Research Article

Insect Faunal Diversity and Relative Abundance Associated with *Trifolium alexandrinum* (Berseem) in District Sialkot, Pakistan

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Abstract | Biodiversity is defined as the variety of life. In agricultural system, biodiversity refers to estimation of number of species that are present in that ecosystem. In an interrelated ecosystem, different species of insects perform different functions; act as pollinators, recycle nutrients, water and energy in many ways. In this kind of mutually dependent systems, the loss of even single species can affect the entire system very severely. Therefore, diversity estimation of insects help in conservation of insect fauna and stabilization of ecosystem. Present study focused on estimation of diversity and also the relative abundances of insect fauna associated with *Trifolium alexandrinum* (Berseem) in District Sialkot. Total of six orders such as, Orthoptera, Hemiptera, Lepidoptera, Diptera, Hymenoptera and Coleoptera belonging to 12 families i.e., Acrididae, Gryllidae, Aleyrodidae, Pentatomidae, Noctuidae, Syrphidae, Culicidae, Formicidae, Braconidae, Coccinellidae, Staphylinidae and Chrysomelidae were sampled from district Sialkot. Coleoptera was recorded as most abundant order with 39% relative abundance. By statistical analysis significant difference (p-value< 0.005) was found in presence of diversity in different months of sampling. Shannon index was maximum in the month of May (H'=2.819). Canonical Corresponding Analysis (CCA) determines the impact of different environmental factors on presence of different species.

Received | October 13, 2023; **Accepted** | December 17, 2023; **Published** | December 22, 2023
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Citation | Suneela, S. Mushtaq, S. Maalik, A.W. Qureshi and M. Batool. 2023. Insect Faunal diversity and relative abundance associated with *Trifolium alexandrinum* (Berseem) in District Sialkot, Pakistan. *Biologia (Lahore),* 69(2): 40-47.
DOI | https://dx.doi.org/10.17582/journal.Biologia/2023/69.2.40.47
Keywords | *Trifolium alexandrinum,* Biodiversity, Insect Fauna, Predator, Biological control, Sialkot

Introduction

Variety of life is known as biodiversity (Frank, 2022). In agro ecosystem, it refers to the estimation of number of species that are present in that agricultural system (Amber *et al.*, 2015). The class Insecta is the largest group of the animal kingdom. They are extensively dispersed in the world and adopt almost every kind of environment. Today, approximately 1.25 million species of insects are known (Al-Mrammathi and Al-Hasnawi, 2021). The insect's species are found in all environments such as

terrestrial, desert and aquatic (Nandini and Murali, 2014). Many climatic factors such as temperature, humidity, rainfall and wind have effect on population dynamics, feeding behaviors, life cycle, diversity and abundance of insects (Okoro, 2015). In maintaining biodiversity insects have chief role as they keep balance between flora and fauna (Rekha *et al*., 2019). In an interrelated ecosystem, different species of insects perform different functions; they act as pollinators, they recycle nutrients, water and energy in many ways. In this kind of mutually dependent systems, the loss of even single species can affect the entire system very severely (Cardinale *et al*., 2006). Many insects' species cause severe damage to different upper and lower parts of plants. The most important pollinators and predators include the syrphid fly, Coccinellidae and honeybees (Hameed *et al*., 2016).

The insect species is completely dependent upon cultivated fields as they feed on roots, stem, leaf, flower and seed. The insect's larvae are voracious feeder and cause a heavy damage to farming crops (Patel *et al*., 2021). More than 10,000 species of insects cause damaged to food plants worldwide (Dhaliwal *et al*., 2010).

Berseem (*Trifolium alexandrinum)* is an annual leguminous fodder crop. Annual leguminous fodder is considered as the major nutrient source for the livestock because they fulfil the requirement of energy, minerals, proteins and fiber of the animal (Balazadeh *et al*., 2021). As it is a leguminous crop, they are mostly grown in moistened belts of Pakistan. They play a role in the fixation of nitrogen (Stewart *et al*., 2008) they also reduce the pH of soil, reduce the alkalinity of the soil (Murtaza and Murtaza, 2012), soil salinity, and increase the water-holding capacity of the soil. From 1980 to 2017, huge increase in human population growth had occurred (i.e., from 82 to 197 million people). Pakistan needs to increase the production of milk and meat. It can only be possible by the production of forage of high yield example such as berseem. Milk production increases in animals by providing good quality berseem. *Trifolium alexandrinum* is affected by different kinds of pests which include the important armyworm, leaf miner and aphid (Hameed *et al*., 2016). Pakistan is having a rich and diverse fauna this is due to intensive agriculture in the southeast of Asia since 3000 BCE. That's why parasitoids and predators of these pests are also very common in this region.

Insects can be grouped in beneficial insects and harmful insects in broad sense. Insects play an important part in the proper functioning of ecosystem and are also an integral part of the food web (Busse *et al*., 2021). Both of these groups should be treated in a different manner as we have to keep harmful insects under threshold level so they can't attain the status of pest and render economic losses. On the other hand, such environment should be provided to beneficial insects which are favorable to enhance their populations (Yi *et al*., 2012). This can only be carried out when status of these organisms will be known. Study of eco-biology, diversity and relative abundance of insect fauna is very helpful tool in identification of insect fauna and assessing their status, leads towards conservation of beneficial insects and sustainability of ecosystem. The importance of present study is that there is no previous research conducted for the estimation of diversity and relative abundance of insect fauna associated with *Trifolium alexandrinum* (Berseem) in District Sialkot. The need of present study is to estimate the accurate diversity of insect's fauna in selected agro-ecosystem and it will be beneficial for the stability and maintenance of the ecosystem.

Materials and Methods

For collection of insect fauna, Barseem crop was selected due to its importance as fodder (Hameed *et al*., 2016). Selection of site for sampling was conducted by considering GC Women University, Sialkot as zero point and fixing an area of 50 km approximately from this point. Sites for sampling were selected randomly within 50 km from GC Women University, Sialkot on any side. The sampling area comprised of 4 randomly selected sites in Sialkot, Punjab, Pakistan.

Localities	Latitude	Longitude
Wario	32.41°N	74.58°E
Dalowali	32.52°N	74.60°E
Kanpur	32.58°N	74.61°E
Sambrial	32.47°N	74.35°E

Sampling was carried out periodically for a period of four months from February 2017 to May 2017. To collect ground fauna of crop quadrate method was applied (Mushtaq *et al*., 2014). Total of four quadrat of 1m^2 were considered to collect data, two quadrat from edge and two from center randomly. Collected

samples were placed in vials having 70% alcohol as preservative and few drops of glycerin were also added. Each of the vials was labeled with date, location, and time of sampling, field no. and number of samples collected per field.

The original tagging was maintained while sorting. The sampled data was carried out towards Zoology Research Laboratory of GC Women University, Sialkot. All specimens were washed with tap water and preserved again in formalin with few drops of glycerin in separate vials with same labeling. Specimen from each of the vials was sorted out for identification on the basis of morphology. Similar specimens were kept in same vials and number of specimens was mentioned there. Specimens were identified up to species level with the help of available information, related to taxonomy in "Fauna of British India" by Talbot (1978), Borror and Delong (2005). These were also confirmed from online electronic keys which are available on different web sites. Identified specimens were further confirmed by the Museum of Department of Agri-Entomology, University of Agriculture Faisalabad.

Statistical analysis

Relative abundance of collected insects was calculated by using MS Excel 2013. Shannon Diversity indices (Magurran, 1988) were applied to check the species diversity (H'), richness (D) and evenness (E). Differences in the selected crop were then compared by applying t-test. Canonical Corresponding Analysis (CCA) was applied to check the meteorological effect on the distribution of species (Rana *et al.*, 2010).

Results and Discussion

In present study, a total of six orders, Orthoptera, Hemiptera, Lepidoptera, Diptera, Hymenoptera and Coleoptera belonging to 12 families were collected from *Trifolium alexandrinum*. Coleoptera was most abundant order containing 350 specimens belonging from 6 species. Relative abundance of order Coleoptera was highest as (39.21%) followed by order Lepidoptera (19.05%). Species richness was also highest for order Coleoptera (H'=1.6429) (Table 1, Figure 1).

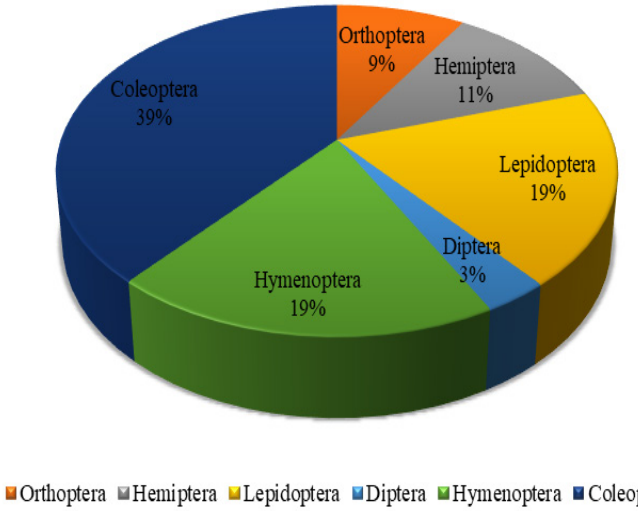

Figure 1: *Order wise abundance of insects from Trifolium alexandrinum.*

The relative abundance of sampled families was as following: Coccinellidae (19.72%), Noctuidae (19.05%), Formicidae (17.94%), Staphylinidae (13.29%), Braconidae (9%), Acrididae (6.86%), Aleyrodidae (6.31%), Chrysomelidae (6.2%), Pentatomidae (4.87%), Culicidae (2.55%), Gryllidae (1.99%), and Syrphidae (0.66%), (Figure 2).

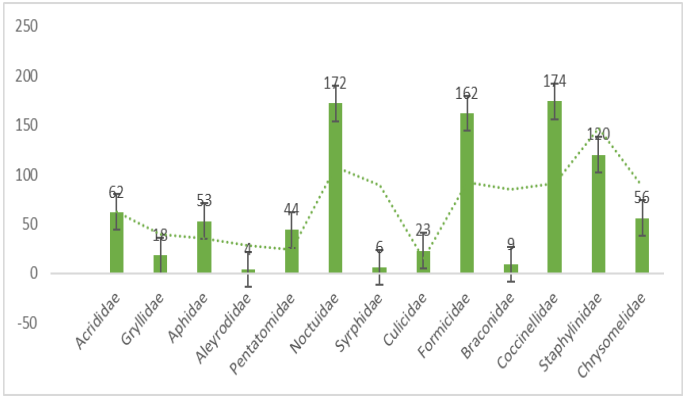

Figure 2: *Number of individuals belonging from different families collected from Barseem.*

Table 1: *Diversity Parameters of collected insect orders from Barseem crop.*

	Orthoptera	Hemiptera	Lepidoptera	Diptera	Hymenoptera	Coleoptera
Species	5	3	3	3	3	6
number	80	101	172	29	171	350
Relative abundance	8.85	11.18	19.05	3.21	18.94	39.21
richness (H')	1.5149	0.82824	0.97525	1.0222	0.86221	1.6429

A total of 903 specimens were collected from berseem. Maximum insect's specimen (359) were collected in the month of April while minimum insect's specimen (80) were sampled in the month of February. *Paederus fuscipes* was dominant species (13.29% relative abundance) throughout the sampling duration. The species of order Orthoptera remained less dominant during February to March, but later they become dominant i.e., in May. Among them *Acrida exaltata* (2.55%) showed dominancy followed by *Mermiria bivittata* (2.21%) and *Oxya hyla* with (2.1% relative abundance), respectively. While members of family Gryllidae (*Acheta domesticus* (1.44%) *Gryllus pennsylvanicus* (0.55%) remained less dominant (Table 2).

Species of family Noctuidae, *Agrotis ipsilon* (2.33%), *Spodoptera litura* (7.53%) and *Spodoptera exigua* (9.19%) also abundantly present during month of March and April, while in May their number started to decrease. The members of family Coccinellidae, i.e., *Coccinella septempunctata* (8.86%) remained dominant throughout the study time, other species like *Cheilomenes sexmaculatus* (2.88%), *Menochilus sexmaculatus* (4.1%), *Coccinella undecimpunctata* (3.43%) were also present. Family Braconidae species

Table 2: *Relative abundance of insect fauna sampled from Barseem in sampling duration.*

Order	Family	Species	February	March	April	May	Total
Orthoptera	Acrididae	Acrida exaltata	0	1.6	2.51	3.97	2.55 (23)
		Oxya hyla	2.5	1.6	1.39	3.25	2.1 (19)
		Mermiria bivittata	2.5	1.6	1.67	3.25	2.21 (20)
		Total	5	4.8	5.57	10.47	6.86 (62)
	Gryllidae	Acheta domesticus	2.5	1.6	1.39	1.08	1.44 (13)
		Gryllus pennsylvanicus	0	0	0.56	1.08	0.55 (5)
		Total	2.5	1.6	1.95	2.16	1.99 (18)
Hemiptera	Aleyrodidae	Rhopalosiphum maidis	6.25	6.42	4.74	6.86	5.87 (53)
		Bemisia tabaci	0	0.53	0.28	0.72	0.44 (4)
		Total	6.25	6.95	5.02	7.58	6.31 (57)
	Pentatomidae	Nezara viridula	0	3.74	5.57	6.14	4.87 (44)
Lepidoptera	Noctuidae	Agrotis ipsilon	1.25	1.6	2.51	2.89	2.33 (21)
		Spodoptera litura	8.75	13.37	7.52	3.25	7.53 (68)
		Spodoptera exigua	7.5	9.09	12.53	5.42	9.19 (83)
		Total	17.5	24.06	22.56	11.56	19.05 (172)
Diptera	Syrphidae	Episyrphus balteatus	0	0.53	0.84	0.72	0.66 (6)
	Culicidae	Anopheles gambiae	1.25	1.07	1.11	0.36	0.89 (8)
		Culex pipiens	0	2.67	1.67	1.44	1.66 (15)
		Total	1.25	3.74	2.78	1.8	2.55 (23)
Hymenoptera	Formicidae	Formica lemani	8.75	4.81	10.58	10.83	9.3 (84)
		Camponotus itoi	11.25	6.42	8.64	9.39	8.64 (78)
		Total	20	11.23	19.22	20.22	17.94 (162)
	Braconidae	Cotesia flavipe	0	1.07	1.39	0.72	1 (9)
Coleoptera	Coccinellidae	Cheilomenes sexmaculatus	2.5	2.67	2.79	3.25	2.88 (26)
		Menochilus sexmaculatus	3.75	3.21	3.62	5.42	4.1 (37)
		Coccinella undecimpunctata	2.5	4.81	3.34	2.89	3.43 (31)
		Coccinella septempunctata	11.25	8.02	8.64	9.03	8.86 (80)
		Total	20	18.71	18.39	20.59	19.72 (174)
	Staphylinidae	Paederus fuscipes	23.75	15.51	9.19	14.08	13.29 (120)
	Chrysomelidae	Chrysochus cobaltinus	3.75	8.02	7.52	3.97	6.2 (56)
Total			100	100	100	100	100 (903)

Cotesia flavipe (1.00) showed least numbers, while some species of family Formicidae, i.e. *Formica lemani* (9.3%), *Camponotus itoi* (8.64%) were also observed. Some specimens of order Diptera (*Episyrphus balteatus* (0.66%), *Anopheles gambiae* (0.89%), and *Culex pipiens* (1.66%) were also observed during study period. The insect pest of order Lepidoptera *Spodoptera litura* (7.53%), *Spodoptera exigua* (9.19%) (family: Noctuidae), Order Hemiptera *Rhopalosiphum maidis* (5.87%) (family: Aphidae), *Nezara viridula* (4.87%) (family: Pentatomidae) order Orthoptera *Acrida exaltata* (2.55%) and *Mermiria bivittata* (2.21%) (family: Acrididae) was the serious pest of berseem (Table 1, Figure 3).

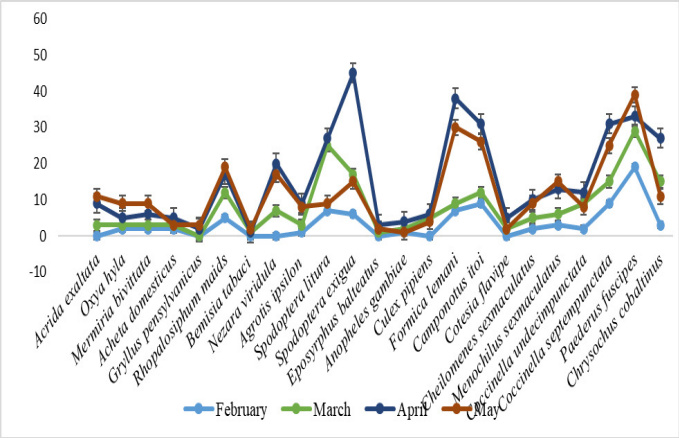

Figure 3: *Month wise diversity of insects in Barseem crop.*

Shannon diversity indices

Data was subjected to statistical analysis by applying Shannon diversity indices. Significant outcomes ($p<0.005$) were there for diversity (H'=2.814), species evenness (E= 0.725), and dominance (0.061). Difference in diversity among sample months was also recorded. Significant differences among diversity were recorded. Overall results showed the presence of highly significant differences in the diversity for fodder crop in the month of May (H'=2.819), followed by April (H'=2.804), March (H'=2.73) and February (H'=2.44) (Table 3).

Canonical corresponding analysis (CCA)

The ordination of canonical corresponding analysis showed that different factors of environment i.e. (temperature, humidity, rainfall, and wind speed) have different effect on species diversity. The present results showed that temperature have direct effect on *Agrotis ipsilon* and *Formica lemani*. Wind speed had significant effect on *Chrysochus cobaltinus, Coccinella undecimpunctata* and *Spodoptera exigua*. A strong association was also showed by the *Nezara viridula* and *Cotesia flavipes* towards the temperature and wind speed. Some other species like *Oxya hyla, Mermiria bivittate* and *Rhopalosiphum maids* showed least significance towards the environment variables. In other words, these species are least effected by temperature, humidity, rainfall and wind speed (Figure 4).

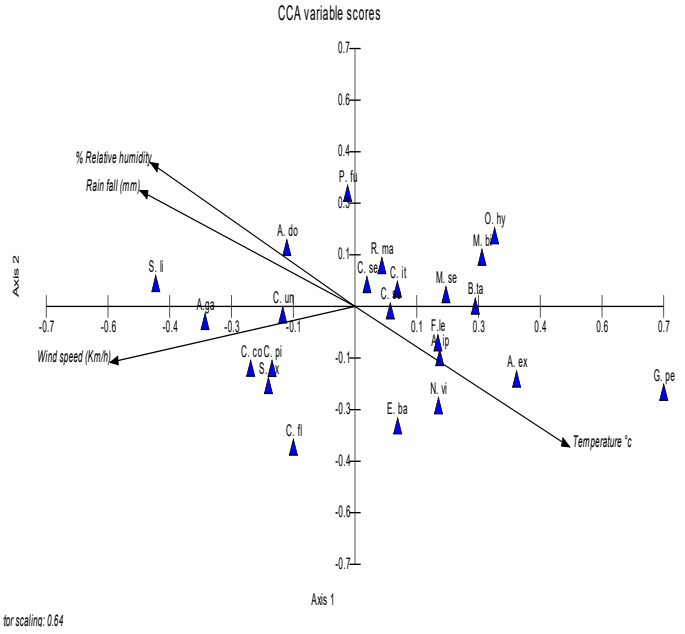

Figure 4: *Canonical corresponding analysis (CCA), ordination biplot showing the association of abundant species towards Fodder crop.*

Table 3: *Statistical Analysis of insect fauna sampled in different months from Berseem by Shannon diversity index.*

Months	N1	H1	E1	N2	H2	E2	t-test	DF	P-value
Feb vs March	80	2.444	0.72	187	2.737	0.701	-2.66	>120	0.00*
Feb vs April	80	2.444	0.72	359	2.803	0.717	-3.56	>120	0.000*
Feb vs May	80	2.444	0.72	277	2.819	0.729	-3.62	>120	0.000*
March vs April	187	2.736	0.7	359	2.804	0.717	-0.96	>120	0.33
March vs May	187	2.736	0.7	277	2.819	0.729	-0.96	>120	0.33
April vs May	359	2.804	0.72	277	2.819	0.729	-0.26	>120	0.789

Barseem is most important fodder crop, cultivated for domestic cattle and for profitable purposes (Sarwar *et al*., 2002). The present study highlights the richness of insect species comprising of 903 specimens representing 23 species belonging to six orders and 12 families associated with crop land in district Sialkot. A sharp difference in insect number is seen in different months. In Barseem crop, maximum number of insects was recorded in the month of April, while maximum numbers of species were found in the month of May. A sharp decline in the abundance of insects was recorded in the month of February (80 specimens) and in March (187 specimens) as compared to April (359 specimens) for berseem crop. Overall, relatively low diversity has been recorded in district Sialkot as compared to the other districts of Punjab (Inayat *et al*., 2010). The reason for this is mainly the excessive use of pesticides and insecticides. The excessive use of pesticide not only kills the pest species but also affects the other ketone species of ecosystem, resulting more harm. The widespread application of pesticides and chemical fertilizers controls the pests and weeds but can also affect the natural ecosystem very severely (Aslam *et al*., 2022). *Paederus fuscipes* belonging to the order Coleoptera was found as dominant species throughout the study period. Presence of insects belonging to order Orthoptera, Hemiptera, Lepidoptera, Diptera, Hymenoptera and Coleoptera was also reported by Inayat *et al*. (2010). The most common species in berseem crop is the *P. fuscipes*. According to Nasir *et al*. (2011), Basedow (1994) the reason of abundantly present *P. fuscipes* is moisture microclimate created by higher dense crop masses. Results of Maalik *et al*. (2013) for fodder crops are also in line with our findings.

Impact of environmental factors on abundance and distribution of species was also checked by applying CCA. Some species i.e., *Agrotis ipsilon, Acrida exaltata, Rhopalosiphum maids, Oxya hyla* and *Paederus fuscipes* all showed significant interactions with temperature. Pronounced effect of wind on insect's distribution while least effect of relative humidity has been recorded. The present findings coincide with the results of Siddiqi (2005) who related the effect of relative humidity with the emergence of insects and with the results of Maalik *et al*. (2013) who reported significant impact of temperature on Lepidopteran species. Fluctuation in temperature significantly affects the abundance and richness of insect fauna. Kutschbach-Brohl *et al*. (2010) also reported that variation in temperature had adverse effects on different insect orders like Hemiptera and Orthoptera.

One of the reasons for supporting high diversity in agro- ecosystems lands is the presence of weeds. Weeds naturally grow on crop edges, and it supports several micro and macro fauna (Hof and Bright, 2010). And it was also supported by Burgio *et al*. (2007) who found the presence of biodiversity significantly higher on weeds present on the edges of crops. During the present study, presence of weeds with supporting biodiversity has also been recorded in the fields of Sialkot. Different chemicals were used to control the growth of these weeds, which affects the biodiversity as well. These findings were also in line with Ruby *et al*. (2010). Present results will enhance the stability of agriculture ecosystem by using the selected species of insect as biological control agent against pest species. It will not only control the pest species but also increase the production of these crops.

Conclusions and Recommendations

The variety of living organisms indicated the health of an ecosystem. Among agroecosystem, barseem is proved to be a good shelter for many insect species. By decreasing the application of agrochemicals, the diversity and relative abundance of the insects can be increased.

Acknowledgements

Authors are highly thankful to Govt. College Women University for providing opportunity to conduct this research.

Novelty Statement

By exploring the diversity of insects in agroecosystem, insect conservational programs or pest control programs can be initiated.

Author's Contribution

Suneela collected the insect specimens and prepared the manuscript. S. Mushtaq and S. Maalik helped in identification of insects and formulation of manuscript. A. W. Qureshi proofread the manuscript. M. Batool helped in statistical analysis.

Conflict of interest

The authors have declared no conflict of interest.

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