A STUDY ON MOLECULAR TAXONOMY AND HOST SPECIES INTERACTION OF AGRICULTURALLY IMPORTANT INSECTS OF VADODARA DISTRICT

Research Synopsis for Ph. D.

Submitted to

Department of Zoology

Faculty of Science

The Maharaja Sayajirao University of Baroda, Vadodara

By

Ms. Linta Paulson

Guide

Prof. P. H. Parikh

Department of Zoology

Faculty of Science

The Maharaja Sayajirao University of Baroda

Vadodara - 390002

Gujarat, India.

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Introduction

Around 30 million species are found worldwide, of which about 1.4 million have briefly described; of these, about 750,000 are insects. Insects now comprise >75% of all described animal species and exhibit not only a wide variety of form, color, and shape but also a range of ecological adaptations unexcelled by any other group (Cigliano *et al.,* 2000). Because of their diversity and many roles, they are familiar to the general public. However, their conservation is a challenge (Jalali *et al.*, 2015). Since long, it has recognized and documented that insects are the most diverse group of organisms and, most authorities agree that more insect species have not described (named by science) than there are insect species that have been previously named (Vartan, 2016).

So far, about 100,000 species of insects known from India, which constitute two-third of the total fauna (Alfred *et al.*, 1998), but global biodiversity assessment estimates it to be 10-15 times more than this number (Thakur *et al.,* 2008). However, 59,353 species under 619 families of 27 orders known in India, which represents 6.08% of global insect diversity (Joshi *et al.,* 2016). Insects comprise the most diverse and successful group of multicellular organisms on the planet, and they contribute significantly to vital ecological functions such as pollination, pest control, decomposition, and maintenance of wildlife species (Losey and Vaughan, 2006). More than 10,000 species of insects damage worldwide food plants. Insect pests are a significant concern for farmers across the world, and more than 10,000 species of insects have recorded damaging crops (Dhaliwal *et al.,* 2007). Despite using various control methods, the control of agriculture pests continues to be critical for farmers. The yield loss by insects reaches as high as 60-70%. In India, agriculture is currently suffering an annual loss of about Rs. 8, 63, 884 million due to insect pests (Dhaliwal *et al.,* 2010). This massive crop loss causes the farmer to use an enormous amount of pesticides.

Among the many challenges in sustaining crop productivity and nutritional security, direct and indirect damages by insect pests is of paramount importance. The population of insect pest outbreaks has enormous potential to damage the agricultural economy. Recognizing the early signs of pests and diseases to deal with the problem is of crucial importance. Therefore, the accurate taxonomic identification is the pivotal issue in biological research, to allow the implementation of adequate measures to contend with species of agricultural concern (Karthika *et al.,* 2016).

The accurate identification of pest species is a prerequisite for the deployment of appropriate management strategies; it is critical to identify pest species accurately during the early phases of an invasion. Correct identification to the species level is also essential for the implementation of selective pest control measures. However, rapid identification of pests often impaired by uninformative morphological traits and a lack of available molecular data, such as species-specific DNA barcode. The main advantage of DNA barcoding is the rapid acquisition of molecular data (Monaghan *et al*., 2005). DNA barcoding is a diagnostic technique in which short DNA sequence(s) is used for species identification and is the most accurate and systemic tool for estimation of species diversity. (Hebert *et al.,* 2003; Floyd *et al*. 2009; Karthika *et al.,* 2016). DNA barcoding built by performing cytochrome oxidase subunit 1 (*COX 1)* sequencing on specimens previously identified by a taxonomist. Molecular identification and phylogeny using *COX 1* of the mitochondrial region regarded as efficient. Insect mitochondria contain two ribosomal RNA (rRNA) genes, 12S rRNA and 16S rRNA. 12s rRNA is highly conserved in insects and used for the study of genetic diversity in phyla, and the large subunit of 16S rDNA often used for families or genera (Mandal *et al.,* 2014). It estimated that over 900,000 species of insects known across the globe, with over 60,000 species described from India with nearly as many species remaining to be named. Barcode of Life Data system (BOLD) Systems populated with nearly 142,398 insect species barcodes out of which India has only 2758 barcodes; NBAII had 110 barcodes as in November 2013(Jalali *et al.,* 2015).

Domestication of nutritionally superior crops and monoculture of vegetables has surrogated ecological diversity of pests and has consequently led to their introduction and outbreak into new favorable areas resulting to the destruction of natural biotic communities, altered behaviors and population distribution (Brown *et al.,* 2009; Mishra *et al.,* 2015). There exists a robust ecological link between pest and their host plants. Host associations can be established by direct observations of feeding or by morphological or chemical studies of gut content (Jurado-Rivera *et al.,* 2009), but require precise identification of host plants. In plants, several 'barcode' loci have proposed (Kress and Erickson, 2007; Fazekas *et al.,* 2008; Lahaye *et al.,* 2008) for which representation in databases increases rapidly, improving the accuracy and speed of host plant identification. When used in comparative studies, e.g., for the analysis of host plant associations, the sequence fragments used by linking them to a named species or DNA-based group to which ecological information from literature or field observations has

associated (Hebert *et al.,* 2003). These groups and their host information provide the starting point for analysing co-evolutionary relationships of plants and pests.

Host plant selection by insect pests often divided into 'host plant finding' and 'host plant acceptance.' While the two are easy to separate conceptually, in practice, they are part of a continuum of three, rather than two, inextricably bonded links. Nonetheless, the central relationship of host plant finding thought previously to be governed by volatile chemicals, has, until now, proved intractable to scientific experimentation. On the other hand, to combat this problem, a new scientific approach in the 21st century has been developed where barcodes of plants and individual insect species used to find the homology between the two. Plants and their associated insect herbivores represent more than 50% of all known species on earth. The first step in understanding the mechanisms generating and maintaining this vital component of biodiversity is to identify plant-herbivore associations (García-Robledo *et al.,* 2013). Hence, we hypothesize that there has to be some sequence homology between various genes (conserved region), which can lead to a specific intrinsic interaction.

At the international level, several DNA-based field studies on intra-guild predation have reported (Gagnon *et al.,* 2011; Hautier *et al.,* 2011; Thomas *et al.,* 2012). Reports documenting predation on exotic pests in the field via molecular gut-content analysis are also available (Gardner *et al.,* 2013; Greenstone *et al.,* 2013; Opatovsky *et al.,* 2013; Grasswitz, 2016). Further, the importance of abiotic factors, as well as human interference with relation to plant-insect interaction at the molecular level, is well documented at the national level (Ali and Agrawal, 2012; Dawkar *et al.,* 2013; Kumar *et al.,* 2014; Mishra *et al.,* 2015). At the regional level, Singhal *et al.,* (2018) have made and attempt to unravel the diversity, phylogeny, and ecological role of cryptic Coleopteran species of Vadodara district. However, there is a lacuna as far as genetic studies on insects of agricultural fields and its host plant interaction are concerned. A key motivation of the present study is the economic significance of agricultural pests that are estimated to cause worldwide crop losses amounting to hundreds of billions of dollars annually (Kerchev *et al.,* 2012). ***Hence the present study aims to taxonomically identify important pest species and characterize them using the DNA barcode approach and to set a link between pest and host plant association by sequence homology approach.***

Objectives

Objective I: To Study the insect Diversity Pattern of agricultural fields of Vadodara.

Objective II. Composition and infestation rate of pest species

Objective III: To Study the Species Level identification of the economic important pest insect species using the DNA Barcode Approach.

3(a): Molecular characterization of important pest

Objective IV: To study phylogenetic correlation of host plants and pest interaction with particular reference to Coleopterans.

4(a): Correlation of insect DNA and plant DNA sequences

Material and Methods

Objective I:

(A) Collection of insects

A preliminary survey carried out for the presence of agriculture fields based on the crop pattern and type. Taking into the consideration of the accessibility, location and cropping pattern, four sites were selected, i.e., Ajwa (22.3751° N, 73.3851° E), Chhani (22.3633° N, 73.1658° E), Karjan (22.0535° N, 73.1202° E) and Padra (22.2394° N, 73.0848° E) areas of the Vadodara district.

Site 1: Ajwa: - Area has good vegetation, and in most of the agriculture fields, the farmers follow a multi-cropping pattern. The dominant crops and flora are Cotton, Chickpea, Pigeon pea, Maize, Ladies finger, Beans, Cabbage, Banana, Wheat, paddy, Drumstick, Sponge gourd and Ivy gourd.

Site 2: Chhani:- Region is more of herbs, and dominant crops are Cotton, Castor, Pigeon pea, Sorghum, Bajra, Brinjal, Spinach, Cabbage, Cauliflower, Beans, Mango, Banana, Hibiscus, Nerium, Marigold, Calotropis, Nerium, Hibiscus and Drumstick.

Site 3: Karjan:- Area is found with a great cover of vegetation, and the prominent type includes Pigeon pea, Castor, Sorghum, Cabbage, Spinach, Nerium, Hibiscus, Marigold, Canna, Calotropis, Datura, Thuver, Sponge gourd, Cotton, Pomegranate and Guava.

Site 4: Padra:- The fields are covered to be flourished with herbage, and the flora were Cotton, Castor, Sugarcane, Brinjal, Radish, Banana, Guava, Nerium, Hibiscus, Vinca, Calotropis, Pearl millet, Paddy, Jowar, Spinach, Mango, Lemon, Ladies finger and Fodder grass.

All the four sites were visited twice a month, and the sampling was done twice in a day: a) Morning hrs (6:30 am to 9:30 am) b) Evening hrs (4:30 pm to 6:30 pm). Along with direct observation and photo documentation, insects were collected manually through scientific methods.

(B) Methods of collection

i. **Sweeping net:** Sweeping net was used for capturing active flying insects. Insects trapped in the insect collecting net were then processed for further study.

ii. **Light trap:** Positively phototaxis insects or nocturnal insects were pulled together by the light trap method, where halogen bulb was kept at the study site, and the insect thus attracted were collected in the plastic container.

iii. **Pitfall trap:** Small Plastic cups filled with a mixture of 70% Ethyl alcohol and Glycerine were buried up to the rim in the ground so that passing insects may fall. This method was used to sample surface-active hymenopterans like ants.

iv. **Hand-picking:** Insects were collected from the barks of the tree by handpicking method and also leaf miners. Soil insects were also collected by handpicking and using berlese funnel.

(C) Preservation and Morphological identification of the insects

All the collected insects were then processed for further identification; the specimens were narcotized by exposure to Cyanide vapors, for maintaining its original color. Different features like the pinning of insects, spreading, and mounting of insect specimens were done before going for taxonomic study. Identification was done by using standard reference books, published articles and was confirmed by comparing with the authentic samples at Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Bombay Natural History Society (BNHS) Mumbai and Zoological Survey of India (ZSI) Kolkata, India.

The occurrence of the insects was checked during the entire study span of the study period was noted down of four study sites. Based on the number of times they were encountered, they were given an abundance rating. Those species sighted 32 of visits were rated Common, less than 15 of the visits were Uncommon, and less than of 5 visits were rated as Rare.

(D) Data Analysis:

All the four site samples were analysed separately and then the data were pooled to get year wise comparison of each site. Data was quantitatively analysed using standard analytical and statistical methods with computer software packages vis. Excel and Past 3x.

(E) Seasonal variations of insect Orders and its comparisons.

Data were analysed and the seasonal occurrence of the insects was checked concerning summer, monsoon, and winter seasons. Yearly comparison of each order has carried out with consideration of seasonal occurrence, and considering their occurrence, Insects species were categorized into various habits like; Bioindicators, Pests, Pollinators, Predators, and Scavengers.

Objective II:

(A)Assessment of insect species occurrence and Infestation Rate of Pests: The assessment of infestation of insect pests on agricultural fields of selected sites on various crops was done as per the scale given by Nagrare and his coworkers in the year 2011. (Central Institute for Cotton Research, Nagpur).

0-4 Scale infestation

0 Grade: No insect/ indecently seen

1 Grade: the scattered appearance of few insect pests on the plants

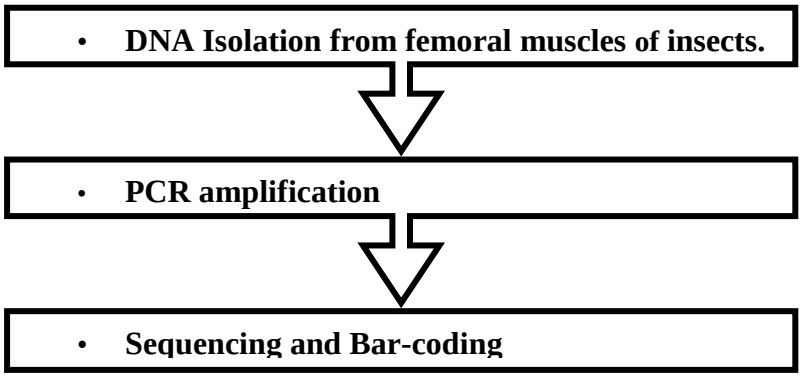
2 Grade: Severe incidence of insect pests on only one branch

3 Grade: Severe incidence of insect pests on more than one branch

4 Grade: Severe incidence of insect pests on whole plants were recorded

Objective III:

Overall experimental design:



Genomic DNA samples were prepared from fresh insects or insects preserved in 95% ethanol. Total genomic DNA was extracted from the femoral muscle of dissected legs of

specimens or complete specimens using Insect DNA Purification kit method and DNA quantification was done spectroscopically.

PCR Amplification

❖ **PCR primers for COI and 16SrRNA:**

Name of DNA marker and primer	**Primer sequence (5' to 3')**	**Reference**
LCO-1490 *(COX I)*	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.*, 1994
HCO-2198 *(COX I)*	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.*, 1994
16s rDNA		
16SA (reverse)	5'-CGC CTG TTT ATC AAA AAC AT-3'	(Simon *et al.*, 1994)
16SB (forward)	5'-CCG GTT GAA CTC AGA TCA-3'	(Kambhampati and Smith, 1995)

❖ **PCR Conditions:**

- 94°C for 3 min.
- 39 cycles of :
  - 94°C for 20 sec.
  - 50°C for 20 sec.
  - 72°C for 30 sec.
  - 72°C for 5 min.
- Hold at 4°C

❖ **Electrophoresis of PCR reactions**

Sequencing was carried out using the Sanger sequencing method. The generated barcode sequences were compared with the previous sequences at Gene bank data for confirmation of the morphometric identifications.

Sequence Validation and Bioinformatics Analysis

- %GC content analysis

  The sequence was validated, and specific contigs of each insect sequence were carried out by using BioEdit 7.0.5.3. Software

  Further, the sequences were submitted to NCBI/ BOLD v.4 having process Id
- AT and GC skew analysis

The sequenced data were subjected to strand asymmetric analysis which was calculated using the formula(s) AT-skew = (A−T)/(A C T), GC skew = (G−C)/(G C C).

- Phylogenetic Analysis

  Phylogenetic analysis was carried out with the help of MEGA X software for maximum parsimony likelihood

Objective IV:

- Correlation of insect DNA and plant DNA sequences.

  For plant – pest interaction genomic sequence (trnL) was compared and the minimum distance calculated to prove their ecological association

❖ **PCR primers for trnL:**

Name of DNA marker and primer	**Primer sequence (5' to 3')**	**Reference**
trnL intron		
c A49325	5'CGAAATCGGTAGACGCTACG	Taberlet *et al.*, 1991
d B49863	5'GGGGATAGAGGGACTTGAAC	Taberlet *et al.*, 1991

❖ **PCR Conditions:**

- 60°C for 3 min.
- 16 cycles with decreasing annealing temperature from 60 to 43°C 60 sec.
- 27 cycles
  - 42°C 60 sec.
  - 94°C for 30 sec.
  - 72 °C for 60sec.
- Hold at 4°C

❖ **Electrophoresis of PCR reactions**

Sequencing was carried out using the Sanger sequencing method.

Homology Analysis: The sequence obtained carried out blast using NCBI/BOLD system, and minimum distance was calculated using neighborhood analysis.

Results

Objective I:

Order	Family	Species	Species			
			Site I	Site II	Site III	Site IV
Thysanura	1	2	2	1	0	0
Odonata	4	13	8	5	7	7
Orthoptera	7	67	31	23	14	30
Isoptera	1	5	0	2	3	2
Dictyoptera	4	4	3	2	2	3
Hemiptera	16	31	24	15	17	18
Thysanoptera	1	3	1	1	1	1
Neuroptera	2	3	2	0	2	0
Coleoptera	26	153	78	68	66	64
Diptera	15	31	16	14	18	15
Lepidoptera	13	36	18	27	21	15
Hymenoptera	9	42	28	23	23	26

Table 1.1: Total number of Insect Orders with number of Species and families

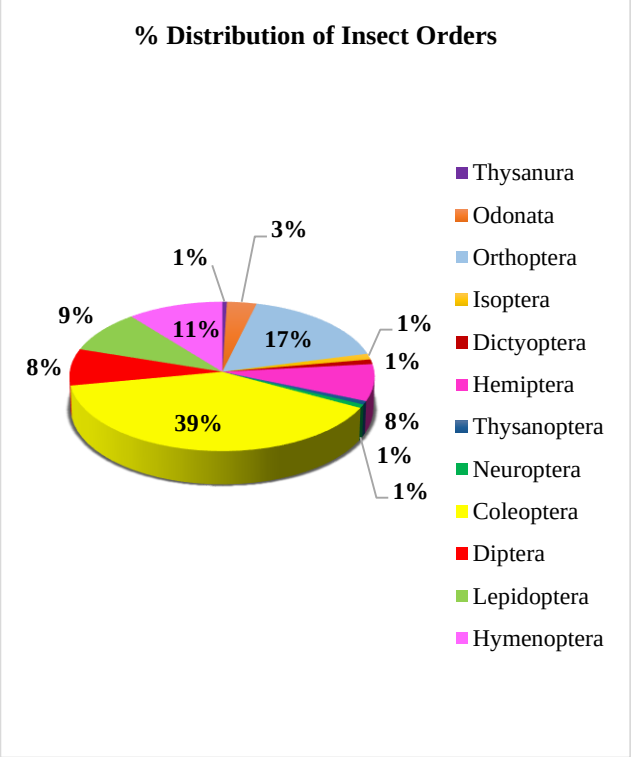


Figure1.1: Percentage Distribution of Insect Orders in the four Sites of Vadodara

In the present study, a total of 390 species of insects representing 12 orders and 99 families are recorded. Table 1.1 describes the list of insect Orders with Species and families. An analysis of % distribution of the species richness is presented in Figure 1.1, which reveals that the maximum species belongs to order Coleoptera with 39% followed by Orthoptera (17%), Hymenoptera (11%), Lepidoptera (9%), Diptera and Hemiptera (8%), Odonata (3%)and the least represented by Isoptera, Neuroptera, Thysanoptera and Dictyoptera (1%). All the four agricultural fields enriched with the insect species of different orders. An analysis of the total number of the individuals collected exhibited marked variations.

Based on field observations and specimens collected, insect orders were divided into three broad categories viz. Common, Uncommon and Rare. A total of 12 insect orders were recorded in the present study. Five orders (Orthoptera, Coleoptera, Hemiptera, Lepidoptera and Hymenoptera) were Common in all four sites, orders Tysanura was Uncommon at Site I and II and was Rare at Site III and IV. Order Odonata was found to be Common at Site I and IV and was recorded Uncommon at Site II and Rare at Site III. Representatives from

Dictyoptera was Uncommon at Site I and Site IV and was Rare at Site II and III. Termites representing order Isoptera was Common at Site I and III and Uncommon at Site II and IV. Order Thysanoptera was Common at Site I and II and was Rare at Site III and IV. Order Neuroptera was Uncommon at Site I and III and Rare at Site II and IV. Order Diptera was Common at Site III and was Uncommon at Site I, II and IV. (Table 1.2).

Order	SiteI	SiteII	SiteIII	SiteIV
Thysanura	UC	UC	R	R
Odonata	C	UC	R	C
Orthoptera	C	C	C	C
Dictyoptera	UC	R	R	UC
Isoptera	C	UC	C	UC
Hemiptera	C	C	C	C
Thysanoptera	C	C	R	R
Neuroptera	UC	R	UC	R
Coleoptera	C	C	C	C
Diptera	UC	UC	C	UC
Lepidoptera	C	C	C	C
Hymenoptera	C	C	C	C

Table 1.2: The Insect species occurrence with respect to Common(C), Uncommon (UC) and Rare (R) in four sites

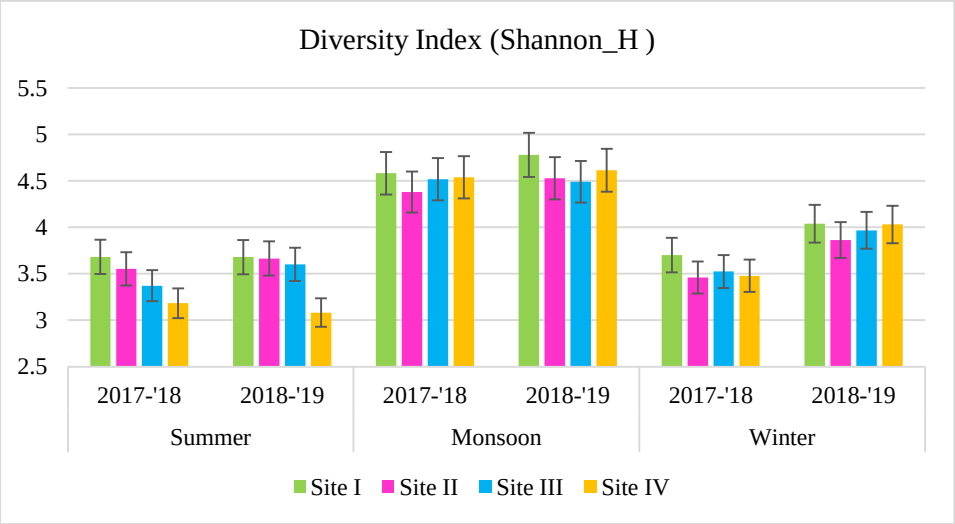


Figure.1.2 Comparative study of Diversity Index of four sites with year variation

Diversity Index						
Area	Summer		Monsoon		Winter	
	2017-'18	2018-'19	2017-'18	2018-'19	2017-'18	2018-'19
Site I	3.681	3.68	4.582	4.78	3.701	4.039
Site II	3.553	3.665	4.38	4.528	3.46	3.863
Site III	3.372	3.601	4.518	4.49	3.524	3.967
Site IV	3.183	3.082	4.538	4.614	3.478	4.032

Table1.3. Comparative study of Diversity Index of four sites with year variation

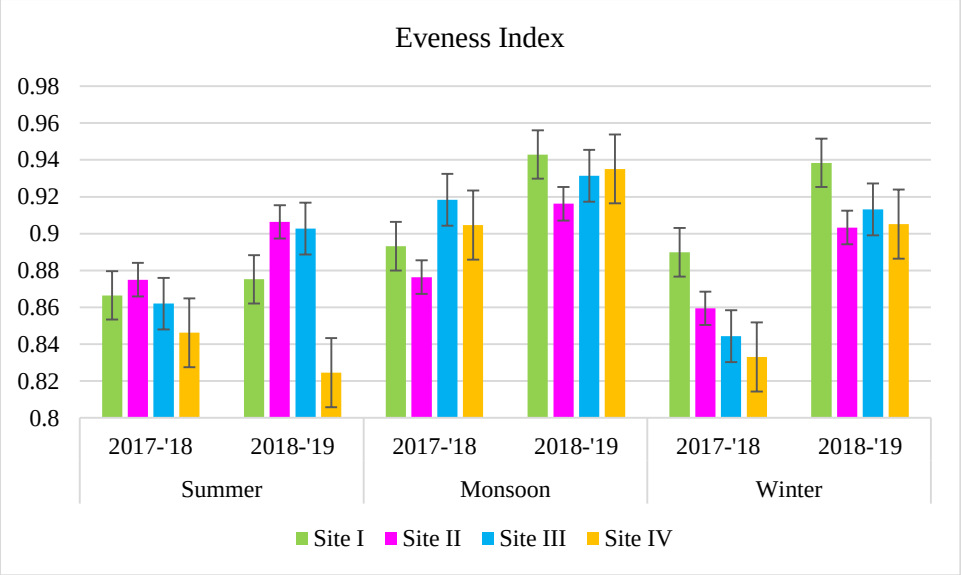


Figure.1.3 Comparative study of Evenness Index of four sites with year variation

Evenness Index						
	Summer		Monsoon		Winter	
	2017-'18	2018-'19	2017-'18	2018-'19	2017-'18	2018-'19
Site I	0.8665	0.8752	0.8932	0.9429	0.8899	0.9384
Site II	0.875	0.9064	0.8764	0.9162	0.8595	0.9033
Site III	0.862	0.9027	0.9183	0.9314	0.8443	0.9132
Site IV	0.8462	0.8245	0.9046	0.9351	0.8331	0.9052

Table1.4. Comparative study of Evenness Index of four sites with year variation

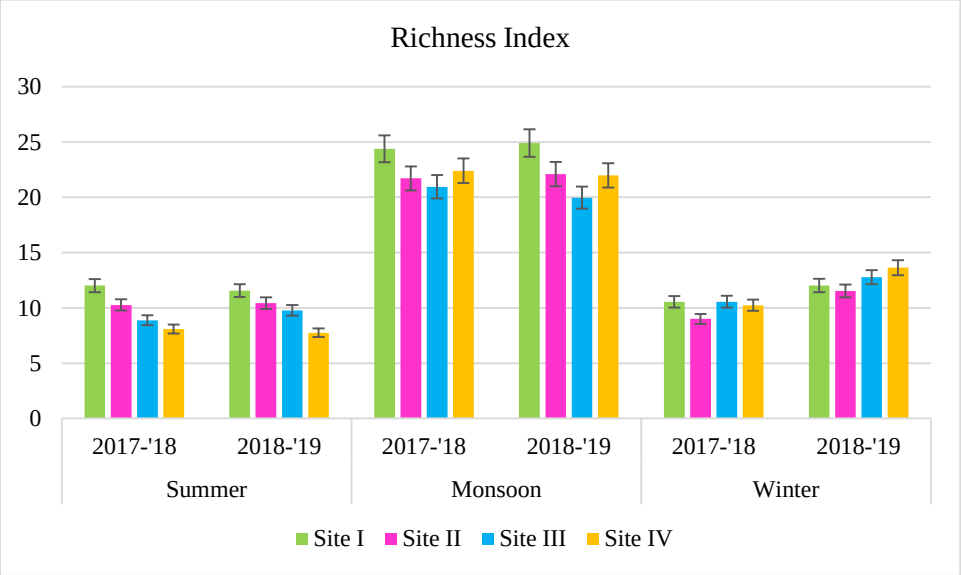


Figure.1.4 Comparative study of Richness Index of four sites with year variation

Richness index						
	Summer		Monsoon		Winter	
	2017-'18	2018-'19	2017-'18	2018-'19	2017-'18	2018-'19
Site I	12.01	11.56	24.38	24.91	10.55	12.03
Site II	10.27	10.43	21.71	22.1	9.006	11.53
Site III	8.874	9.778	20.95	19.96	10.56	12.77
Site IV	8.088	7.753	22.4	21.99	10.24	13.63

Table1.5. Comparative study of Richness Index of four sites with year variation

During the current study, the Shannon Weiner index (Species' diversity), Marglef's index (Species' richness), and Buzas and Gibson's index (Species' evenness) were computed using the data to facilitate comparison between the sites and season over two years. The results revealed that the maximum diversity was in Monsoon season for both the years at all the sites. Site I (Ajwa) was much diverse and rich in terms of insect fauna compared to other three Sites and in winter as well as summer there was declining. (Figure 1.2 & Table 1.3). Year wise results revealed that there was a maximum diversity of insects in the year 2018-'19 compared to 2017 – '18. Evenness of the insect fauna was parallel with the diversity and thus had similar spatial and temporal changes. During summer, Site II showed maximum evenness in both the years. During Monsoon, Site III resulted with more even group of insects in the year 2017 – '18, and Site I showed maximum in the year 2018- '19 . During winter, Site I revealed to be maximum even insect fauna both the years of study period. Richness of insect species is seen more during monsoon season in both the years. Comparing the Sites with seasons of both years Site I got the maximum richness of insect species both in summer as well as Monsoon. During winter, Site III showed more richness of insect fauna in the year 2017- '18 and Site IV has maximum richness in the year 2018 – '19.

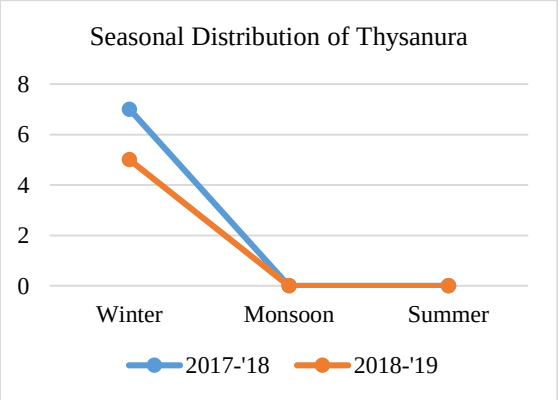


Figure 1.5: Seasonal variation of order Thysanura from 2017 to 2019

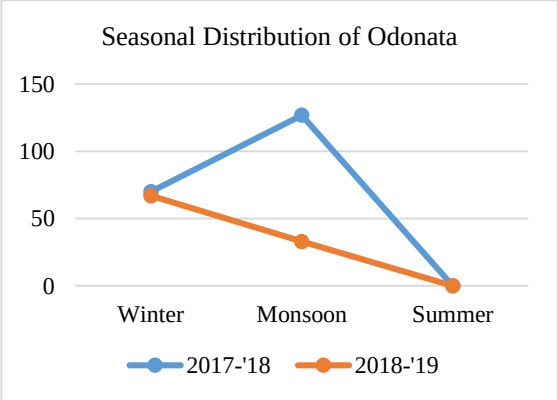


Figure 1.6: Seasonal variation of order Odonata from 2017 to 2019

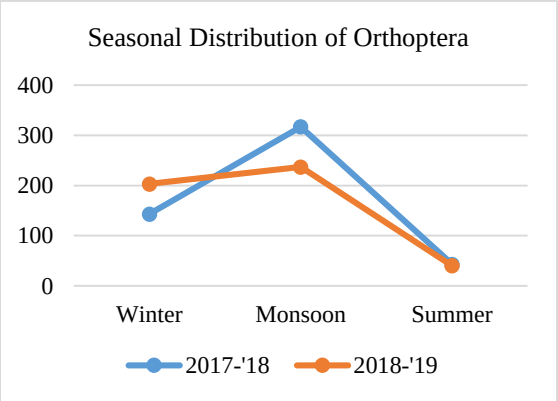


Figure 1.7: Seasonal variation of order Orthoptera from 2017 to 2019

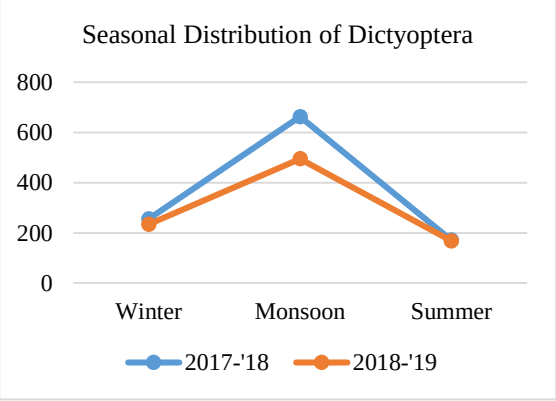


Figure 1.8: Seasonal variation of order Dictyoptera from 2017 to 2019

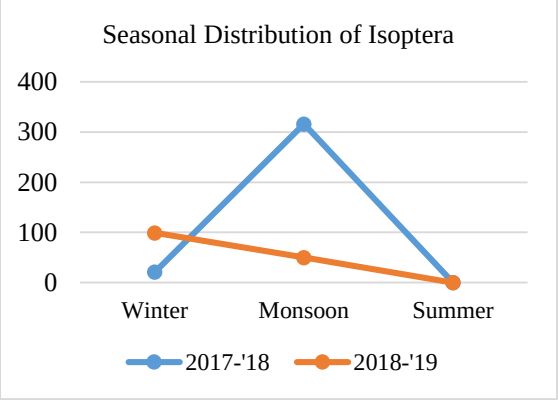


Figure 1.8: Seasonal variation of order Isoptera from 2017 to 2019

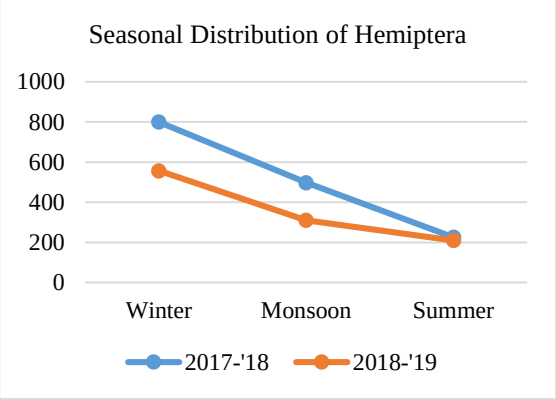


Figure 1.9:Seasonal variation of order Hemiptera from 2017 to 2019

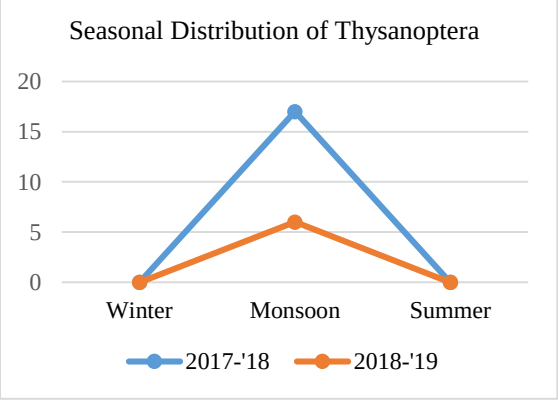


Figure 1.10: Seasonal variation of order Thysanoptera from 2017 to 2019

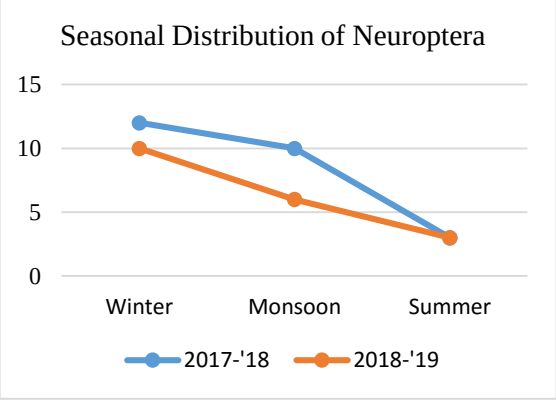


Figure 1.11: Seasonal variation of order Neuroptera from 2017 to 2019

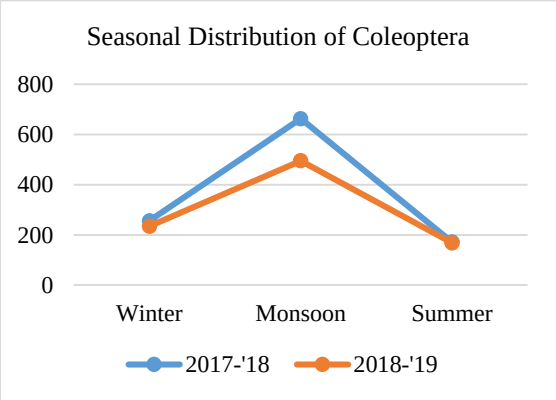


Figure 1.12: Seasonal variation of order Coleoptera from 2017 to 2019

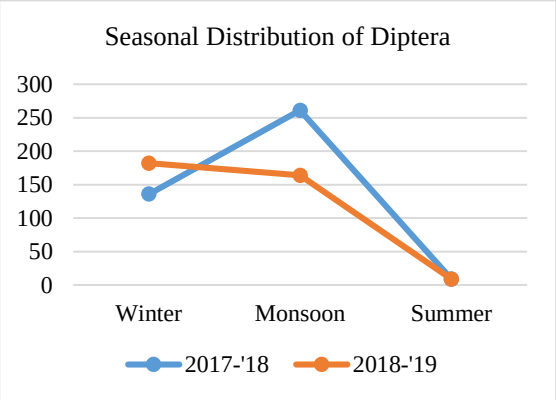


Figure 1.13: Seasonal variation of order Diptera from 2017 to 2019

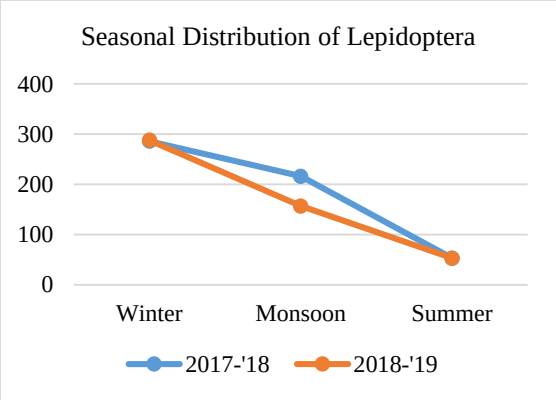


Figure 1.14: Seasonal variation of order Lepidoptera from 2017 to 2019

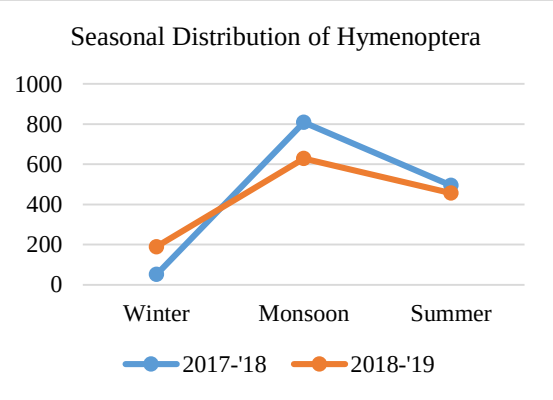


Figure 1.15:Seasonal variation of order Hymenoptera from 2017 to 2019

There was a distinct seasonal variation of the insects, the majority of the orders were found to be monsoon dominant (Odonata, Dictyoptera, Orthoptera, Isoptera, Thysanoptera, Diptera, Coleoptera and Hymenoptera), and year wise comparison revealed that 2017-‘18 had a good number of the individuals compared to 2018-‘19. The winter dominant orders were (Thysanura, Hemiptera, Neuroptera and Lepidoptera). Overall summer season had the minimum number of the representatives from Orthoptera, Odonata, Diptera, Coleoptera, Hemiptera, Lepidoptera, Hymenoptera, and Neuroptera. No representatives were found from the orders, Thysanura, Isoptera, Thysanoptera and Dictyoptera.

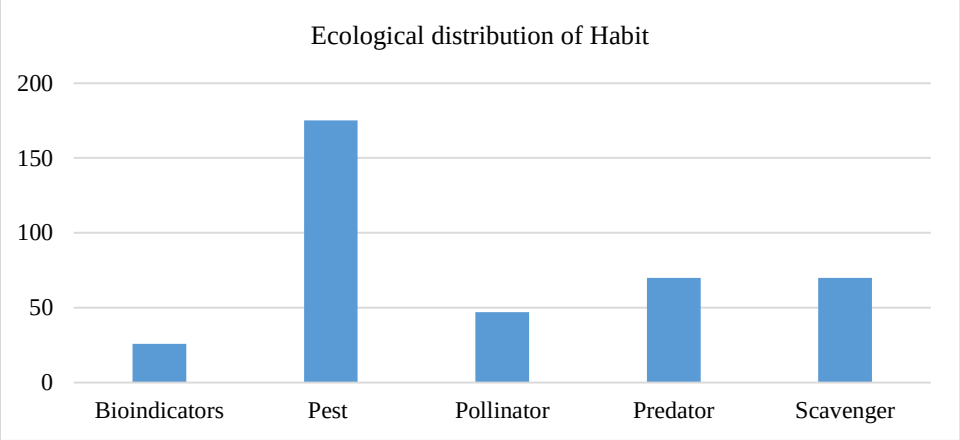


Figure 1.16: Distribution of insects according to their Ecological role

Based on the ecological role of the insects, the collected species were categorized as Bioindicators, Pests, Pollinators, Predators, and Scavengers. When the distribution pattern was studied it revealed that the Pest species were maximum in number, followed by Predators and Scavengers, whereas the Pollinators and Bio-indicators were found as less in number.

Objective II:

A total of 173 pest species belonging to 7 orders (Coleoptera, Hemiptera, Orthoptera, Lepidoptera, Diptera, Isoptera, Thysanoptera, Thysanura and Hymenoptera) were recorded during the study period. Overall a considerable number of the pests were seen in only four orders viz. Coleoptera, Orthoptera, Lepidoptera and Hemiptera were taken into consideration for the infestation rate grading. Members of order Coleopteran were found to be the most dominant with 65 pest species spread in 18 families, next in order of the number of representatives was Order Orthoptera with 35 species belonging to 4 families. Lepidoptera was recorded with 30 species spread in 11 families and last in the order of number of pest species was Hemiptera with 27 species represented by 13 families (Figure 2.1)

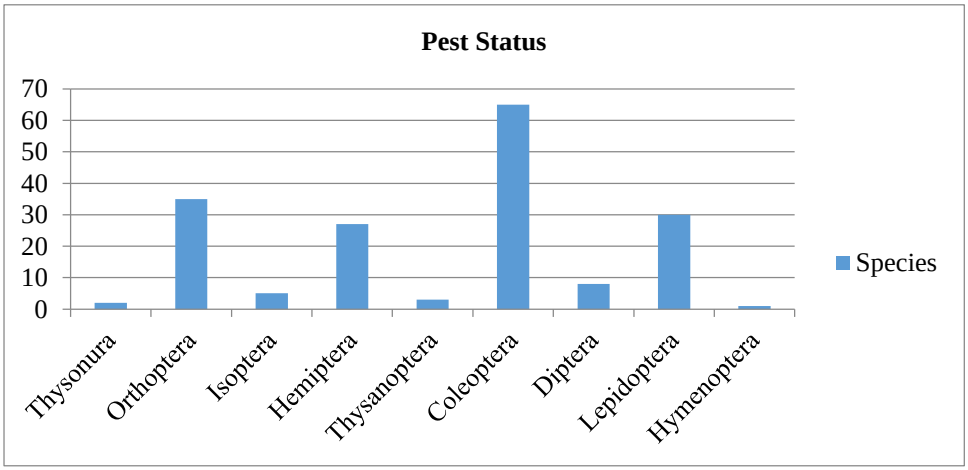


Figure 2.1: Pest status of the insect orders

Order	*Scientific Name*	Host Plant	2017 -'18			2018 - '19		
			Summer	Monsoon	Winter	Summer	Monsoon	Winter
Orthoptera	*Acorypha glaucopsis*	Fodder grass	0	2	0	0	2	0
	Acrida conica (Fabricius, 1781)	Paddy, Maize	0	4	3	0	3	4
	Acrida exaltata (Walker, 1859)	Paddy, Maize	0	4	4	0	3	4
	Acrida ungarica (Herbst , 1786)	Paddy, Fodder grass	0	4	0	0	1	3
	Acrida willemsei	Millet	0	0	0	0	2	0
	Acrotylus umbertianus	Fodder grass	0	4	2	0	0	2
	Aiolopus thalassinus (Fabricius, 1781)	Paddy, Millet	0	4	3	0	0	2
	Calliptamus sp.	Pegion pea	0	1	0	0	0	0
	Catantops humilis (Bolivar, 1902)	Maize, Banana	0	3	2	0	2	3
	Choroedocus robustus (Serville, 1838)	Maize, Banana	0	2	1	0	1	1
	Chorthippus curtipennis (Harris, 1835)	Fodder grass	0	3	0	0	2	0
	Euthystria brachyptera	Fodder grass	1	4	2	1	1	2
	Hieroglyphus banian (Fabricius , 1798)	Paddy	0	4	3	0	0	2
	Locusta migratoria (Linnaeus, 1758)	Wheat, Millet	3	2	2	3	1	1
	Melanoplus femurrubrum (De Geer, 1773)	Paddy,Maize	2	4	2	2	3	2
	Metaleptea brevicornis (Johannson, 1763)	Paddy, Maize	0	3	2	0	3	2
	Omecestus sp.	Fodder grass	0	4	0	0	1	0
	Omocestus viridulus (Linnaeus, 1758)	Fodder grass	0	3	0	0	1	0
	Orphulella pelidna (Burmeister 1838)	Fodder grass	0	4	0	0	3	0
	Oxya hyla hyla (Serville, 1831)	Paddy	0	4	0	0	3	0
	Oxya hyla intricata (Stål, 1861)	Paddy	0	2	0	0	2	0
	Schistocera gregaria (Forskål, 1775)	Maize, Sugarcane	0	4	4	0	4	4
	Schistocera sp	Paddy	0	0	0	0	0	0
	Sphingonatus sp	Fodder grass	0	1	1	0	1	1
	Trilophidia annulata (Thunberg, 1815)	Paddy	0	2	0	0	2	0
	Xenocatantops humilis (Serville, 1838)	Paddy, Fodder grass	1	3	3	1	3	2
	Acheta domesticus (Linnaeus, 1758)	Paddy	0	3	0	0	3	0
	Chrotogonus sp	Millet, Wheat	0	1	0	0	1	0
	Poekilocerus pictus (Fabricius, 1775)	Calotropis	0	4	0	0	3	0
	Amblycorypha rotundifolia (Scudder, 1862)	Cotton	0	0	0	0	0	0
	Mecopoda elongata (Linnaeus, 1758)	Cotton	0	1	0	0	1	0
	Neoconocephalus velox (Rehn & Hebard, 1914)	Fodder grass	0	1	0	0	1	0
	Ducetia japonica (Thunberg, 1815)	Brinjal	0	1	0	0	1	0
	Scudderia furcata (Wattenwyl, 1878)	Citrus	2	1	0	2	1	0
	Trigonocorypha unicolor (Stoll, 1787)	Fodder grass	1	3	0	2	2	0
Hemiptera	*Aleurodicus disperses* (Russell, 1965)	Spinach, Cabbage	0	2	0	0	4	0
	Aphis gossypii (Glover, 1877)	Cotton	1	4	4	1	4	4
	Empoasca decipiens (Paoli, 1930)	Brinjal Cabbage	4	4	4	4	4	4
	Drepanococcus cajani (Maskell, 1891)	Pigeonpea, Guava	4	4	4	4	4	4
	Phenacoccus madeirensis	Tomato,Brinjal	4	4	4	4	4	4
	Acanthocephala femorata (Fabricius 1775)	Sponge gourd	0	3	0	0	2	0
	Cletomorpha Benita (Kirby, 1891)	Raddish	0	3	0	0	0	0
	Cletus punctiger (Dallas, 1852)	Sugarcane, Paddy	0	3	4	0	3	4
	Homoeocerus signatus (Walker, 1871)	Spinach	0	0	0	2	2	4
	Pamendanga sp.	Banana	0	0	0	0	0	0
	Proutista moesta (Westwood, 1851)	Maize, Sugarcane	3	4	4	3	4	4
	Rhynchomitra microrhina (Walker, 1851)	*Asclepias syriaca*	0	1	0	0	0	0
	Coridius janus (Fabricius, 1775)	Sponge gourd, Brinjal	0	3	0	0	2	4
	Leptocentrus moringae	Drumsticks, Cotton,	0	0	0	0	2	1
	Pyrilla perpusilla (Walker, 1851)	Maize, Wheat, Paddy	4	4	3	4	3	4
	Acanthuchus trispinifer (Fairmaire, 1846)	*Acacia decurrens*	2	4	2	1	4	1
	Oxyrachis tarandus	Mulberry	0	4	4	0	4	4

	Bagrada hilaris (Burmeister, 1835)	Cauliflower, Cabbage	0	3	0	0	2	0
	Eysarcoris guttiger (Scopoli, 1763)	*Murraya koenigii*	0	0	0	0	3	0
	Halyomorpha halys (Stål, 1855)	Tomato, Spinach	0	4	0	0	2	0
	Nezara viridula (Linnaeus, 1758)	Cotton, Okra, Castor	0	4	0	0	2	0
	Palomena prasina (Linnaeus, 1761)	Tomato	0	3	2	0	1	2
	Megacopta cribraria (Fabricius, 1798)	Pigeonpea, Cowpea	4	4	4	4	4	4
	Plautia affinis (Dallas, 1851)	Spinach,Brinjal	0	0	3	0	0	3
	Phelanococcus sp.	Spinach, Cabbage	0	0	4	0	0	0
	Dysdercus koenigii (Fabricius, 1775)	Cotton	2	4	1	2	2	3
	Dysdercus cingulatus (Fabricius, 1775)	Cotton	0	4	0	0	2	0
Coleoptera	*Lasioderma serricorne* (Fabricius, 1792)	Paddy	0	0	0	0	1	0
	Formicomus sp.	Paddy, Sunflower	1	0	1	0	0	0
	Apion clavipes	Green gram	0	3	0	0	2	0
	Paratrachelophorus sp.	Tobacco	0	0	1	0	0	0
	Acmaeodera sp.	Ivy gourd	0	0	1	0	0	0
	Acmaeodera viridaenea (Eschscholtz, 1829)	Mango tree	3	0	2	3	0	2
	Craspedophorus saundersi (Chaudoir, 1869)	Guava fruits	0	1	0	0	0	0
	Acanthophorous serraticornis (Olivier, 1795)	Mango tree	0	3	0	0	2	0
	Batocera rufomaculata (De Geer, 1775)	Mango tree	0	4	0	0	2	0
	Celosterna scabrator (Fabricius)	Mango, Pomegranate	4	0	0	3	0	0
	Dectes texanus	Sunflower	0	0	0	0	1	3
	Derobrachus hovorei (Santos-Silva, 2007)	Citrus	1	4	3	1	2	3
	Macrotoma palmate (Fabricius)	Acacia stem borer	0	3	0	0	3	0
	Prionus californicus (Motschulsky)	Cotton root borer	0	3	0	0	2	0
	Trachysida sp.	Sponge gourd	0	2	0	0	0	0
	Xylotrechus stebbingi (Gahan 1906)	Mulberries	0	2	0	0	2	0
	Altica sp (Woods 1917)	Radishes, Cabbage,	0	2	0	0	1	0
	Aspidomorpha miliaris (Fabricius, 1775)	*Ipomoea carnea*	0	0	0	0	0	0
	Aulacophora lewissi (Baly, 1886)	Sponge gourd	0	2	0	0	2	0
	Aulacophora nigripennis (Motschulsky, 1857)	*Ipomoea carnea*	0	4	4	0	4	4
	Aulacophora foveicollis (Lucas, 1849)	Bottle Gourd	0	1	0	0	1	0
	Cassida circumdata	Sweet potato	0	1	1	0	0	0
	Cassida sp.	Sugar beet, Spinach	0	0	0	0	0	0
	Chiridopsis bipunctata (Linnaeus, 1767)	Sweet potato	0	1	0	0	1	0
	Chrysochus cobaltinus (LeConte, 1857)	*Apocynum cannabinum*	0	2	0	0	1	0
	Clytra laeviuscula (Ratzeburg, 1837)	Sweet potato	0	0	0	0	0	0
	Metriona bicolor (Fabricius)	Sweet potato	0	3	0	0	2	0
	Oides bipunctata (Fabricus, 1781)	*Cayratia trifolia*	2	0	0	2	0	0
	Oides palleata (Fabricius, 1781)	*Cayratia trifolia*	0	4	0	0	0	0
	Podagrica fuscicornis (Linnaeus, 1767)	Drumstick	0	3	0	0	2	0
	Sindia clathrata (Olivier,1808)	Drumstick	0	4	0	0	2	0
	Epilachna ocellata (Redtenbacher)	Brinjal, Bitter gourd	0	0	0	0	4	0
	Cleonus sp.	Sugar beet	2	0	0	2	0	0
	Cosmopolites sordidus (Germar, 1824)	Banana	3	0	0	2	0	0
	Hypera postica (Gyllenhal, 1813)	Castor	4	0	0	4	0	0
	*Myllocerus subfasciatus (*Guerin-Meneville, 1843)	Drumsticks, Brinjal	4	4	4	4	4	4
	Myllocerus viridanus	Maize, Castor	0	3	0	0	0	0
	Polydrusus formosus (Mayer, 1779)	Castor	0	4	0	0	3	0
	Sitophilus oryzae	Paddy, Maize	2	2	0	2	2	0
	Lanelater fuscipes (Fabricius, 1775)	Coconut	0	2	0	0	2	0
	Cryptolestes pusillus (Schönherr, 1817)	Paddy, Maize	0	4	0	0	3	0
	Lytta caraganae (Pallas, 1798)	Pea	0	2	0	0	0	0
	Mylabris cichorii (Linnaeus, 1767)	Sponge gourd	2	4	4	2	4	4
	Mylabris pustulata (Thunberg, 1821)	Sponge gourd, Okra	0	4	0	0	3	0
	Mylabris variabilis (Pallas, 1782)	Sponge gourd	0	4	0	0	3	0
	Psalydolytta rouxi	Maize, Paddy	0	1	0	0	2	0
	Cetonia funesta (Poda, 1761)	Millet	1	2	0	1	2	0
	Chiloloba sp	Millet	0	0	0	0	2	0

	Chiloloba acuta (Wiedemann, 1823)	Millet, Maize	0	0	0	0	4	2
	Cyclocephala pasadenae (Casey, 1915)	Maize	0	0	0	0	3	0
	Holotrichia reynaudi (Blanchard, 1851)	Ground nut	0	0	0	0	2	0
	*Oryctes nasicora (*Linnaeus, 1758)	Coconut	0	0	0	0	2	0
	Oryctes rhinoceros	Coconut	0	0	0	0	2	0
	Oxycetonia jucunda (Falderman, 1835)	Citrus	0	4	0	0	4	0
	Oxycetonia versicolor (Fabricius, 1775)	Brinjal, Green gram	0	3	0	0	3	0
	Phyllophaga nebulosa (Polihronakis, 2007)	Conifers	0	4	0	0	3	4
	*Phyllophaga obsolete (*Blanchard, 1851)	Fodders grass	0	0	0	0	0	0
	Phyllophaga sp.	Fodders grass	0	4	2	0	0	0
	*Protaetia alboguttata (*Vigors, 1826)	Maize, Brinjal	0	4	0	0	3	0
	Protaetia aurichalcea (Fabricius, 1775)	Cluster bean	1	3	0	1	0	2
	Protaetia squamipennis (Burmeister, 1842)	Maize, Brinjal	0	1	0	0	1	0
	Oryzaephilus surinamensis (Linnaeus, 1758)	Maize, Ground nut	0	0	0	0	0	0
	Gonocephalum sp.	Ground nut	0	0	1	0	0	1
	*Tenebrio molitor (*Linnaeus, 1758)	Ground nut	0	0	0	0	0	0
	Agrilus acutus (Thunberg, 1787)	Hibiscus	0	0	0	0	0	0
Lepidoptera	*Cnaphalocrocis madinalis* (Guenée, 1854)	Paddy	3	0	0	3	0	0
	Hellula undalis (Fabricius, 1794)	Cabbage, Cauliflower	0	4	0	0	4	0
	*Leucinodes orbonalis (*Guenée, 1854)	Brinjal	0	0	0	0	0	0
	Noorda blitealis (Walker, 1859)	Drumsticks	0	0	4	0	0	4
	Noorda moringae	Drumsticks	0	3	4	0	1	4
	Protrigonia zizanialis (Swinhoe, 1886)	Drumsticks	0	0	3	0	0	3
	Scirpophaga incertulas (Walker, 1863)	Paddy	0	0	3	0	0	2
	*Asota caricae (*Fabricius, 1775)	*Ficus sp., teak*	0	0	0	0	0	0
	Spilosoma oblique (Walker, 1855)	Castor	0	0	3	0	0	2
	Eudocima materna	Citrus	2	4	2	2	2	2
	Hemerocampa leucostigma	Castor	0	4	2	0	2	2
	Eupterote germinate	Drumsticks	0	3	0	0	3	0
	Eupterote mollifera (Walker, 1865)	Drumsticks	0	4	2	0	2	2
	Pectinophora gossypiella (Saunders, 1844)	Cotton	0	0	4	0	0	4
	*Euproctis lunata (*Walker, 1855)	Castor	0	4	4	0	1	4
	Spodoptera exigua	Cabbage	2	4	4	2	1	4
	Helicoverpa armigera	Tomato,Cotton, Paddy	0	4	4	0	4	4
	Olene mendosa	Castor	0	2	3	0	2	3
	Spodoptera frugiperda	Cabbage, Cauliflower	0	4	4	0	4	4
	Trichoplusia ni	Cabbage	0	0	0	4	0	0
	Earias insulana (Boisduval, 1833)	Cotton, Okra	0	4	4	0	2	4
	Ariadne ariadna indica	Castor	3	4	0	3	2	0
	*Ariadne merione (*Cramer, 1777)	Castor	2	1	4	2	1	4
	Danaus chrysippus (Linnaeus, 1758)	*Calotropis, Hibiscus*	2	3	2	2	3	2
	Hypolimnas misippus (Linnaeus,. 1764).	Okra	0	0	0	0	0	0
	Junonia almanac (Linnaeus, 1758)	Citrus	0	3	2	0	3	3
	Eurena hecabe (Linnaeus, 1758)	Pea, Beans.	3	0	0	3	0	0
	Plutella xylostella (Linnaeus, 1758)	Cabbage, Cauliflower	0	4	0	0	4	0
	Euzophera perticella (Ragonot, 1888)	Brinjal	0	4	2	0	4	2
	Earias vitella	Cotton, Okra	0	3	4	0	2	4

Table.2.1. List of pest species with its seasonal infestation grade

The rate of infestation was also monitored. On observation, it was found that a wide range of insect pests was damaging the agricultural fields. Preference of the vegetation type by the pest species revealed that Orthopterans had a dominant choice for Paddy, Millet, Wheat, Fodder grass, and Maize; it was the Site I and IV with a significant number of the Orthopteran pests due to the dominance of the preferred host plants. Polyphagous

Hemipterans were found to infest Cotton, Brinjal, Cauliflower, Cabbage, Chickpea, and Sponge Gourd. All the host plants occur in the study areas, and due to their small size as well as polyphagous feeding habit, a good assemblage was seen. Order Coleoptera had the highest number of pest representatives. Irrespective of the host preference, the Coleopteran pests were seen at all the Sites with a significant amount. Lepidopteran pests were found to be most dominant at Site II and III, where the majority of the agriculture fields includes the seasonal vegetables such as Cabbage, Cauliflower, and Brinjal as well as the Paddy and Maize fields (Table 2.1)

Objective III:

Sequence Validation and bioinformatics analysis

Sequence Annotation

Universal primers were used in this study, which perfectly amplified a 720 bp fragment of the mitochondrial COI gene when applied to template DNA. Fifteen Orthopteran species barcode sequences were obtained from the three selected families. Table 3.1 represents the process ID of the species submitted on BOLD v.4.

Family	**Species**	**Process ID**
Acrididae	*Acrida conica*	GJCST 2
	Acrida exaltata	GJCST 3
	Aiolopus thalassinus	GJCST 4
	Choroedocus robustus	GJCST 6
	Euthystria brachyptera	GJCST 11
	Hieroglyphus banian	GJCST 14
	Locusta migratoria	GJCST 15
	Metaleptea brevicornis	GJCST 17
	Oxya hyla hyla	GJCST 19
	Oxya hyla intricate	GJCST 20
	Trilophidia annulata	GJCST 26
	Chorthippus curtipennis	GJCST 28
Gryllidae	*Acheta domesticus*	GJCST 1
Tettigoniidae	*Neoconocephalus velox*	GJCST 18
	Scudderia furcata	GJCST 21

Table 3.1: List of species with their families submitted to BOLDv.4 along with their processID

%GC content of Orthoptera species analysis was performed to find the sequence composition, which resulted in decreasing order as follows *S. furcata* (37.9%), *A. domesticus*

(37.4%), *N. velox* (37.4%), *H. banian* (35.7%) and so forth. However, the least GC content was found in *T. annulata* (28.9%) among all 15 Species. Further, the sequence was analysed for GC and AT skews, where GC skew was found the maximum in *A. exaltata* (0.065), whereas the lowest was found in *T. annulata* (-0.2) among the selected species. However, overall comparison with other species, the maximum GC skew was shared between *C. discolour*, *T. oceanicus*, *E. brachyptera*, respectively. AT skew analysis revealed that A. *thalassinus* (0.05) has the highest AT value, whereas the lowest one was *S. furcata* (-0.129) (Figure 3.1).

The %GC content analysis of Coleopteran four species were carried out to find out the sequence composition, which resulted in decreasing order as follows; *Lanelater fuscipes* (46%), *Oxycetonia versicolor* (35.3%), *Myllocerus viridanus* (35.2%) and *Aulacophora foveicollis* (34%). However, the least GC content was found in *A.foveicollis* (28.9%) among four Species. Further, the sequence was analyzed for GC and AT skews, where GC skew was found the maximum in *A. foveicollis* (0.08), whereas the lowest was found in *L. fuscipes* (-0.07) among the selected species. AT skew analysis revealed that *A. foveicollis* (0.05) has the highest AT value, whereas the lowest one was *L. fuscipes* (-0.18) (Figure 3.2).

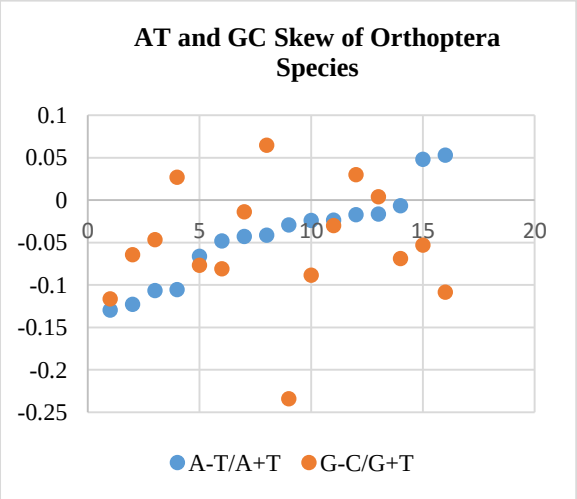


Figure 3.1: AT and GC skew of barcoded Orthoptera species

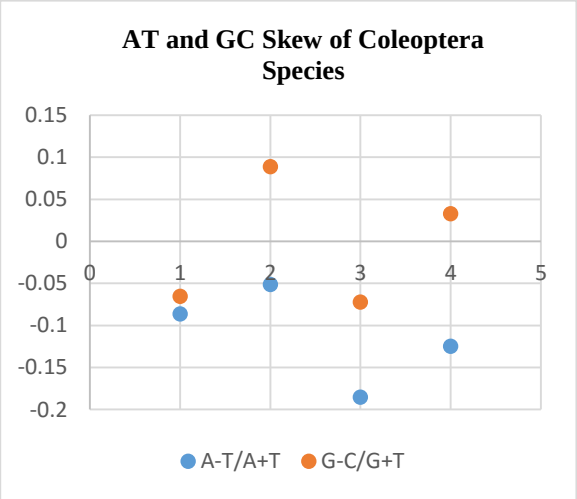


Figure 3.2: AT and GC skew of barcoded Coleoptera species

The neighbor-joining statistical analysis for phylogeny reconstruction was performed, which yielded the maximum likelihood among the selected species. The sequence analysis also revealed that the computed overall mean distance was 5.19 among the species; however, the pairwise mean distance showed a significant range of 0.16 (*C. curtipennis*) to 9.3 (*M. fasciatasulcata*) of individual species comparison.

Nucleotide substitution with maximum composite likelihood method with Gamma distribution (G) yielded the sequence similarity suggesting that among all the species selected for the study were of Orthoptera, Coleoptera, Hemiptera, and Lepidoptera. A total of 7 species belong to suborder Ensifera, while the other 22 species belong to Caelifera of Orthoptera. The present study also revealed that the Gryllidae family has close relations with Tettigoniidae in Ensifera suborder. On the other hand, in Caelifera, Acrididae was found to be closer to Pyrgomorphidae (Figure3.3). Coleoptera revealed that family Anobiidea is having maximum parsimony with Elateridae compared to other family and also Tenebrionidae family was located closer to Meloidae compared to other families (Figure 3.4).

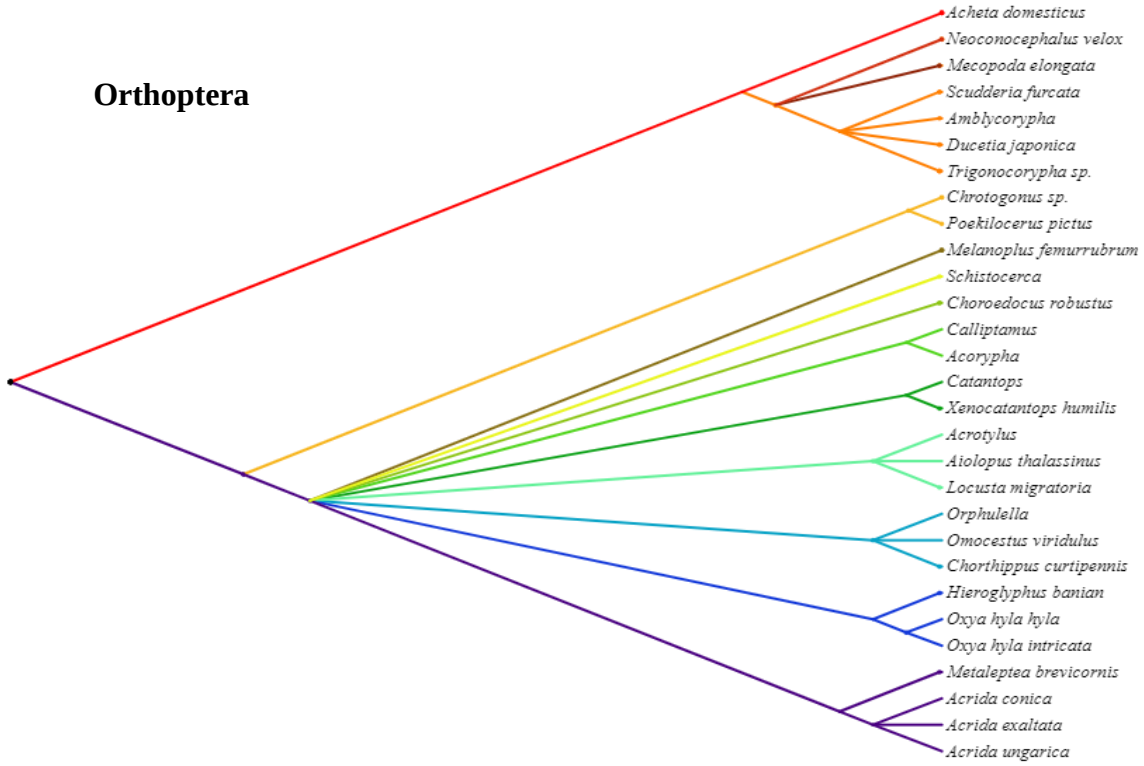


Figure3.3: Diagrammatic representation of the phylogenetic tree of Orthoptera pest species

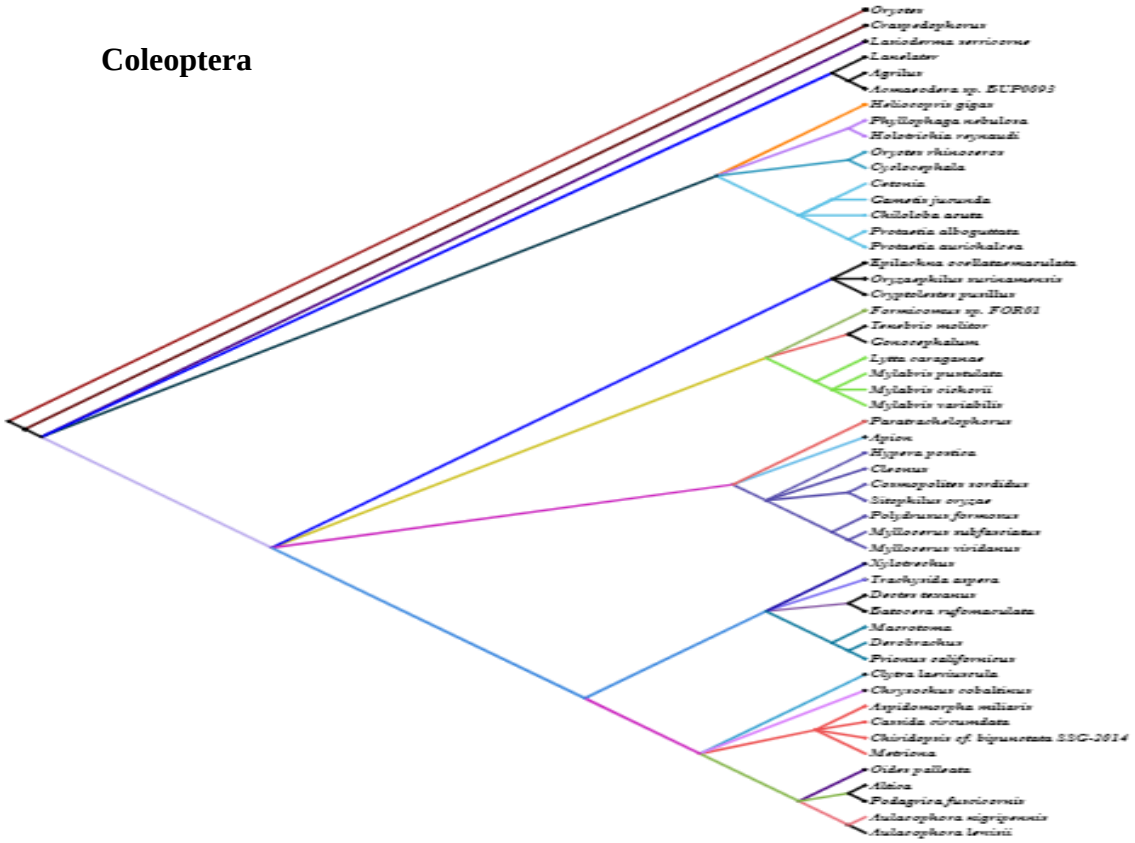


Figure3.4: Diagrammatic representation of the phylogenetic tree of Coleopterapest species

Discussion

In the present study, the maximum assemblage of the insects in the agricultural fields was reported at Ajwa (Site I). Ajwa is blessed with good vegetation cover and water bodies. The most dominant agriculture fields visited were paddy, maize, cotton, millet, and pigeon pea. Various types of vegetation supports and a good variety of insects. In complex landscapes, natural vegetation harbors greater, more diverse populations of service-providing species associated with higher agricultural yields (Henri *et al.*, 2015). Morover, an increase in the abundance/ diversity of vegetation leads to a decrease in pest abundance (Paredes *et al.*, 2015). Our work is in agreement with the earlier reported work where the insects were found more prevalent in the presence of a mixed type of vegetation. (Hahs *et al.*, 2009; Lambert *et al.*, 2016; El-Sabaawi, 2018). Of all the orders of class Insecta, it was Coleoptera, which was reported to be the most dominant. Ground beetles are species-rich and abundant in agricultural land all over the world (Zou, 2019). As one of the most abundant and diverse groups overwintering within cultivated fields (Holland and Reynolds, 2003), they are often used in cultivation experiments (Lemic *et al.*, 2017). Crop type affects ground beetles through the modification of microclimatic factors and from disturbance factors (O´Rourke *et al.*, 2008). The ground beetles population in the agricultural landscape can also be influenced by

chemical pest control (Jeschke *et al.,* 2011; Szczepaniec *et al.,* 2011; Varvara *et al.,* 2012; Douglas *et al.,* 2014). A good number of coleopterans has been reported by number of scientists in different agricultural fields/ crops which includes on sugar beet (Kos *et al.,* 2013), maize (Kos *et al*., 2006; Bažok *et al.,* 2007; Kos *et al.,* 2011) and barley (Kos *et al.,* 2010), where they have focused and have confirmed with the conventional, organic and integrated systems. Thus a dominant assemblage of Coleoptera observed in the present study is following the above authors (Balog *et al.,* 2009; Park *et al.,* 2013; Chapman, 2014; Pywell *et al.,* 2015; Hanson *et al.,* 2016; Pizzolotto *et al.,* 2018; Rizal *et al.,* 2019).

Order Orthoptera is one of the most vital groups of herbivorous insects living in the grassland systems. The availability of host plants of the grasshoppers in the habitat is essential for its colonization. Many investigators have reported the importance of vegetation for the nutritional and ecological needs of grasshoppers (Bhusnar, 2015; Aktar *et al.,* 2018; Zergoun *et al.,* 2019). Of all the families Acrididae was recorded to be the most dominant in all four study sites and was seen to be colonized in more diverse habitats such as grasses, agriculture fields as well as shrubs, however, of all the different habitats, herbs were found to be the most common habitats for grasshoppers. Our findings are parallel with the studies of Koli, (2014), Waghmare, (2013) and Bhusnar, (2015) in Maharashtra; Anbalagan *et al.*,(2015) in Tamil Nadu, Usmani *et al.,* (2012) in Bihar and Jharkhand and Saha and Haldar, (2009) in West Bengal.

Hemiptera is known to be found in a large variety of niches, where they perform a range of ecological functions and services affecting nearly every aspect of the environment (Henry 2009). Terrestrial bugs are associated with plants, and floristic composition and vegetation structure are the environmental factors, which explain the best true bug biodiversity and distribution patterns. Hemiptera was next in the order of dominant insects found in the present study with a wide range of diversity, and most of its species belonging to the families such as Lygaeidea, Pentatomidae, Reduviidae, Membracidae, and Coreidae were found to be pests. Site wise distribution revealed that they were the maximum at Site II and the least was found to be present at Site III, the less number of the Hemipteran may be attributed to the presence of Coleoptera in this region which does not allow the bugs to get flourished (Kataria and Kumar, 2012; Singh and Gandhi, 2012; Rebijith *et al.,* 2013; Feledyn-Szewczyk *et al.,* 2016).

A common phytophagous insect group in agricultural landscapes is Lepidoptera, which includes moths and butterflies. The presence and abundance of butterflies mark the importance of the plant resources available on the site and show a healthy and suitable

environment for insect diversity. In general, caterpillars are the feeding stage in Lepidoptera and caterpillars of most species feed on plant material, such as leaves, flowers, fruits, seeds, or roots. Caterpillars seem especially vulnerable to stressors in agricultural landscapes because they are immobile compared with most adult moths and dependent on the availability of suitable host plants a loss of plant diversity or changes in plant communities most likely affect caterpillars if the host plants are involved (Hahn, 2015; Salunke and More, 2017). Specialized Lepidoptera species can be especially vulnerable to changes in their caterpillar host plant's abundance and appear to decline more strongly compared with less specialized species (Kotiaho *et al.,* 2005). A literature search revealed that high plant species richness and flower abundance have a predominantly positive effect on Lepidoptera (Hahn *et al.,* 2013). In the present study, a considerable number of species were found to be present, and of the four different Sites, it was Site (III) and Site (II), which had the maximum number of the Lepidopterans. Overall there was a comparatively fewer number of Lepidopterans supported by the work of Gandhi and Kumar (2015), where they have suggested accelerating the conservation campaign in cultivating more of such complementary plants for holding up the life of butterflies.

Seasonal distribution of the insects' species was found attaining maximum level during monsoon season, throwing open a plethora of various forms of insects. During the winter season, the environmental circumstances change, leading to a change in the insect population, which showed a decreasing trend. Seasonal changes leading to the summer were remarkably noticeable by a general decrease in insect species richness and abundance. Due to dry condition and defoliation insect population start dwindling during the summer season. However, there was a significant seasonal variation caused due to climatic change, which potentially affected insects both directly through plant association. Similar observations made by Wardhaugh *et al.,* (2018) reported that altered abiotic conditions resulting from human-induced climate change are already driving changes in the spatial and temporal distributions of many insects. Silva *et al.,* (2011) reported the seasonal abundance and variation of insects. Most of the insect orders were found dominant in monsoon season that may be due to the availability of food material they feed on. During the study period in the year 2018-'19, comparatively, there was a variation in the monsoon, the seasonal occurrence of insect species due to the massive flood in all the four sites. Abhishek *et al.,* (2017) reported similar kinds of work where due to heavy rain, reduce the insect population. Nevertheless, the present study has a span of two years in which deals with the species distribution, diversity,

richness, evenness, ecological role, and seasonal variation of insects in four selected areas of Vadodara district.

As far as the pest status is concerned, mainly four insect orders had prominent pest species. The maximum numbers of pests were from order Coleoptera (65) > Orthoptera (35) > Lepidoptera (30) > Hemiptera (27). Pest species found inclusively in four sites and the main crops of these sites were cabbage, cowpea, spinach, onion, bitter gourd, sponge gourd, ivy gourd, pomegranate, ladies finger, tomato, millet, banana, sweet potato, paddy, fodder grass, maize, sugarcane, cotton, brinjal, pigeon pea, drumstick, lemon tree and mango tree in various season. Singh and Sharma, (2014) reported insect pests from Talwandi Sabo, Punjab, were Hemiptera and Lepidoptera insects causing damage to both Kharif and Rabi crops. Sathe *et al.,* (2015) studied color attractively and the occurrence of some cell sucking pests on crop plants from the Kolhapur region and reported four sap-sucking insect pests. Sathe *et al.,* (2016) reported pest species of Brinjal from the Kolhapur region. Patil *et al.* (2016) studied on diversity and biology and control insect pests from Western Maharashtra and reported 30 species. UlAne and Hussain (2016) reported Lepidoptera, Hemiptera, Coleoptera, Diptera, Orthoptera, and Thysanoptera from major rice-growing areas of the world. Salunke and More (2017) reported that in Chandgad Tahsil, the farmers were facing various agricultural insect pests especially in the case of Rice, Red gram, Brinjal, and Cowpeas and observed other pests such as Aphids, Mealybug, and whiteflies are damaging various crops in winter and summer season.

Overall, plant-herbivore interactions represent one of the most widespread and dominant ecological interactions in the conservation of natural and also play a pivotal role in ecosystem functioning (Stam *et al.,* 2014; Turcotte *et al.,* 2014). The enormous number of insect species is often exceedingly difficult to recognize using only morphological approach (Witt *et al.,* 2006) and thus produces an insurmountable barrier for cataloguing total biodiversity by only traditional taxonomy (Blaxter, 2004; Pentinsaari *et al.,* 2014) for which morphological documentation have reduced short and the DNA barcoding has filled the gap (Bourke *et al.,* 2013; Laurito *et al.,* 2013). Several scientists are now using DNA-barcoding to understand the biodiversity of insects (Hebert, *et al.,* 2003; Hajibabaei, *et al.,* 2007). Barcoding and sequence analysis were based on mitochondrial *COX I* (Persis *et al.,* 2008).DNA barcoding significantly enables and complement taxonomic studies; the sequencing data coupled with traditional taxonomy is a model that can be functional in numerous areas and will allow systematic and analytical needs to be scaled to match the enormity of the current biodiversity crisis (Jalali and Ojha, 2015). It will help in the identification and conservation of the

evolutionary processes that generate and preserve biodiversity. The first time from Gujarat, mitochondrial *COX I* gene sequence of 15 pest species belonging to Orthoptera and four pest species of Coleoptera were successfully sequenced, and the phylogeny tree of all the four orders of Coleoptera, Orthoptera, Hemiptera and Lepidoptera for the evolutionary analysis. Similar study have carried out for irrespective of pest species of Coleopterans by Singhal, *et al.,* (2018). Our studies were incongruent with earlier mitochondrial genomes studies of Yuan *et al.,* (2015a, b, 2016). Molecular identification was made for several pests worldwide, in Orius (Hemiptera: Anthocoridae) (Gomez-Polo *et al.,* 2013) and potato flea beetles (Coleoptera: Chrysomelidae) (Germain *et al.,* 2013). Despite optimistic views, the taxonomic impediment remains the main concern and thus demands an urgent need for comprehensive biodiversity assessments due to biodiversity crises: the risk of human activity causing mass extinction. Thus, barcoding can accelerate the process of taxonomic inventory. However, the present study combines morphological, ecological and molecular data which specify its species distribution, richness, and diversity in different sites of Vadodara, Gujarat, and the molecular study derived from present work unravels the status of pest species. And till now the results obtained in this research is the first comprehensive study of pest species with its sequence in Vadodara District.

Work to be carried out:

Sanger Sequencing of three orders and insect-plant correlation (objective 4) is under evaluation, which will be incorporated in the thesis.

Conclusion

From the present study the Agricultural important insects of Vadodara district was obtained by estimating relative abundance and dynamics of related species in space and time. From the collected insects pests' species were extracted and their morphological and molecular identification were obtained. Further the infestation rate revealed that there was a site specific as well as plant specific infestation. The findings has proved to be one of the backbone studies for morphological and molecular identification and has verified the usefulness of barcode data. Further the application of the data obtained can be reliably used for developing reference libraries for species identification via sequence matches. Hence, the present study is given anew and significant insights into both the unique molecular determinants of plant-insect interactions, therefore, a prerequisite for genetic investigations in this study was the technical step of constructing a database of insect pests.

References

Aktar, M., Md. Islam, A. and Md. Alim, A. (2018). Orthopteran Diversity at Keraniganj, Dhaka, Bangladesh. *J. Zool*., **46**(1): 21-31.

Alfred, J. R. B., Das, A. K. and Sanyal, A. K. (1998). Faunal Diversity in India Zoo. *Survey of India*. 479.

Ali, J. G. and Agrawal, A. A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends Plant Sci*. **17**: 293–302.

Anbalagan, V., Paulraj, M. G. and Ignacimuthu, S. (2015). Biodiversity of Insects in Organic and Chemical Vegetable Fields in Tiruvallur District, Tamil Nadu, India. *International Journal of Pure and Applied Zoology*, **3**(2): 2320–9577.

Balog, A., Marko´, V., Kutasi, Cs. and A´ da´m, L. (2003) Species Composition of Ground Dwelling Staphylinid (Coleoptera: Staphylinidae) Communities in Apple and Pear Orchards in Hungary. *Acta Phytopathologica et Entomologica Hungarica*, **38**(1–2): 181–198.

Bažok, R., Kos T., Igrc Barčić, J., Kolak, V., Lazarević, B. and Čatić, A. (2007). Abundance and distribution of the ground beetles *Pterostichus melanarius* (Illiger, 1798) and Pseudoophonus rufipes (DeGeer, 1774) in corn fields in Croatia. *Entomologia Croatica*, **11**(1-2): 39-51.

Bhusnar, A. (2015). Acridid (Orthoptera) diversity of agriculture ecosystem from Solapur District of Maharashtra, India. *Biolife*, **3:** 461–468.

Blaxter, M. L. (2004). The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society of London B : Biological Sciences*, ***359***(1444): 669–679.

Bourke, B. P., Oliveira, T. P., Suesdek, L., Bergo, E. S. and Sallum, M. A. M. (2013). A multi-locus approach to barcoding in the *Anopheles strodei* subgroup (Diptera: Culicidae). *Parasites and Vectors*, **6**(1): 111.

Brown, T. A., Jones, M. K., Powell, W. and Allaby, R. G. (2009). The complex origins of domesticated crops in the Fertile Crescent. *Trends Ecol Evol,* **24**:103 -109.

Chapman, A. (2014). The Influence of Landscape Heterogeneity - Ground Beetles (Coleoptera: Carabidae) in Fthiotida, Central Greece. *Biodiversity data journal*, **2**.

Cigliano, M. M., Wysiecki, M. L. and Lange, C. E. (2000). Grasshopper (Orthoptera: Acridoidea) species diversity in the Pampas, Argentina. *Diversity and Distributions,***6:** 81–90.

Dawkar, V. V., Chikate, Y. R., Lomate, P. R., Dholakia, B. D., Gupta, V. S. And Giri, A. P. (2013). Molecular insights into resistance mechanisms of lepidopteran insect pests against toxicants. *J Proteome Res.***12:** 4727- 4737.

Dhaliwal, G. S., Jindal, V. And Dhawan, A. K. (2010). Insect pest Problems and crop losses: changing trends. *Indian Journal of Ecology*. **37**: 1-7.

Dhaliwal, G.S., Dhawan, A. K. and Singh, R.2007. Biodiversity and ecological agriculture: Issues and perspectives. Indian J. Ecol., **34** (2):100- 109

Douglas, M. R., Rohr, J. R., Tooker, J. F. (2015): Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. - *Journal of Applied Ecology*, **52**: 250–260.

El-Sabaawi, R. (2018). Trophic structure in a rapidly urbanizing planet. *Funct. Ecol.***32**: 1718–1728.

Fazekas, A. J., Burgess, K. S., Kesanakurti, P. R., Graham, S. W., Newmaster, S. G., Husband, B. C., Percy, D. M., Hajibabaei, M. and Barrett, S. C. H. (2008). Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *Plos One* **3**, e2882.

Feledyn-Szewczyk, B., Ku´s, J., Stalenga, J., Berbe´c, A. K. and Radzikowski, P. (2016). The role of biological diversity in agroecosystems and organic farming. In Organic Farming - A Promising Way of Food Production; IntechOpen: London, UK.

Floyd, R. M., Wilson, J. J. and Hebert, P. D. N. (2009). DNA barcodes and insect biodiversity. In: Insect Biodiversity: Science and Society, Foottit R.G., Adler P.H. (Eds), Wiley-Blackwell, Oxford, 417-432.

Folmer, O. M., Black, W., Hoen, R., Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3:** 294-299.

Gagnona, J., Raskinb, M., Remacheb, J. and Sack, B. (2011). The Financial Market Effects of the Federal Reserve's Large-Scale Asset Purchase. *International Journal of Central Banking,* **7** (1): 3-41.

Gandhi, S. and Kumar, D. (2015). Butterfly Species Abundance in Agricultural fields of Vadodara, Gujarat with Special Emphasis on the Conservation of Complementary Plantations. *International Journal of Science and Research*, 2319-7064

García-Robledo, C., Erickson D. L., Staines, C. L., Erwin, T. L. and Kress, W. J. (2013). Tropical Plant–Herbivore Networks: Reconstructing Species Interactions Using DNA Barcodes. *Plos ONE*, **8**(1): e52967.

Gardner, W. A., Blount, J. L., Golec, J. R., Jones, W. A., Hu, X. P., Talamas, E. J., Evans, R. M., Dong, X., Ray, C. H. and Buntin, G. D. (2013). Discovery of Paratelenomus *saccharalis* (Dodd) (Hymenoptera: Platygastridae), an egg parasitoid of *Megacopta cribraria* F. (Hemiptera: Plataspidae) in its expanded North American range. *J. Entomol. Sci.*, **48**: 355-359.

Germain, J., Chatot, C., Meusnier, I., Artige, E., Rasplus, J. and Cruaud, A. (2013). Molecular identification of Epitrix potato flea beetles (Coleoptera: Chrysomelidae) in Europe and North America. *Bulletin of Entomological Research*, **103**(3), 354-362.

Gomez-Polo, P., Alomar, O., Casta~n_e, C., Riudavets, J. and Agustí, N. (2013). Identification of Orius spp. (Hemiptera: Anthocoridae) in vegetable crops using molecular techniques. *Biol. Control*, **67.**

Grasswits, T. R. (2016). Laboratory evolution of predation rates of two native natural enemies on the exotic pest *Bagrada hilaris*. *Journal of Applied Entomology*, **140:** 744-756.

Greenstone, M. H., Tillman, P. G. and Hu, J. (2013). Predation of the Newly Invasive Pest *Megacopta cribraria* (Hemiptera: Plataspidae) in Soybean Habitats Adjacent to Cotton by a Complex of Predators. *Biological and microbial control*, **107** (3): 943-954.

Hahn, M., Schotthöfer, A., Schmitz, J., Franke, L. A., and Brühl, C. A. (2015). The effects of agrochemicals on Lepidoptera, with a focus on moths and their pollination service in field margin habitats. *Agric. Ecosys. Environ.* 207, 153–162.

Hahs, A. K., Mcdonnell, M. J. Mccarthy, M. A., Vesk, P. A., Corlett, R. T., Norton, B. A., Clemants, S. E., Duncan, R. P., Thompson, K., Schwartz, M. W. and Williams, N. S. G. (2009). A global synthesis of plant extinction rates in urban areas. *Ecology Letters*, **12**: 1–9.

Hajibabaei, M., Singer, G. A., Hebert, P. D. and Hickey, D. A. (2007). DNA barcoding: How it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics*, **23**(4): 167–172.

Hanson, H. I., Palmu, E., Birkhofer, K., Smith, H. G. and Hedlund, K. (2016) Agricultural Land Use Determines the Trait Composition of Ground Beetle Communities. PLoS ONE **11**(1): e0146329.

Hautier, L., Lebrun, R., Saksiri, S., Michaux, J. And Vianey-Liaud, M. (2011). Hystricognathy vs Sciurognathy in the Rodent Jaw: A New Morphometric Assessment of Hystricognathy Applied to the Living Fossil Laonastes (Diatomyidae). *Plos ONE*, **6**(4): e18698.

Hebert, P. D., Cywinska, A., and Ball, S. L. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B: Biological Sciences*, **270**(1512): 313–321.

Henri, D. C., Jones, O., Tsiattalos, A., Thébault, E., Seymour, C. L. and van, Veen, F.J.F. (2015), Natural vegetation benefits synergistic control of the three main insect and pathogen pests of a fruit crop in southern Africa. *J Appl Ecol*, **52**: 1092-1101.

Henry T. J. (2009). Biodiversity of Heteroptera. In Foottit R. G. and Adler, P. H. (Eds) Insect Biodiversity: Science and Society. Chichester, UK: Wiley-Blackwell, 223–263.

Holland J. M. and Reynolds C. J. M., 2003 - The impact of soil cultivation on arthropod (Coleoptera and Araneae) emergence on arable land. *Pedobiologia*, **47**: 181-192

Huang, J. Z., Mao, S. and Huang, Y. (2013). DNA Barcoding and Species Boundary Delimitation of Selected Species of Chinese Acridoidea (Orthoptera: Caelifera). *Plos ONE,* **8**(12): e82400.

Jalali, S. K., Ojha, R. And Venkatesan, T. (2015). DNA Barcoding for Identification of Agriculturally Important Insects. *New horizons in insect science*, **XXVII**: 93.

Jeschke, P., Nauen, R., Schindler, M. and Elbert, A. (2011) Overview of the status and global strategy for neonicotinoids. *Journal of Agricultural and Food Chemistry*, **59**: 2897– 2908.

Joshi, N. C., Joshi, P. C., Kumar, S., Nath, P., Singh, V. K. and Mansotra, D. (2016). Entomofaunal diversity in fruit orchards along the altitudinal gradients of district Nainital, Uttarakhand. *International Journal of Fauna and Biological Studies,* **3**(2): 113-120.

Jurado-Rivera J., Vogler A. P., Reid C. M., Petitpierre E. and Go´mez-Zurita J. (2009). DNA barcoding insect–host plant associations. *Proceeding the Royal Soceity B*., **276**: 639–648.

Kambhampati, S., and Smith, P. T. (1995). PCR primers for ampli-fication of four insect mitochondrial gene fragments. *Insect Mol. Biol*., **4**: 233–236.

Karthika, P., Krishnaveni, N., Vadivalagan, C., Murugan, K., Nicoletti, M. and Benelli, G. (2016). DNA barcoding and evolutionary lineage of 15 insect pests of horticultural crops in South India, Karbala. *International Journal of Modern Science,* **2**: 156-168

Kataria, R., & Kumar, D. (2012). Occurrence and Infestation Level of Sucking pests: Aphids on various host plants in Agricultural Fields of Vadodara, Gujarat (India). *International Journal of Scientific and Research Publications*, ***2***.

Kerchev, P. I., Fenton, B., Foyer, C. H. and Hancock, R. D. (2012). Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. *Plant, Cell and Environment*, **35**: 441–453.

Koli, V. K. (2014). Diversity and status of avifauna in Todgarh-Raoli Wildlife Sanctuary, Rajasthan, India. *Journal of Asia-Pacific Biodiversity*, **7**(4): 401–407.

Kos, T., Bažok, R., Drmić, Z., Graša, Ž. (2013). Ground beetles (Coleoptera: Carabidae) in sugar beet fields as the base for conservation biological control. - Insect Pathogens and Entomoparasitic Nematodes" Biological Control- its unique role in integrated and organic production. *IOBC-WPRS Bulletin,* **90**: 353-357.

Kos, T., Bažok, R., Igrc Barčić, J. (2011): Abundance and frequency of ground beetles in three maize fields in Croatia. *Journal of Environmental Protection and Ecology,* **12(**3): 894-902.

Kos, T., Bažok, R., Kozina, A., Šipraga, J., Dragić, S., Tičinović, A. (2010): Ground beetle (Carabidae) fauna at untreated and treated barley fields in Croatia. *Pesticides and Beneficial Organisms IOBC/wprs Bulletin*, **55**: 79-84

Kotiaho, J. S., Kaitala, V., Komonen, A. and Päivinen J.(2005). Predicting the risk of extinction from shared ecological characteristics. *PNAS,* **102** (6): 1963-1967.

Kress, W. J. and Erickson, D. L. (2007). A two locus global DNA barcode for land plants: the coding rbcl gene complements the non-coding trnh-psba spacer region. *Plos One,* **2**: e508.

Kumar, P., Pandit, S. S., Steppuhn, A. And Baldwin, I. T. (2014). Natural history-driven plant-mediated rnai-based study reveals CYP6B46's role in a nicotine-mediated antipredator herbivore defense. *Proc Natl Acad Sci USA***, 111**:1245–1252.

Lahaye, R. (2008). DNA barcoding the floras of biodiversity spots. *Proc. Natl Acad. Sci. USA.* **105**: 2923–2928.

Lambert, K., Kumar, L., Reid, N., and Mcdonald, P. (2016). Habitat selection by a despotic passerine, the Bell Miner (*Manorina melanophrys*): When restoring habitat through Lantana (*Lantana camara*) removal is not enough. *Ecological Management & Restoration*, **17**: 81–84.

Laurito, M., de Oliveira, T. M., Almiron, W. R. and Sallum, M. A. M. (2013). COI barcode versus morphological identification of Culex (Culex) (Diptera: Culicidae) species: A case study using samples from Argentina and Brazil. *Memórias Do Instituto Oswaldo Cruz*, **108**: 110–120.

Lemic, D., Cacija, M., Viric Gasparic, H., Drmić, Z., Bažok, R. and Pajač Živković, I. (2017). The ground beetle (Coleoptera: Carabidae) community in an intensively managed agricultural landscape. *Applied Ecology and Environmental Research*, **15**: 661-674.

Losey, E. L. and Vaughan, M. (2006). The Economic Value of Ecological Services Provided by Insects. *Bio Science*. **56**(4): 331-323.

Mandal, S. D., Chhakchhuak, L., Gurusubramanian, G. and Kumar, N. S. (2014). Mitochondrial markers for identification and phylogenetic studies in insects – A Review. *DNA Barcodes*, **2**: 1–9.

Mishra M., Lomate R., Joshi R., Punekar S., Gupta V. and Giri A. (2015). Ecological turmoil in evolutionary dynamics of plant–insect interactions: defense to offence. *Planta,* **242**:761–771.

Monaghan, M. T., Balke, M., Gregory, T. R. and Vogler, A.P. (2005). DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. *Philos. Trans. R. Soc.*, B.360.

Opatovsky, I., Chapman, E. G., Weintraub, P. G., Lubin, Y. And Harwood J. D. (2013). Molecular characterization of the differential role ofimmigrant and agrobiont generalist predators in pest suppression. Biol. Control, **63**: 25-30.

O'Rourke, Megan & Liebman, Matt & Rice, Marlin. (2008). Ground Beetle (Coleoptera: Carabidae) Assemblages in Conventional and Diversified Crop Rotation Systems. *Environmental entomology*, **37**: 121-130

Paredes D, Cayuela L, Gurr GM, Campos M (2015) Is Ground Cover Vegetation an Effective Biological Control Enhancement Strategy against Olive Pests?. PLOS ONE **10**(2): e0117265. Https://doi.org/10.1371/journal.pone.0117265

Pareek, A., Meena, B. M., Sharma, S., Teterwal, M. L., Kalyan, R. K. and Meena, B. L. (2017). Impact of Climate Change on Insect Pests and Their Management Strategies. 253 - 286.

Park, J. K., Lyu, D. and Park, J. (2013). Two new species of the genus Pterostichus (Coleoptera: Carabidae) from Korea. Journal of Asia-Pacific Entomology. **16**: 385 - 388. 10.1016/j.aspen.

Patil, S. S., Sutar, M. V and Sathe, T. V. (2016). Diversity, Biology and Control of Insect pests of teak *Tectona grandis (Linnaeus)* from western Maharashtra. *Biolife*, **4**(1):141-146.

Pentinsaari, M., Hebbert, P. D. and Mutanen, M. (2014). Barcoding beetles: a regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. *PLOS ONE*, **9**(9): e108651.

Persis, M., Acs, R., Rao, L., Khedkar, G., Kondadhasula, R. and Nasruddin, K. (2008). COI (cytochrome oxidase-I) sequence based studies of Carangid fishes from Kakinada coast, India. *Molecular Biology Reports*, **36**: 1733–1740.

Pizzolotto, R., Mazzei, A., Bonacci, T., Scalercio, S., Iannotta N, et al. (2018) Ground beetles in Mediterranean olive agroecosystems: Their significance and functional role as bioindicators (Coleoptera, Carabidae). PLOS ONE **13**(3): e0194551.

Pywell, R. F., Heard M. S., Woodcock, B. A., Hinsley, S., Ridding L., Nowakowski M. and Bullock, J. M. (2015) Wildlife-friendly farming increases crop yield: evidence for ecological intensification. *Proc. R. Soc.*, **282.**

Rebijith, K. B., Asokan, R., Kumar, N. K. K., Krishna, V., Chaitanya, B. N. and Ramamurthy, V.V. (2013). DNA barcoding and elucidation of cryptic aphid species (Hemiptera: Aphididae) in India, *Bull. Entomol. Res*. **103**.

Rizal , A., Suryana , A. A. H., Rosidah , R. and Nurruhwati I. (2019). Diversity and the abundance of the insect of Coleoptera orders at Mamberamo river bank of Papua Province, Indonesia. *World Scientific News*, **133**:132-144.

Saha, H.K., & Haldar, P. (2009). Acridids as indicators of disturbance in dry deciduous forest of West Bengal in India. *Biodiversity and Conservation*, **18**: 2343–2350.

Salunke, R. N. and More, S. V., (2017). Diversity of insect pests in agriculture and forest areas from Chandgad Tehsil, Kolhapur district of Maharashtra. *Indian Journal of Scientific Research*. **13**(1): 263-267

Sathe, T.V, Gophane, A. and Sendage, N., 2015. Colour attractivity and occurrence of some cell sap sucking pests of crop plants. Biolife, **3**(2):540- 546.

Sathe, T.V., Patil, S.S., Bhosale, A.M., Devkar, S.S., Govali, C.S. and Hankare, S.S., (2016). Ecology and Control of Brinjal insect pests from Kolhapur region, India. Biolife, **4**(1):147-154.

Silva, N. A. P., Frizzas, M. R. and Oliveira, C. M. (2011). Seasonality in insect abundance in the "Cerrado" of Goiás State, Brazil. *Revista Brasileira de Entomologia*, **55**(1), 79-87.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. **87**: 651–701.

Singh A. and Sharma A. L., (2014). Agriculturally important insects diversity in Kharif and Rabi crops, Talwandi Sabo, Pujab. International Journal of Scientific and Research Publications. ISSN 2250-3153.

Singh, A., and Gandhi, S. (2012). Agricultural Insect Pest: Occurrence and Infestation Level in Agricultural Fields of Vadodara, Gujarat. *International Journal of Scientific and Research Publications*, **2**(1): 2250–3153.

Singh, H. K., Parveen, I., Raghuvanshi, S., and Babbar, S. B. (2012). The loci recommended as universal barcodes for plants on the basis of floristic studies may not work with congeneric species as exemplified by DNA barcoding of Dendrobium species. *BMC research notes*, ***5***: 42.

Singhal, S., Thakkar, B., Pandya, P. and Parikh, P. (2018). Unraveling the diversity, phylogeny, and ecological role of cryptic Coleopteran species of Vadodara district: a first comparative approach from India. *Jobaz,* **79:**53.

Stam, J. M., Kroes, A., Li, Y., Gols, R., Van Loon, J. J. A, Poelman, E. H. and Dicke, M. (2014). Plant interactions with multiple insect herbivores: from community to genes. *Annual Review of Plant Biology* **65**: 689-713.

Symondson, W.O.C. (2002). Molecular identification of prey in predator diets. *Mol. Ecol.* **11**: 627-641.

Szczepaniec, A., Creary, S. F., Laskowski, K. L., Nyrop, J. P. and Raupp M. J. (2011). Neonicotinoid insecticide imidacloprid causes outbreaks of spider mites on elm trees in urban landscapes. *PLoS One*, **6**, e20018.

Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. (1991). Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Mol. Biol.* **17**: 1105–1109.

Thakkar, B., and Parikh, P. H. (2016). A Study on diversity and abundance of coleopterans in Gujarat, India. *Journal of Entomology and Zoology* Studies, **4**(5): 1082-1089**.**

Thakur, M. S., Mattu, V. K. and Mehta, S. L. (2008). Distribution record of insect diversity in different altitudes of Shimla Hills, Himanchal Pradesh, India. *J. Ent. Res.* **32**(4): 317- 321.

Thomas, A., Lee, P. J, Dalton, J. E., Nomie, K. J., Stoica, L., Costa-Mattioli, M., Chang, P., Nuzhdin, S., Arbeitman, M. N., Dierick, H. A. (2012). A Versatile Method for Cell-Specific Profiling of Translated mrnas in Drosophila. *Plos ONE*. **7**(7): e40276.

Turcotte, M. M., Davies, T. J., Thomsen, C. J. M., and Johnson, M. T. J. (2014). Macro ecological and macro evolutionary patterns of leaf herbivory across vascular plants. *Proc. R. Soc.* B 281.

Ul Ane N. and Hussain M., (2016). Diversity of insect pestsin major rice growing areas of the world. *Journalof Entomology and Zoology Studies*, **4**(1): 36-41.

Usmani, M. K. Nayeem, M. R. and Andakhtar, M. H. (2012). Field observations on the incidence of Grasshopper fauna (Orthoptera) as a pest of Paddy and pulses. *Eur. J. of Exp. Bio.*, **2**(5):1912- 1917.

Vartan, S. (2016, February 2). This gorgeous cricket chorus sounds like humans singing a haunting song. Retrieved from https://www.mnn.com/earth-matters

Varvara, M., Chimisliu, C. and Šustek, Z. (2012): Distribution and abundance of Calosoma uropunctatum Herbst 1784. (Coleoptera: Carabidae) in some agricultural crops in Romania, 1977-2010. Oltenia. Studii și comunicări. - Ştiinșele Naturii **28**(1): 79–90.

Waghmare, S., Waghmare, D. and Andbhatnagar, P. S. (2013). Species Diversity of Short Horned Grasshopper (Orthoptera: Acrididae) in Selected Grasslands of Solapur District, Maharashtra, India. *J Biodivers Endanger Species*, **1**:110.

Wardhaugh, C. W., Stone, M. J., and Stork, N. E. (2018). Seasonal variation in a diverse beetle assemblage along two elevational gradients in the Australian Wet Tropics. *Scientific reports*, **8**(1): 8559.

Witt, A. B. R., Steenk, H. E., and Palmer, W. A. (2006). Initial screening of Pseudomalegia cf. Lefevrei (Coleoptera: Chrysomelidae), a potential biological control agent for Acacia nilotica (L.) Willd. Ex Del. Ssp. Indica (Benth.) Brenan (Mimosaceae) in Australia. *Afr. Entomol.*, **14**: 384-386.

Yuan, M. L., Zhang, Q. L., Guo, Z. L., Wang, J., & Shen, Y. Y. (2015b). The complete mitochondrial genome of Corizustetraspilus (Hemiptera: Rhopalidae) and phylogenetic analysis of Pentatomomorpha. *Plos One*, **10**(6): e0129003.

Yuan, M. L., Zhang, Q. L., Guo, Z. L., Wang, J., and Shen, Y. Y. (2015a). Comparative mitogenomic analysis of the superfamily Pentatomoidea (Insecta: Hemiptera: Heteroptera) and phylogenetic implications. *BMC Genomics*, **16**(1): 460.

Yuan, M. L., Zhang, Q. L., Zhang, L., Guo, Z. L., Liu, Y. J., Shen, Y. Y., & Shao, R.(2016). High-level phylogeny of the Coleoptera inferred with mitochondrial genome sequences. *Molecular phylogenetics and evolution*, **104**: 99 – 111.

Zergoun, Y., Guezoul, O., Sekour, M., Bouras, N. and Holtz, M. (2019). Acridid (Orthoptera: Caelifera) diversity in agriculture ecosystems at three locations in the Mzab valley, Septentrional Sahara, Algeria. *Journal of Insect Biodiversity*, **9**: 18.

Zou, Y., Sang, W., Bai, F., Brennan, E., Diekman, M., Liu, Y., Li, L., Marples, A., Shi, H., Sui, Z., Sun, X., Wang, C., Wang, X., Warren-Thomas, E., Yang, X., Yu, Z. and Axmacher, J. C. (2019). Large-scale α-diversity patterns in plants and ground beetles (Coleoptera: Carabidae) indicate a high biodiversity conservation value of China's restored temperate forest landscapes. *Divers Distrib*. **25**: 1613 – 1624.

Candidate Signature
(Ms. Linta Paulson)

Research Guide Signature
(Prof. Pragna H. Parikh)