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)

Fatemeh Tabebordbar¹*, Parviz Shishehbor¹ and Ebrahim Ebrahimi²

1. Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Iranian Research Institute of Plant Protection (IRIPP), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

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 *Ephesia 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
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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., 2010).

Two factitious hosts, Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and Mediterranean flour moth, *Ephhestia 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. kuehniella* 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 (31°38’03”N, 49°53’05”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 Moomd 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 those 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 TWOPSEX 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 ($\lambda$), 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):

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

The finite rate of increase ($\lambda$) were calculated as follows:

$$\lambda = e^r$$

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

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

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 =
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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 (TOPP), 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 parasites reared from different egg ages examined. Host age significantly influence the total pre-oviposition period (TOPP), oviposition period, longevity 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. kuehniella 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 (R0), 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_{ij}) 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_{ix}) age-stage specific fecundity (f_{ij}) and age-specific fecundity (m_{ix}) of T. evanescens reared on different age of E. kuehniella egg are presented in Figure 2. The maximum value of f_{ij} 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Host age (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Number of parasitized eggs</td>
<td>27.60 ± 0.49^a</td>
</tr>
<tr>
<td>Female development time</td>
<td>9.83 ± 0.09^a</td>
</tr>
<tr>
<td>Male development time</td>
<td>8.15 ± 0.15^a</td>
</tr>
<tr>
<td>Sex ratio (% female)</td>
<td>0.81 ± 0.016^a</td>
</tr>
<tr>
<td>Survival rate</td>
<td>83.33 ± 1.36^a</td>
</tr>
</tbody>
</table>

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.

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

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

Table 3 Life table parameters of *Trichogramma evanescens* reared on different age of *Ephestia kuehniella* eggs.

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

$R_0$: Net reproduction rate, $r$: Intrinsic rate of increase, $\lambda$: 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 ($S_{i,j}$) of *Trichogramma evanescens* reared on different age of *Ephestia kuehniella* eggs.
Suitability of *E. kuehniella* for *T. evanescens*  

**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
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 pricipium* 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, Tunebilek 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. Supressalis* (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* (Rezniket 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.

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**References**

Suitability of E. kuehniella for T. evanescens


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اثر سن تخم بر رشد و پارامترهای جدول Ephesia kuehniella (Lepidoptera: Pyralidae) و Trichogramma evanesens (Hymenoptera: Trichogrammatidae) 

فاظهه تابی بردار، پرویز شیشه بر و ابراهیم ابراهیمی 

1- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه شهید جهان امیر اهواز، اهواز، ایران.
2- مؤسسه تحقیقات گیاهپزشکی کشور تهران، ایران.

پست الکترونیکی: نوبستگان مسئول مکاتب: fatemeh.tabebordbar@yahoo.com

تاریخ: 22 مرداد 1397; پذیرش: 4 شهریور 1398

چکیده: زنبور تریکرگکا Trichogramma evanesens Westwood بیولوژیکا تخم بالموند داران می‌باشد که در سرتاسر ایران گسترش دارد. در این مطالعه اثر سنین بر تعداد تخم‌های پاپازشته شده، طول دوره رشد پیش از بلخ، نسبت جنسی، طول عمر و زادآوری ارزیابی شد. درک این اثر در تکوین Trichogramma evanesens از این نظر، به‌عنوان یکی از مهم‌ترین عوامل کنترل به‌شمار می‌رود. این اثر طی اثرات مثبتی در محیط ساختن می‌باشد و به‌ویژه در سنین پس از بلوغ، به‌صورت مثبتی در پرورش و زادآوری این زنبور تنظیم می‌شود.

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