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Parasitization Preference of *Hyposoter didymator* (Thunberg) (Hymenoptera: Ichneumonidae) on the Larval Stages of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)*

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ABSTRACT

The young instars (1-3rd instars) of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) were used in order to determine parasitization preference of the parasitoid *Hyposoter didymator* (Thun.) (Hymenoptera: Ichneumonidae) which may develop inside *H. armigera*. One of 1st, 2nd and 3rd instar larvae and an experienced *H. didymator* female were inserted into a petri dish and then observed for parasitization. The parasitized larva that *H. didymator* inserted her ovipositor was immediately removed from the petri dish and another group of 1st, 2nd and 3rd instar larvae were inserted into the petri dish. This procedure was continued until 20 host larvae were parasitized. Parasitized larvae were inserted into transparent 100 ml cylinders with artificial insect diet. All cylinders were stored in the growth chamber at 25±1°C temperature and 65%±5 humidity. The parasitized larvae were dissected at Ringer's solution under a stereomicroscope and the eggs of parasitoid were searched since the parasitoid eggs could easily be seen after 24 hours later than the parasitization. After dissection, the larvae with parasitoid egg were accepted as preferred by the parasitoid and the parasitization ratios (%) for 1st, 2nd and 3rd instar *H. armigera* larvae by *H. didymator* were calculated. Results show that the host instar preferences of *H. didymator* females were significantly different ($F_{(2,24)}$=38,055; P<0,05) and the most preferred *H. armigera* instar was the 3rd instar (52%), then the 2nd instar (32%). The least preferred *H. armigera* instar was the 1st instar (16%). We believe that these results may be useful in *H. didymator* breeding programs.

Keywords: *Hyposoter didymator*, *Helicoverpa armigera*, larval stages preference, parasitoid

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae)'nın larva parazitoiti *Hyposoter didymator* (Thunberg) (Hymenoptera: Ichneumonidae)'un tercih ettiği larva dönemlerinin tespiti

ÖZ

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae)'nın larva parazitoiti *Hyposoter didymator* (Thun.) (Hymenoptera: Ichneumonidae)'un tercih ettiği dönemlerin tespiti amacıyla ele alınan bu çalışmada, konukçu olarak *H. armigera'*nın larvaları kullanılmıştır. *H. armigera'*nın 1'inci, 2'inci ve 3'üncü dönemdeki larvalarından birer adet alınarak petriye yerleştirildikten sonra parazitleme bakımından deneyimli 1 adet *H. didymator* dişisi verilerek gözlenmiştir. *H. didymator* dişisinin ovipozitörünü sokarak parazitlediği konukçu larvası hemen ortamdan uzaklaştırılmıştır. Bunun yerine, aynı kategoriden olmak üzere 1'er larva (1'inci, 2'inci ve 3'üncü dönem) verilerek 20 adet konukçu larvası parazitleninceye kadar çalışmaya devam edilmiştir. Parazitlenen larvalar, içerisinde yapay böcek yemi konulmuş 100 ml'lik şeffaf kutulara teker teker yerleştirilmiştir. Bu kutular 25±1°C sıcaklık ve %65±5 orantılı neme ayarlı iklim odasına tutulmuştur. Parazitlenmeden 24 saat sonra buradan alınan larvalar, petride Ringer ortamında stereo-mikroskop altında dissekte edilerek parazitoitin yumurtası incelenmiştir. Parazitlenmeden 24 saat sonra yapılan incelenmede, parazitoitin konukçu larvası içerisindeki açılmamış yumurtalarının kolaylıkla görülebileceği anlaşılmıştır. İnceleme sonucunda, parazitoitin yumurtası bulunan larvalar, parazitoit tarafından tercih edilmiş larva dönemi olarak

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değerlendirilmiştir. Yapılan değerlendirme sonucunda, *H. didymator* dişilerinin konukçu tercihinin *H. armigera* larva dönemlerine göre önemli düzeyde farklılık gösterdiği belirlenmiştir ($F_{(2,24)}$=38,055; P<0,05). *H. didymator* dişilerinin yumurtasını koyması bakımından en çok 3'üncü dönem *H. armigera* larvalarını tercih ettiği (%52), bunu sırasıyla 2'inci dönem larvalarının izlediği (%32), 1'inci dönemdeki konukçu larvalarını ise en az düzeyde tercih ettiği (%16) tespit edilmiştir. Elde edilen sonuçların *H. didymator*'un toplu olarak yetiştirilmesinde faydalı olacağı düşünülmektedir.

Anahtar Sözcükler: Hyposoter *didymator*, *Helicoverpa armigera*, larva dönemi tercihi, parazitoit

1. Introduction

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is one of the most serious insect pests of over 181 plant species out of 45 families (Bahena *et al.*, 1999; Srivastava *et al.*, 2005). One of the parasitoids of this pest is Hyposoter didymator (Thun.) (Hymenoptera: Ichneumonidae). This parasitoid is a polyphagous solitary endoparasitoid which may parasite various Noctuidae species other than *H. armigera*. Typically, adult females of *H. didymator* oviposit an egg onto a dorsolateral area of the host. The larvae of the parasitoid develop inside the host (Bahena *et al.*, 1999). After completing three instars within 8-12 days inside the host (Jalali *et al.*, 1988), *H. armigera*, the larva kills and leaves the host. Then, the parasitoid larva forms a silken protective cocoon and emerges as an adult after pupation of 6-7 days (Bahena *et al.*, 1999).

There are certain studies in the literature that *H. didymator* is with lepidopterous pests which are harmful to many cultural plants in many regions in Turkey, and this parasitoid as playing a major role in controlling pests (Karaat and Göven, 1987; Göven and Özgür, 1990; Özdemir and Kılınçer, 1990; Göven and Efil, 1994; İkincisoy *et al.*, 1994; Koçlu and Karsavuran, 1999; Atlıhan *et al.*, 2003; Sertkaya *et al.*, 2004; Sertkaya and Bayram, 2005; Kaya and Korşonor, 2008; Gözüaçık and Mart, 2009). Our literature search showed that *Hyposoter* genus is common in all of the zoogeographical regions (Yu *et al.*, 2005) and *H. didymator* is known in Egypt, Israel, Iran, and Azerbaijan (King *et al.*, 1981; Karimpour *et al.*, 2005; Ghadiri *et al.*, 2007). Also, it is an important parasitoid of *H. armigera* in the cotton fields in Azerbaijan and the parasitoid attacks the young instars (5-15 mm) of the pest (Bar *et al.*, 1979; Abidinbekova and Mustafina, 1991). In the U.S., parasitoid species of the genus *Hyposoter* is found where *Helicoverpa* species are found (Neunzig, 1963), it exists in Australia, eastern and western Palearctic regions (Yu *et al.*, 2005), and also it is also the most important parasitoid of *H. armigera* in Greece and Bulgaria (Carl, 1978). The literature about *H. didymator* which is a natural enemy of the noctuids both in agriculture and forest areas show that the previous studies are limited both in Turkey and other countries with regard to the literature given above. We believe that the limited literature about *H. didymator* is due to the mass rearing of this parasitoid species in laboratory and maintaining the parasitoid population being extremely difficult. A recent study by Şimşek (2017) carried out in laboratory conditions regarding the biological relationships between *H. didymator* and its host *H. armigera* is one of the most detailed studies in Turkey (Şimşek, 2017). However, more studies are needed on this subject. Thus, this study is carried out in order to determine the host preference of the parasitoid *H. didymator* when the young instars (1-3) of the host *H. armigera* are present in the laboratory conditions since we could not find any literature on this subject.

2. Materials and methods

The main material of this study were the young instars (1-3) of the host *H. armigera* and also male and female *H. didymator* adults. Stereomicroscope, Petri dishes, transparent plastic jars with 100 ml volume, artificial insect diet (Southland Products Inc., USA), honey, smooth brushes, ethanol, aspirator, and Ringer solution were used as auxiliary materials.

All experiments were carried out at growth chamber that was set at 25±1°C temperature and 65%±5 humidity with 16:8 Light: Darkness conditions.

The laboratory colonies of *H. didymator* and its alternative host *H. armigera* were used in the experiments. Both the parasitoid and its host were reared in Çankırı Karatekin University Faculty of Forestry laboratory according to the literature (Harrington *et al.*, 1993; Bahena *et al.*, 1999; Mironidis and Savopoulou-Soultani, 2009; Yassin and Özkan, 2011; Şimşek, 2017). The young instars (1-3) of *H. armigera* were used as hosts and 5-6 day-old *H. didymator* adult females with parasitization experience were used in the experiments (Figure 1).

Figure 1 The adult female of *Hyposoter didymator* (Thunberg)

The instars of *H. armigera* larvae were determined after measuring the head capsule width under a stereomicroscope as 1st instar (0.257-0.314 mm), 2nd instar (0.400-0.485 mm) or 3rd instar (0.600-0.743 mm)(Mohammadi *et al.*, 2010). One of each instar of *H. armigera* were moved to 5 cm diameter Petri dish with a smooth brush. Then, a 5-6 day-old *H. didymator* female with parasitization experience was introduced into the petri dish with hosts and observed for parasitization. Parasitoid females were given a chance of choosing according to own decision of one out of the given three instars. After the parasitoid inserted its ovipositor into a host, the parasitized larva removed from the petri dish with a smooth brush and another larva of the same instar was introduced into the petri dish. Since there is a difference between ovipositor insertion and oviposition numbers (Glynn and Powell, 1992), removed larvae which were supposed to be parasitized were inserted into 100 ml transparent plastic jars separately and 1 cm^3 of the artificial insect diet was inserted for the larva to feed. All movement process of the larvae was conducted with a smooth brush. Then all the jars were closed with covers with holes for air ventilation. The parasitized larva and non-parasitized larvae were removed and then new larvae of the three instars were inserted into the petri dish when parasitization had occurred. The experiments were continued until 20 larvae were parasitized for each parasitoid females. When the host larvae were dissected after 24 hours later than the parasitization, the parasitoid eggs may easily be seen within Ringer's solution under a stereomicroscope and determine whether the host larvae were parasitized or not (Bertil, 1984; Tillman and Powell, 1989). Thus, the eggs of the parasitoid were searched within the host larvae in Ringer's solution under a stereomicroscope in order to check whether the host larvae are parasitized or not. When an *H. didymator* egg was found in a host body, it was accepted as a preferred host (Figure 2).

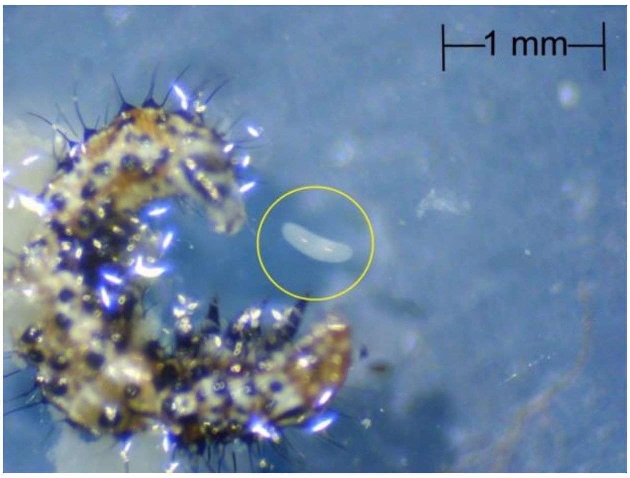

Figure 2 *Hyposoter didymator* (Thun.) egg found inside the 2nd instar *Helicoverpa armigera* (Hübner) host after dissection

The possible reason for *H. didymator to prefer the 1st* instar larvae the least may be the difficulty of insertion of the ovipositor into the host body since the 1st instar host larvae have very small bodies and the most probable reason for *H. didymator* to prefer 3rd instar host larvae may be the abundance of the food for the development of the parasitoid larva in the host body and also be much easier parasitization.

The experiment was designed according to random plots experimental design with 3 replicates. In each replicate, 3 parasitoid females were used and for each parasitoid female, 20 of each instar larvae. A total of 9 parasitoid females and 540 *H. armigera* larvae were prepared however only 180 of the larvae were used. Parasitization ratios of *H. didymator* for first, second and third instars of *H. armigera* were calculated. ANOVA and Tukey's-b statistical tests were applied to determine the parasitoid preference. Statistical evaluation was carried out in SPSS Software.

3. Results and discussion

The larval host, *Helicoverpa armigera* (Hübner) instar preference of the parasitoid, *Hyposoter didymator* for parasitization in a petri dish is given in Table 1 and Figure 3.

Table 1 The host preference of *Hyposoter didymator* by the larval instars of *Helicoverpa armigera*.

Helicoverpa armigera Larval Instars	Parasitization Ratio (%)±Std.Err.		$F_{(2,24)}$	P
1	15,89 ± 3,51	c	38,055	0,000
2	32,00 ± 2,26	b		
3	52,44 ± 2,81	a		

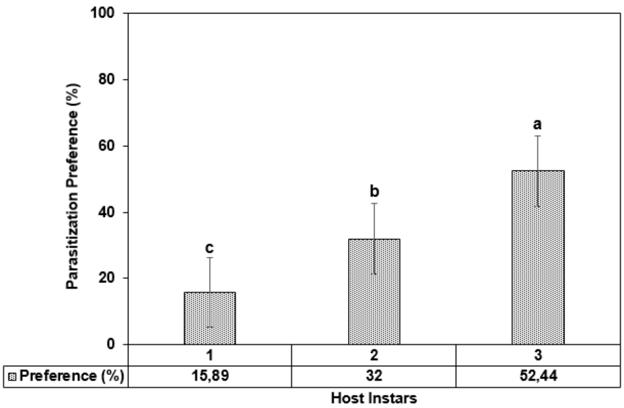

Figure 3 The host preference of *Hyposoter didymator* (Thunberg) by various instar (1-3) larvae of *Helicoverpa armigera* (Hübner)

Table 1 and Figure 3 show that the host preferences of *H. didymator* females on various *H. armigera* instar larvae were 15.89%±3.51 at 1st instar, 32.00%±2.26 at 2nd instar, and 52.44±2.81 at 3rd instar. The host instar preference of *H. didymator* changes significantly by the larval instar of *H. armigera* ($F_{(2,24)}$=38,055; P<0,05). These results indicate that *H. didymator* prefers the 3rd instar larvae of *H. armigera* most, then the 2nd instar larvae and the 1st instar larvae the least. Results of a survey study carried out in Spain between 1995 and 1998 show that parasitization ratios were found as 5%, 48% and 46% at the first, second and the third instars of *H. armigera* which were collected from the field and fed with an artificial insect diet in the laboratory (Torres-Vila *et al.*, 2000). The possible reason for *H. didymator* to prefer the 1st instar larvae the least may be the difficulty of insertion of the ovipositor into the host body since the 1st instar host larvae have very small bodies and the most probable reason for *H. didymator* to prefer 3rd instar host larvae may be the abundance of the food for the development of the parasitoid larva in the host body and also be much easier parasitization.

Parasitization ratios of the young instar (1-3) *H. armigera* larvae by *H. didymator* female when the host larvae are introduced separately in a petri dish and comparison results from another study (Şimşek, 2017) are given in Table 2.

Table 2 Comparison of the parasitization ratios of the young (1, 2 and 3) instar *Helicoverpa armigera* (Hübner) larvae by *Hyposoter didymator* (Thunberg) (Şimşek, 2017).

Parasitized *Helicoverpa armigera* Larval Instars	Parasitization Ratio (%) Mean±Std. Error		$F_{(2,6)}$	P
1	83.71 ± 1.46	a	2,076	0,206
2	90.15 ± 0.84	a		
3	86.74 ± 2.68	a		

The evaluation of the parasitization ratios of the young instar *H. armigera* larvae by *H. didymator* (Şimşek, 2017) show that the parasitization ratios were 83.71%±1.46, 90.15%±0.84 and 86.74%±2.68 with regard to larval age, and the parasitization difference between larval instars are not significant ($F_{(2,6)}$=2,076; P>0,05), and *H. didymator* may successfully parasitize three instars of *H. armigera* while three instars have sufficient food resource for the development of the parasitoid larva (Şimşek, 2017). Higher parasitization ratios at young instars may be due to the introduction of various instars of the host individually and due to the forced parasitization activity for the given host. This also suggests that when *H. didymator* female inserts its ovipositor, the parasitoid oviposits only one egg. In literature, *H. didymator* is reported to insert its ovipositor into *H. virescens* and oviposited an egg 10.3 out of 11 times on average (Glynn and Powell, 1992). In another study, *H. didymator* is reported to have a parasitization ratio of 80% without reporting any host instar data (Schneider and Vinuela, 2007), and also *H. didymator* is reported to accept the young instars of *H. virescens* by 85-100% in another study (Tillman and Powell, 1989). These reports also support our results.

We believe that high parasitization at 2nd and 3rd instar (32% and 52.44% respectively) larvae of the pest which burrow the tomato fruit is beneficial by killing the pest larvae directly (Figure 4) and decrease the pest population density (Schneider and Vinuela, 2007), and also decrease the yield loss indirectly since the parasitized larvae were observed not to feed. It is an important parameter with regard to the development of the parasitoid that the parasitoid larva does not allow its host to die while it is feeding inside the host (Lawrence, 1986; Lawrence, 1990). It is also reported that biological agents decrease their hosts' population densities thus decrease the yield loss of many agricultural pests. Parasitization removes the parasitized larvae out of the population (the indirect effect of decreasing the next generation) and decreases the feeding damage of the pest larvae by changing the feeding habits of the host (the direct effect on the current generation). So, it is important for decreasing the larval damage on the cultural plants (Powell, 1989). Results of another study carried out on *Spodoptera littoralis*, another host of *H. didymator* show that non-parasitized larvae consume much more food than parasitized ones (Morales *et al.*, 2007). The food consumption of the 3rd instar larvae of *S. littoralis* that parasitized by *H. didymator* decreases 4 days after the parasitization and the 6th instar *S. littoralis* larvae loses 13% of their weights (Morales *et al.*, 2007). Results of another study on the same host species suggest that parasitized larvae are advantageous in yield loss since parasitization had a negative effect on the parasitized larvae (Kumar and Ballal, 1992; Kaeslin *et al.*, 2005).

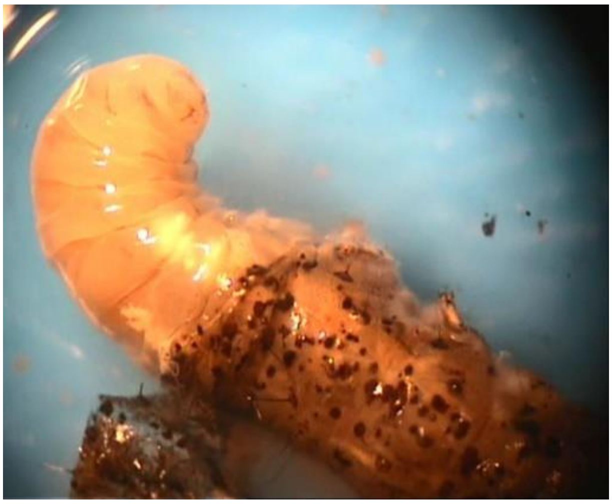

Figure 4 Exit of the last instar of *Hyposoter didymator* (Thunberg) from *Helicoverpa armigera* larva which the parasitoid larva after consuming its host

H. didymator may parasitize all larval instars (1-5) of *H. armigera* (Mironidis and Savopoulou-Soultani, 2009), however, in general, the larvae of the pest feed on the leaves of the host plant when in 1st and 2nd instars and then enters the fruit by burrowing (Anonymous, 1995). Thus, parasitization of the host larvae at latter instars does not seem possible. The healthy larvae are protected in the tomato fruit and also cause the fruit to lose its market value by causing the fruit to rot. Also, it is observed that the old (4 and 5) instars of *H. armigera* attack the parasitoid adult, and even kill parasitoids by attacking. Due to these reasons, the parasitization of the old instars does not have any practical importance and also parasitization of the old instars does not seem possible.

Finally, we believe that the results of this study that have been carried out in the laboratory may be useful at maximizing the utilization of *H. didymator* as a biological control agent at Integrated Pest Management (IPM) programs from which is an important parasitoid of *H. armigera*, and also may be useful in mass production of the parasitoid species in the laboratory conditions.

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