General Consideration

Around 30 million species are found worldwide, of which about 1.4 million have briefly described; about 750,000 are insects. Insects now comprise >75% of all described animal species and exhibit not only a wide number of form , colour and form, but also a number of ecological adaptations that are unmatched by any other community (Balakrishnan, 2016). Because of their diversity and many roles, they are familiar to the general public. However, their conservation is a challenge (Jalali *et al.,* 2015). It has been recognised and recorded for a long time that insects are the most complex group of organisms, and most people accept that no more insect species have been identified than there are previously known insect species. (Vallee, 2020).

The role of insects in the functioning of ecological systems is crucial. As a primary source of animal biomass and life on earth, insects contribute to their natural habitats with a broad variety of critical ecological functions, including feeding herbivores, carnivores, and detritus. Unfortunately, because insects are mostly perceived as pests or potential pests, their real ecological importance often goes unnoticed. Hence, Insect biodiversity should not be viewed as an add-on but an integral part of our agricultural ecosystems and paramount importance to food security. Pests existed for as long as humans conducted crop cultivation and insects were often regarded as rivals in the survival battle. At present, herbivorous insects are responsible for 18 percent of the declines in world agricultural production. Most of these insects get chemically regulated. However, less than 0.5% of known insect species are considered pests. For human life, insects are important since crops cannot be developed in an environment without the functions of insects. About 72 % of the world 's crops are pollinated by insects. Hundreds of solitary bees, bumblebees, bugs, beetles and butterflies are insect pollinators. Insect pollinators include hundreds of solitary bees, bumblebees, flies, beetles, and butterflies (Losey and Vaughan, 2006). The greatest danger to natural biodiversity lies in agriculture. Crop farmers should be aware of the role that insects play in sustainable farming. More than 10,000 species of insects damage worldwide food plants. Insect pests are a significant concern for farmers worldwide, and more than 10,000 species of insects have recorded damaging crops (Koul and Dhaliwal, 2010). Despite using various control methods, the control of agriculture pests continues to be critical for farmers. The loss of insect production is 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). Insect diversity is a fundamental trait of agroecosystems and is the key to sustainable agricultural production. (Jankielsohn, 2018).

Despite recording a high growth rate by the country's economy, Indian agriculture has not been in good shape. There are several topics of interest to those directly or indirectly connected with Indian agriculture's growth and development. Since the independence of India, agriculture has remained the bedrock of the economy of the country, and the majority of the population depends on agriculture for their livelihood (Iqbal, 2018). Agriculture is a dominant human activity and occupies about 40% of available land space globally (World Development Indicators online database, World Bank), even more in India (World Bank Report 2010). Modern farming practices (mechanization, monocropping, hybrid varieties, and genetically modified GM crops) combined with the heavy use of agri-chemicals (fertilizers, pesticides, and herbicides) have resulted in a loss of biodiversity in agricultural landscapes and surrounding areas (Benton *et al.,* 2003; Stoate *et al.,* 2009; Benton, 2010)

Gujarat has a record of 1446 species belonging to 147 families of orders Orthoptera (Thakkar *et al.,* 2015); Coleoptera (Thakkar and Parikh, 2016); Lepidoptera (Kataria and Kumar, 2012; Bhatt and Nagar, 2017; Kumar *et al.,* 2019); Hemiptera (Kataria and Kumar, 2012); Hymenoptera (Thakkar and Parikh, 2018); Diptera (Parikh and Sonavane, 2008); Odonata (Parikh, and Sonavane, 2010; Rathod *et al.,* 2016; Patel *et al.,* 2016; Patel, and Ghetiya, 2018). To date, the study has focused on general diversity; however, there is incredibly inadequate information regarding Gujarat's agriculturally important insect diversity in general and Vadodara. ***Hence, the first aim of present work was to have an insight into the distribution and diversity of agriculturally essential insects of the Vadodara District (Chapter I).***

A total of 423 species of insects representing 12 orders and 101 families were recorded. Orders Odonata, Orthoptera, Coleoptera, Hemiptera, Lepidoptera, Diptera, and Hymenoptera were Common at all the study sites, and Orders Thysanoptera and Neuroptera, Thysanura, Dictyoptera, and Isoptera were Uncommon. Percentage distribution of the species richness revealed that the maximum species belonged to order Coleoptera (38%) followed by Orthoptera (17%), Hymenoptera (11%), Diptera and Lepidoptera (9%), Hemiptera (8%), Odonata (4%) and the least representatives were observed in Thysanura, Dictyoptera, Isoptera, Thysanoptera and Neuroptera (1%). An analysis of the total number of the individuals collected exhibited marked variations among the study sites. The proliferation of insects revealed a distinct seasonal variation. The majority of the orders were monsoon dominant (Odonata, Orthoptera, Dictyoptera, Thysanoptera, Coleoptera, Diptera, and Hymenoptera), dominant winter orders were (Thysanura, Hemiptera, Neuroptera, and Lepidoptera), and summer season had the minimum representatives. Year-wise comparison revealed that 2017-'18 had a good assemblage compared to 2018-'19. Based on the insects' ecological role, the collected species were categorized as Bioindicators, Pests, Pollinators, Predators, and Scavengers. The percentage distribution pattern revealed that the Pest species were maximum in number, followed by Scavengers and Predators, whereas the Pollinators and Bio-indicators were found less in number.

This study provides insights on insects of the agriculture fields of Vadodara. Altogether 423 species of insects representing 12 orders and 101 families were recorded. Our results documented differences in insect diversity and abundance between four different study sites, characterized by the vegetation types and cropping. Relatively a good assemblage of the insect fauna was observed at Ajwa (Site I), Chhani (Site II), and Padra (Site IV). The reason for a relatively high number of species may be due to higher crop density in these fields, which provides a more humid microclimate, or the presence of more suitable food resources. Whereas, Karjan (Site III) had relatively low species diversity. The farmers were following the mono-cropping pattern, and the majority of them were organic farms, thus reducing diversity.

This work concludes that the insects' diversity and assemblage were dependent on the vegetation type, and site-specific distribution was very distinct. The study also acquired the seasonal occurrence, and that majority of the species were monsoon dominant. The comprehensive list of insects from the present study is the first time reported in Insects in and around Vadodara's agriculture fields. This study has also derived that the pest status of insects is high in the agricultural fields. Hence, the present study's next objective was to gain insight into the pest status and infestation rates at the study sites. The information gained through the present study can be utilized in developing IPM strategies against helpful and harmful insects within the agroecosystem.

Insects are responsible for two significant kinds of damage to growing crops. ‘First is a next’, the feeding bug that eats leaves or burrows in plants, seeds, or roots is a direct injury caused to the vine. Hundreds of this type of pest species occur among Orthopterans, Homopterans, Heteropterans, Coleopterans, Lepidopterans, and Dipterans, both in larvae and adults. The second form is indirect injury, in which the insect does little to no injury, but transmits a bacterial viral, or fungal infection into a crop. Examples include the viral diseases of sugar beets and potatoes, carried from plant to plant by aphids.

Among the many threats to maintain crop production and nutritional welfare, the direct and indirect harm to insect pests is of vital importance. The population of insect pest outbreaks has enormous potential to damage the agricultural economy. Insects co-evolved millions of years with plants and are of enormous importance for agriculture. Some insects can damage crops, but others also provide pollination and pest control services or improve the soil fertility by feeding on and assisting the decomposition of organic matter. Conventional agricultural pest-management practices often lead to altered community structure (Macfadyen *et al.*, 2009; Macfadyen *et al.*, 2012), and communities dominated by a few species, contributing to pest outbreaks. ***Hence, the second objective of the present work was to insight the pest population and the rate of infestation and its severity in the Vadodara district's agriculture fields. (Chapter II).***

A total of 163 pest species belonging to four significant orders (Coleoptera, Hemiptera, Orthoptera, and Lepidoptera) were recorded during the study period (2017 - 2019). Members of order Coleopteran were found to be the most dominant with 69 pest species spread in 16 families, next in order of the number of representatives was Order Orthoptera with 34 species belonging to 4 families. Lepidoptera was recorded with 31 species spread in 12 families, and last in the order of the number of pest species was Hemiptera, with 29 species represented by 13 families. Site wise occurrence of the pests revealed that the Site II showed maximum followed by Site I, Site IV, and Site III. Although the diversity of Coleopteran pest was maximum, the highest Percentage incidence and the Severity Index were recorded maximum with Hemiptera and

Lepidoptera's order compared to the other two orders years. The site-wise Percentage Incidence and Severity Index of pest depicted that Site IV had a higher occurrence of all the orders than the other three Sites. Year-wise Percentage Incidence and Severity Index of pests were recorded highly significant during 2017 - '18 compared to 2018 - '19. Jaccard similarity index of both the years concludes that there is a maximum similarity of pest species between Site I and IV.

This report is the first of its kind where the pest status of the agriculture fields around Vadodara has been reported and hence can be interpreted as more of a baseline data which will help entomologists and agriculturalists to gain more insights and measures for better yield of the crops. However, there is a need to complement the existing information with additional studies where a detailed understanding of the trophic interaction and population dynamics will affirm how crop pests can be controlled with more directed measures. Further, one can adopt the alternative forms of vegetation diversity, and as an alternative for pest management, specific predators can be released, which reduce pest species abundance, and alleviates the cost of pest control measures.

Recognition of initial symptoms 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 agricultural concern (Karthika *et al.,* 2016). Precise identification of insect species is a requirement for the application of effective management strategies. deploying appropriate management strategies; it is critical to identify pest species accurately during an invasion's early phases. Correct identification to the species level is also essential for the implementation of selective pest control measures.

Identification has historically been based on morphological diagnosis, where taxa can be correctly described only by taxonomists and qualified technicians because it requires specific expertise gained by training (Kress and Erickson, 2007). Moreover, this approach to the task of routine species identification has four significant limitations. First, both phenotypic plasticity and genetic variability in the characters employed for species recognition can lead to incorrect identifications. Second, this approach overlooks morphologically cryptic taxa (Vodă *et al.,* 2015). Third, since morphological keys are often significant only for a particular life stage or gender, many individuals cannot be identified. Finally, although modern versions represent a significant advance, the use of keys often demands (Jarman and Elliott, 2000) such a high level of expertise that misdiagnoses are common.

Thus, researchers have tested the idea that species could be identified quickly and rapidly using only a short DNA sequence, representing a standardized position in the genome and is called a DNA barcode. The main advantage of DNA barcoding is the rapid acquisition of molecular data (Vohra and Khera, 2013). DNA barcoding is a diagnostic technique where the sequence(s) of short DNA is used for species identification and is the most accurate and systemic tool for estimating species diversity. (Floyd *et al.,* 2009; Karthika *et al.,* 2016). DNA barcoding was 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 to study genetic diversity in phyla, and the large subunit of 16S rDNA is often used for families or genera (Mandal *et al.,* 2014).

The motivations for the sequencing genome of any particular insect are nearly as diverse as insects themselves, though major themes emerge around species that have direct medical or agriculture significance for humans (Li *et al.,* 2019). Additionally, many species are sequenced in advancing basic research, often in ecological and evolutionary studies. The progress of insect genome sequencing has been recently reviewed for several well-studied groups, including beetles (McKenna *et al.,* 2019), dipterans (Wiegmann and Richards, 2018), Hymenoptserans (Branstetter *et al.,* 2018), and lepidopterans (Triant *et al.,* 2018).

After making an inventory on the diversity of Insects in the agriculture fields and observing the pest status of Vadodara and its surrounding (Chapter I and II), ***DNA barcoding is used for identification of insect pests belonging to four significant orders, i.e., Coleoptera, Orthoptera, Lepidoptera, and Hemiptera by using mitochondrial sequence information from COI genes to shed light on molecular ecology of insect pests affecting the economically important vegetable crops, which will help in evaluating the determinants in order to study their divergences and phylogenetic relationships (Chapter III).***

A total of 163 insect pest species belonging to 45 families of 4 orders (Coleoptera, Hemiptera, Lepidoptera, and Diptera) were recorded from Vadodara's agricultural fields, from which 51 pest species were chosen based on its infestation on economically important crops. The quality of the genomic DNA indicated an appreciable amplification process with an approximate 720 bp and 620 bp size for COI and 16s rRNA, respectively. The rate of amplification attainment was higher in pests belonging to Coleoptera and Orthoptera over Lepidoptera and Hemiptera. The sequences' similarity was checked using BLAST, and the identity ranged between 95and 100%. A total of 102 samples were sequenced from which 96 barcodes were obtained (51 barcodes of COI and 46 barcodes 16s rRNA), and the accession number (47 for COI and 44 for 16s rRNA) was obtained from NCBI. Of the total, 5 (COI) sequences and 18 (16s rRNA) sequence were novel and first time recorded in the NCBI GenBank database. Overall, our results reveal that CO1 based DNA barcoding has helped unambiguous identification of ecologically and economically important insect species. As this is the first attempt to include four major orders of agricultural importance at global databases, viz., NCBI-GenBank, data generated will not only help conservation planners, taxonomists, and graduate students but also will be useful at commercials ports to encounter any invasive insect species.

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 favourable areas resulting to the destruction of natural biotic communities, altered behaviours, and population distribution (Brown *et al.,* 2010; Mishra *et al.,* 2015). There exists a strong 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 been proposed (Fazekas *et al.,* 2008; Lahaye *et al.,* 2008) for which representation in databases increases rapidly, improving the host's accuracy speed plant identification. For the analysis of host plant associations, the sequence fragments are used by linking them to a named species or DNA-based group to which ecological information from literature or field observations has been associated; such information provides the starting point for analyzing co-evolutionary relationships of plants and pests (Occhipinti, 2013)

Host plant selection by insect pests is often divided into 'host plant finding' and 'host plant acceptance.' The central relationship of host plant finding thought previously to be governed by volatile chemicals, has, until now, proved intractable to scientific experimentation. To combat this problem, a new scientific approach in the 21st century has been developed where barcodes of plants and individual insect species are used to find the homology between the two. More than 50 percent of all recognised species on earth represent plants and their related insect herbivores. Identifying plant-herbivore interactions is the first step in identifying the processes that produce and sustain this essential component of biodiversity. (García-Robledo *et al.,* 2013).

Several DNA-based field studies on intra-guild predation have been reported (Gagnon *et al.,* 2011; Aebi *et al.,* 2011; Ingels *et al.,* 2013). Descriptions documenting predation on exotic pests in the field via molecular gut-content analysis are also available (Gardner, *et al.,* 2013; Greenstone *et al.,* 2014; Opatovsky *et al.,* 2012; Grasswitz, 2016). 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. Main inspiration for this research 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 essential 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.*** **(Chapter IV).**

A total of 51 insect pest species belonging to 4 orders were sequenced from the pest species, from which 13 polyphagous Coleoptera pest species were chosen for the genetic correlation with crops and vegetables.

- *Altica cyanea* shared the homology with *L. siceraria*, *Spinacia oleracea* and *Oryza sativa* indica however, maximum homology was exhibited with *L. siceraria.*
- *Aulacophora indica* and shared the homology with *L. aegyptiaca*, *Moringa oleifera*, and *L. siceraria*; however, maximum homology was exhibited with *L. siceraria.*

- *Chiloloba orientalis* shared the homology with *Zea mays*, *Cenchrus americanus*, *Triticum monococcum*, and *Oryza sativa*; however, maximum homology was exhibited with *O. sativa*
- *Lanelater fuscipes* shared the homology with *Zea mays*, *Solanum tuberosum*, *Triticum monococcum,* and *Daucus carota*, and maximum likelihood resulted in the closest homology were exhibited with *D.carota.*
- *M. pustulata* shared the homology with *Cajanus cajan, Cyamopsis tetragonoloba, S. melongena,* and *L. aegyptiaca;* however, maximum homology was exhibited with *Solanum melongena.*
- *Monolepta signata* shared the homology with *O. sativa*, *L. siceraria*, and *Triticum monococcum*; however, maximum homology was exhibited with *T. monococcum.*
- *Myllocerus dorsatus, M. undecimpustulatus*, shared the homology with *Zea mays*, *Abelmoschus esculentus*, *Ricinus communis*, and *Gossypium hirsutum* however, *M. dorsatus* was exhibited maximum homology with *A.esculentusa.*
- *Oxycetonia versicolor* revealed that it shared the homology with *L.aegyptiaca*, *Vigna unguiculate, Abelmoschus esculentus,* and *Lagenaria siceraria,* and maximum homology was exhibited with *L. aegyptaca.*
- *Protaetia aurichalcea* shared the homology with *L. aegyptiaca*, *L. siceraria*, *V. unguiculate*, and *A. esculentus*; however, maximum homology was exhibited with *L. siceraria.*
- *Sitophilus oryzae* shared the homology with *Z. mays, T. monococcum, Pennisetum glaucum,* and *Oryza sativa;* however, maximum homology was exhibited *Z. mays.*

The trnL and COI from the coleopteran- host plants using standard extraction protocols provide the information on its evolutionary, ecological, and herbivore–host plant interactions. The new research assesses the value of that method when applied to a highly complex group. Marked limitations for host identification are the localhost database's insufficient ability and the lack of clearly known species limits in the hosts, i.e., the trnL sequences do not always provide an unambiguous host status. In contrast, a future increase of the database and use of additional chloroplast and mitochondrial markers will improve the precision, some apparent precincts of host plant inferences independent of the study method.

The current inventory concludes that species-specific homology modeling concerning plant host species using trnl and COI accurately deciphers the phylogeny and unravels intraspecies and interspecies divergence nucleotide distance. The documentation of A, C, G, T suggests an increase in AT bias in interspecies association and may play a major in driving the pest towards the host plant. Moreover, it also demonstrates the mutation rates i.e., the occurrence of transition, transversion, synonymous to non-synonymous changes occurring in the mitochondrial, and chloroplast markers. However, detailed analysis using the CYP P450 marker will help us understand the mutation rates generated due to pesticide exposure, which will help us control the pest population.

Feeding studies of agroecological importance to date have not questioned taxonomy and species limits of host plants, nor assessed population differentiation and geographic turnover. DNA-based analyses will contribute vital information on host populations and spatial differentiation of host use due to this more excellent resolution. Therefore, the technique permits the reinvestigation of pertinent hypotheses explaining agro diversity, the factors that influence the community characteristics like density-dependent factors that maintain high diversity, or maybe the correlation of Coleoptera diversity with the phylogeny of host plants. However, the metagenome analysis on the host-plant use and host specificity opens a new avenue for validation above mentioned hypotheses.

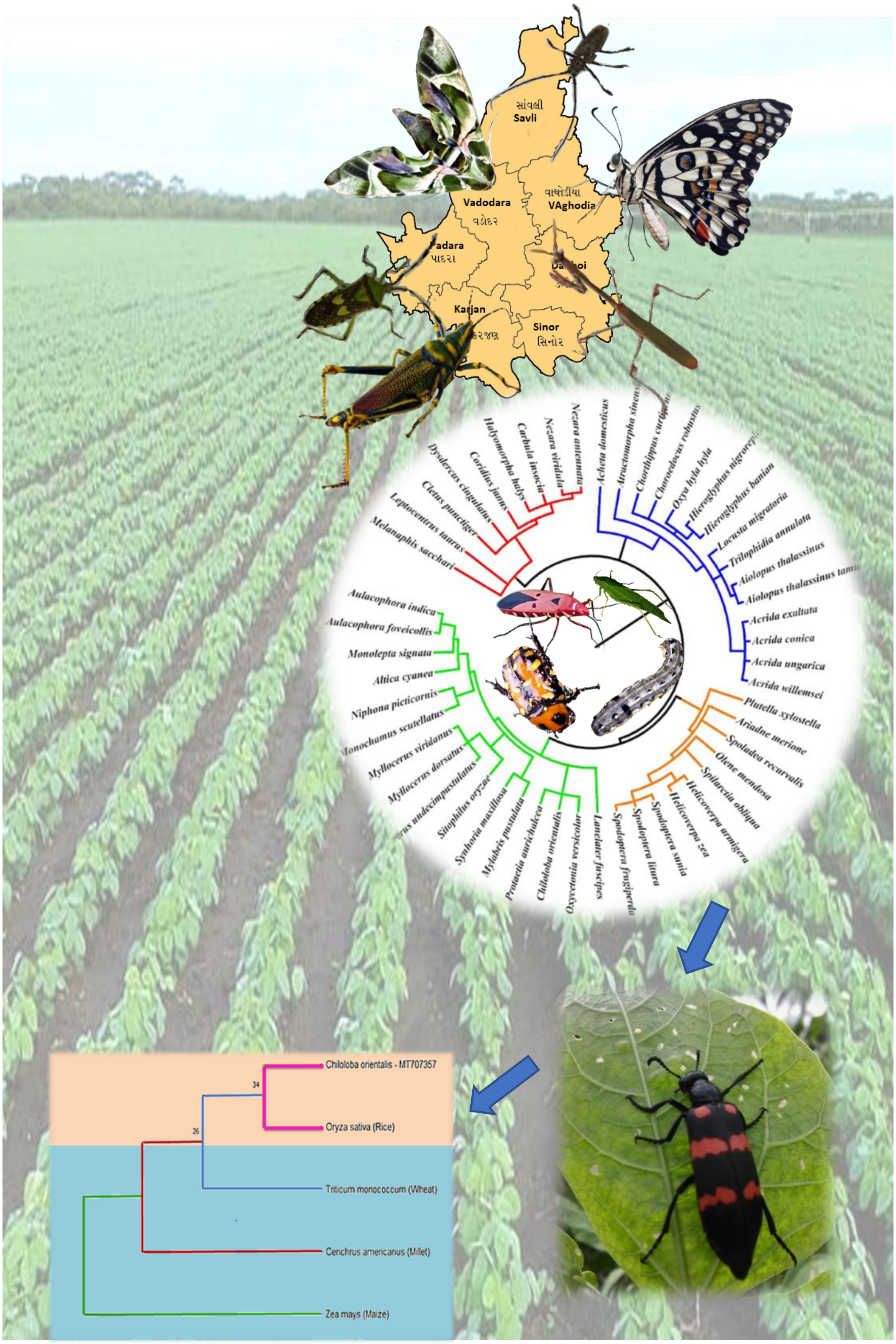

Figure G. C: Summary diagram of Insect diversity, infestation, molecular taxonomy and insect plant molecular correlation in the agricultural fields of Vadodara.

Highlights of the Work

- In the present study, **423** species of insects representing **12** orders and **101** families are recorded. Orders Coleoptera, Orthoptera, Hymenoptera, Lepidoptera, Hemiptera, Diptera, and Odonata were the more dominant orders in the study. Of all the sites studied, Site I (Ajwa) was found to be the most dominant in diversity as well as the richness due to good vegetation cover.
- The study also acquired the species occurrence in the three main seasons, and the result showed the richness of species in the monsoon season.
- As far as the pest status is concerned, a total of **163** insect pest species belonging to **45** families of 4 orders (Coleoptera, Hemiptera, Lepidoptera, and Othoptera) were recorded from the agricultural fields of selected sites. A maximum number of pest species were reported from order Coleoptera, followed by Orthoptera, Lepidoptera, and Hemiptera. Order Hemiptera and Lepidoptera showed more incidence and severity index and were highest in the 2017- ‘18 compared to the year 2018-‘19 due to extreme climate variation.
- Phylogenetic analysis of a total of **102** samples (**51** species) were sequenced from which **97** barcodes were obtained (**51** barcodes of COI and **46** barcodes 16s rRNA) NCBI has provided the Accession number for **91** sequence. ***Out of which 5 COI sequences and 18 of 16s rRNA sequence of pest species were novel and first time recorded in the NCBI GenBank database.***
- A homology modeling for interrelationship with the plants and Coleoptera by using trnl and COI was deciphered by analyzing the phylogeny, intra- species and inter-species divergence and nucleotide distance.
- AT bias was found for interspecies association which plays a major role in driving the pest towards the host plant.
- Analysis of nucleotide substitution exposed the mutation rates i.e. the occurrence of transition, transversion, synonymous to non-synonymous changes occurring in the mitochondrial and chloroplast markers.

Future prospects

- The collected data of insects for the agriculture fields which forms a baseline studies should be done continuously to understand the precise pattern of Insect diversity of agriculture fields. So that, the accurate estimation of relative abundance dynamics of the species over time and space. Further, effects of biotic and abiotic factors should be studied so as to understand its influence on the infestation rate.
- Characterization of multitrophic interactions can reveal novel perspectives on the complexity of induced signalling networks between plants and pest. Hence, Identification of species-specific molecular markers (trnH-psbA, rbcL, ITS2) will help in accurately management of pest insects with more directed measures.
- A detail analysis of CYP P450 marker of the phylogenetically analyzed species will help us in understanding the mutation rates, and further the interaction CPY with CPR will enrich the knowledge of p450 enzymes and assist in elucidation of the function of P450 and CPR in the pest insects.
- The advance techniques have proven to be a major breakthrough in understanding the insect systematics at molecular level. Thus, metagenome sequencing will elucidate the species-specific taxonomic details and will throw more light on the conservation status of agriculturally important insects. Moreover, A major application of bioinformatics will help the modeling of genetic and metabolic networks, and comparative genomics will be useful for pinpointing common and different genes across species. Genome comparisons between different organisms can be focused on to explore gene functions. Thus, in future, there will be a growing tendency for insect molecular scientists to reach out to the broader molecular biology community with all the benefits that such interactions can have for the application of molecular tools in insect science.