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Effect of Urbanization and Season on the Spatiotemporal Distribution of Mosquitoes in Bahawalpur, Pakistan

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FM and MO conceptualized and designed the study. RS performed field work and lab work and prepared the first draft. FA analyzed the data. HR reviewed and revised the manuscript. All authors approved the final manuscript to be submitted.

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Spatiotemporal, Urbanization, *Culex, Anopheles, Aedes*

ABSTRACT

Mosquitoes are disease-causing vectors that carry a variety of pathogens. Their diversity and spatiotemporal distribution are highly affected by urbanization and seasons. In this study, the mosquitoes were collected from six tehsils (Hasilpur, Ahmedpur East, Khairpur Tamewali Yazman Bahawalpur City and Bahawalpur Saddar,) of district Bahawalpur from September 2020 to August 2021. All the mosquitoes were morphologically identified by using standard identification keys. The data was analyzed using the Shannon-Wiener diversity Index, one-way ANOVA, and unpaired t-test. The highest number of mosquitoes was found in tehsil Bahawalpur Saddar while the lowest number was observed in tehsil Ahmedpur East. Of all the three genera collected, the genus *Culex* was highly abundant (75.89%), followed by *Anopheles* (19.08%) and *Aedes* (5.03%). *Culex* and *Anopheles* mosquitoes were found in all the six tehsils of Bahawalpur while *Aedes* mosquitoes were found in Bahawalpur City and Bahawalpur Saddar, only. The number of mosquitoes was higher in rural areas as compared to urban areas of four tehsils of district Bahawalpur calculated through t test.

INTRODUCTION

Pakistan is a subtropical country that is rich in mosquito fauna (Imran *et al*., 2022). Mosquitoes can cause deadly diseases like dengue (Knowlton *et al*., 2009), malaria (Lawler and Lanzaro, 2005) West Nile fever (Styer *et al*., 2011), Chikungunya (Higgs and Vanlandingham 2015), Japanese Encephalitis, etc. (Attaullah *et al*., 2023).

Bahawalpur, a highly populated district of Pakistan, is also experiencing rapid mosquito-borne disease outbursts due to the high level of urbanization which causes mismanagement and deterioration in solid waste disposal (Mohsin and Chinyama, 2016). Due to rapid urbanization a change in anthropogenic landscape i.e., the development of parks, gardens, swimming pools, urban sewage systems, etc. is occurring which is affecting the diversity, spatiotemporal distribution, and abundance of mosquitoes (Ferraguti *et al*., 2016).

The influence of seasons on the breeding habitats of mosquitoes affects mosquito abundance. In the different seasons, temperature plays a key role. Temperature changes the period of maturity of adult mosquitoes (Tun-Lin *et al*., 2000; Bayoh and Lindsay, 2004). Rainfall in different seasons directly affects their breeding sites (Ceccato *et al*., 2005; Byun and Webb, 2012). The relative humidity is also one of the factors in the seasonal effects that regulate the oviposition and metabolism of adult mosquitoes (Ceccato *et al*., 2005; Reiter, 2001). All these climatic variables are therefore responsible for controlling many mosquito-borne diseases. (Wongkoon *et al*., 2013; Bashar and Tuno, 2014).

The present study aims to determine the specific patterns of diversity, abundance, and community composition of mosquitoes in different tehsils of district Bahawalpur. The effect of urbanization and seasons on the spatiotemporal distribution of mosquitoes is also investigated in this study. This study would be helpful in developing strategy for control of mosquito-borne diseases.

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MATERIALS AND METHODS

Study area

Sampling was done from district Bahawalpur (29.418° N and 71.670° E). The total area of this district is 24,830 km^2. It is located 117 m above sea level. Administratively, it is divided into six tehsils; Bahawalpur City, Yazman, Bahawalpur Saddar, Hasilpur, Khairpur Tamewali, Ahmadpur East, and Khairpur. These tehsils are further divided into 78 rural and 29 urban areas. It is a highly populated district with a total population of 3,668,106. The population density of district Bahawalpur is 153.4 persons per km^2. The climate is extremely dry and hot during summers and dry and cold during winters. The mean annual rainfall of Bahawalpur district is 169.8 mm. The rainfall usually occurs during the monsoon season i.e., July and August (https://bahawalpur.org/tehsils/).

Collection of mosquitoes

Adult mosquitoes were collected from specific sites on monthly basis from September 2020 to August 2021 from inside the houses outside gutters, cattle sheds, lawns, graveyards, zoos, etc., from different tehsils of Bahawalpur. The adult mosquitoes were collected with a CDC sweeper and mechanical aspirators.

Identification of mosquitoes

Mosquitoes were identified based on their morphological features using taxonomic keys (Christophers, 1933; Rueda, 2004; Barraud, 1934).

Statistical analysis

The Shannon diversity index (*H*) was applied to characterize species diversity in the six tehsils of district Bahawalpur. Shannon's index accounts for both the abundance and evenness of the species present (Strong, 2016). Adult mosquito abundance between the six tehsils was compared using a one-way analysis of variance test (ANOVA) where significant differences were observed in an ANOVA test, Tukey's post hoc analysis was used to separate the means. One-way ANOVA also helped to find the relationship between the abundance of mosquitoes with the season (Kim, 2017). The comparison of the abundance of mosquitoes between rural and urban areas of different tehsils of district Bahawalpur was analyzed through a student t-test (Kim, 2015).

RESULTS

A total of 18,354 mosquitoes were collected from the six tehsils of Bahawalpur. Among all the three genera, the genus *Culex* was highly abundant (75.89%), followed by *Anopheles* (19.08%) and *Aedes* (5.03%). Genus *Culex* and *Anopheles* were found in all six tehsils. However, *Aedes* mosquitoes were found in two tehsils only i.e., Bahawalpur city and Bahawalpur Saddar (Fig. 1). It was observed that the highest number of mosquitoes were found in the tehsil Bahawalpur Saddar while the lowest number of mosquitoes were found in tehsil Ahmedpur East. A total of five species from the genus *Culex* were identified i.e., *Culex quinquefasciatus* (30.49%). *Culex tritaenorhychus* (27.42%), *Culex pipiens* (11.83%), *Culex pseudovishnui* (4.23%), and *Culex vagans* (1.92%). Similarly, five species of the *Anopheles* genus were identified i.e., *Anopheles subpictus* (10.5%), *Anopheles culcifacies* (5.76%), *Anopheles stephensi* (2.8%), *Anopheles pulcherrimus* (1.72%), and *Anopheles nigerrimus* (0.87%). Only two species, *Aedes albopictus* (3.4%) and *Aedes aegypti* (1.6%) were identified from the genus *Aedes* (Fig. 2).

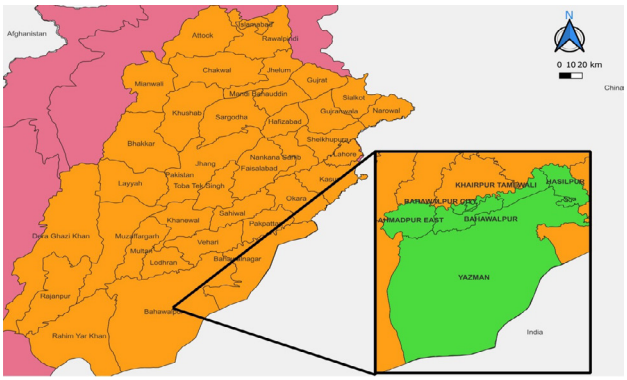

Fig. 1. Map of district Bahawalpur showing different tehsils.

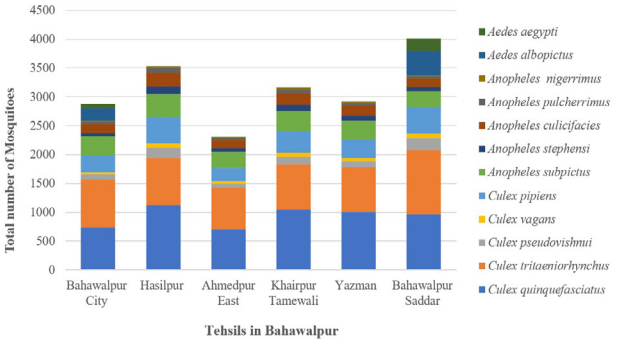

Fig. 2. Total number of mosquitoes in different tehsils of Bahawalpur.

Species diversity and abundance

In general, there were considerable differences between tehsils in terms of the diversity of species identified (n), Shannon diversity (H), and Simpson diversity (D) (Table I).

Table I. Diversity index of number of individuals of different species of mosquitoes collected from six tehsils of District Bahawalpur.

Name of the species	Saddar			Bahawalpur city			Hasilpur			Khairpur			Yazman			Ahmed Pur Sharqia		
	% age	Shannon's index Pi*(logPi)	Simpson's index n (n-1)	% age	Shannon's index Pi*(logPi)	Simpson's index n(n-1)	% age	Shannon'sindex Pi*(logPi)	Simpson's index n(n-1)	%age	Shannon's index Pi*(logPi)	Simpson's index n(n-1)	% age	Shannon's index Pi*(logPi)	Simpson's index n(n-1)	% age	Shannon's index Pi*(logPi)	Simpson's index n(n-1)
Culex quinquifasciatus	24	0.343	928332	26	0.349	546860	32	0.364	1264500	33	0.366	1116192	34	0.367	1007012	31	0.362	499142
Culex tritaenorhychus	28	0.356	1246572	29	0.358	683102	23	0.339	669942	25	0.344	598302	27	0.352	601400	31	0.363	516242
Culex pseudovishnui	5	0.149	39800	3	0.110	8372	5	0.148	29756	4	0.133	17030	4	0.119	10712	3	0.112	5700
Culex Vagans	2	0.079	6642	1.3	0.055	1260	2	0.081	5402	2	0.082	4422	2	0.078	3306	2	0.064	1190
Culex pipiens	11.6	0.249	214832	10	0.233	86730	13	0,265	211140	12	0.256	147840	11	0.246	107256	10	0.235	57360
Anopheles subpictus	6.8	0.183	73712	11	0.245	103362	11	0.246	158006	11	0.243	120756	11	0.241	99540	12	0.252	74256
Anopheles stephensi	1.8	0.071	4830	2	0.084	3906	4	0.124	17822	3	0.113	10920	3	0.100	6642	3	0.098	3782
Anopheles culcifacies	3.7	0.121	21462	5.5	0.159	24492	7	0.181	55932	6	0.176	40602	6	0.169	31152	6	0.168	18632
Anopheles pulcherrimus	1.	0.046	1560	1	0.058	1482	2	0.086	6320	2	0.079	4160	2	0.072	2652	2	0.070	1560
Anopheles negirrimus	0.5	0.026	380	0.8	0.037	462	1	0.049	1406	1	0.046	992	1	0.042	650	1	0.043	420
Aedes albopictus	10	0.236	174306	7	0.192	44732	0		0							0		
Aedes aegypti	5	0.158	46872	3	0.096	5700	0		0							0		

Table II. Abundance (Mean ± SD) of mosquitoes in different Tehsils of District Bahawalpur.

Species	Bahawalpur	Hasilpur	Ahmadpur east	Khairpur	Yazman	Saddar	F	P
C. quinquefasciatus	738.67 ± 17.04^{e}	1128.33 ± 15.28^{a}	$705^{e}\pm16.09$	105.67 ± 10.5^{b}	1005.3 ± 18^{c}	963.33 ± 12.01^{d}	394.56	0.001
C. tritaenorhychus	828.67 ± 16.56^{b}	$816b\pm14.73^{c}$	$717.67^{d}\pm17.04$	773.7 ± 17.5^{e}	774 ± 17.09^{e}	1118.7 ± 17.6^{a}	217.62	0.001
C. pseudovishnui	91.33 ± 17.01^{d}	171.67 ± 15.04^{ab}	$75.67^{d}\pm11.5$	134 ± 15.72^{bc}	102 ± 13.11^{cd}	201.67 ± 14.57^{a}	34.1	0.001
C. pipiens	293 ± 13.11^{c}	460 ± 12^{a}	238.67 ± 16.04^{b}	383.67 ± 13.05^{b}	327.67 ± 11.5^{c}	464 ± 11^{a}	150.3	0.001
C. vagans	35.67 ± 2.52^{c}	$74.33a\pm14.5^{b}$	35 ± 2^{c}	65.67 ± 5.13^{ab}	58.33 ± 3.51^{b}	82.67 ± 3.06^{a}	26.26	0.001
A. subpictus	324 ± 17.09^{b}	396.3 ± 17.6^{a}	273 ± 14^{c}	346.67 ± 12.06^{b}	316.33 ± 16.5^{b}	272.33 ± 13.5^{c}	28.45	0.001
A. stephensi	64.33 ± 5.13^{d}	134.67 ± 5.03^{a}	$64^{d}\pm5.29$	103.33 ± 7.64^{b}	84 ± 5.29^{c}	17.33 ± 4.51^{cd}	74.73	0.001
A. culcifacies	159.67 ± 16.17^{bc}	237.3 ± 19.5^{a}	137.67 ± 17.01^{c}	201.67 ± 10.5^{ab}	178.67 ± 13.58^{bc}	146.67 ± 14.5^{c}	17.6	0.001
A. pulcharimus	39.33 ± 1.52^{d}	80.67 ± 3.06^{a}	$39.67^{d}\pm1.52$	64.33 ± 3.06^{b}	53.33 ± 4.16^{c}	40.33 ± 2.52^{d}	108.69	0.001
A. negirrimus	$21.67b\pm1.52^{c}$	37.67 ± 2.52^{a}	21 ± 1^{bc}	32.67 ± 2.08^{a}	26 ± 3^{b}	20 ± 1^{c}	38.82	0.001
A. albopictus	217.3 ± 30.4^{b}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	419.3 ± 30^{a}	307.17	0.001
A. aegypti	77.33 ± 15.04^{a}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	214.33 ± 16.17^{a}	278.61	0.001

The Shannon and Simpson diversity indices for the six tehsils, respectively, ranged from 2.01 to 1.76 and 0.82 to 0.77. The tehsil Saddar has the greatest and tehsil Ahmedpur East had the lowest values for both indices, respectively. Significant variations in mosquito abundance were found in all of the tehsils after analysis of variance (ANOVA) tests were performed. Hasilpur had the highest concentration of *Culex quinquefasciatus* ($F = 394.56$, $df = 5$, $P < 0.001$), followed by Khairpur Tamewali, Yazman, Saddar, Bahawalpur, and Ahmedpur East with the lowest concentration (Table II). *Aedes aegypti* was a rare species in this district that was absent from Hasilpur, Khairpur Tamewali, Yazman, and Ahmedpur East tehsils but highly dispersed in Bahawalpur and Saddar tehsils ($F = 278.61$, $df = 5$, $P < 0.001$). In the other tehsils of Bahawalpur, every species displayed a similar trend of abundance.

Relationship of the abundance of mosquitoes with seasons

Interesting findings were obtained via a one-way ANOVA on the seasonal abundance of mosquitoes. The research revealed that each tehsil's mosquito population was at its highest during the monsoon season, followed by fall, and at its lowest during the winter. In comparison to the summer, there were more mosquitoes in the spring. The Hasilpur tehsil had the greatest *Culex quinquefasciatus* count ($F = 85.81$, $df = 4$, $P = 0.001$) (Table III). Its peak levels of abundance were noted in the autumn (Mean = 299, SD= 15.72) and monsoon seasons (Mean = 299.33, SD= 12.06), with a sharp decline in abundance throughout the winter (Mean = 125, SD= 15.01). In the spring, it increases once more (Mean = 237, SD = 17), and in the summer, it decreases (Mean = 160, SD = 14.1) (Table III). *Anopheles subpictus* of the genus *Anopheles* displayed the same

Table III. Relationship of abundance of species of mosquitoes with seasons in Tehsil Hasilpur of District Bahawalpur.

Species	Mean ± Standard Deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
Culex quinquefasciatus	299± 15.72^{a}	125±15.01^{c}	237±17^{b}	160±14.11^{c}	299.33±12.06^{a}	85.81	0.001
Culex tritaenorhychus	243.67±11.02^{a}	79.3±18.5^{c}	149±14^{b}	109.33±13.01^{c}	238.67±11.02^{a}	88.01	0.001
Culex pseudovishnui	46±3^{b}	15±3^{c}	32.33±2.52b^{c}	26.66±1.52^{c}	54.33±15.04^{a}	14.4	0.001
Culex pipiens	120±16.52^{a}	37.67±2.52^{b}	103.33±13.5^{a}	64±17.09^{b}	133±12.53^{a}	26.06	0.001
Culex vagans	20.66±1.52^{a}	6±1^{c}	10.33±2.08b^{c}	11.33±1.52^{b}	25.33±2.52^{a}	58.49	0.001
Anopheles subpictus	141.33±14.05^{a}	22±3^{b}	116±16^{a}	0^{b}	119.7±17.5^{a}	79.98	0.001
Anopheles stephensi	45.67±2.52^{a}	9.76±2.08^{c}	38.33±1.52^{b}	0^{b}	41.67±2.08a^{b}	372.42	0.001
Anopheles culcifacies	67.3±18^{a}	7±1^{b}	61.7±17.5^{a}	0^{b}	66.67±15.5^{a}	19.82	0.001
Anopheles pulcherrimus	19.33±1.52a^{b}	6.33±1.52^{c}	17.33±2.52^{b}	0^{b}	23.67±2.08^{a}	94.29	0.001
Anophels nigerrimus	11.66±1.52^{a}	2.67b±2.08^{c}	6.3±1.52^{b}	0^{c}	11.33±2.08^{a}	30.09	0.001
Aedes albopictus	0	0	0	0	0	0	0
Aedes aegypti	0	0	0	0	0	0	0

Table IV. Relationship of abundance of species of mosquitoes with seasons in Tehsil Saddar of District Bahawalpur.

Species	Mean ± Standard deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
Culex quinquefasciatus	295.67±14.57^{a}	80±20^{e}	139.6±16.17^{b}	167.3±12.01^{b}	283.33±15.57^{a}	104.06	0.001
Culex tritaenorhychus	320±17^{a}	98.67±13.01^{c}	171.6±14.01^{b}	200.6±17.04^{b}	325.67±14.5^{a}	125.1	0.001
Culex pseudovishnui	48±15.72^{b}	11±3^{d}	36.67±2.08b^{c}	19.33±2.52^{d}	81.67±13.05^{a}	26.41	0.001
Culex pipiens	138.6±12.01^{a}	35±3^{c}	69.67±14.5^{b}	74±14.53^{b}	149.67±13.58^{a}	47.26	0.001
Culex vagans	15±3b^{c}	2.67±2.08^{d}	16.66±1.52^{b}	10.33±2.08^{c}	37.33±2.52^{a}	94.79	0.001
Anopheles subpictus	87.33±16.01^{a}	16.67±2.52^{b}	78±^{a}14.73	0^{b}	93±18.5^{a}	34.25	0.001
Anopheles stephensi	19±3^{b}	5±1^{c}	21±1a^{b}	0^{d}	24.33±2^{a}	11.93	0.001
Anopheles culcifacies	52.33±13.05^{a}	10.33±1.52^{b}	37±3^{a}	0^{b}	48.66±1.52^{a}	44.48	0.001
Anopheles pulcherrimus	10.33±1.52^{a}	3.33±1.52^{b}	13.66±1.52^{a}	0^{b}	12.67±2.52^{a}	40.81	0.001
Anopheles negirrimus	5±1^{b}	2±1c^{d}	4±1b^{c}	0^{d}	9.33±1.52^{a}	34.75	0.001
Aedes albopictus	146.6±16.17^{a}	16.33±2.52^{c}	71±15.52^{b}	62.33±15.01^{b}	125±14.11^{a}	43.51	0.001
Aedes aegypti	72.33±14.5^{a}	9±1^{c}	35.67±2.52^{b}	26.33±2.08b^{c}	73±13^{a}	31.15	0.001

Table V. Relationship of abundance of species of mosquitoes with seasons in Tehsil Khairpur East of District Bahawalpur.

Species	Mean ± Standard deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
Culex quinquefasciatus	289.67±13.58^{a}	109.67±15.18^{d}	222.67±10.5^{b}	152.67±13.01^{c}	284.67±16.62^{a}	98.2	0.001
Culex tritaenorhychus	226.3±19.5^{a}	71.67±15.57^{c}	152.67±17.01^{b}	99±14^{c}	226.67±15.5^{a}	56.49	0.001
Culex pseudovishnui	36.33±2.08^{a}	13.66±1.52^{c}	27.33±2.52^{b}	18.67±2.08^{c}	35.67±2.08^{a}	70.5	0.001
Culex pipiens	102.33±15.5^{a}	32.66±1.52^{c}	84.67±13.65a^{b}	51.67±15.28b^{c}	108.3±17.5^{a}	16.58	0.001
Culex vagans	16.66±1.52^{b}	5.33±1.52^{d}	11±2^{c}	10.33±2.08c^{d}	23±3^{a}	31.19	0.001
Anopheles subpictus	120±18^{a}	18.67±2.08^{b}	101±16^{a}	0^{b}	107.67±16.04^{a}	55.31	0.001
Anopheles stephensi	33±3^{a}	8.67±2.08^{b}	30±1^{a}	0^{c}	34.67±2.08^{a}	201.8	0.001
Anopheles culcifacies	67.3±18^{a}	7±1^{b}	61.7±17.5^{a}	0^{b}	66.67±15.5^{a}	19.82	0.001
Anopheles pulcherrimus	19.33±1.52a^{b}	6.33±1.52^{c}	17.33±2.52^{b}	0^{d}	23.67±2.08^{a}	94.29	0.001
Anopheles nigerrimus	11.66±1.52^{a}	2.67±2.08b^{c}	6.33±1.52^{b}	0^{c}	11.33±2.08^{a}	30.09	0.001
Aedes albopictus	0	0	0	0	0	0	0
Aedes aegypti	0	0	0	0	0	0	0

Table VI. Relationship of abundance of species of mosquitoes with seasons in Tehsil Yazman of District Bahawalpur.

Species	Mean ± Standard deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
Culex quinquefasciatus	282±12.53^{a}	89.3±17.5^{d}	216.67±16.5^{b}	136.7±19^{c}	277.33±14.57^{a}	83.74	0.001
Culex tritaenorhychus	211.67±16.01^{a}	59±11.53^{c}	183.67±13.58^{a}	108.3±18^{b}	59±13.5^{c}	66.25	0.001
Culex pseudovishnui	29.33±2.52^{a}	10.33±1.52^{c}	21.67±2.08^{b}	15.67±2.52^{c}	27.33±2.08a^{b}	39.95	0.001
Culex pipiens	87.67±11.02^{a}	25.33±2.08^{c}	77.7±17.5a^{b}	50±1b^{c}	85.67±13.05^{a}	18.02	0.001
Culex vagans	14.33±2.52^{b}	5.67±2.08^{c}	10.33±1.52b^{c}	10±1b^{c}	19.33±1.52^{a}	24.36	0.001
Anopheles subpictus	107±14.53^{a}	14±1^{b}	94.33±12.5^{a}	0^{b}	99±18.5^{a}	55.79	0.001
Anopheles stephensi	45.67±2.52^{a}	9.67±2.08^{c}	88.33±1.52^{b}	0^{d}	41.67±2.08^{b}	372.42	0.001
Anopheles culcifacies	74±19^{a}	7±1^{b}	69.67±16.62^{a}	0^{b}	83±11.53^{a}	30.86	0.001
Anopheles pulcherrimus	27±3^{a}	9±1^{c}	19.33±2.52^{b}	0^{d}	24.33±2.08a^{b}	91.91	0.001
Anopheles nigerrimus	15±1^{a}	3±1^{c}	8±1^{b}	0^{c}	12.33±2.52^{a}	62.77	0.001
Aedes albopictus	0	0	0	0	0	0	0
Aedes aegypti	0	0	0	0	0	0	0

Table VII. Relationship of abundance of species of mosquitoes with seasons in Tehsil Ahmadpur East of District Bahawalpur.

Species	Mean ± Standard deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
Culex quinquefasciatus	229.33±12.01^{a}	80±15.1^{b}	89.67±17.01^{b}	80.67±11.02^{b}	230±20^{a}	81.52	0.001
Culex tritaenorhychus	227±15.52^{a}	77±18^{b}	99.67±11.02^{b}	93.33±17.04^{b}	220.33±14.57^{a}	69.04	0.001
Culex pseudovishnui	23.66±1.52^{a}	9.33±1.52^{b}	7±1^{b}	9.67±2.08^{b}	27±3^{a}	67.72	0.001
Culex pipiens	77.33±10.5^{a}	26.33±2.52^{b}	30.67±2.08^{b}	29.66±1.52^{b}	77.33±16.5^{a}	26.79	0.001
Culex vagans	9.67±2.08^{a}	2±1^{b}	6.67±2.08^{a}	8.33±1.52^{a}	10.33±1.52^{a}	11.59	0.001
Anopheles subpictus	86.67±16.56^{a}	13±1^{b}	86.67±15.28^{a}	0	85.3±18.5^{a}	3.99	0.001
Anopheles stephensi	116.67±2.08^{b}	4±1^{c}	18±3^{b}	0^{c}	23.66±1.52^{a}	90.18	0.001
Anopheles culcifacies	43.33±2.08a^{b}	5.67±2.08^{c}	40±2^{b}	0^{d}	47.67±2.52^{a}	403.9	0.001
Anopheles pulcherrimus	10±1^{b}	4.67±2.08^{c}	15±2^{a}	0^{d}	10.66±1.52^{b}	43.46	0.001
Anopheles nigerrimus	5.33±1.52^{a}	2±1^{b}	7±1^{a}	0^{b}	7±1^{a}	27.72	0.001
Aedes albopictus	0	0	0	0	0	0	0
Aedes aegypti	0	0	0	0	0	0	0

Table VIII. Relationship of abundance of species of mosquitoes with seasons in Tehsil Bahawalpur of District Bahawalpur.

Species	Mean ± Standard deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
Culex quinquefasciatus	227±15.72[a]	74±15.52[b]	104±17.09[b]	98±12[b]	234.33±12.06[a]	82.78	0.001
Culex tritaenorhychus	251±15[a]	108±14[b]	108±14.73[b]	103.33±11.02[b]	251±15.1[a]	95.29	0.001
Culex pseudovishnui	31.33±2.52[a]	13.67±2.08[b]	10±1[b]	11±2[b]	27±3[a]	59.1	0.001
Culex pipiens	95.67±16.5[a]	34.33±1.528[b]	37.67±2.08[b]	38.67±2.52[b]	92±14.73[a]	29.18	0.001
Culex vagans	10±1[a]	2±1[b]	6.67±2.08[a]	8.33±1.52[a]	10.33±1.52[a]	15.65	0.001
Anopheles subpictus	96.67±16.04[a]	14±3[b]	104±14.11[a]	0	110±13.11[a]	66.93	0.001
Anopheles stephensi	19.33±2.52[a]	5±1[b]	19.67±2.08[a]	0[c]	20±2[a]	87.69	0.001
Anopheles culcifacies	50.33±1.52[a]	6.67±2.08[b]	46.33±1.52[a]	0[b]	55±19[a]	27.73	0.001
Anopheles pulcherrimus	11.33±1.52[b]	3±1[c]	16±2[a]	0[c]	9.67±2.08[b]	53.64	0.001
Anopheles nigerrimus	6±1a[b]	2.67±2.08b[c]	6±1a[b]	0[c]	8.67±2.08[a]	15.94	0.001
Aedes albopictus	64±12.53[a]	16.33±2.08[b]	24±2[b]	23.33±2.08[b]	83.33±14.05[a]	36	0.001
Aedes aegypti	28.33±2.08[a]	6.33±1.52[c]	9.67±2.08[c]	11±2[c]	21±1[b]	78.04	0.001

pattern of abundance as that of *Culex quinquefasciatus*. It was highest in the autumn season (Mean =119.33, SD=17.5), and it was lowest in the winter (Mean =0, SD = 0). It was highest during the monsoon season (Mean =119 SD=17.5) and spring (Mean =116, SD=16). This tehsil's *Aedes aegypti* population was found to be missing. Its existence in Tehsil Saddar (F = 31.15, df = 4, P = 0.001) demonstrated a consistent seasonal pattern, with monsoon season occurrence being highest (Mean =73, SD=13) and winter season occurrence being lowest (Mean=9, SD=1) (Table IV) The association between abundance and the season of the other species displayed the similar trend as that of *Culex* and *Anopheles* (Tables III-VIII).

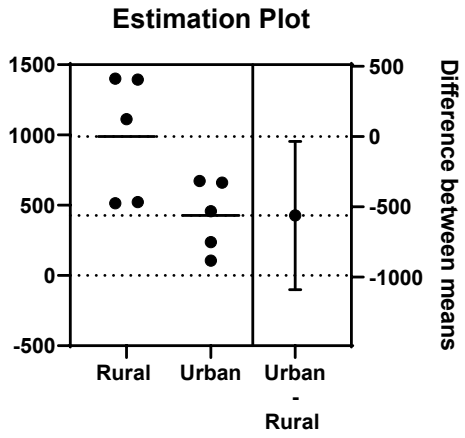

Fig. 3. Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Hasilpur of District Bahawalpur.

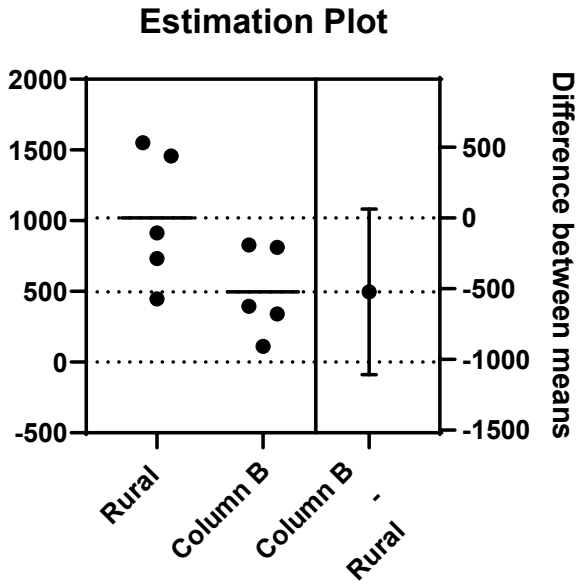

Fig. 4. Non- significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Saddar of District Bahawalpur.

Comparative analysis between rural and urban areas

The unpaired t-test was used to compare mosquito populations in different tehsils of the Bahawalpur district between rural and urban areas. According to the findings, the abundance of mosquitoes in the tehsils of Hasilpur, Ahmedpur East, Khairpur Tamewali, and Yazman were significantly high in rural as compared to urban areas (Fig. 3 and Table IX). Tehsil Yazman recorded a highly significant outcome (t= 2.64, p= 0.028, F= 3.530). Results in the

tehsils of Bahawalpur (t= 1.617, p= 0.144) and Saddar (t= 2.059, p= 0.073) were not statistically significant (Fig. 4), indicating that these areas are heavily urbanized with little distinction between rural and urban areas.

Table IX. Comparative analysis between rural and urban areas of different tehsils of district Bahawalpur.

Tehsils	t	p	F	Difference between Means± Standard error
Hasilpur	2	0.0396	3.117	561.6±228.7
Ahmedpur east	2.38	0.044	3.163	410.4±171.9
Khairpur	2.50	0.0347	3.247	528±207.9
Yazman	2.664	0.0286	3.530	521.2±195.6
Saddar	2.059	0.0734	2.291	523.2±245
Bahawalpur	1.617	0.1446	1.86	326.4±201.9

DISCUSSION

This is the first time in Pakistan that the spatiotemporal distribution of the mosquitoes in all the tehsils of Bahawalpur is studied along with the comparative analysis of diversity, composition, and abundance of all the genera of mosquitoes between rural and urban areas of different tehsils of Bahawalpur. This study also describes the effect of urbanization and environmental variables on the spatiotemporal distribution of mosquitoes in the Bahawalpur district.

Mashaal (1964) has reported only 2 species of *Anopheles*, *Anopheles stephensi* and *Anopheles culcifacies* as malaria vectors in South Punjab. Different species of *Anopheles* i.e., *Anopheles subpictus, Anopheles culicifacies, Anopheles stephensi, Anopheles pulcherrimus, Anopheles Peditaeniatus,* and *Anopheles nigerrimus* have also been reported from South Punjab (Herrel, 2001). All species except *Anopheles Peditaeniatus* were found in this current study. Similarly, *Anopheles subpictus*, *Anopheles stephensi*, *Anopheles culcifacies,* and *Anopheles pulcherrimus* have also been reported, previously. *Culex quinquefasciatus, Culex tritaeniorhynchus,* and *Aedes* mosquitoes were also found in wastewater in South Punjab (Mukhtar *et al*., 2003).

In the current study, the species of genus *Culex* (5 species) and genus *Anopheles* (5 species) were reported in all the rural and urban areas of Bahawalpur. Mosquito abundance and species richness were higher in rural areas than in urban areas. These results agreed with those from previous studies conducted in Europe (Hay *et al*., 1997; Johnston *et al*., 2014) where anthropogenic habitats usually show the lowest abundance of mosquitoes. Rural areas, with freshwater, are more favorable breeding environments, despite those urban areas that may provide suitable habitats for some particular mosquito species (Cox *et al*., 2007).

Aedes mosquitoes were found absent from Tehsil Hasilpur, Khairpur Tamewali, Yazman, and Ahmedpur East. These are periurban areas that are in transition from rural to urban. zone. The species of *Aedes* were sparsely distributed in tehsils Bahawalpur and Saddar. The main reason for the occurrence of Aedes in these tehsils was heavy urbanization which was not found in other tehsils. With time, urbanization has increased in these areas due to the conversion of agricultural, forest, and fallow land into urban land (Hussain *et al*., 2020). With exceptional population growth and rapid urban development, the number of hospitals and clinics has increased several folds which has resulted in huge hospital waste and poor drainage providing favorable conditions for mosquito breeding, especially *Culex* spp. (Khan *et al*., 2020). The high number of animals in the Bahawalpur Zoo also benefits a variety of mosquito species (Derraik, 2004), especially the *Aedes* mosquitoes which require artificial containers for their breeding (Tuten *et al*., 2012). In highly urban areas, used tires are not disposed of properly. They effectively hold rainwater and rapidly warm in sunlight even during winter thus providing an ideal breeding habitat for *Aedes* (Khan *et al*., 2017). Similar results were found in a study conducted in North India where it was observed that tires provide favorable breeding sites for *Aedes aegypti* (Sikhon and Minhas, 2014).

Our study showed that the mosquito species occurred in all six tehsils of Punjab throughout the year in all seasons. The highest abundance was found in the season of monsoon that was during July and August. This has been proved in Pakistan (Resin and Milby, 1986; Mukhtar *et al*., 2003; Ashfaq *et al*., 2014; Akram *et al*., 2009; Fatima *et al*., 2016) and other countries (Roiz *et al*., 2014; Valentine et al., 2020). We found the positive effect of precipitation on the relative abundance of mosquito species. The precipitation increases the number of habitats of larvae (Evans *et al*., 2019). It also has a profound effect on the relative humidity which in turn increases the richness, life span, and host-seeking behavior of mosquitoes (Asigau and Parker, 2018). On the other hand, excessive rain may flush larvae from their habitats and decrease adult mosquito populations (Dieng, 2012).

The current study showed that a high number of species were also found during spring and autumn. During early spring and winter, few species were recorded. The number of species increased again in late April with an

especially high peak in May. In addition, the population density was higher in autumn, especially in September and October. The same results were observed in Pakistan, previously (Akram *et al*., 2009) and around the globe (Alten *et al*., 2000; Santos *et al*., 2020).

It is concluded through this study that changes in the landscape due to urbanization strongly affect the abundance, community composition, and diversity of mosquitoes. Mosquito populations are also affected by changes in climate. This information in the future would be helpful for the public health to design surveillance programs to control the diseases caused by mosquitoes in the reported areas.

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