BJNRD (2024), 11(2): 43-56
Bhutan Journal of Natural Resources & Development

Research Article

www.bjnrd.org

Open Access

ISSN 2409–2797 (Print)
ISSN 2409–5273 (Online)

DOI: https://doi.org/10.17102/cnr.2024.100

Diversity and Distribution of Ladybird Beetles (Coleoptera: Coccinellidae) under Different Habitats in Zhemgang, Bhutan

Jigme Wangchuk[1], Wangchuk Blon[1*] Dorji Phuntsho[1], Karma Tenzin[1], Cheten Dorji[2]

Abstract

The diversity of ladybird beetles is of great importance due to their worldwide utilization as biological control and indicator. The present study was conducted to evaluate the diversity and distribution of ladybird beetles in three different habitats and regions of Nangkor Gewog under Zhemgang district. The stratified line transect sampling technique was employed for the collection of specimens using sweep net and beating tray. A total of 13 species under three tribes with 402 individuals were recorded. Widely found and used biological control species, *Coccinella septempunctata* was the most abundant species found in the study area with its relative abundance of 34.33% when compared to other species. Overall rank distribution based on relative abundance showed that 15 species distributed within 14 ranks. The study area had an overall diversity, $H' = 2.04$, $D_{mg} = 2.33$ and $J = 0.75$. A series of diversity comparisons across different regions within the study area, including habitats and habits showed variable species diversity and host plant preferences. As anticipated, the forest area harboured the most diverse ladybird beetle species, with *Brassica juncea* being the most favoured plant, hosting 84 individuals. The present study offers insights into the ladybird beetle diversity, that enhance our understanding and potential future use of these insects as a biological control.

Keywords: Diversity, abundance , ladybird beetle, ladybug, Coccinellidae, Bhutan

Introduction

Coccinellidae (ladybirds or ladybugs) is a well-known, abundant and diversified coleopteran family comprising of about 6000 described species worldwide (Vandenberg, 2002; Ali et al., 2018) placed in 370 different genera (Ślipiński & Tomaszewska, 2010). After the reclassification of several coccinellid subfamilies to tribe level and the consolidation of multiple tribes, the family now comprises two subfamilies and 38 tribes (Vandenberg, 2002; Seago et al., 2011; Szawaryn et al., 2020). Currently, Bhutan has 96 recorded species (Fig. 1) of which nine species and two sub-species are endemic (Dorji, Loday and Vorst 2019, Dorji, Lhamo & Wangchuk, 2021).

Ladybird beetles are amongst the most visible and best known beneficial predatory insects, except for the tribe Epilachnini, which is phytophagous in nature (Biranvand et al., 2018). They are often regarded as the farmers' friends by many agriculturists as they play a vital role in natural biological pest control. Their economic importance in

[1]Department of Forest and Park Services, Ministry of Energy and Natural Resources
[2]Department of Forest Science, College of Natural Resources, Royal University of Bhutan
*Corresponding author: wblon@moenr.gov.bt
Received: November 12, 2024
Accepted: December 16, 2024
Published online: December 31, 2024
Editor: Ugyen Dorji

agriculture is further emphasized by the fact that both adults and larvae feed on soft-bodied herbivorous pests like aphids, leafhoppers, mealybugs, and mites, helping regulate nuisance pests and reduce crop damage (Poorani, 2002; Albaaj, 2017). However, Epilachnini are the most serious crop pests within the family causing significant damages to the agricultural crops around the world especially *Henosepilachna vigintioctopunctata* and *H. chrysomelina* in eastern and central Asia (Davidson & Evans, 2010; Tomaszewska & Szawaryn, 2016).

Recognizing the ecological and agricultural benefits of ladybird beetles, it is crucial to deepen our understanding of these insects. However, research on their diversity and economic importance remains limited, particularly in Bhutan, where they are among the least-studied insect groups. To address this gap, we conducted a study in remote villages of Nangkor gewog under Zhemgang district. Primarily, we aimed to document ladybird beetle diversity and investigate distribution across different habitats and host plants by using diversity indices statistical tools.

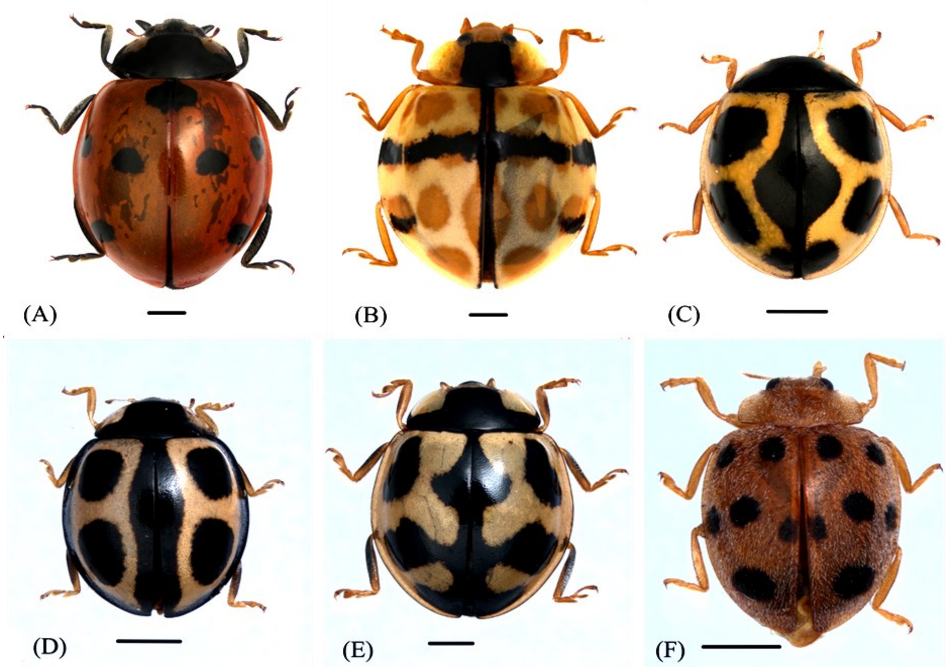

Figure 1: Some representative ladybird beetles of Bhutan. *Coccinella septempunctata* (A); *Harmonia eucharis* (B); *Oenopia mimica* (C); *Oenopia kirbyi* (E); *Propylea luteopustulata* and *Afidentula manderstjernae* (F). (Scale:1mm)

Materials and Methods

Study area

The study was conducted in the remote Nangkor Gewog of Zhemgang district, covering an area of 494 sq. km at an elevation ranging from about 1100 m to 2000 m asl (Fig. 2). This region experiences a tropical to temperate climate, with the moderate weather characterized by summer temperature reaching up to 24°C and in winter ranging from 2°C to 14°C. The area receives an average annual rainfall between 900 mm and 1200 mm.

The landscape is predominantly covered by dense subtropical broad-leaved forests (approximately 90%), interspersed with fruit orchards and agricultural lands. For sampling purposes, the study area was divided into three regions: Kikhar, Tali, and Buli. A wide variety of habitats were selected to ensure a comprehensive assessment of ladybird beetle diversity, including grassy fields, croplands, orchards, residential areas, lawns, grazing fields, bushy areas, forest with tall trees, and mountain peaks (Fig. 2). These diverse habitats and land use types within the study area make it an ideal location for evaluating the diversity and ecological associations of ladybird beetle fauna.

Sampling

The samples were collected between January and March by visiting each region on a bi-weekly basis. Based on convenience, either sweep net or beating tray, and direct hand-picking methods were used for the collection of samples. Every collection was followed by detail recording of habits of hosts along with the host species, coordinates, slopes and elevation.

The stratified line transects sampling technique, incorporating the Pollard Walk method (Pollard, 1977) was employed. Three sampling regions were stratified into three different habitats, namely, forests, meadows and agricultural lands. Further, the habitats were stratified into three sampling habits, namely, agricultural crops, herbs and shrubs, and trees. 300 m transects was laid in each of these habitat type (Ramesh et al., 2010). Samples were observed and collected within a five-meter width on either side of the transect line, as well as within five-meters in front (Mayur et al., 2013). To ensure their preservation, all samples were initially stored in the field using 70–100% ethanol and were later identified using literature but not limited to Bielawski (1979); Poorani, (2002); Dorji et al (2017); Dorji, et al. (2019). Currently, all the specimens studied are stationed in the collection of College of Natural Resources, Royal University of Bhutan.

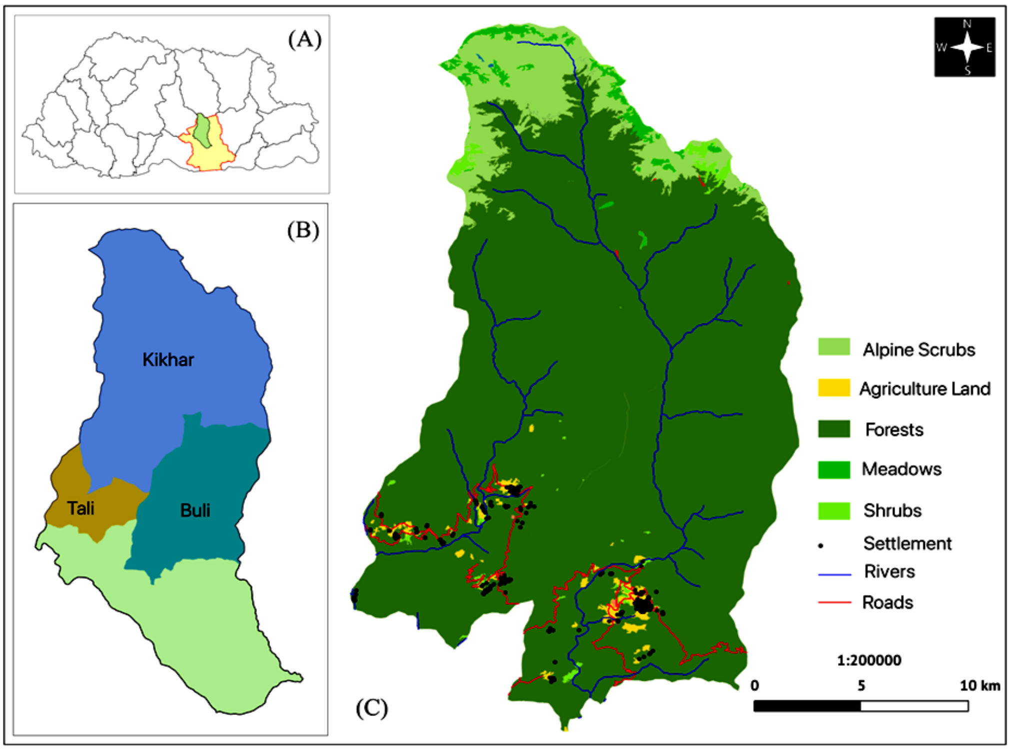

Figure 2: A: Bhutan map showing Zhemgang district (yellow fill) with Nangkor geog (green fill), B: Nangkor geog with three regions, C: Main study map with different habitats of ladybird beetles.

Data analysis

The statistical analyses were performed to infer species diversity, abundance, and similarity in different habitats and different ladybird beetle communities. The Shannon's Diversity (H′) = -∑ *Pi x Ln (Pi)*, Relative Abundance (RA) = *ni / N x 100,* Margalef's Richness Index (D_{mg}) = *(S - 1) / Ln (N),* Pielou's Evenness Index (J) = *H′ / H_{max},* and Berger-Parker Dominance (D_{BP}) = *Pi_{max}* were used, where *Pi* = RA of each species, or proportion, *ni* = Number of individuals in species, i, *N* = Total number of individuals of all species, and *Ln* is a natural log and ∑ is a summation of the calculation, S = Total number of species, N = Total number of individuals of all species, $H_{max} = Ln (S)$. Sorensen's Similarity (QS) = $2 J / (a + b)$, where J = Number of similar species in both communities; a = Total number of species in community, A, b = Total number of species in community, B, was used to find the similarity between two communities.

Other statistical tools

The cluster analysis was performed to find the ladybird beetle assemblages among different sampling habitats, sampling habits and sampling regions, using Sorenson and Bray-Curtis similarity index, using PAST ver. 3. The test for normality was conducted using Shapiro-Wilk's W test in Statistical Package for the Social Sciences (SPSS) ver. 25, followed by Kruskal-Wallis test to check the differences in species diversity and Spearman's correlation was done to check the correlation between the ladybird beetles abundance and host species.

Results and Discussion

Species composition

A total of 402 individuals were recorded across different habitats in three sampling regions of Nangkor Gewog. Currently, Bhutan harbours around 96 species (Dorji et al.,2017), of which 13 species under 10 genera were resampled from the study area (Table 1). Among the taxa observed, the tribe Coccinellini (n = 378, RA = 94.03%) were the most abundant and observed in all the sampling regions, followed by Epilachnini (n = 20, RA = 4.98%). Chilocorini (n = 1, RA = 1.00%) was found to be the least abundant of the three sub-families of ladybird beetles in the study areas (Fig. 3A). These findings align with similar studies conducted in Pakistan by Abbas et al. (2013) and in India by Murali et al. (2017), where Coccinellinae (now classified under the tribe *Coccinellini*) was identified as the most abundant sub-family, representing 79.83% and 75.27% of observed individuals, respectively.

Table 1: Relative abundance (RA in percentage) of 13 species of ladybird beetles in the study area represented by their count

Species	Count	RA
Brumoides daldorfi (Crotch, 1874)	4	1
Calvia sykesii (Crotch, 1874)	7	1.74
Coccinella septempunctata Linnae-	138	34.33
C. transversalis Fabricius, 1781	72	17.91
Harmonia eucharis (Mulsant, 1853)	3	0.75
Illeis sp.	2	0.5
Oenopia kirbyi Mulsant,1850	51	12.69
O. mimica Weise, 1902	15	3.73
O. sauzeti Mulsant, 1866	36	8.96
O. sexareata (Mulsant, 1853)	35	8.71
Propylea dissecta (Mulsant, 1850)	8	1.99
Micraspis discolor (Fabricius, 1798)	11	2.74
Henosepilachna vigintioctopunctata (Fabricius, 1775)	6	1.49
H. septima (Dieke, 1947)	11	2.74
Epilachna sp.	3	0.75
	402	100

Species composition under different habitats

Agricultural fields had the highest relative abundance of ladybird beetle (n = 187, RA = 46.52%), followed by meadows (n = 136, RA = 33.83%), and forests (n = 79, RA = 19.63%), which had the lowest abundance (Fig. 3B). The higher abundance in agricultural crops is due to the presence of generalist feeders, such as *Coccinella septempunctata* and *C. transversalis*, which were particularly prevalent in these fields. This finding also aligns with those of Ahmed et al. (2017), who also observed C. *septempunctata* and *C. transversalis* as abundant in agricultural ecosystems and grasslands. During our study, these species were observed to be the most common and predominant in agricultural fields, resulting in high relative abundance. Similarly, Sharma et al. (2017) reported that the agricultural ecosystems had a higher relative abundance of ladybird beetle compared to forest ecosystems. In the study area, *C. septempunctata* and *C. transversalis* demonstrated notable adaptability and robustness across all habitats. This observation is consistent with the findings of Abbas et al. (2013), who highlighted the ability of these species to thrive in diverse environments, showing their ecological versatility.

Sharma et al. (2017) further emphasized that they are predominantly predatory in nature, depending on the agricultural pests and even resorting to cannibalism in absence of their prey. However, cannibalism was not assessed in this study.

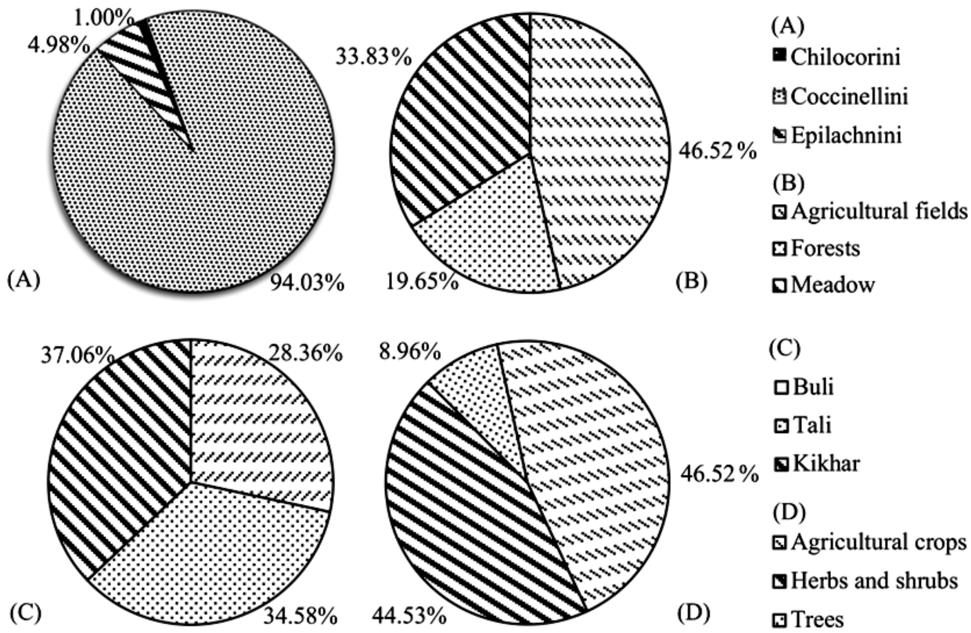

Figure 3: Pie chart showing relative abundance (RA) of tribes of Coccinellidae (A), species composition under different habitats (B), sampling regions (C), different host habits (D)

Species composition under different host habits

The pie chart (Fig. 3D) shows that the agricultural crops had the highest abundance of ladybird beetles (*n* = 187, *RA* = 46.52%) compared to herbs and shrubs (*n* = 179, *RA* = 44.53%), and trees (*n* = 36, *RA* = 8.96%). This higher abundance in agricultural crops may be subjected to availability of food resources and the prevalence of generalist feeders. Sharma et al. (2015) claimed that most of the ladybird beetle species present in the agricultural crops are either generalist predators feeding on the aphids and other soft-bodied insects or phytophagous species dependent on the agricultural crops such as Brassicaceae and Solanaceae.

Species composition under different sampling regions

Figure 3C illustrates that Kikhar (*n* = 149, *RA* = 37.06%) had a higher species abundance than Tali (*n* = 139, *RA* = 34.58%) and Buli (*n* = 114, *RA* = 28.36%). This variation could be potentially attributed by the differences in elevation and temperature among the sampled regions. Kikhar being located at a lower elevation (1,500 m asl) experiences higher temperature compared to Buli and Tali, which are situated between 1,650 m asl. to 1,936 m asl. As temperature decreases with increasing elevation, Kikhar's warmer conditions likely contributed to its greater species abundance.

Species ranking

The relative abundance ranking of 13 ladybird beetle species showed 14 distinct ranks (Fig. 4). *Coccinella septempunctata* had the highest abundance (*n* = 138, *RA* = 34.33%), followed by *C. transversalis* (*n* = 72, *RA* = 17.91%), and *Oenopia kirbyi* (*n* = 51, *RA* = 12.69%), which were ranked 1st, 2nd, and 3rd respectively. Unipal and Mathur (1998) recorded *C. septempunctata* over the snow surface at an elevation of 3,700 m, highlighting its ability to withstand harsh climatic conditions. Similarly, it is noted that the high abundance of *C. septempunctata*, *C. transversalis* and *O. kirbyi* is unsurprising being generalist in their feeding habits and ability to maintain stable population throughout the year (Majunder et al., 2013; Hayat et al., 2017). The remaining 12 species ranked between 4th and 14th, together accounted for about 35.10% of the total abundance. *Epilachna* sp. and *Harmonia eucharis* shared the 13th rank due to their identical relative abundance (*RA* = 0.75%).

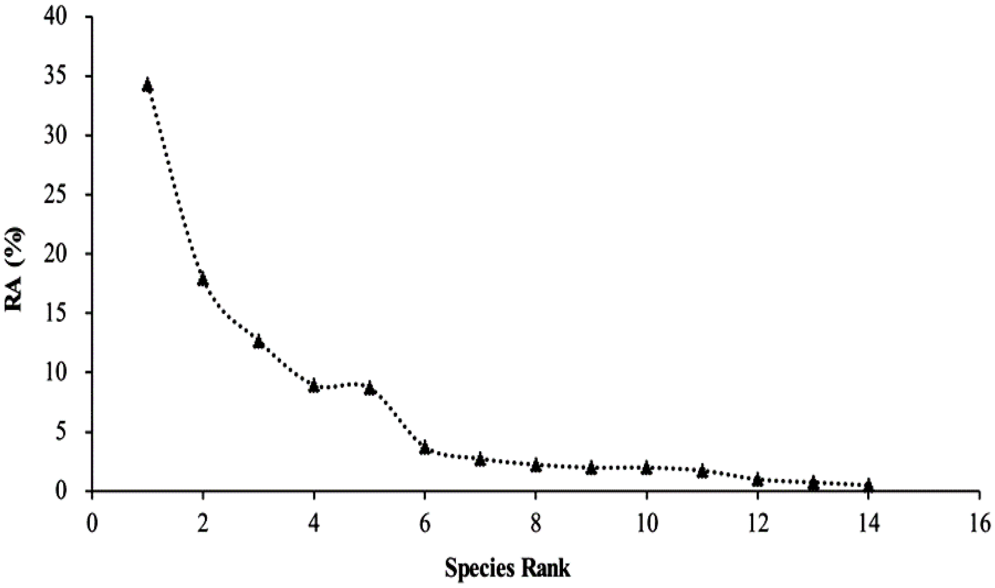

Figure 4: Rank abundance (RA) scatter graph showing the ladybird beetle distribution in the study area

Overall diversity of ladybird beetles

The diversity was computed based on three criteria: habitat types, sampling regions, and sampling habits. Additionally, the overall diversity of the study area was also calculated. Coccinella septempunctata dominated the study area (D_{BP} = 0.34). The overall diversity indices for the study area were: diversity (*H′* = 2.04), richness (D_{mg} = 2.33), and evenness (*J* = 0.75), indicating a diverse and evenly distributed ladybird beetle community.

Diversity under different sampling regions

The specimens collected from different sampled regions exhibited variations in the diversity (H′), richness (D_{mg}) and evenness (J) of ladybird beetle (Fig. 5). However, the Kruskal Wallis test showed no significant difference in diversity of ladybird beetles among different habitats, X^2 (2) = 2.107, p = .349, with mean diversity rank of 26.20, 34.10 and 31.20 for Buli, Tali and Kikhar, respectively. Buli exhibited the highest diversity indices (H′ = 2.06, D_{mg} = 2.53, and J = 0.80) followed by Kikhar (H′ = 1.95, D_{mg} = 2.00, and J = 0.81), and then Tali (H′ = 1.88, D_{mg} = 2.03, and J = 0.78). The species were more evenly distributed in Kikhar than in the other two regions. Overall, these results suggest a fairly even distribution across the sampling regions.

Diversity under different forest structure (host habits)

Figure 5B shows some differences in the diversity of ladybird beetles under different host habits. However, the Kruskal Wallis test revealed no significant difference in diversity among different habitats, X^2 (2) = 1.470, p = .479, with mean diversity rank of 27.45, 30.85 and 36.25 for agricultural crops, herbs and shrubs, and trees respectively. Herbs and shrubs have the highest diversity indices (H' = 2.10, D_{mg} = 2.51, J = 0.79) of ladybird beetles, followed by trees (H' = 2.03, D_{mg} = 2.51, and J = 0.88), then agricultural crops (H' = 1.41, D_{mg} = 1.91 and J = 0.59). The herbs and shrubs harboured a greater diversity owing to a diverse winter hosts compared to the other types of host habits. Sushko (2017) reported similar findings, noting that the habitats covered with herbs and shrubs tended to support a greater diversity. While species are fairly homogeneous across all host habitats, agricultural crops showed lower evenness, likely because they support fewer species in abundance.

Diversity under different habitats

The diversity, dominance, richness and evenness also vary among the habitats (Fig. 5C). The Kruskal-Wallis test showed a significant difference in diversity across different habitats, X^2 (2) = 10.18, p = .006, with mean diversity rank of 33.13, 37.68 and 20.70 for agricultural fields, meadows and forests, respectively.

Forests had a greater diversity indices (H' = 2.11, D_{mg} = 2.52, and J = 0.85), followed by meadows (H' = 1.90, D_{mg} = 2.44, and J = 0.74), and then the agricultural field (H' = 1.41, D_{mg} = 1.91, and J = 0.59). In all cases, forests provided the most suitable habitat for ladybird beetles, due to the natural habitats that they offer, in contrast to the agricultural fields and meadows, which are more frequently disturbed by the human interferences. Majumder et al. (2013) and Egerer et al. (2016) noted that like many other coleoptera, inhabit the natural habitats with minimal human interferences. Similarly, Grez and Alaniz (2019) reported that urbanisation affects their abundance and richness, emphasizing undisturbed nature supports more diverse communities.

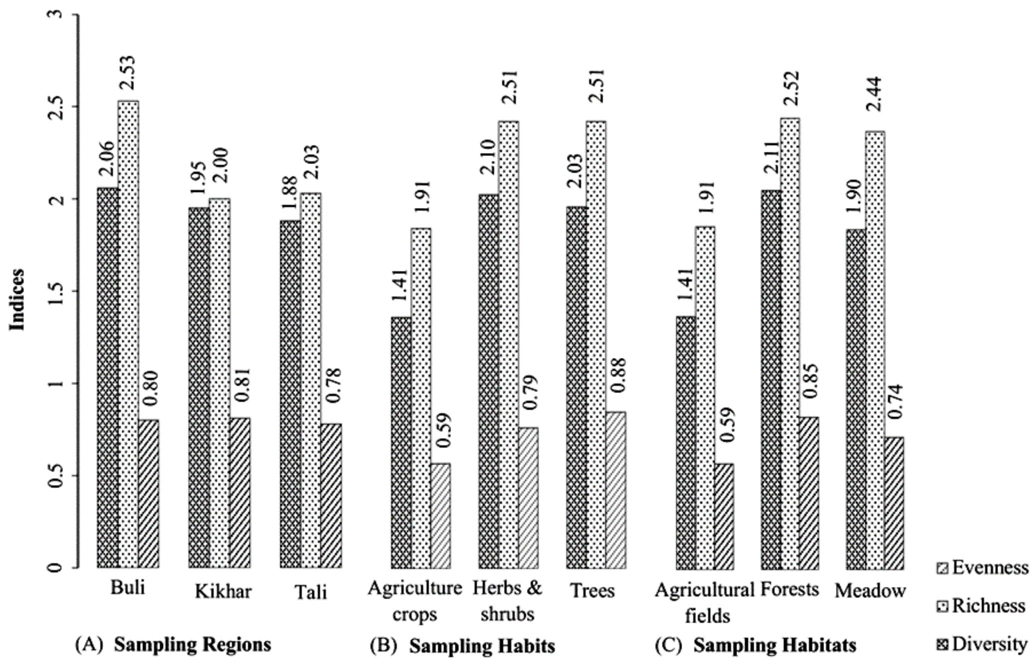

Figure 5. Bar graphs showing species diversity, richness and evenness under different sampling regions (A), sampling habits (B) and Sampling habitats (C)

Beta diversity

Sorenson similarity (*QS*) was deployed to assess the beta diversity between sampling habitats, sampling regions and forest structure (host habits). The Sorenson (*F1*) scores revealed the highest similarity index between agricultural fields and meadows (*F1* = 0.83) while agricultural fields and forests exhibited the lowest similarity index (*F1* = 0.78). Meadows and forests shared a moderate similarity index (*F1* = 0.80), suggesting a higher overlap of species between these two habitats compared to agricultural fields and forests (Fig. 6A). This is due to the proximity of these communities, which facilitates the sharing of similar species which align with the observations of Burns and Strauss (2011). The cluster analysis performed using Sorenson and Bray-Curtis indices with group average linkage method further showed that forest and agricultural fields support a richer assemblage of ladybird beetles compared to meadows.

The *F1* scores indicate that herbs and shrubs exhibited the highest similarity index with agricultural crops (*F1* = 0.88) whereas agricultural crops and trees displayed the lowest similarity index (*F1* = 0.67). Trees and herbs and shrubs showed an intermediate similarity (*F1* -= 0.75) (Fig. 6B). The cluster analysis further revealed a notable similarity in ladybird beetle assemblages between herbs, shrubs and agricultural crops. In contrast, trees showed the least similarity with other host habits. However, a closer relationship was observed between trees, herbs and shrubs compared to the similarity between trees and agricultural crops. The *F1* scores between Buli and Tali exhibited the highest similarity index (*F1* = 0.92) whereas Buli and Kikhar showed the lowest similarity index (*F1* = 0.75) (Fig. 6C). These observations align with findings of Ceryngier (2015), who emphasized the significant role of temperature in influencing the abundance of similar species, thereby stating that the communities experiencing comparable temperatures support a greater overlap of these insects. This holds true as Buli and Tali share a similar elevation range (1,600–1,900 m asl) and comparable temperature conditions, which likely contributed to their high species similarity. In contrast, Kikhar is relatively warmer, resulting in a distinct coccinellid assemblage. Besides, the two areas had a similar temperature. The cluster analysis further confirmed the similarities in their assemblages between Buli and Tali, while Kikhar demonstrated the lowest similarity with the other regions.

Host species preferred by the ladybird beetles

A total of 22 species with 151 individuals were recorded as the host plant species from the study area (Table. 3). Among these species, *Ageratina adenophora* (*n* = 22, RA = 14.57 %) was the most abundant species, followed by *Artemisia myriantha* (*n* = 18, RA = 11.92 %) and *Brassica juncea* (*n* = 16, RA = 10.60 %). Out of 22 host species recorded (Fig. 7), *B. juncea* was the most preferred host species with 84 individuals of ladybird beetles inhabiting on it, followed by *A. adenophora* (*n* = 56) and *Artemisia myriantha* (*n* = 38). *Psidium guajava* was the least preferred host species, supporting only two individuals. The variation in host preferences among different species of ladybird possibly attributed to their differences in habitat requirements. Some species are habitat generalists – thriving across diverse habitats, while others are habitat specialists with more specific ecological needs (Rasheed & Buhroo, 2018). Furthermore, studies by Rahatullah et al. (2012) and Vanderycken et al. (2013) emphasized that host selection is closely linked to dietary preferences, with different ladybird beetle species requiring specific host plants based on the availability and type of food resources.

In the study area, *Coccinella septempunctata*, *C. transversalis* and *Oenopia kirbyi* were identified as the habitat generalists, capable of thriving on a broad range of habitats. On the other hand, *Epilachna cuonaensis, Harmonia eucharis* and *Illeis* sp. were classified as the habitat specialists, restricted to a narrower habitat range. Similar finding was made by

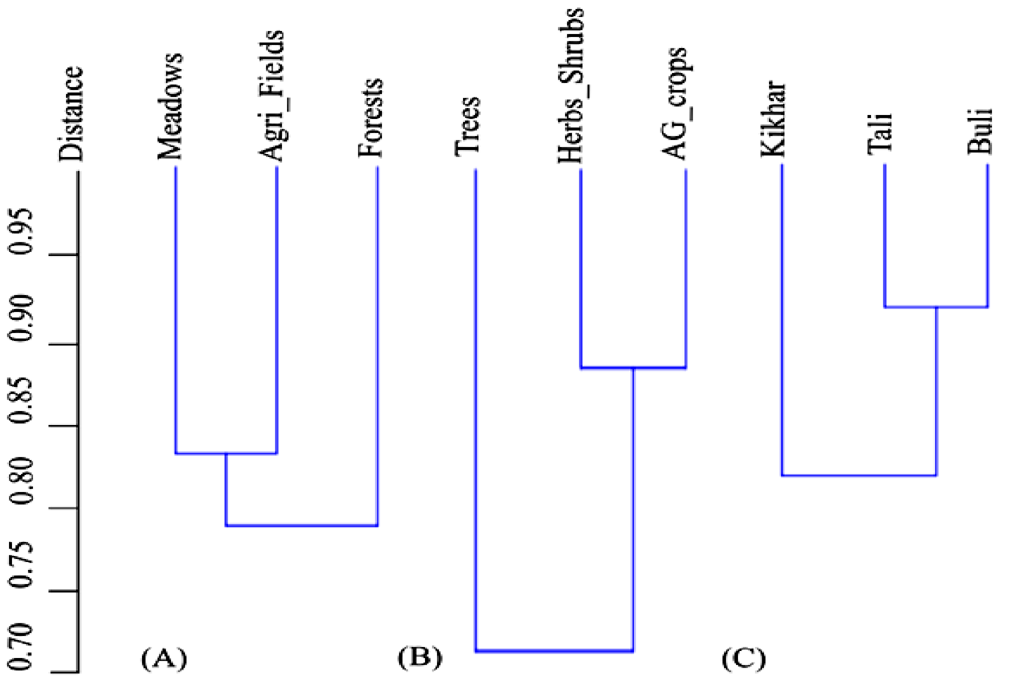

Figure 6: Cluster dendrogram showing similarities between sampling habitats (A), host species (B) and sampling regions (C).

Table 2: Distribution of ladybird beetles under different sampling regions, different habitats and different sampling habits.

Species	Count	Sampling Regions			Habitat Types			Host Habits		
		Buli	Tali	Kikhar	AGF	MD	FO	AGC	Herbs-Shrubs	Trees
Brumoides daldorfi (Crotch, 1874)	4	+	+	+	-	+	+	-	+	+
Calvia sykesii (Crotch, 1874)	7	+	+	-	+	+	+	+	+	-
Coccinella septempunctata Linnaeus, 1758	138	+	+	+	+	+	+	+	+	+
C. transversalis Fabricius, 1781	72	+	+	+	+	+	+	+	+	+
Harmonia eucharis (Mulsant, 1853)	3	+	-	-	-	-	+	-	-	+
Henosepilachna septima (Dieke, 1947)	11	+	+	+	+	+	+	+	+	-
H. vigintioctopunctata (Fabricius, 1775)	6	+	+	+	+	-	+	+	+	+
Illeis sp. Timberlake, 1943	2	+	-	-	+	+	-	+	+	-
Oenopia kirbyi Mulsant,1850	51	+	+	+	+	+	+	+	+	+
O. mimica Weise, 1902	15	+	+	+	-	+	+	-	+	+
O. sauzeti Mulsant, 1866	36	+	+	+	+	+	+	+	+	+
O. sexareata (Mulsant, 1853)	35	+	+	+	+	+	+	+	+	+
Propylea dissecta (Mulsant, 1850)	8	+	+	-	+	+	+	+	+	+
Micraspis discolor (Fabricius, 1798)	11	-	-	+	+	+	-	+	+	-
Epilachna sp.	3	-	-	+	-	+	-	-	+	-
Total no. of individuals	402	114	139	149	187	136	79	187	179	36
Total no. of species	15	13	11	11	11	13	12	11	14	10

Table 3. Checklist of ladybird beetles and host plant species recorded in the study area. List of plant species is based on the presence of ladybird beetles during the time of field visit.

Ladybird beetle species	Host species
Brumoides daldorfi	*Artemisia myriantha, Schima khasiana, Symplocos lucida*
Calvia sykesii	*Ageratina adenophora, Artemisia myriantha, Brassica juncea, Citrus reticulata, Psidium guajava, Spinacia oleracea*
Coccinella septempunctata	*Ageratina adenophora, Ageratum conyzoides, Artemisia myriantha, Brassica juncea, B. oleracea, Edgeworthia gardneri, Elettaria cardamomum, Persia americana, Poaceae, Pteridophytes, Raphanus sativa, Solanum esculentum, S. lycopersicum, S. tuberosum, Spinacia oleracea, Symplocos lucida,*
C. transversalis	*Ageratum conyzoides, Artemisia myriantha, Brassica juncea, B. oleracea, Edgeworthia gardneri, Elettaria cardamomum, Raphanus sativa, Spinacia oleracea, Symplocos lucida, Solanum esculentum, S. tuberosum*
Epilachna sp.	*Ageratum conyzoides, Artemisia myriantha, Pteridophytes*
Harmonia eucharis	*Symplocos lucid, Schima khasiana, S. wallichii*
Henosepilachna septima	*Solanum tuberosum, Ageratina adenophora, Brassica oleracea, Edgeworthia gardneri, Pteridophytes, Solanum esculentum*
H. vigintioctopunctata	*Brassica oleracea, Persia americana, Psidium guajava, Schima wallichii, Solanum esculentum, Artemisia myriantha*
Illeis sp.	*Artemisia myriantha, Brassica juncea*
Micraspis discolor	*Ageratina adenophora, Ageratum conyzoides, Brassica juncea, Solanum tuberosum*
Oenopia kirbyi	*Ageratina adenophora, Ageratum conyzoides, Artemisia myriantha, Daphne bholua, Edgeworthia gardneri, Elettaria cardamomum, Poaceae, Schima khasiana, S. wallichii, Symplocos lucida*
O. mimica	*Artemisia myriantha, Daphne bholua, Engelhardia spicata, Pteridophytes*
O. sauzeti	*Ageratina adenophora, Ageratum conyzoides, Artemisia myriantha, Brassica juncea, Edgeworthia gardneri, Pteridophytes, Symplocos lucida,*
O. sexareata	*Ageratina adenophora, Artemisia myriantha, Edgeworthia gardneri, Engelhardia spicata, Persia americana, Poaceae, Pteridophytes, Symplocos lucida*
Propyla dissecta	*Citrus reticulata, Poaceae, Pteridophytes, Schima wallichii*

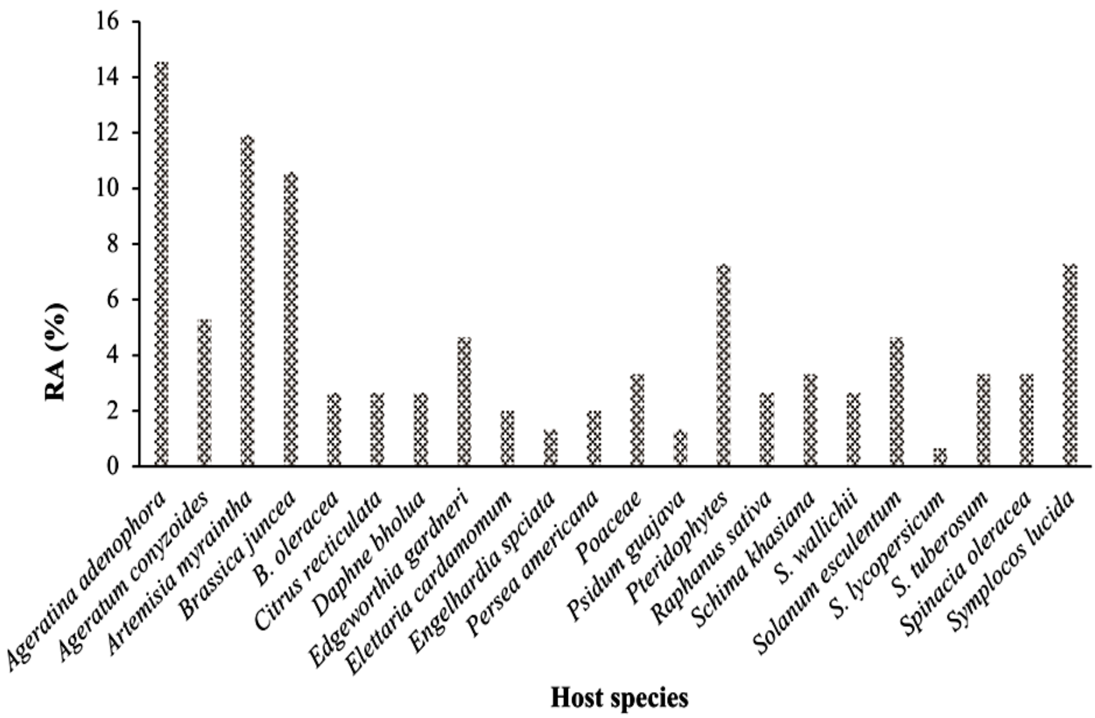

Figure 7: Bar graph showing the relative abundance of host species of ladybird beetles

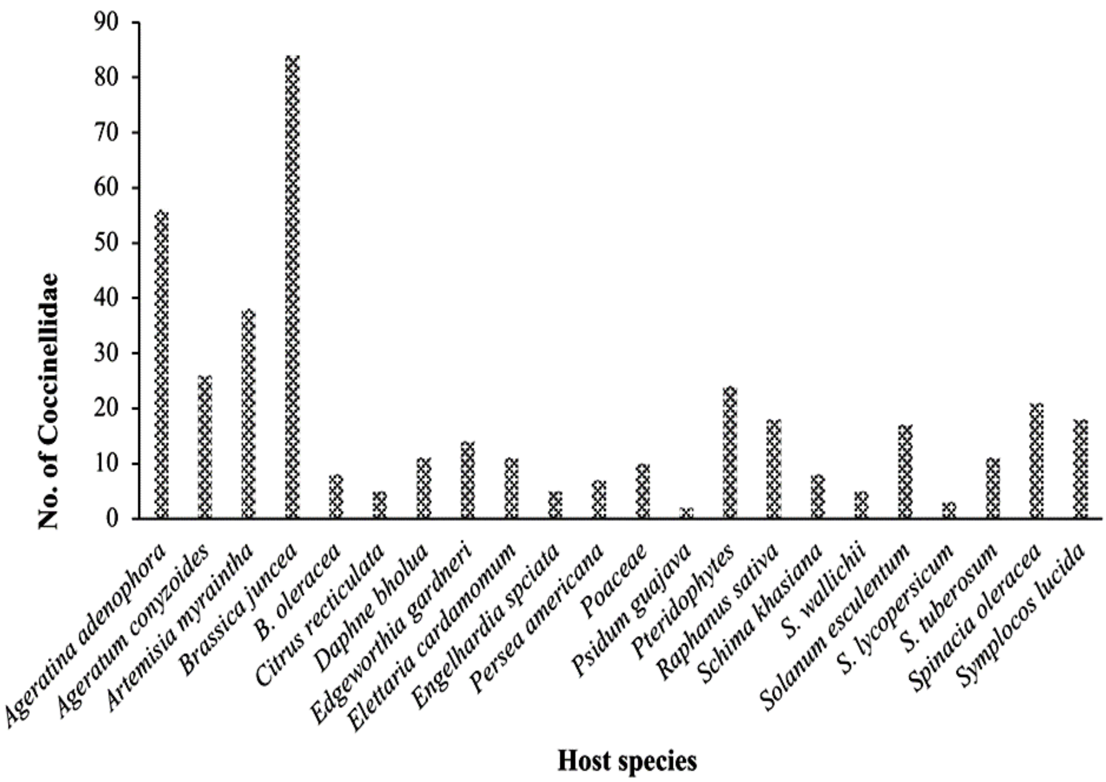

Figure 8: Bar graph showing the association between the number of ladybird beetles and the host species.

Vanderycken et al. (2013), where *C. septempunctata*, and *C. transversalis* are classified as habitat generalists, having a wider range of habitats.

Table 4: Spearman's correlation between abundance of host species and ladybird beetles

			Abundance of Coccinellidae
Spearman's rho	Abundance of host species	Correlation Coefficient	.671**
		Sig. (2-tailed)	.000
		N	60

** Correlation is significant at the .001 level (2-tailed).

Conclusions

This study contributes to understanding the diversity, distribution, and ecological preferences of ladybird beetles (Coccinellidae) in Nangkor Gewog, Zhemgang district. Through a systematic survey conducted across diverse habitats and host plants, we identified 13 species under 10 genera within the subfamily Coccinellinae. Notably, *Coccinella septempunctata, C. transversalis,* and *Oenopia kirbyi* emerged as the most abundant species, underscoring their critical role as biological pest control in agricultural ecosystem. On contrary, *Epilachna* sp., *Harmonia eucharis,* and *Illeis* sp. were the least abundant species in the study area. Our findings also reveal that agricultural fields serve as the most abundant habitats for ladybird beetles due to the high availability of prey, while forests support the greatest species diversity. This highlights the critical need to conserve both agricultural and natural ecosystems to sustain populations of these beneficial insects. Additionally, they exhibited a preference for *Brassica juncea* as a host plant, demonstrating their ecological interdependence with agricultural crops. While this study primarily focused on species composition, it did not delve into the factors influencing the assemblages of ladybird beetle. Future research should prioritize exploring the complex interactions with host plants, their associated pests, and the resulting impacts on the diversity and distribution of ladybird beetles.

References

Abbas, M. N., Kausar, S. & Rana, S. A. (2013). Diversity and distribution of ladybird beetles (Coccinellidae) in the cropland of Faisalabad District, *International Journal of Advanced Research*. 1(1): 27-33.

Ahmed, K. S., Majend, M. Z., Rafi, M. A., Sellami, F. & Afzal, M. (2017). Biodiversity and distribution of Coccinellids (Coccinellidae: Coleoptera) in District Sarjodha (Punjab), Pakistan, *Pakistan Journal of Zoology*. 49(5): 1748-1759. doi: 10.17512/journal.pjz/2017.49.51149.1749-1759.

Albaaj, M. (2017). A Study on the biodiversity of ladybird beetles in soybean agroecosystems of Northwestern Ohio, *Honours Projects*. 218. Retrieved from https://scholarworks.bgsu.edu/honorsprojects/218.

Ali, M., Raza, G., Ali, S. & Anjum, S. I. (2018). The tribe Coccinellini Weise (Coleoptera: Coccinellidae) in Sindh, Pakistan, *The Coleopterists Bulletin*. 72(3): 629-638. doi: 10.1649/0010-065X-72.3.629.

Ali, M., Ahmed, K., Ali, S., Raza, G., Hussain, I., Nafees, M. A. & Anjum, S. I. (2018). An annotated checklist of Coccinellidae with four new records from Pakistan (Coleoptera, Coccinellidae), *ZooKeys*. 803: 93-120. doi: 10.389//zookeys.803.22543.

Ankalgi, M. & Jadesh, S. (2016). Diversity and distribution of Coccinellidae (Coleoptera) in Ankalga, *International Journal of Basic and Applied Sciences.* 5(6): 1-5.

Bielawski, R. (1979) Ergebnisse der Bhutan-Expedition 1972 des Naturhistorischen Museums in Basel. Coeleoptera [sic]: Fam. Coccinellidae. Entomologica Basiliensia, 4, 83–125.

Biranvand, A., Hesami, S., Gheibi, M., Fekrat, L., Nevdev, O. & Shakarami, J. (2018). Contribution to the knowledge of Coccinellidae (Coleoptera) of Iran, *Oriental Insects.* 2157-8745. doi: 10.1080/00305316.2018.1482804.

Burns, J. H. & Strauss, S.Y. (2011). More closely related species are more ecologically similar in an experimental test, *PNAS.* 108(13): 5302-5307. doi: 10.1073/pnas.1013003108.

Chakrabarti, S., Sarkar, S., Debnath, M. & Kalyani. (2012). Diversity, bioecology and biosystematics of aphidophagous predators of eastern Himalaya and Northeast India, *Biodiversitat und Naturausstattung im Himalaya*. 4: 129-147.

Davidson, L. N. & Evans, E. W. (2010). Frass analysis of diets of aphidophagous lady beetles (Coleoptera: Coccinellidae) in Utah Alfalfa fields, *Environmental Entomology*. 39(2): 576–582. doi:10.1603/en08308.

Dorji, C., Loday, P. & Vorst, O. (2017). *A field guide to the common lady beetles of Bhutan.* Bhutan: National Biodiversity Centre.

Dorji, C., Lhamo, T. & Wangchuk, J. (2021) Six species of Coccinellidae new to Bhutan (Insecta: Coleoptera). *Oriental Insects* 56, 187–197. https://doi.org/10.1080/00305316.2021.1930604

Dorji, C., Loday, P. & Vorst, O. (2019) A preliminary checklist of the Coccinellidae of Bhutan (Insecta: Coleoptera). *Zootaxa* 4712, 497–530. https://doi.org/10.11646/zootaxa.4712.4.2

Egerer, M. H., Bichier, P. & Philpott, S. M. (2016). Landscape and local habitat correlates of lady beetle abundance and species richness in urban agriculture, *Annals of the Entomological Society of America*, 110 (1): 1-7. doi: 10.1093/aesa/saw063.

Grez, A. A. & Alaniz, A. J. (2019). Urbanization filters coccinellids composition and functional trait distributions in green spaces across greater Santiago, Chile, *Urban Forestry and Urban Greening*. 38: 337-345. doi: 10.1016/j.ufug.2019.01.002.

Hayat, A., Khan, M. R., Naz, F. & Rafi, M. A. (2014). Ladybird beetles of subfamily Chilocorinae (Coccinellidae: Coleoptera) of Azad Jammu and Kashmir, *Journal of Pakistan Entomologist*. 36(2): 135 -143.

Hussain, M., Malik, M. F., Siddique, S., Umar, M., Zainab, T. & Zafar, F. (2017). Diversity and distribution of Coccinellid beetle in irrigated and rainfed fields of Gujrat, Punjab, Pakistan, *Punjab University Journal of Zoology.* 33(1): 1-6. doi: 10.17582/pujz/2018.33.1.1.6.

Khamashon, L. (2013). A comparative study on systematics and population dynamics of coccinellids (Coleoptera: Coccinellidae) from the fruit orchards and oak forest of district Nainital (Uttarakhand) and their feeding potentials, *PhD thesis*. Kauman University, India: Nainital.

Majumder, J., Bhattacharjee, P. P. & Agarwala, B. K. (2013). Diversity, distribution and habitat preference of predacious Coccinellids (Coleoptera: Coccinellidae) in agro- and forest habitats of Tripura, Northeast India, *International Journal of Current Research*. 5(5): 1060-1064.

Murali, S., Jalali, S. K., Shylesha, A. N., Shivalinga S. T. M. & Gandhi G. R. (2017). Relative abundance and species composition of predatory coccinellids fauna in different seasons of Brinjal crop, *Journal of Entomology and Zoology Studies*. 5(5): 682-686.

Mayur, A. M., Hattappa, S., Mahadevamurthy, M., & Chakravarthy, A. K. (2013). The impact of newly established Bangalore International Airport on local biodiversity. *Global Journal of Biology, Agriculture and Health Sciences, 2*(2), 49-53.

Pang, H., Ślipiński, A., Wu, Y. & Zuo, Y. (2012). Contribution to the knowledge of Chinese *Epilachna* Chevrolet with a description of new species (Coleoptera: Coccinellidae: Epilachnini), *Zootaxa.* 3420: 1-37. doi: 10.11646/Zootaxa.3420.1.1.

Pollard, E. (1977). *A Method for Assessing Changes in the Abundance of Butterflies*. Journal of Applied Ecology.

Poorani, J. (2002). An annotated checklist of the Coccinellidae (Coleoptera: Coccinellidae) from the Indian Sub-continent, *Oriental Insects*. 36: 97-116.

Rahatullah, Haq, F., Ahmad, H., Inayatullah, M., Saeed, K. & Khan, S. (2012). Morphological characteristics of ladybird beetles collected from District Dir Lower, Pakistan, *African Journal of Biotechnology.* 11 (37): 9149-9155. doi: 10.5897/AJB11.2363.

Rahatullah, R., Khan, M. S., Inayatullah, M. & Haq, F. (2016). Multivariate analysis of coccinellids fauna of Malakand division, Pakistan, *Journal of Entomology and Zoology Studies.* 4(6): 485-489.

Rasheed, R. & Buhroo, A. A. (2018). Diversity of coccinellid beetles (Coccinellidae: Coleoptera) in Kashmir, *Entomological Journal*. 43(20): 129-134.

Sarwar, M. (2016). Recognition of some lady beetles (Coleoptera: Coccinellidae) deadly sighted for insect and mite pests in agroecosystems, *International Journal of Entomology Research.* 1(2): 29-34.

Seago, A.E., Giorgi, J.A., Li, J. & Ślipiński, S.A. (2011) Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. *Molecular Phylogenetics and Evolution*, **60**(1), 137–151.

Shah, M. A. & Khan, A. A. (2014). Assessment of Coccinellid biodiversity under pesticide pressure in horticulture ecosystem, *Indian Journal of Entomology.* 76(2): 107-116.

Shantibala, K. & Singh, T. K. (1991). Studies on the vertical distribution of aphidophagous coccinellids (Coleoptera: Coccinellidae) of Manipur and Nagaland, *Journal of Aphidology*. 5: 39-43.

Sharma, P. L., Chauhan, U. & Sharma, K. C. (2015). Studies on the diversity of predatory coccinellid beetles (Coleoptera) in different agro-climatic zones of Himachal Pradesh, *International Quarterly Journal of Life Sciences.* 10(3): 981-985.

Sharma, P. L., Verma, S. C., Chandel, R. S. & Thakur, P. (2017). An inventory of the predatory Coccinellidae of Himachal Pradesh, India, *Journal of Entomology and Zoology Studies*. 5(6): 2503-2507.

Ślipiński, A. & Tomaszewska, W. (2010). Coccinellidae. In *Handbuch der Zoologie. Coleoptera, Beetles*, eds. Leschen, R. A. B., Beutel, R. G., and Lawrence, J. F. 2: 454-472. Berlin: De Gruyter Publishers.

Szawaryn, K., Větrovec, J., & Tomaszewska, W. (2020). A New Tribe of the Ladybird Beetle Subfamily Microweiseinae (Coleoptera: Coccinellidae) Discovered on an Island in the North Atlantic Ocean. *Insects*, *11*(6), 367. https://doi.org/10.3390/insects11060367

Sushko, G. G. (2017). Diversity and species composition of beetles in the herb-shrub layer of a large isolated raised bog in Belarus, *Mires and Peat*. 9(10): 1–14. doi: 10.19189/MaP.2017.OMB.266.

Tomaszewska, W. and Szawaryn, K. (2016). Epilachnini (Coleoptera: Coccinellidae)-revision of the world genera, *Journal of Insect Science,* 16 (1): 1-91. doi: 10.1093/jisesa/iew082

Unipal, V. P. & Mathur, P. K. (1998). A study on the species diversity among selected insect groups. India: Dehradun.

Vandenberg, N. J. (2002). Coccinellidae Latreille 1807. In *American Beetles*, eds. R. H. Arnett, Jr., M. C. Thomas, P. E. Skelley, & J. H. Frank. 2: 371-389, Boca Raton: CRC Press.

Vanderycken, A., Durieux, D., Joie, E., Sloggett, J. J., Haubruge, E. & Verheggan, F. J. (2013). Is the Multicoloured Asia lady beetle, *Harmonia axyridis*, the most abundant natural enemy to aphids in agroecosystem, *Journal of Insect Science*. 13(158): 1-14.