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Field Evaluation of Lethal Ovitraps for the Control of Dengue Vectors in Lahore, Pakistan

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Abstract.- Field evaluation of lethal ovitraps (LOs) containing various concentrations of *Bacillus thuringiensis serovar israelensis* (*Bti*), buprofezin (insect growth regulator) and integration of *Bti*+buprofezin (1:1) in water and 10% hay infusion against dengue vectors was carried out during August-October 2009, at two municipalities (Samanabad and Mughalpura) of Lahore, Punjab-Pakistan. Each municipality was divided in two blocks; control versus treatment block, with 18 randomly selected houses. Each block received 36 ovitraps/LOs with and without any treatment. In total 15 weekly collections, 10,152 *Aedes aegypti* eggs were recovered with 5,351 and 4,801 from treatment and control blocks, respectively, indicating that different treatments did not affect oviposition. However, hay infusion recovered more eggs (6,548) as compared to tap water (3,604). Ovitrap positive index (OPI) was higher in hay infusion as compared with tap water. Moreover, there was no significant difference in egg density index (EDI) in tap water compared with hay infusion. Effect of lethal ovitraps treated with different concentrations of *Bti* indicated that 100 and 10 ppm of this biocide completely inhibited pupal formation, as against 1ppm where 41 and 60% pupae were formed in Samanabad and Mughalpura, respectively. LOs treated with buprofezin indicated that different concentrations of buprofezin were more effective in inhibiting the pupae-adults emergence as compared to *Bti* where lethal affect was more on larval stage. Integration of *Bti*+buprofezin in hay infusion was highly effective in reducing pupal formation and inhibiting adult emergence. There was complete inhibition of adult emergence at all concentrations (100-1 ppm) in integrated lethal ovitraps, indicating the most effective tool for controlling *Aedes* populations under natural conditions.

Key Words: *Aedes aegypti*, Dengue vectors, lethal ovitraps, *Aedes albopictus*, *Anopheles*, *Bti*, buprofezin.

INTRODUCTION

Mosquitoes are important vectors of a number of human and animal diseases. *Aedes aegypti* is an important vector of yellow fever, dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS) (Gubler, 1998). DF and DHF caused by a flavivirus of four different serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) (Westaway and Blok, 1997). These are the most widespread tropical infectious diseases (Gubler and Kuno, 1997).

In Pakistan, the first outbreak of DHF was reported during August-November 1994 from Karachi (Chan *et al.*, 1995). Most of the patients had IgM DEN-2 tested by IgM captures ELISA (Burney, 1996; Chan *et al.*, 1995) with no reports of confirmed death. Another outbreak occurred in district Lasbella, Baluchistan, between September-November 1995 with 1800 positive cases. Recent outbreaks of DF and DHF in Pakistan have occurred annually on regular basis since 2006 to date but no virus isolation was reported during these outbreaks. In 2006, approximately 5,522 cases were declared positive for dengue virus along with 50 deaths. Vast majority of the deaths (43) occurred in Karachi and 4 deaths reported from Lahore. In 2008, more than 900 cases along with 7 deaths were reported from Lahore only. More recently 2010-11, dengue epidemic occurred all over the country with more than 18000 positive cases and >300 deaths from province Punjab. Dengue situation in Pakistan is alarming with tremendous risk of future epidemics.

In recent years, the incidence of dengue has significantly increased in Pakistan. The capital city of Lahore in the Punjab province of Pakistan has been reported to support rather large populations of competent dengue vector mosquitoes; *Ae. aegypti* and *Ae. albopictus* (Hameed and Jahan, 2007), of the two species *Ae. aegypti* plays a crucial role in the transmission of dengue infections (Nogueira *et al.*, 1999; Rodhain and Rosen, 1997). *Ae. aegypti* is exclusively a human blood feeder. In the past two decades, there has been an increase in population and urbanization, which has led to the development

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of breeding places of *Aedes* mosquitoes in Pakistan and other tropical and sub-tropical areas of the world. This in turn has increased incidence of dengue cases (Thavara *et al.*, 2001).

Therapeutic interventions are unavailable for the prevention of DF and DHF (Burney, 1996). Therefore, control of disease depends upon control of dengue vectors. To control mosquito vectors, the elimination of mosquito breeding sources could be an effective approach (Dame and Fasulo, 2003). Insecticides are used for mosquito larval control but their application is expensive in water bodies of large volume and difficult for small artificial breeding sites (Hopkins *et al.*, 2002). Besides the bioaccumulation of organic insecticides and their adverse effect on non-target and aquatic biota are of great concern. Additionally, mosquito vectors are developing resistance against chemical insecticides throughout the world (Chandre *et al.*, 1999). Therefore, alternate strategies are needed to control diseased vectors. Biological control and the use of insect growth regulators (IGRs) are now being recommended. Biological control is the use of living organisms such as bacteria, fungi, nematodes, copepods and some fishes for the control of mosquito vectors. IGRs are chemical compounds that alter growth and development in insects through interaction with hormones. There are two types of IGRs: juvenile hormone analogues (JHAs) and chitin synthesis inhibitors (CSIs). JHAs interfere with normal function of glands that produce juvenile hormones. They include methoprene, hydroprene and pyriproxifen etc. CSIs interfere with chitin production, leading to moulting disturbances and resulting death of insects. Buprofezin (Applaud®) is a thiadiazine IGR, CSI in insects. It is known to have both contact and vapor activity against some sucking insects and has been used in the control of whitefly (De Cock *et al*., 1990).

Various control agents on combination enhance potential and efficacy for the control of long period of time as compared to use alone (Andrande and Modolo, 1991; Riviere *et al.*, 1987). Many combination strategies were evaluated using *Bti*, *Bs* or methoprene (Tietze *et al.*, 1994). It has been documented that a mixture of "vectobac 12 AS and Actellic 50 EC (Pirimiphos-methyl)" was an effective larvicide (Chung *et al.*, 2001). A formulation of *Bti WDG* "water-dispersible-granules of VectoBac ABG 6511, a liquid formulations of VectoBac 12AS" and pyriproxyfen insect growth regulator, Sumilarv 0.5% were applied for the control of larvae of *Aedes* mosquitoes. Water dispersible *Bti* granules provided greater larval mortality than did liquid *Bti* formulation against *Aedes* mosquitoes when integrated with pyriproxyfen. Pyriproxyfen (79.5 and 159 mg/liter) on its own showed low larvicidal activity but provided very effective control of adult emergence (Lee *et al.*, 2005).

Ovitrap was primarily developed in the US to monitor the population of *Ae. aegypti* (Fay and Eliason, 1966; Fay and Perry, 1965). In 1969, it was used first time to control *Ae. aegypti* population at Singapore Airport (Chan, 1973). The US centers for disease control and prevention (CDC) designed an ovitrap which is most commonly used to control *Aedes* mosquitoes. To increase the efficiency of ovitraps some attractants, such as hay infusions in different concentrations have been used as attractants for oviposition. As a result the greater number of eggs was obtained in the ovitrap without the effect of seasonal variation (Reiter *et al.*, 1991). According to Chadee *et al.* (1995) high yields of eggs have been obtained by the ovitraps designed with wooden paddles. Zeichner and Perich (1999) suggested the most effective ovitraps should contain insecticide-treated oviposition strip.

The present study was designed to evaluate *Bti* WDG (microbial mosquito larvicide), Buprofezin 25% WP (a chitin synthesis inhibitor IGR) and a combination of the above two as lethal ovitraps for the control of *Ae. aegypti* populations in two selected municipalities of Lahore. The main objective was to evaluate the efficacy of LOs in controlling immatures and adult population of dengue vectors.

MATERIALS AND METHODS

Study area

The study was carried out in two different municipalities (Samanabad and Mughalpura) of Lahore, Punjab, Pakistan (August-October 2009). Lahore is spread over 1,722 km^2 and support a human population nearly 10 million with density of

8,029 persons/km^2 (Mazhar and Jamal, 2009). Samanabad (31°32'18"N, 74°17'54"E and elevation 694 feet) have a population of 59,101 with density 34,400 persons/km² and Mughalpura (31°34'10"N, 74°22'26"E and elevation 717 feet) have a population of 47,717 with density 13,200 persons/km² with respect to census, 1998 and both located 10.1km apart in North West and North region of Lahore, respectively. Climate of Lahore city is characterized as summer season of long duration with dry atmosphere as compared to winter season of short span.

Dengue vectors surveillance

Lahore is one of the districts in the Punjab that has been infested with large population of dengue vectors (*Ae. aegypti* and *Ae. albopictus*). Almost all sources of *Aedes* mosquitoes breeding habitats and resting sites were situated in two selected areas of Lahore. During preliminary survey in first week of August 2009, mosquito larvae were collected from natural containers (potted plants, plastic pots, cans, jars, cement tanks and grinding stones) in selected municipalities and kept in containers labeled with date and site of collection. Containers were carried to entomology laboratory of GC University, Lahore for recording their numbers and nomenclature. In selected municipalities *Aedes* mosquito density was high (87%) as compared to genus *Culex* (13%). All the *Aedes* were identified as *Ae. aegypti*. *Ae. albopictus* and *Anopheles* mosquitoes were not found anywhere. People living in these areas use coils and other mosquito repellents to protect themselves from mosquito bites and some houses have screen windows and/or doors in this area.

Ovitrap and ovipaddle design

Lethal ovitraps (LOs) treated with *Bti*, buprofezin and integrated *Bti*+ buprofezin was used as a lure and kill device for container breeding dengue vectors. Ovitraps were made according to illustrations of Fay and Perry (1965), consisted of 400 ml capacity plastic cups (height 10.5 cm, upper and Bottom diameter 7.5 cm and 5.5 cm respectively). All ovitraps were wrapped by black polythene sheet in order to attract mosquitoes. Two holes (3-4 mm wide) were drilled equidistant 2 cm below the rim. To these holes double string of 50 cm length was tied in order to hang the ovitrap when required. A wooden tongue depressor (1.8 x 15 cm) was used as ovipaddle, wrapped by layers of paper towel with 11.15 X 07.43 cm size (Paseo® elegant, Indonesia), which acts as a suitable rough surface, preferred for oviposition by mosquitoes. Ovipaddle was placed in inclined position inside the ovitrap.

Test formulations

Test strain of *Bti* WDG (water dispersible granules) VectoBac® by Valent Bioscience Corporation: IL, USA with 3000 ITU (international toxic unit) was used as lethal ovitraps in different concentrations (100, 10 and 1 ppm) to evaluate its affect in field assays against *Ae.* mosquitoes. Different formulations were prepared by serial dilution of 100 ppm solution. Two replicas of each concentration were made in distilled water as well as in 10% hay infusion in water. Test strain of buprofezin 25% WP (wettable powder) was kindly provided by Agriculture Department, Lahore. Buprofezin was used as lethal ovitraps in two replicas of each concentrations (100, 10 and 1 ppm) to evaluate its effect as larvicide.

These two larvicides; synthetic chemical compound IGR and microbial larvicides *Bti* WDG, were used in 1:1 ratio in order to achieve efficient mosquito control and to avoid the development of resistance. Two replicas of different concentrations (100, 10 and 1 ppm) were made in distilled water and 10% hay infusion.

Ovitrap placement

Each municipality was divided into two blocks; control (untreated) and treatment block. The two blocks of houses were separated by a minimum of 450 meters distance. One block of houses was randomly assigned as control block and other as treatment block. In each block of each municipality, 18 houses were selected randomly. Each selected house received two ovitraps hanged by string bound to the holes near rim of ovitraps in front lawn (outdoor) or on a convenient support (window or shade) at the height of 1.5-2 m inaccessible to children and domestic animals. In all instances, traps were set sheltered from direct sunlight and rain to avoid the excessive evaporation and dilution of

different treatments. The 2 ovitraps placed in a house were separated by available distance between them.

Ovitraps without any treatment containing distilled water only (18 ovitraps) or 10% hay infusion in distilled water (18 ovitraps) were installed in control (untreated) block of each municipality and each house received one pair alternatively. Total 18 houses received 36 ovitraps of 10% hay infusion and water alternatively. Treatment block of each municipality received 36 lethal ovitraps. Pairs of 9 lethal ovitraps with different concentrations (100, 10 and 1 ppm of *Bti*, buprofezin and *Bti*+buprofezin) in distilled water and 10% hay infusion each were installed.

Evaluation of lethal ovitraps using field bioassays

Two replicas of each concentration (100, 10, 1 ppm) as lethal ovitrap with *Bti* WDG, buprofezin 25% WP, and buprofezin and *Bti* (integrated) in 1:1 ratio were made in distilled water and 10% hay infusion and installed as described above in treatment block of each municipality. Untreated ovitraps in distilled water and 10% hay infusion in equal number were placed in control block of each municipality. The ovitraps were observed weekly for *Ae. aegypti* eggs, and 10% hay infusion or water added in respective ovitrap up to fix level as needed. Ovipaddle or entire ovitrap was replaced if found missing anyone. At collection site each ovipaddle was examined by a magnifying glass and egg counts were made if having eggs on any face of ovipaddle and it was labeled with site location, folded by aluminium foil and placed in an individual plastic bag and brought carefully to entomological research center GCU for species and number identification. To prevent the fungal contamination, all ovipaddles were replaced with fresh ovipaddles after one week. In the laboratory, egg counts were confirmed using a magnifying glass or dissecting microscope. Each ovipaddle was left to dry at room temperature (28±2°C; 80±10% RH) at diagonal angle as sometime each face was bearing eggs.

Dried paper towel was removed from tongue depressor and shifted to respective plastic cup (capacity 250 ml, height 5 cm, upper and bottom diameter 8.5 cm and 7.8 cm, respectively) filled with 200 ml of distilled water to allow egg eclosion. Cup was labeled and covered with a net and rubber band in order to block adult mosquito release or intervention. In each cup larvae formed were counted and recorded. Pupae were counted and separated with the help of a separate dropper (for each treatment) to another cup containing 200 ml of water for the emergence of adult. In each cup adults emerged were counted and recorded.

Larvae transformed to pupae were counted and separated with the help of a separate dropper (for each treatment) to another cup containing 200 ml of water for the emergence of adult. In each cup adults emerged were counted and recorded. Mortality in each concentration at each level was recorded every day. Mortality was counted by separating dead larvae or pupae 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.

Data analysis

The experimental design was a randomized design (Winer *et al.,* 1991). Infestation by *Ae. aegypti* eggs was estimated using conventional indicators; ovitrap positive index (OPI) and egg density index (EDI) (Gomes, 1998). Choice of LO for oviposition (OPI) was estimated as the percent LOs positive for eggs from the total number of ovitraps inspected.

$$\text{OPI} = \frac{\text{No. of Los positive for eggs}}{\text{Total no. of Los inspected}} \times 100$$

The efficiency of LOs of each treatment in egg collection (EDI) was calculated as average number of eggs laid per positive control/lethal ovipaddle.

$$\text{EDI} = \frac{\text{Total no. of } \textit{Ae. aegypti} \text{ eggs on vipaddles}}{\text{No. of positive ovitraps}}$$

To evaluate changes in the toxic effect of LOs of different treatment groups (*Bti*, Buprofezin and integrated *Bti*+buprofezin) after field exposure, percent larvae, pupae and adults emergence in water

and hay was calculated. Test results compared with their respective controls were statistically analyzed by ANOVA, Tukey's mean separation procedure and Student's t-distribution test at 95% confidence interval of the difference (SPSS version 16.0; SPSS Inc., Chicago, IL).

RESULTS

Field evaluation of lethal ovitraps treated with different concentrations of *Bti*, buprofezin and integration of *Bti*+buprofezin (1:1) with respect to number of ovitraps installed (in each treatment and control), number of eggs along with OPI and EDI in Samanabad and Mughalpura were presented in Table I. It was found that both selected municipalities were highly infested (more than 85%) with dengue vector (*Ae. aegypti*) as compared to other species. Total 15 weekly collections were made and 10,152 eggs of *Ae. aegypti* were harvested from 144 ovitraps of both municipalities indicating that 10% hay infusion yielded more eggs (6,548) as compared to that of simple water medium (3,604). Mughalpura was found 1.3 times more infested with *Ae. aegypti* eggs (5,727) as compared to Samanabad (4,425). It was observed that ovitraps with hay infusion were 1.82 times more attractive for oviposition of *Ae. aegypti* than that of simple water (Fig. 1). Average 19.3 eggs were harvested per ovipaddle in water and 23.17 eggs from hay infusion (Table I).

ANOVA indicated that there was no significant difference ($P > 0.05$) between different treatment groups (*Bti*, buprofezin and integration of *Bti*+buprofezin 1:1) compared with their respective control with respect to number of eggs, OPI and EDI in both municipalities (Table I). However, percent larvae, pupae formation along with adult emergence was significantly lowered ($p=0.000$) in all concentrations (100-1 ppm) as compared to their respective control (Table II). OPI was higher in LOs with hay infusion as compared with that of water. There was no considerable difference with respect to EDI in LOs with hay infusion and water in both municipalities (Table I).

Lethal ovitraps treated with *Bti*, buprofezin and integration of both in water (pooled in all concentrations) formed 65, 78 and 70% larvae 14, 20 and 2% pupae and 14, 9 and no (zero percent) adult emerged at Samanabad respectively (Fig. 2). The same treatments with 10% hay infusion indicated no significant difference in hatching of larvae whereas, no pupae or adults formed in integrated group (Fig. 3). In Mughalpura the same treatments in water formed 70, 81 and 68% larvae, 19, 26 and 6% pupae while 15, 8 and 2% adults respectively (Fig. 4). In hay infusion 11 and 8% adults emerged with *Bti*, Buprofezin respectively and no adult emerged in integrated group (Fig. 5).

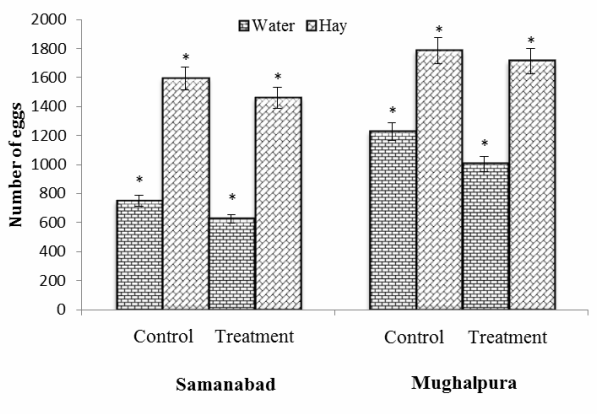

Fig. 1. Evaluation of lethal ovitraps in comparison of number of eggs in water and hay infusion (control and treatment groups) in two selected municipalities of Lahore, Pakistan. *showing that values are not significantly different at $p < 0.05$.

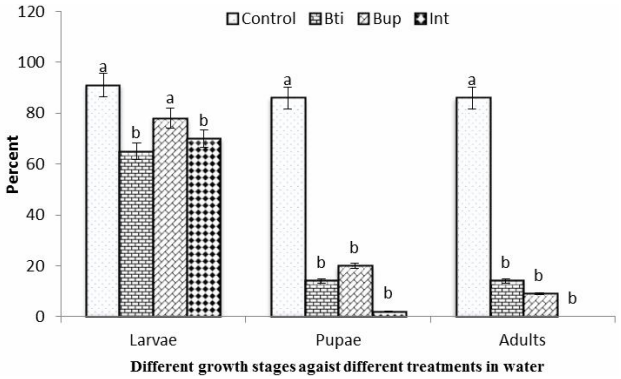

Fig. 2. Evaluation of lethal ovitraps with different treatments of *Bti*, buprofezin alone and integration in water as percent larvae, pupae and adults emergence of *Aedes aegypti* in Samanabad. Means with different letters are significantly different ($P<0.05$) within each growth stage.

Table I.- **Evaluation of lethal ovitraps with different treatments of *Bti*, buprofezin and integrated *Bti*+buprofezin (1:1) in water and hay infusion, as number of LOs installed, ovitrap positive index (OPI), number of eggs and egg density index (EDI) for *Aedes aegypti* in Samanabad and Mughalpura.**

Treatments	No. of LOs			Samanabad						Mughalpura					
				Positive LOs		Total eggs	Eggs / LO			Positive LOs		Total eggs	Eggs / LO		
	Installed	Collections (n)	Total (n)	(n)	OPI	(n)	EDI	±SEM	(Range)	(n)	OPI	(n)	EDI	±SEM	(Range)
Control (W)	6	15	90	17	18.9	253	14.9	±2.13	(03-35)	15	16.7	301	20.07	±2.30	(08-40)
W+*Bti*	6	15	90	15	16.7	219*	14.6	±1.71	(07-25)	11	12.2	236*	21.45	±2.46	(18-30)
Control (W)	6	15	90	17	18.9	276	16.2	±2.03	(06-28)	19	21.1	484	25.47	±2.19	(12-43)
W+Bup	6	15	90	16	17.8	191*	11.9	±1.87	(06-32)	16	17.8	421*	26.31	±1.77	(07-31)
Control (W)	6	15	90	16	17.8	219	13.7	±2.62	(05-48)	24	26.7	442	18.42	±2.18	(03-56)
W+*Bti*+Bup	6	15	90	11	12.2	216*	19.6	±4.05	(05-48)	17	18.9	346*	20.35	±2.00	(03-29)
Control (H)	6	15	90	26	28.9	504	19.4	±2.39	(06-56)	28	31.1	568	20.29	±2.56	(03-74)
H+*Bti*	6	15	90	20	22.2	468*	23.4	±3.34	(08-52)	20	22.2	544*	27.20	±2.78	(04-37)
Control (H)	6	15	90	25	27.8	510	20.4	±2.17	(07-55)	32	35.6	752	23.50	±1.92	(03-49)
H+Bup	6	15	90	25	27.8	487*	19.5	±1.91	(07-30)	34	37.8	776*	22.82	±1.71	(07-56)
Control (H)	6	15	90	27	30.0	578	21.4	±2.43	(07-55)	26	28.9	464	17.85	±1.85	(06-44)
H+*Bti*+Bup	6	15	90	22	24.4	504*	22.9	±2.87	(13-66)	21	23.3	393*	18.71	±1.84	(09-39)

W: Water, H: Hay

*ANOVA with Tukey's mean separation procedure, at 95% confidence interval of the difference indicated that there was no significance difference ($p > 0.05$) between different treatments (*Bti*, buprofezin and integrated *Bti*+buprofezin 1:1) compared with their respective control with respect to number of eggs in both municipalities.

Table II.- Evaluation of lethal ovitraps with different treatments of *Bti*, buprofezin and integrated *Bti*+buprofezin (1:1) in water and hay infusion, as percent larvae, pupae and adults emergence for *Aedes aegypti* in Samanabad and Mughalpura.

Treatments	Conc. (ppm)	Samanabad						Mughalpura					
		Water			Hay			Water			Hay		
		L	P	A	L	P	A	L	P	A	L	P	A
Control	-	91±2.1^{a}	86±3.1^{a}	86±3.1^{a}	89±3.9^{a}	85±3.3^{a}	85±3.3^{a}	94±4.1^{a}	85±3.9^{a}	85±3.6^{a}	89±3.9^{a}	82±3.3^{a}	82±3.6^{a}
Bti	100	54±1.8^{b}	0±0.0^{b}	0±0.0^{b}	58±4.2^{b}	0±0.0^{b}	0±0.0^{b}	55±3.3^{b}	0±0.0	0±0.0^{b}	60±4.0^{a}	0±0.0^{b}	0±0.0^{b}
	10	64±2.4^{b}	0±0.0^{b}	0±0.0^{b}	70±3.0^{a}	5±0.1^{b}	4±0.9^{b}	68±5.1^{b}	0±0.0	0±0.0^{b}	71±1.3^{b}	7±1.4^{b}	7±1.4^{b}
	1	78±1.8^{a}	41±2.3^{b}	41±2.3^{b}	71±4.4^{a}	38±3.2^{b}	38±3.2^{b}	84±1.3^{a}	60±1.6	44±2.2^{b}	69±3.5^{b}	47±4.0^{b}	47±4.0^{b}
Control	-	92±2.7^{a}	87±4.1^{a}	87±4.1^{a}	91±1.5^{a}	84±1.8^{a}	84±1.8^{a}	91±2.9^{a}	81±3.1^{a}	81±3.1^{a}	93±4.8^{a}	87±2.6^{a}	87±2.6^{a}
Bup	100	*	*	*	76±4.0^{a}	0±0.0^{b}	0±0.0^{b}	76±4.7^{a}	0±0.0^{b}	0±0.0^{b}	68±2.7^{a}	2±0.3^{b}	0±0.0^{b}
	10	77±3.1^{a}	0±0.0^{b}	0±0.0^{b}	71±3.3^{a}	28±4.8^{b}	0±0.0^{b}	*	*	*	74±3.7^{a}	33±3.9^{b}	0±0.0^{b}
	1	79±3.9^{a}	40±2.0^{b}	18±2.3^{b}	77±2.1^{a}	47±0.5^{b}	19±1.2^{b}	83±3.7^{a}	45±3.0^{b}	17±4.5^{b}	84±4.1^{a}	48±3.9^{b}	19±4.0^{b}
Control	-	90±3.2^{a}	84±3.1^{a}	84±3.2^{a}	89±2.1^{a}	87±2.0^{a}	87±2.2^{a}	94±4.5^{a}	87±2.0^{a}	87±2.0^{a}	89±3.3^{a}	81±2.8^{a}	81±2.8^{a}
Bti+Bup	100	61±4.0^{b}	0±0.0^{b}	0±0.0^{b}	67±5.1^{a}	0±0.0^{b}	0±0.0^{b}	53±4.5^{b}	0±0.0^{b}	0±0.0^{b}	67±4.8^{a}	0±0.0^{b}	0±0.0^{b}
	10	70±1.5^{b}	0±0.0^{b}	0±0.0^{b}	78±3.3^{a}	0±0.0^{b}	0±0.0^{b}	70±3.8^{b}	0±0.0^{b}	0±0.0^{b}	*	*	*
	1	79±3.9^{a}	6±0.4^{b}	0±0.0^{b}	*	*	*	72±2.6^{b}	16±1.4^{b}	0±0.0^{b}	79±2.6^{a}	20±2.0^{b}	1±0.0^{b}

A: Adults, Bup: Buprofezin, *Bti*: *Bacillus thuringiensis israelensis*, L: Larvae, P: Pupae

* indicates that no egg was observed in the respective treatment group.

Data showing mean values of two Replicates as percentage in all treatment groups.

Means with different letters are significantly different ($p<0.05$) within each growth stage compared with respective control group.

Overall integrated group was most effective in controlling adult population of *Ae. aegypti* in both selected localities.

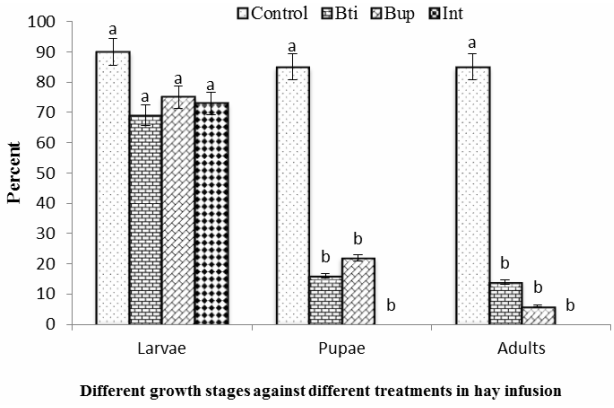

Fig. 3. Evaluation of lethal ovitraps with different treatments of *Bti*, buprofezin alone and integration in hay as percent larvae, pupae and adults emergence of *Aedes aegypti* in Samanabad. Means with different letters are significantly different (P<0.05) within each growth stage.

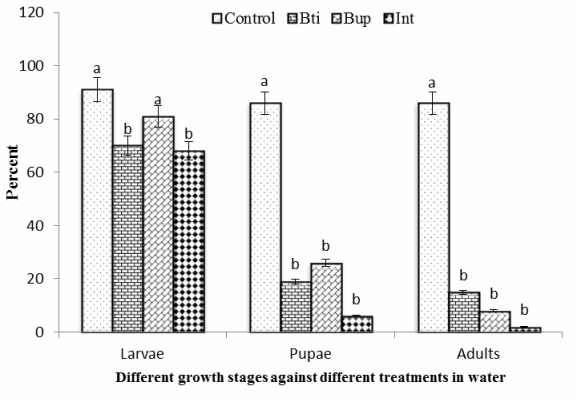

Fig. 4. Evaluation of lethal ovitraps with different treatments of *Bti*, buprofezin and integrated *Bti*+buprofezin (1:1) in water as percent larvae, pupae and adults emergence of *Aedes aegypti* in Mughalpura. Means with different letters are significantly different (P<0.05) within each growth stage.

DISCUSSION

As outbreaks of dengue continue to re-emerge in Pakistan from the last few years, efforts should be made to control its vector population by targeting not only adults but also immature stages. Results from current study in two different municipalities of Lahore suggest that the lethal ovitraps (LOs) treated with different concentrations of *Bti*, buprofezin alone and a mixture of these (1:1) reduced effectively natural population of adult *Ae. aegypti*. It is found that lethal ovitraps may be an integral part of eradicating and surveillance tools for dengue vectors. There is no previous study related to the use of LOs for controlling mosquito vectors in Pakistan.

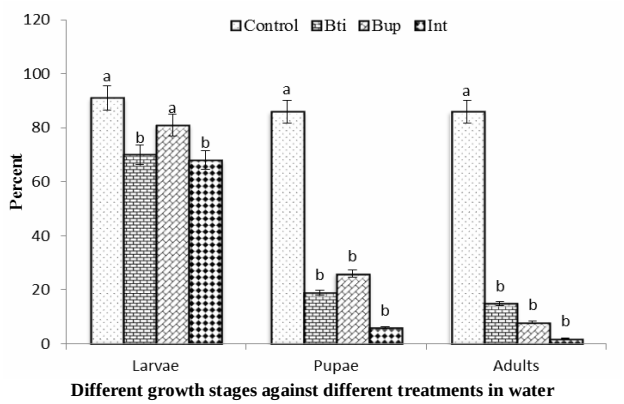

Fig. 5. Evaluation of lethal ovitraps with different treatments of *Bti*, buprofezin and integrated *Bti*+buprofezin (1:1) in hay as percent larvae, pupae and adults emergence of *Aedes aegypti* in Mughalpura. Means with different letters are significantly different (P<0.05) within each growth stage.

In current study, field evaluation of lethal ovitraps treated with different biological and synthetic agents (*Bti* and buprofezin) along with the mixture of *Bti*+buprofezin in water and 10% hay infusion was made. There was no significant difference (P > 0.05) between paired ovitraps of each treatment compared with their respective control with respect to OPI (ovitrap positive index) and EDI (egg density index). These results indicated that the choice of ovitrap as an oviposition site and average number of eggs per ovipaddle were not affected by selected control agents. Similar findings were documented by Santos *et al.* (2003) where there was no significant difference with respect to OPIs between paired ovitraps treated with *Bti* in tap

water and 10% or 30% grass infusion. Polson *et al.* (2002) found that OPI with 10% hay infusion was more (15.56-54.55%) than ovitraps with tap water (6.67-34.88%). Our results are comparable with these authors where OPI in ovitraps with 10% hay infusion was more (22.2 – 37.8%) than ovitraps with tap water (12.2 – 26.7%). However, there was no considerable difference in EDI suggesting that hay infusion attract more *Ae. aegypti* as a choice of oviposition than water but directly it did not affect the average number of eggs laid on each ovipaddle. No dead adult was observed in any LO, indicating that the control agents used in LOs had no detrimental direct effect on the adult *Ae. aegypti* population.

Effectiveness of different concentrations of hay infusion to attract mosquitoes for oviposition in the use of ovitraps appeared to be controversial. In this study it is observed that ovitraps with 10% hay infusion were 1.81 times more attractant for oviposition than that of simple water. Reiter *et al.* (1991) also found that 10% hay infusion was more effective in attracting *Ae. aegypti* as compared to 100% hay infusion. On the other hand Chadee *et al.* (1995) observed no difference between different concentrations of hay infusion (10%, 20%, 60% or 80%) on oviposition. Whereas Santos *et al.* (2003) found that 30% hay infusion harvested more eggs (5,263) as compared to 10% hay infusion (3,456).

In South East Asia, control of dengue vectors with chemical insecticides shifted to biological and integrated control (Gubler and Kuno, 1997). Microbial larvicides such as *Bacillus thuringiensis israelensis* have been used effectively against different mosquito species in different regions of the world (Becker, 1992; Fillinger *et al.*, 2003; Jahan and Abbas, 2006; Jahan and Hussain, 2011). Although their safety to the environment and their efficacy against a variety of mosquito species have been demonstrated by several researchers, both in laboratory and field conditions, their use in vector control program is less studied (Floore *et al.*, 1991) particularly in Pakistan.

In current study, *Bti* WDG was used as lethal ovitraps alone and in combination with buprofezin. Various concentrations of *Bti* indicated high larval mortality and complete inhibition in adult emergence in all treated groups in water and hay infusion. There was a significant ($P < 0.05$) high mortality in *Bti*, buprofezin and integrated treated groups in both media (water and 10% hay infusion) with their respective controls. The effect was most pronounced lethal in integrated groups where ANOVA indicated ($P = 0.000$) highly significant difference as compare to respective control.

Overall results indicated that integration of *Bti* and buprofezin (1:1) in water/10% hay infusion was the most effective group in controlling immature and adult stages of *Ae. aegypti* population in two selected municipalities. No adult emerge in these groups ranged from 100 – 1 ppm. This could be due to mode of action of the two control agents used in this study. *Bti* affect at larval stages and cause high larval mortality after entering inside the body of larvae on the other hand buprofezin affect larval to pupal stage and inhibit pupae formation along with adult emergence. Perich *et al.* (2003) suggested that the LOs would be most useful when incorporated in an integrated control program. Our data support this suggestion as integrated groups with *Bti*+buprofezin were most successful in suppressing *Ae. aegypti* population in both municipalities.

Data on laboratory and field evaluation of different biolarvicides against dengue vectors in Pakistan is limited. Recently the work in our laboratory indicated *Bti* WDG was highly effective against wild caught *Ae. albopictus* larvae in laboratory and field assays (Hameed and Jahan, 2007; Hanif and Jahan, 2007). In current study mixture of two larvicides (buprofezin 25% WP + *Bti* WDG 1:1) evaluated as lethal ovitraps in field bioassays showed a considerable effect on suppressing the adult population of *Ae. aegypti*. Adult emergence was completely inhibited by 100, 10 and 1 ppm as compared to *Bti*/buprofezin alone.

In conclusion, in a comparison of two municipalities the lethal ovitraps in Mughalpura harvested 1.3 times more eggs as compared to Samanabad. This could be due to more breeding habitats in the Mughalpura and better sanitary and social conditions in Samanabad. Lethal ovitraps in integrated group were most efficient among all treatments than the products alone against *Ae. aegypti* immature stages (from larvae-pupae-adults). Integrated lethal ovitraps were found most effective

tool that targets dengue vector breeding sites along with little or no wastage of insecticides and with non-target effects. Furthermore, the minimum effective dosages to kill 100% of the larval population and adults emergence inhibition in a habitat have shown to be extremely low (1 ppm) and product delivery by lethal ovitraps may have great potencies for inclusion in integrated vector management operations. We recommend that lethal ovitraps can act as a simple, inexpensive and sensitive tool for both monitoring oviposition and controlling *Ae. aegypti* population under natural condition.

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Table I.- **Evaluation of lethal ovitraps with different treatments of *Bti*, buprofezin and integrated *Bti*+buprofezin (1:1) in water and hay infusion, as number of LOs installed, ovitrap positive index (OPI), number of eggs and egg density index (EDI) for *Aedes aegypti* in Samanabad and Mughalpura.**

Treatments	No. of LOs			Samanabad						Mughalpura					
				Positive LOs		Total eggs	Eggs / LO			Positive LOs		Total eggs	Eggs / LO		
	Installed	Collections (n)	Total (n)	(n)	OPI	(n)	EDI	±SEM	(Range)	(n)	OPI	(n)	EDI	±SEM	(Range)
Control (W)	6	15	90	17	18.9	253	14.9	±2.13	(03-35)	15	16.7	301	20.07	±2.30	(08-40)
W+*Bti*	6	15	90	15	16.7	219*	14.6	±1.71	(07-25)	11	12.2	236*	21.45	±2.46	(18-30)
Control (W)	6	15	90	17	18.9	276	16.2	±2.03	(06-28)	19	21.1	484	25.47	±2.19	(12-43)
W+Bup	6	15	90	16	17.8	191*	11.9	±1.87	(06-32)	16	17.8	421*	26.31	±1.77	(07-31)
Control (W)	6	15	90	16	17.8	219	13.7	±2.62	(05-48)	24	26.7	442	18.42	±2.18	(03-56)
W+*Bti*+Bup	6	15	90	11	12.2	216*	19.6	±4.05	(05-48)	17	18.9	346*	20.35	±2.00	(03-29)
Control (H)	6	15	90	26	28.9	504	19.4	±2.39	(06-56)	28	31.1	568	20.29	±2.56	(03-74)
H+*Bti*	6	15	90	20	22.2	468*	23.4	±3.34	(08-52)	20	22.2	544*	27.20	±2.78	(04-37)
Control (H)	6	15	90	25	27.8	510	20.4	±2.17	(07-55)	32	35.6	752	23.50	±1.92	(03-49)
H+Bup	6	15	90	25	27.8	487*	19.5	±1.91	(07-30)	34	37.8	776*	22.82	±1.71	(07-56)
Control (H)	6	15	90	27	30.0	578	21.4	±2.43	(07-55)	26	28.9	464	17.85	±1.85	(06-44)
H+*Bti*+Bup	6	15	90	22	24.4	504*	22.9	±2.87	(13-66)	21	23.3	393*	18.71	±1.84	(09-39)

W: Water, H: Hay

*ANOVA with Tukey's mean separation procedure, at 95% confidence interval of the difference indicated that there was no significance difference ($p > 0.05$) between different treatments (*Bti*, buprofezin and integrated *Bti*+buprofezin 1:1) compared with their respective control with respect to number of eggs in both municipalities.

Table II.- Evaluation of lethal ovitraps with different treatments of *Bti*, buprofezin and integrated *Bti*+buprofezin (1:1) in water and hay infusion, as percent larvae, pupae and adults emergence for *Aedes aegypti* in Samanabad and Mughalpura.

Treatments	Conc. (ppm)	Samanabad						Mughalpura					
		Water			Hay			Water			Hay		
		L	P	A	L	P	A	L	P	A	L	P	A
Control	-	91±2.1[a]	86±3.1[a]	86±3.1[a]	89±3.9[a]	85±3.3[a]	85±3.3[a]	94±4.1[a]	85±3.9[a]	85±3.6[a]	89±3.9[a]	82±3.3[a]	82±3.6[a]
Bti	100	54±1.8[b]	0±0.0[b]	0±0.0[b]	58±4.2[b]	0±0.0[b]	0±0.0[b]	55±3.3[b]	0±0.0	0±0.0[b]	60±4.0[a]	0±0.0[b]	0±0.0[b]
	10	64±2.4[b]	0±0.0[b]	0±0.0[b]	70±3.0[a]	5±0.1[b]	4±0.9[b]	68±5.1[b]	0±0.0	0±0.0[b]	71±1.3[b]	7±1.4[b]	7±1.4[b]
	1	78±1.8[a]	41±2.3[b]	41±2.3[b]	71±4.4[a]	38±3.2[b]	38±3.2[b]	84±1.3[a]	60±1.6	44±2.2[b]	69±3.5[b]	47±4.0[b]	47±4.0[b]
Control	-	92±2.7[a]	87±4.1[a]	87±4.1[a]	91±1.5[a]	84±1.8[a]	84±1.8[a]	91±2.9[a]	81±3.1[a]	81±3.1[a]	93±4.8[a]	87±2.6[a]	87±2.6[a]
Bup	100	*	*	*	76±4.0[a]	0±0.0[b]	0±0.0[b]	76±4.7[a]	0±0.0[b]	0±0.0[b]	68±2.7[a]	2±0.3[b]	0±0.0[b]
	10	77±3.1[a]	0±0.0[b]	0±0.0[b]	71±3.3[a]	28±4.8[b]	0±0.0[b]	*	*	*	74±3.7[a]	33±3.9[b]	0±0.0[b]
	1	79±3.9[a]	40±2.0[b]	18±2.3[b]	77±2.1[a]	47±0.5[b]	19±1.2[b]	83±3.7[a]	45±3.0[b]	17±4.5[b]	84±4.1[a]	48±3.9[b]	19±4.0[b]
Control	-	90±3.2[a]	84±3.1[a]	84±3.2[a]	89±2.1[a]	87±2.0[a]	87±2.2[a]	94±4.5[a]	87±2.0[a]	87±2.0[a]	89±3.3[a]	81±2.8[a]	81±2.8[a]
Bti+Bup	100	61±4.0[b]	0±0.0[b]	0±0.0[b]	67±5.1[a]	0±0.0[b]	0±0.0[b]	53±4.5[b]	0±0.0[b]	0±0.0[b]	67±4.8[a]	0±0.0[b]	0±0.0[b]
	10	70±1.5[b]	0±0.0[b]	0±0.0[b]	78±3.3[a]	0±0.0[b]	0±0.0[b]	70±3.8[b]	0±0.0[b]	0±0.0[b]	*	*	*
	1	79±3.9[a]	6±0.4[b]	0±0.0[b]	*	*	*	72±2.6[b]	16±1.4[b]	0±0.0[b]	79±2.6[a]	20±2.0[b]	1±0.0[b]

A: Adults, Bup: Buprofezin, *Bti*: *Bacillus thuringiensis israelensis*, L: Larvae, P: Pupae
* indicates that no egg was observed in the respective treatment group.
Data showing mean values of two Replicates as percentage in all treatment groups.
Means with different letters are significantly different ($p<0.05$) within each growth stage compared with respective control group.