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Natural parasitism of maize stemborers, *Sesamia* spp. (Lepidoptera: Noctuidae) eggs by *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) in Southeastern Turkey

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Abstract

Maize, *Zea mays* L., is currently among the leading cultivated crops in Turkey. Several lepidopteran stemborer species especially, *Sesamia nonagrioides* Lefèbvre and *S. cretica* Lederer (Lepidoptera: Noctuidae) may cause high maize yield losses. This study is the first to report parasitism of *Sesamia* spp. eggs by *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) in Southeastern Turkey. During our extensive 3-years region-wide survey program (2018-2020), we collected *Sesamia* spp. eggs laid between leaf sheets and cobs/stalks. *T. evanescens* adults hatched from the host eggs collected from two locations in Diyarbakır: one in the central county (L10) and the other in Bismil county (L11). The discovery efficiency, parasitism efficiency, and parasitoid impact indexes of *T. evanescens* were 77.5%, 38.92%, and 32.87% in L10 and 50.0%, 33.45%, and 12.42% for L11, respectively. *Sesamia* spp. eggs collected from other counties of Diyarbakır province and other provinces such as Batman, Mardin, and Şanlıurfa did not yield in *T. evanescens* emergence. To our best knowledge, this study is the first to report the presence of *T. evanescens* in Southeastern Turkey. Possible factors involved in the restriction of *T. evanescens* are discussed. Special attention should be devoted to increase the potential of conservative biological control strategies to support egg parasitoids.

Keywords: Discovery efficiency, Diyarbakır, Egg parasitoid, Parasitism efficiency, Parasitoid impact

Introduction

Maize, *Zea mays* L. currently ranks among the most widely sown crops in Turkey with an approximate annual production of 6.000.000 tons (TUIK, 2020). In the southeastern region of Turkey, maize is either sown as the main (first) or the second crop by the majority of large-scale farmers. Maize yield is under high suppression by insect pests especially, lepidopteran stemborers.

Sesamia spp. are leading multivoltine maize stemborers in Turkey. The presence of *Sesamia* spp. in Southeastern Turkey was reported ~50 years ago which was restricted to some counties in which maize cultivation was performed by smallholders (Adıgüzel and Ergül, 1969). These pests may infest all maize growth stages and organs except plant roots (Bayram, 2003). *Sesamia* spp. larval feeding on maize plants in earlier growing stages causes dead heart and the host plant is eventually dead. Their larvae can tunnel into maize stems and cobs resulting in high yield losses reaching up to 100%. The nitrogen and protein uptake of grains are also reduced after larval feeding (Bayram, 2003). In addition, the higher abundance and feeding performance of *Sesamia* spp. are positively correlated with maize disease, *Fusarium* species producing toxic compounds like fumonisin (Avantaggiato et al. 2002).

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The control of *Sesamia* spp. under field conditions is mostly based on insecticide practices performed by farmers in Turkey and 2-3 sprays are recommended when the abundance of the pests exceeds the economic threshold (Bayram, 2003). Insecticide practices may not provide a successful control of *Sesamia* spp. after the larvae enter the host plant stems and cobs. Furthermore, many natural enemies inhabiting maize could be negatively affected by insecticide treatments. Egg parasitoids seem to be the best candidate natural enemies to both prevent the entrance of larvae into maize stems/cobs and provide a unique solution to environmental concerns.

The egg parasitoids parasitizing *Sesamia* spp. eggs mainly belong to two Hymenopteran families, Scelionidae and Trichogrammatidae. For example, the presence of *Telenomus busseolae* has been reported for many countries in Europe, Africa, and the Middle East being recovered from *Sesamia* species and *Busseolae fusca* (Lepidoptera: Noctuidae) (Gahan 1922, Nixon 1935, Bedford, 1935, Moutia and Courtois, 1952, Harris, 1962, Hafez et al, 1977, Scheibelreiter, 1980 Fergusson, 1983). In addition, some *Trichogramma* species were reported to parasitize *Sesamia* spp. eggs under natural conditions in African and Mediterranean countries (Nagarkatti and Nagaraja, 1977; Sertkaya et al.,1999; Sertkaya and Kornoşor, 2002). However, we were unable to locate any study reporting *Trichogramma* species in Southeastern Turkey, especially in maize fields. *Trichogramma* species are polyphagous and can parasitize many insect pests. Surveys targeting the detection of native *Trichogramma* spp. populations in maize fields could suggest useful information on biological control programs in other crops.

The main purpose of the current study is to report the presence and the natural parasitism of *T. evanescens* on *Sesamia* spp. eggs in Southeastern Turkey maize fields between 2018 and 2020. Possible factors involved in the restriction of *T. evanescens* spatial distribution in maize fields are discussed.

Materials and Methods

Survey locations

Regular samplings were performed in maize fields in Diyarbakır, Mardin, and Şanlıurfa Provinces while irregular sampling efforts were devoted to other Southeastern provinces namely Adıyaman, Batman, Siirt, and Şırnak where *Sesamia* spp. egg specimens were collected between 2018-2020 (Figure 1). The summers in the region remain warm during a long-lasting period resulting in semi-arid climatic conditions which are climatically classified as Csa (Köppen, 1936).

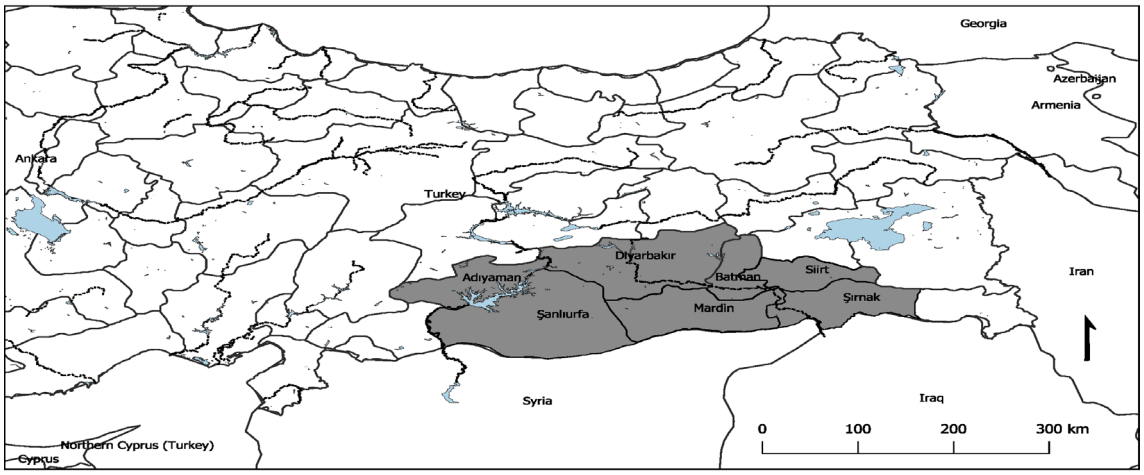

Figure 1. Map of Southeastern Turkey provinces surveyed for the presence and parasitism efficiency of *T. evanescens* during study.

Field sampling procedure

Samplings were performed starting from earlier maize growth stage "leaf development" to "end of fruit development" between maize growth stages: 13-79 according to BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie, Weber and Bleiholder, 1990; Lancashire et al., 1991). Fields were randomly chosen for samplings. In each field, a total of 100 plants were checked, randomly distributed among 20 sampling spots (5 adjacent plants per spot), representing 1 ha area as a survey unit (Bayram and Tonğa, 2016, Tonğa et al., 2021). The distance between spots was not less than 5 meters. Each plant was carefully inspected from root collar to top of the plants since *Sesamia* spp conceal their eggs between maize leaf sheets and stems/cobs. *Sesamia* spp. eggs were put into glass tubes (1 × 10 cm), labeled and protected via cotton stopper until transferred to the laboratory. A climate chamber was set to 26 ± 1 °C, 60 ± 5 RH, 16:8 L:D condition for incubation process of *Sesamia* egg batches. The incubated host eggs were daily checked until the emergence of the parasitoid adults completed. *Trichogramma evanesces* adults were fed with a 20% honey solution in the same tubes for at least 24 hours. The Mediterranean stem borer, *Sesamia nonagrioides* (Lepidoptera: Noctuidae) eggs were provided after feeding and mating. Thus, the laboratory populations

of *T. evanescens* were multiplied for further examinations. Specimens were morphologically identified by Prof. Dr. Gennaro Viggiani (Institute for Sustainable Plant Protection, Italy) and molecularly characterized by Dr. Nicolas Ris and Dr. Sylvia Warot (Institute Sophia Agrobiotech, France).

Statistical Analysis

Trichogramma evanescens adults emerged from the hosts eggs collected from two sampling locations from Diyarbakır province (central and Bismil counties) in 2019. Since no parasitism by *T. evanescens* was observed in 2018 and 2020, these years were discarded from analysis as well as other plant growth stages in 2019. *T. evanescens* is facultative gregarious and, several parasitoid larvae can develop in a single host egg. Natural parasitism of *T. evanescens* on *Sesamia* spp. eggs was assessed employing the parasitism indexes proposed by Bin and Vinson (1990).

The discovery efficiency expresses the number of discovered egg batches (in each location) divided by the total number of sampled egg batches and is presented as simple percent raw data. The parasitism efficiency represents the number of host eggs parasitized by *T. evanescens* divided by the total number of eggs in discovered egg batches (1).

The parasitoid impact was calculated as the number of parasitized eggs divided by the total number of host eggs (2).

$$Parasitism\ efficiency\ (\%) = \frac{the\ number\ of\ host\ eggs\ parasitized\ by\ the\ parasitoid}{the\ total\ number\ of\ host\ eggs\ in\ the\ egg\ batches\ discovered\ by\ the\ parasitoid} \times 100 \text{---------(1)}$$

$$Parasitoid\ impact\ (\%) = \frac{the\ number\ of\ host\ eggs\ parasitized\ by\ the\ parasitoid}{the\ total\ number\ of\ host\ eggs\ in\ the\ collected\ egg\ batches} \times 100 \text{----------(2)}$$

The arcsin square root transformed data of the parasitism efficiency and the parasitoid impact were subjected to analysis of variance (ANOVA) via "*car*" package (Fox and Weisberg, 2019). Differences between percent parasitism of different counties were compared with Tukey multiple comparison tests using "*lsmeans*" package (Lenth, 2016). All statistical analysis was performed in the statistical software environment R (v-4.0.4) (R Core Team, 2021).

Results

Sesamia spp. egg samplings yielded in no parasitism by *T. evanescens* in Southeastern Turkey in 2018 and 2020. *T. evanescens* adults hatched from eggs collected in central (L10) and Bismil (L11) counties of Diyarbakır in the second survey year (2019) only (Table 1, Figure 1). Therefore, *T. evanescens* was not recorded in any surveyed provinces in this study except Diyarbakır (Table 1, Figure 1).

In total, the number of *Sesamia* spp. eggs collected in L10 and L11 were 3109 (from 40 plants) and 789 (from 8 plants) while the number of *T. evanescens* adults hatched host eggs were 1022 (from 31 plants) and 98 (from 4 plants) respectively. The number of *Sesamia* spp. eggs from other locations are not presented due to no parasitism by *T. evanescens*.

There were statistical differences between the parasitism efficiency of *T. evanescens* and its impact on *Sesamia* spp. eggs among surveyed locations ($F_{efficiency}$ = 51.97, df= 11, P < 0.001; F_{impact} = 8.52, df= 11, P < 0.001). The discovery efficiency, parasitism efficiency, and parasitoid impact of *T. evanescens* were 77.5%, 38.92%, and 32.87% in L10 whereas 50.0%, 33.45%, and 12.42% in L11, respectively. The parasitism efficiency of *T. evanescens* did not differ between L10 and L11 while its impact was higher in L10 when compared with L11.

Discussion

This study is the first to report the presence and natural parasitism of *S. nonagrioides* and *S. cretica* eggs by *T. evanescens* in Southeastern Turkey. The incubation of their eggs confirmed the emergence of *T. evanescens* from eggs collected from two locations in Diyarbakır province namely central (L10) and Bismil counties of Diyarbakır province (L11). *Sesamia nonagrioides* and *S. cretica* stand among the most destructive maize yield-reducing organisms in Southeastern Turkey. The morphological identification of their eggs at the species level is difficult. Due to their simultaneous presence in Southeastern Turkey maize fields, the parasitism of *T. evanescens* on *Sesamia* spp. eggs under field conditions is evaluated at the genus level. The natural parasitism of *Sesamia* spp. eggs by *T. evanescens* is calculated based on three different variables i.e., the discovery efficiency, the parasitism efficiency, and the parasitoid impact for these two locations (Bin and Vinson, 1990).

The discovery efficiency referring the searching ability of *T. evanescens* on *Sesamia* spp. eggs under field conditions represents the raw data simply dividing the number of discovered egg batches (considering all eggs on a plant as a single batch) to all egg batches in each location. Thus, *T. evanescens* were able to discover 77.5% of *Sesamia* egg batches (oviposited plants) in L10 while 50% of egg batches were discovered by the parasitoid in L11.

As another calculated variable, the parasitism efficiency of *T. evanescens* was not statistically different between two locations, L10 and L11 and the parasitoid was able to exploit 38.92% and 33.45% of the discovered host eggs in both locations respectively. The parasitism efficiency was calculated based on the number of *Sesamia* spp. eggs exploited by *T. evanescens* rather than the number of parasitoid adults. Therefore, in both locations, the parasitoid was not capable of exploiting all host eggs in a discovered egg batches because of several reasons. For example, depending on temperature, *Sesamia* spp. eggs may normally hatch 4-8 days in the

Table 1. The discovery efficiency, the parasitism efficiency, and the parasitoid impact of *T. evanescens* on *Sesamia* spp. eggs collected in Southeastern Turkey Provinces.

Location	Sampling date	Province	County	Latitude	Longitude	Discovery efficiency (%)	Parasitism efficiency (%)	Parasitoid impact (%)
L1	10.07.2019	Diyarbakır	Bismil	37.860000	40.880556	0	0a	0a
L2	30.07.2019	Mardin	Nusaybin	37.095833	41.339722	0	0a	0a
L3	07.08.2019	Mardin	Mazıdağı[a]	37.487778	40.447222	0	0a	0a
L4	27.08.2019	Mardin	Mazıdağı	37.503889	40.448889	0	0a	0a
L5	27.08.2019	Mardin	Mazıdağı[a]	37.487778	40.447222	0	0a	0a
L6	11.09.2019	Şanlıurfa	Ceylanpınar	36.970278	39.984444	0	0a	0a
L7	18.09.2019	Diyarbakır	Silvan	37.127222	41.222778	0	0a	0a
L8	18.09.2019	Batman	Batman	38.039167	41.178611	0	0a	0a
L9	27.09.2019	Diyarbakır	Diyarbakır	37.890113	40.275188	0	0a	0a
L10	02.10.2019	Diyarbakır	Diyarbakır	37.850556	40.451944	77.5	38.92±3.27b	32.87±3.95c
L11	02.10.2019	Diyarbakır	Bismil	37.840556	40.518333	50.0	33.45±7.61b	12.42±5.36b
L12	08.10.2019	Mardin	Nusaybin	37.093889	41.184167	0	0a	0a
			F				51.97	8.52
			df				11	11
			P				<0.001	<0.001

[a] Same location sampled in different sampling dates. *Sesamia* spp. eggs were found in both sampling dates.
Means followed by different letters in each column depict differences between sampling locations.

postoviposition process (Orang et al., 2014; Sedighi et al., 2017). Therefore, the host egg acceptance by the parasitoid is possible for a time period shorter than hatching. The parasitism capacity of a female Trichogrammatid parasitoid is limited to at most 25 eggs per day (Muli et al., 2010.). Because *Sesamia* spp. egg batches are relatively large, it seems quite normal that parasitism efficiency of *T. evanescens* remained in the levels revealed by this study. In field conditions, several female *T. evanescens* may discover a host egg batch which is expected to result in higher parasitism. However, the facultative gregarious reproduction behavior by *T. evanescens* on host eggs of *Sesamia* spp. size of which is larger than parasitoid female leads self and conspecific superparasitism that limits the host egg exploitation during parasitism in nature (van Dijken and Waage, 1987; Doyon and Boivin, 2005).

Trichogramma evanescens impact on *Sesamia* spp. varied and was higher in L10 when compared with L11 with 32.87% of parasitized host eggs in L10 while only 12.42% of host eggs were parasitized in L11. A possible and maybe the most important exploitation limiting factor for *T. evanescens* is the simultaneous host egg batch exploitation by the specialized parasitoid species of *Sesamia* spp., *Telenomus busseolae* (Hymenoptera: Scelionidae) (unpublished data). Possible competition between two egg parasitoid species would likely restrict the parasitism performance of the non-specialist. Therefore, *T. evanescens* may not be able to exploit large egg batches while they could contribute to suppressing the pest infestation by exploiting the host eggs partially.

Trichogramma evanescens is a minute parasitic wasp and plays significant roles in the control of lepidopteran pest populations on different crops many of which are cultivated in Southeastern Turkey during a growing season. This suggests that *T. evanescens* could easily locate different host species on crop plants throughout the growing season. The parasitoid successfully established its population parasitizing *Sesamia* spp. eggs in two maize fields in Southeastern Turkey. Probably, the highest level of *Sesamia* spp. eggs at the end of the season resulted in the successful host location by the parasitoid. Another possible reason to explain successful establishment of the parasitoid could be the non-chemical agricultural practices conducted in *T. evanescens*-present maize fields and surrounding cultivated lands.

The presence and natural parasitism capability of *T. evanescens* was reported for many host species from different regions of Turkey. For example, Sertkaya (1999), reported natural parasitism of *Sesamia nonagrioides* eggs in the Mediterranean region by *T. evanescens*. Another important maize pest, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) egg parasitism in the Black Sea region, the Marmara region, and the Mediterranean region was associated with *T. evanescens* emergence (Özdemir, 1981; Özpınar ve ark., 1996; Öztemiz, 2007). However, to our knowledge, the presence of *T. evanescens* in Southeastern Turkey was not reported previously. Further research is required to reveal the natural parasitism capacity of Trichogrammatid species on important lepidopteran pests infesting cultivated host plants, for example, *O. nubilalis, Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), *Earias insulana* (Boisd.), *Helicoverpa armigera* (Hübner), *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae).

The spatial distribution of *T. evanescens* parasitism on the eggs of *Sesamia* spp. was restricted to two relatively close

locations (~8 km between). Many factors may be involved in the restriction of *T. evanescens* parasitism on *Sesamia* spp. in Southeastern Turkey. For example, in general, maize fields require 3-4 times insecticide treatments in Southeastern Turkey due to *Sesamia* spp. infestation while these two maize fields were not chemically treated in 2019. Another factor could be the presence of other cash crops cultivated in short-scale farming in surrounding lands that are not chemically treated as well. The chemically untreated crops constitute a good habitat for the above-mentioned lepidopteran pests accordingly for their generalist egg parasitoid, *T. evanescens*.

Conclusion

Trichogramma evanescens adults emerged from *Sesamia* spp. eggs collected from Southeastern Turkey maize fields. Further research programs such as monitoring the natural parasitism performance of this parasitoid species on different host species under field conditions and augmentative biological control should be performed to suppress lepidopteran crop pests in the region. In addition, removing the factors that negatively affect the natural parasitism by egg parasitoids i.e. reducing pesticide usage may help improve conservative biocontrol programs.

Compliance with Ethical Standards

Conflict of interest

The author declares no conflict of interest associated with this study

Author contribution

This study is a part of Ph.D. thesis of Adil Tonğa who designed and performed the study, analyzed the data, and wrote the manuscript in consultation with Prof. Dr. Ahmet Bayram. Both authors read and approved the manuscript. The authors confirm that the data of this study are original and have not been published or in consideration for publication elsewhere.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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