



**The effect of temperature on the bionomics of *Trichogramma euproctidis*  
(Hym.: Trichogrammatidae) parasitizing the tomato fruitworm,  
*Helicoverpa armigera* (Lep.: Noctuidae)**

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**Abstract**

In this study, the life history characteristics of *Trichogramma euproctidis* (Girault), an established egg parasitoid species in southwestern Iran, parasitizing *Helicoverpa armigera* (Hubner) were examined at 18, 21, 24, 27, 30, and 33 °C. The results showed that different constant temperatures significantly affected the number of parasitized eggs, development time, sex ratio, tibial length, number of parasitoids per host egg, progeny longevity, and fecundity. *T. euproctidis* failed to complete development at 18 °C, the lowest temperature tested. The mean developmental duration from egg to adult female decreased from 15.33 days at 21 °C to 7.25 days at 33 °C. An average of 188-degree days was required to complete development above the lower threshold temperature (7.2 °C). Survivorship was 96.20, 97.20, 98.33, 85.46, and 82.22 % at 21, 24, 27, 30, and 33 °C, respectively. The mean longevity of *T. euproctidis* ranged from 11.60 days at 21 °C to 4.57 days at 33 °C. Mean total progeny ranged from 19.50 / female at 33 °C to 168.70 / female at 21 °C. Data analysis demonstrated that different constant temperatures had a significant effect on the net reproductive rate ( $R_0$ ), intrinsic rate of increase ( $r$ ), finite rate of increase ( $\lambda$ ), and generation time ( $T$ ). The intrinsic rate of increase ( $r$ ) improved with temperature from 0.240/day at 21 °C to 0.370/day at 27 °C and then decreased at higher temperatures. Generation time decreased from 16.90 days to 7.53 days with increasing temperature. The optimal temperature for development and reproduction of *T. euproctidis* was 27 °C. The results of this study showed that this strain of *T. euproctidis* appears to have the potential to be utilized in integrated management programs targeting *H. armigera*.

**Keywords:** *Helicoverpa armigera*; *Trichogramma euproctidis*; development; survival; intrinsic rate of increase

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The Khuzestan province in southwestern Iran has a typically hot and dry climate, with mean maximum temperatures of 30 °C from April-June and September- November, relative humidity of 35-40%, and rainfall slightly above 50 mm during these periods (Arianejad & Hosseinpour, 2011). Tomato is one of Khuzestan's leading spring and fall vegetable crops, covering approximately 9,381 ha and yielding 345,774 tons (Ahmadi et al., 2021). The area devoted to tomato cultivation is expanding as more land is irrigated.

The tomato fruitworm *Helicoverpa armigera* (Hubner) is the most significant tomato pest in the Khuzestan province, consuming the interior of the tomato fruit and leaving behind a cavity containing fluid and feces (Khanjani, 2005). Consequently, the damaged tomato decays and rots rapidly. Once tomato fruit has been attacked by fruitworms, it is no longer edible (Behdad, 1982; Metcalf & Metcalf, 1993; Khanjani, 2005; Hemati et al., 2012).

Synthetic chemical insecticides play an important role in *H. armigera* management. However, *H. armigera*'s resistance to conventional insecticides has been reported (Armes et al., 1996; Ahmad et al., 1999; Mossallanezhad et al., 2003; Bues et al., 2005). To this end, using biological control agents as an alternative to conventional insecticides has been recommended to manage *H. armigera* (Cherry et al., 2003). Developing methods to preserve and employ native natural enemies, especially predators and parasitoids that attack egg and larval stages, is crucial for establishing a successful biological control program against *H. armigera* (Cherry et al., 2003). In addition, the chances of biological control of *H. armigera* could be improved by mass rearing and releasing the most important natural enemies.

Parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are important natural enemies in biological control worldwide (Smith, 1996; Mills, 2010) and could be employed as promising candidates for controlling several important lepidopteran pests that attack a variety of agricultural crops (Li, 1994; Greenberg et al., 1996;

van Lenteren, 2000). In more than 30 countries, including China, Switzerland, Canada, and the former Soviet Union, the supplemental release of Trichogrammatid parasitoids can reduce pest damage in various crops by 77 to 92%. These crops include sugarcane, wheat, maize, and cabbage (Li, 1994).

Since native *Trichogramma* species are better adapted to local environmental conditions, their use is preferred (Hassan, 1994), where releasing exotic species has raised safety concerns (van Lenteren et al., 2003).

The parasitoid wasp *Trichogramma euproctidis* (Girault) (previously identified as *Trichogramma turkestanica* Meyer) (Sumer et al., 2009) is commonly found in Khuzestan province, in the southwest of Iran (Modares Awal., 2012), as well as other countries (Hansen & Jensen, 2002; Ferracini et al., 2006; Consoli et al., 2010; Tuncbilek et al., 2012; Lessard & Boivin, 2013; Hegazi et al., 2019). *Trichogramma euproctidis* is prevalent in maize fields and date palm orchards (Tabebordbar, unpublished data) and is an excellent candidate for biological control of several lepidopteran pests (Chailleux et al., 2012; Hegazi et al., 2019).

The temperature significantly impacts the biology, survival, demographic parameters, and effectiveness of biological control agents (Messenger, 1970; Frazier et al., 2006; Moezipour et al., 2008; Iranipour et al., 2009). From late June to early September, the climate in Khuzestan is very hot and dry ( $\approx 45$  °C), where daytime temperatures may briefly exceed 48 °C during the summer. The most significant limitation in Khuzestan province is temperature, and the effectiveness of a released natural enemy will depend on its ability to adapt to these harsh environmental conditions.

Although previous studies have examined the life history characteristics of *T. euproctidis* (e.g., Silva & Stouthamer, 1999; Hansen & Jensen, 2002; Tuncbilek et al., 2012; Atashi et al., 2021; Tabebordbar et al., 2022), literature has neglected the bionomics of *T. euproctidis* parasitizing *H. armigera* concerning

temperature. To this end, the current study was conducted to provide basic information on the influence of different constant temperatures on the biology of *T. euproctidis*, a species well-established in Iran. Consequently, *T. euproctidis* was evaluated for immature development, survival, adult longevity, and reproduction under a range of constant temperatures similar to crop-growing conditions in Khuzestan province.

## Materials and methods

### Insect collection

A laboratory culture of *T. euproctidis* was established from parasitoid wasps collected from a maize field using *Ephestia kuehniella* Zeller eggs pasted on 5 cm × 1 cm cards of papers hung on maize plants on the premises of Shahid Chamran University of Ahvaz (31°17'59"N 48°39'39"E), Ahvaz city, Khuzestan province, Iran, during July 2019. The *E. kuehniella* eggs used in this study were obtained from Golestan Mooud insectary, Ahvaz, Iran and reared continuously according to the method described by Brindley (1930). After 48 h, the cards were collected and kept in growth chambers at 25 ± 1°C, 55 ± 5% RH and a photoperiod of 16:8 (L: D) h. The cards were monitored for three days. Parasitized eggs were distinguished by their black color. Each card was transferred to a glass test tubes (20 cm height and 2 cm diameter) containing 50 *E. kuehniella* eggs (less than 24 h old). Glass tubes were sealed by cotton wool. Glass tubes were maintained in growth chambers set at the temperature mentioned above. Emerged parasitoids were provided a streak of honey that was smeared in the internal part of the tube. Newly emerged parasitoids (10 females and 10 males) were introduced to glass tubes (20 cm height and 2 cm diameter) containing a card (10 × 1 cm) with sufficient number (500 ± 10) of *E. kuehniella* eggs (less than 24 h old). The parasitoid wasps were reared under laboratory conditions at 25 ± 1°C, 55 ± 5% RH and a photoperiod of 16:8 (L: D) h for five generations. The parasitoids were identified

with morphological characteristics described in Pintureau (2008). Voucher specimens are deposited in the collection of the Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Eggs of *H. armigera* were obtained from a laboratory culture reared on bean-yeast based semi-artificial diet (Shorey & Hale, 1965) at University of Guilan, Rasht, Iran and reared on the same diet in the laboratory at 25 ± 1°C, 55 ± 5% RH and a photoperiod of 16:8 h (L: D). Reared and mated *H. armigera* females were confined with two-month-old potted geranium, *Plargonium zonale* (L.), seedlings in a cage (40 cm height and 50 cm diameter) for oviposition. The 14 h old *H. armigera* eggs laid on geranium leaves were collected by a fine paint brush and used for the experiments.

### Experimental procedure

The biological traits of *T. euproctidis* were determined at 18, 21, 24, 27, 30 and 33 ± 1°C, 55 ± 5% RH and photoperiod of 16:8 h (L: D) in the growth chambers. In the beginning of the experiment at least 50 pairs of parasitoids were reared for one generation on *E. kuehniella* eggs in each of the above-mentioned temperatures in order to adapt them to that temperature. A single, mated, < 24 h old female *T. euproctidis* was introduced into a glass test tube (10 cm × 1 cm) containing 40 ± 1 fourteen-hours old of *H. armigera* eggs. *H. armigera* eggs were glued on pieces of white paper (5 cm × 1 cm). The glass tubes were sealed with cotton-wool. The tubes were then kept in the growth chambers set at the temperatures mentioned above. After 24 hours, parasitoids were removed and the parasitized eggs were kept in rearing growth chambers. Each treatment was replicated ten times, leading to 400 eggs/ treatment. For each temperature, the number of parasitized eggs (black eggs), developmental times (female and male), emergence (survival), sex ratio (female %), the size of hind tibia of female and male (as an index of body size) (Waage & Ng, 1984) and number of parasitoids emerged per egg were recorded.

The effect of temperature on longevity and fecundity of adult progeny *T. euproctidis* females were studied by placing newly emerged ( $\pm 24$  h) female and male parasitoids (obtained from above experiment) in glass tubes containing  $40 \pm 1$  fourteen-hours old eggs of *H. armigera*. The glass tubes were kept in growth chambers at the temperatures mentioned above. Every 24 h, parasitoids were transferred to new glass tubes containing similar number of *H. armigera* eggs, until the female parasitoid died. Longevity and fecundity (daily and total) of *T. euproctidis* were recorded for each temperature.

### Statistical analysis

The number of parasitized eggs, developmental time, emergence rate, sex ratio, size of hind tibia, number of parasitoids emerged from each host egg, longevity, daily fecundity and total fecundity were examined for normality using the Kolmogorov-Smirnov test. The data were analyzed using one-way analysis of variance (ANOVA). Survival rate and sex ratio data were transformed to arcsine before analysis. Means were compared using Tukey's honest significant difference (HSD) test ( $P < 0.05$ ). SPSS version 22 statistical software was used to analyze the data (SPSS, 2018).

The lower threshold temperature for development was estimated using a linear regression equation, X intercept method. The parameter t (thermal threshold) and DD (thermal constant) were derived from the regression equation as follows:  $Y = a + bX$ , where Y is the reciprocal of the developmental duration in days (the developmental velocity = developmental rate =  $1/\text{developmental time}$ ), X is the temperature in  $^{\circ}\text{C}$ , a and b are parameters of the linear regression (Campbell et al., 1974). From this the lower developmental threshold (t), i. e. the temperature when the development ceases can be estimated:  $t = a/b$ . The number of degree-days (DD) required for development can be calculated:  $DD = 1/b$ .

Life table parameters were estimated by combining data from the pre-imaginal development, adult survival, and reproduction experiment of different temperature treatments.

The intrinsic rate of population increase was estimated by iteratively solving the following equation:  $\sum e^{-rx} l_x m_x = 1$  (Birch, 1948), where x is the mean age class,  $m_x$  is the mean number of female progenies per female of age x, and  $l_x$  is the probability of survival to age x. A trial number of values for r were substituted into the equation until the r value for which the sum on the left side of the equation approximate unity. The jackknife procedure was used to estimate an SE for the r values of different treatments (Maia et al., 2000). Further data were also calculated for each treatment: net reproductive rate ( $R_0 = \sum l_x m_x$ , number of female offspring produced per female), finite rate of increase ( $\lambda = e^r$ , number of times the population will multiply itself per unit of time), mean generation time ( $T = \ln R_0/r$ ), and doubling time ( $DT = \ln 2/r$ , number of days required for the population to double in number) (Birch, 1948).

### Results

The mean number of *H. armigera* eggs parasitized by *T. euproctidis* was significantly affected by temperature ( $F = 18.89$ ;  $df = 4, 45$ ;  $P < 0.0001$ ). The mean number of parasitized eggs increased with increasing temperature from 21 to 27  $^{\circ}\text{C}$  and then decreased at higher temperatures (Table 1).

*T. euproctidis* developed successfully at temperatures that ranged from 21 to 33  $^{\circ}\text{C}$  (Table 1); *T. euproctidis* failed to complete development at the lowest temperature, 18  $^{\circ}\text{C}$ . The mean developmental time from egg to adult female and male decreased as temperature increased (Table 1). Analysis of variance indicated significant differences in developmental duration for females ( $F = 1431.38$ ;  $df = 4, 834$ ;  $P < 0.0001$ ) and males ( $F = 1063.78$ ;  $df = 4, 472$ ;  $P < 0.0001$ ) among the temperatures examined. The rate of development was more than two times faster at 33  $^{\circ}\text{C}$  than at 21  $^{\circ}\text{C}$ .

The mean emergence (survival) rate of *T. euproctidis* was significantly affected by temperature ( $F = 32.73$ ;  $df = 4, 45$ ;  $P < 0.0001$ ). We observed that as temperature increased from

21 to 27 °C, the mean emergence rate of *T. euproctidis* increased from 96.20 to 98.33 %. At higher temperatures (i. e. 33 °C) the emergence rate decreased to 82.22 % (Table 1).

Analysis of variance indicated that temperature had a significant effect on sex ratio (percentage of females) of *T. euproctidis* ( $F=50.18$ ;  $df=4, 45$ ;  $P < 0.0001$ ). The percentage of females increased with increasing temperature from 21 to 27 °C, where it peaked at 74.62% and then decreased at higher temperatures (Table 1).

Analysis of variance revealed that temperature significantly affected the tibial length of emerging *T. euproctidis* (female:  $F=997.46$ ;  $df=4, 573$ ;  $P < 0.0001$ ; male:  $F=759.27$ ;  $df=4, 341$ ;  $P < 0.0001$ ). We observed that as temperature increased from 21 to 27 °C, the mean tibial length of female emerging parasitoids also increased from 0.178 to 0.194 mm. At higher temperatures (i. e. 33 °C) the mean tibial length of female emerging parasitoids decreased to 0.151 mm (Table 1).

The mean number of *T. euproctidis* progeny emerged from each host egg was significantly influenced by temperature ( $F=45.51$ ;  $df=4, 45$ ;  $P < 0.0001$ ). We found an inverse relationship between the number of emerged progeny and temperature; as temperature increased, numbers of emerged progeny decreased (Table 1).

The lower threshold temperature ( $t$ ) and thermal constant (DD) for the development of *T. euproctidis* parasitizing *H. armigera* eggs at

different temperatures are presented in Table 2. The lower temperature thresholds for the development from egg to adult female and male *T. euproctidis* were 7.20 and 9.97, respectively. According to these thresholds for female *T. euproctidis*, an average of 188.67 degree-days was required to complete development from egg to adults.

We observed significant differences in the longevity of female and male progeny of *T. euproctidis* among the temperature tested (female:  $F=162.39$ ;  $df=4, 145$ ;  $P < 0.0001$ ; male:  $F=145.83$ ;  $df=4, 145$ ;  $P < 0.0001$ ). We found an inverse relationship between the longevity of female and male progeny of *T. euproctidis* and temperature; as temperature increased, longevity decreased (Table 3).

Analysis of variance revealed that temperature significantly affected the mean daily ( $F=89.52$ ;  $df=4, 145$ ;  $P < 0.0001$ ) and total ( $F=75.95$ ;  $df=4, 145$ ;  $P < 0.0001$ ) numbers of progeny produced by the *T. euproctidis* females. Across all of the temperatures we tested, the highest and lowest progeny were produced at 21 °C (168.70 eggs) and 33 °C (19.50 eggs), respectively (Table 3).

Analysis of variance revealed that temperature significantly affected the life table parameters of *T. euproctidis*. Both the finite rate of increase ( $\lambda$ ) and intrinsic rate of natural increase ( $r$ ) reached their peaks at 27 °C and decreased at higher temperatures (Table 4, Figure 1).

**Table 1. Mean ( $\pm$  SE) number of parasitized eggs, pre-adult development time (days), emergence rate (%), sex ratio (female %), size of right hind tibia and number of parasitoids per each host egg of *T. euproctidis* parasitizing *H. armigera* eggs at different constant temperatures.**

Parameter	Temperature (°C)				
	21	24	27	30	33
Mean of parasitized eggs	24.30 $\pm$ 2.72 <sup>bc</sup>	29.70 $\pm$ 1.73 <sup>ab</sup>	33.10 $\pm$ 2.16 <sup>a</sup>	19.10 $\pm$ 1.20 <sup>cd</sup>	13.10 $\pm$ 0.69 <sup>d</sup>
Pre-adult development time (female)	15.33 $\pm$ 0.10 <sup>a</sup>	11.03 $\pm$ 0.05 <sup>b</sup>	9.74 $\pm$ 0.04 <sup>c</sup>	8.39 $\pm$ 0.06 <sup>d</sup>	7.25 $\pm$ 0.29 <sup>e</sup>
Pre-adult development time (male)	14.11 $\pm$ 0.10 <sup>a</sup>	10.26 $\pm$ 0.05 <sup>b</sup>	8.77 $\pm$ 0.06 <sup>c</sup>	7.38 $\pm$ 0.08 <sup>d</sup>	6.29 $\pm$ 0.09 <sup>e</sup>
Emergence rate (survival rate)	96.20 $\pm$ 0.51 <sup>a</sup>	97.20 $\pm$ 1.55 <sup>a</sup>	98.33 $\pm$ 0.59 <sup>a</sup>	85.46 $\pm$ 1.92 <sup>b</sup>	82.22 $\pm$ 1.19 <sup>b</sup>
Sex ratio (% female)	68.50 $\pm$ 1.11 <sup>b</sup>	66.20 $\pm$ 1.64 <sup>b</sup>	74.62 $\pm$ 0.88 <sup>a</sup>	57.11 $\pm$ 1.66 <sup>c</sup>	47.69 $\pm$ 1.88 <sup>d</sup>
Size (mm) of right hind tibia (female)	0.178 $\pm$ 0.46 <sup>c</sup>	0.185 $\pm$ 0.36 <sup>b</sup>	0.194 $\pm$ 0.47 <sup>a</sup>	0.159 $\pm$ 0.47 <sup>d</sup>	0.151 $\pm$ 0.70 <sup>c</sup>
Size (mm) of right hind tibia (male)	0.162 $\pm$ 0.77 <sup>c</sup>	0.172 $\pm$ 0.52 <sup>b</sup>	0.182 $\pm$ 0.41 <sup>a</sup>	0.146 $\pm$ 0.63 <sup>d</sup>	0.139 $\pm$ 0.19 <sup>e</sup>
Number of parasitoids/host egg	1.47 $\pm$ 0.02 <sup>a</sup>	1.19 $\pm$ 0.05 <sup>b</sup>	1.06 $\pm$ 0.01 <sup>c</sup>	1.02 $\pm$ 0.01 <sup>c</sup>	1.01 $\pm$ 0.01 <sup>c</sup>

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

### Discussion

Temperature significantly affected the mean number of *H. armigera* eggs parasitized by *T. euproctidis*. In a similar study Tabebordbar et al. (2022) reported that at 22.5, 25, 27.5, 30, 32.5, 35, 37.5 and 40 °C *T. euproctidis* parasitized mean numbers of 25.85, 32.42, 35.85, 38.42, 44.85, 31.85, 30.28 and 26.14 *Ephestia kuehniella* Zeller eggs, respectively, which are higher than the results obtained in the present study at similar or close temperatures. Hansen and Jensen (2002) found that at 15, 20, 25 and 30 °C *T. euproctidis* (= *T. turkestanica*) parasitized mean number of 61, 56.3, 62.4 and 32.2 *E. kuehniella* eggs, respectively, which are higher than the findings of the present study. These dissimilarities may be explained by the superparasitism phenomenon, which is related to the disparities in size of host species. The size of *H. armigera* eggs was reported to be 0.42 to 0.60 mm (Ali et al., 2009) compared to the eggs of factitious host *E. kuehniella*, which were approximately 0.3 mm (Zuim et al., 2017). These results suggest that availability of a larger amount of nutrients in the host egg could support the development of more than one parasitoid.

However, when more than one parasitoid develops in the same host egg, there may be competition for nutrients resulting in smaller progeny, which in turn could result in lower longevity and reproduction of emerged wasps (Bai et al., 1992).

Temperature also had a significant effect on the developmental time of female *T. euproctidis*. An inverse relationship was observed between temperature and the immature developmental time of *T. euproctidis*. The female developmental time of *T. euproctidis* decreased from 15.33 days at 21 °C to 7.25 days at 33 °C as the temperature rose from 21 to 33 °C. Tabebordbar et al. (2022) reported developmental durations of 13.81, 10.0, 8.28, 7.17, and 7.02 days for *T. euproctidis* on *E. kuehniella* eggs at 22.5, 25, 27.5, 30 and 32.5 °C, respectively, which are consistent with the results of the current study.

The egg-to-adult development time of male *T. euproctidis* was shorter at all tested temperatures than that of females. A similar trend was reported for *Trichogrammatoidea bactrae* Nagaraja, *Trichogramma pretiosum* Riley (Naranjo, 1993), and *Trichogramma dendrolimi* Matsumura (Park et al., 2000).

**Table 2. The lower threshold temperature (t) and thermal constant (DD) for development of *T. euproctidis* parasitizing *H. armigera* eggs at different constant temperatures.**

Sexuality	Parameters			
	Temperature threshold	R <sup>2</sup>	DD	Regression
Female	7.20	0.99	188.67	Y= 0.0053X - 0.0381
Male	9.97	0.99	147.05	Y= 0.0068X - 0.0678

**Table 3. Mean (± SE) longevity (days), daily fecundity and total fecundity of *T. euproctidis* female reared on *H. armigera* eggs at different constant temperature.**

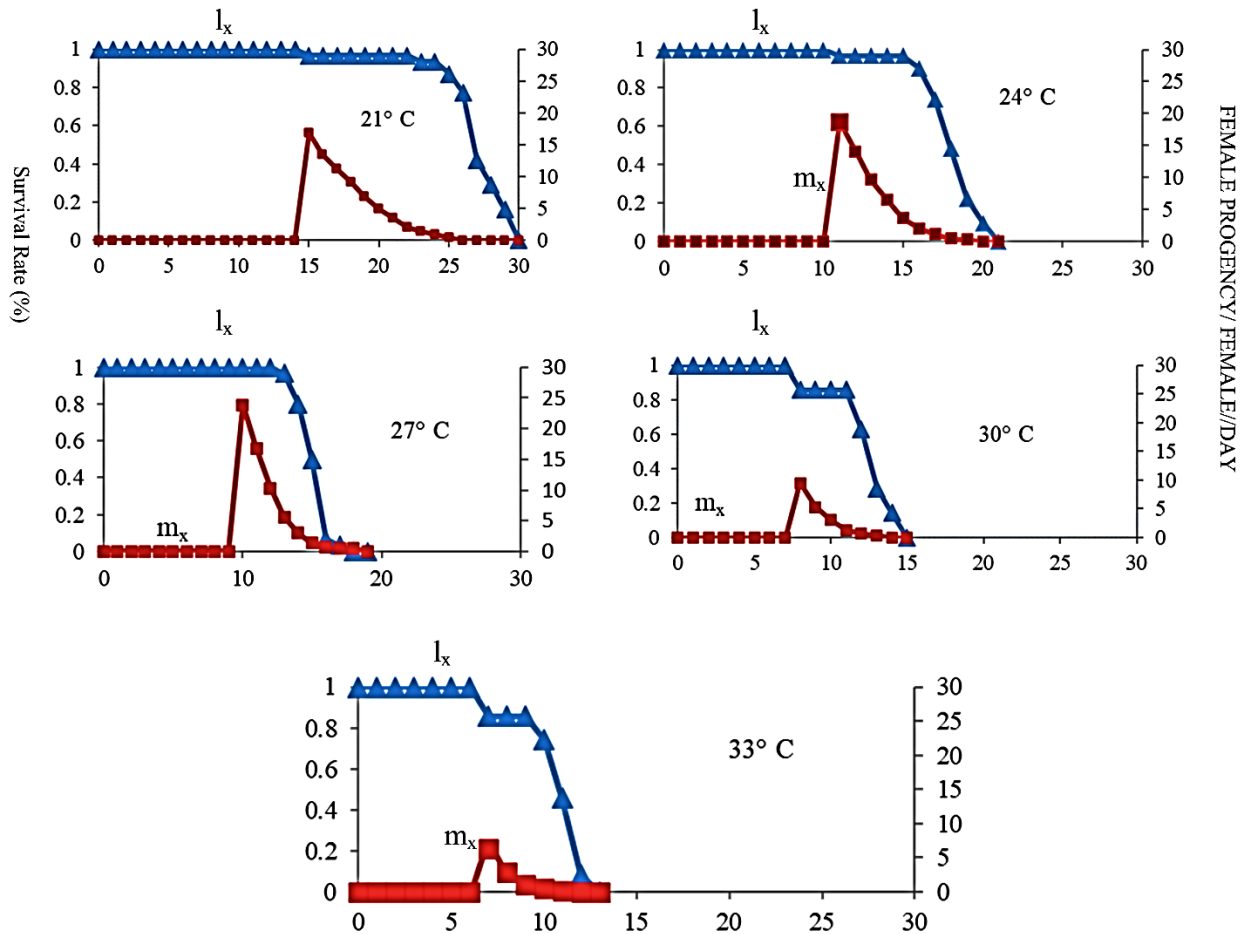
Parameter	Temperature (C)				
	21	24	27	30	33
Female longevity	11.60 ± 0.28 <sup>a</sup>	7.60 ± 0.15 <sup>b</sup>	6.33 ± 0.17 <sup>c</sup>	5.33 ± 0.18 <sup>d</sup>	4.57 ± 0.14 <sup>d</sup>
Male longevity	9.30 ± 0.28 <sup>a</sup>	6.63 ± 0.23 <sup>b</sup>	5.53 ± 0.18 <sup>c</sup>	4.17 ± 0.13 <sup>d</sup>	3.03 ± 0.10 <sup>e</sup>
Daily fecundity (black eggs)	10.70 ± 0.71 <sup>b</sup>	11.65 ± 0.68 <sup>b</sup>	13.03 ± 0.47 <sup>a</sup>	7.22 ± 0.23 <sup>c</sup>	5.66 ± 0.30 <sup>c</sup>
Daily fecundity (parasitoid emerged)	15.45 ± 0.91 <sup>a</sup>	14.18 ± 0.43 <sup>a</sup>	14.02 ± 0.45 <sup>a</sup>	7.19 ± 0.15 <sup>b</sup>	4.89 ± 0.21 <sup>c</sup>
Total fecundity (black eggs)	119.70 ± 3.56 <sup>a</sup>	87.10 ± 2.91 <sup>b</sup>	83.40 ± 4.06 <sup>ab</sup>	34.17 ± 0.98 <sup>c</sup>	22.73 ± 1.22 <sup>c</sup>
Total fecundity (parasitoids emerged)	168.70 ± 3.67 <sup>a</sup>	100.86 ± 5.60 <sup>b</sup>	86.50 ± 2.20 <sup>b</sup>	30.23 ± 0.89 <sup>c</sup>	19.50 ± 1.15 <sup>c</sup>

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

**Table 4. Life table parameters of *T. euproctidis* reared on *H. armigera* eggs at different constant temperatures.**

Parameter	Temperature				
	21	24	27	30	33
$R_0$ (female generation <sup>-1</sup> )	68.23 ± 0.15 <sup>a</sup>	53.96 ± 0.09 <sup>c</sup>	59.61 ± 0.09 <sup>b</sup>	17.02 ± 0.01 <sup>d</sup>	9.50 ± 0.01 <sup>e</sup>
$r$ (days <sup>-1</sup> )	0.24 ± 0.0001 <sup>d</sup>	0.32 ± 0.0001 <sup>b</sup>	0.37 ± 0.0001 <sup>a</sup>	0.33 ± 0.0007 <sup>b</sup>	0.29 ± 0.0002 <sup>c</sup>
$\lambda$ (days <sup>-1</sup> )	1.28 ± 0.0001 <sup>d</sup>	1.38 ± 0.0001 <sup>b</sup>	1.45 ± 0.0001 <sup>a</sup>	1.39 ± 0.012 <sup>b</sup>	1.34 ± 0.0003 <sup>c</sup>
$T$ (days)	16.90 ± 0.001 <sup>a</sup>	12.18 ± 0.001 <sup>b</sup>	10.88 ± 0.0007 <sup>c</sup>	8.61 ± 0.123 <sup>d</sup>	7.53 ± 0.0008 <sup>e</sup>
$DT$ (days)	2.77 ± 0.0013 <sup>a</sup>	2.11 ± 0.0008 <sup>c</sup>	1.84 ± 0.0006 <sup>d</sup>	2.10 ± 0.030 <sup>c</sup>	2.32 ± 0.001 <sup>b</sup>

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



**Figure 1. Daily proportion of female progeny per female ( $m_x$ ) and survival ( $l_x$ ) of *Trichogramma euproctidis*.**

The emergence rate (survival rate) of *T. euproctidis* reared on *H. armigera* eggs at 21, 24, and 27 °C exceeded 90% in the present study. Similar to our findings, Silva and Stouthamer (1999) reported the emergence rate (survival rate) of *T. euproctidis* on *H. armigera* eggs at 25 °C to be over 90%. However, in a laboratory experiment with *T. euproctidis* on *E. kuehniella*, Tabebordbar et al. (2022) observed an emergence

rate of 49.64, 81.18, 73.94, 90.01, 93.06, 72.54, 67.74 and 53.26% at 22.5, 25, 27.5, 30, 32.5, 35, 37.5 and 40 °C, respectively, which interlinks at 30 and 32.5 °C with the results obtained in the current study. Differences in host species and experimental conditions, such as relative humidity or photoperiod, may account for variations in emergence rate at other temperatures evaluated.

In the current study the lower temperature threshold for female *T. euproctidis* was 7.2°C which is close to 8.23 °C reported by Tabebordbar et al. (2022) for female *T. euproctidis* on *E. kuehniella* eggs. However, higher threshold temperatures have been reported for other *Trichogramma* species e. g. 9.23 °C for *T. evanescens* (Haile et al., 2002), 10- 15 °C for *T. pretiosum* and *T. exiguum* (Harrison et al., 1985) and 13.6 °C for *T. galloi* (Consoli & Parra, 1995).

The sex ratio of *T. euproctidis* was biased for females at 21-30 °C. These findings are in line with the results of Tabebordbar et al. (2022), who reported 54, 64, 75 and 84% females for *T. euproctidis* on *E. kuehniella* eggs at 25, 27.5, 30, and 32.5 °C, respectively. Hansen and Jensen (2002) also reported 61, 59, 70, and 70% of females for *T. euproctidis* on *E. kuehniella* eggs at 15, 20, 25 and 30 °C, respectively. Our findings revealed that the proportion of male progeny was higher at elevated (33 °C) temperatures. The sex ratio of insects may be altered by unfavorable environmental conditions (such as extreme temperatures and food shortages) (Lauge, 1985). Thus, the observed shift in sex ratio toward males at 33 °C may be the parasitoid's response to unfavorable rearing conditions.

The results of the current study indicated that the tibial length of male and female parasitoids increased as temperature increased up to 27 °C and then decreased as temperature increased. The size of egg parasitoids directly correlates with female longevity or reproduction (Waage & Ng, 1984; Ruberson et al., 1988; Los-Den Hartogh et al., 1989; Ruberson & Kring, 1993); consequently, the temperature at which *T. euproctidis* develop may have long term effects on female performance, in addition to the obvious immediate effects on developmental times and survival of parasitoids.

Our results demonstrated that the number of parasitoids emerging from each host egg decreased steadily and significantly as the temperature increased at all temperatures

examined. Each *H. armigera* egg consistently produced more than one parasitoid offspring. This phenomenon was attributed to the size of *H. armigera* eggs, which ranged between 0.42 to 0.60 mm (Ali et al., 2009) in comparison to the *E. kuehniella* eggs, which were approximately 0.30 mm (Zuim et al., 2017). These results suggest that greater availability of nutrients (i.e., yolk) in the host egg facilitates the development of multiple parasitoids. However, when more than one progeny develops in a single host egg, there may be competition for nutrients, resulting in smaller progeny, which leads to lower longevity and fecundity of emerged parasitoids (Bai et al., 1992).

The findings of our experiments revealed that the longevity of female progeny of *T. euproctidis* was also affected by temperature. Hansen and Jensen (2002) determined the mean longevity of female *T. euproctidis* on *E. kuehniella* eggs at 25 °C to be 8.9 days. The current value of female progeny longevity at 24 °C (7.6 days) is comparable to these results. However, the longevity of female *T. euproctidis* obtained in this study at various constant temperatures was shorter than those reported by Tabebordbar et al. (2022) in a nearly identical study, where *T. euproctidis* on *E. kuehniella* eggs had respective longevity values of 12.74, 9.88, 9.40, 8.66, 7.68, 7.42, 6.85, and 5.74 days at temperatures of 22.5, 25, 27.5, 30, 32.5, 35, 37.5, and 40 °C. These disparities suggest that environmental factors and parasitoid host species substantially impact the longevity of female offspring.

The maximum fecundity (black eggs) of *T. euproctidis* was 119.70 in the current study, which is comparable to the 121.40 parasitized *E. kuehniella* eggs reported by Tabebordbar et al. (2022) for the same parasitoid on *E. kuehniella* eggs. However, in our experiment *T. euproctidis* produced significantly more progeny (168.7 progeny) than results reported by Tabebordbar et al. (2022). Notably, in the cited study, only one parasitoid emerged from each host egg at each temperature examined. In addition, it should be noted that the optimal temperature for fecundity



and offspring production in our study was 21 °C. In contrast, Tabebordbar et al. (2022) found that the highest fecundity occurred at 32.5 °C. As previously stated, the superparasitism phenomenon is likely the primary cause of this difference. *T. euproctidis* is adapted to the hot and dry climate of Khuzestan province, southwestern Iran; therefore, when a female parasitoid finds herself in a cold condition (21 °C) with a sufficient number of suitable host eggs (*H. armigera*), she lays multiple eggs in each host egg to increase the likelihood of survival of her offspring at such a low temperature. Hansen and Jensen (2002) reported that *T. euproctidis* laid an average of 82.1 eggs on *E. kuehniella* at 25 °C.

The highest value of the intrinsic rate of increase ( $r$ ) (0.370 days<sup>-1</sup>) of *T. euproctidis* parasitizing *H. armigera* eggs was calculated at 27 °C, according to the results of the current study. In an almost identical study, Tabebordbar et al. (2022) found that *T. euproctidis* parasitizing *E. kuehniella* eggs at 32.5 °C had the highest  $r$  value of 0.532 day<sup>-1</sup>. This disparity may be due to differences in ecological factors, such as host species and measurement methods.

The present study's findings indicated that 27 °C is the optimal temperature for development and reproduction of *T. euproctidis*. Similar to our results, approximately 25 °C has been reported as the optimal temperature for *Trichogramma brevicapillum* Pinto and Planter (Pak & Oatman, 1982), *Trichogramma cordubensis* Vargas and Cabello (Cabello & Vargas, 1988), *Trichogramma ostrinae* Pang et Chen (Gou, 1988), and *Trichogramma cacoeciae* Marchal (Scholler & Hassan, 2001). However, an optimal developmental temperature of approximately 31 °C has been reported for *T. pretiosum* (Butler & Lopez, 1980; Calvin et al., 1984) and *T. bactrae* (Hutchison et al., 1990; Naranjo, 1993).

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Moreover, the results of the current study indicated that 27 °C is the optimal temperature for the best performance of *T. euproctidis* parasitizing *H. armigera* eggs. However, Tabebordbar et al. (2022) observed that 32.5 °C was the optimal temperature for development and reproduction of *T. euproctidis* on *E. kuehniella* eggs. Differences in host species and differences in thermal constant and lower temperature threshold of host species may explain the disparities in optimal temperature of *T. euproctidis* on the two host species. Thermal constant (K) and lower temperature threshold (t) for development were reported to be 513.6 DD and 11.6 °C for *H. armigera* (Noor-ul-Ane et al., 2017), while 1111 DD and 9 °C were reported for *E. kuehniella* (Pakyari et al., 2019), respectively. To this end, our results indicate that when evaluating the effect of temperature on the biological characteristics of *T. euproctidis* to determine the optimal temperature, the host species is one of the most important factors; in fact, *T. euproctidis* may adjust its optimum temperature based on the temperature-related parameters of its host species.

Overall, the current study evaluated the development and reproduction of *T. euproctidis* parasitizing *H. armigera* eggs at a series of constant temperatures. Information on an organism's life history, developmental thresholds, and thermal requirements can be used to predict developmental rates under varying temperature conditions. These data are essential for an integrated system to optimize the application of *Trichogramma* for the biological control of lepidopteran pests.

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## اثر دما بر بیواکولوژی زنبور (*Trichogramma euproctidis* (Hym.: Trichogrammatidae)) انگل واره کرم میوه گوجه فرنگی (*Helicoverpa armigera* (Lep.: Noctuidae))

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### چکیده

خصوصیات تاریخچه زندگی *Trichogramma euproctidis* (Girault)، یک گونه زنبور استقرار یافته در جنوب غربی ایران، انگل واره تخم شب پره *Helicoverpa armigera* (Hubner) در دماهای ۱۸، ۲۱، ۲۴، ۲۷، ۳۰ و ۳۳ درجه سلسیوس مورد مطالعه قرار گرفت. نتایج نشان داد که دماهای ثابت مختلف اثر معنی داری روی تعداد تخم پارازیت شده، دوره رشد، نسبت جنسی، طول ساق، تعداد انگل واره در هر تخم، طول عمر و زادآوری نوزادان داشت. میانگین دوره رشد از تخم تا بالغ از ۱۵/۳۳ روز در ۲۱ درجه به ۷/۲۵ روز در ۳۳ درجه سلسیوس کاهش یافت. برای تکمیل رشد در بالای آستانه پایین حرارتی (۷/۲ درجه سلسیوس) میانگین ۱۸۸ روز-درجه مورد نیاز بود. میانگین بقای پیش از بلوغ در دماهای ۲۱، ۲۴، ۲۷، ۳۰ و ۳۳ درجه سلسیوس به ترتیب ۹۶/۲۰، ۹۷/۲۰، ۹۸/۳۳، ۸۵/۶۶ و ۸۲/۲۲ درصد بود. میانگین طول عمر *T. euproctidis* دامنه ای از ۱۱/۶۰ روز در دمای ۲۱ درجه تا ۴/۵۷ روز در دمای ۳۳ درجه سلسیوس داشت. میانگین کل نوزادان دامنه ای از میانگین ۱۹/۵۰ نوزاد در هر ماده در ۳۳ درجه تا ۱۶۸/۷۰ نوزاد در هر ماده در ۲۱ درجه سلسیوس داشت. تجزیه و تحلیل داده ها نشان داد که دماهای ثابت مختلف اثر معنی داری بر نرخ خالص تولید مثل ( $R_0$ )، نرخ ذاتی افزایش جمعیت ( $r$ )، نرخ منتهای افزایش جمعیت ( $\lambda$ ) و طول دوره یک نسل داشت. نرخ ذاتی افزایش جمعیت ( $r$ ) با افزایش دما از ۰/۲۴۰ بر روز در ۲۱ درجه تا ۰/۳۷۰ بر روز در ۲۷ درجه سلسیوس افزایش یافت و سپس در دماهای بالاتر کاهش یافت. با افزایش دما طول دوره یک نسل از ۱۶/۹۰ روز به ۷/۵۳ روز کاهش یافت. دمای بهینه برای رشد و تولید مثل *E. euproctidis* ۲۷ درجه سلسیوس بود. به نظر می رسد که این استرین از *T. euproctidis* دارای این پتانسیل هست که در برنامه های مدیریت تلفیقی علیه *H. armigera* مورد استفاده قرار گیرد.

کلیدواژه‌ها: *Trichogramma euproctidis* *Helicoverpa armigera*، نشو و نما، بقاء، نرخ ذاتی افزایش جمعیت

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