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SPECIES DIVERSITY AND DISTRIBUTION OF LADYBIRD BEETLES (*COLEOPTERA*: *COCCINELLIDAE*) IN SOUTH WAZIRISTAN, PAKISTAN

Ata Ur Rehman[1*], Abdul Haleem Shah[1], Sanaullah Khan[2], Muhammad Waqas[1], Alamgir Khan[3], Nisar Muhammad [4]

[1*]Institute of Biological Sciences, Gomal University Dera Ismail khan 29050,Pakistan
[2]Institute of Zoological Sciences, University of Peshawar, Peshawar 25120,Pakistan.
[3]Department of Sports Sciences and Physical Education, University of Punjab, Lahore 54590,Pakistan
[4]Department of Environmental Sciences, Gomal University 29050,Pakistan

***Corresponding author:** Dr. Ata Ur Rehman
***E-mail:(**a.rehmanbio92@gmail.com)

ABSTRACT. This study presents the first detailed record of ladybird beetle diversity from South Waziristan, a region previously unexplored due to prolonged geopolitical instability. The present study was designed to identify species richness, distribution patterns across eight selected localities in South Waziristan, Pakistan. An extensive survey was conducted from January to December 2022 in these areas. A total of 530 ladybird beetle specimens were collected from all types of agro-ecosystems using sweep netting and hand-picking methods. Samples were then taken to the laboratory, assigned a unique identification label and the ladybird beetle species were identified using published literature and identification keys. Among the collected specimens, fourteen species were identified, belonging to thirteen genera, three tribes and one subfamily. Ten of these species, viz., *Coccinella septempunctata*, *Hippodamia variegata*, *Cheilomenes sexmaculatus*, *Micraspis allardi*, *Propylea dissecta*, *Adalia bipunctata*, *Psyllobora bisoctonotata*, *Oenopia conglobata* and *Llleis confusa* belonged to the tribe Coccinellini, while three species were *Parexochomus nigripennis*, *Parexochomus pubescens* and *Chilocorus nigrita* classified under the tribe Chilocorini. One species, *Chnootriba elaterii*, represented the tribe Epilachnini within the subfamily Coccinellinae. Among all the species, *Coccinella septempunctata* was found to be widely distributed across all the studied localities, whereas *llleis confusa* was identified as a rare species, present in only a few locations. Sholam Kot exhibited the highest species richness (14), the highest Shannon-Wiener's Diversity Index (H = 2.38), and the highest Margalef's Index (2.88), indicating the greatest diversity among the sites. Kaniguram showed moderate diversity, while Sarwekai had the lowest diversity across multiple indices. Additionally, similarity indices between the localities revealed the highest similarity between Zarmilan and Sholam Kot (0.83) and the lowest similarity between Wana and Tiarza (0.33). This study revealed significantly different species diversity among all sites, with Sholam Kot exhibiting the highest species richness and diversity, characterized by orchard trees, wild vegetation, and agricultural crops. This finding provides baseline data for ladybird beetle diversity in understudied areas and is valuable for ecological conservation and agricultural sustainability planning worldwide.

Key words: Coccinellid beetles; Species Richness; Diversity indices; Agro-ecosystem; Khyber Pakhtunkhwa

Introduction

Ladybird beetles play a crucial role in agricultural ecosystems as natural enemies of invasive pests such as aphids, mealy bugs, sweet potato, whitefly, scale insects as well as larvae of other insect (Veeravel and Baskaran, 1997). Their predatory behavior makes them highly valuable for biological pest control, reducing pesticide usage and improving crop yields in advanced agricultural systems like those in the USA (*Obrycki et al.,* 1998). These beetles also serve as bio-indicators, reflecting ecosystem health through their sensitivity to environmental changes, including pollution, habitat loss, and climate impacts (Koch et al., 2008). However, invasive species of ladybird beetles can threaten native populations and disrupt ecological balance (Ware et al., 2010).

The *Coccinellidae* family, the largest of the *Coccinelloidea* superfamily, with about 6,000 described species (Vandenberg, 2002; Slipinski & Tomaszewska, 2010). They are smaller in size not longer than 4-9 mm than or even as small as 1 mm (Koren *et al*., 2012). Their body structure is round but sometime may be elongate, oval and flattened. Furthermore, color of its body is variable, mostly reddish, black, orange and straw. These species are extremely diverse in their habitat and live in all types of terrestrial ecosystems ranging from temperate regions to tropical zones and even reaching high elevations in mountains areas (Halim *et al.,* 2017).

More than 300 species have been described from the Indo-Pakistan sub-continent (Poorani 2002; Kovář 2007; Rahatullah et al., 2010). In Pakistan, 111 species were reported (Poorani, 2002; Irshad & Khan, 2005; Rafi et al. 2005; Irshad & Haq 2010; Ashfaque, 2013; Hayat & Khan, 2014; Ali et al., 2018;Iqbal etal., 2024a,b). Ashfaque and Farman Ullah (2013) documented 29 Coccinellidae species in Gilgit-Baltistan across four subfamilies. Abbas et al. (2013) reported 12 species in Faisalabad, while Ahmed et al. (2017) identified nine in Sargodha. Ali et al. (2012) recorded 29 species in Sindh, including four new to Pakistan. Khan et al. (2006) found 12 species in Chitral, and Rahatullah et al. (2011) identified 14 in Dir Lower. Rehman et al. (2018) documented seven species in Bannu,

Despite vast exploration of the ladybird beetles in Pakistan, very little attention has been given to the ladybird fauna of southern zone of Khyber Pakhtunkhwa particularly in South Waziristan, due to security challenges which made it a data-deficient area for ecological and entomological survey. This left significant gaps in understanding its diversity, distribution and conservation status of ladybird beetles. This study was thus aimed to explore the ladybird beetles diversity and its distribution in South Waziristan, Pakistan.

Material and Method

Study area

This research was carried out in South Waziristan (Lower & Upper), districts of Dera Ismail Khan Division, enveloped in a mountainous region. Covering an area of 3805 km^2, the district lies between 33ºN latitude and 71ºE longitude. Eight different sampling sites were selected across both districts (Figure 1). In each study area, samples were collected from various agro-ecosystems, including Agricultural crops, Orchard tress, Lower vegetation and wild bushes plants.

Collection and Preservation of ladybird beetles

Ladybird Beetle specimens were collected from January to December 2022 by using sweep netting, and hand-picking across randomly collecting sites. Collections occurred bi-monthly, and the search techniques remained uniform throughout the sampling duration at all study regions. All collected ladybird beetles specimens were killed in a killing jar. To ensure accuracy for subsequent analyses, each specimen was assigned a unique identification label. These labeled specimens were then deposited in a wooden box within the Entomology Laboratory of the Institute of Biological Sciences (ELIBS) at Gomal University, Dera Ismail Khan.

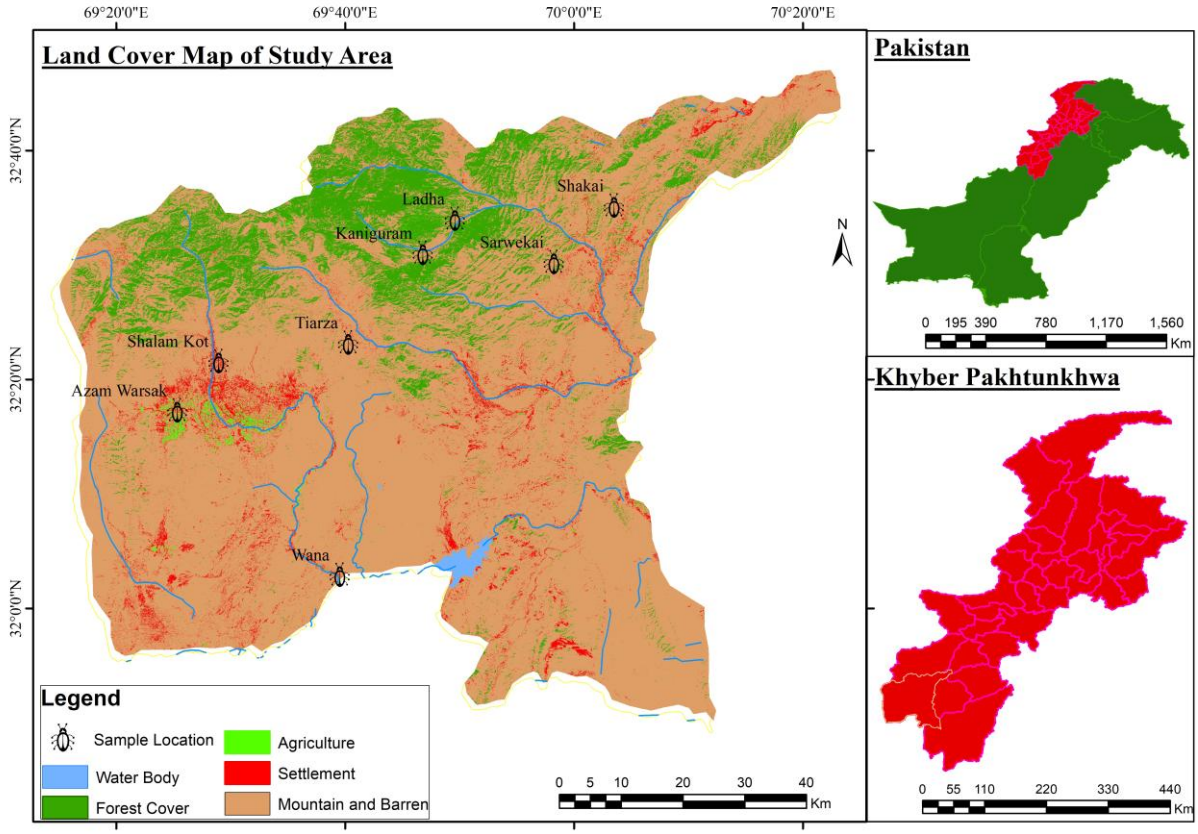

Figure 1. Distribution Map of the study area showing ladybird beetles sampling locations and Key Land Cover Types (Agriculture, Water Bodies, Settlements, Forest Cover, and Mountain/Barren Areas)

Morphological Identification

The morphological characteristics of ladybird beetles were examined using stereo microscopes (Olympus Zoom Stereomicroscope CX23LEDR (S1/S2) Japan). Some specimens of ladybird beetles were dissected for male genitlia for species confirmations. The ladybird beetles species were identified from the published literature and identification keys (Rafi etal.,2005;Hayat etal.,2017;Ahmad etal.,2017;Iqbal etal.,2024a,b)

Statistical analysis

The Alpha diversity indices such as the Simpson diversity index, Shannon diversity index, Margalef index, Shannon Equitability, Evenness, and Similarity indices of the different habitats were computed using PAST version 4.02(Hammer and Harper.2001). An abundance distribution model was used to determine the significance of ladybird beetle species composition from the different habitats during the spring and summer seasons at a 5% probability level (Ojianwuna and Enwemiwe. 2022).

RESULTS AND DISSCUSSION

During the present study, 530 specimens of lady bird beetles were collected from study sites. These specimens were identified into fourteen species belonging to thirteen genera under three tribes and one subfamily (Table S1). A Total of 192 specimens were collected from South Waziristan upper, while 338 specimens were collected South Waziristan Lower from January to December. Among these, the highest number of individuals was found in Wana (131)**,** followed by Kaniguram (90)**,** Zarmilan (90)**,** and Makeen (47) and the lowest number of individuals were recorded in Sarwekai (29) and Tiarza (26)**.** A total of fourteen species were documented from across all the sampling sites. The highest number of species was recorded in Sholam Kot (14) and Wana (13).The lowest number of species was found in Sarwekai (6) and Tiarza (7**)**(Table 1).

Table1. Collective Ranking and List of species Collected from Different Localities in South Waziristan Upper and Lower.

Species	Abundance	South Waziristan Upper				South Waziristan Lower			
		Makeen	Kaniguram	Sarwekai	Tiarza	Wana	Zarmilan	Azam Warsak	Sholam Kot
C. septempunctata	130	14	22	13	08	25	20	10	18
C. undecimpunctata	77	06	15	07	04	17	12	05	11
H. variegate	60	04	10	05	07	13	10	04	07
O. conglobata	27	03	07	0	0	09	03	0	05
P. dissecta	37	0	9	01	0	12	06	02	07
Che. Sexmaculata	39	05	0	0	04	15	9	02	04
M.allardi	21	0	05	01	01	07	04	0	03
P.pubescens	33	01	07	02	01	10	06	01	05
Ch. Nigrita	30	05	05	0	0	0	8	0	12
H. elaterii	08	02	03	0	01	01	0	0	01
A.bipunctata	21	03	02	0	0	05	01	06	04
P. bisoctonotata	33	01	02	0	0	12	8	0	10
P. nigripensis	09	02	03	0	0	03	0	0	01
L. confusa	05	01	0	0	0	02	0	0	02
No. of individual	530	47	90	29	26	131	87	30	90
No. of Species	14	12	12	06	07	13	11	07	14

The highest relative abundance, 189 individuals (35.6%), was recorded in orchard trees, while the lowest abundance, 69 individuals (13%), was observed in lower vegetation (Figure 2).

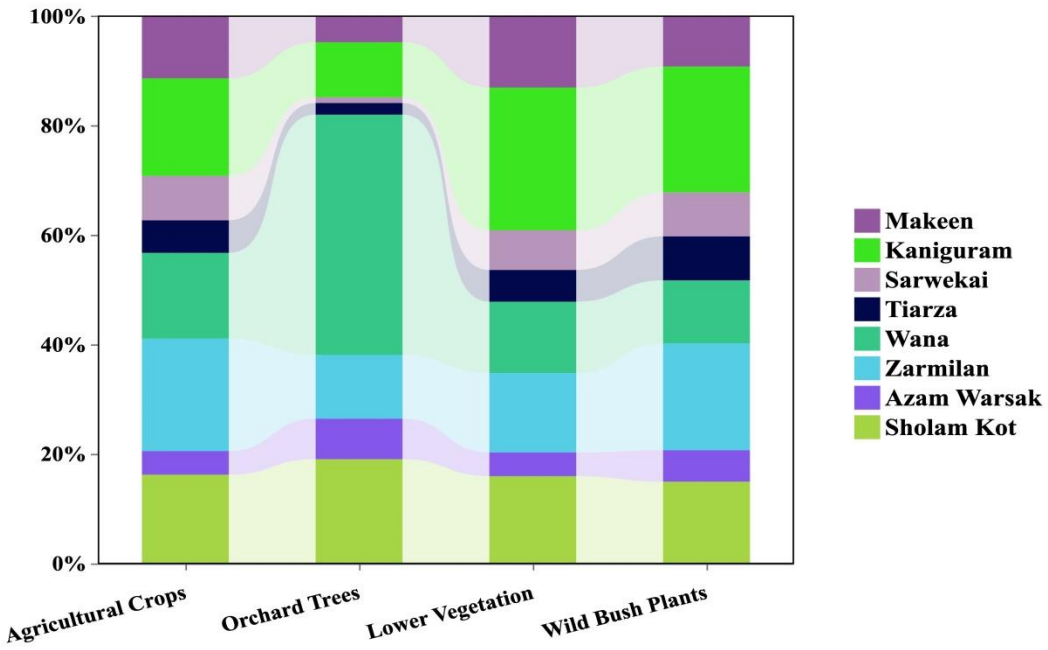

Figure 2. Relative abundance of ladybird beetles collected from different agro-ecosystems across various sampling localities in South Waziristan.

The seasonal collection of various ladybird beetles species from different host plants indicates that most species were recorded between February and October during the study period. Notably, *Coccinella septempunctata*, *Coccinella undecimpunctata*, *Micraspis allardi*, and *Hippodamia variegata* was the most frequently observed species. These species shows distinct preferences with specific host plants like *Triticum aestivum*, *Malus domestica*, *Brassica juncea*, *Citrullus lanatus and Morus spp* being the most preferred (Table 2).

Table 2. Host plant associations of ladybird beetle species in South Waziristan Upper and Lower.

Name of Species	Host plant	Sampling period
Coccinella septempunctata	*Triticum aestivum, Hordeum vulgare, Malus domestica, Brassica juncea, Vitis vinifera,*	March-May
Coccinella undecimpunctata	*Punica granatum, Solanum lycopersicum,Cucumis sativus, Medicago sativa*	March-April
Hippodamia variegate	*Triticum aestivum, Brassica oleracea, Malus domestica, Raphanus sativus, Solanum lycopersicum*	February-May
Oenopia conglobata	*Solanum lycopersicum, Solanum melongena, Daucus carota*	April-July
Propylea dissecta	*Zea mays, Phaseolus vulgaris, Brassica juncea, Brassica rapa, Triticum aestivum*	June-August
Cheilomenes Sexmaculata	*Citrullus lanatus, Abelmoschus esculentus, Solanum lycopersicum, Trichosanthes cucumerina*	March-April
Micraspis allardi	*Brassica juncea, Citrus spp, Malus domestica ,Trichosanthes cucumerina, Medicago sativa*	February-June
Parexochomus pubescens	*Brassica juncea, Brassica oleracea,Brassica rapa ,Solanum tuberosum, Vitis vinifera*	February-April
Chilocorus Nigrita	*Cucumis sativus, Trichosanthes cucumerina , Triticum aestivum,*	March-July
Chnootriba elaterii	*Solanum melongena, Solanum tuberosum, Brassica rapa, Citrullus lanatus, Triticum aestivum*	May-July
Adalia bipunctata	*Malus domestica, Punica granatum, Prunus armeniaca,*	February- October
Psyllobora bisoctonotata	*Lagenaria siceraria, Poaceae spp, Morus alba*	March-July
Parexochomus nigripensis	*Tamarix indica, Poaceae spp*	March-July
llleis confusa	*Morus spp, Parthenium hysterophorus, Malus domestica*	March-April

The relative abundance of each species (Figure 3) shows that the highest abundance was recorded in *Coccinella septempunctata*, with 130 individuals (24.5%), while the lowest was observed in *llleis confusa*, with 5 individuals (0.94%). *Cheilomenes sexmaculata* (7.35%), *Propylea dissecta* (6.98%), and *Parexochomus pubescens* (6.22%) exhibited moderate relative abundances.

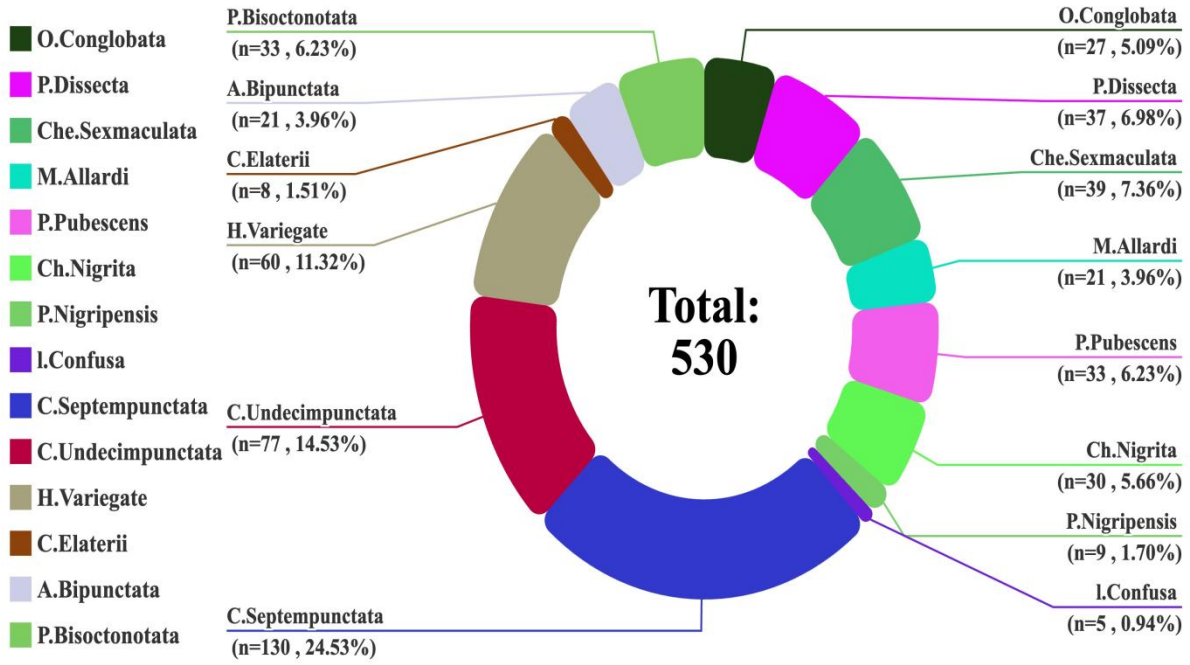

Figure 3. Relative abundance of each species across different localities in the study area.

The collective rank list of ladybird beetles (Table 3) shows that the species *Coccinella septempunctata, Coccinella Undecimpunctata, Hippodamia Variegata* and *Parexochomus pubescens* were found in all the localities representing about 57% of the total individuals collected during the study.

Table 3. The collective rank list of ladybird beetles collected from different localities of the studied area during 2021-2022 (Present=+; absent= -).

Species	Abundance	Makeen	Kaniguram	Sarwekai	Tiarza	Wana	Zarmilan	Azam Warsak	Sholam Kot
Coccinella septempunctata	130	+	+	+	+	+	+	+	+
Coccinella undecimpunctata	77	+	+	+	+	+	+	+	+
Hippodamia variegate	60	+	+	+	+	+	+	+	+
Oenopia conglobata	27	+	+	-	-	+	+	-	+
Propylea dissecta	37	-	+	+	-	+	+	+	+
Cheilomenes Sexmaculata	39	+	-	-	+	+	+	+	+
Micraspis allardi	21	-	+	+	+	+	+	-	+
Parexochomus pubescens	33	+	+	+	+	+	+	+	+
Chilocorus Nigrita	30	+	+	-	-	-	+	-	+
Chnootriba elaterii	08	+	+	-	+	+	-	-	+
Adalia bipunctata	21	+	+	-	-	+	+	+	+
Parexochomus bisoctonotata	33	+	+	-	-	+	+	-	+
Parexochomus nigripensis	09	+	+	-	-	+	-	-	+
llleis confusa	05	+	-	-	-	+	-	-	+
No. of individual	530	47	90	29	26	131	87	30	90
No. of Species	14	12	12	06	07	13	11	07	14

Among the collected genera, *llleis confusa* showed a narrow range of habitat and was captured from three localities (Makeen, Wana and Sholam Kot). Sholam Kot, Wana and Kaniguram localities showed maximum species richness (12-14 species, respectively), while the localities of Sarwekai and Tiarza had the least (6-7 species). *C. septempunctata* was the dominating species in both these localities. Similarity data regarding similarity indices (Table 3) revealed the highest similarity was found between Zarmilan and Sholam Kot (0.83) and the lowest similarity between Wana and Tiarza (0.33) localities. The high similarity between Zarmilan and Sholam Kot is due to their geographical proximity and similar habitat features, such as vegetation type and plant diversity. In contrast, the low similarity between Wana and Tiarza is likely due to significant differences in their ecological characteristics.

Table 3. Similarity indices of ladybird beetle species collected from different localities of South Waziristan.

Sampling localities	Makeen	Kaniguram	Sarwekai	Tiarza	Wana	Zarmilan	Azam Warsak	Sholam Kot
Makeen	1							
Kaniguram	0.58	1						
Sarwekai	0.61	0.47	1					
Tiarza	0.6	0.37	0.7	1				
Wana	0.46	0.75	0.35	0.33	1			
Zarmilan	0.59	0.77	0.48	0.44	0.72	1		
Azam Warsak	0.64	0.4	0.72	0.67	0.36	0.42	1	
Sholam Kot	0.64	0.74	0.47	0.44	0.7	0.83	0.46	1

Alpha diversity indices, used to evaluate species richness and evenness of ladybird beetles fauna among studied localities (Table 4), show that Sholam Kot exhibited the highest values of Evenness (e=0.77), Shannon (H = 2.38), Simpson index (1-D=0.89) and Margalef's (2.88) diversity indices, while Sarwekai and Tiarza had the lowest diversity indices. Collectively, these results suggest that ladybird beetles are fairly evenly distributed across the selected localities in the South Waziristan districts, as indicated by the Shannon Equitability values ranging from 0.79 to 0.92 (Table 4).

Table 4. Alpha diversity indices of different ladybird beetles species collected from different localities of the South Waziristan districts.

Indices	Makeen	Kaniguram	Sarwekai	Tiarza	Wana	Zarmilan	Azam Warsak	Sholam Kot
Simpson(1-D)	0.85	0.86	0.7	0.78	0.89	0.87	0.79	0.89
Shannon(H)	2.17	2.23	1.42	1.66	2.34	2.21	1.73	2.38
Evenness (e)	0.73	0.77	0.69	0.75	0.8	0.82	0.8	0.77
Margalef's Index	2.85	2.44	1.48	1.84	2.46	2.23	1.76	2.88
Shannon Equitability(J')	0.87	0.89	0.79	0.85	0.91	0.92	0.88	0.9

This study emphasizes the diversity of ladybird beetles in various agro-ecosystems across South Waziristan Upper and Lower. The identification of 14 species and 13 genera highlights the significant species richness in the region. Earlier studies have reported similar findings, such as Gilani (1976), who documented ladybird beetle in Faisalabad, Pakistan. Shah (1983) described the geographical distribution of 16 species of ladybird beetles, along with their host plants, in Peshawar Valley. Din (2002) also recorded comparable species in Chitral. Furthermore, Khan *et al.* (2007) conducted a survey of predatory ladybird beetle in District Chitral, identifying 12 species.

In cultivated fields, continuous cropping occurs throughout the year. Through continuous cultivation, food availability increases, which leads to higher pest populations and, consequently, more ladybird beetles (Hayat *et al.,* 2014).Our results showed variation in ladybird beetle populations, with peak numbers recorded during March-April. Previous studies also identified these months as favorable for ladybird beetles (Abbas *et al.,* 2013; Hussain *et al.,* 2018). Higher abundance was observed on agricultural crops and orchard trees, which hosted more species and greater numbers of ladybird beetles compared to lower vegetation and wild bushes. The results showed *Coccinella septempunctata*, *Coccinella undecimpunctata*, and *Hippodamia variegata* as the most abundant and dominant ladybird beetle species across all the localities in South Waziristan upper and Lower. Similar abundance and dominance patterns were reported in Pakistan (Rahatullah *etal.,* 2011; Hussain *etal.,* 2018). Studies from Khyber Pakhtunkhwa (Swabi, Mardan, and Nowshera) also confirmed these trends in species diversity, dominance, and richness (Urooj and Ali, 2016).

CONCLUSION

Present study is the first of this type of study on the diversity and distribution of ladybird beetles in this region, which previously remain unexplored. A total of 14 species were identified, belonging to 13 genera, three tribes, and one subfamily. Margalef's Index values, indicating species richness, revealed that the highest number of species was recorded around Sholam Kot and Makeen, areas characterized by orchard trees, wild vegetation, and agricultural crops. While Sarwekai and Tiarza showed minimum species richness. A comprehensive survey should be conducted in inaccessible and under-sampled areas, as well as across different seasons, to gain a deeper understanding of the ecological roles of ladybird beetles. Such efforts could also increase the chances of discovering novel species in this underexplored region, ultimately contributing to the development of conservation-based bio-control strategies

Author Contributions: Conceptualization, A.R.; Methodology, S.U.K; Software, A.R.; Formal Analysis, A.R.; Data Archiving, A.K; Writing—Original Draft Preparation, A.R.; Writing—Review & Editing, M.N.K, and M.W; Supervision, A.H.S. and S.U.K

Data Availability Statement: The authors confirm that the data supporting this study are accessible. Raw data and additional information can be obtained from the corresponding author.

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