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Original Article

Diversity, distribution and relative abundance of the mosquito fauna (Diptera: Culicidae) of Malakand and Dir Lower, Pakistan

Diversidade, distribuição e abundância relativa da fauna de mosquitos (Diptera: Culicidae) de Malakand e Dir Lower, Paquistão

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Abstract

The present study was conducted to evaluate the diversity, distribution (C) and relative abundance (RA) of the mosquito fauna (Diptera: Culicidae) of Malakand and Dir Lower, Pakistan. Collection of specimens (n = 1087) was made during September 2018 to July 2019 at six different habitats including freshwater bodies, rice fields, animal sheds, indoors, drains and sewage waters. Specimens were collected through light traps, pyrethrum spray, aspirators and nets and subsequently killed, preserved and then arranged in entomological boxes for identification. Three genera were identified namely *Culex*, *Anopheles* and *Aedes.* A total of fourteen species were identified namely: *Cx. quinquefasciatus* (Say, 1823), *An. stephensi* (Liston, 1901), *Cx. tritaeniorhynchus* (Giles, 1901), *Ae. vittatus* (Bigot, 1861), *An. maculatus* (Theobald, 1901), *An. fluviatilis* (James, 1902), *Cx. vishnui* (Theobald, 1901), *Ae. aegypti* (Linnaeus, 1762*) An. subpictus* (Grassi, 1899), *An. dthali* (Patton, 1905), *An. culicifascies* (Giles, 1901), *An. pallidus* (Theobald, 1901), *Ae. albopictus* (Skuse, 1894) and *An. annularis* (van der Wulp, 1884). *Cx. quinquefasciatus* was found constantly distributed in the study area with RA = 16.5% and C = 100%. *An. annularis* was found as a satellite species, sporadically distributed in the study area having RA = 0.9% and C = 17%. Diversity indices of mosquitoes in the studied habitats were found as, Shannon-Wiener Index (2.415), Simpson Index (9.919), Fisher's Index (2.269) and Margalef's Index (1.859). A statistically significant difference was recorded in mosquito diversity in the six habitats (Kruskal-Wallis, chi-squared, H = 17.5, df = 5, P = 0.003 at $\alpha = 0.05$). The present study encompasses mosquito fauna of Malakand, Pakistan with respect to diversity, relative abundance and distribution in diverse habitats and all seasons of the year. This will assist scientists working in various fields related with epidemiology, medical and veterinary entomology, ecology and allied areas of biological sciences.

Keywords: insect vectors, entomology, *Anopheles*, *Culex*, *Aedes*.

Resumo

O presente estudo foi conduzido para avaliar a diversidade, distribuição (C) e abundância relativa (RA) da fauna de mosquitos (Diptera: Culicidae) de Malakand e Dir Lower, Paquistão. A coleta de espécimes (n = 1087) foi feita durante o período de setembro de 2018 a julho de 2019 em seis habitats diferentes, incluindo corpos d'água, campos de arroz, galpões de animais, ambientes internos, ralos e águas residuais. Os espécimes foram coletados por meio de armadilhas luminosas, spray de piretro, aspiradores e redes e posteriormente mortos, preservados e depois dispostos em caixas entomológicas para identificação. Três gêneros foram identificados, nomeadamente *Culex*, *Anopheles* e *Aedes*. Um total de 14 espécies foi identificado, a saber: *Cx. quinquefasciatus* (Say, 1823), *An. stephensi* (Liston, 1901), *Cx. tritaeniorhynchus* (Giles, 1901), *Ae. vittatus* (Bigot, 1861), *An. maculatus* (Theobald, 1901), *An. fluviatilis* (James, 1902), *Cx. vishnui* (Theobald, 1901), *Ae. aegypti* (Linnaeus, 1762), *An. subpictus* (Grassi, 1899), *An. dthali* (Patton, 1905), *An. culicifascies* (Giles, 1901), *An. pallidus* (Theobald, 1901), *Ae. albopictus* (Skuse, 1894) e *An. annularis* (Van der Wulp, 1884). *Cx. quinquefasciatus* foi encontrado constantemente distribuído na área de estudo com AR = 16,5% e C = 100%. *A. annularis* foi encontrada como espécie satélite, distribuída esporadicamente na área de estudo com RA = 0,9% e C = 17%. Os índices de diversidade de mosquitos nos habitats estudados foram encontrados como índice de Shannon-Wiener (2,415), índice de Simpson (9,919), índice de Fisher (2,269) e índice de Margalef (1,859). Uma diferença estatisticamente significativa foi registrada na diversidade de mosquitos nos seis habitats (Kruskal-Wallis, qui-quadrado, H = 17,5, df = 5, P = 0,003 em $\alpha = 0,05$). O presente estudo abrange a fauna de mosquitos de Malakand, Paquistão, com respeito à diversidade, abundância relativa e distribuição em diversos habitats e em todas as estações do ano. Isso ajudará os cientistas que trabalham em vários campos relacionados com a epidemiologia, entomologia médica e veterinária, ecologia e áreas afins das ciências biológicas.

Palavras-chave: vetores de insetos, entomologia, *Anopheles*, *Culex*, *Aedes*.

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1. Introduction

Mosquitoes (Diptera: Culicidae) pose a major risk to human health globally (WHO, 2017). They are the main vectors of many human and livestock diseases caused by viruses and parasites (Qasim et al., 2014). They transmit many diseases such as dengue, malaria, filariasis, arboviruses, West Nile Virus, Japanese Encephalitis and Western Equine viruses etc. (Turell et al., 2005; Ashfaq et al., 2014; Caraballo and King, 2014; Negev et al., 2015; Dad et al., 2019). Larval and pupal stages of mosquitoes live in fresh water and play important role in food chain and as indicators of water quality (Qasim et al., 2014). 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. Mosquitoes' feeding behavior and breeding places are dependent on temperature. Some mosquitoes are attracted to host with their choices, for example odor of skin, temperature, moisture or visual cues (Steib et al., 2001). Mosquitoes bite almost any animal being 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 disease transmission. The great diversity of mosquitoes (31%) is 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). The average life cycle of mosquitoes is fourteen days with four stages, egg, larval and pupal stage (aquatic) and an adult stage (terrestrial) (Kumar and Nattuthurai, 2011).

Studies on the diversity, distribution, relative abundance and surveys of mosquitoes are important due to the loss of natural habitats of mosquitoes (Debrot et al., 2018) and the worldwide spread of invasive mosquitoes (Kraemer et al., 2019). Some of the researchers have studied mosquitoe diversity in the outskirsts of the present study area including Ali et al. (2013), Ilahi and Suleman (2013), Khan, I.A. et al. (2015) and Ud Din and Khan (2015). The present study area and adjacent study areas have witnessed several dengue outbreaks in the past. Malaria is also prevalent in the study area and adjacent localities. Global climate change, enhanced anthropogenic activities and fast communication means has increased the chances of vector-borne disease outbreaks. To cope with future challenges of vector-borne diseases, it is necessary to evaluate the current status of mosquito fauna in various habitats of different geographical localities. The aim of this study was to evaluate the current diversity, distribution and relative abundance of the mosquito fauna of Malakand and Dir Lower, Pakistan.

2. Materials and Methods

2.1. Study area

The present study was conducted at two districts of Pakistan namely Dir Lower with a total area of 1582 Km^2, located at 34° 92' N, 71° 78' E and Malakand with a total area of 952 Km^2 and located at 34°49'N, 71°84'E (see Figure 1). Collection of mosquitoes was made from six different habitats including freshwater bodies, rice fields, animal sheds, indoors, drains and sewage. The average temperature in the study area ranges from 21.6 °C to 34.6 °C in Summer while the relative humidity in December ranges from 21% to 83% recorded at 5:00 pm and from 41% to 94% recorded in January at 08:00 am (Ali et al., 2018).

2.2. Sampling

Sampling was carried out from 15th September 2018 to 15th July 2019. Two to three visits of the habitats were made per week. Adults were mostly collected through light traps and pyrethrum spray. Most of the collection was made at dawn and dusk from various habitats using mosquito nets. The collection was carried out between 7:00 PM to 9:00 AM by applying pyrethrum spray on mosquitoes in various domestic and peri-domestic areas. Mosquitoes collected alive in nets were killed with the help of cotton

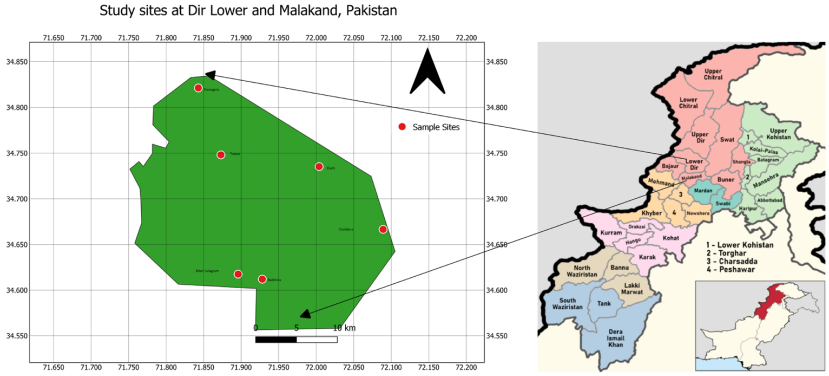

Figure 1. Map of the study area, District Malakand and District Dir Lower, Pakistan.

swab soaked in ethyl acetate. Sweep nets were used for collection according to previously described methods (Herrel et al., 2001; Shortall et al., 2009; Florencio et al., 2014). Larval and pupal stages were collected with standard dipper (440 ml, 1 m handle) according to WHO (2005).

2.3. Identification of mosquitoes

Preservation of the collected specimens was made in plastic vials. Larval and pupal stages were reared in enamel trays in the laboratory. After emergence, the adults were collected in plastic vials. Identification was made based on morphological characters using taxonomic keys and literature (Christophers, 1933; Barraud, 1934; Harbach, 1985; Darsie and Pradhan, 1990; Rueda, 2004; Thielman and Hunter, 2007; Becker et al., 2010; Darsie et al., 2010).

2.4. Pinning and labeling

After collection of adult mosquitoes, killing was carried out with the help of cotton swab soaked in ethyl acetate placed inside a glass jar. Representative specimens of each mosquito species were pinned through the thorax to a small stage (see Figure 2). The specimens were labelled with the information of collector name, specimen number, name, habitat and date of collection.

2.5. Data analysis

The collected specimens (n = 1087) were categorized into genera and species based on the reported literature on taxonomic keys of mosquitoes. The specimens were analyzed in terms of diversity indices, relative abundance and distribution. Relative abundance (RA) and distribution (C) were calculated according to the equations reported by Ali et al. (2013); Rydzanicz and Lonc (2003) and Sengil et al. (2011). Relative abundance was calculated as Equation 1:

$$RA = \frac{l}{L} \times 100 \quad (1)$$

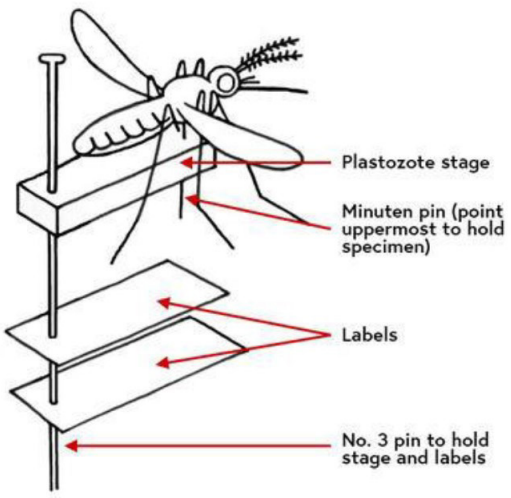

Figure 2. Pinning and labelling of adult mosquitoes for study. Source: Future Learn (2020).

where "l" is the number of specimens of a particular species and "L" is the total number of specimens collected. Based on the values of RA, the mosquito species were categorized according to Trojan (1992) into the given classes: Satellite (RA < 1%), Sub-dominant (RA < 5%) and Dominant species (RA > 5%).

Distribution was calculated as Equation 2:

$$C = \frac{n}{N} \times 100 \quad (2)$$

where "n" is the number of sites where mosquito species were found, and "N" is the total number of sites analyzed. Based on the values of C, the species were categorized according to Dziêczkowski (1972) into the given classes: C = 0 to 20% (Sporadic); C = 20.1 to 40% (Infrequent); C = 40.1 to 60% (Moderate); C = 60.1 to 80% (Frequent), and C = 80.1 to 100% (Constant).

Diversity was evaluated by using Shannon-Wiener Index, Simpson Index, Fisher's Index and Margalef's Index. Kruskal-Wallis test statistic was used for finding out any statistically significant difference in mosquito diversity among the studied habitats at $\alpha = 0.05$ through Microsoft Excel Worksheet (Version 2016).

3. Results

A total of 1087 mosquitoes were trapped and examined during the study period. Fourteen species of mosquitoes were identified belonging to three genera. Description of the identified mosquito genera and species with their size, relative abundance and distribution is given as follows.

3.1. Culex

The size of adult *Culex* ranged from 4-10 mm (0.2-0.4 in). In the female *Culex*, the palps are as long as proboscis. *Culex* was the dominant genus (RA = 34%) with constant distribution in the study area (C = 100%).

3.1.1. Culex quinquefasciatus

Medium body size ranging from 3.96 to 4.25 mm long, brown in color while the thorax, proboscis, tarsi and wings are darker compared with rest of the body. Length of the proboscis and antennae is almost same, in some cases, the antennae are shorter than the proboscis. Flagellum consists of 13 segments with few or no scales. It was found as a dominant species (RA = 16.5%) with constant distribution (C = 100%).

3.1.2. Culex tritaeniorhynchus

A relatively small, reddish brown mosquito with characteristic dark brown scales on the scutem and vertex, the ventral surface of the proboscis has accessory pale patches, and the hind femora possess dark, narrow apical rings. The species was dominant (RA = 11%) and constantly distributed in the study area (C = 83%).

3.1.3. Culex vishnui

Cx. vishnui are based on identifying specific appearance of the legs, abdomen, palpi, wings, proboscis and tarsi.

Anterior surface of the hind femora with pale stripes distinct from the dark scaled area. The species was dominant (RA = 6.6%) and moderately distributed (C = 50%).

3.2. *Anopheles*

Anopheles mosquitoes have palps and proboscis of similar length and have spotted wings with discrete blocks of black and white scales. Abdomens of male and female stick up in the air during rest. They are highly anthropophilic. Most *Anopheles* mosquitoes are crepuscular (active at dawn and dusk), some feed indoors while others feed outdoors. This genus was found dominant in the study area (RA = 48.7%) with a constant distribution (C = 83%).

3.2.1. *Anopheles stephensi*

Size of adults is medium ranging from 2.5 - 4.0 mm. Antennae with small pale scales on the torus. Palps are thick, cylindrical, and smooth except those towards the base. Thorax consists of a few pale scales. The first tarsal segments usually have some spots while other segments have dark and narrower apical banding. Abdomen have narrow scales increasing in extent towards posterior segments. The species was dominant with RA = 14.7% and constantly distributed in the study area (C = 83%).

3.2.2. *Anopheles maculatus*

Antenna with minute white scales on torus, palps are thick, with vertical scales towards the base. The thorax has one or two pale scales, median area covered with soft white scales. Pleurae with some scales on sterno-pleuron. The front femora are slightly swollen in the basal half portion. Cerci have black scales with some pale scales on the dorsal sides between the bases of cerci. This species was dominant (RA = 9%) and frequently distributed (C = 67%).

3.2.3. *Anopheles fluviatilis*

Head with normal scales, antennae devoid of scales and dark in color. Palpi thin, straight. Wings elongated with a length twice or more than the length of petiole. Front femora not swollen in basal half. There are no scales on abdomen and cerci and the color of abdomen is dark having darkish hairs. The species was dominant with RA = 8.2% and frequently distributed (C = 67%).

3.2.4. *Anopheles subpictus*

Head scales are normal, with a prominent vertical area. Cones and rods are about 20 in number. Wings base of costa usually consist of three small dark color spots. Abdomen thickly dressed in golden hairs and some narrowest yellow scales, or with some darkish scales. The species was dominant (RA = 5.5%) and moderately distributed (C = 50%).

3.2.5. *Anopheles dthali*

It is a light-colored, delicate species with pale head, black eyes and a shiny thorax. Palpi are thin and of uniform thickness with marginal hairs making a single row on the dorsal side and a double row on the ventral side. Legs are uniformly darkish in color. There are no scales on abdomen, but dark and light patches of hairs are found. It was a sub-dominant species (RA = 4.5%) with infrequent distribution (C = 33%).

3.2.6. *Anopheles culicifacies*

Palpi with a very short terminal segment having a narrow pale apical band. Rods tapering rapidly from expanded base. The base of costa has a disruption external to the humeral. Fringe usually with ordinary spots and sometimes absent. There are narrow, elongated, and noticeable plume-scales on the wings. Front femora not swollen in basal half. No scales on the entire abdomen and cerci. The species was sub-dominant (RA = 3.6%) and infrequently distributed (C = 33%).

3.2.7. *Anopheles pallidus*

Resembles *An. annularis* but the wings are lighter and having a brown coloration instead of black. A number of plae scales are found on the sternopleuron. Extensive pale areas in wing markings. Abdomen with fewer scales from segment 4th and onwards. This species was sub-dominant (RA = 1.8%) and infrequently distributed (C = 33%).

3.2.8. *Anopheles annularis*

Adults are medium sized with broad filament and one or two dark scales on the thorax. The mesonotum has a uniform black color and is covered with short, broad and oval shaped white scales. Pleura with occasional pale scales. Front femora swollen in basal half. Tibia dark, usually with pale stripes. Front tarsus broad apically and somewhat banded basally. Abdomen with dark hairs and dark scales apically. This was a satellite species (RA = 0.9%) and sporadic in distribution (C = 17%).

3.3. *Aedes*

Adult *Aedes* mosquitoes have a narrow and black body with a peculiar pattern of dark and light scales on the thorax and abdomen, and dark bands on the legs. *Aedes* mosquitoes hold their bodies low and almost parallel to the ground at an angled downward during rest with the help of the proboscis. *Aedes* mosquitoes are day biting insect with maximum feeding rate during dawn and dusk. The genus was dominant (RA = 17%) with frequent distribution (C = 66%).

3.3.1. *Aedes vittatus*

Wing veins consist of narrow scales, three pairs of small round silvery white spots on the scutum, dark tibiae with white spots and a white band on the base of tibiae. The species was dominant (RA = 10%) and frequently distributed (C = 67%).

3.3.2. *Aedes aegypti*

Small to medium sized mosquitoes about 4-7 mm long and having white markings on its legs and a white stripe on the upper surface of its thorax. White scales on the dorsal side of the thorax arranged like in the form of a violin or lyre differentiating it from *Ae. albopictus*. Proboscis is completely black colored, tips of palps are

white, a dorsal pattern of white scales is found on the scutum, and the wings are darkly scaled. *Ae. aegypti* are mostly crepuscular, indoor feeders in shady areas, or in cloudy weather. They breed in stagnant water. This was a dominant species (RA = 5.7%) and moderately distributed (C = 50%).

3.2.3. Aedes albopictus

Also known as Asian tiger mosquito. It has white bands on its legs and body and with a striped appearance like a tiger. It is smaller in size than *Ae. aegypti* about 2 to 6 mm long having a single, silvery-white stripe down the middle of the dorsal side of the thorax. Females are larger than males but both sexes are very similar morphologically. Proboscis dark colored with the upper surface of the last segment covered with silvery scales. The species was sub-dominant (RA = 1.1%) and infrequently distributed (C = 33%).

Relative abundance, distribution and status of the fourteen identified mosquito species is given in Table 1. Highest prevalence of mosquito fauna was recorded in the month of May while lowest was recorded in the month of March (Table 2). Mosquitoes were more abundant in

Table 1. Relative abundance and distribution of mosquito fauna at Malakand and Dir Lower, Pakistan (2018-19).

S.No.	Species	No. of Specimens	RA*	Status	C*	Status
1.	*Cx. quinquefasciatus*	180	16.5%	Dominant	100%	Constant
2.	*An. stephensi*	160	14.7%	Dominant	83%	Constant
3.	*Cx. tritaeniorhynchus*	120	11%	Dominant	83%	Constant
4.	*Ae. vittatus*	110	10%	Dominant	67%	Frequent
5.	*An. maculatus*	100	9%	Dominant	67%	Frequent
6.	*An. fluviatilis*	90	8.2%	Dominant	67%	Frequent
7.	*Cx. vishnui*	72	6 .6%	Dominant	50%	Moderate
8.	*Ae, aegypti*	63	5.7%	Dominant	50%	Infrequent
9.	*An. subpictus*	60	5.5%	Dominant	50%	Infrequent
10.	*An. dthali*	50	4.5%	Sub-dominant	33%	Infrequent
11.	*An. culicifascies*	40	3.6%	Sub-dominant	33%	Infrequent
12.	*An. pallidus*	20	1.8%	Sub-dominant	33%	Infrequent
13.	*Ae albopictus*	12	1.1%	Sub-dominant	33%	Infrequent
14.	*An. annularis*	10	0.9%	Satellite	17%	Sporadic

*Abbreviations: RA = Relative Abundance; C = Distribution.

Table 2. Seasonal variation of mosquito fauna (September 2018 to June 2019).

Mosquito species	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
Cx. quinquefasciatus	30	21	19	11	0	0	7	18	42	32	180
An. stephensi	25	20	18	7	0	0	5	20	35	30	160
Cx. tritaeniorhynchus	23	15	14	3	0	0	3	12	30	20	120
Ae. vittatus	20	11	11	5	0	0	4	13	26	20	110
An. maculatus	18	10	12	4	0	0	3	10	24	19	100
An. fluviatilis	17	10	10	5	0	0	4	9	17	18	90
Cx. vishnui	15	9	6	5	0	0	2	7	14	14	72
Ae. aegypti	13	7	5	5	0	0	3	5	10	15	63
An. subpictus	12	8	6	4	0	0	2	5	13	10	60
An. dthali	10	5	5	2	0	0	3	5	12	8	50
An. culicifascies	5	5	4	2	0	0	3	4	12	5	40
An. pallidus	4	2	2	1	0	0	1	2	3	5	20
Ae. albopictus	3	2	1	0	0	0	0	1	2	3	12
An. annularis	2	1	1	0	0	0	0	1	2	3	10
Total	197	126	114	54	0	0	40	112	242	202	1087

indoor habitats followed by animal sheds and rice fields (as shown in Table 3; see Figure 3). *Cx. quinquefasciatus* was the most prevalent mosquito species followed by *An. stephensi* and *Cx. tritaeniorhynchus* (as shown in Table 2 and 3). Abundance of the identified species was found in the order of: *Cx. quinquefasciatus* > *An. stephensi* > *Cx. tritaeniorhynchus* > *Ae. vittatus* > *An. maculatus* > *An. fluviatilis* > *Cx.vishnui* > *Ae. aegypti* > *An. subpictus* > *An. dthali* > *An. culicifascies* > *An. pallidus* > *Ae. albopictus* > *An. annularis* (as shown in Table 1, 2 and 3).

A Kruskal-Wallis Test was performed to determine any significant difference in the diversity of mosquitoes in the six studied habitats. A total of 14 species were identified in the six habitats with sample size (n = 1087). The value of the Kruskal-Wallis test statistic (H) was, H = 17.561 and the corresponding p-value was p = 0.003. Since this p-value was less than $\alpha = 0.05$, we have sufficient evidence to conclude that the diversity of mosquito fauna was significantly different in the studied habitats.

Diversity indices of mosquitoes in six habitats were found as, Shannon-Wiener Index (2.415), Simpson Index (9.919), Fisher's Index (2.269) and Margalef's Index (1.859) (as shown in Table 4). Highest diversity of mosquitoes was detected indoors followed by animal sheds and rice fields (as shown in Table 4). Minimum diversity was recorded in sewage and drains.

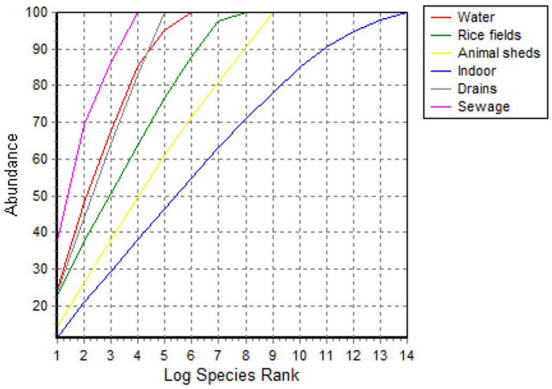

Figure 3. Abundance (K-Dominance) of the fourteen identified mosquito species in the six habitats.

Table 3. Abundance of mosquito fauna in various habitats.

Mosquito Spp.	Fresh Water	Rice Fields	Animal Sheds	Indoor	Drains	Sewage	Total
Cx. quinquefasciatus	24	20	22	40	30	44	180
An. stephensi	0	23	30	35	35	37	160
Cx. tritaeniorhynchus	20	17	25	28	30	0	120
Ae. vittatus	0	35	25	30	0	20	110
An. maculatus	25	0	20	30	25	0	100
An. fluviatilis	0	20	24	30	0	16	90
Cx. vishnui	0	0	24	20	28	0	72
Ae. aegypti	18	0	20	25	0	0	63
An. subpictus	10	20	0	30	0	0	60
An. dthali	0	0	20	30	0	0	50
An. culicifascies	0	15	0	25	0	0	40
An. pallidus	5	0	0	15	0	0	20
Ae. albopictus	0	4	0	8	0	0	12
An. annularis	0	0	0	10	0	0	10
Total	102	154	210	356	148	117	1087

Table 4. Diversity indices of the mosquito fauna in different habitats at Malakand and Dir Lower, Pakistan (2018-19)

Habitat Type	No. of Species	Shannon-Wiener Index	Simpson Index	Fisher's Index	Margalef's Index
Water	6	1.686	5.289	1.395	1.081
Rice fields	8	1.981	7.076	1.793	1.39
Animal sheds	9	2.189	9.19	1.913	1.496
Indoor	14	2.57	12.91	2.91	2.213
Drains	5	1.603	5.076	1	0.8004
Sewage	4	1.306	3.531	0.8024	0.63
Total	14	2.415	9.919	2.269	1.859

4. 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. Malaria and dengue are the two common diseases that are prevalent in the local public and the disease spread is associated with the abundance of mosquito vectors. Fourteen species of mosquitoes were identified belonging to three genera with a detailed description on diversity, distribution, and relative abundance in different habitats at Malakand and Dir Lower, Pakistan. Mosquitoes were found more prevalent in indoor domestic habitats and animal sheds (as shown in Table 3; see Figure 3). The anthropophilic and zoophilic species rely on animal blood for oviposition and hence are abundant mostly in the domestic and peri-domestic areas. The number of mosquito species recorded in the present study were different from earlier recorded species in different parts of the Khyber Pakhtunkhwa Province, Pakistan (Ali et al., 2013; Ilahi and Suleman, 2013; Khan, I.A. et al., 2015; Ud Din and Khan, 2015; Usman et al., 2017). Average temperature, relative humidity and rainfall changes is one of the main reasons for the differences in the diversity of mosquito fauna and other insect species. The difference in abundance and species richness may also be due to differences in sampling techniques or due to differences in ecological conditions. The overall climate change recorded in the last few years has an immense impact on the variations in biodiversity and a paradigm shift in the course of seasonal abundance of fauna. Most of the species recorded in the present study were however similar with those reported in the cited studies.

Cx. quinquefasciatus was found the dominant species and constantly distributed throughout the study area. This conforms to the previous reports cited above and with a recent report by Manzoor et al. (2020). It was found in all habitats with higher abundance in sewage and indoor habitats (as shown in Table 3). *Cx. tritaeniorhynchus* was the third most abundant species with dominant status and constant distribution throughout the study area. This is an important vector for the transmission of West Nile Virus beside other *Culex* spp. in Pakistan (Akhter et al., 1982; Hayes et al., 1982; Zohaib et al., 2015; Khan et al., 2018).

An. stephensi was found as the second most abundant species with constant distribution in the present study (as shown in Table 1, 2 and 3). *An. culicifacies* was found sub-dominant species with sporadic distribution (as shown in Table 1). These two Anopheline mosquitoes are the important vectors of malaria as reported by earlier studies (Leslie et al., 2009; Rowland et al., 2000; Sinka et al., 2010; Jahan and Hussain, 2011; Gayan Dharmasiri et al., 2017).

The overall distribution of the medically important disease vector species of mosquitoes varied across the habitats and seasons throughout the year correlated with ecological and environmental factors. This may have serious implications for disease transmission in the study area. *Ae. aegypti* and *Ae. albopictus* are the vectors for the four different serotypes of dengue. *Ae. aegypti* was found a dominant species with infrequent distribution while *Ae. albopictus* was found sub-dominant with infrequent distribution (as shown in Table 1). These species have been reported from various parts of Pakistan and are associated with dengue epidemics (Khanani et al., 2011; Mukhtar et al., 2011; Rasheed et al., 2013; Jabeen et al., 2021). Abundance of mosquito fauna increases with increase in favorable environmental conditions. In such cases, the mosquito populations may be enhanced and mostly results in disease outbreaks. *Ae. aegypti* was most prevalent in indoor habitats which shows its anthropophilic behavior as reported by Halstead (2008). This strengthens potential of these mosquitoes as disease vectors and may be a risk factor for future dengue epidemics in the study area.

The seasonal varaiation of mosquitoes has shown a differential trend with highest prevalene in the month of May followed by June, Spetember and October (as shown in Table 2). This differs from a recent report on mosquito fauna at Lahore, Pakistan by Manzoor et al. (2020) where they reported higher prevalence of mosquitoes in the month of September followed by August, April and May. No mosquito fauna was reported in the months of January, February, March, November and December. The seasonal difference in the mosquito fauna of the two studies may be due to different climatic and ecological conditions which ultimately determine the course of disease spread in a different temporat pattern in these areas.

It has been concluded from the present study that fourteen mosquito species are presently prevalent in the study area with *Cx. quinquefasciatus* as the most dominant species followed by *An. stephensi* and *Cx. tritaeniorhynchus*. Maximum abundance of the mosquit fauna was detected during May followed by June, September and October. Indoor habitats and animal sheds are the major harbors for most of the mosquito species represting their hematophagic behavior and potential for disease spread. Major disease vector species are reproted with variable diversity, distributiona and abundance representing their potential as disease vectors and possibility of future disease outbreaks in the study area. Future studies are recommended to have an in-depth analysis of the msoquito diversity by incorporation of all possible habitats and all seasons of the year. Moreover, latest molecular and biotechnological tools should be applied for a more reliable and true picture of the mosquito diversity in the stddy area. 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.

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