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

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Abstract: Limited success has been attained using long-established mosquito vector control methods to prevent dengue transmission. Integrated disease control programs making use of alternative tools, e.g. *Lethal ovitraps* may provide greater prospects for monitoring and reducing vector populations and disease transmission in order to provide new robust data on the efficiency of entomological surveillance methods to control important dengue and other disease vectors in Pakistan and other geographic regions. The purpose of this study was to figure out the efficiency of Lethal ovitraps in eggs collection baited with grass infusion. This study also aimed at exploring *Aedes* infestation indices and generation of baseline data by indoor and outdoor ovi-trapping. Field evaluation of a Lethal ovitraps containing Deltamethrin-treated strip was carried out for monitoring the dengue vector (s) *Aedes* mosquitoes during November-February, 2015 in Rawal Town, Islamabad, Pakistan. The study site was divided into treatment and control blocks with 20 randomly selected houses for each block. Each block received 40 Lethal ovitraps (LOs) with and without treatment. The oviposition response by *Aedes* mosquitoes was measured using the Ovitrap Positive Index (OPI) and the Eggs Density Index (EDI). There were six weekly eggs collections made. Which yielded 510 *Aedes* eggs with 32 and 478 from the treatment and the control blocks, respectively, indicating the damaging effect of Deltamethrin on the treatment group. The weekly egg collections yielded 510 *Aedes* eggs with 32 and 478 from the treatment and the control blocks, respectively, indicating the damaging effect of Deltamethrin on the treatment group. The OPI response of treatment and control ovitraps was different. OPI was higher in the controls than in the treatment groups. Moreover, there was a significant difference in EDI of treatment compared to control. There was complete inhibition of larvae emergence in Lethal ovitraps in comparison to the control, where 50.20% larvae were formed. The results indicated that the Lethal ovitraps proved to be a very effective tool for monitoring and controlling *Aedes* populations under natural conditions. Furthermore, a significant decrease in the number of eggs was obtained in the treated group. At lower operational costs and consistency, these LOs can be practically used as a benign tool for measuring infestation rates for entomological surveillance of *Aedes* species.

Keywords: *Aedes Aegypti, Aedes Albopictus*, Lethal Ovitrap, Deltamethrin, Dengue

1. Introduction

Dengue is the most rapidly spreading mosquito-borne viral disease in the world [1] that has proved damaging. The dengue incidence has increased 30-fold with an average rate of 50-100 million new infections / year in more than 100 countries (WHO, 2012) [2]. Approximately 2.5 billion people live in dengue endemic countries [3] along with 500,000 cases of dengue fever (DHF), resulting in around 24,000 deaths annually [4].

Dengue or dengue-like epidemics were reported throughout the nineteenth and early twentieth centuries all over the globe [5, 6] including Pakistan. In Pakistan, the dengue infections are becoming more frequent and severe in large cities [1]. Pakistan was first hit by dengue fever in 1994 while the worst epidemic was observed in 2011 in Punjab resulting in 203 deaths. The Disease Early Warning System (DEWS) in Pakistan reported 4,388 suspected cases from 01 January to 11 September 2013. The worst hit provinces were Khyber Pakhtunkha (3,177 cases) and Sindh (1098 cases) [7].

Domestic populations of the mosquito *Ae. aegypti* are the primary vectors of dengue (Lane & Crosskey, 1993) [8], while *Ae. albopictus* is considered a secondary vector. Both species are sensitive to environmental conditions [9-12]. The dengue virus has also been isolated from species such as *Ae. albifasciatus*, *Ae. polinensis* and several species of the *Ae. scutellaris* complex [13, 14]. These mosquitoes primarily breed in artificial containers like earthen jars, plastic and metal drums, used car tyres, potted plants and man-hole covers and other types (Chareonviriyaphap *et. al.,* 2003) [15].

Dengue virus is transmitted to humans through the bites of infective female *Aedes* mosquito [5, 6, 16]. Most countries in SouthEast Asia region (SEAR) bear a high burden of DF (Dengue fever)/DHF and experience frequent and cyclical epidemics [10, 17]. The dengue virus (DENV) belongs to the genus *Flavivirus* and has four antigenically distinct virus serotypes or genotypes (DENV I to DENV IV) (Westaway & Blok, 1997) [10, 17, 18].

While a vaccine is under process, vector control remains the most effective and affordable method (Chandre, *et. al.,* 1999; Nauen, 2007) [19] to prevent dengue transmission through integrated control approach including community participation. This requires a behavioural change about or toward the vector species and the disease [11] along with the tactical approach to target *Aedes* at their developmental stages in all settings where human-vector contact occurs [1].

The common methods used for *Ae. aegypti* surveillance include the inspection of premises for larvae and pupae and the use of ovitraps. Most operational surveillance systems depend on such surveys to gather their house, Breteau and larval-density indices [20], while others require source reduction through environmental sanitation or employ insecticide treatment (PAHO, 1994). It is not possible to eradicate diverse mosquito breeding sites completely. In addition, no adulticide or larvicide has proven fully successful against *Aedes* vectors (PAHO, 1994) [20, 21].

The Global Strategy for the Prevention and Control of DF and DHF also recommends the applications of integrated vector control measures with community and inter-sectoral involvement [22]. Therefore, in this regard, the development of a Lethal ovitrap (LO) as an alternative and emerging control technology [23], proved to be a practically cost-effective and suitable method for integrated vector control [20, 21].

Ovitraps have been used to present useful data for *Aedes* control operations along with revealing low mosquito populations. Although ovitraps are used to attract mosquito females for eggs lying [20], they have many limitations as they themselves might become potential breeding places if not monitored regularly in less-than-a-week intervals [24].

The first ovitrap made in the United States has been used in many parts of the world for monitoring *Ae. aegypti* populations (Service, 1993). It was practically used against *Ae. aegypti* in 1969 at Singapore International Airport (Chan, 1973). Later, Chan *et al*., (1977) came up with a design of an autocidal screened ovitrap with greater efficacy in the field [8, 21, 25].

Addition of 'hay infusion' as an attractant (Reiter *et. al.,* 1991) yielded higher number of eggs without altering its attractiveness regardless of seasonal variations [25]. Then, Zeichner and Perich (8 & 21) used an insecticide-treated oviposition strip in order to make the trap lethal to both larvae and adult *Ae. aegypti*. Based on these studies, an ovitrap can be modified for controlling *Aedes* vectors. Therefore, we decided to use an insecticide-treated ovistrip to be evaluated against *Aedes* in a field trial [8].

The LO using 25% wettable granulated Deltamethrin is being described for the first time in Pakistan. These ovitraps collect mosquito immature stages for various research purposes and serve as suitable surveillance and monitoring tools. Not much work has been done on LOs except their use in Lahore by Jahan N. [15, 17, 19, 25]. Realizing the need of time, this study was planned to use LOs for reducing vector density sufficiently in an area as a part of integrated vector management strategy. An additional advantage of using LOs is to shorten the longevity of the vector, which ultimately reduces the number of infective mosquitoes in natural populations with the long-term implications for the mosquito vectorial capacity.

The overall aim of this study was to decrease the disease burden of dengue by using economical and benign surveillance tools for monitoring dengue vectors while the objectives fulfilled were; 1) to compare the efficiency of Lethal ovitraps in eggs collection baited with 10% grass infusion and water solution against standard ovitraps, 2) to explore the relationship between different *Aedes* infestation indices [26], & 3) to generate baseline data about infestation by evaluating indoor and outdoor ovitraps.

2. Materials and Methods

2.1. Study Design and Populations

This study was Clustered-Randomized (Winer *et. al.*, 1991) in which a group of 20 houses was used as a treatment block, while another group of 20 houses was used as a control block.

2.2. Study Area

The study was carried out in Rawal town of Islamabad, Pakistan during November 2014–February, 2015. Islamabad (*33°43'N / 73°04'E*) and has an area of 906 km^2 [27] with a human population of 8052035 (census 1998). Rawal town (*33.685 N / 73.117 E*) of Islamabad district has a population of 17,292 people (census 1998).

Islamabad is a part of a semi-arid-sub-tropical climate zone with a large variation in temperature. The mercury here sometimes falls below 0°C during winter and sometime touches 48°C during summer. The average rainfall ranges from 990 to 1000 mm. Thunder, wind and hailstorm are also common [28].

2.3. Dengue Mosquito Vectors Surveillance

During a preliminary survey of two weeks, an adults collection was made using mechanical aspiration [29] collecting mosquitoes within the house for 10 minutes [21] during morning hours (0700–0900 hrs) [30]. The collections were made both inside and outside the selected houses. The captured adult mosquitoes were placed in entomological boxes and brought back to the laboratory for species identification [29] using standard identification keys [30].

In addition, mosquito larvae and pupae were collected from natural containers using larva-fishing nets. The contents collected were transferred to a beaker containing water and transported to the entomological laboratory for species identification [31]. Conventional indices i.e. the House (HI), the Container (CI), the Breteau (BI) and the Pupal (PI), were calculated according to the WHO guidelines [16, 32]. After intervention of six weeks, a post survey was also carried out for collecting mosquito adults, larvae and pupae in the last two weeks of the study.

2.4. Ovitrap and Ovipaddle Design

LOs were used as a lure-and-kill device for dengue vectors using illustrations of Fay and Perry (1965) [33], consisting of 500 ml capacity black-painted plastic cups. Two holes were drilled equidistant at 2 cm below the cup rim to hang the ovitrap using a 50-cm string [25]. A wooden tongue depressor (16 cm x 2.5 cm) [21] wrapped by layers of filter paper served as the ovistrip. The ovistrips were treated with 1.0 mg active ingredient/strip of 25% wettable granulated deltamethrin [34, 35] found to be most effective in prior laboratory testing (Zeichner & Perich, 1999) [8, 21]. Ovistrips were pre-treated with insecticide solution pipetted evenly over the paper strip and were left to dry to be ready for use [8].

To enhance the attractiveness of the LOs, they were filled with a 10% hay infusion-water solution as described by Reiter *et al.*, (1991) to approximately 2 cm of the top [21]. Hay infusion was made by steeping 125 g of dried lawn grass in 15 liters of tap water in a tightly-closed plastic garbage container and incubated for seven days [36].

2.5. Ovitrap Placement

The selected area was divided into two blocks separated by 100-200 m distance. One block of 20 houses was randomly assigned as a control block and other as a treatment block. For all experiments, ovitraps were set between 0900 and 1200 hours, the time of least oviposition activity (Chadee and Corbet 1987, Gomes *et. al.,* 2005) [37].

Each house received 2 ovitraps, one indoor and other outdoor placed at the height of 1.5-2 m [38]. These traps were set sheltered from direct sunlight and rain.

2.6. Evaluation of LETHAL Ovitraps

Ovitraps without any treatment containing 10% hay infusion and water solution were installed in 20 houses (1 pair for each house) of control block alternatively while lethal ovitraps with deltamethrin 25% wettable granulated (1mg/strip) in 10% hay infusion and water solution were installed in 20 houses (1 pair for each house) of the treatment block. All ovitraps (n= 80) were observed weekly for *Aedes* eggs and 10% hay infusion and water solution was replaced in respective ovitrap. Ovipaddle or entire ovitrap was replaced if anyone was found missing. To prevent fungal contamination, all ovipaddles were replaced with fresh ovipaddles weekly.

From collection site, each of one week-old ovipaddle was brought carefully at the entomological insectary after placing in an individual labeled plastic bag. The eggs on pallets were counted using a stereoscopic microscope [31]. Ovitraps were considered positive when at least one egg was detected [39]. Each ovipaddle was left to dry at room temperature (28±2°C; 80±10% RH) at diagonal angle [25]. They were maintained in laboratory conditions until embryonic development was complete [31]. Identification of the species was carried out after adult stage [40] using Zoo taxa keys (Rueda, 2004) [41].

The total dried filter paper strips were shifted to the respective larval tray filled with sufficient amount of tap water to allow egg eclosion. The larval trays were labeled and covered with a net to block intervention. In each tray larvae formed were counted and recorded. Pupae were counted and separated a separate dropper (for each treatment) to a cup containing 200 ml of water for adult emergence. In each cup, the adults emerged were counted and recorded. Mortality in each stage was recorded daily, was and counted by separating dead larvae or pupae from live ones with using camel hair brush. No food was added during the whole experiment period. Water was daily added to balance the water loss by evaporation [25]. Weekly temperature and accumulated rainfall was also taken into consideration during the studied period.

2.7. Data Entry and Analysis

The data collected were analyzed in SPSS (Statistical Package for Social Sciences) version 16.

The Ovitrap Positivity Index (OPI) and the Eggs Density Index (EDI) were used as indicators of oviposition level. These indices were calculated on a weekly basis (Gomes, 1998). The mean numbers of eggs were calculated for the total trial period and also for individual weeks according to the ovitrap installation blocks. The results were also calculated with reference to percentage emergence and SEM (Standard error of the mean). Where required, cross tabulation was done and association was seen by checking the significance of the association using Chi-square statistical test and by applying ANOVA (Analysis of Variance).

Infestation by *Aedes* eggs was estimated using conventional indicators; OPI and EDI (Gomes, 1998) [24, 25]. Choice of Lethal ovitrap for oviposition (OPI) was estimated as the percent Lethal ovitraps positive for eggs from the total number of ovitraps inspected (or distributed).

OPI= No. of Lethal ovitraps positive for eggs / Total no. of Lethal ovitraps inspected × 100

The efficiency of Lethal ovitraps in eggs collection (EDI) was calculated as average number of eggs laid per positive control/Lethal ovipaddle.

EDI= Total no. of *Aedes* eggs on ovipaddles / No. of positive ovitraps

To evaluate the toxic effect of Lethal ovitraps after field exposure, percent larvae, pupae and adults emergence was calculated. Test results compared with their respective controls were statistically analyzed using Excel and SPSS version 16.0; SPSS Inc., Chicago, IL)

3. Results

The studied period was characterized by a mean monthly temperature of 72.2°F (62.4 – 81.5°F) and rainfall of 12.93 inches (11.9–13.9 inches).

3.1. Adults Density

Six weeks after placing LO, the number of female *Aedes* collected was notably fewer from the treatment houses with only five mosquitoes aspirated in comparison to 13 mosquitoes from the controls. This indicates that the LO over time significantly reduced adult female *Aedes* production. The mean number of female *Aedes* aspirated from the treatment houses (0.25) compared to the mean number collected from the control houses (0.65) was not distinctively different.

Although there was no change in the mean of pre-treatment (0.25) to the mean number in post-treatment (0.25) in treatment block, but at least, this value did not elevate. This might be due to the presence of a large number of breeding places available competing with the LO for the oviposition-seeking *Aedes* females. This result is consistant with the study of Perich *et al.,* in Brazil at Nilopolis. The overall mean of both the blocks was 0.2875. This manifests a significant result ($P < 0.05$) $P = 0.022$

3.2. Prevalence Indices of Aedes Species

The four indices indicated different levels of infestation by *Ae. aegypti* and *Ae. albopictus*. HI and BI registered identical values in treatment blocks before and after the study started. In total, 80 houses were searched for *Aedes* breeding. *Aedes* breeding was only detected in 5 houses. About 411 water containers were searched, out of which only 9 were found positive for *Aedes* breeding. During a preliminary survey, the HI, the CI, the BI and the PI of the treatment block were found to be 5, 0.8, 5 and zero, respectively. There was no larvae or pupae found during a preliminary survey in the control block. After treatment of six weeks, the HI, the CI, the BI and the PI of the treatment block remained 5, 1.06, 5 and 0.01, respectively in comparison to the HI, the CI, the BI and the PI of the control block. (Figure 1)

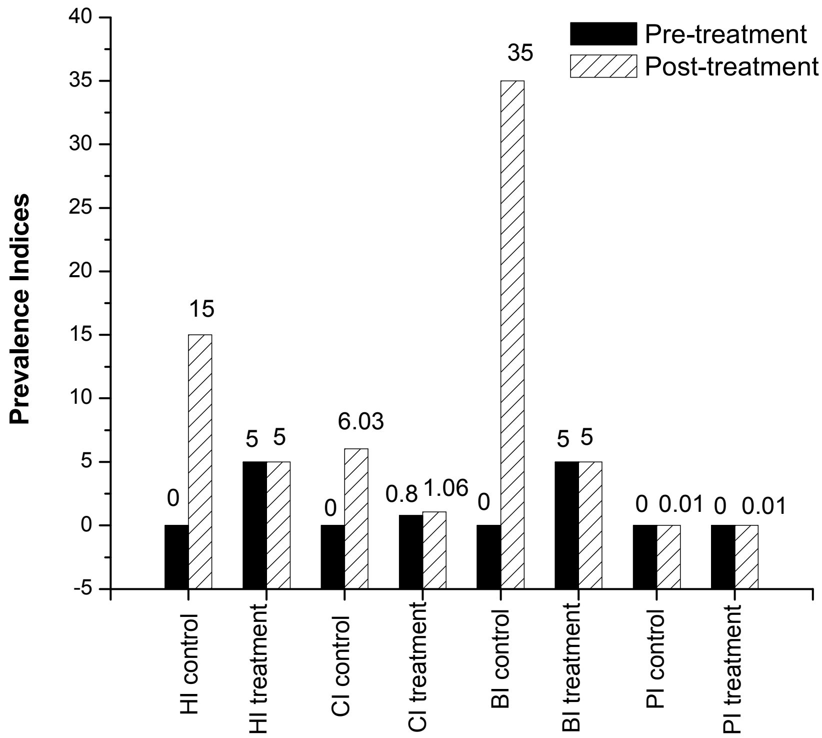

Figure 1. *Graph showing effect of treatment on prevalence indices of Aedes aegypti and Ae. Albopictus.*

The CI of the treatment block (1.06) after the treatment was significantly different compared to the pre-treatment (0.8) of the treatment block rather it was higher. This may be linked to peak activity period of the mosquitoes (Figure 1).

3.3. Eggs Density

During the four months of the experiment, a total of 510 eggs of *Ae. albopictus* and *Ae. aegypti* were collected in the ovitrap out of 480 containers for both blocks. Eggs were collected for 6 weeks, while a very small number of eggs were collected throughout the collection time period.

3.4. Ovitrap Positive Index and Egg Density Index

Out of 480 containers, only 41 were found positive for *Aedes* eggs. Average 15.93 eggs were harvested per ovipaddle in the control block, while 2.90 eggs were obtained per ovipaddle from treatment block. Collections of eggs differed between two blocks chosen, which gave the OPI of 8.54 while EDI was 12.439 (Table 1). OPI and EDI were also calculated for each week per block (Figure 2).

Table 1. *Eggs obtained in Rawal town.*

		No. of LOs			Positive LOs		Total no of eggs	% of Total Sum	Eggs/LO			
		Installed	Collections (n)	Total (n)	(n)	OPI			EDI	± SEM	95% Confidence Interval for Mean: Lower Bound	Upper Bound
Blocks	Control	40	6	240	30	12.5	478	93.70%	15.93	3.103	9.58	22.28
	Treatment	40	6	240	11	4.583	32	6.30%	2.9	0.638	1.48	4.33
	Total	80	12	480	41	8.542	510	100.00%	12.43	2.443	7.5	17.37
Position of the ovitraps	Indoor ovitraps	40	6	240	7	2.91	37	7.30%	5.28	3.307	2.8	13.37
	Outdoor ovitraps	40	6	240	34	14.16	473	92.70%	13.91	2.815	8.18	19.64
	Total	80	12	480	41	17.08	510	100.00%	12.43	2.443	7.5	17.37

There was a significant difference ($P < 0.05$) P=0.016 between two blocks in terms of the number of eggs, OPI and EDI.

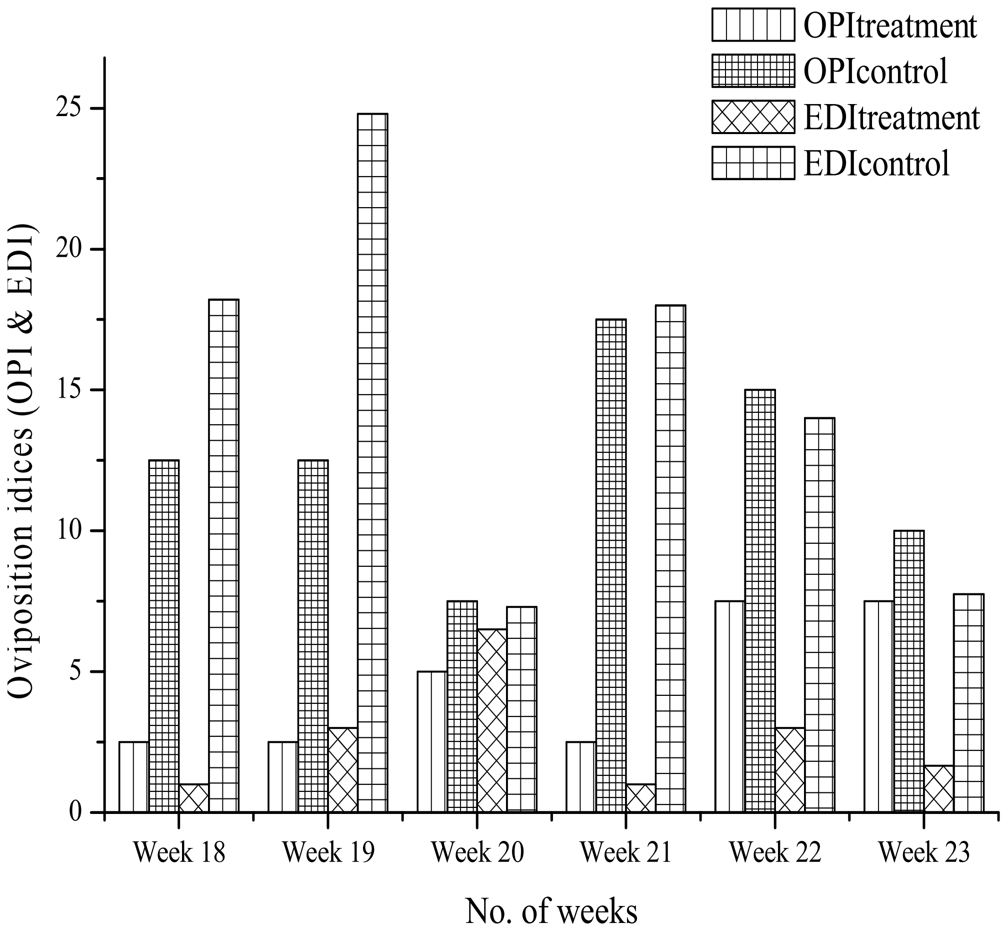

Figure 2. *Graph showing OPI and EDI indices of treatment and control block.*

There were significant differences in the presence and density of eggs between outdoor and indoor ovitraps with higher values for outdoor ovitraps (OPI= 14.16; EDI = 13.91) (Figure 3). The gravid female *Aedes* mosquitoes had a significant preference to oviposit eggs in ovitraps placed outside (F= 1.8; df 1 39; P=0.187).

Only one outdoor ovitrap was positive for mixed breeding which accounted for 2.9% of the total 34 outdoor ovitraps collected. Two species were identified after adult emergence as *Ae. aegypti and Ae. albopictus.* However, there was no mixed breeding found in ovitrap surveillance in treatment.

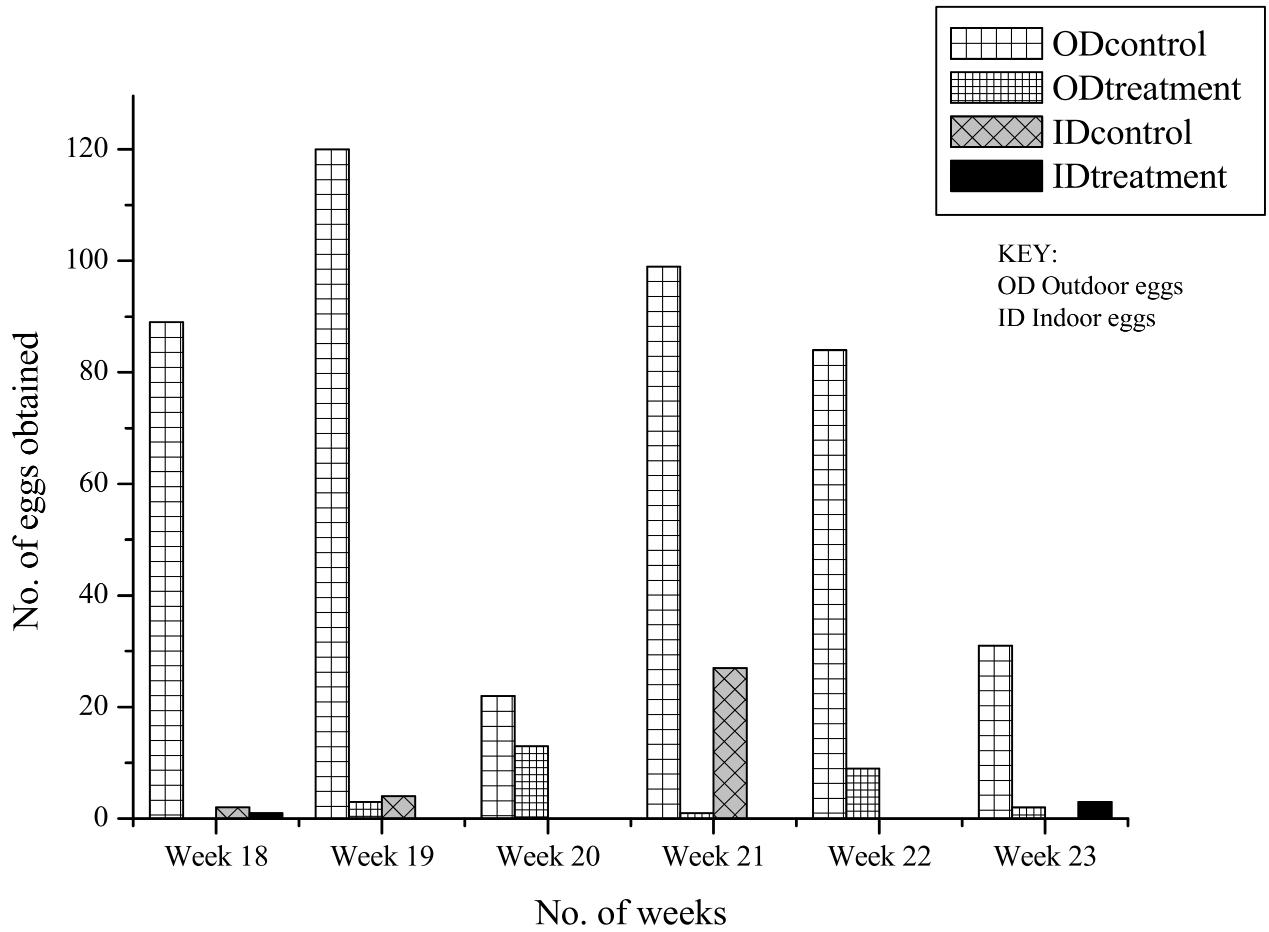

Figure 3. *Graph showing indoor and outdoor oviposition preference of Aedes.*

3.5. Percentage Emergence Rate

Ovitraps collected the largest number of *Ae. albopictus* eggs. Only *Ae. aegypti* eggs were obtained in entomological week no. 18**.** Out of 510 eggs, 240 larvae hatched (47.05%) of which 5 five were *Ae aegypti* (2.08%) and the rest (97.91) were *Ae. albopictus* (%). Percent larvae and pupae formation along with adult emergence was reduced to zero in treatment block as compared to control. (Figure 4)

The control ovitraps yielded 50.20% larvae, 90% pupae and 98.61% adult. Overall treatment group was found to be most effective in controlling adult population of *Aedes* in the selected locality.

3.6. Species Identification of Emerged Adults

Generally, *Ae. albopictus* was found at a higher frequency than *Ae. aegypti* in these ovitraps.

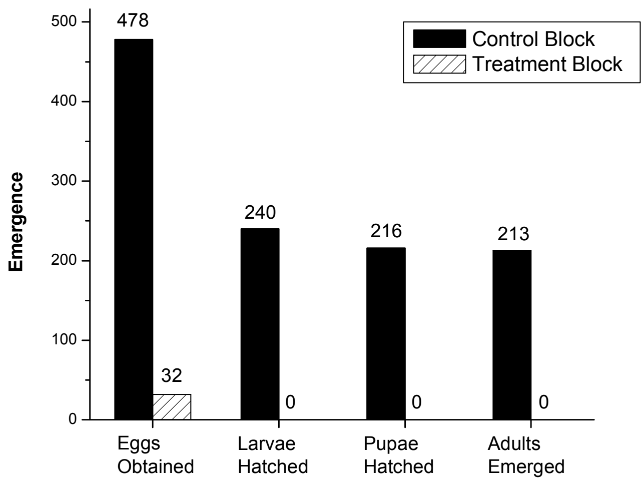

Figure 4. *Graph showing the emergence rates of various developmental stages.*

Out of 213 adults emerged, 208 (97.65%) were *Ae. albopictus*, and only five (2.34%) were identified as *Ae. Aegypti*. The overall mean for both species was 5.19. None of the eggs hatched in the treatment group. (Figure 4) Therefore, no adult identification was possible for the treatment block.

There were significant differences in the presence of *Ae. albopictus* compared to *Ae. aegypti* (F= 2.265; df 3 37; P= 0.097), with *Ae. albopictus* was the predominant species.

4. Discussion

The infestation indices indicated the presence of *Aedes* species during the study period in Rawal Town, Islamabad. The LOs did effectively compete with the other domestic containers for oviposition as they reduced the number of positive containers (Figure 2). The results of our study are consistent to the results of Chan *et. al.*, (1977) and Perich *et. al.,* [21]. The PI values recorded were above 1% and BI above 5%, indicate the risk areas where dengue transmission is likely to occur (FUNASA, 2002).

In the present study, the four larval surveys conducted showed a positive correlation between HI and BI. (Figure 1) Therefore, the larval indices recorded from the study area indicate that such high index (HI= 15%) may lead to an outbreak if no immediate actions are taken. For the index of pupa per person (Focks *et al.,* 1995), our results indicated the value of 0.01 where dengue vector remains unable to sustain dengue transmission.

There are almost no control actions taken by the municipalities to control potential dengue vectors. From the positivity of the ovitraps and the number of eggs deposited throughout the study period, it can be assumed that female *Aedes* were constantly multiplying. Therefore it indicates the urgent demand of at least reducing the mosquito number from attaining the threshold toward the increased risk of dengue transmission.

The presence of *Aedes* females, but not of larvae, indicated fewer breeding places for larvae. The positivity of the studied area in terms of EDI and negativity in terms of HI, CI, BI and PI indicates the accuracy of ovi-trapping at measuring infestation level of *Aedes*. We observed in our study that despite poor prevalence indices recorded, ovitrap surveillance produced quantitative results (Figure 2). Similar results were reported [31, 42]. These studies considered LOs strategy as an effective tool to detect and prevent *Ae. aegypti* population growth, which lowers the risk of potential disease outbreaks.

The current study evaluated LOs impregnated with deltamethrin. It was found that the treated ovitraps received significantly lower number of *Aedes* eggs as compared to their respective control groups (Figure 2 and 3). The effect of deltamethrin impregnated LOs was also studied in Thailand (Sithiprasasna, *et. al*., 2003) and Brazil to observe *Ae. aegypti* population (Perich *et. al*., 2003) [21]. Deltamethrin-treated ovitraps used in Manawann, Lahore [25] also indicated that LOs containing 1.5% EC deltamethrin could serve as an effective dengue vectors suppression tool but the control of mosquito vector will only be attained if it is used effectively as a part of an IVM programme. Perich *et. al.,* [21] & Jahan N, Sarwar MS [25] also suggested the incorporation of LOs in integrated disease control programs.

We found that black ovitraps come with good yield for the surveillance of gravid *Ae. albopictus*, but not for *Ae. aegypti* as only five mosquitoes of this species were found. Similar results were depicted by in North-Central Florida, USA [40]. We also found the mixed breeding of *Ae. aegypti* and *Ae. albopictus* in our study where *Ae. albopictus* were dominant compared to *Ae. aegypti*. Sulaiman *et. al.,* (1991), Norzahira *et. al.,* Rozilawati *et. al.,* Shi CH, Sallehudin *et. al.* and Chen *et. al.*, [38] also reported similar results.

We also found that that *Ae. albopictus* preferred to breed outdoors (473 eggs, mean/trap or collection site±SEM)) rather than indoors (37 eggs) in Rawal Town, Islamabad (Table 1). This was also found by Rozilawati *et. al.,* & Chen *et. al.,* & Dibo *et. al.,* [38, 42]. This may explain the preference of *Ae. albopictus* to breed outdoors rather than indoors.

The positive correlation between OPI and EDI (Table 1) found for both indoor and outdoor placement of the ovitrap is in agreement with that reported by Chadee (1992), Dibo *et. al.,* (2005) [42] & Burroni *et. al.,* (2013) [39]. Although there was a large number of natural containers present in the studied area for breeding but some traps became positive within the first week, that is, during the dry season. Thus, this method was confirmed to be efficient in detecting of *Aedes (Stegomyia)* as has been described by Fay and Eliason (1966), Marques *et. al.,* (1993), Braga *et. al.,* (2000) & Dibo *et. al.,* (2005) [42].

The geographical distribution of *Aedes* in Rawal Town, Islamabad gave only a rough estimate of the infestation, which cannot be applied to the entire geographical region of Islamabad. The number of eggs harvested was very less due to commencement of the study in early dry season in the area and also we faced the problem of closed houses.

5. Conclusion

The LO placed in Rawal Town had a significant damaging effect on *Aedes* populations within three months after their placement inside and outside the treatment houses. In comparison of two blocks, the LOs in the control block harvested eggs about 14.9 times the numbers harvested by the treatment block.

Lethal ovitraps were found most effective tool as it directly targeted dengue vectors with minimal use of insecticides. Furthermore, the minimum effective dosages to inhibit 100% larval emergence and subsequent stages in a habitat have shown to be extremely low (1 mg/ovistrip).

Mixed breeding indicates that more than one mosquito species can oviposit in a single ovitrap. Therefore it can be assumed from our study and other cited that LO is highly sensitive to attract gravid females of more than one mosquito species to oviposit in the container.

Based on the data acquired from LO surveillance, it is concluded that LOs are useful in planning anti-*Aedes* campaign, insecticide application and use of new technologies for vector control. Its effectivity in *Aedes* control demands IVM by using a set of effective interventions rather than sole reliance on LOs. For a country like Pakistan, LOs are more practical surveillance tools as a useful indicator for assessing the impact of control programmes as they are inexpensive, simple to handle and non-disturbing.

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