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Species composition, relative abundance and habitats of mosquito fauna of District Upper Dir, Khyber Pakhtunkhwa-Pakistan

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Abstract
A study was conducted to determine the species composition, relative abundance and seasonal variation of mosquito fauna of District Upper Dir in 2014. A total of 1241 species; 712 male (57.37%) and 529 female (42.63%), comprising of nine species of four genera, were collected from four localities, namely Barawal (278), Sheringal (381), Wari (356) and Dir City (226) of the District Upper Dir. Three species each of *Anopheles* and *Culex*, two species of *Aedes* and one species of *Culiseta* were recorded. The insect species recorded were *Culex quinquefasciatus, Culex mimeticus, Culex theileri, Anopheles maculates, Anopheles stephensi, Anopheles annularis, Aedes albopictus, Aedes shortii* and *Culiseta longiareolata*. Mosquito species were found in water, field crops, animal sheds and human dwellings habitats. Monthly variation and abundance studied from April to November revealed that out of total number of 1241 samples recorded there were highest number of 357 of *Cu. quinquefasciatus* and lower number of 3 of *Cu. longiareolata*. The present results might be helpful in devising pest management strategies against the mosquito species of District Dir Upper.

Keywords: *Mosquito fauna, Relative abundance, Seasonal variation, Species composition.*

1. Introduction
Mosquitoes belong to family *Culicidae* of Order Diptera. There are about 3,500 species of mosquitoes in the world [1]. Most of the species are ectoparasites of vertebrates. In particular females of some species are blood feeders of humans and other animals. The female mosquitoes require blood meal of vertebrates. The blood protein is necessary for egg maturation [2]. Mosquitoes are economically very important. They are vectors for transmission of various diseases of humans and livestock. Mosquito transmitted diseases like malaria, dengue fever and filariasis cause enormous life threatened problems across the world [3].

Mosquitoes' adult females lay eggs in water or near to water's edge. Some species breed in fresh water, others in saline water and marshes [4]. The genera *Coquillettidia*, *Culex*, *Culiseta* and *Uranotaenia* lay eggs in cluster form, floating on water surface or on a wetland on the ground and the eggs form water floating layers called "rafts". *Anopheles* also lays eggs in clusters on water but apparently they are not compactly glued [5]. The larvae are called wigglers. The pupae of mosquitoes are called tumblers [6]. The adults have piercing-sucking type mouth parts [7].

Mosquitoes are cosmopolitan. They are found everywhere in the world except for Antarctica and a few islands. They live in shady and moist places usually. In some species the adults can survive in winter by taking shelter in suitable microhabitats such as buildings or hollow trees [8].

Mosquitoes are responsible for the transmission of various pathogens, such as viruses, bacteria, protozoans and nematodes. Mosquito vectored diseases include malaria, chikungunya, filariasis, dengue fever, West Nile virus, encephalitis, tularemia and yellow fever. Various species of mosquitoes are estimated to transmit various types of diseases to more than 700 million people annually in Africa, South America, Central America, Mexico, Russia, and much of Asia, with millions of resultant deaths. At least two million people annually die of these diseases, and the morbidity rates are many times higher still [9].

Dengue fever is increasingly becoming an epidemic in Pakistan. Last year due to the long and optimum environmental conditions for *Aedes* spp. breeding season, the disease spread more rapidly than in previous years. Swat Valley was badly affected with 29 deaths and more than 9000 infections the last year [10].

The adult mosquitoes feed on decaying matter, flower nectar and juices. The larvae are filter feeders of organic particulates. The female mosquitoes of some species bite mammals' especially human beings. They locate their blood host by scent, sight and heat. Mosquitoes can smell scent and detect exhaled carbon dioxide about 300 meters away. Actually the female mosquitoes do not feed on blood, they require a blood meal for development of their eggs [11].

Keeping in view the importance of mosquitoes as medical pests, the present study aimed to determine species composition, seasonal variations and habitats of different species of mosquito of fauna in the District Dir Upper in 2014.

2. Materials and Methods

2.1 Study Area Description

District Upper Dir is one of the 25 districts in Khyber Pakhtunkhwa Province of Pakistan. The district was formed in 1996 when Dir District was divided in to Upper and Lower Dir. Upper Dir is spread over 3,699 square kilometers. It is situated in the North-West of Khyber Pakhtunkhwa, where it is bounded by long chain of Koh-e-Hindukush. It lies along the Afghanistan border between Chitral, Bajaur Agency and Lower Dir. It is connected with the Kohistan District via Barwani Pass and connected with Chitral District through Lowari Pass and with Afghanistan through Binshahi Pass. It is situated at Latitude 35^0 06 51" N and Longitude 72^0 01 59" E. The climate of Upper Dir is cold and damp. Its mountains are usually covered with snow and receives snow fall in the months of December, January and February. The summer in Dir Upper is very pleasant, which attracts tourists from many countries of the world. The average rainfall in the district is 700 mm and the temperature varies from -6 ^{0}C to 38 ^{0}C [12].

2.2 Research Methodology

The research study was conducted from April to November 2014. Adult mosquitoes were collected from four localities, namely Barawal, Sheringal, Wari and Dir City. The mosquitoes were collected by means of hand net, aspirator and light trap. Light trap was used for night collection and hand net for day time collection. Sampling was regularly made two times in a month for 3-4 hours each time. The collection was made from 7:00 am to 9:00 am and 5:00 pm to 7:00 pm regularly for three days in a weak. During sampling, time, habitat and location was recorded. Each species was given a specific code on the basis of habitat, time and location. For example, a sample collected from water in May from Dir City was given the code WMD-1.

Adult mosquitoes were collected indoor as well as outdoor. Indoor collection was made from homes and cattle-sheds. Outdoor collection was made from plants, water habitats and water-containing automobile tires. During indoor collection pyrethrum-spray method was used. Indoor resting mosquitoes were sprayed with pyrethrum. Paper sheets were spread before spraying. Mosquitoes were collected from sheets by using entomological forceps.

Hand net, aspirator and light trap were used during outdoor collection. The collected species were killed in killing jar with the help of cotton swab impregnated with ethyl acetate. The killed mosquitoes were transferred to glass tubes containing dry silica gel before identification in order to prevent desiccation of the mosquito body.

The collected species were identified on morphological features by using binocular microscope, model and taxonomic identification was carried out by using taxonomic keys provided in "The Fauna of British India, including Ceylon and Burma" [13]. The identification was made up to species level. Identification work was carried out at the Entomology Laboratory of the University of Agriculture, Peshawar and Department of Zoology, Shaheed Benazir Bhutto University Sheringal, Dir. The identified species were then labeled, mounted and pinned. These labeled species were preserved in the insect collection box and deposited at the Entomology Museum of the University of Agriculture, Peshawar.

3. Results and Discussion

3.1 Mosquito species composition and relative abundance

In the present research work a total of 1241 species, in which 712 male (57.37%) and 529 female (42.63%) comprising of nine species of four genera, were collected from four localities, namely Barawal (278), Sheringal (381), Wari (356) and Dir City (226) of the District Upper Dir. Three species each of *Anopheles* and *Culex*, two species of *Aedes* and one species of *Culiseta* was recorded.

The insect species recorded were *Culex quinquefasciatus, Culex mimeticus, Culex theileri, Anopheles maculates, Anopheles stephensi, Anopheles annularis, Aedes albopictus, Aedes shortii* and *Culiseta longiareolata* (Table 1). Out of total number of 1241 samples recorded there were highest number of 357 (28.77%) of *Cu. quinquefasciatus* and lower number of 3 (0.24%) of *Cu. longiareolata.*

The present results are comparable to those of some earlier researchers who reported different species of *Anopheles*, *Culex* and *Aedes* from different regions of the country. Investigation on prevalence of mosquito genera in Tandojam and its surroundings revealed that. *Culex* was the most abundant 89 (81.7%), while *Anopheles* 20 (18.3%) least abundant [14]. Twenty one species of 5 genera: *Anopheles, Aedes, Armigeres, Culex* and *Culiseta* had been reported from Swat [15]. Fifteen species of five genera: *Culex* and *Anopheles* (6 spp. each) and *Armigeres, Aedes* and *Culiseta* (1 sp. each) have been reported from Swat Ranizai of District Malakand [16]. All the Anopheline and Culicine species recorded in the present study were also reported from Murree Hills, Punjab province [17]. Entomological surveillance study conducted in four selected sites: Peshawar, Nowshera, Mardan and Charsadda of Khyber Pakhtunkhwa, Pakistan during 2012-13 revealed mean percent relative abundance of *Culex* species in different districts as: Peshawar (32.3%), Nowshera (18.8%), Mardan (20.3%) and Charsadda (21.0%). Higher numbers of *Aedes* mosquitoes were observed in Nowshera (19.3%), Peshawar (16.4%), Charsadda (13.1%), and Mardan (9.8%) [18].

Table 1: Species composition and percent relative abundance of mosquito species in District Upper Dir in 2014.

Species	Total No.	Percent Relative Abundance
Culex quinquefasciatus	357	28.77
Culex mimeticus	104	8.38
Culex theileri	112	9.02
Anopheles maculates	303	24.42
Anopheles stephensi	136	10.96
Anopheles annularis	181	14.58
Aedes albopictus	33	2.66
Aedes shortii	12	0.96
Culiseta longiareolata	3	0.24
Total: 9 species	1241	99.99

3.2 Mosquito species habitats

The results of habitats of the different mosquito species are presented in Table 2. Out of the total 278 water habitats studied, 84% of the habitats were occupied by *Cx. quinquefasciatus* and 9% by *Ae. shortii*. Of the total 226 field crops habitats sampled, *Cx. mimeticus* was found in higher

number of 63 field crops, while *Ae. shortii* in lower number of 3. Of the total number of 381 animal shed habitats investigated *Cx quinquefasciatus* was found in higher number of 100 habitats and *Cu. longiareolata* in lower number of 3. Of the total 356 human dwellings habitats studies, *An. maculates* was found in higher number of 117 habitats while *Ae. albopictus* in lower number of 11. Of the total number of 1241 habitats investigated, *Cx. quinquefasciatus* were found in higher number of 357 habitats, while *Cu. longiareolata* in lower number of 3. The present results are comparable to those of some earlier researchers. Two genera: *Anopheles* and *Culex* containing 9 species were reported from polluted water of Palosai stream, Peshawar [19]. The *Culex* species was abundant in turbid water with foul smell while the *Aedes* and *Anopheles* were higher in comparatively fresh and clear water with low turbidity. They recommended removal of artificial containers, monitoring of irrigation water and channels and ultimate control of breeding of dengue vectors in the target sites [18]. Mosquitoes prefer stagnant water within which to lay their eggs. They most commonly infest ponds, marshes, swamps and other wetland habitats. However, they are capable of thriving in a variety of locations and can successfully grow in numbers even when not in their natural habitat. Many species of mosquitoes use containers of water as egg-deposit sites. Hot, humid environments are most amenable to mosquito growth and survival. Infestations can occur easily in tropical areas. Some species have also been known to inhabit freezing locations such as the Arctic Circle. Mosquito larvae can be found in various habitats. Some larvae are active in transient waters such as floodwater, ditches and woodland pools. The *Anopheles, Culex, Culiseta, Coquillettidia* and *Uranotaenia* species breed in permanent bodies of water and can survive in polluted water as well as freshwater, acid water and brackish water swamps. Other mosquito larvae may be present in container water sources such as puddles upon leaves and stagnant water within small pools [20].

Table 2. Habitats of different mosquito species in Dir Upper during 2014.

Species	Water	Crops fields	Animal sheds	Human dwellings	Total No.
An. maculates	56	29	101	117	303
An. stephensi	22	23	58	33	136
An. annularis	71	----	69	41	181
Cx. quinquefasciatus	84	60	100	113	357
Cx. mimeticus	----	63	----	41	104
Cx. theileri	36	26	50	-----	112
Ae. albopictus	----	22	----	11	33
Ae. shortii	9	3	-----	-----	12
Cu. longiareolata	----	-----	3	----	3
Total	278	226	381	356	1241

3.3 Mosquito species monthly variations and abundance

The results of monthly variation and relative abundance of mosquito species showed that *Cx. quinquefasciatus* (49) and *Cu .longiareolata* (2) were the only species found in the month of April, where the former dominated the later. *Cx. quinquefasciatus* (81) also dominated the other species, namely *An. maculates* (33), *Cx. mimeticus* (24) and *Cu. longiareolata* (1) in the month of May. In June *An. maculates* (74) dominated the other species, while *Ae. shortii* (3) was least observed. *Cx. mimeticus, Ae. albopictus* and *Cu. longiareolata* were not recorded in June. *Cx. mimeticus* was dominant (63) while *Ae. shortii* (5) was least observed in July. *An. maculates* and *Cu. longiareolata* were not recorded in July. In August *Cx. quinquefasciatus* (96) dominated the other species, while *Ae. Albopictus* (17) was least observed. *Cx. mimeticus, Ae. shortii and Cu. longiareolata* were not found in August. In September *Cx. quinquefasciatus* (43) was dominant while *Ae. shortii* (4) was least observed. *Cx. mimeticus, Ae. albopictus and Cu. longiareolata* were not found in September. *An. maculates* (86) dominated the other species in October, while *An. annularis* (7) was least observed. *Cx. theileri, Ae. shortii* and *Cu. longiareolata* were not recorded. *Cx. quinquefasciatus* (16), *An. annularis* (11) and *An. stephensi* (6) were the only species recorded in September. In total *Cx. quinquefasciatus* (357) was the dominant while *Cu. longiareolata* (3) was the least observed species.

Table 3. Monthly variation and abundance of mosquito species in District Dir Upper during 2014.

Species	April	May	June	July	August	Sept.	Oct.	Nov.	Total
An. maculates	0	33	74	0	88	22	86	0	303
An. stephensi	0	0	67	12	23	09	19	06	136
An. annularis	0	0	59	17	77	10	07	11	181
Cx. quinquefasciatus	49	81	13	19	96	43	40	16	357
Cx. mimeticus	0	24	0	63	0	0	17	0	104
Cx. theileri	0	0	44	21	39	08	0	0	112
Ae. albopictus	0	0	0	06	17	0	10	0	33
Ae. shortii	0	0	03	05	0	04	0	0	12
Cu. longiareolata	02	01	0	0	0	0	0	0	03

There was no published data available on the mosquito fauna of District Upper Dir. The present research was the first of its kind to investigate the species composition, relative abundance, seasonal variation and habitats of mosquito fauna of district. Mosquito fauna was represented by 3 species of *Anopheles*; *An. maculatus, An. stephensi* and *An. annularis,* 3 species of *Culex*; *Cx. quinquefasciatus, Cx. mimeticus* and *Cx. theileri,* 2 species of *Aedes*; *Ae. albopictus* and *Ae. shortii* and 1 species of *Culiseta*; *Cu. longiareolata.*

Cu. longiareolata was the only species recorded from Sheringal in the month of April and May. *Ae. albopictus* was the only species recorded from water containers and water-containing automobile tires in Dir City and Warai. During the study the most preferred habitats of adult mosquitoes were found to be water and human dwellings.

Mean monthly positive ovitraps of species high in May and

October collected from Peshawar (32.5, 31.5), Nowshera (25.3, 26.8), Mardan (22.2, 16.8) and Charsadda (27, 26.9), respectively. The overall abundance of Culex species was high as compared with Aedes and Anopheles species collected from various outdoor breeding habitats [18].

The findings of the present research revealed that District Dir Upper is rich in mosquito fauna of the genera *Anopheles*, *Culex*, *Aedes* and *Culiseta*, containing also the *Anopheles stephensi* which is a malarial vector.

Anopheline species composition in the northern Kruger National Park varies by geographical location. Members of the *An. gambiae* complex occur across this region depending on habitat. Of the five sites sampled, Malahlapanga and Sirheni Dam had the highest anopheline species diversity due to the perennial availability of suitable breeding sites. Mosquito population density fluctuated with seasonal weather dynamics. Prevailing weather conditions, especially wind speed, influenced the productivity of mosquito sampling. Malahlapanga supported a perennial and geographically isolated population of *An. arabiensis* that presents a unique opportunity for assessing SIT as a malaria vector control option in a small pilot study. However, this site is not suitable for evaluating the effect of SIT on malaria transmission due to the lack of local transmission in this part of the Kruger National Park [21].

The variation in the number and species composition recorded in the present and earlier studies might be due to differences in sampling procedures, ecological, climatic and habitat conditions. However, many species identified in the present and earlier study were similar regarding their habitats. Further research work may explore diversity in the mosquito fauna of District Dir Upper.

Conclusion

In the present research work a total of 1241 species comprising of nine species of four genera, were collected from four localities of Barawal, Sheringal, Wari and Dir City of the District Upper Dir. Three species each of *Anopheles* and *Culex*, two species of *Aedes* and one species of *Culiseta* was recorded. The insect species recorded were *Culex quinquefasciatus, Culex mimeticus, Culex theileri, Anopheles maculates, Anopheles stephensi, Anopheles annularis, Aedes albopictus, Aedes shortii* and *Culiseta longiareolata. Cu. quinquefasciatus* dominated the other species. Of the total no. of 1241 habitats investigated, *Cx. quinquefasciatus* were found in higher no. of 357 habitats. Out of the total no. of 1241 samples collected at the four cities, *Cx. quinquefasciatus* was found in higher no. of 357. *Cx. quinquefasciatus* was relatively abundant at Brawal and Sheringal. *Cx. mimeticus* at Dir city and *An. Maculates* at Warai were relatively abundant.

Cx. quinquefasciatus dominated the other species in the month of April, May, August and September. In June *An. maculates* and in July *Cx. mimeticus* were dominant. *An. maculates* dominated the other species in October. During the study the most preferred habitats of adult mosquitoes were found to be water and human dwellings.

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