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Identifying Diversity of Thrips on Lablab (*Lablab purpureus*): A Potential Vegetable Crop

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ABSTRACT

Background: Lablab (*Lablab purpureus*) is a potential vegetable crop cultivated throughout India. It is a tropical and subtropical vegetable adaptive to different climatic conditions and grown in all seasons. Thrips are one of the important pests of lablab as they affect all stages of the crop and transmit plant viruses. The current study aimed at identifying the thrips diversity present in lablab as a base for monitoring and management purposes.

Methods: An exploratory survey was conducted to identify the diversity of thrips on lablab in its growing areas of Coimbatore. Furthermore, continuous monitoring of the lablab crops in TNAU orchard, Coimbatore, was done to explore all the life stages of thrips species and their diversity. Morphological and molecular characterization of the collected species was done using taxonomic keys and mtCOI DNA sequencing approach.

Result: *Thrips palmi* and *Thrips tabaci* were observed during the crop's early stages before blossoming. *Frankliniella schultzei* is a polyphagous pest species that has been found infesting all phases of crop development, from early leaf through pod formation. During and after blooming, the four species *Megalurothrips usitatus*, *Thrips parvispinus*, *Chaetanaphothrips orchidii* and *Haplothrips gowdeyi* began to infest. Shannon and Simpson diversity indices were determined to be -1.739 and -0.1979. According to Shannon index, *C. orchidii* and *T. parvispinus* were the first and second predominant species after flowering. Taxonomic investigations of the collected species were done and substantiated with molecular identification of those analyzed species. A phylogenetic tree of the analyzed sequences was constructed to denote species divergence based on their specific sequences.

Key words: Lablab, Molecular characterization, Morphological identification, Phylogeny, Thrips species.

INTRODUCTION

India is emphasizing nutritional security rather than food security since a sizeable percentage of its population has adopted vegetarianism. The most crucial component of a diet that ensures nutritional security is produce, a source of vitamins and minerals. The main barriers to the effective growth of crops are thought to be pests and diseases. In a tropical environment, insects are the main parts that harm crops by consuming them, which weakens crop configurations and yields (Roy *et al*., 2006).

Hyacinth bean, also known as field bean or dolichos bean, is widely grown in tropical Asia, Africa and America. It is farmed as a field crop in India in different states of Tamil Nadu andhra Pradesh, Karnataka, Madhya Pradesh and Maharashtra. Fresh pods and dry beans are often used as a vegetable and the dried plant parts are used as manure. The fresh pod of 100 g contains 6.7 g carbohydrates, 3.8 g proteins, 1.8 g fiber, 210 mg calcium, 68.0 mg phosphorus, 1.7 mg iron and other nutrients. It is known as "poor man's meat" because it provides several nutritional advantages and is the most excellent source of digestible vegetable protein (20-25%) essential for human health (Joshi *et al*., 2015).

It can fix atmospheric nitrogen in the soil, distinguishing it from other leguminous crops. India has 227.78 ′000 hectares of Indian bean farming, producing 2,276.95 ′000 metric tonnes of vegetable beans and yielding 10 metric tonnes per hectare (DAFW, GOI, 2023). Adult and immature thrips feeding damage to the lablab may result in reduced development and delayed maturity. Thrips feeding causes

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stunting, silvering and distortion of the leaves, as well as harm to or death of the terminal buds, which can affect the quality and quantity of the produce (Sridhar *et al*., 2022).

Thrips (Insecta: Thysanoptera) are the most significant sucking pest on various crops, including vegetables, ornamentals and fruits, in both greenhouse and open field environments across the world. Only around 1% of the 6436 species of thrips known are pests of crops (Mound and Morris 2007; Thripswiki, 2023), but they are more significant because of the severe damage they cause. Thrips are becoming a significant phytosanitary problem internationally and in India because of their prevalence in horticulture systems, particularly in vegetable crops. Thrips like reside in plants' nonwoody aerial regions, where they can also be seen foraging for food by predatory thrips (Kirk, 1997; Crespi *et al*., 1998).

Plants are harmed directly by the immature and adult stages of thrips feeding on them while indirectly by the viruses they spread. Some species like western flower thrips, *Frankliniella occidentalis,* whose existence and establishment in India are still doubtful, exhibit a pretty complicated role on plants, can damage crops directly, transmit plant viruses, control mite populations, or even pollinate (Tyagi *et al*., 2017). As a plant pest, thrips exhibit a high degree of population-level genetic variations and divergence, a more cryptic character and vast disparities in virus vector connections and their function in the epidemiology of various viruses. As vectors of plant viruses, they cause more significant economic losses in vegetables, legumes and ornamental crops in tropical and subtropical regions. Thrips are the sole vector of tospoviruses (Bunyaviridae). Of the 15 global vector species of thrips, 6 are recorded in India (Brunner *et al*., 2002; Pappu *et al*., 2009).

Although many scientific studies have dealt with recording injurious thrips species associated with crops, there is a parallel need to enrich our knowledge with faunistic surveys over cultivated plant species and or even re-examine the thysanopteran fauna of crops in a region and how this change over time (Brunner *et al*., 2002; Asokan *et al*., 2007). The recording of thrips species usually reveals new potential crop pests and shows the diversity of habitats in which thrips can be found. Apart from studying the role of thrips on plants and other habitats (e.g., litter, fungi), the knowledge of the population dynamics and spatial distribution of these insects on host plants is indispensable for planning strategies to control them, particularly in crop monocultures, where these pests are recorded in high population densities (Kirk 1997; Brunner *et al*., 2002).

Accurate and timely identification of thrips vectors in the early developmental stages is vital for understanding the epidemiology of tospoviruses, their management and quarantine because the adults of thrips are the primary transmitters of viruses as a vector. Production of horticultural crops throughout the year in the southern peninsular part of India ensures that thrips have suitable and continuous breeding hosts (Iftikhar *et al*., 2016). With this overview, the current study aimed to identify different thrips species associated with lablab through survey and monitoring. During monitoring, the population of each species was counted and the diversity indices were calculated. The collected thrips species were processed for taxonomic identification and seven thrips associated with lablab were identified. Furthermore, the collected specimens were utilized for molecular characterization to support the taxonomic characterization by constructing a dendrogram.

MATERIALS AND METHODS

A field investigation was carried out to determine the variety of thrips species found in Lablab (*Lablab purpureus*). Thrips samples were gathered from Coimbatore lablab growing regions between 2020 and 2022 to monitor and collect species. Further, the lablab fields at Orchard of TNAU, Coimbatore, were sequentially monitored. The latitude and longitude of the research area is 11.0069°N and 76.9309°E. Thrips adults and various life phases were gathered by tapping the plant parts over a white sheet and placed in AGA fluid (6 parts 60% ethanol, 1 glycerine and 1 acetic acid) using a camel hair brush (Size:000′) to prepare permanent slides. The collected samples of thrips, were used for additional processing, including preservation, maceration (cleaning), dehydration, mounting, identification and analysis. The number of samples for each species was also recorded when examining taxonomic identification to compute the Shannon Diversity Index using the formula:

$$P_i \ln [P_i]$$

Where,

P_i = Proportion of each species in the whole population.

And Simpson Index -

$$\frac{\Sigma_i n_i (n_i - 1)}{N (N - 1)}$$

Where,

n- Number of individuals of single species.

N- Number of individuals in the total population.

Taxonomic characterization

Thrips in AGA samples were placed in an embryo dish with 60% ethanol using a camel hair brush (Size 000) for 24 hours. For the maceration process, the specimens were placed in a 5% NaOH solution for an hour and washed with sterile water to remove the NaOH residues. For the last step in the maceration process, the thrips were immersed in 60% ethanol for 24 hours. The samples were then dried by immersing them in an ethanol series. Samples in 60% ethanol were moved to 70% (1h), then 80% (20 mins), 95% (5 mins) and 100% (5 mins) before being stored in clove oil for 24 hours before mounting on slides. Mounting of the thrips specimen was done using Hoyer's medium. The mounted specimens were dried for 5-7 days in a 40°C hot air oven and saved and reused. The thrips on the slide were identified

using relevant chaetotaxy and other taxonomy keys using a compound microscope (Euromex iScope, The Netherlands).

DNA isolation and Sequence analysis

Total genomic DNA was extracted from a single female thrips using the Hotshot technique (De Barro and Driver, 1997; Montero Pau *et al*., 2008). The thrips samples were crushed and homogenized with 20 µl of tissue lysis buffer (10 N NaOH and 0.5 M Na EDTA; pH 8.0) through vortexing and incubated at 65°C for 20 min. An equal volume of neutralizing buffer (10 mM Tris-HCL, pH 5.0) was added to the homogenate and incubated at 95°C for 15 min, followed by centrifugation for 5 min at 12000 rpm for neutralizing the lysis buffer. Finally, DNA was re-suspended in 20 µl of sterile H_2O. The DNA was quantified in a spectrophotometer at A260 nm before polymerizing reactions.

The polymerase chain reaction (PCR) amplification of the mitochondrial cytochrome oxidase I (mtCOI) gene was performed in a thermal cycler (Eppendorf Mastercycler™, Hamburg, Germany) (Leão *et al*., 2018). The PCR products obtained after purification were sequenced in both directions at Biokart India Pvt. Ltd., Bengaluru sequencing facility, using double-pass Sanger DNA sequencing. The raw sequence species identity was determined through homology search using the BLASTn search tool and submitted to the NCBI website. In this study, 5 sequences with a size of 490 bp were generated and submitted to NCBI (accession numbers: *Megalurothrips usitatus*_OR147158, *Frankliniella schultzei*_OQ975968, *Thrips palmi*_OP315665, *Thrips parvispinus*_OP186455 and *Thrips tabaci*_OP278843).

Phylogenetic tree construction

Generated mitochondrial COI gene sequences were edited in Bioedit software. The maximum likelihood (ML) statistical method-based phylogenetic analysis was performed using MEGA X Software to analyze the genetic relationship (Nei and Kumar, 2000; Kumar *et al*., 2018). The evolutionary history was inferred using the Maximum Likelihood method and General Time Reversible model. The *Spodoptera litura* (accession number: KF022223) sequence from NCBI GenBank served as the outgroup for depicting the difference in analyzed thrips species as the species is distant from the thrips sequences.

RESULTS AND DISCUSSION

Seven different thrips species were discovered on lablab, of which *T. palmi* and *T. tabaci* were found on stem nodes and leaves and at the early stages of the crop. *In contrast, M. status*, *T. parvispinus*, *H. gowdeyi and C. orchidii* were found in flowers. *F. schultzei* was found to be infecting all the stages. Below are diagnostic characters and keys (Mound and Kibby, 1998) for these:

***Frankliniella schultzei* Trybom**

Female macropterous; Males are identical to females but smaller. Existing in two distinct color morphs: body color yellow with light shading on the tergites or body color brown with pronotum tibiae and tarsi that are lighter. Eight segmented antennas with forked sense cones on III and IV. Three pairs of ocellar setae are present, with pair III growing closely between the anterior borders of the hind ocelli. A pair of minor setae are found medially between the postero-marginal sub-median setae on the pronotum, which has five major setae slightly shorter than the antero-angulars. The metanotum lacks campaniform sensilla and has two pairs of setae along the front border. With two entire rows of veinal black setae, the forewing is pale. Tergites VI-VIII have a pair of lateral ctenidia; on VIII, the postero-marginal comb is not formed (Fig 1).

***Thrips parvispinus* karny**

Complete wings are seen in both sexes. Brownish in color, adult females have a lighter head and thorax than the abdomen. Their forewings are brown with a sharply light base and their legs are predominantly yellow. Antennas feature seven segments, with the III and IV segments having a forked sensory cone. Ocellar setae II are shorter than post-ocular setae pair I and III. The ocellar triangle's front borders are the location of the small Ocellar Setae Pair III. Two pairs of long postero-angular setae and three pairs along the posterior border are seen at the pronotum. Reticulate and occasionally display internal sculptured patterns, the metanotum. Long medial setae protrude from the anterior margin. There are no campaniform sensilla. The first and second veins of the forewing have entire rows of setae. Tergites V-VIII have lateral ctenidia behind the spiracles in the abdomen. In tergite VIII, the posterior-marginal comb is completely missing (Fig 2).

***Megalurothrips usitatus* bagnall**

Female macropterous; body color brown; tarsi, apices of mid and hind tibiae yellow. Antennae 8-segmented, III and IV with constricted apical neck, sense cone forked. Antennal segment III is yellow to light brown. Three pairs of ocellar setae are present, pair III on the front borders of the ocellar triangle and longer than the distance between compound eyes; post-ocular setae are tiny. Two pairs of long postero-angular setae on the pronotum, three pairs on the posterior

Fig 1: *Frankliniella schultzei* adult.

margin and well-developed antero-angular setae. Median setae arise at the anterior border of the metanotum, which has a poor sculpture and campaniform sensilla. Forewings are dark with a large pale patch subapically and a pale basal quarter. The first vein of the forewing has a long row of setae and a conspicuous subapical gap, while the second has an entire row of setae. Tergites VIII has an irregular group of microtrichia, although this is mainly missing medially. Tergites VIII also lack sculpture and ctenidia in the middle (Fig 3).

***Thrips tabaci* lindeman**

Female macropterous; body varying in color from yellow to dark brown. The ocellar pigment is typically grey and never red. Antennal segments III and IV are brown, with the basal half pale; antennal segments III and IV are 7-segmented, with a small forked sense cone at the tip. Two pairs of ocellar setae are on the head; pair III is tiny and arises from the front of the ocellar triangle. Ocellar setae III and post-ocular setae pairs of I to III are around the same length. Two pairs of postero-angular setae on the pronotum. The median setae of the metanotum are short and emerge from beyond the front edge; the campaniform sensilla are lacking. The metanotum is irregularly reticulated medially. Forewings pale, the second vein has a row of approximately 15 setae and the first vein typically has 4 (2-6) setae on the distal half. Tergite II has three lateral marginal setae, while tergites V through VIII has lateral ctenidia. There is a posterior margin of VIII with a complete comb of long slender microtrichia (Fig 4).

***Chaetanaphothrips orchidii* moulton**

Macropterous female with a yellow body and light forewing with brown crossbands at the base and medially. Eight-segmented antennae with slender VII and VIII and a forked, slender sensing cone at III and IV. Antennal segments V and VI have brown apexes. Only two pairs of ocellar setae, pair III within an ocellar triangle, a head that is broader than long. Two pairs of prominent postero-angular setae on the pronotum. The median setae of the metanotum are tiny and situated far behind the anterior edge. The first vein in the distal half of the forewing has three setae, while the second has three or four. Tergites' posterior borders are fully grasped, but their medial sculpture is poor (Fig 5).

***Thrips palmi* karny**

Female macropterous; body and legs yellow. Antennal segments IV and V are brown distally, whereas segments VI and VII are brown. Antennae are seven-segmented, with segments III and IV somewhat constricted at the tip and a short-forked sense cone. Two pairs of ocellar setae are in the wider-than-long head, with pair III being tiny and emerging slightly outside the ocellar triangle. Three pairs of long, postero-angular setae are seen on the posterior border of the pronotum. With bending transverse lines at the anterior and irregular longitudinal lines that converge at the posterior boundary of the metanotum, median setae emerge from behind the anterior margin and campaniform sensilla are present. Forewings pale, the second vein has a row of around 15 setae and the first has three (or two) setae on the distal half. Tergite V-VIII with ctenidia present laterally, posterior margin of VIII with complete comb of long slender microtrichia (Fig 6).

Fig 2: *Thrips parvispinus* adult.

Fig 3: *Megalurothrips usitatus* adult.

Fig 4: *Thrips tabaci* adult.

***Haplothrips gowdeyi* franklin**

Female macropterous; body and legs dark brown, fore tarsi light brown. Antennae are 8-segmented, with segment III having 2 sensoria and segment IV having 4 sensoria; antennal segments III-IV are yellow. Post-ocular setae are weakly capitate and roughly as long as the breadth of a compound eye, originating towards the eye's posterior edge. Maxillary stylets retracted to post-ocular setae, approximately half the width of the head apart, with the prominent maxillary bridge. The pronotum has 5 pairs of thin, capitate main setae. Mesopresternum is complete and boat-shaped but medially relatively thin. A tiny tooth at the top of the fore tarsus. The forewing is light, with the extreme base darkened. The forewing is constricted medially, with approximately 8 duplicated cilia; all three sub-basal setae are capitate. Tergite IX setae are sharply acute and as long as the tube; the tube is shorter than the breadth of the head (Fig 7).

Diversity of thrips on lablab

From the total population of thrips collected from the lablab field, *C. orchidii* and *T. parvispinus* were higher in proportion with the Shannon diversity of -00.362 and -00.35, respectively. *T. palmi* (-00.257) and *T. tabaci* (-00.201) were present on fresh pods and stem nodes, while *F. schultzei* (-00.24) was found present on leaves, flower buds and flowers, fresh pods, stem nodes which represent its presence all stages of the crop. *C. orchidii*, *M. status* (-00.231) *and H. gowdeyi* (-00.102) were found to infest after flowering; thus, they were collected from flower buds and flowers. Based on the results of the Shannon diversity index, *C. orchidii*, followed by *T. parvispinus, was* found to be dominant in the flowering stage, whereas *H. gowdeyi* was found to be the lowest in proportion to the total population during flowering (Table 1). They breed on plant blossoms and cause direct harm by puncturing flowers. The species causes significant damage in the bud state, resulting in fewer flowers, petals filled with life stages of thrips and feeding scars (Ananthakrishnan, 1993). *F. schultzei* is a major polyphagous pest in the genus Frankliniella. It economically damages various ornamental and vegetable crops (Milne and Walter, 2000). The females and larvae were always present in flowers (where oviposition occurs). *T. parvispinus*, one of South East Asia's most infamous pest species, is a significant pest on various agricultural and horticulture crops. This species was initially discovered in India on papaya in Bangalore (Tyagi *et al*., 2015).

Phylogenetic tree analysis

Genetic analysis of the lablab thrips species complex based on the mtCOI gene confirmed that five out of seven collected species were with their molecular data. The identified sequences were similar to the well-known species *T.palmi, T.parvispinus, T.tabaci, F. schultzei and M. usitatus*. Those sequences were submitted to NCBI and accession numbers were obtained. Fig 8 represents the phylogenetic relationship

Fig 5: *Chaetanapothrips orchidii* adult.

Fig 6: *Thrips palmi* adult.

Fig 7: *Haplothrips gowdeyi* adult.

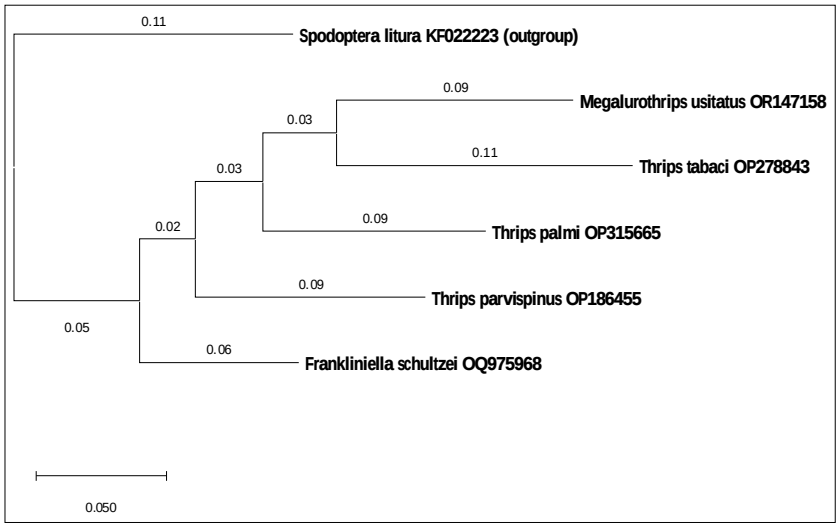

Fig 8: Maximum likelihood tree constructed based on the available sequences. *S. litura* sequence is utilized as an outgroup.

Table 1: Shannon diversity index of the identified thrips species.

Category	Habitat of thrips	No. of thrips found	P_i	P_i^2	$P_i \ln [P_i]$
T. palmi	Fresh pods stem nodes	17	00.122	0.015	-00.257
T. tabaci	Fresh pods, stem nodes	11	0.079	0.006	-00.201
T. parvispinus	Leaves, Flower buds and flowers, Fresh pods	36	00.259	0.067	-00.35
F. schultzei	Leaves, Flower buds, flowers, Fresh pods, stem nodes	15	00.108	0.012	-00.24
M. status	Flower buds and flowers	14	00.101	0.01	-00.231
C. orchidii	Flower buds and flowers, leaves	42	00.302	0.091	-00.362
H. gowdeyi	Flower buds and flowers	4	0.029	0.001	-00.102
Total		139	1		

Pi- Proportion of individuals; Shannon index-1.739; Simpson index-0.1979.

of collected thrips species. It is well-denoted that the species of thrips were far from related to the *S. litura* by showing a well-separated clade. All the species except the outgroup converged into a single clade. However, each species of collected thrips specimens formed a separate subclade, showing the species identity divergence among the analyzed specimens. The sequence analysis was utilized to compare the morphological approach in determining the species makeup of adult thrips (Wang *et al*., 2018; Farkas *et al*., 2020).

CONCLUSION

Thrips are polyphagous and more than one species can live in the same habitat. The current study has concluded that seven species of thrips were associated with different crop stages of lablab either individually or simultaneously. Taxonomic and molecular characterization can provide evidence for the results. Furthermore, the diversity indices can describe the proportion of each species in the total population. Pest management practices need to be formulated based on the dominance of thrips species. *T. parvispinus* is a notorious pest species of South East Asia and a severe pest of quarantine importance. *F. schultzei*, *T. palmi and T. tabaci* are notorious pests and vectors of plant viruses. Since these species may attain major pest status, the report demands regular monitoring and surveys for them on lablab.

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