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Original article (Orijinal araştırma)

First record and parasitism of egg parasitoid *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) on eggs of *Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae) in Turkey[1]

Türkiye'de *Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae)'un yumurta parazitoiti olarak *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae)'in ilk kaydı ve parazitleme oranı

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Abstract

Chilo partellus Swinhoe, 1885 (Lepidoptera: Crambidae), which is currently found in many parts of the world, is a very damaging maize stem borer in Indian subcontinent and South and Eastern Africa. In 2014, it was recorded for the first time in Turkey. This pest is normally controlled by insecticides. Concerns of the negative effects of incessant use of insecticides have necessitated exploration of more benign and natural control methods. This study investigated the occurrence and parasitism rates of some native egg parasitoids of *C. partellus.* In 2018, first and second maize crops were planted in the research field of the Department of Plant Protection, Çukurova University (39º01'50.5"N, 35º21'06.7"E), in Adana, Turkey. Field was scouted once a week for parasitized egg masses. An egg parasitoid was recorded and morphologically identified as *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae). The egg parasitoid was recorded in August-September 2018 for the first time on *C. partellus* in maize (second crop) with rate of parasitism reaching 100% on 16 August. It is suggested that *T. evanescens* can be used for future development of biological control programs against *C. partellus*.

Keywords: Biological control, *Chilo partellus*, egg parasitoid, maize, *Trichogramma evanescens*

Öz

Chilo partellus Swinhoe, 1885 (Lepidoptera: Crambidae), şu an dünyanın birçok bölgesine yayılmış, orijini Hindistan Yarımadası ve Güney ve Doğu Afrika olan oldukça zararlı bir mısır sap kurdudur. Türkiye'de ilk kez 2014 yılında saptanmıştır. Bu zararlının kontrolünde genellikle insektisit kullanılmaktır. Sürekli insektisit kullanımının olumsuz etkileriyle ilgili endişeler daha çevre dostu ve doğal kontrol yöntemlerinin araştırılmasını gerektirmiştir. Bu çalışma ile *C. partellus*'un mevcut yerli parazitoitlerinin varlığı ve parazitleme oranları araştırılmıştır. 2018 yılında Çukurova Üniversitesi, Bitki Koruma Bölümü, Araştırma ve Uygulama Alanı (39º01'50.5"N, 35º21'06.7"E)'nda birinci ve ikinci ürün olarak yetiştirilen mısır tarlalarında haftada bir defa örneklemeler yapılmış ve parazitlenmiş yumurtalar kaydedilmiştir. Morfolojik karakterler incelenerek *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) olarak tanımlanan yumurta parazitoitinin, 2018 Ağustos- Eylül aylarında ilk kez mısırda *C. partellus*'un parazitoiti olarak kaydedildiği ve 16 Ağustos'ta parazitlenme oranının %100 olduğu belirlenmiştir. *Trichogramma evanescens*'in gelecekte *C. partellus*'un biyolojik mücadele programlarında kullanılabileceği düşünülmektedir.

Anahtar sözcükler: Biyolojik mücadele, *Chilo partellus*, yumurta parazitoiti, mısır, *Trichogramma evanescens*

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Introduction

The invasive spotted maize stem borer *Chilo partellus* Swinhoe,1885 (Lepidoptera: Crambidae) is a dangerous maize pest and it is believed to be originated from India (Kfir, 1997), from there it spread to Eastern and Southern parts of Africa (Mathez, 1972; Melaku et al., 2006). Recently *C. partellus* was reported as a serious maize pest in several countries of the Mediterranean Basin, including Turkey (Sertkaya et al., 2014; Yonow et al., 2017). *Chilo partellus* is known to cause very severe damage on maize and sorghum wherever found (Kfir et al., 2002). It damages the vegetative and reproductive parts of the plant, and the losses may range between 24-75% (Kumar, 2002). The severe pest infestation has been reported to cause 80-100% crop losses in Asia and Africa (Overholt et al., 2000). Management of *C. partellus*is principally by insecticide use (Rauf et al., 2017) although other benign methods are also being exploited. The egg parasitoid *Trichogramma chilonis* Ishii, 1941 (Hymenoptera: Trichogrammatidae) and *Bacillus thuringiensis* Berliner, 1915 (Bacillales: Bacillaceae) were used to reduce population of *C. partellus* in India in a joint application (Jalali & Singh, 2006; Shera et al., 2017). Another species, *Trichogrammatoidea lutea* Girault, 1911 (Hymenoptera: Trichogrammatidae), was reported as an egg parasitoid of *C. partellus* in Africa (Kfir, 1990). Many species of the genus *Trichogramma,* including *Trichogramma cacoeciae* Marchal, 1927, *T. chilonis*, *Trichogramma chilotraea* Nagaraja & Nagarkatti, 1969, *Trichogramma dendrolimi* Matsumura, 1926, *Trichogramma brassicae* Bezdenko, 1968, and *Trichogramma japonicum* Ashmead, 1904 (Hymenoptera: Trichogrammatidae), were reported as egg parasitoids of *C. partellus* in the different regions of world (Polaszek, 2010). The egg parasitoid *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) has been recorded to control many lepidopteran pests. Özpınar & Kornoşor (1994) recorded 75.5% parasitism rate of *T. evanescens* on egg masses of *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Crambidae) on the second crop of maize in Adana, Turkey. Adarkwah et al. (2015) determined between 65-90% parasitism by *T. evanescens* on eggs of *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae) in paper and jute bags. According to Dix et al. (2009), biological control (biocontrol) with native natural enemies and/or coevolved natural enemies of the invasive pest can be very successful in the new habitat. Consequently, it is necessary to investigate possible native natural enemies for *C. partellus* in the agroecosystems in Turkey. Can Cengiz (2016) recorded *Telenomus busseolae* Gahan, 1922 (Hymenoptera: Scelionidae) and *T. brassicae*as egg parasitoids of *C. partellus* in Hatay Province, Turkey. Morphological parameters are successfully being used to identify many insect species. The male genitalia are a very vital diagnostic character of the *Trichogramma* genus (Nagarkatti & Nagaraja, 1971). Pinto (1999) established very concise procedures, terminologies of morphological characters and ratios of male antennae and genitalia of *Trichogramma*. Some of these characters include: length of aedeagus, length of basal part of aedeagus, length of dorsal aperture, width of dorsal lamina, length of genital capsule, length of longest seta of flagellum, maximum width of flagellum and more (Woelke et al., 2019). There are many reports of successful identification of *Trichogramma* spp. using morphological characters. Birova & Kazimirova (1997) used these male genitalia and antenna characters to identify *Trichogramma danubiense* Birova & Kazimirova, 1997 (Hymenoptera: Trichogrammatidae). Woelke et al. (2019) used these characters to describe two new *Trichogramma* species. Sorokina (1993) and Pintureau (2008) used these characters and identified *T. evanescens* and *T. brassicae*.

The main goal of this study was to determine the occurrence and identify native egg parasitoids of *C. partellus* in the southwestern part of Turkey especially in Adana Province, analyze its influence on the host population, and to check the opportunity of its future application for biological control of *C. partellus*.

Materials and Methods

Study site

The study was conducted in the fields of Çukurova University, near Adana, Turkey (39º01’50.5”N, 35º21’06.7”E), in 2018 (Figure 1). Two maize crops per year is the predominant practice in the

Mediterranean areas of Turkey; the first crop in March-June and the second crop in July-October. A maize (Pioneer Hybrid 1/2013) crop of 0.4 ha was established on 15 March 2018 as the first crop of maize. The second crop of maize was planted on 12 June 2018. Interrow and intra row spacing was 45 cm and 20 cm, respectively. No pesticides were applied during the entire period of the study.

Figure 1. Sampling sites (stations) of the study (Anonymous, 2019).

Sampling

Once a week between 08.00-10.00 am, 60 maize stalks were selected randomly and cut at ground level and transported to the Laboratory of Entomology in the Department of Plant Protection, Çukurova University. The leaves were meticulously examined for egg masses. The egg masses of *C. partellus* were incubated and the egg parasitoids that emerged were identified. Preliminary studies conducted in 2017 showed it is easier to find egg masses of *C. partellus* in August and September, when there is a peak in the population. Thus, much of the emphasis in these studies was during this period. A total of 60 egg masses (each containing 35-50 eggs) of maize stem borer *C. partellus* were collected within this period. About 15 females and 10 males of *Trichogramma* sp. were reared from these parasitized eggs. These newly hatched egg parasitoids *Trichogramma* sp. were isolated and supplied with a large number of eggs of laboratory host, *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) attached on paper cards. A culture of an egg parasitoid, *Trichogramma* sp., was maintained at the laboratory (25.0±2ºC, 68±2% RH, 16:8 h L:D h photoperiod) on eggs of *E. kuehneilla*. Although *E. kuehniella* is a very destructive pests of stored grains (Tarlack et al., 2015), its eggs and larvae are generally used as laboratory host for rearing of many entomophagous insects (Samara et al., 2008). Two samples containing over 200 dead adult specimens of *Trichogramma* sp. from the laboratory culture were sent to Dr. Victor Fursov (Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Kiev, Ukraine) for detailed morphological study and species identification.

Species identification

Standard methods of slide mounting were used for the preparation of microscope slides on glasses (Pinto, 1999; Platner et al., 1999). About 200 specimens of *Trichogramma* were macerated in 10% liquid of potassium hydroxide, then washed in distilled water and mounted in Faure's liquid on glass slides under small cover slips. Thirty males of *Trichogramma* were dissected, then mounted on slides and used for the identification. Microscope Olympus CX-40 with digital photo-camera Olympus CX4040 was used to receive digital photos of morphological characters. Voucher specimens mounted on microscope slides are deposited at the Department of entomophagous insects and biocontrol, Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine (Kiev, Ukraine).

Rate of parasitism

The egg masses were incubated in the Laboratory of Entomology, Department of Plant Protection, Çukurova University. The egg masses were checked daily under a stereomicroscope (x10) for parasitism. Two days before wasp emergence, parasitized egg masses become black and were easily differentiated from unparasitized egg masses (Woelke et al., 2019). The rate of parasitism (RP) was estimated as the fraction of the number of parasitized egg masses (P) of the total number of egg masses (T) collected per sampling date: RP = (P/T) x 100.

Results and Discussion

Species identification

The species of *Trichogramma* was identified on the base of morphological study of male genitalia and male antennae (Nagarkatti & Nagaraja, 1977; Pinto, 1999). Morphological keys of Pintureau (2008) and Sorokina (1993) were used for the identification. The parasitoid species was identified as *T. evanescens*.

Diagnosis. The species *T. evanescens* can be distinguished from other species by the occurrence of long setae on male antennae. Setae are at least 2.5 to 3 times longer than the width of clava. Male genitalia of *T. evanescens* have a wide, clearly-visible tip and wide lateral lobes of dorsal extension of phallobase of genitalia, and a long, narrow and sharp intervorsellar process, or ventral extension of phallobase. Figure 2 shows these morphological details of the male antennae and genitalia of *T. evanescens*. We consider the species *T. brassicae* to be the morphologically closest species to *T. evanescens,* with only minor morphological differences (Pintureau, 2008).

Trichogramma brassicae has been widely suggested and used as biological control agent for many lepidopterous pests (Moezipour et al., 2008; Lundgren et al., 2009; Thubru et al., 2018). Since *T. brassicae* has been found in different regions of Turkey (Can Cengiz et al., 2016), it appears that local natural enemies can be adaptable for control of *C. partellus*.

Parasitism rate

The egg parasitoid *T. evanescens* was first reared from egg masses of *C. partellus* on 9 August 2018. The rate of parasitism gradually increased from 71.4% (9 August) to 100% (16 August). The rate of parasitism then dropped and rose again as is shown in Table 1. According to the original results, we suggest that the recorded high level of parasitism of *T. evanescens* in the egg masses of *C. partellus* indicates that this parasitoid is an important natural biological control agent of *C. partellus* in the southwestern part of Turkey. In Turkey, *T. brassicae* and *T. evanescens* have been used against lepidopterous pests. *Trichogramma brassicae* has been used in maize fields and hazelnut orchards in Düzce Province to achieve almost complete control of *O. nubilalis* and *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Noctuidae) (Kutuk 2017). Öztemiz et al. (2017) reported that *T. evanescens* reduced *Cydia pomonella* (L., 1758) (Lepidoptera: Tortricidae) in apple fruit to 9.66% compared to 34.0% in control plots in Pozantı. Also, low to medium parasitism rates of *T. evanescens* on eggs of *Sesamia nonagrioides* Lefebvre,1827 (Lepidoptera: Noctuidae) have been recorded in Adana (Sertkaya et al.,1999; Sertkaya & Kornoşor, 2002). Kayapınar & Kornoşor (1992) and Özpınar & Kornoşor (1994) also recorded very high parasitism of *O. nubilalis* by *T. evanescens* in many other parts of Turkey. It is known that excessive use of insecticides is not only damaging for the environment but it is also costly (Achiri et al., 2016). Alternative methods, such as biological control, are actively being investigated in many parts of the world for the control of *C. partellus* (Jalali & Singh, 2006; Shera et al., 2017; Thubru et al., 2018). This study provided the first report of *T. evanescens* in *C. partellus* in Turkey, and this finding along with the previous report of *T. brassicae* in *C. partellus* (Can Cengiz et al., 2016) are supportive of adaptive local biological control of invasive pests. In

the findings of Can Cengiz et al. (2016), parasitism of *C. partellus* was recorded only in the second crop, probably emphasizing the general parasitoid nature of the parasitoids which are often in the field in the second crop of maize alongside native maize stem borers.

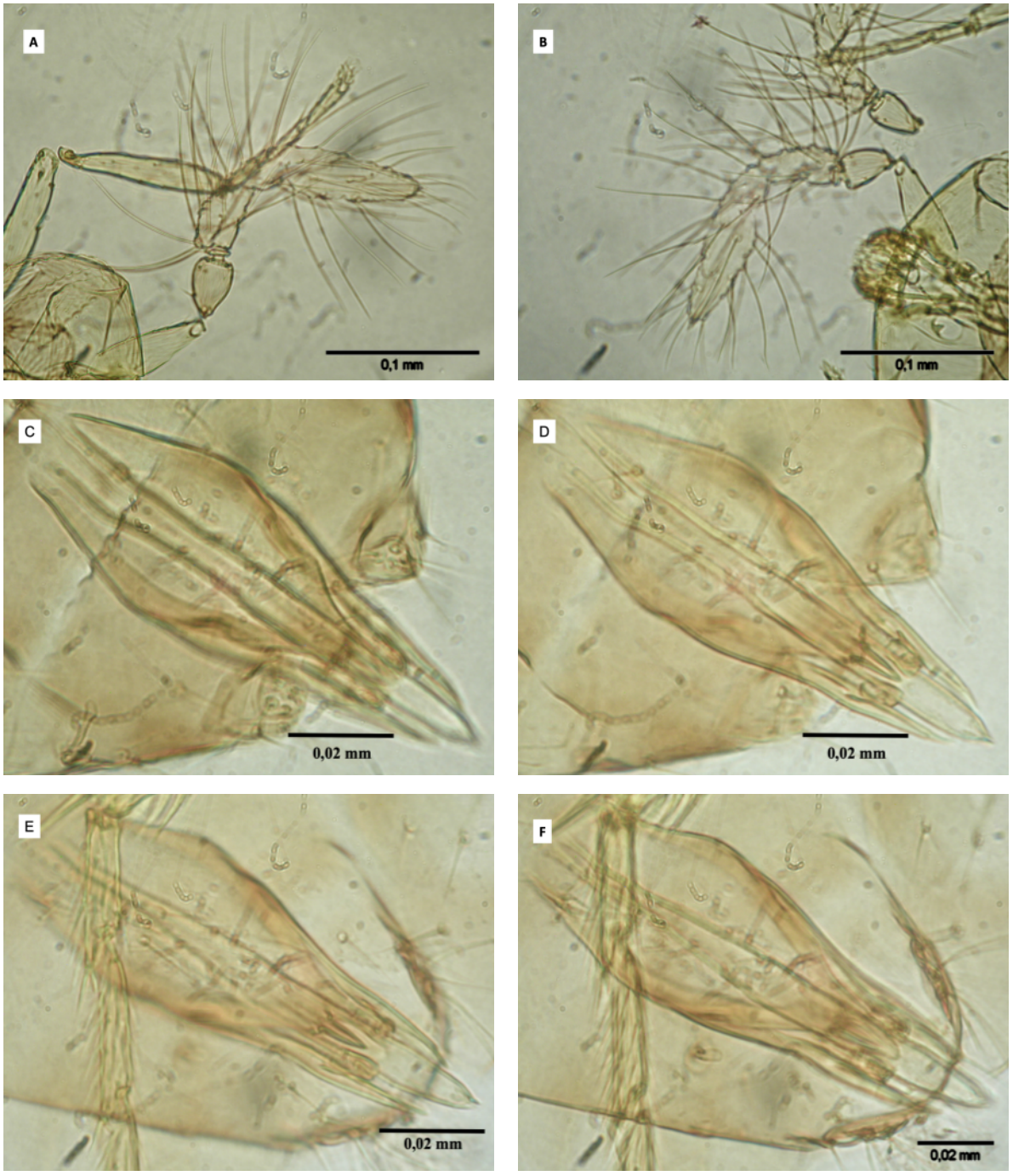

Figure 2. Morphological characteristics of male *Trichogramma evanescens*, reared from eggs of *Chilo partellus* in Adana, Turkey: A) setae at least 2.5 to 3 times longer than the width of clava, B) setae at least 3 times longer than the width of setae, C) wide tip and wide lateral lobes of dorsal extension of phallobase, D) ventral extension of phallobase, E) wide tip and wide lateral lobes of dorsal phallobase enlarged, and F) ventral extension of phallobase enlarged.

Table 1. Rate of parasitism of *Trichogramma evanescens,* reared from eggs of *Chilo partellus* at studied maize fields in Balcali, Adana Province, Turkey in 2018

	Date of collection					
	9 Aug	16 Aug	23 Aug	31 Aug	6 Sep	11 Sep
Egg masses collected	14	6	11	8	12	10
Egg masses parasitized	10	6	0	6	0	10
Rate of parasitism (%)	71.4	100	0.0	75.0	0.0	100

Conclusions

On the base of this study, we suggest that further studies should be conducted, on a large scale, to ascertain the impact of native parasitoids on *C*. *partellus* in the agroecosystems of Turkey. The percentage of egg parasitism and its influence on the population density of *C. partellus* must be taken into the consideration. In addition, an integrated control strategy for *C*. *partellus* should be considered, with natural enemies, such as *T*. *evanescens*, having a pivotal role.

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