Original Article

Spatial Distribution, Seasonal Abundance and Physio-Chemical Assessment of Mosquito Larval Breeding Sites in Mardan District, Khyber Pakhtunkhwa, Pakistan

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Abstract

Background: Mosquitoes (Diptera: Culicidae) are haemotophagus insects and are vectors of many arthropod-borne diseases. Present study aimed to explore species composition, seasonal abundance, spatial distribution and physio-chemical properties of larval breeding sites of mosquitoes in District Mardan, Khyber Pakhtunkhwa, Pakistan.

Methods: Both adults and larvae of mosquitoes were collected through light traps, insecticide spray, mouth aspirator and larval standard dipping method in District Mardan from May to November 2017. Water samples from larval sites were physio-chemically analysed.

Results: 5078 (3704 adults and 1374 larvae) mosquito specimens were collected in Mardan, Katlang and Takhtbhai tehsils. Six species in four genera were reported. *Culex pipiens* (89.80%) and *Armigeres subalbatus* (9.20%) were the most abundant species. Diversity was high in Takhtbhai (0.29) followed by Katlang (0.28) and Mardan (0.25). Greater number of specimens were recorded in peridomestic sites (93.97%) as compared to domestic habitats (6.03%). *Culex pipiens* larval abundance had negative correlation with pH whereas it correlated positively with electric conductivity, salinity, and TDS (total dissolved sulphur). Mosquito abundance peaked in August and July while the lowest was in May. Their monthly abundance had positive correlation with rainfall (r= 0.5069), relative humidity (r= 0.4439) and mean minimum temperature (r= 0.2866). Number of mosquitoes was highest at low elevation < 347m asl (above sea level) in agriculture land and near to water bodies (streams).

Conclusion: *Culex pipiens* being the most abundant species, was susceptible to high pH. Mosquitoes preferred habitats were at low elevation in agriculture land.

Keywords: Diptera; Culicidae; Takhtbhai; Temperature; Salinity

Introduction

Mosquitoes (Diptera: Culicidae) are hematophagous insects distributed throughout the tropical and temperate regions of the globe. To date, a total of 3,500 species and subspecies of mosquitoes have been recorded and are placed in 4 genera, among which important ones are *Culex*, *Anopheles* and *Aedes*. Some species of mosquitoes are the biological vectors of many human and livestock diseases including malaria, dengue, yellow fever and filariasis. Malaria is considered a serious mosquitoes born infection that has greatly impacted human health over a century. In addition to their medical importance as vectors of human pathogens, female mosquitoes can attack humans and other animals causing nuisance of a magnitude that negatively affects individuals and society (1).

Mosquitoes lay their eggs (approximately 100–200 per adult life) in a variety of water sources ranging from small containers to marsh-

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land ecosystems. Among them *Anopheles* species prefer clean and unpolluted water while *Culex* species breed in water with high organic content. Majority of them breed in drainage ditches, ponds, tin cans, old tires, and tree holes. Some floodwater mosquitos eggs withstand desiccation for year/s and hatch when flooded again (2). In larval stages they feed on micro-fauna in aquatic habitats and after emergence their survival depends upon plant nectars with addition females take blood from a variety of animals.

Understanding mosquito species composition, distribution, abundance and seasonality is one of the essential prerequisites for surveillance and epidemiology of the infectious diseases (3, 4). Their pattern of community composition are useful aspects for medical entomologists to gain broader insight and projections regarding disease dynamics, spread and maintenance of globally spreading pathogens (5). In mosquitoes ecological studies, geographic information system (GIS) and remote sensing (RS) tools are used to identify environmental conditions associated with vector-borne diseases (6). These techniques have been used to identify conditions favourable for larval mosquitos development and factors particularly vegetation, elevation as well as landscape for mosquitos distribution (7). Pakistan have a rich fauna of mosquitoes and so far, 134 species of mosquitoes were identified while two species (*Anopheles culicifacies s.l* and *An. stephensi*) have been reported as malaria vectors (2, 8-11). Inadequate research exists on mosquitoes in Khyber Pakhtunkhwa province of Pakistan (2, 12). The previous published literature of the country mainly addressed aspects of mosquito identification, classification, vector potential and breeding sites of some species. Limited researchers documented the general ecology, composition, seasonal variations, and breeding habitats of mosquitoes which is useful for both medical entomologists as well as epidemiologists including health workers. Therefore, the present study aimed to explore species composition, habitats preferences, seasonal abundance, and spatial distribution of mosquitoes in district Mardan, Khyber Pakhtunkhwa, Pakistan.

Materials and Methods

Study area

The present study was carried out in Mardan District (34.20 N and 72.05 E) with total surface area of 1,632Km^2, Khyber Pakhtunkhwa, Pakistan (Fig. 1). The district is bordered in east with Buner and Swabi Districts, in north with Malakand District, in south with Nowshera District and in west with Charsadda District. The region has distinct summer and winter seasons with average temperature 22.2 °C as well annual rainfall 559mm. Topographically the area has plain in the south and west while mountainous terrains in the north-east. Sugarcane, wheat, maize, tobacco and vegetables are the yielding crops where Kalpani (entered from north) is the main stream. Various industries, factories and mills are sporadically established in the district.

Study design, data collection and analysis

The entomological study was conducted in 59 localities of three tehsils (administrative subunit of the district) of Mardan including Mardan, Katlang and Takhtbhai from May to November 2017. Adult mosquito specimens were collected using insecticide flit method, light traps, mouth aspirators and hand net. Larvae were captured through standard dipping method (13). Light traps were used at nighttimes (8pm to 6am) while flit method was used for indoor sites collection in early morning. Larval collection was made from water bodies (runny, stagnant, ponds, water tanks, tyres with 300ml water from the spot to ensure food supply in plastic jars). Net cloth was used for closing jar openings and were later shifted to Entomology research laboratory where the larvae in jars were reared at room temperature until they emerge into adults. During sampling, coordinates of each collection site was recorded

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through GPS (global positioning system) device (Garmin Etrex, USA).

During the field study adults and larvae were collected once per month in 59 fixed sites in Mardan from May to November 2017. Mosquitoes were identified up to species level through taxonomic keys as described by Christopher (14) and Barraud (14, 15). Water samples of 500ml of each larval site were collected in triplicate and analysed for total dissolved sulphur, salinity, electric conductivity, and pH following Ma et al. (15). All data related to mosquitoes were arranged in Microsoft Excel for further analysis. Annual rainfall, minimum, maximum temperature, and relative humidity data for the respective year (2017) were obtained from Meteorological Department, Regional Office, Peshawar (KP), Pakistan. GPS co-ordinates data were entered into ArcGIS version 10.5.0 for spatial distribution where digital elevation model (DEM) was extracted from Advance Spaceborne Thermal Emission and Reflection Radiometer (RASTER). Land-use map of the district was obtained from National Centre of Excellence in Geology University of Peshawar, KP, Pakistan.

Results

A total of 5078 mosquito specimens (adults and larvae) representing four genera (*Culex, Anopheles, Armigers* and *Aedes*) containing six species were collected in 59 sites in Mardan (35), Katlang (14) and Takhtbhai (10) tehsils. Among the collected species *Cx. pipiens* (N= 4560, 89.80%) was the most abundant species followed by *Ar. subalbatus* (467, 9.20%) while *Ae. albopictus*, *Cx. vishnui*, *Cx. tritaeniorhynchus* and *An. walkeri* showed least abundance <1%. Highest mosquito abundance was recorded in Mardan (3481, 68.55%) followed by Takhtbhai (801, 15.77%) and Katlang (796, 15.68%). A total of 1684 males and 3394 females were recorded and male to female ratio was 1:2 (Table 1). Variations in diversity of mosquitoes among the studied tehsils was observed whereas Shannon diversity index H= 0.29 was highest for Takhtbhai while species richness was in peak in Katlang 0.141 and Takhtbhai 0.106 (Table 1).

Greater number of *Culex pipiens* was collected in outdoor habitats (N=3009) as compared to indoor sites (N=105) in Mardan Tehsil. Similarly, number of larvae (N=1089) was highest in Mardan as compared to Takhtbhai (N=204) and Katlang (N=81). *Culex pipiens* larvae were most abundant in Mardan (N=1013) followed by Takhtbhai (N=186) and Katlang (N=77) (Table 2).

During the present study mosquitoes were collected from May to November 2017 and their peak abundance was observed in August and September while least abundance in May and November (Fig. 2) Positive correlation was observed between climatic variables and mosquito abundance in all studied tehsils (Table 3).

Culex pipiens larval abundance had negative correlation with pH whereas it correlated positively with electric conductivity, salinity, and TDS. Majority of mosquito larvae were collected in pH of greater than 7.0 (Table 4 and 5).

Overall mosquito abundance was high at elevation < 347m above sea level (asl) while their abundance was observed at elevation ranging from 347 to 1159m asl in the district. In addition, *Cx. pipiens* abundance was also high at < 347m elevation in the region. *Culex. pipiens* abundance was clustered on agriculture land of the district (Fig. 3).

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Table 1. Species composition, diversity and distribution of mosquitoes (adults and larvae) in Mardan District, 2017

Species	Tehsils						Grand Total
	Mardan		Katlang		Takhtbhai		
Gender	♂/♀	♂:♀	♂/♀	♂:♀	♂/♀	♂:♀	
Cx. pipiens	1041/2073	0.5	182/523	0.34	236/505	0.46	4560
Ar. subalbatus	152/185	0.82	36/49	0.73	19/26	0.73	467
Ae. albopictus	8/18	0.45	1/1	0	0/0	0	28
Cx. vishnui	0/0	0	2/2	1	5/10	0.5	19
Cx. tritaeniorhynchus	1/1	1	0/0	0	0/0	0	2
An. walkeri	1/1	1	0/0	0	0/0	0	2
Sub total	1203/2278		221/575		260/541		5078
Species per site	5		4		3		
Species Richness	0.084		0.141		0.106		
Diversity index (H)	0.25		0.28		0.29		

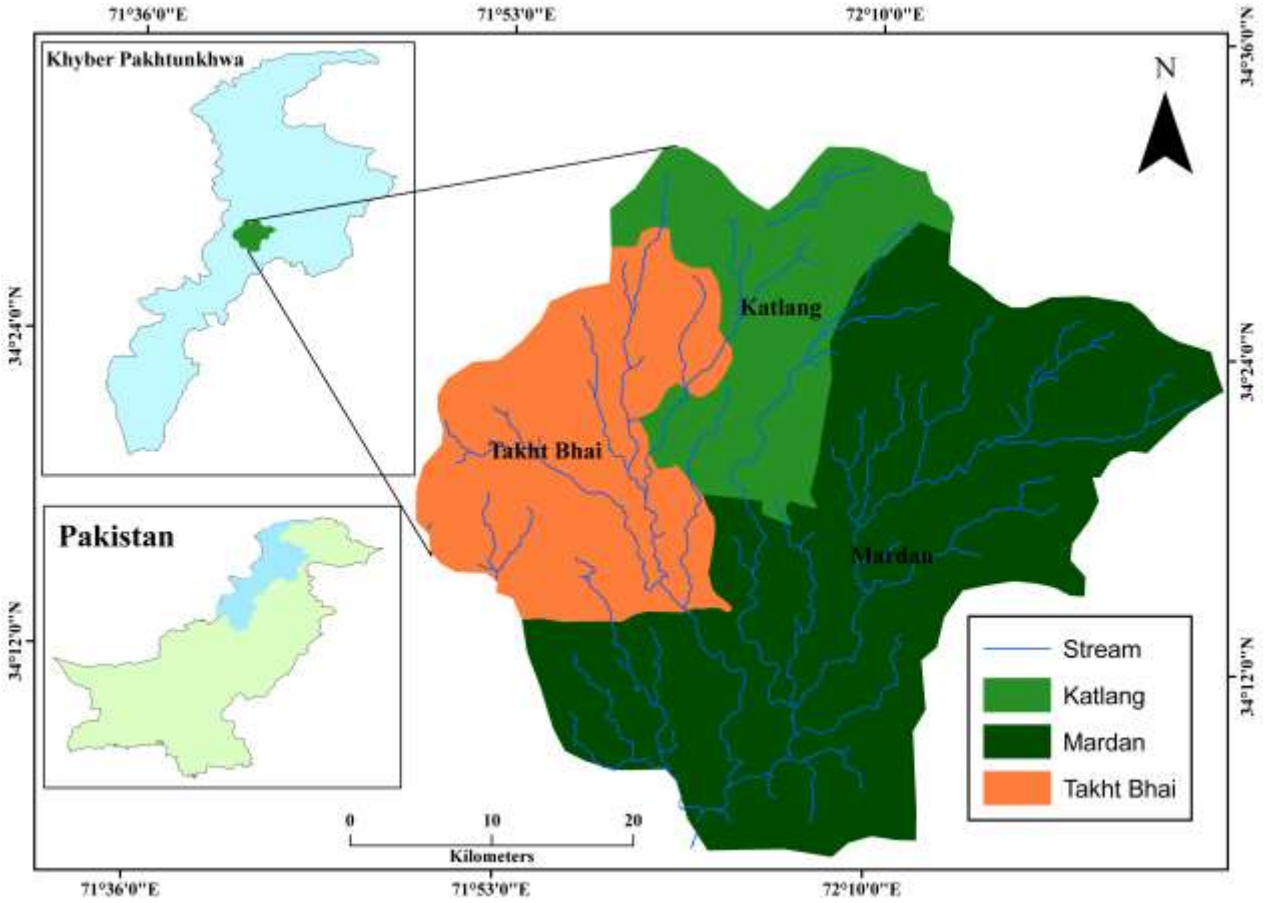

Fig. 1. Study area location map

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Table 2. Mosquito adult/larvae and indoor/outdoor abundance in Mardan District, 2017

Tehsils	Species	*Cx. pipiens*	*Ar. subalbatus*	*Ae. albopictus*	*Cx. vishnui*	*Cx. tritaeniorhynchus*	*An. walkeri*	Sub total
Mardan (N/%)	Indoor	105(53.29)	73(37.05)	15(7.61)	0	2(1.01)	2(1.01)	197
	Outdoor	3009(91.62)	264(8.03)	11(0.33)	0	0	0	3284
Katlang (N/%)	Indoor	27(24.77)	78(71.55)	2(1.83)	2(1.83)	0	0	109
	Outdoor	678(98.68)	7(1.01)	0	2(0.29)	0	0	687
Takhtbhai (N/%)	Indoor	0	0	0	0	0	0	0
	Outdoor	741(92.5)	45(5.617)	0	15(1.87)	0	0	801
Mardan (N/%)	Adult	2101(85.30)	334(13.56)	24(0.97)	0	2(0.08)	2(0.08)	2463
	Larvae	1013(93.08)	74(6.73)	2(0.18)	0	0	0	1089
Katlang (N/%)	Adult	628(97.52)	12(1.86)	2(0.31)	2(0.31)	0	0	644
	Larvae	77(95.06)	2(2.65)	0	2(2.65)	0	0	81
Takhtbhai (N/%)	Adult	555(92.96)	42(7.04)	0	0	0	0	597
	Larvae	186(91.18)	3(1.47)	0	15(7.35)	0	0	204
	Grand Total	4560	467	28	19	2	2	

Table 3. Tehsil wise correlation between abundance and climatic parameters in Mardan, 2017

Tehsils	Monthly mean Min. Temp	Monthly mean Max. Temp	Monthly Total Rain Fall (mm)	Relative Humidity (Average)
Mardan	0.2704	0.0033	0.5666	0.5127
Katlang	0.2591	0.12	0.1723	0.2182
Tkhtbhai	0.3538	0.1266	0.5054	0.2759
Total Abundance	0.2866	0.0396	0.5069	0.4439

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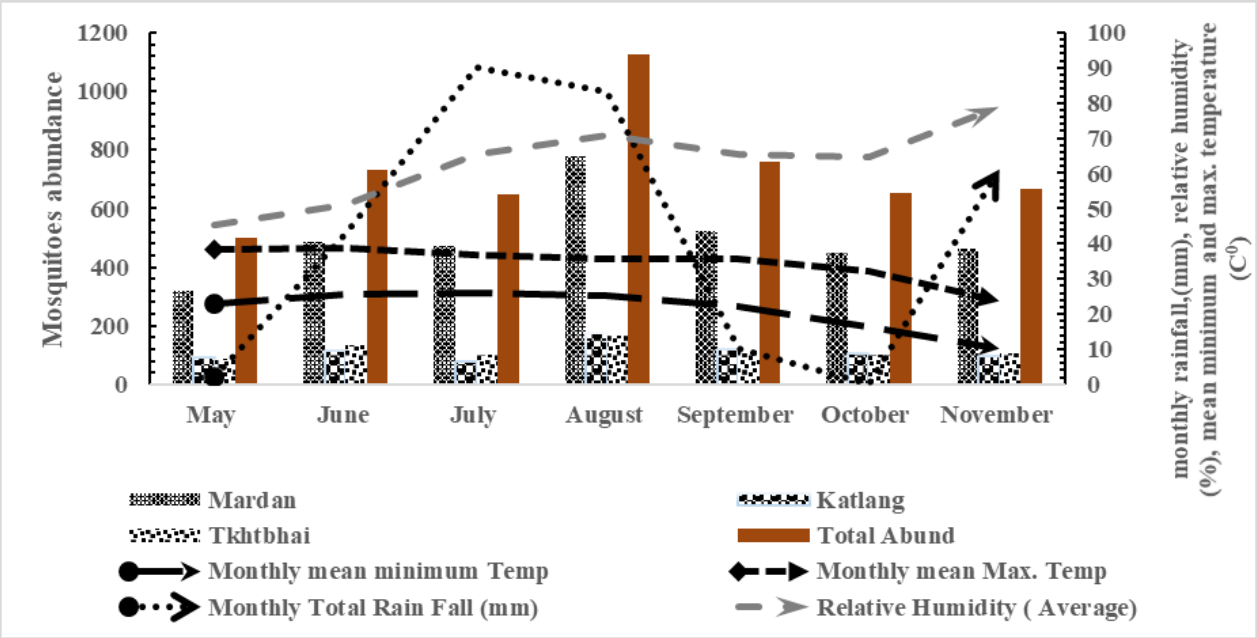

Fig. 2. Tehsil wise seasonal abundance of mosquitoes in Mardan District, 2017

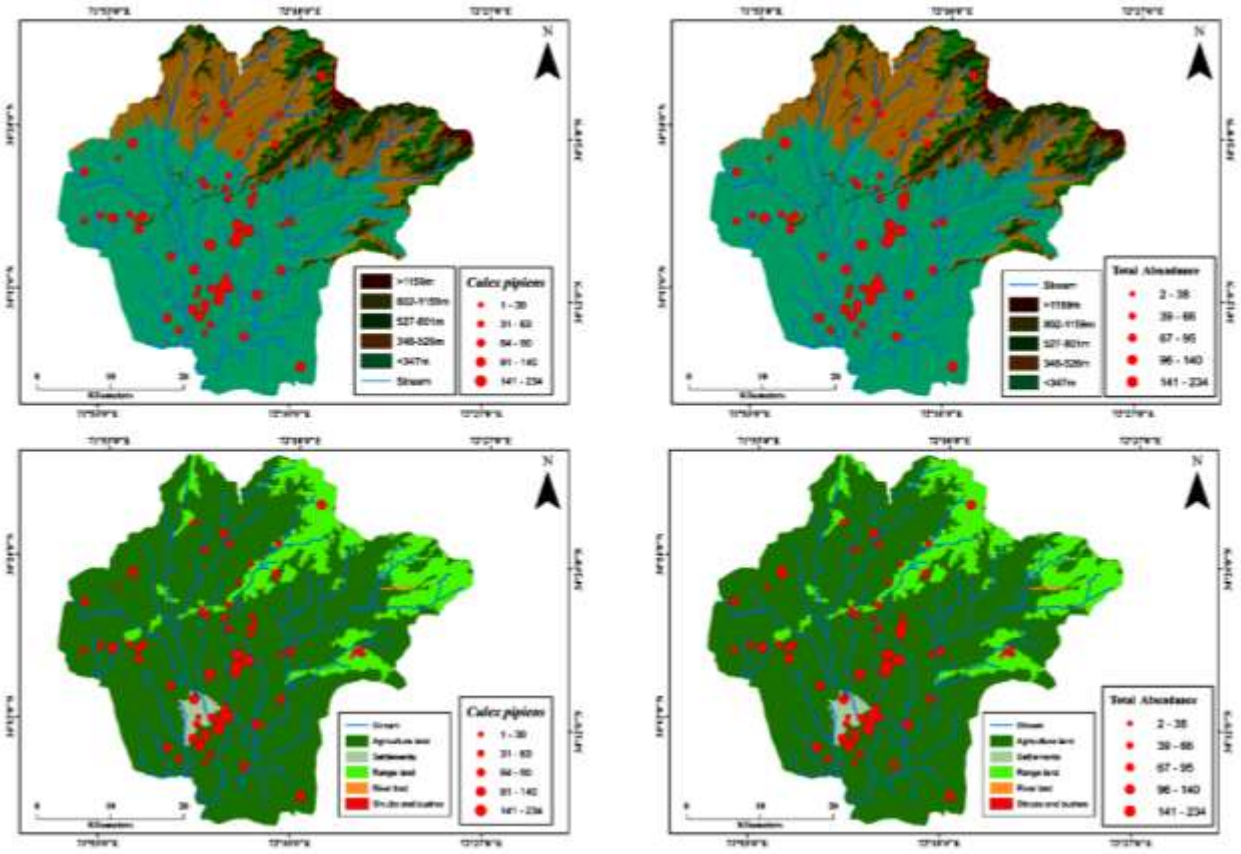

Fig. 3. Mosquito abundance projected on elevation and land cover maps, 2017

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Table 4. Larvae collection and physiochemical properties of water parameters of the studied sites in Mardan, 2017

Tehsil	Collection sites	Average pH	Average EC (µS/cm)	Average TDS	Average Salinity (g/L)	Mosquitoes larvae (number)
Mardan	Stagnant water near houses	7.33	1488.33	997.2	1.00	*Cx. pipiens* (234), *Ar. subalbatus* (15)
	Stagnant water under shrubs/ herbs	7.51	1684	1128.3	1.13	*Cx. pipiens* (200), *Ar. sublbatus* (31)
	Stagnant field water	7.39	1946.1	1303.9	1.3	*Cx. pipiens* (224), *Ar. subalbatus* (10), *Ae. albopictus* (2)
	Sewerages	7.3	2213	1482.7	1.48	*Cx. pipiens* (220), *Ar. subalbatus* (2)
	Stagnant water bodies at dung places/ stockyard and garbage's	7.58	1495.85	1002.23	1	*Cx. pipiens* (135), *Ar. subalbatus* (16)
Katlang	Clean water ditches in crop field	7.48	1569	1051.25	1.05	*Cx. pipiens* (77), *Ar. subalbatus* (2), *Cx. vishuni* (2)
Takhtbhai	Drainages	7.4	1886	1263.6	1.26	*Cx. pipiens* (186), *Ar. sulbalbatus* (3), *An. vishnui* (15)
Mean		7.450	1697.916	1137.614	1.136	
Average SDV		0.107	412.523	276.392	0.276	

TDS: total dissolved sulphur, EC; Electric conductivity, Sd: standard deviation

Table 5. Species wise correlation between larval abundance and water parameters in Mardan, 2017

	Total larvae	*Cx. pipiens*	*Ar. subalbatus*	*Ae. albopictus*
Average pH	-0.42	-0.4064	0.4642	-0.1628
Average EC	0.36	0.3395	-0.4352	0.3113
Average TDS	0.36	0.3396	-0.4351	0.3114
Average Salinity	0.37	0.3474	-0.4296	0.3077

TDS: total dissolved sulphur, EC: electric conductivity

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Discussion

Mosquito biodiversity and ecological preferences are poorly known despite the fact that there is a need to explore the diverse mosquitos fauna in South Asia including Pakistan (16). Recent dengue epidemics revealed the need for more detailed understanding of the diversity, habitat preferences and distribution of these arthropods in the region (17, 18). Studies on species composition and abundance of local mosquito populations have helped to develop better management strategies for mosquito-borne diseases (19-21). Therefore, this study was aimed to provide information related to species composition, seasonal variation, larval breeding sites and spatial distribution of mosquitoes in District Mardan, Khyber Pakhtunkhwa, Pakistan.

During the study period total of 5078 mosquito's specimens (adults and larvae) belonged to four genera and six species including *Cx. pipiens*, *Ar. subalbatus*, *Ae. albopictus*, *Cx. vishnui*, *Cx. tritaeniorhynchus* as well as *An. walker* were recorded in the Mardan. Among the collected mosquitoes, medical importance of *Cx. pipens* is not yet investigated in Pakistan. Although this species is source of nuisance due to its biting pattern. The high abundance of this species was reported in different countries globally (22) and locally in Pakistan (23). This species could transmit filarial parasites to humans and in immunocompromised individuals. In the present study *Ar. subalbatus* were anthropophilic mosquitoes and are known to feed on birds blood showing an opportunistic feeding behaviour (24). However, their medical importance is not adequately studied in Pakistan. Although, this mosquito has the vectorial capability of Brugian filariasis (25), Japanese encephalitis virus (26) and dog heart worm (*Dirofilaria immitis*) (27).

In the current study we reported one species of *Aedes* genus, *Ae. albopictus* primarily because the collection sites were rural, and the species has been reported to be preferentially rural. It's been known to transmit at least 20 arboviruses, filarial worms and a main vector of dengue in Asia (28). In Pakistan previous studies established that, *Ae. albopictus* is the vector of dengue and is chiefly responsible for the maintaining the disease transmission in rural areas (12). *Culex tritaenorhynchus*, *Cx. vishnui* and *An. walkeri* were captured in least number in the study area. Among them *Cx. tritaenorhynchus* usually prefer human settlement (24). It is responsible for the transmission of WNV (West Nile virus) in Pakistan (29). Other two species, *Cx. vishnui* and *An. walkeri* are suspected to be the vectors of JE (30) and WNV to humans respectively (31).

Mosquitoes were collected in three tehsils of Mardan District whereas, highest number of specimens were recorded in tehsil Mardan as compared to Katlang and Takhtbai. This is probably because the collection had *Cx. pipiens* in highest number, which has been known to prefer polluted and urban landscape for existing and breeding (32). Tehsil Mardan has congested human settlements, polluted environment and is a main business hub while other two tehsils have freshwater habitats. Other reason for variations in species composition and distribution is the variation in various biotic factors (food) and abiotic factors (habitat diversity, water bodies, weather conditions and elevation) (33).

Investigating seasonal variation has a key importance in the control of vector-borne diseases. In our study we found that mosquitoes had a single peak from July to September in the region. In Pakistan, heavy rainfall occurs in these months that provides suitable breeding sites and climate for their existence. During the collection period maximum number of specimens were recorded from stagnant water bodies which are reported to be the breeding sites for *Culex*, *Aedes* and *Anopheles* in the province (12, 34).

All ground water breeding mosquito larvae require optimal water temperature, TDS and

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pH for their survival. We observed that mosquito larvae favoured pH of 7.4± (6.8-7.4± preferable for breeding) as recorded by others (35, 36). There was a positive correlation of larval abundance with TDS. Its known that TDS in its higher concentration reduces water transparency and increases oxygen deficiency (37). In our spatial analysis we observed mosquito abundance at low elevation < 347m asl, in agriculture land and near water bodies (streams). Utilization of vector distribution maps help to predict the risk for transmission of various diseases (38, 39).

The current study highlights the mosquito diversity of Mardan region which is poorly explored in the past. The importance of these findings may help medical entomologist and health departments for implementing effective control and surveillance of mosquitoes borne diseases.

Conclusion

A total of sex (06) mosquitoes were collected whereas, *Culex pipiens* being the most abundant species, was susceptible to high pH. Mosquitos species preferred habitats at low elevation in agriculture land in the area. This study will help the medical entomologists and health experts for allocation of the budget.

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Ethical considerations

This study was approved by the Board of Studies, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan.

Conflict of interest statement

Authors declare that there is no conflict of interest.

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