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RESEARCH NOTE

Report on the occurrence of South American Tomato moth, *Tuta absoluta* (Meyrick) in Punjab, India as evident from trap catches and molecular diagnosis

SANDEEP KAUR SIDHU*[1], V. SRIDHAR[2], ABHISHEK SHARMA[1] and R. ASOKAN[3]

[1]Department of Vegetable Science, Punjab Agricultural University, Ludhiana - 141 004, Punjab, India
[2]Division of Entomology and Nematology, [3]Division of Biotechnology, ICAR-Indian Institute of Horticultural Research, Bengaluru - 560 089, Karnataka, India
*Email: sandeepentomologist@pau.edu

ABSTRACT: South American Tomato moth, *Tuta absoluta* (Meyrick), was reported as a new invasive pest on tomato from Ludhiana and Patiala districts of Punjab, India during 2016-17. The molecular identification of the pest was based on the core principle of generating DNA barcode using mitochondrial cytochrome c oxidase 1 (mt COI) gene. Though the incidence observed was very low on tomato from the surveyed areas during the observational period, considering the severity of the pest in other states, and its ability to spread rapidly into newer tomato growing areas, there is a need for constant vigil on the pest, for preventing its further spread within and the adjoining the states of Punjab.

Keywords: Punjab, *Tuta absoluta*, tomato

Tomato (*Solanum lycopersicum* L.) is one of the most popular and economically important vegetables inIndia. It is grown in almost all states of India in an area of 0.767 M hectares with a production of 16.384 M tonnes (Anonymous, 2015). As a part of "Real Time Pest Dynamics in Tomato" under ICAR funded project, "National Innovations in Climate Resilient Agriculture", regular surveillance was carried out to study the insect pests scenario in response to climate change on tomato crop. The observations on incidence of a recently reported invasive pest, South American tomato moth, *Tuta absoluta* (Meyrick) from India (Sridhar *et al.*, 2014)were recorded for two consecutive years (2015-17) in *kharif* (at experimental blocks of Punjab Agricultural University (PAU), Ludhiana (30.9019° N, 75.8078° E) and in 10 farmers' fields of Patiala district (30.3398° N, 76.3869° E) and *rabi* seasons (PAU, Ludhiana only). For regular surveillance of pests, a total of five villages in Patiala district (*i.e*. Sanour, Poonio Jattan, Bossar Khurd, Kartarpur and Asarpur) were selected with two fixed fields from each village. For the monitoring the incidence of *T. absoluta*, sex pheromone lures using water traps from Pest Control India, Mumbai were used at the rate of two traps per acre. Weekly observations on larval incidence on foliage, fruits and male adult catches per trap were recorded.

Till 2015-16, no larval incidence, fruit and foliar damage and male adult catches of *T. absoluta* were noticed in any of the tomato fields surveyed. However, during 2016 *Kharif* season, few *T. absoluta* adults (1-2/trap/week) were observed in the months of September-October at two farmers' fields in the villages Kartarpur and Bossar Khurd in the Patiala district. Similarly, at the end of January, 2017 trap catches were observed in Ludhiana also (Fig. 1). Though trap catches of *T. absoluta* were observed during this period, no foliage or fruit damage was noticed in the nearby fields till February, 2017 which may be attributed to very low incidence of the pest. During *Rabi* season 2017 at PAU, Ludhiana 2 to 5 moths of *T. absoluta*/trap/week were recorded in the month of February onwards and the moth catches increased to 6-8 numbers/trap/week by early April and came down to 1-2 adults/trap/week by end April, 2017. During this period also very low incidence of *T. absoluta i.e.*, < 1 per cent infestation of tomato plants was observed (Fig. 2). The trapped adults were sent to Division of Entomology and Nematology, ICAR-Indian Institute of Horticultural Research, Bengaluru for further confirmation. The identification of the insect specimens was confirmed morphologically as well as using molecular tools as *T. absoluta*.

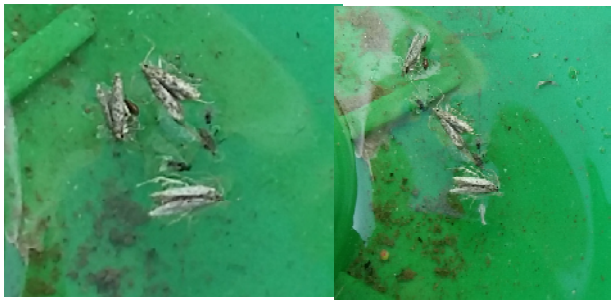

Fig. 1. Moths of *T. absoluta* in pheromone traps

Fig 2. *T. absoluta* damage on tomato fruits

Field damage symptoms:

On leaves, *T. absoluta* caused blotch mines which are visible from both adaxial and abaxial surfaces. Dark coloured excrement was observed inside the blotches and sometimes larva is found feeding on mesophyll tissue. Larvae also bore apical buds and stems. On fruits, usually larva enter into fruit near to the calyx and tunnel into the flesh leaving galleries clogged with frass. More than one hole is seen near to the calyx on fruit.

Identification of *T. absoluta*:

The adult moths were confirmed as *T. absoluta* on the basis of external morphology and male genitalia. It is a small moth measuring 5-6 mm body length and a wing span of 8-10 mm. The adult moth is greyish or silvery gray with darker patches on fore wings. Labial palp prominent, projected forward, up curved and with apical segment long and acute. Head vertex covered with appressed scales, antenna fili form, long and banded with gray and dark brown. Forewing narrow, apex fringed and speckled with brown silvery gray and black patches. Hind wing narrow, margins fringed with long hairs, silvery gray in color and outer margin concave posterior of apex.Valve of male genitalia are digitate and setose apically and inner margin is convex medially. It has broad and well-developed vinculum and long and stout phallus.

Insect DNA isolation and mt COI gene amplification

DNA Isolation

The molecular identification of insect was based on the core principle of generating DNA barcode using mitochondrial cytochrome c oxidase 1 (mtCOI) gene. Total DNA was isolated from individual insect using modified CTAB method (Saghai Maro of *et al.,* 1984). The specimen was ground with 1ml of 2% cetyltri methyl ammonium bromide (CTAB), 100mM Tris-HCl (pH-8.0), 1.4 M sodium chloride, 20mM EDTA and 2% of 2-mercapto ethanol. The suspension was incubated at 65°C for 1-2 hours and then an equal volume of chloroform: isoamyl alcohol (24:1) solution was added. The suspension was centrifuged at 6000 rpm for 15 minutes. The aqueous layer was transferred to a fresh 2ml micro centrifuge tube taking care not to disturb the middle protein interface. DNA was precipitated by the addition of 20µl of 0.3 M sodium acetate and equal volume of ice-cold 95% ethyl alcohol. The precipitated DNA was spun at 8000 rpm for 10 minutes and the resultant DNA pellet was washed with 70% ethyl alcohol. This was centrifuged at 8000 rpm for 10 minutes and finally the pellet was dissolved in 50µl DNase, RNase free molecular biology water. The genomic DNA was visualized using 1% agarose gel and diluted with sterile water to get a working solution of 20-25ng/µl.

Polymerase chain reaction (PCR)

It was carried out in a thermal cycler (Veriti; ABI-Applied Biosystems, Foster City, CA) with the following cycling parameters: 94°C for 4 min as initial denaturation followed by 35 cycles of 94°C C for 30 s, 47°C for 45 s, 72°C for 45 s, and 72°C for 20 min as final extension by using universal CO-I primers (LCO-1490- 5-GGT CAA CAA ATCATA AAG ATA TTG G-3 and HCO-2198- 5- TAAACT TCA GGG TGA CCA AAA AAT CA-3. PCR was performed in a 25-µl total reaction volume containing 20 picomoles of each primer, 10-mM Tris HCl (pH-8.3), 50-mM KCl, 2.5-mM MgCl2, 0.25 mM of each dNTP, and 0.5U of Taq DNA polymerase from Fermentas Life Sciences (Thermo Scientific, Swedesboro, NJ). The amplified products were resolved in 1.0% agarose gel, stained with ethidium bromide (10 g/ml), and visualized in a gel documentation system (Fig. 3).

Molecular Cloning and Sequencing

The PCR amplified fragments were eluted using Nucleospin Extract II according to the manufacturers protocol (MN, Germany) and ligated into the general

purpose-cloning vector, Ins T/A clone and transformation by using *Escherichia coli* (DH5α) cells was carried out (Fermentas GMBH, St. Leon-Rot, Germany; Thermo Scientific, Swedesboro, NJ) according to the manufacturers protocol. Blue/white selection was carried out and all the white colonies (colonies harboring the insert) were maintained on Luria-Bertani Agar (LBA) containing ampicillin (100 mg/ml), incubated at 37°C overnight and stored at 4°C until further use. Plasmids were isolated using Gene JET Plasmid Miniprep Kit (Fermentas, Germany) according to manufacturer's protocol, from overnight cultures of the randomly selected clones multiplied in LB broth. Sequencing was carried out in an automated sequencer (ABI Prism 310; Applied Bio systems, Foster City, CA) by using M 13 universal primers both in forward and reverse directions and also validated using Gen Bank and BOLT database and confirmed as *T. absoluta*.

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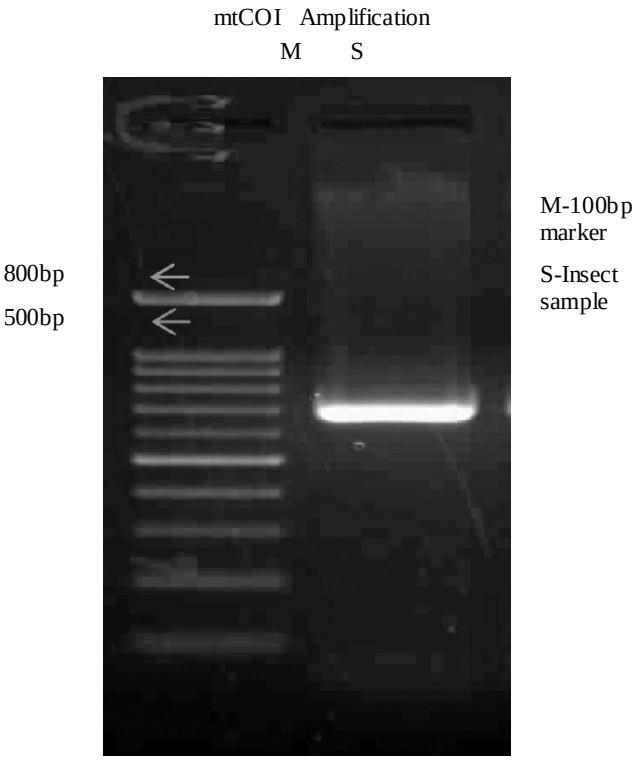

Fig. 3. Amplification of mtCOI in *T. absoluta*

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