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Full Length Article

Efficacy of *Bacillus thuringiensis israelensis* in Different Qualities of Water Positive against Larvae of *Aedes aegypti* (Diptera: Culicidae) under Laboratory Conditions

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Abstract

Mosquitoes can breed in such varieties of water grades as fresh, turbid, foul and sewage water. Different water grades are found, on which mosquitoes can breed, due to environmental pollution caused by industry, inappropriate sewage system and lack of awareness. Therefore, the present studies demonstrated the efficacy of *Bacillus thuringiensis israelensis* (*Bti*) Dunks against *Aede aegypti* larvae in different water grades under lab conditions. Different concentrations of *Bti* dunks (10, 20, 50 and 100 ppm) were used in six water grades *viz*., rich organic, foul, sewage, industrial, turbid and useable waters against *Ae. aegypti* larvae with control treatments in each experiment. Mortality of larvae was checked after 2, 4, 6, 12, 24 and 48 h. The mortality was highest (89%) in useable water followed by foul water (88%) after 24 h while low mortality (62 and 55%) was noted in turbid water and rich organic water due to the presence of organic matter, pollution and mud in water. Highest LC_{50} (6.08 ppm) was observed in rich organic water while lowest LC_{50} (3.63 ppm) was observed in sewage water after 48 h. It is concluded that use of *Bti* is an effective method to combat problem of mosquito borne diseases or mosquitoes themselves in all types of water but more effective in clear water. © 2018 Friends Science Publishers

Keywords: *Aedes* larvae; *Bacillus thuringiensis israelensis*; Water grades; Mortality

Introduction

Mosquitoes are vectors of the world's most hazardous pathogens of diseases such as filariasis, yellow fever, malaria, encephalitis, chikungunya, Zika virus and dengue (Reinert *et al*., 2004; Radhika *et al*., 2011). Dengue fever is an emerging health issue in Pakistan presently. *Aedes albopictus* and *A. aegypti* mosquitoes are the vectors of dengue fever. This disease emerged as an endemic all over the country (Pakistan) during 2011 particularly in Punjab province where >20,000 confirmed cases were found with 207 deaths in the city of Lahore only (Anonymous, 2013). Major reasons for outbreak of this disease were overloaded cities, inadequate water management and poor sanitary system. Prevention is the only way to avoid dengue fever; both at social and managerial levels (Junwei *et al*., 2006). Mosquito population has been reduced through chemicals or source reduction or biological methods. The use of chemicals and source reduction cause severe burden on the ecosystem and the environment, the application of *Bacillus thuringiensis israelensis* (biological method) is an eco-friendly for the management of its juvenile stages. Toxicity to non-target flora and fauna was remarkably low (Lacey and Siegel, 2000; Lacey, 2007).

Mosquitoes can breed in a variety of habitats like polluted waters with organic and/or inorganic matter, small containers, tires and tree holes, roadside and irrigation drains, grasslands, woodland pools, tidal waters, salt marshes, dumped pools, bird baths, roofs, clogged gutters, etc. nevertheless stagnant water is prerequisite for successful breeding (Lacey and Siegel, 2000).

A number of biotic and abiotic factors (water temperature, sunlight, quantity of larvae, larval instar, application of larval food, and some water chemistry elements such as water pollution, dissolved oxygen, dust particles and concentration of dissolved organic matter) affect the action of *B. thuringiensis* var. *israelensis* (*Bti*) (Becker *et al*., 2010). Generally water temperature affects the toxic activity of *Bti* due to larval feeding rate. The *Bti* efficacy increases as a larvicide at higher temperature up to certain limit (35°C) due to high feeding rate of mosquito larvae (Majambere *et al*., 2010). But the high temperature beyond a certain limit (>35°C) may decrease the activity of *Bti* due to the biodegradation of *Bti* (Karch *et al*., 1992). Moreover, the developmental time of the mosquito vector shortened with increase in temperature and the possibility

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for retreatment of breeding places increases (Becker *et al*., 1992). Every geographical locality attributes a specific combination of above said factors that urge the need for testing the efficacy of *Bti* under those situations (Romi *et al*., 1993). In Punjab, the environmental pollution exists due to industrialization. So, variety of water grades are present in different localities of Punjab. Therefore, the current study was proposed to investigate efficacy of *Bti* in various water grades under laboratory conditions.

Materials and Methods

Collection of Mosquitoes

Adult mosquitoes were collected with aspirator from Faisalabad (Nasir *et al*., 2017) during monsoon season. The collected specimens were brought on the same day to the Department of Zoology, Government College University, Faisalabad, by means of a plastic bottle (250 mL) knotted up with muslin cloth to the Department of Zoology, Government College University, Faisalabad, Pakistan.

Rearing of Mosquitoes

After identification with the help of keys (Darsi and Pradhan, 1990), the mosquitoes were reared (Ahmad *et al*., 2017) under laboratory conditions; 25±2°C and 75% RH in rearing cages. Female mosquitoes (Nasir *et al*., 2015) were fed with blood meals from rat and males were fed on 10% sucrose solution. The larvae were shifted in plastic trays (30 x 15 x 6 cm) after hatching of eggs and used for bioassays (20 larvae in 250 mL of water).

Collection of Water Samples

Different water grades including water with rich organic matter (from water channel in a park), tap water (from tap), turbid water (from water channel), foul water (from a small deserted pond along bank of water channel), sewage water (from sewage pond) and industrial water (from the pond near an industry) were collected and their physico-chemical properties (Table 1) were studied in the laboratory before experiment. Mosquito larvae were found in all waters except industrial water.

Test Material for Larval Bioassays

The test strains of bacterial larvicide *Bti* dunks\briquets by Summit, USA by 7000 ITU (International Toxic Unit/mg) (Fansiri *et al*., 2006) were tested for their efficacy in different water grades against *A. aegypti* larvae. *Bti* EPA Registration No is 6218-47 and EPA Est. No is 6218-MD-2 was used. The net weight is 0.458 oz. per Briquet/Dunk or 13 grams per Briquet/Dunk. Different concentrations (10, 20, 50, 100 ppm) of *Bti* dunks were prepared in distilled water and used accordingly against mosquito larvae (Jahan *et al*., 2011).

Bioassay

For each treatment, twenty larvae were taken into glass beakers having 250 mL of water. These larvae were acclimatized for 1 h before adding *Bti*. Each experiment was replicated three times with a control (containing only water of respective grade with no *Bti*) under lab conditions (25 ± 1°C temperature & 70 ± 5% R.H.) Dead larvae were counted after 2, 4, 6, 12, 24 and 48 h. Death of mosquito larvae was determined as a complete lack of movement, even with gentle prodding with a probe (Fansiri *et al*., 2006).

Statistical Analysis

Abbott's Formula was used to calculate the corrected mortality percentage. Then data was examined by using statistical method, software Design Expert 9 and Minitab 15. Probit Analysis was used to evaluate the LC_{50}, LT_{50}, slope, standard error; chi square and probability.

Results

Hundred percent (100%) mortality occurred in industrial water during acclimatizing stage, so only five types of water are included. No effect of *Bti* was seen during first 2 h except in foul water (20%) and maximum mortality (85%) was noticed after maximum time interval (48 h) with 10 ppm. Less mortality was observed with low concentrations (10 and 20 ppm) than high concentrations (50 and 100 ppm). So, 100% mortality was observed with 100 ppm in almost all waters in case of 2^{nd} instar larvae. Higher mortality was observed in 2^{nd} instar larvae than 3^{rd} instar larvae in almost all waters. Less than 6% mortality was also seen in control treatments. Relatively low mortality was seen in rich organic water than other water grades and maximum mortality was seen in sewage water.

In concentration response experiments where water grades were different and larvae were checked at 2, 4, 6, 12, 24 and 48 h, respectively after the start of experiment, the mortality was observed in both instars (2^{nd} and 3^{rd}) at all dosages including control treatment. In different water grades the lethal concentration of *Bti* to kill 50% population (LC_{50}) of *Aedes* larvae was calculated in laboratory conditions as shown in Table 2, 3 and 4. The highest LC_{50} value (34.83 ppm) with (0.034) p-values after 2 h and the lowest (6.08 ppm) after 48 h in rich organic water for 3^{rd} instar larvae were almost double from the values of sewage water as shown in Table 2 and 4.

In our experiment *Bti* showed highest efficacy in sewage water. In foul water, LC_{50} value was 29.82 ppm after 2 h while the LC_{50}, chi square, and p-values were 10.83 ppm, 0.0573 and 0.97 respectively in case of 3^{rd} instar larvae after 24 h as shown in Table 2 and 4. In sewage water 17.52 ppm (12.52-23.56) was required to kill 50% population of 3^{rd} instar larvae after 2 h. The least LC_{50} (3.63 ppm) was noted after 48 h in sewage water that is almost

Table 1: Physico-chemical status of water in the treated and control trials

Water grades	pH	DO (%)	DO (ppm)	TDS (ppm)	EC (m bar)
Rich organic water	7.84	8.32	0.58	2550	977
	8.82	11	0.84	2358	975
	8.51	23	1.61	1840	969
Foul Water	8.64	1.40	1.48	3189	976
	8.74	19.6	1.38	2400	989
	8.33	9.2	1.02	2728	970
Sewage water	8.54	7.10	0.58	1659	976
	8.50	7.05	0.09	2006	960
	8.16	11.8	0.85	2000	969
Useable Water	7.80	11.6	0.81	1557	976
	7.50	23	1.87	1793	975
	8.00	19.5	1.24	1670	969
Turbid Water	8.15	12.45	0.94	317	976
	5.70	26	1.88	240	975
	8.54	22	1.25	230	970
Industrial water	0.90	72	4.13	80.55	976
	0.80	96	3.70	41.25	980
	0.65	87	3.65	69.27	956

Table 2: Susceptibility of laboratory reared *A. aegypti* larvae to *B. thuringiensis israelensi*s in different water grades after 2 and 4 h

Water grades	Larval instar	2-h lethal concentration (ppm)		4-h lethal concentration (ppm)	
		LC_{50}	P-value	LC_{50}	P-value
Rich organic water	2nd instar	29.08 (22.80-31.72)	0.012	22.99 (19.32-25.28)	0.006
	3rd instar	34.83 (28.41-36.22)	0.034	27.63 (23.90-31.60)	0.147
Foul water	2nd instar	26.01 (23.03-29.25)	0.004	19.46 (16.01-21.01)	0.03
	3rd instar	29.82 (27.61-34.77)	0.23	24.09 (17.48-26.86)	0.023
Sewage water	2nd instar	12.01 (10.07-14.20)	0.013	9.98 (7.59- 12.05)	0.023
	3rd instar	17.52 (12.52-23.56)	0.459	14.90 (9.99-19.09)	0.238
Useable water	2nd instar	25.01 (22.64-28.87)	0.031	24.22 (22.09-28.05)	0.003
	3rd instar	29.31 (23.07-34.55)	0.012	28.89 (23.69-37.15)	0.039
Turbid water	2nd instar	20.01 (18.01-23.35)	0.013	12.02 (2.89-5.05)	0.003
	3rd instar	24.25 (17.09-28.64)	0.423	15.10 (12.22-17.15)	0.091

Table 3: Susceptibility of laboratory reared *A. aegypti* larvae to *B. thuringiensis israelensis* in different water grades after 6 and 12 h

Water grades	Larval instar	6-h lethal concentration (ppm)		12-h lethal concentration (ppm)	
		LC_{50}	P-value	LC_{50}	P-value
Rich organic water	2nd instar	16.08 (12.28-18.72)	0.013	13.99 (11.34-15.28)	0.003
	3rd instar	21.93 (18.51-24.83)	0.869	18.65 (15.93- 21.72)	0.45
Foul water	2nd instar	15.09 (12.03-19.25)	0.004	11.46 (9.01-13.61)	0.039
	3rd instar	19.43 (17.72-25.71)	0.006	15.66 (10.93-19.32)	0.209
Sewage water	2nd instar	5.99 (6.07-9.20)	0.014	3.99 (4.59- 7.05)	0.028
	3rd instar	9.95 (5.16-13.05)	0.493	6.48 (4.71-9.60)	0.136
Useable water	2nd instar	19.21 (18.04-24.87)	0.003	16.92 (15.09-18.65)	0.003
	3rd instar	23.36 (18.72-27.72)	0.002	21.81 (16.90-27.82)	0.003
Turbid water	2nd instar	8.51 (6.01-10.35)	0.014	5.02 (3.89-6.05)	0.003
	3rd instar	11.15 (8.63-15.85)	0.297	8.90 (5.80-11.14)	0.017

three times less than turbid and useable water. The highest value of LC_{50} (29.31 and 21.81 ppm) was noted after 2 and 12 h in useable water.

Table 4 showed that lowest LC_{50} value of *Bti* was noted in sewage water against 2nd instar (3.31 ppm) and 3rd instar (5.20 ppm) larvae while its highest values (10.21 and 15.42 ppm) were noted in useable water, that were almost triple as compared to sewage water. After 48 h, highest LC_{50} value was noted in turbid water as shown in Table 4.

Discussion

B. thuringiensis israelensis is safe and effective biocontrol agent used widely to control *Culex*, *Anopheles* and *Aedes* mosquitoes for the last 20 years (Lacey and Siegel, 2000; Mittal, 2003; Junwei *et al*., 2006; Lacey, 2007). The situation of Dengue fever and dengue hemorrhagic fever has become worst in Pakistan and *Aedes* is responsible for these endemic diseases due to unplanned urbanization and pollution. In

Table 4: Susceptibility of laboratory reared *A. aegypti* larvae to *B. thuringiensis israelensis* in different water grades after 24 and 48 h

Water grades	Larval instar	24-h lethal concentration (ppm)		48-h lethal concentration (ppm)	
		LC_{50}	P-value	LC_{50}	P-value
Rich organic water	2nd instar	8.08 (6.28-9.72)	0.012	3.99 (3.34-6.28)	0.007
	3rd instar	11.17 (8.47-14.60)	0.869	6.08 (2.94-11.13)	0.397
Foul water	2nd instar	7.09 (6.03-9.25)	0.003	3.46 (2.01-5.61)	0.04
	3rd instar	10.83 (8.07-15.08)	0.972	5.21 (3.01-9.71)	3.01
Sewage water	2nd instar	3.31 (2.07-5.20)	0.015	1.99 (1.59- 2.05)	0.038
	3rd instar	5.20 (2.38-7.59)	0.012	3.63 (1.25-7.19)	0.003
Useable water	2nd instar	10.21 (8.64-14.87)	0.003	6.02 (5.99-8.65)	0.002
	3rd instar	15.42 (13.93-19.54)	0.453	8.12 (7.67-10.94)	0.031
Turbid water	2nd instar	7.51 (4.01-8.35)	0.012	6.92 (4.89-8.05)	0.003
	3rd instar	9.63 (7.21-11.91)	0.001	8.38 (5.98-11.99)	0.021

Punjab, the climatic conditions fluctuate whole the year. The changes in temperature and wide range of water grades may have a significant effect on the life cycle of mosquitoes (Tun-lin *et al*., 2000) and biological control agents.

Thus, the efficacy of *Bti* was checked in different water grades against *A. aegypti* larvae in the current study. In this research work, different concentrations (10, 20, 50, 100 ppm) of *Bti* dunk were prepared as done by previous workers (Jahan and Shahid, 2012; Abdalmagid *et al*., 2012). Abdalmagid *et al*. (2012) checked the efficacy of *Bti* dunks in field water and studied the physico-chemical properties of water. They concluded that these properties have no impact on the efficacy of *Bti* ($P > 0.05$). Mulla (1990) reported high mortality rate in 1st instar larvae during handling. Due to this reason, 2nd and 3rd instar larvae were used in the present experiments. High mortality rate in case of early instar larvae (2nd) and less mortality in case of late instar larvae (3rd) was also concluded by Rodrigues *et al.* (1999). Ramathilaga *et al*. (2012) studied the impact of *Bti* against 3rd instar larvae of *A. aegypti* as was recorded in the present study against 2nd & 3rd instar larvae. In the present study, 63% and 79% mortality was recorded for 50 and 100 ppm of *Bti* respectively after 24 h in useable water while Ramathilaga *et al*. (2012) recorded (16%) mortality at 1mg concentration of *Bti* for 24 h treatment in tap water. Haung *et al.* (1993) recorded 52.1, 69.5 and 78.2% mortality after 12, 24 and 48 h respectively in 0.10 ppm against *Ae. aegypti* larvae while 97.1% mortality each after 12, 24 and 48 h in 0.20 ppm. Gbehou *et al*. (2010) compared the efficacy of *Bti* on mosquitoes (*Aedes*, *Culex* and *Anopheles*) and observed 40, 80 & 100% mortality after 2, 4 and 6 h against *Aedes*. Many other factors (genera susceptibility, feeding behavior of larvae, instar susceptibility to *Bti*, suspended organic matter, water temperature, larval density, water depth) also affect the efficacy of *Bti* against mosquitoes (Boisvert, 2005). Some of these factors like organic, inorganic, muddy, food and floating particles decreased the efficacy of *Bti* due to adsorption of *Bti* onto suspended particles followed by a slow sedimentation (Margalit and Bobroglo, 1984; Margalit *et al*., 1985). Hence, fewer toxic crystals are ingested by larvae due to suspended particles. So, higher concentration of *Bti* will be needed for larval control in case of high density of suspended particles and pollution (Ohana *et al*., 1987; Mulla, 1990). A pause in the efficacy of *Bti* was by bacterial adsorption to soil particles, but the inactivation could be inverted by washing the mud away (WHO, 2004). Due to these reasons, mean value of LC_{50} was higher in rich organic water (34.83 ppm) and turbid water (24.25 ppm) while low in sewage water (17.52 ppm) after 2 h in the present study. Mean LC_{50} values were 5.20 and 10.83 ppm after 24 h for sewage water (full of organic matter) and foul water, respectively. The results of the present study revealed the higher mortality in sewage water and useable water because these were free of any particles while least was observed in rich organic water due to suspended particles. *Bti* formulation along with these factors affects its efficacy. *Bti* formulations originated as a water dispersible powder (WDP) formulation using fly ash (FA) as a carrier material was developed and was found effective against the larval stages of major mosquito vector species (Tamilselvan *et al*., 2017).

Conclusion

Use of *Bti* in different water grades is an effective method to control larvae of dengue mosquito, *A. aegypti*.

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