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First report of invasive thrips, *Thrips parvispinus* (Karny) infestation on chilli from Eastern part of India

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ABSTRACT

The South East Asian thrips, *Thrips parvispinus* (Karny) was first reported in India on Papaya in 2015 later it spread to many host plants such as cowpea, *Dahlia rosea,* cotton etc. The severe infestation on chilli was reported from southern states during last two years and the extent of loss was more than 50 percent. Therefore, a quick roving survey was conducted to monitor the thrips infestation in chilli at farmer fields of Varanasi and Mirzapur districts of Uttar Pradesh, India. The molecular barcoding of thrips infesting chilli at Varanasi and Mirzapur district of Uttar Pradesh confirmed to be the species as *Thrips parvispinus*. Therefore, it's a first report of extended distribution to eastern part of India (Uttar Pradesh). The average number of thrips per three leaves and per flower was 9.1 and 3.1 in Varanasi while in Mirzapur it was 9.3 and 5.1 respectively. The *T. parvispinus* attained pest status in eastern part of India. The neighbor joining tree indicated certain level of genetic diversity in the species reported from different host plants. Farmers complained about severe crop loss and failure of many insecticides to control them, it may be attributed insecticide resistance.

Key words: *Invasive thrips, Chilli, Molecular barcoding, Pest, Survey*

Introduction

Chilli (*Capsicum annum* L.) is one of the commercial vegetable crop grown in India for vegetable, condiments,sauces, pickles and spice purpose. India is the world leader in production, consumption and export of chilli. In India,Andhra Pradesh, followed by Telangana, Madhya Pradesh, Karnataka and West Bengal are top chilli growing states. Due to climate change and global trade several insect pests introduced in India and across the globe. Fall army worm on maize recent invasive is one of the such examples (Vinay *et al.,* 2022). The pest introduction not only happens by trade of agricultural crops/produce but also due to passive and active migration of insects. The invasive pest known to cause huge loss to local crops due to absence of natural enemies and competitors. So far there are 23 invasive insect pests introduced in India. In India *Thrips parvispinus* was first reported in 2015 on papaya from Karnataka (*Carica papaya*) (Tyagi *et al.,* 2015). The *T. parvispinus* is a cosmopolitan and polyphagous pest reported to be present in Indonesia, Australia, Thailand, North America, Europe, Malaysia and Africa (Waterhouse, 1993; Zhang, *et al.,* 2011; Lim, 1989). The severe infestation of *T. parvispinus* caused 23 percent loss to pepper in Indonesia (Johari and Natalia, 2018) and economic loss to papaya in Hawaii (Sugano *et al.,* 2013). The first reported authors of the pest in India expressed concern over as papaya is commercial crop and the reported thrips may attain serious pest status. Subsequently, the re-

ports of infestation on *Dahlia rosea* (Rachana *et al.,* 2018), *Brugmansia* sp., (Roselin *et al.,* 2021), cotton (Amutha and Rachana, 2022) and on cow pea (*Vigna unguiculata*) (Nagdev *et al.,* 2022) was followed. Later the severe infestation on chilli (*Capsicum annum*) was reported from Karnataka (Basavaraj *et al.,* 2022), Telangana and Andhra Pradesh (Hulagappa *et al.,* 2022; Veerannaet *al.,* 2022), Gujarat (Patel *et al.,* 2022) (Fig. 3). Similarly the farmers of Uttar Pradesh especially the Varanasi and Mirzapur reported severe curling of leaf and heavy flower drop in chilli in the month of August to September-2022. Therefore a quick roving survey was conducted to know the pest responsible for the chilli crop loss and to calculate the extent of crop loss. This survey mainly aims at documenting the pests responsible for crop loss and its molecular characterization using barcoding.

Hebert *et al.* (2003) proposed the mitochondrial cytochrome oxidase I (COI) gene can be used for animal bio-identification. The COI is having faster evolution rate than the nuclear genes (Medina and Walsh, 2000), allows discrimination of closely related species as well as phylo-geographic species within a single species. Now COI is a well-established gene in animal phyla to study molecular systematics (Twinkle *et al.,* 2020), genetic diversity and migration (Tyagi *et al.,* 2022; Narayana *et al.,* 2020) discrimination of closely related taxa (Hebert *et al.,* 2003; Monti *et al.,* 2005), phylogenetic studies (Wells *et al.,* 2007) development of species specific markers (Asokan *et al.,* 2007; Sun *et al.,* 2016). Therefore, we used COI gene sequence based quick identification of thrips infesting chilli in Varanasi and Mirzapur districts of Uttar Pradesh.

Materials and Methods

Roving survey and sample collection

Some chilli growing farmers of Varanasi and Mirzapur districts, Uttar Pradesh complained about heavy infestation and damage by sucking insect pests during chilli growing season, 2022. Therefore, to identify the insect pest and its level of damage a rapid roving survey was conducted in two locations i.e., Varanasi and Mirzapur districts of Uttar Pradesh (Fig. 2). For this purpose, twenty plants were randomly selected for sampling from each location. In a plant, top, middle and bottom leaf was collected and observed under microscope for counting. Number of thrips per flower was observed and calculated on the spot.

Sample collection and analysis

The samples of thrips were collected from the horticulture experimental field (Banaras Hindu University, Varanasi) (25.2677° N, 82.9913° E) and from Mirzapur (25.1337° N, 82.5644° E) district of Uttar Pradesh. The thrips were collected using camel hair brush and transferred into 2 ml centrifuge tubes containing 90% ethanol. Later, the samples were brought to Insect Molecular Laboratory, Division of Entomology and Agricultural Zoology, Institute of Agricultural Sciences, Banaras Hindu University, for molecular analysis. Thrips were stored in 90% ethanol were dried and permanent slides were prepared using Canada balsam for photography. The adult and antennae photographs of thrips were taken at 4X and 20X using Nikon stereo zoom microscope respectively. However, in order to identify precisely molecular barcoding was performed.

DNA Extraction, PCR and Sequencing

The thrips were carefully examined under stereo zoom microscope at 4X and were cleaned using 90% ethanol. Two thrips were dried on tissue paper to evaporate ethanol and then transferred to 1.5 ml centrifuge tube which is coded with place identity. Later, the DNA was extracted from thrips samples using DN®easy Blood and Tissue kit (Qiagen) according to manufactures protocol with little modification. The genomic DNA visualized on 0.8% agarose electrophoresis to check the quality. The samples yielded good quality bands were used as template DNA in polymerase chain reaction (PCR). The PCR was performed to amplify the universal Barcode gene, COI using the primers such as LCO1490(52-GGTCAACAAATCATAAAGATATTGG-32) and HCO 2198(52TAAACTTCAGGGTGACCAAAAAATCA-32) designed by Folmer *et al.,* 1994. The PCR mixture and thermo cyclic conditions were followed as per the protocol of Tyagi *et al.* (2022). Later, the quality of amplified products were checked using Nanodrop Spectrometer. Only those samples having value close to 1.9 at 260/280 readings were considered for purification. The PCR products were purified using QIA®quick PCR purification kit according to manufacturers protocol. The purified product sequenced in both the direction at Bio-Kart, Pvt. Ltd. Bengaluru, India.

Determination of quality of sequences and identity of species

The sequences obtained were analysed using BioEdit.7.0 program (Hall, 2004) for quality and ends were trimmed to get quality sequence for the both direction. The forward and reverse direction was merged to get a single sequence using same software. The thrips sample under investigation identified by using Basic local alignment search tool (BLAST) program of National center for biotechnology information (NCBI) (blast.ncbi.nlm.nih.gov/Blast.cg) and barcode of life data systems (BOLD) search engine (https://www.boldsystems.org/index.).

Phylogenetic analysis

The sequence generated in the present study and the *Thrips parvispinus* COX1 gene sequences from different locations of India and other countries were retrieved from NCBI. The neighbor joining (NJ) (Saitou and Nei, 1987) tree was constructed using Kimura-2-parameter (Tamura *et al.,* 2013) with 2000 replications using MEGA11 (Tamura *et al.,* 2021) with *Scirtothrips dorsalis* was used as an out-group.

Results and Discussion

Morphological identification of thrips: The thrips were identified using the characters presented in research papers (Rachana *et al.,* 2021 and Tyagi *et al.,* 2022) and certain key characters presented in scanning electron images (Hulagappa *et al.,* 2022). Out of the ten specimens examined, all ten found to be females. The key characters observed such as Abdomen brown to black in colour, thorax and head light yellow with seven segmented antennae (Fig. 1f).

Molecular identification and Phylogenetic analysis of *Thrips parvispinus*

The COI sequence of thrips sample subjected to homology search in BLAST (NCBI) resulted in 99-100% similarity to *Thrips parvispinus* COI sequences. Thus, confirmed the identity of thrips as invasive thrips, *Thrips parvispinus*. Later, the sequence was submitted to NCBI and accession number (OQ255621) was obtained. The COI gene is a well-established gene used for species identification, evolutionary studies, systematics in Arthropoda because of low sequence divergence within the species but high sequence divergence between the species

Fig. 1. Chilli invasive thrips, *Thrips parvispinus* infestation in Chilli and Bell pepper at Varanasi and Mirzapur Uttar Pradesh. (a,b). Thrips on the flower, (c). Thrips infested fruits and branch (d). Severely stunted plant, (e). Collection of chilli leaves from field (f). Adult thrips (Female), (g) Thrips antennae (7 segmented)

and mitochondrial in origin (Harvey *et al.,* 2003; Arya *et al.,* 2022). The neighbor joining (NJ) phylogenetic tree was constructed using *Scirtothrips dorsalis* as an out group. The NJ tree (Fig. 2) revealed two major clades one for outgroup and another one for *Thrips parvispinus* population. The Varanasi isolate formed a close association with other isolates from different parts of India. However, out of five Indonesian population three found to be clustered separately indicating certain level of genetic distinctness. While Ghana population formed a clade with Indian population. This implies that the population still at expanding geographical area and no separate genetic groups were formed.

Roving survey to ascertain the incidence and to assess the extent of loss

Roving survey on incidence of thrips revealed severe to heavy severe infestation in two districts of Uttar Pradesh (Table 1). According to farmers obser-

Table 1. Invasive thrips, *Thrips parvispinus* infestation in two districts of Uttar Pradesh, India.

State	Name of the district	Latitude	Longitude	Infestation level	
				Mean no. of thrips per 3 leaves	Mean no. of thrips per flower
Uttar Pradesh	Varanasi	25.2677°	82.9913°	9.1	3.1
Uttar Pradesh	Mirzapur	25.1337°	82.5644°	9.3	5.5

vation the yield loss due to chilli thrips is around 50% in Rabi-2022. Thrips preferred to suck the sap from flowers (Fig.1a, b) and young leaves. Due to sucking of sap, flowers were observed to be brown, dried and drop off. In farmers' fields of Mirzapur the young plants had no flowers due to thrips infestation. Whereas the young leaves curled upward, bronze and silvery in appearance on lower portion Overall, the infested plants observed to be curled, bronze in appearance (Fig. 1d, 1e), scars on fruits (bell pepper) (Fig.1g) and dropped flowers are the typical symptoms. The average number of thrips per three leaves and per flower observed to be 9.1 and 3.1 in Varanasi and 9.3, and 5.1 in Mirzapur respectively.

Similar reports of severe incidence on chilli reported from Karnataka, Andhra Pradesh, Telangana, Chhattisgarh and Gujarat. The *T. parvispinus* was first reported on papaya in India subsequently on *Dahlia* sp., cotton, chilli, bell pepper while recent report on extended host range such as red gram, black gram, beans, cotton, mango, coriander, watermelon etc. is more worrisome (Sridhar *et al.*, 2021). There are 25 insect pest found to be pest on

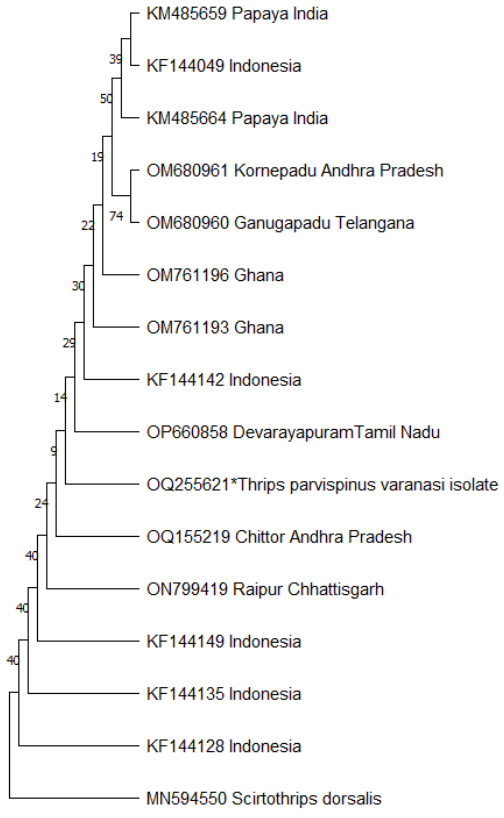

Fig. 2. Neighbor joining phylogenetic tree constructed using Kimura-2-parameter model. Sequence having * sign after accession number is the sequence generated in the current study and *Scirtothrips dorsalis* used as out group. Indonesia indicates isolates reported from that country and Papaya indicates thrips reports on the host Papaya

Fig. 3. Map showing the present distribution record of *Thrips parvispinus* in India. Red colour points indicate the particular location reported. Mirzapur and Varanasi districts of Uttar Pradesh are the new report of distribution. It is marked with a yellow rectangular box on red points for identification.

chilli including fruit borers, whitefly, aphids, mites, hoppers and thrips. Among the thrips, *Scirtothrips dorsalis* was the dominant one. However, in recent survey, *Thrips parvispinus* found to replace the *S. dorsalis* and became a major pest of chilli in Soutern India (Sridhar *et al.,* 2021). Similarly, we noticed only *T. parvispinus* population on chilli in our study area. Some of the farmers of Mirzapur removed the chilli and sown the wheat crop due to severe leaf curl and there is no profitable yield. Similar, reports of crop loss reported earlier in Telangana, Andra Pradesh and Karntaka (Basavaraj *et al.,* 2022; Hulagappa *et al.,* 2022). Since the thrips is expanding its geographical area and host range in India, monitoring on other host plants and non-reported area must be under taken on priority basis. Failure of insecticides to control the pest may attributed to insecticide resistance but the level of resistance and to which group of insecticides is need to be assessed through Bio-assay. For management of thrips the recommendations of MOEFCC-DPPQS technical booklet (2022) may be referred.

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Conflict of Interest: All authors declare that there is no complicit of interest.

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