

## MORPHOLOGY AND MOLECULAR CHARACTERIZATION OF TOMATO LEAF MINER *TUTA ABSOLUTA* DIAGNOSED IN DUHOK GOVERNORATE / IRAQ

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### ABSTRACT:

Tomato leaf miner *Tuta absoluta* (Meyrick) is a highly destructive and invasive pest affecting tomato and other Solanaceae crops, causing severe economic damage. A comprehensive investigation into the presence of the tomato leaf miner *T. absoluta* was undertaken in this study via a field survey across tomato cultivation zones throughout all districts of Duhok Governorate in the Kurdistan Region of Iraq from July to September 2022. Instances of *T. absoluta* infestation were documented in Zakho, Amadiya, Summel, Dohuk, Shekhan, Aqrah, and Bardarash districts. The collected moths were described morphologically depending on the phenotypic characteristics and features of the male genitalia; the phallus was cylindrical; the valva was weakly curved and covered with long setae. The apex of the uncus extends almost to the tip of the valve. They were also confirmed at the molecular level through PCR analysis using mitochondrial cytochrome c oxidase subunit I (COI) as a molecular marker. The sequences of the mtCOI gene of *T. absoluta* specimens were analyzed and compared with the available COI sequence data members from GenBank. All studied specimens of *T. absoluta* were clustered together in a single group with a high support value, suggesting the presence of high homogeneity in the *T. absoluta* specimens. This work represents the first attempt at molecular identification of obtained specimens of *T. absoluta* in Iraq.

**KEYWORDS:** MtCOI, Molecular Markers, Tomato Leaf Miner, *Tuta absoluta*,

### 1- INTRODUCTION

The tomato leaf miner (TLM), *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is one of the most serious pests attacking tomatoes in many tomato-producing locations across the world, both in open fields and greenhouses (Desneux *et al.*, 2010). The pest *T. absoluta* was described for the first time by Meyrick in 1917 from a single adult male captured in Peru. Ever since, it has been steadily spreading throughout South America until 2006, when it was reported for the first time in Castellon, eastern Spain (Urbaneja *et al.*, 2007) when its spread began to expand to the countries of the Mediterranean basin and Europe (Potting, 2009). Currently, it is reported in 14 Latin America and the Caribbean countries, 38 European countries, 34 African countries, and 25 Asian countries (Lata *et al.*, 2024). The tomato leaf miner was introduced to Iraq less than a decade and a half

ago, where it was detected for the first time in the autumn of 2010 in the Rabia region of Nineveh province (Abdul Razaq *et al.*, 2010). Although the tomato crop is the main host of the tomato leaf miner, it attacks many crops and weeds belonging to the Solanaceae family (Lietti *et al.*, 2005; Bloem and Spaltenstein, 2011).

As well as some plants belonging to Convolvulaceae, Fabaceae, Amaranthaceae, and Cucurbitaceae families (Abdul-Rassoul, 2014; Mohamed *et al.*, 2015; Portakaldalı *et al.*, 2017). The tomato leaf miner can cause significant economic losses to tomato crops unless managed properly. According to Assaf *et al.* (2013, 2015), even though farmers implemented pest management strategies weekly and relied heavily on insecticides to control tomato leaf miners, the infection rate ranged between 72 and 100 percent in some districts of Dohuk Governorate. Ali (2023) also indicated that the percentage of fruits infected with

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this pest in some areas of Nineveh Governorate in 2022 was up to 25 percent. The economic impact of *T. absoluta* includes direct harm to tomato leaves and fruits, decreased crop production, and higher expenses for control measures (Han *et al.*, 2019). Oztemiz (2014) illustrated that Turkey expends roughly US\$180 million each year on chemical treatments for *T. absoluta*, whereas in Iran, annual crop losses due to *T. absoluta* amount to approximately US\$35 million (Han *et al.*, 2019). Generally, the larvae of this pest mostly feed on the mesophyll layer inside the host leaves. Still, under significant infestation, they can also pierce through the fragile stems, buds, flowers, and fruits, leaving obvious surface holes in the fruits. Additionally, the infestation of secondary pathogens through the wounds caused by the pest also contributes to damage (Mirza, 2014).

The ability of this pest to adapt to new biotic and abiotic factors enabled it to successfully spread over a wide geographical area (Cifuentes *et al.*, 2011). According to numerous studies, most species' population genetic structure and molecular diversity may change in new habitats; this has been observed clearly in several invasive species (Hoos *et al.*, 2010; Rubinoff *et al.*, 2011). Since mitochondrial DNA has strict maternal inheritance and no genetic recombination, it is typical for examining the genetic structure of populations and tracing the history of variations in organisms (Trewick, 2000; Gissi *et al.*, 2008; Shashank *et al.*, 2014; Sarma *et al.*, 2016). Molecular characterization and DNA barcoding are an exemplary taxonomic method that uses a short genetic marker in an insect's DNA to recognize a species. (Jalali *et al.*, 2015). Furthermore, mitochondrial DNA (mtDNA) is an excellent tool for

determining variations within and among populations. (Vogler *et al.* 1993; Margam *et al.* 2011). Studying the genetic variation of the invasive pest *T. absoluta* is essential for developing effective Integrated Pest Management (IPM) programs (Bettaïbi *et al.*, 2012). caused significant economic losses in both open and protected fields (Abdul-Rassoul, 2014).

Despite the rapid spread of *T. absoluta* throughout the tomato growing areas in greenhouses and open fields and its well-establishment in Iraq (Abdul-Rassoul, 2014), limited research work has been done on it; additionally, there is no molecular study has been done on it in Iraq. This study aimed to conduct a field survey including all districts of Dohuk Governorate to collect *T. absoluta* samples to describe it phenotypically and study the molecular identification and genetic relationship among samples using mitochondrial Cytochrome C oxidase (COI) as a molecular marker.

## 2- MATERIAL AND METHODS

### Study Area

This survey was conducted in the seven districts that belong to Dohuk Governorate in the Kurdistan Region of Iraq in 2022 to identify the areas that cultivate tomatoes. Accordingly, eight tomato-planting fields from seven districts were chosen (Table 1). The actual field survey began from July to September. One to three dunums were randomly chosen from each district based on the agricultural area as experimental units for collecting samples (insects). Water pan traps with pheromone lures were used to detect the tomato leaf miner pest with one trap/dunum (Zink *et al.*, 2020). The pheromone lures were replaced monthly.

Table 1: Survey locations of tomato leaf miner, *T. absoluta* in the Dohuk Governorate.

Subdistrict	Districts	Latitude-Longitude	Experiment area
Chamishku	Zakho	37°11'38.0"N 42°39'55.0"E	Three Dunums
Sersink	Amadiya	36°59'46.2"N 43°16'37.2"E	One Dunum
Khanke	Summel-1	36°47'49.6"N 42°48'00.3"E	Three Dunums
Sharya	Summel-2	36°49'09.3"N 42°55'33.8"E	Three Dunums
Zawita	Dohuk	36°56'56.9"N 43°05'40.3"E	One Dunum
Ba'adra	Shekhan	36°40'28.5"N 43°13'49.4"E	Three Dunums
Denarta	Aqrah	36°47'29.1"N 43°59'12.6"E	One dunum
Rovia	Bardarash	36°38'06.3"N 43°42'10.2"E	One dunum

### Sampling Collection and storage

The samples of infected leaves were collected and individually packed in labeled plastic bags, then transported to the laboratory for rearing to obtain insect stages and diagnose

them morphologically. The alive moths captured with pheromone traps were placed into plastic tubes labeled with the date and locations from which they were collected. The tubes were kept in a refrigerator at 4 °C for further molecular identification. (Zink *et al.*, 2020; Mukwa *et al.*, 2021).

### Morphological Identification

The tomato leaf miner was identified morphologically based on the phenotypic characteristics of the adults and the shape of the male genitalia. The adult abdomen was carefully removed and then macerated in 10% KOH for about 15 minutes. The male genitalia were extracted and examined under a microscope (Toševski *et al.*, 2011). Only the shape of the male genitalia gives a precise definition of this species (Ramos, 2015; Karlsson *et al.*, 2018). The valve, uncus, and phallus shapes were studied as the most appropriate characters for *T. absoluta* recognition (Chang *et al.*, 2021).

### Molecular Identification (Genomic DNA extraction, PCR analysis, and DNA sequencing)

Extraction of genomic DNA for selected sample populations of moth (*T. absoluta*) adults/site was performed using Jena Bioscience Kit (Cat #PP-208S) according to the manufacturer's instructions after grounding samples (8-10 adults) with liquid nitrogen. The concentration and purity of each DNA sample were determined using NanoDrop. Mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified using one pair of COI primer. Master-mix was prepared for eight samples populations of moth (*T. absoluta*) isolates plus control in each test. The amplification reaction consists of 25µl as the final volume for each sample containing 12.5 µl of master mix, 1µl of each primer (10 pmol/µl) including forward primer (LCO1490) 5'-GGTCAACAAATCA TAAAGATATTGG-3' and reverse primer (HCO2198) 5'-TAAACTTCAGGGTGACCAAAAAATCA-3', 4µl of genomic DNA (25 ng/ µl) and 6.5 µl of sterile deionized distill water (Folmer *et al.*, 1994). This work has been done in the Scientific Research Center/ College of Science/ Dohuk University. For DNA sequences, the amplification PCR product of the COI gene (25 ul) was prepared for eight samples. Raw sequences were visualized using Chromas 3.5V software to generate the coting of each target gene using forward and reverse sequences. The sequences of the COI gene of *T. absoluta* samples were confirmed through BLAST in the GenBank database in the National Centre of Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>). Moreover, additional sequences of COI of *T. absoluta* of different regions of the world were obtained from the GenBank database. The phylogenetic tree studied the population samples of *T. absoluta* collected from various locations in Duhok Governorate and compared with other

sequences of *T. absoluta* at neighbor countries available in the Genbank by using software MEGA 11 for neighbor-joining (NJ) multiple alignments (Tamura *et al.*, 2021). Symmetrischema lectulifera (KY951829) was used as an outgroup (Mehrkhoh *et al.*, 2021).

## 3. RESULTS AND DISCUSSION

### Morphological study

The collected tomato leaf miner samples were identified depending on morphology; the male and female length was around 5.6 and 6.4 mm, respectively, and 10.3 and 10.7 mm in the wingspan, respectively. The general color of the adult is silvery grey to brown with dark patches on the front wing. The antennae are filiform, covered with alternating rows of black and brown scales. The labial palpi consist of three prominent, up-curved segments, with the apical segment long and acute. The male's abdominal color is grey, and its end is round and covered with a dense row of cream-colored scales, while the female's abdominal color is cream, and its end is tapered with two rows of scales on either side of the end of the abdomen. The phallus of male genitalia is cylindrical and has an inflated coecum; the valva is weakly curved and covered with long setae. The apex of the uncus extends almost to the tip of the valve (Fig. 1 and 2; A). These results were found to be consistent with the results and characteristics used to diagnose adults of *T. absoluta* by Hayden *et al.* (2013) and Karlsson *et al.* (2017).

The insect egg is cylindrical-shaped, creamy white to yellow, and the egg chorion has an engraving in the form of an unequal-sided pentagon (Fig. 2; B). Arati *et al.* (2018) indicated that the egg chorion of *T. absoluta* has a net-like pattern that gives a taxonomic characteristic for distinguishing the egg of this pest. The fully-grown larva is bluish-green to light pink with a brown head. The prothoracic shield is pale, with dark shading along the posterior margin. The pupa is cylindrical and green, later becoming dark brown. The female pupa has a short longitudinal suture in the middle of the eighth abdominal segment on its ventral side, whereas, in the male pupa, the suture is found in the middle of the ninth abdominal segment. This is consistent with the results of Sannino and Espinosa (2010). Moreover, the results of this morphological study in identifying *T. absoluta* were identical to the results of the Iraq Natural History Research Center and Museum.

**Molecular Identification**

For molecular analysis, the concentration of genomic DNA for selected eight sample populations of *T. absoluta* was

determined at an average of 514.6 ng/ul with purity (1.9). All samples were successfully amplified PCR products with a molecular weight of approximately.



Figure. 1: Genitalia of the male tomato leaf miner (*T.absoluta*). VI =valva; Un=uncus; A.sp=apical spine of the phallus; Cp=corpus phallus; Cc=caecum.

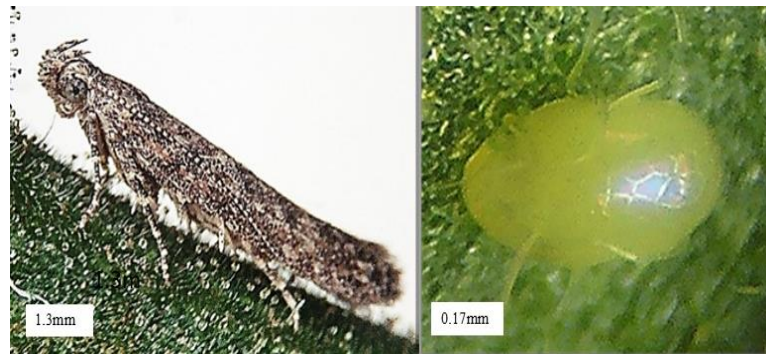


Figure. 2: Shows the stages of tomato leaf miner. A= Adult, B= The engraving is in the form of an unequal-sided pentagon on the egg.

710 bp, as shown in Figure (3). The sequence fragments were trimmed with 638 bp for each sample to a uniform length that corresponded with the region used to identify the target COI gene to ensure the identification of the genus. They were confirmed through BLAST in the NCBI database GenBank. Obtained sequences were submitted in the GenBank with accession numbers as follows: (PP526751) Zakho, (PP526752) Amadiya, (PP526753, PP526754) Summel, (PP526755) Duhok, (PP526756) Shekhan, (PP526757) Aqrah, and (PP526758) Bardarash district, respectively as the first records in Iraq.

Many DNA markers like mtDNA, RAPD, AFLP, microsatellites, and ESTs are widely used in insect genetics research. The choice to select the marker system usually depends on the specific goals of the study (Behura, 2006). Traditionally, mitochondrial DNA (mtDNA) has been a choice marker for studying genetic variations in insect species (Zhaoke *et al.*, 2021). It has been demonstrated as a highly informative and useful marker for estimating inter-intra-specific levels (Mehrkhou *et al.*, 2021)

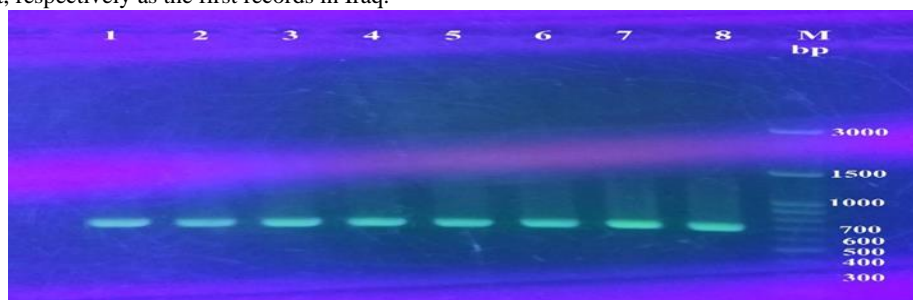


Figure 3: The result of the amplification product of PCR analysis with 710 bp of COI gene marker using Agarose gel electrophoresis with the concentration of 1% Agarose and run on 8-5V/CM, line C is a negative control.

Based on the sequence samples obtained in this study, the relationship between them and the results available in the GeneBank was found, as shown in Figure 4. The evolutionary history was inferred using the UPGMA method (Sneath and Sokal 1973). The optimal tree is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree (Figure 4). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura and Kumar 2004) and are in the units of the number of base substitutions per site. This analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 708 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.*, 2021). Phylogenetic analysis of the present study (Figure 4) showed there are six clusters as follows:

1. The first cluster includes the accession numbers MK189156.1 from Nigeria, OM230176.1 from India, MK116968.1 from Senegal, and PP526756.1:3-681 from Shekhan-Iraq.
2. The second cluster includes the accession numbers PP526752.1 from Amadiya, Iraq, and PP526757.1 from Aqrah, Iraq.
3. The third cluster includes the accession numbers PP526755.1 from Duhok, Iraq; PP526758.1 from Bardarash, Iraq; and MW714930.1 from Saudi Arabia.

The fourth clusters include the accession numbers PP526754.1 from Summel (Sharya)-Iraq, MZ475335.1 from France, and MZ475336.1 from Algeria.

The fifth cluster includes the accession number PP526751.1 from Zakho- Iraq.

The sixth cluster includes the accession number PP526753.1 from Summel (Khanke)-Iraq.

Also, there are inter-lapping among these clusters.

This demonstrates the genetic diversity among *T. absoluta* populations, which was collected from eight different locations in Duhok Governorate, indicating no high genetic homogeneity among them. Similarly, a study on Tunisian *T. absoluta* resulted in high genetic diversity and significant differentiation between populations (Bettaïbi *et al.*, 2012). Guillemaud *et al.* (2015) reported that the native population of *T. absolute* in South America is far from genetically homogeneous based on microsatellite markers and illustrated the source of the invasive population with the hypothesis of single versus multiple introductions. In addition, eight Brazilian populations of *T. absoluta* showed differences in the population's responses to insecticides as well as host plants (Suinaga *et al.*, 2004).

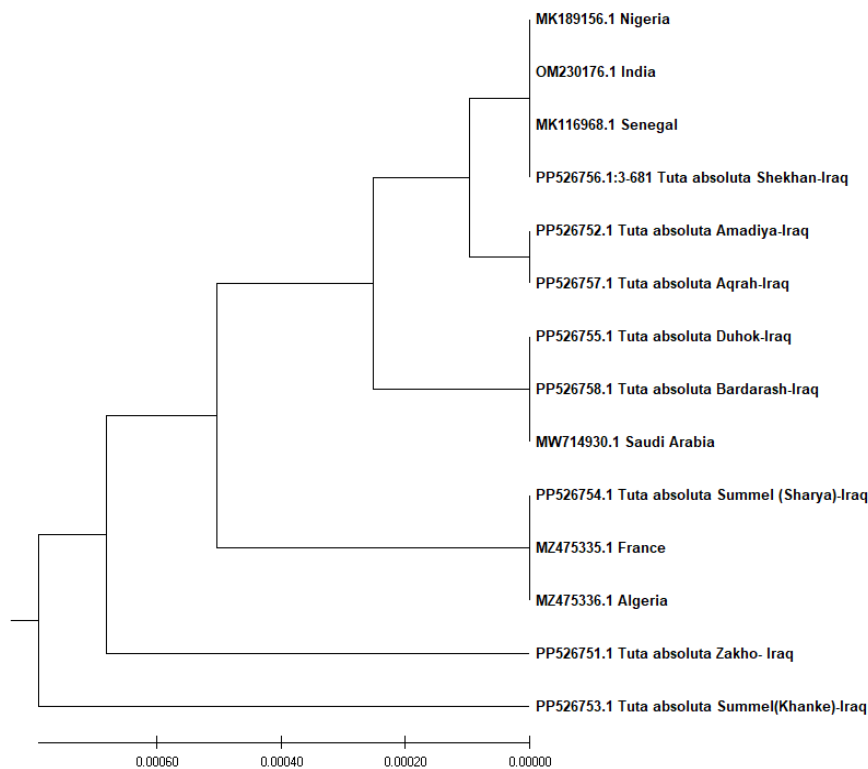


Figure (4): The phylogenetic relationships among fourteen specimens of *T. absoluta*, based on their COI genes deposited in GenBank, including their evolutionary taxa.

On the other hand, Cherif *et al.* (2017) found high genetic homogeneity using mtCOI sequences of seven Tunisian populations of *T. absoluta* and concluded that this was introduced from a single source in Tunisia. In addition, high genetic homogeneity was observed in *T. absoluta* samples collected from five locations in India and one from Nepal based on the mtCOI analysis (Shashank *et al.*, 2018).

Based on the above, this genetic diversity indicates that the pest was introduced from different sources into Iraq through the import of tomato crops from different countries. It is worth noting, the first recorded instance of this pest in Iraq was in 2010. It was found near Rabia (Ninawa Governorate, northern part of the country neighbouring Syria) (Abdul Razzak, 2010). However, no molecular identification of this pest has been conducted until now.

This is the first molecular study, and the first new record of eight population samples collected from different locations in Duhok Province submitted to GenBank. Furthermore, the outcome of this study would be vital for future research on this insect pest.

## CONCLUSION

The current research identified the pest *T. absoluta* morphologically and confirmed it at the molecular level in Duhok Governorate/Iraq. These references were recorded in Gene Bank. The genetic relationship among our samples shows the absence of genetic variation between populations across different districts of our region and with other countries. In Iraq, this is the first attempt to record of *T. absoluta* in gene bank, we hope that our work will be vital for future scientific research on this insect pest and open a new door in the use of molecular techniques for new invasive pest detection in our country.

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