Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Species diversity pattern of mosquitoes (Diptera: Culicidae) breeding in different permanent, temporary and natural container habitats of Peshawar, KP Pakistan

Padrão de diversidade de espécies de mosquitos (Dípteros: Culicidae) reproduzidos em diferentes habitats permanentes, naturais temporárias e contentores de Peshawar, KP Paquistão

Lubna[a]*, S. B. Rasheed[b] and F. Zaidi[b]

[a]Islamia College University Peshawar, Department of Zoology, Khyber Pakhtunkhwa, Pakistan
[b]University of Peshawar, Department of Zoology, Khyber Pakhtunkhwa, Pakistan

Abstract

To determine the species composition, relative abundance and seasonal variation of different mosquitoes Genera (*Aedes, Anopheles, Armigeres, Culex,* and *Culiseta)* in different habitats the present research work was carried out in Entomology Research Laboratory of The University of Peshawar. Sampling performed from variety of permanent and temporary breeding habitats was carried out on monthly basis from targeted breeding sites for two consecutive years through dipping method. Species diversity in the survey sites was noted. Collection from these seventeen various types of potential larval habitats, yielded a total of **42,430** immature constituting **41,556** larvae and **874** pupae. Among these only **19,651** adult mosquitoes emerged comprising **11,512** female and **8,139** male mosquitoes. 78% (n= 15333) of mosquito larvae were from permanent and 22% (n=4318) were from temporary breeding sites. This study showed that Peshawar valley harbours 15 species from the genera *Aedes, Anopheles, Armigeres, Culex* and *Culiseta.* When the density of each species was examined, *Culex quinquifasciatus* was found to be dominant (79%) and constant in distribution. Among the temporary habitats *Aedes albopictus* was found as the most prevalent species particularly from tree holes and water cisterns. The highest intensity of mosquitoes was in June (2243 emerged adults) and November (2667 emerged adults) while the lowest was in January (203 emerged adults). A perfect positive correlation (r = +0.8) was found between temperature and population of mosquitoes (df 10 and α 0.05). The species diversity index for mosquitoes remained between 0.12 and 1.76. The Margalef's richness components was noticeably low for bamboo traps (0.2) and fairly high for rice fields, Percolating water and Animal tracks (1.3) which shows the abundance of mosquito species in these habitats. Similarly Pielou's Evenness was highest for bamboo traps (E=1) showing species uniform distribution. Animal tracks were presumed not only the diverse habitat rather also possessed high value for species richness and species evenness. Temperature, rainfall, humidity and other related attributes responsible for species variation and abundance need to be analysed further to pave way for controlling vector species in their oviposition targeted sites.

Keywords: *Aedes*, *Anopheles*, *Armigeres*, *Culex*, habitat, mosquito larvae, Peshawar.

Resumo

Para determinar a composição de espécies, abundância relativa e variação sazonal de diferentes gêneros de mosquitos (*Aedes*, *Anopheles*, *Armigeres*, *Culex* e *Culiseta*) em diferentes habitats, o presente trabalho foi realizado no Laboratório de Pesquisa em Entomologia da Universidade de Peshawar. A amostragem coletada a partir de uma variedade de habitats de reprodução permanentes e temporários foi realizada mensalmente a partir de locais de reprodução alvo por 2 anos consecutivos através do método de imersão. A diversidade de espécies nos locais de pesquisa foi anotada. A coleta desses 17 tipos diferentes de habitats larvais potenciais rendeu um total de 42.430 imaturos, constituindo 41.556 larvas e 874 pupas. Destes, emergiram apenas 19.651 mosquitos adultos, sendo 11.512 fêmeas e 8.139 machos. 78% (n = 15333) das larvas do mosquito eram de criadouros permanentes e 22% (n = 4318) de criadouros temporários. Este estudo mostrou que o vale de Peshawar abriga 15 espécies dos gêneros *Aedes*, *Anopheles*, *Armigeres*, *Culex* e *Culiseta.* Quando a densidade de cada espécie foi examinada, *Culex quinquifasciatus* foi considerado dominante (79%) e constante na distribuição. Entre os habitats temporários, o *Aedes albopictus* foi encontrado como a espécie mais prevalente, principalmente em ocos de árvores e cisternas de água. A maior intensidade de mosquitos foi em junho (2.243 adultos emergidos) e novembro (2.667 adultos emergidos), enquanto a menor foi em janeiro (203 adultos emergidos). Uma correlação positiva perfeita (r = +0,8)

*e-mail: lubnawadood@yahoo.com
Received: January 27, 2023 – Accepted: March 15, 2023

foi encontrada entre temperatura e população de mosquitos (DF 10 e α 0,05). O índice de diversidade de espécies de mosquitos permaneceu entre 0,12 e 1,76. Os componentes de riqueza de Margalef foram visivelmente baixos para armadilhas de bambu (0,2) e razoavelmente altos para campos de arroz, água percolada e rastros de animais (1,3), o que mostra a abundância de espécies de mosquitos nesses habitats. Da mesma forma, a uniformidade de Pielou foi maior para armadilhas de bambu (E = 1), mostrando distribuição uniforme das espécies. As pegadas de animais foram presumidas não apenas como habitat diverso, mas também possuíam alto valor para riqueza e uniformidade de espécies. Temperatura, chuva, umidade e outros atributos relacionados responsáveis pela variação e abundância das espécies precisam ser analisados mais a fundo para abrir caminho para o controle de espécies de vetores em seus locais de oviposição alvo.

Palavras-chave: *Aedes*, *Anopheles*, *Armigeres*, *Culex*, habitat, larvas de mosquito, Peshawar.

1. Introduction

Mosquitoes, the deadliest insects on earth are vector agents to bring forth mosquito-borne diseases like Malaria, Dengue, Elephantiasis, Yellow and West Nile fever and many more, critically disturbing human being and animal's health. Many countries round the world are affected yearly by the mosquito borne diseases bringing about excessive economic deficits globally; certainly half of the earth's population is affected by it (WHO, 2004). These bloodthirsty creatures cause serious harm to livestock, domestic animals and even pets (Service, 1993).

In Pakistan, the most common mosquito borne diseases include dengue (Qamash et al., 2021; Shabbir et al., 2020; Gul et al., 2019), filariasis (Beg et al., 2001; Ilahi and Suleman, 2013; Hussain et al., 1981; Khan and Pervez, 1981), Malaria (Karim et al., 2021; Qureshi et al., 2019; WHO, 2017, 2018; Khatoon et al., 2010), West Nile virus (Zohaib et al., 2014; Khan et al., 2018), Chikungunya Fever (WHO, 2017, 2019; Afzal et al., 2015; Mallhi et al., 2017; Rauf et al., 2017; Meraj et al., 2020). Japanese encephalitis was documented in 1980s and 1990s, Pakistan remains at risk for JE due to presence of mosquito vector, amplifying hosts, rice irrigation and poorly developed diagnostic infrastructure. It occurs in less than 1% of JE virus infections often with catastrophic sequelae including death and neuropsychiatric disability (Fatima et al., 2020).

There are about 3,500 described species of mosquitoes in the world (Sathe and Tingare, 2010). Indo-Pakistan mosquitoes were primarily studied by Christophers (1933) v. IV and Barraud (1934) v. V available in "the fauna of British India". Ramachandra Rao (1981), Sathe and Girhe (2001, 2002), Jagtap et al. (2008, 2009) and Sathe et al. (2010) etc. also worked on biodiversity and ecology of mosquitoes.

A comprehensive checklist of mosquitoes of Pakistan by Aslamkhan (1971) reported 134 mosquitoes from both east and West Pakistan, listing 23 *Anopheles* species and 63 *Culicines* with *Aedes* signified by 32 species in 10 subgenera, three species of *Culiseta*, two *Mansonia* species and one species each of *Tripteroides, Uranotaenia, Coquilletidia, Ficalbia* and *Armigere.*

24 species of the genus *Anopheles* have been reported in Pakistan (Alemu et al., 2011) of which ten species usually recovered in KP dwell in different habitats display seasonal fluctuation influenced by ecological factors. Eight *Aedes* species, nine *Culex*, two *Culiseta* and one each of *Armigeres* and *Mansonia* were recovered from Peshawar valley (Suleman et al., 1993).

Mosquito's inhabit various sorts of environments for breeding. Some breed in standing water others in permanent breeding sites especially natural sites i.e. lakes, pools, ponds, ditches. Some mosquito species prefer artificial containers whereas other species prefer to breed in temporary places and some species breed preferably in water rich in Ammonia content (Reisen et al., 1977) like *Anopheles stephensi*, a chief vector of urban malaria (Ramachandra Rao, 1989) while others are adapted to clean and clear water like *Aedes* mosquitoes. Entomological studies prove that *Aedes albopictus,* a rural vector (Hawley, 1988) was responsible for dengue transmission (Gubler and Kuno, 1997) in some countries of S.E Asia (Smith, 1956; Hammon, 1966; Rudnick, 1967; Stephenson et al., 2003; Gratz, 2004) and thought to be a possible vector of encephalitis (Beaman and Turell, 1991), Rift Valley Fever (Turell et al., 1988) and Chikungunya virus (Turell et al., 1992).

The population expansion and breeding pattern of medically significant mosquitoes are influenced by physico-chemical features of their habitats (Amini et al., 2020) as oviposition is regarded as one of the most important component of mosquito-borne infections (Bentley and Day, 1989). These factors not only influence mosquito's oviposition selection sites, but correspondingly effect larval mass and species composition (Hanafi-Bojd et al., 2012; Nikookar et al., 2017).

The current study aimed to update existing status of mosquitoes and deduce the seasonal abundance and habitat preference of mosquitoes. In the course of study the species constitution, reproductive biology and population dynamics determined will help in proposing suitable control measures against mosquito borne diseases.

2. Material and Methods

2.1. Study area

The study of mosquito population dynamics was conducted in different areas of Peshawar valley including 14 localities (City, Hayatabad, Islamia College Peshawar, Nasir bagh, Ring road, Reggi, Shoba Bazar, Sherabad, Saddar, Shahi Bala, Shahi Payan, Tehkal, Univ Town, Warsak) representing urban (eight) and rural areas (six) throughout Peshawar district. The distance between the stations varied from 7 km to 15 or 20 Km (Figure 1, Map).

2.2. Survey of mosquito larvae

Seasonal larval collection was made from January to December for two consecutive years (2016-17) at twenty

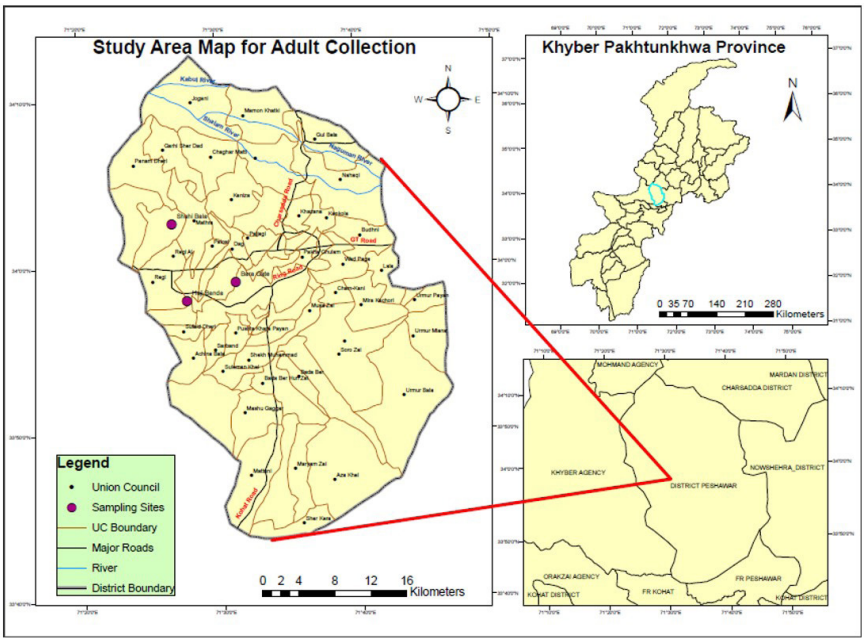

Figure 1. Study Area Map for Mosquito Adult Sampling.

different temporary and permanent breeding sites such as Cemented Construction Ponds, Ditches, Drainage Channels, Irrigation Channel, Percolating water, Polluted water bodies, Seepage Tank, Animal tracks, Bamboo traps, Boggy Ponds, Flooded Swamps, Rain pools, Rice fields, Tires, Tree holes, Water cisterns and Water fountains.

Collection of immature mosquito was undergone monthly between 9:00 to 12:00 hrs on each sampling day, using **dipping method**. Ten dips (200 ml) were reserved per capture station depending on the density of larvae.

2.3. Rearing/preservation of mosquitoes

Samples in plastic cans were transferred to the laboratory where larvae (Early 1st/2nd and late 3rd/ 4th) and pupae were counted and maintained. All the immature were reared on bakers' yeast until adult emergence. Emerging adults were collected with manual aspirator and killed by chloroform fumes in a cotton swab thereafter, preserved in silica tubes.

2.4. Laboratory processing of mosquitoes

Collected specimens were segregated gender wise and the key morphological distinctions within species were noticed and documented. Accurate taxonomic identification under stereo zoom microscope was made by means of standard taxonomic keys available in "Fauna of British India" by Christophers (1933) and Barraud (1934); Knight and Stone (1977).

2.5. Meteorological data and habitat characteristics

Weather conditions like Environmental temperature, Relative humidity, heat index and altitude were noted at each sampling station using Kestrel pocket weather tracker Model 4500 NV. All the relevant information regarding the physicochemical parameters i.e., colour, odour, temperature, pH, turbidity, presence or absence of plants, predators and sunlight penetration of the targeted water reservoir were determined. Some other characteristics of larval habitat like area, sampling site, type of reservoir, vegetation cover, number of samples for each locality along with the number of larvae and pupae in each sample were also recorded.

2.6. Data analysis

Periodic alteration of mosquito species in terms of relative abundance and distribution was calculated by their respective Formulae 1 and 2 (Rydzanicz and Lonc, 2003; Sengil et al., 2011).

$$D = I / L \, x \, 100\% \quad (1)$$

$$C = n \, b / N \, x \, 100\% \quad (2)$$

The dominance pattern and diversity of mosquito species in different localities were calculated by Shannon-Wiener diversity Index (H'), Simpson dominance Index (D), Pielou's Evenness Index (E), Margalefs Richness Index (Me) and Berger Parker dominance Index (d).

Shannon Index $H' = \Sigma$ **pi In pi**

Pielou's Evenness Index $(E) = H'/ Hmax = H'/ \ln S$
Margalefs Richness Index $(Me) = S - 1 / \ln N$
Simpson Dominance Index $(D) = \Sigma \, n(n-1) / N(N-1)$
Berger Parker dominance Index $(d) = N \, max / N$
Jaccard's Coefficient (Cj) compute similarity among the sites and localities (Magurran, 2004; Aslan and Karaca, 2012). **Jaccard's Similarity Index** $(Cj) = j / (a + b - j)$
Sorenson's similarity Coefficient (CC) $CC = 2C / S1 + S2$

3. Results

3.1. Sampling of immature

Sampling performed from variety of permanent and temporary breeding habitats yielded a total of **42,430** immature constituting **41,556** Larvae and **874** pupae. Among these **19,651** adult mosquitoes emerged comprising **11,512** female and **8,139** male mosquitoes.

3.2. Mosquito species composition

Taxonomic account of adult mosquito revealed the presence of fifteen species of mosquitoes belonging to five genera viz. *Aedes*, *Anopheles*, *Armigeres*, *Culex* and *Culiseta*. Genus *Aedes* was represented by three species *Aedes aegypti*, *Aedes albopictus* and *Aedes unilineatus*. Genus *Culex* (*Culex bitaeniorhyncus*, *Culex quinquifasciatus*, *Culex theleri*, *Culex tritaeniorhyncus*, *Culex vishnui*) and Genus *Anopheles* (*Anopheles annularis*, *Anopheles culicifacies*, *Anopheles fluviatilus*, *Anopheles pulcherrimus* and *Anopheles stephensi*) comprised of five species each, while Genus *Armigeres* (*Armigeres subalbatus*) and Genus *Culiseta* (*Culiseta longiareolata*) included a sole species each (Table 1).

3.3. Mosquito's seasonal dynamics as per relative abundance and distribution status

Seasonal dynamics of mosquito immature sampled from various temporary and permanent habitats were reared to adults and were then analysed by density and distribution formulae.

As far as the criteria of relative abundance of the recovered species was concerned, *Culex quinquifasciatus* (78.5%), *Culex tritaeniorhyncus* (6.8%) and *Aedes albopictus* (5.71%) belongs to the **dominant** class; three species *Anopheles stephensi* (4%), *Anopheles culicifacies* (1.77%) and *Culex vishnui* (1%) were included in the **sub-dominant** class and the rest of the nine were among the **satellite species** adopted after Trojan (1992) (Table 2).

Table 1. Mosquito species gathered from various temporary and permanent habitats of Peshawar KP (January, 2016- December, 2017).

Genus	*Aedes*	*Anopheles*	*Armigeres*	*Culex*	*Culiseta*
Species	*Aedes aegypti*	*Anopheles annularis*	*Armigeres subalbatus*	*Culex bitaeniorhyncus*	*Culiseta longiareolata*
	Aedes albopictus	*Anopheles culicifacies*		*Culex quinquifasciatus*	
	Aedes unilineatus	*Anopheles fluviatilus*		*Culex theleri*	
		Anopheles pulcherrimus		*Culex tritaeniorhyncus*	
		Anopheles stephensi		*Culex vishnui*	

Table 2. Relative abundance and distribution of mosquito species collected from different localities of Peshawar district during Jan, 2016 to Dec, 2017.

Species	Total (%)	Relative abundance	Relative abundance status	Distribution C=nb/Nx100%	Distribution status
Aedes aegypti	39	0.20%	Satellite	1/20= 5%	Sporadic
Aedes albopictus	1123	5.71%	Dominant	5/20= 25%	Infrequent
Aedes unilineatus	1	0.005%	Satellite	1/20= 5%	Sporadic
Anopheles annularis	42	0.21%	Satellite	7/20= 35%	Infrequent
Anopheles culicifacies	347	1.77%	Subdominant	12/20= 60%	Moderate
Anopheles fluviatilus	123	0.62%	Satellite	9/20= 45%	Moderate
Anopheles pulcherrimus	4	0.02%	Satellite	2/20= 10%	Sporadic
Anopheles stephensi	784	4%	Subdominant	14/20= 70%	Frequent
Armigeres subalbatus	7	0.04%	Satellite	1/20= 5%	Sporadic
Culex bitaeniorhyncus	128	0.65%	Satellite	9/20= 45%	Moderate
Culex quinquifasciatus	15,429	78.5%	Dominant	18/20= 90%	Constant
Culex theleri	21	0.11%	Satellite	3/20= 15%	Sporadic
Culex tritaeniorhyncus	1336	6.8%	Dominant	13/20= 65%	Frequent
Culex vishnui	193	1%	Subdominant	9/20= 45%	Moderate
Culiseta longiareolata	74	0.38%	Satellite	2/20= 10%	Sporadic
TOTAL	**19,651**	**100%**			

As per distribution criteria, *Culex quinquifasciatus* (90%) was found to be **constant** (80.1- 100%); that is occurring in most of the habitats, two species *Anopheles stephensi* (70%) and *Culex tritaeniorhyncus* (65%) were **frequent** (60.1- 80%) while four mosquito species *Anopheles culicifacies* (60%), *Anopheles fluviatilus* (45%), *Culex bitaeniorhyncus* (45%) and *Culex vishnui* (45%) were **moderate** (40.1- 60%) in distribution. Among the rest of species, *Aedes albopictus* and *Anopheles annularis* were **infrequent** (20.1-40%) while the remaining six were regarded as **sporadic** (0-20%) in distribution (Table 2).

3.4. Monthly distribution of mosquito species

A total of 19,651 mosquitoes recovered from immature collected during the field portray a tri-modal distribution with considerable reduction in winter and gradual increased in spring season leading to highest densities in April and climax was attained in November (14%) probably due to the limiting effects of high temperature in summers and cold temperature in winter. Some mosquito species preferably appears in dusk and dawn like *Aedes* mosquitoes that over crowds the abundance of mosquitoes. From March to November species richness and diversity among mosquitoes were also noticeable possessing more than nine species monthly (Table 3).

Occurrence of mosquitoes belonging to various species in different months is shown in Table 4. *Culex quinquifasciatus,* the dominant and constant species was collected throughout the study period therefore, regarded as a cold tolerant species. *Ae. albopictus, An. stephensi and Cx. tritaeniorhyncus* were also gathered in all the studied months except the coldest months of January and February. The next abundant species was *An. culicifacies* (347 individuals) reported from March to November followed by *Cx. vishnui* (193), *Cx. bitaeniorhyncus* (128) and *An. fluviatilus* (123) that all were reported co-occuring in same months but there was a significant difference in their numbers, the former being more abundant than the later ones.

Among the genus *Aedes*, a sole male specimen of *Aedes unilineatus* was found in November whereas, *Aedes aegypti* comprise 39 adult mosquitoes caught in the month of June till November from artificial containers. *Culiseta longiareolata* comprising 74 individuals was captured in February, March, August, September and November from waste water bodies only.

Anopheles pulcherrimus reported in June and July and *Armigeres subalbatus* recovered in April, May and June were found the least dominant species.

Table 3. Mosquito species recovered throughout the survey months from district Peshawar during the survey period 2016-2017.

Months	Relative Abundance of Mosquitoes (%)	No. of Species
January	203	1
February	641	2
March	2,046	9
April	2,227	10
May	1,690	10
June	2,243	12
July	2,196	12
August	2,170	11
September	1,442	10
October	1,537	10
November	2,667	13
December	589	4
TOTAL	**19,651**	**15**

3.5. Habitat specificity of different mosquito species

Twenty aquatic habitats including both temporary and permanent water bodies were assessed for the distribution of mosquitoes. The present results demonstrated a total of fifteen different mosquito larva species in these water sources in the study area. Mosquito larvae species harvested belonging to five genera were potential vectors of five human disease. Genera *Armigeres* and *Culiseta* were reported solely from Permanent Habitats while Genus *Aedes* from Temporary habitats only. Genus *Anopheles* and Genus *Culex* was found to co-occur equally in both temporary and permanent habitats.

In the current survey the highest collection (78%) was discovered in permanent habitats while 22% collection was from temporary habitats. Regarding species richness, the maximum numbers of species (9 species) were encountered from both sorts of habitats so the species richness was comparable in both types of habitats.

3.6. Habitat preference of mosquitoes

Among all studied habitats, drainage channel, percolating water body and animal tracks (9 species) were recorded to be the most preferred breeding habitats of mosquito species during this survey. Former two were permanent water bodies while later represent a temporary water habitat. Species number was low in container habitats like flooded swamps, Rain pools, water cisterns, water fountains and Bamboo glasses comprising of three or two mosquito species.

With regard to relative abundance, polluted water reservoirs contributed 40.4% to the total number of mosquitoes emerged followed by road side ditches (13%), Construction ponds (11.2%), drainage channels (6%) and tree holes (5.2%). Irrigation Canal and water cisterns were comparable constituting 3.6% of the total mosquitoes but the former possess high species richness enclosing eight species while the later resides only three mosquito species. Rest of the species were accounting less than 3% to the total mosquitoes but again a Percolating water body that was contributing very little to the totals included 9 species so was the richest water reservoir (Table 5).

Species confine to one sort of habitat included the *Aedes* species, that were caught absolutely (100%) from temporary habitat. Current finding reported these species in Bamboo sections, tires, tree holes, water cisterns and

Table 4. Distribution of mosquitoes immature in respective months collected from different areas of Peshawar during 2016-2017 at Peshawar District.

Species/Months	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**TOTAL**	
Aedes aegypti	0	0	0	2	0	5	6	12	4	6	4	0	28-11	39
Aedes albopictus	0	0	87	92	54	43	52	50	86	285	333	41	728-395	1123
Aedes unilineatus	0	0	0	0	0	0	0	0	0	0	1	0	0-1	1
Anopheles annularis	0	0	0	0	8	11	11	9	0	1	2	0	28-14	42
Anopheles culicifacies	0	0	30	43	17	108	19	24	23	10	73	0	192-155	347
Anopheles fluviatilus	0	0	13	19	7	4	5	29	16	24	6	0	85-38	123
Anopheles pulcherrimus	0	0	0	0	0	1	3	0	0	0	0	0	3-1	4
Anopheles stephensi	0	0	81	119	98	129	91	90	60	93	19	4	501-283	784
Armigeres subalbatus	0	0	0	2	2	3	0	0	0	0	0	0	7-0	7
Culex bitaeniorhyncus	0	0	22	8	2	0	64	21	3	6	2	0	64-64	128
Culex quinquifasciatus	203	631	1738	1839	1248	1714	1605	1606	1108	1078	2149	510	8949-6480	15429
Culex theleri	0	0	0	0	0	14	5	0	0	0	2	0	9-12	21
Culex tritaeniorhyncus	0	0	11	89	218	192	315	295	120	32	30	34	774-562	1336
Culex vishnui	0	0	38	14	36	19	20	33	19	2	12	0	109-84	193
Culiseta longiareolata	0	10	26	0	0	0	0	1	3	0	34	0	35-39	74
TOTAL	136-67	393-246	1051-995	1311-916	980-710	1216-1027	1293-903	1266-904	871-571	962-575	1642-1025	391-198	11512-8139	
	203	631	2046	2227	1690	2243	2196	2170	1442	1537	2667	589		19651

Table 5. Percentage of mosquitoes collected from Permanent and Temporary habitats along with relative abundance surveyed during 2016-17 at Peshawar district.

Genera	Mosquito Species	Total mosquitoes captured	Permanent Habitat contribution (%)	Temporary Habitat contribution (%)
Aedes	*Aedes aegypti*	39	0	39 (100%)
	Aedes albopictus	1123	0	1123 (100%)
	Aedes unilineatus	1	0	1 (100%)
Anopheles	*Anopheles annularis*	42	24 (57%)	18 (43%)
	Anopheles culicifacies	347	203 (59%)	144 (41%)
	Anopheles fluviatilus	123	106 (86%)	17 (14%)
	Anopheles pulcherrimus	4	1 (25%)	3 (75%)
	Anopheles stephensi	784	292 (37%)	492 (63%)
Armigeres	*Armigeres subalbatus*	7	7 (100%)	0
Culex	*Culex bitaeniorhyncus*	128	78 (61%)	50 (39%)
	Culex quinquifasciatus	15,429	13509 (88%)	1920 (12%)
	Culex theleri	21	19 (90%)	2 (10%)
	Culex tritaeniorhyncus	1336	941 (70%)	395 (30%)
	Culex vishnui	193	79 (41%)	114 (59%)
Culiseta	*Culiseta longiareolata*	74	74 (100%)	0

water fountains that were all clear water bodies (Table 6). Similarly, *Armigeres subalbatus* and *Culiseta longiareolata* were solely reported from permanent breeding grounds. Rest of the species found breeding in both of the habitats appeared more or less in one or the other sort of habitat. *Anopheles pulcherrimus, Anopheles stephensi* and *Culex vishnui* were the only species that were richly found in temporary water bodies; here also a malarial vector has been reported abundantly in temporary habitat. *Culex* species were found richly occupying permanent habitats because of the wide surface areas and the accumulation of water throughout irrespective of rainy spell. *Culex quinquifasciatus* was the most abundant species suitably occurring in polluted water though recorded from all surveyed habitats except from rain pools and water fountains which were found to be the ideal sites for *Anopheles* mosquitoes (Table 6).

3.7. Breeding habitats for mosquito species and their co-occurrence

As far as *Aedes* species are concerned, bamboo traps, tires, tree holes, water fountains and water cisterns were found to be the acceptable breeding habitats and all these reservoirs of *Aedes* are temporary water bodies. *Aedes albopictus* inhabited all of these sites whereas; *Aedes aegypti* and *Aedes unilineatus* were reported from single habitats. *Aedes aegypti* and *Aedes albopictus* were found to be co-breeding in tires, however, *Aedes albopictus* and *Aedes unilineatus* were found to be co-breeding in tree holes.

Concerning *Anophelines*, Seepage tank was found as the preferred habitat bearing all five *Anopheline* followed by Drainage Channel, irrigation canal, Percolating water, Animal track and Rice fields (enclosing four species each), Boggy pond (three species each) and the rest with two or one species, with flooded swamps and tires devoid of *Anophelines*. In contrast to *Anophelines, Culicines* cover wide range of acceptable habitats, reported in all surveyed habitats, the most favourable habitat with all the five species include Drainage Channel, Percolating water and Animal track followed by Ditches and Irrigation Canal (four species each), Construction Pond, Polluted water and flooded swamps (three species each) others habitats bears few species with the only water fountains devoid of any species. The most frequent, dominating and widely distributed species *Culex quinquifasciatus* was found positive in fifteen out of the total seventeen surveyed habitats.

Armigeres subalbatus of Genus *Armigeres* was represented by only specimen caught from Ring road Peshawar area ditches which was the richest habitat in terms of enclosing four different Genera, and this species co breeds with two *Anopheline* and four *Culicine* species along with a very rare specimen of *Culiseta longiareolata* that was also captured in trivial number from the same habitat.

3.8. Species diversity, richness and evenness of mosquitoes in different habitats.

In terms of species diversity of different habitats the Animal tracks assessed at Regi area possesses diversified mosquito fauna with comparatively larger index value (H^{-}=1.76) followed by rice fields (H^{-}=1.63), Percolating water (H^{-}=1.61) and Drainage channel (H^{-}=1.1) (Table 7).

Margalef's Richness of mosquito fauna was found maximum for rice fields, Percolating water and Animal tracks (1.3), which showed the abundance of mosquito species in these habitats was fairly high (Table 7).

Table 6. Habitat wise distribution and abundance of emerged adult mosquitoes in all targeted habitats during the study period 2016-2017 from district Peshawar .

	Habitats	*Aedes aegypti*	*Aedes albopictus*	*Aedes unilineatus*	*Anopheles annularis*	*Anopheles culicifacies*	*Anopheles fluviatilus*	*Anopheles pulcherrimus*	*Anopheles stephensi*	*Armigeres subalbatus*	*Culex bitaeniorhyncus*	*Culex quinquifasciatus*	*Culex theleri*	*Culex tritaeniorhyncus*	*Culex vishnui*	*Culiseta longiareolata*	TOTAL
Permanent	Construction Pond					32			146		24	1868		54		73	2197
	Ditches				5				28	7	17	2020		486	16	1	2580
	Drainage Channel				2	128	71		9		15	787	5	107	24		1148
	Irrigation Canal				9	3	3		17		12	591		71	7		713
	Percolating water				7	36	30		69		10	261	14	99	10		536
	Polluted water								5			7782		124	22		7933
	Seepage Tank				1	4	2	1	18			200					226
	TOTAL	0	0	0	24	203	106	1	292	7	78	13509	19	941	79	74	15333
Temporary	Animal tracks				3	69		3	169		32	79	2	137	81		575
	Bamboo traps		135									144					279
	Boggy Ponds					16	6		85			541					648
	Flooded Swamps										18	239		221			478
	Rain pools					23			91						28		142
	Rice fields				15	5	7		28			3		30	5		93
	Tires	39	14									140		7			200
	Tree holes		545	1			3					478					1027
	Water cisterns		414				1					296					711
	Water fountains		15			31			119								165
	TOTAL	39	1123	1	18	144	17	3	492		50	1920	2	395	114		4318

Table 7. Comparison of Shannon Wiener diversity Index (H^-), Margalef's Richness Index and Pielou's Evenness Index in all surveyed habitats.

	Shannon-Wiener diversity Index (H^-)	Margalef's Richness Index (Me)	Pielou's Evenness Index (E)
Construction Pond	0.62	0.7	0.4
Ditches	0.66	0.9	0.3
Drainage Channel	1.1	1.1	0.5
Irrigation Canal	0.45	1.1	0.2
Percolating water	1.61	1.3 *	0.7
Polluted water	0.12	0.3	0.1
Seepage Tank	0.47	0.9	0.3
Animal tracks	1.76 *	1.3 *	0.8
Bamboo traps	0.69	0.2	1 *
Boggy Ponds	0.55	0. 5	0.4
Flooded Swamps	0.84	0.3	0.8
Rain pools	0.89	0.4	0.8
Rice fields	1.63	1.3 *	0.8
Tires	0.88	0.6	0.6
Tree holes	0.72	0.4	0.5
Water cisterns	0.69	0.3	0.6
Water fountains	0.78	0.4	0.7

*Shows maximum values for all the three indices.

Similarly, species evenness (Pielou's Evenness) was highest for bamboo traps (1), which indicated that species were uniformly distributed in this habitat followed by Animal tracks, flooded swamps, rain pools and rice fields (0.8) (Table 7).

3.9. Simpson's diversity index

In parallel with species number, Simpson's diversity index values were higher for Animal tracks (1/D=5) regarded as the richest habitat whereas, for polluted water (1/D=1) the Simpson's diversity was least. Percolating water and drainage channel also reside maximum number of species but the former possessed a higher diversity index as compare to later on the account of different number of mosquitoes collected from the two habitats (Table 8).

3.10. Jaccard's (CJ) similarity index for habitats

The Jaccard's similarity analysis based on species composition in their respective habitats revealed top highest relationship of 100% in **Drainage Channel ≈ Percolating Water** reflecting all the reported species in both the habitats followed by chief similarity of 90% between **Drainage Channel ≈ Irrigation Canal**, **Irrigation Canal ≈ Percolating Water**, **Irrigation Canal ≈ Rice Field** (Table 9).

3.11. Efficiency indicator/affinity index

Fager and McGowan (1963) test was used to compute the indices of affinity between sets of Culicidae species in inspected breeding habitat of the study area using Formula 3:

Table 8. Number of Species, the adults and Simpson's Diversity Index (D) in different surveyed habitats of Peshawar during 2016-2017.

	S	N	Simpson's Diversity Index D (1/D)
Construction Pond	6	2197	0.7 (**1.4**)
Ditches	8	2580	0.7 (**1.5**)
Drainage Channel	9	1148	0.5 (**2**)
Irrigation Canal	8	713	0.7 (**1.4**)
Percolating water	9	536	0.3 (**3.3**)
Polluted water	4	7933	1 (**1**)
Seepage Tank	6	226	0.8 (**1.3**)
Animal tracks	9	575	0.2 (**5**) *
Bamboo traps	2	279	0.5 (**2**)
Boggy Ponds	4	648	0.7 (**1.4**)
Flooded Swamps	3	478	0.5 (**2.2**)
Rain pools	3	142	0.5 (**2.1**)
Rice fields	7	93	0.2 (**4.5**)
Tires	4	200	0.5 (**1.9**)
Tree holes	4	1027	0.5 (**2**)
Water cisterns	3	711	0.5 (**2**)
Water fountains	3	165	0.6 (**1.8**)

*Shows maximum values for Simpson's diversity Index.

$$\mathbf{I} = \left[\mathbf{J} / \left(\mathbf{Na} + \mathbf{Nb}\right)^{1/2}\right] - \left[\mathbf{1} / \mathbf{2}\left(\mathbf{Nb}\right)^{1/2}\right] \quad (3)$$

The highest affinity of 2.0 was found among *Anopheles stephensi* and *Anopheles culicifacies* followed by a value of 1.8 which occurs in *Culex quinquifasciatus* with *Anopheles stephensi* as well as with *Culex tritaeniorhyncus* (Table 10).

The italic values * of affinity between the pairs of species indicates the highest values between the studied pairs of species respectively.

4. Discussion

Mosquitoes as disease vector and nuisance pest are of remarkable significance but their occurrence in strangely huge numbers during some parts of the year also awards them special status. Vector borne diseases together with dengue have shown a magnificent expansion (Jones et al., 2008) and pose tremendous commercial and public health complications. To consider the availability of diverse breeding grounds for ovipositing mosquitoes in different areas is of great essence before implementing anti-mosquito processing.

The only available information on mosquitoes of KP was presented by Suleman et al. (1993). Here, it may be pointed out that the species composition, relative abundance and seasonal prevalence of mosquitoes may change from year to year at the same site, reflecting great complexities of mosquito ecology (Suleman et al., 1993). So, it requires long term studies to figure out seasonal patterns in species composition and relative abundance of mosquitoes in various ecological zones.

The present study was aimed to identify mosquitoes, characterize their larval grounds and to know their seasonal dynamics in different areas of Peshawar city. This survey revealed that human interventions offer various ideal breeding grounds for mosquitoes to breed that thereby aids in increasing mosquito populations.

This survey has the peculiarity of being the extensive entomological study in the area that reported the existence of both dengue vectors i.e., *Ae. aegypti* and *Ae. albopictus*, primary Malarial vectors, i.e., *An. stephensi* and *An. culicifacies* and filariasis and West Nile virus vector, i.e., *Cx. quinquifasciatus* in Peshawar. Recently dengue vectors species were recovered from thirteen towns of Sindh, Punjab and KP including Peshawar (Rasheed et al., 2013) using larval catch.

Current finding revealed that Culicine were more abundant than Anophelines, this difference may be attributed to habitat variability in the localities. So, *Culex* was considered as the common, the largest important

Table 9. Jaccard's coefficient for different targeted habitats of Peshawar during 2016-2017.

No. of species →	Construction Pond	Ditches	Drainage Channel	Irrigation Canal	Percolating water	Polluted water	Seepage Tank	Animal tracks	Bamboo traps	Boggy Ponds	Flooded Swamps	Rain pools	Rice fields	Tires	Tree holes	Water cisterns	Water fountains
	6	8	9	8	9	4	6	9	2	4	3	3	7	4	4	3	3
Construction Pond																	
Ditches	0.6																
Drainage Channel	0.5	0.7															
Irrigation Canal	0.6	0.6	*0.9**														
Percolating water	0.5	0.5	**1**	*0.9**													
Polluted water	0.4	0.5	0.4	0.4	0.4												
Seepage Tank	0.2	0.3	0.5	0.6	0.5	0.3											
Animal tracks	0.5	0.5	0.8	0.7	0.8	0.4	0.5										
Bamboo traps	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1									
Boggy Ponds	0.4	0.2	0.4	0.5	0.4	0.3	0.7	0.3	0.2								
Flooded Swamps	0.5	0.4	0.3	0.4	0.3	0.4	0.1	0.3	0.3	0.2							
Rain pools	0.3	0.2	0.3	0.4	0.3	0.4	0.3	0.3	0	0.4	0						
Rice fields	0.4	0.5	0.8	*0.9**	0.8	0.6	0.6	0.6	0.1	0.6	0.3	0.4					
Tires	0.3	0.2	0.2	0.2	0.2	0.3	0.1	0.2	0.5	0.1	0.4	0	0.2				
Tree holes	0.1	0.1	0.2	0.2	0.2	0.1	0.3	0.1	0.5	0.3	0.2	0	0.2	0.3			
Water cisterns	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.1	0.7	0.4	0.2	0	0.3	0.4	0.8		
Water fountains	0.3	0.1	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.4	0	0.5	0.3	0.2	0.2	0.2	

→The bold italic value indicates the highest similarity between mosquito species inhabiting study habitats based on Jaccard's Index. →The italic values * of similarity between the mosquito species indicates the highest values of mosquito species inhabiting study habitats based on Jaccard's Index.

Table 10. Efficiency Index values among pair of species revealing strength of affinity.

Efficiency Indices value	Affinity among species
2.0	• *Anopheles stephensi + Anopheles culicifacies*
*1.8**	• *Culex quinquifasciatus + Anopheles stephensi*
	• *Culex quinquifasciatus + Culex tritaeniorhyncus*
*1.7**	• *Culex vishnui + Anopheles stephensi*
*1.6**	• *Culex quinquifasciatus + Anopheles fluviatilus*
	• *Culex tritaeniorhyncus + Anopheles stephensi*
*1.5**	• *Anopheles stephensi + Anopheles annularis*
	• *Culex quinquifasciatus + Anopheles culicifacies*
	• *Culex tritaeniorhyncus + Culex bitaeniorhyncus*
	• *Culex tritaeniorhyncus + Culex vishnui*
1.4	• *Culex quinquifasciatus + Anopheles annularis*
	• *Culex quinquifasciatus + Culex bitaeniorhyncus*
	• *Culex quinquifasciatus + Culex vishnui*
1.3	• *Anopheles culicifacies*
	• *Anopheles annularis +Culex bitaeniorhyncus*
	• *Anopheles annularis +Culex vishnui*
	• *Culex bitaeniorhyncus + Anopheles stephensi*
1.2	• *Culex bitaeniorhyncus + Anopheles annularis*
	• *Anopheles fluviatilus + Anopheles culicifacies*
	• *Culex vishnui + Anopheles culicifacies*
	• *Anopheles stephensi + Anopheles fluviatilus*
1.1	• *Anopheles fluviatilus + Anopheles annularis*
	• *Culex tritaeniorhyncus + Anopheles culicifacies*
	• *Culex vishnui + Culex bitaeniorhyncus*
1.0	• *Culex bitaeniorhyncus + Anopheles culicifacies*
0.8	• *Culex tritaeniorhyncus + Anopheles fluviatilus*
	• *Culex vishnui + Anopheles fluviatilus*
0.7	• *Culex quinquifasciatus + Aedes albopictus*
	• *Culex theleri + Culex bitaeniorhyncus*
	• *Culex theleri + Culex vishnui*
0.6	• *Culex bitaeniorhyncus + Anopheles fluviatilus*
	• *Culex theleri + Culex quinquifasciatus*
	• *Culex tritaeniorhyncus + Culex theleri*
0.5	• *Culiseta longiareolata + Culex bitaeniorhyncus*

The bold italic value indicates the highest affinity between the studied species (*Anopheles stephensi* and *Anopheles culicifacies*).

and abundant genus of the tribe Culicini (Service, 1993). Larvae of *Culex pipiens quinquifasciatus* have been reported from variety of natural as well as artificial water reservoirs (Cranston et al., 1987; Harbach 1988; Service 1993) therefore it has been reported as the dominant and abundant species in several areas (Sengil et al., 2011; Ali and Rasheed, 2009; Aditya et al., 2006; Alten and Bosgelmez, 1996; Suleman et al., 1993). This species can with stand huge mass of pollutants and dwells in wide range of habitats like 18/ 20 habitats were found positive for it in the present study. Hamidian (2007) also reported great number of this mosquito species from manmade larval sites in Iran. Suleman et al. (1993) also revealed the same breeding habitats of the species and reported its larvae even in metal drums holding rain water. Similar results regarding breeding pattern of the species were documented by Ali and Rasheed (2009), Ali et al. (2013), Ilahi and Suleman (2013).

Cx. pipiens quinquifasciatus showed a bimodal distribution with a climax in late autumn (November) and a second peak in spring (March) with a declining population in cold winter months. Reisen and Aslamkhan (1978) and Reisen and Milby (1986) reported the same seasonal bimodal pattern for *An. stephensi* and *An. culicifacies* in accordance with the present study.

Temporary habitats i.e. pit, crevices and hoofs have been observed to enclose rich *Anophlelines* (Gillies and Meillon, 1968; Minakawa et al., 1999; Gimnig et al., 2001; Minakawa et al., 2004) that is comparable with the current findings to collect more *Anopheles* particularly *Anopheles stephensi* and *Anopheles pulcherrimus* in the container reservoirs. In temporary habitats, six mosquito species belonging to *Aedes*, *Armigeres*, *Culex* and *Toxorhynchites* were recovered by Aditya et al. (2006) while inspecting ditches, pools, tanks and polluted drains in Darjeeling sharing two mosquito species *Culex pipiens quinquifasciatus* and *Culex bitaeniorhyncus* with the current study. Likewise, Mwangangi et al. (2009) also assessed temporary water bodies i.e. pools, puddles, water tanks and tire tracks in Kenya reported six species (*Aedes*, *Anopheles* and *Culex*) of which only *Culex pipiens quinquifasciatus* was shared with the present study. Mwangangi et al. (2009) further concluded that both Anopheline and Culicine mosquitoes breed in temporary pools as indicated by this study. Among temporary habitats Animal tracks were presumed not only the diverse habitats as revealed by $\bar{H}$=1.76, rather also possessed high value for species richness and species evenness (Table 7).

Devi and Jauhari (2007) explore both permanent and temporary habitats in India, reported thirty four mosquito species belonging to Genera (*Aedes*, *Anopheles, Armigeres*, *Culex* and *Uranotaenia*) and determined that the zones of lower elevations reside rich mosquito fauna in term of species compared to the higher elevations. No such correspondence of species and elevations was observed in the current study. Mosquito (Culicidae) larvae belonging to Genera *Aedes*, *Anopheles*, *Culex, Culiseta* and *Ochlerotatus* were recovered in these habitats by Sengil et al. (2011) in Istanbul, Turkey. Common species shared with the present survey include, *Culex theleri, Culex tritaeniorhynchus* and *Culiseta longiareolata.* They reported *Culex pipiens* the dominant species in temporary as well as permanent habitats and a second dominant *Culex tritaeniorhynchus* that is comparable with our result. Same Genera of mosquito were revealed by Rydzanicz and Lonc (2003) in Poland reporting *Culex pipiens* as most abundant species in accordance with present results.

The vector species, *Culex tritaeniorhynchus* in current study was reported from all permanent habitats except seepage tanks but among temporary reservoirs it was got

positive in hoofs, swamps, rice fields and tires signifying its inclination for small or large clean water habitats as stated by Alten and Bosgelmez (1996).

The assessment of immature mosquitoes in permanent habitat reported a hazardous water body, the percolating water reservoir that encloses nine specie in two Genera (*Anopheles* and *Culex*) residing top vector species.

Current survey also include many natural habitats like irrigation canals, ditches, drainage channels, percolating water bodies, animal tracks, boggy ponds, flooded swamps, rice fields, tree holes that were wide and spacious possessing usually fresh water with dense vegetation at the shores so inter species competition trims down as mosquitoes thrives in their natural habitats. In all these habitats richest collection was observed. Moosa-Kazemi et al. (2009) while assessing natural habitats reported almost the same *Culicines* in Chabahar country, Southeastern Iran. Likewise, Hamidian (2007) while surveying same natural micro-habitats described species corresponding to Genus *Culex* in Guilan Province, Iran.

Artificial habitats are more troublesome as it can be anything that retains water and progress into a breeding area for mosquitoes. Several studies have reported pest species *Aedes albopictus* from such habitats (Bartlett-Healy et al. 2012; Okogun et al. 2005) that is in accordance with the current study while surveying seepage tanks, bamboo sections, tires, water cisterns and fountains.

Bartlett-Healy et al. (2012) assessed about 276 various types of artificial containers in New Jersey during peak mosquito season and reported *Aedes albopictus* as abundant and *Aedes japonicus* as slightly abundant species in urban and rural sites respectively. Richards et al. (2008) reaches to the opinion that such habitats contribute significantly to *Aedes albopictus* in suburban habitats. Okogun et al. (2005) in Nigeria reported seventeen species in the same genera (*Aedes*, *Anopheles*, *Culex*) as collected in the current study.

Anopheline mosquitoes were targeted by Jude et al. (2010) in Sri Lanka reporting the major (*An. culicifascies*) and secondary malarial vectors (*An. subpictus*) in brackish waters. Yadav et al. (1989) depicted sixteen species from canal irrigated, non-canal irrigated zones and riverine region of Kheda District and reported *Anopheles annularis, Anopheles culicifacies, Anopheles fluviatilus, Anopheles pulcherrimus* and *Anopheles stephensi* in accord with our findings. Abdoon and Alshahrani (2003) reported species that do not coincide with current findings.

All Anopheline mosquitoes reported in the current study were also reported by Yadav et al. (1989) in irrigated and non-irrigated zones of Kheda Kheda District and solely and *Anopheles culicifacies* of the current survey was reported by Jude et al. (2010) in brackish waters of Sri Lanka.

Anopheles annularis and *Anopheles culicifacies* were found co-occuring as reported currently in drainage channel, irrigation canal, percolating waters, seepage tank, animal tracks and rice fields (Reisen et al., 1981; Devi and Jauhari, 2007). Likewise, *Anopheles culicifacies* and *Anopheles fluviatilus* were found co-occuring in drainage canal, irrigation canal, percolating waters, seepage tanks, boggy ponds and rice fields in the current study and also reported by Yadav et al. (1989); Devi and Jauhari (2007); Ali and Rasheed (2009); Ilahi (2001) and many more local studies. Jaccards coefficient (Cj) and affinity index was also found maximum for *Anopheles stephensi* and *Anopheles culicifacies*. Fager and McGowan (1963) test revealed that (Table 10) species occurring together in more than 50% of the targeted sites perhaps have the same environmental requirements or needs.

5. Conclusion

The current study has investigated different temporary and permanent larval breeding habitats and seasonal dynamics of mosquito species in different rural and urban areas of Peshawar. Information on the population dynamics of mosquitoes and particularly of vectors is necessary in order to develop an environment friendly control strategy. Environmental factors Temperature, rainfall, humidity and other related climatic attributes affecting the breeding of mosquitoes can help in detecting ovipositional site selection and distribution of vector species thereby provide a way for controlling vectors with great accuracy.

Acknowledgements

This article is a part of the first author's dissertation for fulfilment of a PhD degree in Medical Entomology from Department of Zoology, University of Peshawar.

References

ABDOON, O.M.A. and ALSHAHRANI, M.A., 2003. Prevalence and distribution of Anopheline mosquitoes in malaria endemic area of Asir region, Saudi Arabia. *La Revue de Santo de la Mediterranee Orientale*, vol. 9, no. 3, pp. 240-246.

ADITYA, G., PRAMANIK, M.K. and SAHA, G.K., 2006. Larval habitats and species composition of mosquitoes in Darjeeling Himalayas, India. *Journal of Vector Borne Diseases*, vol. 43, no. 1, pp. 7-15. PMid:16642780.

AFZAL, M.F., NAQVI, S.Q., SULTAN, M.A. and HANIF, A., 2015. Chikungunya fever among children presenting with nonspecific febrile illness during an epidemic of dengue fever in Lahore, Pakistan. *Merit Research Journal of Medicine and Medical Sciences*, vol. 3, no. 3, pp. 69-73.

ALEMU, A., ABEBE, G., TSEGAYE, W. and GOLASSA, L., 2011. Climatic variables and malaria transmission dynamics in Jimma town, south west Ethiopia. *Parasites & Vectors*, vol. 4, no. 1, pp. 30. http://dx.doi.org/10.1186/1756-3305-4-30. PMid:21366906.

ALI, N. and RASHEED, B., 2009. Determination of species composition of mosquitoes found in Palosai stream, Peshawar. *Pakistan Entomologist*, vol. 31, no. 1, pp. 47-51.

ALI, N., MARJAN, A., KHAN, K. and KAUSAR, A., 2013. Study on mosquitoes of Swat Ranizai subdivision of Malakand. *Pakistan Journal of Zoology*, vol. 45, no. 2, pp. 503-510.

ALTEN, B. and BOSGELMEZ, A., 1996. Investigations of the bio-ecology of the *Culex* species (Diptera: Culicidae) in Ortaca-Dalaman Region, Mugla I Doga. *Tropical Zoology*, vol. 20, pp. 27-53.

AMINI, M., HANAFI-BOJD, A.A., AGHAPOUR, A.A. and CHAVSHIN, A.R., 2020. Larval habitats and species diversity of mosquitoes (Diptera: Culicidae) in West Azerbijan Province, Northwestern Iran. *BMC Ecology*, vol. 20, no. 1, pp. 60. http://dx.doi.org/10.1186/s12898-020-00328-0. PMid:33213441.

ASLAMKHAN, M., 1971. The mosquitoes of Pakistan. I. A checklist. *Mosquito Systematics Newsletter*, vol. 3, pp. 147-159.

ASLAN, B. and KARACA, I., 2012. Comparitive diversity of insects in various habitats of Kovada Lake National Park Basin (Isparta, Turkey). *Scientific Research and Essays*, vol. 7, no. 24, pp. 2160-2167.

BARRAUD, P.J., 1934. *The Fauna of British India, Including Ceylon and Burma. Diptera. Family Culicidae. Tribe Megarhinini and Culicinae, 5*. London: Taylor & Francis.

BARTLETT-HEALY, K., UNLU, I., OBENAUER, P., HUGHES, T., HEALY, S., CREPEAU, T., FARAJOLLAHI, A., KESAVARAJU, B., FONSECA, D., SCHOELER, G., GAUGLER, R. and STRICKMAN, D., 2012. Larval Mosquito habitat utilization and community dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: culicidae). *Journal of Medical Entomology*, vol. 49, no. 4, pp. 813-824. http://dx.doi.org/10.1603/ME11031. PMid:22897041.

BEAMAN, J.R. and TURELL, M.J., 1991. Transmission of Venezuelan Equine Encephalomyelitis virus by strains of *Aedes albopictus* (Diptera: Culicidae) collected in North and South America. *Journal of Medical Entomology*, vol. 28, no. 1, pp. 161-164. http://dx.doi.org/10.1093/jmedent/28.1.161. PMid:2033608.

BEG, A., NAQVI, A., ZAMAN, V. and HUSSAIN, R., 2001. Tropical pulmonary eosinophilia and filariasis in Pakistan. *The Southeast Asian Journal of Tropical Medicine and Public Health*, vol. 32, no. 1, pp. 73-75. PMid:11485099.

BENTLEY, M.D. and DAY, J.F., 1989. Chemical ecology and behavioural aspects of mosquito oviposition. *Annual Review of Entomology*, vol. 34, no. 1, pp. 401-421. http://dx.doi.org/10.1146/annurev.en.34.010189.002153. PMid:2564759.

CHRISTOPHERS, S.R., 1933. *The Fauna of British India, Including Ceylon and Burma. Diptera. Family Culicidae. Tribe Anophelines, 4*. London: Taylor & Francis.

CRANSTON, P.S., RAMSDALE, C.D., SNOW, K.R. and WHITE, G.B., 1987. *Adults, Larvae and Pupae of British Mosquitoes (Culicidae): a key*. Cumbria: Freshwater Biological Association, vol. 48.

DEVI, N.P. and JAUHARI, R.K., 2007. Mosquito species associated within some western Himalayas phytogeographic zones in the Garhwal region of India. *Journal of Insect Science*, vol. 7, no. 32, pp. 1-10. http://dx.doi.org/10.1673/031.007.3201. PMid:20233101.

FAGER, E.W. and MCGOWAN, J.A., 1963. Zooplankton species groups in the Northern Pacific. *Science*, vol. 140, no. 3566, pp. 453-460. http://dx.doi.org/10.1126/science.140.3566.453. PMid:17829536.

FATIMA, T., RAIS, A., KHAN, E., HILLS, S.L., CHAMBERS, T.V., HOTWANI, A., QURESHI, S., SHAFQUAT, S., MALIK, S., QAMAR, F., MIR, F., MARFIN, A.A., ZAIDI, A., KHOWAJA, A.R. and SHAKOOR, S., 2020. Investigation of Japanese encephalitis virus as a cause of acute encephalitis in southern Pakistan, April 2015- January 2018. *PLoS One*, vol. 15, no. 6, e0234584. http://dx.doi.org/10.1371/journal.pone.0234584. PMid:32530966.

GILLIES, M.T. and MEILLON, B.D., 1968. *The Anophelinae of Africa South of the Sahara*. 2nd ed. South Africa: South Africa Institute of Medical Research, 54 p.

GIMNIG, E., OMBOK, M., KAMAU, L. and HAWLEY, W., 2001. Characteristics of larval anopheline (Diptera: Culicidae) habitats in western Kenya. *Journal of Medical Entomology*, vol. 38, no. 2, pp. 282-288. http://dx.doi.org/10.1603/0022-2585-38.2.282. PMid:11296836.

GRATZ, N.G., 2004. Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology*, vol. 18, no. 3, pp. 215-227. http://dx.doi.org/10.1111/j.0269-283X.2004.00513.x. PMid:15347388.

GUBLER, D.J. and KUNO, G., 1997. *Dengue and dengue hemorrhagic fever*. New York: CAB International, pp. 1-22.

GUL, R., TABASSUM, I., ULLAH, I. and RAHMAN, F., 2019. Incidence of dengue in the highland District Swat, Pakistan: a major shift in the geographical prevalence of the disease. *Pakistan Academy of Sciences*, vol. 56, no. 2, pp. 27-38.

HAMIDIAN, S.A., 2007. Checklist of Iranian mosquitoes (Diptera: culicidae). *Journal of Vector Ecology*, vol. 32, no. 2, pp. 235-242. http://dx.doi.org/10.3376/1081-1710(2007)32[235:COIMDC]2.0.CO;2. PMid:18260513.

HAMMON, W.M., 1966. History of mosquitoes-borne haemorrhagic fever. *Bulletin of the World Health Organization*, vol. 44, pp. 643-649.

HANAFI-BOJD, A., VATANDOOST, H., OSHAGHI, M., CHARRAHY, Z., HAGHDOOST, A., SEDAGHAT, M., ABEDI, F., SOLTANI, M. and RAEISI, A., 2012. Larval habitats and biodiversity of *Anopheline* mosquitoes (Diptera: Culicidae) in malarious areas of Southern Iran. *Journal of Vector Borne Diseases*, vol. 49, no. 2, pp. 91-100. PMid:22898481.

HARBACH, R.E., 1988. The mosquitoes of the subgenus *Culex* in southwestern Asia and Egypt (Diptera: culicidae). *Contributions of the American Entomological Institute*, vol. 24, pp. 1-240.

HAWLEY, W.A., 1988. The biology of *Aedes albopictus*. *Journal of the American Mosquito Control Association*, vol. 1, pp. 1-39. PMid:3068349.

HUSSAIN, R., HAMILTON, R.G., KUMARASWAMI, V., ADKINSON JUNIOR, N.F. and OTTESEN, E.A., 1981. IgE responses in human filariasis. I. Quantification of filarial-specific IgE. *Journal of Immunology*, vol. 127, no. 4, pp. 1623-1629. http://dx.doi.org/10.4049/jimmunol.127.4.1623. PMid:7276575.

ILAHI, I., 2001. *Ecology and Biodiversity of Mosquitoes in Swat*. Pakistan: University of Peshawar, 60 p. M. Sc in Zoology.

ILAHI, I. and SULEMAN, M., 2013. Species composition and relative abundance of mosquitoes in Swat, Pakistan. *International Journal of Innovation and Applied Studies*, vol. 2, no. 4, pp. 454-463.

JAGTAP, M., SALE, L.S., BHOSALE, A.B., ASAWARI, S.A.T.H.E. and SATHE, T.V., 2009. Incidence of Dengue and Shifting trend to rural in Kolhapur district, India. *Biological Forum: an International Journal*, vol. 1, no. 2, pp. 69-72.

JAGTAP, M., SALE, L.S. and SATHE, T.V., 2008. Studies of *P. falciparum* (PF) resistance to chloroquine in Etapali Block, Dist. Gadchiroli. *National Journal of Life Sciences*, vol. 5, no. 3, pp. 55-60.

JONES, K.E., PATEL, N.G., LEVY, M.A., STOREYGARD, A., BALK, D., GITTLEMAN, J.L. and DASZAK, P., 2008. Global trends in emerging infectious diseases. *Nature*, vol. 451, no. 7181, pp. 990-993. http://dx.doi.org/10.1038/nature06536. PMid:18288193.

JUDE, P.J., DHARSHINI, S., VINOBABA, M., SURENDRAN, S.N. and RAMASAMY, R., 2010. *An. culicifacies* breeding in brackish waters in Sri Lanka and implications for malarial control. *Malaria Journal*, vol. 9, no. 1, pp. 106. http://dx.doi.org/10.1186/1475-2875-9-106. PMid:20409313.

KARIM, A.M., YASIR, M., ALI, T., MALIK, S.K., ULLAH, I., QURESHI, N.A., YUANTING, H., AZHAR, E.I. and JIN, H.J., 2021. Prevalence of Clinical Malaria and household characteristics of patients in tribal districts of Pakistan. *PLoS Neglected Tropical Diseases*, vol. 15, no. 5, e0009371. http://dx.doi.org/10.1371/journal.pntd.0009371. PMid:33939717.

KHAN, E., BARR, K.L., FAROOQI, J.Q., PRAKOSO, D., ABBAS, A., KHAN, Z.Y., ASHI, S., IMTIAZ, K., AZIZ, Z., MALIK, F., LEDNICKY, J.A. and LONG, M.T., 2018. Human West Nile virus disease outbreak in Pakistan 2015-2016. *Frontiers in Public Health*, vol. 6, pp. 20. http://dx.doi.org/10.3389/fpubh.2018.00020. PMid:29535994.

KHAN, M.A. and PERVEZ, S.D., 1981. Imported filariasis in Pakistan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*,

vol. 75, no. 6, pp. 869-871. http://dx.doi.org/10.1016/0035-9203(81)90435-1. PMid:7036440.

KHATOON, L., BALIRAINE, F.N., BONIZZONI, M., MALIK, S.A. and YAN, G., 2010. Genetic structure of *Plasmodium vivax* and *Plasmodium falciparum* in the Bannu district of Pakistan. *Malaria Journal*, vol. 9, no. 1, pp. 112. http://dx.doi.org/10.1186/1475-2875-9-112. PMid:20416089.

KNIGHT, K. and STONE, A., 1977. *A catalog of the Mosquitoes of the World (Diptera: Culicidae)*. 2nd ed. College Park: Entomological Society of America, 611 p. Thomas Say Foundation, no. 6.

MAGURRAN, A.E., 2004. *Measuring biological diversity*. Oxford: Blackwell Science Ltd.

MALLHI, T.H., KHAN, Y.H., KHAN, A.H., TANVEER, N. and QADIR, M.I., 2017. First Chikungunya outbreak in Pakistan: a trail of viral attacks. *New Microbes and New Infections*, vol. 19, pp. 13-14. http://dx.doi.org/10.1016/j.nmni.2017.05.008.

MERAJ, L., SALEEM, J., MANZOOR, S., ASHFAQ, A. and KHURRAM, M., 2020. First report of Chikungunya fever in Rawalpindi, Pakistan. *Eastern Mediterranean Health Journal*, vol. 26, no. 6, pp. 744-747. http://dx.doi.org/10.26719/emhj.19.095. PMid:32621511.

MINAKAWA, N., MUTERO, C.M., GITHURE, J.I., BEIER, J.C. and YAN, G., 1999. Spatial distribution and habitat characterization of anopheline mosquito larvae in western Kenya. *The American Journal of Tropical Medicine and Hygiene*, vol. 61, no. 6, pp. 1010-1016. http://dx.doi.org/10.4269/ajtmh.1999.61.1010. PMid:10674687.

MINAKAWA, N., SONYE, G., MOGI, M. and YAN, G., 2004. Habitat characteristics of *Anopheles gambiae* s.s. larvae on a Kenyan Highland. *Medical and Veterinary Entomology*, vol. 18, no. 3, pp. 301-305. http://dx.doi.org/10.1111/j.0269-283X.2004.00503.x. PMid:15347399.

MOOSA-KAZEMI, S.H., VATANDOOST, H., NIKOOKAR, H. and FATHIAN, M., 2009. Culicine (Diptera: Culicidae) mosquitoes in Chabahar Country, Sistan and Baluchistan Province, Southern Iran. *Journal of Arthropod-Borne Diseases*, vol. 3, no. 1, pp. 29-35. PMid:22808369.

MWANGANGI, J.M., MUTURI, E.J. and MBOGO, C.M., 2009. Seasonal mosquito larval abundance and composition in Kibwezi, lower eastern Kenya. *Journal of Vector Borne Diseases*, vol. 46, no. 1, pp. 65-71. PMid:19326710.

NIKOOKAR, S., FAZELI-DINAN, M., AZARI-HAMIDIAN, S., MOUSAVINASAB, S., ARABI, M., ZIAPOUR, S., SHOJAEE, J. and ENAYATI, A., 2017. Species composition and abundance of mosquito larvae in relation with their habitat characteristics in Mazandaran Province, northern Iran. *Bulletin of Entomological Research*, vol. 107, no. 5, pp. 598-610. http://dx.doi.org/10.1017/S0007485317000074. PMid:28956526.

OKOGUN, G.R.A., ANOSIKEB, J.C., OKEREB, A.N. and NWOKE, B.E.B., 2005. Ecology of mosquitoes of Midwestern Nigeria. *Journal of Vector Borne Diseases*, vol. 42, no. 1, pp. 1-8. PMid:15999454.

QAMASH, T., JAMIL, J., KALSOOM., KHAN, F.A., SAIRA., SULTAN, A., BEGUM, N. and DIN, S.U., 2021. Epidemiological study of dengue fever in District Swabi, Khyber Pakhtunkhwa, Pakistan. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 81, no. 2, pp. 237-240. http://dx.doi.org/10.1590/1519-6984.216284. PMid:32696850.

QURESHI, N.A., FATIMA, H., AFZAL, M., KHATTAK, A.A. and NAWAZ, M.A., 2019. Occurrence and seasonal variation of human *Plasmodium* infection in Punjab Province, Pakistan. *BMC Infectious Diseases*, vol. 19, no. 1, pp. 935. http://dx.doi.org/10.1186/s12879-019-4590-2. PMid:31694574.

RAMACHANDRA RAO, T., 1981. *The anophelines of India.* New Delhi: Indian Council of Medical Research.

RAMACHANDRA RAO, T., 1989. *The Anophelines of India*. New Delhi: Malaria Research Centre, 518 p.

RASHEED, S.B., BUTLIN, R.K. and BOOT, S.M., 2013. A review of dengue as an emerging disease in Pakistan. *Public Health*, vol. 127, no. 1, pp. 11-17. http://dx.doi.org/10.1016/j.puhe.2012.09.006. PMid:23219263.

RAUF, M., ZAHRA, F., MANZOOR, S., MEHMOOD, A. and BHATTI, S., 2017. Oubreak of Chikungunya in Pakistan. *The Lancet. Infectious Diseases*, vol. 17, no. 3, pp. 258. http://dx.doi.org/10.1016/S1473-3099(17)30074-9. PMid:28244384.

REISEN, W.K., ASLAM, Y. and SIDDIQUI, T.F., 1977. The swarming and mating of Pakistan mosquitoes. *Annals of the Entomological Society of America*, vol. 70, pp. 988-995. http://dx.doi.org/10.1093/aesa/70.6.988.

REISEN, W. and ASLAMKHAN, M., 1978. Biting rhythms of some Pakistan mosquitoes (Diptera: culicidae). *Bulletin of Entomological Research*, vol. 68, no. 02, pp. 313-330. http://dx.doi.org/10.1017/S0007485300007392.

REISEN, W.K. and MILBY, M.M., 1986. Population dynamics of some Pakistan mosquitoes: changes in adult relative abundance overtime and space. *Annals of Tropical Medicine and Parasitology*, vol. 80, no. 1, pp. 53-68. http://dx.doi.org/10.1080/00034983.1986.11811984. PMid:2873798.

REISEN, W.K., SIDDIQUI, T.F., ASLAMKHAN, M. and MALIK, G.M., 1981. Larval interspecific associations and physicochemical relationships between the ground water-breeding mosquitoes of Lahore. *Pakistan Journal of Scientific and Industrial Research*, vol. 3, pp. 1-23.

RICHARDS, S.L., GHOSH, S.K., ZEICHNER, B.C. and APPERSON, C.S., 2008. Impact of Source reduction on the spatial distribution of larvae and pupae of Aedes albopictus (Diptera: Culicidae) in suburban neighborhoods of a Piedmont community in North Carolina. *Journal of Medical Entomology*, vol. 45, no. 4, pp. 617-628. http://dx.doi.org/10.1093/jmedent/45.4.617. PMid:18714860.

RUDNICK, A., 1967. *Aedes aegypti* and haemorrhagic fever. *Bulletin of the World Health Organization*, vol. 36, no. 4, pp. 528-532. PMid:5299445.

RYDZANICZ, K. and LONC, E., 2003. Species composition and seasonal dynamics of mosquito larvae in the Wroclaw, Poland area. *Journal of Vector Ecology*, vol. 28, no. 2, pp. 255-266. PMid:14714675.

SATHE, T.V., ASAWARI, S.A.T.H.E. and MAHENDRA, J.A.G.T.A.P., 2010. *Mosquito borne diseases*. New Delhi: Mangal Publishers, 342 p.

SATHE, T.V. and GIRHE, B.E., 2001. Biodiversity of Mosquitoes (Order: Diptera) in Kolhapur district, Maharashtra. *Rivista di Parassitologia*, vol. 18, no. LXVII-3, pp. 189-194.

SATHE, T.V. and GIRHE, B.E., 2002. *Mosquitoes and diseases*. New Delhi: Daya Publishing House, 96 p.

SATHE, T.V. and TINGARE, B.P., 2010. *Biodiversity of mosquitoes*. New Delhi: Mangal Publishers, 200 p.

SENGIL, A.Z., AKKAYA, H., GONENC, M., GONENC, D. and OZKAN, D., 2011. Species composition and monthly distribution of mosquito (Culicidae) larvae in the Istanbul metropolitan area, Turkey. *International Journal of Biological and Medical Research*, vol. 2, no. 1, pp. 415-424.

SERVICE, M.W., 1993. Mosquitoes (Culicidae): medical importance. In: R.P. LANE and R.W. CROSSKEY, eds. *Medical insects and arachnids.* London: Chapman & Hall, vol. 5, pp. 196-208.

SHABBIR, W., PILZ, J. and NAEEM, A., 2020. A spatial-temporal study for the spread of dengue depending on Climate factors in Pakistan (2006-2017). *BMC Public Health*, vol. 20, no. 1, pp. 995. http://dx.doi.org/10.1186/s12889-020-08846-8. PMid:32586294.

SMITH, C.E.G., 1956. The history of Dengue in tropical Asia and its probable relation to the mosquuitoes *Aedes aegypti. The Journal of Tropical Medicine and Hygiene*, vol. 59, no. 10, pp. 243. PMid:13368255.

STEPHENSON, I., ROPER, J., FRASER, M., NICHOLSON, K. and WISELKA, M., 2003. Dengue fever in febrile returning travellers to a UK regional infectious diseases unit. *Travel Medicine and Infectious Disease*, vol. 1, no. 2, pp. 89-93. http://dx.doi.org/10.1016/S1477-8939(03)00061-9. PMid:17291893.

SULEMAN, M., KHALID, M. and KHAN, S., 1993. Ecology of mosquitoes in Peshawar and adjoining areas species composition and relative abundance. *Pakistan Journal of Zoology*, vol. 25, pp. 321-328.

TROJAN, P., 1992. Analiza struktury fauny [The analysis of the fauna's structure]. *Memorabilia Zoologica*, vol. 47, pp. 1-120.

TURELL, M.J., BAILEY, C.L. and BEAMAN, J.R., 1988. Vector competence of a Houston, Texas strains of *Aedes albopictus* for Rift Valley Fever virus. *Journal of the American Mosquito Control Association*, vol. 4, no. 1, pp. 94-96. PMid:3193106.

TURELL, M.J., BEAMAN, J.R. and TAMMARIELLO, R.F., 1992. Susceptibility of selected strains of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) to Chickungunya virus. *Journal of Medical Entomology*, vol. 29, no. 1, pp. 49-53. http://dx.doi.org/10.1093/jmedent/29.1.49. PMid:1313111.

WORLD HEALTH ORGANIZATION – WHO, 2004. *Report on second malaria cross border meeting for Afghanistan, Islamic Republic of Iran and Pakistan.* Geneva: WHO Press.

WORLD HEALTH ORGANIZATION – WHO, 2017. *World malaria report 2017.* Geneva: WHO Press.

WORLD HEALTH ORGANIZATION – WHO, 2018. *World malaria report 2018.* Geneva: WHO Press.

WORLD HEALTH ORGANIZATION – WHO, 2019 [viewed 3 December 2019]. *Chikungunya* [online]. Available from: http://www.who.int/mediacentre/factsheets/ fs327/en/

YADAV, R.S., SHARMA, R.C., BHATTI, R.M. and SHARMA, V.P., 1989. Studies on Anopheline fauna of Kheda district and species specific breeding habitats. *Indian Journal of Malariology*, vol. 26, no. 2, pp. 65-74. PMid:2792472.

ZOHAIB, A., SAQIB, M., BECK, C., HUSSAIN, M.H., LOWENSKI, S. and LECOLLINET, S., 2014. High prevalence of West Nile Virus in equines from two provinces of Pakistan. *Epidemiology and Infection*, vol. 31, pp. 1-5. PMid:25358382.