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## Occurrence of South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): An invasive pest in Tamil Nadu, India

DR Balaji, S Jeyarani, K Ramaraju, S Mohankumar and PS Shanmugam

#### Abstract

In this study, extensive survey was carried out for the occurrence of *T. absoluta* in different vegetable growing regions of Tamil Nadu during August, 2015 to April, 2016. Among the forty one localities surveyed, *T. absoluta* infestation was found nearly in 25 localities of Tamil Nadu. Among them, the incidence was highest in a polyhouse of Tamil Nadu Agricultural University (TNAU) orchard (92.50 %) followed by 89.70 per cent in Echampatti village of Krishnagiri district and 82.40 per cent in Magendra mangalam village of Dharmapuri district. Apart from tomato, *T. absoluta* damage was also recorded on brinjal at TNAU orchard (9.72%) and potato at wood house, Horticultural Research Station, Ooty (26.67%). Characterisation of *Tuta absoluta* populations from Coimbatore, Dharmapuri and Krishnagiri Districts using cytochrome c oxidase subunit I (COI) revealed that the Dharmapuri and Krishnagiri populations were closely related with NCBI accessions sourced from Pune, Maharashtra (Accession No. KP814055) and the Coimbatore population was closely related with Florida (KJ657679) and Bonsia (KC852871).

**Keywords:** South American tomato pinworm, *Tuta absoluta*, survey, molecular characterisation, COI gene

#### Introduction

Tomato is the world's largest vegetable crop grown after potato and sweet potato, but it tops the list of canned vegetables [19]. India is the second largest producer of tomato next to China with an area of 9.93 million hectares with an annual production of 1.63 lakh million MT (14.4% of total production) and 21.2 MT of productivity ha<sup>-1</sup> [18].

Tomato is ravaged by several insect pests of which the South American tomato pinworm, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is now getting a status of key pest in India [10]. Several cultivated and wild species viz., *Solanum melongena* L., *S. tuberosum* L., *Nicotiana glauca* Graham, *Datura stramonium* L., *Capsicum annuum* L. *Phaseolus vulgaris* L. [9], *Lycium* sp., *Malva* sp. [6], Cape gooseberry and *Physalis peruviana* L. were reported as host plants for *T. absoluta*. Crop losses up to 100 per cent were reported both under greenhouse and open-field tomato [1, 5].

The tomato pinworm, *T. absoluta* was first described in Peru in 1917 and spread to South America, where it is considered as one of the most devastating pests for tomato since 1960s [4, 8, 11, 12]. In Europe, *T. absoluta* presence was initially reported in the Eastern Spain in late 2006 [27] and recently it was introduced into India [23]. In view of the invasive nature of this pest, the present investigation was carried out to document the spread of this dreadly pest in various districts of Tamil Nadu. The identity of the pest was also confirmed through molecular characterization and the results are presented hereunder.

#### Materials and Methods

##### Survey

An extensive survey was carried out from August, 2015 to April, 2016 in 41 localities covering thirteen major vegetable growing districts of Tamil Nadu viz., Coimbatore, Krishnagiri, Dharmapuri, Erode, Salem, Tirupur, The Nilgris, Dindigul, Tirunelveli, Theni, Madurai, Trichy and Cuddalore to collect and document the occurrence of tomato leaf miner, *T. absoluta*. The tomato leaf miner was identified morphologically as described by Harizanova *et al.* (2009) [14] and Sridhar *et al.* (2014) [23] who described the egg as oval-cylindrical, creamy-white to yellow, 0.4 mm long.

The larva was creamy-white in the beginning and becomes green or pinkish with dark brown head in later instars. The prothoracic shield has paler patches with variable markings. The pupa was greenish-brown at the beginning and later becomes dark brown and the length was up to 6 mm. The adult moths were quite small, less than 7 mm in length with grey to brown scales and black spots on anterior wings. Antennae filiform with bicoloured segments, legs and palps ringed with black and brown colour, labial palpi prominent, projected forward, up-curved, with the apical segment long and acute; head vertex covered with appressed scales, hindwings with outer margin concave posterior of apex. During the survey, *T. absoluta* damage was recorded from top, middle and bottom leaves of five randomly selected plants and the per cent damage was worked out. Based on the damage percentage, it was grouped into six damage rating scale viz., 0 = 0% of injury in the plant, 1 = small lesions, no coalescing, 0.1 to 5% injury, 2 = small lesions, not coalescing, 5.1 to 20% injury, 3 = medium and large lesions, from 20.1 to 50% of injury, 4 = numerous lesions, large and coalescent, from 50.1 to 80% of injury, 5 = plant completely deformed, more than 80.1 % of injury<sup>[17, 20]</sup>.

### Molecular characterisation of pinworm

Being an invasive pest, the occurrence of *T. absoluta* in Tamil Nadu was confirmed by DNA barcoding technique using standardized cytochrome c oxidase subunit I (COI) sequencing approach that enables us to identify the pest at species level<sup>[15]</sup>. In the present study, populations of *T. absoluta* from three districts viz., Coimbatore, Dharmapuri and Krishnagiri were characterized. Genomic DNA was isolated from single adult of *T. absoluta* following the CTAB method<sup>[2]</sup>.

### Quality and quantity check of genomic DNA

Quality of genomic DNA was checked by 0.8 per cent agarose gel. Agarose at 0.8gm was dissolved in 100 ml of 1X TBE buffer. After cooling, 1-2 µl ethidium bromide was added from the stock (10 mg ethidium bromide / ml H<sub>2</sub>O). Then the mixture was poured into a preset template kept with appropriate comb to make wells. 2µl of DNA added with 2 µl of loading dye (6X loading dye) were loaded in each well. Electrophoresis was carried out at 65 V for 1 h. Amplified genomic DNA was visualized on UV transilluminator (Bio-Rad, USA) and documented using Gel documentation system (GELSTAN 1312). The quantification of DNA was done using Nanodrop Spectrophotometer (ND-1000). Based on the nanodrop readings, DNA dilutions were made in TE buffer or double distilled water to make a final concentration of 50-60 ng µl<sup>-1</sup> and stored in 40C for further use<sup>[21]</sup>.

### mtDNA (COI) sequencing and phylogenetic analysis

A fragment of the mitochondrial gene (Cytochrome Oxidase 1 (COI)) was amplified across the populations of *T. absoluta* using Folmer primers LCOI490 (Forward) and HCO2198 (Reverse) by adopting the procedure described by Hebert *et al.* (2003)<sup>[15]</sup>.

Forward primer (5'-3'):

GGTCAACAAATCATAAAGATATTGG

Reverse primer (3'-5'):

TAAACTTCAGGGTAACCAAAAAATCA

Polymerase chain reactions were performed in 25µl volumes in PCR machine (Sure cycler 8800, Agilent Technologies). Amplified products of COI gene were screened using agarose gel electrophoresis (1.5%), 5 µl of PCR product along with 2

µl of loading dye loaded on the agarose gel, electrophoresed at 65 v voltage for 1 hour. The products were then visualized on UV transilluminator and the gel was documented using gel documentation system (GELSTAN, 1312).

PCR products of (20 µl) and their respective forward and reverse primers (10 µl each per sample) were labelled appropriately and sent to Scigenom Labs Pvt. Ltd., Kerala for sequencing. The PCR products of *T. absoluta* were sequenced by double pass method in both forward and reverse direction. The PCR products were purified using Pure Link PCR purification Kit and the sequencing PCR were set up by using Big Dye Terminator V3.1 Cycle Sequencing Kit. The COI fragments of *T. absoluta* were sequenced bi-directionally at Scigenom, Kerala, India and then the sequences were aligned, edited and trimmed using the program Geneious and outgroups obtained from GenBank using the blastn algorithm to search the nucleotide (nr/nt) data base.

### Similarity analysis / Statistical analysis

The nucleotide sequence was compared to identify the similarity between each host by Basic Local Alignment Search Tool (BLAST) and Barcode of Life Database (BOLD). The gene sequences were aligned using the ClustalW algorithm<sup>[27]</sup>. The phylogenetic tree was constructed by using MEGA version 5 and the tree was drawn using Bootstrap method.

### Results and Discussion

#### Survey and documentation of tomato pinworm incidence in major vegetable growing regions of Tamil Nadu

Extensive survey was carried out conducted in 41 localities covering thirteen major vegetable growing districts of Tamil Nadu viz., Coimbatore, Krishnagiri, Dharmapuri, Erode, Salem, Tirupur, The Nilgris, Dindigul, Tirunelveli, Theni, Madurai, Trichy and Cuddalore Among the 13 districts surveyed, the occurrence of *T. absoluta* was recorded in 8 districts viz., Coimbatore, Krishnagiri, Dharmapuri, Tirupur, The Nilgris, Dindigul, Theni and Madurai. Severe incidence of *T. absoluta* (92.50% leaf damage) was recorded in Tamil Nadu Agricultural University (TNAU) orchard, Coimbatore followed by Echampatti village (89.70%) of Krishnagiri, Mahendra mangalam village (82.40%) of Dharmapuri and Ambilikai village (82.00%) of Dindigul districts. Apart from tomato, *T. absoluta* damage was also recorded on brinjal at TNAU orchard (9.72%) and potato at wood house, Horticultural Research Station, Ooty (26.67%) (Table 1). The present findings were in accordance with Sridhar *et al.* (2014)<sup>[23]</sup> who reported the presence of *T. absoluta* in all the 10 villages of Bengaluru rural and Bengaluru urban districts through the explorative survey. Their survey results also revealed that the regions of Kalyani (West Bengal), Ludhiana (Punjab), Bhubaneswar (Odisha), Raipur (Chattisgarh), Hyderabad (Telangana), Rahuri (Maharashtra), Varanasi (Uttar Pradesh) and New Delhi (Delhi) were not infested with *T. absoluta*. Similarly, Shashank *et al.* (2015)<sup>[22]</sup> surveyed 18 major tomato growing localities in Maharashtra and reported the occurrence of *T. absoluta* in majority of the localities surveyed except for 5 localities viz., Manmad, Shahada, Dhule, Chopda and Amalner. They also reported severe infestation of the *T. absoluta* in four localities viz., Pune, Ghargaon, Saptashrunji and Malegaon with more than 50 per cent damage, followed by 10 to 50 per cent damage in Shirpur and Satara. Kumari *et al.* (2015)<sup>[16]</sup> reported the incidence of *T. absoluta* (97.9%) for the first time on tomato at Vegetable Research Station, Rajendranagar, Telangana State during

regular surveys from January to April 2015. Similarly, surveys conducted by ICAR-National Bureau of Agricultural Insect Resources (ICAR-NBAIR) in Karnataka, Tamil Nadu and Gujarat from January to March 2015 revealed 0.5 to 13.5 per cent fruit damage by *T. absoluta* in Tamil Nadu, 2.0 to 100 per cent in Karnataka and 5 to 12 per cent in Gujarat (Ballal *et al.*, 2016) [3].

Surveys carried out by several workers revealed the occurrence of *T. absoluta* in almost all the localities of Karnataka, Maharashtra, Gujarat and Tamil Nadu [24]. The severity of this pest in the surveyed areas indicates that the *T. absoluta* may also be present in other states of India which needs to be monitored and documented at national level.

### Molecular Identification

Identification system based on DNA barcoding facilitates the identification of known as well as discovery of new species [15]. In present study, Populations of *T. absoluta* from three districts *viz.*, Coimbatore, Dharmapuri and Krishnagiri were characterised using standardized cytochrome c oxidase subunit I (COI) sequencing approach. The DNA of the *T. absoluta* populations from three different locations were amplified using mitochondrial Cytochrome Oxidase I (mtCOI) primer. The amplified product length was approximately 670 bp in all the three populations (Plate 1). The three location population sequences were compared with reference sequences available in NCBI GENBANK and analysed by BLAST. Based on the dendrogram, Coimbatore, Krishnagiri and Dharmapuri populations were grouped under single cluster and were more closely related. Similarly, Sridhar *et al.* (2014) [23] also analysed the diversity

of *T. absoluta* from different locations *viz.*, Shivakote, Madhugirihalli, Thirumalapura, Thamarasanahalli, Agrahara, Gudadhalli, Ivarakandapura (IIHR), Kalenahalli, Linganhalli and Hessaraghatta of Karnataka using mtCOI. According to them, all the *T. absoluta* populations were grouped under single clade in Neighbour-Joining (NJ) tree revealing no genetic variation within populations. Tosevski *et al.* (2011) [26] also analysed the diversity of *T. absoluta* from different villages *viz.*, Donjivrtogos and Navalín villages of South Serbia using mtCOI gene. According to them, COI barcode region of their population had cent per cent identity with the barcode sequence of *T. absoluta* from France (HQ968678; BOLD: PHLAB662-10).

### Conclusion

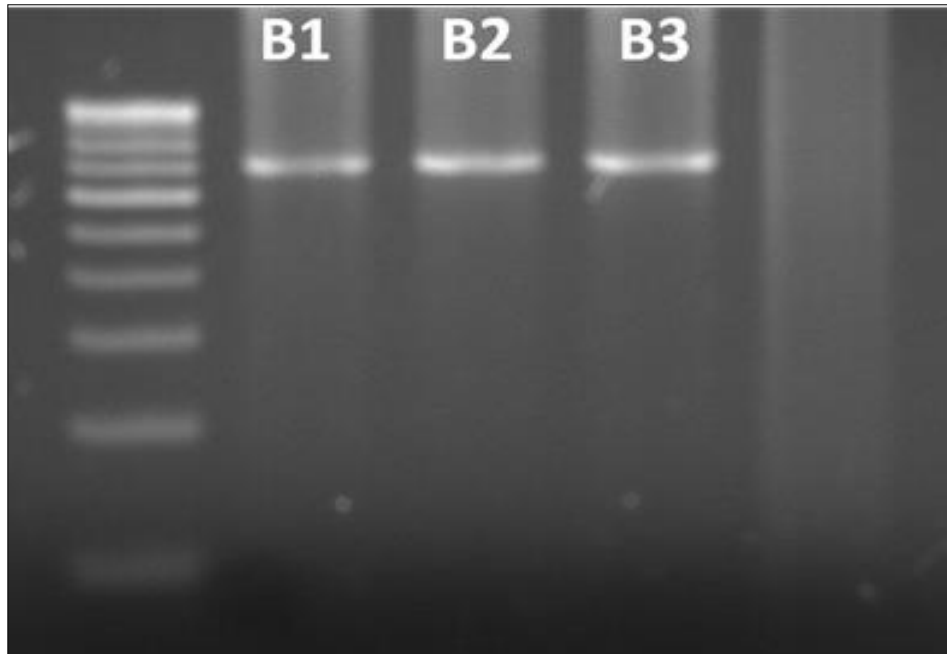
Since the initial detection, *T. absoluta* has become the most serious pest causing severe damage to tomato in many areas [13]. Due to its rapid population growth, potential dispersal through environment and expressed resistance to insecticides [7], this pest has been classified as the most serious threat for tomato production worldwide. The present investigations are also indicative that the *T. absoluta* can soon become a major pest of concern, since the environmental conditions available in India particularly in Tamil Nadu is conducive for the development of the pest.

Adaptation of rapid response strategies against its invasion should be the focus by educating farmers, extension entomologists and other stakeholders. The major challenge lies in adopting better management options from the previous experiences of successful management of this pest, worldwide.

**Table 1:** Survey and documentation of South American tomato pinworm, *T. absoluta* incidence in Tamil Nadu

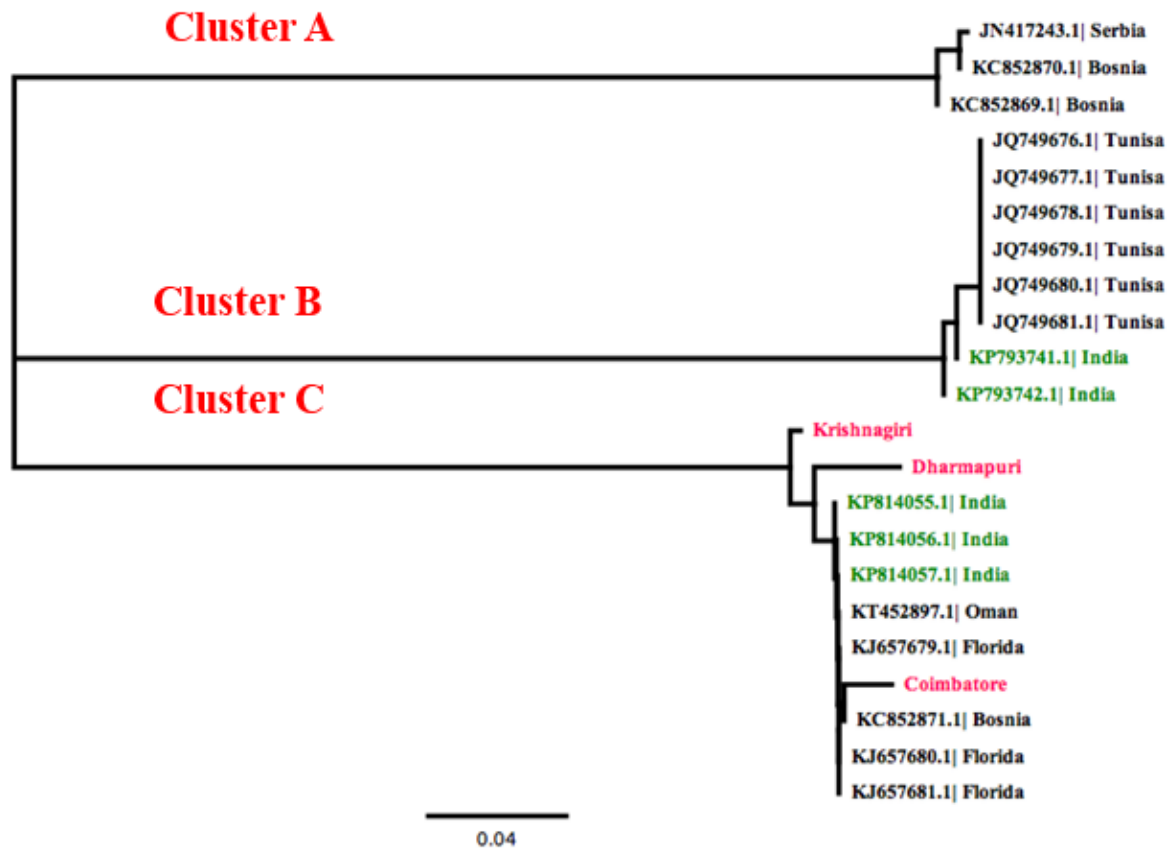
District	Location	Latitude	Longitude	Variety/hybrid	Stage of the crop	Incidence of <i>T. absoluta</i> (%)	Damage rating scale
<b>Tomato</b>							
Krishnagiri	Haleseebam	12.588296°N	77.968189°E	Sivam, Sagar	Fruiting	58.50	4
	Ellappan patti	12.508405°N	78.046053°E	Bangalore local	Fruiting	3.70	1
	Echampatti	12.493164°N	78.026919°E	Sivam	Fruiting	89.70	5
Dharmapuri	Mahendra mangalam	12.410696°N	78.063589°E	Sivam, Sagar	Fruiting	82.40	5
	Kaadusetti patti	12.455130°N	78.026157°E	Sivam	Fruiting	12.90	2
	Jittanda halli	12.425473°N	78.049663°E	Sivam	Fruiting	28.89	3
	Papparpatti	12.219491°N	78.059838°E	Arka rakshath	Flowering and fruiting	1.70	1
	Kettu halli	12.096523°N	78.010968°E	UPS 800	Flowering and fruiting	64.21	4
	Ramar koodal	12.099872°N	78.011734°E	Sivam	Flowering and fruiting	48.48	3
Coimbatore	Madampatti	10.971569°N	76.863152°E	Sivam	Seedling, flowering and fruiting	2.57	1
	kinathukadavu	10.829207°N	77.026854°E	Anaga, 5024- Rasi, Aishwarya and Suguna	Flowering and fruiting	0.00	0
	TNAU orchard	11.009349°N	76.929942°E	COTH 1	Flowering and fruiting	92.50	5
Salem	Pannapatti	11.829466°N	78.076102°E	Lakshmi 5005	Fruiting	0.00	0
Tirupur	Thaneer pandal	10.911179°N	77.690712°E	Sivam	Flowering and fruiting	0.5	1
	Nanjiyampalayam	10.772491°N	77.508911°E	Lakshmi 5005	Fruiting	0.00	0
	Ruthravathi	10.862114°N	77.421093°E	unknown	Fruiting	0.00	0
Dindigul	Ambilikai	10.564532°N	77.725521°E	Lakshmi 5005	Flowering and fruiting	82.00	5
	Gavadalvalsi	10.563631°N	77.713918°E	Sivam	Fruiting	20.2	3
	Pannaipatti	10.415705°N	77.814500°E	Lakshmi 5005	Fruiting	0.00	0
	Poothipuram	10.245709°N	77.882888°E	Local	Flowering	0.00	0
	Kamalapuram	10.245054°N	77.882750°E	Local	Flowering	0.00	0
	Sempatti	10.310552°N	77.862260°E	unknown	Fruiting	2.60	

Tirunelveli	Naduvakurichi	9.107891°N	77.459820°E	Local	Flowering and fruiting	0.00	0
Theni	Thamarkamnayakam	9.920845°N	77.668948°E	Lakshmi 5005	Fruiting	6.67	2
	T. Bomminaykkan patti	9.997244°N	77.665902°E	Local	Flowering and fruiting	0.00	0
	Arappadidevan patti	10.00121°N	77.539197°E	Lakshmi 5005	Flowering and fruiting	2.11	1
	Narayanadevan patti	9.715621°N	77.303000°E	Local	Seedling	1.03	1
	Pulanandapuram	9.863755°N	77.390637°E	Local	Flowering and fruiting	28.45	3
	Kokilapuram	9.784777°N	77.343247°E	Local	Fruiting	1.33	1
	Sediyampatti	9.871706°N	77.396014°E	Vellai thakkali	Fruiting	0.52	1
	D. Vadipatti	10.132606°N	77.618280°E	Unknown	Flowering and fruiting	0.00	0
	Periyakulam	10.11774°N	77.549447°E	PKM 1	Flowering and fruiting	1.30	1
Parasurampuram	10.163861°N	77.722409°E	Unknown	Fruiting	0.00	0	
Madurai	Insectary, AC & RI	9.970634°N	78.203128°E	PKM 1	Flowering and fruiting	28.90	3
Trichy	Pulutheri	10.730610°N	78.545696°E	Local	Fruiting	0.00	0
Nilgris	Havukal	11.441028°N	76.900389°E	Local	Flowering and fruiting	17.41	2
Cuddalore	Thozhuthur	11.415694°N	78.987026°E	Local	Flowering and fruiting	0.00	0
Erode	Thaalavaadi	11.774939°N	77.008495°E	Local	Fruiting stage	0.00	0
<b>Brinjal</b>							
Coimbatore	TNAU orchard	11.009349°N	76.929942°E	Trichy oodha	Flowering and fruiting	9.72	2
<b>Potato</b>							
Ooty	Wood house, HRS	11.417880°N	76.722662°E	Kufri swarna	Tuber formation	26.67	3
Ooty	Kodappamandu	11.424340°N	76.722736°E	Local	Tuber formation	0.00	0



L – Ladder (100 bp)  
 B1 - Coimbatore  
 B2 - Krishnagiri  
 B3 - Dharmapuri

**Plate 1:** *mtCOI* amplicon profile of *T. absoluta*



**Fig 2:** Dendrogram obtained from *mCOI* gene sequences using neighbour joining method

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