Research Article

Suitability of different egg ages of *Ephestia kuehniella* (Lep.: Pyralidae) for the development, reproduction and life table parameters of *Trichogramma evanescens* (Hym.: Trichogrammatidae)

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Abstract: *Trichogramma evanescens* Westwood is an important biological control agent of lepidopteran pests and is widely distributed throughout Iran and neighboring countries. Laboratory studies were conducted to determine the influence of *Ephestia kuehniella* Zeller eggs age on the number of parasitized eggs, development time, sex ratio, progeny longevity and fecundity. Understanding this influence is important for developing biological control programs. Mated female parasitoids (24 h age) were provided with 1, 2, 3, and 4-days-old *E. kuehniella* eggs in no-choice experiments, individually. *T. evanescens* developed on *E. kuehniella* eggs of all ages tested, while showing a better adaptation to younger host eggs with significantly faster developmental time, higher survival and more female progeny on 1-day-old eggs. Progeny emerged from 1-day-old eggs had also higher longevity and fecundity than those emerging from other host ages tested. The intrinsic rate of increase (*r*) values of *T. evanescens* reared on 1, 2, 3 and 4-days-old *E. kuehniella* eggs were 0.345, 0.322, 0.281 and 0.233 day^{-1} and the mean generation time (*T*) was 12.19, 12.13, 12.01 and 11.82 days, respectively. The current study provides useful information to use suitable host age of *E. kuehniella* for mass production of *T. evanescens*.

Keywords: egg parasitoid, host age, development time, sex ratio, the intrinsic rate of increase, the mean generation time

Introduction

The egg parasitoids belonging to the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) are important biological control agents extensively used against several lepidopterous pests. Augmentation of *Trichogramma* species is used in more than 32

million hectares of different agricultural systems for controlling various lepidopteran pests (Li, 1994; Smith, 1996). These parasitoids are commercially produced and used in China, Columbia, USA, various European countries and India (Wajnberg and Hassan, 1994). The augmentative release of Trichogrammatid parasitoids has reduced pest damage by 77-92% in some crops such as sugarcane, wheat, corn and cabbage in several countries including China, Switzerland, Canada and the former USSR (Li, 1994, Parra, 2010).

Parasitoids of the genus *Trichogramma* are important natural enemies in biological control

and widely distributed in Iran. Modarres Aval (2012) listed 14 species for *Trichogramma* genus from Iran. This parasitoid is mass produced and released against several lepidopteran pests including *Chilo suppressalis* Walker in rice, *Helicoverpa armigera* Hubner in cotton and tomato, *Tuta absoluta* (Meyrick) in tomato and *Ectomyelois ceratoniae* (Zeller) in pomegranate (Ahmadipour *et al*., 2015; Ebrahimi *et al*., 1998; Ebrahimi, 2004; Poorjavad *et al*., 2011).

Vinson (1976) stated that the age of the host is an important factor which has a considerable effect on the biological and physiological aspects of parasitoids. Pak (1986) demonstrated that the age of the host eggs had a significant influence on its acceptability and suitability for *Trichogramma* species. In the majority of *Trichogramma* species, the number of parasitized hosts decreased as host age increased (Hintz and Andow, 1990; Reznik and Umarova, 1990; Pizzol *et al*., 2012).

Two factitious hosts, Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) are mostly used as hosts for mass production of *Trichogramma* species. Several countries including France, Canada and Iran use *E. kuehniella* as host because of better production from rearing medium and improving sanitation condition (Smith, 1996). Furthermore, better performance of *Trichogramma* species that reared on *E. kuehniella* has been attributed to the larger size of their egg compared with the eggs of *S. cerealella* (Hassan *et al*., 1978; Bigler *et al*., 1987; Bigler, 1988; Corrigan and Laing, 1994).

Trichogramma evanescens Westwood and *Trichogramma brassicae* Bezdenko are the most important biological control agents of lepidopteran pests in Iran. These parasitoids are sibling species and in morphological characteristic are similar (Ebrahimi *et al*., 1988). Review of literature shows that the biological characteristics of *T. brassicae* parasitizing *E. kuehiniella* eggs have been studied by several researchers (Bigler*et al*., 1987; Fleury and Bouletreau, 1993; Iranipour *et al*., 2009; Ozder and Kara, 2010; Lashgari, *et al*., 2010), however, no detailed information regarding the effect of different factors such as *E. kuehniella* egg age on

life history and life table parameters of *T. evanescens* is available. In the present study, the effect of host-egg age of *E. kuehniella* on host selection and suitability by *T. evanescens* was evaluated to determine the appropriate host age for mass production of this parasitoid.

Materials and Methods

Insect collection
A laboratory culture of *T. evanescens* was established from parasitoid wasps, collected from a citrus orchard using *E. kuehniella* eggs during July 2017 in Baghmalek city (31º38′03″N, 49º53′05″E), Khuzestan province. Parasitoids were reared at 25 ± 1 °C, a photoperiod of 16:8 (L: D) h and 55 ± 5% RH using *E. kuehniella* eggs as host. The *E. kuehniella* eggs used in this study were obtained from a colony kept at Golestan Mooud insectary company, Ahvaz, Iran. Using morphological and molecular characteristic (unpublished data), the third author identified the parasitoids to be *T. evanescens*. Parasitoids were reared for five to six generations in laboratory conditions and then were used for the experiment. Voucher specimens of *T. evanescens* were deposited in the Shahid Chamran University of Ahvaz arthropod collection (20-25 °C, 40-50% RH).

Experimental procedure
A preliminary experiment showed that at 25 °C, *E. kuehniella* eggs take 5 days to hatch. Consequently, 1, 2, 3 and 4-days-old *E. kuehniella* eggs were selected as test hosts (Tabebordbar, unpublished data). The newly emerged *T. evanescens* adult was allowed to mate for 8 h and then introduced into a clear glass tube (Diameter 1 cm, Height 10 cm) containing egg mass of *E. kuehniella*. The glass tubes were sealed with cotton-wool. Egg masses of *E. kuehniella* used in our experiments contained 40 ± 1 eggs. Egg masses were glued on a piece of white paper (5 by 1 cm). Parasitoids had no contact with host eggs before the tests. Female parasitoids were fed with droplets of honey deposited in the internal part of each tube during the experiments. The female parasitoid was allowed to parasitize *E.*

kuehniella eggs in a growth chamber (25 ± 1 °C, 55 ± 5% and a photoperiod of 16: 8 L: D h) for 24 h. The parasitoid was removed after 24 h and tubes were kept in the incubator until all the parasitoid's progeny emerged. Five replications were used for each egg age. The experiment was carried out in a completely randomized design. Parameters recorded were the number of parasitized eggs, juvenile developmental time of female and male parasitoids, number of emerged parasitoids and percentage of females in the progeny.

The effect of different host age on longevity and fecundity of progenies was studied by placing one adult female and one adult male (< 24 h old), obtained from the previous experiment in a glass tube (similar to tubes as described in the previous experiment) containing an egg mass of 40 ± 1-day-old *E. kuehniella* eggs and honey-water solution (10%). Males were replaced in case they died in the experiment. New eggs were offered daily to each female until all females died. A completely randomized design with 38 replications for each treatment (host age) was used. This experiment was also conducted in a growth chamber (25 ± 1 °C, 55 ± 5% R. H. and 16: 8 L: D). Longevity and fecundity of adult parasitoids were calculated. Those females which were injured during daily handling or the ones that died because of being submerged in honey droplets were excluded from data analysis.

Statistical analysis
The effect of egg age on the number of parasitized eggs, developmental time of offspring, the percentage of adult emergence and percentage of female progeny were analyzed by a one-way ANOVA using general linear model (PROC GLM), and the means were separated using Tukey's honestly significant difference test at P < 0.05. The percentage values of adult emergence (survival rate) and female progeny were arcsine square root transformed to homogenize variances before an ANOVA was performed. All statistical analysis was performed using statistical software package SPSS Version 21.

The life history data from each host age were analyzed based on the age-stage, two sex life table

(Chi & Liu, 1985; Chi, 1988) using TWOSEX MSchart computer program (Chi, 2016). For each host age, we determined the age-stage specific survival rate (l_x), the age-stage specific fecundity (m_x) and other stage and age Chi (1988). The age-specific survival rate (l_x) was then calculated as:

$$l_x = \sum_{j=1}^{K} S_{xj}$$

Where k is the number of stages. The age-stage specific fecundity (m_x) was calculated as:

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}}$$

The population parameters, the intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), mean generation time (T), were estimated in sequence. The intrinsic rate of increase was determined by iteratively solving the Euler-Lotka equation with age indexed from 0 (Goodman, 1982):

$$\sum_{x=0}^{\omega} e^{-r(x+1)} l_x m_x = 1$$

The finite rate of increase (λ) were calculated as follows:

$$\lambda = e^r$$

The mean generation time (T) is then calculated using the following equation:

$$T = \frac{L_n R_0}{r}$$

The bootstrap technique was used to estimate the means, variances, and standard errors of the population parameters (Efron and Tibshirani, 1993). As bootstrap uses random resampling, a small number of replications will generate variable means and standard errors. To generate less variable results, 100,000 replications were used in this study (Huang and Chi, 2013; Chi, 2016).

Results

The host age had a significant effect on the mean number of parasitized eggs by *T. evanescens* (F =

62.27; df = 3, 12; P < 0.001) (Table 1). The number of parasitized eggs decreased as egg age increased. Mean number of 1-day-old parasitized eggs by *T. evanescens* was more than two times higher than 4-days-old eggs.

Egg-to-adult development times for *T. evanescens* varied significantly with host age (females: F = 7.48; df = 3, 116; P < 0.05; males: F = 23.21; df = 3, 116; P < 0.001) (Table 1). Developmental time of *T. evanescens* females and males were shortest on 1-day-old host eggs. There were no significant differences in development times of females between 1-day-old and 2-day-old host eggs and also between 3-day-old and 4-day-old (Table 1).

Egg-to-adult survivorship was significantly affected by host age (F = 107.15; df = 3, 12; P < 0.001) (Table 1). Survival rate decreased as egg age increased.

The percentage of females was significantly affected by host age (F = 161.44; df = 3, 12; P < 0.001) (Table 1). A female-biased sex ratio was observed on all egg ages tested, except 4-days old eggs.

The adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), oviposition period, female and male longevity, daily and total fecundity of *T. evanescens* reared on different age *E. kuehniella* eggs are presented in Table 2. No preoviposition period was observed for the female parasitoids emerged from different egg ages examined. Host age significantly influence the total pre-oviposition period (TPOP), oviposition period, longevities of females and males. Longest lived female parasitoid emerged from 1-day-old eggs (11 days). The mean daily and total number of eggs

oviposited by *T. evanescens* females emerged from different host ages were significantly different (Table 2). Female *T. evanescens* oviposited mean number of 85.76 eggs on 1-day-old *E. kuehnilella* eggs compared to 19.75 eggs on 4-day-old host eggs (about 4 times higher).

Life table parameters statistics emphasized that 1-day-old eggs were a preferable host age for *T. evanescens* compared with other host ages tested (Table 3). As a consequence of accelerated development duration and great fecundity early in adulthood, *T. evanescens* reared on 1-day-old eggs had a significantly greater gross reproduction rate (*GRR*), net reproductive rate (R_0), the intrinsic rate of natural increase (*r*) and finite rate of increase (*λ*) than those bred on other host ages (Table 3). In addition, the longest and shortest mean generation time of *T. evanescens* were obtained on the 1-day-old eggs and 4-day-old eggs, respectively.

The age-stage-specific survival rate (s_{xj}) of *T. evanescens* which represents the probability of a newborn surviving to age x and stage j, is shown in Figure 1 on different age of *E. kuehniella* eggs. The survival rate of *T. evanescens* on 1-day-old egg was relatively higher than on the others.

Age-specific survivorship (l_x), age-stage specific fecundity (f_x) and age-specific fecundity (m_x) of *T. evanescens* reared on different age of *E. kuehniella* egg are presented in Figure 2. The maximum value of f_x was 26.75, 21.4, 15.5 and 9 for the *T. evanescens* reared on 1, 2, 3 and 4 days, respectively, which occurred on the 9th, 9th, 9th and 10th day of the life span, respectively.

Table 1 Mean number of parasitized eggs, development time, survival rate and sex ratio (Female %) (± SE) of *Trichogramma evanescens* parasitizing *Ephestia kuehniella* eggs of different ages.

Parameters	Host age (day)			
	1	2	3	4
Number of parasitized eggs	27.60 ± 0.49[a]	21.67 ± 0.65[b]	18.86 ± 0.76[c]	13.54 ± 0.96[d]
Female development time	9.83 ± 0.09[a]	10.00 ± 0.04[ab]	10.23 ± 0.10[bc]	10.50 ± 0.12[c]
Male development time	8.15 ± 0.15[a]	8.25 ± 0.12[a]	9.00 ± 0.12[b]	9.45 ± 0.05[c]
Sex ratio (% female)	0.81 ± 0.016[a]	0.65 ± 0.011[b]	0.55 ± 0.009[c]	0.44 ± 0.012[d]
Survival rate	83.33 ± 1.36[a]	62.02 ± 2.18[b]	51.03 ± 2.12[c]	33.39 ± 2.37[d]

Means in each row followed by the same letter(s) are not significantly different at *P*< 0.05 (Tukey test).

Table 2 Mean (± SE) of adult preoviposition period (APOP), total preoviposition period (TPOP), oviposition period, female and male longevity (day), daily and total fecundity of *Trichogramma evanescens* reared on different age *Ephestia kuehniella* eggs.

| Parameters	Host age (day)			
	1	2	3	4
APOP	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
TPOP	9.82 ± 0.09^b	9.96 ± 0.07^{ab}	9.96 ± 0.06^{ab}	10.17 ± 0.06^a
Oviposition period	6.93 ± 0.10^a	5.79 ± 0.02^b	5.03 ± 0.00^c	4.47 ± 0.09^c
Female longevity	10.23 ± 0.11^a	10.16 ± 0.18^a	9.73 ± 0.10^b	9.03 ± 0.11^c
Male longevity	7.46 ± 0.15^a	7.30 ± 0.17^a	6.76 ± 0.12^b	6.56 ± 0.13^b
Daily fecundity	8.67 ± 0.15^a	6.04 ± 0.12^b	4.40 ± 0.16^c	2.30 ± 0.12^d
Total fecundity	85.76 ± 1.01^a	59.79 ± 1.06^b	40.77 ± 0.86^c	19.75 ± 0.80^d

Means in each row followed by the same letter(s) are not significantly different at $P < 0.05$ (paired bootstrap test).

**Table3** Life table parameters of *Trichogramma evanescens* reared on different age of *Ephestia kuehniella* eggs.

Parameters	Host age (day)			
	1	2	3	4
GRR (offspring/individual)	68.77 ± 3.19^a	49.19 ± 1.99^b	31.66 ± 2.08^c	16.93 ± 1.80^d
R_0 (offspring/individual)	67.70 ± 4.59^a	48.17 ± 2.99^b	30.57 ± 2.28^c	15.92 ± 1.17^d
r (day^{-1})	0.345 ± 0.006^a	0.322 ± 0.005^b	0.281 ± 0.006^c	0.233 ± 0.007^d
λ (day^{-1})	1.41 ± 0.008^a	1.38 ± 0.007^b	1.32 ± 0.008^c	1.26 ± 0.008^d
T (day)	12.19 ± 0.07^a	12.13 ± 0.07^a	12.01 ± 0.08^a	11.82 ± 0.01^b

R_0: Net reproduction rate, *r*: Intrinsic rate of increase, *λ*: Finite rate of increase, *T:* Mean generation time.
Means in each row followed by the same letter are not different using paired bootstrap procedure ($P < 0.05$).
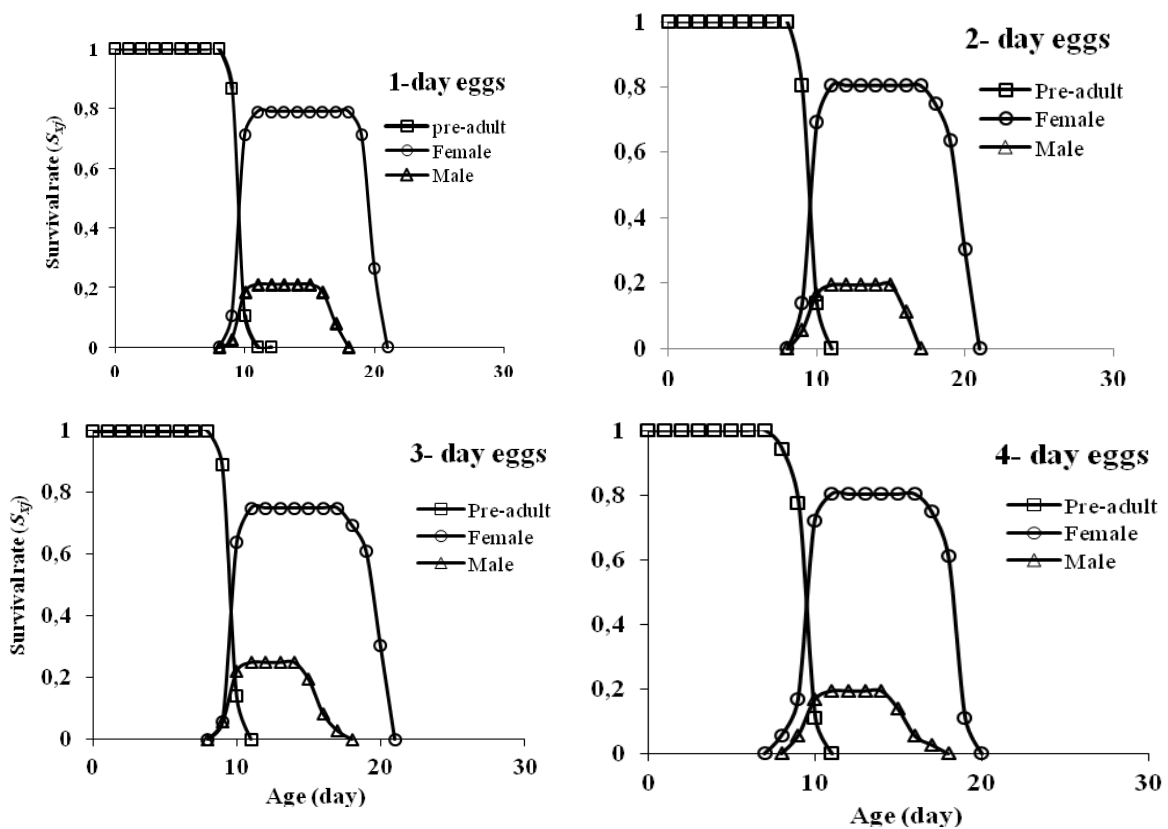
Figure 1 Age-stage survival rate (s_{xj}) of *Trichogramma evanescens* reared on different age of *Ephestia kuehniella* eggs.
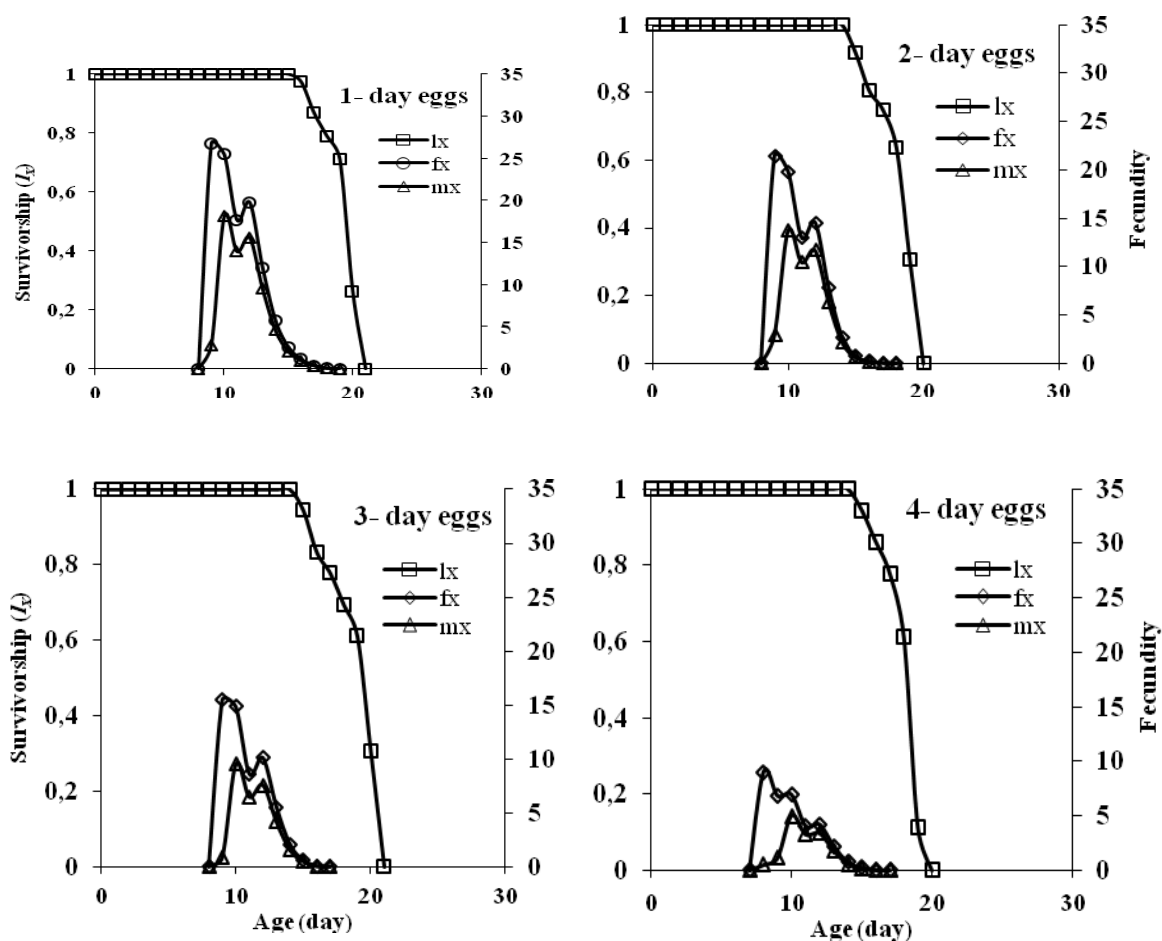
Figure 2 Age-specific survivorship (l_x), age-stage fecundity (f_x), and age-specific fecundity (m_x) of *Trichogramma evanescens* reared on different age of *Ephestia kuehniella* eggs.

Discussion

Age of the host egg is an important factor which affects the availability, acceptability and suitability of various hosts for several *Trichogramma* species (Pak, 1986). Generally, younger host eggs are more frequently accepted for parasitism than older host eggs. In the current study, the differently aged host eggs of *E. kuehniella* were all accepted by *T. evanescens*. However, the number of parasitized eggs by *T. evanescens* on 1-day-old eggs of *E. kuehniella* were up to 2 fold higher compared with all other egg age categories, showing that 1-day-old host eggs are more easily used by *T. evanescens* for oviposition. Similar results have been reported for other Trichogrammatid species (Schmidt, 1994).

T. evanescens was able to develop faster in young eggs compared with old eggs. Similarly, Godin and Boivin (2000) also found that *Trichogramma* spp. developed and survived best in young eggs. As an egg gets older and during the different stages of embryogenesis, the nutrients available in the host egg are gradually incorporated and assimilated by the embryo, as a consequence, egg suitability for parasitoid development decline (Ruberson and Kring, 1993). Furthermore, Guang and Oloo (1990) stated that *Trichogramma* is sometimes unable to develop in old eggs. This phenomenon has been attributed either to the rotation of the host embryo or sclerotization of the head capsule (Pak, 1986). Also Pak reported that eggs of most lepidopetran host species are

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suitable for *Trichogramma* development only if less than seventy-five percent of their development has taken place.

In accordance with our findings, survival rate (emergence rate) of *T. evanescens* was also negatively affected by host age. Ruberson and Kring (1993) reported that *Trichogramma pretiosum* (Riley) developing in old *Heliothis zea* (Boddie) eggs had lower survival than those in younger eggs. Reduction in survival rate as host eggs get older may or may not result from components such as delayed development duration. For instance, no significant differences in *Trichogramma cacoeciae* Maechl, *Trichogramma principium* Sugonjaev & Sorokina and *Trichogramma evanescence* Westwood emergence from different age eggs of *Phthorimaea operculella* (Zeller) was detected, although the lowest rates of emergence were documented for the oldest eggs (Saour, 2004).

Sex ratio of *T. evanescens* reared on different age of *E. kuehniella* egg are presented in Table 1. Our findings indicated that the age of *E. kuehniella* eggs had a significant effect on progeny sex ratio of *T. evanescens*. It should be noted that the sex ratio of *T. evanescens* was biased towards females for all host egg ages tested except 4-days-old eggs. Similar to our results, Tuncbilek and Ayvaz (2003) reported that egg ages of *E. kuehniella* and *S. cerealella* had a significant effect on the sex ratio of *T. evanescens* and that younger eggs produced more female parasitoids. However, no significant effect of host egg age on sex ratio was reported for *T. brassicae* on *Trichoplusia ni* (Hubner) and *Pieris rapa* (L.) (Godin and Boivin, 2000), *Trichogramma dendrolimi* Matsumura on *Ch. Suppressalis* (Walker) (Zhang *et al*., 2013), *T. dendrolimi* on *Mamestra brassicae* (Linnaeus) (Takada *et al*., 2000), *T. cacoeciae* on *Lobesia botrana* Den. (Moreno *et al*., 2009), *T. pretiosum* (Riley) on *Diatraea grandio* sella Dyar (Calvin *et al*., 1997), *Trichogramma fuentesi* Torre on *Cactoblastes cactorum* (Berg) (Paraiso *et al*., 2012), and *Trichogramma pricipium* Sug et Sor on *S. cerealella* (Reznik*et al*., 1997) which is

different from our findings. Which is different than our findings. These differences may be explained by difference in host species, experimental conditions and different population.

At the same temperature Iranipour *et al*. (2010) reported *r* of 0.354/day for *T. brassicae* on the same host species, and same host age which is very close to the results obtained in the present study ($r = 0.345$/day) for *T. evanescens*. However, other laboratory studies have reported a variety of *r* values for *T. brassicae* including 0.504/day (Ozder and Kara, 2010) and 0.309/day (Lashgari *et al*., 2010) on the same host, same host age and at the same temperature. The discrepancy between the results of mentioned studies with current study may be due to differences in species or differences in the experimental conditions such as humidity.

T. evanescens developed faster on 1-day-old eggs and produced significantly more offspring than other egg ages tested. Therefore, it is recommended that 1-day-old eggs of *E. kuehniella* be used in mass production for *T. evanescens*. Furthermore, *T. evanescens* was able to parasitize a range of egg ages around its most preference; for a certain age of a host species may be a limiting factor in the performance of parasitoids under field conditions. The relatively broad host-age preference is helpful for the effectiveness of the parasitoids since different host egg ages usually exist simultaneously in the field.

Acknowledgements

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اثر سن تخم (Lepidoptera: Pyralidae) *Ephestia kuehniella* بر رشد و پارامترهای جدول زندگی *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae)

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چکیده: زنبور تریکوگراما Westwood *Trichogramma evanescens* یکی ازمهم‌تـرین عوامـل کنتـرل بیولوژیک تخم بال‌پولک‌داران می‌باشد که در سرتاسر ایـران گـسترش دارد. در ایـن مطالعـه اثـر سـنین مختلف تخم شب‌پره آرد Zeller *Ephestia kuehniella* بر تعداد تخم‌های پارازیته شده، طول دوره رشـد پیش از بلوغ، نسبت جنسی، طول عمر و زادآوری *T. evanescens* ارزیابی شد. درک این اثر در تکـوین برنامه‌های کنترل بیولوژیک اهمیت دارد. چهار سن تخم شامل ۱، ۲، ۳ و ۴ روزه *E. kuehniella* (بـدون قدرت انتخاب) در اختیار یک ماده *T. evanescens* جفت‌گیری کرده با عمـر کـم‌تـر از ۲۴ سـاعت قـرار گرفت. زنبور *T. evanescens* در تمامی تخم‌های مورد آزمایش رشد نموده و خارج گردیـد. درحـالی‌کـه زنبور تطابق بهتری با تخم‌های میزبان جوان داشت و این حالت را با زمان رشد سریع‌تر، بقای بیش‌تـر و نوزادان ماده بیش‌تر در تخم‌های یک روزه نشان داد. هم‌چنین نوزادان خارج شده از تخـم‌هـای میزبـان یک روزه طول عمر و زادآوری بیش‌تری نسبت به نوزادان خارج شده از سایر سنین تخـم داشـتند. نـرخ ذاتی افـزایش جمعیـت (*r*) زنبـور در سـنین ۱، ۲، ۳ و ۴ روزه بـه‌ترتیـب ۰/۰۰۶ ± ۰/۳۴۵، ۰/۰۰۵ ± ۰/۳۲۲، ۰/۰۰۶ ± ۰/۲۸۱ و ۰/۰۰۷ ± ۰/۲۳۳ ماده/ماده/روز و طول دوره یک نسل (*T*) به‌ترتیب ۰/۰۷ ± ۱۲/۱۹، ۰/۰۷ ± ۱۲/۱۳، ۰/۰۸ ± ۱۲/۰۱ و ۰/۰۱ ± ۱۱/۸۲ روز به‌دسـت آمـد. نتـایج ایـن مطالعـه اطلاعات مفیدی در مورد استفاده از سن مناسب تخم *E. kuehniella* جهت پرورش انبوه *T. evanescens* فراهم می‌نماید.

واژگان کلیدی: پارازیتوئید تخم، رشد و نمو، نسبت جنسی، نرخ ذاتی افزایش جمعیت، طول دوره یک نسل