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New record of the invasive South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in India

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ABSTRACT : The occurrence of the South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is being reported for first time as an invasive pest on tomato and potato in India. *T. absoluta* was observed during regular surveillance on tomato at Indian Institute of Horticultural Research (IIHR) and adjoining farmers' fields in Bengaluru, Karnataka during November 2014 as a part of Real Time Pest Surveillance of Tomato (RTPST) of National Initiative on Climate Resilient Agriculture (NICRA) Project. Subsequent surveys revealed the presence of the pest in six districts of Karnataka State *viz.*, Bengaluru Rural & Urban, Kolar, Chikkaballapur, Ramanagar and Tumkur. Incidence of the *T. absoluta* has also been recorded on potato. Larvae were generally found to feed on leaves, creating blotch-like mines visible from both sides of the leaf and several mines were observed on a single leaf. The larvae were also observed feeding on apical buds, stalks and boring the fruits. The affected fruits carried distinct holes mainly in the upper half towards fruit stalk and usually covered with faecal mass. The infestation of *T. absoluta* ranged from low to high (up to 15 mines/plant) in different tomato fields surveyed. In some of the fields up to 87% of the tomato plants were infested by *T. abosoluta*. A zoophytophagous mirid bug, *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) was recorded as predator on eggs and early larval stages of *T. absoluta* under field conditions. Hence, there is an urgent need to deploy suitable management practices to contain further spread of this potential pest.

Keywords: *Tuta absoluta*, tomato, invasive pest, potato, *Nesidiocoris tenuis*

INTRODUCTION

The increased international trade and the movement of plant materials have increased the risk of invasive alien pest species, threatening cultivation of crops globally (Satyanarayana and Satyagopal, 2013). Incidence of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was recorded for the first time on tomato at the Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bengaluru (13°8'12"N 77°29'45"E, altitude 890 m), Karnataka, India during the *rabi* season of 2014 (transplanted in October 2014). *Tuta absoluta* has several common names like tomato borer, South American tomato moth, tomato leaf miner and South American tomato pinworm. Tomato is one of the important vegetable grown in India with 8.8 lakh ha area with a production of 18.2 M mt. Similarly, potato is another important solanaceous vegetable grown in 19.9 lakh ha with a production of 40.3 M mt (NHB, 2014).

Tuta absoluta, is a neotropical, oligophagous pest infesting many solanaceous crops. Since the 1960s, this moth has become one of the key pests of tomato in South America (Garcia and Espul, 1982). In Europe, *T. absoluta* presence was initially reported in the Eastern Spain in the late 2006 (Urbaneja *et al.*, 2007), thereafter, it was recorded in Morocco, Tunisia, France, Italy, Netherlands, Albania, Portugal, Bulgaria , Cyprus, Germany, Israel, Hungary, Greece, Bahrain, Iraq, Isreal, Japan, Jordan, Kuwait, Qatar, Saudi Arabia, Syria, Turkey, Yemen, Ukraine and other countries (CABI, 2014: Figure 1). Explosive spread and dissemination of *T. absoluta* is mainly correlated with fruit import and further distribution (Potting, 2009). One of the possible pathways for a long distance dissemination of *T. absoluta* could be through packaging materials (boxes) from infested countries (EPPO, 2010). Since the initial detection, this has become the most serious pest causing severe damage on tomato in many areas (Germain *et al.*, 2009). Cost-benefit analysis showed that *T. aboluta* significantly increased costs of pest management, primarily as a result of increased use of insecticides (Thomas, 1999; Lietti *et al.*, 2005). The tomato leafminer can cause crop losses up to 100% and it is considered a key pest of greenhouse and open-field tomato (Arturo *et al.*, 2012).

The pest is multivoltine having nearly 12 generations per year. According to its rapid population growth this

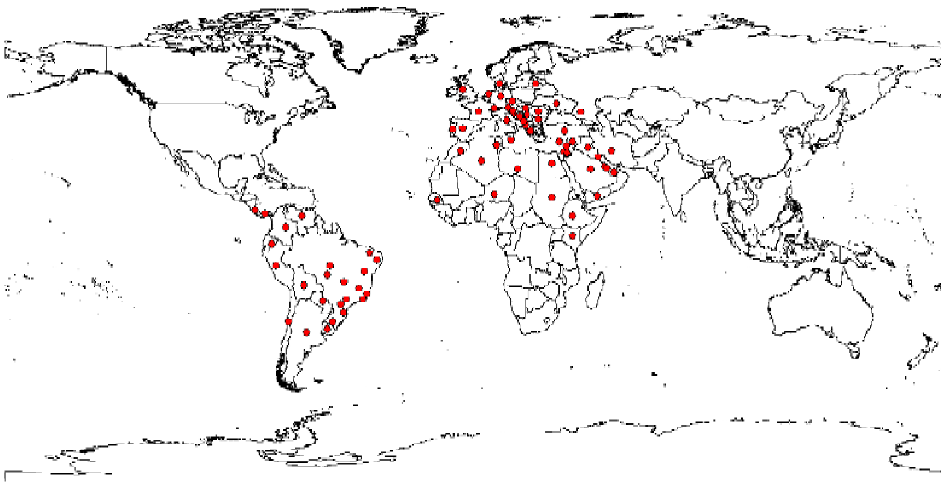

Fig. 1. Global distribution of *T. absoluta*

pest should be treated as r-selected species (Pereyra and Sanchez, 2006). The rapid growth, potential natural dispersal and resistance to insecticides (Desneux *et al.*, 2010) render this pest as the most serious threat for tomato production systems worldwide.

The present study reports identification, occurrence and damage of *T. absoluta* on tomato and potato in Bengaluru and its adjoining districts of Karnataka.

MATERIALS AND METHODS

Surveillance of the tomato pests was carried out through weekly observations during *Rabi* (October-February) season of 2014-15 in the experimental plots of the Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bengaluru (13°8'12"N 77°29'45"E, altitude 890 m), Karnataka, India for study of their population dynamics. Observation on the hitherto unrecorded insect at its larval stage from tomato leaf mines was made during November 2014. The larvae were brought to the laboratory and reared till adult emergence at Division of Entomology and Nematology, IIHR for further identification.

Species identification based on morphological characters

Morphological characters of the adults, larvae, pupae and eggs were examined under Leica® stereo microscope. Male genetalia of the moths was carefully dissected out, and macerated in 10% KOH for about 15 min. After maceration the male genitalia were examined under the microscope. The shape of valva and vinculum were studied as most appropriate characters for identification of the Gelechiidae. The identification of the pest was confirmed by keys provided by Roditakis *et al.* (2010) and other authentic web sources (http://caps.ceris.purdue.edu/screening/tuta_absoluta_id & http://idpools/id/leps/micro/about.php).

Molecular identification

Total DNA was extracted from individual larva and adult of the insect using CTAB method (Asokan *et al.,* 2010). A portion of thoracic tissue was homogenized with liquid nitrogen using a sterile micropestle in 1.5 mL microcentrifuge tube filled with 100 µL STE buffer (100 mM NaCl, 10 mM Tris HCl (pH-8.0), and 1 mM EDTA (pH-8.0)). The homogenate was heated at 65°C for 60 min before being centrifuged at 6000 rpm for 15 minutes at room temperature followed by precipitation of DNA and dissolution in molecular biology grade water (DNAase-free and RNAase free) (Eppendorf, Germany). 2µL was used as the template for Polymerase Chain Reaction (PCR).

PCR was carried out in a thermal cycler (AB-Applied Biosystems, Veriti 96 wells) with the following cycles: 94°C for 4 min as initial denaturation followed by 35 cycles of 94°C 40 s, 47°C for 45 s 72°C for 45 s and 72°C for 10 min as final extension. Use of primers specific to mitochondrial cytochrome oxidase I (CO-I), (LCO-1490- 5' - GGT CAA CAA ATC ATA AAG ATA TTG G -3'; HCO-2198- 52 - TAA ACT TCA GGG TGA CCA AAA AAT CA -32 ; Hebert *et al.* 2003) resulted in the amplification of an approximately 700bp fragment. PCR was performed in 25 µL total reaction volume containing 20 pmoles of each primer, 10 mM Tris HCl (pH-8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP and 0.5 U of Taq DNA polymerase (Thermo Scientific, Fermentas). The amplified products were resolved in 1.0% agarose gel, stained with ethidium

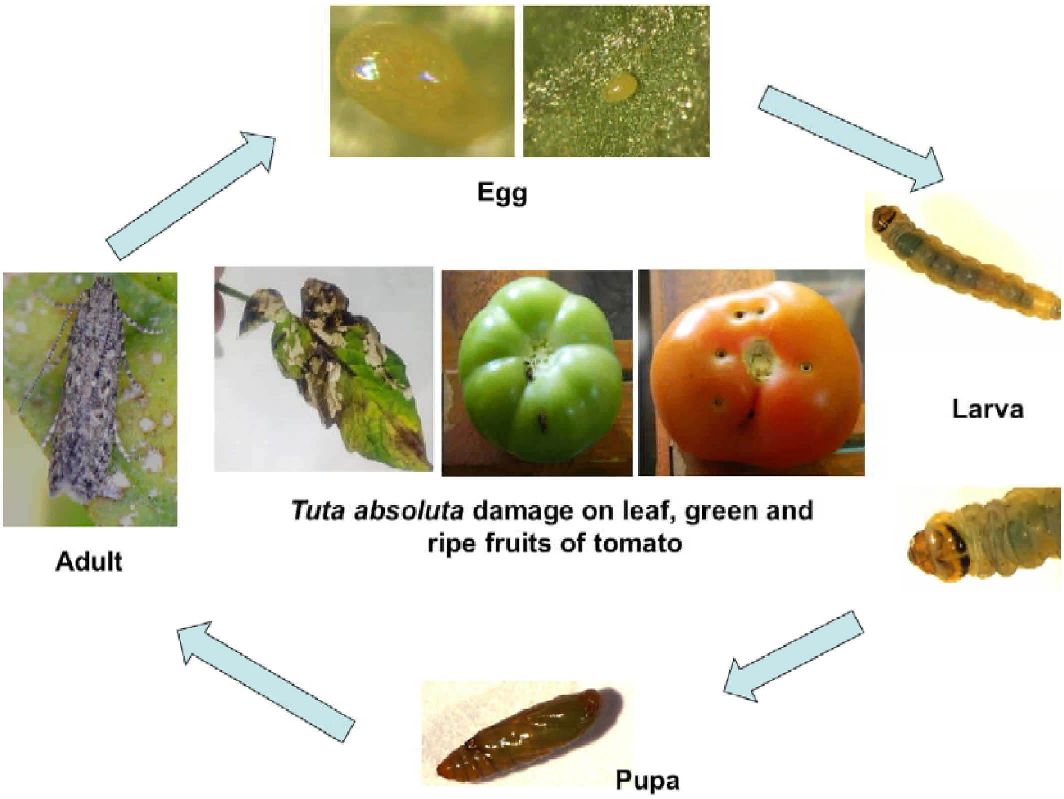

Fig. 2. Life cycle and damage symptoms of *T. absoluta* on tomato

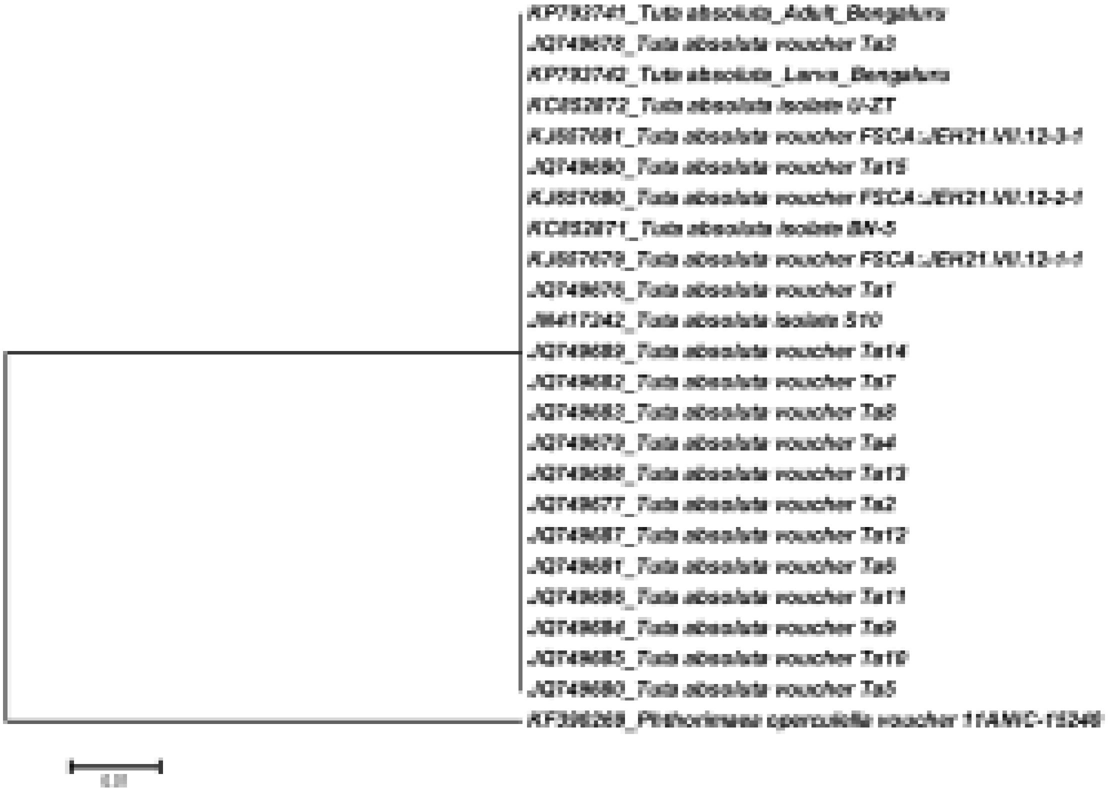

Fig. 3. NJ tree showing single clade for *T. absoluta* CO-I sequences. *P. operculella* was used as an outgroup

bromide (10 μg/ml) and visualized in a gel documentation system (UVP).

Cloning and sequence analyses

The amplified products were eluted using gel elution kit (Nucleospin® Extract II, Macherey Nagel, Germany) according to the manufacturer's protocol. The eluted products were ligated into general purpose cloning vector, InsT/A clone (Fermentas Life Sciences, EU). Blue-white selection was carried out and plasmids were isolated from the overnight culture of positive clones, incubated in LB broth using GenJET™ plasmid MiniPrep kit (Fermentas Life Sciences, EU) according to manufacturer's protocol. Sequencing was carried out for triplicates of the same clones in an automated sequencer (ABI prism® 3730 XL DNA Analyzer; Applied Biosystems, USA) using M13 universal primers both in forward and reverse directions. Homology search was done using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/) and sequence alignment carried out using BioEdit version 7.0.9.0 (Hall, 1999). Phylogenetic analyses were carried out in MEGA v.6.0 (Tamura *et al*, 2013). All the sequences generated in this study were deposited in NCBI-GenBank.

Pheromone trapping

Pheromone lure specific to *T. absoluta* (Supplier: Ponalab, Bengaluru: Commercial name: Catch *Tuta*-9) was used along with water trap to confirm the identity of the insect. One trap per acre was installed in the tomato plots of IIHR and nearby farmers' fields in Shivakote, Gudadahalli and Hessaraghatta villages for monitoring *T. absoluta*.

Explorative surveys

Surveys for the occurrence of *T. absoluta* were carried out in adjacent districts of Bengaluru after confirming the insect identity. Infestation of *T. absoluta* was surveyed on other crops and weeds to document the alternate hosts, if any in the ecosystem during the *rabi* season of 2014-15. Standard sampling procedure adopted for the leaf miner (*Liriomyza trifolii*) was followed as per the manual of tomato pest surveillance (NICRA, 2012) for assessing the intensity of damage.

Roving surveys were also carried out in Bengaluru Rural, Bengaluru Urban, Kolar, Chikkaballapur, Ramanagara and Tumkur districts of Karnataka for this pest. Occurrence of T. *absoluta* was also communicated to other tomato workers in the States of West Bengal, Punjab, Odisha, Chhattisgarh, Telangana, Maharashtra, Uttar Pradesh and New Delhi. Information and alerts were sent to several entomologists across India through e-mails to make them aware about the presence of the new pest on tomato.

RESULTS AND DISCUSSION

Morphological characterisation based on the taxonomic keys confirmed the species identity as *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), the South American tomato leaf miner. Life stages of the insect and its damage symptoms are described below. (Figure 2).

Egg: Oval-cylindrical, usually observed singly on the underside of leaves, on buds, or on the calyxes of green fruit. Eggs were cream-coloured and small, about 0.2 mm in diameter and less than 0.4 mm in length.

Larva: Early instars are white or cream with a black head. As larvae grew older, they turned greenish to pink with a brown head. The prothoracic shield is pale, with darker shading along posterior margin.

Pupa: Less than 6 mm long, light to dark brown, formed in forks or under rolled/folded tomato leaves, singly.

Adult: Moths are small with a body length of about 5-7 mm. They were brown or silver with black spots on the narrow wings. Antennae are filiform with bicoloured segments. Legs and palps were ringed with black and brown, labial palpi prominent, projected forward, up-curved, and with the apical segment long and acute; head vertex covered with appressed scales (scales flattened and against the surface); hindwings with outer margin concave posterior of apex. The male genetalia have a broad, horseshoe/ovate shaped gnathos and digitate valva with a medial hump and constriction. The vinculum is broad, deeply excavated medially and with paired trapezoid-shaped processes with outwardly curved tips.

Nature of damage: After hatching, young larvae of *T. absoluta* immediately mined into tomato leaves, apical buds, stalks or fruits. Feeding resulted in conspicuous mines (blotches) and galleries on leaves and pinhole sized holes on fruits from the stalk end generally covered with the frass. Larvae mainly attacked leaves, creating blotch/leaf mines visible from both sides of the leaf. Several mines were observed on a single leaf. The mines have dark frass (excrement) visible inside and the mined areas turned brown and dried over time.

Molecular identification

CO-I was successfully sequenced from *T. absoluta*, where a total product size of 658 bp was analyzed for the diversity, of which no characters were variable or parsimony informative. There were no pseudogene sequences observed, which was supported by the absence of stop codons within the sequences and the base composition was similar with no indels (Rebijith *et al.,* 2012). The nucleotide frequencies were 39.28 % (A), 27.62 % (T), 17.50 % (C) and 15.61 % (G). The base composition of the CO-I gene fragment biased toward Adenine and Thymine, which together constituted 66.90 % of the total as expected from earlier studies on insects (Wang *et al.,* 2011). The overall transition (ti)/ transversion (tv) bias of the nucleotide sequence was R= 0.456. The CO-I data set yielded one NJ tree representing *T. absoluta* sequences, which formed one single clade (Figure 3) revealing no genetic variations within population. *Phthorimaea operculella* (Zeller) was used as an outgroup.

Surveillance/survey observations

Explorative surveys revealed the presence of *T. absoluta* in all the 10 villages of Bengaluru Rural and Bengaluru Urban districts. The intensity of damage due to *T. absoluta* was as low as 0.08 mines/plant at Thirumalapura and Gudadhalli to the highest of 14.08 mines/plant Ivarakandapura. The intensity of the pest was severe in Shivakote and Ivarakandapura villages recording up to 9 and 14 mines per plant, respectively. Peak damage of 30-40 total mines/plant were recorded in these villages (data not shown). Apical buds and tender new leaflets were preferred sites of larval infestation.

In Shivakote, up to 87 per cent of tomato plants were observed to be infested with this pest. Up to 3.5 % of tomato fruits were damaged by this pest. In Thavarekere village (Bengaluru Rural District) and Alambadi village (Kolar District) incidence of *T. absoluta* was observed on potato plants also.

The regions of Kalyani (West Bengal), Ludhiana (Punjab), Bhubaneswar (Odisha), Raipur (Chhattisgarh), Hyderabad (Telangana), Rahuri (Maharashtra), Varanasi (Uttar Pradesh) and New Delhi (Delhi) did not have the occurrence of *T. absoluta* at the time of this reporting.

However, *T. absoluta* was recorded in all the districts of Karnataka where the survey was carried out. The severity of the pest in these surveys indicates that the pest may be present in other states of India also and needs to be monitored at national level.

In the pheromone traps installed for monitoring of *T. absoluta* up to 65 males were trapped/day/trap indicating the widespread prevalence of the pest in tomato fields (Figure 4) in the study area.

Host range

In the present study, incidence of *T. absoluta* was recorded on two hosts *viz*., tomato as well as potato and the incidence was higher on tomato than potato. Reports indicate the main host plant of *T. absoluta* as tomato (*Lycopersicon esculentum*) although the insect has also been reported on solanaceous weeds, including *Solanum nigrum* and *Datura stramonium*. Damage has also been reported on egg plant (*Solanum melongena*), pepper (*Capsicum annuum*) and potato (*Solanum tuberosum*) (Pereyra and Sanchez, 2006). Even though, tomato trade is one of the main aspect for a long distance dissemination of *T. absoluta*, short and medium natural spread immediate to invasion is a matter of great concern. Tomato leaf miner may multiply during summer months in outdoors but it is not expected to survive winter conditions because its development stops between 6 and 9^{O}C (Barrientos *et al*., 1998). It is most likely that different development stages can survive in greenhouse conditions (Potting, 2009). Alternative host plants, especially *S. nigrum* may play important role in rapid and continuous spread of this pest. There needs to be a

Table 1. Incidence of *T. absoluta* in different villages in Bengaluru Rural/Urban Districts of Karnataka

Village/Experimental plot**	Latitude and Longitude	(No of mines/plant)*
Shivakote	13° 08' 33" N; 77° 30' 11"E	9.40
Madhugirihalli	13° 07' 34.8" N; 77° 30' 12.3"E	0.12
Thirumalapura	13° 07' 24.1" N; 77° 28' 57.9"E	0.08
Thamarasanahalli	13° 07' 18.8" N; 77° 30' 03.3"E	0.12
Agrahara	13° 07' 51.8" N; 77° 27' 13.5"E	0.16
Gudadhalli	13° 08' 28.5" N; 77° 27' 43.2"E	0.08
Ivarakandapura (IIHR)**	13° 08' 05.6" N; 77° 29' 53.8"E	14.08
Kalenahalli	13° 10' 00.7" N; 77° 31' 37.7"E	0.20
Linganahalli	13° 09' 25.8" N; 77° 30' 56.6"E	0.28
Hessaraghatta	13° 08' 36" N; 77° 28' 45"E	0.20

*Average of 25 plants ** Experimental plot in IIHR

Fig. 4. *T. absoluta* pheromone trap catch in tomato field

continuous watch for the spread of *T. absoluta* on the already recorded and any of the new host plants in India.

Natural enemies

Nesidiocoris tenuis (Reuter) (Hemiptera: Miridae) was found predating on eggs and early instars of *T. absoluta* under field conditions. Presence of this natural enemy feeding on whitefly in tomato ecosystem in India was earlier reported (Sridhar *et al.* 2012a, Sridhar *et al.*, 2012b).

CONCLUSIONS

Although it is not certain at this point of time to pinpoint the exact route of entry of the South American tomato leaf miner, *T. absoluta* into India, it is speculated that the import/export of agricultural commodities in containers from/to north-western neibouring countries, might have carried the pest into northwestern States of India and spread further. Besides these inadvertant means, intentional introduction of the pest for either research or commercial interest also cannot be ruled out. Passive movement of pest through wind and crossing the borders is also a possibility. Since tomato being the preferred host, its incidence has been noted first on tomato followed by potato at Bengaluru (Karnataka State of India). Further studies are required regarding its spread within India although its containment through pest alerts to other regions and its proper management need attention. As the insect has also been recorded on potato, chances are high for its occurrence on other solanaceous crops, weeds and wild plants. While the specific studies on the bioecology and management of *T. absoluta* are underway at IIHR, Bengaluru, the high reproductive capacity, fecundity of 260 eggs per female with shorter generation cycle involving four larval instars (Uchoa-Fernandes *et al.*, 1995) and the reported ability to overwinter as egg, pupa or adult (EPPO, 2005) indicate the potential threat of invaded insect. There is an urgent need for containing the pest from further spread and also to take-up management strategies in the locations of its occurrence. Setting up of pheromone traps for monitoring *T. absoluta* at new localities for observations on its spread, deployment of pheromone traps for mass trapping at the locations where its incidence is seen, removal and destruction of infested plants with insect stages upon its occurrence in nursery and main fields is recommended for its management. Organising awareness camps to the tomato growers with suitable eco-friendly management measures like use of Bt should be the priority of the researchers in coordination with the field extension agencies and tomato growers. There is also an urgent need for domestic quarantine measures to curtail the pest from spreading further to other tomato growing regions of India.

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