

ROLE OF URBANIZATION IN SPATIOTEMPORAL DISTRIBUTION, DIVERSITY AND ABUNDANCE OF MOSQUITO IN PUNJAB, PAKISTAN



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A THESIS SUBMITTED TO LAHORE COLLEGE FOR WOMEN UNIVERSITY IN
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CERTIFICATE

This is to certify that the research work presented in this thesis, is entitled "**Role of urbanization in spatiotemporal distribution, diversity and abundance of mosquito in Punjab, Pakistan**" was conducted by **Ms. Robeela Shabbir** under the supervision of **Dr. Farkhanda Manzoor**. No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the department of zoology, lcwu in partial fulfillment of the requirements for the degree of doctor of philosophy in zoology, department of zoology, Lahore College for women university, Lahore.

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DEDICATE THIS EFFORT TO**MY LOVING FATHER**

My father, Shabbir Ahmed Tahir not only brought me up and cared for me but gave me the greatest gift anyone could give another person: he believed in me! He is the anchor upon which I stand strong.

MY DOTING MOTHER

Tousif Qamar, her influence in my life is beyond calculation. The inspiration she provided for knowledge has been a beacon of light, and I owe everything I possess to her faithful prayers and unheralded efforts.

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TABLES OF CONTENTS

Chapter 1 Introduction.....	1
1.1 INTRODUCTION	2
1.2 AIM AND OBJECTIVES.....	11
Chapter 2 REVIEW OF LITERATURE.....	12
Chapter 3 METHODOLOGY	25
3.1 Study Area and Duration	26
3.1.1 Rice Tract Zone.....	26
3.1.2 Mixed Cropping Zone.....	26
3.1.3 Cotton Zone	27
3.1.4 Thal Zone	27
3.1.5 Barani Zone.....	28
3.2 Collection of Mosquito	28
3.3 3.3: Morphological Identification of mosquitoes.....	29
3.4 Molecular Identification.....	29
3.4.1 Gel Electrophoresis.....	29
3.4.2 Polymerase Chain reaction	30
3.4.3 Species discrimination using DNA barcodes.....	30
3.4.4 Making phylogenetic tree	31
3.5 Statistical Analysis.....	31
3.6 GIS Mapping of mosquito population	31
Chapter 4 RESULT	33
4.1 Rice tract Zone District Lahore.....	34
4.1.1 Species Diversity and Abundance of Mosquitoes in District Lahore	36
4.1.2 Relationship of abundance of mosquito with seasons in different tehsils of District Lahore	39
4.1.3 Effect of climatic factors on the abundance of mosquitoes in District Lahore.....	45
4.1.4 GIS Mapping for District Lahore.....	46
4.2 Mixed Cropping Zone District Faisalabad.....	46
4.2.1 Species Diversity and Abundance of mosquitoes in district Faisalabad.....	47
4.2.2 Relationship of the abundance of mosquitoes with seasons in District Faisalabad	53
4.2.3 Effect of climatic factors on the abundance of mosquitoes in District Faisalabad.	61
4.2.4 Comparative analysis of abundance of mosquitoes between rural and urban areas of tehsils of Faisalabad	61

4.2.5 GIS Mapping.....	65
4.3 Cotton Zone District Bahawalnagar.....	66
4.3.1 Species Diversity and Abundance of mosquitoes in district Bahawalnagar.....	67
4.3.2 Relationship of the abundance of mosquitoes with seasons in District Bahawalnagar	74
4.3.3 Effect of climatic factors on the abundance of mosquitoes in District Bahawalnagar	81
4.3.4 Comparative analysis between rural and urban areas of tehsils of Bahawalnagar .	81
4.3.5 GIS Mapping.....	84
4.4 Cotton Zone District Bahawalpur	84
4.4.1 Species Diversity and Abundance of Mosquitoes in District Bahawalpur	85
4.4.2 Relationship of the abundance of mosquitoes with seasons in District Bahawalpur	36
4.4.3 Effect of climatic factors on the abundance of mosquitoes in District Bahawalpur	47
4.4.4 Comparative analysis between rural and urban areas of tehsils of Bahawalpur....	48
4.4.5 GIS Mapping.....	50
4.5 Thal Zone District Layyah	51
4.5.1 Species Diversity and Abundance of mosquitoes in district Layyah.....	52
4.5.2 Relationship of the abundance of mosquitoes with seasons in district Layyah.....	59
4.5.3 Effect of climatic factors on the abundance of mosquitoes in District Layyah.....	63
4.5.4 Comparative analysis between rural and urban areas of tehsils of Layyah	63
4.5.5 GIS Mapping.....	65
4.6 Barani Zone District Rawalpindi	66
4.6.1 Species Diversity and Abundance of mosquitoes in district Rawalpindi	67
Figure	82
4.6.2 Relationship of the abundance of mosquitoes with seasons in District Rawalpindi	82
4.6.3 Effect of climatic factors on the abundance of mosquitoes in District Rawalpindi	90
4.6.4 Comparison of abundance of mosquito between rural and urban areas in district Rawalpindi	90
4.6.5 GIS Mapping in District Rawalpindi:	96
4.7 Consolidated Result of Agroecological Zones of Punjab	96
4.7.1 Species Diversity and Abundance of mosquitoes in all Agroecological Zones of Punjab	98
4.8 Polymerase Chain Reaction:	107
Chapter 5 : DISCUSSION.....	111

REFERENCES	120
ANNEXURES	xi
PLAGIARISM REPORT	xxi
LIST OF PUBLICATIONS	xxii

LIST OF TABLES

Table 3.4.4.1: Total Number of mosquitoes in different tehsils of Lahore	Error! Bookmark not defined.
Table 3.4.4.2: Diversity index of a number of individuals of different species of mosquitoes collected from five tehsils of District Lahore.	Error! Bookmark not defined.
Table 0.1:Abundance of Culex tritaeniorhynchus	Table 0.2:Abundance of Culex
quinquefasciatus	37
Table 0.3:Abundance of Culex psedovishnui	Table 0.4:Abundance of Culex pipiens
	38
Table 0.5:Abundance of Culex siiens	Table 0.6:Abundance of Aedes albopictus
	38
Table 0.7:Abundance of Aedes aegypti	39
Table 4.1.2.1:Relationship of abundance of species of mosquitoes with seasons in Tehsil Lahore City of District Lahore	Error! Bookmark not defined.
Table 4.1.2.2:Relationship of abundance of species of mosquitoes with seasons in Tehsil Model Town of District Lahore	41
Table 4.1.2.3:Relationship of abundance of species of mosquitoes with seasons in Tehsil Shalimar Town of District Lahore	42
Table 4.1.2.4:Relationship of abundance of species of mosquitoes with seasons in Tehsil Raiwind of District Lahore	43
Table 4.1.2.6: Effect of climatic variables with relative abundance of mosquito in district Lahore	Error! Bookmark not defined.
Table 4.1.4.1:GIS map of different tehsils showing mosquito abundance in district Lahore	Error! Bookmark not defined.
Table 4.1.4.1:Total no of mosquitoes in district Faisalabad	Error! Bookmark not defined.
Table 4.2.1.1:Diversity index of a number of individuals of different species of mosquitoes collected from tehsils of District Faisalabad.....	48

Table 4.2.1.2: Comparative analysis of the abundance of mosquitoes in different Tehsils of District Faisalabad	50
Table 4.2.1.3: Abundance of Culex quinquefasciatus	Table 4.2.1.4: Abundance of Culex tritaeniorhynchus
	50
Table 4.2.1.5:Abundance of Culex pseudovishnui	Table 4.2.1.6:Abundance of Culex sitiens
	51
Table 4.2.1.7:Abundance of Culex pipiens	Table 4.2.1.8:Abundance of Anopheles subpictus
	51
Table 4.2.1.9:Abundance of Anopheles stephensi.....	Error! Bookmark not defined.
Table 4.2.1.10:Abundance of Anopheles culicifacies.....	52
Table 4.2.1.11:Abundance of Aedes albopictus	Table 4.2.1.12:Abundance of Aedes aegypti
	53
Table 4.2.2.2:Relationship of abundance of species of mosquitoes with seasons in Tehsil Samundari of District Faisalabad.....	55
Table 4.2.2.3:Relationship of abundance of species of mosquitoes with seasons in Tehsil Sadar of District Faisalabad	56
Table 4.2.2.5:Relationship of abundance of species of mosquitoes with seasons in Tehsil Tandlianwala of District Faisalabad	60
Table 4.2.2.6:Relationship of abundance of species of mosquitoes with seasons in Tehsil Jaranwala of District Faisalabad	60
Table 4.2.3.1: Effect of climatic variables on relative abundance of mosquito species in district Faisalabad	Error! Bookmark not defined.
Table 4.2.4.1:T test between the rural and urban areas of different Tehsils of district of Faisalabad	62
Table 4.2.4.2: Significant results of difference of Abundance of mosquito in rural and urban areas of Tehsil Chak Jhumra of District Faisalabad	Error! Bookmark not defined.

Table 4.2.4.3: Significant results of difference of Abundance of mosquito in rural and urban areas of Tehsil Jaranwala of District Faisalabad..... **Error! Bookmark not defined.**

Table 4.2.4.4: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Faisalabad of District Faisalabad **Error! Bookmark not defined.**

Table 4.2.4.5: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Tandiawala of District Faisalabad..... **Error! Bookmark not defined.**

Table 4.2.4.6: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Saddar of District Faisalabad **Error! Bookmark not defined.**

Table 4.2.4.7: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Sammundri of District Faisalabad **Error! Bookmark not defined.**

Table 4.2.5.1: Total no of mosquitoes in district Bahawalnagar **Error! Bookmark not defined.**

Table 4.3.1.1: Diversity index of a number of individuals of different species of mosquitoes collected from tehsils of District Bahawalnagar **Error! Bookmark not defined.**

Table 4.3.1.2: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of Bahawalnagar 69

Table 4.3.1.3: Relative abundance of different species of mosquito in different tehsils of district Bahawalnagar..... **Error! Bookmark not defined.**

Table 4.3.2.1: Relationship of abundance of species of mosquitoes with seasons in Tehsil Fortabbas of District Bahawalnagar..... 74

Table 4.3.2.3: Relationship of abundance of species of mosquitoes with seasons in Tehsil Haroonabad of District Bahawalnagar..... 76

Table 4.3.2.5: Relationship of abundance of species of mosquitoes with seasons in Tehsil Minchanabad of District Bahawalnagar..... 80

Table 4.3.3.1: Effect of climatic variables on relative abundance of mosquito species in district Bahawalnagar..... 81

Table 4.3.4.2: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Bahawalnagar of District Bahawalnagar**Error! Bookmark not defined.**

Table 4.3.4.3: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Haroonabad of District Bahawalnagar **Error! Bookmark not defined.**

Table 4.3.4.4: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Minchanabad of District Bahawalnagar**Error! Bookmark not defined.**

Table 4.3.4.5: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Chishtian of District Bahawalnagar**Error! Bookmark not defined.**

Table 4.3.4.6: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Fortabbas of District Bahawalnagar**Error! Bookmark not defined.**

Table 4.3.5.1: GIS map showing mosquito abundance in different tehsils of district Bahawalnagar.....84

Table 4.4.1.1: Diversity index of a number of individuals of different species of mosquitoes collected from six tehsils of District Bahawalpur..... **Error! Bookmark not defined.**

Table 4.4.1.2: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of Bahawalpur **Error! Bookmark not defined.**

Table 4.4.1.3: Relative abundance of different species of mosquito in different tehsils of district Bahawalpur **Error! Bookmark not defined.**

Table 4.4.2.1: Relationship of abundance of species of mosquitoes with seasons in Tehsil Hasilpur of District Bahawalpur **Error! Bookmark not defined.**

Table 4.4.2.3: Relationship of abundance of species of mosquitoes with seasons in Tehsil Khairpur East of District Bahawalpur.....41

Table 4.4.2.4: Relationship of abundance of species of mosquitoes with seasons in Tehsil Yazman of District Bahawalpur.....42

Table 4.4.2.6: Relationship of abundance of species of mosquitoes with seasons in Tehsil Bahawalpur of District Bahawalpur.....46

Table 4.4.3.1: Effect of climatic variables on relative abundance of mosquito species in district Bahawalpur	47
Table 4.4.4.2: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Khairpur of District Bahawalpur.....	48
Table 4.4.4.3: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Yazman of District Bahawalpur	Error! Bookmark not defined.
Table 4.4.4.4: Non-Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Saddar of District Bahawalpur	49
Table 4.4.4.5: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Ahmed pur East of District Bahawalpur	49
Table 4.4.4.6: Non-Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Bahawalpur of District Bahawalpur	49
Table 4.4.5.1: GIS map showing mosquito abundance in different tehsils of district Bahawalpur	51
Table 4.4.5.1: Total number of mosquitoes in district Layyah Error! Bookmark not defined.	
Table 4.5.1.1: Diversity index of a number of individuals of different species of mosquitoes collected from six tehsils of District Layyah	54
Table 4.5.1.2: Comparative analysis of abundance of mosquitoes in different Tehsils of Layyah.....	Error! Bookmark not defined.
Table 4.5.1.3: Relative abundance of different species of mosquito in different tehsils of district Layyah	Error! Bookmark not defined.
Table 4.5.2.1: Relationship of abundance of species of mosquitoes with seasons in Tehsil Karor Lal Esan of District Layyah.....	Error! Bookmark not defined.
Table 4.5.2.2: Relationship of abundance of species of mosquitoes with seasons in Tehsil Choubara of District Layyah.....	61
Table 4.5.2.3: Relationship of abundance of species of mosquitoes with seasons in Tehsil Layyah of District Layyah	62

Table 4.5.3.1: Effect of climatic variables on relative abundance of mosquito species in district Layyah	Error! Bookmark not defined.
Table 4.5.4.1: T test between the rural and urban areas of different Tehsils of district of Bahawalpur	Error! Bookmark not defined.
Table 4.5.4.2: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Layyah of District Layyah.....	Error! Bookmark not defined.
Table 4.5.4.3: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Choubara of District Layyah	Error! Bookmark not defined.
Table 4.5.4.4: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Karor Lal Esan of District Layyah	Error! Bookmark not defined.
Table 4.5.5.1: GIS map showing mosquito abundance in different tehsils of district Layyah	66
Table 4.5.5.1: Total number of mosquitoes in district Rawalpindi	Error! Bookmark not defined.
Table 4.6.1.1: Diversity index of a number of individuals of different species of mosquitoes collected from six tehsils of District Rawalpindi.....	Error! Bookmark not defined.
Table 4.6.1.2: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of Rawalpindi.....	77
Table 4.6.1.3: Relative abundance of different species of mosquito in different tehsils of district Rawalpindi	Error! Bookmark not defined.
Table 4.6.2.2: Relationship of abundance of species of mosquitoes with seasons in Tehsil Taxila of District Rawalpindi.....	84
Table 4.6.2.3: Relationship of abundance of species of mosquitoes with seasons in Tehsil Kallar Syedan of District Rawalpindi	85
Table 4.6.2.4: Relationship of abundance of species of mosquitoes with seasons in Tehsil Kahuta of District Rawalpindi	86

Table 4.6.2.5: Relationship of abundance of species of mosquitoes with seasons in Tehsil Gujar Khan of District Rawalpindi	87
Table 4.6.2.6: Relationship of abundance of species of mosquitoes with seasons in Tehsil Murree of District Rawalpindi	88
Table 4.6.2.7: Relationship of abundance of species of mosquitoes with seasons in Tehsil Kotli Sattian of District Rawalpindi	89
Table 4.6.3.1: Effect of climatic variables on relative abundance of mosquito species in district Rawalpindi	Error! Bookmark not defined.
Table 4.6.4.1:T test between the rural and urban areas of different Tehsils of district of Rawalpindi	91
Table 4.7.1.1: Diversity index of a number of individuals of different species of mosquitoes collected from six districts of Punjab.....	Error! Bookmark not defined.
Table 4.7.1.2: Comparative analysis of the abundance of mosquitoes in Agroecological zones of Punjab	102

LIST OF FIGURES

Figure 4.6.1: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Murree of District Rawalpindi **Error! Bookmark not defined.**

Figure 4.6.2: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Rawalpindi of District Rawalpindi..... **Error! Bookmark not defined.**

Figure 4.6.3: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Gujar Khan of District Rawalpindi **Error! Bookmark not defined.**

Figure 4.6.4: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Taxla of District Rawalpindi..... **Error! Bookmark not defined.**

Figure 4.6.5: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Kahuta of District Rawalpindi..... **Error! Bookmark not defined.**

Figure 4.6.6: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Kallar Syedan of District Rawalpindi . **Error! Bookmark not defined.**

Figure 4.6.7: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Kotli of District Rawalpindi **Error! Bookmark not defined.**

Figure 4.6.8: GIS map showing mosquito abundance in different tehsils of district Rawalpindi **Error! Bookmark not defined.**

Figure 4.7.2: GIS map showing mosquito abundance in Agroecological zones of Punjab 107

Figure 4.8.1: PCR results of the species of genus Anopheles and Culex108

Figure 4.8.2: PCR results of the species of genus Aedes, Anopheles and Culex108

Figure 4.8.3: PCR results of the species of mosquitoes exclusively found in district
Rawalpind 109

Figure 4.8.4: Phylogenetic tree of the sequences of the species collected from Punjab 110

LIST OF ABBREVIATIONS

Ae	Aedes
Ahpr	Ahmedpur
ANOVA	Analysis of variance
AEZ	Agroecological Zones
An	Anopheles
Bhwp	Bahawalpur
Bhwng	Bahawalnagar
Chtn	Chishtian
CO1	cytochrome coxidase subunit 1
Chj	Chak jumra
Cx	Culex
DyMSiM	Dynamic Mosquito Simulation Model
WRF	Weather Research and Forecasting
Fsl	Faisalabad
FtAb	Fortabbas
GLMM	generalized linear mixed model
GIS	Geographic Information System
Gjr	Gujar Khan
Hrnbd	Haroonabad
Hspr	Hasilpur
Jrn	Jaranwala
Kht	Kahuta
Kst	Kotli Sattian
Khpr	Khairpur
Klsy	Kallar Syedan
Mnchbd	Minchanabad
MEGA X	Molecular Evolutionary Genetics Analysis
Mur	Mureec
PCR	Polymerase Chain Reaction

x

Rwp	Rawalpindi
SD	Standard Deviation
Smun	Samundari
Sdr	Saddar
Tand	Tandlianwali
Txl	Taxila
Yzmn	Yazman

ABSTRACT

Mosquitoes are carriers of disease, carrying a wide range of infections. Urbanization, climate, and seasonal variations all have a significant impact on their diversity and spatial distribution. Mosquitoes were collected for this study between September 2019 and August 2021 from six districts in Punjab: Lahore, Fais^alabad, Bahawalnagar, Bahawalpur, Layyah, and Rawalpindi. All of the mosquitoes were identified morphologically using common identification keys and genetically by polymerase chain reaction. The data was analyzed by using the Shannon-Wiener diversity Index, one-way ANOVA and unpaired t-test. Using GIS mapping, the mosquito population's dispersion was investigated. District Rawalpindi has the most diversity of mosquitoes, whereas district Lahore had the lowest diversity. The most prevalent genus was *Culex* (75.37%), with *Anopheles* (18.11%), *Aedes* (6.17%), and *Armigeres* (0.34%) following closely behind. *Culex perezi*, was firstly reported from the Rawalpindi district using genetic and morphological analysis. Our findings demonstrated a distinct and well-defined pattern of diversity, abundance, and spatiotemporal distribution of mosquitoes in relation to anthropogenic changes in the landscape of the areas observed the species like *Aedes albopictus* and *Culex quinquefasciatus* were more detected in those areas that which were considered more urbanized.

Key words: Spatiotemporal, urbanization, *Culex*, *Anopheles*, *Aedes*, *Armigeres*, Geographic Information System, *Culex perezi*

Chapter 1 Introduction

1.1 INTRODUCTION

Various arthropods including bugs, mites, fleas, flies, ticks, and mosquitoes are responsible for transferring deadly pathogens to animals, birds, and humans, as epidemics or pandemics, through different arthropods like bugs, ticks, mosquitoes, and flies. These arthropods act as a natural reservoir for vector-borne diseases (Mehlhorn *et al.*, 2012). Among all arthropods, mosquitoes are thought to be the first renowned vector for the transmission of vector-borne diseases. Their hematophagous lifestyle enables them to transmit a large number of pathogens and parasites to humans as well as animals and birds. The most common mosquito-borne diseases are Malaria, Chikungunya fever, West Nile fever (WNV), Yellow fever, Dengue fever, Rift Valley fever, Japanese Encephalitis, and Lymphatic Filariasis (Anoopkumar and Aneesh, 2022).

Mosquitoes belong to Phylum Arthropoda, Class Insecta, Order Diptera, and Family *Culicidae* (Balakrishnan *et al.*, 2011). Family *Culicidae* comprises 41 genera and 3,541 identified species. It is divided into three sub-families: *Toxorhynchitinae*, *Culicinae*, and *Anophelinae* (Harbach, 2011). Based on the current scheme of classification, the subfamily *Toxorhynchitinae* consists of only one genus known as *Toxorhynchites*. The subfamily *Culicinae* is the largest and most diverse, consisting of 38 genera. Some well-known genera within this subfamily are *Culex*, *Psorophora*, *Haemagogus*, *Aedes*, *Sabettas*, and *Mansonia*. It consists of more than 3000 species, worldwide. Subfamily *Anophelinae*, with its cosmopolitan distribution, is categorized into three genera i.e., *Chagasia*, *Anopheles*, and *Bironella*. It, collectively, consists of 465 formally recognized species (Foster *et al.*, 2017).

Mosquitoes are found on

The *Culex* genus comprises the majority of mosquito species found worldwide. There are over 400 subgenera in the genus *Culex*, and there are about 1000 known species of *Culex*. Numerous researchers have reported on a variety of *Culex* species, including *Culex quinquefasciatus*, *Culex pipiens*, *Culex bitaeniorhynchus*, *Culex vishnui*, *Culex theileri*, *Culex tritaeniorhynchus*, *Culex flavicornis*, *Culex gelidus*, *Culex nigropunctatus*, *Culex fuscitarsis*, *Cx. univittatus*, *Cx. bailyi*, and *Culex brevipalpis*. (Dutta *et al.*, 2010; Nchoutpouen *et al.*, 2019; Qasim *et al.*, 2014). The vast majority of mosquito species identified globally belong to the genus *Culex*. The genus *Culex* contains more than 400 subgenera, and there are roughly 1000 recognized species of the genus. *Culex quinquefasciatus*, *Culex pipiens*, *Culex bitaeniorhynchus*, *Culex vishnui*, *Culex theileri*, *Culex tritaeniorhynchus*, *Culex flavicornis*, *Culex gelidus*, *Culex nigropunctatus*,

Culex fuscitarsis, *Cx. univittatus*, *Cx. bailyi*, and *Culex brevipalpis* are just a few of the numerous *Culex* species that have been studied by numerous researchers. (Gorris *et al.*, 2021).

Anopheles mosquitoes are believed to be the deadliest of all anthropophilic insects (Konopka, *et al.*, 2021). High ecological variety is exhibited by *Anopheles* mosquitoes, which are most frequently found close to rain pools, saline water, and rice fields. They are widely recognized as the main Plasmodium parasite vectors. Worldwide, more than 30 *Anopheles* species are known to transmit malaria. Malaria kills around 0.5 million people worldwide each year, according to reports. (WHO, 2019). They are also accountable for the transmission of *Wuchereria bancrofti*, Eastern and Western Equine Encephalitis, and Malaria. There are 465 recognized species in the genus *Anopheles*. The most prevalent species worldwide are *An. stephensi*, *An. subpictus*, *An. arabiensis*, *An. gambiae*, *An. culicifacies*, etc. (Sinka *et al.*, 2012).

Aedes mosquitoes expand as a result of increased trade, globalization, and environmental changes because their eggs can endure harsh conditions for extended periods. Numerous illnesses, including Dengue fever, yellow fever, West Nile fever, Chikungunya, and La Crosse encephalitis, are mostly spread by *Aedes* mosquitoes. In Europe, Latin America, and Africa, *Aedes* mosquitoes are also known to be active carriers of the Zika virus (Medeiros *et al.*, 2018). Over 100 countries, dengue fever is the only cause of the health crisis. Three billion people are in danger. *Aedes albopictus* (Skuse) and *Aedes aegypti* (Linnaeus) are the two most prevalent species in the genus *Aedes*. Unlike *Ae. albopictus* (Skuse), which is found in natural containers like tree holes, bamboo, etc., *Ae. aegypti* (L) is found in clear water and household containers. *Ae. aegypti* is typically associated with dengue fever epidemics. Although *Ae. albopictus* controls rural endemics in human populations and has a minor impact on the spread of disease (Higa, 2011). Many countries do not currently have access to vaccines or medications that could be useful against dengue or other diseases spread by *Aedes* mosquitoes. Nonetheless, a few nations are using the CYD-TDV dengue virus vaccine. It is a recombinant, attenuated vaccination. However, it is not advised for those who have never contracted the dengue virus because it could increase the risk of infection.

Mosquitoes are found on every continent in the world except for Antarctica because the harsh and extreme environmental conditions there make it impossible for them to survive (Goubert *et al.*, 2016). Numerous mosquito species have their highest populations in the Arctic tundra. In tropical woods, their diversity is at its peak. Their higher abundance is most likely found in

the Arctic and Antarctic regions where wetlands have proliferated due to spring and early summer snowmelt. (Kraemer *et al.*, 2015).

The life cycle and morphological properties of mosquito species are greatly impacted by a number of factors that also affect their spread. Land cover, climate, urbanization, and other environmental factors are some of these influences. The land covers serve as environmental reservoirs, giving vectors and hosts opportunities to interact. Climate influences mosquito dispersal patterns, vector transmission, and the temporal and geographic distribution of mosquitoes. Rainfall, temperature, humidity, and precipitation are a few of the main environmental variables that affect mosquito transmission. They have an impact on mosquito transmission as well (Weicheld, 2015). Temperature and humidity are two of these variables that significantly affect mosquito development, lifespan, and mortality, resulting in differences in illness patterns (Ciota *et al.*, 2014; Jemal and Al-Thukair, 2016).

The physical and biological characteristics of mosquitoes such as their growth rates, development periods, body size, reproductive capacity, and lifespan can be significantly impacted by various environmental factors during both their larval and adult stages. Temperature, particularly, serves as a fundamental abiotic factor for various arthropods, including mosquitoes. Its influence on their mortality, physiology, behavior, ecology, lifespan, and developmental rates can lead to alterations in their morphology (Loetti *et al.*, 2011). Mosquitoes are ectotherms so; their body temperature is regulated by surrounding environments. The metabolism of mosquitoes decreases at very low and high temperatures. Mosquitoes have a narrow tolerance for temperature fluctuations, and exposure to temperatures outside of their optimal range can cause developmental disturbances and variations in their growth patterns (Gunay *et al.*, 2011). The tolerance to temperatures in mosquitoes is species-specific. Some species are capable of performing across a wide range of temperatures and are known as thermal generalists. On the other hand, others require a much narrower temperature range to maintain their activity and are called thermal specialists. The optimal temperature of mosquitoes in terms of distance and duration of the flight is 21 °C (Reinhold *et al.*, 2018). The frequency of blood feeding is also positively correlated with temperature. Female mosquitoes bite their hosts for blood feeding at warmer temperatures as compared to cooler temperatures. In addition to mosquito development, temperature also affects their vector competence. It also influences the incubation period of pathogens within the mosquitoes (Brady *et al.*, 2014).

Precipitation plays an important role in mosquito development. It determines the quality and quantity of larval habitats by creating or expanding them thus, determining the adult population size. It can modify the existence of suitable larval habitats, and the viability of eggs and larvae. It can also increase the near-surface humidity, which enhances the flight activity and host-seeking behavior of mosquitoes (Baril *et al.*, 2023). The variations in precipitation result in complex hydrological and vegetative landscapes (Reisen *et al.*, 2008). Similarly, rainfall can play a critical role in predicting the density of mosquitoes. The amount and geographical distribution of breeding grounds for mosquitoes are influenced by the level of rainfall (Valdez *et al.*, 2017). Rainwater is also responsible for the dispersal of mosquitoes to new areas. However, heavy rains and flooding have a significant impact on the populations of mosquitoes. The excess water or flooding can effectively destroy the eggs and aquatic stages of mosquitoes, preventing them from thriving. By causing overflow water, thus, destroying the immature stages of mosquitoes (Yi *et al.*, 2014).

Like temperature, precipitation, and rainfall, humidity plays a significant role in mosquito populations. It influences the biology and ecology of mosquitoes however, it highly depends on the mosquito species and the microclimate (Drakou *et al.*, 2020) mosquitoes prefer to live in areas with high humidity levels. The optimum levels of humidity for the development and survival of mosquitoes range between 60-90%. Low levels of humidity may reduce the lifespan of mosquitoes and decrease their geographic distribution. Humidity also influences the feeding behavior of mosquitoes. Mosquitoes tend to be more active in high humidity conditions because it allows them to fly for longer periods and find hosts more efficiently. However high humidity can also weaken their flight performance. Humidity also affects the vector competence of mosquitoes by influencing the development and survival of pathogens in the mosquitoes (Yamana and Eltahir, 2013). Since variation in environmental factors affects mosquito diversity spatiotemporally, various species can occur in the habitat by simply emerging at different times of the year. Co-occurrence of species can also occur when one species does not out-compete another for resources, or if intraspecific competition is greater than intraspecific (Cunze *et al.*, 2016).

In addition to environmental influences, urbanization has a significant impact on mosquito diversity. According to the US Bureau of the Census, an urbanized area is defined as a location and its surrounding, densely populated area that together have a minimum population of 50,000 people. The area composed of one or more nearby blocks with a population density of at least 1,000 persons per square mile is referred to as the "density settled surrounding territory" next

to the location. The term "urbanization" describes the shift in the size, density, and diversity of cities. It also describes the large-scale population shift from rural to urban areas and the ensuing physical modifications to metropolitan areas. Urbanization is primarily influenced by population mobility, land cover growth, division, and industrial expansion. Therefore, the dynamics of an individual's agglomeration rather than the notion of urban as such determine the process of urbanization. (Vlahov and Galea, 2002).

It is estimated that 55% of the world's population live in urban areas in 2018, while by 2050, 68% of the world's population will be projected to be urban (Ritchie and Roser, 2018). Due to continuous urbanization, land cover is increasing rapidly. It has a significant impact on the number of breeding habitats for mosquitoes. The primary reason for this is the alteration of the local topography, which creates more areas where water can accumulate, such as ditches, puddles, and other water-holding structures. Furthermore, this transformation can lead to an increase in peri-domestic water storage practices, such as the use of water jars and barrels, as well as an increase in waste containers that can harbor standing water. These changes, in turn, provide ideal breeding grounds for mosquitoes to thrive and multiply (Samson *et al.*, 2015).

Pakistan is a multiethnic country located in the tropical and subtropical zone in South East Asia. Pakistan is situated at a latitude of 24 and 37° and a longitude of 61 and 75° stretching over 1,600 km from North to South and 885 km from east to west. The total area of Pakistan is 881,913 km². To the East, it shares its border with the Republic of India. While the Islamic Republic of Iran and the Islamic Republic of Afghanistan are situated to the West of Pakistan. At its Northeastern frontier, it shares its border with the People's Republic of China. Pakistan has a coastline along the Arabian Sea towards the South (Ziring and Burki, 2023). It has a subtropical and semi-arid climate. The annual rainfall ranges from 125 mm in the extreme southern plains to 500 to 900 mm in the sub-mountainous and northern plains. About 70 percent of the total rainfall occurs as heavy downpours in summer from the month of July to September, originating from the summer monsoons, and 30 percent in winter. Summers, except in the mountainous areas, are very hot with a maximum temperature of more than 40 °C, while the minimum temperature in winter is a few degrees above the freezing point (www.climatestotravel.com).

Agroecological zone (AEZ) refers to the division of an area of land into land resource mapping units, each possessing a unique association of landform, climatic conditions, soil, and land covers for agricultural use (FAO, 1996). At a regional scale, AEZs are influenced by latitude, elevation, and temperature, as well as seasonality, and rainfall amounts and distribution during

the growing season. Pakistan is divided into 10 agroecological zones. Punjab province of Pakistan is further divided into 5 zones on the basis of the type of crop production, climate, rainfall, and the quality of the soil according to the Director General Agriculture Punjab, Lahore. These zones include Arid irrigated, Cholistan desert, Cotton Sugarcane, Rod-i-Kohi, Semi-desert irrigated, Cotton, mix cropping, Thal, Rice-tract, Barani zones. (Ahmad *et al.*, 2019).

The rice tract zone of Punjab, Pakistan is a major rice-producing region in the country. The zone is located in the central and southern parts of the province. It is characterized by a hot, humid climate and fertile soil. The rice tract zone is facing several challenges, including climate change, water scarcity, and disease vectors. Climate changes are causing the region to become hotter and drier. The majority of the population of this zone lives in urban areas. (Rahim *et al.*, 2010). In this zone, the main districts are Lahore, Sheikhupura, Gujrat, and Narowal. The major crop rotation is rice-wheat-rice. This zone covers about 59% of the total rice-harvested area in Pakistan. The climate of this zone is cold and sub-humid and the annual rainfall is 250-600 mm (Asghar and Arshad, 2015).

The mixed cropping zone of Punjab Pakistan covers about 28% of the cultivated land of the province. Faisalabad, Sargodha, Jhang, and Chiniot are a few of its main districts. Wheat, Sugar cane, rice, cotton, and maize are the major crops of the mixed cropping zone. Here the climate is warm and semi-arid while the annual rainfall is 200-400 mm (Saeed *et al.*, 2022).

The cotton zone is located in the southern part of Punjab, Pakistan. It covers about 36% of the cultivated area of the province. Cotton and wheat are the main crops of this zone. The climate and the annual rainfall are not found uniform among all districts of this zone. For instance, in Multan, Pakpattan, and Bahawalnagar, the climate is hot with rainfall of 100-200 mm per annum while in Dera Ghazi Khan, Bahawalpur, and Rahim Yar Khan, the climate is hot and arid with an annual rainfall of 80-150 mm (Nazeer *et al.*, 2023).

The Thal zone is located in Thal desert on the western side of Punjab, Pakistan. This zone is a vast expanse of sandy dunes, with occasional patches of vegetation. It comprises 6% of the agricultural land of the province. It has quite arid and semi-arid climatic conditions with rainfall of 150-250 mm per annum. Grams, wheat, millets, barley, pulses, oilseed, and Guara are the prominent crops of this zone. Bhakkar, Layyah, and Muzaffargarh are some of the important districts of this zone. Lack of rainfall, a high degree of daily temperature change, and a high wind speed, are characteristics of the area. Perennial grasses account for a large portion of the vegetation, and aridity is a frequent feature. The population, who reside in sparsely populated communities dispersed around the desert, relies primarily on agriculture and animal rearing for

their subsistence. The farming system of this zone depends on water availability and soil fertility (Amin *et al.*, 2020)

Barani zone has the crop rotation of groundnut and wheat. Crops grown in this zone are fodders, oilseeds, millet, groundnuts, and wheat. Some of the major crop rotations are wheat-fallow, wheat-millet, wheat-groundnut, and wheat-maize. This zone has heavy rainfall of 500-100mm annually with cool sub humid climate. Rawalpindi, Chakwal and Jhelum districts fall in this zone. (Arif and Ahmed, 2001).

With a more humid subtropical climate, Pakistan, being a subtropical country, is a hotspot for mosquito-borne diseases, including mosquitoes and other insects. Due to the presence of extensive agricultural lands, and uncovered large systems of rivers, dams, and irrigation networks, mosquitoes can find various breeding sites easily (Gadahi *et al.*, 2012). In Pakistan, various mosquito genera like *Culex*, *Anopheles*, *Aedes*, *Psorophora*, and *Uranotaenia* are found and around 134 different mosquito species have been reported (Aslamkhan, 1971). Being the largest genus found in Pakistan, the most common species belonging to the genus *Culex* are *Cx. quinquefasciatus*, *Cx. pipiens*, *Cx. bitaeniorhynchus*, *Cx. vishnui*, and *Cx. tritaeniorhynchus*. Species from other genera like *Ae. Aegypti*, *Ae. albopictus*, *Mansonia unifasciata*, *Phagomyia cogilli*, *Ochlerotatus pulcritarsis*, *Ochlerotatus caspius*, *An. subpictus*, *An. stephensi*, *An. pulcherrimus*, *An. annularis*, *An. dravidicus*, *An. splendidus*, and *An. culicifacies* have also been reported (Ilahi and Suleman, 2013; Manzoor *et al.*, 2017; Qasim *et al.*, 2014; Ashfaq *et al.*, 2014).

Pakistan has faced several outbreaks in different years over the last few decades. However, in 2011, dengue disease caused a major threat to public health in Punjab province, and district Lahore was considered the center of the disease. Currently, Pakistan is considered endemic for this disease with more than 24,000 dengue cases reported from different districts of Punjab, during the year 2016 (Ahmad, *et al.*, 2017). It is estimated that more than 7 lac malarial cases were reported over the span of 3 years i.e., 2013-2015, in Pakistan (Umer, *et al.*, 2019). Filarial encephalitis cases have also been reported in Pakistan in the past few years (Fatima, *et al.*, 2020).

Mosquitoes are mainly identified based on their morphological characteristics. It is an easy process to identify mosquitoes but this method is not entirely reliable due to potential human error and limitations of morphological keys in identifying new species haplotypes. To effectively combat diseases transmitted by mosquitoes, it is important to accurately identify these vectors. While there have been numerous taxonomic studies conducted on mosquitoes that serve as vectors for human illnesses, many other species have been largely overlooked.

Furthermore, there are several closely related mosquito species that possess distinct ecological and host preferences, making it difficult to differentiate between them based on their morphological characteristics alone. As a result, identifying mosquitoes to a specific species or even genus can often be a challenging task. So, Mosquitoes can be identified accurately using molecular techniques, with Polymerase Chain Reaction (PCR) being the most commonly utilized method. In particular, PCR utilizes specific gene markers like *COX* and *ITS* to differentiate between mosquito species. The *COXI* gene marker is typically preferred due to its superior accuracy and lower probability of mutation errors. Additionally, genetic barcoding and DNA barcoding techniques are implemented to aid in mosquito identification (Ashfaq et al. 2014) A phylogenetic tree is an estimate of the relationships among taxa (or sequences) and their hypothetical common ancestors (Nei and Kumar 2000; Felsenstein 2004; Hall 2011). Today most phylogenetic trees are built from molecular data: DNA or protein sequences. Originally, the purpose of most molecular phylogenetic trees was to estimate the relationships among the species represented by those sequences, but today the purposes have expanded to include understanding the relationships among the sequences themselves without regard to the host species, inferring the functions of genes that have not been studied experimentally (Hall et al. 2009), and elucidating mechanisms that lead to microbial outbreaks (Hall and Barlow 2006) among many others.

Modern ground-based vector surveillance techniques rely less on antiquated techniques and more on sophisticated technologies like geographic information systems (GIS), remote sensing (RS), and geographic positioning systems (GPS). We can map larval habitats more effectively thanks to these techniques. The study of spatial patterns and processes can be accomplished with the use of geographic information systems (GIS), which are effective computer mapping and analysis tools that can be used in a wide range of fields, including mosquito ecology. The spatial distribution of mosquitoes' preferred hosts, their flight range, and the locations of their larval breeding grounds all play a significant role in determining their overall spread. Because of their spatial and temporal heterogeneity, GIS offers a wide range of possible uses in the study of mosquitoes and the diseases they carry. The spatial distribution of mosquitoes may be mapped, analyzed, and the ecological factors influencing the distributions seen can all be done with GIS. A thorough comprehension of the factors influencing variations in mosquito distribution and the diseases they transmit can aid in the development of more effective control strategies that make the most use of the little resources available. (Gimming *et al.*, 2005; Ahmad *et al.*, 2011; Jemal and Al-Thukair, 2018).

In order to combat mosquito-borne diseases (MBDs), it is crucial to not only assess the areas that are most vulnerable to these diseases but also to map out the spatial information of these locations. One of the most effective methods for reducing the prevalence of mosquito-borne diseases is to destroy the vectors. However, in order to fully comprehend the spatial distribution of these diseases, it is essential to use a decision hierarchy that takes into account the growth and development of parasitic mosquitos, among other closely associated factors. It has been determined that the best way to prepare a decision hierarchy when weighing multiple factors in MBDs is through the application of the analytic hierarchy process. Furthermore, the use of GIS techniques and high-resolution satellite data can be incredibly useful in identifying areas that are particularly vulnerable to these diseases (Ali and Ahmad, 2019).

The goal of the current study was to discover patterns of relative abundance in Punjab's several agroecological zones as well as specific patterns of abundance, species richness. The comparison of the abundance of mosquitoes in urban and rural environments is also analysed. We also looked at the relationships that mosquitoes have with their surroundings, particularly with regard to habitat and environmental (meteorological) factors. Thus, research was done on the relationship between humidity, rainfall, and temperature and how it affects mosquito abundance. This study will aid in the management of mosquitoes, which pose a serious threat to human health and could spread diseases.

1.2 AIM AND OBJECTIVES

The present study mainly focused on the spatiotemporal distribution of mosquitoes in Punjab, Pakistan. The study also dealt with the molecular identification of mosquitoes. The aims and objectives of the present study are as follows:

This research will help to:

- Identify the mosquito and to determine the role of habitat in shaping populations of mosquitoes in Punjab.
- Investigate the specific patterns of abundance, species richness, shared species, and the relative abundance of dominant species, by identifying mosquitoes at molecular level.
- Find the temporal changes in mosquito populations within the same habitats.
- Investigate the relationship between mosquito abundance and the environmental variables with reference to available meteorological data.
- Have comparative analysis of urban and rural areas with reference to the abundance of mosquitoes.
- Study the role of urbanization in spatiotemporal distribution of mosquitoes in Punjab.
- Prepare the GIS mapping to find the distribution of different mosquito species.

Chapter 2 REVIEW OF LITERATURE

Many diseases that are spread by vectors harm humans, animals, and birds. Yellow fever, Japanese encephalitis, West Nile fever, dengue fever, and other illnesses spread by ticks are examples of vector-borne illnesses. The main disease-transmission vectors include ticks, houseflies, bugs, and mosquitoes. Mosquito-borne infections are the most concerning of all vector-borne illnesses because of the steadily rising number of disease cases. One of the primary reasons for the rise in disease incidence is the challenge of managing mosquitoes. The distribution of mosquitoes is caused by a variety of factors, including climatic, environmental, and human influences. (Schmidt *et al.*, 2013).

It has been observed that *Anopheles gambiae* may survive the hot temperatures by aestivating. In the study, *Anopheles gambiae* mosquitoes were collected from Riparian and Sahelian areas. The mosquitoes were then studied to measure their flight activity and body size. Metabolic rates were also measured. A difference in body size from both populations was observed in different seasons. The Sahelian population of mosquitoes showed an increase in flight activity during the humid season as compared to the dry season. Various seasonal factors also affected the metabolic rates of mosquitoes from both areas. The effect of season was perfectly explained by assay temperature in the riparian mosquito population. Significant seasonal variation persisted despite adjusting for all measured factors in the Sahelian mosquito population. However, a reduction in mean metabolic rates was not observed in dry weather. It was assumed that mosquitoes would preserve energy by slowing their metabolism during the dry season thus increasing their lifespan.

In 2014, Coita *et al.* analyzed that in the future, climate changes may cause significant changes to the populations and distributions of mosquito-borne pathogens. To understand how these changes could impact the epidemiological patterns of vector-borne diseases, it was essential to study the effect of temperature shifts on specific life history traits of mosquitoes. The impact of various temperatures on the size, time, blood feeding, fertility, and immature and adult survival of *Culex* mosquitoes in both field and lab populations were calculated. For this purpose, *Culex pipiens* L., *Culex quinquefasciatus* Say, and *Culex restuans* Theobald were collected from Washington, DC, and California. The findings revealed that temperature significantly affected all the characters studied. However, the effect varied among temperatures, species, and populations. Comparing colonized mosquitoes with field populations, it was found that colonies were not fully representative of natural populations, with decreased adult and immature survival, increased blood feeding and egg production, and

significant variation in the effects of temperature. Among field populations, increases in temperature were likely to increase mosquito development. This effect was greater at temperatures below 24°C. However, increased temperature also resulted in increased mortality. Temperature showed the greatest impact on *Cx. restuans* which had shorter life spans as compared to other species. It was also observed that *Cx. restuans* had higher larval and adult mortality with each small rise in temperatures. Although *Cx. quinquefasciatus* and *Cx. pipiens* were found in regions with distinctive climates, there was no evidence of considerable species-specific adaptation to temperature variations. When viewed collectively, these findings showed that geographic region, species, and population variations must be taken into account when estimating the impact of temperature on mosquito populations.

Ashfaq *et al.* (2014) analyzed the mosquito diversity in Pakistan by DNA barcoding. Recent epidemics have shown that there must be a detailed study on the distribution and diversity of mosquitoes in Pakistan. Thus, the DNA barcoding technique was used to determine the mosquito diversity in this region. For this purpose, 450 sites from the province of Punjab and 41 sites from the KPK province were selected. Mosquitoes were collected from homes, ponds, marshes, junkyards, forests, and construction sites. Light traps and aspirators were used to catch adult mosquitoes. Larvae were collected by using sieves and pipettes. GPS coordinates were also noted down. After collection, a total of 1942 mosquitoes were selected randomly. DNA was extracted from these randomly selected mosquitoes. After PCR amplification, samples were subjected to sequencing. Results showed that 61% of the mosquito collection was comprised of *Cx. quinquefasciatus*. 15% species were from the genus *Aedes*. However, only 6% of the total collected mosquitoes were from the genus *Anopheles*. In conspecific species, 0 to 2.4% divergence was observed. However, 2.3 to 17.8% divergence was observed in congeneric species. A global haplotype analysis was also performed. It showed the presence of multiple haplotypes. In terms of geographic distribution, *Ae. Albopictus* was highly abundant in both urban and rural areas.

Roiz *et al.* (2014) showed that the climatic effects on mosquitoes are non-linear and vary from species to species. These effects are also place-specific. The effect of climatic conditions on mosquitoes is a debatable aspect. For this purpose, data on *Culex pipiens*, *Ochlerotatus caspius*, and *Ochlerotatus detritus* mosquitoes from 2003 to 2012 were collected from Spain. Factors including rainfall, temperature, relative humidity, photoperiod, and tide heights were studied. The results showed that both mosquito species were highly prevalent during rainfall in the

winter season. A positive relation was observed between mean temperature and *Ochlerotatus caspius*. However, a non-linear relation was studied between *Culex* mosquitoes and maximum temperatures. Generalized Circulation Models (GCM) were also used for modeling the effect of climatic alterations on mosquitoes from 2011 to 2100. It was predicted that *Ochlerotatus caspius* would increase abundantly. No changes in the abundance of *Ochlerotatus detritus* and *Culex pipiens* were predicted. It was also concluded that warm climates in wetlands do not inevitably result in an increase in mosquito populations.

In 2016, Marini *et al.* studied that *Culex pipiens* is a dominant species all across Europe. It acts as a primary vector for many arboviruses e.g., West Nile Virus. WNV has been spread in many European countries in the past few years. The study was conducted in Piedmont, Italy to identify the primary factors that influence the fluctuating levels of *Cx. pipiens* mosquito populations during different seasons. A model was developed to determine the impact of temperature on the development and mortality rates of mosquitoes at different stages of their life cycle. To calibrate the model, data was collected between 2000 and 2011 in the study area and employed a Markov chain Monte Carlo approach. The research findings suggested that seasonal variations in mosquito populations were primarily driven by changes in temperature and seasonal-specific larval carrying capacities. It was studied that high temperatures in early spring could result in a surge in population size, while high temperatures during summer could decrease it due to adult mortality. Furthermore, it was identified that higher densities of adult mosquitoes were associated with higher larval-carrying capacities, which, in turn, were positively linked to spring precipitations. Ultimately, an increase in larval carrying capacity was likely to lead to a proportional increase in adult mosquito populations.

Bostan *et al.* (2016) reported different climatic factors involved in the outbreaks of dengue fever in Pakistan. They highlighted high-risk zones by studying other factors related to host and vector. Since its first outbreak, dengue fever has been considered one of Pakistan's most prominent diseases. Dengue fever epidemic may be promoted by interactions between *Aedes* mosquitoes to their hosts and favorable environmental conditions. It was observed that various factors like temperature, humidity, precipitation, colonization, urbanization, vector population, and global warming are responsible for the spread of disease.

Valdez *et al.* (2017) studied the effects of rainfall on *Culex* mosquitoes. The mosquito population dynamics are highly dependent on climatic conditions such as rainfall and

temperature. It is important to understand how the frequency and intensity of rainfall impact mosquito populations due to the predicted impact of global warming on these variables. A dynamics model was used to study the effect of rainfall on *Cx. quinquefasciatus* mosquitoes. Mean monthly precipitation on maximum annual abundance (M_{max}) was also studied. The effect of any change in daily rainfall on M_{max} was investigated. There is an optimal number of rainy days at which M_{max} is a maximum, given a constant monthly precipitation? The findings indicated that higher variability in daily rainfall has a significant impact on the relationship between M_{max} . And the number of rainy days. Specifically, an increase in daily rainfall variability led to a reduction in the dependence of M_{max} . On the number of rainy days. This reduction in dependence results in a higher abundance of mosquitoes, especially in cases of low mean monthly precipitation. Therefore, it is important to consider the effects of daily rainfall variability when analyzing the relationship between M_{max} . And mosquito abundance in different precipitation regimes. After examining the rainfall patterns leading up to the wettest season, it was discovered that a consistently high level of precipitation, combined with significant variability, can cause a slight acceleration in the peak mosquito population time. Additionally, this type of rainfall pattern can significantly impact the overall mosquito population throughout the year.

Ebrahim and Dawood (2018) conducted a study to determine the mosquito diversity and their biting patterns between unlogged and logged forests in Sabah, Malaysia. For this purpose, mosquitoes were collected from the forests near the logged forest area and Maliau Basin area. Bi-monthly sampling was done from June 2016 to April 2017. Human Landing Catch was used to capture the mosquitoes. 807 mosquitoes from 17 species were captured. Only 9 species were collected from the logged forest however, 15 species were captured from the Maliau Basin area. By using GLMMs, a significant difference in mosquito abundance in both areas was observed. A significant difference in mosquito day biting time was also observed. During the study, more than 500 mosquitoes were collected from the Maliau basin area while only 244 mosquitoes were collected from the logged forest. In the Maliau area, *Ae. Albopictus*, *Cx. vishnui*, *Heizmannia scintillans*, and *An. balabacensis* were highly abundant. *Armigrerres jugraensis*, *An. umbrosus*, *Ae. Albopictus*, and *Heizmannia scintillans* were highly abundant in the Maliau area. The biting pattern was also observed which showed that in logged forest mosquito populations, the peak biting hours were 2, 5, 7, and 9 p.m. The peak hours for Malau populations were 2-3 and 6 p.m.

Tokaraz and Novak (2018) studied the spatio-temporal distribution of *Anopheles* larvae in Uganda by using GIS, spatial autocorrelation models, and remote sensing technologies. In response to the prevalent mosquito-borne diseases in Papoli Parish, a comprehensive mosquito surveillance program was designed and executed over 4 months. Sampling was done from the larval habitats of *Anopheles* mosquitoes. Daily, detailed surveys were conducted to identify habitats and assess their productivity. The data collected was then plotted spatially, resulting in a comprehensive map of different habitats and their productivity levels. These daily outputs were combined and analyzed to create a weekly habitat time series, providing a clear understanding of how the habitats change over time. A spatial analysis including Global and Anselin's Local Moran's *I* statistics was also conducted by using to assess habitat spatial autocorrelation. The spatial models were used to identify the most important habitats of mosquitoes for controlling the growth of larvae. By analyzing the data collected from these models, it was possible to determine which areas required immediate attention to prevent the proliferation of larvae. Through the use of weekly time-series analysis, the models were able to precisely identify the various locations of each habitat and accurately gauge their levels of productivity. Significant clusters and outliers were identified by Local Moran's *I* cluster maps. High clusters and High outliers were identified for control priority.

In 2018, Asigau and Parker determined the effect of ecological factors on mosquito abundance in the Galapagos. Mosquitoes were collected from 18 different sites from the north to the south of Isla Santa Cruz, Galápagos. *Cx. Quinquefasciatus* and *Ae. Taeniorhynchus* were collected. Two GLMMs were used in the study. The 1st model determined the effect of environmental factors on *Ae. Taeniorhynchus*. While, the effect of environmental factors on *Cx. quinquefasciatus* was evaluated by the 2nd model. It was observed that the mosquito populations decreased as the elevation increased. The results found that the proximity to mangroves and the level of humidity played a significant role in the number of *Ae. Taeniorhynchus* mosquitoes found in the area. Additionally, it was observed that the presence of *Cx. quinquefasciatus* was significantly influenced by the level of humidity in the surrounding environment. In the areas of the Galapagos where both species were observed, the environmental conditions were favorable for the growth and development of mosquitoes, as well as the parasites responsible for causing avian malaria. These conditions included appropriate levels of temperature, precipitation, and humidity, which are necessary for the complete life cycle of both mosquitoes and blood parasites. As a result, avian malaria could be transmitted through mosquito bites to both species.

Wilke *et al.* (2019) identified the most productive habitats for *Ae. Aegypti* in Florida. For this purpose, larval stages of mosquitoes were collected from different areas of Miami-Dade County. Almost 45,000 mosquitoes were collected from 2018 to 2019. More than 75 different habitats were analyzed for this study. Out of all the collected mosquitoes, *Ae. Aegypti* mosquitoes were highly abundant (43%). *Ae. Aegypti* larvae were abundant in artificial breeding areas, especially plant pots and buckets. Bromeliad plants were considered highly responsible for providing a suitable habitat for the development of *Ae. Aegypti* mosquitoes.

Hernandez-Triana *et al.* (2019) recognized British mosquitoes by using the technique of DNA barcoding and discovered the genetic diversity of these mosquitoes. For this purpose, a partial sequence of the cytochrome *c* oxidase unit I (*COI*) gene was used. 3 different methods were used to extract whole genome DNA. 42 species from were analyzed in the study. 21 species from the genus *Aedes*, 1 from the genus *Coquillettidia*, 6 from the genus *Culex*, 7 species from the genera *Anopheles* and *Culiseta* each, and 1 from the genus *Orthopodomyia* were analyzed. Conspecific species divergence was 0-5.4%. The highest congeneric divergences (24.6%) were observed between *Culiseta litorea* and *Ae. geminus*. An analysis of the taxonomic classification of *An. messeae* and *An. daciae* revealed a significant discrepancy. This indicated that the *COI* DNA barcoding region, which is commonly used for species identification, has poor resolution in distinguishing between these two taxa. The findings suggested that the genetic marker currently used to identify certain mosquito species may have limitations. Specifically, *Ae. Cantans* and *Ae. Annulipes* exhibit discrepancies that hinder accurate identification.

Uusitalo *et al.* (2019) studied the predictive mapping of mosquito distribution in Kenya. The study used species distribution modeling techniques to investigate the relationship between various environmental, anthropogenic, and distance-related factors affecting the presence of two mosquito genera, namely *Culex* and *Aedes*, in the Taita Hills region of southeastern Kenya. The objective of the research was to determine the effectiveness of the statistical prediction models generated by the Biomod2 package in R for accurately predicting the distribution patterns of mosquitoes belonging to certain genera in the Taita Hills. Additionally, the study aimed to identify the factors that have the greatest influence on the presence of these mosquitoes. Mosquitoes were collected in 2016 from 122 locations throughout the Taita Hills. The study used geospatial data from the Taita Hills geo-database, which included satellite and aerial imagery, to analyze the impact of both environmental and anthropogenic factors on the

region. The data was processed using advanced GIS software, enabling a detailed assessment of the area's unique characteristics and the factors influencing them. To generate predictive models and forecast species distributions, the Biomod2 package in R was used. This package was designed to provide ensemble modeling capabilities, allowing for improved accuracy and reliability in predicting species distributions. The terrain slope, vegetation health, density of human population, elevation, and distance to roads were all considered as factors in estimating the distribution of *Culex* mosquitoes using a generalized additive model. The model was able to achieve an area under the curve (AUC) value of 0.791, indicating its effectiveness in accurately predicting the distribution of *Culex* mosquitoes. Among the various factors analyzed for predicting the distribution of *Aedes* mosquitoes, a random forest model determined that mean radiation, normalized difference vegetation index, human population density, mean temperature, and distance to roads were the most significant. The model yielded an impressive AUC value of 0.708, indicating its strong predictive power.

Ezeakacha and Yee (2019) conducted a study to determine the role of temperature on *Ae. Albopictus* mosquitoes. Mosquitoes being ectotherms have also evolved different mechanisms or responses to combat temperature variations. In the study, they investigated how temperature affects larval stages and all life stages of *Ae. Albopictus*. For this purpose, larval stages of mosquitoes were reared at 3 different temperatures and densities. The experiment included the rearing of 10, 20, and 40 mosquitoes at 21, 27, and 34 °C. Fecundity data was also obtained. Results indicated a significant relation between the density of mosquitoes and temperature. Adult mosquitoes in higher temperatures showed great differences in survival and fecundity. It was also observed that temperature altered the adult fitness of *Ae. Albopictus* mosquitoes.

Wang *et al.* (2020) studied the effect of urbanization on the mosquito population in China. The effect of climatic change was also studied. It was discussed that urbanization may alter local climatic conditions which could have an impact on mosquitoes. The Delta region of the Pearl River in China has experienced significant urbanization and is likely to undergo climatic changes. A model known as DyMSiM (Dynamic Mosquito Simulation Model) was used in the study. WRF (Weather Research and Forecasting model was used to get the climatic data. A decrease of almost 13% was observed in newly formed urban sites. However, an increase of 6% was observed in already existing urban areas. Around 16% decline in mosquito population due to climate was observed. A decline in mosquitoes was noted in the months of Peak season. On the contrary, a considerable increase in mosquitoes was observed in the months of non-peak season.

In 2020, Zettle determined that the diversity and abundance of mosquitoes could be analyzed by the abundance of larval mosquitoes. He studied the effects of urbanization gradient on mosquito distribution. Over a span of five years i.e., from 2011 to 2015, a study was conducted in Baltimore, Maryland to observe how mosquito composition and abundance are affected by temperature variation along an urbanization gradient. The standardized ovitraps paired with iButton temperature loggers to collect data were utilized. The focus of the study was on the four most commonly observed species - two *Culex* species - *Culex restuans* and *Culex pipiens* and two *Aedes* species - *Aedes albopictus* and *Aedes japonicus*. The results revealed that all species were highly abundant in areas with high waterproof surfaces and vegetation cover. However, *Aedes japonicus* and *Culex restuans* were also abundant in rural sites. *Aedes albopictus* and *Culex pipiens* were most commonly found in urban sites. It was found that *Aedes albopictus* and *Culex pipiens* mosquitoes were positively correlated with waterproof surface cover. All species were positively affected by temperature but with the moistening association at higher temperatures. It was also discovered that the species that bite humans, *Aedes albopictus* and *Culex pipiens* were more likely to be found in urban areas with warm climates. This could lead to an increase in the population sizes of these species because of the increase in urbanization and climatic mosquito variations.

Camara *et al.* (2020) evaluated the diversity of mosquitoes in an endemic area for arboviruses. It was observed that in several American countries including Brazil, various mosquito-borne diseases have been coexisting for many years. So, they decided to understand the relationship between mosquitoes and their landscape. The key factors on which this relationship exists are distribution patterns and ecology of mosquitoes. For this purpose, 14 sites from various landscapes in the state of Rio de Janeiro were selected, randomly. These sites were then characterized as rural, peri-urban, and urban. Two endemic cities including Cachoeiras de Macacu and Itaborai were also selected. Mosquitoes were collected from each site by using different types of mosquito traps. Dry and rainy seasons were selected for the collection of mosquitoes. Species accumulation curves were generated to determine the mosquito diversity in each area. Various indexes like Chao1-bc and rarefied species richness were used. Partial conical correspondence analysis also known as pCCA was used to study the division of mosquito habitats through various land uses. Likewise, the chance of the most abundant species existing through urban forest areas was modeled by using GLMMs. Results showed that more than 13,000 mosquitoes were captured including 10 genera and more than 40 species. *Ae.*

Albopictus, *Ae. Aegypti*, and *Cx. quinquefasciatus* were the most abundant species. It was noted that a significant association between species richness and landscape was present. Most species show division through various land uses. Only 6 mosquito species were fit for prediction models.

In 2021, Attaullah *et al.* conducted a survey to determine the diversity of mosquitoes in Lower Dir and Malakand. Relative abundance and distribution patterns were also studied. Six habitats were selected for the collection of mosquitoes. Mosquitoes were collected from September 2018 to July 2019. For the collection of adult mosquitoes, light traps and aspirators were used. Aquatic stages of mosquitoes were collected with the help of a dipper. Mosquitoes were identified morphologically. Shannon-wiener Index was used to evaluate the mosquito diversity. Results showed the identification of 3 commonly found genera in Pakistan i.e., *Aedes*, *Culex*, and *Anopheles*. 14 species including *Anopheles stephensi*, *Anopheles maculatus*, *Aedes aegypti*, *Anopheles annularis*, *Anopheles dthali*, *Culex vishnui*, *Aedes vittatus*, *Culex quinquefasciatus*, *Anopheles fluviatilis*, *Culex tritaeniorhynchus*, *Anopheles culicifacies*, *Aedes albopictus*, *Anopheles pallidus*, and *Anopheles subpictus* were identified. It was observed that *Culex quinquefasciatus* was persistently distributed in the study area. Mosquito diversity was significantly different in all six habitats.

In 2021, Adeniran, *et al.* identified mosquitoes from Mexico using DNA barcoding. When it comes to identifying mosquito species, the most common method used is to look at their morphological traits. However, this approach may not always be enough, especially when dealing with immature specimens, poorly stored samples, or complex species groups that are difficult to distinguish using morphological traits alone. Therefore, complementary identification methods are often necessary to accurately identify mosquito species. DNA barcoding is a molecular technique that uses a specific gene, namely *cytochrome c oxidase subunit 1 (COI)*, to identify species. This method provides a reliable and accurate means of species identification. For this purpose, a comprehensive entomological survey of mosquito species in Mexico State identified by *COI DNA barcoding* was conducted. Mosquitoes were collected from all the provinces in Mexico State from 2017 to 2019. A total of 2,218 specimens were gathered from 157 areas, including both subfamilies Anophelinae and Culicinae. 342 mosquitoes were subjected to *COI* sequencing after DNA extraction and PCR amplification. The distance between conspecific species was 0-3.9%. However, 1.2-25.3% distance was observed in congeneric species. New *COI* sequences were produced for 8 species. These

species included *Ae. Gabriel*, *Ae. Ramirezi*, *Ae. Vargasi*, *Ae. Guerrero*, *Uranotaenia geometrica*, *Ae. Chionotum*, *Cx. restrictor*, and *Haemagogus mesodentatus*

Mehmood *et al.* (2022) worked on the abundance, distribution, and diversity of mosquitoes in Punjab, Pakistan. The 365 mosquitoes were collected from the district Jhelum from 2014 to 2016. Mosquitoes were collected from scrapyards, graveyards, streams, animal sheds, homes, cropping areas, parks, and forests with the help of various types of traps. 21 mosquito species were identified during the study from the district Jhelum. Two species from *Aedes*, 6 from *Anopheles*, 9 from *Culex*, 2 from *Armigeres*, and 2 from *Lutzia* were recorded. Overall, *Anopheles* mosquitoes were found in high prevalence in animal sheds and homes. However, a low prevalence of mosquitoes was found in streams. *Aedes* mosquitoes were found more prevalent in homes, parks, graveyards, and forests. *Culex* mosquitoes were highly prevalent in scrapyards and graveyards. Simpson index was calculated and it showed the highest value for scrapyards while the lowest for forest areas. However, evenness was documented as the highest in graveyards and the lowest in fields of crops.

Barrientos-Roldan *et al.* (2022) described the effects of landscape anthropization on mosquito diversity in southeastern Mexico. It has been known that urbanization, land use alterations, and defaunation have a great impact on mosquito diversity. This effect can be a positive effect or a negative effect. To study these effects on mosquitoes, two habitats were selected including anthropized areas and deciduous forest in southeastern Mexico. Mosquitoes were collected from 2014 to 2016 by using CDC traps. Mosquitoes were captured during 3 seasons i.e., dry, cold, and rainy. More than 750 mosquitoes from 22 species were collected. Rank-abundance curves were formed to analyze the temporal and spatial alterations in mosquito populations. Shannon exponential, Simpson dominance, and Shannon index were used to measure alpha diversity. Beta diversity was measured with the help of Jaccard's coefficient of similarity.

In 2022, Khan determined the effect of seasonal variations on mosquito fauna in district Bannu, KPK, Pakistan. For this purpose, mosquitoes were collected from cattle sheds, washrooms, and human dwellings. Results showed that 5 genera of mosquitoes were found. 2 species from *Aedes*, 6 from *Anopheles*, 5 from *Culex*, 1 from *Mansonia*, and 1 from *Culiseta* were found. Overall, it was observed that the highly abundant species from the *Culex* genus was *Cx. quinquefasciatus* (88%). Among all *Anopheles* species, *An. stephensi* was prevalent. It was also observed that the highest density of mosquito fauna was found in animal sheds as compared to

other collection sites and habitats of mosquitoes. Mosquitoes were abundant in the early summer season while their number dropped in the late winter. *Cx. Vagans* and *Culiseta lagiaerolata* were collected in their immature form (larval form) only.

In 2023, Baril *et al.* studied the effect of weather on the population dynamics of common mosquitoes in Manitoba Canada. A conducted weekly mosquito monitoring was done over a period of 2 years in Canadian Prairies, during the mosquito breeding season. The associations between weather variables over the time of two weeks and mosquito trap counts were then investigated using Generalized Linear Mixed Models (GLMMs). The four most commonly found vector species were collected i.e., *Culex tarsalis*, *Aedes vexans*, *Ochlerotatus dorsalis*, and *Coquillettidia perturbans*. More than 250,000 mosquitoes were collected in the duration of 2020 and 2021. *Ae. Vexans* was found highly abundant followed by *Cx. tarsalis*. It was observed that high humidity, moderate temperatures, and precipitation were preferred by *Ae. Vexans*. Meanwhile, high humidity and rainfall enhanced the activity of *Cq. perturbans* and *Oc. dorsalis*, respectively. The association between the number of mosquitoes collected and precipitation showed distinct patterns between years, with *Cx. tarsalis* mosquitoes prefer high temperatures. The trap counts were affected by the minimum trapping temperature for *Ae. Vexans* and *Cq. perturbans*, only.

Ullah *et al.* (2023) designed a study to determine the distribution of mosquitoes in six agroecological zones in Punjab, Pakistan. The mosquito fauna of these zones is affected by seasons, cropping, urbanization, and topographical structures. These factors play an important role in the spread of mosquito-borne diseases in these ones. Thus, a surveillance study was conducted to study the distribution patterns of mosquitoes in these zones. Mosquito samples were collected during the monsoon, winter, and summer seasons. Mosquito larvae were collected from various habitats for example paddy fields, wetlands, fish farms, tires, parks, sewerage drains, pools, catch basins, rock pools, tree holes, and irrigation channels. Results showed the presence of 24 mosquito species in these zones. From the subfamily Anophelinae and Culicinae, 3 species each were found. However, 8 species from Culicini, 1 from Mansoniini and Ficalbiini each, and 11 from Aedini were found during the study. It was also observed that the Changa Manga forest is rich in Aedine mosquito species. Anopheline mosquitoes were abundant in wetlands and rice paddies. A few commonly found species were *Ae. Aegypti*, *Ae. Vittatus*, *Ae. Caspius*, *Ae. Albopictus*, *An. stephensi*, *An. subpictus*, *Cx. pseudovishnui*, *Cx. tritaeniorhynchus*, *Cx. malayi*, and *Cx. quinquefasciatus*.

Cevidan et al. (2023) studied the increase in invasive *Aedes* mosquitoes in urban and peri-urban areas of northern Spain. *Aedes japonicus* was first reported in 2020 from the Basque province of Spain. *Aedes albopictus* was reported in 2014 from Spain. Thus, the study was proposed to study the distribution of *Aedes* mosquitoes and the effect of urbanization on them. From June 2021 to November 2021, 568 ovitraps were installed in 113 sampling areas to get the eggs of *Aedes* mosquitoes. Eggs were hatched after counting until they reached their adult stage. Non-hatched eggs were subjected to PCR technique to confirm the species of *Aedes* mosquitoes. The results showed that more than 65% of sampling sites were positive for *Aedes* eggs. Almost 32% of ovitraps in the Basque province presented the eggs. Both species of *Aedes* mosquitoes were prevalent in study sites. At more than 10 sites, co-occurrence of both species was observed. The results also showed that 4.39 times high possibility was observed for *Aedes albopictus* mosquitoes of being found in urban areas. *Aedes albopictus* was also found in abundance in areas with high human population density. The study concluded that the distribution of *Aedes* mosquitoes has increased in northern Spain.

Chapter 3 METHODOLOGY

The selection of the study sites was based on environmental factors like temperature and rainfall, ecological zones (urban and rural) and populations since variations in species, abundance, and spatiotemporal distribution of mosquitoes could be influenced by these parameters.

3.1 Study Area and Duration

The present study was carried out in five major agroecological zones of Punjab i.e., Rice tract zone, Mixed cropping zone, Cotton zone, Thal zone and Barani zone. One district from each agroecological zone of Punjab was selected as the representative of the respective zone and the mosquitoes were collected accordingly. District Lahore was chosen for Rice tract zone, Faisalabad for Mixed cropping zone, Layyah for Thal zone and Rawalpindi for Barani zone. Two districts were preferred from the Cotton zone due to the climatic and rainfall variations i.e. district Bahawalnagar having hot climate and Bahawalpur for hot arid climate. Urban and rural areas from each zone were selected on the basis of population, availability of habitat of mosquitoes and anthropogenic landscape. Every month a random sampling was conducted across the five zones from 2018 to 2020. The rural and urban areas of each tehsil were visited accordingly. The coordinates of each area were noted at the time of investigation. The climatic variables of each tehsil were taken from the local meteorological department stations located in Punjab, Pakistan.

3.1.1 Rice Tract Zone

The most populated, entirely urban district in the rice tract zone, Lahore, is bordered by India. It has five tehsils Shalamar town, Raiwind, Model town, Lahore City and Cantt. It has an area of 1772 square kilometers and is located between 31°15' N and 31°42' N as well as between 74°01' E and 74°-39' E (Nasar-u-Minallah 2020). According to Nasar-u-Minallah and Ghaffar (2020), Lahore has an average elevation of 150 to 200 meters above sea level, an average annual mean temperature that ranges from 18°C to 38.8°C, and an average annual rainfall of roughly 628.7 mm. The weather in Lahore is really harsh. The warmest months are May, June, and July. The coldest months of the year are December, January, and February. The average temperature is 39 °C with relative humidity 60%. The annual rainfall ranges from 250-600 mm (Rana and Bhatti, 2018).

3.1.2 Mixed Cropping Zone

The district Faisalabad, which has been selected from this zone, is located at 184 meters above sea level at coordinates of 31.4 degrees North latitude and 73.1 degrees East longitude. Over 3

million people live in the district, which has a total area of 58.56 km². Faisalabad experiences summer temperatures of 26.9° C–45.5 °C and winter temperatures of 4.1°C–19.4 °C on average. There is 375 mm of rain on average. Although there is some disparity in the population of this zone, most people reside in rural areas. The sampling was done from six tehsils Faisalabad, Saddar, Chak Jhumra, Jarawala, Samundri, and Tandiawala.

3.1.3 Cotton Zone

The cotton zone, which is found in Punjab's central and southern regions, is characterized by extremely hot and dry weather. The bulk of people living in the zone are from rural areas. The district of Bahawalnagar was chosen for the purpose of collecting mosquitoes from the cotton zone. Bahawalnagar's geographic coordinates are 30.33 degrees North latitude, 73.23 degrees East longitude, and 165 meters above sea level. Summers can reach temperatures of up to 50°C, and they are extremely hot and dry. Winters are brief and bitterly cold, with lows of up to -1 °C. The average rainfall is 45 mm. This district spans 8,878 Km² and has a population of around 1.5 lac people. The cotton zone in this district is well-known for its livestock breeding and agricultural crops. Samples were collected from Bahawalnagar City, Haroonabad, Chishtian, Fort Abbas, and Minchanabad, including rural and urban locations. (Rahim *et al.*, 2010)

The other district from cotton zone was Bahawalpur district which covers 24,830 square kilometers. The most recent census, conducted in 2017, found that there are 3,668,106 people living there overall, or 153.4 people per square kilometer. It is separated administratively into six tehsils: Ahmadpur East, Hasilpur, Kairpur Tamewali, Yazman, and Bahawalpur City. Bahawalpur can be roughly classified into three topographic regions: the plains, the desert, and the riverine sections. The Sutlej River is nearby the riverine area. Compared to the riverine area, the irrigated tract is higher. The majority of the land has been bought under cultivation. The region known as Cholistan is a desert. It is located north of the Indian deserts of Bikanir and Jaisalmer, and south and east of the irrigated tract. Bahawalpur has a very hot and dry summer climate and a chilly, dry winter climate. Bahawalpur district experiences 169.8 mm of mean annual rainfall, most of which falls in July and August during the monsoon season (Khan *et al.*, 2020).

3.1.4 Thal Zone

District Layyah in Punjab's Thal zone was chosen for the mosquito collection. Layyah has a total size of 1,772 km² and a population of around 1.12 million. The district is located 143 meters above sea level, between latitudes of 30.96 ° North and longitudes of 70.93 ° East. The

district experiences quite harsh weather. The average temperature in a desert climate is between 2° and 48 °C, and there is essentially no rainfall. (Rahim *et al.*, 2010). The adult and larval mosquito sample locations were Main Layyah City, Chaubara, and Karor Lal Esan.

3.1.5 Barani Zone

District Rawalpindi of Barani zone occupies 5,285 square kilometers in total. It consists of seven administrative regions: Gujar Khan, Kalar-Syedan, Kahuta, Kotli Sattian, Rawalpindi, Taxila, and Murree. The location of the district Rawalpindi is 33.4095 °N latitude and 72.9933 °E longitude. The height above sea level varies from 300 to 2790 meters. The district's climate varies from place to place. The district's southern portion experiences hot summers and pleasant winters, whereas the district's northern portion experiences harsh winters and mild summers. The average maximum temperature is 25.6 °C to 39.4 °C. Highest precipitation occurs in Tehsil Murree, with an annual mean of 1550 mm. The southern portion of the district is primarily plain, whereas the majority of the area is made up of hills (EIA, 2005; Khan *et al.*, 2019).

3.2 Collection of Mosquito

Every month, both adult mosquitoes and their larvae were collected. Larvae were collected using the conventional dipping procedure. The mosquito larvae were gathered from various oviposition sites, including gutters, marshes, ponds, puddles, and abandoned tires. A traditional handle-mounted water dipper was used to sample the water. The dipper was lowered gradually to a point somewhat below the surface at a 45° angle. Because too much disturbance of the water will cause the larvae to dive below, care was taken. It was careful not to spill the larvae-containing water when lifting the water. Once the larvae in the dipper were elevated to the water's surface, the dipper was held steadily. Each habitat was subjected to at least ten dips at various sites where mosquito larvae are anticipated. The collected larvae were transferred to the laboratory for additional research using bottles or vials labeled with the date and the name of the sampling habitat. To bring the larvae to adulthood, they were placed in the net.

Every month, adult mosquitoes were collected using the CDC aspirators as well as mouth and mechanical aspirators. The mosquitoes were found to be inhabitant of room coolers, inside houses, animal sheds, gardens, parks, and graveyards. They were kept frozen and preserved in dry ice until they could be identified.

3.3 3.3: Morphological Identification of mosquitoes

Using taxonomic keys and literature, the identification was based on morphological features (Christophers, 1933; Barraud, 1934; Rueda, 2004). After being recognized and labeled, each mosquito was placed in a different petri dish and frozen at -80°C.

3.4 Molecular Identification

Five mosquitoes were taken in a mortar and pestle. A sterile pestle was used to homogenize 400 microliters of TEN extraction buffer [10 mM Tris-HCl (Applichem, Darmstadt, Germany), 2 mM EDTA (Sigma-Aldrich, Missouri, U.S.A), 0.4 M NaCl pH 8.0 (Applichem, Darmstadt, Germany)]. This powdered substance was transferred to Eppendorf tubes. Subsequently, the tube was filled with 10 µl of 10% SDS (Applichem, Darmstadt, Germany) and 8 µl of 20 mg/ml Proteinase K. (GeneJET Genomic DNA Purification Kit K0721) After thorough mixing, the solution was incubated for one hour at 55°C. Then, each tube received 300 µl of 6 M NaCl. Following a 20-minute centrifugation at 14,000 rpm, 500 µl of the supernatant was moved into a tube, and 500 µl of 2-propanol was added to precipitate DNA. Following 20 minutes at 14,000 rpm centrifugation, the pellet was dried and then rinsed with ice-cold 70% ethanol. 50 µl of water was used to suspend the pellet. (Huang *et al.*, 2004)

3.4.1 Gel Electrophoresis

In a 100 ml bottle, 25 ml TBE buffer (Thermo Fischer Scientific TBE Buffer B52) was combined with 25g of agarose. This solution was microwave for approximately 50 seconds to dissolve. Solution mixed twice during the microwaving, cooled down to ~ 50°C, and 1.0 µl of ethidium bromide (Thermo Fischer scientific ultrapure ethidium bromide) was added and mixed. In order to make wells in the gel, the horizontal gel apparatus was sealed and the comb was inserted until its base was 2 mm from the gel's base. A gel tray was filled with molten agarose, being careful not to create bubbles. After the gel stand was filled with buffer TBE until it completely covered the gel, it was let to harden and the gel tray was inserted into the electrophoresis chamber in the correct position. After the comb was taken out, the gel was prepared to accept samples for electrophoresis. 5 µl of DNA samples were mixed with 2 µl of 6X loading dye (Thermo scientific 00830783) the material was well mixed before being injected into the wells. Using a 100 base pair DNA ladder marker (Thermo scientific 00796174) from 100-3000 bp MW as a standard (3µl loaded), which is loaded and moved on the same gel with samples, the molecular weight (size) of each DNA fraction was calculated. For about an hour, the gel was operated at a continuous current of 100V until the bromophenol blue passed about

two thirds of the gel distance. After that, a UV Tranilluminator was used to view the gel, and a digital camera was used to take pictures.

3.4.2 Polymerase Chain reaction

An approximately 710-bp fragment including primers of the barcoding region of COI was amplified using the universal primers

LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') Forward primer

HCO2198 (5'- TAAACTTCAGGGTGACCAAAAAATCA-3') Reverse primer (Folmer *et al.*, 1994).

In a 25 µl volume of solution, 0.1 µl of Taq Polymerase (Thermo scientific 00855243), 1 µl of primer, 0.5 µl of DNTPs (Thermo scientific 00857406), 5.0 µl of 10x buffer, 3.0 µl of template DNA, and the remaining volume was made up of deionized water (Ambion AM9932), until the desired volume was reached. A Standard thermo cycler with various temperature profiles was used to conduct the PCR reaction. With an initial denaturation at 94 °C for 3 minutes, 5 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s, and extension at 72 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 51 °C for 1 min, and 72 °C for 1 min, with a final 10 min extension step at 72 °C. PCR amplification was carried out using the suggested PCR program. The 1.5% agarose gel (was used to separate the PCR results in order to verify that the DNA amplification was effective. (Becker *et al.*, 2021)

After gel electrophoresis, PCR products with visible bands were shipped to Singapore for Sanger Sequencing through Advance Bioscience International (ABI), Lahore.

3.4.3 Species discrimination using DNA barcodes

Using "BLAST" and the "Identification Request" function, the sequence of the obtained specimen was compared on GenBank. The majority of Diptera species exhibit >2% sequence divergence at COI, according to earlier research (Herbert *et al.*, 2003). For the purpose of separating taxa, researchers have employed a 2% distance criterion (Strutzenberger *et al.*, 2011). Next, the accession number is assigned to the particular sequence. Using normal parameters, ClustalTM (McWilliam *et al.*, 2013) was used to compare and align the barcode sequences with Genbank sequences. Using MEGA version X (Tamura *et al.*, 2011), a neighbor-joining phylogenetic tree based on K2P genetic distance (Kimura, 1980) was constructed. This was done in order to compare the discovered divergence in sequence with sequences stored in Genbank and to visually represent and illustrate it.

3.4.4 Making phylogenetic tree

The program MEGA (Molecular Evolutionary Genetic Analysis) X was utilized to create the phylogenetic tree. Selecting an alignment technique opened ClustalW's default settings, the sequence was pasted on it. The OK button was hit to begin the alignment process. It took several seconds to several hours, depending on how many sequences were involved and how the alignment process was carried out. The session was saved after the alignment was finished. Since MEGA X cannot directly estimate a phylogenetic tree from a .mas file, alignment from the Data menu is chosen, and the file is exported in MEGA X format and given a.meg extension. The data's title has been entered. Phylogenetic tree was prepared by choosing the neighbor joining option. (Hall, 2013). The evolutionary distances were expressed in base substitutions per site and were calculated using the Maximum Composite Likelihood approach. There were eight nucleotide sequences in this investigation. For every sequence pair, all unclear places were eliminated (pairwise deletion option). The final dataset contained 678 locations in total.

3.5 Statistical Analysis

The species diversity in each of Punjab's five agroecological zones' tehsils and several districts was assessed using the Shannon diversity index (H) and Weiner diversity index (D). According to Strong in 2016 Shannon's index takes into consideration both the species' evenness and abundance. One-way analysis of variance test (ANOVA) was performed to evaluate and compare the mosquito abundance at tehsil and district level. If significant differences were found in the ANOVA test, Tukey's post hoc analysis was employed to separate the means. The association between mosquito abundance and season was also determined with the aid of one-way ANOVA (Kim, 2017). A Student t-test was used to compare the mosquito abundance in rural and urban areas of various tehsils across all six districts (Kim, 2015). Graphs on the abundance of mosquito and effect of climatic variables on it were prepared on excel sheets.

3.6 GIS Mapping of mosquito population

In the process of creating the map, the renowned Geographic Information System (GIS) software ArcGIS 10.8 was employed integrating a range of diverse datasets to craft a comprehensive visual representation of Genus Type. The fundamental datasets utilized in the generation of this map encompassed the Shapefile of Punjab, sourced from Diva.gis, as well as Elevation data acquired from the United States Geological Survey (USGS). Additionally, a crucial component of this map was the Genus Type Dataset converted into CSV file. The map creation technique was carried out with meticulous attention to detail and in accordance with a

disciplined workflow. The Punjab shapefile was initially imported into the ArcGIS environment, enabling for the identification of the exact area of interest. The USGS elevation data was then carefully cut to match to the Shapefile bounds, ensuring the smooth integration of topographic information into the research region. To render the elevation variations within the study area perceptible, a symbology analysis was conducted. This involved the utilization of a color ramp to symbolize the elevation values, allowing for a clear and intuitive representation of the topographical variations, ranging from low to high elevations. The Genus Type Dataset, which is provided in CSV format, is then uploaded; this dataset provides critical information about the placements of tehsil points within the study area. Symbology techniques were used to visually display this data, allowing for the assignment of separate symbols to each genus type. Furthermore, for each feature, bar charts were constructed, providing an instructive portrayal of the distribution of genus types among distinct tehsils within the study area. In the final stages of map composition, meticulous attention was devoted to the inclusion of essential cartographic elements. These elements encompassed an inset map, scale, legend, north arrow, and a grid, serving to enhance the overall clarity and interpretability of the map. These elements contribute to the map's utility and effectiveness as a communication tool for conveying complex geographical and ecological information.

Chapter 4 RESULT

4.1 Rice tract Zone District Lahore

Mosquitoes were collected from Model Town, Shalimar Town, Lahore City, Raiwind and Lahore Cantt. A total of five tehsils in Lahore yielded $n = 5004$ mosquitoes with seven species. The genus *Culex* was the most prevalent of the three, accounting for 82% of the total, followed by *Aedes* (18%) while *Anopheles* were not found from this district. It was noted that tehsil Cantt had the lowest abundance as compared to Tehsil Lahore City where it was large in number. The five species of the genus *Culex* were observed including *Culex tritaeniorhynchus* (30%) *Culex quinquefasciatus* (19%).*Culex pseudovishnui* (14%), *Culex pipiens* (10%) and *Culex sitiens* (5%).There were only two known species in the *Aedes* genus: *Aedes albopictus* (12%), and *Aedes aegypti* (6%). (Figure 4.1).

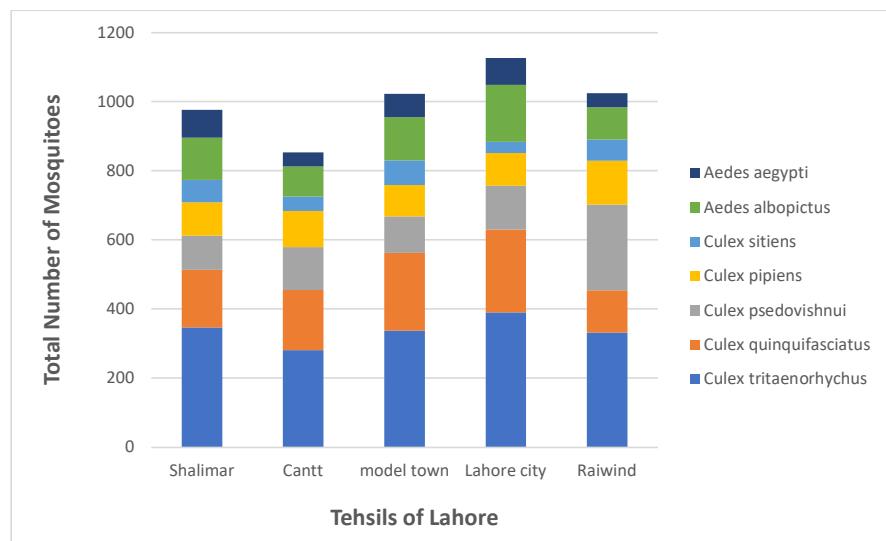


Figure 4.1-1: Total Number of mosquitoes in different tehsils of Lahore

	Model Town				Shalimar Town				Lahore Cantt				Raiwind				Lahore City			
Name of the species	Total counts	%age	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)	Total counts	%age	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)	Total counts	%age	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)	Total counts	%age	Shannon's Index Pi*(logPi)	Raiwind	Total counts	%age	Shannon's Index Pi*(logPi)	Lahore City
Culex tritaeniorhynchus	338	33.04	0.3659	113906	347	35.52	0.3677	120062	281	32.94	0.3658	78680	332	32.42	0.3652	3652	1560	7.8	0.1848	6006
Culex quinquefasciatus	225	21.99	0.3331	50400	166	16.99	0.3012	27390	174	20.40	0.3243	30102	122	11.91	0.2535	2535	1560	7.8	0.1848	6006
Culex pseudovishnui	105	10.26	0.2337	10920	100	10.24	0.2333	9900	124	14.54	0.2803	15252	249	24.32	0.3438	3438	1560	7.8	0.1848	6006
Culex pipiens	91	8.90	0.2152	8190	96	9.83	0.2280	9120	105	12.31	0.2579	10920	126	12.30	0.2578	2578	1560	7.8	0.1848	6006
Culex sitiens	71	6.94	0.1852	4970	65	6.65	0.1803	4160	42	4.92	0.1483	1722	61	5.96	0.1680	1680	1560	7.8	0.1848	6006
Aedes albopictus	125	12.22	0.2569	15500	122	12.49	0.2598	14762	86	10.08	0.2313	7310	94	9.18	0.2192	2192	1560	7.8	0.1848	6006
Aedes aegypti	68	6.65	0.1802	4556	81	8.29	0.2064	6480	41	4.81	0.1459	1640	40	3.91	0.1267	1267	1560	7.8	0.1848	6006
Total	1023		1.7701	0.8006	977		1.7766	0.7988	853		1.7537	0.7996	1024		1.7342					

Table 4.1.1: Diversity index of a number of individuals of different species of mosquitoes collected from five tehsils of District Lahore.

4.1.1 Species Diversity and Abundance of Mosquitoes in District Lahore

Table 4.1.1.1 indicated that there were notable variations throughout the tehsils for the diversity of species identified (n), Shannon diversity (H), and Simpson diversity (D). For the five tehsils, the Shannon and Simpson diversity indices varied from 1.77 to 1.71 and 0.80 to 0.78. The diversity index was highest in tehsil Model Town and Shalimar Town (H=1.77) then in Lahore Cantt (H=1.75) and Raiwind (H=1.732) and least in tehsil Lahore city (H=1.71). Analysis of variance (ANOVA) tests revealed significant differences in mosquito abundance across all tehsils in district Lahore. *Culex tritaeniorhynchus* had the highest abundance ($F = 19.85$, $DF = 4$, $P < 0.001$) (**Table 4.1.1.2**). Of all the species collected from district Lahore especially in tehsil Lahore city (Mean = 390, SD = 11) followed by tehsil Shalamar (Mean = 344.47, SD = 16.62), Model Town (Mean = 341, SD = 14.73), Raiwind (Mean = 334, SD = 15.10), and Cantt (Mean = 284, SD = 15.72) (**Figure 4.1.1.2.1**). For *Culex quinquefasciatus* (**Figure 4.1.1.2.2**) and *Aedes aegypti*, the same pattern was noted (**Figure 4.1.1.1.7**). Different results were obtained for *Culex pseudovishnui* (**Figure 4.1.1.2.3**) and *Culex pipiens* (**Figure 4.1.1.2.4**) which were more prevalent in Tehsil Raiwind and less in Model Town tehsil.

No.	ANOVA	Mean± Standard Deviation						F	P
		Species	Shalamar	Cantt	Model Town	Lahore City	Raiwind		
1	Cx. quinquefasciatus	166 ^b ±15	173 ^b ±14.53	227 ^a ±14.11	242 ^a ±13.11	120 ^c ±17.09	33.24	0.001	
2	Cx. tritaeniorhynchus	244.67 ^b ±16.62	284 ^c ±15.72	341 ^b ±14.73	390 ^a ±11	334 ^b ±15.10	19.58	0.001	
3	Cx. pseudovishnui	101.67 ^b ±13.58	122.67 ^b ±13.05	105 ^b ±17	125.33 ^b ±12.58	248.67 ^a ±3.50	57.21	0.001	
4	Cx. pipiens	95.67 ^b ±4.51	104.67 ^b ±4.51	91.33 ^c ±4.51	94.33 ^b ±4.51	125.33 ^a ±5.03	26.85	0.001	
5	Cx. sitiens	64.67 ^a ±4.51	41.67 ^b ±3.51	71 ^a ±5	33.33 ^b ±4.51	61 ^a ±5	37.52	0.001	
6	Ae.albopictus	119.67 ^b ±16.62	82.67 ^b ±15.28	122.33 ^b ±16.17	166 ^a ±11.53	93 ^b ±17.5	12.98	0.001	
7	Ae.aegypti	81 ^a ±7	41 ^b ±4	67.67 ^a ±6.51	77.67 ^a ±5.51	39.67 ^b ±3.51	39.41	0.001	

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4.1.1 4.1.1: Species Diversity and Abundance of Mosquitoes in District Lahore

Table 4.1-2: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of Lahore

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Culex sitiens was one of the species in this district which was highly abundant in tehsil Raiwind and leastly found in tehsil Lahore city. (Figure 4.1.1.2.5)

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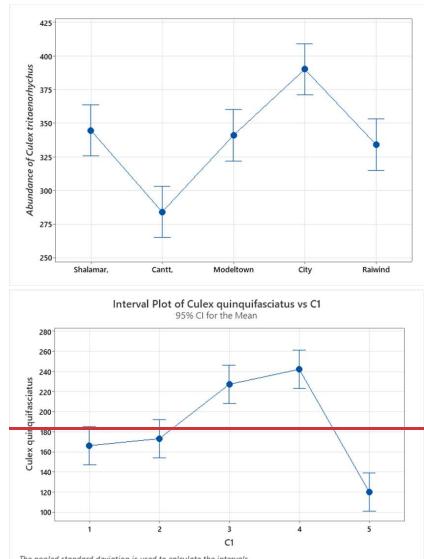


Figure 4.1-3:Abundance of *Culex tritaeniorhynchus quinquefasciatus*

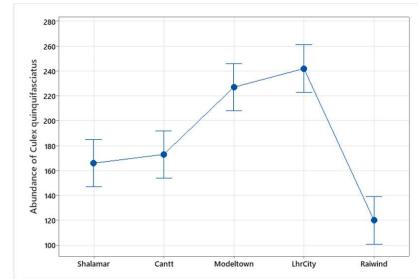
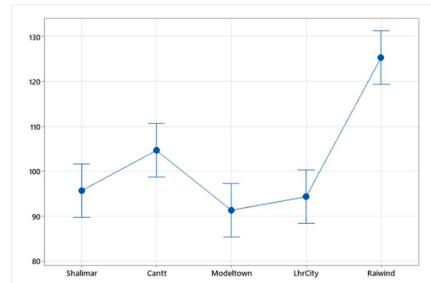
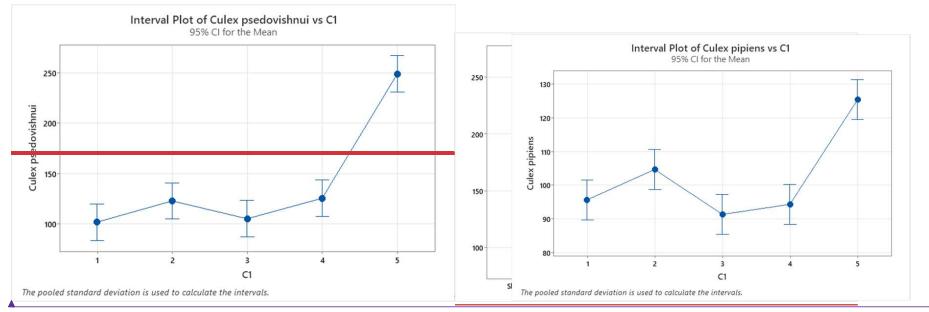


Figure 4.1-4:Abundance of *Culex quinquefasciatus*





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Figure 4.1-5: Abundance of *Culex psedorvishnui*
with-season

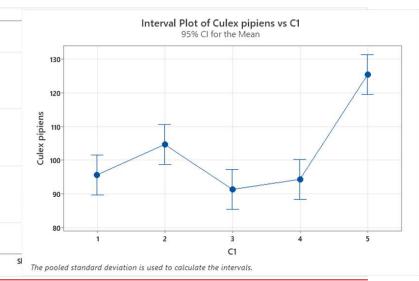


Figure 4.1-6: Abundance of *Culex pipiens*

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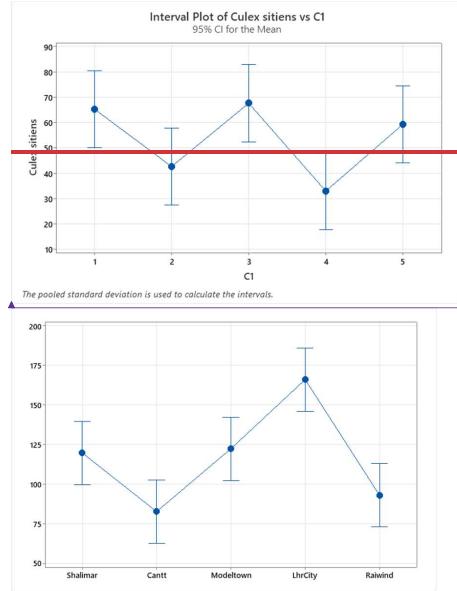


Figure 4.1-7: Abundance of *Culex sitiens*
Abundance of

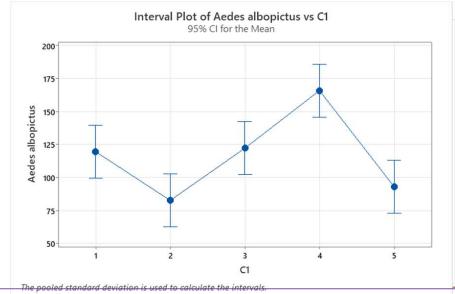
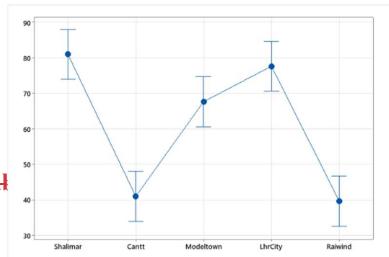


Figure 4.1-8: Abundance of *Aedes albopictus*

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Figure 4.1.1.1 : Relative abundance of different species in different tehsils



Relative abundance of different species in different tehsils

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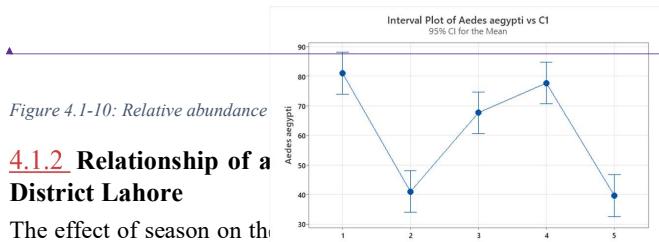


Figure 4.1-10: Relative abundance

4.1.2 Relationship of a District Lahore

The effect of season on the relative abundance of mosquito was calculated through one-way ANOVA. The monsoon season was found to be highly favorable for all the species of mosquitoes. The autumn season was also conducive. The highest fall of the reproduction of the mosquito was observed in winter season. Genus *Culex* was richly distributed in all the tehsils where Lahore city showed the greatest abundance of mosquito as compared to all the tehsils. It had the greatest abundance of *Culex tritaeniorhynchus* count ($F = 136.8$, $df = 4$, $P = 0.001$). Its peak levels of abundance were noted in autumn season (Mean = 106.33, SD = 2.52) then in monsoon (Mean = 104.33, SD = 4.04) then in spring (Mean = 57, SD = 4) decreasing in summer (Mean = 79, SD = 6.56) falling in winter (Mean = 42.33, SD = 2.52) (Table 4.1.2.1). The genus *Aedes* showed different pattern of occurrence ($F = 95.57$, $df = 4$, $P = 0.001$). It was highest in monsoon (Mean = 75.33, SD = 5.51) then in autumn (Mean = 62, SD = 11.79) then in spring (Mean = 11, SD = 1) falling in winter (Mean = 0, SD = 0). This pattern of abundance in different season was also observed for all the other species in tehsil Model Town (Table 4.1.2.2), Tehsil Shalamar Town (Table 4.1.2.3) Tehsil Raiwind (Table 4.1.2.4) and tehsil Cantt (Table 4.1.2.5)

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Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<u>Cx. quinquefasciatus</u>	<u>58.67^b±4.51</u>	<u>25^d±2</u>	<u>38.33^c±1.52</u>	<u>45.33^c±3.06</u>	<u>72.67^a±3.06</u>	<u>111.82</u>	<u>0.001</u>
<u>Cx. tritaeniorhynchus</u>	<u>106.33^a±2.52</u>	<u>42.33^d±2.52</u>	<u>57^c±4</u>	<u>79^b±6.56</u>	<u>104.33^a±4.04</u>	<u>136.8</u>	<u>0.001</u>
<u>Cx. pseudovishnui</u>	<u>32.33^a±2.52</u>	<u>12.67^d±2.52</u>	<u>20.67^c±2.08</u>	<u>25.33^b±1.52</u>	<u>35.67^a±5.13</u>	<u>27.76</u>	<u>0.001</u>
<u>Cx. pipiens</u>	<u>26.67^a±3.06</u>	<u>9.67^b±5.03</u>	<u>15.33^a±5.03</u>	<u>19^a±6</u>	<u>23.67^a±4.51</u>	<u>5.84</u>	<u>0.001</u>
<u>Cx. Sitiens</u>	<u>7^a±1</u>	<u>1.66^b±0.57</u>	<u>6.33^a±1.52</u>	<u>7.33^a±1.52</u>	<u>10^a±2</u>	<u>13.72</u>	<u>0.001</u>
<u>Ae.albopictus</u>	<u>62^a±11.79</u>	<u>0^c±0</u>	<u>11^b±1</u>	<u>17.33^b±2.08</u>	<u>75.33^a±5.51</u>	<u>95.57</u>	<u>0.001</u>
<u>Ae.aegypti</u>	<u>36^a±5.57</u>	<u>0^c±0</u>	<u>7^b±1</u>	<u>12.33^b±2.52</u>	<u>30.33^a±6.51</u>	<u>44.14</u>	<u>0.001</u>

Figure 4.1-11:Relationship of abundance of species of mosquitoes with seasons in Tehsil Lahore City of District Lahore

Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<u>Cx. quinquefasciatus</u>	<u>57.67^a±7.09</u>	<u>22.67^c±2.52</u>	<u>37.33^b±2.52</u>	<u>42.67^b±4.04</u>	<u>64^a±6</u>	<u>5.26</u>	<u>0.001</u>
<u>Cx. tritaeniorhynchus</u>	<u>100.23^a±4.51</u>	<u>35.33^c±4.73</u>	<u>43.33^c±4.16</u>	<u>58.33^b±7.02</u>	<u>97.67^a±2.52</u>	<u>124.04</u>	<u>0.001</u>
<u>Cx. pseudovishnui</u>	<u>29^a±2</u>	<u>3.66^c±1.52</u>	<u>20^b±3</u>	<u>22.33^b±2.52</u>	<u>29^a±1</u>	<u>71.31</u>	<u>0.001</u>
<u>Cx. pipiens</u>	<u>26^b±1</u>	<u>4^d±1</u>	<u>13^c±1</u>	<u>13^c±1</u>	<u>35^a±2</u>	<u>280.69</u>	<u>0.001</u>
<u>Cx. Sitiens</u>	<u>16.66^b±1.52</u>	<u>7^d±1</u>	<u>11^c±1</u>	<u>14^b±1</u>	<u>22^a±2</u>	<u>51.84</u>	<u>0.001</u>
<u>Ae.albopictus</u>	<u>36.67^a±2.52</u>	<u>0^c±0</u>	<u>15^b±3</u>	<u>33.33^a±3.51</u>	<u>40.67^a±4.04</u>	<u>100.22</u>	<u>0.001</u>
<u>Ae.aegypti</u>	<u>20^b±2</u>	<u>0^e±0</u>	<u>6^d±1</u>	<u>15^c±2</u>	<u>25.33^a±2.52</u>	<u>103.39</u>	<u>0.001</u>

Figure 4.1-12:Relationship of abundance of species of mosquitoes with seasons in Tehsil Model Town of District Lahore

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Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	48 ^a ±8	17.33 ^b ±1.52	21 ^b ±3	29.33 ^b ±2.52	59 ^a ±8	33.09	0.001
<i>Cx. tritaeniorhynchus</i>	97 ^a ±7.21	21.67 ^b ±9.61	40 ^b ±9	85 ^a ±7.21	101 ^a ±5	63.82	0.001
<i>Cx. pseudovishnui</i>	27 ^a ±3.61	8.33 ^c ±1.52	16 ^b ±2	18.33 ^b ±5.03	29 ^a ±2	21.92	0.001
<i>Cx. pipiens</i>	22.67 ^a ±2.52	6 ^c ±2	10.67 ^b ±5.03	17 ^b ±5	32 ^a ±8	12.51	0.001
<i>Cx. Sitiens</i>	19.33 ^b ±3.51	6.33 ^c ±1.52	10.67 ^c ±3.06	9.33 ^b ±3.06	31.33 ^a ±4.04	31.01	0.001
<i>Ae.albopictus</i>	36 ^a ±15.52	0 ^c ±0	11 ^b ±7	20.33 ^b ±11.5	53.33 ^a ±11.02	12.16	0.001
<i>Ae.aegypti</i>	29.33 ^a ±2.52	0 ^d ±0	7.66 ^c ±1.15	15 ^b ±3	31 ^a ±3.61	91.95	0.001

Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx.</i>							
<i>Cx. tritaeniorhynchus</i>	97 ^a ±7.21	21.67 ^b ±9.61	40 ^b ±9	85 ^a ±7.21	101 ^a ±5	63.82	0.001
<i>Cx.</i>							
<i>Cx. quinquefasciatus</i>	48 ^a ±8	17.33 ^b ±1.52	21 ^b ±3	29.33 ^b ±2.52	59 ^a ±8	33.09	0.001
<i>Cx.</i>							
<i>Cx. pseudovishnui</i>	27 ^a ±3.61	8.33 ^c ±1.52	16 ^b ±2	18.33 ^b ±5.03	29 ^a ±2	21.92	0.001
<i>Cx. pipiens</i>	22.67 ^a ±2.52	6 ^c ±2	10.67 ^b ±5.03	17 ^b ±5	32 ^a ±8	12.51	0.001
<i>Cx. Sitiens</i>	19.33 ^b ±3.51	6.33 ^c ±1.52	10.67 ^c ±3.06	9.33 ^b ±3.06	31.33 ^a ±4.04	31.01	0.001
<i>Ae.albopictus</i>	36 ^a ±15.52	0 ^c ±0	11 ^b ±7	20.33 ^b ±11.5	53.33 ^a ±11.02	12.16	0.001
<i>Ae.aegypti</i>	29.33 ^a ±2.52	0 ^d ±0	7.66 ^c ±1.15	15 ^b ±3	31 ^a ±3.61	91.95	0.001

Figure 4.1-13:Relationship of abundance of species of mosquitoes with seasons in Tehsil Shalimar Town of District Lahore

Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P

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<i>Cx. quinquefasciatus</i>	<u>66.33^b±7.09</u>	<u>22.6⁷±5.03</u>	<u>40^c±4</u>	<u>40.33^c±5.51</u>	<u>82.67^a±6.51</u>	<u>51.88</u>	<u>0.001</u>
<i>Cx. tritaeniorhynchus</i>	<u>85.67^b±7.51</u>	<u>26.33^d±2.52</u>	<u>64.33^c±7.57</u>	<u>56.67^c±6.51</u>	<u>105.67^a±8.33</u>	<u>58.41</u>	<u>0.001</u>
<i>Cx. pseudovishnui</i>	<u>26.66^b±1.52</u>	<u>13^c±1</u>	<u>22.67^b±3.06</u>	<u>23^b±2.65</u>	<u>35^a±4</u>	<u>26.48</u>	<u>0.001</u>
<i>Cx. pipiens</i>	<u>27.33^b±3.06</u>	<u>12.33^c±2.08</u>	<u>22.33^b±3.21</u>	<u>23.33^b±2.08</u>	<u>39.33^a±4.04</u>	<u>32</u>	<u>0.001</u>
<i>Cx. Sitiens</i>	<u>13^b±1</u>	<u>6^c±1</u>	<u>8.66^c±1.52</u>	<u>13.33^b±1.52</u>	<u>20.33^a±2.52</u>	<u>34.32</u>	<u>0.001</u>
<i>Ae.albopictus</i>	<u>33.67^a±3.51</u>	<u>0^d±0</u>	<u>8b^c±1</u>	<u>15^b±2</u>	<u>39^a±9</u>	<u>42.45</u>	<u>0.001</u>
<i>Ae.aegypti</i>	<u>13^a±1</u>	<u>0d^d±0</u>	<u>3^c±1</u>	<u>9b^b±1</u>	<u>15a^a±1</u>	<u>153.75</u>	<u>0.001</u>

Mean ± Standard Deviation							
Species	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Culex tritaeniorhynchus</i>	<u>85.67^b±7.51</u>	<u>26.33^d±2.52</u>	<u>64.33^c±7.57</u>	<u>56.67^c±6.51</u>	<u>105.67^a±8.33</u>	<u>58.41</u>	<u>0.001</u>
<i>Culex quinquefasciatus</i>	<u>66.33^b±7.09</u>	<u>22.67⁷±5.03</u>	<u>40^c±4</u>	<u>40.33^c±5.51</u>	<u>82.67^a±6.51</u>	<u>51.88</u>	<u>0.001</u>
<i>Culex pseudovishnui</i>	<u>26.66^b±1.52</u>	<u>13^c±1</u>	<u>22.67^b±3.06</u>	<u>23^b±2.65</u>	<u>35^a±4</u>	<u>26.48</u>	<u>0.001</u>
<i>Culex pipiens</i>	<u>27.33^b±3.06</u>	<u>12.33^c±2.08</u>	<u>22.33^b±3.21</u>	<u>23.33^b±2.08</u>	<u>39.33^a±4.04</u>	<u>32</u>	<u>0.001</u>
<i>Cx. Sitiens</i>	<u>13^b±1</u>	<u>6^c±1</u>	<u>8.66^c±1.52</u>	<u>13.33^b±1.52</u>	<u>20.33^a±2.52</u>	<u>34.32</u>	<u>0.001</u>
<i>Ae.albopictus</i>	<u>33.67^a±3.51</u>	<u>0^d±0</u>	<u>8b^c±1</u>	<u>15^b±2</u>	<u>39^a±9</u>	<u>42.45</u>	<u>0.001</u>
<i>Ae.aegypti</i>	<u>13^a±1</u>	<u>0d^d±0</u>	<u>3^c±1</u>	<u>9b^b±1</u>	<u>15a^a±1</u>	<u>153.75</u>	<u>0.001</u>

Figure 4.1-14: Relationship of abundance of species of mosquitoes with seasons in Tehsil Raiwind of District Lahore

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Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	<u>46.33^b±1.52</u>	<u>16.33^d±2.52</u>	<u>20.67^d±4.51</u>	<u>33.67^c±4.04</u>	<u>57.33^a±5.03</u>	<u>62.77</u>	<u>0.001</u>
<i>Cx. tritaeniorhynchus</i>	<u>72.67^a±3.06</u>	<u>15^c±1</u>	<u>54.33^b±3.06</u>	<u>55.67^b±5.03</u>	<u>53^b±3</u>	<u>124.9</u>	<u>0.001</u>
<i>Cx. pseudovishnui</i>	<u>34.33^a±3.06</u>	<u>9^c±1</u>	<u>21^b±1</u>	<u>23^b±1.73</u>	<u>25^b±1</u>	<u>80.96</u>	<u>0.001</u>
<i>Cx. pipiens</i>	<u>26^a±5</u>	<u>6^c±1</u>	<u>12.66^b±1.52</u>	<u>20.67^a±2.08</u>	<u>20a^b±4</u>	<u>18.74</u>	<u>0.001</u>
<i>Cx. Sitiens</i>	<u>±</u>	<u>±</u>	<u>±</u>	<u>±</u>	<u>±</u>	<u>-</u>	<u>0.001</u>
<i>Ae.albopictus</i>	<u>29^a±6.24</u>	<u>0^c±0</u>	<u>11.66^b±1.52</u>	<u>18.33^b±1.52</u>	<u>28.67^a±5.51</u>	<u>30.3</u>	<u>0.001</u>
<i>Ae.aegypti</i>	<u>15.33^a±3.06</u>	<u>0^d±0</u>	<u>5^c±1</u>	<u>11a^b±1</u>	<u>9.67b^c±2.08</u>	<u>33.12</u>	<u>0.001</u>

Figure 4.1-15: Relationship of abundance of species of mosquitoes with seasons in Tehsil Cantt of District Lahore

Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. tritaeniorhynchus</i>	<u>72.67^a±3.06</u>	<u>15^c±1</u>	<u>54.33^b±3.06</u>	<u>55.67^b±5.03</u>	<u>53^b±3</u>	<u>124.9</u>	<u>0.001</u>
<i>Cx. quinquefasciatus</i>	<u>46.33^b±1.52</u>	<u>16.33^d±2.52</u>	<u>20.67^d±4.51</u>	<u>33.67^c±4.04</u>	<u>57.33^a±5.03</u>	<u>62.77</u>	<u>0.001</u>
<i>Cx. pseudovishnui</i>	<u>34.33^a±3.06</u>	<u>9^c±1</u>	<u>21^b±1</u>	<u>23^b±1.73</u>	<u>25^b±1</u>	<u>80.96</u>	<u>0.001</u>
<i>Cx. pipiens</i>	<u>26^a±5</u>	<u>6^c±1</u>	<u>12.66^b±1.52</u>	<u>20.67^a±2.08</u>	<u>20a^b±4</u>	<u>18.74</u>	<u>0.001</u>
<i>Cx. Sitiens</i>	<u>±</u>	<u>±</u>	<u>±</u>	<u>±</u>	<u>±</u>	<u>-</u>	<u>0.001</u>
<i>Ae.albopictus</i>	<u>29^a±6.24</u>	<u>0^c±0</u>	<u>11.66^b±1.52</u>	<u>18.33^b±1.52</u>	<u>28.67^a±5.51</u>	<u>30.3</u>	<u>0.001</u>
<i>Ae.aegypti</i>	<u>15.33^a±3.06</u>	<u>0^d±0</u>	<u>5^c±1</u>	<u>11a^b±1</u>	<u>9.67b^c±2.08</u>	<u>33.12</u>	<u>0.001</u>

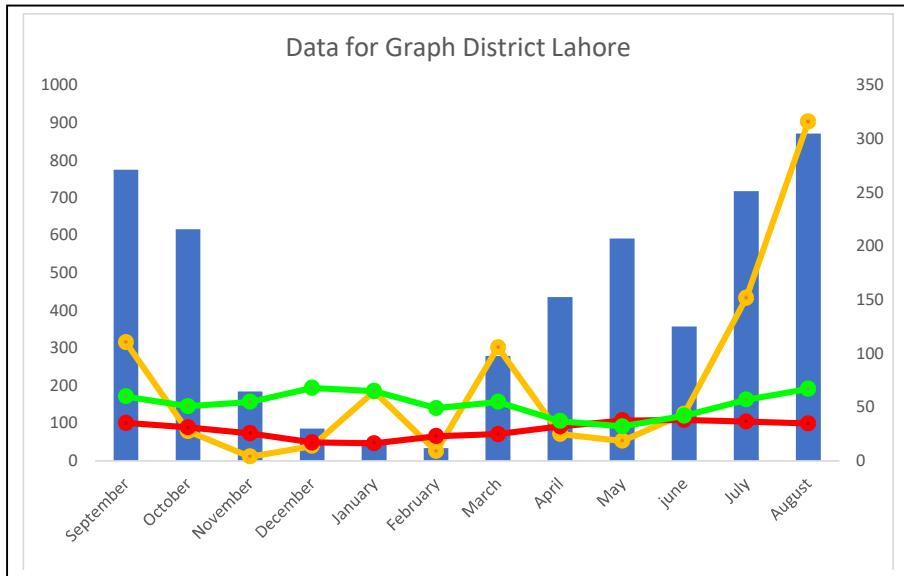


Figure 4.1-16: Effect of climatic variables with relative abundance of mosquito in district Lahore

4.1.3 Effect of climatic factors on the abundance of mosquitoes in District Lahore

The weather data and the monthly relative mosquito abundance for the entire Lahore district was studied was (studied (Figure 4.1.3.1) 4.1.3.1). The district's data interpretation showed the same pattern of mosquito abundance as previously mentioned, with the highest abundance occurring in August when temperature was high and humidity was moderate. This was followed by a decline in mosquito abundance in the months of March and September, which has been lowered been lowered during the winter. However, once the summer officially began in May, the average rainfall and mean temperature fluctuated, leading to a rise a rise in the mosquito population.

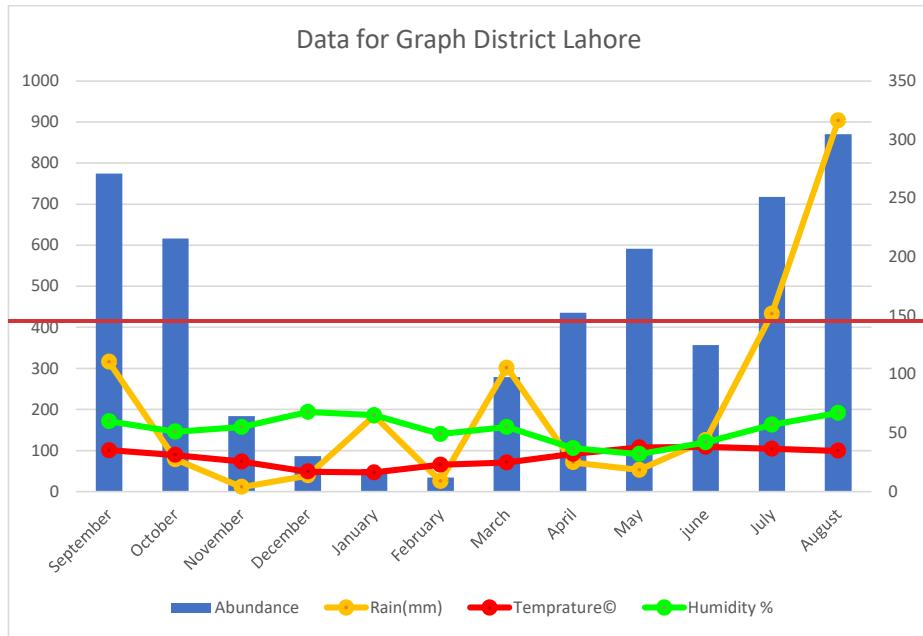


Figure 4.1.3.1: Effect of climatic variables with relative abundance of mosquito in district Lahore

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4.1.4 GIS Mapping for District Lahore

GIS mapping from several tehsils in the district of Lahore revealed that there was a significant abundance of mosquitoes in every tehsil, with the genus *Aedes* being particularly prevalent in Lahore City. This tehsil is well-known for its tire business, which deals with both new and old tires which may help in invading the *Aedes* species.

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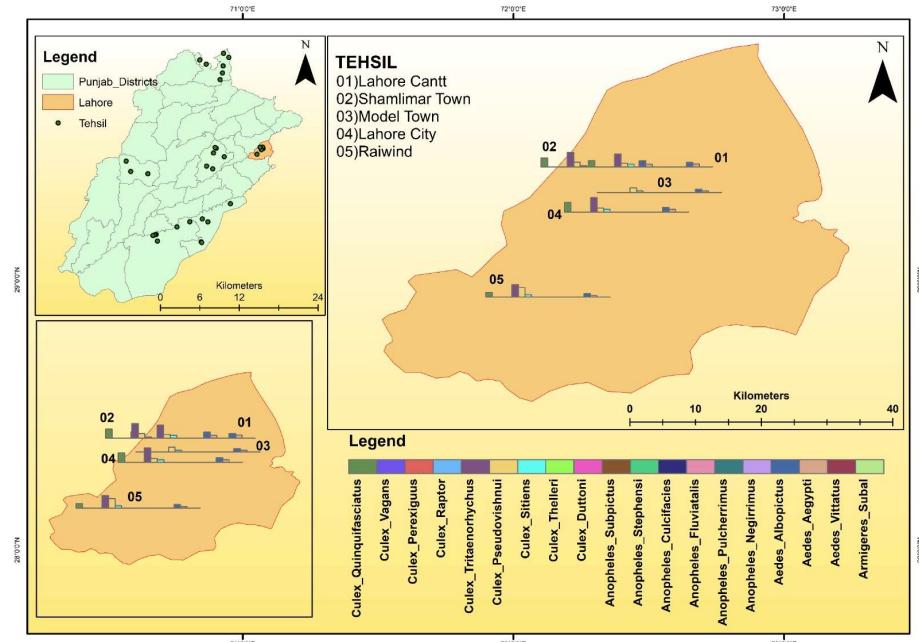


Figure 4.1-17: GIS map of different tehsils showing mosquito abundance in district Lahore

4.2 Mixed Cropping Zone District Faisalabad

The sampling area of the mixed cropping zone was ~~district Faisalabad~~ district Faisalabad considering the rural and urban regions ~~of tehsils of tehsils~~ Chak Jhumra, Saddar, Jarawala, Samundri, and Tandiwala. The total ~~of nof n=17,199~~ of 17,199 of 10 mosquito species ~~were species were collected~~ collected. *Culex* was the most common of the three genera, accounting for 78.9%, followed by *Anopheles* (13.97%) and *Aedes* (6.07%). Tehsil Chak Jhumra was found to have the highest number of mosquitoes, with Faisalabad, Samundri, Sadar, and Tandiawala and Jarawala tehsils following suit. Five species in all were identified from the genus *Culex*: *Culex sitiens* (5.59%), *Culex pipiens* (3.91%), *Culex quinquefasciatus*

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(34.37%), *Culex tritaeniorhynchus* (25.26%), and *Culex pseudovishnui* (10.81%) respectively. *Anopheles stephensi* (4.81%), *Anopheles subpictus* (6.44%), and *Anopheles culcifacies* (2.7%) were the only three species of the *Anopheles* genus that were discovered. There were only two species identified in the genus *Aedes*: *Aedes albopictus* (4.51%) and *Aedes aegypti* (1.55%) (Figure 4.2)

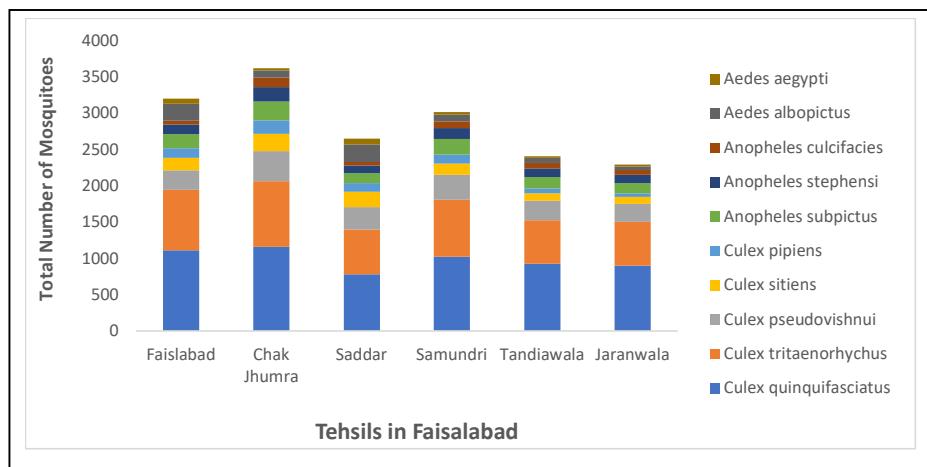


Figure 4.2-1: Total no of mosquitoes in district Faisalabad

4.1.24.2.1 Species Diversity and Abundance of mosquitoes in district Faisalabad

The diversity of species identified (n), Shannon diversity (H), and Simpson diversity (D) varied significantly across tehsils, on average. For the six tehsils, the Shannon and Simpson diversity indices varied from 1.96 to 1.72 and 0.84 to 0.75, respectively. Tehsil Chak Jhumra ($H=1.89$), Faisalabad ($H=1.86$), Samundri ($H=1.84$), Tandiawala ($H=1.77$), and Tehsil Jarawala ($H=1.72$) had the lowest diversity index, while Tehsil Saddar had the highest ($H=1.96$). (Table 4.2.1.1)

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Name of the species	Saddar						Chak Jhumra						Faisal Abad						Summandari						Tandia wala						Jaranwala					
	Tot al	cou nts	%a ge	Pi*(lo gPi)	Shann on's Index	Simps on's	Tot al	cou nts	%a ge	Pi*(lo gPi)	Shann on's Index	Simps on's	Tot al	cou nts	%a ge	Pi*(lo gPi)	Shann on's Index	Simps on's	Tot al	cou nts	%a ge	Pi*(lo gPi)	Shann on's Index	Simps on's	Tot al	cou nts	%a ge	Pi*(lo gPi)	Shann on's Index	Simps on's						
	cou nts	Index n(n-1)			cou nts	Index n(n-1)			cou nts	Index n(n-1)	cou nts	Index n(n-1)			cou nts	Index n(n-1)	cou nts	Index n(n-1)		cou nts	Index n(n-1)	cou nts	Index n(n-1)	cou nts	Index n(n-1)	cou nts	Index n(n-1)	cou nts	Index n(n-1)	cou nts	Index n(n-1)					
<i>Culex quinquefasciatus</i>	29.49	0.3601	610742	1161	32.04	0.3647	13467	1117	34.85	0.3674	12465	1025	33.99	0.3668	10496	92852	38.52	0.3675	86025	8996	39.21	0.3671	807302													
<i>Culex tritaeniorhynchus</i>	23.34	0.3396	382542	90806	25.8	0.3468	82355	82883	25.83	0.3497	68475	7856	26.03	0.3503	61544	59987	24.0	0.3460	358202	6062	26.43	0.3517	366630													
<i>Culex pseudovishnui</i>	11.61	0.2500	94556	41442	11.8	0.2478	170982	2729	8.49	0.2093	73712	345	11.44	0.2480	118680	27021	11.3	0.2453	72630	251	10.95	0.2422	62750													
<i>Culex sitiens</i>	7.9	0.201	4431		6.5	0.177			5.2	0.155			5.1	0.151			4.1	0.132			4.0	0.129														
<i>Culex pipiens</i>	2116	4	0	2361	9	55460	1697	2	28392	1541	9	23562	1005	1	9	23562	1005	1	9	9900	921	0	0	8372												
<i>Anopheles subpictus</i>	4.4	0.138	13805	61848	5.0	0.1513			4.1	0.1314			4.0	0.1308			2.7	0.0996			2.1	0.0824														
<i>Anopheles stephensi</i>	5.1	0.1523	18360		7.2	0.1899			6.0	0.1703			7.1	0.1883			6.6	0.1790			6.1	0.1723														
<i>Anopheles culicifacies</i>	1363	0.1303	11556	2002	5.5	0.1599			4.1	0.1314			5.0	0.1506			5.0	0.1502			5.0	0.1501														
<i>Aedes albopictus</i>	1087	4	6	2002	9	39800	1322	4	17292	1524	8	22952	1212	2	14520	1152	1	131101																		
<i>Aedes aegypti</i>	2652			1.9692	0.8232	3624		1.8966	0.8046	3205		1.8608	0.7889	3016		1.8496	0.7909	2401		1.7720	0.7669	2293		1.7263	0.7550											

Figure 4.2-2: Diversity index of a number of individuals of different species of mosquitoes collected from tehsils of District Faisalabad

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Analysis of variance (ANOVA) tests revealed significant differences in mosquito abundance across all tehsils. This district had the highest concentration of *Culex quinquefasciatus* (**Figure 4.2.1.1.1**) ($F = 272.61$, $df=5$, $P < 0.001$) and the largest abundance of genus *Culex*. With the lowest concentration in tehsil Jaranwala (Mean=896.67, SD=14.64) and the least in Saddar (Mean=781.33, SD=14.01), Tehsil Chak Jhumra had the highest value of occurrence (Mean=1162, SD=13.53), followed by Faisalabad (Mean=1117, SD=18), Samundari (Mean=1024.67, SD=16.50), and Tandiawala (Mean=926.67, SD= 13.05) (**Table 4.2.1.2**). *Culex tritaeniorhynchus* (**Figure 4.2.1.1.2**) *Anopheles subpictus* (**Figure 4.2.1.1.6**) *Anopheles stephensi* (**Figure 4.2.1.1.7**) and *Anopheles culicifacies* (**Figure 4.2.1.1.8**) all displayed the same pattern of abundance as that of *Culex quinquefasciatus*. *Culex pseudovishnui* (**Figure 4.2.1.1.3**), *Culex sitiens* (**Figure 4.2.1.1.4**) and *Culex pipiens* (**Figure 4.2.1.1.5**) were quite prevalent in tehsil Saddar while *Aedes albopictus* (**Figure 4.2.1.1.9**) and *Aedes aegypti* (**Figure 4.2.1.1.10**) were highly abundant in tehsil Saddar and Faisalabad.

ANOVA		Mean± Standard Deviation							
Species	Faisalabad	Chak jhumra	Saddar	Samundari	Tandiawala	Jaranwala	F	P	
Cx. quinquefasciatus	1117 ^b ±18	1162 ^a ±13.53	781.33 ^e ±14.01	1024.67 ^c ±16.50	926.67 ^d ±13.05	896.67 ^d ±14.64	272.61	0.001	
Cx. tritaeniorhynchus	827.7 ^b ±19.5	908.33 ^a ±14.50	621 ^d ±17.09	784 ^c ±12.53	600.67 ^d ±14.57	604.33 ^d ±2.58	225.81	0.001	
Cx. pseudovishnui	272.7 ^c ±18	413.7 ^a ±17.5	306.33 ^b ±13.58	347 ^b ±14.11	269.7 ^c ±19.5	251.7 ^d ±18	38.86	0.001	
Cx. pipiens	132.3 ^b ±17.5	185.67 ^a ±14.57	119.67 ^b ±15.57	123.67 ^b ±15.01	70 ^c ±14.73	48.66 ^c ±1.528	35.23	0.001	
Cx. sitiens	169.3 ^b ±17.5	238 ^a ±13.11	212 ^a ±14.53	153.33 ^b ±11.02	97 ^c ±14.73	90 ^c ±14.11	52.21	0.001	
Anopheles subpictus	195.67 ^b ±15.01	261 ^a ±12.53	137.67 ^d ±16.56	214 ^b ±12.53	159 ^c ±d13	141.33 ^d ±3.01	36.11	0.001	
Anopheles stephensi	130.33 ^b ±12.58	199.33 ^a ±13.01	108 ^c ±14	150.67 ^b ±15.04	121 ^b ±12	112.67 ^c ±5.63	18.36	0.001	
Anopheles culicifacies	55 ^b ±13.53	130 ^a ±14	48.66 ^c ±1.528	93.67 ^a ±14.50	72.3 ^b ±18	66.7 ^b ±19	12.68	0.001	
Ae.albopictus	232.33 ^a ±12.06	97.3 ^b ±18	242 ^a ±18	88.33 ^b ±13.01	68.7 ^b ±17.5	49.66 ^c ±1.528	101.93	0.001	
Ae.aegypti	75.67 ^a ±11.02	33 ^b ±3	79 ^a ±12	34.33 ^b ±1.528	23 ^b ±2	23 ^b ±2	42.01	0.001	

Figure 4.2-3:

		Mean \pm Standard Deviation							
Species	Faisalabad	Chak jhumra	Saddar	Samundri	Tandiwala	Jaranwala	F	P	
Cx. quinquefasciatus	1117b \pm 18 3.53	1162a \pm 1 14.01	781.33e \pm 14.01	1024.67c \pm 16.50	926.67d \pm 13.05		896.67d \pm 14.64	272 .61	0.0 .01
Cx. tritaeniorhynchus	827.7b \pm 1 9.5	908.33a \pm 14.50	621d \pm 17. 09	784c \pm 12. 53	600.67d \pm 14.57		604.33d \pm 12.58	225 .81	0.0 .01
Cx. pseudovittatus	272.7cd \pm 18	413.7a \pm 17.5	306.33bc \pm 13.58	347b \pm 14. .11	269.7cd \pm 19.5		251.7d \pm 18	38. 86	0.0 .01
Cx. pipiens	132.3b \pm 1 7.5	185.67a \pm 14.57	119.67b \pm 15.57	123.67b \pm 15.01	70c \pm 14. 73		48.667e \pm 1.528	35. 23	0.0 .01
Cx. sitiens	169.3b \pm 1 7.5	238a \pm 13 .11	212a \pm 14. 53	153.33b \pm 11.02	97c \pm 14. 73		90c \pm 14. .11	52. 21	0.0 .01
Anopheles subpictus	195.67bc \pm 15.01	261a \pm 12 .53	137.67d \pm 16.56	214b \pm 12 .53	159c \pm d1 3		141.33d \pm 13.01	36. .11	0.0 .01
Anopheles stephensi	130.33bc \pm 12.58	199.33a \pm 13.01		150.67b \pm 15.04	121bc \pm 1 2		112.67e \pm 15.63	18. 36	0.0 .01
Anopheles culicifacies	55bc \pm 13. 53	130a \pm 14	48.667c \pm 1.528	93.67ab \pm 14.50	72.3bc \pm 18		66.7bc \pm 19	12. 68	0.0 .01
Ae. albopictus	232.33a \pm 12.06	97.3b \pm 1 8		88.33bc \pm 24.2a \pm 18	68.7bc \pm 13.01		49.66c \pm 1.528	101. .93	0.0 .01
Ae. aegypti	75.67a \pm 1 1.02	33b \pm 3	79a \pm 12	34.33b \pm 1.528	23b \pm 2		23b \pm 2 42. .01	42. .01	0.0 .01

Comparative analysis of the abundance of mosquitoes in different Tehsils of District Faisalabad

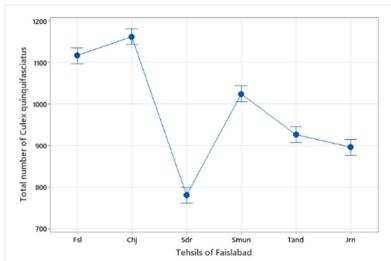


Figure 4.2-4: Abundance of Culex quinquefasciatus

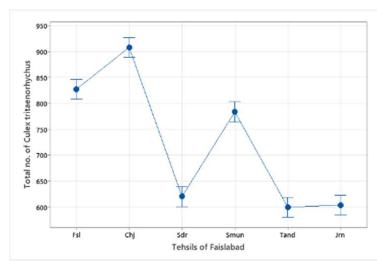
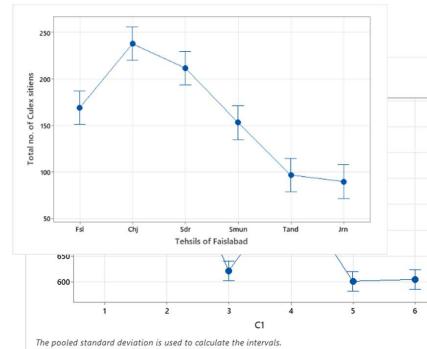
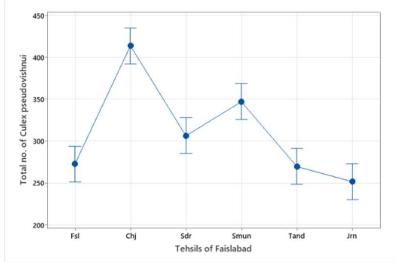


Figure 4.2-5: Abundance of Culex tritaeniorhynchus



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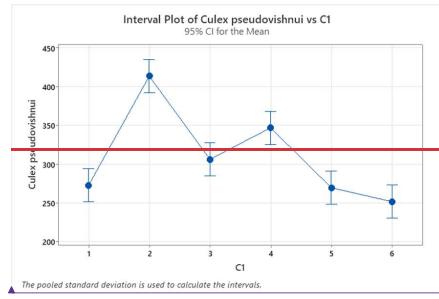


Figure 4.2-6: Abundance of *Culex pseudovishnui*

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Figure 4.2-7: Abundance of *Culex sitiens*

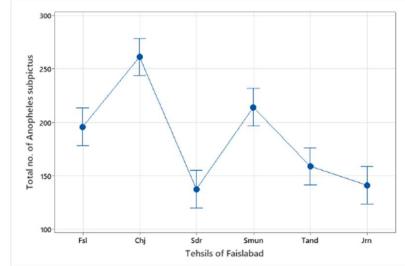


Figure 4.2-8: Abundance of *Culex pipiens*

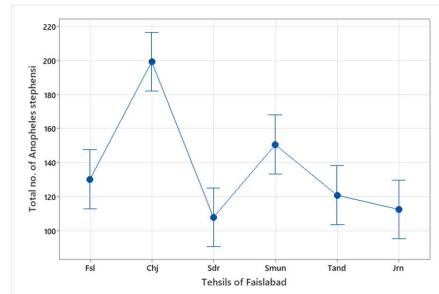
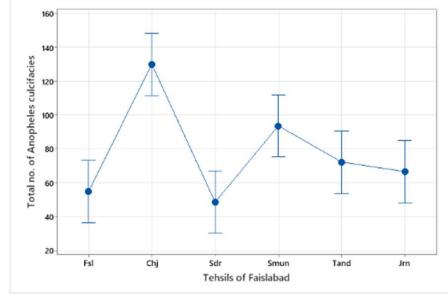
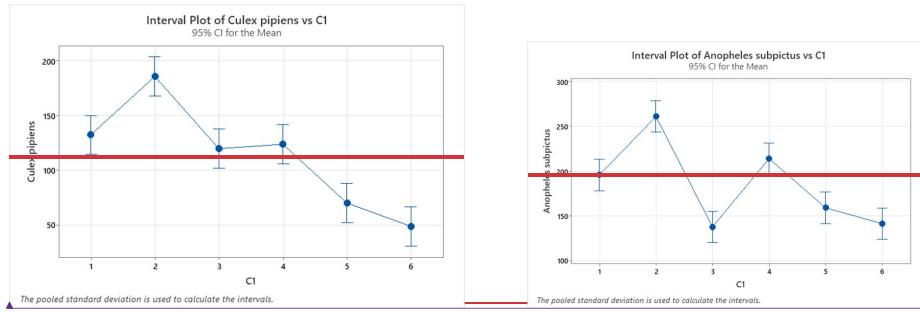
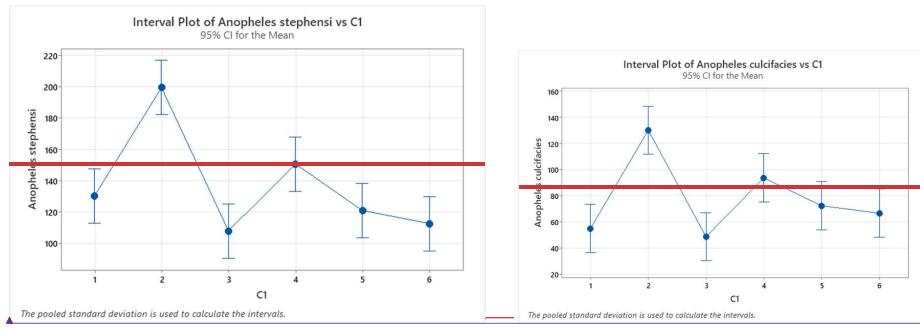


Figure 4.2-9: Abundance of *Anopheles subpictus*





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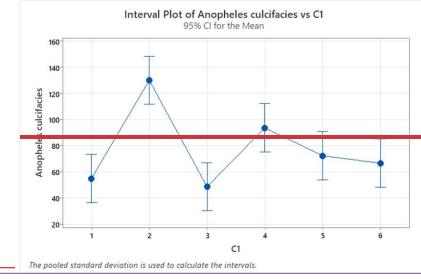
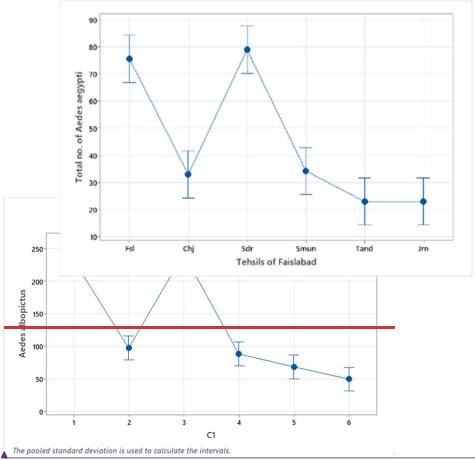
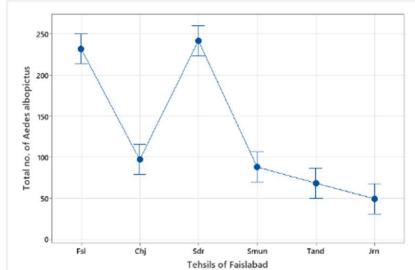


Figure 4.2-10: Abundance of *Anopheles stephensi*

Figure 4.2-11: Abundance of *Anopheles culicifacies*



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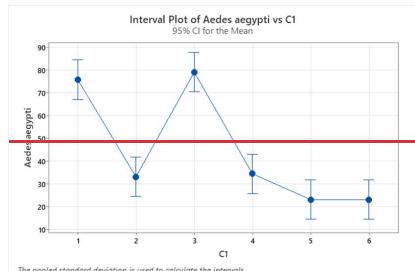


Figure 4.2-12: Abundance of Aedes albopictus

Figure 4.2-13: Abundance of Aedes aegypti

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Figure 4.2-14: Relative abundance of different species of mosquito in different tehsils of district Faisalabad

4.2.2 Relationship of the abundance of mosquitoes with seasons in District Faisalabad

A one-way ANOVA on the seasonal abundance of mosquitoes produced intriguing results.
According to the study, the number of mosquitoes in each tehsil belonging to the species *Culex*

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peaked during the monsoon season, then in the fall, and finally in the winter. The spring brought with it a greater number of mosquitoes than in the summer. Out of all the tehsils in Fais~~a~~labad, Tehsil Chak Jhumra had the highest mosquito abundance. *Culex quinquefasciatus* count was most abundant ($F = 33.97$, $df = 4$, $P = 0.001$), autumn (Mean = 305, SD = 16.09) was when its abundance peaked, followed by the monsoon (Mean = 269.67, SD = 15.04), spring (Mean = 202, SD = 19), summer (Mean = 208.3, SD = 15.01), and winter (Mean = 174.67, SD = 14.05). (**Table 4.2.2.1**). *Anopheles* displayed a distinct pattern of incidence. It peaked during the monsoon season (mean = 98.33, SD = 15.63), then declined significantly during the fall season (Mean = 86.3, SD = 18.5), spring season (Mean=59, SD = 15.01), and winter season (Mean=19.33, SD = 2.08). The same trend was observed for all other species in tehsils Samundri (**Table 4.2.2.2**) Saddar (**Table 4.2.2.3**) Faisalabad. (**Table 4.2.2.4**) Tandliawala (**Table 4.2.2.5**) and Jaranwala. (**Table 4.2.2.6**)

Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<u>Cx. quinquefasciatus</u>	<u>305^a±16.09</u>	<u>174.67^b±14.05</u>	<u>202^b±19</u>	<u>208.3^b±15.01</u>	<u>269.67^a±15.04</u>	<u>33.97</u>	<u>0.001</u>
<u>Cx. tritaeniorhynchus</u>	<u>218^a±15.1</u>	<u>108^c±15.52</u>	<u>174^b±15</u>	<u>176.33^b±15.28</u>	<u>230.33^a±14.57</u>	<u>30.28</u>	<u>0.001</u>
<u>Cx. pseudovishnui</u>	<u>96^a±11.53</u>	<u>56.67^b±8.08</u>	<u>64^b±11.53</u>	<u>68^b±11.53</u>	<u>113.33^a±8.33</u>	<u>16.24</u>	<u>0.001</u>
<u>Cx. pipiens</u>	<u>45.66^a±1.52</u>	<u>18^c±2</u>	<u>30.67^c±2.08</u>	<u>34^b±3</u>	<u>57^a±12.53</u>	<u>18.79</u>	<u>0.001</u>
<u>Cx. Sitiens</u>	<u>52.67^a±14.05</u>	<u>22.67^c±2.08</u>	<u>37.66^b±1.52</u>	<u>47.66^b±1.52</u>	<u>75.33^a±16.04</u>	<u>12.25</u>	<u>0.001</u>
<u>An.subpictus</u>	<u>86.3^a±18.5</u>	<u>19.33^c±2.08</u>	<u>59^b±15.01</u>	<u>0^c±0</u>	<u>98.33^a±15.63</u>	<u>32.84</u>	<u>0.001</u>
<u>An.stephensi</u>	<u>68.67^a±15.18</u>	<u>13.67^b±2.52</u>	<u>19.66^a±1.52</u>	<u>0^b±0</u>	<u>72.33^a±14.64</u>	<u>35.16</u>	<u>0.001</u>
<u>An.culicifacies</u>	<u>33.67^b±2.52</u>	<u>6.33^c±1.52</u>	<u>35.67^b±2.52</u>	<u>6.33^c±1.52</u>	<u>47.33^a±2.08</u>	<u>238.98</u>	<u>0.001</u>
<u>Ae.albopictus</u>	<u>43^a±2</u>	<u>11.66^b±1.52</u>	<u>0^c±0</u>	<u>2.33^c±1.52</u>	<u>39.33^a±1.52</u>	<u>573.29</u>	<u>0.001</u>
<u>Ae.aegypti</u>	<u>17.67^a±2.08</u>	<u>4.67^b±2.08</u>	<u>0^d±0</u>	<u>0^d±0</u>	<u>11.66^b±1.52</u>	<u>81.41</u>	<u>0.001</u>

Figure 4.2-15: Relationship of abundance of species of mosquitoes with seasons in Tehsil Chak Jhumra of District Faisalabad

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Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<u>Cx. quinquefasciatus</u>	<u>282.0^a±18.5</u>	<u>151^b±19</u>	<u>155.67^b±12.06</u>	<u>167.67^b±16.01</u>	<u>271.33^a±16.62</u>	<u>46.32</u>	<u>0.001</u>
<u>Cx. tritaeniorhynchus</u>	<u>196.33^a±13.58</u>	<u>86.67^c±13.08</u>	<u>150.33^b±16.56</u>	<u>142.67^b±16.62</u>	<u>86.67^c±14.11</u>	<u>30.38</u>	<u>0.001</u>
<u>Cx. pseudovishnui</u>	<u>87.33^a±12.05</u>	<u>42.33^c±2.52</u>	<u>55.67^b±13.5</u>	<u>61.3^b±18</u>	<u>100^a±13.53</u>	<u>10</u>	<u>0.001</u>
<u>Cx. pipiens</u>	<u>31.33^b±2.52</u>	<u>9.67^d±2.08</u>	<u>19.67^c±2.08</u>	<u>25.33^b±2.52</u>	<u>38.66^a±1.52</u>	<u>77.73</u>	<u>0.001</u>
<u>Cx. Sitiens</u>	<u>38.67^b±2.52</u>	<u>13.33^d±1.52</u>	<u>28.33^c±1.52</u>	<u>27^c±3</u>	<u>46.67^a±2.52</u>	<u>91.03</u>	<u>0.001</u>
<u>An.subpictus</u>	<u>75.67^a±16.5</u>	<u>15.33^c±1.52</u>	<u>47.33^b±1.52</u>	<u>0^c±0</u>	<u>75.33^a±13.58</u>	<u>38.57</u>	<u>0.001</u>
<u>An.stephensi</u>	<u>52.3^a±18.5</u>	<u>8.67^b±2.08</u>	<u>38.33^a±2.08</u>	<u>0^c±0</u>	<u>52^a±16.52</u>	<u>14.47</u>	<u>0.001</u>
<u>An.culicifacies</u>	<u>29.67^a±2.08</u>	<u>4^c±1</u>	<u>20.33^b±2.52</u>	<u>8.33^c±1.52</u>	<u>32.33^a±2.08</u>	<u>29.29</u>	<u>0.001</u>
<u>Ae.albopictus</u>	<u>37.67^a±2.08</u>	<u>9.67^b±2.08</u>	<u>0^c±0</u>	<u>3.33^c±1.52</u>	<u>39.67^a±2.52</u>	<u>316.89</u>	<u>0.001</u>
<u>Ae.aegypti</u>	<u>15^a±1</u>	<u>3.33^b±1.52</u>	<u>0^c±0</u>	<u>0^c±0</u>	<u>16.67^a±2.08</u>	<u>131.52</u>	<u>0.001</u>

Figure 4.2-16: Relationship of abundance of species of mosquitoes with seasons in Tehsil Samundari of District Faisalabad

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Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
Cx. quinquefasciatus	<u>266.33^a±15.01</u>	<u>142.33^c±15.5</u>	<u>72^d±15.72</u>	<u>82.67^d±16.62</u>	<u>222^b±14.72</u>	<u>90.62</u>	<u>0.001</u>
Cx. tritaeniorhynchus	<u>212^a±17.5</u>	<u>109.0^b±15</u>	<u>52^c±11.53</u>	<u>60.67^c±17.01</u>	<u>188.33^a±10.5</u>	<u>75.03</u>	<u>0.001</u>
Cx. pseudovishnui	<u>83.33^a±14.05</u>	<u>26.33^b±1.52</u>	<u>65.33^a±14.62</u>	<u>55.33^ab±15.01</u>	<u>83^a±13.11</u>	<u>10.27</u>	<u>0.001</u>
Cx. pipiens	<u>30^a±3</u>	<u>8.67^c±2.08</u>	<u>222.67^b±2.52</u>	<u>20.67^b±2.52</u>	<u>36^a±1</u>	<u>59.33</u>	<u>0.001</u>
Cx. Sitiens	<u>53.33^ab±15.01</u>	<u>20.66^c±1.52</u>	<u>39.67ab^c±2.08</u>	<u>36.33b^c±1.52</u>	<u>61.33^a±11.5</u>	<u>10.17</u>	<u>0.001</u>
An. subpictus	<u>42.33^b±2.52</u>	<u>16^d±1</u>	<u>30.33^c±1.52</u>	<u>0^e±0</u>	<u>48.67^a±2.08</u>	<u>419.08</u>	<u>0.001</u>
An.stephensi	<u>33.67^b±2.52</u>	<u>10^d±1</u>	<u>23.67^c±2.52</u>	<u>0^e±0</u>	<u>39.67^a±2.52</u>	<u>201.97</u>	<u>0.001</u>
An.culicifacies	<u>19.33^a±2.52</u>	<u>3^c±1</u>	<u>10.33^b±1.52</u>	<u>0^c±0</u>	<u>16.67^a±2.52</u>	<u>65.76</u>	<u>0.001</u>
Ae.albopictus	<u>93.67^a±14.64</u>	<u>23.33^b±2.52</u>	<u>15.33^b±2.52</u>	<u>22.33^b±2.08</u>	<u>86^a±17.5</u>	<u>40.85</u>	<u>0.001</u>
Ae.aegypti	<u>36.33^a±2.52</u>	<u>5.33^c±1.52</u>	<u>5.33^c±1.52</u>	<u>7^c±1</u>	<u>25.33^b±2.08</u>	<u>185.74</u>	<u>0.001</u>

Figure 4.2-17: Relationship of abundance of species of mosquitoes with seasons in Tehsil Sadar of District Faisalabad

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Table:4.2.2.2 **-Relationship of abundance of species of mosquitoes with seasons in Tehsil Samundari of District Faisalabad**
Relationship with Seasons

Species	Mean				Standard Deviation				F	P	
	Autumn	Winter	Spring	Summer	Monsoon	Autumn	Winter	Spring	Summer	Monsoon	
Cx. quinquefasciatus	282.0 ^a	151 ^b	155.67 ^b	167.67 ^b	271.33 ^a	18.5	19	12.06	16.01	16.62	46.32
Cx. tritaeniorhynchus	196.33 ^a	86.67 ^c	150.33 ^b	142.67 ^b	86.67 ^c	13.58	13.08	16.56	16.62	14.11	30.38
Cx. pseudovishnui	87.33 ^a	42.33 ^c	55.67 ^b c	61.33 ^b c	100 ^a	12.05	2.52	13.5	18	13.53	10
Cx. pipiens	31.33 ^b	9.67 ^d	19.67 ^c	25.33 ^c	38.66 ^a	2.52	2.08	2.08	2.52	1.52	77.73

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Cx.Sitiens	38.67b	13.33d	28.33c	27e	46.67a	2.52	1.52	1.52	3	2.52	91.03	0.00 1
An.subpictus	75.67a	15.33c	47.33b	0e	75.33a	16.5	1.52	1.52	0	13.58	38.57	0.00 1
An.stephensi	52.3a	8.67bc	38.33ab	0e	52a	18.5	2.08	2.08	0	16.52	14.47	0.00 1
An.culicifacies	29.67a	4e	20.33b	8.33c	32.33a	2.08	1	2.52	1.52	2.08	29.29	0.00 1
Ae.albopictus	37.67a	9.67b	0e	3.33e	39.67a	2.08	2.08	0	1.52	2.52	316.8	0.00 9 1
Ae.aegypti	15a	3.33b	0e	0e	16.67a	1	1.52	0	0	2.08	131.5	0.00 2 1

Table 4.2.2.3
Relationship of abundance of species of mosquitoes with seasons in Tehsil Sadar of District Faisalabad
Relationship with Seasons

Species	Mean					Standard Deviation					F	P
	Autum n	Winter	Spring	Summe	Mensoe n	Autum n	Winte r	Sprin g	Summe	Mensoe n		
Cx. quinquefasciatus	266.33a	142.33 e	72d	82.67d	222b	15.01	15.5	15.72	16.62	14.72	90.62	0.00 1
Cx. tritaeniorhynchus	212a	109.0b	52e	60.67e	188.33a	17.5	15	11.53	17.01	10.5	75.03	0.00 1
Cx. pseudovishnui	83.33a	26.33b	65.33a	55.33ab	83a	14.05	1.52	14.62	15.01	13.11	10.27	0.00 1
Cx. pipiens	30a	8.67e	222.67b	20.67b	36a	3	2.08	2.52	2.52	1	59.33	0.00 1
Cx.Sitiens	53.33ab	20.66c	39.67ab e	36.33bc	61.33a	15.01	1.52	2.08	1.52	11.5	10.17	0.00 1
An.subpictus	42.33b	16d	30.33e	0e	48.67a	2.52	1	1.52	0	2.08	8	0.00 1

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An.stephensi	33.67b	10d	23.67c	0e	39.67a	2.52	1	2.52	0	2.52	201.9	0.00
An.culicifacies	19.33a	3e	10.33b	0e	16.67a	2.52	1	1.52	0	2.52	65.76	0.00
Ae.albopictus	93.67a	23.33b	15.33b	22.33b	86a	14.64	2.52	2.52	2.08	17.5	40.85	0.00
Ae.aegypti	36.33a	5.33c	5.33c	7e	25.33b	2.52	1.52	1.52	1	2.08	185.7	0.00

Table:4.2.2.4 Relationship of abundance of species of mosquitoes with seasons in Tehsil Faisalabad of District Faisalabad
Relationship with Seasons

Species	Mean					Standard Deviation					F	p
	Autumn	Winter	Spring	Summer	Menses n	Autumn	Winter	Spring	Summer	Menses n		
Cx. quinquefasciatus	264a	123.7b	157b	141b	246a	15	17.6	15.1	15.52	13.01	52.73	0.00
Cx. tritaeniorhynchus	171.67a	65.3b	98.67b	100b	159a	10.5	17.6	13.01	13.11	18.5	27.29	0.00
Cx. pseudovishnui	75a	31.33b	41.33b	47b	69a	11.53	2.08	1.528	1	8.19	24.94	0.00
Cx. pipiens	19a	4.33e	14b	10b	19.67a	2	1.52	1	1	2.52	42.12	0.00
Cx. Sitiens	25.33b	6.33e	21b	15.67e	31a	2.08	1.52	1	2.52	2	74.15	0.00
An.subpictus	75.67a	15.33c	47.33b	0e	75.33a	16.5	1.52	1.52	0	13.58	38.57	0.00

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<i>An.stephensi</i>	52.3 ^a	8.67 ^{b,c}	38.33 ^a ^b	0 ^c	52 ^a	18.5	2.08	2.08	0	16.52	14.47	0.00 1
<i>An.culcifacies</i>	29.67 ^a	4 ^c	20.33 ^b	8.33 ^c	32.33 ^a	2.08	1	2.52	1.52	2.08	129.2 9	0.00 1
<i>Ae.albopictus</i>	37.67 ^a	9.67 ^b	0 ^c	3.33 ^c	39.67 ^a	2.08	2.08	0	1.52	2.52	316.8 9	0.00 1
<i>Ae.aegypti</i>	15 ^a	3.33 ^b	0 ^c	0 ^c	16.67 ^a	1 1.52	0	0	0	2.08	131.5 2	0.00 1

Mean ± Standard Deviation

Species	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	264 ^a ±15	123.7 ^b ±17.6	157 ^b ±15.1	141 ^b ±15.52	246 ^a ±13.01	52.73	0.001
<i>Cx. tritaeniorhynchus</i>	171.67 ^a ±10. 5	65.3 ^b ±17.6	98.67 ^b ±13.01	100 ^b ±13.11	159 ^a ±18.5	27.29	0.001
<i>Cx. pseudovishnui</i>	75 ^a ±11.53	31.33 ^b ±2.08	41.33 ^b ±1.528	47 ^b ±1	69 ^a ±8.19	24.94	0.001
<i>Cx. pipiens</i>	19 ^a ±2	4.33 ^c ±1.52	14 ^b ±1	10 ^b ±1	19.67 ^a ±2.52	42.12	0.001
<i>Cx. Sitiens</i>	25.33 ^b ±2.08	6.33 ^c ±1.52	21 ^b ±1	15.67 ^c ±2.52	31 ^a ±2	74.15	0.001
<i>An.subpictus</i>	75.67 ^a ±16.5	15.33 ^c ±1.52	47.33 ^b ±1.52	0 ^c ±0	75.33 ^a ±13.58	38.57	0.001
<i>An.stephensi</i>	52.3 ^a ±18.5	8.67 ^b ±2.08	38.33 ^{a,b} ±2.08	0 ^c ±0	52 ^a ±16.52	14.47	0.001
<i>An.culcifacies</i>	29.67 ^a ±2.08	4 ^c ±1	20.33 ^b ±2.52	8.33 ^c ±1.52	32.33 ^a ±2.08	129.29	0.001
<i>Ae.albopictus</i>	37.67 ^a ±2.08	9.67 ^b ±2.08	0 ^c ±0	3.33 ^c ±1.52	39.67 ^a ±2.52	316.89	0.001
<i>Ae.aegypti</i>	15 ^a ±1	3.33 ^b ±1.52	0 ^c ±0	0 ^c ±0	16.67 ^a ±2.08	131.52	0.001

Figure 4.2-18: Relationship of abundance of species of mosquitoes with seasons in Tehsil Faisalabad of District Faisalabad

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Species	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	264 ^a ±15	123.7 ^b ±17.6	157 ^b ±15.1	141 ^b ±15.52	246 ^a ±13.01	52.73	0.001
<i>Cx. tritaeniorhynchus</i>	171.6 ^a ±10.5	65.3 ^b ±17.6	98.67 ^b ±13	100 ^b ±13.11	159 ^a ±18.5	27.29	0.001
<i>Cx. pseudovishnui</i>	75 ^a ±11.53	31.33 ^b ±2.08	41.33 ^b ±1.52	47 ^b ±1	69 ^a ±8.19	24.94	0.001

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<u>Cx. pipiens</u>	<u>19^a±2</u>	<u>4.33^c±1.52</u>	<u>14^b±1</u>	<u>10^b±1</u>	<u>19.67^a±2.52</u>	<u>42.12</u>	<u>0.001</u>
<u>Cx. Sitiens</u>	<u>25.33^b±2.08</u>	<u>6.33^d±1.52</u>	<u>21^b±1</u>	<u>15.67^c±2.52</u>	<u>31^a±2</u>	<u>74.15</u>	<u>0.001</u>
<u>An.subpictus</u>	<u>55.33^a±17</u>	<u>13.33^b±2.52</u>	<u>35.33^a±2.52</u>	<u>0^c±0</u>	<u>56.67^a±16.56</u>	<u>16.5</u>	<u>0.001</u>
<u>An.stephensi</u>	<u>40.66^a±1.52</u>	<u>9^c±1</u>	<u>27^b±3</u>	<u>0^d±0</u>	<u>44.33^a±2.52</u>	<u>301.54</u>	<u>0.001</u>
<u>An.culcifacies</u>	<u>17.33^b±2.08</u>	<u>2^d±1</u>	<u>18^b±3</u>	<u>10.67^c±2.08</u>	<u>25.33^a±1.52</u>	<u>55.08</u>	<u>0.001</u>
<u>Ae.albopictus</u>	<u>25.67^a±2.52</u>	<u>7.33^b±1.52</u>	<u>0^c±0</u>	<u>7.33^b±1.52</u>	<u>29.33^a±1.52</u>	<u>184.52</u>	<u>0.001</u>
<u>Ae.aegypti</u>	<u>8^b±1</u>	<u>3.33^c±1.52</u>	<u>0^d±0</u>	<u>0^d±0</u>	<u>12.33^a±1.52</u>	<u>76.24</u>	<u>0.001</u>

Figure 4.2-19:Relationship of abundance of species of mosquitoes with seasons in Tehsil Tandlianwala of District Faisalabad

<u>Species</u>	<u>Mean ± Standard Deviation</u>						
	<u>Autumn</u>	<u>Winter</u>	<u>Spring</u>	<u>Summer</u>	<u>Monsoon</u>	<u>F</u>	<u>P</u>
<u>Cx. quinquefasciatus</u>	<u>262^a±15.52</u>	<u>109^c±16.09</u>	<u>152^b±18</u>	<u>128b^c±13.53</u>	<u>250.67^a±16.01</u>	<u>60.1</u>	<u>0.001</u>
<u>Cx. tritaeniorhynchus</u>	<u>169^a±15.52</u>	<u>64^b±17.09</u>	<u>110.7^b±17.5</u>	<u>97.3^b±19.5</u>	<u>164.3^a±18.5</u>	<u>19.44</u>	<u>0.001</u>
<u>Cx. pseudovishnui</u>	<u>70^a±12.49</u>	<u>30.33^c±1.52</u>	<u>40.66b^c±1.52</u>	<u>41b^c±2</u>	<u>60.33a^b±13.61</u>	<u>11.25</u>	<u>0.001</u>
<u>Cx. pipiens</u>	<u>15^a±1</u>	<u>4^d±1</u>	<u>10b^c±1</u>	<u>8^c±1</u>	<u>12.33a^b±1.52</u>	<u>41.66</u>	<u>0.001</u>
<u>Cx. Sitiens</u>	<u>24.67^b±2.08</u>	<u>6.33^d±1.52</u>	<u>19^c±2</u>	<u>12.33^d±1.52</u>	<u>31^a±1</u>	<u>101.9</u>	<u>0.001</u>
<u>An.subpictus</u>	<u>49.33^a±2.52</u>	<u>12^c±1</u>	<u>30.33^b±2.08</u>	<u>0^d±0</u>	<u>49.66^a±1.528</u>	<u>527.58</u>	<u>0.001</u>
<u>An.stephensi</u>	<u>36.33^a±2.52</u>	<u>9^c±1</u>	<u>29.67^b±2.08</u>	<u>0^d±0</u>	<u>41^a±2</u>	<u>304.28</u>	<u>0.001</u>
<u>An.culcifacies</u>	<u>16^b±1</u>	<u>3.67^c±2.08</u>	<u>16^b±2</u>	<u>7^c±1</u>	<u>24.33^a±2.52</u>	<u>60.47</u>	<u>0.001</u>
<u>Ae.albopictus</u>	<u>19.67^a±2.08</u>	<u>5.67^b±2.08</u>	<u>0^c±0</u>	<u>5.67^b±2.08</u>	<u>21^a±1</u>	<u>94.07</u>	<u>0.001</u>
<u>Ae.aegypti</u>	<u>8^b±1</u>	<u>3.33^c±1.52</u>	<u>0^d±0</u>	<u>0^d±0</u>	<u>12^a±1</u>	<u>95.38</u>	<u>0.001</u>

Figure 4.2-20:Relationship of abundance of species of mosquitoes with seasons in Tehsil Jarawala of District Faisalabad

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4.1.34.2.3 Effect of climatic factors on the abundance of mosquitoes in District Faisalabad

The comparison of meteorological data with the month-wise relative abundance of mosquito abundance in whole of the district Faisalabad is shown in (Figure 4.2.3.1-4.2.3.1). The highest abundance of the mosquito was observed in the month of August with high average temperature and humidity. The second rise in the number of mosquito was observed in the month of March and September declining in the months of January and February that is in winter season. The months of April and May again found to be conducive for the proliferation of mosquito population with favorable meteorological variables.

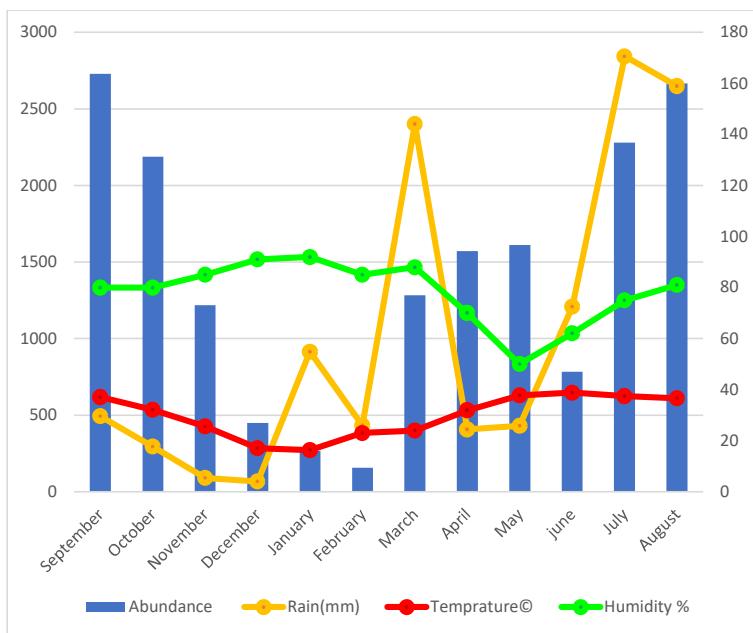


Figure 4.2-21: Effect of climatic variables on relative abundance of mosquito species in district Faisalabad.

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4.2.4 Comparative analysis of abundance of mosquitoes between rural and urban areas of tehsils of Faisalabad

The mosquito populations in all the tehsils of the Faisalabad district were compared between rural and urban areas using the unpaired t-test. The results showed that there were

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significantly more mosquitoes in rural than in urban regions in the tehsils of Jaranwala (**Figure 4.2.4.1**) Chak Jhumra (**Figure 4.2.4.2**)—**Tandiawala** (**Figure 4.2.4.3**)—**Faisalabad** (**Figure 4.2.4.4**)—**Samundri** (**Figure 4.2.4.5**)—and **Saddar** (**Figure 4.2.4.6**). A highly significant result was reported in **Tehsil in Tehsil** Jaranwala ($t=3.28$, $p=0.0111$, $F=2.597$). Findings in the Saddar tehsils ($t=2.371$, $p=0.0452$, $F=2.139$) were found to be significantly less than those in other tehsils, suggesting that there is less variation in mosquito abundance between rural and urban areas in this tehsil.

Tehsils	T value	P value	F	Difference between Means ±Standard Error
Jaranwala	3.28	0.0111	2.597	217.4±66.13
Chak Jhumra	3.257	0.0116	1.796	268.8±82.53
Tandiawala	3.229	0.0121	5.362	325.6±100.8
Faisalabad	3.189	0.0128	3.399	227.4±71.31
Samundri	2.98	0.0174	2.210	234.8±78.62
Saddar	2.371	0.0452	2.139	215.6±90.95

Figure 4.2-22: T test between the rural and urban areas of different Tehsils of district of Faisalabad

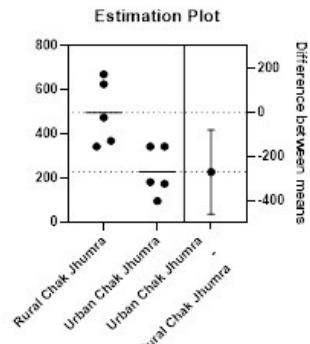


Figure 4.2-23: Significant results of difference of Abundance of mosquito in rural and urban areas of Tehsil Chak Jhumra of District Faisalabad

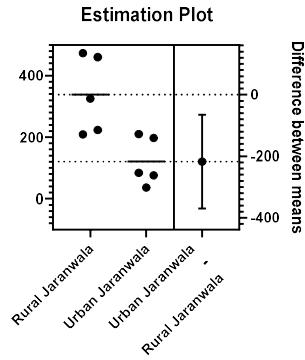
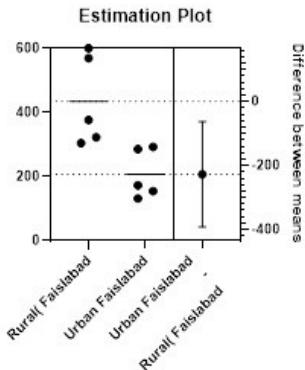
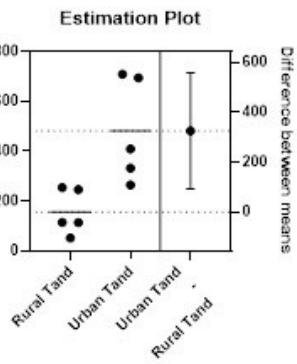


Figure 4.2-24: Significant results of difference of Abundance of mosquito in rural and urban areas of Tehsil Jaranwala of District Faisalabad



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Figure 4.2-25: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Faisalabad of District Faisalabad

4.2.4.1:Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Jarawala of District Faisalabad

Figure 4.2.4.2:Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Chak Jhumra of District Faisalabad

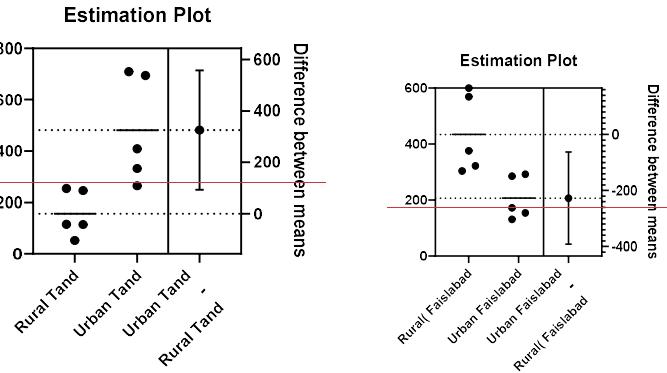


Figure 4.2.4.3: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Tandiawala of District Faisalabad

Figure 4.2.4.4: Significant results of difference of abundance of mosquito in rural and urban areas of Faisalabad of District Faisalabad.

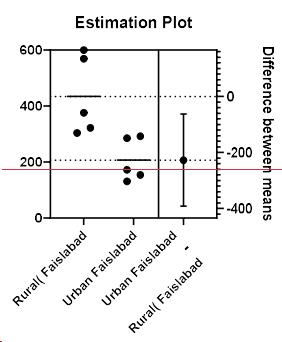
Figure 4.2-28: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Saddar of District Faisalabad

Figure

Figure 4.2-26: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Tandiawala of District Faisalabad

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Figure 4.2-27: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Sammundri of District Faisalabad

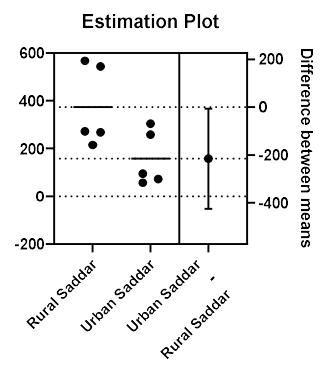
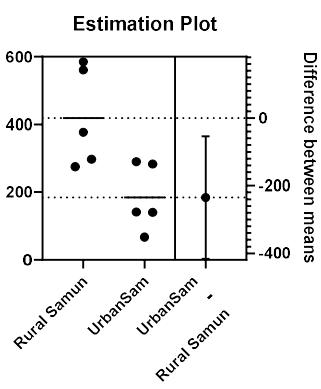


Figure 4.2.4.5: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Sammundri of District Faisalabad

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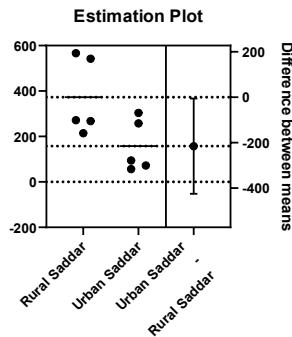


Figure 4.2.4.3: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Saddar of District Faisalabad

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4.2.5 — 4.2.5 : GIS Mapping

GIS mapping of mixed cropping zone showed all the three genus *Culex*, *Anopheles* and *Aedes* of mosquitoes were found in all the tehsils of Faisalabad where all the species reported in this zone were found to be spatially distributed.

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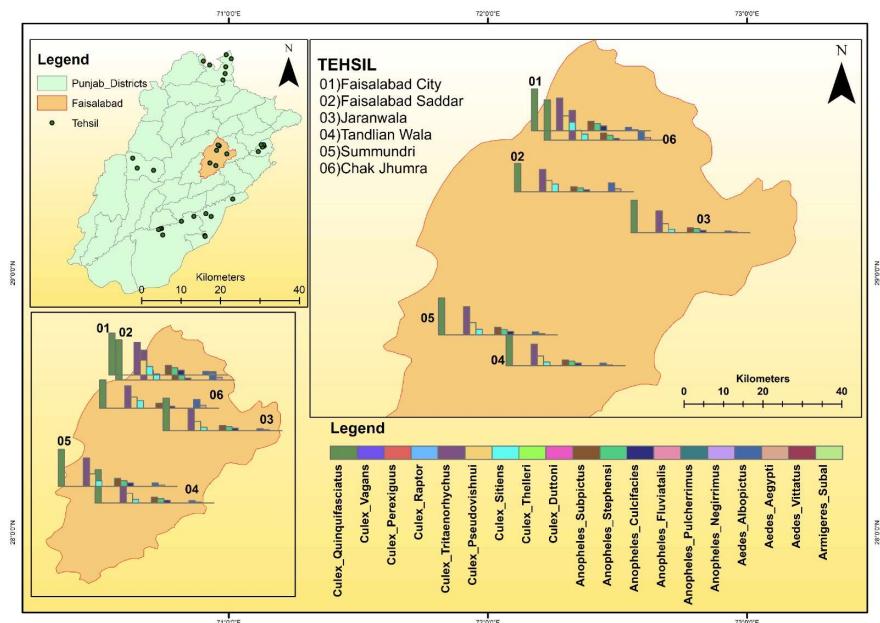


Figure 4.2-29: GIS map showing mosquito abundance in different tehsils of district Faisalabad

4.2

4.3 Cotton Zone District Bahawalnagar

Samples were collected from Bahawalnagar City, Haroonabad, Chishtian, Fort Abbas, and Minchanabad, including rural and urban locations. A total of $n = 2120$ of eleven species were collected from this district. *Anopheles* and *Culex* were the only two genera discovered. Out of the two genera, *Culex* was the most common (80%), with *Anopheles* coming in second (20%). There were no *Aedes* mosquitoes observed. Tehsil Fortabbas had the greatest number of mosquitoes, whereas the tehsil Chishtian had the lowest number. Six species in the genus *Culex* were found, with *Culex quinquefasciatus* accounting for (27%) of the total. *Culex tritaeniorhynchus* (24%), *Culex thelleri* (16%) and *Culex pseudovishnui* (10%), *Culex sitiens* and *Culex pipiens* (2%) both. Five species of the *Anopheles* genus were identified i.e., *Anopheles subpictus* (9%), *Anopheles culicifacies* (5%), *Anopheles stephensi* (3%), *Anopheles pulcherrimus* (2%), and *Anopheles nigerrimus* (1%). (Figure 4.3.).

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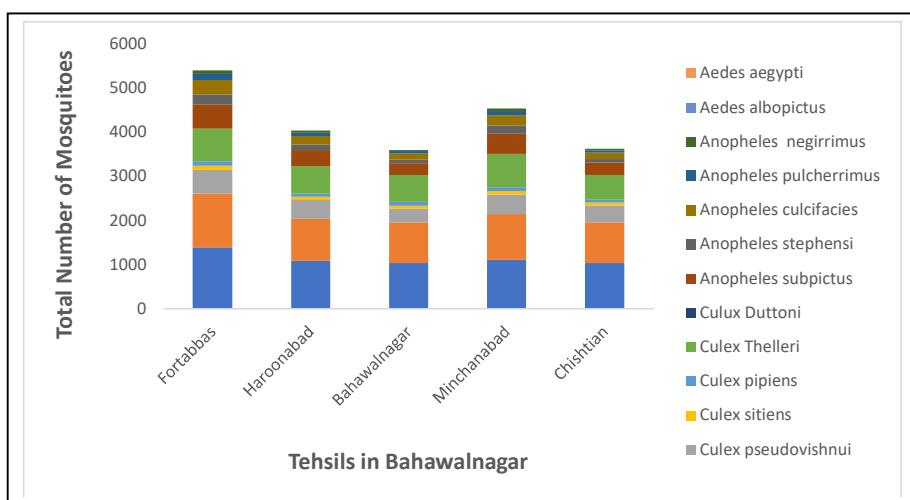


Figure 4.3-1: Total no of mosquitoes in district Bahawalnagar

4.2.14.3.1 Species Diversity and Abundance of mosquitoes in district Bahawalnagar

There were notable variations throughout the district for the diversity of species identified (n), Shannon diversity (H), and Simpson diversity (D). For the five tehsils, the corresponding Shannon and Simpson diversity indices were 2.02 to 1.88 and 0.836 to 0.805. Tehsil Bahawalnagar had the lowest values for both indices, and tehsil Fortabbas had the highest values. (**Table 4.3.1.1**).

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Name of the species	Fortsabas				Minchanabad				Haroonabad				Chistian				Bahawalnagar			
	Total count	%age	Shanno n's Index	Simpson's Index	Total count	%age	Shanno n's Index	Simpson's Index	Total count	%age	Shanno n's Index	Simpson's Index	Total count	%age	Shanno n's Index	Simpson's Index				
<i>Culex quinquefasciatus</i>	25.7	0.3494	193905	6	1114	24.5	0.3448	2	1090	26.9	0.3535	0	1039	28.6	0.3581	2	1043	28.9	0.3589	6
<i>Culex tritaeniorhynchus</i>	22.5	0.3358	148230	6	1035	22.8	0.3371	0	955	23.6	0.3410	911070	924	25.4	0.3484	852852	920	25.5	0.3487	845480
<i>Culex pseudovishnui</i>	539	9.97	0.2299	289982	439	9.68	0.2260	192282	420	10.4	0.2354	175980	385	10.6	0.2381	147840	305	8.47	0.2091	92720
<i>Culex sitiens</i>	96	1.78	0.0716	9120	81	1.79	0.0719	6480	73	1.81	0.0725	5256	61	1.68	0.0687	3660	67	1.86	0.0741	4422
<i>Culex pipiens</i>	97	1.79	0.0722	9312	89	1.96	0.0771	7832	79	1.96	0.0770	6162	69	1.90	0.0754	4692	86	2.39	0.0892	7310
<i>Culex Thellleri</i>	746	0	0.2733	555770	754	2	0.2982	567762	620	5	0.2877	383780	555	15.3	0.2872	307470	617	4	0.3023	380072
<i>Culux Duttoni</i>	0	0.00	0.0000	0	0	0.00	0.0000	0	0	0.00	0.0000	0	0	0.00	0.0000	0	0	0.00	0.0000	0
<i>Anopheles subpictus</i>	10.0	0.2313	296480	462	8	10.1	0.2326	212982	368	9.11	0.2183	135056	282	7.78	0.1986	79242	266	7.39	0.1925	70490
<i>Anopheles stephensi</i>	225	4.16	0.1323	50400	184	4.06	0.1300	33672	117	2.90	0.1026	13572	82	2.26	0.0857	6642	81	2.25	0.0854	6480
<i>Anopheles culicifacies</i>	323	5.98	0.1684	104006	233	5.14	0.1525	54056	197	4.88	0.1473	38612	143	3.94	0.1275	20306	137	3.81	0.1244	18632
<i>Anopheles pulcherrimus</i>	140	2.59	0.0946	19460	96	2.12	0.0816	9120	71	1.76	0.0710	4970	54	1.49	0.0626	2862	54	1.50	0.0630	2862
<i>Anopheles negirmaculata</i>	83	1.54	0.0641	6806	50	1.10	0.0497	2450	49	1.21	0.0535	2352	33	0.91	0.0428	1056	24	0.67	0.0334	552
<i>Aedes albopictus</i>	0	0.00	0.00	0	0	0.00	0.0000	0	0	0.00	0.0000	0	0	0.00	0.0000	0	0	0.00	0.0000	0
<i>Aedes aegypti</i>	0	0.00	0.00	0	0	0.00	0.0000	0	0	0.00	0.0000	0	0	0.00	0.0000	0	0	0.00	0.0000	0
Total	5405	2.0230	0.8369	4537		2.0015	0.8349	4039		1.9597	0.8244	3627		1.8930	0.8095	3600		1.8810	0.8058	

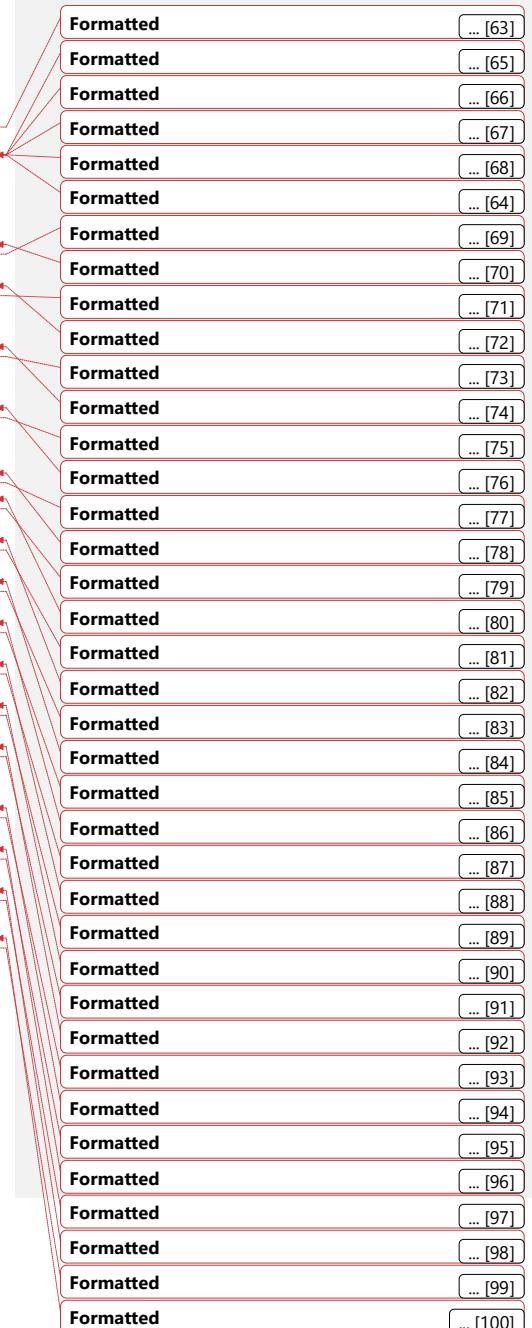


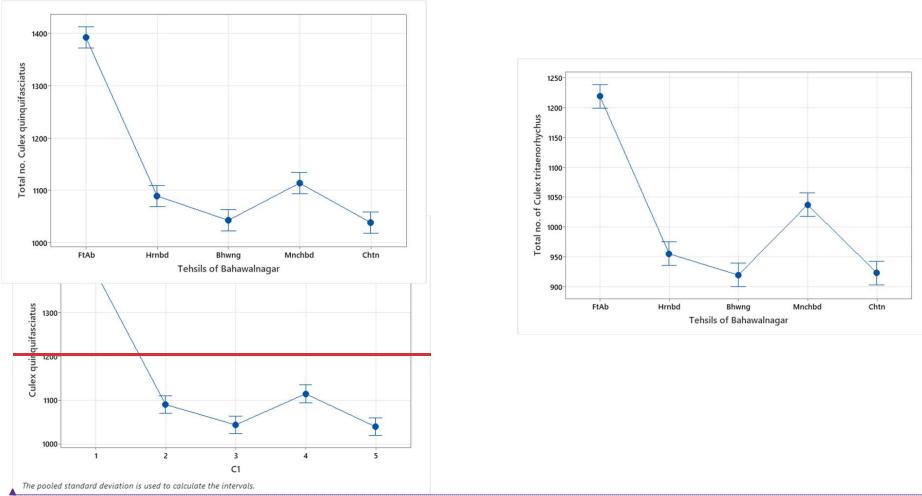
Figure 4.3-2: Diversity index of a number of individuals of different species of mosquitoes collected from tehsils of District Bahawalnagar

Analysis of variance (ANOVA) tests revealed significant differences in mosquito abundance across all ~~the tehsils~~ the tehsils. This district has the greatest number of *Culex quinquefasciatus* ($F = 262.67$, $df=4$, $P < 0.00$) within the genus Culex, which was discovered in abundance .With the lowest concentration in tehsil Chishtian (Mean=1039.00, SD=14), Tehsil Fortabbas had the highest value of occurrence (Mean=1392.33, SD=17.01), followed by Minchanabad (Mean=1114.33, SD=12.50), Haroonabad (Mean=1089.67, SD=14.50), and Bahawainagar (Mean=1043.3, SD= 19.5). (Table: 4.3.1.2). The abundance pattern of *Culex quinquefasciatus* was replicated by *Culex tritaeniorhynchus* (Figure 4.3.1.1.2) *Culex sitiens* (Figure 4.3.1.1.4) *Culex pipiens* (Figure 4.3.1.1.5) *Anopheles subpictus* (Figure 4.3.1.1.7) *Anopheles stephensi* (Figure 4.3.1.1.8) *Anopheles culicifacies* (Figure 4.2.1.1.9), *Anopheles pulcherrimus* (Figure 4.2.1.1.10) and *Anopheles negerrimus* (Figure 4.2.1.1.11). The results for *Culex theileri* (Figure 4.2.1.1.6) were completely different; it was discovered to be quite common in the tehsils of Fortabbas and Minchanabad (Fig. The Aedes genus was discovered absent from this district.

<u>Species</u>	<u>Mean ± Standard Deviation</u>						<u>F</u>	<u>P</u>
	<u>Fortabbas</u>	<u>Haroonabad</u>	<u>Bahawalnagar</u>	<u>Minchanabad</u>	<u>Chishtian</u>			
<u><i>Cx. quinquefasciatus</i></u>	<u>1392.33^a±17.01</u>	<u>1089.67^b±14.5</u>	<u>1043.3^c±19.5</u>	<u>1114.33^b±12.5</u>	<u>1039^c±14</u>	<u>262.67</u>	<u>0.00</u>	<u>0.00</u>
<u><i>Cx. tritaeniorhynchus</i></u>	<u>1219.33^a±12.06</u>	<u>955.67^b±16.01</u>	<u>920^c±13</u>	<u>1037.67^b±15.18</u>	<u>923.7^c±19.5</u>	<u>200.25</u>	<u>0.00</u>	<u>0.00</u>
<u><i>Cx. pseudovishnui</i></u>	<u>537.33^a±13.58</u>	<u>419.7^{bc}±19.5</u>	<u>305.33^d±11.5</u>	<u>438.67^b±10.5</u>	<u>385^c±12</u>	<u>112.14</u>	<u>0.00</u>	<u>0.00</u>
<u><i>Cx. pipiens</i></u>	<u>97^a±5</u>	<u>77.67^{bc}±6.11</u>	<u>84^a±7.21</u>	<u>88.33^{ab}±4.04</u>	<u>69.33^c±2.52</u>	<u>12.05</u>	<u>0.00</u>	<u>0.00</u>
<u><i>Cx. theileri</i></u>	<u>744^a±15.1</u>	<u>618.67^b±15.04</u>	<u>617^b±16</u>	<u>756.67^a±15.18</u>	<u>555.7^c±18</u>	<u>91.39</u>	<u>0.00</u>	<u>0.00</u>
<u><i>Cx.sitiens</i></u>	<u>96^a±4</u>	<u>73.33^b±3.51</u>	<u>66.33^c±5.03</u>	<u>80.33^b±6.03</u>	<u>61^a±5</u>	<u>24.21</u>	<u>0.00</u>	<u>0.00</u>
<u><i>An.subpictus</i></u>	<u>544.33^a±13.01</u>	<u>366.33^c±12.58</u>	<u>264.33^d±12.58</u>	<u>461.3^b±18</u>	<u>281^d±13.53</u>	<u>214.91</u>	<u>0.00</u>	<u>0.00</u>
<u><i>An.stephensi</i></u>	<u>222.67^a±16.62</u>	<u>114.67^b±14.64</u>	<u>82^b±18.5</u>	<u>185.33^b±15.04</u>	<u>82^b±16</u>	<u>46.23</u>	<u>0.00</u>	<u>0.00</u>
<u><i>An.culicifacies</i></u>	<u>323.67^a±15.01</u>	<u>197^b±12</u>	<u>135.33^c±15.57</u>	<u>232^b±13.53</u>	<u>145^c±15.1</u>	<u>85.49</u>	<u>0.00</u>	<u>0.00</u>
<u><i>An.pulcharimus</i></u>	<u>139.33^a±11.02</u>	<u>69.33^{bc}±13.58</u>	<u>54.7^a±19</u>	<u>95.67^b±13.5</u>	<u>57.33^{bc}±15.28</u>	<u>17.26</u>	<u>0.00</u>	<u>0.00</u>
<u><i>An.negirrimus</i></u>	<u>81.67^a±13.05</u>	<u>49^b±3</u>	<u>23.33^c±2.08</u>	<u>49.67^b±2.52</u>	<u>33.33^{bc}±1.52</u>	<u>38.19</u>	<u>0.00</u>	<u>0.00</u>

Figure 4.3-3: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of Bahawalnagar

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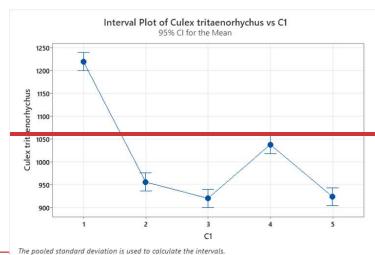
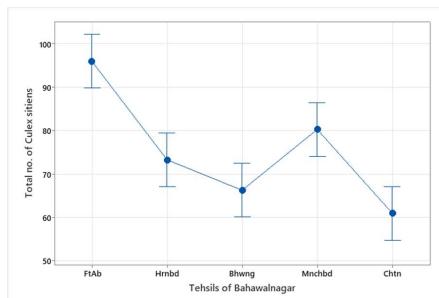
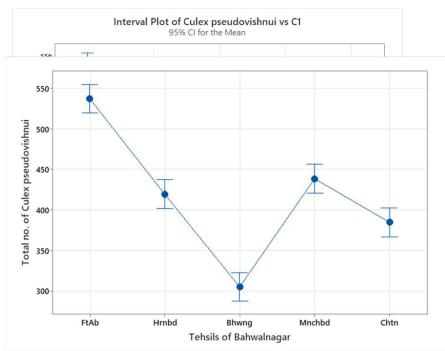


Figure 4.3.1.1.1: Abundance of *Culex quinquefasciatus*

Figure 4.3.1.1.2: Abundance of *Culex tritaeniorhynchus*

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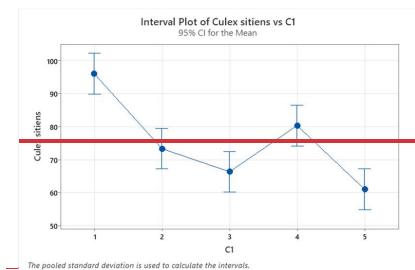
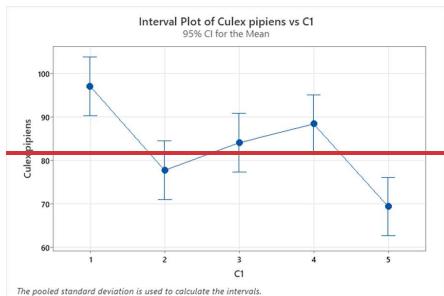


Figure 4.3.1.1.3: Abundance of *Culex pseudivishnui* *sitiens*



The pooled standard deviation is used to calculate the intervals.

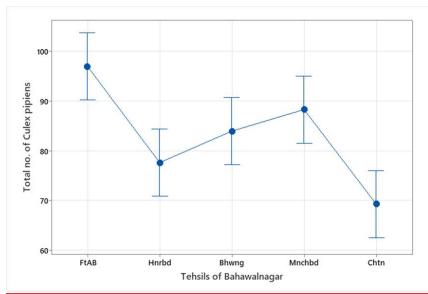


Figure 4.3.1.1.5: Abundance of *Culex pipiens*

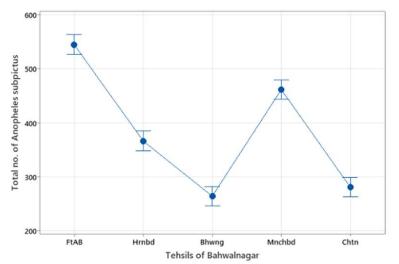


Figure 4.3.1.1.6: Abundance of *Culex thelleri*

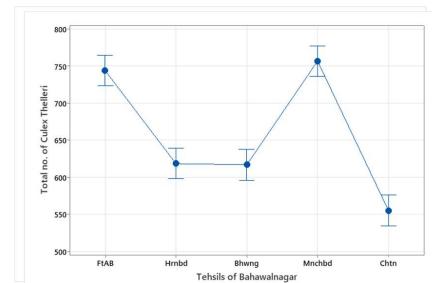
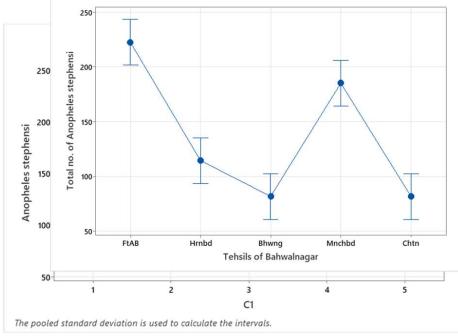


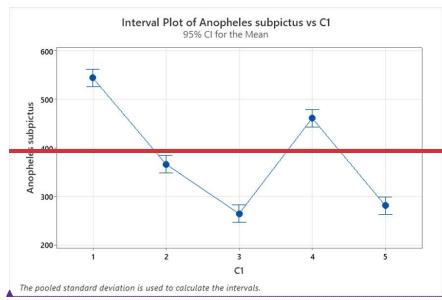
Figure 4.3.1.1.6: Abundance of *Culex thelleri*



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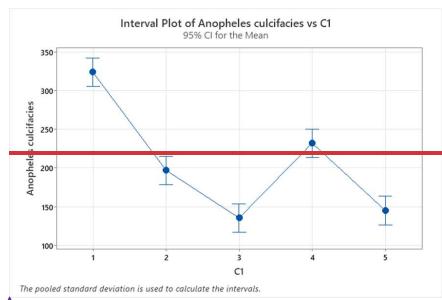
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Figure 4.3.1.1.7: Abundance of *Anopheles subpictus*

Figure 4.3.1.1.8: Abundance of *Anopheles stephensi*

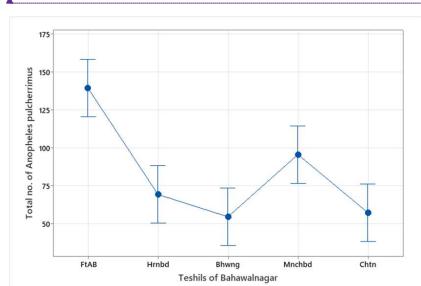
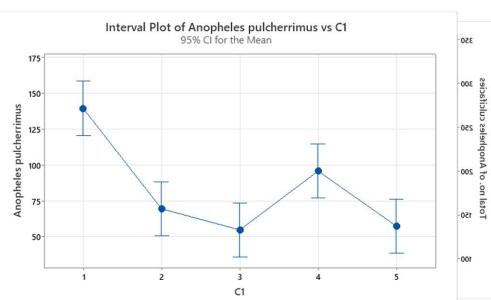


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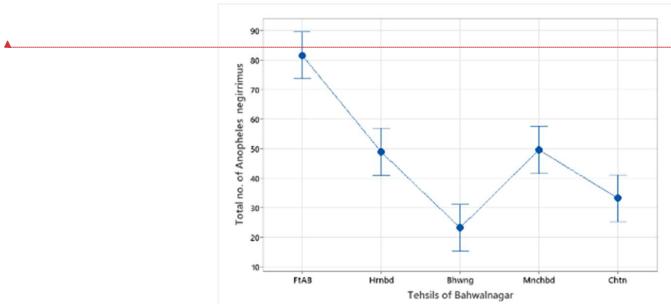
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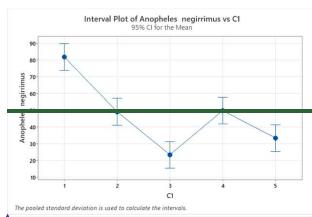
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Figure 4.3.1.1.9: Abundance of *Anopheles culicifacies*—Figureculicifacies

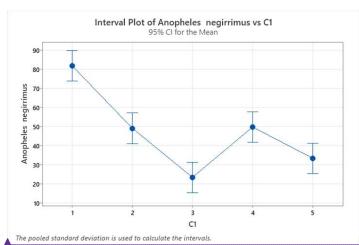
Figure 4.3.1.1.10: Abundance of *Anopheles pulcherrimus*



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Figure 4.3.1.11: Abundance of *Anopheles negirrimus*

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Figure 4.3-4: Relative abundance of different species of mosquito in different tehsils of district Bahawalnagar

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4.3.2 Relationship of the abundance of mosquitoes with seasons in District Bahawalnagar

The results were obtained via one-way ANOVA on the seasonal abundance of mosquitoes. The research revealed that in each tehsil the genus *Culex* 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. Tehsil Fortabbas (**Table 4.3.2.1**) had the greatest *Culex quinquefasciatus* count ($F = 111.1$, $df = 4$, $P = 0.001$). Its peak levels of abundance were recorded during the monsoon seasons (Mean = 367, SD = 14), followed by the autumn season (Mean = 364, SD = 17), while the winter season (Mean = 119, SD = 20) saw a severe decrease in abundance. It rises again in the spring (Mean = 262.33, SD = 19), and again in the summer (Mean = 279.67, SD = 11.5). *Anopheles* displayed a distinct pattern of incidence. The monsoon season (Mean = 174.33, SD = 14.5), spring (Mean = 172.67, SD = 15.01), and fall (Mean = 155.33, SD = 14.57) were the times when it peaked. Winter saw a significant drop in abundance (Mean = 40, SD = 3), with summer seeing the biggest fall (Mean = 0, SD = 0). (**Table 4.3.2.1**). This district was confirmed to be devoid of *Aedes*. *Anopheles* and *Culex* species demonstrated the same pattern of occurrence in all the other tehsils Bahawalnagar (**Table 4.3.2.2**) Haroonabad, (**Table 4.3.2.3**) Chishtian (**Table 4.3.2.4**) and Minchanabad (**Table 4.3.2.5**) as found in tehsil Fortabbas.

Species	Mean ± Standard Deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
<i>Cx. quinquefasciatus</i>	364 ^a ±17	119 ^c ±20	262.3 ^b ±19	279.67 ^b ±11.5	367 ^a ±14	111.1	0.001
<i>Cx. tritaeniorhynchus</i>	283 ^a ±12.53	170.33 ^c ±12.01	213.33 ^b ±12.5	237.33 ^b ±15.28	319.3 ^a ±18	50.2	0.001
<i>Cx. pseudovishnui</i>	129a ^b ±14.73	44.66 ^d ±1.52	88 ^c ±18	114.33b ^c ±14.01	157 ^a ±17.01	36.47	0.001
<i>Cx. pipiens</i>	19.33 ^b ±2.08	11 ^c ±1	20 ^b ±1	17.66 ^b ±1.52	28 ^a ±3	31.35	0.001
<i>Cx. sitiens</i>	21.33 ^b ±1.52	2.33 ^c ±1.52	18.67 ^b ±2.52	21.66 ^b ±1.52	32 ^a ±2	99.36	0.001
<i>Cx. theileri</i>	189.7a ^b ±19.5	86.33 ^c ±12.06	103 ^a ±11.53	155 ^b ±15	212.33 ^a ±15.57	39.11	0.001
<i>An. subpictus</i>	155.33 ^a ±14.57	40 ^b ±3	172.67 ^a ±15.01	0 ^c ±0	174.33 ^a ±14.57	154.26	0.001
<i>An. stephensi</i>	66.33 ^a ±15.57	11.66 ^b ±1.52	64.33 ^a ±15.01	0 ^b ±0	77.67 ^a ±13.65	28.72	0.001
<i>An. culicifacies</i>	19.7 ^a ±18	19.33 ^b ±2.52	99.7 ^a ±17.5	0 ^b ±0	114.3 ^a ±17.5	42.16	0.001
<i>An. pulcherrimus</i>	39.66 ^a ±1.52	9.67 ^b ±2.08	40 ^a ±1	0 ^b ±0	52.33 ^a ±14.05	36.43	0.001
<i>An. nigerinus</i>	25.33 ^b ±1.52	5.67 ^c ±2.08	20.67 ^b ±2.52	0 ^d ±0	31.33 ^a ±2.08	152.52	0.001

Figure 4.3-5: Relationship of abundance of species of mosquitoes with seasons in Tehsil Fortabbas of District Bahawalnagar

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Figure 4.3.1.1 : Relative abundance of different species of mosquito in different tehsils of d

Species	Mean ± Standard Deviation						
	Fortabbas	Hareenabad	Bahawalnagar	Minchanabad	Chishtian	F	p
<i>Cx. quinquefasciatus</i>	1392.33a±17.01	1089.67b±14.5	1043.3e±19.5	1114.33b±12.5	1039c±14	262.6	0.001
<i>Cx. tritaeniorhynchus</i>	1219.33a±12.06	955.67c±16.01	920c±13	1037.67b±15.18	923.7c±19.5	5	0.001
<i>Cx. pseudovishnui</i>	537.33a±13.58	419.7bc±19.5	305.33d±11.5	438.67b±10.5	385c±12	4	0.001
<i>Cx. pipiens</i>	97a±5	77.67bc±6.11	84ab±7.21	88.33ab±4.04	69.33c±2.52	12.05	0.001
<i>Cx. thellleri</i>	744a±15.1	618.67b±15.04	617b±16	756.67a±15.18	555.7c±18	91.39	0.001
<i>Cx. sitiens</i>	96a±4	73.33bc±3.51	66.33c±5.03	80.33b±6.03	61c±5	24.21	0.001
<i>An. subpictus</i>	544.33a±13.01	366.33c±12.58	264.33d±12.58	461.3b±18	281d±13.53	4	0.001
<i>An. stephensi</i>	222.67a±16.62	114.67b±14.64	82b±18.5	185.33±15.04	82b±16	46.23	0.001
<i>An. culicifacies</i>	323.67a±15.01	197b±12	135.33c±15.57	232b±13.53	145c±15.1	85.49	0.001
<i>An. pulcharimus</i>	139.33a±11.02	69.33bc±13.58	54.7c±19	95.67b±13.5	57.33bc±15.28	17.26	0.001
<i>An. negirimimus</i>	81.67a±13.05	49b±3	23.33c±2.08	49.67b±2.52	33.33bc±1.52	38.19	0.001

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Species	Mean ± Standard Deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
<i>Cx. quinquefasciatus</i>	295.33 ^a ±12.06	96.67 ^c ±15.04	208 ^b ±13.11	216 ^b ±17.5	276 ^a ±14.53	85.37	0.001
<i>Cx. tritaeniorhynchus</i>	233 ^a ±15.1	145 ^b ±11	163.67 ^b ±11.02	177 ^b ±16.09	236.7 ^a ±18.5	24.17	0.001
<i>Cx. pseudovishnui</i>	102.67 ^a ±12.5	44.33 ^c ±2.52	72.67 ^b ±16.17	89.33 ^a ±13.65	119 ^a ±15.72	14.39	0.001
<i>Cx. pipiens</i>	17 ^a ±1	12 ^c ±1	16.33 ^b ±1.52	14 ^b ±1	19.66 ^a ±1.52	16.8	0.001
<i>Cx.sitiens</i>	16.33 ^b ±2.52	3 ^c ±1	14 ^b ±3	16 ^b ±2	24 ^a ±1	40.12	0.001
<i>Cx. theileri</i>	159.67 ^a ±16.17	71.33 ^c ±15.5	110.67 ^b ±15.01	127 ^a ±17.09	154.67 ^a ±14.64	15.72	0.001
<i>An.subpictus</i>	115 ^a ±15.1	17.67 ^b ±2.52	112 ^a ±11	0 ^b ±0	126.33 ^a ±16.04	88.83	0.001
<i>An.stephensi</i>	34.33 ^b ±2.08	6 ^c ±1	34 ^b ±2	0 ^d ±0	41.67 ^a ±2.52	338.86	0.001
<i>An.culicifacies</i>	55 ^a ±11.53	9.67 ^b ±2.08	61 ^a ±15.72	0 ^b ±0	69 ^a ±15.52	24.12	0.001
<i>An.pulcherrimus</i>	19.33 ^b ±2.08	3 ^c ±1	20.66 ^b ±1.52	0 ^c ±0	26.66 ^a ±1.52	206.37	0.001
<i>An.nigerimus</i>	12.33 ^b ±2.08	2 ^c ±1	14.33 ^b ±1.52	0 ^c ±0	20 ^a ±1	124.56	0.001

Figure 4.3-6: Relationship of abundance of species of mosquitoes with seasons in Tehsil Haroonabad of District Bahawalnagar

Species	Mean ± Standard Deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
<i>Cx. quinquefasciatus</i>	295.33 ^a ±12.06	96.67 ^c ±15.04	208 ^b ±13.11	216 ^b ±17.5	276 ^a ±14.53	85.37	0.001
<i>Cx. tritaeniorhynchus</i>	233 ^a ±15.1	145 ^b ±11	163.67 ^b ±11.02	177 ^b ±16.09	236.7 ^a ±18.5	24.17	0.001
<i>Cx. pseudovishnui</i>	102.67 ^a ±12.5	44.33 ^c ±2.52	72.67 ^b ±16.17	89.33 ^a ±13.65	119 ^a ±15.72	14.39	0.001
<i>Cx. pipiens</i>	17 ^a ±1	12 ^c ±1	16.33 ^b ±1.52	14 ^b ±1	19.66 ^a ±1.52	16.8	0.001
<i>Cx.sitiens</i>	16.33 ^b ±2.52	3 ^c ±1	14 ^b ±3	16 ^b ±2	24 ^a ±1	40.12	0.001
<i>Cx. theileri</i>	159.67 ^a ±16.17	71.33 ^c ±15.5	110.67 ^b ±15.01	127 ^a ±17.09	154.67 ^a ±14.64	15.72	0.001
<i>An.subpictus</i>	115 ^a ±15.1	17.67 ^b ±2.52	112 ^a ±11	0 ^b ±0	126.33 ^a ±16.04	88.83	0.001
<i>An.stephensi</i>	34.33 ^b ±2.08	6 ^c ±1	34 ^b ±2	0 ^d ±0	41.67 ^a ±2.52	338.86	0.001
<i>An.culicifacies</i>	55 ^a ±11.53	9.67 ^b ±2.08	61 ^a ±15.72	0 ^b ±0	69 ^a ±15.52	24.12	0.001
<i>An.pulcherrimus</i>	19.33 ^b ±2.08	3 ^c ±1	20.66 ^b ±1.52	0 ^c ±0	26.66 ^a ±1.52	206.37	0.001
<i>An.nigerimus</i>	12.33 ^b ±2.08	2 ^c ±1	14.33 ^b ±1.52	0 ^c ±0	20 ^a ±1	124.56	0.001

Figure 4.3-7: Relationship of abundance of species of mosquitoes with seasons in Tehsil Haroonabad of District Bahawalnagar

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Table 4.3.1.2: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of

4.3.2 :Relationship of the abundance of mosquitoes with seasons in District Bahawalnagar

The results were obtained via one way ANOVA on the seasonal abundance of mosquitoes. The research revealed that in each tehsil the genus *Culex* 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. Tehsil Fortabbas (**Table 4.3.2.1**) had the greatest *Culex quinquefasciatus* count ($F = 111.1$, $df = 4$, $P = 0.001$). Its peak levels of abundance were recorded during the monsoon seasons (Mean = 367, SD = 14), followed by the autumn season (Mean = 364, SD = 17), while the winter season (Mean = 119, SD = 20) saw a severe decrease in abundance. It rises again in the spring (Mean = 262.33, SD = 19), and again in the summer (Mean = 279.67, SD = 11.5). *Anopheles* displayed a distinct pattern of incidence. The monsoon season (Mean = 174.33, SD = 14.5), spring (Mean = 172.67, SD = 15.01), and fall (Mean = 155.33, SD = 14.57) were the times when it peaked. Winter saw a significant drop in abundance (Mean = 40, SD = 3), with summer seeing the biggest fall (Mean = 0, SD = 0). (**Table 4.3.2.1**) This district was confirmed to be devoid of *Aedes*. *Anopheles* and *Culex* species demonstrated the same pattern of occurrence in all the other tehsils Bahawalnagar (**Table 4.3.2.2**) Haroonabad, (**Table 4.3.2.3**) Chishtian (**Table 4.3.2.4**) and Minchanabad (**Table 4.3.2.5**) as found in tehsil Fortabbas (**Table 4.3.2.1**) Relationship of abundance of species of mosquitoes with seasons in Tehsil Fortabbas of District Bahawal Nagar

Species	Mean ± Standard Deviation					F	P
	Autumn	Winter	Spring	Summer	Monsoon		
<i>Cx. quinquefasciatus</i>	264±17	119±20	262.33±19	279.67±11.5	367±14	111.1	0.001
<i>Cx. tritaeniorhynchus</i>	283±12.53	170.33±12.01	213.33±12.5	237.33±15.28	319.3±18	50.2	0.001
<i>Cx. pseudovishnui</i>	129±14.73	44.66±1.52	88±18	114.33±14.01	157±17.01	36.47	0.001
<i>Cx. pipiens</i>	19.33±2.08	11±1	20±1	17.66±1.52	28±3	31.35	0.001

Relative abundance of species of mosquitoes in different seasons in Tehsil Fortabbas of District Bahawal Nagar

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<i>Cx. sitiens</i>	21.33b±1.52	2.33c±1.52	18.67b±2.52	21.66b±1.52	32a±2		99.36	0.001	
<i>Cx. theileri</i>	189.7ab±19.5	86.33c±12.06	103c±11.53	155b±15	212.33a±15.57		39.11	0.001	
<i>An. subpictus</i>	155.33a±14.57	40b±3	172.67a±15.01	0c±0	174.33a±14.57		154.26	0.001	
<i>An. stephensi</i>	66.33a±15.57	11.66b±1.52	64.33a±15.01	0b±0	77.67a±13.65		28.72	0.001	
<i>An. culicifacies</i>	49.7a±18	19.33b±2.52	99.7a±17.5	0b±0	114.3a±17.5		42.16	0.001	
<i>An. pulcharimus</i>	29.66a±1.52	9.67b±2.08	40a±1	0b±0	52.33a±14.05		36.43	0.001	
<i>An. negirmimius</i>	25.33b±1.52	5.67c±2.08	20.67b±2.52	0d±0	31.33a±2.08		152.52	0.001	
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Table 4.3.2.2

Species		Mean						Autumn
		Autumn	Winter	Spring	Summer	Monsoon	Aut	
<i>Cx. quinquefasciatus</i>		258.33a	167.33b	184b	183.7b	256.33a		
<i>Cx. tritaeniorhynchus</i>		234.67a	150.7b	149.33b	154.67b	231.33a		

Relationship

<i>Cx. pseudovishnui</i>		87.33^a	25.67^c	47^bc	61.33^{ab}	87.33^a	1
<i>Cx. pipiens</i>		22^a	10.67^c	12.33^{bc}	16^b	25.67^a	
<i>Cx. sitiens</i>		13^b	2.33^c	11.33^b	14^b	26^a	
<i>Cx. theileri</i>		160^a	80.33^c	105.33^c	115.67^{bc}	150.33^b	
<i>An. subpictus</i>		89.67^a	14.33^b	71.67^a	0^b	92.33^a	1
<i>An. stephensi</i>		26.67^a	5^c	20.66^b	0^d	27.67^a	
<i>An. culicifacies</i>		40^b	8.67^c	40.33^b	0^d	49^a	
<i>An. pulcharimus</i>		14.33^b	2^c	16.33^b	0^c	22^a	
<i>An. nigerrimus</i>		6.67^a	2^b	6.67^a	0^b	10.67^a	

Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	275^a±12.53	91.33^c±16.62	196.67^b±14.64	200^b±14.11	270.33^a±16.17	75.03	0.001
<i>Cx. tritaeniorhynchus</i>	226.3^a±70.6	143.7^b±18	157^b±14.11	166^b±17.5	230^a±13.53	18.67	0.001
<i>Cx. pseudovishnui</i>	94.67^ab±11.5	35.67^c±2.08	67^bc±16.52	74.67^b±13.65	112.3^a±18.5	13.49	0.001
<i>Cx. pipiens</i>	15.33^ab±1.52	10^c±1	12^bc±2	12.33^b±1.52	19.67^a±2.52	13.26	0.001
<i>Cx. sitiens</i>	14.33^b±2.52	2.67^c±2.08	10.67^b±2.08	14^b±1	21^a±2	33.36	0.001
<i>Cx. theileri</i>	142.67^a±13.58	56^c±16.09	102.67^b±14.05	112.33^ab±13.58	137.67^ab±15.5	16.92	0.001
<i>An. subpictus</i>	88.7^a±17.5	10.67^b±2.52	87.67^a±12.58	0^b±0	96.3^a±19.5	13.14	0.001
<i>An. stephensi</i>	25^a±1	5^b±1	25^a±3	0^c±0	26.66^a±1.52	183.44	0.001
<i>An. culicifacies</i>	41.33^b±2.52	8.67^c±2.08	45^ab±2	0^d±0	49.67^a±2.08	412.43	0.001
<i>An. pulcherrimus</i>	16.33^a±2.52	3.67^b±2.08	18.33^b±2.52	0^b±0	17^a±3	42.26	0.001
<i>An. nigerrimus</i>	9.33^a±1.52	2^b±1	10.67^a±2.52	0^b±0	11^a±1	38	0.001

Figure 4.3-8: Relationship of abundance of species of mosquitoes with seasons in Tehsil Chishtian of District Bahawalnagar

Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	312.33^a±17.04	80.33^c±15.5	198.33^b±14.5	226^b±14.73	305^a±15.72	110.99	0.001
<i>Cx. tritaeniorhynchus</i>	255.67^a±17.01	122^c±14.73	182^b±18.5	210.33^b±14.5	260.67^a±17.04	36.41	0.001

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<i>Cx. pseudovishnui</i>	<u>114.3^a±17.5</u>	<u>35.33^c±1.52</u>	<u>71.7b^c±18.5</u>	<u>93.67a^b±15.18</u>	<u>124.67^a±15.63</u>	<u>17.03</u>	<u>0.001</u>
<i>Cx. pipiens</i>	<u>16.33^b±2.08</u>	<u>10^c±1</u>	<u>16^b±1</u>	<u>18.33^b±1.52</u>	<u>28.67^a±2.08</u>	<u>53.24</u>	<u>0.001</u>
<i>Cx.sitiens</i>	<u>17.33^b±1.52</u>	<u>2^c±1</u>	<u>19.67^b±2.52</u>	<u>16.67^b±2.08</u>	<u>25.67^a±2.52</u>	<u>56.2</u>	<u>0.001</u>
<i>Cx. theileri</i>	<u>173^a±11</u>	<u>71^b±15.52</u>	<u>160.67^a±16.17</u>	<u>160.7^a±17.5</u>	<u>193.67^a±12.58</u>	<u>30.63</u>	<u>0.001</u>
<i>An.subpictus</i>	<u>137.67^a±15.63</u>	<u>35^b±3</u>	<u>139^a±11.53</u>	<u>0^c±0</u>	<u>147.67^a±13.05</u>	<u>128.71</u>	<u>0.001</u>
<i>An.stephensi</i>	<u>56^a±14.73</u>	<u>10^b±1</u>	<u>55.3^a±17.6</u>	<u>0^b±0</u>	<u>65.3^a±18</u>	<u>15.84</u>	<u>0.001</u>
<i>An.culicifacies</i>	<u>73^a±18.5</u>	<u>18.33^b±2.52</u>	<u>63.33^a±15.18</u>	<u>0^b±0</u>	<u>76.33^a±14.01</u>	<u>23.34</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>26.67^b±2.08</u>	<u>6.67^c±2.08</u>	<u>29.33^b±2.52</u>	<u>0^d±0</u>	<u>35.33^a±1.52</u>	<u>204.15</u>	<u>0.001</u>
<i>An.nigerrimus</i>	<u>15a^b±3</u>	<u>2.67^c±2.08</u>	<u>13.33^b±1.52</u>	<u>0^c±0</u>	<u>20.33^a±2.5</u>	<u>50.51</u>	<u>0.001</u>

Figure 4.3-9: Relationship of abundance of species of mosquitoes with seasons in Tehsil Minchanabad of District Bahawalnagar

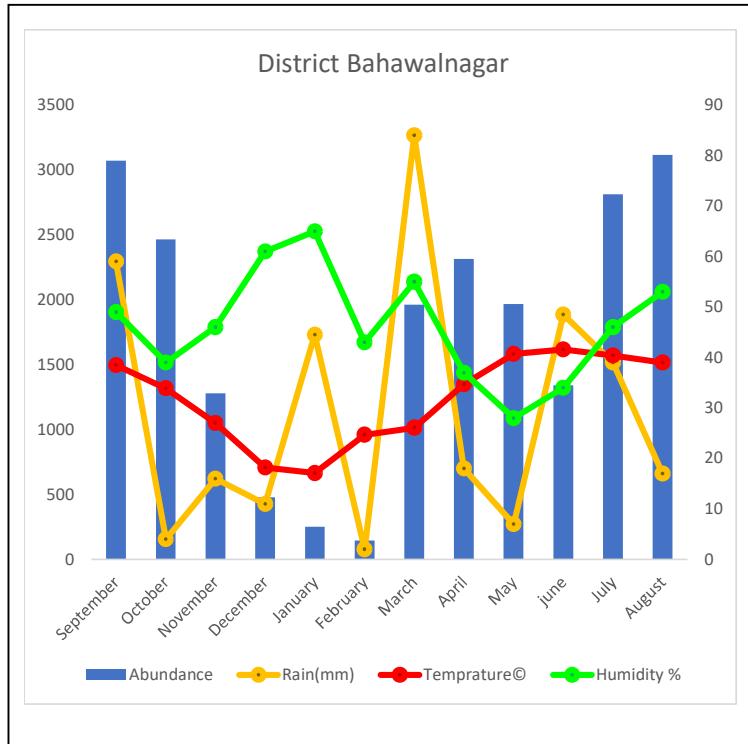
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4.3.3 Effect of climatic factors on the abundance of mosquitoes in District Bahawalnagar

-Highest rain was found in the month of March as compared to August in district Bahawalnagar. The highest abundance was observed in August as temperature and humidity were found to be more favorable for the reproduction of the mosquitoes.

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Figure 4.3-10: Effect of climatic variables on relative abundance of mosquito species in district Bahawalnagar

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4.3.4 Comparative analysis between rural and urban areas of tehsils of Bahawalnagar

-The mosquito populations in various Bahawalnagar district tehsils were compared between rural and urban areas using the unpaired t-test. The results showed that there were significantly more

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mosquitoes in rural than in urban regions in the tehsils of Bahawalnagar, Haroonabad, Chishtian, Minchanabad, and Fortabbas (Table 4.3.4.1). A highly significant result was observed in Tehsil Bahawalnagar ($t=3.793$, $p=0.005$, $F=2.302$). The findings in the Fortabbas tehsils were ($t=2.64$, $p=0.029$, $F=1.992$) was discovered to be statistically lower than those of other tehsils, suggesting that there was less variation in mosquito abundance between rural and urban areas in this tehsil.

Tehsils	T	P	F	Difference between Means ±Standard Error
Bahawalnagar	3.793	0.005	2.302	288.4±75.92
Haroonabad	3.28	0.0110	2.459	314.6±95.64
Chishtian	3.02	0.0164	2.190	257.4±85.05
Minchanabad	2.967	0.0180	2.520	353.4±119.0
Fortabbas	2.64	0.029	1.992	363.8±137.4

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Figure 4.3-11: T test between the rural and urban areas of different Tehsils of district of Bahawalnagar

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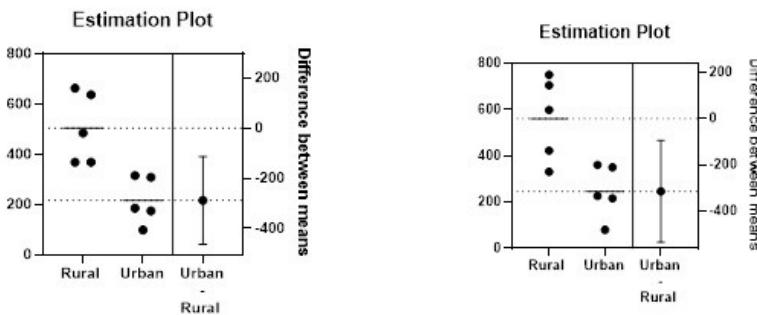


Figure 4.3-13: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Haroonabad of District Bahawalnagar

Figure 4.3-12: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Bahawalnagar of District Bahawalnagar

Figure 4.3.4.1: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Bahawalnagar of District Bahawalnagar

Figure 4.3.4.2: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Haroonabad of District Bahawalnagar

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Figure 4.3-15: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Chishtian of District Bahawalnagar

Figure 4.3-14: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Minchanabad of District Bahawalnagar

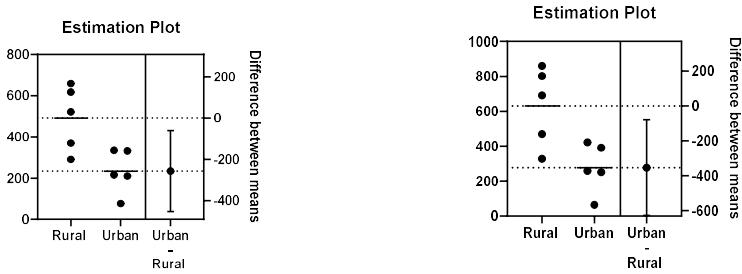
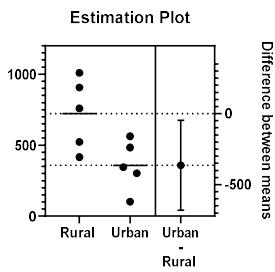


Figure 4.3.4.4: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Minchanabad of District Bahawalnagar

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Figure 4.3-16: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Fortabbas of District Bahawalnagar



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4.3.5 GIS Mapping

According to the map the genus *Culex* and *Anopheles* were conspicuously found in all the tehsils of

Bahawalnagar with the absence of genus *Aedes*.

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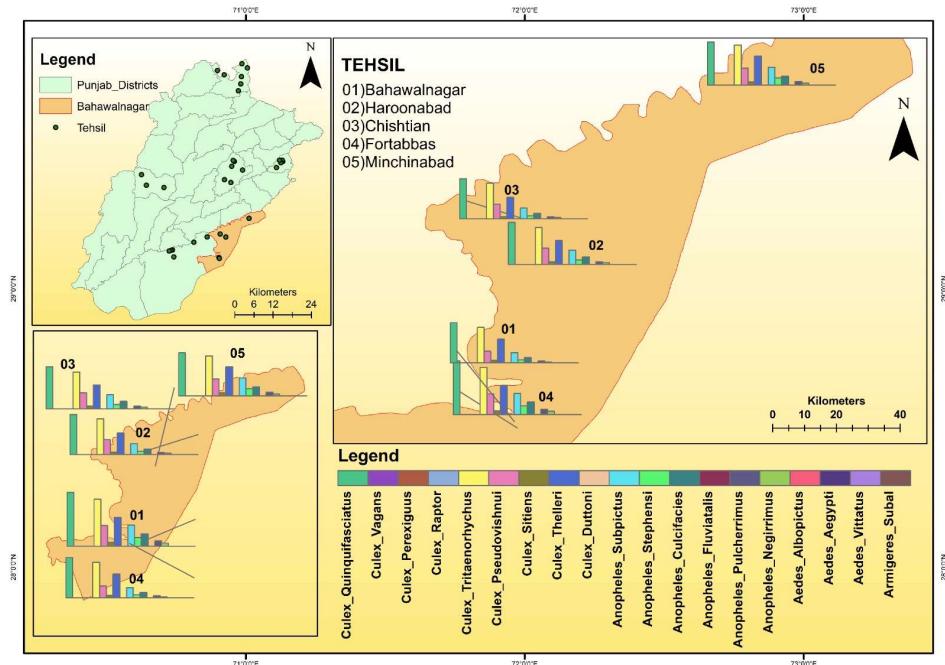


Figure 4.3-17: GIS map showing mosquito abundance in different tehsils of district Bahawalnagar

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4.3

4.4 Cotton Zone District Bahawalpur

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A total of n = 18,354 of 18,354 of 12 mosquito species were gathered from Bahawalpur's six tehsils.

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Culex was the most prevalent of the three genera, with 75.89% of the total, followed by *Anopheles* (19.08%) and *Aedes* (5.03%). *Anopheles* and *Culex* genus were discovered in each of the six tehsils.

While genus *Aedes* were observed in only two tehsils, Bahawalpur City and Bahawalpur Saddar. It was noted that tehsil Ahmedpur East had the lowest abundance of mosquitoes, whereas tehsil Bahawalpur Saddar was found to be rich in mosquito fauna. There were five species found in the genus *Culex*, with *Culex quinquefasciatus* accounting for

30.49% of them. *Culex tritaeniorhynchus* (27.42%), *Culex pipiens* (11.83%), *Culex pseudovishnui* (4.23%), *Culex vagans* (1.92%). The *Anopheles* genus yielded five species, including *Anopheles subpictus* (10.5%), *Anopheles culicifacies* (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*.

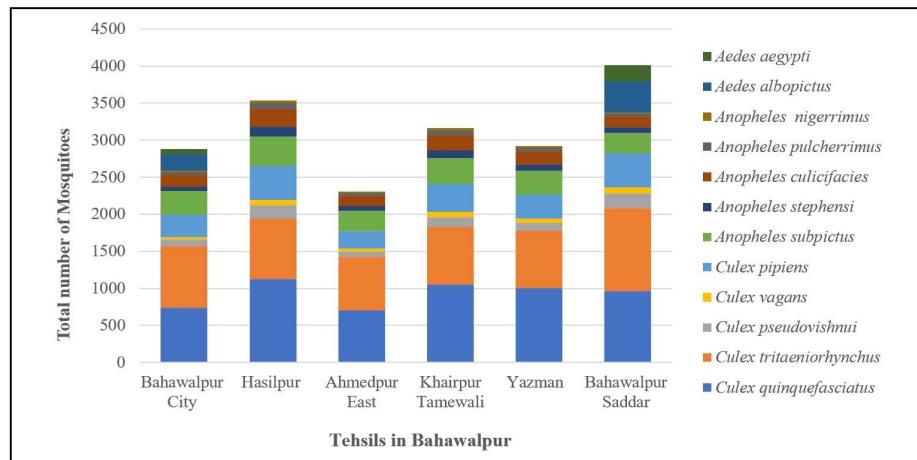


Figure 4.4-1: Total number of mosquitoes in district Bahawalpur

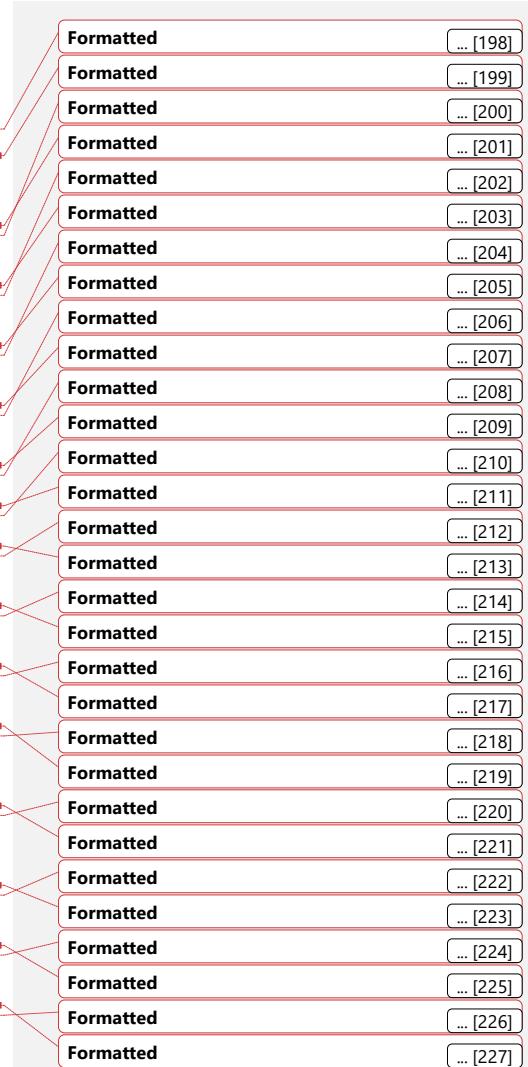
4.3.14.4.1 Species Diversity and Abundance of Mosquitoes in District Bahawalpur

Diversity of the mosquito fauna calculated through Shannon diversity (H), and Simpson diversity (D) indicated that there were notable variations throughout the district. For each of the six tehsils, the corresponding Shannon and Simpson diversity indices varied from 2.01 to 1.76 and 0.82 to 0.77. For both indices, the highest values were found in tehsil Saddar, and the lowest values were observed in tehsil Ahmedpur East (Table 4.4.1.1). Analysis of variance (ANOVA) tests revealed significant differences in mosquito abundance across all the tehsils. The maximum value of *Culex quinquefasciatus* (Figure 4.4.1.1.1) was found in Hasilpur ($F = 394.56$, $df = 5$, $P < 0.001$), while the lowest one was observed in Khairpur Tamewali, Yazman, Saddar, Bahawalpur, and Ahmedpur East. (Table 4.4.1.2). Nearly the similar results were obtained for *Anopheles subpictus* (Figure 4.4.1.1.6) *Anopheles culicifacies* (Figure 4.4.1.1.7) *Anopheles pulcherrimus* (Figure 4.4.1.1.8) *Anopheles stephensi* (Figure 4.4.1.1.9) and *Anopheles negirrimus* (Figure 4.4.1.1.10). *Culex vagans* were only found from district Bahawalpur and Rawalpindi. It was abundantly found in tehsil Saddar and least in Ahmed pur East

(Figure 4.4.1.1.3) — The similar pattern of occurrence was found to be followed by *Culex pipiens* (Figure 4.4.1.4). and And *Culex pseudovishnui* (Figure 4.4.1.5). In this district, *Aedes albopictus* (Figure 4.4.1.11) and *Aedes aegypti* (Figure 4.4.1.12) were uncommon were that uncommon that were absent from Hasilpur, Khairpur Tamewali, Yazman, and Ahmedpur East tehsils but quite scattered in the tehsils of Saddar and Bahawalpur.

Name of the species	SADDAR						BAHAWALPUR CITY						HASILPUR						KHAIRPUR						YAZMAN						AHMEDPUR SHARQIA					
	Tot al	cou nts	Shann on's Index	Simps on's	Tot al	cou nts	Shann on's Index	Simps on's	Tot al	cou nts	Shann on's Index	Simps on's	Total	cou nts	Shann on's Index	Simps on's	Total	cou nts	Shann on's Index	Simps on's	Total	cou nts	Shann on's Index	Simps on's	Total	cou nts	Shann on's Index	Simps on's								
	%a ge	Pi*(lo gPi)	Index n(n-1)	%a ge	Pi*(lo gPi)	Index n(n-1)	%a ge	Pi*(lo gPi)	Index n(n-1)	%a ge	Pi*(lo gPi)	Index n(n-1)	coun ts	%a ge	Pi*(lo gPi)	Index n(n-1)	coun ts	%a ge	Pi*(lo gPi)	Index n(n-1)	coun ts	%a ge	Pi*(lo gPi)	Index n(n-1)	coun ts	%a ge	Pi*(lo gPi)	Index n(n-1)								
<i>Culex quinquefasciatus</i>	24, 964	03	0,342	92833	72	25, 740	69	0,349	54686	112	31, 80	0,364	12645	30	33, 1057	39	0,366	11161	92	34, 1004	35	0,367	10070	12	30, 707	61	0,362	49914	2							
<i>Culex tritaeniorhynchos</i>	111, 7	27, 85	0,356	12465	72	28, 827	71	0,358	68310	23	0,338	66994	24,	0,344	59830	2	26, 776	55	0,352	60140	1	31, 719	13	0,363	51624	2										
<i>Culex pseudovittatus</i>	4, 200	9	0,149	39800	92	3, 9	0	0,110	8372	173	4, 9	0,147	29756	131	4, 8	17030	104	3, 6	10712	7	3, 76	9	0,112	5700	3											
<i>Culex Vagans</i>	2, 82	4	0,079	6642	36	1, 5	8	0,054	1260	74	9, 9	0,080	2, 9402	67	2, 2	4422	58	8, 8	3306	35	2, 1	1190	5	2, 5	1190	5										
<i>Culex pipiens</i>	11, 464	1, 57	0,249	21483	2	10, 295	24	0,233	86730	460	0,265	21114	13,	0,256	14784	12,	11, 0,245	10725	10,	10, 0,235	10725	10,	10, 240	39	0,235	57360	3									
<i>Anopheles subpictus</i>	6, 272	6, 8	0,182	73712	322	11, 18	0,9	0,244	10336	2	11, 25	0,245	15800	6	10, 348	99	0,242	12075	7	10, 316	81	0,240	99540	5	11, 273	82	0,252	74256	4							
<i>Anopheles stephensi</i>	1, 70	1, 5	0,070	4830	63	2, 9	6	0,083	3906	134	3, 9	0,124	17822	105	2, 0	10920	82	3, 1	6642	3	2, 8	62	0,097	3782	1											
<i>Anopheles culicifacies</i>	3, 6147	3, 6	0,121	21462	157	5, 5	6	0,158	24492	237	6, 0	0,181	55932	202	6, 8	40602	177	6, 6	31152	8	6, 0	137	0,169	18632	5											
<i>Anopheles pulcherimus</i>	1, 040	1, 0	0,046	1560	39	1, 35	2	0,058	1482	80	2, 26	0,085	6320	65	2, 5	4160	52	1, 78	2652	7	1, 740	40	0,070	1560	2											
<i>Anopheles negirmaculatus</i>	0,520	0,5	0,026	380	22	0,76	2	0,037	462	38	1, 07	0,048	1406	32	1, 01	992	26	0,89	650	0	0,921	21	0,042	420	7											
<i>Aedes albopictus</i>	10, 418	42	0,235	17430	6	7, 6	212	0,192	44732	0	0,0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
<i>Aedes aegypti</i>	5, 4217	1	0,157	46872	76	4	9	0,095	5700	0	0,0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
Total	401	1	H=2,0	D=0,8	288		H=1,9	D=0,8	353		H=1,8	D=0,8	820		H=1,8	D=0,7	943		H=1,8	D=0,7	953		H=1,7	D=0,7	810		H=1,7	D=0,7	667		H=1,7	D=0,7	791			

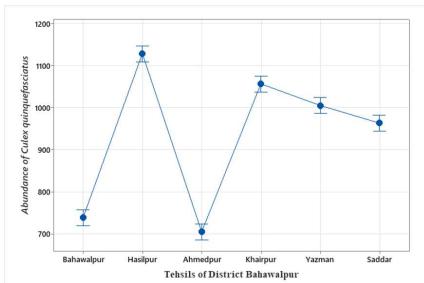
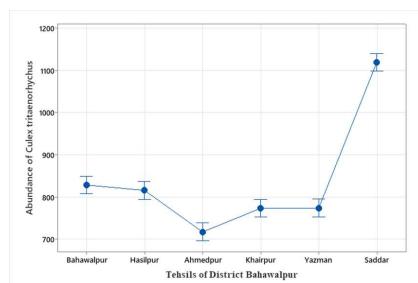
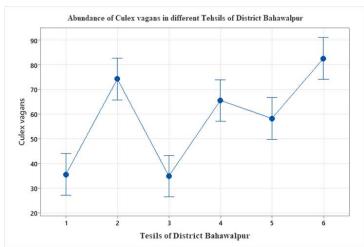
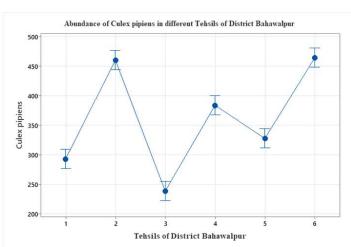
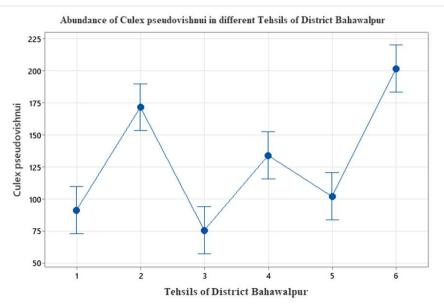
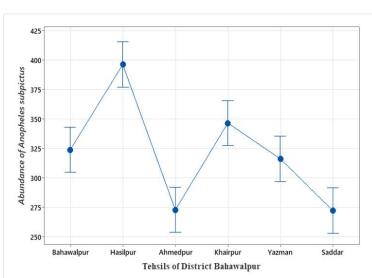
Figure 4.4-2: Diversity index of a number of individuals of different species of mosquitoes collected from six tehsils of District Bahawalpur



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Species	Mean ± Standard Deviation							F	P
	Bahawalpur	Hasilpur	Ahmadpur East	Khairpur	Yazman	Saddar			
<u>Cx.</u> <u>quinquefasciatus</u>	<u>738.67^e±17.04</u>	<u>1128.33^a±15.28</u>	<u>705^e±16.09</u>	<u>105.67^b±10.5</u>	<u>1005.3^c±18</u>	<u>963.33^d±12.01</u>	<u>394.56</u>	<u>0.001</u>	
<u>Cx.</u> <u>tritaeniorhynchus</u>	<u>828.67^b±16.56</u>	<u>ANOVA</u>	<u>717.67^d±17.04</u>	<u>773.7^c±17.5</u>	<u>774^c±17.09</u>	<u>1118.7^a±17.6</u>	<u>217.62</u>	<u>0.001</u>	
<u>Cx.</u> <u>pseudovishnui</u>	<u>91.33^d±17.01</u>	<u>171.67^a±15.04</u>	<u>75.67^d±11.5</u>	<u>134b^c±15.72</u>	<u>102c^d±13.11</u>	<u>201.67^a±14.57</u>	<u>34.1</u>	<u>0.001</u>	
<u>Cx. pipiens</u>	<u>293^c±13.11</u>	<u>460^a±12</u>	<u>238.67^b±16.04</u>	<u>383.67^b±13.05</u>	<u>327.67^c±11.5</u>	<u>464^a±11</u>	<u>150.3</u>	<u>0.001</u>	
<u>Cx. Vagans</u>	<u>35.67^c±2.52</u>	<u>74.33a^b±14.5</u>	<u>35^c±2</u>	<u>65.67a^b±5.13</u>	<u>58.33^b±3.51</u>	<u>82.67^a±3.06</u>	<u>26.26</u>	<u>0.001</u>	
<u>An.subpictus</u>	<u>324^b±17.09</u>	<u>396.3^a±17.6</u>	<u>273^c±14</u>	<u>346.67^b±12.06</u>	<u>316.33^b±16.5</u>	<u>272.33^c±13.5</u>	<u>28.45</u>	<u>0.001</u>	
<u>An.stephensi</u>	<u>64.33^d±5.13</u>	<u>134.67^a±5.03</u>	<u>64^d±5.29</u>	<u>103.33^b±7.64</u>	<u>84^c±5.29</u>	<u>17.33c^d±4.51</u>	<u>74.73</u>	<u>0.001</u>	
<u>An.culcifacies</u>	<u>159.67b^c±16.17</u>	<u>237.3^a±19.5</u>	<u>137.67^c±17.01</u>	<u>201.67a^b±10.5</u>	<u>178.67b^c±13.58</u>	<u>146.67^c±14.5</u>	<u>17.6</u>	<u>0.001</u>	
<u>An.pulcharimus</u>	<u>39.33^d±1.52</u>	<u>80.67^a±3.06</u>	<u>39.67^d±1.52</u>	<u>64.33^b±3.06</u>	<u>53.33^c±4.16</u>	<u>40.33^d±2.52</u>	<u>108.69</u>	<u>0.001</u>	
<u>An.negirimus</u>	<u>21.67b^c±1.52</u>	<u>37.67^a±2.52</u>	<u>21b^c±1</u>	<u>32.67^a±2.08</u>	<u>26^b±3</u>	<u>20^c±1</u>	<u>38.82</u>	<u>0.001</u>	
<u>Ae.albopictus</u>	<u>217.3^b±30.4</u>	<u>0^c±0</u>	<u>0^c±0</u>	<u>0^c±0</u>	<u>0^c±0</u>	<u>419.3^a±30</u>	<u>307.17</u>	<u>0.001</u>	
<u>Ae.aegypti</u>	<u>77.33^a±15.04</u>	<u>0^c±0</u>	<u>0^c±0</u>	<u>0^c±0</u>	<u>0^c±0</u>	<u>214.33^a±16.17</u>	<u>278.61</u>	<u>0.001</u>	

Figure 4.4-3: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of Bahawalpur

Figure 4.4.1.1.1: Abundance of *Culex quinquefasciatus*Figure 4.4.1.1.2: Abundance of *Culex tritaeniorhynchus*Figure 4.4.1.1.3: Abundance of *Culex vagans*Figure 4.4.1.1.4: Abundance of *Culex pipiens*Figure 4.4.1.1.5: Abundance of *Culex pseudovishnui*Figure 4.4.1.1.6: Abundance of *Anopheles subpictus*

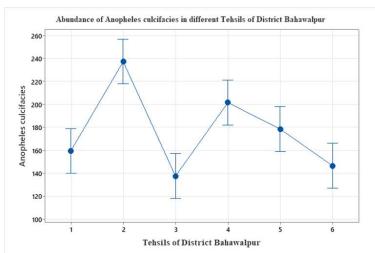
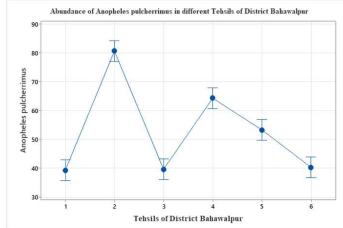
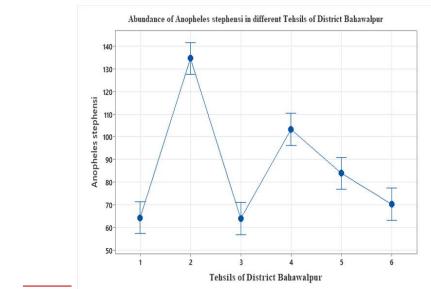
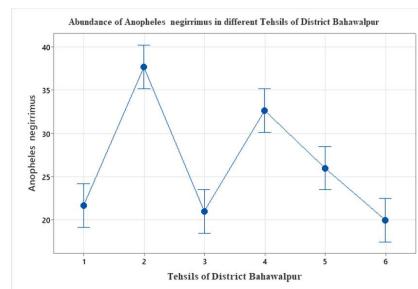
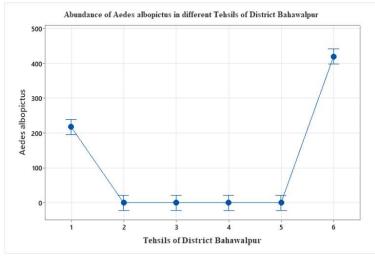
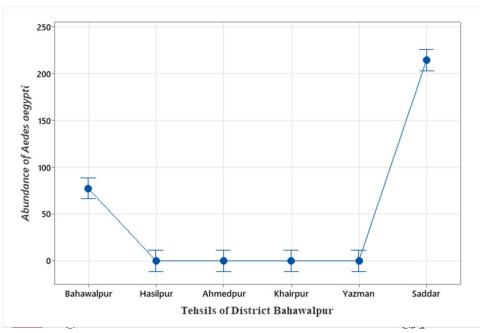
Figure 4.4.1.1.7: Abundance of *Anopheles culicifacies*Figure 4.4.1.1.8: Abundance of *Anopheles pulcherrimus*Figure 4.4.1.1.9: Abundance of *Anopheles stephensi*Figure 4.4.1.1.10: Abundance of *Anopheles negirrimus*Figure 4.4.1.1.11: Abundance of *Aedes albopictus*

Figure 4.4.4: Relative abundance of different species of mosquito in different tehsils of district Bahawalpur

	SADDAR				BAHAWALPUR CITY				HASHI PUR				KHAIR PUR				YAZMAN		AHMED PUR SHARQIA	
Name of the species	Total counts	%age	Shannon's Index	Simpson's Index	Total counts	%age	Shannon's Index	Simpson's Index	Total counts	%age	Shannon's Index	Simpson's Index	Total counts	%age	Shannon's Index	Simpson's Index	Total counts	%age	Shannon's Index	Simpson's Index
<i>Culex quinquefasciatus</i>	964	24.03	0.3427	0.28332	740	25.68	0.3491	0.28660	1125	31.80	0.3643	0.264500	1052	33.39	0.3663	0.266182	1116132	29.20	0.3663	0.266182
<i>Culex tritaeniorhynchus</i>	1117	27.95	0.3560	0.246572	827	28.71	0.3583	0.23102	819	23.15	0.3387	0.269942	724	24.45	0.3444	0.298207	703	22.77	0.3444	0.298207
<i>Culex pseudovishnui</i>	200	4.99	0.1495	0.38800	93	3.19	0.1100	0.3772	173	4.89	0.1476	0.29756	131	4.14	0.1318	0.27036	142	4.00	0.1318	0.27036
<i>Culex vagans</i>	82	2.04	0.0795	0.6642	36	1.25	0.0548	0.2660	74	2.00	0.0804	0.5402	67	3.12	0.0816	0.4422	74	2.00	0.0816	0.4422
<i>Culex pipiens</i>	464	11.52	0.2495	0.214822	295	10.24	0.2222	0.26730	460	12.00	0.2652	0.211140	385	12.16	0.2562	0.147842	425	11.52	0.2562	0.147842
<i>Anopheles subpictus</i>	272	6.78	0.1825	0.27712	222	11.18	0.2449	0.193262	298	11.25	0.2458	0.158006	248	10.99	0.2422	0.120754	282	11.25	0.2422	0.120754
<i>Anopheles stephensi</i>	70	1.75	0.0707	0.4820	63	2.19	0.0826	0.3906	124	3.79	0.1240	0.17822	105	3.22	0.1130	0.1092	115	3.79	0.1130	0.1092
<i>Anopheles culicifacies</i>	147	3.66	0.1212	0.21462	152	5.45	0.1586	0.24492	222	6.70	0.1811	0.15922	202	6.38	0.1756	0.14602	232	6.70	0.1756	0.14602
<i>Anopheles pulcherrimus</i>	40	1.00	0.0469	0.1560	39	1.35	0.0582	0.1482	80	2.26	0.0857	0.6220	65	2.05	0.0798	0.4160	75	2.26	0.0798	0.4160
<i>Anopheles negrivittata</i>	30	0.50	0.0264	0.280	22	0.76	0.0372	0.462	38	4.07	0.0487	0.1406	32	4.01	0.0464	0.992	38	4.07	0.0464	0.992
<i>Aedes albopictus</i>	418	10.42	0.2257	0.174306	212	7.26	0.1920	0.47222	0	0.00	0	0								
<i>Aedes aegypti</i>	237	5.41	0.1578	0.46872	76	2.64	0.0959	0.5706	0	0.00	0	0								
Total	4011		II=2.0174	D=0.8284	3881		II=1.9759	D=0.8180	3538		II=1.8820	D=0.8066	3166		II=1.8277	D=0.7985	2847		II=1.7945	D=0.7895

Table 4.4.1.1: Diversity index of a number of individuals of different species of mosquitoes collected from six tehsils of District Bahawalpur

Table 4.4.1.2: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of Bahawalpur

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4.4.2 Relationship of the abundance of mosquitoes with seasons in District Bahawalpur

Riveting results were noticed when noticed one when one-way ANOVA was applied on applied on the relationship of season on the abundance of mosquitoes in district Bahawalpur. The study found that the mosquito population in each tehsil peaked in the monsoon season, then declined in the fall, and then peaked again in the winter. The spring brought with it a greater number of mosquitoes than in the summer. The tehsil of Hasilpur had the most *Culex quinquefasciatus* count ($F = 85.81$, $df = 4$, $P = 0.001$) in autumn (Mean = 299, SD = 15.72) and monsoon (Mean = 299.33, SD = 12.06) seasons where seasons where its abundance peaked, winter, winter (Mean = 125, SD = 15.01) saw a steep drop in abundance. It rises again in the spring (Mean = 237, SD = 17) and falls (Mean = 160, SD = 14.1) in the summer (Table 4.4.2.1). The the genus *Anopheles subpictus* showed an identical trend of abundance as *Culex quinquefasciatus*. It was lowest in the winter (Mean = 0, SD = 0) and greatest in the fall (Mean = 119.33, SD = 17.5). It peaked during the monsoon season 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 4.4.2.2) the association between abundance and the season of the other species displayed the similar trend as that of Culex and Anopheles in all the tehsil Khairpur (Table 4.4.2.3) tehsil Yazman (Table 4.4.2.4) tehsil Ahmedpur east (Table 4.4.2.5) and tehsil Bahawalpur district (Table 4.4.2.6).

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

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Anophels nigerrimus	11.66 ^a	\pm	1.52	2.67 ^b	\pm	2.08	6.3 ^b	\pm	1.52	0 ^c	\pm	0	11.33 ^a	\pm	2.08	30.09	0.001
Aedes albopictus	0	\pm	0	0	\pm	0	0	\pm	0	0	\pm	0	0	\pm	0	0	0
Aedes aegypti	0	\pm	0	0	\pm	0	0	\pm	0	0	\pm	0	0	\pm	0	0	0

Figure 4.4-5: Relationship of abundance of species of mosquitoes with seasons in Tehsil Hasilpur of District Bahawalpur

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<u>Species</u>	<u>Mean ± Standard Deviation</u>												<u>F</u>	<u>P</u>			
	<u>Autumn</u>		<u>Winter</u>		<u>Spring</u>		<u>Summer</u>		<u>Monsoon</u>								
<u>Culex quinquefasciatus</u>	295.67 ^a	\pm	14.57	80 ^c	\pm	20	139.6 ^b	\pm	16.17	167.3 ^b	\pm	12.01	283.33 ^a	\pm	15.57	104.06	0.001
<u>Culex tritaeniorhynchus</u>	320 ^a	\pm	17	98.67 ^c	\pm	13.01	171.6 ^b	\pm	14.01	200.6 ^b	\pm	17.04	325.67 ^a	\pm	14.5	125.1	0.001
<u>Culex pseudovishnui</u>	48 ^b	\pm	15.72	11 ^d	\pm	3	36.67 ^b	\pm	2.08	19.33 ^d	\pm	2.52	81.67 ^a	\pm	13.05	26.41	0.001
<u>Culex pipiens</u>	138.6 ^a	\pm	12.01	35 ^c	\pm	3	69.67 ^b	\pm	14.5	74 ^b	\pm	14.53	149.67 ^a	\pm	13.58	47.26	0.001
<u>Culex vagans</u>	15b ^c	\pm	3	2.67 ^d	\pm	2.08	16.66 ^b	\pm	1.52	10.33 ^c	\pm	2.08	37.33 ^a	\pm	2.52	94.79	0.001
<u>Anopheles subpictus</u>	87.33 ^a	\pm	16.01	16.67 ^b	\pm	2.52	78 ^a	\pm	14.73	0 ^b	\pm	0	93 ^a	\pm	18.5	34.25	0.001
<u>Anopheles stephensi</u>	19 ^b	\pm	3	5 ^c	\pm	1	21a ^b	\pm	1	0 ^d	\pm	0	24.33 ^a	\pm	2	11.93	0.001
<u>Anopheles culicifacies</u>	52.33 ^a	\pm	13.05	10.33 ^b	\pm	1.52	37 ^a	\pm	3	0 ^b	\pm	0	48.66 ^a	\pm	1.52	44.48	0.001
<u>Anopheles pulcherrimus</u>	10.33 ^a	\pm	1.52	3.33 ^b	\pm	1.52	13.66 ^a	\pm	1.52	0 ^b	\pm	0	12.67 ^a	\pm	2.52	40.81	0.001
<u>Anopheles negirrimus</u>	5 ^b	\pm	1	2c ^d	\pm	1	4b ^c	\pm	1	0 ^d	\pm	0	9.33 ^a	\pm	1.52	34.75	0.001

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<i>Aedes albopictus</i>	<u>146.6^a</u>	\pm	<u>16.17</u>	<u>16.33^c</u>	\pm	<u>2.52</u>	<u>71^b</u>	\pm	<u>15.52</u>	<u>62.33^b</u>	\pm	<u>15.01</u>	<u>125^a</u>	\pm	<u>14.11</u>	<u>43.51</u>	<u>0.001</u>
<i>Aedes aegypti</i>	<u>72.33^a</u>	\pm	<u>14.5</u>	<u>9^c</u>	\pm	<u>1</u>	<u>35.67^b</u>	\pm	<u>2.52</u>	<u>26.33^b^c</u>	\pm	<u>2.08</u>	<u>73^a</u>	\pm	<u>13</u>	<u>31.15</u>	<u>0.001</u>

Figure 4.4-6: Relationship of abundance of species of mosquitoes with seasons in Tehsil Saddar of District Bahawalpur

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<u>Species</u>	<u>Mean ± Standard Deviation</u>										<u>F</u>	<u>P</u>					
	<u>Autumn</u>		<u>Winter</u>		<u>Spring</u>		<u>Summer</u>		<u>Monsoon</u>								
<i>Culex quinquefasciatus</i>	<u>289.67^a</u>	\pm	<u>13.58</u>	<u>109.67^d</u>	\pm	<u>15.18</u>	<u>222.67^b</u>	\pm	<u>10.5</u>	<u>152.67^c</u>	\pm	<u>13.01</u>	<u>284.67^a</u>	\pm	<u>16.62</u>	<u>98.2</u>	<u>0.001</u>
<i>Culex tritaeniorhynchus</i>	<u>226.3^a</u>	\pm	<u>19.5</u>	<u>71.67^c</u>	\pm	<u>15.57</u>	<u>152.67^b</u>	\pm	<u>17.01</u>	<u>99^c</u>	\pm	<u>14</u>	<u>226.67^a</u>	\pm	<u>15.5</u>	<u>56.49</u>	<u>0.001</u>
<i>Culex pseudovishnui</i>	<u>36.33^a</u>	\pm	<u>2.08</u>	<u>13.66^c</u>	\pm	<u>1.52</u>	<u>27.33^b</u>	\pm	<u>2.52</u>	<u>18.67^c</u>	\pm	<u>2.08</u>	<u>35.67^a</u>	\pm	<u>2.08</u>	<u>70.5</u>	<u>0.001</u>
<i>Culex pipiens</i>	<u>102.33^a</u>	\pm	<u>15.5</u>	<u>32.66^c</u>	\pm	<u>1.52</u>	<u>84.67^a^b</u>	\pm	<u>13.65</u>	<u>51.67^b^c</u>	\pm	<u>15.28</u>	<u>108.3^a</u>	\pm	<u>17.5</u>	<u>16.58</u>	<u>0.001</u>
<i>Culex vagans</i>	<u>16.66^b</u>	\pm	<u>1.52</u>	<u>5.33^d</u>	\pm	<u>1.52</u>	<u>11^c</u>	\pm	<u>2</u>	<u>10.33^c^d</u>	\pm	<u>2.08</u>	<u>23^a</u>	\pm	<u>3</u>	<u>31.19</u>	<u>0.001</u>
<i>Anopheles subpictus</i>	<u>120^a</u>	\pm	<u>18</u>	<u>18.67^b</u>	\pm	<u>2.08</u>	<u>101^a</u>	\pm	<u>16</u>	<u>0^b</u>	\pm	<u>0</u>	<u>107.67^a</u>	\pm	<u>16.04</u>	<u>55.31</u>	<u>0.001</u>
<i>Anopheles stephensi</i>	<u>33^a</u>	\pm	<u>3</u>	<u>8.67^b</u>	\pm	<u>2.08</u>	<u>30^a</u>	\pm	<u>1</u>	<u>0^c</u>	\pm	<u>0</u>	<u>34.67^a</u>	\pm	<u>2.08</u>	<u>201.8</u>	<u>0.001</u>

<u>Anopheles culicifacies</u>	<u>67.3^a</u>	\pm	<u>18</u>	<u>7^b</u>	\pm	<u>1</u>	<u>61.7^a</u>	\pm	<u>17.5</u>	<u>0^b</u>	\pm	<u>0</u>	<u>66.67^a</u>	\pm	<u>15.5</u>	<u>19.82</u>	<u>0.001</u>
<u>Anopheles pulcherrimus</u>	<u>19.33a^b</u>	\pm	<u>1.52</u>	<u>6.33^c</u>	\pm	<u>1.52</u>	<u>17.33^b</u>	\pm	<u>2.52</u>	<u>0^d</u>	\pm	<u>0</u>	<u>23.67^a</u>	\pm	<u>2.08</u>	<u>94.29</u>	<u>0.001</u>
<u>Anopheles nigerrimus</u>	<u>11.66^a</u>	\pm	<u>1.52</u>	<u>2.67b^c</u>	\pm	<u>2.08</u>	<u>6.33^b</u>	\pm	<u>1.52</u>	<u>0^c</u>	\pm	<u>0</u>	<u>11.33^a</u>	\pm	<u>2.08</u>	<u>30.09</u>	<u>0.001</u>
<u>Aedes albopictus</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	<u>0</u>
<u>Aedes aegypti</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	\pm	<u>0</u>	<u>0</u>	<u>0</u>

Figure 4.4-7: Relationship of abundance of species of mosquitoes with seasons in Tehsil Khairpur East of District Bahawalpur

<u>Species</u>	<u>Mean ± Standard Deviation</u>										<u>-</u>	<u>-</u>	<u>F</u>	<u>P</u>			
	<u>Autumn</u>	<u>-</u>	<u>-</u>	<u>Winter</u>	<u>-</u>	<u>-</u>	<u>Spring</u>	<u>-</u>	<u>-</u>	<u>Summer</u>							
<u>Culex quinquefasciatus</u>	<u>282^a</u>	\pm	<u>12.53</u>	<u>89.3^d</u>	\pm	<u>17.5</u>	<u>216.67^b</u>	\pm	<u>16.5</u>	<u>136.7^c</u>	\pm	<u>19</u>	<u>277.33^a</u>	\pm	<u>14.57</u>	<u>83.74</u>	<u>0.001</u>
<u>Culex tritaeniorhynchus</u>	<u>211.67^a</u>	\pm	<u>16.01</u>	<u>59^c</u>	\pm	<u>11.53</u>	<u>183.67^a</u>	\pm	<u>13.58</u>	<u>108.3^b</u>	\pm	<u>18</u>	<u>59^c</u>	\pm	<u>13.5</u>	<u>66.25</u>	<u>0.001</u>
<u>Culex pseudovishnui</u>	<u>29.33^a</u>	\pm	<u>2.52</u>	<u>10.33^c</u>	\pm	<u>1.52</u>	<u>21.67^b</u>	\pm	<u>2.08</u>	<u>15.67^c</u>	\pm	<u>2.52</u>	<u>27.33a^b</u>	\pm	<u>2.08</u>	<u>39.95</u>	<u>0.001</u>
<u>Culex pipiens</u>	<u>87.67^a</u>	\pm	<u>11.02</u>	<u>25.33^c</u>	\pm	<u>2.08</u>	<u>77.7a^b</u>	\pm	<u>17.5</u>	<u>50b^c</u>	\pm	<u>1</u>	<u>85.67^a</u>	\pm	<u>13.05</u>	<u>18.02</u>	<u>0.001</u>
<u>Culex vagans</u>	<u>14.33^b</u>	\pm	<u>2.52</u>	<u>5.67^c</u>	\pm	<u>2.08</u>	<u>10.33b^c</u>	\pm	<u>1.52</u>	<u>10b^c</u>	\pm	<u>1</u>	<u>19.33^a</u>	\pm	<u>1.52</u>	<u>24.36</u>	<u>0.001</u>
<u>Anopheles subpictus</u>	<u>107^a</u>	\pm	<u>14.53</u>	<u>14^b</u>	\pm	<u>1</u>	<u>94.33^a</u>	\pm	<u>12.5</u>	<u>0^b</u>	\pm	<u>0</u>	<u>99^a</u>	\pm	<u>18.5</u>	<u>55.79</u>	<u>0.001</u>
<u>Anopheles stephensi</u>	<u>45.67^a</u>	\pm	<u>2.52</u>	<u>9.67^c</u>	\pm	<u>2.08</u>	<u>88.33^b</u>	\pm	<u>1.52</u>	<u>0^d</u>	\pm	<u>0</u>	<u>41.67^b</u>	\pm	<u>2.08</u>	<u>372.42</u>	<u>0.001</u>
<u>Anopheles culicifacies</u>	<u>74^a</u>	\pm	<u>19</u>	<u>7^b</u>	\pm	<u>1</u>	<u>69.67^a</u>	\pm	<u>16.62</u>	<u>0^b</u>	\pm	<u>0</u>	<u>83^a</u>	\pm	<u>11.53</u>	<u>30.86</u>	<u>0.001</u>

<u>Anopheles pulcherrimus</u>	<u>27^a</u>	<u>±</u>	<u>3</u>	<u>9^c</u>	<u>±</u>	<u>1</u>	<u>19.33^b</u>	<u>±</u>	<u>2.52</u>	<u>0^d</u>	<u>±</u>	<u>0</u>	<u>24.33a^b</u>	<u>±</u>	<u>2.08</u>	<u>91.91</u>	<u>0.001</u>
<u>Anopheles nigerrimus</u>	<u>15^a</u>	<u>±</u>	<u>1</u>	<u>3^c</u>	<u>±</u>	<u>1</u>	<u>8^b</u>	<u>±</u>	<u>1</u>	<u>0^c</u>	<u>±</u>	<u>0</u>	<u>12.33^a</u>	<u>±</u>	<u>2.52</u>	<u>62.77</u>	<u>0.001</u>
<u>Aedes albopictus</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>0</u>
<u>Aedes aegypti</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>0</u>

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Figure 4.4-8: Relationship of abundance of species of mosquitoes with seasons in Tehsil Yazman of District Bahawalpur

Species	Mean ± Standard Deviation										F	P						
	Autum n			Winter		Spring		Summe n		Menses n								
<i>Culex</i> <i>quinquefasciatu</i> <i>s</i>	289.67 ^a	±	13.5	109.67 ^d	±	15.1	222.67 ^b	±	10.5	152.67 ^e	±	4	284.67 ^a	±	2	16.6	98.2	0.00
<i>Culex</i> <i>tritaeniorhynchus</i>	226.3 ^a	±	19.5	71.67 ^e	±	7	15.5	152.67 ^b	±	17.0	99 ^e	±	14	226.67 ^a	±	15.5	56.49	0.00
<i>Culex</i> <i>pseudovishnui</i>	36.33 ^a	±	2.08	13.66 ^e	±	1.52	27.33 ^b	±	2.52	18.67 ^e	±	2.08	35.67 ^a	±	2.08	70.5	0.00	
<i>Culex pipiens</i>	102.33 ^a	±	15.5	32.66 ^e	±	1.52	84.67 ^a ^b	±	13.6	51.67 ^b ^e	±	8	108.3 ^a	±	17.5	16.58	0.00	
<i>Culex vagans</i>	16.66 ^b	±	1.52	5.33 ^d	±	1.52	11 ^c	±	2	10.33 ^c ^d	±	2.08	23 ^a	±	3	31.19	0.00	
<i>Anopheles</i> <i>subpictus</i>	120 ^a	±	18	18.67 ^b	±	2.08	101 ^a	±	16	0 ^b	±	0	107.67 ^a	±	4	16.0	55.31	0.00
<i>Anopheles</i> <i>stephensi</i>	33 ^a	±	3	8.67 ^b	±	2.08	30 ^a	±	±	0 ^c	±	0	34.67 ^a	±	2.08	201.8	0.00	
<i>Anopheles</i> <i>culicifacies</i>	67.3 ^a	±	18	7 ^b	±	1	61.7 ^a	±	17.5	0 ^b	±	0	66.67 ^a	±	15.5	19.82	0.00	

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<i>Anopheles pulcherrimus</i>	19.33 ^{a,b}	±	1.52	6.33 ^c	±	1.52	17.33 ^b	±	2.52	0 ^d	±	0	23.67 ^a	±	2.08	94.29	0.00 1
<i>Anopheles nigerrimus</i>	11.66 ^a	±	1.52	2.67 ^{b,c}	±	2.08	6.33 ^b	±	1.52	0 ^e	±	0	11.33 ^a	±	2.08	30.09	0.00 1
<i>Aedes albopictus</i>	0	±	0	0	±	0	0	±	0	0	±	0	0	±	0	0	0
<i>Aedes aegypti</i>	0	±	0	0	±	0	0	±	0	0	±	0	0	±	0	0	0

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Species	Mean ± Standard Deviation										F	P					
	Autumn			Winter		Spring		Summer		Monsoon							
<i>Culex quinquefasciatus</i>	229.33 ^a	±	12.01	80 ^b	±	15.1	89.67 ^b	±	17.01	80.67 ^b	±	11.02	230 ^a	±	20	81.52	0.001
<i>Culex tritaeniorhynchus</i>	227 ^a	±	15.52	77 ^b	±	18	99.67 ^b	±	11.02	93.33 ^b	±	17.04	220.33 ^a	±	14.57	69.04	0.001
<i>Culex pseudovishnui</i>	23.66 ^a	±	1.52	9.33 ^b	±	1.52	7 ^b	±	1	9.67 ^b	±	2.08	27 ^a	±	3	67.72	0.001
<i>Culex pipiens</i>	77.33 ^a	±	10.5	26.33 ^b	±	2.52	30.67 ^b	±	2.08	29.66 ^b	±	1.52	77.33 ^a	±	16.5	26.79	0.001
<i>Culex vagans</i>	9.67 ^a	±	2.08	2 ^b	±	1	6.67 ^a	±	2.08	8.33 ^a	±	1.52	10.33 ^a	±	1.52	11.59	0.001
<i>Anopheles subpictus</i>	86.67 ^a	±	16.56	13 ^b	±	1	86.67 ^a	±	15.28	0	±	0	85.3 ^a	±	18.5	3.99	0.001
<i>Anopheles stephensi</i>	116.67 ^b	±	2.08	4 ^c	±	1	18 ^b	±	3	0 ^c	±	0	23.66 ^a	±	1.52	90.18	0.001

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<u>Anopheles culicifacies</u>	<u>43.33^{a,b}</u>	<u>±</u>	<u>2.08</u>	<u>5.67^c</u>	<u>±</u>	<u>2.08</u>	<u>40^b</u>	<u>±</u>	<u>2</u>	<u>0^d</u>	<u>±</u>	<u>0</u>	<u>47.67^a</u>	<u>±</u>	<u>2.52</u>	<u>403.9</u>	<u>0.001</u>
<u>Anopheles pulcherrimus</u>	<u>10^b</u>	<u>±</u>	<u>1</u>	<u>4.67^c</u>	<u>±</u>	<u>2.08</u>	<u>15^a</u>	<u>±</u>	<u>2</u>	<u>0^d</u>	<u>±</u>	<u>0</u>	<u>10.66^b</u>	<u>±</u>	<u>1.52</u>	<u>43.46</u>	<u>0.001</u>
<u>Anopheles nigerrimus</u>	<u>5.33^a</u>	<u>±</u>	<u>1.52</u>	<u>2^b</u>	<u>±</u>	<u>1</u>	<u>7^a</u>	<u>±</u>	<u>1</u>	<u>0^b</u>	<u>±</u>	<u>0</u>	<u>7^a</u>	<u>±</u>	<u>1</u>	<u>27.72</u>	<u>0.001</u>
<u>Aedes albopictus</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>0</u>
<u>Aedes aegypti</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>±</u>	<u>0</u>	<u>0</u>	<u>0</u>

Figure 4.4-9: Relationship of abundance of species of mosquitoes with seasons in Tehsil Ahmadpur East of District Bahawalpur

Table 4.4.2.4: Relationship of abundance of species of mosquitoes with seasons in Tehsil Yazman of District Bahawalpur

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

Table 4.4.2.5: 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	227 ^a	<u>±</u>	15.72	74 ^b	<u>±</u>	15.52	104 ^b	<u>±</u>	17.09	98 ^b	<u>±</u>	12	234.33 ^a	<u>±</u>	12.06	82.78	0.001

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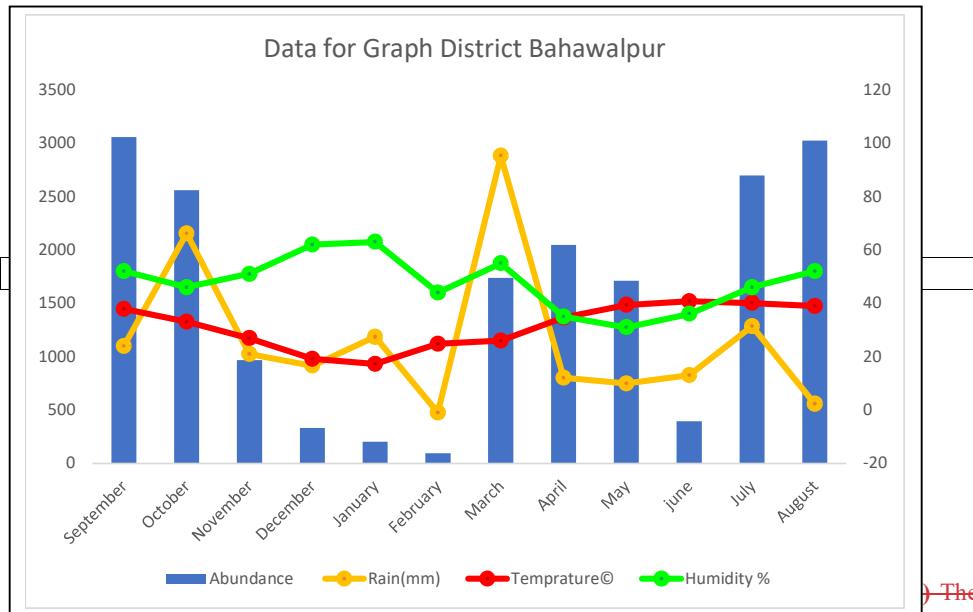
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Culex tritaeniorhynchus	251 ^a	±	15	108 ^b	±	14	108 ^b	±	14.73	103.33 ^b	±	11.02	251 ^a	±	15.1	95.29	0.001
Culex pseudovishnui	31.33 ^a	±	2.52	13.67 ^b	±	2.08	10 ^b	±	1	11 ^b	±	2	27 ^a	±	3	59.1	0.001
Culex pipiens	95.67 ^a	±	16.5	34.33 ^b	±	1.528	37.67 ^b	±	2.08	38.67 ^b	±	2.52	92 ^a	±	14.73	29.18	0.001
Culex vagans	10 ^a	±	1	2 ^b	±	1	6.67 ^a	±	2.08	8.33 ^a	±	1.52	10.33 ^a	±	1.52	15.65	0.001
Anopheles subpictus	96.67 ^a	±	16.04	14 ^b	±	3	104 ^a	±	14.11	0	±	0	110 ^a	±	13.11	66.93	0.001
Anopheles stephensi	19.33 ^a	±	2.52	5 ^b	±	1	19.67 ^a	±	2.08	0 ^c	±	0	20 ^a	±	2	87.69	0.001
Anopheles culcifacies	50.33 ^a	±	1.52	6.67 ^b	±	2.08	46.33 ^a	±	1.52	0 ^b	±	0	55 ^a	±	19	27.73	0.001
Anopheles pulcherrimus	11.33 ^b	±	1.52	3 ^c	±	1	16 ^a	±	2	0 ^c	±	0	9.67 ^b	±	2.08	53.64	0.001
Anopheles nigerrimus	6a ^b	±	1	2.67b ^c	±	2.08	6a ^b	±	1	0 ^c	±	0	8.67 ^a	±	2.08	15.94	0.001
Aedes albopictus	64 ^a	±	12.53	16.33 ^b	±	2.08	24 ^b	±	2	23.33 ^b	±	2.08	83.33 ^a	±	14.05	36	0.001
Aedes aegypti	28.33 ^a	±	2.08	6.33 ^c	±	1.52	9.67 ^c	±	2.08	11 ^c	±	2	21 ^b	±	1	78.04	0.001

Figure 4.4-10: Relationship of abundance of species of mosquitoes with seasons in Tehsil Bahawalpur of District Bahawalpur

4.4.3 Effect of climatic factors on the abundance of mosquitoes in District Bahawalpur

The months of March and April were found to be conducive for the replication of the mosquitoes due to high rainfall optimum temperature and humidity and humidity in district Bahawalpur. High abundance of mosquitoes were observed in the month of October as there was moderate amount of rainfall. The month of August were observed to be highly favorable with reference to the reproduction of mosquitoes (Figure 4.4.3)



The association between abundance and the season of the other species displayed the similar trend as that of Culex and Anopheles in all the tehsil Khaipur (Table 4.4.2.3) tehsil Yazman (Table 4.4.2.4) tehsil Ahmedpur east (Table 4.4.2.5) and tehsil Bahawalpur district (Table 4.4.2.6).

4.4.3 Effect of climatic factors on the abundance of mosquitoes in District Bahawalpur

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Figure 4.4-11: Effect of climatic variables on relative abundance of mosquito species in district Bahawalpur

4.3.2

4.3.3

4.4.4 Comparative analysis between rural and urban areas of tehsils of Bahawalpur

-The mosquito populations in various Bahawalpur district tehsils were compared between rural and urban areas using the unpaired t-test. The results showed that, in comparison to urban regions, the tehsils of Yazman (**Figure 4.4.4.1**), Khaipur (**Figure 4.4.4.2**), Ahmedpur East (**Figure 4.4.4.3**) and Saddar (**Figure 4.4.4.4**) had significantly higher mosquito abundances. A very significant result was noted by Tehsil Yazman ($t=2.64$, $p=0.028$, $F=3.530$). The lack of statistical significance in the data Bahawalpur ($t=1.617$, $p=0.144$) suggests that these regions are substantially urbanized and do not distinguish between rural and urban areas. (**Table 4.4.4.1**).

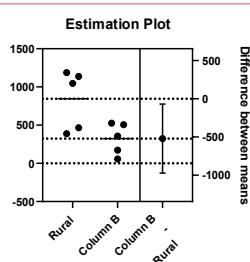
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Tehsil	t	p	F	Difference between Means ±Standard Error
Yazman	2.664	0.0286	3.530	521.4±195.6
Khairpur	2.50	0.0347	3.247	528±207.9
Ahmedpur East	2.38	0.044	3.163	410.4±171.9
Saddar	2.059	0.0734	2.291	523.2±245
Hasilpur	2	0.0396	3.117	561.6±228.7
Bahawalpur	1.617	0.1446	1.86	326.4±201.9

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Figure 4.4-12: T test between the rural and urban areas of different Tehsils of district of Bahawalpur



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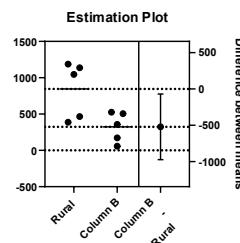


Figure 4.4-13: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Khaipur of District Bahawalpur

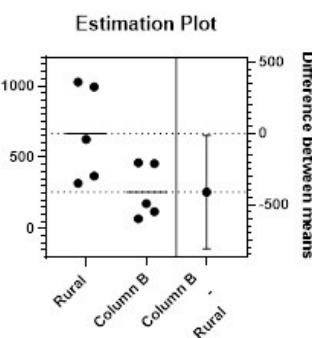


Figure 4.4-14: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Yazman of District Bahawalpur

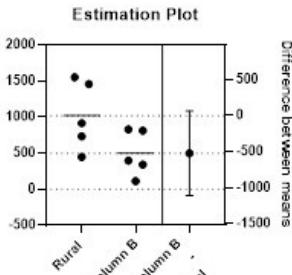


Figure 4.4.4.3: Significant results of difference of abundance of mosquito in Rural and Urban areas of Tehsil Saddr of District Bahawalpur

Figure 4.4-16: Non-Significant results of difference of abundance of mosquito in Rural and Urban areas of Tehsil Saddr of District Bahawalpur

abundance of mosquito in rural and urban areas of Tehsil Ahmed pur East of District Bahawalpur

Figure 4.4-15: Significant results of difference of abundance of mosquito in Rural and Urban areas of Tehsil Ahmed pur East of District Bahawalpur

Figure 4.4.1: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Yazman of District Bahawalpur

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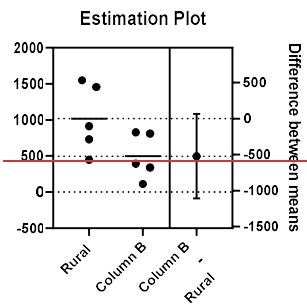


Figure 4.4.4.18: Non-Significant results of difference of abundance of mosquito in Rural and Urban areas of Tehsil Bahawalpur of District Bahawalpur

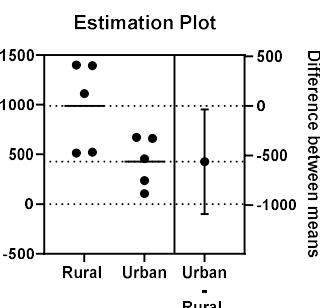


Figure 4.4.17: Significant results of difference of abundance of mosquito in Rural and Urban areas of Tehsil Hasilpur of District Bahawalpur

Figure 4.4.1: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Yazman of District Bahawalpur

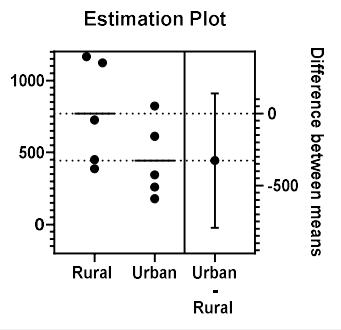


Figure 4.4.4.4: Non-Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Saddar of District Bahawalpur

Figure 4.4.4.5: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Hasilpur of District Bahawalpur

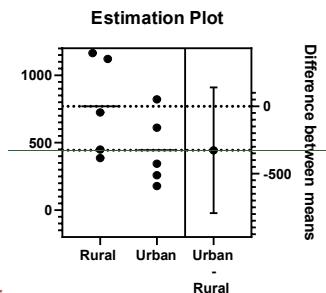
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4.3.4



4.3.5

Figure 4.4.4.6: Non-Significant results of difference of

abundance of mosquito in rural and urban areas of Tehsil Bahawalpur

4.3.6

of District Bahawalpur

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4.4.5 GIS Mapping

According to the [GIS map](#) all the tehsils of Bahawalpur are found to be rich in mosquito [genus Anopheles](#) [genus Anopheles](#) and [Culex](#). Nonetheless, it was discovered that the genus [Aedes](#) was also widely distributed in the tehsils of Saddar and Bahawalpur.

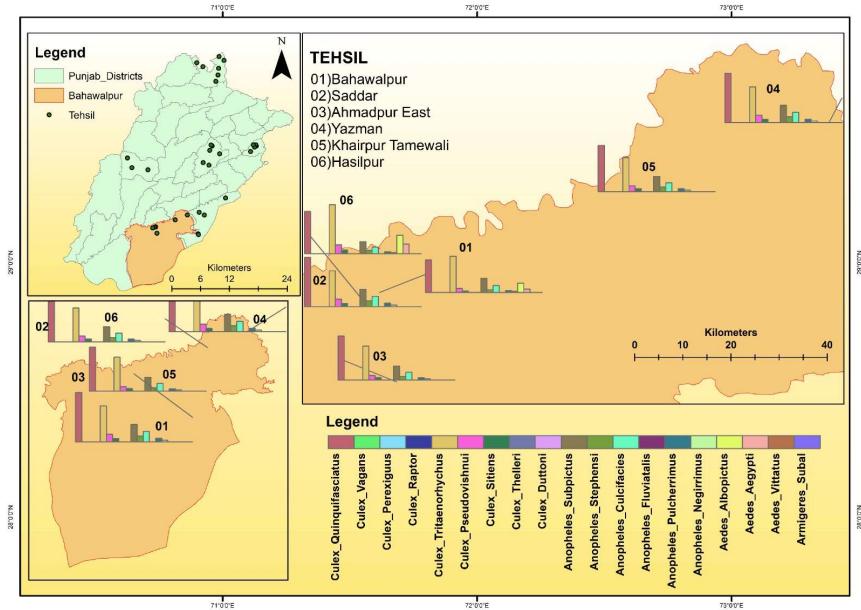


Figure 4.4-19: GIS map showing mosquito abundance in different tehsils of district Bahawalpur.

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4.4

4.5 Thal Zone District Layyah

Main Layyah City, Choubara, and Karor Lal Esan were the sampling sites for adult mosquitoes and larvae. In district Layyah 9200 mosquitoes—~~of~~of mosquitoes of eleven species were collected. *Culex* was the ~~most~~more common of the two genera (76%), with *Anopheles* coming in second (24%). There was no presence of the genus *Aedes* in any of the three tehsils. It was noted that tehsil Karor Lal Esan had the greatest abundance of mosquitoes, while tehsil Choubara had the lowest abundance. There were six species found in the genus *Culex*, including *Culex quinquefasciatus* (29%) *Culex tritaeniorhynchus* (21%), *Culex pseudovishnui* (13%), *Culex pipiens* (6%), *Culex Theilleri* (4%), *Culex Duttoni* (3%). Similarly five *Anopheles* species were discovered, namely, *Anopheles Subpictus* (10%), *Anopheles Culcifacies* (6%), *Anopheles Stephensi* (4%), *Anopheles Pulcherrimus* (3%), and *Anopheles Nigerrimus* (1%). (**Figure 4.5.**)

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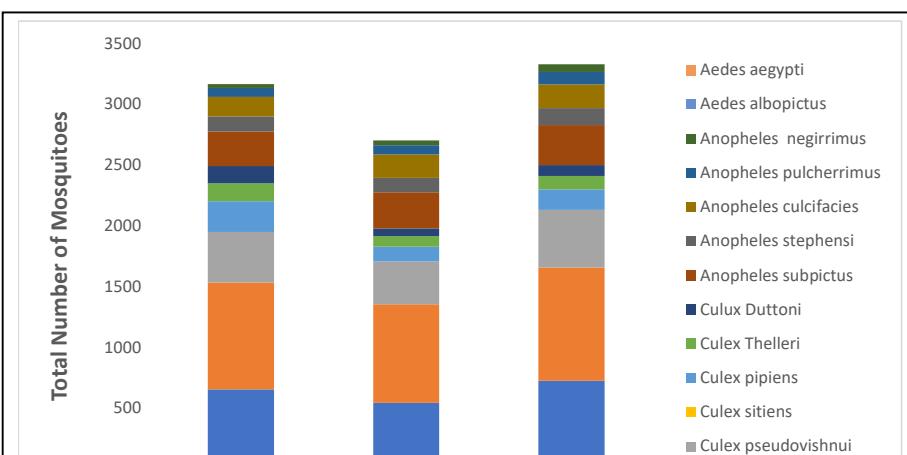


Figure 4.5-1: Total number of mosquitoes in district Layyah

4.4.14.5.1 Species Diversity and Abundance of mosquitoes in district Layyah

There were notable variations throughout the tehsils for the diversity of species identified. For the three tehsils, the Shannon and Simpson diversity indices varied from 2.05 to 2.01 and 0.89 to 0.82. Tehsil Layyah has the highest values for both indexes, whereas Tehsil Choubara has the lowest values. (**Table 4.5.1.1**)

Name of the species	Layyah Total counts				Karor Lal Esan Total counts				Choubara Total counts				53
	Total counts	%age	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)	Total counts	%age	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)	Total counts	%age	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)	
Name of the species	Total counts	%age	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)	Total counts	%age	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)	Total counts	%age	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)	
Culex quinquefasciatus	655	20.69	0.3260	428370	727	21.83	0.3322	527802	546	20.19	0.3231	2975	Formatted
Culex tritaeniorhynchus	881	27.83	0.3560	775280	932	27.99	0.3564	867692	810	29.96	0.3611	6552	Formatted
Culex pseudovishnui	416	13.14	0.2667	172640	472	14.17	0.2769	222312	352	13.02	0.2654	1235	Formatted
Culex sitiens	0	0.00	0.00	0.00	0	0.00	0.0000	0	0	0.00	0.0000	0	Formatted
Culex pipiens	251	7.93	0.2010	62750	168	5.05	0.1507	28056	122	4.51	0.1398	147	Formatted
Culex Thelleri	146	4.61	0.1419	21170	112	3.36	0.1141	12432	86	3.18	0.1097	731	Formatted
Culux Duttoni	145	4.58	0.1412	20880	91	2.73	0.0984	8190	64	2.37	0.0886	403	Formatted
Anopheles subpictus	281	8.88	0.2150	78680	327	9.82	0.2279	106602	296	10.95	0.2422	873	Formatted
Anopheles stephensi	125	3.95	0.1276	15500	141	4.23	0.1339	19740	120	4.44	0.1382	142	Formatted
Anopheles culicifacies	162	5.12	0.1521	26082	194	5.83	0.1656	37442	192	7.10	0.1878	366	Formatted
Anopheles pulcherrimus	72	2.27	0.0860	5112	101	3.03	0.1060	10100	76	2.81	0.1004	570	Formatted
Anopheles negirrimus	32	1.01	0.0464	992	65	1.95	0.0768	4160	40	1.48	0.0623	156	Formatted
Aedes albopictus	0	0.00	0.00	0	0	0.00	0.0000	0	0	0.00	0.0000	0	Formatted

... [228] ... [231] ... [232] ... [234] ... [236] ... [233] ... [235] ... [237] ... [229] ... [230] ... [238] ... [239] ... [240] ... [241] ... [242] ... [243] ... [244] ... [245] ... [246] ... [247] ... [248] ... [249] ... [250] ... [251] ... [252] ... [253] ... [254] ... [255] ... [256] ... [257] ... [258] ... [259] ... [260] ... [261] ... [262] ... [263]

Aedes aegypti	0	0.00	0.00	0	0	0.00	0.0000	0	0	0.00	0.0000	0
Total	3166		H=2.0598	D=0.8396	3330		2.0390	0.8336	2704		2.0186	0.82

Figure 4.5-2

: Diversity index of a number of individuals of different species of mosquitoes collected from six tehsils of District Layyah

Analysis of variance (ANOVA) tests revealed significant differences in mosquito abundance across all the tehsils. *Culex quinquefasciatus* (Figure 4.5.1.1.1) had the largest abundance of all the mosquitoes ($F = 79.73$, $df = 5$, $P < 0.001$), which were detected in large quantities in Karor Lal Esan (Mean = 726, SD = 17), Layyah (Mean = 653, SD = 17.09), and Choubara (Mean = 547.7, SD = 17.6) (Table 4.5.1.2). *Culex tritaeniorhynchus* (Figure 4.5.1.1.2), *Culex pseudovishnui* (Figure 4.5.1.1.3), *Culex pipiens* (Figure 4.5.1.1.4), *Culex theileri*, (Figure 4.5.1.1.5) and *Culex duttoni* (Figure 4.5.1.1.6) all showed the same outcomes. Given their high prevalence in the tehsil Karor Lal Esan, *Anopheles subpictus* (Figure 4.5.1.1.7) *Anopheles stephensi*, (Figure 4.5.1.1.8) *Anopheles culcifacies* (Figure 4.5.1.1.9) *Anopheles pulcherrimus* (Figure 4.5.1.1.10) and *Anopheles nigerrimus* (Figure 4.5.1.1.11) displayed distinct patterns of abundance.

<u>Species</u>	<u>Mean ± Standard Deviation</u>						<u>F</u>	<u>P</u>
	<u>Layyah</u>		<u>Choubara</u>		<u>Karor Lal Esan</u>			
<i>Cx. quinquefasciatus</i>	653 ^b	± 17.09	547.7 ^c	± 17.6	726 ^a	± 17.5	79.7 ³	0.00 ¹
<i>Cx. tritaeniorhynchus</i>	884 ^b	± 14.73	812.6 ^c	± 15.1	931.7 ^a	± 19.5	39 ³	0.00 ¹
<i>Cx. pseudovishnui</i>	416 ^b	± 11	350.3 ^c	± 15.5	470 ^a	± 9	49.3 ²	0.00 ¹
<i>Cx. pipiens</i>	254 ^a	± 14.73	122.3 ^b	± 14.5	167 ^b	± 2	57.6 ²	0.00 ¹
<i>Cx. Thelleri</i>	146.33 ^a	± 13.5	83.33 ^b	± 15.1	113.67 ^a	± 16.5	13 ¹	0.00 ¹
<i>Cx. Duttoni</i>	143 ^a	± 13.11	65.33 ^b	± 13.0	91.3 ^b	± 18.5	20.5 ⁴	0.00 ¹
<i>An. subpictus</i>	281.33 ^c	± 3.51	295.3 ^b	± 5.03	326.33 ^a	± 5.03	75.7 ⁶	0.00 ¹
<i>An. stephensi</i>	-	± -	-	± -	-	± -	-	0.00 ⁴
<i>An. culcifacies</i>	162 ^b	± 4	191.3 ^a	± 6.03	194.67 ^a	± 5.03	37.4 ⁴	0.00 ¹
<i>An. pulcherrimus</i>	72 ^b	± 4	75.67 ^b	± 4.51	101 ^a	± 5	36.5 ⁹	0.00 ¹
<i>An. nigerrimus</i>	32 ^b	± 1	39.66 ^b	± 1.52	62.67 ^a	± 5	12.0 ⁹	0.00 ¹

<u>Mean ± Standard Deviation</u>								
<u>Species</u>	<u>Layyah</u>	<u>Choubara</u>	<u>Karor Lal Esan</u>	<u>F</u>	<u>P</u>			
<i>Cx. quinquefasciatus</i>	653 ^b ±17,09	547.7 ^c ±17,6	726 ^a ±17,5	79.73	0.001			
<i>Cx. tritaeniorhynchus</i>	884 ^b ±14,73	812.67 ^c ±15,18	931.7 ^a ±19,5	39	0.001			

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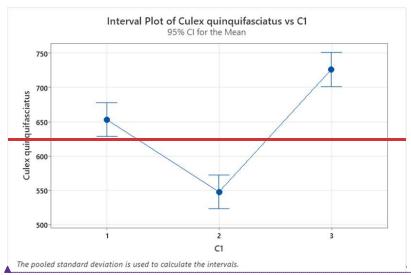
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<u>Cx. pseudovishnui</u>	<u>416^b±11</u>	<u>350.33^c±15,57</u>	<u>470^a±17,09</u>	<u>49.32</u>	<u>0.001</u>
<u>Cx. pipiens</u>	<u>254^a±14,73</u>	<u>122.33^c±14,5</u>	<u>167^b±16,52</u>	<u>57.62</u>	<u>0.001</u>
<u>Cx. theileri</u>	<u>146.33^a±13,</u>				
<u>Cx. duttoni</u>	<u>143^a±13,11</u>	<u>65.33^b±13,05</u>	<u>91.3^b±18,5</u>	<u>20.54</u>	<u>0.001</u>
<u>An.subpictus</u>	<u>281.33^c±3,51</u>	<u>295.33^b±5,03</u>	<u>326.33^a±5,03</u>	<u>75.76</u>	<u>0.001</u>
<u>An.stephensi</u>	<u>±</u>	<u>±</u>	<u>±</u>	-	<u>0.001</u>
<u>An.culcifacies</u>	<u>162^b±4</u>	<u>191.33^a±6,03</u>	<u>194.67^a±5,03</u>	<u>37.44</u>	<u>0.001</u>
<u>An.pulcherrimus</u>	<u>72^b±4</u>	<u>75.67^b±4,51</u>	<u>101^a±5</u>	<u>36.59</u>	<u>0.001</u>
<u>An.nigerrimus</u>	<u>32^b±1</u>	<u>39.66^b±1,52</u>	<u>62.67^a±13,65</u>	<u>12.09</u>	<u>0.001</u>

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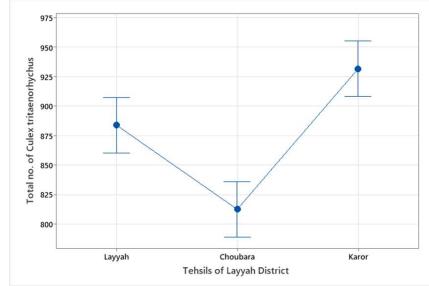
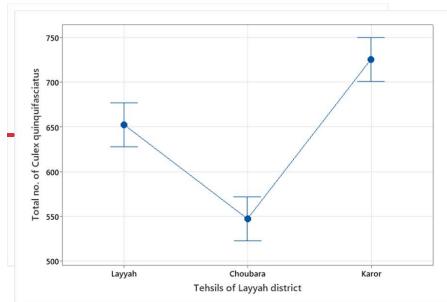
Table 4.5.1.2: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of Bahawalpur



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Figure 4.5-3: Comparative analysis of abundance of mosquitoes in different Tehsils of Layyah



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Figure 4.5.1.1.1: Abundance of *Culex quinquefasciatus*

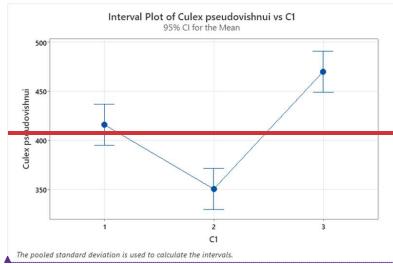
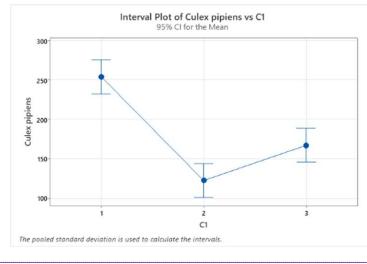


Figure 4.5.1.1.2: Abundance of *Culex tritaeniorhynchus*



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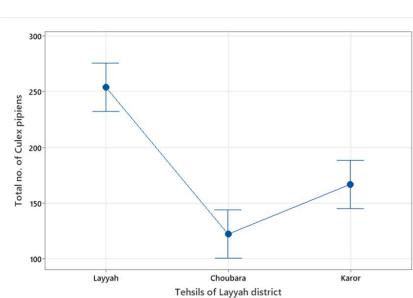
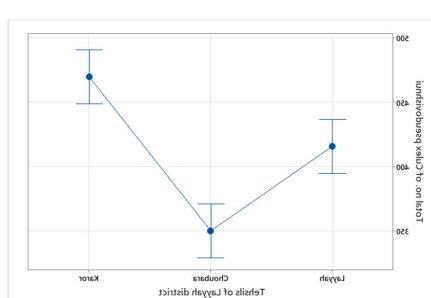


Figure 4.5.1.1.3: Abundance of *Culex pseudovishnui*

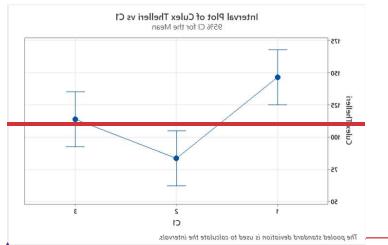
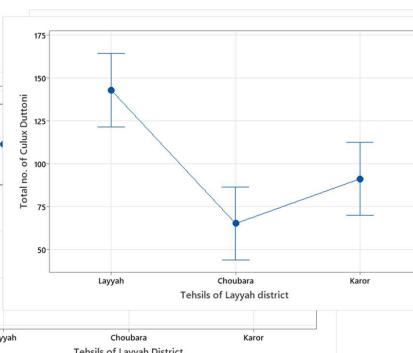


Figure 4.5.1.1.4: Abundance of *Culex pipiens*



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Figure 4.5.1.1.5: Abundance of *Culex theileri*

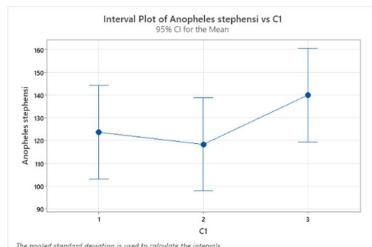
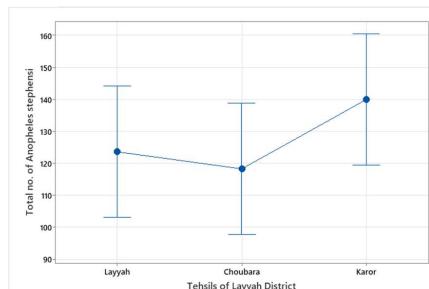


Figure 4.5.1.1.6: Abundance of *Culex duttoni*



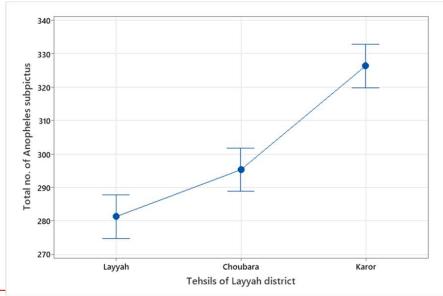


Figure 4.5.1.1.7: Abundance of *Anopheles subpictus*

Figure 4.5.1.8: Abundance of *Anopheles stephensi*

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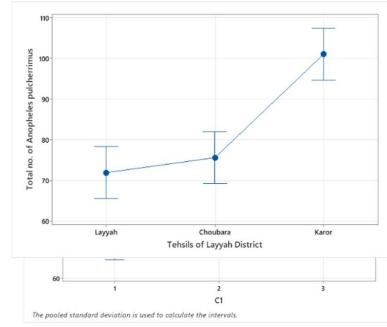
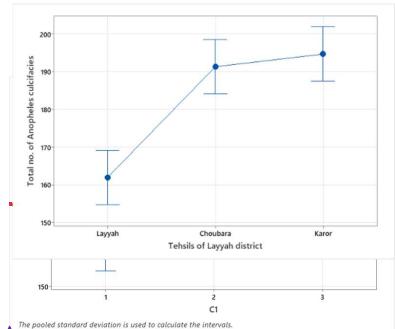


Figure 4.5.1.9: Abundance of *Anopheles culicifacies*

Figure 4.5.1.10: Abundance of *Anopheles pulcherrimus*

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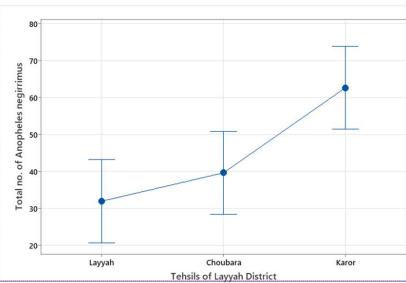
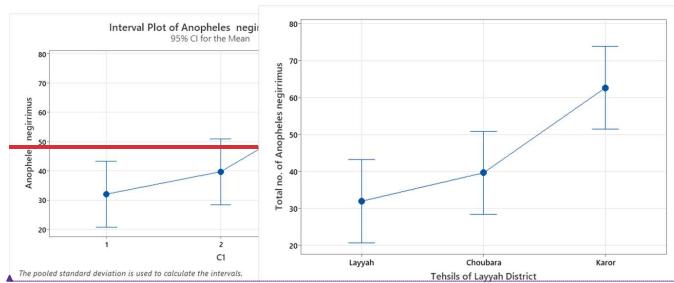


Figure 4.5.1.11: Abundance of *Anopheles nigerrimus*

Figure 4.5-4: Relative abundance of different species of mosquito in different tehsils of district Layyah

4.5.2 Relationship of the abundance of mosquitoes with seasons in district Layyah

Different outcomes were observed by applying one-way ANOVA on the effect of season abundance of mosquitoes. The study found that the mosquito population in each tehsil peaked in the monsoon season and then declined in the fall. The spring brought with it a greater number of mosquitoes than in the summer. The lowest abundance was observed in winter. The highest *Culex quinquefasciatus* count was discovered in Tehsil Karor Lal Esan (**Table 4.5.2.1**) ($F = 181.67$, $df = 4$, $P = 0.001$). In this tehsil the monsoon season (Mean = 214.33, SD = 14.19) and the autumn season (Mean = 181.67, SD = 13.5) saw its highest level of abundance, while the winter saw a steep drop in abundance (Mean = 71.33, SD = 11.5). It rises once more in the spring (Mean = 76, SD = 20), then it falls in the summer (Mean = 109, SD = 11.5). In the same way *Anopheles subpictus* of the genus *Anopheles* showed a similar pattern of abundance: highest in the monsoon (Mean = 95.3, SD = 14.51), followed by autumn (Mean = 91.67, SD = 11.5), spring (Mean = 60.33, SD = 13.58), summer (Mean = 25.0, SD = 1), and lowest in the winter (Mean = 5.0, SD = 1). In all the other tehsils in Choubara (**Table 4.5.2.2**) and district Layyah (**Table 4.5.2.3**) the relationship between the abundance and season of the other species showed a similar trend to that of *Culex* and *Anopheles*.

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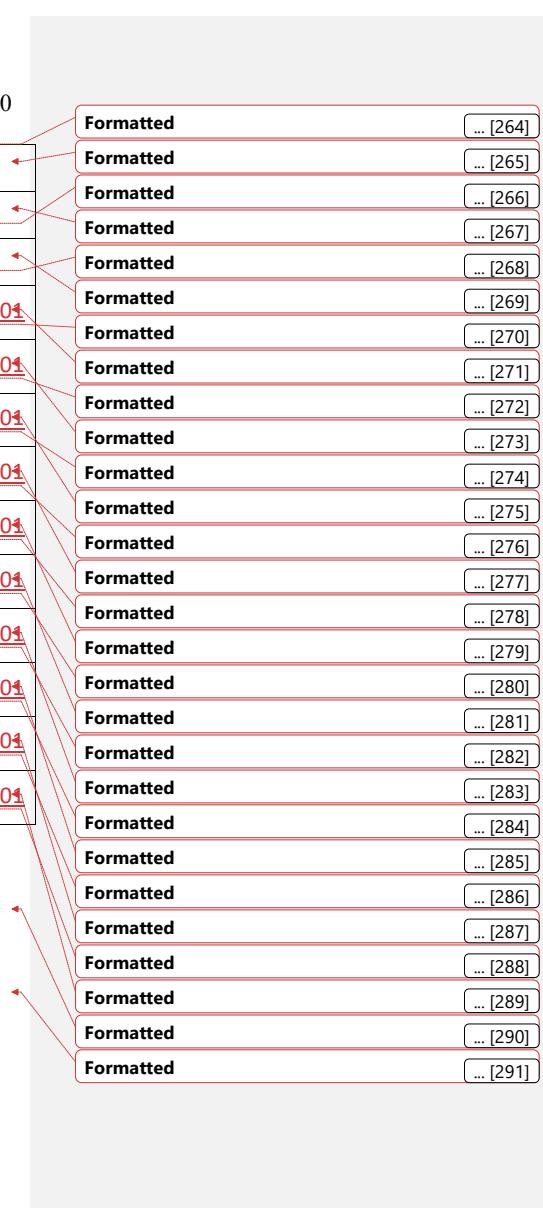
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Species	Mean ± Standard Deviation							
	Autumn	Winter	Spring	Summer	Monsoon	F	P	
<i>Cx. quinquefasciatus</i>	-	-	-	-	-	-	-	
<i>Cx. tritaeniorhynchus</i>	<u>246.67^b±12,58</u>	<u>122.67^c±15,18</u>	<u>120^d±14,73</u>	<u>156.67^c±12,5</u>	<u>292^a±12,53</u>	<u>98.47</u>	<u>0.001</u>	
<i>Cx. pseudovishnui</i>	<u>1222^b±17,5</u>	<u>39^d±1</u>	<u>61.67^c±17,01</u>	<u>89^b±17,5</u>	<u>163.3^a±14,5</u>	<u>32.57</u>	<u>0.001</u>	
<i>Cx. pipiens</i>	<u>13.33^b±1,52</u>	<u>15.33^c±1,52</u>	<u>32^b±1</u>	<u>39.6a^b±1,52</u>	<u>5.3^a±11,02</u>	<u>19.24</u>	<u>0.001</u>	
<i>Cx. theileri</i>	<u>26^b±3</u>	<u>11.33^c±2,52</u>	<u>23a^b±2</u>	<u>22.33^b±2,52</u>	<u>29.67^a±2,52</u>	<u>22.1</u>	<u>0.001</u>	
<i>Cx. duttoni</i>	<u>21^b±1</u>	<u>6.3^d±1,52</u>	<u>16.3^c±1,52</u>	<u>18.3b^c±2,52</u>	<u>30.33^a±1,52</u>	<u>78.23</u>	<u>0.001</u>	
<i>An. subpictus</i>	<u>103.33^a±14,01</u>	<u>12^b±2</u>	<u>99^a±11,53</u>	<u>0^b±0</u>	<u>112.67^a±15,5</u>	<u>77.99</u>	<u>0.001</u>	
<i>An. stephensi</i>	<u>46.33^a±2,52</u>	<u>5.3^b±1,52</u>	<u>37.66^a±1,52</u>	<u>0^b±0</u>	<u>54^a±15,1</u>	<u>37.67</u>	<u>0.001</u>	
<i>An. culicifacies</i>	<u>62^a±11,53</u>	<u>7.67^b±2,08</u>	<u>54.67^a±14,01</u>	<u>0^b±0</u>	<u>70.0^a±17,09</u>	<u>25.4</u>	<u>0.001</u>	
<i>An. pulcherrimus</i>	<u>34.33^a±2,08</u>	<u>3^c±1</u>	<u>24.33^b±2,08</u>	<u>0^c±0</u>	<u>37.33^a±2,08</u>	<u>325.04</u>	<u>0.001</u>	
<i>An. nigerrimus</i>	<u>19.67^b±2,52</u>	<u>2^c±1</u>	<u>16^b±1</u>	<u>0^c±0</u>	<u>26.33^a±2,08</u>	<u>154.25</u>	<u>0.001</u>	

Figure 4.5-5: Relationship of abundance of species of mosquitoes with seasons in Tehsil Karor Lal Esan of District Layyah



Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	±	±	±	±	±	-	-
<i>Cx. tritaeniorhynchus</i>	<u>229^b±41,53</u>	<u>102.33^c±13,65</u>	<u>88.3^c±18,5</u>	<u>1221^c±15,72</u>	<u>273.6^a±13,5</u>	<u>98.47</u>	<u>0.001</u>
<i>Cx. pseudovishnui</i>	<u>106.7^a±18</u>	<u>21.67^b±2,52</u>	<u>47.6^b±1,52</u>	<u>49.3^b±2,08</u>	<u>123^a±18,5</u>	<u>32.57</u>	<u>0.001</u>
<i>Cx. pipiens</i>	<u>29.6^b±1,52</u>	<u>14^e±1</u>	<u>18.3^d±1,52</u>	<u>25.6^c±1,52</u>	<u>33.6^a±1,52</u>	<u>19.24</u>	<u>0.001</u>
<i>Cx. theileri</i>	<u>20.33^b±1,52</u>	<u>10^c±1</u>	<u>10^c±1</u>	<u>18^b±1</u>	<u>27.6^a±1,52</u>	<u>22.1</u>	<u>0.001</u>
<i>Cx. duttoni</i>	±	±	±	±	±	<u>78.23</u>	<u>0.001</u>
<i>An.subpictus</i>	<u>87.3^a±14,19</u>	<u>10^c±1</u>	<u>44.3^b±1,52</u>	<u>49.3^b±14,19</u>	<u>98^a±14,11</u>	<u>77.99</u>	<u>0.001</u>
<i>An.stephensi</i>	<u>38.3^a±2,52</u>	<u>4^d±1</u>	<u>21.67^c±2,52</u>	<u>26^b±1</u>	<u>30.33^b±2,52</u>	<u>37.67</u>	<u>0.001</u>
<i>An.culcifacies</i>	<u>59.33^a±15,28</u>	<u>8.67^c±2,08</u>	<u>30^b±1</u>	<u>38a^b±2</u>	<u>61.67^a±14,57</u>	<u>25.4</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>21.66^a±1,52</u>	<u>2.33^c±1,52</u>	<u>13.33^b±2,08</u>	<u>20.67^a±2,08</u>	<u>18^a±1</u>	<u>325.04</u>	<u>0.001</u>
<i>An.nigerrimus</i>	<u>10.67^a±2,08</u>	<u>2.33^b±1,52</u>	<u>8.33^a±1,52</u>	<u>10^a±1</u>	<u>10.67^a±2,08</u>	<u>154.25</u>	<u>0.001</u>

Figure 4.5-6: Relationship of abundance of species of mosquitoes with seasons in Tehsil Choubara of District Layyah

Mean ± Standard Deviation							
Species	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	<u>181.67^a±13,5</u>	<u>71.33^b±11,5</u>	<u>76^b±20</u>	<u>109^b±20</u>	<u>214.33^a±14,19</u>	-	-
<i>Cx. tritaeniorhynchus</i>	<u>24.17^b±18</u>	<u>103^c±18,5</u>	<u>107.67^c±16,01</u>	<u>135.33^c±14,01</u>	<u>295.33^a±13,65</u>	<u>98.47</u>	<u>0.001</u>
<i>Cx. pseudovishnui</i>	<u>108^b±17</u>	<u>22.35^d±2,52</u>	<u>51^c±14</u>	<u>66^c±15,72</u>	<u>165^a±14,53</u>	<u>32.57</u>	<u>0.001</u>
<i>Cx. pipiens</i>	<u>42.67^b±1,52</u>	<u>11.33^c±2,08</u>	<u>47^b±2</u>	<u>66^a^b±17,9</u>	<u>81^a±19</u>	<u>19.24</u>	<u>0.001</u>
<i>Cx. theileri</i>	<u>28^b±3</u>	<u>9.67^c±2,08</u>	<u>24.67^b^c±2,08</u>	<u>33.33^b±2,08</u>	<u>52^a±13,53</u>	<u>22.1</u>	<u>0.001</u>
<i>Cx. duttoni</i>	<u>38.33^b±2,5</u>	<u>1.67^d±2,08</u>	<u>24,3^c±1,52</u>	<u>32.6^b±2,52</u>	<u>49.6^a±2,08</u>	<u>78.23</u>	<u>0.001</u>
<i>An. subpictus</i>	<u>91.67^a±11,5</u>	<u>5.0^b±1</u>	<u>60.33^b±13,58</u>	<u>25.0^b±1</u>	<u>95.3^a±14,51</u>	<u>77.99</u>	<u>0.001</u>
<i>An. stephensi</i>	<u>39.33^a±2</u>	<u>2^d±1</u>	<u>24.6^b±1,52</u>	<u>16.6^c±2</u>	<u>42.3^a±2,52</u>	<u>37.67</u>	<u>0.001</u>
<i>An. culcifacies</i>	<u>50.33^a±1,52</u>	<u>3.67^d±2,08</u>	<u>37.33^b±1,52</u>	<u>22^c±2</u>	<u>49.6^a±2,52</u>	<u>25.4</u>	<u>0.001</u>
<i>An. pulcherrimus</i>	<u>26.33^a±1,52</u>	<u>0^d±0</u>	<u>13.67^b±2,52</u>	<u>7.3^c±1,52</u>	<u>25.3^a±1,52</u>	<u>325.04</u>	<u>0.001</u>
<i>An. nigerrimus</i>	<u>11.6^a±2,52</u>	<u>0^c±0</u>	<u>6^b±1</u>	<u>2^b±1</u>	<u>12.67^a±2,08</u>	<u>154.25</u>	<u>0.001</u>

Figure 4.5-7: Relationship of abundance of species of mosquitoes with seasons in Tehsil Layyah of District Layyah

4.5.3 Effect of climatic factors on the abundance of mosquitoes in District Layyah

The results of climatic factors in district Layyah was same as that of district Bahawalnagar that high rain was found in the month of March. Both of the districts lies in the desert areas therefore the climatic conditions were found to be the same affecting the fauna of mosquito (**Figure 4.5.3**).

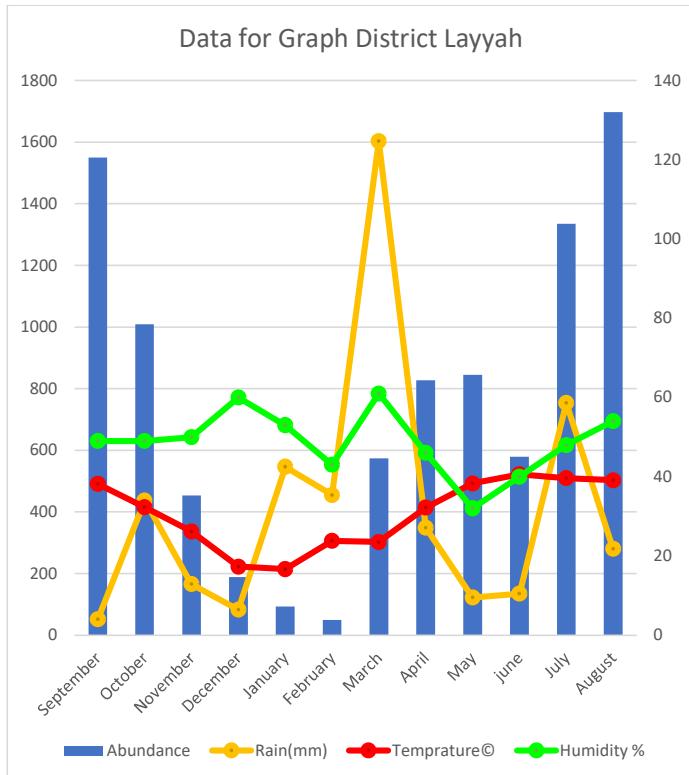


Figure 4.5.8: Effect of climatic variables on relative abundance of mosquito species in district Layyah.

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4.5.4 Comparative analysis between rural and urban areas of tehsils of Layyah

The mosquito populations in the Layyah district were compared between rural and urban areas using the unpaired t-test. Between rural and urban areas, Tehsil Choubara (**Figure 4.4.5.1**) found a significant difference ($t=2.991$, $p=0.0178$, $F=2.132$). The results for tehsil Layyah (**Figure 4.4.5.2**) were likewise quite high ($t=2.94$, $p=0.018$, $F=3.228$). The findings in the Karor Lal Esan (**Figure 4.4.5.3**) tehsils showed a significant disparity in abundance between rural and urban areas ($t=2.38$, $p=0.023$, $F=2.42$) (**Table 4.4.5.1**)

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Tehsils	t	p	F	Difference between Means ±Standard Error
Choubara	2.991	0.0178	2.132	259.6±86.79
Layyah	2.94	0.018	3.228	330.4±112.1
Karor Lal Esan	2.38	0.023	2.42	298.4±106.7

Figure 4.5-9: T test between the rural and urban areas of different Tehsils of district of Bahawalpur

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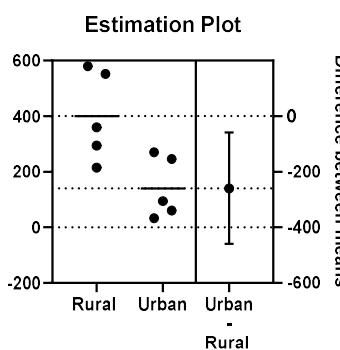


Figure 4.5-11: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Layyah of District Layyah

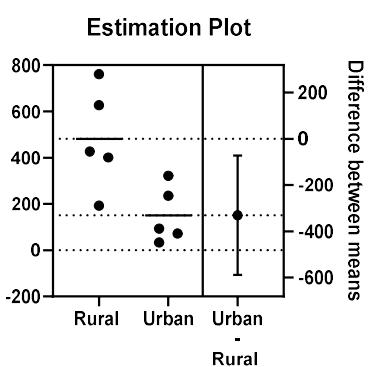


Figure 4.5-10: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Choubara of District Layyah

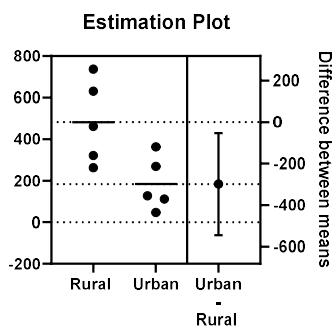


Figure 4.4.5.1: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Choubara of

difference of

Figure 4.4.5.2: Significant results of difference of abundance of mosquito in rural and urban areas of Tehsil Layyah of District Layyah

district Layyah

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4.4.2

4.4.3

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4.4.5

4.4.6 Figure 4.4.5.3: Significant results of difference of

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4.4.7 abundance of mosquito in rural and

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urban areas of Tehsil Karor Lal Esan of District Layyah

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4.5.5 4.4.5 :GIS Mapping

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Genus *Culex* and *Anopheles* ~~were prominent~~were prominent in all the tehsils of Layyah as

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highlighted in GIS mapGIS map. (Figure 4.4.5.1)

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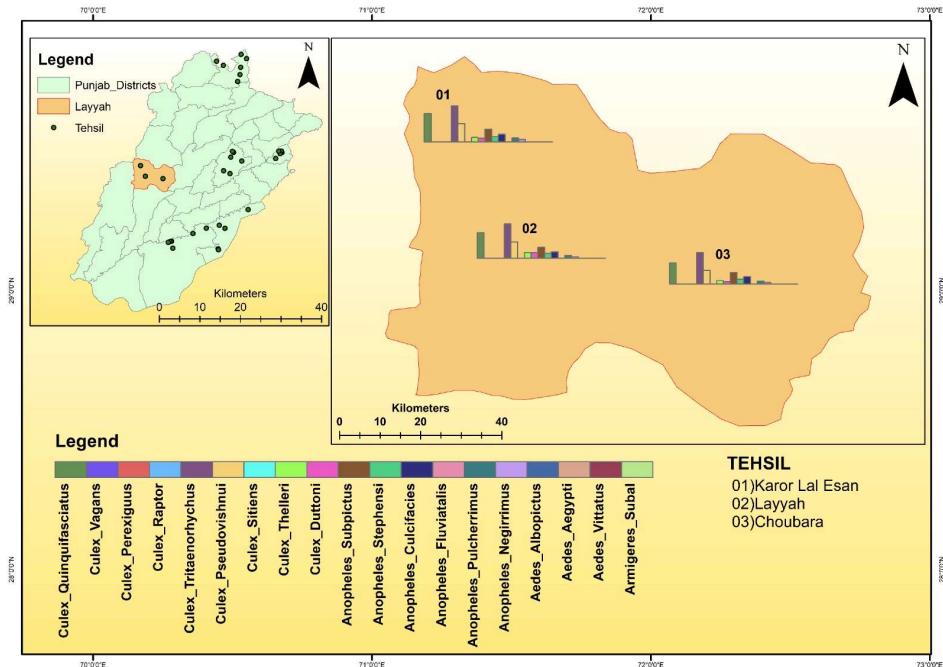


Figure 4.5-13: GIS map showing mosquito abundance in different tehsils of district Layyah.

4.6 Barani Zone District Rawalpindi

It is made up of seven administrative regions which are Gujar Khan, Kallar-Syedan, Kahuta, Kotli Sattian, Rawalpindi, Taxila, and Murree. This district was found to be highly diverse in mosquito fauna as sixteen species were discovered ~~with 23841~~^{with 23841} mosquitoes in number. One new species that was *Culex perexiguus* was discovered for the first time from tehsil Murree and Kotli Sattian of ~~this district~~. The genus *Armigeres* in addition to the three genera *Culex*, *Anopheles*, and *Aedes* were spotted. Similar to previous districts, *Culex* was the most common genus (68%), followed by *Anopheles* (18%), *Aedes* (13%) and *Armigeres* (1%). The tehsil Rawalpindi was noticed to be rich in mosquitoes, followed by Taxila, Gujar Khan, Kallar Syedan, Kahuta, Kotli Sattian, and Murree. Tehsil Murree and Kotli Sattian had a completely different mosquito fauna than the other tehsils in the Rawalpindi district. Only these two tehsils contained the species *Culex perexiguus* and *Anopheles maculipennis*. Six species in all were found to be belonging to the genus *Culex*: *Culex quinquefasciatus* (21%) *Culex vagans* (16%) *Culex tritaeniorhynchus* (13%), *Culex raptor* (11%), *Culex pseudovishnui* (5%), and *Culex perexiguus* (1%). Similarly, ~~six species~~ ^{six species} of the *Anopheles* genus were identified i.e., *Anopheles culicifacies* (6%),

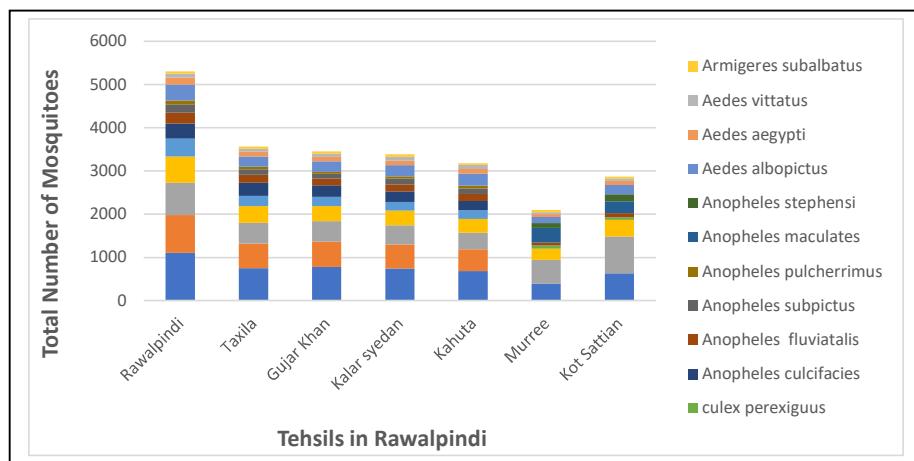
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Anopheles fluviatalis (4%), *Anopheles subpictus* (3%), ~~*Anopheles*~~, *Anopheles maculatus* (3%) *Anopheles stephensi* (1%) and ~~*Anopheles* and *Anopheles*~~ *pulcherrimus* (1%). Three species of genus *Aedes* were observed *Aedes albopictus* (7%) *Aedes vittatus* (7%) and *Aedes aegypti* (3%). The fourth genus *Armigeres* had only one ~~species detected~~ *species detected* *Armigeres subalbatus* ~~with~~*subalbatus* with 1% occurrence. (Figure 2).



4.4.9 Figure 4.6-1: *Total* number of mosquitoes in district Rawalpindi

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4.4.104.6.1 Species Diversity and Abundance of mosquitoes in district Rawalpindi

Table 4.6.1.1 indicated that there were notable variations throughout the tehsils for the diversity of species identified (n), Shannon diversity (H), and Simpson diversity (D). For each of the seven tehsils, the Shannon and Simpson diversity indices varied from 2.31 to 1.985. The tehsil Kahuta had the greatest diversity index ($H=2.313$), followed by Kallar Syedan ($H=2.29$), Taxila ($H=2.28$), Rawalpindi ($H=2.26$), and Gujar Khan ($H=2.25$), Murree ($H=2.05$) and Kotli Sattian ($H=1.98$).

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Name of the species	Kahuta		Kallar Syedan		Taxila		Rawalpindi		Gujar Khan		Murree		Kotli Sattian	
	Shannon's Index Pi*(logPi)	Simpson's Index n(n-1)												
<i>Culex quinquefasciatus</i>	0.3299	463080	0.3331	554280	0.3281	561750	0.3264	1213302	0.3361	606062	0.3148	155630	0.3334	398792
<i>Culex tritaeniorhynchus</i>	0.2932	258572	0.2974	311922	0.2923	319790	0.2974	766500	0.3019	346332	0.0000	0	0.0000	0
<i>Culex Vagans</i>	0.2541	144780	0.2664	196692	0.2718	235710	0.2760	557262	0.2702	215760	0.3509	299756	0.3607	726756
<i>Culex Raptor</i>	0.2310	102080	0.2289	111890	0.2419	150932	0.2489	372710	0.2330	123552	0.2607	68906	0.2704	149382
<i>Culex pseudovishnui</i>	0.1734	39402	0.1666	39402	0.1760	51756	0.1984	169332	0.1741	46872	0.0000	0	0.0000	0
<i>culex perexiguus</i>	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.1125	4692	0.0748	2862
<i>Anopheles culicifacies</i>	0.1847	48180	0.1910	60762	0.2141	98282	0.1793	122150	0.1972	69960	0.0000	0	0.0000	0
<i>Anopheles fluviatalis</i>	0.1491	24806	0.1454	26082	0.1481	30450	0.1443	62750	0.1382	23256	0.1090	4290	0.1163	9702
<i>Anopheles subpictus</i>	0.1300	16512	0.1252	16770	0.1215	17030	0.1197	36290	0.1106	12210	0.0000	0	0.0000	0
<i>Anopheles pulcherrimus</i>	0.0767	3782	0.0669	2970	0.0662	3192	0.0715	8742	0.0576	2070	0.0000	0	0.0000	0
<i>Anopheles maculatus</i>	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.2953	115260	0.2240	74256
<i>Anopheles stephensi</i>	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.1613	13572	0.1611	25440
<i>Aedes albopictus</i>	0.2107	74256	0.1962	66306	0.1832	58806	0.1835	131406	0.1893	61256	0.1818	19740	0.1949	46440
<i>Aedes aegypti</i>	0.1178	12432	0.1077	10920	0.1025	10506	0.1112	29412	0.1019	9702	0.1067	4032	0.1138	9120
<i>Aedes vittatus</i>	0.1056	9120	0.0956	7832	0.0781	4970	0.0656	6972	0.0857	6006	0.0866	2256	0.0769	3080
<i>Armigères subalbatus</i>	0.0571	1722	0.0697	3306	0.0581	2256	0.0460	2756	0.0576	2070	0.0771	1640	0.0596	1560
Total	2.3133	0.8816	2.2900	0.8771	2.2817	0.8781	2.2682	0.8764	2.2533	0.8717	2.0566	0.8423	1.9857	0.8237

Figure 4.6-2: Diversity index of a number of individuals of different species of mosquitoes collected from six tehsils of District Rawalpindi

When compared to the other five districts, very different outcomes were recognized in the district of Rawalpindi because here, a great number of discovered species were found. The species of mosquito with the highest abundance was *Culex quinquefasciatus* (Figure 4.6.1.1.1) ($F = 582.87$, $df = 6$, $P < 0.001$), which was found in large quantities in the following locations: tehsil Rawalpindi (Mean = 1099, SD = 14.73), Gujar Khan (Mean = 780.67, SD = 16.56), Taxila (Mean = 750.67, SD = 14.01), Kallar Syedan (746.33, SD = 16.04), and Kotli Sattian (Mean = 633, SD = 16.56), SD=11.53) and the least count in tehsil Murree (Mean=396, SD=11.53) (Table 4.6.1.2) For *Culex tritaeniorhynchus* (Figure 4.6.1.1.2) the same outcomes were noted. *Culex vagans* (Figure 4.6.1.1.3) was highly populated in tehsil Kotli Sattian and least in Kahuta while *Culex raptor* (Figure 4.6.1.1.4) was abundantly found in tehsil Rawalpindi and least in Murree. There were only two locations where *Culex perexiguus* (Figure 4.6.1.1.6) ($F = 40.89$, $df = 6$, $P < 0.001$) was originally discovered in tehsils Murree (Mean = 71.67, SD = 16.17) and Kotli Sattian (Mean = 55, SD = 15.52) (Table 4.6.1.2). Similar results were obtained for *Anopheles maculatus* (Figure 4.6.1.1.7) and *Anopheles stephensi* (Figure 4.6.1.1.8). Many species of genus Anopheles, *Anopheles culicifacies* (Figure 4.6.1.1.9) *Anopheles fluviatalis* (Figure 4.6.1.1.10) *Anopheles subpictus* (Figure 4.6.1.1.11) and *Anopheles pulcherrimus* (Figure 4.6.1.1.12) were not present in tehsils Murree and Kotli Sattian. According to the Figure 4.6.1.1.13 *Aedes vittatus* was abundant in tehsil Kahuta whereas *Armigeres subalbatus* (Figure 4.6.1.1.14) was densely populated in Tehsil Kallar Syedan.

When compared to the other five districts, very different outcomes were recognized in the district of Rawalpindi because here, a great number of discovered species were found. The species of mosquito with the highest abundance was *Culex quinquefasciatus* (Figure 4.6.1.1) ($F = 582.87$, $df = 6$, $P \leq 0.001$), which was found in large quantities in the following locations: tehsil Rawalpindi (Mean = 1099, SD = 14.73), Gujjar Khan (Mean = 780.67, SD = 16.56), Taxila (Mean = 750.67, SD =

Mean ± Standard Deviation

14.01), Kallar Syedan (746.33, SD = 16.04), and Kotli Sattian (Mean = 633, SD = 16.56).
SD=11.53) and the least count in tehsil Murree (Mean=396, SD=11.53)
(Table 4.6.1.2)
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locations where

Culex

peregrinus

(Figure

4.6.1.1.6) (F =

40.89, df = 6, P <

0.001) was

originally

discovered in

tehsils Murree

(Mean = 71.67,

SD = 16.17) and

Kotli Sattian

(Mean = 55, SD

= 15.52) (Table

4.6.1.2). Similar

results were

obtained for

Anopheles

maculatus

(Figure

4.6.1.1.7) and

Anopheles

stephensi

(Figure

4.6.1.1.8). Many

species of genus

Anopheles,

Anopheles

culexifacies

(Figure

4.6.1.1.9)

Anopheles

flaviatalis

(Figure

4.6.1.1.10)

Anopheles
subpictus
(Figure
4.6.1.1.11) and
Anopheles
pulcherrimus
(Figure
4.6.1.1.12)
were not
present in
tehsils Murree
and Kotli
Sattian.
According to
the Figure
4.6.1.1.13 *Aedes*
vittatus was
abundant in
tehsil Kahuta
whereas
Armigeres
subalbatus
(Figure
4.6.1.1.14) was
densly
populated in
Tehsil Kallar
Syedan, df = 6, P
 ≤ 0.001 , which
was found in
large quantities
in the following
locations: tehsil
Rawalpindi
(Mean = 1099,
SD = 14.73),

Gujar—Khan
(Mean = 780.67,
SD = 16.56),
Taxila (Mean =
750.67, SD =
14.01), Kallar
Syedan (746.33,
SD = 16.04),
and Kotli
Sattian (Mean =
633, SD =
16.56),
SD=11.53) and
the least count
in tehsil Muree
(Mean=396,
SD=11.53)
(Table—4.6.1.2)
For *Culex*
tritaeniorhynchus
(Figure
4.6.1.1.2) the
same outcomes
were noted.
Culex vagans
(Figure
4.6.1.1.3) was
highly
populated in
tehsil—Kotli
Sattian—and
least in Kahuta
while *Culex*
raptor (Figure
4.6.1.1.4) was
abundantly

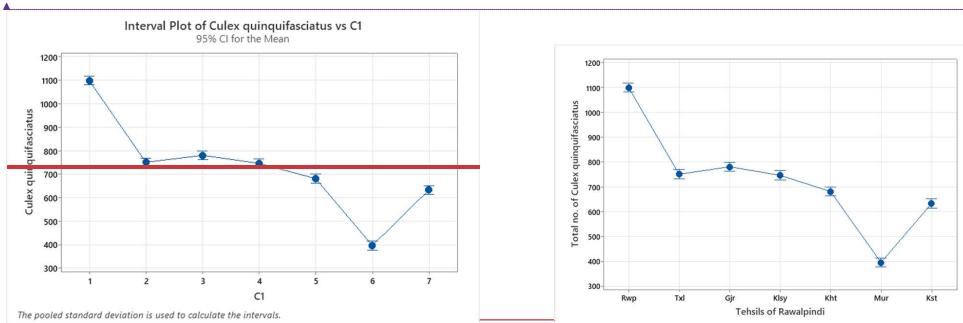
found in tehsil Rawalpindi and least in Murree. There were only two locations where *Culex peregrinus* (Figure 4.6.1.1.6) ($F = 40.89$, $df = 6$, $P < 0.001$) was originally discovered in tehsils Murree (Mean = 71.67, SD = 16.17) and Kotli Sattian (Mean = 55, SD = 15.52) (Table 4.6.1.2). Similar results were obtained for *Anopheles maculatus* (Figure 4.6.1.1.7) and *Anopheles stephensi* (Figure 4.6.1.1.8). Many species of genus *Anopheles*, *Anopheles eulcifacies* (Figure

4.6.1.1.9) *Anopheles fluviatalis* (Figure
 4.6.1.1.10) *Anopheles subpictus* (Figure
 4.6.1.1.11) and *Anopheles pulcherrimus* (Figure
 4.6.1.1.12) were not present in tehsils Murree and Kotli Sattian. According to the Figure 4.6.1.1.13 *Aedes vittatus* was abundant in tehsil Kahuta whereas *Armigeres subalbatus* (Figure 4.6.1.1.14) was densely populated in Tehsil Kallar Syedan.

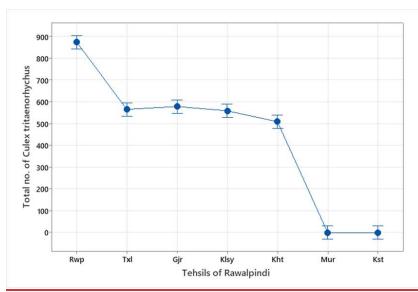
Species	Rawalpindi	Taxla	Gujar Khan	Kallar Syedan	Kahota	Murree	Kotli Satian	F	P
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<i>Cx. quinquefasciatus</i>	1099 ^a ±14,73	750.67 ^b ±14,01	780.67 ^b ±16,56	746.33 ^b ±16,04	681.33 ^c ±12,5	396 ^e ±18,5	633 ^d ±11,53	582.8 7	0.001	
<i>Cx. tritaeniorhynchus</i>	875.3 ^a ±25	566.3b ^c ±23,5	579.7 ^b ±35,9	559.7b ^c ±30	509.7 ^c ±30	^a ±0	^a ±0	517.6 5	0.001	
<i>Cx. pseudovishnui</i>	411.67 ^a ±12,5	229.67 ^b ±12,58	216.3 ^b ±18	199 ^b ±12	200.3 ^b ±18	^a ±0	^a ±0	388.5 1	0.001	
<i>Cx. vagans</i>	748 ^b ±15,52	484 ^d ±16,09	465d ^e ±12	443.67 ^e ±10,5	5	378.67 ^f ±13,6	547 ^c ±14,53	853 ^a ±16 6	0.001	
<i>Cx.raptor</i>	611 ^a ±15	389 ^b ±20	2	351.33b ^c ±11,0	334.33 ^c ±17,01	319 ^c ±12,53	264 ^d ±18,5	84.33 ^b ±15,18 4	0.001	
<i>Cx.peregrinus</i>	^a ±0	^a ±0	^a ±0	^a ±0	^a ±0	71.67 ^a ±16,17	55 ^a ±15,52	40.89	0.001	
<i>An.subpictus</i>	188.33 ^a ±16,1 7	131.3 ^b ±19,5	110 ^b ±15,52	130.33 ^b ±16,5	131 ^b ±16,09	^a ±0	^a ±0	76.12	0.001	
<i>An.stephensi</i>	^a ±0	^a ±0	^a ±0	^a ±0	^a ±0	115.67 ^b ±17,0 4	161 ^a ±12,53	221.9 3	0.001	
<i>An.culicifacies</i>	350 ^a ±16	314.33 ^a ±11,5	266 ^b ±14,53	2	244.67b ^c ±16,6	220.3 ^c ±18,5	^a ±0	351.5 3	0.001	
<i>An.pulcharimus</i>	91.67 ^a ±16,62	55.33 ^b ±14,57	46 ^b ±1	57.67 ^b ±15,18	62.33a ^b ±13,5	^a ±0	^a ±0	26.36	0.001	
<i>An. fluviatalis</i>	249.60 ^a ±13,6	177 ^b ±17,09	156 ^b ±15,72	160.33 ^b ±16,56	1	157.33 ^b ±12,0	68.67 ^c ±15,18	98.33 ^c ±17,01 42.7	0.001	
<i>An. maculatus</i>	^a ±0	^a ±0	^a ±0	^a ±0	^a ±0	343.3 ^a ±25,2	271 ^b ±30	313.0 3	0.001	
<i>Aedes albopictus</i>	363 ^a ±10 4	241.67b ^c ±16,0	245.33b ^c ±15,1 8	257b ^c ±16,52	274 ^b ±14,53	140 ^d ±18,5 1	111.33 ^b ±17,0	219.33 ^c ±15,2 8	56.31	0.001
<i>Aedes aegypti</i>	172.67 ^a ±13,0 1	102.67 ^b ±14,5	100 ^b ±17,5	105.33 ^b ±16,5 1	66 ^b ±16,09	96 ^b ±20	47.67 ^d ±4,51	11.38	0.001	
<i>Aedes vittatus</i>	83.67a ^b ±3,51	71.33 ^c ±3,51	78.33b ^c ±3,51	88.67a ^b ±4,51	95.67 ^a ±4,51	56.33 ^d ±4,51	53.46	0.001		
<i>Armigeres subalbatus</i>	53a ^b ±3	48ab ^c ±4	46.67b ^c ±3,06	57.67 ^a ±4,51	42.33 ^c ±3,51	41 ^c ±3	40.33 ^c ±3,51	10.03	0.001	

Figure 4.6-3: Comparative analysis of the abundance of mosquitoes in different Tehsils of District of Rawalpindi



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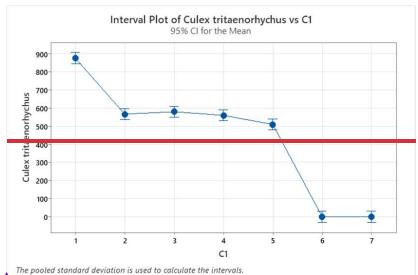
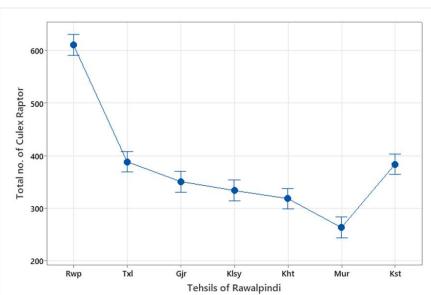
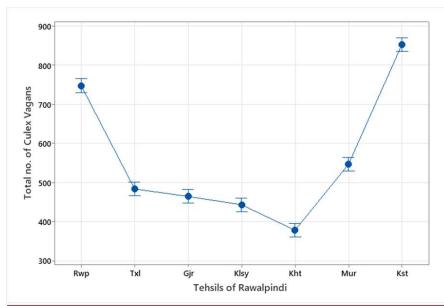
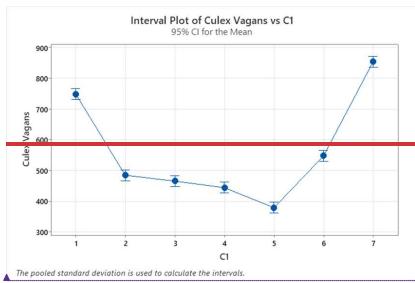
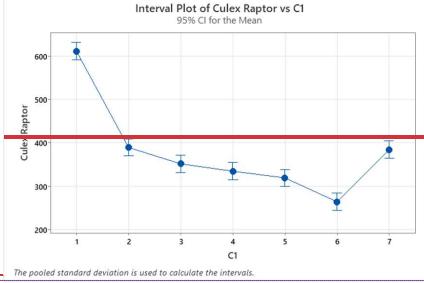


Figure 4.6.1.1.1: Abundance of *Culex quinquefasciatus*

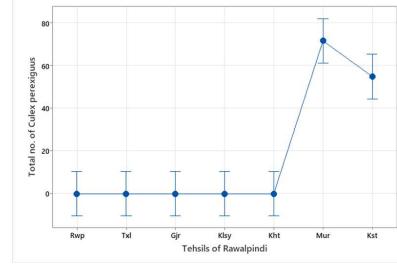
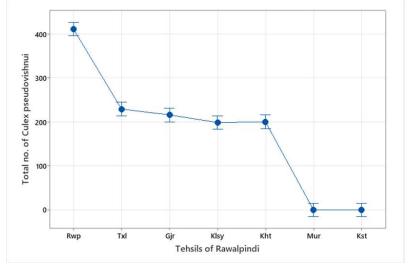
Figure 4.5.1.1.2: Abundance of *Culex tritaeniorhynchus*

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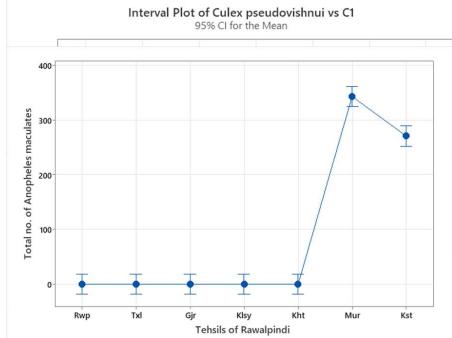
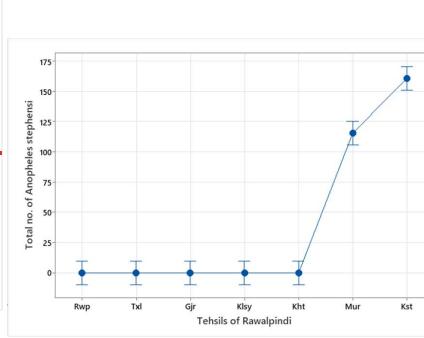


Figure 4.6.1.1.3: Abundance of *Culex vagans*Figure 4.6.1.1.4: Abundance of *Culex raptor*

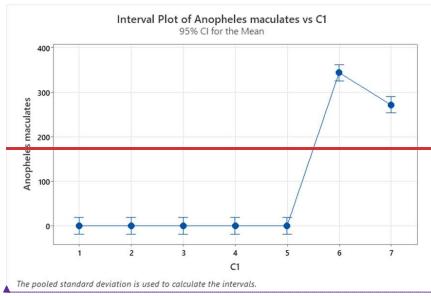
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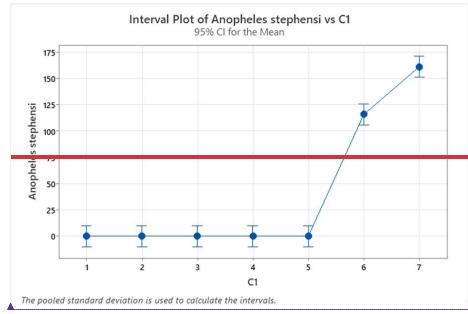


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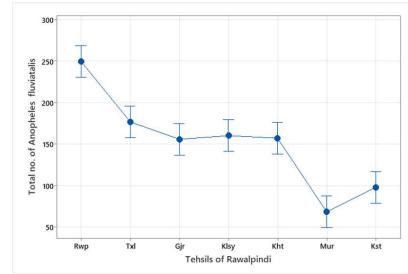
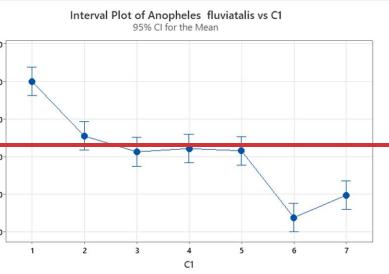
Figure 4.6.1.1.5: Abundance of *Culex pseudovishnui*Figure 4.6.1.1.6: Abundance of *Culex perexiguus*

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**Figure 4.6.1.1.7: Abundance of *Anopheles maculatus***

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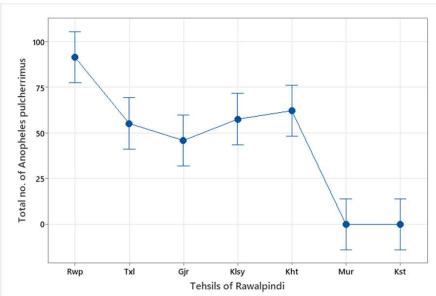
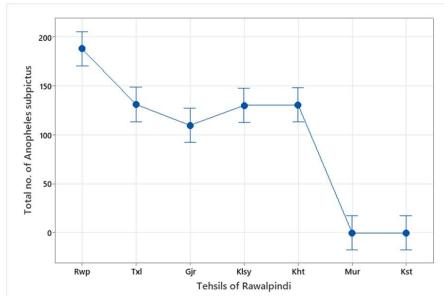
Figure 4.6.1.1.8: Abundance of *Anopheles stephensi***Figure 4.6.1.1.9: Abundance of *Anopheles culicifacies*****Figure 4.6.1.1.9: Abundance of *Anopheles culicifacies***

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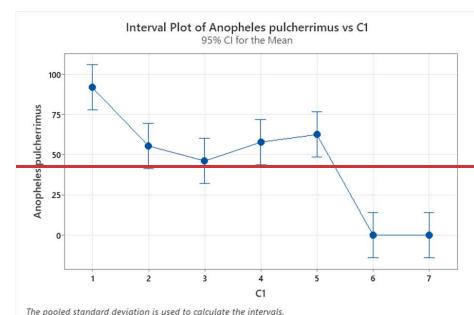
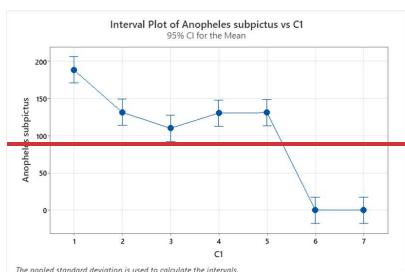
Figure 4.6.1.1.10: Abundance of *Anopheles fluviatalis*

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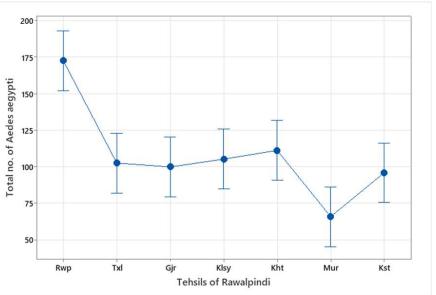
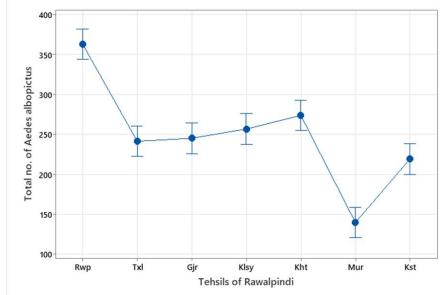
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Figure 4.6.1.11: Abundance of *Anopheles subpictus*

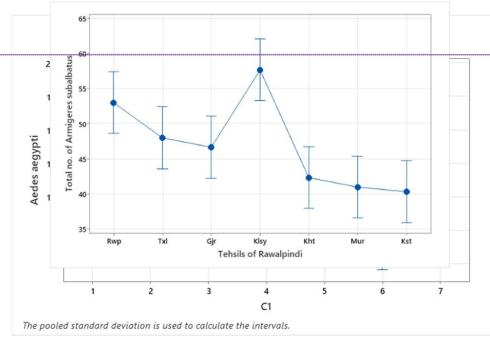
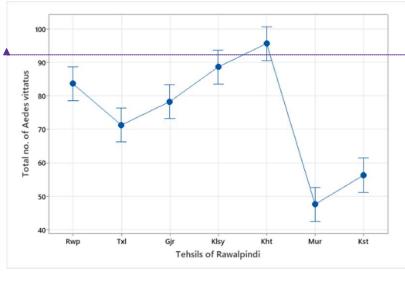
Figure 4.6.1.12: Abundance of *Anopheles pulcherrimus*

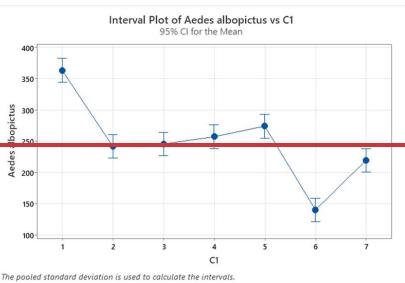
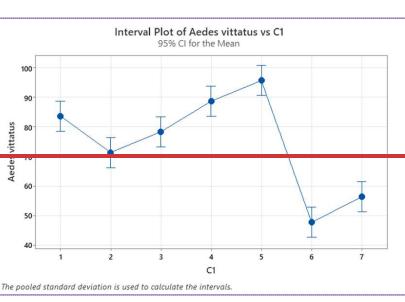
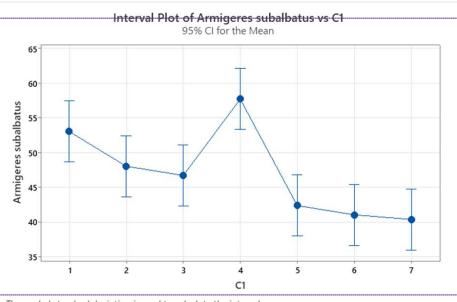
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Figure 4.6.1.13: Abundance of *Aedes albopictus*Figure 4.6.1.14: Abundance of *Aedes aegypti*Figure 4.6.1.15: Abundance of *Aedes vittatus*Figure 4.6.1.16: Abundance of *Armigeres*

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4.6.2 Relationship of the abundance of mosquitoes with seasons in District Rawalpindi

The effect of seasons on the different species of mosquito of district Rawalpindi was same as found in other districts. The maximum levels of abundance occurred during the monsoon season, followed by fall, and the lowest levels throughout the winter. The spring brought with it a greater number of mosquitoes than in the summer. In Rawalpindi district, *Culex quinquefasciatus* ($F = 106.83$, $df = 4$,

Figure 4.6-4: Relative abundance of different species of mosquito in different tehsils of district Rawalpindi
Figure

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$P = 0.001$) peaked during the monsoon (Mean = 336.67, SD = 12.50) and autumn (Mean = 299.33, SD = 15) seasons, and then sharply declined during the winter (Mean = 126.7, SD = 19.5). It climbed up again in the spring (Mean = 177.33, SD = 14.57) and falls in the summer (Mean = 157.33, SD = 15.8) (**Table 4.6.2.1**). Genus *Anopheles*, *Aedes*, and *Armigeres* also showed the same behavior in this tehsil. The mosquitoes in other tehsils Taxila (**Table 4.6.2.2**), Kallar Syedan (**Table 4.6.2.3**) Kahuta (**Table 4.6.2.4**), and Gujar Khan (**Table 4.6.2.5**) of this district also showed the same relationship with seasons as that of tehsil Rawalpindi. As the temperature in Murree and Kotli Sattian remained very low in winters as compared to other tehsils of Rawalpindi so the mosquitoes were

scarcely found in this season (**Table 4.6.2.6 and 4.6.2.7**).

Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	299 ^a ±15	126.7 ^c ±19. 5	177.33 ^b ±14.5 7	157.33 ^b ±15.18	336.67 ^a ±12.5 3	106.8 0	0.00 1
<i>Cx. tritaeniorhynchus</i>	258 ^a ±15.52	88.33 ^b ±16. 5	131.33 ^b ±13.5	122.7 ^b ±18	276.33 ^a ±16.5 0 60.48	85.06 7	0.00 1
<i>Cx. pseudovishnui</i>	130 ^a ±18	14.33 ^c ±2.5 2	70 ^b ±17.5	59.33 ^b ±15.28	144.67 ^a ±15.5 7	38.59 1	0.00 1
<i>Cx. vagans</i>	219.67 ^a ±14.6 4	69 ^c ±17.09	19 ^b ±17.09	109 ^b ±14.73	233.33 ^a ±16.0 1	60.48 1	0.00 1
<i>Cx.raptor</i>	179 ^a ±14.11	42.33 ^c ±1.5 2	101.7 ^b ±18	97.67 ^b ±16.5	188.67 ^a ±16.5 1	52.71 1	0.00 1
<i>An. subpictus</i>	57 ^a ±13.11	8.33 ^c ±1.42	30.33 ^b ±1.528	22 ^b ±1	75 ^a ±12.53	32.76 1	0.00 1
<i>An.fluviatalis</i>	77.33 ^a ±11.02	10 ^c ±1	39.66 ^b ±1.52	35 ^b ±2	89.67 ^a ±14.57	47.76 1	0.00 1
<i>An.culicifacies</i>	110.33 ^a ±16.5 6	16 ^c ±1	54 ^b ±14.53	59.33 ^b ±11.5	108.33 ^a ±16.5	26.86 1	0.00 1
<i>An.pulcherrimus</i>	30 ^b ±1	0 ^c ±0	17.67 ^c ±2.52	8 ^d ±1	38 ^a ±2	293.5 4	0.00 1
<i>Ae.vittatus</i>	21 ^b ±1	0 ^d ±0	18.67 ^b ±2.08	10.33 ^c ±1.52	34.67 ^a ±2.52	177.9 4	0.00 1
<i>Ae.albopictus</i>	117 ^a ±20	44.33 ^b ±2.0 8	37.67 ^b ±2.52	47.67 ^b ±2.08	19.33 ^a ±15.28	39.3 1	0.00 1
<i>Ae.aegypti</i>	55.67 ^a ±12.06	17.66 ^b ±1.5 2	17.66 ^b ±1.52	24.33 ^b ±1.52	55.3 ^a ±19.5	10.92 1	0.00 1
<i>Armigeres subalbitus</i>	11.67 ^b ±2.08	0 ^d ±0	8 ^c ±1	10 ^b ±1	24 ^a ±1	153.4 5	0.00 1

Figure 4.6-5: Relationship of abundance of species of mosquitoes with seasons in Tehsil Rawalpindi of District Rawalpindi

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Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	218 ^a ±10	56 ^c ±19	125.3 ^b ±18.5	104 ^b ±11	249.3 ^a ±13.65	87.95	0.001
<i>Cx. tritaeniorhynchus</i>	176.67 ^a ±17.01	38 ^c ±1	85.33 ^b ±15.63	80.67 ^b ±12.5	188 ^a ±19	61	0.001
<i>Cx. pseudovishnui</i>	71.67 ^a ±11.5	10.67 ^c ±2.52	36.66 ^b ±1.528	36 ^b ±1	73 ^a ±15.52	27.64	0.001
<i>Cx.vagans</i>	139 ^a ±13.53	28 ^c ±1	63b ^c ±16	89 ^b ±12	169.33 ^a ±17.04	55.65	0.001
<i>Cx.raptor</i>	114 ^a ±10	19 ^c ±3	71 ^b ±17.5	66 ^b ±10	119 ^a ±18.5	29.03	0.001
<i>An.subpictus</i>	40 ^b ±3	6.33 ^c ±1.52	24.67 ^c ±2.52	14.67 ^d ±2.08	46.33 ^a ±1.52	173.47	0.001
<i>An.fluviatalis</i>	57.67 ^a ±11.02	9.33 ^b ±1.52	22.33 ^b ±1.52	26 ^b ±2	61 ^a ±16	20.3	0.001
<i>An.culcifacies</i>	100.33 ^a ±12.5	15.67 ^c ±2.52	50.67 ^b ±2.08	42.67 ^b ±2.08	103.33 ^a ±16.17	50.49	0.001
<i>An.pulcherrimus</i>	17.67a ^b ±2.08	0 ^d ±0	13.33 ^b ±2.52	6 ^c ±1	21 ^a ±2	70.39	0.001
<i>Ae.vittatus</i>	22.33 ^b ±2.52	0 ^c ±0	9.33 ^c ±1.52	4.67 ^d ±2.08	36 ^a ±1	229.62	0.001
<i>Ae.albopictus</i>	81 ^a ±13.11	25.33 ^b ±1.52	34.33 ^b ±1.52	31 ^b ±3	74.33 ^a ±10.5	35.03	0.001
<i>Ae.aegypti</i>	36.67 ^a ±2.08	7 ^d ±1	14.33 ^c ±2.08	16.33 ^c ±2.08	28 ^b ±2	115.68	0.001
<i>Armigeres subalbitus</i>	20.33 ^a ±2.52	0 ^b ±0	4.33 ^b ±1.52	3 ^b ±1	21.67 ^a ±2.08	113.55	0.001

Figure 4.6-6: Relationship of abundance of species of mosquitoes with seasons in Tehsil Taxila of District Rawalpindi

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Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	216 ^a ±11.53	52.67 ^c ±16.62	138.3 ^b ±18	104.67 ^b ±14.19	233 ^a ±14.53	74.95	0.001
<i>Cx. tritaeniorhynchus</i>	175.67 ^a ±14.5	40.33 ^c ±2.52	89 ^b ±11.53	81 ^b ±11.53	173 ^a ±15.1	75.99	0.001
<i>Cx. pseudovishnui</i>	54.33 ^a ±5.13	12.67 ^d ±2.52	34.67 ^b ±2.52	31.67 ^c ±2.52	64.33 ^a ±16.56	19.26	0.001
<i>Cx.vagans</i>	127.67 ^a ±15.28	29.33 ^c ±2.52	63.67 ^b ±13.01	82 ^b ±19	141 ^a ±17.09	29.84	0.001
<i>Cx.raptor</i>	91.67 ^a ±13.65	19.33 ^c ±2.08	52.33 ^c ±16.17	57 ^b ±16.52	107 ^a ±17.5	17.38	0.001
<i>An.subpictus</i>	39.66 ^b ±1.52	5.33 ^c ±1.52	25.33 ^c ±2.08	14 ^d ±2	45.67 ^a ±2.08	247.74	0.001
<i>An.fluviatalis</i>	56 ^a ±16	9.67 ^b ±2.08	21 ^b ±3	21.66 ^b ±1.52	54.67 ^a ±15.01	13.69	0.001
<i>An.culcifacies</i>	82.67 ^a ±14.5	14.67 ^c ±2.08	18.33 ^b ±2.08	35.33 ^b ±2.08	75 ^a ±12	33.57	0.001
<i>An.pulcherrimus</i>	17 ^b ±1	0 ^d ±0	9.67 ^c ±2.08	6.67 ^c ±2.08	23.33 ^a ±2.52	77.14	0.001
<i>Ae.vittatus</i>	21.66 ^b ±1.52	0 ^d ±0	18.67 ^b ±2.52	11 ^c ±3	36.33 ^a ±2.08	123.05	0.001
<i>Ae.albopictus</i>	79.33 ^a ±10.5	24.67 ^b ±2.52	38 ^b ±2	32 ^b ±1	84 ^a ±12	43.95	0.001
<i>Ae.aegypti</i>	32.33 ^a ±2.52	10.67 ^b ±2.08	13.67 ^b ±2.52	14.67 ^b ±2.52	34.33 ^a ±2.52	64.06	0.001
<i>Armigeres subalbitus</i>	14.33 ^b ±2.52	0 ^d ±0	9.33 ^c ±1.52	7.33 ^c ±1.52	27.33 ^a ±2.08	100.98	0.001

Figure 4.6-7: Relationship of abundance of species of mosquitoes with seasons in Tehsil Kallar Syedan of District Rawalpindi

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Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	208.67±15.01	53±16.52	127 ^b ±12	80±16.52	212.33 ^a ±16.17	67.61	0.001
<i>Cx. tritaeniorhynchus</i>	158 ^a ±19	34.67 ^c ±2.52	82.67 ^b ±10.5	74.67 ^b ±14.5	159.67 ^a ±13.58	52.08	0.001
<i>Cx. pseudovishnui</i>	55.7 ^a ±17.6	13.33 ^c ±2.08	33 ^b ±1	31 ^b ±2	69 ^a ±13.11	14.76	0.001
<i>Cx.vagans</i>	111.33 ^a ±14.5	22.33 ^c ±2.08	53.67 ^b ±15.28	67.33 ^b ±14.57	121.67 ^a ±13.01	30.67	0.001
<i>Cx.raptor</i>	88 ^a ±20	21.67 ^c ±2.52	60.67 ^{ab} ±13.01	53 ^b ±14.73	93 ^a ±17.5	11.38	0.001
<i>An.subpictus</i>	39.67 ^b ±2.52	5.33 ^e ±1.52	21.67 ^c ±2.52	12.66 ^d ±1.52	49 ^a ±1	273.86	0.001
<i>An.fluvialis</i>	56.33 ^a ±15.63	9.33 ^b ±1.52	19 ^b ±3	23 ^b ±1	52 ^a ±17.5	11.68	0.001
<i>An.culcifacies</i>	75.33 ^a ±15.28	14.33 ^c ±1.52	39 ^b ±3	35.33 ^b ±1.52	58 ^a ±15.1	17.03	0.001
<i>An.pulcherrimus</i>	18.66 ^b ±1.52	0 ^d ±0	10.33 ^c ±2.08	7.33 ^c ±1.52	24.33 ^a ±2.08	102.66	0.001
<i>Ae.vittatus</i>	23.33 ^b ±2.08	0 ^d ±0	20.67 ^b ±2.08	10.33 ^c ±2.08	41 ^a ±2	207.72	0.001
<i>Ae.albopictus</i>	85.33 ^a ±16.62	27 ^b ±3	41.66 ^b ±1.52	33 ^b ±1	87.33 ^a ±15.01	24.89	0.001
<i>Ae.aegypti</i>	31 ^b ±2	11.33 ^c ±2.08	13.33 ^c ±2.52	13.33 ^c ±2.08	42 ^a ±2	121.43	0.001
<i>Armigerae subalbitus</i>	10.67 ^b ±2.08	0 ^e ±0	10.67 ^b ±2.08	7.33 ^b ±1.52	15.33 ^a ±1.52	36.35	0.001

Figure 4.6-8: Relationship of abundance of species of mosquitoes with seasons in Tehsil Kahuta of District Rawalpindi

Species	Mean ± Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>An.pulcherrimus</i>	<u>225.3^a±18</u>	<u>58.67^c±13.5</u>	<u>144.3^b±18</u>	<u>99^c±15.52</u>	<u>250.33^a±17.01</u>	<u>73.12</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>184^a±13.11</u>	<u>38^c±2</u>	<u>93^b±15.1</u>	<u>81.67^b±13.5</u>	<u>198^a±15.1</u>	<u>87.94</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>61.67^ab±14.57</u>	<u>13^c±1</u>	<u>38.67^bc±2.08</u>	<u>35.33^c±1.52</u>	<u>69.67^a±16.04</u>	<u>15.99</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>144.33^a±12.5</u>	<u>28.33^c±1.52</u>	<u>64^b±15.1</u>	<u>82.67^b±16.04</u>	<u>147.33^a±12.5</u>	<u>50.21</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>109^a±15.72</u>	<u>20.33^c±2.52</u>	<u>64.33^b±12.06</u>	<u>59.67^b±11.02</u>	<u>104.7^a±19</u>	<u>22.62</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>35.33^a±2.52</u>	<u>3.67^d±2.08</u>	<u>22.67^b±2.52</u>	<u>13^c±2</u>	<u>36.33^a±2.08</u>	<u>118.51</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>48.67^a±2.52</u>	<u>7.67^c±2.08</u>	<u>24.67^b±2.08</u>	<u>24^b±2</u>	<u>49^a±2</u>	<u>206.98</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>86.3^a±18.5</u>	<u>13.66^c±1.52</u>	<u>44.67^b±2.52</u>	<u>36^b±1</u>	<u>13.66^c±1.52</u>	<u>28.06</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>15^a±3</u>	<u>0^c±0</u>	<u>10^ab±1</u>	<u>6.67^b±2.08</u>	<u>14.33^a±2.08</u>	<u>30.47</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>24^b±2</u>	<u>0^d±0</u>	<u>10.67^c±2.08</u>	<u>6.67^c±2.08</u>	<u>38^a±2</u>	<u>206.88</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>74^a±15.1</u>	<u>22^b±1</u>	<u>35.67^b±2.08</u>	<u>34^b±1</u>	<u>86^a±13.53</u>	<u>28</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>27^a±3</u>	<u>8.67^c±2.08</u>	<u>15.66^b±1.52</u>	<u>15.67^b±2.08</u>	<u>32.66^a±1.52</u>	<u>63.04</u>	<u>0.001</u>
<i>An.pulcherrimus</i>	<u>14^ab±3</u>	<u>0^c±0</u>	<u>8.67^b±2.08</u>	<u>9.67^ab±2.08</u>	<u>14.66^a±1.52</u>	<u>25.85</u>	<u>0.001</u>

Figure 4.6-9: Relationship of abundance of species of mosquitoes with seasons in Tehsil Gujrat Khan of District Rawalpindi

Species	Mean \pm Standard Deviation						
	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	146.67 ^a \pm 11.02	33 ^c \pm 3	61 ^c \pm 15	41 ^c \pm 2	114.33 ^b \pm 14.5	63.93	0.001
<i>Cx. perexiguus</i>	20 ^b \pm 3	0 ^c \pm 0	14 ^c \pm 1	6 ^d \pm 1	28.33 ^a \pm 2.08	122.5	0.001
<i>Cx.vagans</i>	178.67 ^a \pm 13.01	65 ^b \pm 17.5	83.33 ^b \pm 14.57	53.33 ^b \pm 17.5	168.33 ^a \pm 12.5	45.81	0.001
<i>Cx.raptor</i>	87 ^a \pm 11.53	17 ^c \pm 1	40.33 ^b \pm 1.52	38.67 ^b \pm 2.52	82.67 ^a \pm 14.57	39.06	0.001
<i>An.maculatus</i>	53.33 ^b \pm 13.58	12 ^c \pm 1	81.67 ^a \pm 14.05	73.3 ^b \pm 19	113.67 ^a \pm 14.64	22.09	0.001
<i>An.fluvialis</i>	17.67 ^a \pm 2.08	0 ^b \pm 0	14 ^a \pm 3	17.67 ^a \pm 2.52	17.67 ^a \pm 2.08	36.65	0.001
<i>An.stephensi</i>	30 ^b \pm 3	0 ^d \pm 0	29.33 ^b \pm 1.52	21 ^c \pm 3	36.66 ^a \pm 1.52	133.69	0.001
<i>Ae.vittatus</i>	16.67 ^a \pm 2.08	0 ^c \pm 0	7.33 ^b \pm 1.52	9 ^b \pm 1	16 ^a \pm 2	60.54	0.001
<i>Ae.albopictus</i>	49.67 ^a \pm 13.65	2.33 ^c \pm 1.52	21.33 ^b \pm 2.52	16.33 ^b \pm 1.52	50.67 ^a \pm 2.08	33.89	0.001
<i>Ae.aegypti</i>	27 ^a \pm 1	1 ^d \pm 1	9.67 ^c \pm 2.08	6.33 ^c \pm 1.52	21 ^b \pm 2	136.05	0.001
<i>Armigeres subalbitus</i>	10.33 ^b \pm 1.52	0 ^c \pm 0	7.33 ^b \pm 1.52	9 ^b \pm 1	14.67 ^a \pm 2.52	35.93	0.001

Figure 4.6-10: Relationship of abundance of species of mosquitoes with seasons in Tehsil Murree of District Rawalpindi

	Mean \pm Standard Deviation						
Species	Autumn	Winter	Spring	Summer	Monsoon	F	P
<i>Cx. quinquefasciatus</i>	183 ^a \pm 12	53.33 ^c \pm 15.04	115.67 ^b \pm 15.5	96.33 ^b \pm 16.04	188.33 ^a \pm 13.65	47.64	0.001
<i>Cx. perexiguus</i>	15.33 ^a \pm 2.08	0 ^d \pm 0	12.33 ^b \pm 1.52	8.67 ^c \pm 2.08	18 ^a \pm 3	36.73	0.001
<i>Cx.vagans</i>	225.33 ^a \pm 11.5	66.67 ^d \pm 11.02	175 ^b \pm 13	129.33 ^c \pm 13.01	247.33 ^a \pm 14.5	90.81	0.001
<i>Cx.raptor</i>	103 ^a \pm 15.1	30 ^d \pm 2	67.33 ^c \pm 11.5	69.67 ^b \pm 16.17	116.67 ^a \pm 12.06	22.4	0.001
<i>An.maculatus</i>	69.33 ^a \pm 10.5	28.33 ^c \pm 1.52	19.33 ^b \pm 2.52	50.67 ^b \pm 2.08	78 ^a \pm 14.53	16.78	0.001
<i>An.fluviatalis</i>	24 ^b \pm 2	7.33 ^d \pm 1.52	16.67 ^c \pm 2.08	19.33 ^b \pm 2.08	32 ^a \pm 3	51.86	0.001
<i>An.stephensi</i>	43.67 ^a \pm 2.08	17.33 ^d \pm 1.52	22.33 ^c \pm 1.52	29.67 ^b \pm 2.52	48 ^a \pm 1	162.07	0.001
<i>Ae.vittatus</i>	18.66 ^b \pm 1.52	0 ^d \pm 0	6.67 ^c \pm 2.08	7.67 ^c \pm 2.08	24.33 ^a \pm 2.52	83.59	0.001
<i>Ae.albopictus</i>	73.3 ^a \pm 19	10.33 ^b \pm 2.08	22.67 ^b \pm 2.52	38.33 ^b \pm 1.52	72.33 ^a \pm 16.62	18.92	0.001
<i>Ae.aegypti</i>	37.67 ^a \pm 2.08	5.33 ^b \pm 1.52	7.33 ^b \pm 1.52	1.33 ^b \pm 2.08	36 ^a \pm 3	73.77	0.001
<i>Armigerae subalbitus</i>	12.33 ^a \pm 1.52	0 ^c \pm 0	6 ^b \pm 1	7 ^b \pm 1	15 ^a \pm 1	96.16	0.001

Figure 4.6-11: Relationship of abundance of species of mosquitoes with seasons in Tehsil Kotli Sattian of District Rawalpindi

4.6.3 Effect of climatic factors on the abundance of mosquitoes in District Rawalpindi

Figure 4.5.3 indicated that August was the month with the most rainfall and the highest mosquito abundance. March was also discovered to be ~~suitable~~—suitable for the mosquitoes' procreation.

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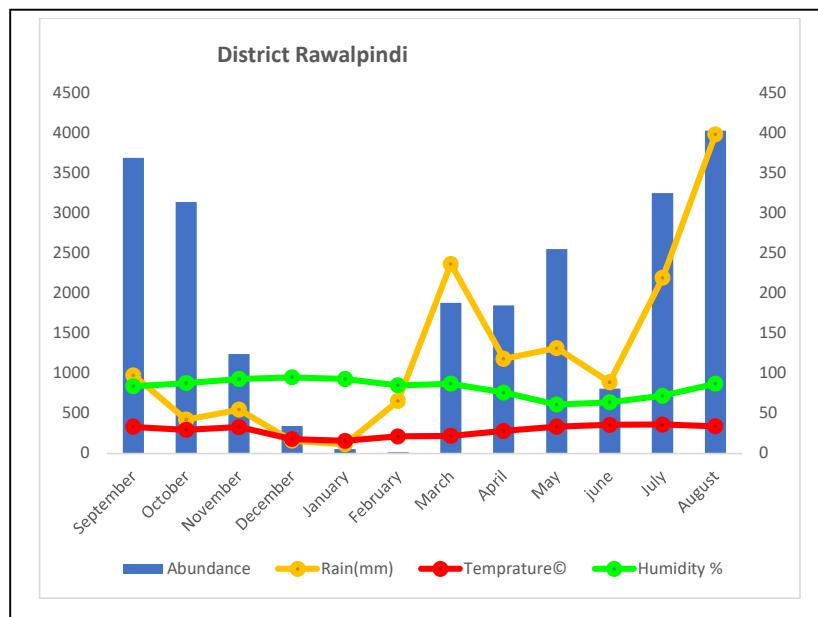


Figure 4.6-12: Effect of climatic variables on relative abundance of mosquito species in district Rawalpindi.

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4.6.4 Comparison of abundance of mosquito between rural and urban areas in district Rawalpindi

The mosquito populations in the Rawalpindi ~~district were~~ compared between rural and urban areas using the unpaired t-test. Between rural and urban areas, Tehsil Kotli Sattian (Figure 4.6.4.1) found ~~a significant~~ a significant difference ($t=3.244$, $p=0.0118$, $F=3.514$). The results for District Murree (Figure 4.6.4.2) were likewise very high ($t=3.200$, $p=0.0126$, $F=6.072$). Findings in the Kahuta tehsil (Figure 4.6.4.3) ~~showed~~ showed a significant disparity in abundance between

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rural and urban areas ($t=3.124$, $p=0.0141$, $F=9.661$). The distinction between Gujar Khan (Figure 4.6.4.4)–Kallar Kallar Syedan, (Figure 4.6.4.5)–Rawalpindi Rawalpindi (Figure 4.6.4.6), and Taxila (Figure 4.6.4.7)–among other tehsils of Rawalpindi, was also quite evident (Table 4.6.5.1)

Table 4.6.5.1: T test between the rural and urban areas of different Tehsils of district of Rawalpindi

Tehsils	T	P	F	Difference between Means \pm Standard Error
Kotli Sattian	3.244	0.0118	3.514	260.8 ± 80.39
Murree	3.200	0.0126	6.072	228.4 ± 71.36
Kahuta	3.124	0.0141	9.661	363.2 ± 116.3
Gujar Khan	2.757	0.0248	5.821	344.0 ± 124.8
Kallar Syedan	2.659	0.0288	5.188	315.8 ± 118.8
Rawalpindi	2.568	0.003	4.118	336.0 ± 130.9
Taxila	2.568	0.0333	4.118	336.0 ± 130.9

Figure 4.6-13:T test between the rural and urban areas of different Tehsils of district of Rawalpindi

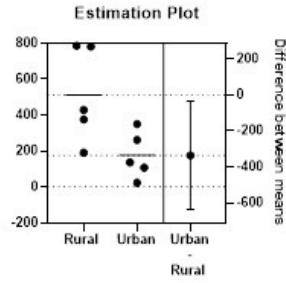


Figure 4.6-14: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Muree of District Rawalpindi

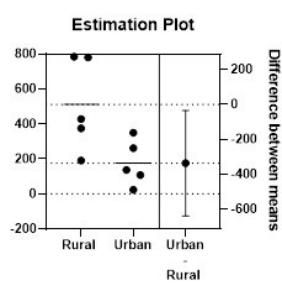


Figure 4.6-15: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Rawalpindi of District Rawalpindi

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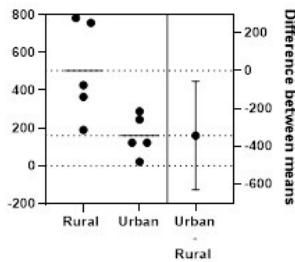
Estimation Plot

Figure 4.6-17: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Gujar Khan of District Rawalpindi

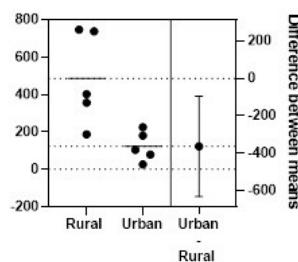
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Figure 4.6-16: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Taxla of District Rawalpindi

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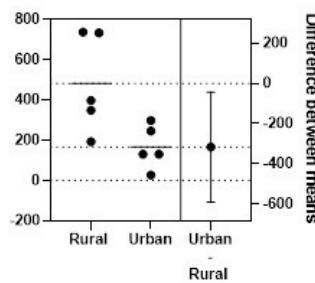
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Figure 4.6-19: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Kahuta of District Rawalpindi

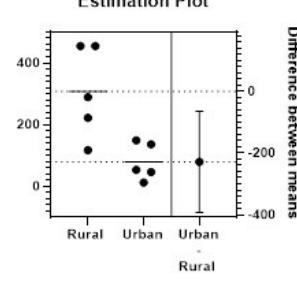
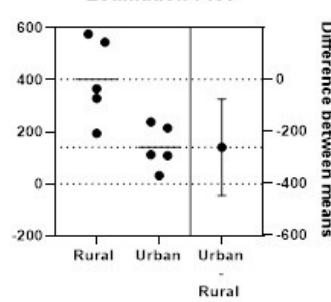
Estimation Plot

Figure 4.6-18: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Kallar Syedan of District Rawalpindi

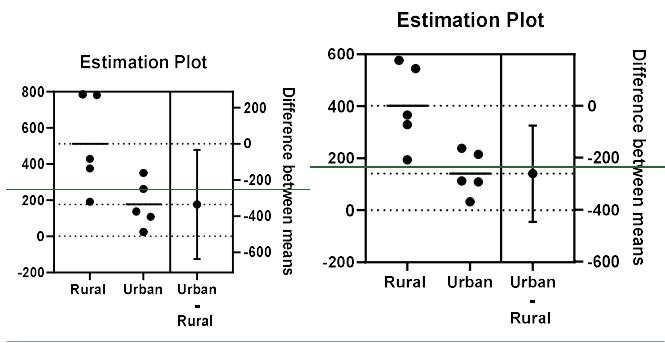
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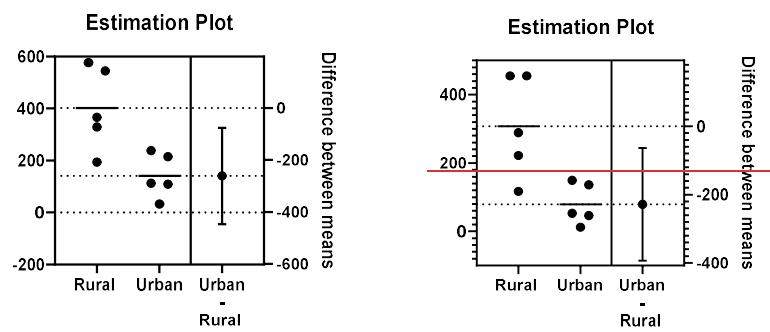
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Figure 4.6-20: Significant results of difference of Abundance of mosquito in Rural and Urban areas of Tehsil Kotli of District Rawalpindi

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4.4.11 Figure 4.6.5.1: Significant results of difference of

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4.4.12 abundance of mosquito in rural and

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urban areas of Tehsil Kotli Sattian of District Rawalpindi

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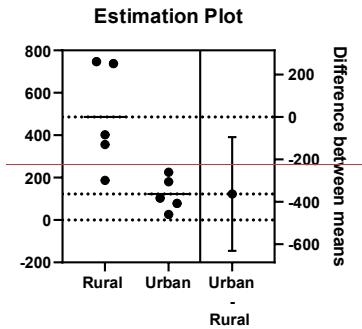
urban areas of Tehsil Muree of District Rawalpindi

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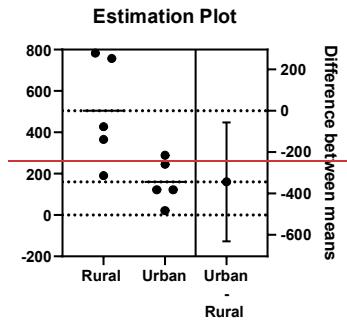
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4.4.16 Figure 4.6.5.3: Significant results of difference of

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4.4.17 abundance of mosquito in rural and

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urban areas of Tehsil Kotli Sattian of District Rawalpindi

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Figure 4.6.5.4: Significant results of difference of

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abundance of mosquito in rural and

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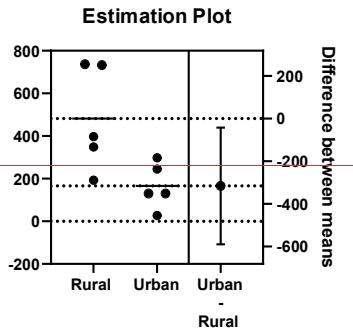
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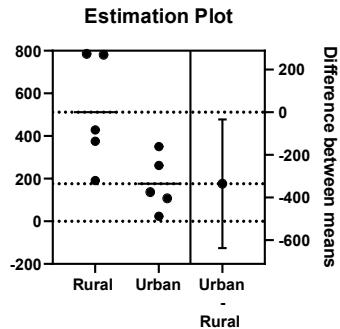
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4.4.22 Figure 4.6.5.5: Significant results of difference of

4.4.23 abundance of mosquito in rural and

urban areas of Tehsil Kallar Syedan of District Rawalpindi

4.4.24

Figure 4.6.5.6: Significant results of difference of

abundance of mosquito in rural and

urban areas of Tehsil Rawalpindi of District Rawalpindi

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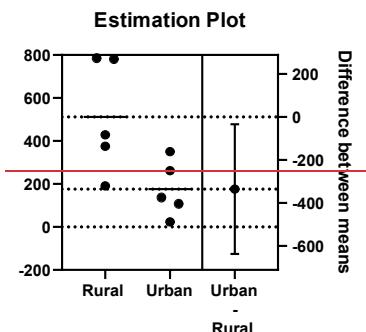
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4.4.27

4.4.28 Figure 4.6.5.7: Significant results of difference of

4.4.29 abundance of mosquito in rural and

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Urban areas of Tehsil Kollar Syedan of District Rawalpindi

4.4.30

4.4.31

4.6.5 GIS Mapping in District Rawalpindi:Rawalpindi:

All the four genera *Culex*, *Anopheles*, *Aedes* and *Armigeres* have been sparsely distributed in seven tehsils of Rawalpindi which are clearly observed in GIS map (**Figure 4.6.5.1**).

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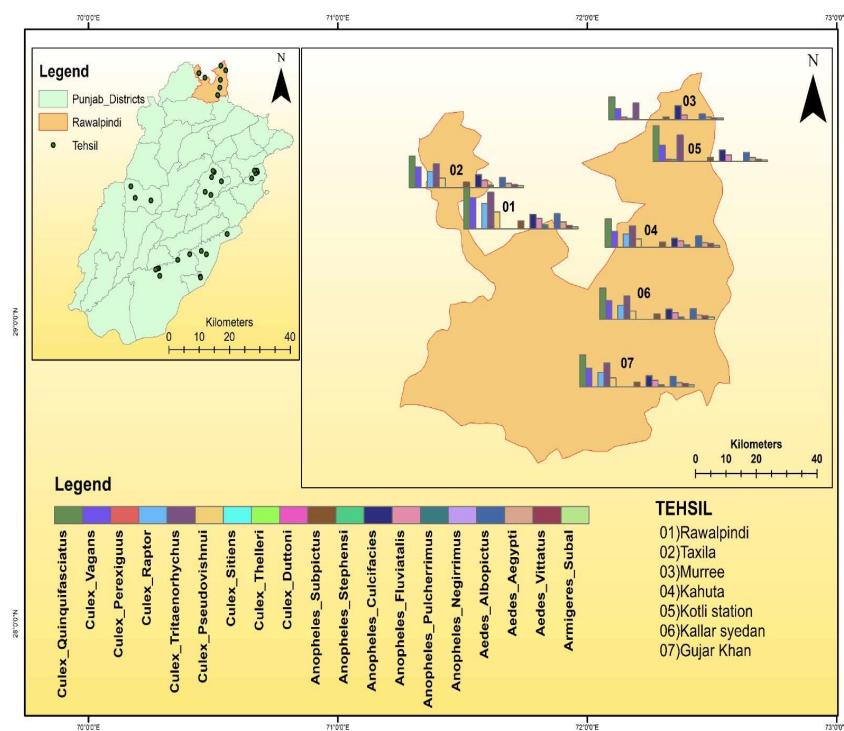


Figure 4.6-21: GIS map showing mosquito abundance in different tehsils of district Rawalpindi

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4.5

4.6

4.7 Consolidated Result of Agroecological Zones of Punjab

A total of 95281 mosquitoes from all over Punjab were identified, representing four genera and 21 species. With 75.37% *Culex* was the most common genus, followed by *Anopheles* (18.11%), *Aedes* (6.17%), and *Armigeres* (0.34%). The following ten species from the genus *Culex* were explored, *Culex quinquefasciatus* (26.37%), *Culex tritaeniorhynchus* (22.92%), *Culex pseudovishnui*

(8.32%), *Culex vagans* (4.49%), *Culex pipiens* (4.53%), *Culex theileri* (3.82%) *Culex raptor* (2.79%) *Culex sitiens* (1.69%), *Culex duttoni* (0.31%), and *Culex perexiguus* (0.13%). Seven species of the genus *Anopheles* were spotted in this study which were *Anopheles subpictus* (6.88%), *Anopheles culicifacies* (4.72%), *Anopheles stephensi* (2.83%) *Anopheles pulcherrimus* (1.36%), *Anopheles flaviatalis* (1.12%), *Anopheles maculipennis* (0.64%), *Anopheles negirrimus* (0.56%). The three species of the genus *Aedes* were identified as *Aedes albopictus* (3.93%), and *Aedes aegypti* (1.70%) and *Aedes vittatus* (0.55%). The fourth genus was *Armigeres* the species of which was *Armigeres subalbatus* (0.34%). (**Figure 4.7**).

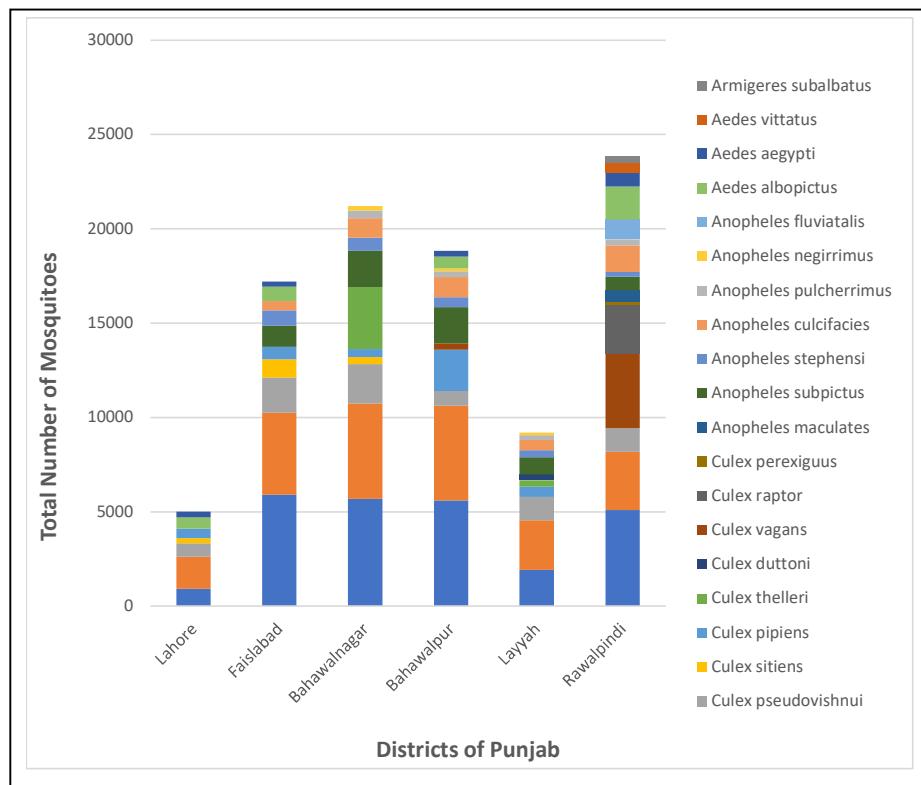


Figure 4.7-1: Total Number of mosquitoes in different districts of Punjab

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4.7.1 Species Diversity and Abundance of mosquitoes in all Agroecological Zones of ~~Punjab~~

In general, there were considerable differences between districts in terms of the diversity of species identified (n), Shannon diversity (H), and Simpson diversity (D) (Table 4.7.1.1). The ~~Shannon indices~~ for the six districts ranged from 2.3717 to 1.7707. The district Rawalpindi had the highest diversity index (H=2.3717) then of Layyah (H=2.0459) then district Bahawalnagar(Bahawalnagar (H=1.9651) then Bahawalpur (H= 1.949) in Faisalabad(Faisalabad (H=1.8685) and the least in district Lahore (H= 1.7707).

<u>Name of the species</u>	<u>Shannon's Index Pi*(logPi)</u>	<u>Simpson's Index n(n-1)</u>										
<i>Culex quinquefasciatus</i>	0.3295	25841972	0.3275	3715256	0.3528	32245362	0.3606	31320812	0.3671	34945832	0.3123	858402
<i>Culex tritaeniorhynchus</i>	0.2652	9600702	0.3578	6877506	0.3417	25517652	0.3527	25315992	0.3476	18874680	0.3666	2847656
<i>Culex pseudovishnui</i>	0.1550	1573770	0.2701	1536360	0.2282	4357656	0.1314	601400	0.2405	3457740	0.2761	496320
<i>Culex sitiens</i>	0.0000	0	0.0000	0	0.0718	142506	0.0000	0	0.1613	924482	0.1583	73712
<i>Culex pipiens</i>	0.0000	0	0.1666	292140	0.0777	175980	0.2491	4715412	0.1268	452256	0.2333	261632
<i>Culex thelleri</i>	0.0000	0	0.1229	117992	0.2892	10833972	0.0000	0	0.0000	0	0.0000	0
<i>Culex duttoni</i>	0.0000	0	0.1116	89700	0.0000	0	0.0000	0	0.0000	0	0.0000	0
<i>Culex vagans</i>	0.2970	15393852	0.0000	0	0.0000	0	0.0744	123552	0.0000	0	0.0000	0
<i>Culex raptor</i>	0.2445	7056992	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.0000	0
<i>Culex perexiguus</i>	0.0272	15006	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.0000	0
<i>Anopheles maculatus</i>	0.0941	375156	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.0000	0
<i>Anopheles subpictus</i>	0.1027	478172	0.2280	816312	0.2177	3696006	0.2334	3719112	0.1768	1228772	0.0000	0
<i>Anopheles stephensi</i>	0.0518	76452	0.1330	148610	0.1113	474032	0.0986	265740	0.1460	684756	0.0000	0
<i>Anopheles culicifacies</i>	0.1662	1947420	0.1680	299756	0.1472	1066056	0.1617	1116192	0.0978	216690	0.0000	0
<i>Anopheles pulcherrimus</i>	0.0570	98282	0.0977	61752	0.0770	171810	0.0686	99540	0.0000	0	0.0000	0

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<i>Anopheles negirimus</i>	0.0000	0	0.0626	18632	0.0506	56882	0.0403	25122	0.0000	0	0.0000	0
<i>Anopheles fluviatalis</i>	0.1388	1131032	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.0000	0
<i>Aedes albopictus</i>	0.1912	3032822	0.0000	0	0.0000	0	0.1137	396270	0.1399	602952	0.2525	349872
<i>Aedes aegypti</i>	0.1089	563250	0.0000	0	0.0000	0	0.0648	85556	0.0647	71022	0.1716	94556
<i>Aedes vittatus</i>	0.0837	271962	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.0000	0
<i>Armigeres subalbatus</i>	0.0590	107256	0.0000	0	0.0000	0	0.0000	0	0.0000	0	0.0000	0
Total	2.3717	0.8811	2.0459	0.8349	1.9651	0.8249	1.9493	0.8088	1.8685	0.7922	1.7707	0.8010

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Figure 4.7-2: Diversity index of a number of individuals of different species of mosquitoes collected from six districts of Punjab

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Significant variations in mosquito abundance were found in all the districts of five agroecological zones of Punjab after analysis of variance (ANOVA) tests performed. The highest abundance of all the mosquitoes was of *Culex quinquefasciatus* ($F = 667.90$, $df = 5$, $P < 0.001$) (Table 4.7.1.2). This species was abundantly found in Mixed Cropping zone (District Faisalabad) (Mean= 5915.3, SD=128) then in cotton zone (district Bahawalnagar) (Mean= 5677.3, SD=128.0) then in district Bahawalpur (Mean= 5590.3, SD=166.1) then in Barani zone (district Rawalpindi) (Mean= 5088.0, SD=172.0) then in Thal zone (district Layyah) (Mean= 1899.3, SD=159.0) and least in rice tract zone (district Lahore) (Mean=929.33. SD=14.64). The same pattern was observed for *Culex tritaeniorhynchus*. *Culex pseudovishnui* showed a little difference as its highest abundance was found in Thal Zone district Bahawalnagar and least in Rice tract zone and district Bahawalpur. *Culex sitiens* was found absent from Thal, Barani and district Bahawalpur of Thal Zone while its highest abundance was observed in Mixed Cropping Zone. *Culex pipiens* was not found from Barani zone while its highest abundance was observed in Thal Zone. *Culex thellleri* was absent from all the zones except Thal zone and district Bahawalnagar of Cotton zone. *Culex duttoni* was only observed in Thal Zone. *Culex vagans* was inhabitant of Barani zone and district Bahawalpur of Cotton zone. Some of the species in our research were exclusively found in Barani zone which were *Anopheles maculatus*, *Anopheles fluviatalis*, *Culex raptor*, *Culex perexiguus* *Aedes vittatus* and *Armigeres subalbatus*. *Culex perexiguus* was first time discovered in Barani Zone in this research from tehsil Murree and Kotli Sattian. *Culex raptor* was found in highest number in Barani Zone a moderate amount was observed in Layyah (Fig 4.7.1.1 - 4.7.1.21).

Species	Mean ± Standard Deviation							
	Lahore	Faisalabad	Bahawalnagar	Bahawalpur	Layyah	Rawalpindi	F	P
Cx. quinquefasciatus	929.33 ^d ±14.64	5915.3 ^a ±128	5677.3 ^a ±171.5	5590.3 ^a ±166.1	1899.3 ^c ±159	5088 ^b ±172	667.9	0.001
Cx. tritaeniorhynchus	1688.33 ^c ±14.5	4335 ^b ±170.2	5032.3 ^a ±164.4	5034 ^a ±154	2641.7 ^d ±147.9	3085.7 ^d ±128.5	288.38	0.001
Cx.pseudovishnui	703.67 ^c ±16.04	1868.3 ^a ±150.7	2091.3 ^a ±172	779 ^c ±15.72	1246 ^b ±123.1	1253.3 ^b ±163.5	59.99	0.001
cx. sitiens	274.3 ^c ±24.6	960.3 ^a ±39.5	366.3 ^b ±60.4	0 ^d ±0	0 ^d ±0	0 ^d ±0	436.6	0.001
cx. pipiens	509b ^c ±14.73	674 ^b ±13.53	420.33 ^c ±13.5	2186 ^d ±176	541.3b ^c ±18.5	0 ^d ±0	320.28	0.001
cx. theileri	0 ^c ±0	0 ^c ±0	3287 ^a ±115.6	0 ^c ±0	348 ^b ±150	0 ^c ±0	875.46	0.001
cx. duttoni	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	301.67 ^a ±16.56	0 ^b ±0	995.17	0.001
cx.vagans	0 ^c ±0	0 ^c ±0	0 ^c ±0	351 ^b ±190	0 ^c ±0	3920.7 ^a ±136	821.04	0.001
cx.raotor	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	2622 ^a ±297	234.63	0.001
cx.perexiguus	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	125 ^a ±14.11	235.55	0.001
An.maculatus	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	608.7 ^a ±125	71.33	0.001
An.subpictus	0 ^d ±0	1075.0 ^b ±68.6	1908.3 ^a ±169.5	1934.3 ^a ±118.1	903b ^c ±16.52	689.33 ^c ±15.18	207.98	0.001
An.stephensi	0 ^d ±0	772 ^a ±113.8	689 ^a ±15	514.33 ^b ±12.58	387b ^c ±12.53	276.33 ^c ±11.02	105.96	0.001
An.culcifacies	0 ^d ±0	465.3 ^c ±18	1041.0 ^b ±155.2	1037.7 ^b ±168.8	547.67 ^c ±11.5	1414 ^a ±163.7	58.29	0.001
An.pulcherrimus	0 ^d ±0	0 ^d ±0	415.3 ³ ±12.5	318.33 ^b ±14.64	248.67 ^c ±12.5	3111.67 ^b ±16.62	688.94	0.001
An.negirimimus	0 ^c ±0	0 ^c ±0	237.7 ^a ±18	157.33 ^b ±15.57	135.33 ^b ±12.58	0 ^c ±0	2661.22	0.001
An.fluvialis	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	1088.7 ^a ±143.6	172.43	0.001
Ae.albopictus	589.67 ^c 14.64	776.67 ^b ±16.5	0 ^d ±0	632b ^c ±17.09	0 ^d ±0	1764.7 ^a ±153.3	312.17	0.001
Ae.aegypti	310 ^b ±17.09	267 ^c ±20	0 ^d ±0	292.7b ^c ±18.5	0 ^d ±0	750 ^a ±14.53	1093.65	0.001
Ae.vittatus	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	508. ⁷ ±129.5	46.27	0.001
Ar.subalbatus	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	0 ^b ±0	332 ^a ±101.1	32.38	0.001

Figure 4.7-3: Comparative analysis of the abundance of mosquitoes in Agroecological zones of Punjab

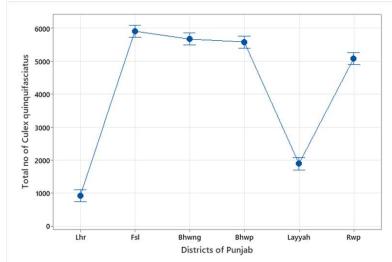


Figure 4.7.1.1: Abundance of *Culex quinquefasciatus* in Different districts of Punjab

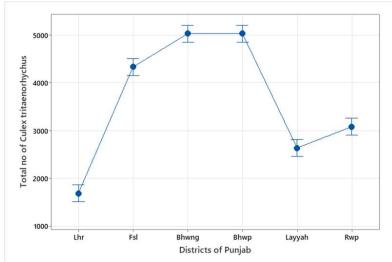


Figure 4.7.1.2: Abundance of *Culex tritaeniorhynchus* in Different districts of Punjab

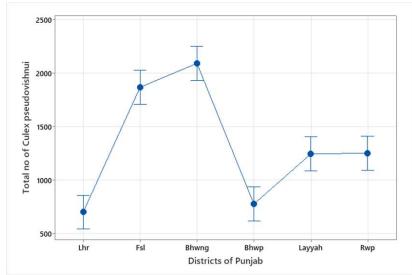


Figure 4.7.1.3: Abundance of *Culex pseudovishnui* in Different districts of Punjab

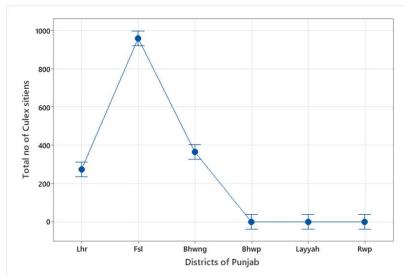


Figure 4.7.1.4: Abundance of *Culex sitiens* in Different districts of Punjab

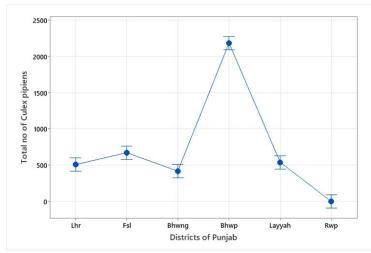


Figure 4.7.1.5: Abundance of *Culex pipiens* in Different districts of Punjab

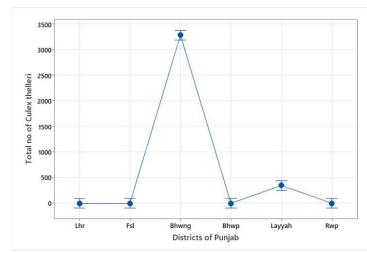


Figure 4.7.1.6: Abundance of *Culex theileri* in Different districts of Punjab

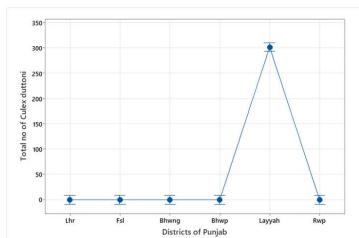


Figure 4.7.1.7: Abundance of *Culex duttoni* in Different districts of Punjab

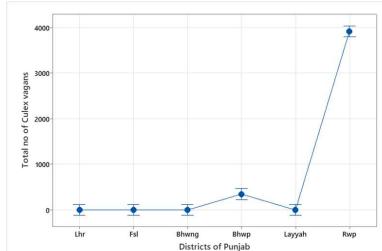


Figure 4.7.1.8: Abundance of *Culex vagans* in Different districts of Punjab

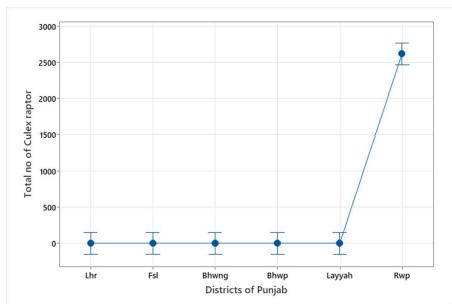


Figure 4.7.1.9: Abundance of *Culex raptor* in Different districts of Punjab

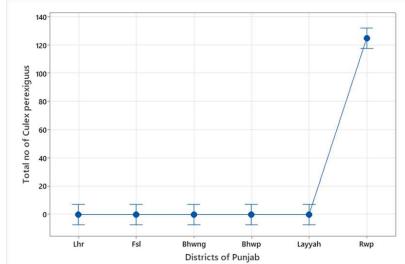


Figure 4.7.1.10: Abundance of *Culex perexiguus* in Different districts of Punjab

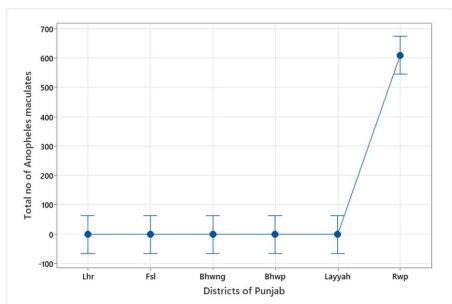


Figure 4.7.11: Abundance of *Anopheles maculatus* in Different districts of Punjab

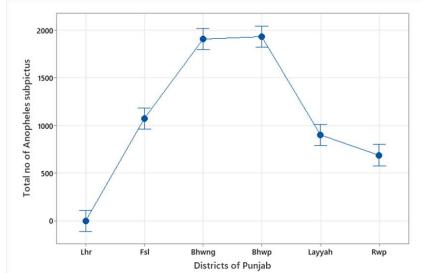


Figure 4.7.1.12: Abundance of *Anopheles subpictus* in Different districts of Punjab

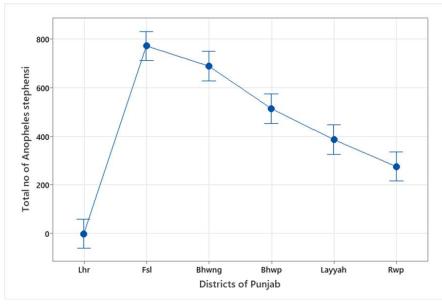


Figure 4.7.13: Abundance of *Anopheles stephensi* in Different districts of Punjab

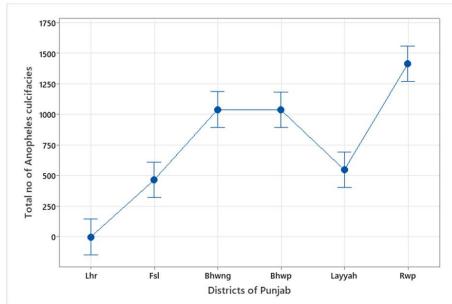


Figure 4.7.14: Abundance of *Anopheles culicifacies* in Different districts of Punjab

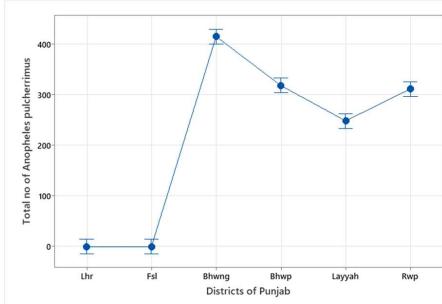


Figure 4.7.15: Abundance of *Anopheles pulcherrimus* in Different districts of Punjab

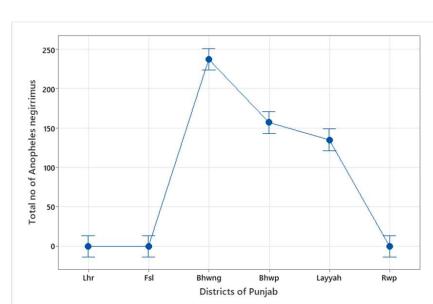


Figure 4.7.16: Abundance of *Anopheles negerrimus* in Different districts of Punjab

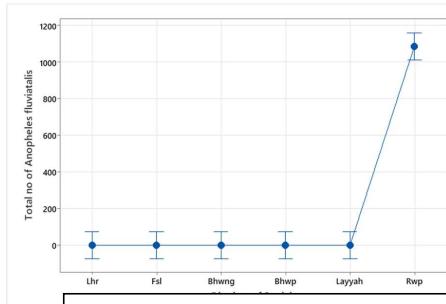


Figure 4.7.17: Abundance of *Anopheles fluviatalis* in Different districts of Punjab

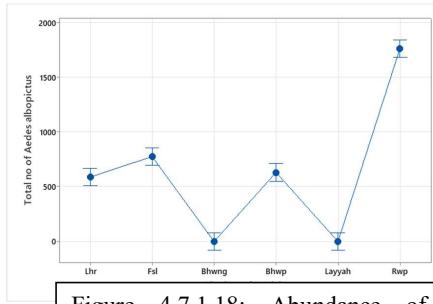


Figure 4.7.18: Abundance of *Aedes albopictus* in Different districts of Punjab

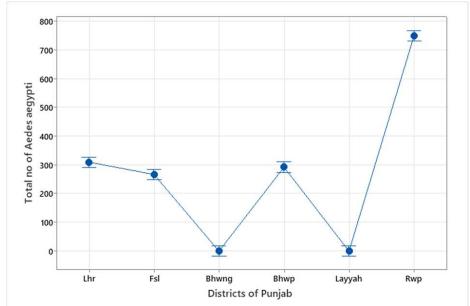


Figure 4.7.19: Abundance of *Aedes aegypti* in Different districts of Punjab

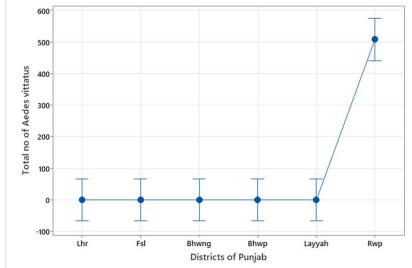


Figure 4.7.1.20: Abundance of *Aedes vittatus* in Different districts of Punjab

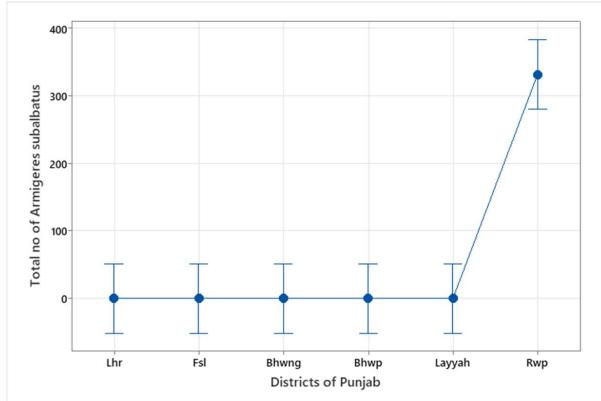


Figure 4.7.21: Abundance of *Armigerae subalbatus* in Different districts of Punjab

4.7.2: GIS Mapping

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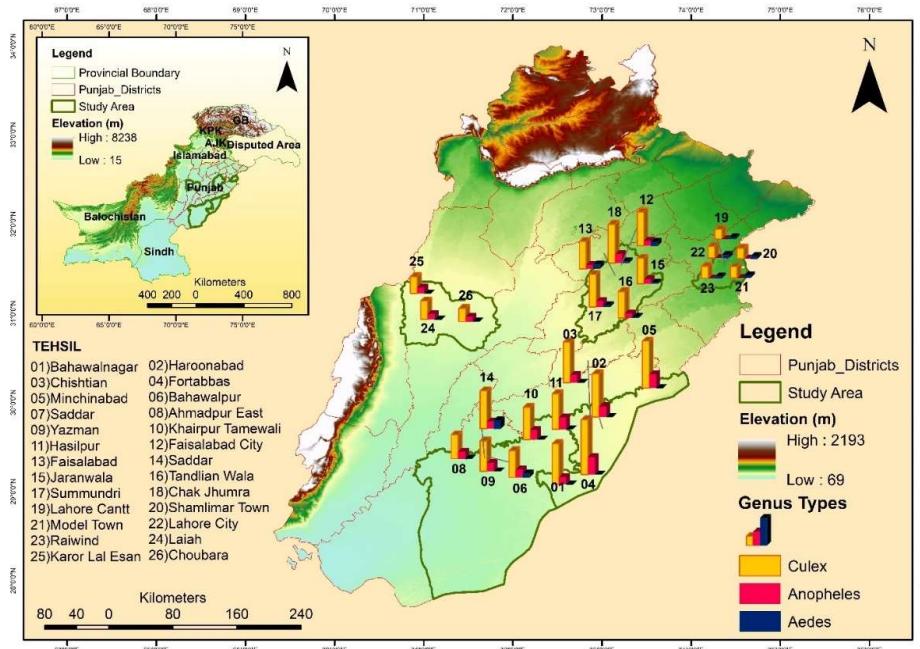


Figure 4.7-4: GIS map showing mosquito abundance in Agroecological zones of Punjab.

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4.8 Polymerase Chain Reaction:

Approximately 710 bp fragments were obtained from all DNA templates during amplification. In figure 4.8.1 the amplification of the DNA of the species were identified *Anopheles subpictus* at no. 1, *Culex sitiens* at 2, *Culex tritaeniorhynchus* at 3, *Anopheles nigerrimus* 4, *Culex pipiens* 5, *Anopheles maculatus* at 6, *Culex theileri* at 7, *Aedes albopictus* at 8, *Culex raptor* at 9 and *Anopheles stephensi* at 10. The result after no. 10 has a weak band of approximately 710-bp, this could be the result of contaminating DNA in the sample causing an unspecific annealing of primers. This result therefore was not considered for further sequencing. The result of number 11 was of *Anopheles fluviatalis*.



Figure 4.8-1: PCR results of the species of genus *Anopheles* and *Culex*

Similarly in **Figure 4.8.2** the result of *Aedes aegypti* was at No.12, *Culex quinquefasciatus* 13, *Culex vagans* 14, *Anopheles pulcherrimus* 15, *Anopheles culicifacies* 16, *Culex duttoni* 17 and *Culex pseudovishnui* at position 18.

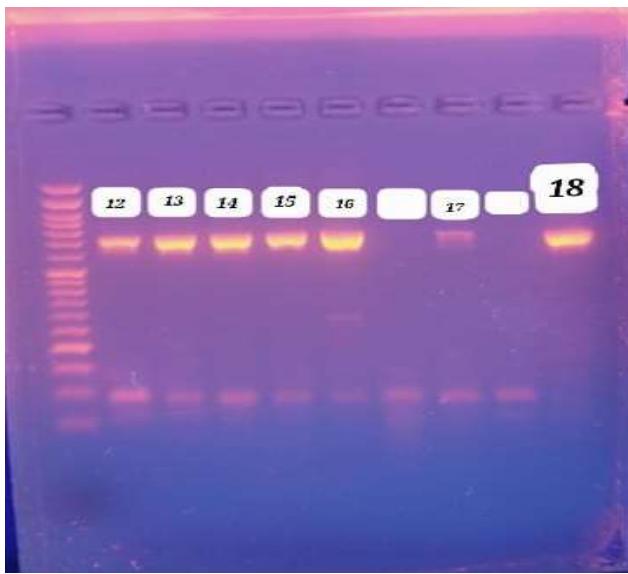


Figure 4.8-2: PCR results of the species of genus *Aedes*, *Anopheles* and *Culex*

In the Figure 4.8.3 is the result of those species which were exclusively found from district Rawalpindi. At 19 the species was *Armigeres subalbatus* while on no. 20 it was *Aedes vittatus*. The result on no. 21 was of *Culex perezius* which was first time discovered from Murree and Kotli sattian and first time to be sequenced in Pakistan in this research.

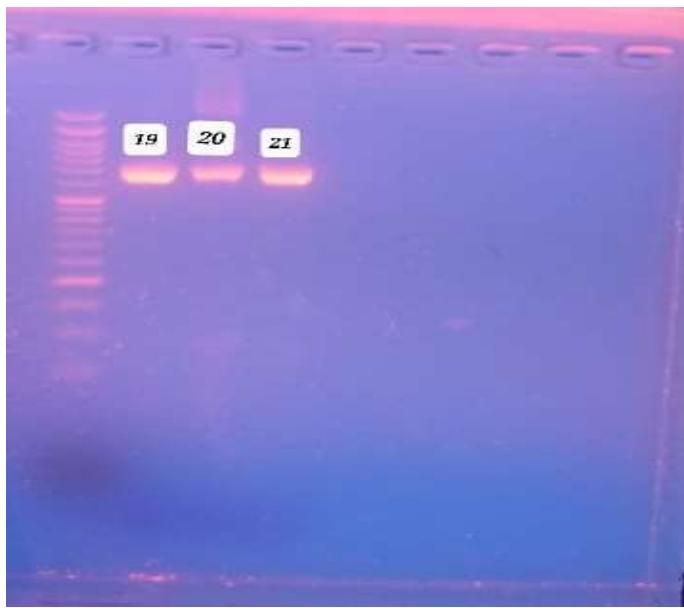


Figure 4.8.3: PCR results of the species of mosquitoes exclusively found in district Rawalpind

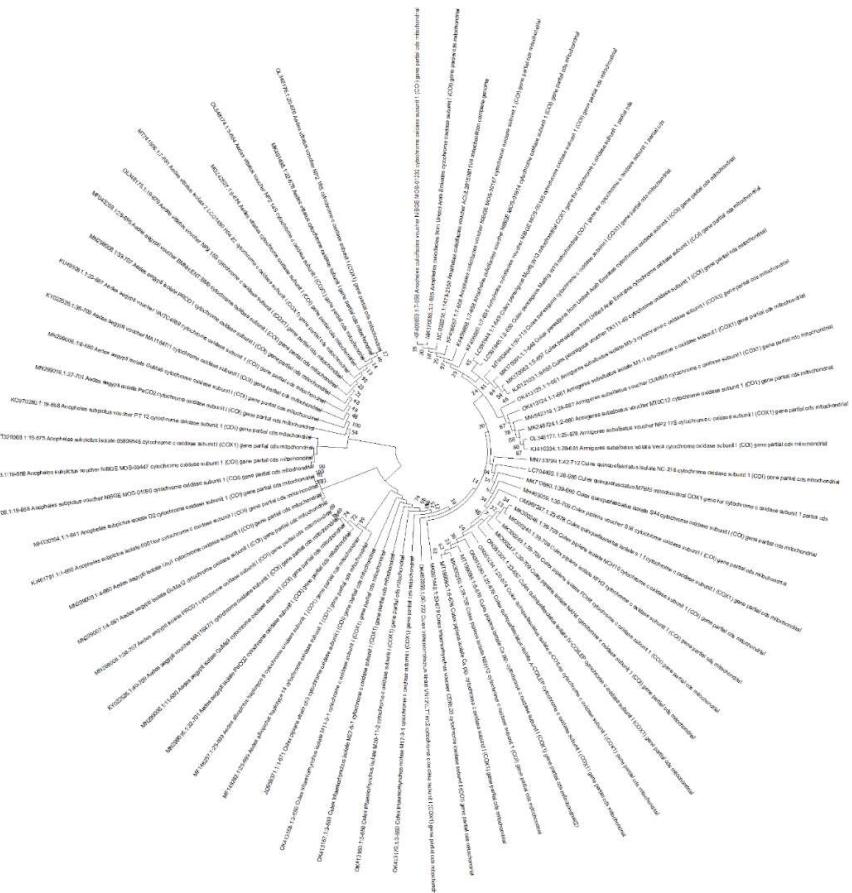


Figure 4.8-4: Phylogenetic tree of the sequences of the species collected from Punjab

Chapter 5 : DISCUSSION

The current study is about the composition, abundance and diversity of mosquito fauna in five agroecological zones of Punjab. Although several workers (Aslamkhan, 1971; Mukhtar *et al.*, 2003; Akram *et al.*, 2009; Ashfaq *et al.*, 2014; Attaullah *et al.*, 2015; Attaullah *et al.*, 2021) have observed all the species while others (Fatima *et al.*, 2020; Herrel *et al.*, 2001; Mashaal, 1964) have reported some of the species discussed in this study. This is the first time in Pakistan that the comparative analysis of *Culex*, *Anopheles*, *Aedes* and *Armigeres* mosquito between rural and urban areas, the role of urbanization on these mosquito species the effect of climate and their distribution in agroecological zones have been analysed. Other than this the effect of seasons on mosquito abundance was also evaluated.

Hafeez *et al* in 2023 had studied the mosquitoes with reference to the agroecological zones of Punjab but he has only studied the distribution of the mosquitoes not the effect of urbanization. Many of the researchers have studied some of the areas of agroecological zone like South Punjab (Mashaal, 1964; Herrel *et al.*, 2001; Mukhtar *et al.*, 2003) but they have only studied two genera that were *Culex* and *Anopheles* but none of them have worked on the genus *Aedes*. Akram in 2009 had discussed the effect of different seasons on the fauna of mosquito with reference to the ecological zones of Pakistan but this study is based on agroecological zones of Punjab (Khan, 2022; Mahmood and Naeem, 2022). The agroecological zones selected in present study were mainly from Punjab, the largest populous province of Pakistan comprising 110 million people (nearly half of the population of Pakistan which is 207.8 million) (PBS 2021).

In the current study eight species were reported from Rice tract zone (**Figure 4.1.1**) while Aslamkhan in 1969 reported 29 species (Aslamkhan and Salman, 1969). The contemporary analysis showed reduction in the number of mosquito species. This decline in Lahore during last forty years is due to drastic changes in environmental conditions, expanding sewage system and diminishing freshwater habitats. Lahore is highly urbanized where human anthropogenic landscape is conspicuous which favors many species of mosquito but denial others like the *Anopheles*. In this study high number of *Aedes* were reported from tehsil Lahore city. The same results were detected by Qureshi and his colleagues in 2017 who found that, Data Ganj Bakhsh, Gulberg and Shalamar Town were the hotspots of *Aedes* mosquitoes due to the favorable environmental conditions (Qureshi *et al.*, 2012 and Qureshi *et al* 2017). Fatima in 2016 has found higher density of *Aedes* mosquitoes particularly in used tyre markets in Data-Ganj-Bakhsh. (Christopher, 2009; Fatima, 2016). The severe outbreak of dengue was also recorded in these urban hubs of the city in 2011 (Mahmood and Rahman 2009; Tahir *et al.*, 2020). The dangerous intimacies of humans and disease-carrying

mosquitoes in urban environments are particularly heightened by ongoing infrastructural fragmentations, economic and social precarity, global climate change, ecological degradation, and changing cycles of land and urban development. (Manzoor *et al.*, 2013; Qureshi *et al.*, 2017; Manzoor *et al.*, 2020 and Rehman, 2020).

With its massive textile unit development and reputation as "the Manchester of Pakistan," Faisalabad is a highly significant region that has contributed significantly to the industrial growth of the surrounding area. The district's changing mosquito fauna is being caused by the release of hazardous chemicals into the environment, excessive aquifer pumping, long-range atmospheric transport of pollutants, and contamination of water bodies with substances that encourage the growth of algae. (Aziz, et al., 2019). The mosquito species reported from this district (**Figure 4.2.1**) agreed with the results of previous studies conducted by Mukhtar and his colleagues in 2002 and Ataullah in 2015. Generally, these species prefer foul and polluted water for breeding as these are pollution tolerant. Carlson and Knight in 1987 had documented the same conditions in Florida.

In district Bahawalnagar the highest number of mosquitoes were discovered from Tehsil Fortabbas which is situated on the border of Pakistan and India (Maplinda.com 2020). This tehsil is bounded by forests, deserts, and agricultural land which favours the growth of mosquitoes (Qureshi *et al* 2022). Of all the species collected *Culex theileri* was found to be the main inhabitant of this district due to the ideal habitats like flooded meadows, stagnant water, tobas, rock pools, swamps and rice paddies which are found usually in rural areas. (Becker 2012). It is considered as one of vector for *Dirofilaria* sp. (Santa-Ana, *et al.*, 2012). The diverse species of *Anopheles* captured from this district strengthened the study of different researchers who had declared it as malaria endemic district. (Mashall, 1964; Herrel, 2000; Mukhtar *et al.*, 2000 and Rehan *et al.*, 2022).

Layyah was selected from the Thal zone adjoining the desert Thal. Mosquito fauna identified from this region was nearly the same as that of district Bahawalnagar lying adjacent to the Cholistan desert. This similarity was due to arid climate and extreme temperatures. (Asif *et al.*, 2023). The species *Culex duttoni* was only reported from district Layyah of all the districts investigated. In 2010 Khater with his colleagues has revealed the presence of *Culex duttoni* in Jazen, the city of Saudi Arabia. It has been observed that the climatic variables of city Jazen was same as that of Layyah. (Khater *et al.*, 2013). The high frequency rate of malaria cases in this district is in consistent with abundant number of *Anopheles* species collected (Rathore in 2013; Khan 2017) .

Rawalpindi was selected from the Barani zone. All the five tehsils of Rawalpindi i.e Rawalpindi, Taxila, Gujrat, Kallar Syedan and Kahuta showed the same diversity of mosquitoes as these districts lies in plain regions having hot and humid summer and mild winter. However, because of their greater elevation, Murree and Kotli Sattian have varied diversity and richness, with harsh winters and moderate summers. (Mazher *et al* 2018) The mosquito fauna of this district had been inquired since 1988 by Aziz and Rehman in 1991. Rehman has reported eight species of *Culex* commonly found all around the district. Apart from the documented *Culex* species, this district was the first to record the presence of *Culex perexiguus*, in this study, a vector of the West Nile virus in Africa and the Middle East. (Benbetka *et al.*, 2018). In 2013, Ilahi and Suleman reported *Culex perexiguus* from Swat. They have identified this species morphologically, but this is the first time that this species has been recorded from the Rawalpindi district in Pakistan and is being identified genetically.

Owing to the high diversity of *Anopheles* species found here, malaria cases are prevalent throughout the district (Aziz *et al.*, 1988; Rehman, 1991; Ahmed, 2012) *Aedes* species were widely distributed throughout the district Rawalpindi, particularly in urban areas (Arslan *et al.*, 2016, Fantinatti *et al.*, 2007, Prophiro *et al.*, 2011 and Gomez *et al.*, 2005). Because of the erratic water supply, it was discovered during the *Aedes* collection that households often had water preserved for domestic usage, mainly in open containers. It was shown that storing water increased the chance of *Aedes* colonies growing and spreading dengue fever (Mukhtar *et al.*, 2012; Fatima, 2016). Phoung *et al* highlighted the same issue, identifying that mosquito larvae in water containers and gardens near the houses were the most important risk factors for dengue transmission (Schmidt, 2011).

This study manifested that rural areas dominated over the urban areas in terms of abundance of mosquitoes. The same results were observed in different countries of Europe by Alkan, Aldemir and Johnston (Alkan and Aldemir, 2010; Johnson *et al.*, 2014). Their reasoning was based on the fact that many mosquito species can readily deposit their eggs in rural locations such as wetlands, marshes, sludges, and estuaries, where there is an abundance of mosquito breeding habitat (Cox *et al.*, 2007). Our results agreed with the results of Johnston who in 2014 had found that abundance of mosquito increased with the decrease in urban spots and human population. (Johnston *et al.*, 2014). Many scientist other than him have found more *Culex* in rural areas (Cetin *et al.*, 2010 and Yaseen *et al.*, 2020) The type of vegetation in the rural and peri-urban areas may account for the observed significant difference in mosquito species richness across habitats (Patz *et al.*, 2003; Brant, 2011).

The genus *Culex* predominated in our study where the species *Culex quinquefasciatus* found abundantly in all the agroecological zones of Punjab (**Table 4.7.1**). Same results were obtained by Ashfaq *et al.*, in 2014, Mukhtar *et al* from South Punjab in 2014, Attullah *et al*, from Faisalabad in 2015, Manzoor *et al* from Lahore in 2020, Attullah *et al*, from Malakand in 2023. A high profusion of this species is due to its inordinate reproductive ability as it reproduces whole of the year. It is widely distributed in indoor as well as outdoor habitats. They are found in polluted water found in ditches, drains, septic tanks, artificial containers, ground pools etc. The same was reported by Badaki in 2010 in North Central Nigeria (Badaki, 2010; Service 2012). Its survival in all the seasons is also one of the reason of its abundance (Bhattacharya and Basu, 2016). *Culex tritaeniorhynchus* was the second 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). This virus is commonly found in all the continents like Asia, Europe and Africa (Campbel *et al.*, 2002). This virus not only affects humans but also animals like horses, cows and birds. (Halstead and Jacobson , 2013; Hemani *et al.*, 2014; Lou *et al*, 2016; Karthika *et al.*, 2018)

This study also included reports of *Anopheles subpictus*, a member of the *Anopheles* genus. This finding was in line with that of Rao (1984), who discovered that *Anopheles subpictus* was the most prevalent Anopheline in the majority of the Indian subcontinent. Its extensive range extended east and south to New Guinea (Cooper *et al.*, 2006), west to Iran (Sedaghat and Harbach, 2005), and north to China. Its propensity to reproduce in a wide range of habitats, including flowing or stagnant waters, turbid or clear waters, water with or without vegetation, unshaded or slightly shaded water bodies, wells, burrow pits, channels, ponds, tanks, ground pools, freshly flooded and fallow rice fields, cement cisterns, tree holes, lake margins, fresh or brackish waters, etc., is related to its abundance. *Anopheles stephensi* and *Anopheles culicifacies* was also found abundantly. According to past research, these two Anopheline mosquitoes are the primary malaria carriers. (Leslie *et al.*, 2009; Rowland *et al.*, 2000; Sinka *et al.*, 2010; Jahan and Hussain, 2011; Gayan Dharmasiri *et al.*, 2017; Khan *et al* 2017). The presence of such kind of vectors in the study area may cause serious consequences. According to WHO, in 2018, 60% of the people in Pakistan lived in the malaria-endemic region, Jalani in 2019 from mixed cropping zone, Khan in 2020 from Thal zone and Rehan in 2023 from cotton zone reported many malaria cases (Jalani *et al.*, 2019; Khan, 2022; Rehan *et al.*, 2023; Tariq-Ali, 2010). The findings for *Anopheles nigerrimus* are in line with earlier research that found the species in agricultural regions, specifically in rice fields in the vicinity of cities, where it

can be found in broad sunlight to moderate shade at elevations of up to 300 meters above sea level. (Harrison & Scanlon, 1975)

The third genus *Aedes* with its two species *Aedes albopictus* and *Aedes aegypti* were found to be abundantly found in Rice tract (**Table 4.1.1.2**) Mixed cropping **Table (4.2.1.2)**and Barani zones(**Table 4.6.1.2**) tehsils of Bahawalpur City and Bahawalpur Saddar (**Table 4.4.1.2**). The primary cause can be the high level of urbanization, which is absent from other districts and tehsils. Due to the conversion of agricultural, forest, and fallow land into urban areas, these areas have seen an increase in urbanization over time (Hussain *et al.*, 2020). The number of hospitals and clinics has expanded multiple times due to exceptional population expansion and rapid urban development. This has led to massive hospital waste and poor drainage, which have created ideal circumstances for mosquito reproduction, particularly *Culex* spp. (Khan *et al.*, 2020). High metropolitan areas are ideal mosquito breeding grounds due to the overuse of air conditioners, room coolers, disposable bags, and cans (Zahouli *et al.*, 2017). Compared to less urbanized locations, *Aedes* mosquitoes were typically found in densely populated areas, where they were typically found in old tires that are found at random (Mukhtar *et al.*, 2003; Khan *et al.*, 2017). Used tires are not disposed of appropriately in densely populated areas. They provide the perfect breeding habitat for *Aedes* because they efficiently store rainfall and warm quickly in the sun, even in the winter (Khan *et al.*, 2017). Similar findings were reported in a study carried out in North India, where it was shown that tires offer *Aedes aegypti* optimal breeding locations (Sikhon and Minhas, 2014) Many mosquito species benefit from the Bahawalpur Zoo's large animal population (Derraik, 2004), particularly the *Aedes* mosquito species, which needs artificial containers to reproduce. (Tuten *et al.*, 2012). Because of the low level of urbanization, *Aedes* was not discovered in the Cotton zone (Table 4.3.1.2) or the Thal zone (Table 4.5.1.2). Less industrialization, buildings, gardens, swimming pools, and piles of trash thrown everywhere are characteristics of less urbanized areas that do not supply *Aedes* with suitable habitats. The seasonal effect on the growth of mosquitoes in the current study was very much evident. The spring and autumn seasons were found to be the most favorable seasons for the proliferation of mosquitoes. On the other hand the extreme temperature of winters and early spring gave negative impact on the expansion of mosquito species. This peak of mosquito growth then again raised in late spring and summers. The same results were obtained in Pakistan (Akram *et al.*, 2009; Khan, 2022) and elsewhere (Alten *et al.*, 2000; Santos *et al.*, 2020).The current study showed that the mosquito species occurred in all the six districts of Punjab throughout the year in all the seasons.

It is demonstrated how the environmental variables of temperature, relative humidity, and rainfall relate to the quantity of larval and adult mosquitoes. (Figure 4.1.3.1, 4.2.3.1, 4.3.3.1, 4.4.3.1, 4.5.3.1 and 4.6.3.1) These numbers also demonstrated the impact of precipitation and rainfall on adult mosquito abundance during the wet season. Compared to the dry seasons, there was an increased amount of adult mosquito activity during the rainy months of July and August. Rainfall has a positive impact on mosquito populations by creating or preserving breeding habitats. (Koenraadt et al., 2004 ; Ceccato et al., 2005; Hu et al., 2006; Murty et al., 2010; Byun and Webb, 2012; ; Alshehri, 2013; Bashar and Tuno, 2014; Tian et al., 2015; Asigau and Parker, 2018; Evans et al., 2019)). However Deing in 2012 had found negative effect of rain as flushing out of the larvae from breeding sites in heavy rain.

This study clearly shows that temperature has a favorable effect on mosquito numbers. Numerous studies have determined that mosquito growth was most favourable at temperatures between 20°C and 29°C. (Bayoh and Lindsay, 2003; Alshehri, 2013; Tian et al., 2015);. Mosquito survivorship and abundance are reduced at temperatures above 30° C (Westbrook et al., 2010; Christiansen-Jucht et al., 2014). In contrast to this study, other researchers have found that high temperatures promote mosquito numbers and accelerate insect growth. (Alahmed, 2012; reference to be added) This study demonstrated that a high relative humidity raises the number of mosquito larvae and adults. The research area's increased mosquito abundance was facilitated by an average relative humidity ranging from 44% to 69% (Fig. 4a and b). Relative humidity has been found to affect mosquito survival and activity in various investigations. It has been observed that a mosquito's lifespan increases with humidity, and that a high humidity level increases the density and number of mosquitoes. (Tian et al., 2015; Bashar and Tuno, 2014; Alshehri, 2013; Alahmed, 2012; Murty et al., 2010; Reiter, 2001).

The GIS maps prepared were helpful in examining the geographical distribution of mosquitoes and the variables that affected the heterogeneities in that distribution. These maps will help us in analyzing the biology of adult mosquitoes by locating the mosquito hotspots that are known to transmit diseases. The creation of larval breeding sites and the production of interpolated measures of transmission as predictors to better understand the relationship between disease transmission and disease outcomes may be influenced by the elevation, slope, and land-use patterns shown on the maps. It may also serve as a forecast for regions with a high risk of disease transmission. With this information, we can create future disease control and surveillance programs (Giming et al., 2005; Jemal et al., 2018).

The fastest DNA extraction technique for DNA barcoding was applied in this investigation. This method is useful tool for classifying and differentiating between species, but there are many problems in this regard. First of all, the DNA is contaminated since RNase and proteinase are not used, and there is no phase separation or magnetic bead separation. In particular, the DNA has a high concentration of PCR inhibitors that may interfere with proper primer annealing or polymerase activity. There are areas of the mosquito where there is insufficient high-quality DNA. It can be challenging to recover DNA of a high enough quality from the mosquito's wings and legs in particular. PCR results are not satisfactory when extracted mitochondrial DNA from a complete mosquito specimen is used. There are several PCR inhibitors in the abdomen and limbs, and a large amount of chitin is present in the shell. (Cywinska *et al.*, 2006; Ruiz *et al.*, 2010).

The idea molecular constraints also apply to DNA barcoding. Because the analysis is focused on a single gene or a portion of a gene, there is a restricted amount of molecular data available. A gene that has evolved quite quickly could be sufficient to determine the species. For a more profound comprehension of evolution in taxonomy, species characterisation, and discrimination, standard DNA taxonomy, transcriptome analyses, behavior, and morphological investigations are favored. A holistic approach should always be used when new species are discovered or when fresh data indicates that the existing Linnaean taxonomy has to be altered. DNA barcoding will be viewed as an addition to that viewpoint and has a lot of promise as a useful diagnostic tool, either (Will *et al.*, 2005; Ebach and Holdrege, 2005).

Our findings demonstrated a distinct and well-defined pattern of diversity, abundance, and spatiotemporal distribution of mosquitoes in relation to anthropogenic changes in the landscape of the areas observed. The species like *Aedes albopictus* and *Culex quinquefasciatus* were more detected in those areas that which were considered more urbanized like Rice tract zone, Mixed cropping Zone and Barani zone. The results of this research add to the conversation about the threats to public health that urbanization poses, particularly with regard to the development of mosquito-borne diseases in regions that are primarily defined by dense populations or quickly expanding urbanization.

CONCLUSION

In this study we provide strong assertion that the urbanization, environmental factors and seasons are important factors in determining the abundance and composition of the mosquitoes. The results of this research add to the conversation about the threats to public health that urbanization poses, particularly with regard to the development of mosquito-borne diseases in regions that are primarily defined by dense populations or quickly expanding urbanization. Urban disease dynamics provide a serious risk to public health systems, necessitating the development and implementation of multidisciplinary, multisectoral approaches for the prevention and control of disease.

The discovery of *Culex perexiguus* focused the significance of more research on the geographic distribution of mosquitoes, ecological factors, the detection of diseases related epidemiological surveillance, and potential epidemiological connections with newly developing and reemerging arboviruses along the Rawalpindi district.

In order to reduce health risks and guarantee the sustainability of their development, project developers may also utilize the GIS maps created in this research that highlight danger areas to relocate the project or fund mosquito control measures. The maps prepared through GIS may be successfully applied to support development and health decision-making.

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<https://bahawalpur.org/tehsils/>

www.doi.punjab.gov.pk/system/files/Rawalpindi

<https://bahawalnagar.org/tehsils/>

ANNEXURES

List of the coordinates of the Tehsils of districts, Punjab			
No.	District	Tehsil	Coordinate
1	Lahore	Cantt	N 31°32'13.6392"
			E 74°22'57.6876"
			Sarfraz Rafique Rd Cantt
		Shalimar town	N 31°34'19.3224"
			E 74°22'13.5552"
			Mughalpura
		Model Town	N 31°30'37.71"
			N 31°30'37.71"
			Gulberg 3
		Lahore city	N 31°34'19.1784"
			E 74°18'20.6352"
			Anarkali
2	Bahawalnagar	Bahawalnagar	N 29°12'35.1936"
			E 72°50'29.9364"
			Chak 274/H.R
		Haroonabad	N 29°42'33.4332"
			E 73°00'18.6876"
			Adda pul murad
		Chishtian	N 29°46'59.0016"
			E 72°52'04.7244"
			Chishtian
		Fortabbas	N 29°11'30.9516"
			E 72°50'59.442"
			Fortabbas
3	Layyah	Layyah	N 30°09'40.7592"
			E 73°34'03.702"
			City link rd
		Karor Lal Esan	N 30°57'54.3888"
			E 71°04'55.9416"
			Azam Road rd
		Chaubara	N 31°13'21.8064"
			E 70°57'54.1836"
			Karor lal esan
		Chaubara	N 30°54'27.3744"
			E 71°30'31.104"
			Chaubara

4	Faisalabad	Faisalabad	N 31°32'35.1024"
			E 73°12'50.7276"
			Chaubara
		Saddar	N 31°25'33.4308"
			E 73°08'47.1984"
			Eden Garden Road
		Jaranwala	N 31°19'56.388"
			E 73°24'43.506"
			Mehmood Colony
		Tandiawala	N 31°01'46.956"
			E 73°07'38.1468"
			Mumtazabad
		Samundri	N 31°05'59.1108"
			E 72°58'23.3004"
			Chaubara
		Chak Jhumra	N 31°33'53.064"
			E 73°11'06.972"
			Chak Jhumra
5	Bahawalpur	Bahawalpur	N 29°23'75.346"
			E 71°72'55.215"
			Chak 42/D.B
		Saddar	N 29°23'09.2364"
			E 71°40'48.4608"
			Bahawalp
			ur Cantt
		Ahmedpur East	N 29°22'07.284"
			E 71°38'07.5624"
			Ahmedpur
		Yazman	N 29°13'41.811"
			E 71°74'49.911"
			Rashid Javed Rd
		Khairpur Tamewali	N 29°35'00.78"
			E 72°14'00.3444"
			Khairpur tamewali
		Hasilpur	N 29°41'59.1108"
			E 72°33'25.8516"
			Jalandhar Colony
		Rawalpindi	N 33°38'31.4772"
			E 72°57'54.3996"
			Golra more
		Taxila	N 33°44'35.6388"
			E 72°48'12.15"

		Taxila
		N 33°54'32.616"
	Murree	E 73°23'43.9944"
		Murree
		N 33°48'25.4592"
6	Rawalpindi	E 73°31'37.3548"
	Kot sattian	Kotli sattian
		N 33°35'49.3584"
	Kahuta	E 73°23'06.5688"
		Thana Rd
		N 33°25'15.15"
	Kallar syedan	E 73°22'07.5576"
		THQ hospital
		N 33°15'13.4604"
	Gujar Khan	E 73°18'34.5672"
		Gujar khan

DATA OF THE CLIMATE OF DISTRICT LAHORE

RRR = MONTHLY TOTAL RAIN (MM)[-1=TRACE] [-100 Means data not available]

R	YEA											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	32.6	83.7	12.8	54.5	23.1	28.5	400.9	106.1	110.5	27.6	3.9	13.7
2020	64.6	9.0	105.7	25.0	18.5	43.7	151.7	316.3	107.7	0.0	21.6	16.8

TXTX = MONTHLY MEAN MAX TEMP. (oC) [-100 Means data not available]

R	YEA											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	19.0	20.0	25.7	34.7	37.6	40.6	35.0	35.1	35.3	31.2	25.6	17.0
2020	16.2	22.9	24.8	32.1	37.6	38.1	36.5	34.8	35.7	33.7	25.5	19.6

TNTN = MONTHLY MEAN MINIMUM TEMPERATURE (oC) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	7.5	10.1	14.6	22.1	24.2	27.7	26.2	27.4	26.7	20.6	15.2	8.1
2020	7.0	10.9	14.9	20.8	25.3	27.0	27.8	27.1	27.4	20.0	12.0	7.7

UU2 = HUMIDITY AT 0800 AM (%) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	81	83	71	57	47	46	80	81	79	79	82	87
2020	85	77	80	63	52	62	74	82	74	66	76	85

UU3 = HUMIDITY AT 0500 PM (%) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	54	55	44	34	26	30	63	64	60	51	55	68
2020	65	49	55	37	32	42	57	67	52	32	44	60

DATA OF THE CLIMATE OF DISTRICT FAISALABAD

RRR = MONTHLY TOTAL RAIN (MM)[-1=TRACE] [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
AN.TOTAL												
2019	14.6	66.0	33.2	37.8	15.6	55.0	87.7	44.4	29.6	17.7	5.4	4.0
2020	54.8	26.0	144.1	24.4	25.8	72.5	170.6	159.0	33.9	0.0	-1.0	7.0

TXTX = MONTHLY MEAN MAX TEMP. (oC) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	18.7	19.6	24.9	34.4	38.2	41.4	37.1	37.2	37.1	32.1	25.6	17.0
2020	16.3	23.0	24.0	31.9	37.7	38.8	37.4	36.6	36.4	34.2	25.5	20.3

UU2 = HUMIDITY AT 0800 AM (%) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	88	88	78	63	46	45	77	79	80	80	85	91
2020	92	85	88	70	50	62	75	81	79	73	83	90

DATA OF THE CLIMATE OF DISTRICT BAWALNAGAR

RRR = MONTHLY TOTAL RAIN (MM)[-1=TRACE] [-100 Means data not available]
=====YEA

R	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	5.0	20.8	2.5	62.0	56.0	6.0	110.0	9.0	59.0	4.0	16.0	11.0
2020	44.5	2.0	84.0	18.0	7.0	48.5	39.0	17.0	109.0	0.0	-1.0	8.0

TXTX = MONTHLY MEAN MAX TEMP. (oC) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	19.9	22.1	28.4	37.5	40.1	43.5	40.4	38.5	38.5	33.9	27.0	18.2
2020	17.1	24.7	26.1	34.7	40.7	41.6	40.4	39.0	38.1	36.2	27.2	21.5

TNTN = MONTHLY MEAN MINIMUM TEMPERATURE (oC) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	6.5	8.1	13.7	20.2	24.4	27.1	28.0	27.8	27.4	20.0	12.6	6.9
2020	7.1	9.8	14.9	21.0	25.0	26.9	28.0	26.5	25.4	19.0	12.0	7.7

UU2 = HUMIDITY AT 0800 AM (%) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	82	82	71	61	51	44	57	69	75	78	80	89
2020	89	84	82	65	56	58	69	74	73	71	75	83

UU3 = HUMIDITY AT 0500 PM (%) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	55	50	38	29	25	23	44	53	49	39	46	61
2020	65	43	55	37	28	34	46	53	47	32	41	52

DATA OF THE CLIMATE OF DISTRICT BAHAWALPUR

RRR = MONTHLY TOTAL RAIN (MM)[-1=TRACE] [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	9.0	38.0	10.3	18.0	22.3	26.8	-1.0	44.4	24.0	66.2	21.0	16.6
2020	27.4	-1.0	95.3	12.1	10.0	13.1	31.4	2.3	74.7	0.0	1.0	10.3

TXTX = MONTHLY MEAN MAX TEMP. (oC) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	19.2	20.2	26.3	36.8	39.1	41.4	39.6	38.2	37.9	33.1	26.9	19.2
2020	17.3	24.8	26.0	34.7	39.4	40.8	40.1	39.0	37.0	35.5	27.6	22.2

TNTN = MONTHLY MEAN MINIMUM TEMPERATURE (oC) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	6.7	7.9	13.4	21.1	24.2	27.3	29.7	28.5	27.4	19.8	14.0	7.1
2020	6.5	10.0	14.6	20.4	24.6	28.7	29.5	29.4	26.2	19.0	12.4	7.4

UU2 = HUMIDITY AT 0800 AM (%) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	92	88	79	65	56	58	69	75	76	85	90	
2020	90	82	86	69	57	62	67	73	76	66	79	91

UU3 = HUMIDITY AT 0500 PM (%) [-100 Means data not available]

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2019	60	55	45	32	28	31	45	53	52	46	51	62
2020	63	44	55	35	31	36	46	52	49	35	48	57

DATA OF THE CLIMATE OF DISTRICT LAYYAH

RRR = MONTHLY TOTAL RAIN (MM)[-1=TRACE] [-100 Means data not available] YEA

R	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
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2019	29.4	9.9	57.9	78.4	5.0	30.2	70.3	93.0	4.0	34.0	12.9	6.4
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2020	42.5	35.4	124.7	27.1	9.5	10.5	58.6	21.8	104.0	0.0	-1.0	3.2
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TXTX = MONTHLY MEAN MAX TEMP. (oC) [-100 Means data not available] YEA

R	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
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2019	18.7	19.9	24.4	33.9	38.3	41.3	38.9	37.3	38.2	32.3	26.2	17.3
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2020	16.7	23.8	23.5	32.2	38.3	40.6	39.6	39.1	36.8	34.4	25.2	21.3
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TNTN = MONTHLY MEAN MINIMUM TEMPERATURE (oC) [-100 Means data not available] YEA

R	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
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2019	4.8	7.1	11.8	19.1	22.6	26.1	27.0	26.4	26.2	18.4	12.0	5.1
------	-----	-----	------	------	------	------	------	------	------	------	------	-----

2020	4.6	7.5	12.5	18.5	23.2	26.8	27.6	28.2	24.4	16.6	10.0	5.7
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UU2 = HUMIDITY AT 0800 AM (%) [-100 Means data not available] YEA

R	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
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2019	85	81	81	71	54	53	71	79	79	80	81	87
------	----	----	----	----	----	----	----	----	----	----	----	----

2020	85	81	85	77	62	63	69	75	80	76	82	84
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UU3 = HUMIDITY AT 0500 PM (%) [-100 Means data not available] YEA

R	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
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2019	51	45	47	38	27	30	49	56	49	49	50	60
------	----	----	----	----	----	----	----	----	----	----	----	----

2020	53	43	61	46	32	40	48	54	53	42	53	51
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DATA OF THE CLIMATE OF DISTRICT RAWALPINDI

RRR = MONTHLY TOTAL RAIN (MM)[-1=TRACE] [-100 Means data not available]
 ====== YEA
 R JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
 ======
 2019 141.3 126.1 74.2 91.0 52.0 31.0 252.4 535.5 97.6 42.2 54.6 16.0
 2020 111.5 65.5 236.8 118.0 131.6 89.0 219.5 398.7 96.0 0.0 92.8 21.2

TXTX = MONTHLY MEAN MAX TEMP. (oC) [-100 Means data not available]
 ======
 YEAR JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
 ======
 2019 15.9 17.4 22.2 30.8 34.4 38.3 35.3 33.5 33.3 29.3 22.8 18.0
 2020 15.8 21.3 22.0 27.9 33.4 35.6 36.2 33.9 33.9 31.8 23.2 19.0

UU2 = HUMIDITY AT 0800 AM (%) [-100 Means data not available]
 ======
 YEAR JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
 ======
 2019 92 89 85 72 58 50 77 84 84 88 93 95
 2020 93 85 87 76 61 64 72 87 76 78 90 93

PLAGIARISM REPORT



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From Department of Zoology.

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LIST OF PUBLICATIONS

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1.	Effect of urbanization and season on the spatiotemporal distribution of mosquitoes in Bahawalpur, Pakistan	Pakistan Journal Of Zoology	X	Published
2.	The Effect of Urbanization And Climatic Factors On The Spatiotemporal Distribution Of Mosquitoes In Different Agroecological Zones Of Punjab, Pakistan	Pakistan Veterinary Journal	X	Under Reviewed

List of Tables

Sr. No.	Topic of the Table
	<u>Table 4.1.1: Diversity index of a number of individuals of different species of mosquitoes collected from five tehsils of District Lahore.</u>

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