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Rapid Communication

First report of the South American tomato pinworm, *Tuta absoluta* (Meyrick), as invasive pest in Udaipur Region of Southern Rajasthan in India

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Abstract

The invasive pest tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), was observed for the first time in the Udaipur district of Southern Rajasthan, India, during 2018–2020 under field and protected cultivation in tomato. Morphological and molecular identification (amplifying *cytochrome oxidase* gene (CO-I) of mitochondrial DNA) was made to confirm the occurrence of this new invasive species. The developmental period of *T. absoluta* has four stages *viz*; egg, larva, pupa and adult. The duration of the incubation period, first, second, third and fourth instar larvae were observed at 4.52 ± 0.68, 2.38 ± 0.50, 3.95 ± 0.80, 3.43 ± 0.51 and 2.57 ± 0.51 days, respectively. The hatching rates of the eggs were 94–97 per cent. The average length of first, second, third and fourth instar larvae was measured at 0.610 ± 0.037, 1.577 ± 0.067, 3.917 ± 0.121 and 7.379 ± 0.176 mm, respectively. A total larval period of 10–15 days was recorded, with a mean of 12.33 ± 1.35 days. The pupal phase lasted 10–12 days on average, with a mean of 10.95 ± 0.86 days. Adult longevity was 12.00 ± 1.41 days on average, with a range of 10–14 days. *T. absoluta* had a life span ranging from 30 to 40 days, with a mean of 35.43 ± 3.56 days.

Key words: *T. absoluta*, morphological and molecular identification, DNA Barcode, cytochrome oxidase subunit I gene (COI)

Introduction

South American tomato pinworm, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), also known as tomato leaf miner and tomato borer, is an oligophagous pest (Babasaheb et al. 2020) feeding on tomato as a primary host and on other solanaceous crops *viz.,* brinjal, chili, sweet pepper, potato, and tobacco (Kanle et al. 2019; Pereyra and Sanchez 2006). Infested tomato plants by *T. absoluta* reduce the quantity and quality of fruit and cause up to 50 to 100 percent losses in protectedas well as open field conditions (Konan et al. 2022). The tomato plant is damaged by the direct feeding of larvae on leaves, stems, buds, and fruits. In India, *T. absoluta* was first reported in Karnataka (Sridhar et al. 2014), and then in different

states, such as Maharashtra, Tamil Nadu, Delhi, Madhya Pradesh, Gujarat, Delhi, Telangana, Chhattisgarh and Meghalaya (Shashank et al. 2016; Taram et al. 2016; Swathi et al. 2017; Sankarganesh et al. 2017; Fand et al. 2020). Similarly, it was reported in Morocco, Tunisia, France, Italy, Netherlands, Albania, Portugal, Bulgaria, Cyprus, Germany, Israel, Hungary, Greece, Jordan, Kuwait, Qatar, Saudi Arabia, Syria, Turkey, Yemen, and Ukraine (Rwomushana et al. 2019). Currently, Mukwa et al. (2021) reported the spreading of *T. absoluta* in many countries of the African continent.

Tomato pinworm *T. absoluta* has been observed for the first time in the Southern region of Rajasthan in both protected and open fields from 2018 to 2020. Hence, preliminary studies were designed on the incidence pattern along with population dynamics of insect pests of tomato, including natural enemies, *in-vitro* biology, and holistic approaches for managing *T. absoluta* under protected cultivation.

Materials and methods

The data on *T. absoluta* infestation on tomato were collected at the Agricultural Research Station, Banswara (23°51′07.24N; 74°37′98.77E) in *Rabi* 2018, and from the Polyhouse at the Horticulture Farm, Rajasthan College of Agriculture, MPUAT, Udaipur (Satellite location: 24°57′61.15N; 73°70′66.66E) in *Rabi* 2020–21. Molecular characterization was done at the College of Fisheries, MPUAT, Udaipur.

In-vitro biology of tomato pinworm

The larvae were collected from the infested tomato plants under poly house/field and reared on tomato leaves under lab conditions at the Department of Entomology, Rajasthan College of Agriculture, MPUAT, Udaipur. The emerged pair of male and female adults were transferred into other Petri dishes filled with paper towels/tomato leaves as an oviposition substrate and 10% honey solution soaked in cotton for adult feeding. Eggs were collected and kept in an insect breeding dish for hatching, and after hatching, the larvae were reared individually on fresh tomato leaf bits. The incubation period, larval and pupal period, adult emergence, and hatching per cent were observed.

Morphological identification of T. absoluta

Adults and larvae were collected from the experimental locations and were morphologically recognized in the laboratory based on form, colour, size, and other exterior features as suggested by Nayana and Kalleshwaraswamy (2015).

Molecular identification of T. absoluta

Molecular identifications were made using amplification and sequencing of the mitochondrial *cytochrome oxidase subunit I* (COI) gene (Mukwa et al.

2021).Total genomic DNA was extracted using a DNA isolation kit (DNeasy® Blood and Tissue kit, Qiagen), in accordance to the manufacturer's instructions. The extracted DNA was subjected to polymerase chain reaction for the amplification of *cytochrome oxidase* subunit I (COI) gene which was of 658–700 bp using the universal primers (forward primer LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') as per previously published methodology (Folmer et al. 1994). The amplified PCR products were analysed by electrophoresis on a 1.2 percent agarose gel and visualized in a gel documentation system (Gel Doc™ EZ Imager, Bio-Rad, USA). The PCR amplified COI region was sequenced using an automated DNA Sequencer (ABI 310 Genetic Analyzer Applied Biosystems, USA) as per the standard procedure.

The raw DNA sequences were edited using BioEdit sequence alignment editor version 7.0.5.2. The COI sequences obtained in the study were compared with previously submitted sequences of the GenBank nucleotide database at National Centre for Biotechnology (NCBI) using the Nucleotide BLAST. Further, the COI sequences were translated using the online software ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and aligned through Protein BLAST. Through this method, the generated sequences were confirmed to the fragments of the mitochondrial COI gene. Further, the identification of the specimen was done using DNA barcode sequences by a comparable match in the BOLD species identification system (BOLD-IDS, www.barcodinglife.org). The sequences obtained were deposited at the gene bank of the National Centre for Biotechnology Information (NCBI), USA and an accession number was obtained.

Results and discussion

Biology: The adults were collected from the research field, and then males and females were separated in the laboratory of the Department of Entomology, Rajasthan College of Agriculture, MPUAT, Udaipur. A single pair of males and females were released into the Petri dishes with tomato leaves under laboratory conditions. The biology of *T. absoluta* consists of four developmental stages: egg, larva, pupa, and adult (Figure 1). The *T. absoluta* finished its life cycle ata temperature ranging from 15 to 35 °C. Previously Mohamed et al. (2022) reported that the egg, larva, and pupa development times were in the range of 4.0–11, 6.3–16.0, and 5.4–20.7 days, respectively, in *T. absoluta.* It was assumed that the low temperatures during winter would prevent *T. absoluta* from successful overwintering. However, in southern Rajasthan, the winter temperature range (8–28 °C) might allow the pest to overwinter. The high reproductive capacity, a shorter life cycle with 10–12 generations and the reported ability to overwinter as an egg, pupa, or adult (Sridhar et al. 2014) indicate the potential threat of tomato pinworm.

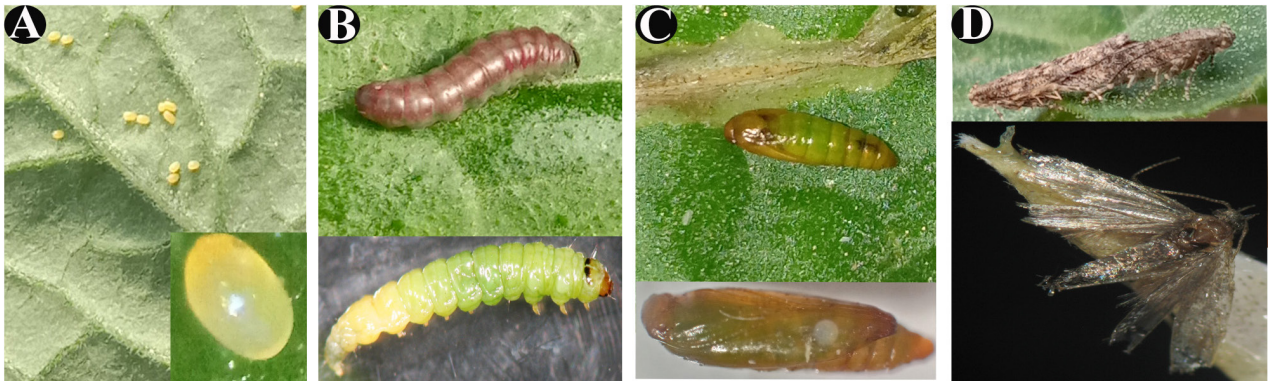

Figure 1. Life stages of tomato pinworm (*T. absoluta*) A. eggs, B. larva, C. Pupa, D. Adult. Photographs by Beerendra Singh.

Table 1. Mean duration of various development stages of *Tuta absoluta* in lab condition.

Stage of Insect	Duration (Days)*	Duration Range (Days)
Incubation period	4.52 ± 0.68	4–6
First instar of larvae	2.38 ± 0.50	2–3
Second instar of larvae	3.95 ± 0.80	3–5
Third instar of larvae	3.43 ± 0.51	3–4
Fourth instar of larvae	2.57 ± 0.51	2–3
Total larval period	12.33 ± 1.35	10–15
Pupal period	10.95 ± 0.86	10–12
Adult period	12.00 ± 1.41	10–14
Total life cycle	35.43 ± 3.56	30–40
Eggs hatch per cent**	95.48 ± 1.03	94–97 Range in %

* Value presented as Mean ± SEM (Standard error of mean).
** Percent of egg hatch.

Egg: The freshly deposited eggs were smooth, shining, and oval-shaped. The pinworm eggs were measured as small cylindrical ones with pale creamy white coloured turning yellow and black before hatching. The results of the present study were comparable to the finding of Halder et al. (2017). The incubation period was 4.52 ± 0.68 days under the laboratory conditions, *i.e.* 26 ± 2 °C temperature and 75 ± 5% relative humidity (Table 1). The eggs hatching percent ranged from (94–97%) with a mean of 95.48% (Table 1). Similarly, the results of the present study were in concurrence with the findings of Shashank et al. (2015) who reported that the egg period of *T. absoluta* was fastest (3.75 days) on tomato followed by potato (5.10 days) and eggplant (6.10 days).

Larva: The average larval period in the present study was 12.33 days ranging between 10–15 days (Table 1). The average period of the larval instar was found 2.38 days, 3.95 days, 3.43 days and 2.57 days with the first, second, third and fourth instars, respectively (Table 1). The 7.38 mm average length recorded in larvae ranged between 7.10–7.65mm (Table 2). The larvae colour changed from whitish with a black head immediately after eclosion to greenish to pink. The larvae were generally feeding on mesophyll content of leaves, the soft part of the leaf, stem; the blotches appearing on the leaf surface and mines were visible on both sides of the leaf. The results of the present study conform with the findings of previous studies (Nayana and Kalleshwaraswamy 2015; Manoj et al. 2017).

Table 2. Mean size of various development stages of *Tuta absoluta* in lab condition.

Stage of Insect	Measurements (mm)*	Range (mm)
Length of eggs	0.356 ± 0.068	0.41–0.48
Width of eggs	0.217 ± 0.011	0.20–0.24
Length of Ist instar larva	0.610 ± 0.037	0.55–0.65
Length of IInd instar larva	1.577 ± 0.067	1.45–1.65
Length of IIIrd instar larva	3.917 ± 0.121	3.75–4.10
Length of IVth instar larva	7.379 ± 0.176	7.10–7.65
Length of pupa	4.981 ± 0.452	4.65–5.25
Breadth of pupa	1.492 ± 0.037	1.45–1.55
Adult length	5.940 ± 0.277	5.25–6.30
Adult wing span	9.541 ± 0.580	8.25–10.00

* Value presented as Mean ± SEM (Standard error of mean).

Pre-pupa: The pre-pupal stage is a lighter body than the feeding larval stage (first to fourth instars) and develops a distinguishing pink coloration on the dorsal surface of the last instar larvae. Pre-pupa leaves the food and make silk cocoons on the leaflets, stems, or in the soil, according to the habitat. The results of the present study were comparable to the finding of Shiberu and Getu (2017). However, if the pupation occurs inside the mined leaves or fruit, the pre-pupae do not build cocoons.

Pupa: The 10.95 days average pupal period was found in the range between 10–12 days in laboratory conditions (Table 1). The average length and diameter measured for larvae were 4.98 mm and 1.49 mm, ranging between 4.65–5.25 mm and 1.45–1.55 mm, respectively (Table 2). The pupae were octet with green in colour at the first time, and then turning chestnut brown to dark brown colour near-adult emergence. Desneux et al. (2010) also studied *T. absoluta* biology on tomato and revealed that the typical pupal duration was 9–11 days. The results of the present study were comparable with the findings of Polat et al. (2016), who reported that the pupal period ranged from 7.52–20.62 days.

Adult: The average development period of the adult was 12 days, ranging from 10–14 days (Table 1). The adult moths of *T. absoluta* were 5.94 mm, long with a wingspan of 9.54 mm, ranging between 5.25–6.30 mm long and 8.25–10 mm wingspan, respectively (Table 2). The adults have silvery grey colour scales with filiform antennae, alternating light or hard segments, and well-developed recurved labial palps. Adults are nocturnal and usually hide between leaves and other suitable places during the day. The pest of *T. absoluta* may overwinter as eggs, pupae, or adults. The adult female lays 250–280 eggs in her lifetime in 10–12 batches. The total life span was an average of 35.43 days, ranging between 30–40 days (Table 1). Our results were contradictory to the findings of Bajracharya et al. (2016), EPPO (2005) and Attwa et al. (2015), who studied the life span of *T. absoluta* on tomato plants and found that its average adult longevity was 12 days.

Morphological and molecular characterization: The identification of *T. absoluta* was confirmed by the morphological and DNA barcoding of mitochondrial *cytochrome oxidase* (CO-I) gene. The larvae and adults

collected from the tomato from southern Rajasthan were observed under a microscope and based on the morphological characters found that they were belonging to *T. absoluta*. This is a new invasive pest in the southern region of Rajasthan and further, the species identification was done at the molecular level. The mt-COI gene sequences were trimmed and finally around 492 bp and 643 bp of high-quality sequences were obtained for Udaipur and Banswara, respectively. Nucleotide BLAST search results showed the sequence homology with the previously submitted *T. absoluta* sequences in the database. The two sequences were deposited in the NCBI with the accession numbers MZ357191 and MK000732. Similarly, Sankarganesh et al. (2017) reported *T. absoluta* for the first time in the northeast region of India by morphological and mitochondrial *cytochrome oxidase* gene (CO-I) DNA barcoding.

Conclusion

This is the first information on the incidence of this new invasive pest in Southern Rajasthan and this would be helpful for the farmers for the timely identification and management of this new pest. As this is a new invasive pest, continuous monitoring is needed to understand the population dynamics and their natural enemies so that the significant economic losses to tomato and other solanaceous plants can be avoided in the future.

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Authors' contribution

Material preparation, data collection, and data analysis were performed by Beerendra Singh have given and S. Ramesh Babu. Trials were conducted under the supervision of M. K. Mahla and A. K Vyas, V. Singh; with assistance by K. Kumar and V. Kumar; the Molecular part was helped by D. Jain, M. L. Ojha, and S. Jagawat. The manuscript was first drafted and written by Beerendra Singh and S. Ramesh Babu and all authors commented on previous versions of the manuscript. All authors read, reviewed, revised, and approved the final manuscript.

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