STUDIES ON DIVERSITY OF COTTON THRIPS IN MAHARASHTRA

THESIS

Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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By
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(A) DECLARATION OF STUDENT

I hereby declare that, the experimental work and its interpretation of the thesis entitled, **“STUDIES ON DIVERSITY OF COTTON THRIPS IN MAHARASHTRA”** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Nagpur	(Baghele Durga Janardan)
Date: 31/05/2019	Enrolment No – LL-03

(B) CERTIFICATE

This is to certify that thesis entitled, **“STUDIES ON DIVERSITY OF COTTON THRIPS IN MAHARASHTRA”**submitted in partial fulfilment of the requirement for the degree of **“Master of Science in Agriculture (Agricultural Entomology)”** of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **BAGHELE DURGA JANARDAN** under my guidance and supervision.

The subject of the thesis has been approved by the Student's Advisory Committee.

Place: Nagpur
Date: 31/05/2019

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THESIS APPROVED BY THE STUDENT’S ADVISORY COMMITTEE INCLUDING EXTERNAL EXAMINAR (AFTER VIVA-VOCE)

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Place: Nagpur **(Baghele Durga Janardan)**
Date: 31/05/2019

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(G) Abbreviations

%	-	per cent
@	-	at the rate
BLAST	-	Basic local alignment search tool
BOLD	-	The barcode of life data
bp	-	Base pair
Cm	-	Centimeter
COI	-	Cytochrome oxidase one
COX	-	Cyclooxygenase
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylenediaminetetraacetic acid
et al.	-	et alia (And others)
etc	-	Etcetera
F	-	Forward
GPS	-	Global Positioning System
G	-	Relative centrifugal force
Fig.	-	Figure
Ha	-	Hectare
HCL	-	Hydrochloric acid
ITS	-	Internal transcribed spacer
Mg	-	Milligram
$MgCl_2$	-	Magnesium chloride Days After
Ml	-	milliliter
mm	-	millimeter
NCBI	-	National Center for Biotechnology Information
PCR	-	Polymerase Chain Reaction
R	-	Reverse
RAPD	-	Random Amplification of Polymorphic
TAE	-	mixture of Tris base, acetic acid and
UV	-	Ultraviolet
V	-	Volt
µl	-	Microliter

(H) THESIS ABSTRACT

a) Title of the thesis : **STUDIES ON DIVERSITY OF COTTON THRIPS IN MAHARASHTRA**

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ABSTRACT

The present study entitled, “Studies on diversity of cotton thrips in Maharashtra” was undertaken by collecting thrips samples from cotton crop from the 11 districts of Maharashtra *viz.,* Nagpur, Wardha, Amravati, Yavatmal, Akola, Washim, Buldhana, Jalgaon, Dhule, Parbhani and Ahmednagar. All the investigations were carried out at ICAR- Central Institute for Cotton Research, Nagpur during *Kharif* 2018. Study was conducted under the objectives: i) Survey and collection of thrips from different cotton growing districts of Maharashtra and ii) To study the diversity of cotton thrips in Maharashtra. Genomic DNA extraction and amplification of COI region was obtained in all samples using the primers C1-J-1751 F and C1-N-2191R.

The results revealed diversity of 4 species of thrips *viz., Ayyaria chaetophora* Karny, *Rhipiphorothrips cruentatus* Hood, *Thrips hawaiiensis* Morgan and *Thrips palmi* Karny belongs to order Thysanoptera and family Thripidae, infest the cotton crop in Maharashtra. *Ayyaria chaetophora* identified from surveyed field of Nagpur and Parbhani districts. *Thrips palmi* was identified from Nagpur, Amravati, Wardha, Buldhana, Akola, Yavatmal, Jalgaon, Dhule, Ahmednagar and Parbhani districts. *Rhipiphorothrips cruentatu*s species identified from the Katol region of Nagpur. *Thrips hawaiiensis* was identified from Washim.

Comparison of the sequences with those in Gene bank and The barcode of life data (BOLD) revealed that 9 samples of *T. palmi* showed the close sequence matches of 100% with the National Center for Biotechnology Information (NCBI) sequences and 3 samples of *T. palmi* species were found 99% similarity. Both *T. hawaiiensis and R. cruentatus* showed 100% similarity with NCBI sequences whereas 3samplesof *A. chaetophora* resembles about 91% similarity with NCBI sequences.

Phylogenetic analysis of molecular data, results into the formation of three major clusters (clades) in 17 thrips samples collected from 11 cotton growing districts of Maharashtra. Abundant species *T. palmi* comes under clade 1 due to similarity in their sequences. Whereas all three identified species *A. chaetophora* shared same clade under the clade 2. However, *T. hawaiiensis* and *R. cruentatus* comes

under clade 3 showed close relatedness or genetic similarity among them.

Identified thrips species from cotton growing districts revealed that *T. palmi* was most abundant species found throughout the cotton growing districts of Maharashtra. *A. chaetophora* thrips species also identified from different locations of Maharashtra from where samples collected. *T. hawaiiensis* and *R. cruentatus* reported from the limited location indicated that the distribution of this thrips species is limited on cotton crop.

Chapter I

INTRODUCTION

1.1 Background information

Cotton is an angiosperm, dicotyledonous plant, belongs to *Gossypium* genus and Malvaceae family. Cotton recognises as the king of fibre and white gold as it is an industrial commodity of World Wide importance. It is one of the most important commercial crop playing economic and social affairs of world. It is multipurpose crop and apart from lint, it also provides various by-products such as cotton seed, cotton seed cakes, meal and biomass. Cotton lint is most important product which provides high quality fibre for textile industry.

Cotton is grown in over 85 countries in the world. India, Russia, USA, China, India, Brazil, Pakistan, Turkey, Egypt, Mexico and Sudan account for about 85 percent of total production. Cotton cultivated over an area of 34.5 m ha in the world. India accounts for about 27% of world cotton area and 14% of world productions. India is the largest producer of cotton in the world. In India cotton is cultivated in 10 Major states *viz.,* Maharashtra, Gujarat, Madhya Pradesh, Punjab, Karnataka, Tamil Nadu, Andhra Pradesh, and Telangana. Cotton production in India during 2017-18 was348 lakh bales (1 bale=170kg lint) on acreage of 124.29lakhhectares (Nagrare *et al.,* 2019).

Cotton is cultivated in 27 districts of Maharashtra and around three millions of farmers and farm labour depend on the cotton crop for their direct and indirect livelihood. Approximately 41.46 lakh hectares, which is about 17 percent of Maharashtra’s net sown area is under cotton (Kulkarni *et al.,* 2017). Cotton is vulnerable to many insect pests due to which yield reduction ranging from 20-90% was recorded (Bhaltilak *et al.,*2018).

1.2 Importance of study

There are 1326 species of insects recorded on cotton from sowing to maturity in different cotton growing areas of world

(Hargreaves, 1948) and in India, 162 species have been reported on cotton crop (Sundramurthy and Chitra, 1992). Cotton bollworms *viz.,* American bollworm (*Helicoverpa armigera* (Hubner)), spottedbollworm (*Earias insulana* (Boisduvel), *Earias vittella* (Fab.)), Pink bollworm (*Pectinophora gossypiella* (Saunders)) and sucking pests *viz.,* jassid (*Amrasca biguttula biguttula (*Ishida)); aphid (*Aphis gossypii* (Glover)); whitefly (*Bemicia tabaci* (Gennadius)) and thrips (*Thrips tabaci* (Lindeman)) contributed significantly in lowering potential yield (Nagrare *et al.,* 2013). Among the sucking pests**,** thrips (Thysanoptera: Thripidae) causes the injury starting from seedling and continues till the boll opening. Both the nymph and adult remains on the lower surface of leaves and feed on the leaves by rasping the epidermis and sucking the cell sap. Symptoms such as curling of leaf, silvery patches under surface of leaves and bronzing of leaves developed. Upward and inward curling of the leaf margin occur towards the main stem (Telford and Hopkins, 1957). Leaves show the twisting appearance and bolls result into development of necrotic patches. Thrips act as a vector of tobacco streak virus of cotton (Vinodkumar *et al*., 2017).

Thrips are weak fliers, having the fringed wing which enable them to remain airborne long enough to travel easily between neighbouring field and blown by wind over long distance (Lewis, 1997). Thrips can be found on various crops as well as weed species, many of which are found within cotton production environment (Cook *et al.,* 2011). Tobacco thrips, *F. fusca* as well as complexes of other thrips species attack on cotton crop (Samler, 2012). Four species, *Thrips tabaci, Frankliniella occidentalis, Scirtothrips dorsalis, and T. palmi* describes in the literatures (Mound, 2002).*Thrips palmi* Karny caused damaged on the leaves of cotton in the Philippines (Bournier, 1983).

Because of cryptic behaviour of thrips and small size it is very difficult to detect them in the field and on plants or plant products transported for international trade. Fresh plant material transported around the world which may readily spread these insects in the form of eggs, larvae or adult (Kirk and Terry, 2003). Therefore, many species spread from their natural habitats. After translocation to new areas outside of the home range, some species may reproduce rapidly and causes the significant economic losses of the crops. Chemical treatments for the control of thrips are often specific, especially for the thrips that have acquired insecticides resistance (Bielza, 2008) and as a fundamental first step, these treatments required correct and unambiguous identification of species (Rugman-Jones *et al.,* 2006).Molecular studies could also elucidate the prevalence of biotypes and cryptic species (Fakrudin *et al.,*2006).

Occassionally thrips species found on cotton doesn’t mean that cotton used as host plant by that species and they may disperse from another host plant through the wind. Understanding the host plant and host range of insect is important for the number of studies in ecology, speciation and coevalution of insect- host plant association (Jurado-Rivera *et al.,* 2009).

Published reports revealed scanty information on the diversity of thrips especially in cotton growing districts of Maharashtra. Understanding its population diversity advances the knowledge about its ecology and management (Nault *et al.,* 2014). Therefore, it becomes necessary to study the genetic diversity of cotton thrips in Maharashtra.

1.3 Objectives of the study

Considering research gaps, it was envisaged to carry out investigation on thrips diversity by using the molecular techniques with the following objectives:

1) Survey and collection of thrips from different cotton growing districts of Maharashtra.

2) To study the diversity of cotton thrips in Maharashtra.

1.4 Scope and limitation of study

Because of small size, cryptic behaviour, polyphagous in nature, sexual dimorphism and misidentification of species occur on the basis of morphological characters. Larval thrips are often mistaken with collembolan and adult are confused with staphylinid beetles (Vierbergen, 1995). Identification of thrips to species level required great expertise and in many cases it is only possible using adult (Brunner *et al.,* 2002). Assessing molecular diversity, extent and spread is essential for understanding the behavior, response to selection pressure, structure and dynamics of different insect population (Yenagi *et al*., 2012). Molecular data provide the means to differentiate sympatric and allopatric species and also modes of evolution.

Thus, study of molecular diversity of thrips on cotton crop helps in accurate identification of thrips at species level which ultimately precedence in designing of correct management practices. Simple and accurate method of identification of species is molecular identification which facilitated the identification of thrips in a cropping system where multiple species co-exist (Biradar, 2015).

Limitations are

Identification of thrips species base on DNA technology is simple and accurate, having limitation such as that it was technical methods it required high skill and management. Molecular methodologies are usually more expensive than standard traditional methodologies.

1.5 Hypothesis

As the identification of insect species is the fundamental step in plant quarantine diagnosis and treatments against exotic insect pest and pathogens (Biradar, 2015). Current research on diversity of cotton thrips in Maharashtra will explore the accurate identification of thrips species in cotton ecosystem.

Chapter II

REVIEW OF LITERATURE

Several researchers have attempted through their research work to explore the various aspects of molecular study of thrips in cotton crop. Various literatures are available from India and abroad on different aspect of thrips which has been reviewed under the following heads:

2.1 Survey and collection of cotton thrips

National status

Gupta *et al.* (1997) recorded incidence of thrips *Thrips tabaci* Lindeman and *Scirtothrips dorsalis* Hood was recorded at four-week stage of cotton crop during 1989 and 1990 in Madhya Pradesh.

Prema *et al*. (2018) used the coloured sticky traps to monitor thrips population in cotton and reported that *Thrips palmi, Thrips tabaci* and *Scirtothrips dorsalis* are the species of thrips found on the cotton ecosystem in Tamil Nadu.

International Status

Gaines (1934) conducted a study of thrips on seedling cotton and reported that a large number of thrips occurred on cotton that causes significant damage. He reported the most common species of thrips on cotton seedling as *Frankliniella tritici* and *Sericothrips variablis* with *F. fusca.*

Bournier (1983) described the damage caused by *Thrips palmi* Karny on the leaves of cotton in the Philippines. However, the species was not recorded in the Philippines until 1981. It was suspected to have been present there for longer than had been supposed and to have been mistaken for *T. tabaci* or *T. flavus,* which closely resemble it.

Mabbett *et al*. (1984) studied the pest scouting techniques in cotton in relation to distributions of thrips *Ayyaria chaetophora* Karny and jassids *Amrasca devastan* on cotton in Thailand.

Wilson and Bauer (1993) studied the species composition and sesonal abundance of thrips on cotton in Namoi Valleyand found that species composition changing from predominantly *T. tabaci* early to *T. imaginis* mid-season and *Frankliniella schultzei* late season.

Faircloth *et al*. (2001) reported that reproductive potential of tobacco thrips *Frankliniella fusca* (Hinds) was greater than western flower thrips, *Frankliniella occidentalis* on cotton seedling.

Cook *et al*. (2003) conducted a survey in different states of United States of America in cotton field to determine the thrips species infesting the cotton and reported seven species of thrips out of which tobacco thrips (*Frankliniella fusca*) was the most common collected species in all states. Other species recorded tobacco thrips (*Frankliniella fusca* (Hinds)); western flower thrips (*Frankliniella occidentalis* (Pergande)); onion thrips (*Thrips tabaci* (Lindeman)); and soybean thrips (*Neohydatothrips variabilis* (Beach)).

Slosser *et al*. (2005) conducted experiment on thrips species associated with cotton in the Northern Texas rolling plains and recorded six species*viz., Frankliniella exigua* (Hood), *F. fusca* (Hinds), *F. genuine* (Hood), *F. occidentale* (Pergande), *F. tritici* (Fitch) and *Thrips tabaci* (Lindeman).

Mailhot *et al*. (2007) described the species of thrips in cotton flowers to determine how they were affected by the insecticides application and recorded that *Frankliniella tritici* was the most common species on cotton.

Greenberg *et al*. (2009) studied the species composition and observed the seven species of thrips and six species of natural enemy on cotton in the Lower Rio Grande Valley of Texas. Among different thrips *Frankliniella occidentalis*, *Caliothrips fasciatus* and *Thrips tabaci* were most abundant.

Akin *et al*. (2010)conducted a survey in Texas and Verginia in the cotton belt to determine the composition of thrips species of cotton and reported five species of cotton *viz.,Frankliniella tritici, Franklinia occidentalis, Thrips tabaci* and soybean thrips (*Neohydatothrips variabilis*) from fifteen to sixteen location.

Cook *et al.* (2011) studied the biology, crop injury and management of thrips infesting cotton seedling in the United States and found that five species are commonly found on cotton seedling. These are western flower thrips *Frankliniella tritici*, soyabean thrips *Neohydato thrips variabilis*, onion thrips*Thripstabaci* and tobacco Thrips *Frankliniella fusca* in entire United States.

Samler (2012) observed the abundances and species diversity of thrips in cotton and peanut in Southeast Virginia concluded that tobacco thrips, *Frankliniella fusca* as well as complex of other thrips species attack cotton and peanut seedlings.

Stewart *et al*. (2013) observed association of six thrips species with cotton in the Southern United States *viz., Frankliniella fusca, Frankliniella williamsi* Hood, *scolothrips paladus* Beach, *Microcephalothrips abdominalis* (D. L. Crawford), *Thrips tabaci* and *Thrips nigriliposus*, out of which *Frankliniella fusca* was most dominant species.

Silva *et al.* (2018) studied the seasonal abundance of cotton thrips across the crop and non- crop vegetation in an Australian cotton producing region and reported thrips species *viz., Thrips tabaci, Frankliniella occidentalis* and *Frankliniella schultzei.*

2.2 Study on diversity of thrips

National status

Meena *et al.* (2005) studied the molecular characterization of tospovirus transmitting thrips population from India and constructed the dendrogram on the basis of RAPD similarity matrix revealed that *S. dorsalis* population from tomato, groundnut and chillies showed the 75% similarity and only 50% similarity found between the *Frankliniella schultzei* population from cowpea and sunhemp. Also *T. tabaci* from cotton was distantly related *S. dorsalis* and *F. schiltzei* with lowest similarity indices.

Asokan *et al.* (2007) studied the molecular diversity of thrips species *viz*., *Thrips tabaci* and *Thrips palmi* collected from onion and watermelon, respectively at Bangalore. Result shows that overall variations observed in *T. palmi* (DQ228495) was 2-11% out of the total length of 484 nucleotides compared to the 24 NCBI accessions and *T. tabaci* (DQ228495) had an overall variation of 8% with references to two other NCBI accessions. Phylogenetic analyses of both *T. tabaci* and *T. palmi* were different clades as compared to the other NCBI accessions.

Rebijith *et al.* (2011) studied molecular diversity of thrips species *viz., Thrips palmi* and *Scirtothrips dorsalis* collected from watermelon and capsicum respectively using mt COI gene. The PCR amplicon of the size 700 bp was amplified for *T. palmi* and *S. dorsalis* and the result show that the total nucleotide length obtained was 706bp for both *T. palmi* and *S. dorsalis*.

Asokan *et al.* (2013) conduct experiment on the molecular diversity of cardamom thrips (*Scirtothrips cardamomi*) using mitochondrial COX-I as marker and universal primers like LCO1490 and HCO2198 for the analysis. The COX-I sequencing resulted in 655 bp sequences for the 45 intraspecific populations. These results indicated that the populations of *S. cardamomi* from the same locations analysed belong to a single species. Such results on *S. cardamomi* showed that there were no appreciable nucleotide differences in its intraspecific populations.

Kadrivel *et al*. (2013) used the molecular approaches to identify the thrips species collected from vegetables crops using the partial cytochrome oxidase I (COI) sequences. The phylogenetic analysis showed that the insects used in that study clustered with five distinct species such as *Thrips palmi*, *Thrips tabaci*, *Frankliniella occidentalis* and *Scirtothrips dorsalis* of thrips species. Higher intraspecific genetic variation was observed in *S. dorsalis*and *T. palmi* followed by *T. tabaci* and *F. occidentalis.*

Rebijith *et al.* (2014) employed the CO-I gene sequences for discriminating 151 species of thrips and analyses of the intraspecific and intrageneric distances of the CO-I sequences was ranged from 0.0 to 7.91% and 8.65% to 31.15% respectively and also found that the cryptic species in *Thrips hawaiiensis* and *Scirtothrips perseae* for the first time along with previously presently species such *Thrips palmi, Thrips tabaci, Frankliniella occidentale* and *Scirtothrips dorsalis.*

Uppar (2014) studied the molecular diversity of cotton thrips in ARS Dharwad and reported that *Thrips tabaci* COI gene show 88 per cent homology and *Scirtothrips dorsalis* COI gene sequence showed 91 per cent homology with NCBI published sequence. The study indicates that there is a wide diversity among the cotton thrips population of four districts of Karnataka.

Biradar (2015) studied the molecular diversity of thirps species in groundnut and based on sequence comparison, sharp amplicon of expected size (700 bp) of DNA band of thrips sample obtained concluded that all the fragment of COI gene sequences of test insects collected from six regions of Karnataka were confirmed as *Scirtothrips dorsalis* cytochrome oxidase subunit I (COI) gene.

Tyagi *et al.* (2017)attempted the DNA barcoding studies on thrips in India and found that cryptic diversity including, two major vector species *Frankliniella schultzei* and *Thrips palmi.* However, four morphospecies *Thrips moundi, Thrips carthami, Haplothrips andersi* and *Haplothrips gowdeyi* showed low genetic distances between them.

International status

Palmer and Wetton (1987) studied the morphometric analysis of *Thrips hawaiiensis* (Morgan) species-group and compared the result with previously published morphometric data concerning the separation of *T. hawaiiensis* and *T. florum*. They also assessed additional distinguishing characteristics.

Jenser *et al*. (2001) applied the RAPD-PCR for observing intraspecific molecular variations between *T. tabaci* population collected from tobacco and from onion. The amplified DNA-banding patterns of tobacco population were significantly different from those propagating on onion. Total 54 DNA bands were amplified, out of that 39 were different and 7 bands were mutual between two population. Slight differences found on the female and larvae of *T. tabaci* population cultivated on tobacco and onion on the basis of PCR fragment.

Bayar *et al.* (2002) studied the molecular polymorphism of six species of Thysanoptera of both sexes, collected from different locations and host plants in Hungary by using RAPD-PCR technique. Dendrogram resulted in two main groups: Aeolothripidae *(Aeolothrips intermedius)* and Thripidae *(Frankliniella intonsa, Kakothrips robustus, Odontothrips confusus, Thrips dilatatus and T. tabaci).* Within the family Thripidae two subgroups were observed including *F. intonsa, T. dilatatus* and *T. tabaci, K. robustus* and *O. confusus.*

Brunner *et al.* (2002) conducted an experiment on molecular identification key for economically important thrips species. The report revealed that 264 individuals of thrips representing 10 named species and detected 17 haplotypes. Variation within species was low and among species was high.

Brunner *et al.* (2004) tested the host associated genetic differentiation in *Thrips tabaci* using the mitochondrial sequence data. Clustering analyses and haplotype networks based on sequence yielded that three major evolutionary linages found in which two were associated with leek and third with tobacco.

Fang *et al*. (2005) evaluated the level of polymorphisms and genetic relationship of eight populations of *Thrips tabaci* and *Frankliniella occidentale* using amplified fragment length polymorphism. They were found few unique bands of characterizing some of population within each species and high level of polymorphism within each species whereas the high level of polymorphism within populations suggests a high level of heterozygosity.

Walsh *et al.* (2005) conducted the study on the random amplified polymorphic DNA analysis for identification of *Thrips palmi* species and found that 21 thrips species including 10 other species of the genus *Thrips* including*T. tabaci*, *T. nigropilosus, T. flavus* and were found to be specific to *T. palmi*.

Toda and Murai (2007) investigate the intraspecific genetic diversity of *Thrips tabaci* and810bp region of the mitochondrial cytochrome oxidase gene subunit I (COI) were analyzed, also tested the eight populations from six foreign countries and 18 Japanese populations and identified the 17 different haplotypes with two distinct groups shown by the phylogenetic tree of the COI gene.

Timm *et al.* (2008) conducted an experiment for the identification of thrips species in southern Africa. From the analysis of 163 individuals there were nine species of thrips recorded *viz.*, *Thrips tabaci, Frankliniella occidentale, F. schultzei, Scirtothrips aurantii, Haplothrips nigricornis, H. clarisetis, Fulmekiola serrata, Synaptorthrips prosaleae, Heliothrips sylavanus* were found on the basis of COI sequences.

Walter *et al*. (2008) conducted the field study for identification of thrips species on cotton crop using the morphological keys. Outcome of field survey was that nine species were found across years and also concluded that thrips were very abundant at the beginning of the season, but abundance slowly decreased both years during the four weeks of sampling.

Glover *et al.* (2010) studied the comparison of five different loci to investigate their ability to discriminate a small number of thrips species. All five loci discriminated the species by neighbour-joining tree and varying degrees of discrimination were determined upon further investigation of the intraspecific and interspecific distances. Two distinct COI clades were observed for *T. palmi* and judged to be COI haplotypes when data from the other four additional loci and geographical collection data were taken into consideration.

Karimi *et al.* (2010) performed an experiment to identify thrips species by using DNA barcodes and found that thrips sequences in six major clades such as *Frankliniella occidentalis, Haplothrips* spp.*, Thripspalmi, T. vulgatissi and T. tabaci* (two clades) *species.*

Kumar *et al.* (2011) conducted a preliminary morphometric analysis of various populations of *Scirtothrips dorsalis* from five different geographical regions including India, Japan, Israel, and St. Vincent and Florida in the United States of America. Comparing fourteen morphometric characteristics of each population indicated that the Japan population was significantly more robust than the other population.

Srinivasan *et al*. (2011) conduct an experiment for analysing variations of *Thrips tabaci* population from Georgia and Peru and observed that new biotypes of *T. tabaci* had been introduced.

Zhang *et al.* (2011) studied a species-specific COI marker for rapid diagnosis of the invasive species of western flower thrips and found that their diagnostic PCR assay provides a quick, simple and reliable molecular technique for the identification of *F. occidentalis.*

Buckman *et al.* (2012) conducted an experiment on molecular study of thrips for their phylogenetic study they observed that ninety-nine thrips species from seven of the nine families, all six subfamilies and 70 genera were sequenced. Four of the six subfamilies recovered as monophyletic *viz*., Phlaeothripidae, Aeolothripidae, Melanthripidae and Thripidae and the two largest subfamilies, Phlaeothripinae and Thripinae, were paraphyletic.

Alana *et al*. (2013) studied the population genetic structure of *T.tabaci* collected from four locations in North Carolina and population genetic structures of *T. tabaci* by mitochondrial COI sequence analysis carried out. Result revealed the presence of two genetically distinct groups.

Westmore *et al.* (2013) conducted an experiment on genetics and host-associated differentiation within *Thrips tabaci* and its link to tomato spotted wilt virus vector competence. Result concluded that 15 Austalian population, there were six variable sites, only 2-3 sites difference separating population from onion and potato. All population from potato had identical sequence to each other.

Fekrat *et al*. (2014) studied the morphometric and molecular variation in *Thrips tabaci* population on onion and tobacco in Iran by collecting the four population of *Thrips tabaci* from tobacco and 18 populations from onion. The result was found that both molecular morphometric analyses showed heterogenecity of *T. tabaci* populations representing at least two different biotypes on tobacco and on onion.

Nault *et al.* (2014) mitochondrial cytochrome oxidase subunit I (COI) gene sequence was used as a molecular marker to analyze *T. tabaci* population from onion and cabbage fields in New York and observed that eight COI haplotypes were identified in 565 *T. tabaci* individuals. All *T. tabaci* were thelytokous and genetically similar to those originating from hosts representing seven plant families spanning five continents.

Yeh *et al.* (2014) developed the species specific primers, nuclear internal transcribed spacer (ITS2) and successfully used the multiple PCR techniques to identify the flower thrips *Frankliniella* *occidentalis* and also found *Thrips tabaci, Thrips hawaiiensis* and *Frankliniella intosa* in the given samples.

Macharia *et al*. (2015) carried out research on the diversity of thrips species in Kenya using the cytochrome oxidase I (COI) collected from tomato and weeds. Phylogenetic analysis shows that among the collected species *Ceratothripoides brunneus* Bagnall was the predominant species of thrips in all areas. *F. occidentalis* and *T. tabaci* were abundant in Nakuru, Kirinyaga, and Loitokitok. Other vectors of tospoviruses identified in low numbers were *Frankliniella schultzei* (Trybom) and *Scirtothrips dorsalis* (Hood).

Nakahara and Minoura (2015) identified four thrips species *viz., Thrips palmi, T. tabaci, F. intonsa* and *F. occidentalis* by using PCR and amplified the internal transcribed spacer 2 regions (ITS2) of nuclear ribosomal DNA using 5 specific primers. For 71 individuals of the four species examined in this study, species-specific single bands were detected.

Jacobson *et al.* (2016) studied the cryptic diversity in *Thrips tabaci* and revealed that genetic structure supports *T. tabaci* as a cryptic species complex; these results also provide evidence that thelytoky and arrhenotoky was not fixed phenotypes among members of different *T. tabaci* lineages that have been generally associated with either reproductive mode.

Iftikhar *et al.* (2016) analyzed the sequence variation of 471 thrips samples collected from various host plant in Pakistan, it was observed that sequences of *T. palmi* revealed close matched >99% with NCBI sequences and some sequences of identified species show >98% with NCBI sequences.

Daniel *et al*. (2018) explored the species diversity of thrips in Mexican avocado orchards based on partial sequences of the mitochondrial gene cytochrome oxidase subunit I (COI). By analysing the forty-four specimens, they were identified six genera *viz.,* Scirtothrips, Neohydatothrips, Frankliniella, Leptothrips, Arorathrips and Caliothrips. The most abundant specimens were those within the genera Scirtothrips and Frankliniella.

Wang *et al.* (2018) conducted the molecular identification of thrips species infesting cotton in the Southeastern United States and result revealed that *Frankliniella fusca* was the dominant species across all the location followed by *F. occidentalis* and *F. tritici* whereas *T. tabaci* were occasionally found.

Chapter III

MATERIALS AND METHODS

The present investigation entitled, “Studies on diversity of cotton thrips in Maharashtra” was carried out at ICAR - Central Institute for Cotton Research, Nagpur during kharif season of 2018-2019 to explore the genetic diversity of cotton thrips in the cotton growing districts of Maharashtra.

Details of materials and methodology followed during the course of studies are described herewith.

3.1 Materials required for the research work

For conducting laboratory studies, camel hair brush, eppendorf tube, thrips samples, PCR tubes stand, ethanol (70 per cent), filter paper, parafilm, micropestle, ice-cold lysis buffer, 5 mM of Tris-HCl, pH 8.0, 0.5 mM of EDTA, 0.5% Nonidet P-40 and 1 mg/mL of proteinase K, PCR tube, micropipette, centrifugation machine, gel casting plate, comb, tissue paper or delicate task wipers and agarose powder, 1X TAE buffer, Ethidium bromide, 2 µL loading dye, UV light source,0.4 µL of dinucleotide triphosphates (dNTPs), 16.4 µL of distilled water and 4 µL of 5X buffer, 1.4 µL of $MgCl_2$ and 0.3 µL of *Taq* DNA polymerase and 0.5 µL of each forward and reverse primers, stereoscopic zoom microscope and slides etc. were utilized for conducting experiment and all these materials were made available by ICAR- CICR, Nagpur.

3.2 Survey and collection of thrips specimens from cotton fields

3.2.1 Survey of cotton growing districts of Maharashtra

To ascertain the infestation and extent of damage due to thrips and for the collection of thrips samples, survey was conducted in 11 cotton growing districts of Maharashtra (Fig. 1) *viz.,* Nagpur, Akola, Amravati, Washim, Wardha, Yavatmal, Buldhana, Dhule, Jalgaon,

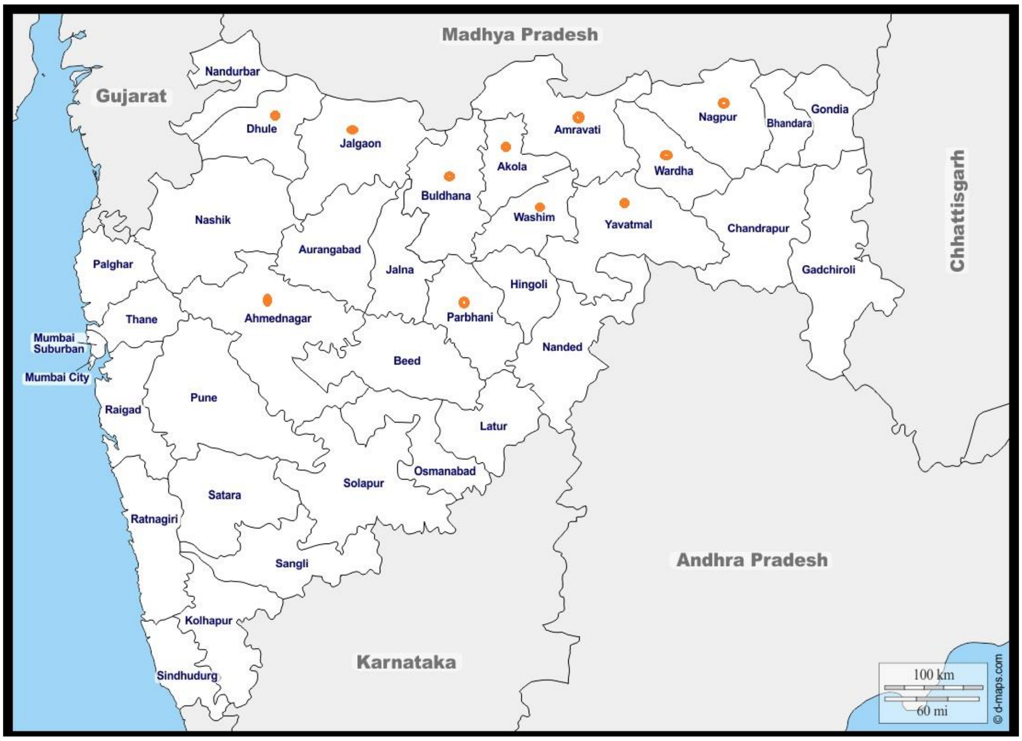

Fig. 1: Map of Maharashtra showing the survey areas of cotton growing districts

Parbhani and Ahmednagar districts during August to September 2018, when the infestation was at its peak. The vials were labelled with sample details such as name of specimen, location, latitude and longitude of collection points (GPS location), date of collection, collected by etc. and after collection of samples they were suitably coded as per given in table 1.

3.2.2Method of collection of specimens and preservation

Before the collection, thrips were observed on leaves, stem and flowers of cotton crop. Then thrips were collected by dipping the small size brush in ethanol and slightly pressing the brush on the thrips observed on the cotton leaves, flowers and again dip the brush in vials containing 70% ethanol, so that thrips settled down at the bottom of vial as shown in the plate 1. Collected samples were then preserved at -20^0C temperature (Deep Freezer Make) till the further analysis.

3.3 Extraction of genomic DNA from the insects

Genomic DNA was extracted by using the protocol given by Frohlich *et al.,* 1999. Before the extraction of genomic DNA from the preserved thrips samples, single adult thrips was rinsed 3-4 times with distilled water to remove ethanol traces. After that thrips specimen placed on filter paper for drying and then place on section of parafilm for the extraction of total genomic DNA. Then thrips specimen was grinded by using the micropestle in 60 µL of ice-cold lysis buffer. Lysis buffer was 5 mM of Tris-HCl, pH 8.0, containing 0.5 mM of EDTA, 0.5% Nonidet P-40 and 1 mg/ml of proteinase K. This extract was transfer into PCR tube with micropipette and was incubated at 65 °C for 15 min and 95 °C for 10 min prior to a brief centrifugation (10000 *g*) to pellet debris. The aqueous supernatant was used as a template in polymerase chain reaction (PCR) amplification and store at -20^0C for further studies.

Table 1: Collection of thrips samples from the different cotton growing districts of Maharashtra.

Sr.	Sample code	Districts	Latitude	Longitude
1.	Th -NAG	Nagpur	21^{0} 8’ 21”	79^{0} 4’ 33”
2.	Th-KAT	KatoL (Nagpur)	21^{0} 15’ 55”	78^{0} 38’ 9”
3.	Th-AMR	Amravati	20^{0} 37’ 53”	77^{0} 50’ 27”
4.	Th-AKL	Akola	20^{0} 42’ 15”	77^{0} 1’ 50”
5.	Th-WAR	Wardha	20^{0} 36’ 22”	78^{0} 40’ 25”
6.	Th-WARU	Warud (Amravati)	21^{0} 25’ 37”	78^{0} 23’ 18”
7.	Th-WAS	Washim	20^{0} 22’ 55”	77^{0} 19’ 10”
8.	Th-YAV	Yavatmal	20^{0} 18’ 28”	78^{0} 6’ 51”
9.	Th- CICR1	Nagpur	21^{0} 2’ 17”	79^{0} 4’ 19”
10.	Th- CICR2	Nagpur	21^{0} 2’ 18”	79^{0} 4’20”
11.	Th-BUL	Buldhana	19^{0} 58’ 55”	76^{0} 9’ 32”
12.	Th-JAL	Jalgaon	21^{0} 02’ 26”	76^{0} 02’ 52”
13.	Th-DHU	Dhule	21^{0} 01’ 30”	74^{0} 46’ 4”
14.	Th-PAR	Parbhani	19^{0} 17’ 4”	76^{0} 40’ 1”
15.	Th-PARB	Parbhani	19^{0} 17’ 4”	76^{0} 40’ 1”
16.	Th- RHA1	Rahuri (Ahmednagar)	19^{0}23’ 20”	74^{0} 38’ 60”
17.	Th- RHA2	Rahuri (Ahmednagar)	19^{0} 23’ 20”	74^{0} 38’ 60”

Plate 1: Thrips sample collection from cotton crop with the help of camel hair brush.

3.4 Amplification of COI gene though polymerase chain reaction

Cytocrome oxidase subunit I (One) (COI) region of mitochondrial DNA was amplified by using following primers Simon *et al.* (1994), Fekrat *et al*. (2014).

1) C1-J-1751 F GGATCACCTGATATAGCATTCCC (CO- I 3F) and

2) C1-N-2191 R CCCGGTAAAATTAAAATATAAACTTC (CO-I 3R)

PCR amplification was conducted using 1.5 µL of each template DNA in a total reaction volume of 25 µL. The PCR reaction mixture contained 0.4 µL of dinucleotide triphosphates (dNTPs), 16.4 µL of distilled water and 4 µL of 5X buffer, 1.4 µL of $MgCl_2$ and 0.3 µLof *Taq* DNA polymerase and 0.5 µL of each forward and reverse primer. For the amplification of mitochondrial region following PCR conditions was used in which are given in the table 2:

Table 2: PCR conditions for the amplification of mitochondrial region

Stage	Step	Temperature	Duration	No. of cycle
1.	Initial denaturation	94 ^{0}C	4 min	35
2.	Denaturation	94 ^{0}C	45 sec	
	Annealing	51 ^{0}C	45 sec	
	Extension	72 ^{0}C	1 min	
3.	Final Extension	72 ^{0}C	7 min	
	Hold	4 ^{0}C	-	-

3.5 Agarose Gel Electrophoresis

Both the gel casting plate and comb were washed with water and wiped with 70 per cent alcohol using tissue paper or delicate task wipers and after that placed on perfectly horizontal leveled platform. The comb was kept inside the gel casting plate. Agarose gel (1.5%) was prepared by adding agarose powder to 1X TAE buffer. It was boiled until the agarose dissolved completely and allowed it to cool upto room temperature. (1X TAE buffer prepared from 50X TAE buffer and distilled water). Ethidium bromide was added to the agarose with micropipette and poured it in the gel plate, kept for solidification. After solidification of the agarose, the combs were removed carefully and the casted gel was placed in the electrophoresis unit with wells facing towards the cathode and pours the 1X TAE buffer till it submerged up to the depth of about 1 cm.

3.6. Loading the PCR product for the confirmation

PCR product (5 µL) was pipetted out in the small PCR tubes. Loading dye (blue 6x) (2 µL) was added to the DNA sample and mixed thoroughly by spinning in spinner. The content were loaded into well of submerged gel using micropipette. DNA ladder (4 µL) of 100 bp was loaded as standard marker. Cathode and anode were connected to a power supply and gel was run at the constant voltage (80 V). The gel was run until the loading dye migrated an appropriate distance through the gel and bands were visualized in a UV light source.

3.7 Sequencing of PCR product

The fragment of the cytochrome oxidase I (COI) gene of about 439 bp amplicon size sequenced by using both forward (CO-I 3F) and reverse (CO-I 3R) primers. For that PCR products were sent to custom-sequencing service of Agrigenome labs (Kochi, India) using COI forward and reverse primers on automated ABI PRISM 3100 Genetic Analyzer bio-edit.

3.8 Analysis of sequence

The sequence obtained using forward and reverse primers were assembled and made contig. The sequences were subjected to BLAST at http://www.ncbi.nlm.nih.gov. and mitochondrial cytochrome oxidase I (COI) sequences of *Thips palmi* Karny*, Thrips hawaiiensis* Morgan, *Rhipiphorothrips cruentatus* Hood and *Ayyaria chaetophora* Karny were determined using the sequence alignment editor ‘Bioedit’.

3.9 Phylogenetic Analysis of molecular data

Phylogenetic relationships amongst the species were assessed using maximum parsimony (MP) and maximum likelihood (ML), both with the nearest neighbour interchange algorithm, and neighbour-joining (NJ) using the boost trap methods. All the analyses were done in the Molecular Evolutionary Genetic Analysis (MEGA) Software ver. 6.0 for windows (Tamura *et al*., 2013) using 1000 replicates.

3.10 Morphometric analysis

Additional study on morphological analysis also conducted using the identification keys and literatures. Study was conducted by mounting specimen and the morphological features of each identified species, on the slides and observed under the stereoscopic zoom microscope (8x).

3.11 Identification of thrips species from experts

Thrips sample were submitted to the Zoological Survey of India (ZSI), Kolkata and National Bureau of Agricultural Insect Resources (NBAIR), Bengalore for the identification of species on the basis of conventional method.

Chapter IV

RESULTS AND DISCUSSION

Cotton, one of the world’s leading fibre crop. In India, cotton enjoys a free eminent status among all cash crops in the country, being a principal raw material for a flourishing textile industry which accounts for nearly 20 percent of the total national industrial production. Maharashtra is one of the important cotton growing states in India. Cotton crop is heavily damaged by about a dozen of major pests starting from seedling stage by jassids, aphids, thrips and white fly and and thereafter the flower and boll development stage by bollworm complex results in yield reduction. Among that, thrips is one of the important sucking pests of cotton crop, since this pest is responsible for reduction in yield by directly feeding on the foliage and also indirectly by acting as vector of tobacco streak virus (Vinodkumar *et al*., 2017).
In the recent years complexity of pest management is increasing due to the insect resistance to various insecticides which is a major problem associated with the chemical control of insect pests. Therefore, it is necessary to take an appropriate control measure which is possible when there is accurate identification of species occurred. Here, diversity of thrips in cotton crop by surveying and collection of thrips from the different cotton growing districts of Maharashtra is presented.

The results of the present investigation on “STUDIES ON DIVERSITY OF COTTON THRIPS IN MAHARASHTRA” are illustrated and discussed under the following heads:

4.1 Survey and collection of thrips specimen from cotton fields

4.2 Isolation of genomic DNA and amplification of COI region

4.3 Genetic diversity of Cotton thrips in cotton growing districts of Maharashtra

4.4 Sequencing of cytochrome oxidase I (COI) gene

4.5 Phylogenetic analysis of molecular data

4.6 Distribution of identified thrips species on cotton crop

4.7 Morphometric analysis

4.8 Identification of thrips species from experts

4.1 Survey and collection of thrips specimen from cotton fields

Survey was conducted and samples were collected from the cotton growing districts of Maharashtra *viz.,* Nagpur, Akola, Amravati, Washim, Wardha, Yavatmal, Buldhana, Dhule, Jalgaon, Parbhani and Ahmednagar districts during August to September 2018, when the infestation was in the peak. From this survey it was found that more than one species found on cotton field on the basis of their morphological appearance. Most of the thrips species were found on lower surface of cotton leaves. Some species collected from flowers also. Some black coloured species were found on topmost expanded leaves. Out of that yellow coloured thrips species found in every region from where the survey and sampling taken. Black coloured thrips species found rarely from some fields of cotton during survey.

Various reports are available with similar findings reporting more than one species of thrips on cotton crop during the survey reported by Cook *et al*., 2003; Stewart *et al.,* 2013; Slosser *et al.,* 2005; Akin *et al.,* 2010. Present findings are in line with Mabbet *et al.,* 1984 who studied the pest scouting techniques in cotton in Thailand reported that the majority of thrips species were found on the top four or five expanded leaves of cotton crop.

4.2 Isolation of genomic DNA and amplification of COI region

Genomic DNA of the single adult thrip was extracted by using the protocol given by Frohlich *et al.* 1999). The obtained DNA was confirmed by running on 1.5 per cent agarose gel electrophoresis. A fragment of the mitochondrial cytochrome oxidase I (COI) gene was amplified (Simon *et al.,* 1994; Fekrat *et al.,* 2014).

1) C1-J-1751 F GGATCACCTGATATAGCATTCCC (CO-I 3F) and

2) C1-N-2191R CCCGGTAAAATTAAAATATAAACTTC (CO-I 3R)

A very sharp amplicon of expected size (439 bp) was obtained in all samples (Plate 2, 3 and 4).

Such sharp amplicon of expected size (700 bp) of DNA band of thrips sample reported by Biradar (2015) who studied the molecular diversity of thrips species in groundnut by amplified mitochondrial cytochrome oxidase I (COI) for all test insects through polymerase chain reaction (PCR). Similarly, Rebijith *et al*. (2011) studied molecular diversity of thrips species *viz*., *T. palmi* and *S. dorsalis* collected from watermelon and capsicum respectively using mitochondrial COI gene and the PCR amplicon of expected size of 700 bp was amplified in all the collected thrips samples.

4.3 Genetic diversity of thrips collected from the cotton growing districts of Maharashtra

Genetic identification results of thrips of cotton growing districts of Maharashtra are presented in table 3. species identification results revealed that composite collection of adult cotton thrips samples of Nagpur, Amravati, Wardha, Warud (Amravati), Buldhana, Akola, Yavatmal, Jalgaon, Dhule, Ahmednagar and some species of Parbhani identified as *Thrips palmi* Karny*, Rhipiphorothrips cruentatu*s Hood species identified from the Katol (Nagpur). Samples collected from ICAR-CICR Nagpur and some samples of Parbhani were identified as *Ayyaria chaetophora* Karny and *Thrips hawaiiensis* Morgan identified from the Washim samples.

Similar research on the diversity of thrips through the molecular was done by Nakahara and Minoura, 2015 and identified the four thrips species *viz., T. palmi, T. tabaci, Frankliniella intonsa, and F. occidentalis.* Wang *et al.* (2018) also identified three species of thrips *viz., F. fusca, F. tritici* and *T. tabaci* in the experiment of molecular

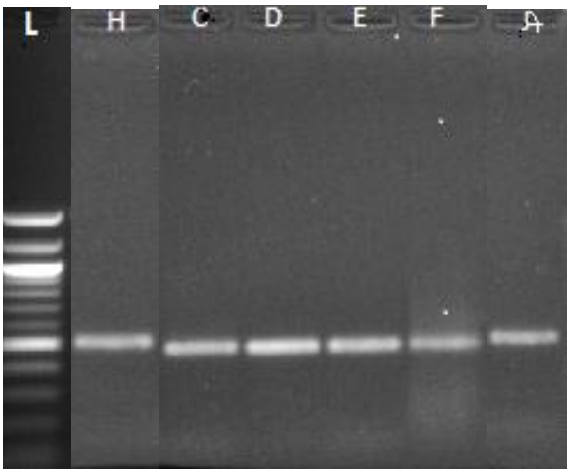

Plate 2: A very sharp amplicon of expected size (439 bp) was obtained in all samples.

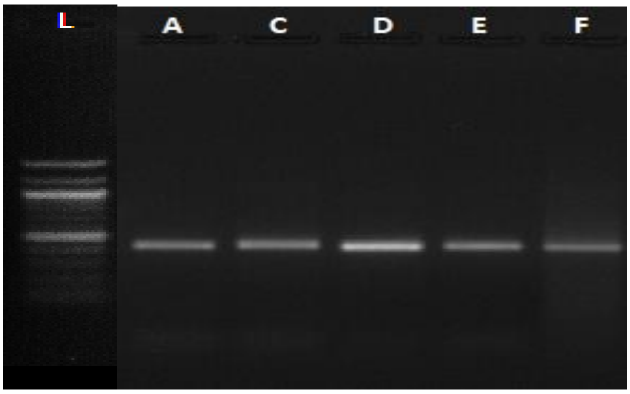

Plate 3: A very sharp amplicon of expected size (439 bp) was obtained in all samples.

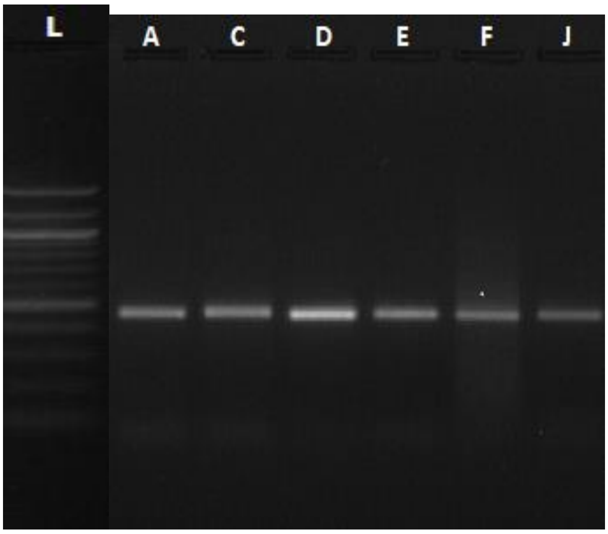

Plate 4: A very sharp amplicon of expected size (439 bp) was obtained in all samples.

Identification of thrips species infesting cotton in the South eastern United States. Yeh *et al.* (2014) identified *T. hawaiiensis* in the thrips samples along with flower thrips *F. occidentalis* and also found *T. tabaci* using the multiple PCR techniques.

Table 3: Identification results of thrips samples collected from the cotton growing districts of Maharashtra.

Sr.	Sample code	Districts	Species identified	Order: family
1.	Th- NAG	Nagpur	*Thrips palmi* Karny	Thysanoptera : Thripidae
2.	Th-KAT	Katol (Nagpur)	*Rhipiphorothrips cruentatus* Hood	
3.	Th-AMR	Amravati	*Thrips palmi* Karny	
4.	Th-AKL	Akola	*Thrips palmi* Karny	
5.	Th-WAR	Wardha	*Thrips palmi* Karny	
6.	Th-WARU	Warud (Amravati)	*Thrips palmi* Karny	
7.	Th-WAS	Washim	*Thrips hawaiiensis* Morgan	
8.	Th-YAV	Yavatmal	*Thrips palmi* Karny	
9.	Th- CICR1	Nagpur	*Ayyaria chaetophora* Karny	
10.	Th- CICR2	Nagpur	*Ayyaria chaetophora* Karny	
11.	Th-BUL	Buldhana	*Thrips palmi* Karny	
12.	Th-JAL	Jalgaon	*Thrips palmi* Karny	
13.	Th-DHU	Dhule	*Thrips palmi* Karny	
14.	Th-PAR	Parbhani	*Thrips palmi* Karny	
15.	Th-PARB	Parbhani	*Ayyaria chaetophora* Karny	
16.	Th- RHA1	Rahuri(Ahmednagar)	*Thrips palmi* Karny	
17.	Th- RHA2	Rahuri(Ahmednagar)	*Thrips palmi* Karny	

4.4 Sequencing of cytochrome oxidase I (COI) gene

The sequences obtained using forward and reverse primers were assembled using Bioedit software. Nucleotide sequences of test insects COI region collected from the different districts of Maharashtra region were analyzed using bioinformatics tools like NCBI database (National Center for Biotechnology Information) and Basic local alignment search tool (BLAST) programmed. Based on sequence comparison, all the fragment of COI gene sequences of thrips specimen collected from 17 locations were confirmed as 4 species of thrips *viz.*, *Thrips palmi* Karny*, Thrips hawaiiensis* Morgan, *Rhipiphorothirps cruentatus* Hood and *Ayyaria chaetophora* Karny.

Comparison of the sequences with those in Gene bank and The barcode of life data (BOLD) revealed that 9 samples of *T. palmi* showed the close sequence matches of 100% with the NCBI sequences and 3 samples of *T. palmi* species were found 99% similarity. *T. hawaiiensis and R. cruentatus* both species showed 100% similarity with NCBI sequences whereas 3samples of *A. chaetophora* resembles about only 91% similarity with NCBI sequences. The sequences have been deposited in Gene bank with accession numbers (Table 4).

Similar findings on comparison of thrips sequences with NCBI sequences given by Uppar, 2014 and found that *T. tabaci* population showed 88 per cent similarity with NCBI published sequence of COI gene and *Scirtothrips dorsalis* COI gene sequences showed 91 per cent homology.

Similarly, Iftikhar *et al.* (2016) analyzed the sequence variation of 471 thrips samples collected from various host plant and observed that some identified sequences of *T. palmi* revealed close matched >99% with NCBI sequences and some sequences of identified *T. palmi* show >98% with NCBI sequences.

Table 4: List of specimens sequenced with their percent similarity and gene bank accession numbers.

Sample code	Locality	Species Identified	Gene bank accession number for COI fragment	% similarity with NCBI submitted sequences
Th-NAG	Nagpur	*Thrips palmi*	MK134705	100
Th-KAT	Katol(Nagpur)	*Rhipiphorothrips cruentatus*	MK192989	100
Th-AMR	Amravati	*Thrips palmi*	MK192984	100
Th- AKL	Akola	*Thrips palmi*	MK192983	100
Th-WAR	Wardha	*Thrips palmi*	MK192986	99
Th-WARU	Warud (Amravati)	*Thrips palmi*	MK192993	100
Th- WAS	Washim	*Thrips hawaiiensis*	MK192985	100
Th- YAV	Yavatmal	*Thrips palmi*	MK192987	100
Th-CICR1	Nagpur	*Ayyaria chaetophora*	MK192998	91
Th-CICR2	Nagpur	*Ayyaria chaetophora*	MK192999	91
Th-BUL	Buldhana	*Thrips palmi*	MK192996	100
Th-JAL	Jalgaon	*Thrips palmi*	MK192995	100
Th-DHU	Dhule	*Thrips palmi*	MK192988	100
Th-PAR	Parbhani	*Thrips palmi*	MK192994	100
Th-PARB	Parbhani	*Ayyaria chaetophora*	MK192997	91
Th-RHA1	Rahuri (Ahmednagar)	*Thrips palmi*	MK193004	99
Th-RHA2	Rahuri (Ahmednagar)	*Thrips palmi*	MK193005	99

4.5 Phylogenetic Analysis of molecular data

Phylogenetic analysis was done using COI sequences from thrips collected from the cotton crop of cotton growing districts of Maharashtra shown in the fig. 2. There were three major clusters (clades) found in 17 thrips samples collected from 11 cotton growing district of Maharashtra. Abundant species *Thrips palmi* comes under clade 1 due to similarity in their sequences. Whereas, all identified species of *Ayarria chaetophora* shared the same clade 2. However, *Thrips hawaiiensis* and *Rhipiphorothrips cruentatus* comes under clade3 showed close relatedness or genetic similarity among them.

Clades of *Thrips palmi* in phylogenetic analysis also reported by Kadrivel *et al.* (2013) who studied the diversity of thrips species from vegetables crops. Phylogenetic analysis also done by Glover *et al.* (2010) where two distinct COI clades observed for *T. palmi*. Present findings are in line with Karimi *et al.* (2010) where they found that thrips sequences form six major clades including clades of *Thrips palmi* in the experiment of identify thrips species by using DNA barcodes.

4.6 Distribution of identified thrips species on cotton crop

Four species were found *viz., Thrips palmi* Karny*, Thrips hawaiiensis* Morgan*, Rhipiphorothrips cruentatus* Hood *and Ayyaria chaetophora* Karny from the different districts of Maharashtra and distribution of molecularly identified thrips species shown graphically in fig. 3. The corresponding distribution of these species was recorded as 70, 6, 6 and 18 % respectively. *T. palmi* was most abundant species found throughout the cotton growing districts of Maharashtra.

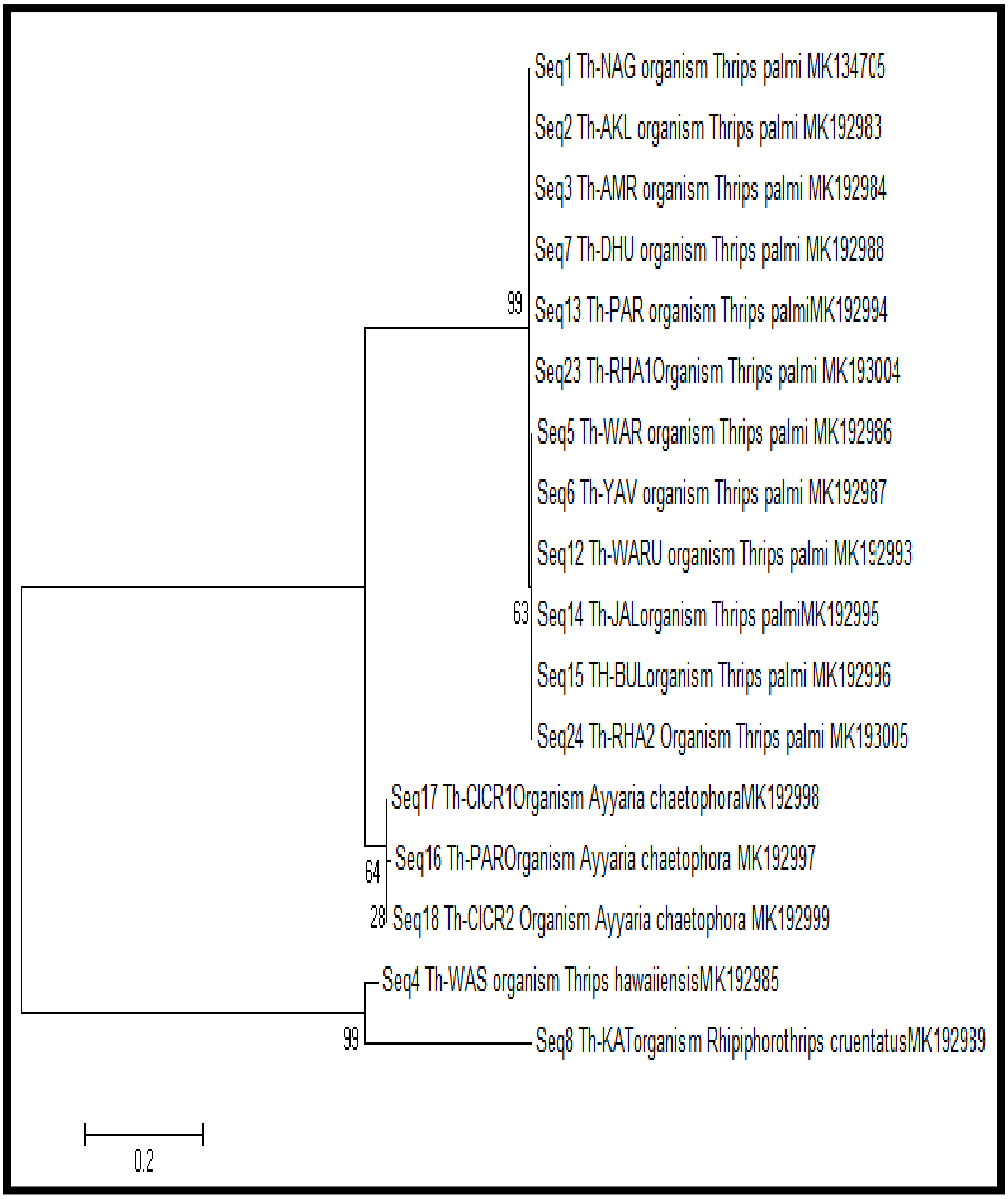

Fig. 2: Dendrogram of phylogenetic relationships amongst the thrips species were assessed using maximum parsimony (MP) and maximum likelihood (ML), both with the nearest neighbour interchange algorithm, and neighbour-joining (NJ) using the boost trap methods.

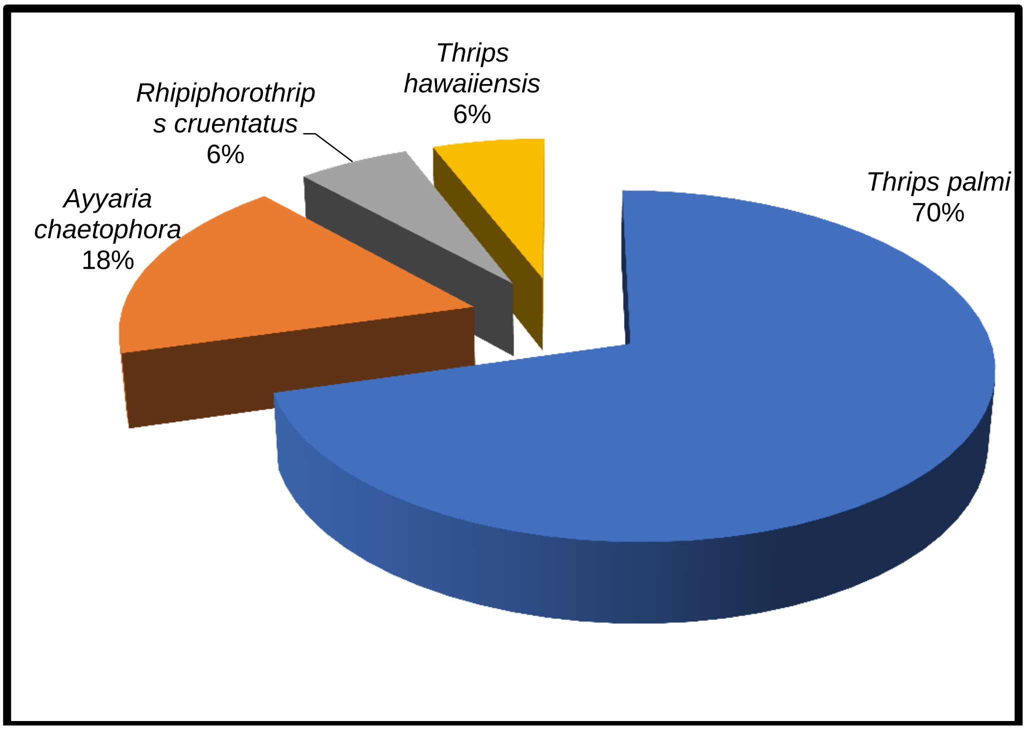

Fig. 3: Graph showing the distribution of thrips species on cotton crop in Maharashtra.

Prema *et al*. (2018) monitor thrips population in cotton using the colour sticky traps and also reported the *T. palmi* along with*T. tabaci* and*S. Dorsalis* in the cotton ecosystem. However, in the present study none of the sample was of *T. tabaci*. Similarly, Bournier (1983) described that *T. palmi* caused damage on the leaves of cotton in the Philippines.

Frankliniella fusca was the dominant species across all the location of cotton growing area in Southeastern United States followed by *F. occidentalis* and *F. tritici* whereas *T. tabaci* were occasionally found by Wang *et al.* (2018). Wangboonkong, 1981 reported*Thrips palmi* had been first recognised in 1978 on cotton and subsequently become the pest of cotton by Rachana *et al*., 2016.

Thrips hawaiiensis also reported from the present study and found from the single location. It was first time reporting that *T. hawaiiensis* found on cotton crop. During the survey and collection it was collected from the cotton flowers in the district of Washim and it was confirmed from the findings of Reynaud*et al*.(2008)that *T. hawaiiensis* is a strict flower-dwelling species and adults are very polyphagous and it can feed on numerous host plants such as tobacco (Kurozawa *et al.,* 1964), rose (Woo & Paik, 1971), gladiolus (Chen and Lo, 1987), *Brassica oleracea (*Chandra and Lal, 1973), tea (Chen, 1979), mango (Lee & Wen, 1982), and banana (Tsai *et al.,* 1992; Jhala *et al*., 2004).

Rhipiphorothrips cruentatus identified from the single location. There is no single report for this thrips that cotton as a host plant in India. Instead this species was reporded on plantational crop such as guava, apple, rose and grapes (Anonymous, 2012).

Ayyaria chaetophora thrips species identified from different location of Maharashtra from where samples collected. There are very scanty reports on this thrips species. Mabbet*et al*. (1984) in Thailand reported that the majority of *A. chaetophora* are found on the top four or five expanded leaves. Similar observation was recorded on cotton crop. In the literature *A. chaetophora* species was recorded from crop belongs to the family Euphorbiaceae (Acalypha and Ricimus) and Malvaceae (Gossypium) (Miyazaki *et al.,* 1984).

4.7 Morphometric analysis

Species identified as *Thrips palmi* when observed under the microscope show the characters such as body was entirely yellow coloured. 1st and 2nd antennal segments clear yellow coloured, 3rd segment was yellow but show brownish shedding on the apex portion. As the antennal segments increases colour of antenna become yellowish to brownish gradually from base to apex. Red coloured triangle was found on the top of the head as shown in plate5 and 6.

Similar characters of *T. palmi* were given in literature of Sakimura *et al*., 1986. Most important identification key given by Capinera, 2008 for the identification of *T. palmi* species that there are three ocelli on the top of the head of red colour in *T. palmi* and in case of *Thrips tabaci,* it was grayish coloured.

Species identified as *Ayyaria chaetophora* when observed under the microscope show the most prominent character that wings showed the banded pattern. This thrips show elongated body as compared to other identified thrips species. Antennal segments were difficult to observe as they are very thin and slender (Plate 7 and 8).

Similar morphological characters of species *Ayyaria chaetophora* were given by Ananthakrishnan and Sen, 1980.

Species identified as *T*. *hawaiiensis* when observed show the characters such as pale yellow head and thorax are as compared to abdomen. Forewings were pale brown from basal portion and remaining portion was dark brown as in plate 9. All the antennal segments were brown except 3rd, 4th which was yellow with brown sheddings. Thrips species identified as *Thrips hawaiiensis* were

Plate 5: Microscopic picture (8x) of *Thrips palmi* a) dorsal view

Plate 6: Microscopic picture (8x) of *Thrips palmi* b) ventral view

Plate 7: Microscopic picture (8x) of *Ayyaria chaetophora* a) dorsal view

Plate 8: Microscopic picture (8x) of *Ayyaria chaetophora* b) ventral view

Plate 9: Microscopic picture (8x) of *Thrips hawaiiensis* a) adult

Plate 10: b) Species on cotton flower

Plate 11: Microscopic picture (8x) of *Rhipiphorothrips cruentatus*: a) immature stage

Plate 12: Microscopic picture (8x) of *Rhipiphorothrips cruentatus*: b) Mature stage.

collected from the flowers of the cotton crop during survey as shown in the plate 10.

These morphological features were confirmed with finding of Marullo and Grazia, 2017.

Species identified as *Rhipiphorothrips cruentatus* when observed under the stereoscopic zoom microscope, characters such as body brown body colour, antennae and legs clearly yellow in colour. Yellow and pale immature stage as compared to mature stage as in the plate 11 and 12 and fore wing with pale yellow veins.

Above characters were confirmed with article (Anonymous, 2012).

4.8 Identification of thrips species from experts

The identification reports received from Zoological Survey of India, Kolkata and National Bureau of Agricultural Insect Resources, Bengalore confirm the correct identification of *Thrips hawaiiensis, Rhipiphorothrips cruentatus* and *Ayyaria chaetophora.* However, in the report of NBAIR and ZSI *Thrips palmi* was identified as *Thrips tabaci* and as *Scirtothrips dorsalis* through conventional taxonomy. Present study results that *T. palmi* is the widespread species on cotton.

As reported by various researchers that the large number of thrips species present on the site may be linked to the diversity of host plants. Indeed, plant species diversity increased the number of possible ecological associations (Sanchez-Monge *et al.,* 2011). Number of captured thrips species is higher when when there are weeds and lower when there is only one plant species by Hernan-ayar *et al.,* 2009. This principle has been used in several crops to increase the insect diversity and foster the presence of natural enemies for pests (Schellhorn and Sork, 1997).

According to Mound(2005), a thrips host plant is commonly defined as “a plant species can successfully maintain a population; thus all the life stages of a thrips species must be able to thrive on the plant species in order for it to be designated as host of the thrips species”.

Presence of large number of adult thrips on plant is not in itself an indication of an insect plant association (Mound, 2013). Even thrips species remain airborne long enough to travel easily between neighbouring field and blown by wind over long distance (Lewis, 1997) because of fringed wing and being weak fliers.

In our study, only adult thrips were collected from cotton growing areas through survey and study of full development of thrips on cotton crop not conducted because of its only short duration study, hence the study did not permit to say identified all thrips species may used the cotton as host. Therefore, there is need of further confirmation of present study for the identification of thrips species on the cotton ecosystem.

Chapter V

SUMMARY AND CONCLUSIONS

Present investigation entitled “Studies on diversity of cotton thrips in Maharashtra” was conducted at ICAR - Central Institute for Cotton Research, Nagpur during 2018-2019 to explore the genetic diversity of cotton thrips in the cotton growing districts of Maharashtra.

The data obtained were compiled and results of the present study are summarized below:

Survey was conducted and samples were collected from the cotton growing districts of Maharashtra *viz*., Nagpur, Akola, Amravati, Washim, Wardha, Yavatmal, Buldhana, Dhule, Jalgaon, Parbhani and Ahmednagar during August to September 2018.

Genomic DNA of the single adult thrip from the sample collected during survey was extracted and obtained DNA was confirmed by running on 1.5 per cent agarose gel electrophoresis. A very sharp amplicon of expected size (439 bp) was obtained in all thrips samples.

Four species were found *viz., Thrips palmi* Karny, *Thrips hawaiiensis* Morgan, *Rhipiphorothrips cruentatus* Hood *and Ayyaria chaetophora* Karny from the different districts of Maharashtra during the survey. Species identification results revealed that composite collection of adult cotton thrips samples of Nagpur, Amravati, Wardha, Warud (Amravati), Buldhana, Akola, Yavatmal, Jalgaon, Dhule, Ahmednagar and some samples of Parbhani identified as *T. palmi,* whereas *R. cruentatu*s identified from the Katol (Nagpur). Samples collected from ICAR-CICR Nagpur and some samples of Parbhani were identified as *A. chaetophora* and *T. hawaiiensis* was identified from the sample collected from Washim.

When the sequence were compare with sequences of those in Gene bank and BOLD revealed that 9 samples of *T. palmi* showed the close sequence matches of 100% with the,comparison of the NCBI sequences and 3 samples of *T. palmi* species were found 99% similarity. *T. hawaiiensis and R. cruentatus* both species showed 100% similarity whereas 3 samples of *A. chaetophora* resembles about only 91% similarity with NCBI sequences.

In phylogenetic analysis three major clades recorded. First clade made by abundant species *Thrips palmi*, second clade consists of all samples of *Ayarria chaetophora* whereas *Thrips hawaiiensis* and *Rhipiphorothrips cruentatus* comes under the third clade.

The corresponding distribution of these species was recorded as 70, 6, 6 and 18 % respectively (Fig. 3). Found that *T. palmi* was most abundant species throughout the cotton growing districts of Maharashtra followed by the *A. chaetophora* but *T. hawaiiensis* and *R. cruentatus identified* from the single location.

Conclusions

I. Four species of thrips *viz., Thrips palmi* Karny*, Thrips hawaiiensis* Morgan*, Ayyaria chaetophora* Karny and *Rhipiphorothrips cruentatus* Hood are identified on the cotton crop from the present study.

II. Distribution of all identified species done graphically and found that *Thrips palmi* was most abundant species found throughout the cotton growing districts of Maharashtra followed by the *A. chaetophora* but *T. hawaiiensis* and *R.cruentatus identified* from the single location.

III. *T. palmi* was most abundant occupy 70 % share among observed species habituating in most of the cotton growing districts of Maharashtra

IV. In phylogenetic analysis three major clades recorded. First clade made by abundant species *T. palmi*, second clade of all species *A. chaetophora* whereas *T. hawaiiensis* and *R. cruentatus* come under third clade.

V. There is very scanty reports were available with regards to diversity of thrips species either on cotton crop or any other crops. Present study help to enhance the knowledge related with presence of thrips species on the cotton crop.

VI. Identification of species on the basis of morphology is not sufficient for such minute and small body insects. Therefore molecular identification in addition with morphological study is essential. These add to the efficiency of IPM programs against thrips.

Chapter VI

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