

Relative Abundance, Distribution, and Diversity of Mosquitoes in District Peshawar, Charsadda, and Swabi

ABSTRACT

Aims: To date, there are 3601 named mosquito species recognized worldwide representing the diverse and medically important fly family *Culicidae*. In this study, after collection and identification of mosquitoes from the study area, their diversity, relative abundance (RA), and distribution (C) were evaluated for the prevention of mosquito-borne diseases.

Place and Duration of Study: Mosquitoes in their immature and adult stages were collected from their habitats in district Peshawar, Charsadda, and Swabi from September 2021 till April 2022.

Study design: The immature samples were collected from various places such as pots, small water containers, bushy places under trees, and from the edges of some open drains. While the adult mosquitoes were collected during the same period by using the light trap and *Pyrethrum spray* methods for outdoor and indoor environment, respectively.

Methodology: 252 and 295 mosquitoes in their larval and pupal, and adult stages, respectively, were collected and brought to the laboratory for rearing and were observed on daily basis. All the adult specimens were preserved using silica gel in test tubes. The specimens were kept in entomological boxes after rearing and preservation for identification. Taxonomic identification of the adult mosquitoes was made using the key provided in the fauna of British India.

Results: The identification showed the presence of four species belonging to three genera: *Culex quinquefasciatus*; *Aedes aegypti*, *Aedes albopictus*; and *Armigeres subalbatus*. *Culex quinquefasciatus* was the most abundant specie found at all the study sites (C=100% and RA=44.24%), while *Armigeres subalbatus* was found to be an infrequent specie (C=25% and RA=20.66%). Several indexes were also calculated to find out the diversity of the mosquitoes. The Shannon-Weiner indexes (H) for the month of April and December are 1.32 and 0.60, respectively, representing highest and lowest diversities among the study period.

Conclusion: The study found that *Culex quinquefasciatus*, *Aedes aegypti*, and *Aedes albopictus* are the most prevalent species in the study area and their presence increases the risk of mosquito-borne diseases such as West Nile virus, Filariasis, and Japanese Encephalitis transmission in the area. The study suggests further research to examine the diversity of mosquitoes more thoroughly to design effective vector control strategies and predict the risk of future disease outbreaks.

Keywords: Mosquitoes; Diversity; Species distribution; Relative abundance; Taxonomic identification.

1. INTRODUCTION

Mosquitoes are the main vectors of many human and livestock diseases caused by viruses and parasites (Qasim et al., 2014). Mosquitoes serves as a vector of various deadly diseases including Malaria, Dengue fever, Zika fever, and Chikungunya. The vector causes deaths of more than one million people annually (WHO, 2009). Mosquitoes are one of the most important dipterous insects, and the significance lies in their transmission capability of pathogens and parasites such as virus, bacteria, protozoans, and nematodes which causes an enormous number of tropical diseases, including malaria, dengue, Japanese encephalitis, yellow fever, Rift valley fever, Chikungunya, West Nile Fever, and lymphatic Filariasis (Kettle, 1995). Mosquitoes play an important role in food chains of various ecosystems as well (Rueda, 2008). Pakistan is a subtropical country, so it is under a great threat of vector borne diseases mainly due to the climate of this country is suitable to harbor the outbreaks of many diseases.

Like other Asian countries, Pakistan is also withstanding substantial climatic changes that are sufficient to harbor the outbreaks of most mosquito borne diseases (Ashfaq et al., 2014). The shift in the climate of South Asian countries tends to escalate the incidence of mosquito-borne diseases (Ramasay and Sunderan, 2012). There are 134 species of mosquitoes found in Pakistan belonging to Anophelinae and Culicinae sub-families. Pakistan is one of those subtropical country that has been under the threat of

vector-borne diseases mainly due to its climate, which is suitable to support the outbreaks of many diseases. Moreover, the abiotic factors like temperature, humidity, and rainfall are positively supporting the population of mosquitoes with increased sustainability of their breeding sites and long survivorship (Sutherst, 2004; Reiter 2001). The massive mosquito population along with the increased sustainability of their breeding sites is significantly supported by the temperature, humidity, and rainfall patterns in Pakistan. Likewise, certain studies have been conducted in various regions of Pakistan reporting the mosquito fauna that have related the relative abundance of different species with the gradual shift in climatic trend (Manzoor et al., 2020).

Mosquitoes are cosmopolitan and are found in all types of habitats such as sewage water, stagnant water, and fresh water. Many species of mosquitoes are adaptable to their specific habitats such as *Aedes* species are adaptable to cooler regions where their eggs are more dominant compared to the warmer regions (Gadahi et al., 2012). Activities of mosquitoes vary from species to species. Some species are diurnal and others are nocturnal while many others are crepuscular. However, some highly aggressive species are known to feed both in day and night. Majority of the mosquitoes are nocturnal in their habitat. Some mosquitoes are attracted to a host with their choices, for example, odor of skin, temperature, moisture or visual cues (Steib et al., 2001). Mosquito bite almost any animal big enough to provide them with a blood meal. Some species are host specific. Host specificity for blood feeding by mosquitoes play an important role in diseases transmission. The great diversity mosquitoes (31%) are found in Neotropical region (Khan, J. et al., 2015). Mosquitoes can be classified on the basis of morphology with the help of specific identification keys and differentiation between male and female species is done from their wings and palps (Das et al., 1990; Shepard et al., 2006).

According to the World Health Organization, globally around one-sixth of the global population is affected by vector-transmitted diseases, and among them, dengue fever is the world's fastest growing disease. It has also been reported that till now approximately 124 countries are affected by DENV, of which about 2.5 billion people (almost one-third population of the world) are considered at high risk (WHO, 2011). *Aedes* genus is responsible for the spread of DENV virus while its most prominent carrier is *Aedes aegypti* (Lai et al., 2000).

In Pakistan, the first outbreak of dengue fever was reported in 1994 in Karachi, affecting around 1000 people while causing 2 deaths (Chan et al., 1995). Particularly, in Khyber Pakhtunkhwa, dengue became uncontrollable in 2017 affecting 74820 people. 54 people died out of 15828 confirmed cases. During the outbreak of dengue in 2011 few Chikungunya cases were also reported (Halstead, 2015). The mosquitoes were identified through taxonomic keys provided in "The fauna of British India."

The main objective of this study is to describe the relative abundance, diversity, and distribution of mosquitoes in some specific areas of district Peshawar, Swabi, and Charsadda. This study is crucial as it quantifies the diversity, distribution, relative abundance, and surveys of various species of mosquitoes in the mentioned districts. In this study, both immature and mature mosquito collection was made from a few localities of three districts of Peshawar (Lalazar colony), Charsadda (Sherpao) and (Malakabad) and Swabi (Sairi) during the active season of mosquito when both immature and adult stages could be found. Taxonomic identification of mosquitoes was based on morphological characters using key characters from fauna of British India. Several indices were also used to identify species richness, diversity, and rarity of species for the prevention of mosquito-borne diseases. Moreover, distribution as well as relative abundance were also calculate to perceive mosquito-borne diseases in the specific study sites. Global climate change, enhanced anthropogenic activities, and fast communication means has increased the chances of vector-borne diseases, making it crucial to evaluate the status of mosquito fauna in various geographical localities. This study will be helpful to the scientists working in the field of epidemiology, entomology, and other relevant fields in the mitigation of mosquito-borne diseases.

2. LOCATION AND CLIMATE OF THE STUDY AREAS

The study was conducted in three different districts of Pakistan namely district Charsadda, which lies between 34.1682° North and 71.7504° East; district Peshawar, which lies between 34.0151° North and 71.5249° East; and district Swabi, which lies between 34.1241° North and 72.4613° East.

District Charsadda experiences an extreme climatic conditions. The summer season persists from the month May to September. The month of June is extremely hot and dry with temperature above 40°C. A rainy monsoon period extends from July to September. The month of July and August are very humid. Changes in the weather are observed in the month of October due to gradual decrease in the temperature. From the month of December to mid of February, this area experiences extreme cold

weather. The pleasant period of the year is spring, which comes in the middle of March. There are three spells of rainy season in a year: the winter rainfall, the spring rain fall in the months of March and April, while there is a highest rainfall in the August. Similarly, district Peshawar has an extreme climate. The summer season is very hot. There is a rise in temperature from May to June. The temperature reaches to its maximum in the month of June. The coldest month is January. Similar to the above two districts, Swabi also experiences an extreme climate. The summer season is very hot. May and June are the hottest and dry months. During the months of May and June, dust storms are frequent at night time. Due to intensive cultivation and irrigation, the tract is humid and heat is oppressive. A rapid fall of temperature is recorded from October onwards. The coldest month is January. Towards the end of the cold weather, there are occasional thunder storms and hill storms. The maximum rainfall is received in July and August during which the weather becomes hot and humid.

3. MATERIAL AND METHODS

As mentioned above, collection of both immature and mature mosquitoes was made from several localities in three districts of Khyber Pakhtunkhwa including: Peshawar: Lalazar Colony (located at 34.0116° North and 71.4759° East), Charsadda (Sherpao and Malakabad, located at 34.2636° North and 71.6985° East and 34.146° North and 71.7642° East, respectively), and Swabi (Sairi, located at 34.1707° North and 72.4060° East) during the active season of mosquitoes, when both the immature and adult stages could be found.

Mosquitoes in their immature stages were collected from all the three studied sites during the months September and April from standing water. The collection was carried out from pots and other small containers with water in places such as near bushes, under the trees, and at the edges of some drains. Regular inspection of these sites was made to look for the presence of mosquito larvae. In case of drains and other small water bodies, a dipper was used for collecting the immature mosquitoes by dipping it into the targeted water, and the entity collected in it was then shifted into a jar. The collected larvae were brought to the laboratory for rearing and were observed on daily basis. While for more efficient collection, a strainer was used for concentrating and cleaning of the larvae and pupae from the dirty contaminated water. These collected larvae and pupae were placed in bottles half filled with water while leaving some space vacant so that upon emergence, the adult will not be drawn in water. A cloth netting was used to cover mouth of the bottle in order to avoid the escape of adult mosquitoes. A rubber band was tied to keep the net tightly onto the bottle. On attainment of adulthood, mosquitoes were collected using aspirator and transferred into the tubes, which were placed in the freezer for a few minutes to kill them.

For the collection of adult mosquitoes, the following two methods were practiced: (i) Light trap method, (ii) Pyrethrum spray catch collection method. The first method was carried out for trapping outdoor mosquitoes, while the second method was performed indoor mosquitoes. In the first method, a light trap was installed outside at the most suitable place for overnight (i.e., from early evening to sunrise). Mosquitoes from nearby environment got attracted towards the light and were successfully trapped upon coming in contact with the device. When enough mosquitoes were trapped, they were collected from the light trap using an aspirator. The aspirator consisted of a glass tube and a rubber tube. The glass tube was kept in the cage while the rubber tube was held in the mouth and upon sucking, the mosquitoes were drawn into the glass tube and then emptied into a bottle. This bottle was then sealed with a cotton swab soaked in chloroform in order to kill the mosquitoes.

With the help of the Pyrethrum spray catch collection method, adult mosquitoes were collected from indoor environments such as bedrooms, washrooms, and cars. Collection from the houses was made by placing white sheets on places such as floor, beds, and other flat surfaces. The targeted area was thoroughly sprayed with a pesticide and made airtight by closing the doors and windows for few hours. The fallen mosquitoes were collected in small tubes using a fine brush with gentle care so that body parts of the mosquitoes were not damaged. Mosquitoes were collected from vehicles parked in houses as well. In such cases, white plain sheets were spread on the seats and the windows were kept open overnight so that the adult mosquitoes could easily enter the car. Early in the morning, the inside of the vehicles was sprayed with pesticide spray while the doors and windows of the vehicles were closed. After a few hours, dead mosquitoes were collected using a brush and placed in preservation tubes.

4. DATA ANALYSIS METHODS

The distribution status of collected mosquito species in different collection sites was calculated by the equation given below:

$$\text{Distribution (C)} = \frac{n}{N} \times 100 \quad \text{Eq. 1}$$

Where n is the number of sites where mosquitoes were found, and N is the total number of sites analyzed. Based on the values of C, the species were categorized into the given classes: Constant species (80-100%), frequent species (60-80%), moderate species (40-60%), infrequent species (20-40%), and sporadic species (0 – 20%).

The relative abundance of mosquitoes was determined by:

$$\text{Relative Abundance (RA)} = \frac{n}{N} \times 100 \quad \text{Eq. 2}$$

Where n is the number of mosquitoes and N is the number of mosquito species. Based on the values of RA, the species were categorized into the given classes: Satellite species (RA < 3%), Sub-dominant species (3-10 %), and Dominant species (RA > 10%).

Several indices were also calculated to find out the diversity of the mosquitoes. In this lieu, Simpson Index (D) was applied to find out the rarity of the mosquito species collected. It can be calculated by the given expression:

$$D = \frac{\sum n(n-1)}{N(N-1)} \quad \text{Eq. 3}$$

Where n is number of mosquitoes of individual species and N is the total number of mosquito species collected.

Similarly, Margalef's Richness Index (Dmg) was calculated to find out mosquito species richness. It is given by the following expression:

$$Dmg = (S - 1) / \log N \quad \text{Eq. 4}$$

Where S is total number of species and N is the total number of individuals in a sample.

Lastly, Shannon-Weaver Index (H) was calculated to find out the diversity of the mosquitoes with regard to monthly variation. It can be calculated by the given expression:

$$H = -\sum [(pi) \times \log (pi)] \quad \text{Eq. 5}$$

Where pi is the proportion of individuals of i-th species in a whole community, pi is equal to $\frac{n}{N}$, where n = importance value index of the species and N = importance value index of all species.

5. PRESERVATIONS AND IDENTIFICATION OF THE MOSQUITOES

All the adult specimens were kept and preserved in test tubes and were studied later on. Silica gel was used as a preservative, which absorbs the moisture from the specimens, and thus perfectly preserving them. In this regard, a small amount of silica gel was added into the bottom of each test tube, which was then covered with a cotton swab. After that, mosquitoes were transferred to these test tubes. Each of the test tube was covered with a cork to avoid the entrance of any further moisture from the external environment. Taxonomic identification of adult mosquitoes was made using the key provided in the fauna of British India. Moreover, some relevant pictures and details of taxonomic description available in the internet were also frequently used for this purpose.



Fig. 1. Sample preserved mosquitoes in test tubes.

6. RESULTS

Taxonomic study of the mosquito samples revealed that the collected mosquitoes belonged to three genera, i.e. *Culex*, *Aedes*, and *Armigeres*. Genus *Aedes* was represented by two species: *Aedes albopictus* and *Aedes aegypti* while the remaining two genera's representation was done by a single specie each, i.e. *Culex quinquefasciatus* and *Armigeres subalbatus*.

In the following, key characteristics of the discovered species after thorough taxonomic identification are discussed in detail. *Culex quinquefasciatus* has palpi that are much shorter than its proboscis having no post spiracular bristles while the first segment of its fore tarsus is as long as the remaining four segments (vid. Fig. 2(a)). Its proboscis is straight, and it lacks lower mesepimeral bristles, a median white band on its proboscis, and white banding on its tarsi. It also lacks a white scale patch in the post-spiracular area, and its abdomen is banded with white and dark colors, pleuron without conspicuous white and black markings. Its mid-femur lacks a longitudinal stripe, and its pleuron lacks parallel black lines, but has scale patches present. Its mesonotum is brown to light brown in color.

Aedes albopictus, which is also known as Asian tiger mosquito, has white bands on its legs and body, giving it a striped appearance, much like a tiger (vid. Fig. 2(b)). Its palpi are much shorter than its proboscis. Post spiracular bristles are also present. Its wing scales are oval, without green stripes on the mesonotum. The second marginal cell of its wings is longer than its petiole, and there is a fine lateral line. Its proboscis is uniform in shape and straight. There is not much conspicuous white scaling on the abdomen sternites. The wing scales are uniformly dark, and the tarsi have conspicuous white bands. The proboscis does not have a median white band, and all the tibiae do not have a median white band. The mesonotum has a narrow white stripe running through its entire length.

The palpi of *Aedes aegypti* are much shorter than its proboscis (vid. Fig. 2(c)). It has post spiracular bristles which are present. The wing scales are oval in shape without any green stripes on the mesonotum. The second marginal cell of the wing is longer than its petiole, found on fine lateral line. The proboscis is uniform in shape and straight, not much conspicuous white scaling on the abdomen sternites. The wing scales are uniformly dark. The tarsi have conspicuous white bands. The proboscis does not have a median white band and all tibia do not have a median white band. The mesonotum has four narrow white stripes of which the median two stripes are straight while the lateral two stripes are curved which can help distinguish it from *Aedes albopictus*.

Armigeres subalbatus is commonly known as the Asian bush mosquito is shown in Fig. 2(d). The female adult mosquito is characterized by its long proboscis, which is used to siphon blood from humans and animals. The wings are broad and have a distinctive white-striped pattern. Its palpi are much shorter than their proboscis. Further, post spiracular bristles are also present. Wing scales are oval in shapes without green stripes on their mesonotum. Second marginal cell of wing is longer than its petiole on fine lateral line. Proboscis curving downward and laterally are compressed. Conspicuous

white scaling is present on their abdomen sternites (bottom), while absent from their abdominal tergites (top).



(a) *Culex quinquefasciatus*



(b) *Aedes albopictus*



(c) *Aedes aegypti*



(d) *Armigeres subalbatus*

Fig. 2. Pictures of the identified species under the microscope.

6.1. Abundance of mosquito species

Based on the scrutiny of the 547 collected mosquitoes, 242 individuals belonged to *Culex quinquefasciatus* specie (vid. Fig. 3). The *Culex quinquefasciatus* specie accounted for 44.4% of the total number of mosquitoes collected. This is one of the most common mosquito species found in the three districts and is known to be a major vector for disease-causing pathogens such as West Nile virus, Filariasis, and Japanese Encephalitis.

The second most abundant mosquito specie was *Aedes* (192 individuals in total) with 110 and 82 individuals belonging to *Aedes albopictus* and *Aedes aegypti*, respectively (vid. Fig. 3). It was found in districts Charsadda and Swabi only. These mosquitoes are known to transmit diseases such as Dengue, Yellow Fever, Zika virus, and Chikungunya.

Genus *Armigeres* was represented by only one specie, i.e. *Armigeres subalbatus* having total of 113 individuals (vid. Fig. 3). It was only found in district Charsadda. *Armigeres subalbatus* is believed to be source of illness like zoonotic *Brugia pahangi* infections and Zika virus, indicating that the study site where it has been identified is at a higher risk of these diseases. It should be kept in mind that areas where a mosquito specie had a frequency less than five (05) were ignored.

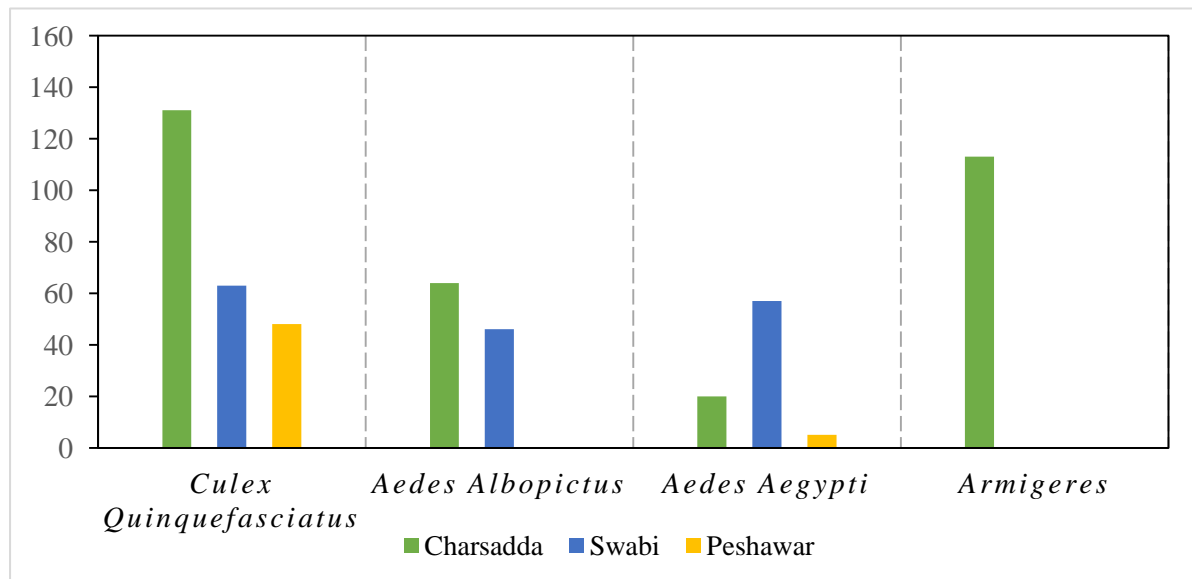


Fig. 3. Relative abundance of the mosquitoes found in the three districts.

6.2. Monthly distribution of mosquito species

6.2.1. *Culex quinquefasciatus*

Fig. 4 illustrates the distribution of *Culex quinquefasciatus* by month and location. From the figure, it is obvious that the distribution of *Culex quinquefasciatus* varies depending on the month (i.e., season) and location. Overall, it can be noted that highest number of *Culex quinquefasciatus* appeared in district Charsadda, while the lowest number of species were found in district Peshawar. Further, highest frequency of the specie was shown in the month of September, while no mosquitoes were found in the months of January and February. Moreover, Swabi showed intermediate number of species as compared to the other two districts.

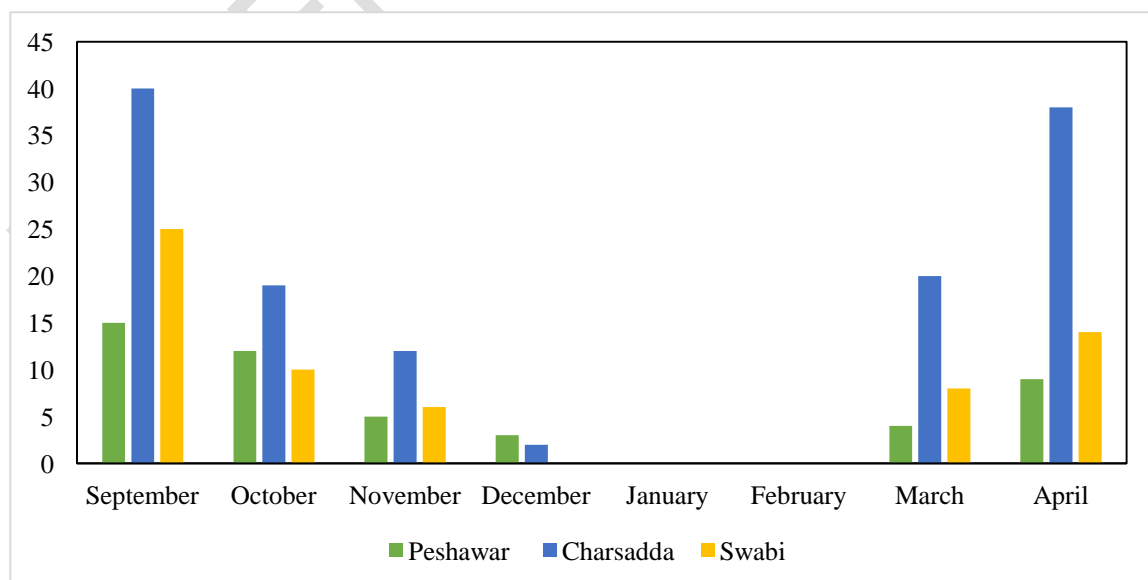


Fig. 4. *Culex quinquefasciatus* monthly and location wise distribution.

6.2.2. *Aedes albopictus*

The monthly and location wise distribution of *Aedes albopictus* shown in Fig. 5. The figure illustrates the abundance of the species in district Charsadda followed by district Swabi, while no mosquitoes were found in district Peshawar. The highest number of mosquitoes were found in September followed by April, October, November, and March. Interestingly, no mosquitoes were detected in the months of January and February. This indicates that the population of *Aedes albopictus* is likely to drop considerably during winter months. This suggests that the species has a specific habitat preference.

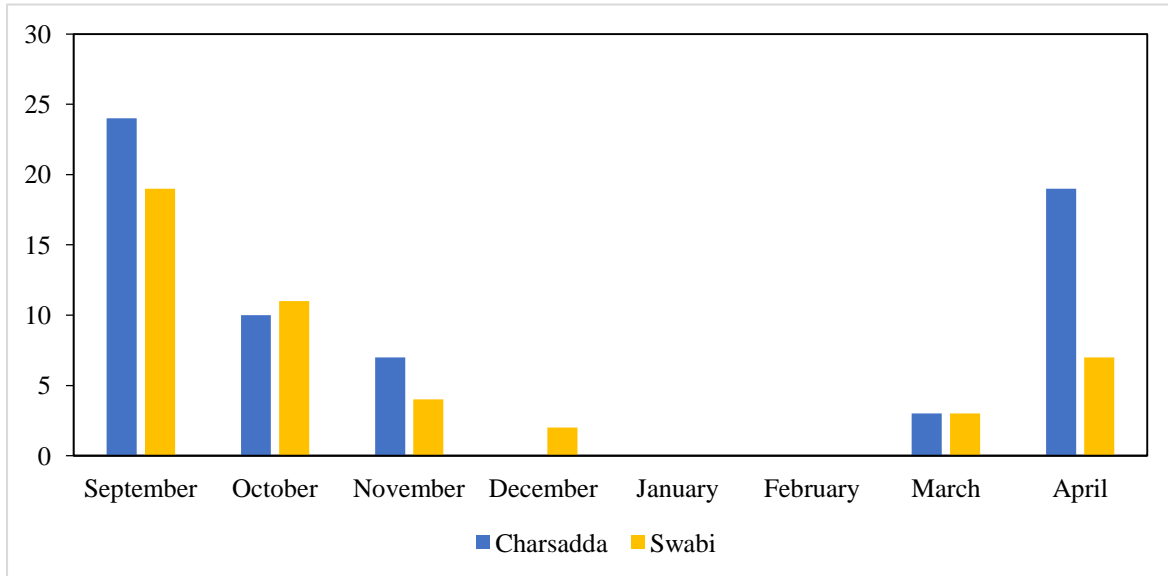


Fig. 5. *Aedes albopictus* monthly and location wise distribution.

6.2.3. *Aedes aegypti*

Aedes aegypti was also among the discovered species across the three districts (vid. **Fig. 6**). The highest concentration of the species was observed in district Swabi. Conversely, the least number of *Aedes aegypti* was recorded in Peshawar, which can be considered insignificant. District Charsadda had an intermediate number of the species when compared to district Swabi and Peshawar.

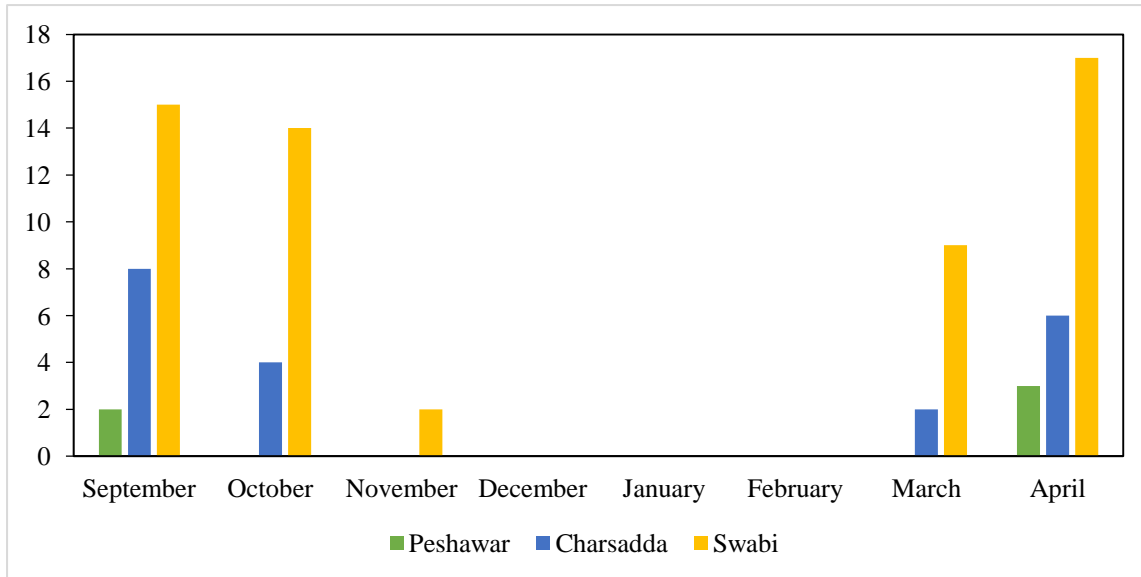


Fig. 6. *Aedes aegypti* monthly and location wise distribution.

6.2.4. *Armigeres subalbatus*

Lastly, *Armigeres subalbatus* was found in district Charsadda only (vid. Fig. 7). The highest population was recorded in the months of September and April, while no individuals were captured in December, January, and February.

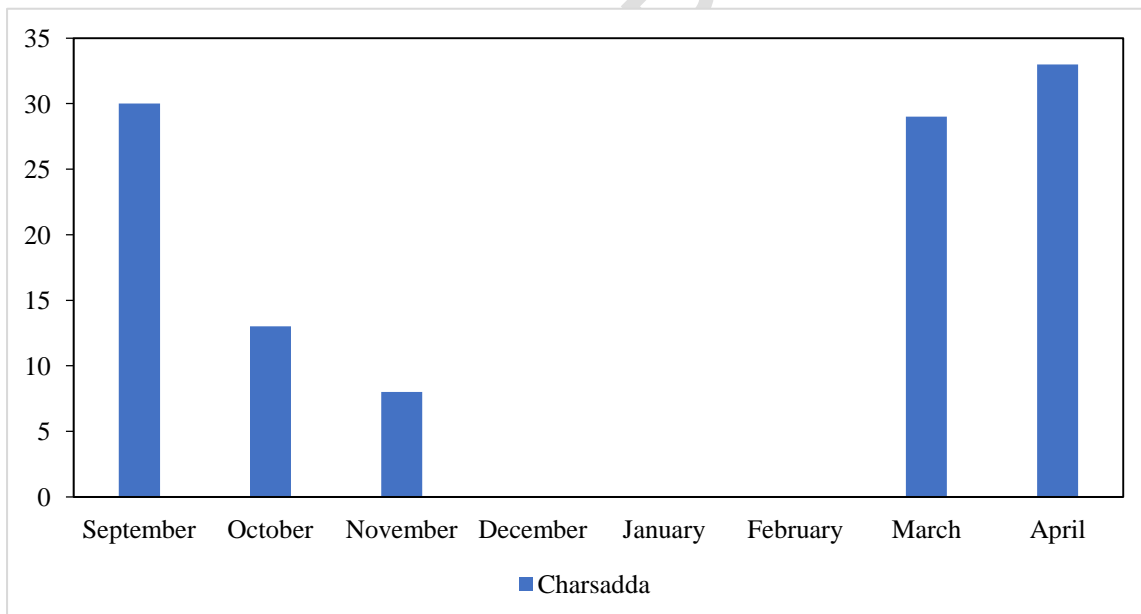


Fig. 7. *Armigeres subalbatus* monthly and location wise distribution.

6.3. Diversity Indices

The information about the diversity, richness, and rarity of the mosquito species is given in Table 1. The largest sample size of mosquitoes was observed in September ($D_{mg} = 1.51$), while the smallest sample size was observed in December ($D_{mg} = 0.2$). According to the Simpson Index (D), the greatest number of rare species were found in December ($D = 0.52$) due to the lowest diversity, and the lowest number of rare species were found in April ($D = 0.25$) due to the highest diversity. Based on the Shannon-weaver Index (H), the highest diversity was observed in April ($H = 1.32$), and the lowest diversity was observed in December. It should be noted that no or very less number of mosquito species were found in the months of January and February. Hence, the values of the calculated indices are written as 0.

Table 1. Diversity indices of the mosquito species in the three districts.

Month	Margalef's Index of Richness (Dmg)	Simpson Index (D)	Shannon- Weaver Index (H)
September	1.51	0.18	1.29
October	0.98	0.29	1.22
November	0.9	0.36	1.14
December	0.2	0.52	0.60
January	0	0	0
February	0	0	0
March	0.76	0.32	1.21
April	1.38	0.25	1.32

The relative abundance (RA) and distribution (C) of the species is shown in **Table 2**. The table illustrates the relationship between the two parameters for each month. The calculations are based on the total population of four species across different months.

Table 2. Distribution and relative abundance of mosquito species in the three districts.

S.No.	Species name	Total number of specimens	Relative abundance (RA)	Distribution (C)	Status
1	<i>Culex quinquefasciatus</i>	242	44.24%	100%	Constant
2	<i>Aedes albopictus</i>	110	20.11%	50%	Moderate
3	<i>Aedes aegypti</i>	82	14.99%	75%	Frequent
4	<i>Armigeres subalbatus</i>	113	20.66%	25%	Infrequent

Culex quinquefasciatus was the most abundant species found at all the study sites with C=100% and RA=44.24%, while *Armigeres subalbatus* was found to be an infrequent species with C=25% and RA=20.66%. Further, *Aedes albopictus* was found to be moderate species with C=50% and RA=20.11%. Lastly, *Aedes aegypti* falls in the category of frequent species with C=75% and RA=14.99%.

7. DISCUSSION

Mosquitoes are important disease vectors, and their diversity and distribution determine the course of disease transmission and the ecological status of the native environment. As discussed above, the taxonomic identification revealed the presence of four species belonging to three genera: *Culex*, *Aedes*, and *Armigeris*, each represented by one species except *Aedes*, which contained two species. The species are *Culex quinquefasciatus*, *Aedes albopictus*, *Aedes aegypti*, and *Armigeres subalbatus*. The most abundant species was *Culex quinquefasciatus*, collected from all the studied sites, followed by *Aedes albopictus* and *Aedes aegypti*, found in two sites, while *Armigeres subalbatus* could only be found at one study site.

Several existing studies have confirmed the presence and abundance of *Culex quinquefasciatus* in various locations in KP province and Pakistan, including Peshawar, Charsadda, Dir Lower, Malakand, and Lahore. The studies were conducted by (Ali and Rasheed 2009; Attaullah et al. 2023; Manzoor et al. 2020; and Khan et al. 2014). As for *Aedes albopictus*, previous studies such as (Khan et al., 2017) has shown its presence in Peshawar, though in other regions of the city as compared to the ones studied in the current study. On the other hand, the specie was widely found in sites at Charsadda and Swabi in the current study. However, no studies could be found pertaining to the presence and distribution of the specie in these two districts. As shown above (vid. **Table 2**), *Aedes aegypti* was found to be a frequent specie in the study sites considered in this research. Existing published literature show that the specie is widely distributed in Peshawar, Charsadda, Lahore, and Swat (clumped distribution

mainly spread by travel and heterogeneous environment), while infrequently distributed in Dir Lower and Malakand owing to the fact that the latter districts have different climatic and ecological conditions (Khan et al., 2017; Khan et al., 2014; Fatima et al., 2016; Attaullah et al., 2023). Lastly, *Armigeres subalbatus*' presence was only restricted to district Charsadda as shown above (vid. Fig. 7). Limited work has been done on the identification and distribution of the specie in the literature.

Some characteristics such as color and size of the mosquitoes observed in the present study were different from those given in the key identification. For example, the color of thorax depends on the food taken at the larval stage. In this study, no artificial food was provided at the immature stages, and they were dependent on the naturally available food in the water collected at the time of sampling. The difference in abundance and species richness may also change due to differences in sampling techniques or due to different ecological conditions.

Some of the species such as *Culex quinquefasciatus* and *Aedes aegypti* were found in unexpected habitats such as bird bath/bushy places and drainage, respectively. Their abundance was very low in such habitats as compared to their favorite habitats. Hence, such data was considered as an outlier and was discarded in the calculation of diversity indices as discussed above.

Average temperature, relative humidity, and rainfall changes are the main reasons for the differences in the diversity of mosquito fauna and other insect species. This is attributed to the fact that the wet season provides more suitable conditions for the development and survival of mosquitoes. The higher abundance of mosquitoes in the wet season is also credited to the increased availability of breeding sites and food sources.

The presence of these species indicate a potential risk of disease transmission in the area. Therefore, public health authorities should put in place effective strategies to reduce the population of mosquitoes and prevent the spread of mosquito-borne diseases. Such strategies can include improving sanitation and hygiene, usage of larvivorous fish to reduce larval populations, insecticides, and bed nets to reduce contact between humans and mosquitoes.

8. CONCLUSION

The current study offers a basis of understanding about the types of mosquitoes found in the study area and their potential as vectors of diseases. The findings of this study can be used for designing effective vector control strategies and for predicting the risk of future disease outbreaks. The main conclusions drawn from this study are given below:

- *Culex quinquefasciatus* is the most prevalent species followed by *Aedes aegypti* and *Aedes albopictus* according to their distribution status. This indicates that the risk of mosquito-borne diseases such as West Nile virus, Filariasis, and Japanese Encephalitis transmission is high around all the specific study area.
- The highest concentration of mosquitoes was observed in September followed by April and October in all the study area.
- District Charsadda and Swabi have a higher likelihood of experiencing diseases such as Dengue, Yellow Fever, Zika virus, and Chikungunya due to the presence of *Aedes aegypti* mosquitoes in those regions.
- The presence of *Aedes albopictus* district Charsadda and Swabi pose a high risk for the contraction of the Chikungunya virus, Dengue, and Dirofilariasis in these areas.
- District Charsadda was the sole location where *Armigeres subalbatus* has been discovered, is at increased risk of zoonotic *Brugia pahangi* infections and Zika virus.

The species found to be potential vectors of diseases showed varying levels of diversity, distribution, and abundance, indicating the potential for disease outbreaks in the study area. Further research is suggested to examine the diversity of mosquitoes more thoroughly by analyzing all potential habitats and throughout all seasons by using advanced molecular and biotechnological techniques. Climatic factors including temperature, humidity, and rainfall need to be addressed with respect to population dynamics of the mosquito fauna which will be helpful for future modelling of the disease course with respect to trends in mosquito diversity, distribution and abundance.

9. REFERENCES

Ali, N. and Rasheed, S.B., 2009. Determination of species composition of mosquitoes found in Palosai stream, Peshawar. *Pak Entomol*, 31(1), pp.47-51.

- Ali, N., Khan, K. and Kausar, A., 2013. Study on mosquitoes of Swat Ranizai sub-division of Malakand. *Pakistan Journal of Zoology*, 45(2).
- Ashfaq, M., Hebert, P.D., Mirza, J.H., Khan, A.M., Zafar, Y. and Mirza, M.S., 2014. Analyzing mosquito (Diptera: Culicidae) diversity in Pakistan by DNA barcoding. *PLoS One*, 9(5), p.e97268.
- Attaullah, M., Gul, S., Bibi, D., Andaleeb, A., Ilahi, I., Siraj, M., Ahmad, M., Ullah, I., Ali, M., Ahmad, S. and Ullah, Z., 2023. Diversity, distribution and relative abundance of the mosquito fauna (Diptera: Culicidae) of Malakand and Dir Lower, Pakistan. *Brazilian Journal of Biology*, 83.
- Chan, M. and Johansson, M.A., 2012. The incubation periods of dengue viruses. *PloS one*, 7(11), p.e50972.
- Das, B.P., Rajagopal, R. and Akiyama, J., 1990. Pictorial key to the species of Indian anopheline mosquitoes. *Pictorial key to the species of Indian anopheline mosquitoes*. 2(3), pp.131-162.
- Fatima, S.H., Atif, S., Rasheed, S.B., Zaidi, F. and Hussain, E., 2016. Species Distribution Modelling of *Aedes aegypti* in two dengue-endemic regions of Pakistan. *Tropical Medicine & International Health*, 21(3), pp.427-436.
- Gadahi, J.A., Bhutto, B., Akhter, N. and Arijo, A.G., 2012. Population diversity of mosquito fauna in and around Tandojam-Pakistan.
- Halstead, S.B., 2015. Reappearance of chikungunya, formerly called dengue, in the Americas. *Emerging infectious diseases*, 21(4), p.557.
- Ilahi, I. and Suleman, M., 2013. Species composition and relative abundance of mosquitoes in Swat, Pakistan. *International Journal of Innovation and Applied Studies*, 2(4), pp.454-463.
- Jabeen, A., Ansari, J.A., Ikram, A., Khan, M.A., Qaisrani, M.I., Khan, S. and Safdar, M., 2021. First Report of *Aedes albopictus* (Diptera: Culicidae) in District Mirpur, Azad Jammu and Kashmir, Pakistan. *Journal of Medical Entomology*, 58(2), pp.943-946.
- Kettle, D.S., 1995. Medical and veterinary entomology. CAB International. Wallingford, UK.
- Khan, G.Z., Khan, I.A., Khan, I. and Inayatullah, M., 2014. Outdoor breeding of mosquito species and its potential epidemiological implications in Khyber Pakhtunkhwa. *Pakistan Journal of Agricultural Research*, 27(4).
- Khan, J., Khan, I., Ijaz, A., Iqbal, A. and Salman, M., 2017. The role of vertical transmission of dengue virus among field-captured *Aedes aegypti* and *Aedes albopictus* mosquitoes in Peshawar, Khyber Pakhtunkhwa, Pakistan. *Pakistan Journal of Zoology*, 49(3).
- Khan, J., Shah, M., Khan, B.T., Naeem, M., Ismail, M., Abbasi, A. and Khan, S.A., 2015. A survey of adult and larval mosquito fauna in Tehsil Daggar and Gagra of District Buner, Khyber Pakhtunkhwa, Pakistan. *Int J Mosq Res*, 2(3), pp.170-4.
- Khanani, R.M., Arif, A. and Shaikh, R., 2011. Dengue in Pakistan: Journey from a disease free to a hyper endemic nation. *Journal of the Dow University of Health Sciences (JDUHS)*, 5(3), pp.81-84.
- Lai, C.H., Tung, K.C., Ooi, H.K. and Wang, J.S., 2000. Competence of *Aedes albopictus* and *Culex quinquefasciatus* as vector of *Dirofilaria immitis* after blood meal with different microfilarial density. *Veterinary Parasitology*, 90(3), pp.231-237.
- Manzoor, F., Shabbir, R., Sana, M., Nazir, S. and Khan, M.A., 2020. Determination of Species Composition of Mosquitoes in Lahore, Pakistan. *Journal of Arthropod-Borne Diseases*, vol. 14, no. 1, pp. 106-115. <http://dx.doi.org/10.18502/jad.v14i1.2717> PMID:32766354.
- Mukhtar, M., Tahir, Z., Baloch, T.M., Mansoor, F. and Kamran, J., 2011. Entomological investigations of dengue vectors in epidemic-prone districts of Pakistan during 2006–2010.
- Muslim, A., Fong, M.Y., Mahmud, R., Lau, Y.L. and Sivanandam, S., 2013. *Armigeres subalbatus* incriminated as a vector of zoonotic *Brugia pahangi* filariasis in suburban Kuala Lumpur, Peninsular Malaysia. *Parasites & vectors*, 6(1), pp.1-5.
- Narvaez, F., Gutierrez, G., Pérez, M.A., Elizondo, D., Nuñez, A., Balmaseda, A. and Harris, E., 2011. Evaluation of the traditional and revised WHO classifications of dengue disease severity. *PLoS neglected tropical diseases*, 5(11), p.e1397.

- Qasim, M., Naeem, M. and Bodlah, I., 2014. Mosquito (Diptera: Culicidae) of Murree Hills, Punjab, Pakistan. *Pakistan Journal of Zoology*, 46(2).
- Ramasamy, R. and Surendran, S.N., 2012. Global climate change and its potential impact on disease transmission by salinity-tolerant mosquito vectors in coastal zones. *Frontiers in physiology*, 3, p.198.
- Rasheed, S.B., Butlin, R.K. and Boots, M., 2013. A review of dengue as an emerging disease in Pakistan. *Public Health*, 127(1), pp.11-17.
- Reiter, P., 2001. Climate change and mosquito-borne disease. *Environmental health perspectives*, 109(suppl 1), pp.141-161.
- Rueda, L.M., 2007. Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. In *Freshwater animal diversity assessment* (pp. 477-487). Springer, Dordrecht.
- Shepard, J.J., Andreadis, T.G. and Vossbrinck, C.R., 2006. Molecular phylogeny and evolutionary relationships among mosquitoes (Diptera: Culicidae) from the northeastern United States based on small subunit ribosomal DNA (18S rDNA) sequences. *Journal of medical entomology*, 43(3), pp.443-454.
- Steib, A., Freys, G., Lehmann, C., Meyer, C. and Mahoudeau, G., 2001. Intraoperative blood losses and transfusion requirements during adult liver transplantation remain difficult to predict. *Canadian Journal of Anesthesia*, 48(11), pp.1075-1079.
- Sutherst, R.W., 2004. Global change and human vulnerability to vector-borne diseases. *Clinical microbiology reviews*, 17(1), pp.136-173.
- ud Din, M. and Khan, I.A., 2015. Species composition, relative abundance and habitats of mosquito fauna of District Upper Dir, Khyber Pakhtunkhwa-Pakistan. *Journal of Entomology and Zoology Studies*, 3(5), pp.447-450.
- Usman, K., Rehman, H.U., Pervaiz, K., Khudadad, S. and Aslam, S., 2017. A study of mosquito fauna of Amin Khel district Karak, Khyber Pakhtunkhwa, Pakistan. *Int. J. Mosq. Res*, 4, pp.47-49.
- World Health Organization, Special Programme for Research, Training in Tropical Diseases, World Health Organization. Department of Control of Neglected Tropical Diseases, World Health Organization. Epidemic and Pandemic Alert, 2009. *Dengue: guidelines for diagnosis, treatment, prevention and control*. World Health Organization.