Research Article

Biodiversity, Seasonal Abundance, and Population Dynamics Assessment of Coccinellidae (Coleoptera) from Maize Crops

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Abstract | Coccinellids play a pivotal role as natural predators providing dynamic services in agriculture. The coccinellid fauna in the agricultural ecosystem of Pakistan, as well as their composition, drivers of assemblages, and species richness, lack comprehensive understanding and remain poorly documented. We studied biodiversity, species richness, and seasonal dynamics of coccinellids in two major maize-growing districts of the Punjab province, i.e., Kasur and Lahore, in 2018-2019. The coccinellids were collected from February to June during both cropping seasons. Visual counting, handpicking, sweep nets, sticky traps, and pitfall traps were employed for collection of specimens fortnightly. In aggregate, 10637 specimens representing 21 species, 16 genera, and 3 subfamilies were identified based on diagnostic morphological characteristics. The sub family Coccinellinae (Latreille, 1807) was the most dominant, constituting 94.72% of the total catch. *Coccinella septempunctata* (Linnaeus, 1758) was the most abundant species (21.09%), followed by *Coccinella undecimpunctata* (Linnaeus, 1758) (17.45%), and *Cheilomenus sexmaculata* (Fabricius, 1781). The study used various indices to estimate species richness (Menhinick and Margalef) and diversity (Shannon-Weiner and Simpson). The estimated species richness of all Coccinellidae species of both districts was about 95%. A significant change in the population dynamics of Coccinellidae was observed at different phenological stages (BBCH Principal Stage) of maize crops during different study months. The highest species density was recorded during April and May in both districts. A positive correlation of the Coccinellidae was observed with temperature and negative for rainfall and humidity. This work is the first to characterize coccinellids biodiversity from Pakistan's maize crops. The research will aid in employing the ladybeetles as bio-control agents for effective Integrated Pest Management (IPM) of maize growing zones in Pakistan and across the globe.

Novelty Statement | In this study, the biodiversity, seasonal abundance, and population dynamics of coccinellids in maize crops are discussed for the better understanding of these predators, which would be helpful in the development of sustainable pest control practices.

Introduction

Pakistan's agriculture sector is the backbone and critical component of economy as it contributes a substantial 22.7% to the Gross Domestic Product (GDP)- (Govt. of Pakistan, 2021). It serves as a significant contributor to both employment opportunities and foreign exchange earnings. The sustainable development of this sector is essential to ensure food security and promote rural development (Ahmad and Farooq, 2010). In Pakistan, the principal crops include wheat, rice, maize, sugarcane and cotton. Among these crops, maize has been imparting a

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critical role by providing food for humans, feed for livestock and poultry, and as industrial raw material (Rehman *et al.*, 2015). Furthermore, maize is utilized to produce a range of dietary products, such as bread, corn flakes, corn starch, corn syrup and corn oil (Khan *et al.*, 2022). Among the major crops of Pakistan, maize holds the third position after wheat and rice regarding the cultivated area and yield (Ali *et al.*, 2017). During the year 2021-2022, maize was cultivated on an area of 1,653 thousand hectares with 10.635 million tons of production. It contributes 3.2% of the total value added in the national agricultural sector and accounts for 0.7% of the Gross Domestic Product (GDP) (GoP, 2021).

Maize crop yield is reduced by multiple biotic and abiotic factor. The damage caused by insect pest is one of the primary factors leading to reduced crop production (Adeyinka *et al.*, 2018). Globally, 22.5% of maize is lost by insect pests and pathogens (Savary *et al.*, 2019; Norgrove, 2021). In Pakistan, maize production has been largely susceptible to various kinds of biological and climatic risks (Akhtar *et al.*, 2018). Various insect pests frequently invade this crop and have continuously changing feeding preferences from seedlings to crop maturity (Chaudhary *et al.*, 2014). Control and management of these damaging insect pests have always been a global challenge due to various biological and climatic factors (Prasanna *et al.*, 2022). Use of chemical pesticides is considered a quick solution to manage these insect pests (Poudel *et al.*, 2020). However, their extensive and irregular use has led to the mortality of non-targeted insects including useful natural predatory insects (Sánchez-Bayo and Wyckhuys, 2019; Brühl and Zaller, 2019). The unwise utilization of synthetic pesticides in agriculture poses a significant risk to the effective application of bio-control plans (Mubushar *et al.*, 2019).

Arthropods, including spiders, ladybugs (Coccinellids), green lacewings, and syrphid flies, serve as natural predators in agroecosystems (Basha *et al.*, 2021). They offer valuable biological control services by preying on insect pests (Losey and Vaughan, 2006). The higher densities of these natural predators ensure the suppression of pests in the agroecosystems (Nadeem *et al.*, 2023). Different species of natural predators utilize various hunting strategies to target pests at various stages of their life cycle resulting in enhanced biological pest control (Hajek and Eilenberg, 2018). The diverse hunting techniques by these predators contribute to more effective management of pest populations (Snyder, 2019). Even if natural predators may not be able to completely suppress the pest fauna below the threshold level, they can still have a significant impact by reducing the reproductive capacity of pests (Michalko *et al.*, 2019).

Lady beetles or ladybugs (Coleoptera: Coccinellidae) represent a significant and diverse group of insects found in various ecosystems (Mora *et al.*, 2020). The known taxonomy includes approximately 6,000 distinct species which are organized into 360 genera. These are widely distributed across various ecosystem globally (Pervez, 2016; Bouchard *et al.*, 2017) and 90% of the specie functioning as predators that consume various insect pest of cereal crops (Pan *et al.*, 2020). They are also among the most well-known generalist predators and their role in the suppression of pest populations has been distinguished for a long time (Riddick, 2023). Due to its predatory nature, lady beetles are considered valuable biological agents for regulating the population density of insect pests (Sentis *et al.*, 2022). Coccinellids utilize a wide range of prey species, like mites, aphids, Coleoptera, and Lepidoptera, as well as non-prey (Hodek and Honěk, 2009; Obrycki *et al.*, 2009; Evans, 2009; Biddinger *et al.*, 2009). Coccinellids contribute to pest suppression by preying upon both larvae and adult pests (Sappington *et al.*, 2018). Plant volatiles and prey abundance help in the regulation of coccinellid populations (Xiu *et al.*, 2019). Different factors like climatic conditions, crop management practices, application of pesticides, side crops, and availability of the prey may affect the diversity and population dynamics of coccinellids in maize crops (Hassan *et al.*, 2016).

Recently, the unwise use of synthetic pesticides especially in developing countries like Pakistan has a number of drawbacks, such as non-target specificity, long-term perseverance, bio-magnification, and loss of biodiversity (Riaz *et al.*, 2017). Considering the harmful effects of synthetic pesticides, it is essential to promote integrated pest control programs as a means to conserve biodiversity and decrease pesticide use. Despite of the ecological and economic importance of coccinellids in agroecosystems, no significant research has been conducted to assist with use of coccinellids in pest control (Rafi *et al.*, 2005; Dhawan and Peshin, 2009). Our objective is to address the knowledge gap concerning coccinellids within the maize agro-ecosystem of Pakistan. Our study objective was to investigate biodiversity, seasonal abundance, and population dynamics of the coccinellids in maize crops. Future IPM strategies can use this information for sustainable practices, improving pest management, and reducing the need for insecticides.

Materials and Methods

Study area and field layouts

The study was conducted in two primary maize-growing districts i.e., Kasur (31.1179° N, 74.4408° E) and Lahore (31.367°N 74.367°E) in the province of Punjab, Pakistan. The sites selected from district Kasur are Khudian Khas (30.9906° N, 74.2708° E), Chunian (30.9698° N, 73.9712° E, and Pattoki (31.0249° N, 73.8479° E), whereas from Lahore are Mustafabad (30.8903° N, 73.4998° E),

Pakki Haveli (31.1188° N, 74.3302° E), and Khana Nou (31.3731° N, 74.3617° E). From each district, the study area of 8,094 square meters was split into four sub-sites, each containing three plots measuring 674 square meters. The study sites in Kasur district were generally situated at an average distance of 20 to 25 kilometers from each other. In contrast, the average distance between the two sites in Lahore district was 25 to 30 kilometers. The combined average distance between the two sites in both districts was 70 kilometers.

Crop management practices

The seedbed was prepared using one deep plow and followed by two cultivations using a tractor-mounted cultivator during both cropping seasons (2018 and 2019). The hybrid maize variety Pioneer-P2848W was chosen and the maize was sown between February 14th and February 25th in both 2018 and 2019. Maize was manually sown with ridges spaced 75 cm apart and with a plant-to-plant distance of 30 cm. Standard N treatments were followed for nitrogen fertilizer application and 172 kg per hectare of phosphorus was applied. During the whole cycle of the maize crop, 12 irrigations (especially water-need-based stages like tasseling, silking, cob, and grain development) were applied through a manual tube well. Manual weed management was applied using hand picking and hoeing. No herbicide was applied at any experimental sites. Notably in 2018, the study sites in the Kasur district were characterized by a monoculture of maize, while in 2019, they were surrounded by alfalfa. Conversely, in the Lahore district, the study sites were surrounded by alfalfa in 2018 and maize monoculture 2019.

Sampling of coccinellids

To study the population dynamics of coccinellids at various growth stages of maize crops, the BBCH principal scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) was employed. This scale is well-known for offering a standardized description of plant developmental stages, primarily determined by their phenological characteristics (Lancashire *et al.*, 1991). Table 1 represents a complete account of the date of observation along with the BBCH scale of maize growth. Data collection began after the germination of the maize seed from March 1, 2018, to March 3, 2019. For data recording, 20 rows of maize plants with 15 plants in each row were selected from each plot. The whole plant was visually observed for the collection of the coccinellids fortnightly from 10 am to 2 pm. First, visual counting was performed for sampling the maize plants. The outer surfaces of plants, including leaf whorls, axils, tassels, and ears were visually inspected on each sampling date for the detection of eggs, larvae, pupae, and adults. The least exposed plant structure was also separated to expose any hidden beetles. Sweep net sampling was also performed to estimate the flying ladybeetles. A 15-inch diameter sweep net was swung in a

180° arc twice from each plant. The sampling was carried out carefully to minimize disturbance and avoid disrupting ladybug activity (Hesler and Kieckhefer, 2008). At least five sweeps from each plant were taken. Hand picking method was also used for the collection of specific ladybugs species from different part of maize plant. Sticky tarps of 200 cm² were hung in the air to capture coccinellids (Kemp and Cottrell, 2015).

Table 1: Sampling date and their corresponding BBCH Code for maize growth stages.

Sampling period	BBCH code	BBCH description
03-March	BBCH-16	5 Leaves or more unfolded
18-March	BBCH-19	10 Leaves or more unfolded
03-April	BBCH-29	Maximum of tillers
18-April	BBCH-39	9 or more nodules detectable
03-May	BBCH-59	Tassel emerges and silking
18-May	BBCH-75	Kernels in the middle of grains
03-June	BBCH-85	Early dough
18-June	BBCH-89	Fully ripening

In order to collect coccinellids located in various parts of maize plots, a three-layered arrangement (outer, middle, and center) of 25 glass jars was also set up in selected fields. The jars were filled with 85% alcohol and small amount of detergent to decrease surface tension. This sampling method ensured thorough coverage of targets located in the field's margins, middle, and central areas. On each sampling day, data regarding the climate was recorded to establish a correlation between the population dynamics of lady beetles and the prevailing weather conditions.

Samples preservation and data storage

Coccinellid samples were transferred into 20 ml vials containing 95% alcohol. Each sample bottle was carefully labeled with a unique field number, collection date, and collector. All specimens were washed with 70% alcohol to remove debris attached to their bodies. Geographical coordinates, elevation data, and other notes of ecological importance such as temperature (LM-8000), humidity (R6001 Thermo-Hygrometer), rainfall data, etc. were also noted using a portable GPS device (Garmin 010-02256-00) and environmental data recorder. The preserved specimens were transferred to the Department of Zoology (Entomology Section) at Government College University Lahore for further examination.

Morphological identification

Coccinellid specimens were identified by carefully inspecting their physical characteristics using an IRMECO GmbH (IM-SZ-500) microscope mounted with a digital camera. With the help of the available published literature,

the sampled specimens were identified (Kapur, 1955; Bielawski, 1972; Majerus and Kearns, 1989; Canepari and Milanese, 1997; Zahoor *et al.*, 2003; Inayatullah *et al.*, 2005; Rafi *et al.*, 2005; Bahlai *et al.*, 2015; Poorani, 2023). Voucher specimens were deposited at the Stephenson Natural History Museum, GC University, Lahore, following appropriate tagging and numbering to facilitate future reference.

Data analysis

Shapiro-Wilk test was used to determine whether the data from the current study had a normal distribution before being subjected to further analysis (Shapiro and Wilk, 1965). Since there was no significant difference (P > 0.05) in coccinellid populations at selected locations during of both cropping seasons (i.e., 2018-2019), it was combined together for further analysis. SPDIVERS.BAS program was used to generate species accumulation curves (Ludwig and Reynolds, 1988). A logarithmic tendency curve (Colwell and Coddington, 1994) was also used to visualize the increase in the number of species. As the biodiversity data collection is time consuming and laborious process, a small portion of the community is characterized by rare species, often which are mostly single-tones (Lawton *et al.*, 1998). Most biodiversity surveys fail to detect them (Chao *et al.*, 2009). To estimate the total species richness of the coccinellid fauna of both districts, the two most widely used estimators Chao 1 and Chao 2 non-parametric richness test were computed in Estimate S 9.1 (Colwell and Elsensohn, 2014). Inventories completeness was analyzed using the ratio between the Chao 1 estimate so that completeness value was comparable with the previous values (Chao, 1984; Hortal *et al.*, 2006; Chao *et al.*, 2009). Chao 1 was used as it is an abundance-based estimator, which can be applied to a single sample. Chao 1 is the most conservative estimator of species richness when there are many single-tone and double-tone species:

$$S1 = S_{obs} + \frac{F_1^2}{2F_2} \quad \dots (1)$$

Where S_{obs} represent species count in the sample, F_1 shows number of single tone species in the sample and F_2 represent double tone species in the sample (Chao, 1984).

$$S2 = S_{obs} + \frac{Q_1^2}{2Q_2} \quad \dots (2)$$

Where S_{obs} is the number of species in the sample, Q_1 the number of single tone species in the sample and Q_2 is the number of double tone species in the sample (Colwell and Coddington, 1994). The diversity of coccinellids at various selected sites was computed using commonly employed diversity indices. viz., the Shannon-Wiener index, which is sensitive to changes in the abundance of rare species within the community, as well as the Simpson index, which is sensitive to the most abundant species in the community (Solow, 1993). The following formula was used to calculate the Shannon-Wiener index (H'):

$$H' = -\sum P_i \log p_i \quad \dots (3)$$

Where; $p_i = n/N$

Species richness was also computed by using Margalef Index, which is based on the relationship between species richness (S) and the total number of individuals observed (N).

$$d' = \frac{S - 1}{\ln N} \quad \dots (4)$$

The Menhinick index was employed to determine the relationship between the species present in the sample and the total number of individuals in the samples (Whittaker and Levin, 1977). The evenness index describes how evenly species are distributed within the sample. High value of the evenness index shows that all species in the sample are equally distributed. A decreasing evenness value approaching zero indicates that the relative abundance of species in the sample has shifted away from an even distribution. The modified Hill's Ratio (E-5) is considered the most reliable evenness index because it is not influenced by the number of species in the sample.

$$E5 = \left(\frac{1}{D}\right) - \frac{1}{e^{H'}} - 1 \quad \dots (5)$$

Here, *D* represents Simpson's index, and H' stands for the Shannon-Wiener index.

Diversity indices were calculated using the statistical software SPDIVERS. BAS (Ludwig and Reynolds, 1988). Cluster analysis was employed to determine the degree of association among the sampling sites. It is a valuable data reduction technique that can be helpful in analysis of grouping of objects. The cluster analysis was performed through MSVP. Version.3.22. The similarity estimates were analyzed using UPGMA, and the resulting clusters were depicted as dendrograms (Mercado *et al.*, 2014).

Results and Discussion

Over two years (2018-2019), a total of 10,637 coccinellid specimens, comprising 21 species, 16 genera, and 3 subfamilies, were documented in districts Kasur and Lahore. Figure 1 indicates the overall relative abundance of coccinellids during sampling districts in 2018 and 2019. The sub family Coccinellinae (Latreille, 1807) was reported as the most abundant, representing 94.72% of the total catch followed by Chilocorinae (Mulsant, 1846)

Table 2: The relative abundance (%) of ladybeetles of the two major maize producing district of Punjab, Pakistan.

Family	Sub-Family	Name of Species	Kasur	Lahore	Total	R.A%
Coccinellidae	Epilachnini Mulsant 1846	*Epilachna varivestis* (Mulsant, 1850)	55	34	89	0.84
Coccinellidae	Chilocorini Costa, 1849	*Brumoides suturalis* (Fabricius, 1798)	32	16	48	0.45
Coccinellidae	Chilocorini Costa, 1849	*Chilocorus nigrita* (Fabricius, 1798)	94	51	145	1.36
Coccinellidae	Chilocorini Costa, 1849	*Exochomus quadripustulatus* (Linnaeus, 1758)	57	213	270	2.54
Coccinellidae	Coccinellini Latreille, 1807	*Adalia bipunctata* (Linnaeus, 1758)	47	77	124	1.17
Coccinellidae	Coccinellini Latreille, 1807	*Adalia tetraspilota* (Hope, 1831)	62	99	161	1.51
Coccinellidae	Coccinellini Latreille, 1807	*Anegleis cardoni* (Weise,1892)	104	79	183	1.72
Coccinellidae	Coccinellini Latreille, 1807	*Cheilomenus sexmaculata* (Fabricius, 1781)	866	302	1168	10.98
Coccinellidae	Coccinellini Latreille, 1807	*Coccinella septempunctata* (Linnaeus, 1758)	987	1256	2243	21.08
Coccinellidae	Coccinellini Latreille, 1807	*Coccinella transversalis* (Fabricius, 1781)	81	765	846	7.95
Coccinellidae	Coccinellini Latreille, 1807	*Coccinella trifasciata* (Linnaeus, 1758)	198	131	329	3.09
Coccinellidae	Coccinellini Latreille, 1807	*Coccinella undecimpunctata* (Linnaeus, 1758)	823	1033	1856	17.45
Coccinellidae	Coccinellini Latreille, 1807	*Cycloneda sanguinea* (Linnaeus, 1763)	78	181	259	2.43
Coccinellidae	Coccinellini Latreille, 1807	*Harmonia dimidiata* (Fabricius)	89	234	323	3.04
Coccinellidae	Coccinellini Latreille, 1807	*Hippodamia convergens* (Guerin-Meneville, 1842)	34	110	144	1.35
Coccinellidae	Coccinellini Latreille, 1807	*Hippodamia variegata* (Goeze, 1777)	91	345	436	4.09
Coccinellidae	Coccinellini Latreille, 1807	*Lemnia bissellata* (Mulsant, 1850)	23	56	79	0.74
Coccinellidae	Coccinellini Latreille, 1807	*Menochilus sexmaculatus* (Fabricius, 1781)	68	142	210	1.97
Coccinellidae	Coccinellini Latreille, 1807	*Micraspis allardi* (Mulsant, 1866)	88	73	161	1.51
Coccinellidae	Coccinellini Latreille, 1807	*Oenopia oncina* (Olivier, 1808)	453	65	518	4.87
Coccinellidae	Coccinellini Latreille, 1807	*Psyllobora bisoctonatata* (Mulsant)	698	347	1045	9.82
Total			5028	5609	10637	100
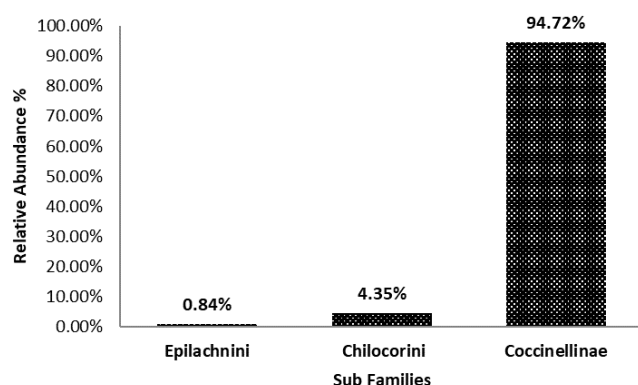
Figure 1: Percent relative abundance sub families of ladybeetles from maize crops of both districts (2018-2019).

which constituted 4.35%, while Epilachninae (Mulsant, 1846) accounted for 0.84% of the total population. *Coccinella septempunctata* was the most abundant species (21.09%), followed by *C. undecimpunctata* (17.45%), *Cheilomenus sexmaculata* (10.98%), *Psyllobora bisoctonatata* (9.82%), *C. transversalis* (7.95%), *Oenopia oncina* (4.87%), and *Hippodamia variegate* (4.09%). Furthermore, *Brumoides suturalis* constituted the smallest proportion (0.45%) among the Coccinellidae fauna (s). More coccinellids were captured from the district Lahore (n= 5609) than Kasur (n=5028). Table 2 provides a comprehensive list of coccinellid species identified based on their morphological diagnostic characteristics.

The pooled species accumulation curves were drawn for coccinellids of both study districts over a period of two years. A positive correlation was observed between the sample size and the number of species in both districts. Figure 2 clearly illustrates that with an increase in sample size, there is also a consistent rise in the number of captured species. The rate of species increase was prominently reduced after the capture of 4,000 specimens. Initially, there was a sharp increase in the total number of species, but in both districts, the accumulation curves did not reach asymptote i.e., additional sampling will not yield any additional species (Colwell *et al.*, 2004). The species richness calculated by Chao-2 estimate of coccinellids for the district Kasur was 22.25 and 21.66 for Lahore, while the average % completeness of both districts was 95% (Table 3).

The total abundance, diversity, richness, and evenness indices were computed using the data combined from the two-year in both Kasur and Lahore districts (Table 4). The Shannon-Wiener index and Simpson indices exhibited nearly identical values for both districts, suggesting comparable levels of diversity and evenness in the populations
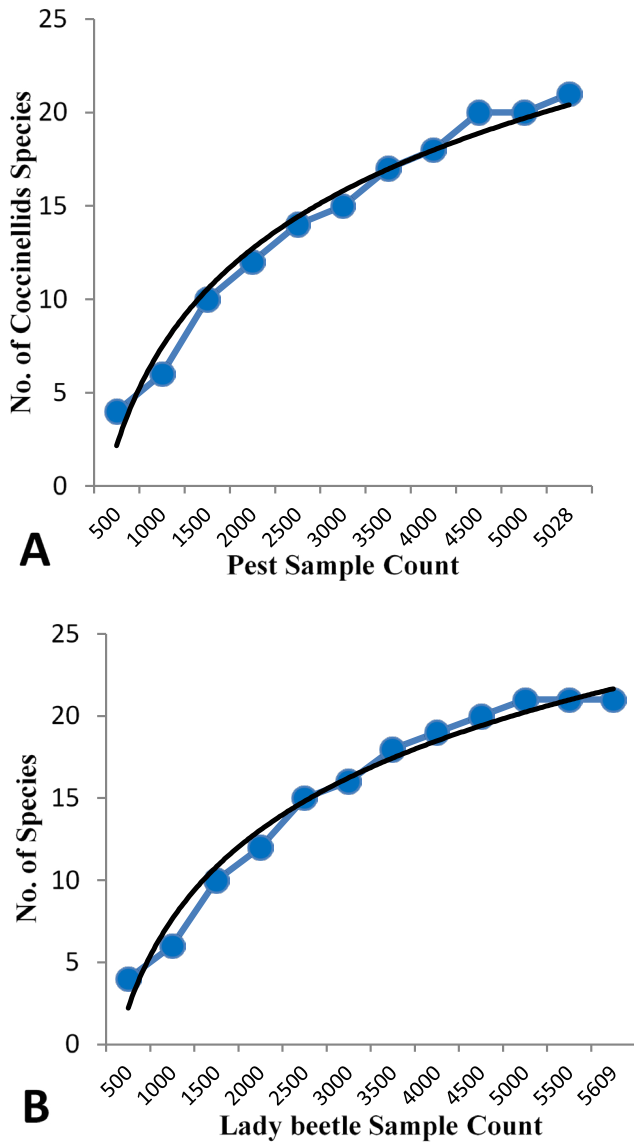
Figure 2: Species accumulation curve for coccinellids collected from district Kasur (A) and Lahore districts (B).

Table 3: Species diversity and inventory completeness for insect pests collected from Kasur and Lahore districts.

Parameters	District Kasur	District Lahore
No. of specimens	5028	5609
Observed richness	21	21
Estimated richness		
No of singletons	3	2
No of Doubletons	3	2
Chao 1	21.75	21.33
Chao 2	22.25	21.66
% Completeness	94	95

of coccinellids within the study areas. The Margalef richness index was slightly higher for district Kasur (2.35) than Lahore (2.32). The dynamics of coccinellids fluctuated throughout the sampling period in both study

districts (2018-2019). Coccinellid population increased in March (BBCH-12 to BBCH-39), peaked in May (BBCH-51 to BBCH-65), and gradually decreased from late May (BBCH-69) to June (BBCH-89) during the ripening stage (Figure 3). The species evenness (E-5) was 0.94 for district Kasur and 0.95 for Lahore indicating a uniform distribution of species abundances, contributing to a more diverse and balanced ecosystem. The species abundance based analysis revealed two main clusters (Figure 4). Coccinellid abundance demonstrated a positive correlation with temperature ($r = 0.703$, $P = 0.023$), while negative correlations with humidity ($r = -0.710$, $P = 0.022$) and rainfall ($r = -0.699$, $P = 0.024$) (Table 5).

Table 4: Total abundance, richness, diversity, and evenness indices for the lady beetles collected from different areas of Kasur and Lahore districts during 2018 and 2019.

Parameters	Study areas	
	Kasur	Lahore
Total abundance	5028	5609
Richness indices		
Margalef index	2.35	2.32
Menhinick Index	0.296	0.284
Diversity indices		
Shannon-wiener index (H')	3.44	3.58
Simpson's index (D)	0.127	0.120
Evenness (E-5)	0.94	0.95

Table 5: The association of the coccinellids abundance with temperature, rainfall and humidity during maize growing seasons (2018-2019).

	Temperature	Rainfall	Humidity
Coccinellids	$r = 0.703$	$r = -0.699$	$r = -0.710$
	$P = 0.023$	$P = 0.024$	$P = 0.022$

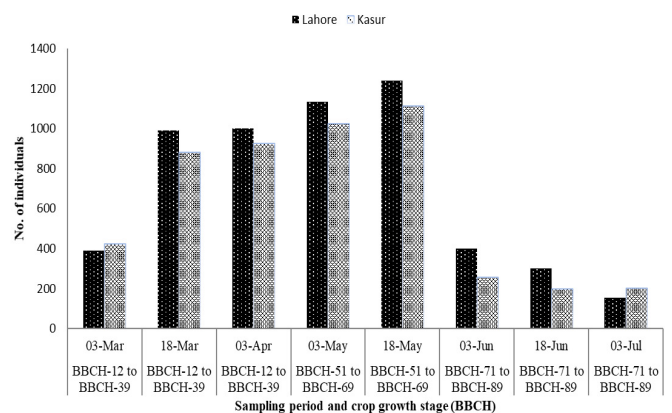* Correlation is significant at the 0.05 level

Figure 3: Population dynamics of coccinellids during different tarping session at both districts according to BBCH stage.
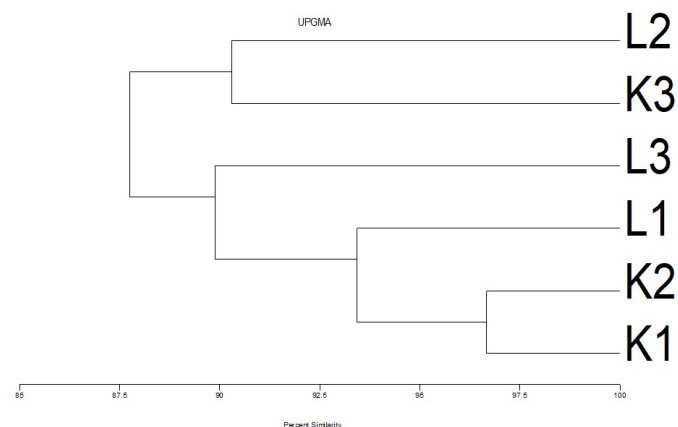
Figure 4: Cluster analysis of different sampling sites showing % similarity on basis of abundance data of coccinellids of Kasur and Lahore (2018-2019). K1, K2 and K3 are sampling sites or Kasur district, where L1, L2 and L3 are sampling sites of district Lahore.
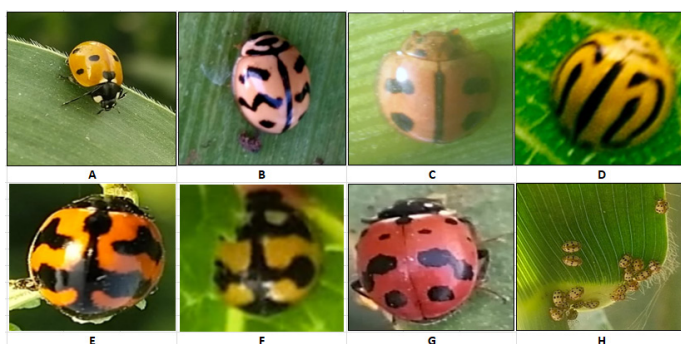

Figure 5: (A) *Coccinella septempunctata* Linnaeus; **(B)** *Cheilomenus sexmaculata* **(Fabricius); (C)** *Micraspis allardi* **(Mulsant); (D)** *Anegleis cardoni* **(Weise); (E)** *Coccinella transversalis* **(Fabricius, 1781); (F)** *Propylea dissecta* **(Mulsant); (G)** *Hippodamia variegata* **(Goeze); (H)** *Psyllobora bisoctonatata* **(Mulsant).**

Increased agriculture production through intensive farming has resulted in more pest problems leading to lower crop yields (Maqbool *et al.*, 2020). Despite pesticide use, crop losses have almost doubled (Wilson and Tisdell, 2001). Now, there is a worldwide resurgence of awareness and understanding regarding the hazards of pesticide use (Oerke, 2006). This growing realization has encouraged a significant decrease in pesticide usage. Also, the gradual shift towards more sustainable and environmentally friendly pest management practices has been noted in the recent past (Wezel *et al.*, 2009). Therefore, it is necessary to adopt a strategy that would help to control pest populations using local natural predators (Deguine *et al.*, 2021).

The ladybeetles of two districts showed dominance from the sub family Coccinellinae (Latreille, 1807) which accounted for 96.62% of total catch during the sampling period (two years). Among these, *C. septempunctata* (Linnaeus, 1758) was the most common and dominant species throughout the sampling period and this finding

is in accordance with the earlier research on Coccinellinae distribution, conducted in the other agricultural crops (rice, wheat, and cotton) in Pakistan (Rahatullah and Inayatullah, 2010; Abbas *et al.*, 2013). Members of this sub family are aphidophagous feeders showing dominance throughout the crop cycle. They are found in all habitats and agriculture ecosystems globally feed on aphids, mealybugs, psyllids, and thrips (Rahat *et al.*, 2012; Zare Khormizi *et al.*, 2013; Sanjta and Chauhan, 2018).

Furthermore, *C. undecimpunctata* (Linnaeus, 1758) was the second dominant species (17.45%) collected from the maize crops. Many other researchers also reported the dominance of this species in various studies worldwide (Kılınçer *et al.*, 2010). The primary reason for their abundance in the agroecosystems could be due to the availability of plentiful prey like aphids. However, this species also feeds on host species like psyllids and small insects (Rahat *et al.*, 2012). The 4th instar and adults (both male and female) are reported to attack equally on leaf aphids and their supremacy leads to the efficacy of pest suppression in field crops (Cabral *et al.*, 2009). In maize crops, *C. undecimpuncta* is reported to be the most abundant during the month of May and the least abundant in June (Mahmoud *et al.*, 2021). *Cheilomenus sexmaculata* (Fabricius, 1781), was the third most abundant species (10.98%) during both cropping seasons and similar results were also reported from other cereal crops in Punjab, Pakistan (Bodlah *et al.*, 2021). This species feeds on aphids including *Rhopalosiphum maidis* (Fitch), *Aphis gossypii* (Glover) and *Lipaphis erysimi* (Kaltenbach). The aphid consumption is highest in 4th instar grubs. The high feeding potential of *Che. sexmaculata* makes it an excellent biological control agent. *Epilachna varivestis* (Mulsant, 1850) was observed during the early tasseling stage of maize crops and it is among the main pest of the maize and bean (Ranum *et al.*, 2014). Moreover, this species exhibited a significant increase in population dynamics, transitioning from maize crops to surrounding weeds. *C. transversalis* (Fabricius, 1781) is a polyphagous coccinellid in agriculture fields of the Oriental regions. It feed on a wide range of aphids (Omkar and Bind, 1993; Navodita *et al.*, 2011).

Variation in the number of coccinellid species was associated with the combined species curves. The results indicated as the sample size increased, the number of coccinellids species also increased. The species accumulation curve for coccinellids across all sites in both districts did not reach an asymptote suggesting there might be more species not yet found. This could be due to different periods of activity for different coccinellid species (Willott, 2001). Around 94–95% of the coccinellid species in the region were identified during the two-year sampling period, while the remaining 5–6% consists of a few uncommon or rare species. These species may not have been recorded during our sampling due to their distinct

activity times (Willott, 2001; Gatti and Carneiro, 2019). Research shown that different species of ladybeetles show their predacious activity during different times of the day due to their varying feeding habits (Siddiqui and Mishra, 2023). This behavior might be due to signals from their prey, which change over the course of the day (Ferreira *et al.*, 2022).

Our study highlights the correlation between ladybeetle abundance and richness with both the local habitat and landscape factors. The landscape design and surrounding habitat highly influence both the composition and diversity of the lady beetles (Egerer *et al.*, 2017). The success of biological pest control with Coccinellidae is intricately linked to the diversity of species within this group (Michaud, 2012). Varied dietary preferences, temporal activity patterns, and habitat adaptations among coccinellid species collectively support pest control efficacy (Kheirodin *et al.*, 2022). A diverse community ensures coverage across different pests, seasons, and locations, as well as mitigates the risk of pest resistance, and enhances the resilience of control efforts against changing environmental conditions (Skendžić *et al.*, 2021).

The crop growth stages and climatic variables like air temperature, humidity level, and precipitation also influence the diversity and richness of coccinellids (Skendžić *et al.*, 2021). Aphidophagous coccinellids face challenges arising directly due to climate changes which may alter prey population (Sloggett, 2021). During the current study, the coccinellid abundance increased from the vegetative stage to the tasseling stage (BBCH-19 to BBCH-65). A rapid decrease was observed at the start of the ripening stage (BBCH-70 to BBCH-89) in both districts. The peak density of coccinellids might be due to the high availability of the aphid population (Obrycki *et al.*, 2009). These results coincide with previous findings that indicate abundance reached its highest point during the vegetative and tasseling stages, but declined during the ripening stage (Svobodová *et al.*, 2015; Pan *et al.*, 2020). Even under high temperature, *C. septempunctata* shows the highest density among the captured coccinellids fauna. Mounting evidence indicates advocated the high abundance of coccinellids even under high temperatures as the maize whorls provide microhabitat refuge to predator lady beetles (Pan *et al.*, 2020). Low temperatures and higher humidity were observed inside the whorl as compared to the outside. Understanding the mechanisms used by natural enemies to look for and capture their prey is essential for the development of successful biological control strategies.

Conclusions and Recommendations

Due to their substantial success in the biological control of various harmful insects especially aphids, coccinellid beetles are considered to hold great economic importance within agroecosystems. The current study aimed to identify and document the coccinellid fauna of the maize crops so that they may be employed for the successful implementation of Integrated Pest Management plans. These outcomes will ultimately pave the way for the development of conservation focused biological strategies for the indigenous plant protection programs. In addition, this research will help to lay the groundwork for a cost effective and eco-friendly pest control strategy suitable for use in developing countries like Pakistan.

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