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We are proud to launch the first issue of *Pakistan Journal of Zoological Sciences*. This new journal provides a platform for scientists to share important research about animals and their environments. Our goal is to publish high-quality studies that help us understand and protect wildlife, especially in Pakistan and surrounding regions. This first issue includes exciting research on two key topics: how microplastics affect fish health and how mosquitoes live in urban areas. These studies show real-world problems that scientists are working to solve. We believe this kind of research can help make better decisions about conservation and public health.

Our journal follows strict scientific standards with careful peer review, but we make sure the research is available to everyone through open access. We want students, researchers, and policymakers to benefit from these findings. We thank all the scientists who submitted their work, the experts who reviewed the papers, and our supporting institutions. Their hard work made this first issue possible.

To researchers everywhere: we invite you to share your zoology studies with us. Together, we can grow our understanding of animals and their ecosystems. This journal belongs to all of us who care about wildlife science and conservation. *Pakistan Journal of Zoological Sciences* is just beginning, but we hope it will become an important resource for zoological research in our region and beyond. We look forward to publishing more valuable studies in future issues.

Ms. Saba Malik

Founder & CEO

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RESEARCH ARTICLE

Assessing The Role of Bioclimatic Variables in Shaping Diptera Biodiversity and Distribution in District Faisalabad, Punjab

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Abstract Agricultural and climatic changes are reshaping insect communities globally, yet the role of bioclimatic factors in determining Diptera diversity remains underexplored. This study examined the effects of temperature, precipitation, humidity, and seasonality on Diptera occurrence across 14 sites in District Faisalabad, Pakistan. Field observations of 11 ecologically and medically relevant species were analyzed alongside 19 WorldClim bioclimatic variables. Principal Component Analysis (PCA) revealed that temperature (PC1) and moisture (PC2) accounted for 61% of the environmental variation. Sites were grouped into four clusters warm, humid, cool, wet, cool and dry, and hot, dry each supporting distinct Diptera assemblages. K-means clustering validated these ecological groupings, highlighting microclimatic gradients as key drivers of species composition. Warm–humid sites harbored the highest richness, while arid and cooler areas supported more specialized or generalist taxa. Correlation analysis showed strong positive associations between Diptera abundance and both Bio5 (maximum temperature) and Bio12 (annual precipitation), suggesting these as primary predictors of species richness. Overall, the findings underscore the importance of temperature and moisture thresholds in shaping Diptera communities and offer a framework for forecasting climate-induced distribution shifts and managing key fly species.

Keywords: diptera diversity, bioclimatic variables, species distribution modeling, principal component analysis (PCA), microclimate gradient

Introduction

True flies (Order: Diptera) display remarkable biodiversity, with their distribution closely linked to bioclimatic factors such as temperature, humidity, precipitation, and seasonal variability. As ectothermic organisms, their development, reproduction, and survival are directly affected by these environmental variables. Species Distribution Models (SDMs) like MaxEnt are widely used to predict their ranges based on such climatic inputs. Temperature and humidity are among the most influential factors. Studies from Pakistan's Malakand and Dir Lower districts showed mosquito populations peaking at summer temperatures of 21.6°C to 34.6°C, with relative humidity from 21% to 94%. Similarly, a metabarcoding study in Eastern China found that Diptera diversity varied significantly with elevation, slope direction, and especially seasonal changes, which had the strongest effect on

species composition.
In temperate regions like Moscow, drosophilid fly populations rose during hot, dry summers, confirming that temperature and precipitation drive abundance. Sandflies exhibit clear climatic preferences: *Phlebotomus* species dominate subtropical areas, while *Lutzomyia* and *Brumptomyia* prefer tropical climates. Seasonal activity patterns also reflect this dependence. For instance, in southern Punjab, Diptera remained active year-round, unlike other pollinators that ceased activity below 20°C. In Egypt, MaxEnt modeling of the gall midge *Schizomyia buboniae* identified temperature seasonality and altitude as critical distribution predictors. In Faisalabad, studies show fluctuations in Diptera biodiversity correspond with changing temperature and moisture. Vector studies reveal that *Phlebotomus major* and *P. hindustanicus* prefer cooler, moist highlands, while *P. papatasi* and *P. sergenti* are more prevalent in dry lowlands. In Sri Lanka, SARIMA modeling showed that *Bactrocera dorsalis* populations peak in wet and intermediate climate zones. In Iran and the U.S., similar models demonstrated that precipitation and temperature changes directly impact mosquito distribution. Climate change is predicted to expand the geographic range of species like *Sarcophaga dux* and *S. haemorrhoidalis*, affecting their forensic utility. In Europe, *Drosophila suzukii* has adapted to new conditions, influenced by factors like annual precipitation and temperature seasonality.
North American studies of frugivorous *Drosophila* and their parasitoids show increasing diversity with latitude, largely driven by temperature and rainfall variations. In Australia, temperature and elevation help explain the coastal confinement of *Anopheles farauti*. Land use and vegetation also impact Dipteran distributions. For instance, Austrian research showed mosquito composition differed between forested and agricultural zones. In Thailand's Doi Pha Hom Pok National Park, black fly diversity responded to stream velocity, canopy height, and vegetation cover, with stream width and conductivity being significant at 500–700 meters altitude. Malaysian studies found altitude, water speed, and dissolved oxygen to influence aquatic Diptera diversity. In Jordan, necrophagous flies increased in humid conditions but declined under temperature stress.
A Mexican study reported slightly higher genus-level Psychodidae diversity in protected forests versus unprotected ones, indicating that anthropogenic land use may reduce biodiversity. In Brazil's Pampa biome, *Drosophila melanogaster* and *Zygothrica vittimaculosa* showed negative correlations with temperature. Pakistani research on *Phlebotomus* species confirms that elevation and humidity are major determinants of distribution. Predictive models like MaxEnt remain essential. The distribution of *Spogostylum royale*, for instance, was strongly tied to maximum temperature and annual temperature range. In Iran, *Anopheles maculipennis s.l.* and *Culex theileri* distributions were linked to temperature seasonality, precipitation, and NDVI. In tropical streams, black flies and other Diptera were strongly affected by temperature, precipitation, and elevation.
Climate projections suggest that pests like *Bactrocera dorsalis* will become more widespread under warming scenarios, especially by 2070. Such changes call for climate-informed strategies in pest control and vector management. Overall,bioclimatic factors—particularly temperature, rainfall, and seasonal dynamics—are crucial to understanding Diptera's distribution and ecological roles as pollinators, decomposers, and disease vectors. Ongoing modeling and field research continue to reveal how climate shapes Diptera biodiversity and distribution patterns globally.

Materials and Methods

Data Collection

This study on Diptera biodiversity and distribution, with emphasis on bioclimatic factors, is conducted in Faisalabad, located in central Punjab, Pakistan. Covering 5,856 square kilometers at 31°24′N and 73°04′E, Faisalabad comprises urban, rural, and agricultural landscapes. The district's semi-arid climate features hot summers with temperatures reaching 40°C and mild winters with nighttime lows near 10°C. Rainfall is concentrated during the monsoon season from July to September, contributing 300–400 mm annually as shown in Fig 1. These climate variations make Faisalabad a suitable site for studying insect biodiversity responses to environmental conditions such as temperature, humidity, and precipitation.

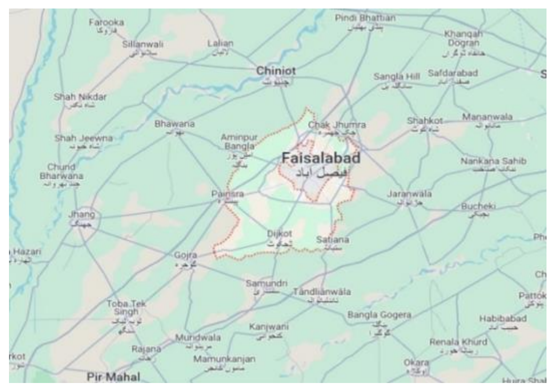

Figure 1. *Collection sites were within and around the Faisalabad region of Punjab, Pakistan.*

The district's diverse habitats including urban parks, sidewalks, residential zones, agricultural fields, and riparian wetlands offer varied ecological conditions for Diptera species. Urban areas, despite human interference, sustain biodiversity in green spaces like parks and gardens. Agricultural regions produce wheat, rice, sugarcane, and cotton, offering suitable conditions for pollinators, decomposers, and pest-regulating flies. Wetlands and canals further enrich habitat diversity by supporting semi-aquatic species. Although urbanization has diminished natural forest cover, scattered forest patches and riverbank reserves continue to offer refuge for many Dipteran species. This environmental gradient, ranging from urban to wetland zones, allows for a detailed analysis of how temperature, moisture, and vegetation influence Diptera distribution. It also provides insight into the impacts of land use and climatic change on local biodiversity.

Diptera species exhibit marked sensitivity to bioclimatic variables. Many displays temperature-dependent seasonal life cycles, with some preferring moist conditions driven by precipitation levels. Relative humidity significantly influences survival and activity, especially in semi-arid zones. Solar radiation affects species behavior and reproduction. By assessing these factors, the study aims to explain Diptera distribution patterns across varied habitats in the Faisalabad district.

Data Processing

To determine Diptera richness and abundance, a comprehensive approach involving sampling, specimen handling, identification, and statistical analysis will be applied:

- Sampling Design
- Insect Pinning
- Identification of Insects
- Data Analysis
- Statistical Analysis

Sampling Design

A stratified random sampling approach will be used to ensure representative coverage of urban, rural, and agricultural habitats. Selected sites will vary by elevation, land use, and vegetation type to reflect different bioclimatic conditions. Collection tools will include pan traps for ground insects, yellow sticky traps for flying adults, Malaise traps for aerial species, and sweep nets for plant-dwelling Diptera. Light traps will be used to attract nocturnal species. Sampling will occur every two weeks over 12 months to capture seasonal fluctuations in species diversity. Simultaneously, temperature, humidity, rainfall, and solar radiation data will be gathered using GIS-based tools and local meteorological records. This will enable correlation between environmental variables and Diptera distribution and diversity.

Pinning of Insects

Standard entomological techniques will be used to preserve collected Diptera specimens. Each insect will be pinned through the thorax, with careful positioning of wings and legs to maintain diagnostic features. Specimens will be stored in insect boxes and labeled with detailed collection information, including date, location, and local climate conditions at the time of capture. A controlled dry environment will ensure long-term preservation and prevent deterioration. These specimens will be maintained as a reference collection for future identification and taxonomic verification.

Identification of Insects

Insect identification will rely on both morphological and molecular approaches. Morphological classification will be based on established taxonomic keys that emphasize traits such as wing venation, antenna structure, and body segmentation (Mullen & Durden, 2002; Freidberg, 2008). When specimens lack distinguishable physical features or fall into cryptic groups, DNA barcoding will be employed. This involves analyzing the mitochondrial cytochrome c oxidase I (COI) gene to confirm species identity. The resulting sequences will be compared with global reference databases like the Barcode of Life Database (BOLD). All identified species will be cataloged, and relevant bioclimatic data will be used to assess species-environment relationships.

Statistical Analysis

Data analysis will include both univariate and multivariate techniques to explore the relationships between Diptera diversity and environmental factors. Diversity indices such as species richness, Shannon-Wiener, and Simpson's index will quantify the species composition in different habitats. Multivariate tools, including Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA), will be used to examine how climate variables—temperature, precipitation, humidity, and solar radiation—influence community structure and species distribution. To forecast the potential distribution of Diptera under various climate scenarios, the MaxEnt (Maximum Entropy) species distribution modeling tool will be used. This model integrates presence-only data with environmental variables to predict suitable habitats. Statistical analysis will be conducted using R software (version 4.0) and SPSS (version 25), ensuring robust interpretation and reproducibility. These tools will help clarify how climatic and ecological factors interact to shape Diptera biodiversity across Faisalabad's heterogeneous landscape.

Results and Discussion

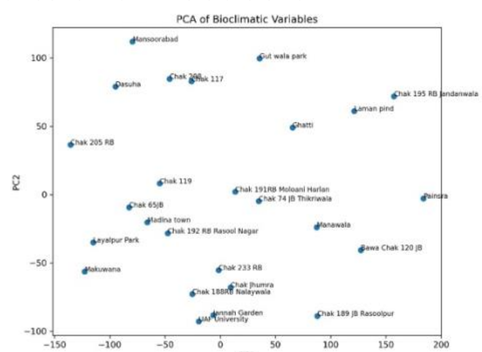

Figure 2. *PCA of Bioclimatic Variables.*

Fig 1. Principal Component Analysis (PCA) revealed key climatic factors influencing Diptera distribution across various sites in District Faisalabad. The first two principal components (PC1 and PC2) explained 61% of total variance. PC1 mainly reflected temperature-related variables (e.g., annual mean temperature, seasonality), while PC2 represented precipitation and moisture (e.g., rainfall in the wettest quarter).

Sites like Chak 195 RB Jandawala and Laman Pind—positioned in the upper-right PCA quadrant—exhibited high temperature and humidity, supporting moisture-dependent

Dipteran species such as mosquitoes and crane flies. Meanwhile, areas like Mansoorabad and Gut Wala Park (top-left quadrant) showed cooler but moist conditions, ideal for species such as non-biting midges and fungus gnats. The lower-left quadrant, including UAF University and Jannah Garden, had both low temperature and precipitation, likely limiting Diptera diversity to generalist species like Muscidae. In contrast, the lower-right quadrant (Painsra, Chak 189 JB Rasoolpur) featured hot, arid conditions favoring thermophilic and xerophilic species, albeit with reduced overall diversity.

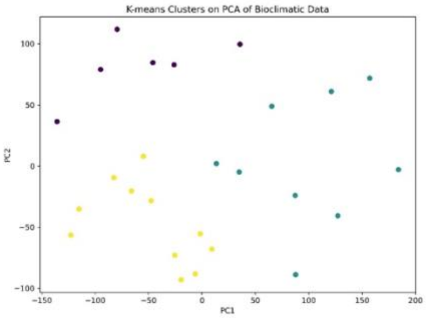

Figure 3. *K-means Cluster on PCA.*

K-means clustering Fig 3, helped group locations by climate similarity. Moderate environments like Chak 119 and Madina Town clustered near the PCA center, indicating balanced conditions that support greater Diptera diversity. More extreme sites such as Painsra and Makuwana, located at PCA margins, exhibited either heat stress or moisture extremes, supporting more specialized Dipteran communities. These groupings validated the importance of microclimatic gradients in determining insect distribution and biodiversity hotspots.

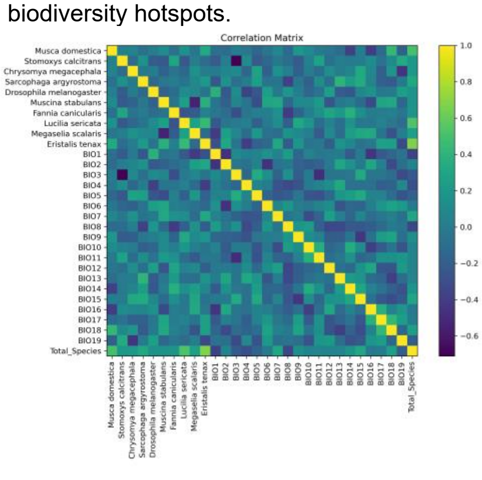

Figure 4. *Correlation Matrix.*

The correlation matrix displayed associations between Diptera abundance, total species richness, and 19 bioclimatic variables (BIO1–BIO19) as shown in Fig 4. Bright yellow tones indicated strong positive correlations, particularly between species richness and variables like Bio10 (mean temperature of the warmest quarter) and Bio18 (precipitation of the warmest quarter), suggesting these factors promote Diptera diversity. Negative correlations highlighted in dark blue indicated climatic stress, such as high maximum temperatures (Bio5) reducing species presence. This matrix provided valuable ecological thresholds and highlighted key drivers affecting Diptera communities, crucial for modeling climate impacts and informing biodiversity conservation in Faisalabad.

Discussion

Study's findings demonstrate how bioclimatic factors particularly temperature and precipitation have great influence on the distribution and abundance of Diptera species in Faisalabad. This is in line with earlier studies that emphasize how climate influences the makeup of insect communities (e.g., Zhang et al., 2020; Hammami et al., 2021). Spatial differences between study sites were well-represented by the PCA plot, which demonstrated that areas with moderate to high humidity and temperature, as Chak 195 RB Jandawala and Laman Pind, support a higher diversity of Diptera. This lends credence to the ecological theory that warm, humid climates promote insect reproduction and survival by providing adequate food supplies and larval homes (Rogers and Randolph, 2006).
Conversely, localities such as UAF University and Chak 188RB Nalyawala, with lower temperatures and limited rainfall, were associated with reduced species richness. Such environments impose physiological stress on insects, restricting survival to only a few generalists or xerophilic taxa like Muscidae or Calliphoridae. These finding parallels previous observations where Diptera biodiversity was reduced in arid or thermally extreme zones (Basset et al., 2012).
The correlation matrix enhances understanding through its capability to detect individual species reactions to different climate variables. The house fly (Musca domestica) exhibited maximal population distribution in urban and semi-urban areas due to its positive connection with Bio10 (mean temperature of the warmest quarter) and Bio12 (annual precipitation) (Grzywacz et al., 2017). The occurrence of Megaselia scalaris together with Eristalis tenax declined because these species need moist and organic-rich areas yet they can't tolerate dry climatic conditions as shown by research from diverse temperature and tropical zones (Brown et al., 2005).
Faisalabad's climate microzones according to the PCA site grouping determine the distribution patterns of Diptera insects in this region. Stomoxys calcitrans along with Chrysomya megacephala showed higher incidence levels in wet and waste-containing locations near agricultural and water resources. Another example of how microhabitats influence species distribution is the presence of Drosophila melanogaster close to ripening and fermenting regions (Markow & O'Grady et al., 2005).
Furthermore, during the wettest times (Bio16 and Bio18), species richness and precipitation showed a positive correlation, indicating that rainfall affects adult emergence rates and dispersal in addition to providing breeding substrates. This supports results from research on tropical entomology, which showed that temporal population surges in Dipteran flies were directly governed by rainfall patterns (Carvalho et al., 2004). The study offers strong

proof that the biodiversity of Diptera in District Faisalabad is largely influenced by bioclimatic factors, particularly temperature and moisture availability. PCA and correlation matrix analysis work well together to show that some areas support great species diversity because of their climatic circumstances, making them ecological hotspots. Harder microclimates, on the other hand, restrict variety to a small number of hardy or specialist taxa.

These discoveries have applications in public health, pest control, and conservation in addition to advancing our ecological knowledge of Diptera distribution in Pakistan. Keeping an eye on Diptera population variations can be a sensitive bioindicator of ecological change as climate patterns alter as a result of global warming. The study emphasizes the necessity of adaptive biodiversity initiatives and targeted climate monitoring, particularly in areas like Faisalabad that are quickly urbanizing.

Conclusions

This study clearly demonstrates that bioclimatic factors, especially temperature and precipitation, play a crucial role in shaping the distribution and diversity of Diptera species in Faisalabad. Areas with warm and humid conditions supported higher species richness, while cooler and drier zones showed reduced diversity, reflecting the physiological constraints these environments impose on insects. The combination of PCA and correlation matrix analyses highlighted how specific climatic variables influence not only overall species abundance but also the presence of particular Diptera taxa with distinct ecological preferences. These findings align with broader ecological theories and previous research, confirming that suitable temperature and moisture levels are essential for the survival, reproduction, and dispersal of many Diptera species.

The results emphasize the importance of microclimatic variability in determining local Diptera communities, with wetter and moderate temperature regions acting as biodiversity hotspots and harsher environments favoring more specialized, tolerant species. This knowledge has practical implications for public health, pest management, and biodiversity conservation in rapidly urbanizing regions like Faisalabad. Monitoring Diptera populations can serve as an effective bioindicator of environmental change amid climate variability and urban expansion. Therefore, ongoing ecological surveillance and adaptive conservation strategies are vital to maintaining insect diversity and ecosystem health in the face of future climate challenges.

Author Contributions

Qi Xue: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Qian Tang: Writing – review & editing, Visualization, Formal analysis, Conceptualization. Lin Deng: Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition. Wei Luo: Writing – review & editing, Conceptualization. Mingle Xia: Writing – review & editing, Conceptualization. Shuang Fu: Writing – review & editing, Conceptualization. Chaoqun Tan: Writing – review & editing, Conceptualization. Jun Hu: Writing – review & editing, Conceptualization. Rajendra Prasad Singh: Writing – review & editing.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Data Availability

Data will be made available on request

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RESEARCH ARTICLE

Factors Responsible for Shaping the Distribution and Biodiversity of Different Species of Lepidoptera in District Faisalabad

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Abstract The analysis examines butterfly distribution and diversity in District Faisalabad, Punjab, Pakistan, and the impacts of ecological (climate variables and land cover) and anthropogenic (population density and urbanization) factors. A diverse assemblage including *Pieris rapae*, *Danaus chrysippus*, and *Euploea core* was recorded. PCA and K-means clustering revealed three major climatic zones in Faisalabad. Species like *Eurema hecabe* and *Pieris rapae* were common in agro-ecosystems due to human activity adaptation and host plant presence. Correlation matrix showed strong positive correlations between butterfly richness and moderate climates (stable temperatures, adequate precipitation), while extreme weather or stress led to negative correlations. Butterflies, as pollinators and bioindicators, are affected by urbanization, habitat fragmentation, and pesticide use. Results emphasize targeted conservation through nectar-rich vegetation and reduced chemical use to sustain butterfly diversity.

Keywords: Climatic factors, diverse assemblage, temperature, precipitation, humidity

Introduction

Moths and butterflies, belonging to the order Lepidoptera, exhibit diverse distribution patterns shaped by ecological and environmental conditions. Climate is a major driver, with seasonal fluctuations, temperature, and humidity significantly affecting species richness. Tropical regions with warm, stable weather support high Lepidoptera diversity, while colder or drier climates restrict survival. Elevation and microclimate also influence habitat suitability and species distribution. Vegetation type and habitat availability are also vital. Plant diversity affects Lepidoptera distribution due to their reliance on specific host plants for feeding and reproduction. Wetlands, forests, grasslands, and agro ecosystems offer varying resources that impact species abundance and community composition. Habitat fragmentation and deforestation further modify distributions.

Pakistan, primarily an agricultural country, features diverse soil types and irrigation systems. Punjab, with 69% of its land under agriculture, is the most productive province. Faisalabad contributes 3985 hectares to Punjab's agriculture. Lepidoptera, the second-largest insect order with over 180,000 species globally (Perveen and Ahmad, 2012), includes butterflies, moths, and skippers. Over 5,000 insect species have been documented in Pakistan, including around 400 moths and butterflies. More than 100,000 species have been studied to date (Richards & Davies, 1977). The agro forest concept integrates agricultural and forest ecosystems (Ishizuka et al., 1995). Lepidoptera dispersal, reproduction, and ecological interactions are highly influenced by global climate change. Species such as Spodoptera frugiperda have expanded their ranges due to shifting climate conditions. In Iran, Zygaenidae moths are predicted to move due to climate change, with endemic species shifting to higher elevations. In Nepal's trans-Himalayas, flora diversity correlates with butterfly richness along elevation gradients. Lower elevations with shrubby vegetation support more species than higher, less diverse zones.

Butterflies like *Pieris rapae* have shown adaptability in fragmented agricultural landscapes, with mobile generalist species adjusting more easily than specialists. Migration correlates with higher genetic diversity in butterflies, potentially enhancing environmental resilience. Habitat conversion, especially through agricultural intensification, poses serious threats to Lepidoptera. Agro ecological practices and local woodland cover influence butterfly richness, while poorly managed pest control can negatively affect populations.

Vegetation structure supports higher butterfly diversity, as a varied plant landscape provides resources for larvae and adults. In Iran's Hyrcanian Forest, a positive correlation was found between Lepidoptera richness and plant diversity. Habitat features such as canopy cover, water sources, and tree density (e.g., in Tanzania's Kihansi Gorge Forest) are closely linked to butterfly community structure. Climate changes, pollution, and land use shifts since the 1800s have driven declines in European butterflies. Species composition responds to pollution gradients, and some serve as bio indicators. Favorable climates boost populations, while droughts reduce them, causing migration to moist refuges. For instance, during the Last Glacial Maximum, cold-adapted mountain butterflies experienced contractions and expansions that shaped current genetic diversity.

Invasive species, pollution, and urbanization continue to threaten native populations. Studies from the UK show drastic butterfly declines due to habitat loss, pesticides, and climate change. A Dutch study with nearly 3 million observations from 6,075 sites revealed that vegetation structure and land use significantly shape butterfly diversity. Dispersal ability is key to Lepidoptera distribution. Habitat fragmentation reduces connectivity and gene flow. Topography such as elevation, slope, and aspect create microclimates that define niches for different species. Human-driven changes like deforestation and urbanization fragment habitats but targeted conservation can aid recovery, as seen in Scotland's dark-bordered beauty moth. Range shifts due to warming climates are evident; in Scotland, generalists like the red admiral expanded while specialists like the grayling declined. Thus, understanding ecological factors and microclimatic conditions is essential for managing Lepidoptera biodiversity.

Materials and Methods

Study Area:

The study was carried out in Punjab, Pakistan's District Faisalabad, which is situated at latitude 31.5204° N and longitude 73.3587° E. The distribution of Lepidoptera species is affected by the semi-arid climate of Faisalabad, which features scorching summers and mild winters. In order to evaluate how various environmental conditions affect Lepidoptera variety, the study sites were chosen based on habitat changes, including urban areas, agricultural fields, forested regions and semi-natural grasslands as shown in Fig 1. Lepidoptera depend on the agricultural crops (wheat, sugarcane, maize, and cotton), fruit orchards and sporadic natural flora that make up the majority of the vegetation in the study area. The Faisalabad Meteorological Department provided temperature, humidity and precipitation data so that the effects of climate on species distribution could be assessed.

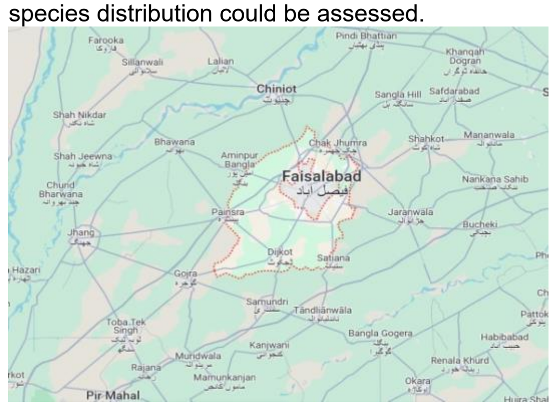

Figure 1. ***Map of Faisalabad Punjab, Pakistan.***

Research Design

To account for seasonal fluctuations in lepidoptera biodiversity, a comprehensive field survey was carried out for a complete year (specify the precise time period). Each chosen location underwent a monthly sampling program that included both nocturnal and diurnal species. Along with environmental variables like temperature, humidity, vegetation type and human disturbances, the study's main objectives were to document species richness, abundance and habitat preferences (Ahmad et al., 2021).

Data Collection

For data collection, a variety of standardized methodologies were used in the sampling of Lepidoptera species in District Faisalabad as shown in Fig 2. In order to attract moths and other night-flying Lepidoptera, light trapping was used for nocturnal species. Mercury vapor and UV light traps were positioned in different habitats from dusk to dawn (Holloway, 1980).

In places with a lot of foliage, sweep netting was utilized for diurnal species, especially butterflies, so that specimens could be collected with little harm (Pollard & Yates, 1993). In order to gain insight into plant-lepidopteran relationships, host plant observations were also carried out by keeping an eye on particular plant species to document the existence of caterpillars and their corresponding butterfly or moth species (Habel et al., 2016). Pitfall traps were set up at specific locations to evaluate ground-dwelling animals, making it possible to capture species that are difficult to gather using other techniques (Spitzer et al., 1997).

By combining these methods, a thorough evaluation of Lepidoptera variety was guaranteed, enabling the determination of species richness, abundance and habitat preferences across various ecological zones.

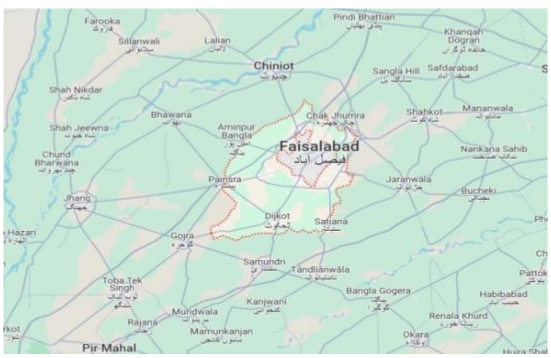

Figure 2. ***Selected Regions in Faisalabad and its Surroundings.***

Identification:

Standard field guides and taxonomy keys were used to identify collected Lepidoptera specimens based on their morphological characteristics (Heppner, 1991; Scoble, 1992). Details such as wing patterns, colors, body structure and antenna form were meticulously inspected for each species. The specimens also identified using the standard literature that was available, including research publications, theses and previously described species (Munir et al., 2008; Abbas et al., 2002).

Analysis of Data

The study examined lepidopteran species richness and diversity using the Shannon-Weiner Diversity Index (H') for species variation, Simpson's Index (D) for dominance patterns, and the Evenness Index (J') to assess distribution across ecosystems (Magurran, 2004). Seasonal population fluctuations and climate impacts were analyzed through species abundance monitoring, with regression models and ANOVA employed to evaluate species-environment relationships (Legendre & Legendre, 2012). Statistical analyses were performed using SPSS v.26 and R for ecological modeling, while ArcGIS enabled spatial visualization of species distributions. The results and discussion present findings from these comprehensive analyses.

Results and Discussion

Climate, vegetation, and the availability of habitat are some of the natural elements that affect butterfly abundance in Faisalabad. Butterfly populations are supported by the host plants and nectar sources found in urban gardens, green spaces, and agricultural environments. Because of their adaptability and correlation with agricultural fields, species such as *Eurema hecabe* (Common Grass Yellow) and *Pieris rapae* (Small White) are frequently seen. Butterfly abundance is also influenced by seasonal changes, especially those related to temperature and rainfall patterns; peaks are frequently seen during the spring and monsoon seasons when floral supplies are abundant. However, the diversity and stability of butterfly populations are threatened by urbanization, pesticide usage, and habitat fragmentation. Butterfly abundance in the area can be preserved and increased using conservation measures like growing plants that attract butterflies and using fewer chemicals.

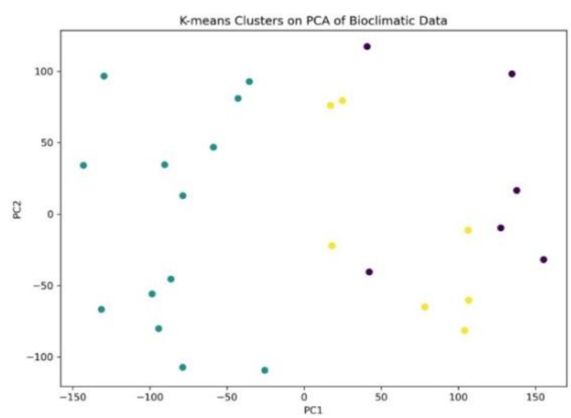

Figure 3. PCA of Climatic Variables for Study Sites

The PCA plot of bioclimatic variables for Lepidoptera sites visually represents the distribution of different locations based on their climatic characteristics. Principal Component Analysis (PCA) is a statistical method used to reduce the complexity of large datasets by identifying key patterns. In Fig 3, the X-axis (PC1) explains the highest variance in the data, while the Y-axis (PC2) explains the second highest variance. Each point on the plot represents a different Lepidoptera site, with their positioning indicating how similar or different their bioclimatic conditions are.

The spread of data points suggests variability in climatic conditions across different locations. Sites such as Jannah Garden, Manawala, and Ghatti are positioned toward the

upper side, indicating distinct bioclimatic properties. Conversely, Chak 65JB and Makuwaana are located at the lower end, implying significantly different environmental conditions. Locations such as Chak Jhumra, Chak 233 RB, and Chak 195 RB are closer to the center, suggesting they have more moderate climatic characteristics. Sites that are clustered together share similar environmental conditions, whereas widely dispersed points indicate notable differences.
The variation captured by PC1 and PC2 may reflect contrasts such as moisture gradients, temperature ranges, or other climate-related factors critical to the distribution and habitat suitability of Lepidoptera species. By analyzing how these sites group or separate in the PCA space, the study identifies which environmental variables most strongly influence species distribution patterns and potentially uncovers ecological niches or regions of particular conservation importance.
This PCA analysis helps in understanding how climatic factors influence the distribution of Lepidoptera populations across various locations. The diversity in site placements suggests that different regions experience unique ecological conditions, which could impact the habitat suitability and distribution of species. This insight can be useful for conservation efforts, ecological studies, and further research on climate impacts on biodiversity.

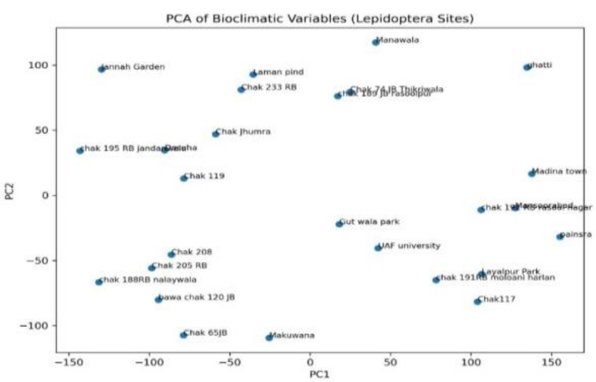

Figure 4. *K-means Clusters on PCA of Bioclimatic Data.*

Figure 4, presents the K-means clustering results applied to PCA of bioclimatic data, helping to identify patterns within ecological datasets. Each point represents a geographical site, and the data has been grouped into three clusters shown in teal, yellow, and purple. PCA reduces data complexity, with PC1 and PC2 capturing major variations. The teal cluster contains the most points, showing shared environmental features, while yellow and purple clusters contain fewer points with unique bioclimatic characteristics.
The clustering indicates three ecological or bioclimatic regions, each shaped by climatic variables like temperature, humidity, precipitation, altitude, and soil. Sites within a cluster share environmental conditions influencing species distribution and biodiversity. Greater distances between clusters show greater environmental differences. This approach is useful in ecological research, conservation planning, and climate studies by revealing similar habitats and areas needing conservation focus.

Moreover, this method supports species distribution modeling, predicting suitable areas for Lepidoptera species. The presence of three distinct clusters shows that the study area includes three major ecological zones with unique environmental influences on species diversity. Understanding these clusters is essential for biodiversity conservation strategies, habitat restoration, and sustainable land-use planning.

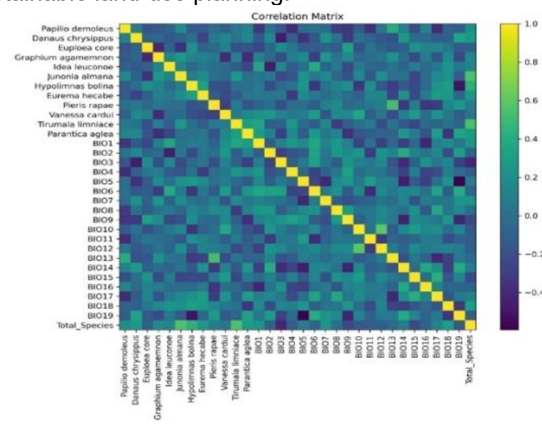

Figure 5. *Correlation Matrix*.

The correlation matrix in Fig. 5, shows relationships between butterfly species and bioclimatic variables (Bio1 to Bio19) with the total species count. Each cell represents a correlation coefficient, color-coded from -0.4 (negative) to 1.0 (strong positive). Diagonal elements are bright yellow, indicating perfect self-correlation.
This matrix helps understand how butterflies respond to climate factors. Strong positive correlations (green to yellow) show which conditions promote butterfly abundance. Negative correlations (blue to purple) show limiting factors. The matrix helps identify favorable and limiting environmental conditions, useful for biodiversity and habitat conservation.

Positive Correlations and Favorable Conditions: If total species has strong positive correlations with certain bioclimatic variables, it suggests those factors such as moderate temperature, adequate rainfall, and climatic stability favor butterfly richness. When multiple species correlate positively with the same variables, it shows they share ecological preferences.

Negative Correlations and Limiting Factors: Negative correlations suggest that certain climatic conditions reduce butterfly richness. These include extreme temperatures, irregular rainfall, or stress factors that impact survival. Species may decline in diversity in harsh or unstable environments, affecting overall abundance.

Discussion

Butterflies are among the most widely studied and admired insects, not only for their aesthetic appeal but also for their ecological and evolutionary significance. Their diversity reflects adaptations to varied habitats, feeding preferences, mimicry mechanisms, and migratory behaviors. Species such as *Eurema hecabe*, *Junonia almana*, *Idea leuconoe*, *Hypolimnas bolina*, *Vanessa cardui*, *Tirumala limniace*, and *Parantica aglea* represent distinct taxonomic groups and ecological strategies, showcasing the richness of Lepidoptera in tropical and subtropical regions.

Eurema hecabe, the common grass yellow, is a widespread Pierid found throughout Asia and Africa, thriving in sunlit, open areas and disturbed habitats like gardens and roadsides. Its erratic flight and preference for low vegetation enable it to adapt to human-altered environments (Kunte, 2000). *Junonia almana* (Peacock Pansy) of the Nymphalidae family is notable for its eyespots, which aid in predator avoidance and mate attraction. This species exhibits seasonal polyphenism, developing different morphs in wet and dry seasons to adapt camouflage or signaling strategies (Brakefield & Larsen, 1984).

Idea leuconoe (paper kite), belonging to the Danainae subfamily, glides through Southeast Asian rainforests with slow, graceful flight and translucent wings. It stores toxic compounds from Apocynaceae host plants, signaling unpalatability to predators an example of aposematism (Ackery & Vane-Wright, 1984). Similarly, *Tirumala limniace* (Blue Tiger) and *Parantica aglea* (Glass Tiger) use Müllerian mimicry and chemical defenses. *Tirumala* often migrates in swarms in southern India, while *Parantica* mimics toxic species with translucent wings and slow flight (Smith et al., 2005).

Hypolimnas bolina (Great Eggfly) shows Batesian mimicry, with polymorphic females mimicking toxic *Danaus chrysippus* for predator avoidance (Clarke & Sheppard, 1975). Males display iridescent blue patches, while females vary in appearance. This species also evolved rapidly in response to male-killing *Wolbachia* infections, illustrating strong natural selection. *Vanessa cardui* (Painted Lady) is cosmopolitan and a long-distance migrator, capable of intercontinental migrations across Europe, Africa, and Asia. It is a generalist in habitat and host plants, contributing to its survival in diverse environments (Stefanescu et al., 2013; Talavera & Vila, 2017).

Mimicry and chemical defense mechanisms are crucial survival strategies. *Tirumala limniace* and *Parantica aglea* use Müllerian mimicry, sharing warning coloration to reinforce predator avoidance (Smith et al., 2005). *Hypolimnas bolina* uses Batesian mimicry by imitating unpalatable species (Clarke & Sheppard, 1975). Danainae members store toxins from host plants, and species like *Idea leuconoe* advertise this through slow, floating flight (Ackery & Vane-Wright, 1984). Phenotypic plasticity is another adaptive feature. *Junonia almana* shows seasonal morphs: cryptic coloration in the dry season and vivid patterns in the wet season, aiding survival through changing environmental conditions and enhancing reproductive success (Brakefield & Larsen, 1984). Migration and ecological flexibility are exemplified by *Vanessa cardui*, which travels across continents in multi-generational journeys. Its broad host range and irregular migration pattern help it adapt to resource availability (Stefanescu et al., 2013). *Tirumala limniace*, though not a long-distance migrator, forms seasonal swarms responding to temperature and food supply (Smith et al., 2005).

Butterflies play vital ecological roles as pollinators, prey species, and environmental indicators. Their larval dependence on specific plants means that biodiversity loss, habitat degradation, and intensive agriculture directly affect them.

In Faisalabad, butterflies like *Eurema hecabe* indicate disturbed habitats, while species like *Idea leuconoe* signal intact ecosystems (Smith et al., 2005). Several factors shape Lepidoptera distribution and biodiversity in District Faisalabad. Habitat availability, floral resources, and breeding areas are key biotic influences. Urbanization and agricultural expansion fragment habitats, limiting movement and genetic exchange. Climatic variables temperature, precipitation, and humidity affect migration, reproduction, and larval development.

Seasonal peaks in diversity occur during spring and post-monsoon months. Intensive agriculture, especially cotton and wheat monoculture, limits host plant diversity and exposes butterflies to harmful pesticides. Mixed farming systems support higher richness. Plant diversity, especially native flowering plants, strongly correlates with Lepidoptera richness. Anthropogenic pressures pollution, deforestation, and light pollution further threaten these populations, particularly moths.

Faisalabad's semi-arid environment and mosaic of natural, agricultural, and urban areas offer insight into Lepidoptera vulnerability and adaptability. These findings align with global trends of insect decline due to habitat loss and climate change, emphasizing the urgent need for conservation actions.

Conclusions

This study highlights the significant diversity and ecological complexity of butterfly species in the District Faisalabad, where both environmental and anthropogenic factors play pivotal roles in shaping their distribution and abundance. The observed species, including *Eurema hecabe*, *Junonia almana*, *Idea leuconoe*, *Tirumala limniace*, *Parantica aglea*, *Hypolimnas bolina*, and *Vanessa cardui*, reflect a wide range of ecological strategies such as mimicry, toxicity, migratory behavior, and phenotypic plasticity. These adaptations enable them to survive in varied habitats, from disturbed urban landscapes to more intact ecosystems. The presence or absence of particular species can serve as a bioindicator of environmental health, with some species thriving in degraded areas while others require stable, resource-rich habitats for survival.

The results of the study confirm that factors such as habitat fragmentation, plant diversity, climatic variability, and intensive agricultural practices significantly influence Lepidoptera diversity in the region. Seasonal changes, particularly during spring and post-monsoon periods, support greater butterfly abundance due to favorable temperature and floral availability. However, threats like pesticide use, monoculture cropping, urbanization, and light pollution are contributing to population declines. Conservation strategies should prioritize preserving native vegetation, promoting sustainable agricultural practices, and mitigating urban pressures. By understanding these complex ecological interactions, we can better protect butterfly populations and maintain the biodiversity and ecological integrity of Faisalabad's semi-arid landscape.

Author Contributions

Qi Xue: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Qian Tang: Writing – review & editing, Visualization, Formal analysis, Conceptualization. Lin Deng: Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition. Wei Luo: Writing – review & editing, Conceptualization. Mingle Xia: Writing – review & editing, Conceptualization. Shuang Fu: Writing – review & editing, Conceptualization. Chaoqun Tan: Writing – review & editing, Conceptualization. Jun Hu: Writing – review & editing, Conceptualization. Rajendra Prasad Singh: Writing – review & editing.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Data Availability

Data will be made available on request

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RESEARCH ARTICLE

Influence of Environmental Factors on the Mosquito Vector Habitat Distribution in Urban Areas of Faisalabad, Punjab

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Abstract The viruses that cause malaria, Dengue hemorrhagic fever, and Rift Valley fever are primarily spread by mosquitoes. Globally, mosquito-borne diseases pose a serious threat to public health, particularly in crowded cities. The goal of the current study was to update knowledge about the mosquito (Diptera: Culicidae) fauna of Punjab region, Pakistan's District Faisalabad, and forecast the distribution of the larvae of the most important mosquito vectors in this area. Environmental factors such as water sources, land use, temperature, and humidity were recorded at each collection location. A perspective for the geographic distribution of dengue vectors in the metropolitan areas of Faisalabad was created using GIS-based spatial analytic tools after the data was gathered. The number of mosquito larvae was assessed in connection with the physiochemical characteristics (pH & TDS) of breeding grounds. Mosquito larvae were collected from January 2024 until December 2024. *Aedes aegypti* was the most important vector discovered in Faisalabad. To predict the species distribution of *Aedes aegypti* in the district of Faisalabad, 19 bioclimatic variables were combined with the data from the collection. More than 100 locations yielded more than 1800 mosquito larvae. The northeastern region of the Faisalabad district was identified as having the best suited lands. The region that was least conducive to the presence of *Aedes aegypti* was the southwest. Precipitation factor (bio8) contributed the most in the presence of this vector, accounting for over 40%, followed by bio19, which contributed 20%.

Keywords: Mosquito vectors, spatial distribution, GIS, dengue, *Aedes aguptii*, urban areas, Faisalabad, Punjab, Pakistan.

Introduction

Mosquito-borne diseases are a growing concern in many urban areas, especially in developing countries like Pakistan. In cities such as Faisalabad, rapid urban growth, poor drainage, stagnant water, and changing weather conditions create ideal breeding grounds for mosquitoes. Factors like temperature, humidity, rainfall, vegetation, and land use play a key role in shaping where mosquitoes can live and thrive. This study aims to explore how these environmental elements influence mosquito habitats across different parts of Faisalabad. Using mapping tools and ecological models, the research will help identify areas at greater risk, ultimately supporting more focused and effective mosquito control and public health strategies.

Due to the change in the climate, urbanization and human activities, Pakistan has to deal with high risk of mosquitoborne illnesses in last few years. In disease transmission, researches from region such as Peshawar and Punjab have highlighted the role of *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus*. Research indicates that these species differ in bacterial diversity and *Wolbachia* infection, with *Ae. albopictus* hosting more diverse strains. Environmental surveys have recognized parks, forests, and scrapyards as key breeding grounds, with mosquito diversity peaking during the rainy season. Advanced tools like GIS and AHP have also been used to map high-risk areas and assess environmental factors. These insights support integrated, targeted mosquito control strategies by combining ecological, microbial, and spatial data.

Ammar et al. (2025), discovered that due to climatic changes in Pakistan, deforestation, urbanization and poor sanitation causes the arboviral diseases at high level. As there is no proper investigation system in Pakistan, it causes the great hurdle in the way effectively tracking the disease prevalence. High prevalence rate of dengue, West Nile virus, chikungunya, and Japanese encephalitis have caused the major health issues along with other hidden arboviral diseases. So improved investigation system, research and control strategies are required to overcome these challenges.

Nayab et al. (2025), completed the research work by focusing on Ae. aegypti and *Ae. albopictus*. Mosquitoes that were collected using Gravitraps, and their species were confirmed through molecular methods, these investigations have pointed out the presence of Wolbachia in Aedes mosquitoes, in Peshawar, the northwest region of Pakistan. With *Ae. albopictus* hosting 921 bacterial species, while *Ae. aegypti* had only 239 species, the 16S rRNA sequencing disclosed remarkable differences in bacterial diversity, Both species were found to carry Wolbachia, with Ae. aegypti infected with Wolbachia pipientis, and Ae. albopictus showing a co-infection of two Wolbachia strains, Wolbachia pipientis and Wolbachia bourtzisii. To control dengue and other mosquito-borne diseases in the area, these researches pointed out the significance for using Wolbachia.

Mehmood et al. (2024), found that, based on behavioral and feeding habit, mosquito species show the great degree of variations in habitat selection. By analyzing microhabitats, species diversity, and habitat web structures through the surveys conducted in the Chakwal district of Punjab, Pakistan, identified the potential mosquito's habitats. A total of 580 mosquito specimens were collected, representing 12 species from five genera. Crop fields had the lowest while parks, forest areas, and scrapyards had the highest mosquito populations. Parks also emerged as the most species-rich habitats, with graveyards showing the least diversity.

Ullah et al. (2023) explored that Mosquito distribution across different agro-ecological zones of Punjab is shaped by seasonal changes, land use, and human activity, all of which influence disease risk. Surveillance was conducted in six zones, collecting samples from stagnant water sources during various seasons. A total of 24 mosquito species were identified, with higher diversity observed during the rainy season. Anopheline mosquitoes were found across both rural and urban areas, while Aedine species were mostly limited to northern irrigated plains, especially around Changa Manga Forest. Culicine mosquitoes were widespread and present year-round. Some species showed specific habitat preferences, such as *C. crassipes* in rainfed areas and *M. chamberlaini* in both urban and rural environments.

Climatic factors significantly influence the presence of *Aedes aegypti* larvae in indoor and outdoor water containers across various districts. Analysis showed a strong link between weather patterns and larval abundance, except in Faisalabad during the summer, where no significant association was found. Containers containing stagnant water placed at specific places also played a significant role in this purpose. These researches provided the remarkable understandings and demonstrations about potential breeding sites of mosquitoes and manipulating the crucial mosquitoes control strategies (Malik et al., 2023).

In 2019, Ali and Ahmed, provided a detailed map that represents the complete sketch of diseases caused by mosquitoes in Kolkata Municipal Corporation, with the help of geographical information system (GIS) and analytical hierarchy technique. To recognize the weightage of chosen items, a pairwise contrasting matrix, a choice-based classifying technique was formulated.

Jemal and Thukair (2018), illustrated that a pairwise comparison matrix was used to assess the weights of ten pertinent environmental components. A geo-database was also created using the weight of each causal element to facilitate overlay analysis. To confirm the significance-measurement and decision-making processes, the consistency ratio was created. Because it is less than 0.1, the compatibility ratio to the select variables, which was determined to be 0.0470, is considered accurate. The study's analysis shows that a number of important factors.

The current study demonstrates the extensive applicability of satellite data and a spatial approach to the zonation of epidemic diseases. Numerous studies across the globe have highlighted the effectiveness of Geographic Information Systems (GIS) and environmental modeling tools in understanding mosquito vector distribution. For instance, research conducted in the Eastern Province of Saudi Arabia used GIS and meteorological data to map mosquito breeding sites, revealing that temperature, humidity, and rainfall play key roles in mosquito abundance. Similar work in Hawaii emphasized the need to include environmental variation—such as rainfall and temperature thresholds—when predicting mosquito habitats, especially in areas with high population density. In Virginia, USA, GIS was used alongside remote sensing and field data to forecast the abundance of mosquito species, showing how environmental factors could guide risk assessments and disease surveillance.

Meanwhile, in Pakistan, although species distribution modeling (SDM) has been used to study mosquitoes, GIS-based studies remain limited. Past outbreaks like the 2017 dengue epidemic in Khyber Pakhtunkhwa highlight the urgent need for spatial modeling. Studies using techniques such as binary logistic drift and binomial kriging have shown promising results in identifying high-risk areas. Traditional mosquito control methods, such as chemical pesticides, have been challenged by rising resistance in mosquito populations, making ecological modeling even more critical.

Materials and Methods

Description of the study area

Pakistan's terrain is defined by a number of regions, including the Indus Plain, the Northern and North-Western Mountains, the Potwar Plateau, the Balochistan Plateau, and Salt Range, and other ecosystems and climate zones. Faisalabad, Pakistan, is a 3.4 million-person city that occupies 3,358 km2 in northeastern Pakistan, situated between the Ravi and Chenab river plains. According to Saleem and Mahmood (2023), the southern half is estimated to be bordered by Sahiwal and Toba Tek Singh, the northern extremity by Chiniot and Hafizabad, Nankana Sahib to the east, and Jhang to the west.

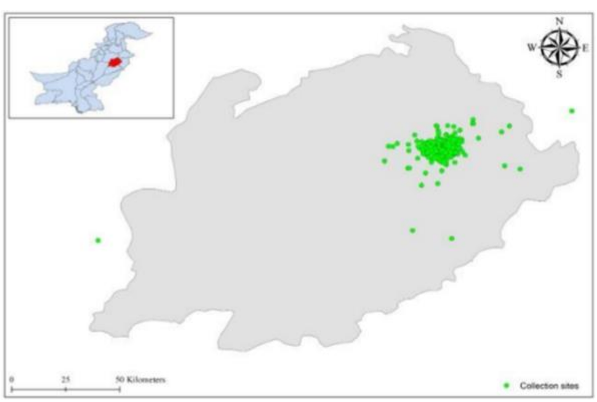

Figure 1. Pakistan map showing collection sites in Faisalabad.3.2.

Data Collection

Field trips and surveillance operations were carried out to collect mosquito larvae. After sampling every breeding location, the most usual and promising habitats were chosen from both lower and higher elevations. From January 2024 to December 2024, the collection of mosquito larvae was conducted continuously. To collect mosquito larvae, a regular 350 ml plastic dipper was utilized. By using a global positioning system unit (GPS), the coordinates of the collection sites were acquired (Garmin, eTrix). Each recovered mosquito larva was identified and submerged in 80% alcohol.

Diverse Breeding Habitats:

Various breeding sites were surveyed for mosquito larvae collection, including streams, wells, water containers, and pools. Here are some images depicting these sites.

Figure 2. Mosquito larvae emerged in the stagnant water present in a tree aperture, Jinnah colony, Faisalabad district.

Figure 3. *Rainwater accumulation in a park of Chak Jhumra, Faisalabad district.*

Identification of Mosquito

Larvae were gathered from several nesting locations using conventional dipping methods in order to identify mosquito species. They were then raised in a controlled laboratory environment until they matured into adult mosquitoes. Morphological keys and taxonomic guides were then used to identify the emerging adults down to the species level (Rueda, 2004; Gillies & Coetzee, 1987). Important morphological traits such wing venation, leg banding patterns, and abdominal markings were used for identification.

Morphological identification is still a valid way to distinguish between mosquito species, especially in field-based research, according to Alahmed et al. (2011). Their study acknowledged that environmental influences can affect morphological changes, but it also emphasized the significance of defined taxonomic keys for precise species confirmation. In order to ensure correctness, we used published taxonomic keys and expert assistance to crossverify our identification method. For use in MaxEnt, Dengue mosquitos were recognized and data was monitored in CSV (Comma separated value) format.

Physiochemical characteristics

The pH and total dissolved solids of every breeding locations were analyzed through the pH meters along with TDS (HANNA Instruments, USA).

Visualisation and analysis

The result of molecular docking was analysed using different software such as AutoDock 4.2, PyMOL and LigPlot program. PyMOL program was mainly used for visualisation of the structures. The main parameters for molecular docking analysis were the binding energy values of the protein-ligand interaction, hydrophobic interaction and the number of hydrogen bond formation. The binding energy value for each compound was shown as a result of molecular docking and the best interacting compound of both enzymes was selected. Besides, the number of hydrogen bonds formation and the residues that formed the hydrogen bonds with the ligand were identified using AutoDock 4.2 software. The distance of hydrogen bonding between the ligand and the interacting residue was being calculated using PyMOL. Also, AutoDock 4.2 software was used to reveal all the interacting residues of the protein, and each ligand; meanwhile, Ligplot software was used to visualise the hydrophobic interaction of the protein and the ligands.

Modeling procedures

Accurate test statistics and independent evaluating data is required due to the nichebased nature of the distributional models. In order to offer distinct test data, the Faisalabad district was selected for the collecting of mosquito larvae kinds from multiple grid cells. Test statistics were then acquired using Maxent software. To describe the environment of mosquito larvae, 19 bioclimatic layers temperature layers and According to Phillips et al. (2006), from the WorldClim database version 1.4 (www.worldclim.org) eight precipitation levels were taken. 30 arc-seconds (1 km) is spatial resolution of these bioclimatic strata. After being shrunk to the size of the Faisalabad district, in ASCII grid format each of these bioclimatic layers was recorded so that MaxEnt could use it. The model builder tool in ArcGIS version 10 was used to reduce these layers. The geographical distribution of the species was modeled using MaxEnt software version 3.3. The program was set up using the "Auto features" option, ASCII output file format, and logical output structure as recommended by Phillips and Dudík (2008).The Maxent software connected predictor characteristics (environmental and topographical) with the distribution of target potential species.

The value assigned to any pixel is = (The probabilities of that pixel + probabilities of all other pixels having equal or lower probability values) X 100

Any pixel with a value of 100 indicates the most adaptability to depict the presence of the species, while a value of 0 indicates the least compatibility (Phillips et al., 2004; Phillips et al., 2006).

Evaluation Metrics

MaxEnt

Maximum Entropy (MaxEnt) originated in statistical mechanics (Jaynes, 1957). It is a common method for predicting topographical dispersion of mosquitoes in the research area. Only presence data and ecological data are required for MaxEnt to simulate species distribution (Phillips et al., 2006). In a large-scale model discrimination investigation, Maxent performed well (Elith et al., 2006). Similarly, the analysis of four species distribution modeling approaches revealed that, even with a small sample size, maxent could produce extremely valuable and accurate results (Hernandez et al., 2006).

The model developed using this approach also shows how climate change impacts the habitat of the target species (Phillips et al., 2004; Hernandez et al., 2006). Only a little number of specimens can be employed for the species compatibility referred by maximum (Pearson et al., 2007; Ortega and Townsend, 2008; Wisz et al., 2008; Kija et al., 2013). Even with sample numbers as little as five, Maxent has proven to have a high success rate (Pearson et al., 2007).

The Genetic Algorithm for Rule-set Production (GARP) performs worse than maxent for sample sizes smaller than 10 (Pearson et al., 2007). The marginal density along the study area region contrasted to the conditional density at the presence sites of predictors to forecast species-suitable habitats using maxent

(Conley et al., 2014). Maxent can generate species distribution models using five occurrence tracks (Hernandez et al., 2006). In any event, for a reliable forecast, at least 30 recurrence data should be present (Wisz et al., 2008). Models produced by Maxent show less omission errors than those produced by GARP (Foley et al., 2010).

Determining the importance of variables in model building

The relative importance and value of each variable in the geographic distribution of species models are analyzed by Maxent using the Jackknife technique. By the Jackknife test, three different model types are produced (Phillips et al., 2006; Phillips and Dudik, 2008):

- Models created with all but one variable considered.
- Models created with one variable and all other variables eliminated at the same time.
- Models that contained all of the variables.

The initial learning gain increases when the model is developed with only one variable, while decreases as the most important variable is eliminated from the model, but. In other words, the least AUC values shown by variables that are eliminated from the model show, while the largest AUC values by variables that are added individually (Phillips et al., 2006). In order to analyze the prediction accuracy, Maxent automatically divides our mosquito sample information into training and test sets. Both threshold-independent and threshold-dependent methods are used to evaluate how accurate the model predictions are (Phillips et al., 2006). The threshold independent approach uses external omission rate (the percentage of test sites that fall in pixels that are not suitable for the species) and proportional projected area (the percentage of all pixels that are favorable for the species) to evaluate the model prediction. Despite the fact that independent threshold assessments utilize the recipient's operational features to measure the area under the curve.

Receiver Operating Characteristics Curves (ROC)

Maxent's performance is indicated by the area under the curve (AUC) value, which is typically the unit square section of the area with a value between 0 and 1. Using this receiver operating feature approach, Maxent predicts the distribution according to species (Fawcett, 2006). This curve shows how well a classifier works when a threshold parameter affects its output. This graph shows the ratio of true and false positive values for each level. The classifier has classifies a faction "x" of negative examples (grids without occurrence localities) as positive and a proportion "y" of positive cases as positive shown by some location on the graph (Phillips, 2004). The points on the graph are joined to create a curve. However, the area under the curve (AUC) shows how likely it is that the classifier will correctly arrange the two points (Phillips, 2004). An AUC value of less than one indicates a good classifier. (Wiley and others, 2003). Threshold unbiased measurements show sensitivity and specificity on a graph. Sensitivity is the accurately true positive predicted sites for a species, while selectivity is the real, exactly determined negative sites (Fielding and Bell, 1997; Phillips et al., 2006; Phillips and Dudik, 2008). The AUC value can be computed by the differential capacity of the model. 0.5 to 0.7, 0.7 to 0.9, or > 0.9 and 1.0 are AUC values of the model's low, fair, high, and perfect discriminative power correspondingly (Brooker et al., 2001; Phillips et al., 2006; Phillips and Dudik, 2008).

Results and Discussion

In species distribution modeling (SDM), visual tools such as response curves and the jackknife test play a vital role in exploring how environmental factors shape the presence or spread of a species. Response curves visually depict the influence of individual environmental variables on species predictions, helping researchers understand which factors are most critical and which have limited impact. Interpreting these curves, however, can be challenging when variables are closely related, as overlapping effects may obscure clear patterns. Alongside these, the jackknife test serves as a powerful method to evaluate the model's reliability by systematically excluding variables or data points to observe changes in prediction accuracy. This process highlights potential overfitting and helps determine how well the model can handle unseen data. Together, these methods provide a clearer picture of ecological relationships and support efforts in species conservation and environmental planning.

Results

Mosquito distribution in Faisalabad

Larvae of *Aedes* were collected from different sites in Faisalabad city. Table discusses their websites in more detail.

Analysis of omission/commission

The forecasted area and exclusion ratio are shown against the progressive approach in the following figure. The original presence records and, if test data is used, the test records as well are used to evaluate the exclusion ratio. For this reason the specification of the progressive approach, the exclusion ratio must be around the predicted omission.

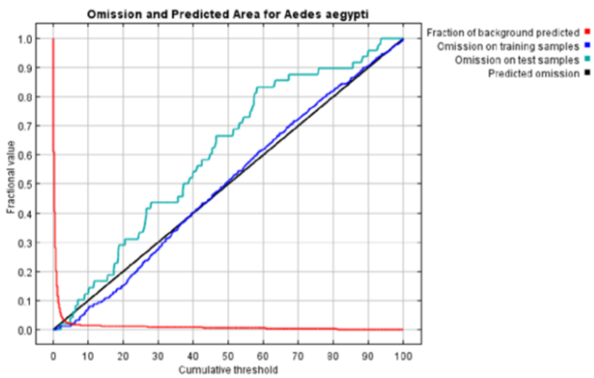

Figure 4. *Omission and predicted are for Aedes aegypti*

In the following figure the receiver operating characteristic (ROC) curve for the similar data is shown. Keep in mind that specificity is determined by expected area rather than actual commission; see the Phillips, Anderson, and Schapire study area to understand about its, on the support page. This suggests that the highest AUC that may be attained is less than 1. The maximum test AUC that could be achieved if test data were taken from the Maxent distribution itself would be 0.986 instead of 1, even if the test AUC may be greater.

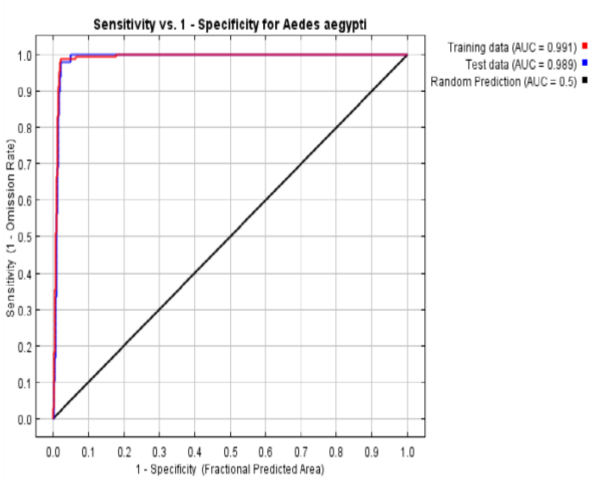

Figure 5. *Sensitivity vs. 1-Specificity foe Aedes aegypti*

Below is a list of some specific criteria along with the corresponding exclusion ratios. When test information is provided, if there are less than 25 test samples ,binomial chances are computed appropriately; if not, a conventional method to the binomial is used. The null hypothesis, which states that test sites can be expected no more accurately than by a random prediction with the same fractional anticipated area, is supported by these one-sided p-values. The total of the following is reduced by the "Balance" threshold: Six * fractional predicted area +.04 * cumulative threshold + 1.6 * training omission rate.

Table 2. *Maxent modeling analysis of Aedes aegypti and its output.*

Cumulative threshold	Logistic threshold	Description	Fractional Predicted area	Training omission rate	Test omission rate	P-value
1.000	0.001	Fixed cumulative value 1	0.169	0.007	0.000	1.42E-53
5.000	0.085	Fixed cumulative value 5	0.021	0.014	0.042	0E0
10.000	0.313	Fixed cumulative value 10	0.016	0.075	0.125	0E0
0.967	0.001	Minimum training presence	0.176	0.000	0.000	3.849 E-51
13.773	0.430	10 percentile training presence	0.014	0.095	0.167	0E0
5.470	0.106	Equal training sensitivity and specificity	0.020	0.020	0.042	0E0
5.004	0.085	Maximum training sensitivity plus specificity	0.021	0.014	0.042	0E0
4.855	0.079	Equal test sensitivity and specificity	0.022	0.014	0.021	0E0
4.855	0.079	Maximum test sensitivity plus specificity	0.022	0.014	0.021	0E0
1.997	0.004	Balance training omission, predicted area and threshold value	0.063	0.007	0.000	0E0
4.256	0.057	Equate entropy of thresholded and original distributions	0.024	0.014	0.021	0E0

Response curves

In these graphs the effect of every environmental variable on the Maxent forecast is shown. When each environmental factor is changed while maintaining a fixed mean sampling value for all other environmental variables, the graphs illustrate how logistical prediction varies. It perhaps be demanding to clarify the curves if the variables that you employ are closely related since the model could rely on the correlations in ways that are not shown in the curves. In other words, the curves demonstrate the minimal effect of changing a single variable, even when the model could advantageous from changing groups of variables collectively.

Molecular Docking Analysis of DPP-4 Enzyme with Polyphenol Compounds

Similar to AG enzyme, all the selected polyphenol compounds and sitagliptin were successfully docked at the targeted binding sites of the DPP-4 enzyme. The results of the molecular docking for each combination that formed a complex with DPP-4 enzyme were summarized in Table 3 with their corresponding binding energy values. In Table 4, it stated the results of the hydrogen bond formation and the interacting residues of DPP-4 with each polyphenol compound and sitagliptin.

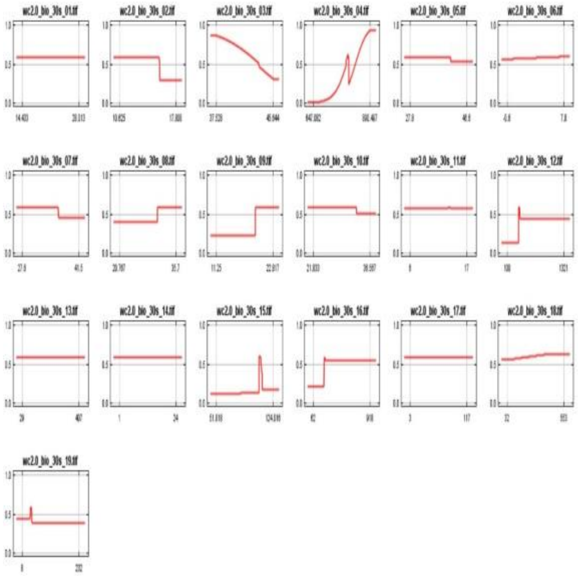

Figure 6. *Responsive curves in accordance to each environmental variable.*

Every following curves represents a different model, compared to the marginal response curves above, i.e., a Maxent model developed using just related variable. The graphs show the weakness of forecasted appropriateness on the variable of choice and weaknesses resulting from association between other factors and the variable of choice. If there are strong relationships between them, the variables may be simple to comprehend.

Analysis of variable contributions:

The given table presents predictions about comparative contribution made by environmental factors to the Maxent model. In each training process iteration, the initial estimate is computed by adding the addition of the established gain to the related variable's contributions, or its removal in the event that fluctuate the true worth of lambda is negative. The learning presence and background data of every single environmental component are permuted at random for the second evaluation. The training AUC drop resulting from reevaluating the prediction model on the permuted data is shown in the table, adjusted to percentages. When the variables that predict are connected, variable contributors should be carefully evaluated, much as the variable jackknife

Table

3. *Estimate for the Relative Contributions of Ecological Factors.*

Variable	Percent contribution	Permutation importance
wc2.0_bio_30s_15.tif	19.9	5.7
wc2.0_bio_30s_16.tif	18.4	12.6
wc2.0_bio_30s_12.tif	11.3	22.1
wc2.0_bio_30s_19.tif	9.8	0
wc2.0_bio_30s_09.tif	9.6	24.1
wc2.0_bio_30s_08.tif	8.6	0.7
wc2.0_bio_30s_03.tif	7.1	7.1
wc2.0_bio_30s_02.tif	5.1	7.2
wc2.0_bio_30s_07.tif	4.6	0
wc2.0_bio_30s_10.tif	3.8	0
wc2.0_bio_30s_04.tif	1.7	20.4
wc2.0_bio_30s_05.tif	0.1	0
wc2.0_bio_30s_18.tif	0	0
wc2.0_bio_30s_13.tif	0	0
wc2.0_bio_30s_11.tif	0	0.2
wc2.0_bio_30s_06.tif	0	0
wc2.0_bio_30s_17.tif	0	0
wc2.0_bio_30s_14.tif	0	0
wc2.0_bio_30s_01.tif	0	0

The Jackknife test of constant relevancy findings are shown in the following image. Since it has the most benefit when utilized alone, the ecological factor wc2.0_bio_30s_12.tif seems to contain the most valuable information. Since Wc2.0_bio_30s_15.tif is the component of the environment when deleted, decreases the gain the most, it looks like to have the most of data that is absent in the other variables.

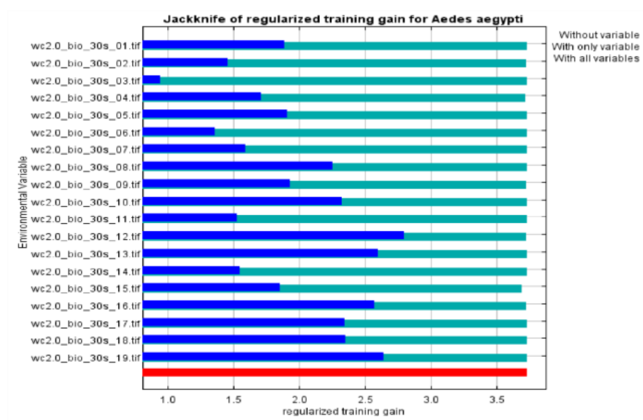

Figure 6. *Jackknife of regularised training gain for Aedes aegypti.*

In the following picture the identical Jackknife test is shown, but test gain is used in place of training gain. Keep in mind that test findings may cause us to change our minds about which aspects are most crucial.

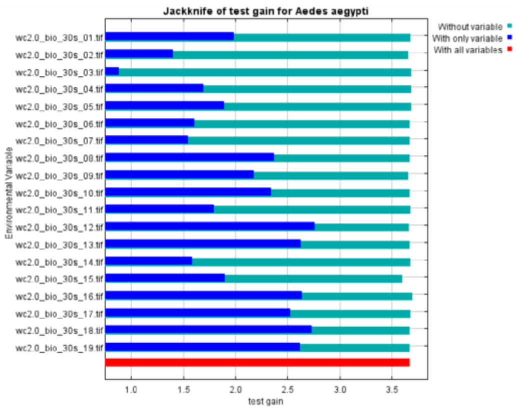

Figure 7. *Jackknife of test gain for Aedes aegypti*

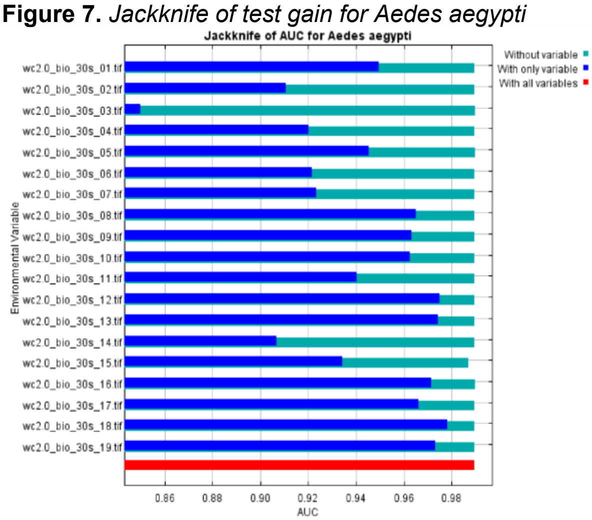

Figure 8. *Jackknife of AUC gain for Aedes aegypti*

Discussion

The graphics that are displayed shed light on a critical aspect of species distribution modeling (SDM) by emphasizing reaction curves and the understanding of Maxent models. These visualization approaches are essential for comprehending how environmental conditions impact the projected distribution of species.

Response curves must be understood in order to comprehend the relationship between environmental conditions and species dispersion forecasts. By examining these response curves, we can ascertain the extent to which changes in a particular environmental variable impact a species' predicted distribution. Interpreting response curves is crucial to understanding the ecological interactions between species and their environments. If a response curve for a specific environmental variable is generally flat through most of range of values, it may not have a powerful effect on distribution of species. In particular, the presence of significant correlations between environmental variables also entangled the interpretation. In this scenario the model may manipulate connections between factors in such ways that response curves do not readily show. Contrastingly to the marginal reaction curves which were addressed previously, these graphs describe how the selected variables expected to appropriately affects selected variables along with the other factors to produce dependencies. The graphs is in depth visually describe how the change of environmental circumstances impact the expected expansion of species. These graphs enable the researchers to understand the pattern of ecological processes in controlling specie distribution.

Concluding that response curves along with Maxent model response curves are the most crucial tools used by researchers to develop relationship between environmental factors on species distribution, how they understand species distribution. This research graphs are a quick way to understand the ecological dynamics which are effecting species distribution and steps can be taken to conserve species and managing the environmental conditions to the extent they can.

The jackknife test is as essential tool as the previously described response curve and Maxent model response are used for the SDM (species distribution modeling). Jackknife test is a resampling method used to determine the statistical estimator's variance and bias. After understanding model on all the other data points, this cross-validation is called leave-one-out, and it is used to test the model on excluded data point. In this process the test is repeated for each data point in the dataset. The jackknife testis used to draw graphs that depicts the Maxent models performance and it's stability in that particular research. By comparing actual and expected values of every missing data point research can evaluate the accuracy of models' predictions. The jackknife test helps in identifying over fitting model by evaluating the model's performance on imagined data points. If prediction across all the data points came out consistent then it model appears to be stable and suitable for handling unknown data. Over fitting, are the model's where new data effect the model's ability to detect its target. If the jackknife test predictions are unlike from the training data predictions than this is over fitting model.

Conclusions

The study provides valuable information about the geographic dispersal region for the important mosquito species that act as vector represent a major health risk. To prevent the spread of illness, selected vector management measures are necessary. The predictive models developed in this work can help direct efforts to manage vectors in the regionof vector mosquitoes in the urban regions of Pakistan especially Faisalabad, using GIS techniques. Since the area is main suitable breeding.

There are various restrictions on this study. Because of the district's terrain in some places, mosquito captures from the allocated locations were insufficient for field validations of these models. Finding high-risk areas close to water sources and mosquito populations was one way to deal with this problem. Positive results were occasionally achieved, even though these efforts were not always successful. In particular, accessibility was a major obstacle to the collection of Ae. aegypti from high-risk areas; just two sites produced positive results, and the rest sites produced negative results because of a lack of water or logistical challenges in getting to them. However, our findings are supported by the two positive locations in high-risk zones, which validate our model predictions.

In Faisalabad, the region of Punjab in Pakistan ,there is spread of diseases affecting its urban areas these diseases are carried by mosquitoes, so some targeted vector control measures are needed to put in that place. Moreover it is needed to analyze the directional proficiency of mosquito species at different places. So in order to check the vector potential and dispersion in metropolis of Faisalabad, Punjab, Pakistan there is need of more larvae collection of mosquito. There are some strategies we can adopt to control this disease spreading by vector by different controlling strategies that includes use of pesticide spray, source reduction of larvae, reduction of mosquito breeding grounds , control of mosquito population , improvement of sanitary conditions, improvement of waste management infrastructure, implementation of IVM(integrated vector management) as it contain a number of control mechanism , public awareness about the causative agents of diseases , carry out a range of mosquito control programs with the help of environmental departments etc.

Author Contributions

S.S: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. M.R. and M.N.: Writing – review & editing, Visualization, Formal analysis, Conceptualization. H.C. and N.H.B.: Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition. Wei Luo: Writing – review & editing, Conceptualization. N.R.: Writing – review & editing, Conceptualization. R.Y.: Writing – review & editing.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Data Availability

Data will be made available on request.

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RESEARCH ARTICLE

Biodiversity and Relative Abundance of Insects Fauna in different Crops of Kamalia Region, Pakistan

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Abstract This study examines insect biodiversity across 28 locations in Kamalia using key ecological indices—Species Richness, Shannon Index, and Simpson Index—to assess species distribution and diversity. The findings highlight significant variations in biodiversity, with areas like the Forest area, Chak No. 740 GB Kamalia exhibiting high species richness and a balanced ecosystem, whereas urbanized locations such as Govt. Girls High School 713 GB Kamalia show lower diversity, dominated by species like Aedes aegypti and Periplaneta americana. The study underscores the impact of human activities, including urbanization, pollution, and habitat fragmentation, on insect populations. It also identifies both beneficial species, such as Apis mellifera linnaeus, and pest species, indicating a complex ecological balance. Key limitations include sampling biases and the exclusion of seasonal variations. To address these gaps, future research should incorporate broader geographic sampling and long-term monitoring. The study emphasizes the need for targeted conservation strategies and sustainable habitat management to preserve biodiversity. Encouraging green spaces and community-based research can help mitigate biodiversity loss. These findings provide valuable insights for researchers, conservationists, and policymakers aiming to balance ecological stability with sustainable development in Kamalia's diverse ecosystems.

Keywords: Biodiversity, species richness, abundance, Shannon index, Simspons Index.

Introduction

The decomposition of organic matter, pollination, pest control, nutrient cycling, and other ecological services are all provided by insects, which are especially important in agroecosystems (Gurr et al., 2012; Majer, 1987). Insects make up over 73% of all known animal species and are one of the most prolific and diverse groups of organisms on

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Earth. They are also essential to maintaining ecological balance (Raghavendra et al., 2022). Although insects are important, several anthropogenic factors, including intense agricultural practices, habitat fragmentation, pesticide usage, and climate change, are contributing to the loss of insect populations worldwide (Hallmann et al., 2017; Dunn, 2005). This reduction puts at risk not only biodiversity but also the long-term viability of natural ecosystems and food systems (Losey & Vaughan, 2006).

The preservation of biodiversity and agricultural productivity must be balanced in agroecosystems. The very ecosystem services that sustain crop production are undermined by agricultural intensification, which frequently results in a decline in arthropod diversity (Swift et al., 1996; Rana et al., 2019). For improving soil fertility and controlling pest populations, insects like pollinators and natural predators are essential (Cardinale et al., 2006; Rathore & Jasrai, 2013). However, the location, abundance, and diversity of these beneficial insect populations are influenced by bioclimatic factors and environmental changes, which make them extremely susceptible (Yi et al., 2012). Studies have looked at how farming techniques affect biodiversity generally, but few have looked at the insect fauna in particular agricultural regions, especially in developing nations where ecological data are sometimes lacking (Nicholls & Altieri, 2013).

Farmlands, orchards, fallow lands, and woodlands make up the diverse environment of Pakistan's Kamalia Region in District Toba Tek Singh (Khan et al., 2020). It is the perfect case study for examining how agricultural practices affect insect biodiversity because of its diversity. Nevertheless, not much research has been done to assess the ecological importance and diversity of insects in this area. The creation of evidence-based conservation plans adapted to regional environmental and agricultural circumstances is hampered by the lack of baseline data on insect abundance and distribution (Saunders et al., 2020).

By investigating the diversity and relative abundance of insect fauna across different crop systems in the Kamalia Region, this study seeks to close this important knowledge gap. Additionally, it looks for bioclimatic elements that affect insect populations. This study will aid in the development of sustainable and biodiversity-friendly farming techniques by examining the effects of various agricultural strategies on insect communities (Altieri & Letourneau, 1982). It is anticipated that the results would aid ecological conservation initiatives and offer recommendations for controlling insect populations in a way that is advantageous to the environment and agriculture (Samways, 2007).

Materials and Methods

This research was carried out at Riphah University, Faisalabad Campus, between July 2024 and March 2025. The primary aim intended to assess the relative abundance of insect fauna in Kamalia city, situated in the district T. T. Singh. Kamalia is geographically located at 30° 44′ 0″ N, latitude & 72° 39′ 0 ″ E, longitude. The city is bordered by the River Ravi and Chichawatni in the south, Pir Mahal in the west, Rajana and Mamu Kanjan in the north, and Harappa and Sahiwal to the east.

The Kamalia region, District Toba Tek Singh, Pakistan, was the site of this study's July–December 2024 fieldwork, which was centered at the Riphah International University Faisalabad Campus. Assessing the relative variety and richness of insect fauna across various agroecosystems was the main goal. The Kamalia region is perfect for assessing biodiversity since it provides a variety of habitats, such as grasslands, woodland borders, and agricultural areas (Khan et al., 2020).

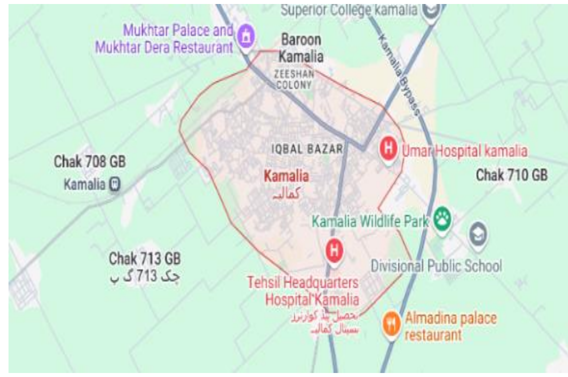

Figure 1. The boundary of Kamalia Tehsil, Punjab, Pakistan.

On a weekly basis sampling took place in 200 m² of well-chosen crop fields. To investigate how climatic factors affected insect populations, digital thermometers and hygrometers were used to collect environmental data, such as temperature and humidity (Yi et al., 2012).

Several techniques of collection were used. A commonly used technique in ecological research, sweep netting entailed swinging a net horizontally across vegetation to catch insects that were above the canopy. Specimens were preserved in jars with 70% alcohol and 30% glycerine, and this procedure was carried out between 5:00 and 7:00 a.m. (Altieri & Letourneau, 1982). In order to ensure accuracy and least harm to the specimen, visible and delicate insects were collected using forceps and handpicking (Majer, 1987).

Pitfall traps were used to catch insects that live on the ground in order to augment canopy-level collection. These traps were double-cup containers filled with a mild detergent solution to immobilize insects, and they were buried flush with the earth (Cardinale et al., 2006). To attract pollinators, fluorescent bowl traps in blue and yellow were positioned at random at each field location. The traps were in operation from 9:00 a.m. to 5:00 p.m., and the contents were gathered at the end of the day (Hallmann et al., 2017).

The insects were taken to the Riphah International University's entomology laboratory so they could be identified. Standard taxonomic keys were used to identify specimens to the species level after morphological characteristics were analyzed under dissecting microscopes (Kumlert et al., 2018). A deeper comprehension of insect population dynamics and the development of regionally specific conservation strategies were facilitated by the analysis of the recorded data for species richness, diversity indicators, and ecological roles (Wilson & Fox, 2021).

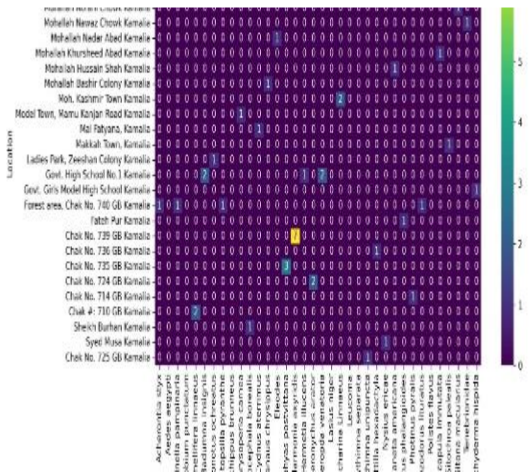

Figure 2. Heatmap illustrating the abundance of insect species across different locations in Kamalia, with locations ordered by K-Means clustering. Species are represented by their scientific names, and cell values indicate observed counts.

The patterns of insect diversity in Kamalia's urban and rural areas differ significantly. Urban locations like *Govt. Girls High School 713 GB Kamalia* and *Govt. Girls Model High School Kamalia* are mostly inhabited by species such as *Aedes aegypti* and *Apis mellifera Linnaeus*, both commonly associated with human-modified ecosystems. These areas often have low plant diversity and are subject to pesticide use, limiting the variety of insect species. In contrast, the *Forest area, Chak No. 740 GB Kamalia* hosts a broader range of insects, including *Acherontia styx*, *Anavitrinella pampinaria*, and *Chrysoperla carnea*, reflecting a richer ecosystem. Urbanized zones also support specialized insects like *Periplaneta americana* and *Polistes flavus*.

Results

Table 1. Species Richness, Shannon Diversity and Simpsons Diversity Index at locations in Kamalia:

No.	Location	Species Richness	Shannon Index	Simpson Index
1	Chak #: 709 GB	1	0	0
2	Bashir Colony	1	0	0
3	Ravi Town	3	1	1
4	Chak #: 710 GB	1	0	0
5	GHS No.1	3	1	1
6	GHS 713 GB	2	1	1
7	Chak #: 724 GB	1	0	0
8	Chak #: 735 GB	1	0	0
9	Chak #: 736 GB	1	0	0
10	Nadar Abad	1	0	0
11	GGMHS	1	0	0
12	Khurshid Abad	1	0	0
13	Makkah Town	1	0	0
14	Model Town	1	0	0
15	Nawaz Chowk	1	0	0
16	Chak #: 714 GB	1	0	0
17	Chak #: 725 GB	1	0	0
18	Chak #: 740 GB	4	1	1
19	Mal Fatyana	1	0	0
20	Chak #: 739 GB	1	0	0
21	Fateh Pur	1	0	0
22	Sheikh Burhan	1	0	0
23	Syed Musa	1	0	0
24	Kashmir Town	1	0	0
25	Zeeshan Colony	1	0	0
26	Ladies Park	1	0	0
27	Hussain Shah	1	0	0
28	Norani Chowk	1	0	0

Species richness, Shannon diversity index, and Simpson diversity index are three ecological metrics that show significant differences in insect diversity across 28 sites in the Kamalia region. In agroecosystems with different degrees of disturbance, these indicators show significant trends in species variety, evenness, and dominance (Morris et al., 2014).

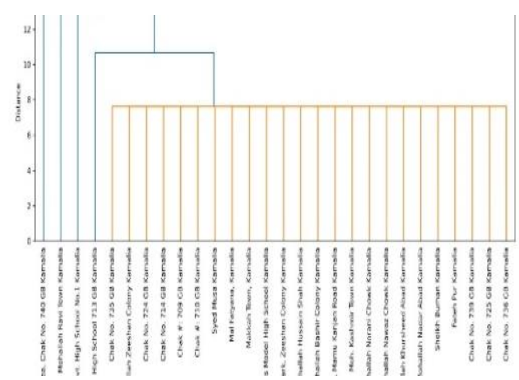

Figure 3. Hierarchical clustering dendrogram of sampling locations in Kamalia based on insect species composition, using Ward's linkage method. The y-axis represents the linkage distance, indicating similarity between locations.

Species Richness

In terms of species richness, Figure 3 & 4 show that most sites, such as Chak 709 GB and Mal Fatyana, only recorded one species, indicating a low level of diversity. On the other hand, Mohallah Ravi Town and Govt. High School No. 1 Kamalia each had three species, while the forest area at Chak No. 740 GB had the highest richness (4 species). According to these findings, a greater variety of insect species can be found in semi-natural or less disturbed habitats because they provide more ecological niches (Cardinale et al., 2006; Hallmann et al., 2017).

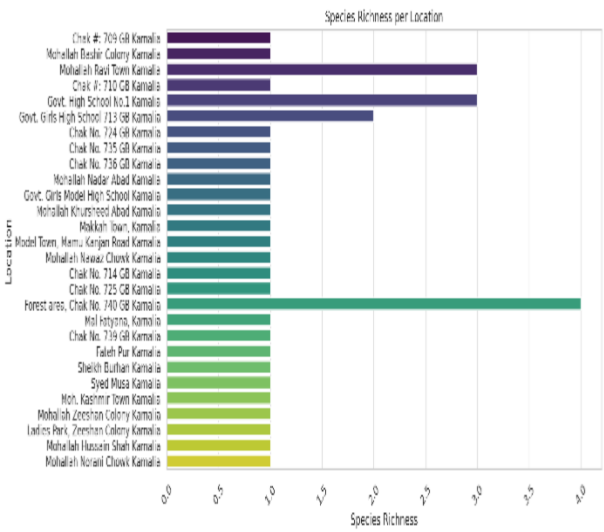

Figure 4. Bar plot representing species richness across different locations in Kamalia. Species richness is defined as the number of distinct species recorded at each site, highlighting variation in biodiversity among sampling locations.

Shannon diversity index

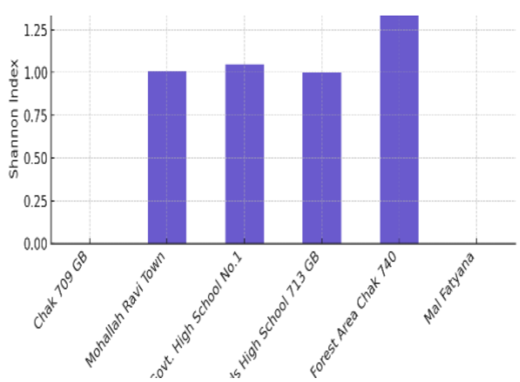

Figure 5. The highest biodiversity was observed at *Forest Area Chak 740* (H' ≈ 1.38), while *Chak 709 GB* and *Mal Fatyana* showed no recorded diversity (H' = 0). Other sites exhibited moderate diversity levels (H' ≈ 1.0–1.05).

A second indication of the poor diversity in many places is the Shannon diversity index (Figure 5), which combines species abundance and evenness. For example, Chak 709 GB has a Shannon index of 0 which indicates a single-species dominance. In contrast, the Forest area near Chak 740 GB received a score of 1.39, whilst Govt. High School No. 1 had a score of 1.05; this suggests that the distribution of coexisting species is more even (Magurran, 2007).

Simpson diversity index

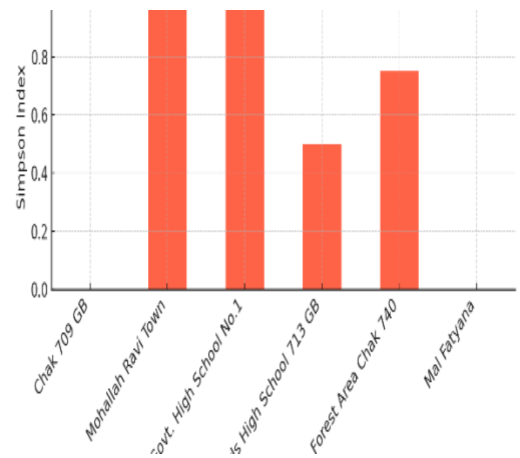

Figure 6. *Mohallah Ravi Town* and *Govt. High School No.1* showed the highest diversity (Index = 1.0), while *Chak 709 GB* and *Mal Fatyana* had no recorded diversity (Index = 0). Other locations exhibited moderate to low diversity.

The Simpson diversity index, shown in Figure 6, exhibits a similar trend. Sites like Forest area Chak 740 GB (0.75) and Govt. Girls High School 713 GB (0.5) exhibit higher index values, indicating better balance and less species dominance. This implies that because of improved habitat structure and a reduction in human stresses, these regions might have higher ecological stability (Altieri & Letourneau, 1982).

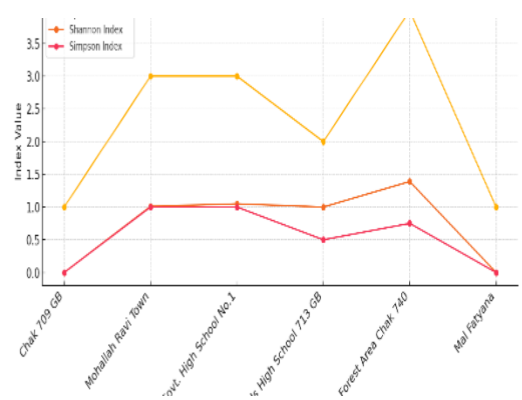

Figure 7. Comparative analysis of Species Richness, Shannon Index, and Simpson Index across six locations, illustrating variation in biodiversity levels.

A comparison of all three indices at specific sites is shown in a composite line graph (Figure 7). It highlights how, on every metric, natural or semi-natural locations do better than intensively cultivated or urbanized areas.
These findings highlight the significance of protecting semi-

natural areas like Chak 740 GB to preserve ecological resilience and biodiversity, showing a strong correlation between reduced insect biodiversity and human disturbance, habitat simplification, and pesticide use (Rathore & Jasrai, 2013; Yi et al., 2012).

Discussion

According to species richness, the Shannon Diversity Index, and the Simpson Diversity Index, the results of this study show significant heterogeneity in insect diversity throughout the Kamalia region. Low species richness (usually one species) was found in most surveyed sites, indicating low ecological complexity and biodiversity. Only a few places showed moderate to high diversity, including Mohallah Ravi Town, Govt. High School No. 1 Kamalia, and the wooded region of Chak No. 740 GB Kamalia. These findings suggest that a greater range of insect species are more common in natural and semi-natural settings, most likely as a result of improved habitat quality, the availability of floral resources, and less anthropogenic disturbance.

These findings are consistent with earlier research that emphasized the influence of land use and habitat structure on the composition of insect communities: A diversified agroecosystem with mixed vegetation and low pesticide use promotes greater arthropod diversity (Altieri & Letourneau, 1982). Natural and less disturbed environments support beneficial insect populations necessary for pollination and pest regulation (Gurr et al., 2012). The low diversity found in urbanized or heavily modified areas of Kamalia, such as residential neighbourhoods and school campuses, documented a global decline in insect populations as a result of urbanization, chemical use, and habitat loss (Hallmann et al., 2017).

The study has a number of limitations in spite of the new information. First, only one six-month period was used for data collection, which would have excluded species having seasonal occurrences. An extended investigation spanning several seasons would probably provide a more thorough comprehension of insect behaviour. Second, although methods such as pitfall traps and sweep nets were employed, the emphasis was mostly on insects that were visible or surface-dwelling, which may have resulted in an underrepresentation of nocturnal or soil-dwelling species (Cardinale et al., 2006). In addition, environmental factors that may affect insect dispersion, such as pesticide residues, soil quality, and microclimatic conditions, were not thoroughly examined.

This study concludes that Kamalia's biodiversity is unevenly distributed and heavily impacted by human activity and habitat quality. In order to preserve insect populations that are essential to the sustainability of agroecosystems, it emphasizes the necessity of conservation and management practices that place a high priority on habitat preservation, biodiversity-friendly farming, and the use of less chemicals.

Conclusions

This study used a variety of sample techniques, including bowl traps, pitfall traps, and sweep nets, to thoroughly examine the insect species in the Kamalia region. With a few notable exceptions, such as the forest area in Chak No. 740 GB Kamalia, which showed comparatively high species richness and balanced diversity indices (Shannon Index = 1.39; Simpson Index = 0.75), the results showed low overall insect diversity throughout the majority of urban and semi-urban areas. The prevalence of pests like Periplaneta americana and Aedes aegypti in cities highlights the impact of human activity on biodiversity. These findings emphasize the importance of ecological preservation, particularly in areas that have been impacted by human activity. Longitudinal studies should be the main focus of future research in order to track seasonal variations in biodiversity and evaluate how agricultural practices affect insect populations. In addition, incorporating molecular identification techniques should improve species resolution and facilitate more accurate conservation plans (Smith et al., 2020).

Author Contributions

Qi Xue: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Qian Tang: Writing – review & editing, Visualization, Formal analysis, Conceptualization. Lin Deng: Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition. Wei Luo: Writing – review & editing, Conceptualization. Mingle Xia: Writing – review & editing, Conceptualization. Shuang Fu: Writing – review & editing, Conceptualization. Chaoqun Tan: Writing – review & editing, Conceptualization. Jun Hu: Writing – review & editing, Conceptualization. Rajendra Prasad Singh: Writing – review & editing.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Data Availability

The data can be made available on request from corresponding authors.

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RESEARCH ARTICLE

Effects of Silver Nitrate ($AgNO_3$) Nanoparticles on the Growth Performance and Liver of *Cyprinus carpio*

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Abstract Water pollutants that contaminate water supplies and harm aquatic life all over the world and impact on their growth and physical parameters. Silver nanoparticles have wide use in industries because of their electrical, optical, and antibacterial qualities. The aquatic life has suffered as a result of its widespread use and application. The objective of the study was to investigate the effect of various concentrations of silver nitrate nanoparticles on growth performance and liver profile of *Cyprinus carpio*. In experimental design, total 60 fishes (15 fingerling/ aquarium) were subjected to different concentrations of $AgNO_3$ NPs 0 mg/L (T_o serve as control) group), 50 mg/L (T_1), 100 mg/L (T_2) and 150 mg/L T_3). The treatments were compared by applying one way Analysis of variance and variation among mean was evaluated by applying Duncan's Multiple Range Test (DMRT) in statistical software R (Version 4.3.3). Results showed that effect of silver nitrate nanoparticles on fish growth rate are toxic and showed non-significant ($P>0.05$) variation in average weight, specific growth rate and condition factor while, significant ($P<0.05$) in weight gain, average length and length gain and liver profile (bilirubin, albumin, total protein, alanine transaminase, alkaline phosphatase and aspartate aminotransferase) of *Cyprinus carpio*. It can be concluded that silver nitrate nanoparticles at higher concentration (150 mg/L) in T_3 have significant ($P<0.05$) toxic effect on fish growth rate and liver profile of *Cyprinus carpio*.

Keywords: antimicrobial**,** aquatic life, $AgNO_3$ NPs, adverse effects, water pollutant

Introduction

The rapid advancement of nanotechnology has revolutionized numerous scientific and industrial domains, leveraging the unique physicochemical properties of materials engineered at the nan scale (1–100 nm). Among these, silver nitrate nanoparticles ($AgNO_3$ NPs) have emerged as particularly significant due to their exceptional antimicrobial, catalytic, and optical characteristics [26,25]. These nanoparticles, composed of ultra-fine particles invisible to the naked eye, exhibit high reactivity and dispensability in aqueous environments, making them invaluable for applications ranging from water purification to biomedical diagnostics. In aquaculture, $AgNO_3$ NPs are increasingly employed to mitigate pollutants, pathogens, and bio fouling, thereby enhancing water quality and operational efficiency [10,25]. Their integration into fish feed formulations has also been explored to augment nutrient absorption, immune response, and growth performance in commerciallyvital species such as Gulfam and *Cyprinus carpio* [2,7].Concurrently, the exponential rise in $AgNO_3$ NP production projected to grow by 63% until 2024 raises critical ecological concerns [20]. Industrial and consumer products containing these nanoparticles often release them into aquatic ecosystems via wastewater discharge, agricultural runoff, and atmospheric deposition [14,19].

Once introduced, $AgNO_3$ NPs interact with biological systems in complex ways potentially disrupting metabolic functions and accumulating in vital organs. Fish, as primary inhabitants of aquatic environments, are especially vulnerable due to direct exposure through gills, skin, and dietary intake [15]. Their role as bio indicators of ecosystem health underscores the urgency of understanding nanoparticle-induced toxicity. Water pollution exacerbates these risks, with contaminants like heavy metals, pesticides, and nanoparticles compromising aquatic biodiversity and human health [1,6]. Nanoparticles, including $AgNO_3$ NPs, enter water bodies through treated sludge and industrial effluents, where their minute size and high surface-area-to-volume ratio facilitate uptake by organisms [11]. The liver, a primary detoxification organ in fish, is particularly susceptible, acting as a biological filter that concentrates 41–88% of circulating nanoparticles [4]. Despite this, the metabolic fate and hepatotoxic mechanisms of $AgNO_3$ NPs remain poorly elucidated, with existing studies highlighting dose-dependent histopathological alterations and enzymatic disruptions [13,31]. The common carp (Cyprinus carpio) serves as an ideal model for such investigations due to its ecological ubiquity, economic importance in global aquaculture, and sensitivity to environmental stressors [18]. Native to Eurasia but widely introduced, this species exhibits marked physiological responses to pollutants, making it a robust sentinel for nano toxicology studies. Prior research indicates that $AgNO_3$ NPs impair growth, alter behavior and induce oxidative stress in fish, yet comprehensive analyses of their hepatotoxic effects specifically on liver morphology, enzyme activity, and growth dynamics are scarce [7,29]. This knowledge gap is critical, as the liver governs essential processes like metabolism, nutrient storage, and toxin neutralization, directly influencing overall health and aquaculture yield. This study aims to evaluate the effects of $AgNO_3$ NPs on the growth performance and liver health of *Cyprinus carpio*. Specifically, we assess changes in growth parameters (weight gain, length gain, and specific growth rate) and liver enzyme activity (ALT, AST, ALP, bilirubin, albumin, and total protein) following exposure to varying concentrations of $AgNO_3$ NPs. The findings will contribute to understanding the ecological risks of nanoparticles in aquaculture and provide insights for sustainable fish farming practices.

Materials and Methods

Data Collection

The experiment was conducted in the Fisheries Laboratory, Department of Zoology, University of Agriculture, Faisalabad, from March to April 2024. Healthy fingerlings of *Cyprinus carpio* (average weight: 15 ± 2 g; length: 10 ± 1 cm) were obtained from the Government Fish Hatchery, Faisalabad. The fish were acclimatized for 10 days in dechlorinated tap water under controlled conditions (temperature: 25 ± 2°C; dissolved oxygen: 6.5 ± 0.5 mg/L; pH: 7.2 ± 0.3).

Nanoparticle preparation and Exposure

Silver nitrate nanoparticles ($AgNO_3$ NPs; 20 nm and 40 nm) were synthesized and characterized by the Physics Department, University of Agriculture, Faisalabad. The fish were divided into four treatment groups (n=15 per group): Control (T_0): 0 mg/L $AgNO_3$ NPs

- T_1: 50 mg/L $AgNO_3$ NPs
- T_2: 100 mg/L $AgNO_3$ NPs
- T_3: 150 mg/L $AgNO_3$ NPs

The experiment lasted 28 days, with fish fed a commercial diet (0.05 g pellets) twice daily. Water quality parameters were monitored weekly.

Growth Performance Analysis

Growth parameters were assessed using the following formulas:

Weight gain (%) = [(Final weight – Initial weight) / Initial weight] × 100

Length gain (%) = [(Final length – Initial length) / Initial length] × 100

Specific growth rate (SGR, %/day) = [(ln Final weight – ln Initial weight) / Days] × 100

Condition factor (CF) = (Body weight (g) / (Total length (cm))^3.

Liver Function Assessment

At the end of the trial, five fish per group were anesthetized (200 ppm clove powder), and blood samples were collected from the caudal vein. Serum was separated via centrifugation (5000 rpm, 4°C, 20 min) and analyzed for:

Alanine transaminase (ALT)

Aspartate aminotransferase (AST)

Alkaline phosphatase (ALP)

Total protein, albumin, and bilirubin Biochemical assays were performed using QCA diagnostic kits (Micro Lab 300® spectrophotometer).

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) in R software (Version 4.3.3). Significance was set at $p < 0.05$.

Results and Discussion

Growth Performance

Cyprinus carpio exposed to silver nitrate nanoparticles ($AgNO_3$ NPs) at concentrations of 0 (T_o), 50 (T1), 100 (T2), and 150 mg/L (T3) for 4 weeks showed concentration-dependent impacts on growth parameters (Tables 1–2). While survival remained 100% across all treatments, significant effects were observed in key metrics: Weight gain decreased markedly at 150 mg/L (T3: 0.19 ± 0.19 g) compared to control (T_o: 0.60 ± 0.10 g; p = 0.00459, ANOVA).

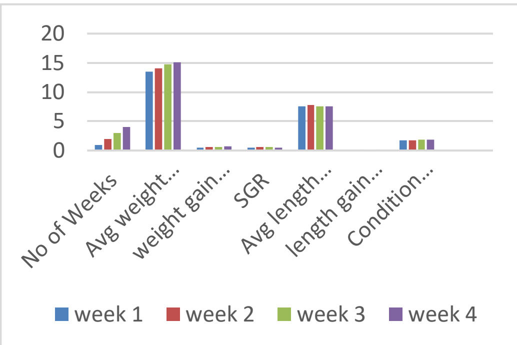

Figure 3.1: A Graphical Demonstration of the Growth Performance of Cyprinus carpio In Terms Of Specific Growth Rate, Length Condition Factor And Weight T Group.

Table 3.1: *Growth Performance of Cyprinus carpio In Terms Of Average Weight, Weight Gain, SGR, Average Length, Length Gain, Condition Factor and Survival Rate in T_1 for 4 weeks.*

Group T_1 50mg/L							
Weeks	Avg W. (g)	Weight Gain (g)	SGR	Avg. L (cm)	Length gain (cm)	Condition factor	Survival Rtae %
1	13.33	0.45	0.45	7.52	0.02	1.76	100
2	13.97	0.63	0.63	7.54	0.03	1.86	100
3	14.52	0.57	0.57	7.57	0.03	1.91	100
4	15.22	0.64	0.64	7.59	0.02	2.0	100
Mean ± SD	14.26 ±0.69	0.57±0.07	0.57±0.07	7.55±0.02	0.02±0.05	1.88±0.08	100±0

Length gain was significantly reduced in T3 (0.01 ± 0.007 cm) versus T_o (0.03 ± 0.007 cm; p = 0.0178).

Specific Growth Rate (SGR) declined at 150 mg/L (T3: 0.41 ± 0.05%) but showed no statistical significance (p = 0.134).

Table 3.3: *Growth Performance of Cyprinus carpio In Terms Of Average Weight, Weight Gain, SGR, Average Length, Length Gain, Condition Factor and Survival Rate in T_3 for 4 weeks.*

			Group T_3				
No. of Weeks	Avg. weight (g)	Weight Gain (g)	SGR	Avg. length (cm)	Length gain (cm)	Condition factor	Survival Rtae %
1	13.33	0.39	0.42	7.51	0.01	1.75	100
2	13.71	0.38	0.45	7.51	0.01	1.81	100
3	13.74	0	0.46	7.53	0.02	1.81	100
4	13.74	0	0.33	7.52	0	1.82	100
Mean ± SD	13.63±0.17	0.19±0.19	0.41±0.05	7.51±0.008	0.01±0.007	1.79±0.02	100±0

Condition factor and average weight exhibited no significant differences across treatments (p > 0.05).

Liver enzymes and Biochemical Markers

$AgNO_3$ NPs induced significant hepatic toxicity (p < 0.001 for all enzymes): ALT decreased in T1 (4.27 ± 0.149 U/L) and T2 (5.70 ± 0.123 U/L) but increased in T3 (15.7 ± 1.51 U/L) versus control (24.23 ± 0.1 U/L).

Table 3.4: *Effect Of Concentrations of Silver Nitrate Nanoparticles on the Weight of Cyprinus carpio*

No. of weeks		T_o		T_1		T_2		T_3
	Avg weight	weight gain	Avg weight	Weight gain	Avg weight	Weight gain	Avg weight	Weight gain
1	13.51	0.45	13.33	0.45	13.34	0.45	13.33	0.39
2	14.07	0.64	13.97	0.63	13.85	0.63	13.71	0.38
3	14.71	0.59	14.52	0.57	14.51	0.45	13.74	0.00
4	15.12	0.75	15.22	0.64	15.93	0.53	13.74	0.00
Mean ± SD	14.35 ±0.61	0.60±0.10	14.26 ±0.69	0.57±0.07	14.40±0.97	0.50±0.06	13.63±0.17	0.19±0.19

Albumin and bilirubin also showed significant elevations at 100–150 mg/LAST and ALP exhibited dose-dependent increases, peaking at T3 (AST: 37.4 ± 2.1 U/L; ALP: 54.83 ± 3.07 U/L).

Table 3.5: *ANOVA Of Various Concentration of Silver Nitrate Nanoparticles on the Average Weight of Cyprinus carpio*

SOV	DF	SS	MS	F-Value	P-Value
Treatment	3	1.254	0.4181	0.994	0.429ns
Error	12	5.049	0.4208		
Total	15	6.303			

Table 3.6: *Mean Comparison Of Test Values Of Effects Of Different Conc. Of Silver Nitrate NPs on the Avg. Weight of Cyprinus carpio*

Treatment	mean comparison test
T_o Control	14.35 ±0.61
T_1 50 mg/L	14.26 ±0.69
T_2 100 mg/L	14.40±0.97
T_3 150 mg/L	13.63±0.17

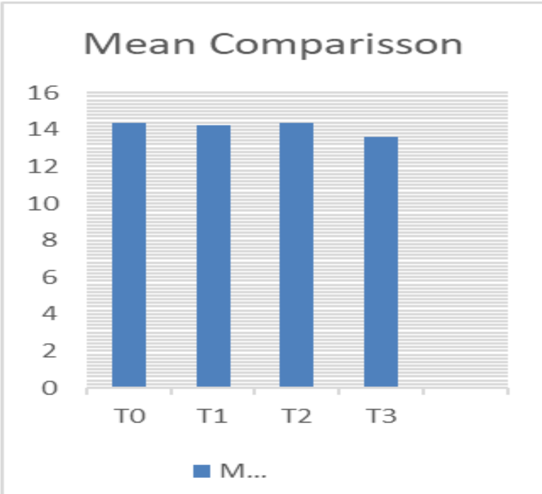

Figure 3.2: *Graph mean comparison of Avg. weight of Cyprinus carpio in various 4 treatments*

Figure showed that (DMRT) Mean Comparison Test Values of effects of different concentrations (0,50,100,150mg/L) of silver nitrate NPs at various 4 treatments on the average weight of *Cyprinus carpio.* Total protein surged at higher concentrations (T2: 14 ± 2.16 g/dL; T3: 20.67 ± 1.63 g/dL).

Water Quality Parameters

Physicochemical properties (pH, dissolved oxygen, temperature) remained stable weekly across all treatments, confirming that growth and liver alterations were nanoparticle-induced.

Discussion

The pervasive introduction of silver nitrate nanoparticles ($AgNO_3$NPs) into aquatic ecosystems via anthropogenic activities represents a critical environmental challenge, with profound implications for ecological stability and aquatic organism health. As highlighted by [1], water contamination from industrial, medical, and technological applications facilitates the accumulation of nanoparticles in aquatic environments, where their high reactivity, extensive surface area, and photolytic properties though beneficial for wastewater treatment [16] also render them persistent pollutants. Silver nanoparticles (AgNPs), a dominant subset of aquatic contaminants, induce severe health disruptions in aquatic life, particularly fish, as evidenced by their detrimental effects on growth metrics and organ function[9,28]. This study corroborates prior findings, demonstrating that $AgNO_3$NPs significantly impair growth performance in Cyprinus carpio (common carp), a species vital to global aquaculture and food security in developing nations [21].

Specifically, dose-dependent exposure resulted in statistically significant reductions ($P<0.05$) in weight gain, average length, and length gain, aligning with observations by Noor and Rasheed [22, 27]. These growth impediments likely stem from altered feeding behaviours and compromised nutrient absorption, as nanoparticles disrupt digestive processes and induce physiological stress. Paradoxically, while intentional incorporation of $AgNO_3$NPs into fish feed may enhance growth and immunity in controlled aquaculture settings [25], their unintended environmental release elicits toxicological consequences that undermine these benefits. Hepatotoxicity emerged as a critical concern in this investigation, with $AgNO_3$NPs inducing marked biochemical alterations in liver enzymes and proteins. Liver parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, and total proteins serve as sensitive biomarkers for pollutant-induced damage, reflecting cellular integrity and metabolic function [3].

Our data revealed significant elevations in AST and ALT activities indicators of hepatocyte injury alongside increased total protein and bilirubin levels in exposed common carp, consistent with findings by Matranga and Corsi [17] and Khorshidi [8]. Conversely, ALP activity decreased significantly, suggesting nanoparticle interference with enzymatic pathways, a phenomenon also reported by Wang [30]. These perturbations underscore a dose-dependent accumulation pattern, wherein $AgNO_3$NPs preferentially bio accumulate in the liver, followed by gills and muscles, with the highest concentration (150 mg/L) inflicting severe damage compared to lower doses (50 mg/L). This bioaccumulation initiates upon nanoparticle entry into the bloodstream, followed by hepatic sequestration [5], ultimately manifesting as chronic toxicity that compromises vital organs, including the heart and gills [24]. The oxidation state of silver further influences its eco toxicological impact. In aquatic systems, AgNPs exist as Ag^0, Ag^+, Ag^{2+}, and Ag^{3+}, with nanoparticulate silver (Ag^0) exhibiting heightened bioavailability and toxicity relative to bulk silver due to its propensity for cellular uptake and ion release [28,12]. This characteristic exacerbates chronic exposure risks, as evidenced by persistent alterations in liver enzymes and growth parameters in our study. Notably, the highest treatment concentration (T3) induced the most pronounced declines in swim performance, growth, and biochemical homeostasis, highlighting the concentration-dependent nature of $AgNO_3$NP toxicity. These findings align with global concerns regarding nanoparticle bioaccumulation and their potential to disrupt aquatic food webs [23].

In conclusion, this study substantiates that $AgNO_3$NPs pose significant threats to aquatic biota, particularly fish, through growth inhibition and hepatotoxic mechanisms. However, the environmental reality involves concurrent exposure to multiple pollutants organic and inorganic which may interact synergistically or antagonistically. Future research must therefore prioritize long-term, multifactorial studies to elucidate the cumulative impacts of mixed contaminants. Advanced simulations and mechanistic models are essential to predict nanoparticle behaviour, transport, and chronic hepatotoxicity in complex aquatic systems. Such efforts will inform regulatory frameworks and mitigation strategies, safeguarding ecological integrity and human health against emerging nano scale pollutants.

Author Contributions

Tehreem Shakoor and Muhammad Saail Abbas designed the study, collected and analyzed data, and drafted the manuscript. Muhammad Naeem (supervisor) and Maryam Riasat (co-supervisor) oversaw the study and reviewed the manuscript. All authors approved the final version.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

This work is part of a research.

Data Availability

Data will be made available on request.

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