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Research Article

Mosquito species, seasonality, abundance, and resting sites in three villages of Peshawar, Pakistan

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Abstract

To study adult mosquito species resting sites selection in the study area, they prefer cattle sheds despite the availability of mixed dwellings and human dwellings. Further, to study the relative abundance of different mosquito genera (*Culex*, *Aedes*, *Anopheles*, and *Mansonia*) in different rural villages, the present research work was carried out in the Entomology Research Laboratory, University of Peshawar, Pakistan. Indoor resting mosquitoes were collected from several shelters (rooms), including human dwellings and cattle sheds in the three rural villages of Peshawar during September 2018 - October 2019. The Flet method was used for capturing all the mosquitoes available in a room. The study conducted in different settlements, including rural areas of Peshawar, yielded a total of 12 species of mosquitoes belonging to four genera (*Aedes*, *Anopheles*, *Culex*, and *Mansonia*). A total of 4,928 (2,306 + 899 + 1,723) adult mosquitoes (2,908 females and 2,020 males) were captured using the Flet method from eighteen shelters, including nine human dwellings and nine cattle sheds, in the three study villages in Peshawar. The abundance of mosquitoes was higher in cattle sheds than in human dwellings; of the total capture, 60% (2,931/4,928) came from the cattle sheds, verifying that cattle sheds were more attractive than the human dwellings. Mosquito abundance was richly reported from cattle sheds, which should be given special attention during any control programme targeting adult mosquitoes. Furthermore, as mosquito increase was apparent during the post-monsoon season, the control measures should be enhanced during this time to ensure maximum protection of the inhabitants.

Keywords: Cattle Sheds, Human Dwellings, Mosquito-Borne Diseases

Introduction

Mosquitoes are economically important as they are vectors for many viruses and other parasitic disease pathogens, seriously affecting human and animal health. Some mosquitoes routinely bite humans, thus serving as vectors for several infectious diseases affecting millions of people per year, while others are vectors for animal diseases that may become disastrous agents for zoonosis of new diseases when their habitats are disturbed (WHO, 2007). Mosquito-borne illnesses include *Plasmodium* spp. causing Malaria, West Nile Virus, Elephantiasis, Dengue Fever, Yellow Fever, Chikungunya, Encephalitis, and Ross River virus, etc. Factors like global warming, periodic flooding, and deforestation cause considerable extension of mosquito breeding habitats and create aquatic sites that support diverse mosquito species, including vectors (Mwangangi et al., 2009). Floodwaters spread beyond rivers and irrigation channels into poorly-drained, low-lying areas, forming stagnant pools that further extend breeding grounds for mosquitoes. Malaria is not the only mosquito-borne disease causing trouble in Pakistan, but recent outbreaks of yellow fever and dengue fever have reached epidemic status, especially in Punjab province, though a large number of cases are also reported in other parts of the country, including Khyber Pakhtunkhwa.

All mosquitoes have four developmental stages egg, larva, pupa, and adult. The egg, larva, and pupa stages are restricted to water. Females of some mosquito species deposit eggs on moist surfaces, such as mud or fallen leaves near water. Later, rain or high tides flood these surfaces, stimulating the eggs to hatch into larvae. Several floodwater mosquito eggs can survive drying, even over winter, and hatch when flooded again (Ali and Rasheed, 2009).

Mosquitoes exhibit species-specific activity patterns throughout their lifecycle (Clements, 1999). During their inactive period, mosquitoes use certain resting sites where they spend most of their time resting and digesting blood meals (Silver, 2008). This was revealed in collections from resting sites, which usually contained a higher proportion of blood-fed and gravid females compared to regular baited traps (Burkett-Cadena, 2008; Sauer, 2020). Selecting an accurate mosquito trapping technique is crucial, as several studies have reported significant differences in capture efficiencies between methods (Kline, 2006). The human bait trap (human landing catch) has been a traditional method for estimating mosquito abundance. Resting site preference is considered the most direct and reliable method for identifying species that are highly anthropophilic and prefer indoor environments; it also demonstrates a good correlation with the human landing catch (Mgbemena, 2015).

Research on mosquitoes in Khyber Pakhtunkhwa province of Pakistan needs further assessment (Ali and Rasheed, 2009; Khan et al., 2014). The most effective method to manage vector-borne diseases in affected areas is larval source management, which targets mosquito larvae during their aquatic stage using chemicals and environmental management. This can be achieved by adding substances to standing water that either kill or inhibit larval development (larviciding) (Tusting et al., 2015). However, measuring mosquito-borne infection or disease transmission requires adult mosquito sampling—specifically through indoor spray catch. The variability and quantity of mosquitoes caught depend on the method used (Mgbemena, 2015). This study aims to determine species composition, resting site preferences, population dynamics, and ecological conditions, which will help in designing effective control measures against this serious problem. Effective control of mosquito-borne diseases through vector management requires detailed information on the distribution, extent, and abundance of vector populations in targeted areas.

Material and methods

Study Village

Peshawar is one of the oldest cities in Asia, the provincial capital, and the largest city of Khyber Pakhtunkhwa. It is situated between 33.44° and 34.01° north latitude and 71.22° and 71.58° east longitude, at an elevation of 340 meters above sea level. Peshawar Valley is located toward the eastern end of the Khyber Pass, with the Afghan border approximately 40 km to its west. It is bounded on the north by Charsadda district, on the east by Nowshera district, on the south by tribal areas adjoining Peshawar and Kohat district, and on the west by Mohmand and Khyber agencies. Peshawar is a semi-arid area, characterized by extreme hot summers and mild winters. The summer season extends from mid-April to the end of September, while winter months range from November to late March. The mean maximum summer temperature exceeds 40°C, and the mean maximum winter temperature surpasses 18.3°C, whereas the mean minimum temperatures are 25°C in summer and 4°C in winter. Although Peshawar is not a monsoon region, it receives rainfall during both summer and winter seasons.

Sampling Sites

In three rural villages of Peshawar, eighteen fixed sampling sites were selected to collect adult mosquitoes, representing nine human dwellings and nine cattle sheds. Collections were made once a month throughout the year, from October 2018 to September 2019. Of these eighteen sites, six (three human dwellings and three cattle sheds) were located in Bara Gate, six (three HD and three CS) in Haji Banda, and six (three HD and three CS) in Shahi Payan (Fig. 1; Table

1). The selected human dwellings and cattle sheds in each location belonged to the same household and shared a boundary wall. The nature and size of the fixed sampling sites in the three villages are shown in Table 1 (Fig. 1). All the human dwellings were 'dirt houses,' except for HD 1 (Bara Gate), HD 3 (Haji Banda), and HD 1 (Shahi Payan), which were constructed with cemented walls and concrete roofs. All cattle sheds were 'dirt houses' with mud walls and thatched roofs.

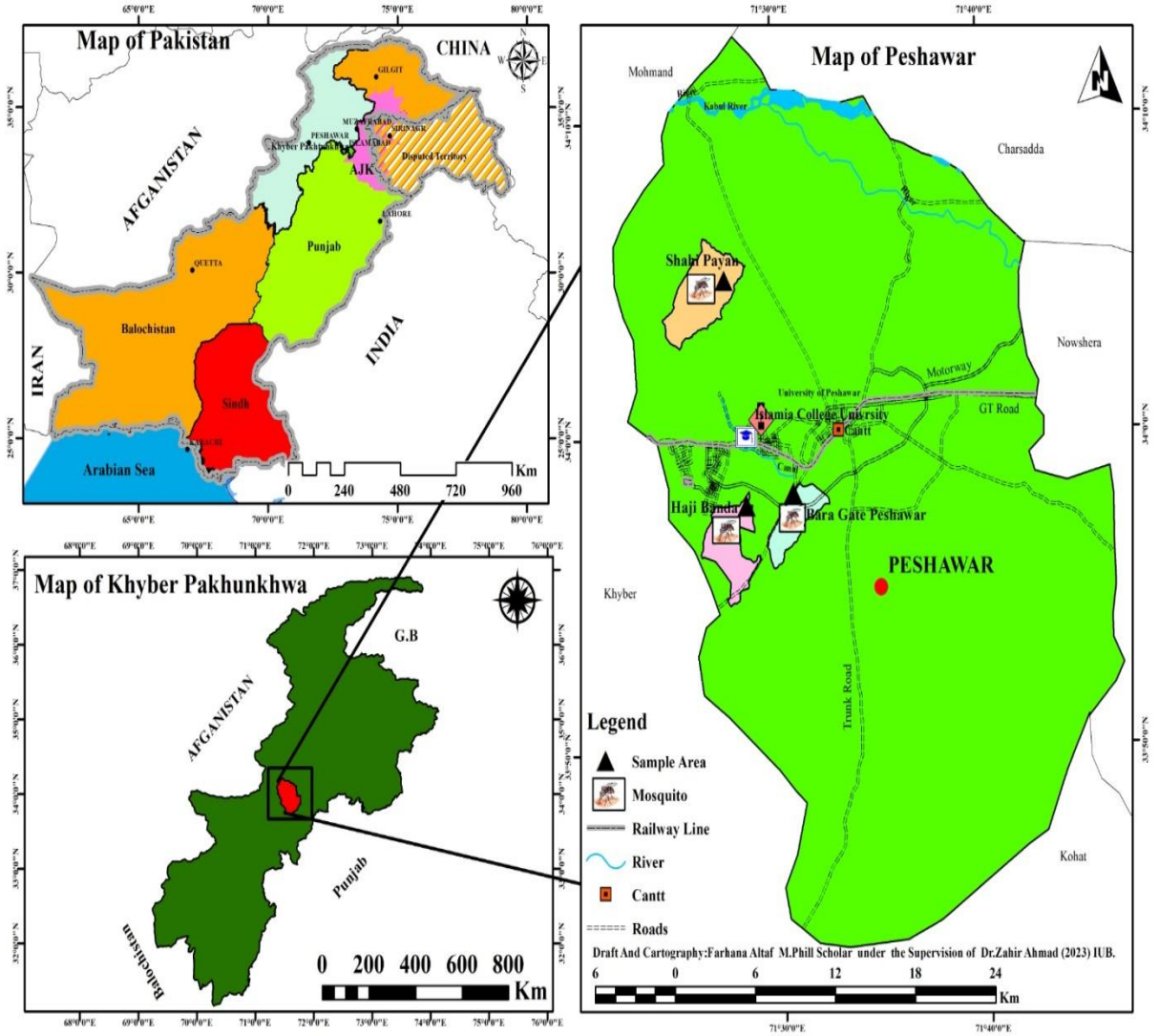

Figure 1. Study Area Map for Mosquito Adult Sampling from three targeted villages of Peshawar (2017- 2018).

Table 1. Nature and size of the fixed sampling sites in the three study villages in Peshawar(HD, Human dwelling; CS, Cattle shed).

A. BARA GATE SWATTI PATAK				
House No.	HD/ CS	Size (Feets)LxWxH	Inhabitants	Texture
1	HD 1	15x 10x 12	10 Persons	Cemented

	CS 1	10x 10x 11	3 cows, 2 Asses, 2 goats	Mud House with one wall, thatched roof
2	HD 2	12x 12x 12	7 Persons	Mud House, thatched roof
	CS 2	20x 10x 12	4 cows, 2 goats, 3 sheeps	Mud House with no wall, thatched roof
3	HD 3	18x 15x 10	13 Persons	Mud House, thatched roof
	CS 3	15x 10x 9	4 cows, 3 goats, 1 Buck, 2 sheep	Mud House with no wall, thatched roof
B. HAJJI BANDA				
House No.	HD/ CS	Size (Feets) LxWxH	Inhabitants	Texture
1	HD 1	15x 12x 10	10 Persons	Mud House, thatched roof
	CS 1	15x 10x 9	3 Cows, 1 Donkey, 4 Goats	Mud House with no walls, thatched roof
2	HD 2	18x 15x 10	12 Persons	Mud House, thatched roof
	CS 2	12x 12x 9	3 Cows, 5 Goats	Mud House with no wall, thatched roof
3	HD 3	15x 12x 10	10 Persons	Cemented
	CS 3	16x 12x 10	4 Cows, 3 Goats, 1 Donkey	Mud House with no wall, thatched roof
C. SHAHI PAYAN				
House No.	HD/ CS	Size (Feets)LxWxH	Inhabitants	Texture
1	HD 1	20x 15x 11	Bethak, Hujra	Cemented
	CS 1	15x 15x 8	Pair of Bullocks, 1 Donkey	Mud House with no wall, thatched roof
2	HD 2	15x 12x 10	Study Room	Mud House, thatched roof
	CS 2	15x 12x 8	3 Cows, 1 Buck, 1 Sheep	Mud House with no wall, thatched roof
3	HD 3	18x 12x 9	Hujra	Mud House, thatched roof
	CS 3	15x 10x 9	3 Cows, 3 Goats, 1 Ram	Mud House with no wall, thatched roof

Pyrethrum Spray Collection

The Flet method, using knockdown spray catches, was employed to capture all mosquitoes available in a room. The collection was typically performed around 10 a.m. In the Flet method, all insects resting inside the room were killed by spraying a fast-acting volatile insecticide. For this purpose, all occupants, including animals, and articles such as tables, chairs, and edibles, were removed from the room or appropriately covered. Cotton cloth sheets were spread over the entire floor, beds, and other miscellaneous objects that had not been removed. The doors and windows were closed before spraying with commercially available aerosol insecticides, such as 'BOP' spray. The doors and windows remained closed for 10 to 15 minutes, after which the room was opened, and mosquitoes were collected from the sheets and transferred into a plastic container. Collections from each room were kept in separate glass tubes and labeled accordingly.

Laboratory Processing of Mosquitoes

The collected specimens were sexed, and the major morphological differences among the species were observed and recorded. Accurate taxonomic identification was performed using a binocular microscope and standard taxonomic keys provided in *Fauna of British India* by Christophers (1933), Barraud (1934), and Knight and Stone (1997).

Statistical Analysis

Analysis of Periodic variation (ANOVA) of mosquito species in different villages was examined in terms of density and distribution (Rydzanicz and Lonc, 2003; Sengil *et al*., 2011).

Relative abundance Rel. Ab =I /L x 100% where, I= no. of specimens of each mosquito species and L= Total number of specimens.

Distribution C = n b/N x 100% where C = Constancy n-number of occurrence sites of the species N- Total number of sites examined.

The dominance pattern and diversity of mosquito species in different villages were calculated by Shannon-Wiener diversity Index (*H'*), Pielou's Evenness Index (E), Margalef's Richness Index (Me) (Magurran, 2004; Aslan and Karaca, 2012).

Shannon Index $H' = -\Sigma$ pi In pi Here, Pi is the proportion (n/N), n= no of entities of the species found, N= Total number of individuals, In=natural log, Σ= Summation

Pielou's Evenness Index (E) = H' / Hmax = H' / ln S, where S= No. of species (Rasool et al ., 2018).

Margalef's Richness Index (Me) =S-1/ ln N, where S= No. of species and N= total no. of specimens collected (Younas et al., 2023).

Results

Mosquito Species Composition

The study conducted in different settlements, including rural areas of Peshawar, yielded a total of 12 species of mosquitoes belonging to four Genera (*Aedes, Anopheles, Culex,* and *Mansonia*). The rural areas targeted for adult indoor catch by the flit method include Bara Gate, Hajji Banda, and Shahi Payan. A total of 4928 (2306+ 899+1723) adult mosquitoes (2,908 females and 2,020 males) were captured using the flit method from eighteen shelters, including nine human dwellings and nine cattle sheds in the three study villages of Peshawar. The abundance of mosquitoes was found to be higher in cattle sheds than in human dwellings, verifying that cattle sheds were more attractive than human dwellings.

Species Composition And Relative Abundance In Baragate

A total of 2306 mosquitoes (1369 female and 937 male) comprising eight species in four Genera (*Aedes, Anopheles, Culex,* and *Mansonia*) were found as indoor resting adults in this village (Table 2). *Anophelines* represented by three species (*Anopheles culicifacies*, *Anopheles fluviatilus,* and *Anopheles stephensi*) constituted 6.1 % of the total specimens collected. Genus *Culex* was represented by three species (*Culex quinquifasciatus*, *Culex tritaeniorhyncus,* and *Culex vishnui*), constituting 94 % of the total fauna collected. The remaining two genera, i.e., *Aedes* (*Aedes albopictus*) and *Mansonia* (*Mansonia uniformis*), were represented by a single species, and a single specimen each contributed very little to the total population.

The global relative abundance of various species specified the usual house mosquito, *Culex quinquifasciatus,* to be the supreme abundant species (92 %) followed by *Anopheles fluviatilus* (3%) and *Anopheles stephensi* (2.1 %). The rest of the species make < 2 of the total mosquito fauna (Table 2). Among the Anopheline group, *Anopheles fluviatilus* was the abundant species, constituting 44.3 % of the total Anophelines, followed by *Anopheles stephensi* (35%) and *Anopheles culicifacies* (21 %).

Resting Site Preference Of Indoor Mosquitoes In Baragate

This village has 2306 indoor mosquitoes, comprising 779 (34 %) mosquitoes captured from human dwellings, whereas 1527 (66 %) mosquitoes were captured from Cattle sheds. Among these species, *Mansonia uniformis* was captured only from Cattle sheds, whereas *Aedes albopictus* was captured from Human dwellings. The rest of the species were collected from both types of resting sites, but found abundance in Cattle sheds (Table 2).

Among the indoor-resting mosquitoes, *Culex quinquifasciatus* was the most abundant species in both categories of resting sites, constituting 98.6% of the total catch from human dwellings and 88% from cattle huts. *Anopheles culicifacies*, *Anopheles fluviatilus*, *Anopheles stephensi, Culex tritaeniorhyncus,* and *Culex vishnui* were relatively more abundant in Cattle sheds than in human dwellings, though their abundance was not significantly different in the two types of resting sites (Table 2). Both *Aedes albopictus* and *Mansonia uniformis* were represented by a single specimen, the former from a human dwelling and the latter from cattle sheds.

Table 2. Comparative assessment of species composition and relative abundance of indoor-resting mosquitoes with their respective totals in human dwellings, HD, and Cattle sheds, CS, in Bara Gate village of Peshawar (2017- 2018).

Species / Locality	HD1	HD2	HD3	Total	%	CS1	CS2	CS3	Total	%	**Overall total**
Ae. Albopictus			1-0	1	0.1						**1**
An. Culicifascies			1-0	1	0.1	6-3	8-1	7-3	28	1.8	**29**
An. Fluviatilis		1-0	1-0	2	0.3	25-6	14-5	5-5	60	4	**62**
An. Stephensi	3-0			3	0.4	18-1	6-1	16-4	46	3	**49**

Cx. quinquifasciatus	289-126	167-69	110-7	768	98.6	335-258	188-142	116-302	1341	88	**2109**
Cx. tritaeniorhynchus			1-0	1	0.1	14-1	12-1	4-0	32	2.1	**33**
Cx. Vishnui		3-0		3	0.4	7-1	4-0	6-1	19	1.2	**22**
Mn. Uniformis							1-0		1	0.01	**1**
	292-126	171-69	114-7	577-202		405-270	233-150	154-315	792-735		**1369-937**
Total	418	240	121	779		675	383	469	1527		**2306**

Species Composition And Relative Abundance In Hajji Banda

A total of 899 mosquitoes (613 female and 286 male) comprising eight species belonging to three Genera (*Aedes, Anopheles, Culex*) were found as indoor resting adults in this village (Table 3). Anophelines, represented by four species, constituted 59% of the total specimens collected. Genus *Culex* was represented by three species, constituting 41% of the total fauna collected. The remaining genus, i.e., *Aedes,* was represented by a sole species (*Aedes unilineatus*) and nine specimens, contributing very little (1%) to the total population.

The global relative abundance of various species specified the usual house mosquito, *Culex quinquifasciatus,* to be the chief abundant species (32 %) followed by *Anopheles culicifacies* (21 %), *Anopheles stephensi* (20 %), *Anopheles fluviatilus* (15 %), and *Culex tritaeniorhynchus* (5 %). The rest of the species make up less than 5% of the total collection.

Resting Site Preference Of Indoor Mosquitoes In Hajji Banda

Of 899 indoor mosquitoes collected from this village, 386 (43 %) mosquitoes were captured from human dwellings, whereas 513 (57 %) mosquitoes were from Cattle sheds. Six species were found common in both types of resting sites but reported abundantly in Cattle sheds except *Culex tritaeniorhynchus,* which was trapped richly from the human dwellings (Table 3). *Anopheles subpictus* was captured solely from Cattle sheds, whereas *Aedes unilineatus* was caught mostly from Human dwellings.

Among the collected mosquitoes, *Culex quinquefasciatus* was the most abundant species in both categories of resting sites, constituting 25 % of the total catch from human dwellings and 37% from cattle huts. *Anopheles stephensi*, *Anopheles culicifacies,* and *Anopheles fluviatilus* were all found abundant in human dwellings, contributing 23%, 22% and 18% of the entire captured mosquitoes as compared to cattle sheds, donating 17%, 20% and 12% in the entire pool. *Culex tritaeniorhynchus* also dominates in human dwellings, adding 41 specimens. The occurrence of *Culex bitaeniorhyncus* was not significantly different in the two types of resting sites. However, *Anopheles stephensi* and *Culex quinquefasciatus* exhibited significant differences; *Anopheles stephensi* was more abundant in human dwellings, and *Culex*

quinquefasciatus was more abundant in cattle sheds. Both *Aedes unilineatus* and *Anopheles subpictus* were represented by limited specimens, the former largely from human dwellings and the latter from cattle sheds (Table 3).

Table 3. Comparative assessment of species composition and relative abundance of indoor-resting mosquitoes with their respective totals in human dwellings, HD, and Cattle sheds, CS, in Hajji Banda village of Peshawar (Oct 2017- Sept 2018).

Species / Locality	**HD1**	**HD2**	**HD3**	**Total**	**%**	**CS1**	**CS2**	**CS3**	**Total**	**%**	**Overall Total**
Ae. Unilineatus	7-1			8	2	1-0			1	0.2	**9**
An. Culicifascies	19-13	18-6	22-8	86	22	14-1	14-8	46-21	104	20	**190**
An. Fluviatilis	18-3	16-4	21-6	68	18	20-8	3-0	20-11	62	12	**130**
An. Stephensi	20-5	34-17	9-5	90	23	14-8	23-9	26-9	89	17	**179**
An. subpictus						6-5	10-6		27	5	**27**
Cx. bitaeniorhyncus	3-4	1-3	2-3	16	4		10-2	9-2	23	4.5	**39**
Cx. quinquefasciatus	16-7	35-18	13-6	95	25	45-44	36-10	32-22	189	37	**284**
Cx. tritaeniorhynchus	10-1	8-3	0-1	23	6	8-4		4-2	18	4	**41**
Total	93-34	112-51	67-29	272-114		108-70	96-35	137-67			**613-286**
	127	163	96	386		178	131	204	513		**899**

Species Composition And Relative Abundance In Shahi Payan

A total of 1723 mosquitoes (926 female and 797 male) comprising seven species in three Genera (*Aedes, Anopheles, Culex*) were found as indoor resting adults in Shahi Payan village (Table 4). *Anopheles,* represented by four species, constituted 24 % of the total specimens collected. Genus *Culex* was represented by two species, constituting 76 % of the total fauna

collected. The remaining genera, i.e., *Aedes,* were represented by a single species and a single specimen contributing 0.1% to the total population.

The global relative abundance of various species specified the usual house mosquito, *Culex quinquifasciatus,* to be the unbeatable abundant species (76%). Among the rest of 24% *Anopheles fluviatilus* (11 %) is on the top, followed by *Anopheles culicifacies* (7 %) and *Anopheles stephensi* (6 %), *Culex bitaeniorhyncus* (0.2 %), *Anopheles pulcherrimus* (0.2 %), and *Aedes albopictus* (0.1 %). Among the Anopheline group, *Anopheles fluviatilus* was the abundant species, constituting 44.3 % of the total Anophelines (Table 4).

Resting Site Preference Of Indoor Mosquitoes In Shahi Payan

This village has 1723 indoor mosquitoes, comprising 832 (48 %) mosquitoes captured from human dwellings, whereas 891 (52 %) mosquitoes were captured from Cattle sheds. Among these species, *Anopheles pulcherrimus* and *Aedes albopictus* were captured solely from Cattle sheds, whereas *Culex bitaeniorhynchus* was caught only from Human dwellings. The rest of the species were collected from both types of resting sites, but *Anopheles culicifacies* and *Culex quinquefasciatus* were trapped richly from the human dwellings, whereas *Anopheles fluviatilus* and *Anopheles stephensi* were found abundant in Cattle sheds (Table 4).

Among the indoor-resting mosquitoes, *Culex quinquefasciatus* was the most abundant species in both categories of resting sites, constituting 84 % of the total catch from human dwellings and 68 % from cattle huts. *Culex quinquefasciatus* and *Anopheles culicifacies* were both found abundant in human dwellings, contributing 84% and 8% of the entire captured mosquitoes as compared to cattle sheds, where they accounted for 68% and 6% of the entire pool. However, *Anopheles fluviatilus* and *Anopheles stephensi* were both found abundant in cattle sheds, contributing 17 % and 9 % of the entire captured mosquitoes, as compared to human dwellings, donating 4 % and 3 % in the entire pool. Conversely, *Anopheles stephensi* and *Culex quinquefasciatus* exhibited significant differences; *Anopheles stephensi* was more abundant in cattle sheds, and *Culex quinquefasciatus* was more abundant in human dwellings (Table 4). A sole female specimen of *Aedes albopictus* was caught from cattle sheds, contributing 0.1% to the total number of mosquitoes from this village.

Table 4. Comparative assessment of species composition and relative abundance of indoor-resting mosquitoes with their respective totals in human dwellings, HD, and Cattle sheds, CS, in Shahi Payan Village (Sept 2017- Oct 2018).

Species / Locality	**HD1**	**HD2**	**HD3**	**Total**	**%**	**CS1**	**CS2**	**CS3**	**Total**	**%**	**Overall Total**
Ae. Albopictus							1-0		1	0.1	**1**
An. Culicifascies	30-1	20-1	18-0	70	8	18-7	17-1	11-1	55	6	**125**
An. Fluviatilis	10-0	12-6	7-0	35	4	40-5	67-3	34-0	149	17	**184**
An. pulcherrimus						1-0	2-0		3	0.3	**3**
An. stephensi	0-5	13-7		25	3	30-4	34-2	8-0	78	9	**103**
Cx. bitaeniorhyncus	1-0		2-0	3	0.3						**3**
Cx. quinquefasciatus	79-263	107-113	56-81	699	84	64-121	123-75	121-101	605	68	**1304**
Total	120-269	152-127	83-81	355-477		153-137	244-81	174-102	571-320		**926-797**
	389	279	164	832		290	325	276	891		**1723**

Mosquito abundances

The overall abundance of mosquitoes as reflected by the average room densities differs significantly in the three study villages (chi-square= 186.31; df=2; $p > 0.05$). The average room density of *Anopheles culicifacies* and *Anopheles fluviatilus* was higher in Hajji Banda than in Shahi Payan, while that of *Culex quinquifasciatus* was excessively higher in Shahi Payan than Hajji Banda. The average room density of *Culex quinquifasciatus* was comparable in the two study villages of Bara Gate and Shahi Payan.

Table 5. Comparison of mosquito abundance in A-human dwellings, B- cattle sheds in the three study villages of Peshawar based on total collections using flit from 2017- 2018.

	A- HUMAN DWELLINGS			
Species / Locality	**BARA GATE**	**HAJJI BANDA**	**SHAHI PAYAN**	**TOTAL**
Ae. Albopictus	1			1
Ae. Unilineatus		8		8
An. Culicifascies	1	86	70	157
An. Fluviatilis	2	68	35	105
An. Stephensi	3	90	25	118
Cx. bitaeniorhyncus		16	3	19
Cx. quinquifasciatus	768	95	699	1562
Cx. tritaeniorhynchus	1	23		24
Cx. Vishnui	3			3
Total	577-202	272-114	355-477	1204-793
	779	386	832	1997
	B- CATTLE SHEDS			
Species / Locality	**BARA GATE**	**HAJJI BANDA**	**SHAHI PAYAN**	**TOTAL**
Ae. Albopictus			1	1
Ae. Unilineatus		1		1
An. culicifascies	28	104	55	187
An. Fluviatilis	60	62	149	271

An. pulcherrimus			3	3
An. stephensi	46	89	78	213
An. subpictus		27		27
Cx. bitaeniorhyncus		23		23
Cx. quinquifasciatus	1341	189	605	2135
Cx. tritaeniorhynchus	32	18		50
Cx. Vishnui	19			19
Mn. uniformis	1			1
Total	792-735	341-172	571-320	
	1527	513	891	2931

Mosquito Abundance in Cattle Sheds

The overall abundance of mosquitoes, as reflected by the average cattle shed densities, differs significantly in the three study villages (chi-square 52.33; df = 2; $p > 0.05$). On the whole, the abundance of mosquitoes was significantly greater in Bara gate, followed by Shahi Payan and Hajji Banda village. The average number of mosquitoes per cattle shed of *Anopheles culicifacies* and *Anopheles stephensi* was higher in Hajji Banda than in the rest of the two villages (Table 5), whereas *Anopheles fluviatilis* was higher in Shahi Payan, and *Culex quinquifasciatus* was higher in Bara gate village. The sole specimens of *Aedes albopictus* were caught from Shahi Payan only, *Aedes unilineatus* from Hajji Banda only, and *Mansonia uniformis* from Bara gate village only. As was the case with collections from resting sites, *Anopheles pulcherrimus, Anopheles subpictus,* and *Mansonia uniformis* were only caught from cattle sheds. The rest of the species were alike in both the study hosts.

Seasonal variations

The study on seasonal variation starts from October, comprising 9% of the total mosquitoes, followed by 9.4% of the total collected mosquitoes in November. The population of mosquitoes decreased markedly in December, January and increased gradually in the spring season. This was obviously due to severe winter conditions The mosquito population shows an increasing trend in February and March with a peak in April enclosing 20% of total mosquitoes followed by 15% in May and gradually declining in June (14%) with a record maximum temperature of 39 ˚C followed by a steep downward decrease of mosquito population in July (8%), August (8%) and September (7%) (Fig 2).

Culex quinquifasciatus was found to be a terribly cold-tolerant species throughout the study period, even at a temperature of 13˚C. It was found to be a Dominant and Constant species after Trojan (1992) and Dzieczkowski *et al*. (1972) levels of density and distribution statuses, respectively (Table 7). Its highest density occurred in early summers, particularly in April, followed by two successive months exceeding the month, and gradually declines in the

monsoon, and thereafter a second wave of increase is apparent in the post-monsoon season. Among *Anopheles* species*, Anopheles culicifacies* (7%) and *Anopheles fluviatilus* (8%) were found throughout the study period, even in the coldest months of December, January, and February, but their densities declined due to extreme cold (Table 6). *Anopheles stephensi* (7 %) was found absent in the coldest period of the study. All of these Anopheline species were Dominant and Constant species, acquiring a Bimodal distribution showing towering peaks in early summers and late Autumn (Table 7).

Table 6. Seasonal variation in species composition and relative abundance of indoor-resting mosquitoes of Peshawar (Oct 2017- Sept 18).

Months/ Species	Temperature ˚C	*Ae. albopictus*	*Ae. unilineatus*	*An. culicifascies*	*An. fluviatilis*	*An. pulcherrimus*	*An. stephensi*	*An. subpictus*	*Cx. bitaeniorhyncus*	*Cx. quinquifasciatus*	*Cx. tritaeniorhynchus*	*Cx. vishnui*	*Mn. uniformis*	Total	%
Oct	19		7	27	43		25	12		315	17			**446**	**9**
Nov	19			39	42		32		5	343				**461**	**9.4**
Dec	15	1		9	13		15			35				**73**	**1.5**
Jan	13			4	9					30				**43**	**0.9**
Feb	18			1	7					161				**169**	**3.4**
March	18			7	41		5		2	123				**178**	**4**
April	19			32	63		41			850			1	**987**	**20**
May	33			24	30		29		4	633				**720**	**15**
June	39	1		52	37		41		5	564	11			**711**	**14**
July	35		2	29	19		34		1	297	14	7		**403**	**8**
Aug	37			83	37	1	71	8	16	144	15	5		**380**	**8**
Sept	36			37	35	2	38	7	9	202	17	10		**357**	**7**
Total		**2**	**9**	**344**	**376**	**3**	**331**	**27**	**42**	**3697**	**74**	**22**	**1**	**4928**	

The only specimen of *Aedes albopictus* was captured in the coldest month of December (15 ˚C) and the hottest month of June (39 ˚C) from cattle sheds. The rest of the months were found unconcerned with their distribution. A species thriving under two extremes, *Aedes unilineatus* (1%), was captured in July at 35˚C and in October at 19˚C from human dwellings as well as from cattle sheds. The least occurring species, *Culex bitaeniorhyncus* (0.2%), was found after hot summers, i.e., June and July, whereas *Anopheles pulcherrimus* (0.2%) was collected in the post-monsoon seasons of the year, i.e., August and September, respectively.

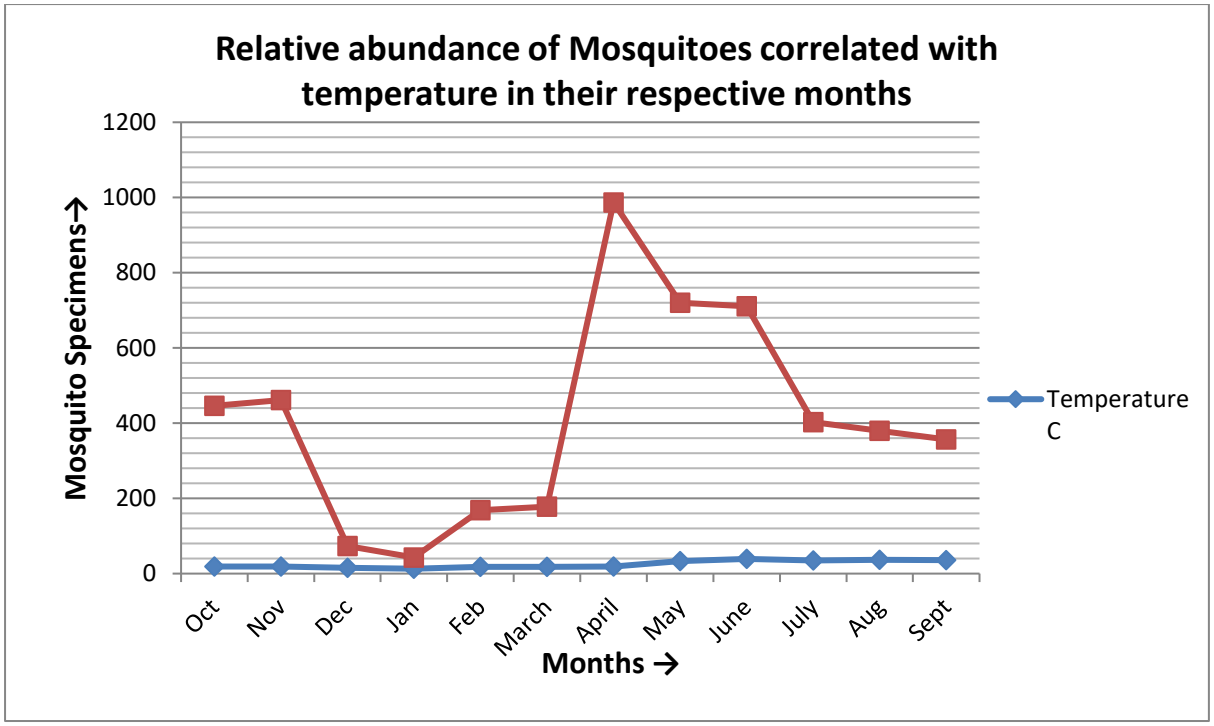

Figure 2. Seasonal Abundance of indoor-resting mosquitoes w.r.t temperature in the study villages of Peshawar (Oct 2017- Sept 18).

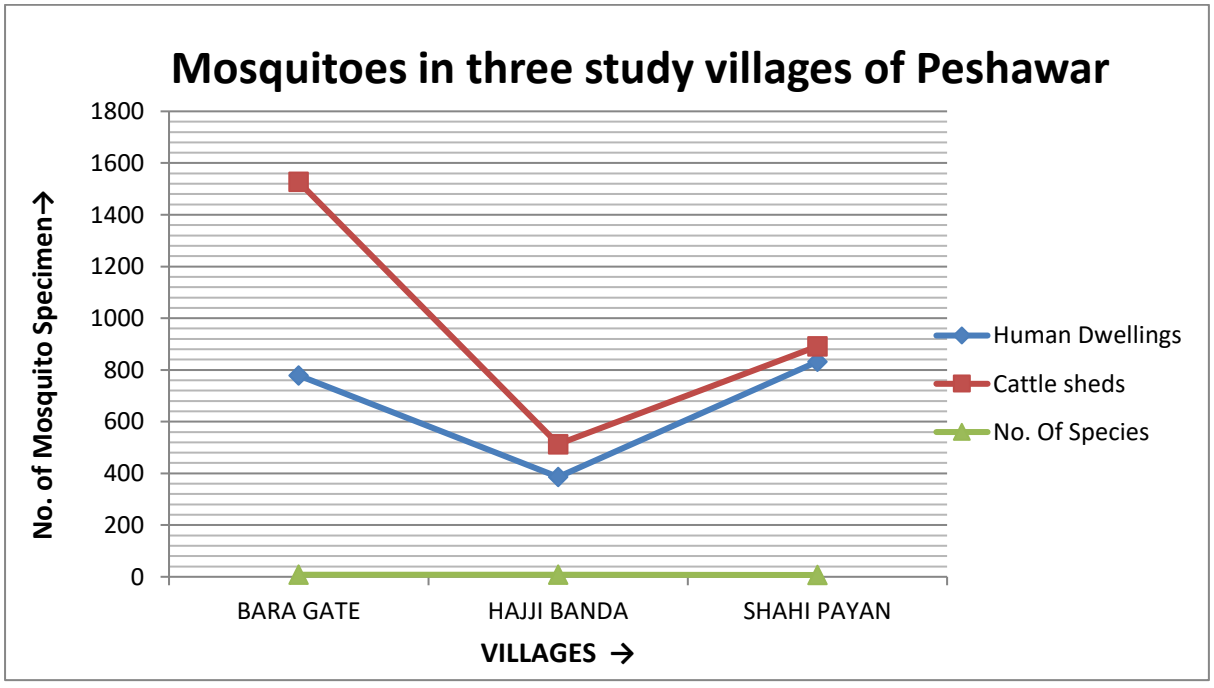

Figure 3. Seasonal Abundance of indoor-resting mosquitoes in Human dwellings and Cattle sheds of three villages of Peshawar (Oct 2017- Sept 18).

Table 7. Comparative assessment of species composition and relative abundance of indoor-resting mosquitoes in the three study villages of Peshawar (Oct 2017- Sept 18).

Species / Locality	BARA GATE	HAJJI BANDA	SHAHI PAYAN	TOTAL	Relative abundance	Relative abundance status	Distribution C=nb/Nx100%	Distribution status
Ae. albopictus	1	-	1	2	0.04%	Satellite	2/3= 67%	frequent
Ae. unilineatus	-	9	-	9	0.2%	Satellite	1/3= 33%	Infrequent
An. culicifascies	29	190	125	344	7%	Dominant	3/3= 100%	Constant
An. fluviatilis	62	130	184	376	8%	Dominant	3/3= 100%	Constant
An. pulcherrimus	-	-	3	3	0.1%	Satellite	1/3= 33%	Infrequent
An. stephensi	49	179	103	331	7%	Dominant	3/3= 100%	Constant
An. subpictus	-	27	-	27	0.5%	Satellite	1/3= 33%	Infrequent
Cx. bitaeniorhyncus	-	39	3	42	0.9%	Satellite	2/3= 67%	Frequent
Cx. quinquifasciatus	2109	284	1304	3697	75%	Dominant	3/3= 100%	Constant
Cx. tritaeniorhynchus	33	41	-	74	1.5%	Subdominant	2/3= 67%	Frequent
Cx. vishnui	22	-	-	22	0.4%	Satellite	1/3= 33%	Infrequent
Mn. uniformis	1	-	-	1	0.02%	Satellite	1/3= 33%	Infrequent
TOTAL	**2306**	**899**	**1723**	**4928**				
Species per site	**8**	**8**	**7**					
Shannon-Wiener Diversity(H^-)	**0.43**	**1.78**	**0.80**					
Margalef's Richness (Me)	**0.90**	**1.03**	**0.8**					
Pielou's Evenness (E)	**0.21**	**0.9**	**0.4**					

Species Diversity

Assessment of mosquito species diversity in various villages reported that Hajji Banda possesses a highly diverse mosquito fauna with a higher index value (H^- = 1.78), followed by Shahi Payan (H^- = 0.80), and Bara Gate (H^- = 0.43). The Shannon-Wiener diversity indices are nearly comparable among the villages (Table 7). Margalef's richness index was highest for Hajji Banda, indicating a fairly high abundance of mosquito species in this village (Table 7). Similarly, species evenness (*Pielou's Evenness*) was also highest in Hajji Banda (0.9), suggesting that species are evenly distributed in this village. The other villages showed lower evenness, indicating less uniform distribution of species (Table 7).

Discussion

The studies conducted in three villages in Peshawar yielded a total of 12 mosquito species from indoor resting sites, including human dwellings and cattle sheds. The mosquito fauna comprised five species of *Anopheles*, four species of *Culex*, two species of *Aedes*, and one species of *Mansonia*. Of these, nine species were recovered from human dwellings and twelve from cattle sheds. Periodic surveys at Bara Gate and Hajji Banda reported eight species each, while Shahi Payan yielded seven species. A comparison of species composition based on indoor-resting collections indicated minimal differences among the localities regarding

common species. However, some variation existed concerning less common species. For example, *Cx. vishnui* and *Ma. uniformis* were encountered in Bara Gate but not in Hajji Banda or Shahi Payan. Conversely, *Ae. unilineatus* and *An. subpictus* were found in Hajji Banda but not in the other villages. Additionally, *An. pulcherrimus* was only found in Shahi Payan, with the other two villages devoid of this species.

The relative abundance of indoor-resting mosquitoes varied as well. *Cx. quinquefasciatus* was the most frequently occurring species across all study areas, followed by *An. fluviatilus* in Bara Gate and Shahi Payan, whereas in Hajji Banda, it was followed by *An. culicifacies*. From the total 4928 mosquitoes collected, *Cx. quinquefasciatus* constituted 75%, likely due to its anthropophilic, nocturnal, and indoor resting behavior. This finding aligns with the mosquito collections reported by Mgbemena et al. (2015) in Nigeria. *Cx. quinquefasciatus* is a primary vector for West Nile Virus and lymphatic filariasis and is highly abundant globally and locally in Pakistan (Ma et al., 2016; Akram et al., 2009; Khan et al., 2015; Gul et al., 2022).

In terms of density, Bara Gate had the highest mosquito abundance, attributed to its dense population, polluted surroundings, and presence of cattle in crowded environments. Shahi Payan was second, while Hajji Banda recorded the lowest, likely due to lower population density and less vegetation or aquatic habitats (Table 7). Human activity influences the formation of breeding habitats, as higher human activity correlates with increased aquatic sites for mosquito larvae (O'Meara, 1997). Seasonally, mosquito prevalence varied. No mosquitoes were found in Bara Gate and Hajji Banda during December and January, but several species, including *Ae. albopictus*, *An. culicifacies*, *An. fluviatilus*, *An. stephensi*, and *Cx. quinquefasciatus*, were present in Shahi Payan year-round. The population peaked in April, declined gradually, and sharply decreased by September. A similar pattern was noted by Gul et al. (2022) in Mardan, KP, probably due to rainfall providing breeding sites. Post-monsoon, *Cx. quinquefasciatus* was particularly abundant, consistent with Gul et al. (2022). Mosquito density per room was higher in cattle sheds than in human dwellings, with about 60% of the total mosquitoes (2931/4928) found in cattle sheds, reinforcing their attractiveness and importance in control efforts.

This study highlights key vectors for disease transmission, including *An. culicifacies*, *An. stephensi*, *An. fluviatilus*, *Cx. quinquefasciatus*, *Aedes albopictus*, and *Cx. tritaeniorhynchus*. *An. culicifacies* is the principal malaria vector in Pakistan, particularly in tribal areas (Sharma and Dev, 2015), while *An. stephensi* is a significant urban vector (Rehman and Muttalib, 1967; Reisen et al., 1982). Both species contribute to the transmission of malaria, often co-occurring and intensifying disease risk. *An. fluviatilus* also plays a role in malaria transmission in some

regions. The genus *Culex* includes important vectors such as *Cx. tritaeniorhynchus*, which transmits Japanese encephalitis, and *Cx. pipiens quinquefasciatus*, responsible for lymphatic filariasis in Pakistan and India (Peiris and Amerasinghe, 1994). *Aedes albopictus* (the Asian tiger mosquito) is a primary vector for dengue and has been shown to transmit chikungunya; while *Aedes aegypti* is traditionally regarded as the main vector of dengue virus, *Ae. albopictus* also poses a significant potential vector (Waldock et al., 2013; Collantes et al., 2015; Rosen et al., 1983; Ryan et al., 2004).

Historical data on mosquitoes in KP is sparse, with Suleman et al. (1993) reporting 15 species via indoor-resting collection, while our study identified 12 species based on spray sheet collection. This variation may be due to different sampling methods. Shah (1997) reported 14 species from indoor collections, with some species like *An. maculatus*, *An. splendidus*, *Cx. theleri*, *Cx. pseudovishnui*, *Ae. caspius*, and *Ar. subalbatus* not encountered here. Conversely, *Ma. uniformis* was found in our study but not by Shah (1997).

It's important to recognize that species composition, abundance, and seasonal patterns can vary annually even at the same site, reflecting complex mosquito ecology (Suleman et al., 1993). Long-term studies are needed to understand seasonal trends and environmental influences. Absence of a species during a survey does not confirm its permanent absence, as distributions can shift spatially and temporally. For example, *Cx. vishnui* and *Ma. uniformis* were observed in Bara Gate but not in Hajji Banda or Shahi Payan, while *Aedes unilineatus* and *An. subpictus* were found only in Hajji Banda. *An. pulcherrimus* was exclusive to Shahi Payan, with other locations lacking this species. This survey provides valuable insights into the species composition, seasonal dynamics, and resting site preferences in rural districts. The high prevalence of *Cx. quinquefasciatus* throughout the villages underscores its dominance, consistent with its role as a major vector in the region. Their adaptation to indoor resting and nocturnal activity makes control challenging. *Ae. albopictus*, which prefers dense vegetation and bites during dusk and dawn, had low representation, possibly due to collection timing, but remains a concern as a dengue vector (Gul et al., 2022). Resting site surveys are essential for formulating effective vector control strategies. Reducing mosquito populations at resting sites, especially cattle sheds and indoor environments, can significantly lower disease transmission risk (Mgbemena et al., 2015). The persistence of mosquito-borne diseases in Peshawar's rural, suburban, and urban areas highlights the need for ongoing surveillance and integrated vector management (IVM). Different trapping techniques influence capture efficiency; for example, human landing catches are considered the gold standard but raise ethical concerns due to exposure risk (Kline, 2006; Mgbemena et al., 2015; Ndiath et al., 2011). Our study used resting

site and spray sheet collection methods, which proved reliable and showed good correlation with human landing data.

Conclusion

This research confirms that District Peshawar harbors diverse mosquito fauna, including medically important vectors like *An. stephensi*, *An. culicifacies*, *Ae. albopictus*, and *Cx. quinquefasciatus*. Notably, *An. Stephensi* and *An. Culicifacies* are primary malaria vectors, *Ae. albopictus* transmits dengue, chikungunya, and potentially encephalitis, and *Cx. quinquefasciatus* is involved in filariasis transmission. The study emphasizes the importance of continued surveillance, particularly focusing on cattle sheds, which house a higher mosquito density. Seasonal peaks, especially in post-monsoon periods, necessitate targeted control efforts during these times to safeguard the population.

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