# OUTDOOR BREEDING OF MOSQUITO SPECIES AND ITS POTENTIAL EPIDEMIOLOGICAL IMPLICATIONS IN KHYBER PAKHTUNKHWA

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**ABSTRACT:- Entomological surveillance study was conducted in four selected sites; Peshawar, Nowshera, Mardan and Charsadda of Khyber Pakhtunkhwa, Pakistan during 2012-13 for development of ultimate control strategies of mosquito breeding in outdoor habitats. Larvae were collected from various mosquito breeding habitats such as irrigation channels, irrigation water leakages, pots, vase, tyres, temporary containers stagnant flood waters, etc. Ovitraps were used as monitoring tools in urban areas of the selected sites. Results showed that mosquitoes are active throughout the year with their most active season during May, September and October. The mean relative abundance of *Culex* species in different districts were: Peshawar (32.3), Nowshera (18.8), Mardan (20.3) and Charsadda (21.0). Higher numbers of *Aedes* mosquitoes were observed in Nowshera (19.3), Peshawar (16.4), Charsadda (13.1), and Mardan (9.8), respectively. Mean monthly positive ovitraps of species was high in May and October collected from Peshawar (32.5, 31.5), Nowshera (25.3, 26.8), Mardan (22.2, 16.8) and Charsadda (27, 26.9), respectively. The overall abundance of *Culex* species was high as compared with *Aedes* and *Anopheles* species collected from various outdoor breeding habitats. The *Culex* species was abundant in turbid water with foul smell while the *Aedes* and *Anopheles* were higher in comparatively fresh and clear water with low turbidity. The study recommends consideration on removal of artificial containers, monitoring of irrigation water and channels and ultimate control of breeding of dengue vectors in the target sites.**

*Key Words: Culex; Aedes; Anopheles; Mosquitoes; Habitats; Surveillance; Site Index; Pakistan.*

## INTRODUCTION

The outdoor aquatic spots play an important role in the establishment of different colonies of mosquitoes. It is further associated with biting action of mosquito vectors that opportunistically bite human outdoors during the day. This may consequently have imperative implications to disease transmission including dengue fever. In spite of the epidemiological importance of mosquito borne diseases, little work has been done on the vectors aspect of mosquitoes including dengue (Hamady et al., 2010). Therefore, developing techniques for effective management of larval habitats of dengue vectors is an essential component of dengue control programmes (Edelman, 2007).

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Among the mosquito borne diseases, dengue fever is a serious problem worldwide (Gubler, 1998). In Pakistan, dengue was not reported until 2005; however, it was mostly confined to the southern area (Karachi) of Pakistan. After severe outbreak in central areas (Lahore) and now in Northern areas (Swat) of Pakistan, the disease is reported from both the rural and urban areas of Pakistan (WHO, 2012). Its continued existence in Khyber Pakhtunkhwa in spite of control measures indicates the potential establishment of dengue vectors in other areas of Pakistan also (Suleman et al., 1996). The life history of mosquitoes need the development of larvae and pupae in habitats containing water with varying physical and chemical properties depending on mosquito species (Muturi et al., 2007). About 3 billion people annually are primarily infected by this viral disease (Guzman et al., 2010). Large population of mosquitoes can survive almost everywhere under favorable ecological conditions. Therefore, prevention and control should be targeted by avoiding human interaction with mosquitoes, reduction of adult mosquito populations and elimination of mosquito's larval habitats (Gubler, 1998).

Entomological surveillance is an effective tool to identify the key breeding areas for the control of *Aedes* species (Chen et al., 2006; Lagrotta, 2008). *Aedes* species are closely associated with human environments, where indoor and outdoor artificial containers like drums, tyres, buckets, plant pots, and vases make adequate habitats for larval development (Focks, 2003; Pages et al., 2006). Therefore, the knowledge about the local larval habitats, population dynamics, distribution trend and relative abundance etc. are important for working out effective management strategies for the mosquito vectors (Khan et al., 2011). The aim of current study was to explore different outdoor preferred sites and make widespread approaches for the control of dengue vector in the rural, semi-urban and urbanized areas of Khyber Pakhtunkhwa province.

## MATERIALS AND METHOD

### Entomological Surveillance

Surveys for exploring prevailing mosquito species were conducted at four selected sites of Peshawar division, Khyber Pakhtunkhwa, Pakistan during 2012-13. Mosquito species namely, *Culex, Aedes* and *Anopheles* were collected from different outdoor habitats i.e., irrigation channels, irrigation water, plant pots vases, tyres and flood water.

### Larvae/Pupae Surveys and Collection

Various breeding sites; irrigation channels, pools, river banks, different containers inside houses and lawns and potential breeding places (water tanks, etc.) were monitored fortnightly. Larval and pupal collections were made with 0.5 liter standard iron dippers. The collected larvae were brought in plastic bottle (2l) into laboratory and were reared following Khan (2011). Identification was made with the help of available taxonomic keys (Rueda, 2004). Site index was calculated as: Number of positive sites/Total number of sites visited x 100. The index was used as criteria for the key habitats of the mosquito species. The larval abundance per site was calculated by dipping the dippers randomly five times by dividing the sites into four sub-sites and one middle portion. The mean number of larvae/pupae collected per dip was recorded after the five dips sampling for each habitat under study.

**Monitoring through Ovitraps**

For monitoring abundance of mosquito species in urban areas, locally fabricated black color plastic ovitraps (25 cm x 37cm) were filled with 300 ml water following Khan et al. (2011). Strips of hard board (15 cm x 5 cm) having rough surface were placed in slanting position in each trap. All traps were placed in 15-20 different outdoor sites near and around the residential places. All the ovitraps were examined and replaced weekly. The number of larvae were recorded individually for each positive ovitrap. Data were analyzed as House Index and was determined as the percent number of positive ovitraps to the total number of recovered ovitraps. Mean number of mosquito species larvae per total number of recovered ovitraps were determined. The recorded data were separated by ANOVA (Steel and Torrie, 1980) using LSD test.

## RESULTS AND DISCUSSION

Three mosquito species i.e., *Culex, Aedes* and *Anopheles* were found during the study. The infantile stage profusion remained high during August, September and October, indicating its regular occurrence during this period.

Significant differences were observed among various species (Table 1). *Culex* species dominated *Aedes* and *Anopheles*. Percentage monthly positive ovitraps of *Culex* was high (32.3) in Peshawar. The mean abundance of *Aedes* in outdoor sites was 16.4%, which started in March. *Culex* and *Anopheles* were highest in September (53.3%) and October (16.3%), respectively. The activities of *Aedes* species started in March, increased up to May, decreased during June-July. It went higher again in August-November and decreased afterwards till December. The behavior of *Anopheles* species started in February, increased up to May, decreased during June-July and increased again in August-October but further decreased in November-December. Activities of *Culex* were high during May and September; *Aedes* in October-November and *Anopheles* were highest in October.

Percentage abundance of mosquito species was significantly different in Nowshera (Table 2). *Culex* (18.8) and *Aedes* species (19.3) dominated *Anopheles*. The mean population of Anopheles was no more than 3.69. Mean abundance of all the three species was highest in October (26.8). Breeding of all the three species started at January (1.95), persisted up to May (25.3) and then decreased during June-July (8.05, 4.43) but further increased in August- October and then decreased till December. It is assumed that *Culex* and *Aedes* were self-motivated species during May, September and October, even as *Anopheles* emerges during September-October.

Significant differences were observed among species collected from various outdoor locations in

**Table 1. Outdoor ovitraps index of different mosquito species in Peshawar during 2012-2013**

(%)

| Species | Month | | | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan. | Feb. | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | |
| *Culex* | 9.3 | 11.0 | 20.8 | 37.5 | 71.7 | 27.5 | 25.8 | 47.5 | 53.3 | 46.7 | 25.0 | 11.7 | 32.3[a] |
| *Aedes* | 0.0 | 0.0 | 14.2 | 20.0 | 20.8 | 5.0 | 1.7 | 17.5 | 19.2 | 31.5 | 44.3 | 22.2 | 16.4[b] |
| *Anopheles* | 0.0 | 0.7 | 2.3 | 3.0 | 5.0 | 1.2 | 1.0 | 7.8 | 13.2 | 16.3 | 5.7 | 0.7 | 4.7[c] |
| Mean | 3.1[h] | 3.8[h] | 12.4[e] | 20.2[d] | 32.5[a] | 11.2[f] | 9.5[g] | 24.3[c] | 28.6[b] | 31.5[a] | 25.0[c] | 11.5[ef] | - |

*LSD for species and months = 0.57 and 1.13, respectively at 0.05% level of probability;*
*Means followed by the same letters do not differ significantly at 0.05% level of probability*

**Table 2. Outdoor ovitraps index of different mosquito species in Nowshera during 2012-2013**

(%)

| Species | Month | | | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan. | Feb. | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | |
| *Culex* | 3.3 | 5.9 | 9.3 | 19.3 | 47.9 | 15.7 | 10.0 | 26.4 | 34.3 | 29.3 | 20.0 | 3.9 | 18.8[b] |
| *Aedes* | 2.6 | 2.6 | 8.6 | 13.3 | 22.1 | 5.4 | 2.6 | 26.0 | 30.0 | 39.9 | 48.3 | 29.9 | 19.3[a] |
| *Anopheles* | 0.0 | 0.1 | 1.1 | 3.7 | 6.0 | 3.0 | 0.7 | 6.0 | 8.4 | 11.3 | 3.4 | 0.4 | 3.7[c] |
| Mean | 1.95[j] | 2.86[i] | 6.33[g] | 12.1[e] | 25.3[b] | 8.05[f] | 4.43[h] | 19.5[d] | 24.2[c] | 26.8[a] | 23.9[c] | 11.4[e] | - |

*LSD for species and months = 0.40 and 0.80, respectively at 0.05% level of probability;*
*Means followed by the same letters do not differ significantly at 0.05% level of probability*

Mardan (Table 3). *Culex* spp. showed higher population than other two species. Maximum larvae of *Culex* (20.3), followed by Aedes (9.80) were found at different outdoor sites in Mardan. The mean population of Anopheles was 2.62. Its mean abundance was at peak during May (22.2). The mean proliferation of all the three mosquito species started in January (2.83), persisted up to May (22.2) and then decreased during June-July (6.06, 3.44) but further increased in August-September and then decreased till December. It is evident from the data that *Culex* species was lively during May and September and then decreased till December.

Mosquito vectors of various species showed significant differences collected from different outdoor sites in Charsadda (Table 4). *Culex* species had the highest population than other two species. Maximum number of *Culex* larvae (21.0), followed by *Aedes* (13.1) was found at various outdoor locations in Charsadda. The mean population of *Anopheles* was no more than 7.83. The mean abundance of all the three species (*Culex*, *Aedes* and *Anopheles*) was at peak stage in May (27.0), September (27.0) and October (26.9). The mean population of species complex started in January (2.5), persisted up to May (27.0) and then decreased during June-July (5.0, 2.9) but further increased in August-October and then decreased till December. Data showed that species complex was highly active during May, September and October-December.

The overall abundance of different species collected from various locations showed significant differences (Table 5). Highest abundance of species (497) was found in irrigation channels, followed by irrigation water (115). The peak incidence of *Culex* species 95, 43, 119 and 11 collected from irrigation channels was found in Peshawar, Nowshera, Mardan and Charsadda, respectively (Table 5). The abundance of *Culex*, *Aedes* and *Anopheles* was found in Peshawar (221, 85, 41), Nowshera (48, 61, 24), Mardan (138, 85, 20) and Charsadda (44, 38, 13), respectively (Table 5). Data showed that abundant *Culex* specimens were found in irrigated and urbanized areas of Peshawar division.

The ovitrap surveillance indicated that number of *Culex* individual were more than *Aedes* and *Anopheles* collected from various outdoor sites in Peshawar showing significant differences among them. More larvae of *Culex* were found in comparison with other two species indicated low population. The abundance of all the three species was at peak stage in May and October. Various species showed significant differences collected from outdoor sites in Charsadda. *Culex* species had the highest population than other two species. The mean abundance of species was at peak stage in May, September and October. The mean proliferation of species complex started in January, decreased during June-July but further increased in August to October. The peak population of mosquitoes in September and October may be due to wet season of June-July resulting in larval breeding thereafter.

The examination of widespread literature indicates that *Aedes albopictus* probably serves as a maintenance vector of dengue in

**Table 3. Outdoor ovitraps index of different mosquito species in Mardan during 2012-2013**

(%)

| Species | Month | | | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan. | Feb. | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | |
| *Culex* | 8.5 | 9.5 | 16.7 | 32.5 | 52.8 | 12.2 | 7.3 | 27.3 | 33.8 | 21.7 | 13.3 | 7.7 | 20.3[a] |
| *Aedes* | 0.0 | 0.17 | 2.3 | 4.3 | 11.0 | 4.5 | 0.8 | 12.5 | 18.3 | 21.7 | 26.3 | 15.3 | 9.80[b] |
| *Anopheles* | 0.0 | 0.0 | 0.0 | 2.2 | 2.8 | 1.5 | 2.2 | 5.0 | 6.8 | 7.0 | 3.3 | 0.7 | 2.62[c] |
| Mean | 2.83[h] | 3.33[h] | 6.33[g] | 13.0[e] | 22.2[a] | 6.06[g] | 3.44[h] | 14.9[d] | 19.7[b] | 16.8[c] | 14.3[d] | 7.89[f] | - |

*LSD for species and months = 0.41 and 0.81, respectively at 0.05% level of probability;*
*Means followed by the same letters do not differ significantly at 0.05% level of probability*

**Table 4. Outdoor ovitraps index of different mosquito species in Charsadda during 2012-2013**

(%)

| Species | Month | | | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan. | Feb. | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | |
| *Culex* | 6.7 | 8.3 | 15.0 | 26.0 | 56.2 | 6.8 | 2.3 | 34.2 | 40.5 | 30.8 | 18.3 | 7.0 | 21.0[a] |
| *Aedes* | 0.0 | 0.0 | 5.0 | 6.5 | 14.5 | 4.3 | 3.2 | 21.0 | 25.0 | 27.0 | 30.0 | 20.2 | 13.1[b] |
| *Anopheles* | 0.8 | 1.5 | 5.2 | 7.2 | 10.3 | 4.0 | 3.3 | 13.3 | 15.5 | 22.8 | 7.7 | 2.3 | 7.83[c] |
| Mean | 2.5[h] | 3.3[h] | 8.4[f] | 13.2[d] | 27.0[a] | 5.0[g] | 2.9[h] | 22.8[b] | 27.0[a] | 26.9[a] | 18.7[c] | 9.8[e] | - |

*LSD for species and months = 0.57 and 1.13, respectively at 0.05% level of probability;*
*Means followed by the same letters do not differ significantly at 0.05% level of probability*

**Table 5. Outdoor ovitraps index of different species collected from different habitats at various locations during 2012-2013**

| Habitat type | Location | | | | | | | | | | | | Total (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Peshawar | | | Nowshera | | | Mardan | | | Charsadda | | | |
| | *Culex* | *Aedes* | *Anophe les* | *Culex* | *Aedes* | *Anophe les* | *Culex* | *Aedes* | *Anophe es* | *Culex* | *Aedes* | *Anophe les* | |
| Irrigation channels | 95 | 50 | 22 | 43 | 49 | 24 | 119 | 31 | 20 | 11 | 22 | 11 | 497 |
| Irrigation water leakages | 39 | 9 | 13 | 0 | 0 | 0 | 0 | 54 | 0 | 0 | 0 | 0 | 115 |
| Pot vase | 63 | 26 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 95 |
| Tyres | 24 | 0 | 0 | 5 | 12 | 0 | 19 | 0 | 0 | 0 | 8 | 0 | 68 |
| Slaghaut flood water | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 8 | 2 | 43 |
| Total | 221 | 85 | 41 | 48 | 61 | 24 | 138 | 85 | 20 | 44 | 38 | 13 | 818 |

rural areas of Pakistan. These results are in accordance with those of previous workers. Minakawa et al. (1999) documented that *Anopheles arabiensis* is a predominant species in habitats characterized on the basis of size, pH, and distance to the nearest houses. Similarly, Chen et al. (2006) indicated that *Aedes aegypti* and *Aedes albopictus* were present both indoor and outdoor locations. Kuslimawathie and Siyambalagoda (2005) reported that breeding sites of *Aedes aegypti* and *Aedes albopictus* differed from one locality to another as well as from one time period to another. Piyaratnea et al. (2005) investigated that *Anopheles culicifacies* was positively related only to temperature and briefly available stream bed pool habitat, to optimize breeding success. Sophie et al. (2005) stated that irrigated fields and orchards were important determinants for recent dengue infection. Chen et al. (2006) indicated that ovitrap was a sensitive tool to attract gravid females of more than one mosquito species to ovipositor in the container. Doherty (2007) reported that agricultural areas had the highest mosquito abundance, likely due to increased irrigation. Harding et al. (2007) found larvae in a wide range of habitats but were particularly abundant in artificial water bodies, e.g., empty concrete water tanks. Hribar (2007) reported that *Culex quinquefasciatus* was the most frequently encountered species. Jonathan et al. (2007) showed the association of *Aedes aegypti* with high density house in urban areas and *Culex quinquefasciatus* with low density house in suburbs. Mcmahon et al. (2008) determined the species composition of mosquitoes in tyres where 95% of the larvae collected for each month of the summer and *Culex tarsalis* reached their greatest numbers in July and August.

It is thus concluded that, *Culex* and *Aedes* species showed high abundance of mosquitoes indicative of essential biting means compared to *Anopheles* species. Majority of mosquitoes were collected from irrigation channels and irrigation water. The population of these two species was maximum during September - November. Results of this research show that health support and education in realizing dengue control programme in the study area where irrigation channels are rough and control practices are not smooth. Therefore, temporary breeding sites consisting of waste water, empty tins, stand water, pots vases and old tyres should be removed for controlling dengue vectors.

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