one of each concentration) to collect live pupae and separate dead larvae and pupae from each tub/used tyre on a daily basis. In each set of experiment 100 larvae of *Aedes albopictus* were replaced daily in the first week and twice a week and from second week onward to evaluate residual activity in each container.

The test procedure and daily observation of the two formulations in two types of containers (tubs/used tyres) were the same. Air and water temperature in the tubs/used tyres was recorded throughout the evaluation (july-september). Temperature recording thermometers were randomly submerged in different tubs/used tyres. Minimum and maximum air temperature varied from 29°C to 37°C, water temperature 26°C to 35°C and relative humidity from 58% to 67% during the experiment (Fig. 17).

The efficacy and residual activity of a formulation in the tub and used tyre was assessed as percent mortality and percent inhibition of pupae/adult emergence in treatments and adjusted for any larval or pupal mortality in corresponding controls with the formula (Mulla *et al.,* 1974).

Results

Field evaluation of *Bti* TP and *Bti* WDG in plastic containers and used tyres 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 – 1.2mg/lit) and *Bti* WDG (0.05 – 0.13mg/lit) from laboratory with fixed volume of water (2 liter) various concentrations were applied in the field using two different containers i-e Plastic containers (2.5 liter) and Used tyres (16 inches) most often recorded as breeding containers of *Aedes* mosquitoes in nature.

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 0.3ppm, while in Used tyres 4, 3, 2 and 1 day respectively, whereas LT_{50} was 17, 15, 11 and 9 days, as compared to used tyres 15, 10, 9 and 8 days at the same concentrations respectively.

Maximum residual activity of *Bti* TP was 35 days recorded against 2.4ppm in plastic containers and minimum 14 days against 0.3 ppm in used tyres (Table 1, Fig 1.). In plastic containers residual effect of *Bti* TP was 35, 28, 21 and 21 days against 2.4, 1.2, 0.6 and 0.3ppm, whereas, in used tyres 28, 21, 21 and 14 days at the same concentrations (Table 1, Fig. 2).

In general residual activity of *Bti* TP was lower in used tyres as compared to plastic containers. However there is no significant difference between the two ($P > 0.05 = 0.27$) types of containers.

Table 1. Comparative field evaluation as percent Larval mortality of *Aedes albopictus* against *Bacillus thuringiensis* TP in Plastic Containers and Used Tyres.

Concentrations in ppm	Plastic Containers						Used Tyres				
	Max days of Residu-al activity	Percent mortality/week					Max days of Residual activity	Percent mortality/ week			
		1st	2nd	3rd	4th	5th		1st	2nd	3rd	4th
2.4	35	94	65	40	17	3	28	89	59	28	9
1.2	28	89	53	21	6	0	21	81	46	17	0
0.6	21	76	41	12	1	0	21	71	34	8	0
0.3	21	69	32	4	0	0	14	65	27	0	0

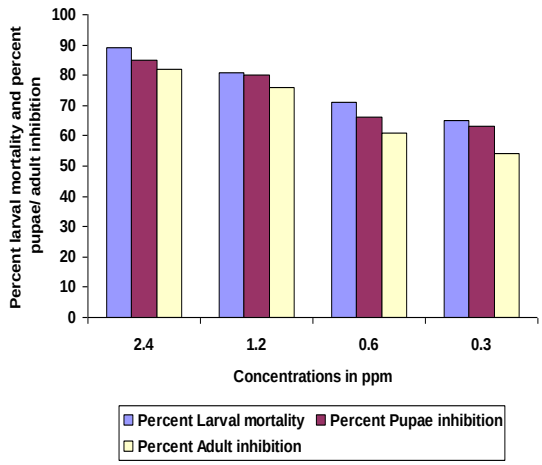

Fig. 1. Field Evaluation as Mean Percent Larval Mortality with Pupae/Adults emergence inhibition of *Aedes albopictus* against *Bacillus thuringiensis* TP in Used Tyres 1 Week Post Exposure.

Bti WDG at 0.26, 0.13 and 0.05ppm induced 100% larval mortality for 5, 2 and 1 day while in used tyres 3, 2 and 1 day at the same concentrations respectively. LT_{50} in plastic containers was 15, 10 and 9 days as compared to used tyres 15, 9 and 8 days with same concentrations respectively.

Residual effect in plastic container with 0.26, 0.13 and 0.05ppm was 35, 28 and 21 days (Table 2, Fig. 2) whereas, in used tyres 28, 21 and 14 days (Table 2, Fig. 2) at the same concentrations respectively.

Maximum residual activity of *Bti* WDG was observed in plastic containers for 35 days at 0.26 ppm and minimum residual effect of 14 days with 0.05 ppm in used tyres (Table 2).

Table 2. Comparative field evaluation as percent larval mortality of *Aedes albopictus* against *Bacillus thuringiensis* WDG in Plastic Containers and Used Tyres.

Concentrations in ppm	Artificial Plastic Containers						Used Tyres				
	Max days of Residual activity	Percent mortality/ week					Max days of Residual activity	Percent mortality/ week			
		1st	2nd	3rd	4th	5th		1st	2nd	3rd	4th
0.26	35	89	65	42	10	2	28	86	63	37	11
0.13	28	78	42	13	1	0	21	73	40	8	1

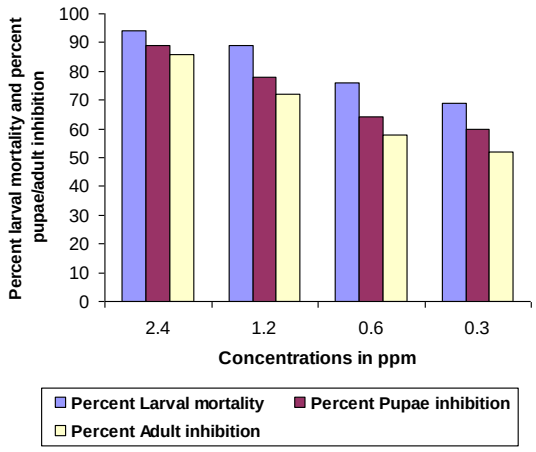

Fig. 2. Field Evaluation as Mean Percent Larval Mortality with Pupae/Adults emergence inhibition of *Aedes albopictus* against *Bacillus thuringiensis* TP in Plastic Containers 1 Week Post Exposure.

Results as percent pupae/adult emergence inhibition/week are presented in tables 16 – 19. Reduction in pupae emergence by the end of 1st – 5th weeks post exposure was 89 and 05% and adult emergence was inhibited by 86 – 04% against 2.4ppm *Bti* TP while 60-6% in pupae emergence inhibition and 52-4% adult emergence inhibition was observed by 1st and 3rd week post exposure against 0.3ppm *Bti* TP in plastic containers (Table 3, Fig 2). Whereas in used tyres pupae percent inhibition was 85-13% and 82-8% adults were inhibited against 2.4ppm by the end of 1st and 4th week post exposure (Table 3, Fig. 1).

No significance difference was observed ($p > 0.05$) between two types of containers with respect to pupae/adult emergence inhibition. (Table 16-17).

Table 3. Field Evaluation as percent Inhibition of Pupae/Adult emergence of *Aedes albopictus* against *Bacillus thuringiensis* TP in Used Tyres.

Concentrations in ppm	Percent Inhibition of Pupae/Week				Percent Inhibition of Adult / Week			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
2.4	85	54	37	13	82	45	26	8
1.2	80	46	25	2	76	37	19	2
0.6	66	34	6	0	61	30	4	0
0.3	63	33	0	0	54	28	0	0

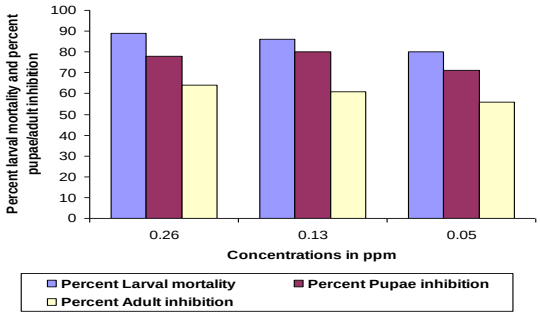

Fig. 3. Field Evaluation as Mean Percent Larval Mortality with Pupae/Adults emergence inhibition of *Aedes albopictus* against *Bacillus thuringiensis* WDG in Plastic Containers 1Week Post Exposure.

Field evaluation against Bti WDG induced 86, 80 and 61% pupae reduction and 80, 71 and 56% adults emergence inhibition using 0.26, 0.13 and 0.05ppm in plastic containers by the end of 1st week post exposure respectively (Table 4, Fig 3). The pupae/adult emergence Inhibition effect was observed for 4 weeks against 0.26ppm and 3 weeks with lowest concentrations i-e 0.05ppm (Table 4, Fig. 4).

Reduction in pupae emergence was 79, 65 and 51% against 0.26, 0.13 and 0.05ppm *Bti* WDG while 76, 65 and 51% adults were inhibited with same concentrations 1 week post exposure in used tyres.

Pupae/adult emergence inhibition was observed upto 4 weeks post exposure against 0.26 ppm and 2 weeks against 0.05 ppm *Bti* WDG in used tyres (Table 4, Fig. 4).

In general comparison of two types of containers residual activity of *Bti* WDG was low in used tyres as compare to plastic containers with respect to larval mortalities-pupae/adult emergence inhibition.

Table 4. Field Evaluation as percent Inhibition of PupaeAdult emergence of *Aedes albopictus* against *Bacillus thuringiensis* WDG in Used Tyres.

Concentrations in ppm	Percent Inhibition of Pupae/week				Percent Inhibition of Adult/week			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
0.26	79	44	24	3	74	34	19	3
0.13	65	32	5	0	65	29	3	0
0.05	51	9	0	0	51	7	0	0

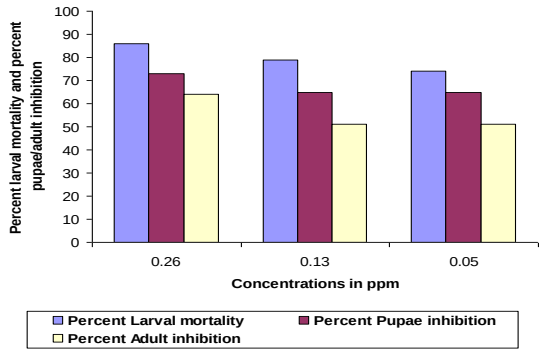

Fig. 4. Field Evaluation as Mean Percent Larval Mortality with Pupae/Adults emergence inhibition of *Aedes albopictus* against *Bacillus thuringiensis* WDG in Used Tyres 1 Week Post Exposure.

The studies indicated that LT_{100} and residual activity was dose dependant. Higher doses of *Bti* enhanced the residual effects against various species of mosquitoes. These values are higher than our values for *Bti* TP and *Bti* WDG at 48 hours post exposure, indicating *Bti* TP and *Bti* WDG was 10X and 12X more toxic than mosquito dunks. Residual activity using 0.3-2.4ppm of the *Bti* TP formulation varied from 3-5 weeks (21-35 days) in both types of containers i-e plastic containers and used tyres whereas, much lower concentrations of *Bti* WDG 0.05-0.26ppm 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.

Discussion

The trend for dengue vector has shifted from insecticides to biological control using biological control agents. 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 and used effectively against different mosquito species in different regions of the world. 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.

In current study 100 % larval mortality was observed upto 5 days against *Bti* TP at 2.4 *Bti* WDG at 0.26 ppm. Similarly pupae/adults emergence was inhibited > 80% at the end of first week with the same concentration of the two *Bti* formulations. In general among the formulations *Bti* WDG has 10X more residual activity as compared to *Bti* TP in both plastic containers and used tyres. These results indicated that *Bti* WDG was more effective at lower rate and inhibit larvae - adult emergence by 10-12X more as compared to *Bti* TP. The residual activity of *Bti* WDG was low in used tyres as compared to plastic containers but there was no significant difference of residual effect between the two ($P > 0.05$) types of containers. Apparently these results indicated that *Aedes albopictus* breeding in used tyres and less susceptible to these larvicides as compared to plastic containers.

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References

Balaraman SL, Hoti, Manomani M. 1993. Susceptibility of An. stephensi, Culex and Aedes against *Bacillus thuringiensisis israelensis* formulation. Curs Sa 150-152.

Becker N. 1992. The use of *Bacillus thuringiensis* subsp. *israelensis* (Bti) against mosquitoes, with special emphasis on the ecological impact. Israel J. Entomol **165,** 170-176.

Charles JF, Nielsen-Le Roux, C. 2000. Mosquitocidal bacterial toxins: diversity, mode of action and resistance phenomena. Memorias Do Instituto Oswaldo Cruz **95,** 201-206.

Dame D, Fasulo TR. 2003. Mosquitoes. Public Health Pesticide Applicator Training Manual.

Eritja R, Escosa R, Lucientes J, Marquès E, Roiz D, Ruiz S. 2005. Worldwide invasion of vector mosquitoes: present European distribution and challenges for Spain. Biological Invasion **7,** 87.

Gubler DJ, Reiter P. 1997. Surveillance and control of urban dengue vectors. In: Dengue and dengue hemorrhagic fever. New York: CAB International. pp. 425-462.

Gubler DJ. 1998. Dengue and dengue hemorrhagic fever. Clinical Microbiology Reviews. 480-496.

Hawley WA. 1988. The biology of *Aedes albopictus*. J. Am Mosq Control Assoc Suppl. **1,** 1-39.

Killeen GF, Fillinger U, Bart , Knols GJ. 2002. Advantages of larval control for African malaria vectors: Low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage: Malaria Journal **1,** 8.

Mulla MS, Su T. 1999. Activity and biological effects of neem products against arthropods of medical and veterinary importance. J Am Mosq Control Assoc **15(2),** 133-52.

Mulla MS. 1974. Laboratory and field evaluation of insect growth regulators against mosquitoes. Proc. Papers Calif. Mosq. Contr. Assoc **42,** 175-176.

O'Meara NM, Sturis J, Blackman JD, Byrne MM, Jaspan JB, Roland DC, Thistlethwaite JR, Polonsky KS. 1993. Oscillatory insulin secretion after pancreas transplant. Diabetes **42(6),** 855-61.

Shouche YS, Patole MS. 2000. Sequence analysis of mitochondrial 16S ribosomal RNA gene fragment from seven mosquito species. J Biosci **25(4),** 361-6.

Strickman D, Miller ME, Kim HC, Lee KW. 2000. Mosquito surveillance in the demilitarized zone, Republic of Korea, during an outbreak of *Plasmodium vivax* malaria in 1996 and 1997. J Am Mosq Cont Assoc **16,** 100-113.

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.