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Two promising larval parasitoids, *Bracon* (*Habrobracon*) *didemie* and *Dolichogenidea appellator* (Hymenoptera: Braconidae) for biological control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

Kamil Karut[1*], Mahmut Mete Karaca[1], İsmail Döker[1] and Cengiz Kazak[1]

Abstract

Background The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most important tomato pests that causes irreversible economic losses. Due to lack of effective control methods, chemical control is still the most preferred approach for management of the pest all over the world. Therefore, researches are mainly focused on implementing a sustainable pest control program against this pest through finding out effective natural enemies. For this purpose, a survey study was conducted to determine the larval parasitoids of *T. absoluta* in field-grown tomatoes in Adana, Turkey, in 2021–2022.

Results Two larval parasitoids, *Bracon* (*Habrobracon*) *didemie* Beyarslan and *Dolichogenidea appellator* (Telenga) (Hymenoptera: Braconidae), were identified morphologically and molecularly. The first parasitoid populations were observed in tomato fields at the end of June and were maintained until the tomato plants were cleared (Middle of July). Throughout the survey, *B. didemie* was determined to be the more prevalent species than *D. appellator*. The highest percentage of *D. appellator* was found in the last week of June in 2021, at 42.8%. When the parasitoid species densities were compared, *B. didemie* accounted for 93.2% of the total parasitoids. Despite the intensive use of insecticides, the highest parasitoid density was achieved by *B. didemie* as 28 individuals per 100 leaves.

Conclusions Based on these results, it was concluded that *B. didemie* can be candidate as a successful biological control agent against *T. absoluta* in tomato field as a part of IPM programs.

Keywords Tomato leaf miner, *Tuta absoluta*, Braconid larval parasitoids, Tomato, Parasitism, Turkey

Background

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), native to South America, has been detected in Spain in 2006 and then spread to various countries in Europe, Asia and Africa (Ferracini et al. 2019). Its integrated pest management programs have been challenging in European countries where it was newly introduced. The main damage in the plant is caused by the larvae during all growing developmental stages, beginning with seedling stage. Infestation of tomato plants by the pest frequently results in a considerable decrease in yield and quality of the fruits (Biondi et al. 2018). However, due to the lack of effective natural enemies, various pest management methods have

*Correspondence:
Kamil Karut
karuti@cu.edu.tr
[1] Agricultural Faculty, Department of Plant Protection, Cukurova University, 01330 Adana, Turkey

been employed to control the pest. Among these methods, pheromones, color and water traps were the most commonly used approaches, but they were insufficient to control the pest populations at a significant level (Erler and Bayram 2021).

Nevertheless, two mirid predators, *Macrolophus pygmaeus* Rambur and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae), which are primarily employed in biological control of whitefly in greenhouses, were reported to be successful in biological control of *T. absoluta*, particularly when combined with the egg parasitoids of *Trichogramma* species (Naselli et al. 2017). However, challenges like prey stage preferences of *M. pygmaeus*, phytophagous feeding behavior of *N. tenuis*, host preference of *Trichogramma* spp. and lack of an efficient larval parasitoid of the pest prevented the development of an effective biological control programs against this pest (Arno et al. 2021). Consequently, chemical control is still the fast and preferred method for management of the pest (Mama Sambo et al. 2022).

Currently, studies are focused on finding out effective larval parasitoids in order to develop a successful biological control program against *T. absoluta* (Arno et al. 2021). A large number of *T. absoluta* larval parasitoids were identified in South America, Europe and Africa, of which only few species were reported as promising for biological control (Ferracini et al. 2019). *Pseudapantales dignus* (Muesebeck), *Dolichogenidea gelechiidivoris* (Marsh). *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae), *Neochrysocharis formosa* (Westwood), *Necremnus tutae* Ribes & Bernardo, *N. cosmopterix* Ribes & Bernardo and *Stenomesius* sp. nr. *japonicus* (Ashmead) (Hymenoptera: Eulophidae) are among the most studied candidates that have significant potentials for use in biological control of *T. absoluta* (Denis et al. 2022).

Turkey is one of the most important tomato-producing countries in the world. Until 2009, whiteflies, leafminers, aphids, mites, thrips and some lepidopterous species were the pests that caused significant economic losses in tomato (Karut and Kazak 2014). However, after 2009, *T. absoluta* has become the most important pest that causes economic losses in tomato production in Turkey, as in many countries (Karut et al. 2011). Similar to the rest of the world, some *T. absoluta* larval parasitoids were reported from Turkey, but no comprehensive data on their efficiency and population development were provided (Topakcı et al. 2022).

Therefore, in this study, determinations of larval parasitoid species of *T. absoluta* as well as their prevalence and population development were carried out in two consecutive years 2021–2022 in Adana, one of the most important open-field tomato growing areas in Turkey.

Methods

Species identification

Morphological and molecular identification was carried out for the collected parasitoid species during the study. Morphological identification was done by using the identification key proposed by Beyarslan (2002) at the Department of Biology, Faculty of Arts and Sciences, University of Thrace, Edirne, Turkey. For molecular identification, DNAs of braconid species were isolated from the third leg of 3 females stored at 96% alcohol using a PureLink® genomic DNA kit (QIAGEN Inc., Dusseldorf, Germany). PCR products were generated from a mitochondrial gene COI region. The extracted DNAs were amplified by using the COI primers, LCO1490 (5′-GGTCAACAAATCATA AAGATATTGG-3′) and HCO2198 (5′-TAAACTTCA GGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). The DNA samples were sequenced by MEDSANTEK (İstanbul, Turkey). Contigs were constructed by alignment of the forward and reverse sequences by using the BioEdit program (Hall 1999). Before phylogenetic analyses, the sequences were subjected to BLAST analyses (NCBI 2022). The phylogenetic tree was constructed using the maximum Neighborhoods Joining (NJ), based on the Kimura model with 1000 bootstraps, in Mega 7 (Kimura 1980; Kumar et al. 2016). The sequences of braconid species, which have already loaded to The National Center for Biotechnology Information (NCBI) (Accession numbers: OP939435 and OP935763 for *B. didemie* and *D. appellator*, respectively), were included in the phylogenetic analyses to improve the precision of the tree. The sequence of another braconids, *Apanteles gelechiidivoris* (MZ298984) and *Bracon nigricans* (MH733585), was included as an out-group. All molecular studies were carried out at Insect Biotechnology Laboratory.

Sampling for prevalence of parasitoids

In 2021, a hundred tomato leaflets were collected weekly from various (five to eight) tomato fields throughout five-week survey period in Adana (37°0′0.0000″N, 35°19′16.8060″E) for the purpose of determining *T. absoluta* parasitoids and their prevalence. In each sampling, leaflets were randomly collected from the inner parts of the field following a zigzag pattern, then placed in paper bags and transported to the laboratory in an ice container. The samples were then placed in clear transparent plastic parasite collection boxes located at room conditions (25 ± 2 °C, 70 ± 5%-RH, 16 L:8 D h photo phase). Afterward, the emerged parasitoids were collected using an aspirator and documented on a daily basis. Collected parasitoids were stored in 70 and 96% alcohol for morphological and molecular identification, respectively. The sampling started on 17 June 2021 and continued until 14 July 2021, the end of tomato growing season.

Sampling for population development and parasitism of *Tuta absoluta*

To determine the population development and parasitism of T. absoluta, two and four different tomato fields were selected and sampled periodically between 17 June and 14 July 2021 and from 22 April to 19 July in 2022, respectively. On each sampling date, a hundred fully matured leaves were collected from each field and transported to the laboratory as described above for assessment of population development and parasitism of *T. absoluta*. Number of *T. absoluta* larvae was determined by examining those sampled leaves under a stereo-binocular microscope. After counting, the leaves were transferred to parasitoid collection boxes; emerged individuals were recorded and stored as above. The survey was carried out under growers' conditions in both years; plant protection and cultural practices were not interfered during the study. However, during the studies, the sampled fields were sprayed with chlorantraniliprole (CTRP) + abamectin active ingredient insecticide in weekly and 3 days interval in 2021 and 2022, respectively. Sampled fields ranged in size from 10 to 50 decares, and the most prevalent tomato variety was Hazera 5656.

Results

Identified Braconid species

All collected braconid species were identified morphologically as belonging to two species, *Apanteles* sp. and *Bracon* (*Habrobracon*) *didemie* (Beyarslan), from the Microgastrinae and braconid subfamilies, respectively (Fig. 1). The species identified as *Apanteles* sp. was revealed to be *Dolichogenidea appellator* (Telenga), after BLAST and phylogenetic analysis (Fig. 2). The sequences of *B. didemie* did not match with any species in the BLAST analysis and located in the same branch with *B. nigricans*, which was previously reported among the parasitoids of *T. absoluta* (Fig. 3).

Prevalence of braconid parasitoid

Throughout the five-week survey, *B. didemie* was determined to be the more prevalent species than *D. appellator*. There was no parasitoid species collected in the first sampling week (17 June); however, 62 *B. didemie* (100%) individuals were obtained in the last week of sampling (14 July). During the survey, a total of 163 individuals were collected and 93.25% was belonging to *B. didemie* (Fig. 4).

Population development and parasitism of *Tuta absoluta*

Tuta absoluta larval populations were higher in both tomato fields during the first two weeks of the sampling but decreased in the subsequent weeks in 2021. When two fields were compared, the mean numbers of *T. absoluta* per leaf were higher in Karataş than in Karagöçer. The highest mean number of *T. absoluta* per leaf was 2.07 and 4.21 for Karagöçer and Karataş, respectively. Only *B. didemie* was found in the two fields, and the trend of total number of parasitoids per hundred leaves was similar. The highest total numbers of parasitoids per hundred leaves were 22 and 28 individuals in Karagöçer and Karataş, respectively (Fig. 5).

In 2022, *T. absoluta* larvae population densities were low at the beginning of the season and started to increase in mid-June in Tuzla I and Karataş. In these two fields, the highest larval densities were determined as 3.07 per leaf. In the other two fields (Yüreğir and

Fig. 1 General habitus of adult females of *Bracon* (*Habrobracon*) *didemie* (**a**) and *Dolichogenidea appellator* (**b**)

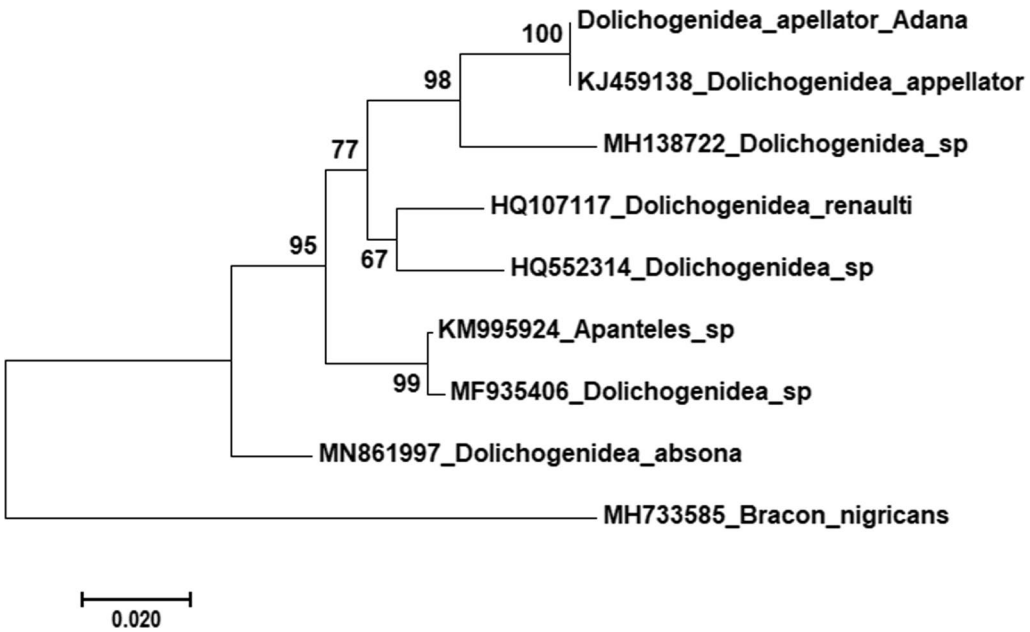

Fig. 2 Phylogenetic tree based on partial mtDNA COI of *Dolichogenidea appellator*

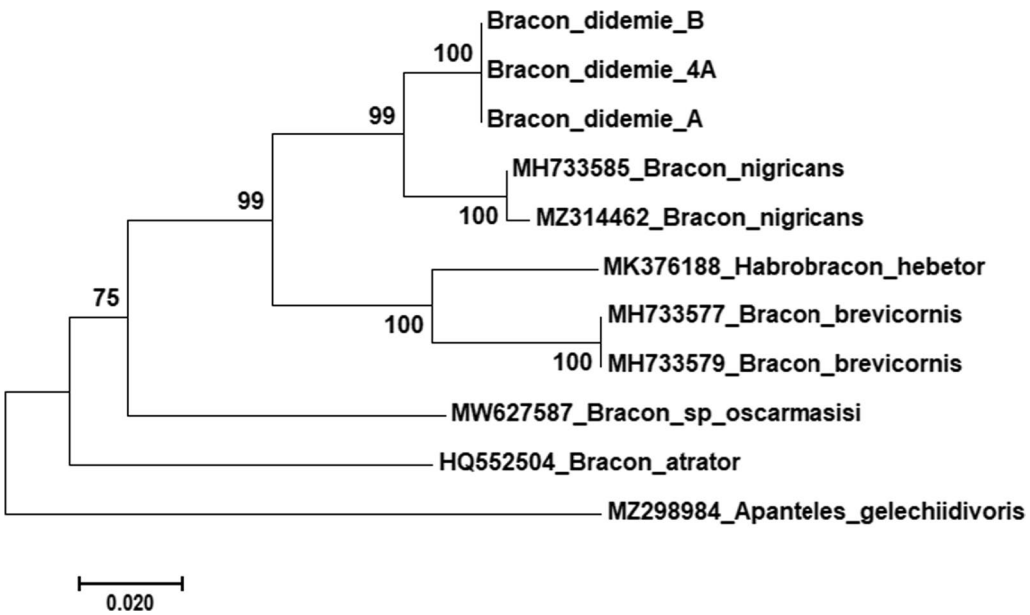

Fig. 3 Phylogenetic tree based on partial mtDNA COI of *Bracon* (*Habrobracon*) *didemie*

Tuzla II), the larval population densities were quite low during the growth season. In contrast to 2021, *B. didemie* was detected only in Karataş in 2022 among the fields in the last sampling week (Fig. 6).

Discussion

Bracon didemie was first identified by Beyarslan in 2002; it was later recorded from Turkey and Iran without any details about the host (Zargar et al. 2019). Later on, *B.*

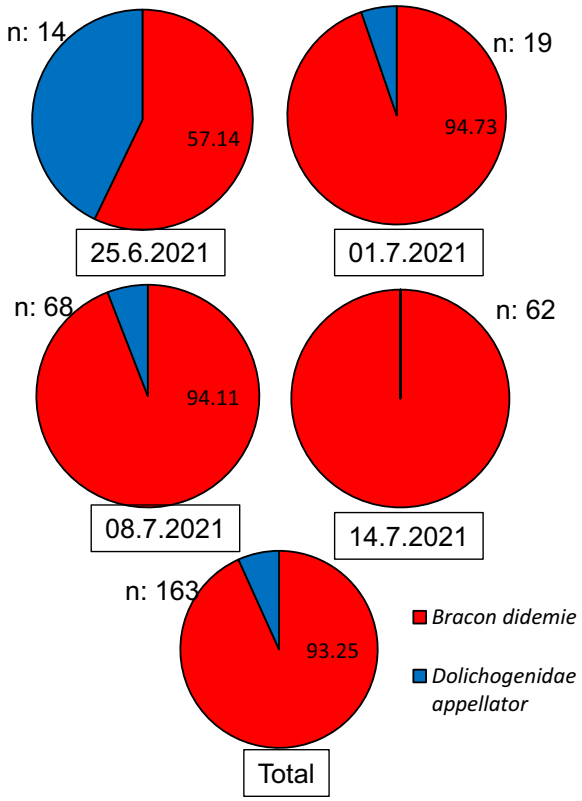

Fig. 4 Prevalence of *Bracon* (*Habrobracon*) *didemie* and *Dolichogenidea appellator* in tomato fields of Adana in 2021. n: Total number of parasitoids

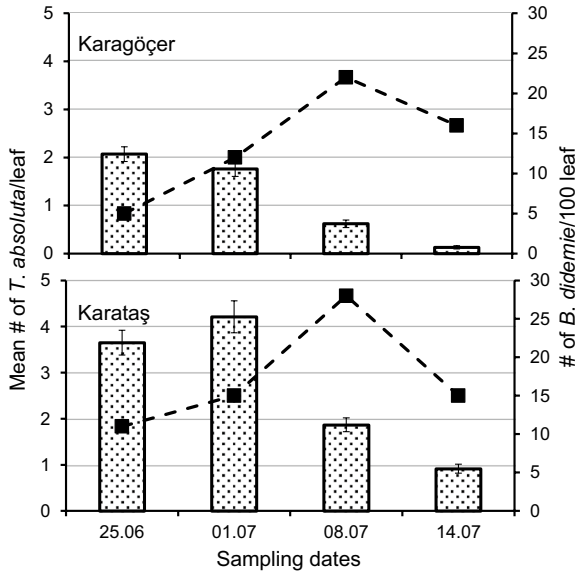

Fig. 5 Population developments of *Tuta absoluta* (Dotted bar, Mean ± SEM) and *Bracon* (*Habrobracon*) *didemie* in two different tomato fields in Adana in 2021

didemie was reported by Doğanlar and Yiğit (2011) on *T. absoluta* larvae in Antakya, then Aksu-Altun and Çıkman (2019) reported the same species as a larval parasitoid of the pest in Şanlıurfa tomato fields. Although several studies were conducted to determine *T. absoluta*'s natural enemies in tomatoes in Adana, no larval parasitoids of the pest were reported (Portakaldalı et al. 2013). Therefore, recent studies have shown that *B. didemie* has adapted and expanded as the larval parasitoid of *T. absoluta* in tomato fields in different parts of Turkey, despite the fact that this parasitoid was not observed in earlier studies in Adana.

Dolichogenidea appellator was the second larval parasitoid found in this study, albeit being less abundant (6.75%) than *B. didemie*. In this study, *D. appellator* was reported for the first time as a *T. absoluta* parasitoid in Turkey, despite being reported as native parasitoid of the pest in Sudan (Idriss et al. 2018). Similar to *B. didemie*, the parasitoid was reported without specification of the host from different regions of Turkey (İnanç and Beyraslan 2001). Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), *Scrobipalpa* spp. (Lepidoptera: Gelechiidae), *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), *Sparganothis pilleriana* (Denis & Schiffermiler) (Lepidoptera: Tortricidae) and *Etiella zinckenella* (Tr.) (Lepidoptera: Phycitidae) were reported among the host of this parasitoid species (Papp 1988).

Bracon didemie was detected in two different tomato fields from the end of June to middle of July in 2021, but only in one of the four fields at the last sampling week in 2022. In Adana, field-grown tomato season begins in February with the planting of seedlings and covering them with polyethylene. After the polyethylene covers are opened in response to the rising temperature (17–19 °C) in April, tomato plants then become attractive hosts for insects. Tomato growing-season lasts until mid-July; during this period, fields were sprayed ten times on average with pesticide mixtures to control *T. absoluta* and other insect pests. The spring season in Adana is generally temperate, with temperatures ranging from 5 to 30 °C. However, in 2022, it was colder with temperatures dropping below zero degrees twice during March. Consequently, we speculate that a colder spring season and frequent spraying may be a reason for delayed population establishment of parasitoid.

Although two *T. absoluta* larval parasitoids were found in this study, *B. didemie* was detected more frequently. This showed that *B. didemie* has adapted to *T. absoluta*, which is the most significant pest in field-grown tomato in Adana and whose management relies solely on chemical control. The capacity of endo- or ecto-parasitoid braconid species to parasitize *T. absoluta* in field conditions

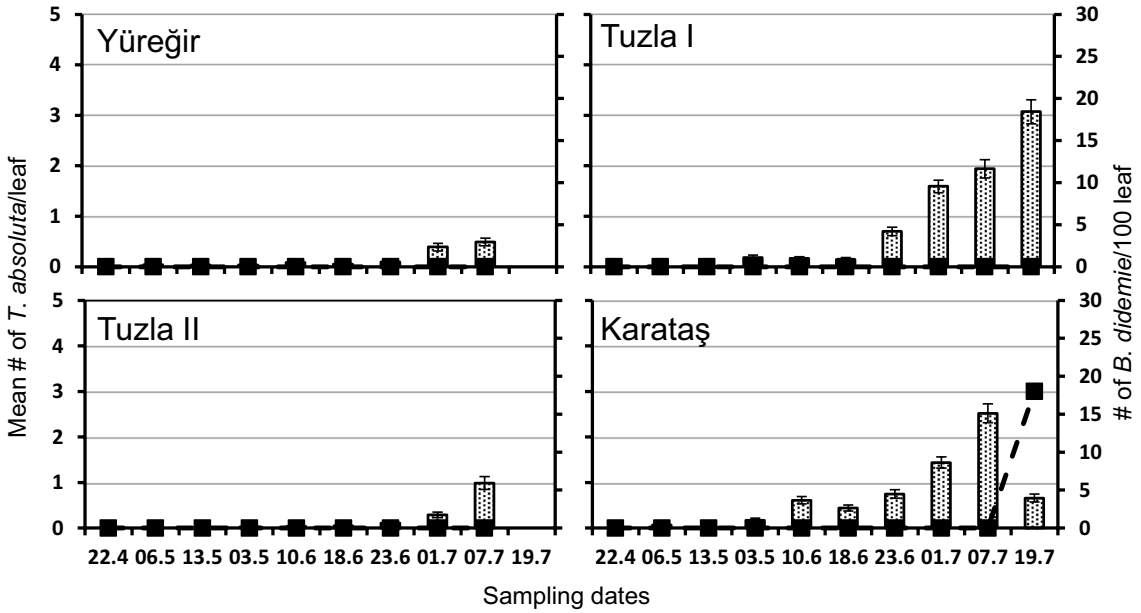

Fig. 6 Population developments of *Tuta absoluta* (Dotted bar, Mean ± SEM) and *Bracon* (*Habrobracon*) *didemie* in different tomato fields in Adana in 2022

has received very little attention. Doğanlar and Yiğit (2011) reported 7% parasitism of *B. didemie* in the samples collected from the semi-open greenhouses and fields where organic farming was practiced without the use of pesticides in Antakya, Turkey. Luna et al. (2010) revealed that endo-parasitoid *P. dignus* parasitized 23.34% of *T. absoluta* larvae in an organic field-grown tomato in Argentina. Similar parasitism rates (23.8 and 20.5%) were reported by Garrido et al. (2017) for *P. dignus* in tomato crops at Alto Valle de Río Negro, Argentina. Abbes et al. (2015) found 10.23% parasitism rate for *Bracon* sp. on sentinel plants in the Ragueda (Kiarouan) site, Tunisia.

All tomato fields in the study area were sprayed with an insecticide containing the active ingredients CTPR + abamectin at three days interval, often in excess of the recommended dosage, except the fungicides. Therefore, it is considered that intensive pesticide application prevented *B. didemie* from establishing population in tomato. Nozad-Bonab et al. (2021) reported that CTPR alone was highly toxic to the parasitoid *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae). Similarly, Pereira et al. (2019) found that CTPR alone caused negative impact on the emergence and progeny size of *Palmistichus elaeisis* Delvare and LaSalle (Hymenoptera: Eulophidae), a generalist gregarious pupal parasitoid of caterpillars. In addition, CTPR + abamectin resulted in 79% mortality and classified as harmful against predatory mirid *N. tenuis* in autumn season (Kaya and Keçeci 2021).

In organic tomato production, where the use of synthetic pesticides was limited, the pest species and their densities were lower and the population density of natural enemies was substantially higher than in conventional production (Bettiol et al. 2004). In addition, while there was no difference in insect damage intensities in organic and conventional tomato growing areas in California, arthropod biodiversity was one-third greater in organic farms than in conventional farms (Letourneau and Goldstein 2001).

Conclusions

Since *B. didemie* is a new species detected on *T. absoluta*, laboratory studies should be conducted on biological characteristics, parasitism and mass-rearing possibilities. Further studies on the role of natural enemies under field conditions are essentially needed.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by KK, CK, MMK and İD. The first draft of the manuscript was written by KK and CK, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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