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Multivariate analysis of coccinellids fauna of malakand division, Pakistan

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Abstract
Coccinellidae (ladybird beetles) fauna of Malakand division was explored during 2013-15. There are 28 species under 19 genera belonging to subfamilies Coccinellinae, Chilocorinae, Scymninae and Epilachninae. The statistical analysis (DCA ordination) of coccinellids fauna showed that different species clustered along different ordination axis having similar habitat types. The maximum eigenvalue were recorded for axis 1 (0.28) with gradient length of 1.32 indicating that the whole data set was dominated by single gradient length. The species which having maximum ordination score with axis 1 included *Chilocorus circumdatus*, *Propylea dissecta*, *Hyperaspis leechi*, *Stethorus gilvifrons*. The sample weighted mean species score were maximum for Dir lower with 132 value followed by Chitral with 85 score on axis 1. The DCA ordination showed that the minimum population size were recorded in November, December, January and February, maximum population size were recorded in September followed by October. The coccinellids fauna population changes with altitude. At high altitude its population size was minimum while at lower altitude its population size was maximum. The coccinellids fauna population was also disturbed by different birds which feed upon it.

Keywords: Coccinellids, multivariate, DCA ordination, population size, habitat types, seasonal distribution, Malakand division

1. Introduction
The coccinellids, commonly called as ladybird beetles, belong to family Coccinellidae superfamily Cucujoidea, series Cucujiformia, suborder Polyphaga and order Coleoptera [1]. Six subfamilies of Coccinellidae have been recognized so for. These include Coccinellinae, Chilocorinae, Coccinellidae, Sticholotidinae, Scymninae and Epilachninae. Among these, the subfamilies Coccinellinae, Chilocorinae, Coccinellidae, Sticholotidinae and Scymninae are mostly predacious while Epilachninae is phytophagous [2]. The family was in to two groups; aphidophagous and phytophagous by Redtenbacher [3].
Coccinellid beetles are more or less worldwide in distribution; many tribes are restricted to particular bio-geographical regions. More than 6000 species of coccinellids have been reported worldwide [4]. Ladybird beetles have bright contrasting colour patterns. About 90% species of coccinellid beetles are predator mainly against homopterous insects and phytophagous mites, which are injurious to various agricultural crops and forest plants. In Coccinellidae, members of a relatively small sub-family Epilachninae, are however phytopagous and are serious pests of brinjal and cucurbits.
In Malakand division very little work has been done on coccinellid beetles. Khan *et al*. in 2007 described twelve species from Chitral District [5], Ullah *et al.* in 2011 and 2012 reported 14 species from Dir lower [6, 7], Naz *et al.* in 2012 reported two Epilachnine ladybird beetles *Henosepilachna vigintioctopunctata* and *H. septima* from Buner and Swat [8].
Keeping in view the importance of these useful predators and poor attention in the past, there is need to explore the coccinellids fauna found in Malakand division. The multivariate approaches will help to find out the similarity in species composition and habitat types as well as its seasonal occurrence. Species having similar habitats will cluster in ordination space and habitats on the basis of common species.

2. Materials and methods
2.1 The study area
Malakand division consists of seven districts i.e. Swat, Buner, Shangla, Lower Dir, Dir Upper, Chitral and Malakand [9]. The maximum altitudinal range of the area is 4876m in the north east

and 3049m along with Afghanistan to the West. Malakand division is bounded by Afghanistan on north, Hazara division on the East, on south east by Mardan, south west by Charsadda and on the west by Mohmand and Bajour Agencies with total 29872km² land area. Its coordinates are 35.5E° and 72N°. There are scenic places and tourist resorts in Malakand division like Jabban, Kalam, Bahrain, Madyan, Malam Jaba, Kumrat and Malakand Hydro-electric Power. The area is a natural hub of high quality Walnut, Honey, Soybean, Trout fish, seasonal and off-season fruits and vegetables [10].

The study area shows distinct seasons, summer, winter, monsoon, autumn and spring. The summer season starts from March to June with maximum temperature recorded up to 45 °C and minimum temperature of 10 °C during the day and night time, respectively. Winter season starts from November to mid of March and the days are short and nights are very long and cold with frost during winter months. The monsoon season starts from July till end of August. The maximum rainfall occurs during July and August. During our study period 120 mm rainfall was recorded, which was the average rainfall recorded across all the seven sampled localities and sub localities, the maximum of which was during the monsoon season [9].

2.2 Sample collection

The collection of coccinellids beetles were carried out from different ecosystems viz, agricultural field, herbal garden and forest of Malakand division located at different altitudes by aerial netting and hand picking method from March 2013 to October 2015. Each specimen was properly labeled with place of collection, date of collection and collector name [11]. The properly fixed specimens were preserved in collection box. Proper protection of the specimens was conducted regularly to avoid damage, from humidity and museum pests attack. Inside the collection boxes phenolphthalein balls were placed for protecting specimens from pest damages. The population status were studied in the active season during 2011 to 2014 and were identified with the help of available literature, taxonomic keys and by comparison with identified species. The identified specimens were deposited in the Zoological Museum, Hazara University, Mansehra. Species were described following Kapur, Katakura *et al*., and Naz [12, 13, 14]. The relationship of coccinellid species in various ecosystem in different seasons were obtained through multivariate analysis. The multivariate analysis included Detrended Correspondence Analysis (DCA). DCA ordination cluster different species having similar habitats and vice versa. DCA ordination also cluster different seasons having common population [15, 16].

3. Results

During the present study on the identification and distribution of coccinellids fauna of Malakand division, 1355 specimens were collected from seven districts and 37 sub-localities. Identification of these beetles showed that there are 28 species under 19 genera belonging to sub-families Coccinellidae, Chilocorinae, Scymninae and Epilachninae.

3.1 Multivariate analysis of coccinellids fauna of Malakand division

The statistical analysis (DCA ordination) of coccinellids fauna showed that different species clustered along different ordination axis having similar habitat types. The maximum eigenvalue were recorded for axis 1 (0.28) with gradient length 1.32 indicating that the whole data set was dominated by single gradient length. The eigenvalue for axis 2 were 0.01 with gradient length 0.51. The explained variation for axis 1 was 44.45 and for axis 2 were 46.63. In unconstrained DCA ordination total variation were 0.62 (Table 1).

The species which having maximum ordination score with axis 1 included *Chilocorus circumdatus* (260), *Propylea dissecta* (260), *Hyperaspis leechi* (260), *Stethorus gilvifrons* (260) while minimum species score were recorded for axis 1 included *Cryptogonus nepalensis* (-125). The maximum species score for axis 2 were recorded for *Halyzia tschitscherini* (365) followed by *Afidentula manderstjernae* (181) while minimum species score were recorded for axis 2 included *Coccinella transversalis* (-133). The species having maximum ordination score with axis 3 includes *Halyzia tschitscherini* (389) followed by *Afidentula manderstjernae* (136) while minimum ordination score were recorded for *Illies confusa* (-178) (Table 2). The sample weighted mean species score were maximum for Dir lower with 132 value followed by Chitral with 85 score on axis 1. The zero sample score were recorded for Malakand on axis 1. The sample weighted mean species score for axis 2 were maximum for Dir lower with 50 score while zero for Buner. The sample weighted mean species score were maximum for Swat having sampled score 46 followed by Dir lower with 45 and Chitral with 44 while zero for Buner. These result showed that the maximum population score were recorded in Dir lower (Table 3).

DCA ordination also showed that different species clustered together and having positive correlation. The species clustered together having strong correlation included *Chilocorus circumdatus*, *Propylea dissecta, Hyperaspis leechi* and *Stethorus gilvifrons*. There were also strong correlation among other species clustered includes *Macroilleis hauseri, Oenopia conglobata*, *Calvia punctata*, *Chilocorus bijugus*, *Exochomus nigripennis*, *Aiolocaria hexaspilota*, *Chilocorus melas*, *Propylea leuteopustulata* and *Oenopia mimica*. The species which has weak correlation in ordination space included *Afidentula manderstjernae*, *Menochilus sexmaculata*, *Cryptogonus nepalensis*, *Illies confusa*, *Coccinella transversalis* and *Brumoides suturalis* while *Halyzia tschitscherini* were at the top of ordination space and having negative correlation with most of the species (Fig. 1).

The DCA ordination also showed that the minimum population size of coccinellids fauna of Malakand division were recorded for the months of November, December, January and February while maximum population size were recorded for the months of September followed by October while intermediate population size were recorded for the months of August, July, June and May (Table 4).

In ordination it was also observed that during unconstrained analysis the samples of Malakand, Shangla and Buner clustered below average position. The other samples Dir lower, Dir Upper Swat and Buner were distributed away from centered position. From Dir lower a total of 27 species were recorded indicating the most suitable locality for coccinellids fauna. It was followed by Chitral with 20 species, Dir Upper with 16 species, Shangla and Swat each contributed 13 species, Malakand and Buner each contributed 12 species. Eleven species were common in all stands while five species showed specific habitat and have restricted population (Fig. 2).

The population of *Coccinella septempunctata* and recorded in all months of the year (all seasons) however its population were restricted during the months of November, December, January and February (Fig. 3). The population of *Propylea*

dissecta was only recorded during months of September and October. The population of *Hyperaspis leechi* was recorded during months of July and September. The population of *Stethorus gilvifrons* was distributed during months of August, September and October. Among all the months the maximum (219) population of coccinellids fauna were recorded during July, followed by August (205), June (203), September (190), May (185), April (126), October (117), March (67), February (13), November (10). The minimum populations were recorded during months of December (6) and January (6). These results showed that for maximum coccinellids fauna of Malakand division require high temperature, humidity and rainfall. In Malakand division the coccinellids fauna population changes with altitude and latitude. At high altitude its population size was minimum while at lower altitude its population size was maximum. The coccinellids fauna population was also disturbed by different birds which feed upon it.

4. Discussion

Coccinellidae ladybird beetles belong to super family Cucujoidea. Generally the family Coccinellidae is known for its predatory nature, however members of subfamily Epilachninae are phytophagous, which are very important pest of tomato, brinjal, potato, bitter gourd, ribbed gourd, melons and cereal crops. The coccinellids fauna of Pakistan has been explored by [17]. They reported 71 and 74 species of predatory coccinellids from all over the country respectively. But the coccinellid ladybird beetles poorly studied in Malakand division. Some information is available on the occurrence of these beetles in Chitral and Dir valley of Malakand division. These include; Ullah *et al*. (2010) reported 14 species from Dir valley while Khan *et al*. reported 12 species from Chitral. However a proper study of Malakand division was lacking despite of its unique climatic condition. A comprehensive work has been done in different part of the world and also in our neighboring countries including Taiwan, China, India and Nepal. The World fauna of coccinellid beetles contains about 6000 species [4].

During the present study the coccinellid ladybird beetles fauna of Malakand division was explored. The study shows that there are 28 different species under 19 genera in the division; belonging to sub families Coccinellinea, Chilocorinae, Scientiae and Epilachninae. Among these species, 5 species are newly recorded from Malakand division while one species reported first time from Pakistan

In DCA ordination maximum eigenvalue were recorded for axis 1 (0.28) with gradient length 1.32 indicating that the whole data set were dominated by single gradient length. The species which have maximum ordination score with axis 1 included *Chilocorus circumdatus* (260), *Propylea dissecta* (260), *Hyperaspis leechi* (260), *Stethorus gilvifrons* (260). The sample weighted mean species score were maximum for Dir lower with 132 value followed by Chitral with 85 score on axis 1. The species clustered together having strong correlation included *Chilocorus circumdatus*, *Propylea dissecta, Hyperaspis leechi* and *Stethorus gilvifrons*. The DCA ordination also showed that the minimum population size of coccinellids fauna of Malakand division were recorded for the months of November, December, January and February while maximum population size were recorded for the months of September. From Dir lower a total of 27 species were recorded indicating the most suitable locality for coccinellids fauna. The population of *Coccinella septempunctata* and having were recorded in all months of year however its population were restricted during the months of November, December, January and February. These results showed that for maximum coccinellids fauna of Malakand division require high temperature, humidity and rainfall. In Malakand division the coccinellids fauna population changes with altitude and latitude.

Table 1: Axis summary table

Axis	Eigenvalue	Gradient length
1	0.28	1.32
2	0.01	0.51
3	0.01	0.46

Table 2: DCA ordination with species score

Name	Axis 1	Axis 2	Axis 3	Ranked 1		Ranked 2	
				EIG= 0.28		EIG= 0.013	
Bru sut	112	-118	-81	Chi cir	260	Hal tsc	365
Chi mel	200	60	58	Pro dis	260	Afi man	181
Chi rub	106	136	120	Hyp lee	260	Chi rub	136
Chi bij	208	60	58	Ste gal	260	Men sex	126
Chi cir	260	60	58	Oen con	225	Cry nep	116
Exo nig	191	60	58	Mac hau	225	Coc sem	71
Coc sem	-1	71	87	Cal pun	223	Pro dis	60
Coc tra	53	-133	-125	Chi bij	208	Pro leu	60
Ada bip	204	0	-36	Aio hex	207	Ada tet	60
Ada tet	167	60	58	Oen mim	205	Ste gal	60
Aio hex	207	60	58	Ada bip	204	Chi bij	60
Cal pun	223	60	58	Pro leu	204	Oen mim	60
Hip var	87	-4	15	Chi mel	200	Oen con	60
Hal tsc	178	365	389	Exo nig	191	Chi mel	60
Mac hau	225	60	58	Hal tsc	178	Chi cir	60
Har dim	-37	-46	-58	Ada tet	167	Aio hex	60
Oen sau	-29	-45	-56	Bru sut	112	Exo nig	60
Oen mim	205	60	58	Chi rub	106	Hyp lee	60
Oen con	225	60	58	Hip var	87	Cal pun	60
Ill con	35	-77	-178	Coc tra	53	Mac hau	60
Men sex	-104	126	102	Ill con	35	Hen vig	42
Pro leu	204	60	58	Hen vig	2	Ada bip	0
Pro dis	260	60	58	Coc sem	-1	Hip var	-4

Hyp lee	260	60	58	Oen sau	-29	Oen sau	-45
Cry nep	-125	116	89	Har dim	-37	Har dim	-46
Ste gal	260	60	58	Afi man	-54	Ill con	-77
Afi man	-54	181	136	Men sex	-104	Bru sut	-118
Hen vig	2	42	59	Cry nep	-125	Coc tra	-133

Table 3: DCA sample scores - which are weighted mean species scores

Name	Axis 1	Axis 2	Axis 3	Ranked 1		Ranked 2	
				EIG= 0.28		EIG= 0.013	
Dir lower	132	50	45	Dir lower	132	Swat	51
Dir upper	68	31	27	Chitral	85	Dir lower	50
Swat	8	51	46	Dir upper	68	Chitral	45
Buner	13	0	0	Buner	13	Dir upper	31
Shangla	1	20	9	Swat	8	Malakand	27
Malakand	0	27	14	Shangla	1	Shangla	20
Chitral	85	45	44	Malakand	0	Buner	0

Table 4: Ordination indicating month wise scores

Name	Axis 1	Axis 2	Axis 3	Ranked 1		Ranked 2	
				EIG= 0.28		EIG= 0.013	
January	233	44	66	January	233	August	72
February	224	49	59	December	233	April	54
March	93	34	54	February	224	November	54
April	60	54	55	November	215	May	52
May	54	52	38	March	93	February	49
June	54	0	42	April	60	September	46
July	33	4	55	May	54	January	44
August	28	72	19	June	54	December	44
September	0	46	49	July	33	March	34
October	13	10	0	August	28	October	10
November	215	54	51	October	13	July	4
December	233	44	66	September	0	June	0

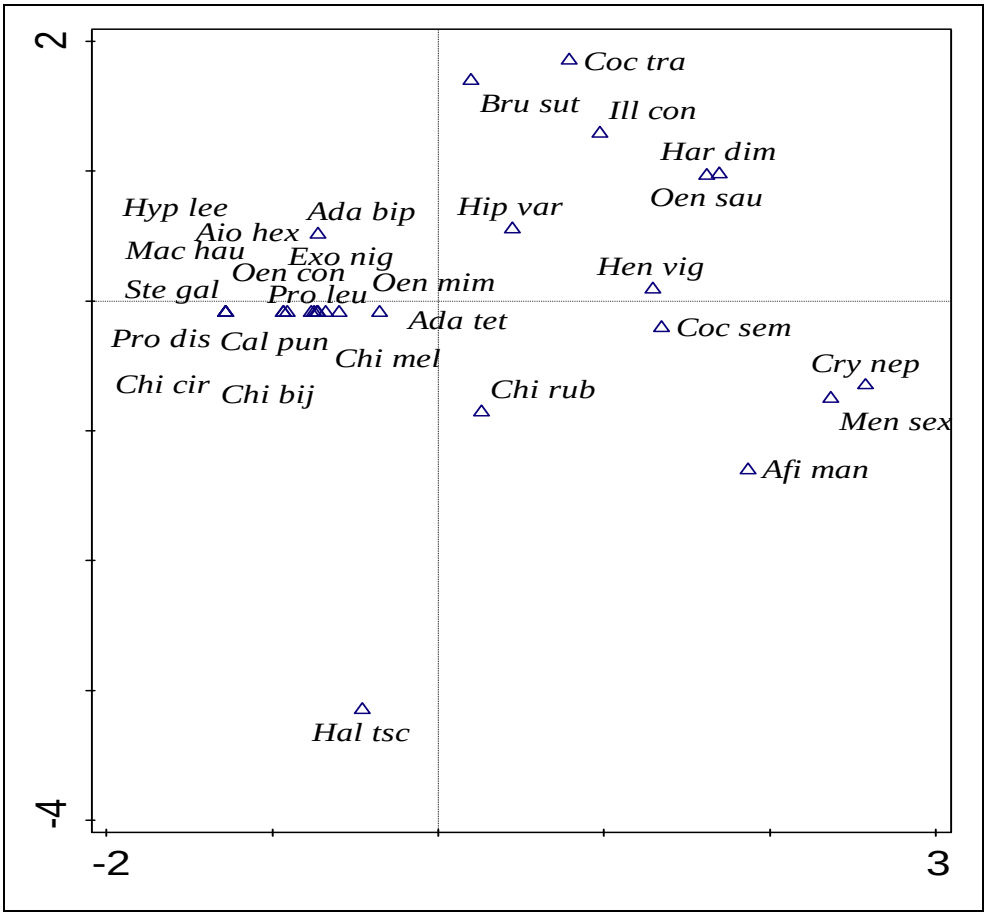

Fig 1: DCA ordination of species of Malakand Division.

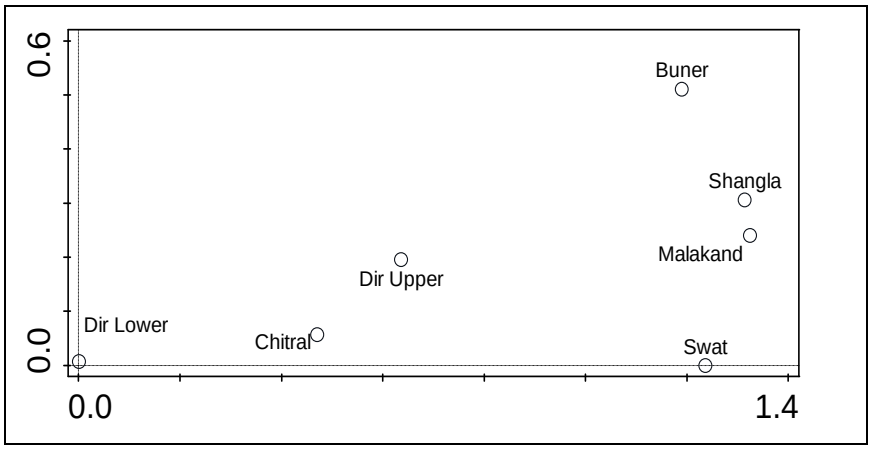

Fig 2: DCA ordination of samples of Malakand division

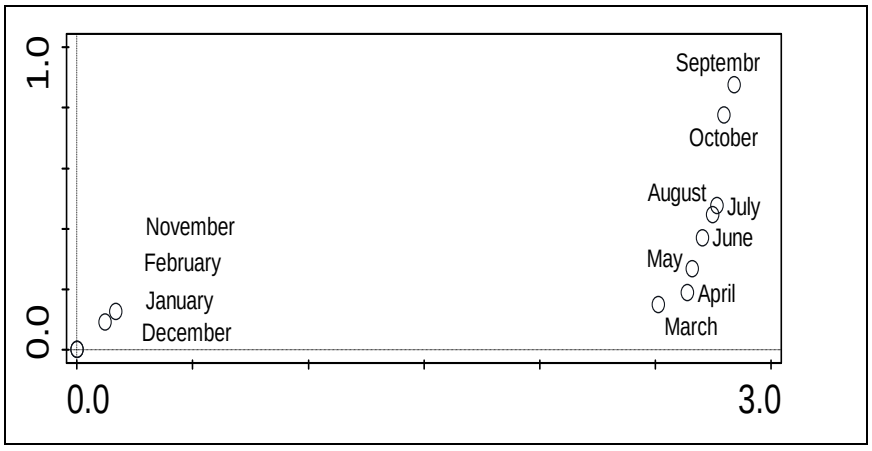

Fig 3: DCA ordination of monthly distribution of coccinellid fauna

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