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**Photoperiod Effect on Fecundity, Longevity and Sex ratio of
Trichogramma brassicae (Hym: Trichogrammatidae)**

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Abstract

The effect of photoperiod on parasitization of the eggs of *Ephestia kuehniella* Zeller (Lep: Pyralidae) by *Trichogramma brassicae* Bezdenko (Hym: Trichogrammatidae) was investigated under several photoperiodic regimes of L: D = 0: 24, 3: 21, 6: 18, 9: 15, 12: 12, 15: 9, 18: 6, 21: 3 and absolute light on. Fecundity of *T. brassicae* females (the number of parasitized *E. kuehniella* eggs) was independent of photoperiod in the whole life time of females. However, photoperiod had a significant influence on the longevity of *T. brassicae* females. The least fecundity and longevity was for those that developed and were kept under 18L: 6D and the highest fecundity and longevity was for those that developed and were kept under 21L: 3D and absolute darkness, respectively. On the other hand, the proportion of *T. brassicae* females was dependent of photoperiod and the highest proportion of females was observed under 18L: 6D. It seems that long term photoperiods may stimulate a particular gland to secrete a special hormone which results in more longevity and female proportion of parasitoid. As a conclusion, long term photoperiods may improve efficiency of the parasitoid by increasing longevity and sex ratio.

Keywords: Egg parasitoid, fecundity, longevity, photoperiod, sex ratio.

بررسی اثر طول دوره روشنایی بر زادآوری، طول عمر و نسبت جنسی زنبور پارازیتوید

***Trichogramma brassicae* (Hym: Trichogrammatidae)**

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چکیده

در این تحقیق اثر رژیم‌های مختلف نوری شامل تاریکی مطلق، ۳، ۶، ۹، ۱۲، ۱۵، ۱۸، ۲۱ و روشنایی مطلق بر میزان پارازیتسیم تخم‌های *Ephestia kuehniella* Zeller (Lep: Pyralidae) توسط زنبور پارازیتوید *Trichogramma brassicae* Bezdenko (Hym: Trichogrammatidae) مورد مطالعه قرار گرفت. بنابر نتایج به دست آمده، زادآوری پارازیتویدهای ماده (تعداد تخم‌های پارازیت شده *E. kuehniella*) پرورش یافته در رژیم‌های نوری متفاوت، مستقل از طول دوره روشنایی بود اما طول دوره روشنایی دارای اثر معنی‌دار بر طول عمر پارازیتویدهای ماده بود. حداقل میزان زادآوری و طول عمر در رژیم نوری ۱۸ ساعت روشنایی: ۶ ساعت تاریکی و حداکثر میزان زادآوری و طول عمر به ترتیب در رژیم‌های نوری ۲۱ ساعت روشنایی: ۳ ساعت تاریکی و تاریکی مطلق مشاهده شد. از طرف دیگر، نسبت ماده‌ها وابسته به رژیم نوری بوده و حداکثر نسبت ماده‌ها در رژیم نوری ۱۸ ساعت روشنایی: ۶ ساعت تاریکی مشاهده شد. بنابر نتایج به دست آمده، به نظر می‌رسد که قرارگیری زنبورهای پارازیتوید در رژیم‌های نوری بلندمدت منجر به افزایش طول عمر و نسبت ماده‌ها از طریق تحریک برخی غدد و به دنبال آن ترشح گروه خاصی از هورمون‌ها می‌شود. بنابراین رژیم نوری بلندمدت می‌تواند منجر به افزایش کارایی پارازیتوید از طریق افزایش طول عمر و نسبت ماده‌ها شود.

واژگان کلیدی: پارازیتوید تخم، زادآوری، طول عمر، طول دوره روشنایی، نسبت جنسی.

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Introduction

Success in biological control depends on the reproductive potential of biocontrol agents. Fecundity, fertility and the number of female offspring are the most important factors affecting reproductive success (Hoffmann *et al.*, 2001; Reznik *et al.*, 2001, 2003; Reznik & Vaghina, 2006a). These factors are different between insect species and could be affected by the environmental factors. Environmental abiotic and biotic factors may influence many aspects of biological control agents' life (Calvin *et al.*, 1984). Among abiotic factors, photoperiod control reproduction of insects both qualitatively and quantitatively (Reznik & Vaghina, 2007). Photoperiod is a much more reliable and stable seasonal signal because a same geographic location will experience the same length of day on a specific day each year (Meuti & Denlinger, 2013). Most previous studies showed the effect of photoperiod on diapause of *Trichogramma* wasps (Denlinger, 2002; Saunders *et al.*, 2002; Reznik *et al.*, 2011) and few studies exposed the influence of photoperiod on biological parameters of *Trichogramma* wasps (Rounbehler & Ellington, 1973; Park *et al.*, 1999; Tuncbilek & Ayvaz, 2003; Shirazi, 2006; Reznik & Vaghina, 2007).

The aim of the present study was to test the hypothesis that the reproductive behaviour of females may modify in different photoperiods. For this purpose, we investigated the effect of photoperiod on various aspects of parasitization by *T. brassicae* females: the percentage of females that oviposited, female's longevity, fecundity, and sex ratio. This wasp is a small egg parasitoid for the biological control of some Lepidopteran key pests such as *Chilo suppressalis* (Lep: Pyralidae), *Ostrinia nubilalis* (Lep: Crambidae) and *Pieris brassicae* (Lep: Pieridae) (Ebrahimi *et al.*, 1998) which is used as a model insect in many studies (Smith, 1996). The results of this study could also be of practical importance because of the effective role of this parasitoid in biological control.

Materials and methods

In our experiments, we used a laboratory line of *Trichogramma brassicae* collected in North of Iran (Mazandaran province) and cultured for more than 100 generations on eggs of *Ephesia kuehniella* under constant laboratory conditions ($20 \pm 1^\circ\text{C}$, 18L: 6D, $70 \pm 5\%$ RH). The species identity was checked by Dr. N. Poorjavad (Plant Protection Department, Isfahan University of Technology, Isfahan, Iran).

To start the experiment, 50 *E. kuehniella* eggs were glued by non-toxic and water-soluble glue (Canco) on 9 cardboard paper strips (8 cm \times 1 cm) and subjected to parasitization by 30 mated *T. brassicae* females for 4 h in transparent, plastic cylinders (approximately 18 cm tall \times 8 cm diameter) with an opening place that covered with a mesh in order to ventilation. We sprayed 20% honey water on the wall of cylindrical to feed *T. brassicae* adults. Then the cards with parasitized host eggs were individually incubated at the same temperature

conditions (20°C) and $70 \pm 5\%$ RH but under different 24-h light-dark conditions (0:24, 3:21, 6:18, 9:15, 12:12, 15:9, 18:6, 21:3, 24:0 L: D).

After the mass emergence of G_1 adults, an equal number of mated females (20) from each photoperiodic regime were individually placed into (1.5 cm \times 10 cm) glass tubes that contained 20% honey water for adult's nutrition. A small paper card with ca 50 *E. kuehniella* eggs was placed in each tube. After 24 h, each female was checked to determine whether it is still alive or not. All females were presented with a possibility to parasitize for 24 h. New portions of fresh *E. kuehniella* eggs were presented for another 24 h. For each replicate of each photoperiod, the percentage of ovipositing females, fecundity, longevity and sex ratio (the number of female/female+male) was calculated. As *Trichogramma* females usually lay only one egg in each *E. kuehniella* egg (Garcia & Tavares, 2001), the number of parasitized hosts (darken *E. kuehniella* eggs) was taken to be the number of eggs laid by *T. brassicae*.

The effect of photoperiod on fecundity, longevity and sex ratio were checked for simple linear regression. All calculations were performed using SAS, version 9.2. statistical software.

Results and discussion

The results revealed only a slight (although significant) increase in longevity of females that were kept under absolute darkness which followed by day length 15L: 9D (Table 1). In this experiment, the mean lifetime of the females that oviposited in the absolute darkness was 20.33 ± 2.45 days as the longest lifetime, while that of the females that did oviposit in the 18L: 6D was 6.75 ± 1.37 as the shortest lifetime (Fig. 1, $F = 3.77$, $R^2 = 0.03$, $P = 0.05$). The average longevity of *T. principium* females under ultra-short (3L: 21D) photoperiod was more than long (15L: 9D) photoperiod (Reznik & Vaghina, 2006b). According to the previous studies, the effect of photoperiod on longevity is different based on *Trichogramma* species or strain. For the first time, Rounbehler & Ellington (1973) demonstrated that one strain of *T. semifumatum* had more longevity at the day length of 14L: 10D however another strain had higher longevity at 10L: 14D.

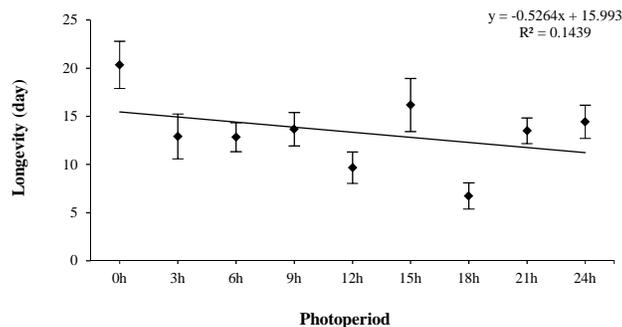


Fig. 1. The effect of different photoperiods (0L: 24D, 3L: 21D, 6L: 18D, 9L: 15D, 12L: 12D, 15L: 9D, 18L: 6D, 21L: 3D and 24L: 0D) experienced during development and during contact with the host on the longevity of *T. brassicae* females.

Mean daily fecundity of *T. brassicae* females usually ranged between 9 up to 21 eggs at the first day of oviposition, and then sharply declined in the second day in all the photoperiod regimes (Fig. 2). Fecundity of *T. brassicae* females (the number of parasitized eggs of *E. kuehniella*) was independent of photoperiod (Fig. 3, $F = 1.09$, $R^2 = 0.01$, $P = 0.29$). The maximum oviposition was observed under ultra-long 21L: 3D (73.92 ± 7.12) photoperiod which followed by absolute darkness (73.83 ± 7.21 eggs/female) indicates a positive relationship between longevity and fecundity of *T. brassicae* females (Table 1). Shirazi (2006) showed that photoperiod significantly affected all the biological parameters in *T. chilonis* and the fecundity, longevity and adult emergence of *T. chilonis* increased with the increase in day length.

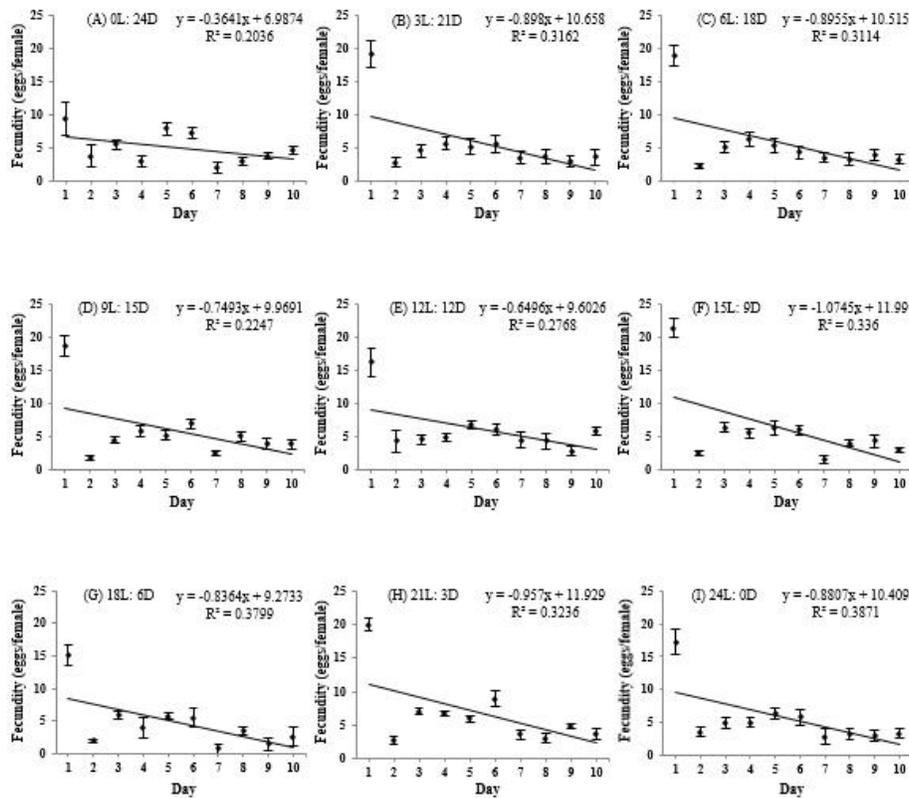


Fig. 2. The effect of photoperiod experienced during development and oviposition on the fecundity of *T. brassicae* females during the first 10 days of their life, (A) 0L: 24D; (B) 3L: 21D; (C) 6L: 18D; (D) 9L: 15D; (E) 12L: 12D; (F) 15L: 9D; (G) 18L: 6D; (H) 21L: 3D and (I) 24L: 0D photoperiods. The symbols represent mean fecundity between the first and tenth days of oviposition.

However an interesting result obtained about the maximum and minimum fecundity in the first day and total, minimum fecundity happened in absolute darkness in the first day while it was the second grade of fecundity in total. The same oviposition pattern was observed in all the day lengths during the first 10 days of their contact with the host (Fig. 2). Zaslavski & Quay (1982) showed that maximum fecundity happened at long-time day length (16L: 8D)

in *T. chilonis* and *T. evanescens*. Similar results were later obtained for *T. dendrolimi* Matsumura (Park *et al.*, 1999). However, our previous study showed that maximum and minimum fecundity of *T. embryophagum* happened in 6L: 18D day length and absolute darkness, respectively which shows opposite results with the *T. brassicae* (Rahimi *et al.*, 2014). Similar to our results, the fecundity of *T. pretiosum* (Calvin *et al.*, 1984) and *T. galloi* Zucchi (Consoli & Parra, 1994) were independent of day lengths. Also, *T. evanescens* adults kept in total darkness showed less fecundity during the 1st day, but during the 3rd day their fecundity was more than females kept in other photoperiod regimes (Tuncbilek & Ayvaz, 2003) while fecundity in *T. minutum* Riley is not affected by developing in darkness (Corrigan *et al.*, 1994).

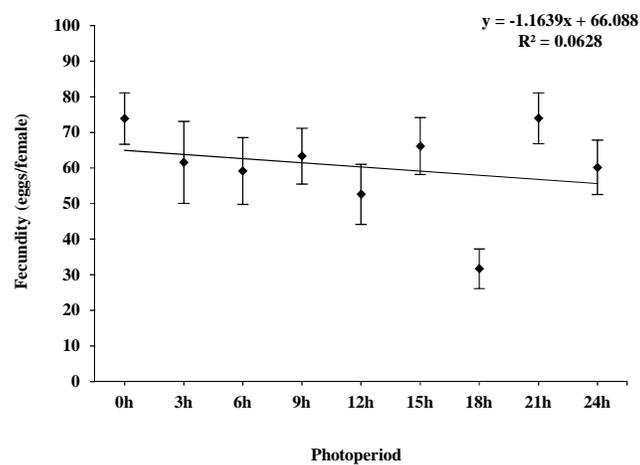


Fig. 3. The effect of different photoperiods (0L: 24D, 3L: 21D, 6L: 18D, 9L: 15D, 12L: 12D, 15L: 9D, 18L: 6D, 21L: 3D and 24L: 0D) experienced during development and during contact with the host on the fecundity of *T. brassicae* females.

The results revealed significant dependence of the number of female progeny (sex ratio) on the photoperiodic conditions experienced during development and oviposition ($F = 5.13$, $R^2 = 0.05$, $P = 0.02$). The sex ratio (number of female progeny/female+male) produced was also significantly higher at day length 18L: 6D (0.69 ± 0.03) in comparison with other day lengths which followed by 12L: 12D (0.64 ± 0.08) and 24L: 0D (0.64 ± 0.08) photoperiods (Fig. 4). The results of the number of female offspring showed significant differences in the third day ($F = 5.09$, $R^2 = 0.05$, $P = 0.02$) of oviposition but it did not show a significant differences in the first, second, fourth, fifth, sixth, seventh, eighth, ninth and tenth day of oviposition. Similar to the results of this study, the maximum number of female offspring in *T. embryophagum* had been seen in 18L: 6D day length (Rahimi *et al.*, 2014). As shown in Figure 5, the number of female offspring decreases and increases alternatively in the darkness, ultra-short (3L: 21D and 6L: 18D) and short (9L: 15D and 12L: 12D) photoperiods but it does not show a particular pattern in long (15L: 9D and 18L: 6D) and ultra-long (21L: 3D and 24L: 0D) photoperiods.

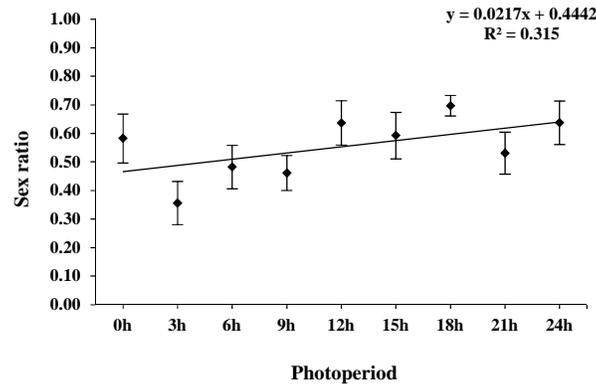


Fig. 4. The effect of different photoperiods (0L: 24D, 3L: 21D, 6L: 18D, 9L: 15D, 12L: 12D, 15L: 9D, 18L: 6D, 21L: 3D and 24L: 0D) experienced during development and during contact with the host on the sex ratio of *T. brassicae* females.

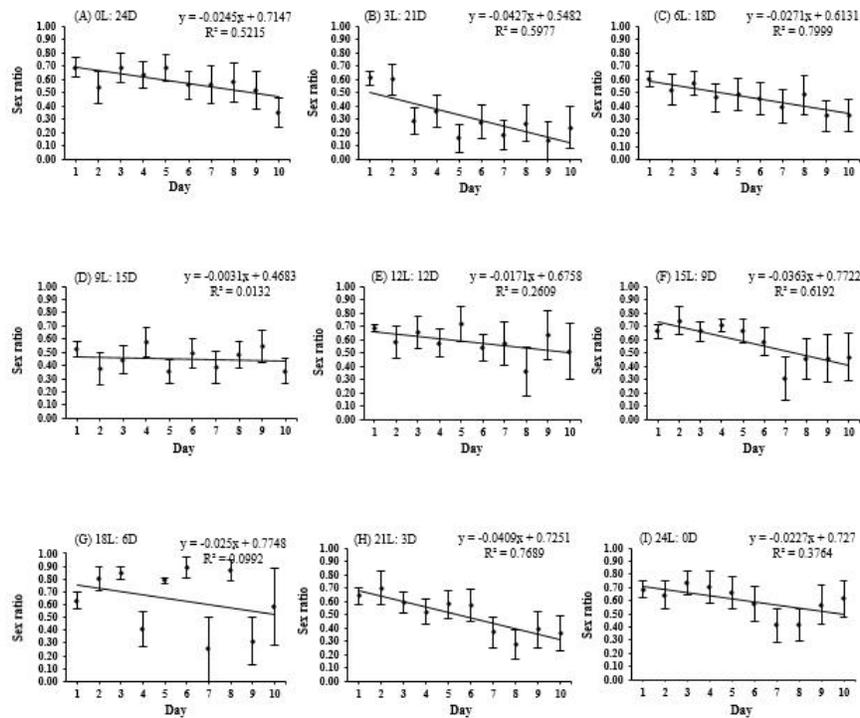


Fig. 5. The effect of photoperiod experienced during development and oviposition on the sex ratio of *T. brassicae* females during the first 10 days of their life, (A) 0L: 24D; (B) 3L: 21D; (C) 6L: 18D; (D) 9L: 15D; (E) 12L: 12D; (F) 15L: 9D; (G) 18L: 6D; (H) 21L: 3D and (I) 24L: 0D photoperiods. The symbols represent mean sex ratio proportion between the first and tenth days of oviposition.

Although most females started oviposition during first days after contact with the host but a few of them especially in the short term photoperiods did not oviposit initially but

started to oviposit 3-5 days later, which shows a delay in oviposition. About 25%, 33.33%, 8.33% and 33.33% of females did not oviposit in the first, second, third and fourth days of oviposition in the absolute darkness. Similar results were observed at 3L: 21D and 6L: 18D photoperiod, about 8.33%, 20%, 10% and 22.22% of females did not oviposit in the first, third, fourth and fifth days of oviposition in 3L: 21D day length and about 18.18%, 18.18%, 9.09% and 20% of females did not oviposit in the second, third, fourth and fifth days of oviposition in day length 6L: 18D. A few females about less than 10% did not oviposit in the first and second days of their life under day length 12L: 12D. Some researchers suggest that this delay is dependent on photoperiod. Similarly, Park *et al.* (1999) indicated that *T. dendrolimi* females that developed under 8L: 16D laid much fewer eggs during the first day of contact with the host than those reared under a longer day length, and their daily fecundity was higher during the following days.

In conclusion, the effect of photoperiod on factors such as fecundity, longevity, sex ratio and the percentage of females that oviposited could be different based on *Trichogramma* species or even strain which must be studied to improve the mass rearing of these useful biological agents.

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