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**ARAŞTIRMA MAKALESİ** **RESEARCH ARTICLE**

# Preference study of *Trichogramma pintoi* (Voegele) (Hymenoptera:Trichogrammatidae) on host eggs of different ages and species

*Trichogramma pintoi* (Voegele) (Hymenoptera: Trichogrammatidae)'nin farklı yaşlardaki ve türlerdeki konukçu yumurtalarını tercih çalışması

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**ABSTRACT**

Eggs of *Ephestia kuehniella* (Zeller), *Cadra cautella* (Walker) and *Plodia interpunctella* (Hübner) are host of parasitoid *Trichogramma pintoi* (Hymenoptera: Trichogrammatidae) which is used in biological control of pests. In this study, the host species and egg age preferences were determined in the parasitization performance of the adult female of *T. pinto*. For host species and egg age preference, 1, 2, 3 and 4 days old eggs from three different hosts were given to the parasitoid which were kept in controlled conditions until they hatched and then counted. Adult *T. pintoi* species were also stored at 4±1 °C up to 4 days. It was determined that the fecundity and life span of female parasitoids decreased as the storage period increased. *T. pintoi* was found to prefer up to 1 day eggs old in all three hosts. Parasitoid preferred 2, 3, and 4 days old eggs of all host species at the similar rates. Eggs of *E. kuehniella* and *C. cautella* were determined as the most preferred host, followed by eggs of *P. interpunctella*. Adult *T. pintoi* females stored for 1 day were found as the most surviving female individuals. The highest parasitization rate was also determined in females stored for 1 day. Host species, egg age preference and storage time of adult parasitoid are thought to be very important in mass production and release studies of *T. pintoi*.

**ÖZET**

*Ephestia kuehniella* (Zeller), *Cadra cautella* (Walker) ve *Plodia interpunctella* (Hübner) yumurtaları biyolojik mücadelede kullanılan parasitoid *Trichogramma pinto* (Voegele)'nin konukçularındandır. Bu çalışmada *T. pinto*'nun ergin dişisinin parazitleme performanslarında konukçı tür ve yumurta yaşı tercihleri belirlenmiştir. Konukçu türü ve yumurta yaş tercihi çalışmalarında, her konukçu türü için, 1, 2, 3 ve 4 yaşındaki yumurtalar dişi parazitoid erginlerine verildikten sonra kontrollü koşullarda parazitoitlerin tümü çıkıncaya kadar bekletilmiş ve sayımları yapılmıştır**.** Ergin *T. pintoi* türleri ayrıca 4 gün süre boyunca 4±1 °C'de depolanmıştır. Depolama süresi uzadıkça dişi parazitoitlerin doğurganlığı ve yaşam sürelerinin azaldığı belirlenmiştir. *T. pintoi*'nin her üç konukçuda da en fazla 1 yaşındaki yumurtaları tercih ettiği belirlenmiş olup, her üç konukçuda da 2, 3 ve 4 yaşındaki yumurtaları istatiksel olarak aynı oranda tercih ettiği görülmüştür. Konukçu tercihlerine bakıldığında ise, istatiksel olarak *E. kuehniella* ve *C. cautella* yumurtalarını en fazla tercih etmiş, bunu *P. interpunctella* izlemiştir. Ergin *T. pintoi* dişilerinin deplanması sonucu en fazla yaşayan dişiler 1 gün süre ile depo edilenlerde belirlenmiştir. En fazla parazitlenmenin yine 1 gün süre ile depo edilen dişilerde olduğu belirlenmiştir. Konukçu türü, yumurta yaşı tercihi ve ergin parazitoidlerin depolanma süresinin *T. pintoi*'nin kitle üretimi ve salımı çalışmalarında oldukça önemli olduğu düşünülmektedir.

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## INTRODUCTION

About the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) the related species are small parasitic bees that parasitize the eggs of numerous economically important pests (Oztemiz et al., 2013). These organisms are group-living endoparasites that are particularly effective on Lepidoptera pests (Pinto & Stouthamer, 1994). *Trichogramma* species are now among the most widely used parasitoids worldwide for the control of moth pests that damage agricultural crops (Steidle et al., 2001) and by laying its eggs in many moth eggs, the host kills their eggs and completes their life stages there. *Trichogramma* species are used worldwide on millions of hectares for biological control of Lepidoptera (Li-Ying, 1994; Smith, 1996; Pintureau, 2009; Agamy, 2010; Desneux et al., 2010; Andrade et al., 2011). Biological pest control is more efficient, sustainable, environmentally friendly and compatible with other pest control methods because it prevents ecological contamination by chemical pesticides (Laba & Kartohardjono, 1998; Maneerat & Suasaard, 2015; Subandi et al., 2017).

*Trichogramma* species parasitize the eggs of moths that cause damage to stored products which are commercially produced and released due to their ease of reproduction in laboratory conditions (Bernardi, 2000). Particularly in mass production of egg parasitoids, host egg age and host type affect many criteria, such as the number of parasitized eggs, hatching rate of parasitized eggs, adult emergence, and body size of parasitoids (Uzun, 1994; Aydin Özder & Kilincer, 1996; Oztemiz 2010). The egg yield of *Trichogramma* species varies depending on the parasitoid species studied, host species, and production conditions (Uzun, 1994). Host quality plays a critical role in determining the growth rate and success of parasitoids (Liu et al., 2013; Farahani et al., 2016). *Trichogramma* species have been successfully used against various lepidopteran species by mass propagation on flour moth, barley moth and some other host eggs in the laboratory (Sertkaya & Kornoşor, 2002).

Host species and host age can have a significant impact on whether potential hosts are successfully infested by parasitoids (Pak, 1986). The selection of appropriate candidate species is essential for successful biological control programs because the host-specific characteristics of candidate species are generally among the factors that determine their effectiveness as control agents (Pak, 1988). This situation demonstrates that a clear determination of the relationships between the host and the parasitoid is essential for mass production studies. In this study, the host species and egg age preferences were determined for the parasitization performance of the adult female of *Trichogramma pinto*.

## MATERIALS and METHODS

The main material of the study was *Ephestia kuehniella, Cadra cautella, Plodia interpunctella* (Lepidoptera: Pyralidae) and the egg parasitoid *Trichogramma pintoi* (Hymenoptera: Trichogrammatidae).

Mass productions of *C. cautella* and *E. kuehniella* were carried out at 25±1 °C, 65-70% relative humidity and a mixture of bran, cracked corn and cracked wheat were used.

Mass production of *P. interpunctella* was carried out at 25±1 °C, 65-70% relative humidity, and a mixture of honey, bran, cracked corn, milk powder and glycerin was used.

Mass production of *T. pintoi* was produced with the host *E. kuehniella* and stock cultures were established in this way. The parasitoid stock cultures were grown in glass tubes at 25±1 °C, 65–70% relative humidity and 16 hours of light and 8 hours of darkness.

### *Host age preference*

Experiments performed at 26±1 °C, 60-70% relative humidity and 16:8 long days. In order to determine the age of the host eggs, the eggs were collected separately for each host every day and accumulated for 4 days. Then the eggs were taken into tubes and kept at 25 °C. Eggs obtained on the first day were accepted as 4 days old, and

those obtained on the last day were accepted as 1 day old. A total of 200 eggs, 50 on each sheet, were pasted in groups on paper strips in various combinations (1-2-3-4; 2-3-4-1; 3-4-1-2; 4-1-2-3; 1-3-4-2; 1-4-3-2; 3-1-2-4; 3-2-1-4; 2-1-3-4; 2-3-1-4; 1-3-2-4; 1-2-4-3; 1-4-2-3; 4-3-2-1; 4-2-3-1). After placing a female in each tube and administering honey, the females were removed after 24 hours, and an attempt was made to determine the preferred host age of the parasitoids by waiting until the eggs darkened. The experiments were performed with 15 repetitions for each pests.

***Host preference***

For host preference; 1-day-old eggs of each host were glued in separate combinations (*E. kuehniella-C. cautella-P. interpunctella; C. cautella-P. interpunctella-E. kuehniella; E. kuehniella-P. interpunctella-C. cautella; C. cautella- E. kuehniella- P. interpunctella; P. interpunctella-E. kuehniella- C. cautella; P. interpunctella-C. cautella- E. kuehniella; E. kuehniella-C. cautella-P. interpunctella; C. cautella-P. interpunctella-E. kuehniella; E. kuehniella-P. interpunctella-C. cautella; C. cautella- E. kuehniella- P. interpunctella; P. interpunctella-E. kuehniella- C. cautella; P. interpunctella-C. cautella-E. kuehniella; E. kuehniella-C. cautella-P. interpunctella; C. cautella- E. kuehniella- P. interpunctella; P. interpunctella-E. kuehniella- C. cautella*) 50 eggs from each host egg, for a total of 150 eggs. After a female parasitoid was placed in each tube, honey was administered, and removed after 24 hours. The dark coloured eggs were counted and recorded by daily observations. The experiments were performed with 15 repetitions for each pests.

***Parasitism performance of adult parasitoid stored at 4 °C***

50 freshly hatched female parasitoids *T. pintoi* were placed individually in tubes and stored at 4 °C in the refrigerator.

Ten tubes of adult female parasitoids stored in the refrigerator for one day were collected and transferred to the laboratory at 25 °C. These adult parasitoids were given fresh *E. kuehniella* eggs and honey as food every day. The eggs administered the previous day were collected in separate tubes, their data were recorded, and they were stored to await adult hatching. Then, kept in the refrigerator for two days, 10 tubes containing the adult female parasitoids were collected, and brought to the laboratory at 25 °C. İn the same way these adult parasitoids were given fresh *E. kuehniella* eggs and honey every day, the eggs administered the previous day were collected in separate tubes, their data were recorded, and they were stored to await adult hatching. The same procedure was applied to parasitoids stored at 4 °C for 3 and 4 days. The adult female belonging to the control group were recorded by giving honey at 25 °C. The experiments were performed with 10 repetitions.

***Statistical evaluation***

Experiments' data obtained were analyzed using the SPSS 21.0 package program according to the random plots trial design. Comparison of the means of the data obtained was performed using the Duncan multiple comparison test.

**RESULTS and DISCUSSIONS**

In the study 1-4 days old eggs were used to investigate the effects of egg age on parasitism. The females of all tested hosts parasitized a significantly higher number of younger hosts in all offered combinations. *T. pintoi* preferred eggs up to 1 day old in all three hosts (Table 1). In a previous study females of all strains tested parasitized a signifcantly higher number of younger hosts in all combinations offered (Monje et al., 1999). While little parasitism was observed in two-day-old eggs, almost no parasitism was observed in three- and four-day-old eggs. In most *Trichogramma* species, the number of parasitized hosts decreases as the host embryo develops.

A study was conducted to investigate the host and egg-age preferences of *T. brassicae, T. cacoeciae,* and *T. evanescens E. kuehniella* and *C. cautella* was found that all three parasitoid species preferred 1-day-old eggs of *E. kuehniella* and *C. cautella* over 2- and 3-day-old eggs (Kara & Özder, 2017). It is known that one of the most important factors in host acceptance of parasitoids is the age of the host (Reznik et al., 1997). The age of the host has a significant effect on the parasitization, development and reproduction of a parasitoid (Navarajan, 1979). *T. pintoi* was found to parasitize *P. interpunctella* 2-, 3-, and 4-day-old eggs statistically to the same extent, preferring mostly 1-day-old eggs.

Table 1. Egg age preference of *Trichogramma pintoi* in different hosts
*Çizelge 1. Trichogramma pintoi'nin farklı konukçularda yumurta yaşı tercihi*

| **Hosts** | **1 old** | **2 old** | **3 old** | **4 old** |
|---|---|---|---|---|
| *Ephestia kuehniella* | 21.33±1.08**b*** | 11.60±1.00 **c** | 1.66±0.43 **d** | 0.40±0.21 **d** |
| *Cadra cautella* | 15.20±1.61 **b** | 7.06±0.98 **c** | 2.80±0.75 **d** | 0.26±0.20 **d** |
| *P. interpunctella* | 12.46±0.74 **b** | 2.80±0.88 **c** | 0.33±0.15 **c** | 0.00±0.00 **c** |

*Mean in a column with the same letters are not significantly different (p<0.05).

A study was conducted in an attempt to determine egg age preferences of four different *Trichogramma* species on *E. kuehniella* and *Cydia pomonella* eggs, accordingly, four different *Trichogramma* species were found to parasitize on *E. kuehniella* eggs at the highest rate on 1-day-old eggs and at the lowest rate on 5 day-old eggs (Bulut, 1990). In another study, young eggs were found to contain more protein, glycogen and triglycerides, making them preferable and more suitable for mass production (Kishani et al., 2016).

Table 2. Host preference of *Trichogramma pintoi*'s on eggs of *E. kuehniella*, *C. cautella and P. interpunctella*
*Çizelge 2. Trichogramma pintoi'nin E. kuehniella, C. cautella ve P. interpunctella yumurtalarındaki konukçu tercihi*

| | **Number of eggs parasitized in different hosts** | | |
|---|---|---|---|
| **Parasitoit** | ***E. kuehniella*** | ***C. cautella*** | ***P. interpunctella*** |
| *T. pintoi* | 20.13±0.98 **a*** | 18.80±0.70 **a** | 4.60±0.58 **b** |

*Mean with the same letters are not significantly different (p<0.05).

When host preference was examined, it was found that the highest parasitism occurred in *E. kuehniella* with 20.13±0.98 and in *C. cautella* with 18.80±0.70, being statistically in the same group. This was followed by *P. interpunctella* with a parasitization rate of 4.60±0.58 (Table 2, $p < 0.05$).

The second preferred host was *P. interpunctella*, which is also one day old, and the parasitoid parasitized 4.60±0.58 eggs. Hassan (1994) parasitoid in the selection of mass production hosts in addition to efficacy was found to depend on the physical and physiological conditions in which they are found. In the selection of the mass rearing, the biological parameters determined in the laboratory are not always sufficient. Considering the hosts used in different parts of the world, economic and local conditions are paramount in the selection of hosts (Özpınar, 1997). In one study, it was reported that host switching decreased parasitoid performance, and that parasitoid performance of *E. kuehniella* decreased significantly when parasitoids reared on *Sitotraga cerealella* began to live on *E. kuehniella* eggs as hosts (Iranipour et al., 2010).

Kara (2006) comparatively investigated the host preferences of parasitoids that were found on eggs of *C. cautella* and *E. kuehniella*, it was found that the adaptation of parasitoids developed on *C. cautella* to the eggs of *E. kuehniella* is much easier and that the adaptation of parasitoids developed on *E. kuehniella* eggs to *C. cautella* eggs was some what weaker.

Our results showed that the parasitoid survived for 1, 2, 3 and 4 days at 4°C. In stored adult *T. cacoeciae* and *T. brassicae*, life span was reduced as a result of cold storage (Ozder, 2008). The female with the shortest life expectancy at four days of storage was calculated as 6.70±2.09 days. The longest lifespan was obtained with one-day storage compared to the control group and was calculated to be 11.40±7.54 days (Table 3, p< 0.05). The total number of eggs parasitized by *T. pintoi* during its lifetime was calculated to be 133.6±1.38 during one-day storage (Table 3).

Table 3. Mean longevity and fecundity of stored *Trichogramma pintoi*
*Çizelge 3. Trichogramma pintoi'nin ergin dişi ömrü ve ömrü boyunca parazitlediği toplam yumurta sayısı*

| Storage period (day) | Female Longevity (day) | Parasitized eggs (*E.kuehniella*) |
|---|---|---|
| 1 | 11.40±7.54 **b*** | 133.60±5.32 **b** |
| 2 | 9.20±3.20 **c** | 96.20±2.20 **c** |
| 3 | 8.60±6.70 **c** | 72.10±5.10 **c** |
| 4 | 6.70±2.09 **d** | 52.40±2.40 **d** |
| Control | 14.40±2.41**a** | 196.40±5.32**a** |

*Mean in a column with the same letters are not significantly different (p<0.05).

In one study, 4 °C was found to be the most suitable temperature for storage of adult *T. cacoeciae* (Ozder, 2008). Also the fecundity of *T. pintoi* statistically decreased after the first day. Female longevity among the adults stored for two and three days were not statistically different. The lowest parasitization was observed in the adults stored for four days (52.4±2.4). The adults stored for two and three days were statistically similar. According to this study, adult *T. pintoi* can be stored for 1 to 4 days (Table, 3).

**STATEMENT OF CONFLICT OF INTEREST**

The authors declare no conflict of interest for this study. This study was supported by Tekirdag Namık Kemal University Scientific Research Projects (BAP) with the project numbered NKUBAP.03.GA.17.089.

**AUTHOR'S CONTRIBUTIONS**

The contribution of the authors is equal.

**STATEMENT OF ETHICS CONSENT**

Ethical approval is not required as there are no studies with human or animal subjects in this article.

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