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Parasitoids of *Tuta absoluta* (Meyrick) in open field tomato crop in Iraq[1]

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ABSTRACT: The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is an invasive pest in Iraq, that represents a global threat to commercial tomato production, in both open field and greenhouse. Field survey was conducted to identify parasitoids of *T. absoluta* in tomato open field in three sites: Suweera, Azeezia and Kut in Wasit province, central Iraq during the 2019 cropping season. Five hymenopterans parasitoid species were identified on *T. absoluta* as: two egg parasitoids *Trichogramma pintoi* Voegele (Trichogrammatidae) and *Telenomus* sp. (Platygastridae); two larval parasitoids *Habrobracon concolorans* Marshall (Braconidae) and *Closterocerus* sp. (Eulophidae), and one parasitoid of pupa, *Proconura* sp. (Chalcididae). The present study reports the first record of these parasitoids of *T. absoluta* on tomato open field in Iraq.

Key words: *Closterocerus*; *Habrobracon*; *Proconura*; *Telenomus*; *Trichogramma*

Parasitóides de *Tuta absoluta* (Meyrick) na cultura do tomate em campo aberto no Iraque

RESUMO: A traça-do-tomateiro, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), é uma praga invasora no Iraque, que representa uma ameaça global à produção comercial de tomate, tanto em campo aberto quanto em casa de vegetação. A pesquisa foi conduzida para identificar parasitóides de *T. absoluta* em campo aberto de tomate em três locais: Suweera, Azeezia e Kut na província de Wasit, Iraque Central durante a safra de 2019. Cinco espécies de parasitóides de himenópteros foram identificadas em *T. absoluta* como: dois parasitóides de ovos, *Trichogramma pintoi* Voegele (Trichogrammatidae) e *Telenomus* sp. (Platygastridae); dois parasitóides larvais *Habrobracon concolorans* Marshall (Braconidae) e *Closterocerus* sp. (Eulophidae), e um parasitóide de pupa, *Proconura* sp. (Chalcididae). O presente estudo relata o primeiro registro desses parasitóides de *T. absoluta* em campo aberto de tomate no Iraque.

Palavras-chave: *Closterocerus*; *Habrobracon*; *Proconura*; *Telenomus*; *Trichogramma*

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Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelelchiidae), is one of the most devastating pest species of tomato crops in South America. Until late 2006, when it was first detected in Spain, *T. absoluta* was only present in the area of origin. However, this invasive pest has since spread quickly throughout the Mediterranean Basin and to other countries in Europe (Desneux et al., 2010). The tomato leaf miner was first detected in Mosul province in North of Iraq in September 2010, it caused large damage on tomato open field, then spread into other provinces to infest tomato, both open field cultivation and greenhouses (Abdul Razzak et al., 2010).

Tuta absoluta attacks the tomato plant at any stage of its development, and the infestation may lead to a great loss in the yield. The larvae mine into the leaves, shoots and feed on fruits causing rot and decaying it; while the infestation on the stems can significantly delay plant growth (Desneux et al., 2010). The loss of tomato yield in South America because of a tomato infestation by tomato leaf miner ranges between 80 and 100 % (Lobez, 1991), while in Iraq, the loss of the tomato yield in plastic houses was 49.14% (Al-Gerrawy et al., 2017).

The tomato leaf miner larval feeding behavior, most time staying inside the leaves, shoots, stems and tomato fruits reduce their contact with insecticides applied on the crop. Furthermore, the pest has high frequency of resistance selection to insecticides (Siqueira et al., 2001). However, the control against this pest since its entry into the Mediterranean basin countries has generally focused on the use of chemical insecticides (Sannino & Espinosa, 2012). This has led to the confusion of integrated pest management programs on tomatoes, especially those based on biological control of pests, because of the negative effects of these insecticides on natural enemies (Zappalà et al., 2012).

There are many parasitoids recorded parasitizing various developmental stages of the tomato leaf miner. Ferracini et al. (2019) indicated that more than 50 species of hymenopterans were recorded as parasitoids of the *T. absoluta* in Europe. Among them, the most important was the egg parasitoid *Trichogramma achaeae*, which was found to have the ability to reduce the crop damage rate by 91.74%, which has been commercially produced in Spain to use against the tomato leaf miner (Cabello et al., 2009). Gallego et al. (2019) also noted that there is a compatibility in the use of parasitoid *T. achaeae* with some of selective insecticides within IPM programs toward to the control *T. absoluta*. In addition, many species of larval parasitoids were identified parasitizing *T. absoluta* such as *Bracon* (*Habrobracon*) *nigricans* (Braconidae) and *Necremnus artynes* (Eulophidae) (Zappalà et al., 2012(. These results support the need to preserve local natural enemies through environmental management techniques when planning an integrated pest management strategy (Zappalà et al., 2012(. In south America, Krechemer & Forester (2019) found four parasitoids of *T. absoluta* in organic growth of tomato in Brazil: *Conura* sp., *Earinus* sp., *Myosoma* sp., and *Casinaria* sp. In Egypt, Abdelmaksoud et al. (2016) identified three parasitoids of *T. absoluta*: *Diglyphus* sp., *Telenomus* sp., and *Elasmus* sp. Al-Jboory et al. (2012) recorded the parasitoid wasp *Bracon* (*Habrobracon*) *concolorans* (Braconidae) parasitizing tomato leaf miner larvae in Jordan.

The present study aimed to survey parasitoids of *T. absoluta* infesting tomato open fields in different sites in center of Iraq, in order to benefit from them in the future for setting their conservation into IPM programs.

Materials and Methods

A survey was conducted using tomato open fields at three sites in Wasit governorate (central Iraq): Suweera, Aziziya, and Kut. Infested tomato leaves were collected 12 times throughout the 2019 tomato cropping season. The surveys were run every two weeks for each site from March to mid-June 2019. The samples were placed in transparent bags and taken to the Insect Laboratory in the Plant Protection Department, Faculty of Agriculture, Wasit University. All samples were examined under at stereomicroscope 40X magnification (KRUSER). The leaves were opened for checking the larvae of *T. absoluta*, also check the upper and lower surfaces of the leaves to observe of eggs and pupae. The host larvae were isolated with eggs, larvae and pupae of ecto-parasitoid, and each one larva placed in small Durham tubes (2 x 1cm in Ht x Diam) to allow the parasitoid development until the adults' emergence. The other larvae were reared in the laboratory, placed in the growth chamber type (GEOTECH), fed tomato leaves at 25 ± 1 °C, relative humidity of 70 ± 5 %, and 14:10 (L:D) photoperiod (Marcano, 1995). The larvae were monitored until molting to pupae and to adult's emergence. These monitoring allowed the identification of the endoparasitoids.

The eggs found were collected from the tomato leaves and placed in the petri-dishes by a soft brush, laid on a filter paper with the humidification to following of non hatching eggs until the parasitoid adults' emergence. Parasitoid adults were kept in glass tubes (10 × 1.4 cm H x D) containing 70% alcohol. The specimens were sent to the Natural History Museum in UK for identification after numbering each tube and writing information such as the time and place of collection, the type of plant host and some other important ecological notes. The specimen of parasitoid *Trichogramma pintoi* Voegele was also sent to the laboratory of the California Institute of the USA for confirming identification by test of DNA molecular analysis by PCR technology, while the larval parasitoid specimen *Habrobracon concolorans* was sent to the Hungarian Natural History Museum for a species-level identification.

Results and Discussion

Egg parasitoids

Through the inspection of collected tomato leaves, were found dark eggs of the tomato leaf miner, *T. absoluta*, from one of the tomato fields (i.e., Suweera site) in Wasit province,

central Iraq. The eggs were isolated in the laboratory, four days later, the parasitoid adult emerged from parasitized eggs. The specimen was identified as *Trichogramma pintoi* Voegele (Hymenoptera: Trichogrammatidae). The egg parasitoid *T. pintoi* was found in the present study only in Suweera site. This occurrence may relate to many factors. However, the environmental conditions in Suweera are seem more appropriate for this parasitoid than other sites.

Twelve species of *Trichogramma* spp. have been recorded from *T. absoluta* eggs including the found species in our site, *T. pintoi*. This species is also recorded from tomato leaf miner eggs in Peru (Desneux et al., 2010). This species is spread in China, India, Iran, Japan, Pakistan, Tajikistan, Turkey and Uzbekistan in Asia; but in Europe it is recorded in Belarus, Bulgaria, France, Greece, Moldova, Poland, Portugal, Romania, Russia, Spain and Ukraine; while it is registered in Africa only in Tunisia. It is also reported from Argentina, Cuba, Peru, Canada and the United States of America (Pinto, 2006; Polaszek, 2010). The parasitoid, *T. pintoi*, has been observed on eggs of many economic insect pest species from different orders and families, but species within Lepidoptera was the most noted. For instance, it was found on *Sitotroga cerealella* (Gelechiidae), *Agrotis egetum*, *Helicoverpa armigera*, *H. zea* (Noctuidae), *Pieris brasicae* (Pieridae), *Ephestia kuehniella*, *Ostrinia nubilale* (Pyralidae), *Cydia pomonella* (Totricidae) and *Plutella xylostella* (Yponomeutidae) (Polaszek, 2010). In Iraq, Al-Gerrawy et al. (2012) recorded for the first time *T. pintoi* parasitizing tomato leaf miner eggs in greenhouses. This parasitoid was reared in the laboratory on eggs of *Ephestia kuehniella*, then released as biological control tool against the tomato leaf miner in greenhouses (Al-Gerrawy et al., 2014).

The egg parasitoid *Telenomus* sp. (Hymenoptera: Platygastridae) was also identified on eggs of *T. absoluta* from survey run in both sites, Azizia and Kut during April, but not in Suweera site. Parasitoids from this genus is of particular importance in biological control of Lepidoptera pest species. The most successful example is the release of *Telenomus remus* imported from Papua New Guinea to control of the armyworm on corn and vegetable crops in India. Furthermore, it was released to control *Spodoptrera frugiperda* in Venezuela (Polaszek, 2010). In Iraq, Al-Ali (1977) indicated that the egg parasitoid *Telenomus* sp. was observed parasitizing Sunn' pest, *Eurygaser integriceps*. Razak & Al-Rubeai (2011) also recorded the parasitoid *T. chlurpus* parasitizing *Eurygaster testudimaria* during April in the Najaf Province of Iraq. *Telenomus busseolae* Gahan is an effective egg parasitoid against stalk borer *Sesamia cretica* in maize and sorghum crops in Iraq (Al-Karboli & Al-Nakhli, 2006; Mohammed et al., 2012). Al-Gerrawy et al. (2012) recorded *Telenomus* sp. parasitizing the tomato leaf miner eggs in greenhouses in Iraq.

Larval parasitoids

The larval ectoparasitoid *Habrobracon concolorans* Marshall (Hymenoptera: Braconidae) parasitizing *T. absoluta* was found at high population density across all the three surveyed sites from April to mid June, despite the high temperature during this period. This may be due to its high adaptation to environmental conditions. Furthermore, from observations regarding parasitoid behavior in both field and laboratory, female's parasitoid laid 1 to 4 eggs on or near the larva of *T. absoluta*, making it a gregarious parasitoid on the third and the fourth instars of the host larva. At this stage, when the parasitoid cripples of host movement from outside the mine through the ovipositor. After hatching, parasitoids larvae attach to the host larvae and feed from the outside, with pupation occurring next to the dead larva. The presence of *H. concolorans* was in high population density at all sites (Table 1). Initially, when this species was sent to the Natural History Museum in UK, it was identified to only subgenus- level as *Bracon* (*Habrobracon*) sp. Although the taxonomist in the museum was referred to a great similarity to the species *Habrobracon concolorans* = *nigricans* and the species was the closest to the sample sent to them. However, the observation of a few significant differences between the submitted sample and the last species makes them difficult to accept as one species. Characteristics such as the ovipositor is proportionately longer than the last specimens; the mesosoma is all black while with yellow on the mesoscutum in *H. concolorans* species; the 2nd metasomal tergite is dull, rather than brown while it was black in *H. concolorans* specimens. The same sample was identified by Dr.Jeno Papp in Hungarian Natural History Museum as *Habrobracon concolorans*. Most of taxonomists consider *Habrobracon* as sub-genus related with the genus of *Bracon*. Some of taxonomist, however, deal with it as a separate genus (Darwish et al., 2003).

In South America, four species of Bracon were found parasitizing larvae of *T. absoluta*: *Bracon tutus*, *B. lulensis*, *B. lucileae*, and *Bracon* sp. (Desneux et al., 2010). Moreover, two species of *Bracon* was reported from *T. absoluta* collected south of Italy, *B.* (*Habrobracon*) *nigricans* and *B. osculator* (Zappalà et al., 2012). Al-Jboory et al. (2012) recorded the larval parasitoid *B.* (*Habrobracon*) *concolorans* parasitizing tomato leaf miner in greenhouses in Jordan. In Iraq, Al-Gerrawy et al. (2014) also recorded *Habrobracon concolorans* on *T. absoluta* larvae in greenhouses for the first time.

The larval endoparasitoid *Closterocerus* sp. (Hymenoptera:Eulophidae) was identified from *T. absoluta* larvae collected in tomato open field only in Suweera site. The occurrence took place at the end of April and continued until the end of May 2019. This parasitoid was observed parasitizing first and second larval instars of tomato leaf miner. Adults of the parasitoid lay their eggs inside the host's larvae inside the

Table 1. Identified parasitoids of T. absoluta and % parasitism in three sites in Iraq during March-June.

Parasitoids	Host stage attacked	% parasitism per site		
		Suweera	Azizia	Kut
Trichogramma pintoi	Egg	4	0	0
Telenomus sp.	Egg	0	2	4
Habrobracon concolorans	Larva	23.5	18	12
Closterocerus sp.	Larva	11.5	0	3
Proconura sp.	Pupa	1.66	0	0

mine. The pupa is black and small, often one larva comes out parasitized larvae for pupation, and the other larvae often die because of the competition for food. It was noted that this parasitoid is less present and less active than *H. concolorans*. The endoparasitoid *Closterocerus* sp. was identified for the genus-level, because of the specimen available to the museum was only a single male, while the taxonomists would need more specimens including a female for a species-level identification.

The larval parasitoid *Closterocerus formosus* W estwood was recorded from tomato leaf miner in tomato organic in open field in Argentine during the season 2004-2005 with parasitism rates varying from 1.5 to 5 % (Luna et al., 2005). Furthermore, it was found on the same pest in both crops (open field and greenhouses) in south of Italy in the period 2009-2011 (Zappalà et al., 2012). Additionally, it was found on other pest species, citrus leafminer *Phyllocnistis citrella* (Massa et al., 2001). As well, Gumovasky (2001) referred to the parasitoid *C. formosus* found on many pest species such as; leaf miners, scale insects, and psyllid. In Iraq, Al-Gerrawy et al. (2012) recorded for the first time the endo-larval parasitoid on *T. absoluta* on tomato in greenhouses.

Pupal parasitoid

The parasitoid *Proconura* sp. (Hymenoptera:Chalcididae) was observed parasitizing pupae of T. absoluta in the tomato open field only in the Suweera site, during the period from mid-May to mid-June. This species usually behaves as secondary parasitoid on the pupae of the primary parasitoids, *Habrobracon concolorans* Marshall. The adult of *Proconura* sp. enters the tunnel created by the tomato leaf miner larvae to find the pupa of the first parasitoid *H. concolorans* inside a silk cocoon, and then lay their eggs inside. The secondary parasitism has increased with increasing of primary parasitism during June. The appearance of the pupal parasitoid *Proconura* sp. occurred only in Suweera site (Table 1), despite the high population density of *T. absoluta* in this site, or it is not adapted to the environmental conditions in the other two sites, or because of both reasons. Furthermore, studies of pupal parasitoids on tomato leaf miner are necessary.

A few of pupal parasitoids have been recorded from *T. absoluta*. Field survey to search for parasitoids on generally different pests, including *T. absoluta* rarely care about them in the sampling program. However, the parasitism rate of *T. absoluta* pupae in South America has been more than 30% (Desneux et al., 2010). Furthermore, two species of pupal parasitoids from Chalcididae were found from pupae of tomato leaf miner: *Invreia* sp. in Colombia and *Conura* sp. in Argentina and Brazil (Desneux et al., 2010). Liu (2001) was referred to endoparasitoid *Proconura* sp. on pupae of moths and butterflies in China. Additionally, the parasitoid *Proconura* sp. was recorded on pupae of the moth of diamond back *Plutella xylstella*. In South Africa, this parasitoid behaves as a hyperparasitoid of the pupal of parasitoids *Cotesia plutella* and *Apanteles eriophyes* (Kfir, 1997). Eheteshami et al. (2019) recorded the species *Proconura persicae* in Shiraz, south of Iran. Al-Gerrawy et al. (2012) found the parasitoid *Proconura* sp. parasitizing pupae of *T. absoluta* collected from tomato crop cultivated in greenhouses in Iraq.

Conclusions

This study reports for the first time the egg parasitoids (*Trichogramma pintoi* and *Telenomus* sp.) in Iraq.

The larval ectoparasitoid (*Habrobracon concolorans*), the larval endoparasitoid (*Closterocerus* sp.), and the pupal parasitoid (*Proconura* sp.) were found parasitizing tomato leaf miner in open field tomato crop in Iraq.

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Compliance with Ethical Standards

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