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Quality Control of the Parasitoid Wasp *Trichogramma euproctidis* (Hymenoptera: Trichogrammatidae) over 50 Continuous Generations of Rearing on *Ephestia kuehniella*

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ABSTRACT

Trichogramma euproctidis (Girault) (Hymenoptera: Trichogrammatidae), an economically important biocontrol agent of lepidopterous pests, was continuously reared on *Ephestia kuehniella* Zeller for 50 generations. The effects of continuous rearing on this host were evaluated by comparing biological parameters among different generations (G1–G50). The number of *E. kuehniella* eggs parasitized by *T. euproctidis* was significantly affected by generation. Female preadult developmental time was significantly different among consecutive generations, ranging from 9.66 (at G1) to 12.18 d (at G10). Significant differences were detected in female longevity of *T. euproctidis* among subsequent generations and the highest longevities were found in G5 and G30. However, no significant difference was found in adult emergence rate (survival rate) and sex ratio (female%) among different generations. The highest intrinsic rate of increase (r = 0.373 day^{-1}), finite rate of increase (λ = 1.452 day^{-1}), and net reproductive rate (R_0 = 91.57 females/female) were recorded in G30, whereas lower values were observed after prolonged rearing. Our results indicate that *T. euproctidis* colonies maintained acceptable biological quality under laboratory rearing conditions for up to 30 generations, whereas a decline in some performance parameters was observed after prolonged rearing.

Keywords: Biological pest control, Egg parasitoids, Generational fitness, Mass-rearing performance, *Trichogramma euproctidis*.

INTRODUCTION

The members of Trichogrammatidae family are among the most important parasitoids used for biological control of lepidopterous pests (Parra, 2010). In countries including China, Canada, Switzerland, and Russia, the application of trichogrammatid wasps has been shown to decrease

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damage caused by lepidopteran pests in crops such as sugarcane, wheat, maize, and cabbage by approximately 77–92% (Dodiya *et al.*, 2023).

The parasitoid wasp *Trichogramma euproctidis* (Girault) is naturally found in Khuzestan province, located in southwestern Iran (Modares Awal, 2012), as well as in several other countries (Hansen and Jensen, 2002; Ferracini *et al*., 2006; Cônsoli *et al*., 2010; Tuncbilek *et al*., 2012; Lessard and Boivin, 2013; Hegazi *et al*., 2019). Within Khuzestan, this species is frequently observed in various crops, including maize fields and date palm orchards (Tabebordbar, 2022), making it an effective candidate for the biological control of multiple lepidopteran pests (Chailleux *et al*., 2012; Hegazi *et al*., 2019).

Mass production of biological control agents generally requires continuous colony maintenance over multiple generations to ensure a reliable supply of high-quality individuals for augmentative biological control programs (Ghaemmaghami *et al.*, 2021a,b; Gorji *et al.*, 2024). Various *Trichogramma* species have been successfully mass reared on factitious hosts worldwide; however, only a limited number of host species are considered economically and biologically suitable for large-scale production (Laurentis *et al.*, 2019; Gowda *et al.*, 2021; Ningthoujam *et al.*, 2025). Among these, the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), remains one of the most widely used factitious hosts because of its ease of mass production, relatively low production cost, compatibility with mechanized rearing systems, and consistent production of high-quality parasitoids (St-Onge *et al.*, 2014; Moghaddassi *et al.*, 2019; Ghaemmaghami *et al.*, 2021b).

The first step in biological control of a pest species through augmentative release of a natural enemy, is to examine the key life history traits of the biological control agent and ascertain whether it can be effectively produced in long-time periods. Nevertheless, long-term rearing can affect biological characteristics of parasitoids, including developmental time, survival and parasitism rate of reared agents (Bellutti, 2011; Sørensen *et al*., 2012; Naranjo-Guevara *et al.*, 2020; Ghaemmaghami *et al.*, 2021a,b; Badran *et al.*, 2022). Therefore, understanding how to mass-produce biological control agents over extended periods without compromising their biological traits is a crucial step in successful large-scale rearing programs (Sørensen *et al*., 2012). It is well established that long-term rearing of natural enemies can lead to inbreeding depression and random genetic drift, which reduce genetic diversity and may diminish the effectiveness of the biological control agent. According to available studies, such challenges have sometimes undermined

biological control initiatives (van Lenteren, 2003; Cônsoli *et al*., 2010; Bellutti, 2011). Consequently, implementing rigorous quality control measures is essential in large-scale production schemes (Pratissoli *et al*., 2004; Parra, 2010).

Although considerable information is available regarding different biological characteristics of *Trichogramma* species, Only a limited number of studies have investigated the parasitism potential and demographic traits of parasitoid wasps when reared continuously over multiple generations (Nordlund *et al*., 1997; Pratissoli *et al*., 2004; Lü *et al*., 2015, 2017; Ghaemmaghami *et al*., 2021a, b). For instance, Pratissoli *et al*. (2004) evaluated the life-history traits of *Trichogramma pretiosum* Riley reared on *E. kuehniella* for 23 generations and observed a significant reduction in adult female longevity in the later generations. Similarly, Lü *et al*. (2017) examined the biological performance of *Trichogramma dendrolimi* Matsumura over 30 generations on both an artificial diet and a natural host, reporting that individuals reared on the artificial diet exhibited higher pupation and adult emergence rates compared to those reared on natural hosts. Ghaemmaghami *et al*. (2021a) investigated biological traits of *Trichogramma brassicae* (Bezdenko) breed on *Sitotroga cerealella* (Olivier) for 45 generations and found that reproduction of the parasitoid reduced significantly after 15 generations. Moreover, multiple studies have highlighted the importance of maintaining strict quality control for the successful long-term rearing of biological control agents under laboratory or other controlled conditions (Miyatake, 1998; Bellutti, 2011; Khanamani *et al*., 2017).

Although previous studies have investigated the long-term rearing performance of several Trichogramma species, comparable information for *Trichogramma euproctidis*, an important parasitoid used in biological control programs, remains unavailable. Moreover, no study has comprehensively assessed changes in both life-history and demographic parameters of this species over an extended period of continuous laboratory rearing. Therefore, the present study evaluated the quality of *T. euproctidis* over 50 consecutive generations reared on *Ephestia kuehniella* by examining parasitism performance, life-history traits, and life-table parameters. The results provide practical quality-control criteria for long-term mass rearing and identify an appropriate generation interval for colony renewal to maintain the biological performance of this parasitoid.

MATERIALS AND METHODS

Insect Culture

A culture of Mediterranean flour moth was stablished in the entomology laboratory of the Plant Protection Department of the Shahid Chamran University of Ahvaz, Ahvaz, Iran. The *E. kuehniella* eggs used in our study were gained from Golestan Mooud insectary, Ahvaz, Iran and breed continually according to the method described by Brindley (1930), with routine laboratory practices commonly adopted in recent mass-rearing studies (e.g., Pakyari *et al.*, 2018; Moghaddassi *et al.*, 2019).

A colony of *T. euproctidis* was initiated from parasitoids collected in a maize field at Shahid Chamran University of Ahvaz (31°17'59"N, 48°39'39"E) in July 2022. *Ephestia kuehniella* eggs were placed on small paper cards (5 × 1 cm) and attached to maize plants for 48 hours to allow parasitism. Afterward, the cards were removed and kept in growth chambers at 25 ± 1°C, 55 ± 5% relative humidity, with a 16:8 h light:dark cycle. Parasitized eggs were identified by their black coloration over the following three days. Each card was then transferred into a 20 cm × 2 cm glass tube, sealed with cotton wool, and the emerged wasps were supplied with a thin layer of honey inside the tube. Newly emerged adults (10 females and 10 males) were subsequently placed into fresh tubes containing a card (10 × 1 cm) with 50 ± 10 *E. kuehniella* eggs, less than 24 hours old, to continue the colony. The parasitoid colony was maintained continuously under laboratory conditions for 50 generations. During colony maintenance, individuals for the next generation were randomly selected from the available population without any selection based on parasitism capacity, developmental rate, fecundity, longevity, or other biological traits.

The laboratory colony used in the present study originated from the same field population that had been previously identified as *T. euproctidis* using an integrative taxonomic approach combining morphological and molecular analyses. Morphological identification was based on slide-mounted adult specimens following the procedures of Platner *et al.* (1999) and the diagnostic characters described by Pintureau (2008), whereas molecular confirmation was achieved by sequencing the ITS2 region of the nuclear rDNA. Detailed information on the identification procedure has been published previously (Tabeborbar *et al.*, 2023). The present study was conducted using the same continuously maintained laboratory colony, and therefore species identification was not repeated.

Experimental Procedure

At the start of the main experiment, a single mated female *T. euproctidis* (< 24 h old) was introduced into a glass tube (10 cm × 1 cm) containing 50 one-day-old *E. kuehniella* eggs. Experimental females were randomly selected from each generation colony and were used only for evaluation of biological and demographic parameters. The eggs were fixed onto small pieces of white paper (5 × 1 cm). Tubes were sealed with cotton wool and placed in growth chambers maintained at 25 ± 1°C, 55 ± 5% relative humidity, and a 16:8 h light:dark photoperiod. After 24 hours, the female was removed, and the parasitized eggs were left in the chambers for further development. This setup was replicated ten times using a completely randomized design. For each tube, data were collected on the number of parasitized (black) eggs, developmental duration of males and females, emergence (survival) rates, and female percentage of the sex ratio.

To evaluate the longevity and fecundity of adult female *T. euproctidis*, newly emerged pairs of females and males (< 24 h old) from the previous experiment were placed in glass tubes containing 50 ± 1 one-day-old *E. kuehniella* eggs. A thin layer of honey was applied inside each tube to supply carbohydrates for the parasitoids. The tubes were kept in growth chambers under the same environmental conditions as described earlier. Every 24 hours, females were transferred to fresh tubes with a similar number of eggs until they died, and males were replaced if they died prematurely. This procedure was replicated ten times using a completely randomized design. Daily and total fecundity, along with female longevity, were recorded. Any females that were damaged during handling or died due to contact with honey were excluded from the analysis. This protocol was repeated for generations 5, 10, 20, 30, 40, and 50 of the *T. euproctidis* colony.

Statistical Analysis

Life table parameters were estimated using the conventional female age-specific life table proposed by Birch (1948), and the jackknife procedure described by Maia *et al.* (2000) was used to estimate the standard errors of demographic parameters. In this approach, demographic parameters are calculated from female survival and fecundity; therefore, male longevity was not included in the life table analysis. The conventional female age-specific life table (Birch, 1948) was adopted in the present study because it has been widely used in previous demographic studies on *Trichogramma* spp., allowing direct comparison with earlier published data.

Data on the number of parasitized eggs, developmental duration, emergence rate, sex ratio, female longevity, as well as daily and total fecundity, were subjected to one-way analysis of variance (ANOVA). Prior to analysis, survival rates and sex ratio values were arcsine-transformed. Differences among means were assessed using Tukey's HSD test at a significance level of $P < 0.05$. All statistical analyses were performed using SPSS software, version 22 (SPSS 2018).

Data on pre-imaginal development, survival, adult longevity, and reproduction were integrated to estimate the life table parameters across various treatments. The equation: $\Sigma e^{rx}\ lx\ mx = 1$ was used to calculate the intrinsic rate of increase (Birch, 1948), where x represents the mean age class, mx denotes the mean number of female progenies per female of age x, and lx is the probability of survival to age x. This equation was iteratively solved, and a range of r values was tested in the equation until the left side approximated one. The jackknife procedure was employed to estimate the standard error of the r values across different treatments (Maia *et al*., 2000). For each treatment, additional parameters were calculated, including the net reproductive rate ($R_0 = \Sigma lxmx$, representing the average number of female offspring per female), the finite rate of increase ($\lambda = e^r$, indicating the population multiplication rate per unit time), the mean generation time ($T = \ln R_0 / r$), and the doubling time ($DT = \ln 2 / r$, representing the number of days required for the population to double) (Birch, 1948).

RESULTS

Significant differences were found among the mean number of *E. kuehniella* eggs parasitized by *T. euproctidis* reared in different generations ($F = 4.723$; $df = 6, 71$; $p < 0.0001$) (Table 1). The highest and lowest mean number of *E. kuehniella* eggs parasitized by *T. euproctidis* were recorded in G10 and G50, respectively. *T. euproctidis* successfully developed on *E. kuehniella* eggs even after being reared for fifty generations on *E. kuehniella* eggs (Table 1). The developmental time of *T. euproctidis* female and male was significantly affected by increasing the duration of rearing on *E. kuehniella* eggs (female: $F = 29.40$; $df = 6, 1169$; $p < 0.0001$; male: $F = 25.59$; $df = 6, 400$; $p < 0.0001$). The female pre-adult duration in G10 was significantly longer than other treatments. In G1, developmental durations of *T. euproctidis* females were the shortest, followed by those in G40 (Table 1). The emergence rate (survival rate) ($F = 1.80$; $df = 6, 70$; $p = 0.111$) and sex ratio (female %) ($F = 0.804$; $df = 6, 69$; $p = 0.571$) of *T. euproctidis* were not significantly affected by the time of rearing on *E. kuehniella* eggs (Table 1).

The female longevity of *T. euproctidis* was significantly different among various treatments ($F = 8.948$; $df = 6, 192$; $p < 0.001$) (Table 2). The longest female lifespan was observed in generation 5 (G5), although no significant difference in longevity was found between G5 and generation 30 (G30) (Table 2). Both mean daily and total fecundity of *T. euproctidis* varied significantly across generations (daily fecundity: $F = 4.95$; $df = 6, 186$; $p < 0.001$; total fecundity: $F = 21.55$; $df = 6, 184$; $p < 0.001$). As shown in Table 3, the life table parameters of *T. euproctidis* were affected by the time of rearing on *E. kuehniella* eggs (Table 3). The highest values of R_0 (91.57 ± 0.28 eggs/individual), r (0.373 ± 0.0002 day^{-1}), and λ (1.452 ± 0.0003 day^{-1}) were recorded in the 30th generation. In addition, the shortest and longest mean doubling times (*DT*) occurred in G30 with 1.85 ± 0.001 and G10 with 2.49 ± 0.001 days, respectively. Age-specific survival (*lx*) and fecundity (*mx*) curves derived from these data for each generation are illustrated in Figure 1.

DISCUSSION

The parasitoids belong to the family Trichogrammatidae are among the most well-known natural enemies and the most widely used biological control agents with a worldwide distribution. These parasitoids are mass produced and released annually in order to control various lepidopterous pests on different agricultural systems (Dodiya *et al.*, 2023). However, such natural enemies may lose their quality over long term mass rearing and become ineffective (Pratissoli *et al.*, 2004; Parra, 2010; Badran *et al.*, 2022). Several substantial changes may occur during long-lasting mass production of natural enemies, among them are the deprivation of tolerance to severe climatic conditions and changes in life history traits and host priority (Sadat *et al.*, 2021; Badran *et al.*, 2022). Therefore, evaluating the quality of natural enemies during mass rearing or prior to their application is crucial, as reductions in the performance of mass-reared biological control agents can compromise pest management programs (van Lenteren, 2003; Sørensen *et al.*, 2012).

The findings of the present study showed that the longevity of female *T. euproctidis* decreased as it was breed on *E. kuehniella* for 50 generations. Similar results have been reported for *T. minutum* after 10 generations of rearing on *Helicoverpa zea* (Boddie) (Nordlund *et al.*, 1997), *T. dendrolimi* after 30 generations of rearing on *Antheraea pernyi* (Guérin- Méneville) (Lü *et al.*, 2017), *T. pretiosum* after 22 generations of rearing on *E. kuehniella* (Pratissoli *et al.*, 2004), and *T. brassicae* after 45 generations of rearing on *E. kuehniella* (Ghaemmaghami *et al.*, 2021b). Several factors affect longevity of trichogrammatid wasps, among them is nutrition. Gurr and

Nicol (2001) reported that nutrition had a profound effect on longevity of *Trichogramma carevera* Oatman and Pinto. i.e. those fed with honey had higher longevity than those deprived of carbohydrates.

Our results showed that fecundity of *T. euproctidis* deminished over 50 generations of breeding on *E. kuehniella*. Similar to our results, decrease in fecundity have been reported for *T. pretiosum* after 22 generations of raising on *E. kuehniella* (Pratissoli *et al*., 2004), *T. dendrolimi* after 30 generations of rearing on *A. pernyi* (Lü *et al*., 2017), and *T. brassicae* after 45 generations of rearing on *E. kuehniella* (Ghaemmaghami *et al*., 2021b). However, in contrast to our findings, Nordlund *et al*. (1997) stated increase in oviposition of *Trichogramma minutum* after 10 generations of breeding on *H. zea*. Fecundity is a standard measure used to evaluate the quality of natural enemies such as trichogrammatid parasitoids mass produced in insectaries (van Lenteren *et al*., 2002). Fecundity can be affected by several factors including factitious or natural hosts, host species, egg volume and oldness, and diet of adult female (Cônsoli *et al*., 2010). According to the International Organization for Biological Control (IOBC) procedure at least 40 progeny per female is a required level of fecundity for high quality *T. brassicae* (van Lenteren *et al*., 2002). In our study, *T. euproctidis* fecundity declined over consecutive generations. However, the fecundity was higher than 47 in all generations tested.

The gradual decline in the performance of *T. euproctidis* observed after approximately 30 generations is likely the result of several interacting factors rather than a single cause. Long-term rearing under constant laboratory conditions may promote adaptation to the artificial rearing environment, favoring traits that enhance performance under laboratory conditions but not necessarily overall biological fitness. In addition, continuous laboratory culture may lead to the accumulation of genetic changes through genetic drift and, to a lesser extent, inbreeding, potentially reducing adaptive variation within the colony. Furthermore, prolonged exposure to a single factitious host (*E. kuehniella*) may alter host acceptance behavior or physiological adaptation, which can influence important fitness-related traits such as longevity, fecundity, and parasitism capacity. Similar concerns regarding laboratory adaptation and genetic deterioration have been highlighted as important challenges in long-term mass-rearing programs for biological control agents (van Lenteren, 2003; Bellutti, 2011; Sørensen *et al.*, 2012; Cherif *et al.*, 2021).

The results of the present study indicated that the sex ratio of *T. euproctidis* was not affected by rearing over 50 generations on *E. kuehniella*. In line with our results, sex ratio of *T. minutum* after

10 generations of rearing on *H. zea* (Nordlund *et al*., 1997), *T. pretiosum* after 22 generations of breeding on *E. kuehniella* (Pratissoli *et al*., 2004), and *T. brassicae* after 45 generations of rearing on *E. kuehniella* (Ghaemmaghami *et al*., 2021b) were not affected by generations. The significance of sex ratio of egg parasitoids is mostly associated to the different expenses of produced males, which have no role in pest control (Cônsoli *et al*., 2010). For profitable rearing and also for efficient biological control, an excess of fit and productive females is extremely required (Cônsoli *et al*., 2010). Based on the IOBC protocol, in mass rearing programs the sex ratio of *Trichogramma* spp. should be higher than 50% (van Lenteren *et al*., 2002). Our results showed that sex ratio was not affected by rearing over numerous generations and always was higher than 75% female.

The highest r value (0.373 day^{-1}) was recorded in G30, which was higher than the values of 0.311 day^{-1} reported by Atashi *et al.* (2021) and 0.354 day^{-1} reported by Tabeborbar *et al.* (2020) for *T. euproctidis* reared for a single generation on *E. kuehniella*. These differences may be partly attributed to the number of laboratory generations. However, other factors, including differences in environmental conditions, host quality and age, colony history, genetic background of the parasitoid population, and methodological differences in demographic analyses, may also have contributed to the observed variation among studies.

Our findings indicated that when *T. euproctidis* was breed continuously on *E. kuehniella* eggs, the highest r value was documented in G30 after which the r value decreased. Dissimilar to our results the highest r value of *T. dendrolimi* reared on an artificial diet was in G20 (Lü *et al*., 2017), *T. brassicae* reared on *Sitotroga ceralella* in G15 (Ghaemmaghami *et al*., 2021a), and *T. brassicae* reared on *E. kuehniella* in G10 (Ghaemmaghami *et al*., 2021b). In addition, Pratissoli *et al*. (2004) studied the parasitism rate and female longevity of *T. pretiosum* reared on *E. kuehniella* for 23 generations and found that the highest parasitism rate and female longevity was recorded in G8 and G2, respectively. The comparisons of our results with findings of above-mentioned studies clearly indicated that *T. euproctidis* is well adapted to be reared continuously on *E. kuehniella* eggs under laboratory conditions.

Although the age-stage, two-sex life table and bootstrap resampling methods are increasingly recommended for demographic analyses because they incorporate both sexes and stage differentiation, the present study employed the conventional female age-specific life table to maintain methodological consistency with previous studies evaluating long-term laboratory

rearing of *Trichogramma*. Future investigations may benefit from applying the age-stage, two-sex life table to provide a more comprehensive assessment of population dynamics.

Although the present study demonstrated that *T. euproctidis* maintained acceptable quality parameters during long-term laboratory rearing, the results should be interpreted within the context of controlled laboratory conditions. Continuous rearing on a factitious host may impose selective pressures that favor individuals better adapted to laboratory environments. Consequently, improvements or stability in laboratory-measured traits may not necessarily correspond to enhanced performance after release under greenhouse or field conditions. Furthermore, prolonged laboratory maintenance may influence genetic diversity and behavioral characteristics of parasitoid populations. Therefore, additional studies under semi-field and field conditions are required to determine whether long-term laboratory-reared colonies maintain their effectiveness against target pests.

In conclusion, the present study demonstrates that *T. euproctidis* can be successfully maintained on *E. kuehniella* for multiple generations under laboratory conditions without substantial deterioration in key biological and demographic traits up to approximately 30 generations. However, a gradual decline in several fitness-related parameters beyond this point highlights the importance of continuous quality control and periodic colony rejuvenation through the introduction of wild individuals. Although the present findings provide valuable guidance for laboratory mass-rearing programs, further studies under greenhouse and field conditions are required to determine whether the observed laboratory performance is maintained after release and to improve the practical application of this parasitoid in biological control programs.

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References

1. Atashi, N., Shishehbor, P., Seraj, A. A., Rasekh, A., Hemmati, S. A. and Riddick, E. W. 2021. Effect of *Helicoverpa armigera* egg age on development, reproduction and life table parameters of *Trichogramma euproctidis*. *Insects* **12**: 569. https://doi.org/10.3390/insects12070569

2. Badran, F., Y. Fathipour, A. Bagheri, M. Attaran, G. V. P. Reddy. 2022. Effects of prolonged mass rearing on life history traits of *Habrobracon hebetor* (Hymenoptera: Braconidae). *Int J Pest Manag*, **68**(3). 246–255.

3. Bellutti, N. 2011. Effects of mass rearing on life-history traits of an invasive fruit moth species. BOKU Vienna, Grapholita molesta (Busck) Institute of Forest Entomology, Forestathology and Forest Protection, p. 35.

4. Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. *Anim. Ecol.,* **17**(1): 15-26. https://doi.org/10.2307/1605

5. Brindley, T. A. 1930. The growth and development of *Ephestia kuehniella* Zeller (Lepidoptera) and *Tribolium confusum* Duval (Coleoptera) under controlled conditions of temperature and relative humidity. *Ann. Entomol. Soc. Am.,* **24**(1): 1-17. https://doi.org/10.1093/aesa/23.4.741

6. Carey, J. R. 1993. Applied Demography for Biologists, with Special Emphasis on Insects; Oxford University Press: Oxford, UK. 1993

7. Chailleux, A., Desneux, N., Seguret, J., Khanh, H. D. T., Maignet, P. and Tabone, E. 2012. Assessing European egg parasitoids as a mean of controlling the invasive South American tomato pinworm *Tuta absoluta*. *PLoS ONE* **7**: e48068.

8. Consoli, F. L., Parra, J. R. P. and Zucchi, R. A. 2010. Egg parasitoids in agroecosystems with emphasis on *Trichogramma*. Springer, New York, USA.

9. Dodiya, R.D., Barad, A.H., Pathan, N.P. and Raghunandan, B.L. 2023. *Trichogramma*: A promising biocontrol agent. *Int J Econ Plants* **10**: 192–199.

10. Ferracini, C., Boivin, G. and Alma, A. 2006. Costs and benefits of host feeding in the parasitoid *Trichogramma turkestanica. Entomol. Exp. Appl.,* **121**: 229-234. https://doi.org/10.1111/j.1570-8703.2006.00479.x

11. Ghaemmaghami, E., Fathipour, Y., Bagheri, A., Talebi, A. A. and Reddy, G. V. P. 2021a. Quality control of the parasitoid wasp *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) over 45 generations of rearing on *Sitotroga cerealella. Insect Sci.,* **28**: 180-190.

12. Ghaemmaghami, E., Fathipour, Y., Bagheri, A., Talebi, A. A. and Reddy, G. V. P. 2021b. Continuous rearing on *Ephestia kuehniella* reshaped quality of the parasitoid wasp *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae). *J. Asia-Pacific Entomol.,* **24**: 166- 174.

13. Gorji, Z., Shishehbor, P., Kocheili, F., Riahi, E. 2024. Quality control of the predatory mite *Euseius scutalis* (Acari: Phytoseiidae) against *Tetranychus turkestani* (Acari: Tetranychidae) over 30 generations of rearing on cattail pollen. *Acarologia* **64**(3): 1-14. https://doi.org/10.24349/gz0z-vqgh

14. Gowda, B.G., Pandi, G. G.P., Ullah, F., Patil, N.B., Sahu, M., Adak, T., Pokhare, S., Yadav, M.K., Mahendiran, A., Mittapelly, P., Desneux, N., Rath, P.C. 2021. Performance of *Trichogramma japonicum* under field conditions as a function of the factitious host species used for mass rearing. *PLoS One* **19**: 16(8):e0256246. doi: 10.1371/journal.pone.0256246.

15. Gurr, G. and Nicol, H. I. 2001. Effect of food on longevity of adults of *Trichogramma carverae* Oatman and Pinto and *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae). *Aust. J. Entomol.,* **39**: 185–187.

16. Hansen, L. S. and Jensen, K. M. V. 2002. Effect of Temperature on Parasitism and Host-Feeding of *Trichogramma turkestanica* (Hymenopetra: Trichogrammatidae) on *Ephestia kuehniella* (Lepidopeta: Pyralidae). *J. Eco. Entomol.,* **95**(1): 50-56. https://doi.org/10.1603/0022-0493-95.1.50

17. Hegazi, E., Alder, C., Khafagi, W. and Agamy, E. 2019. Host preference and parasitic capacity of new candidates of *Trichogramma* species (Hym.: Trichogrammatidae) against some stored products moths. *J. Stored Product Res.,* **80**: 71-78. https://doi.org/10.1016/j.jspr.2018.11.006

18. Khanamani, M., Fathipour, Y., Talebi, A. A. and Mehrabadi, M. 2017. Quantitative analysis of long-term mass rearing of *Neoseiulus californicus* (Acari: Phytoseiidae) on almond pollen. *J. Econ. Entomol.,* **110**: 1442–1450.

19. Laurentis, V.L., Ramalho, D.G., Santos, N.A. *et al.* 2019. Performance of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) on eggs of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Sci Rep* **9**: 1156. https://doi.org/10.1038/s41598-018-37797-9

20. Lessard, E. and Boivin, G. 2013. Effect of age and huger on host-feeding behavior by female *Trichogramma euproctidis* (Hymenoptera: Trichogrammatidae). *Can. Entomol.,* **145**: 53-60. https://doi.org/10.4039/tce.2012.86

21. Li, L. L. 1994. Worldwide use of *Trichogramma* for biological control in different crops. In: Wajnberg E, Hassan S A (eds) Biological control with egg parasitoids. CAB International, Wallingford, UK, pp 37–54.

22. Lü, X., Han, S. and Li, L. 2015. Biochemical analyses of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) in vitro and in vivo rearing for 10 generations. *Fla. Entomol.,* **98**: 911–915.

23. Lü, X., Han, S., Li, Z. and Li, L. 2017. Biological characters of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) reared *in vitro* versus *in vivo* for thirty generations. *Scientific Rep.,* **7**: 17928.

24. Maia, A. H. N., Alfredo, J. B. and Campanhola, C. 2000. Statistical influence on associated fertility life table parameters using Jackknife technique: computational aspects. *J. Econ. Entomol.,* **93**: 511-518. https://doi.org/10.1603/0022-0493-93.2.511

25. Miyatake, T. 1998. Genetic changes of life history and behavioral traits during mass-rearing in the melon Fly, *Bactrocera cucurbitae* (Diptera: Tephritidae). *Res. Popul. Ecol.,* **40**: 301–310.

26. Modares Awal, M. 2012. List of agricultural pests and their natural enemies in Iran. Third edition, Ferdowsi University Press, 285p.

27. Moghaddassi Y, Ashouri A, Bandani AR, Leppla NC, Shirk PD. 2019. Effect of *Ephestia kuehniella* (Lepidoptera: Pyralidae) larval diet on egg quality and parasitism by *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae). *J Insect Sci* **19**(4):10. doi: 10.1093/jisesa/iez076.

28. Naranjo-Guevara, N., dos Santos, L. A. O., Barbosa, N. C.C.P., Castro, A. C.M.C.C., and Fernandes, O.A. 2020. Long-Term Mass Rearing Impacts Performance of the Egg Parasitoid *Telenomus remus* (Hymenoptera: Platygastridae). *J Entomol Sci* **55**(1): 69-86. https://doi.org/10.18474/0749-8004-55.1.69

29. Ningthoujam, K., Moirangthem, Z., Pathak, M. *et al.* 2025. Host suitability and parasitism of *Trichogramma pretiosum* on *Galleria melonella* and *Corcyra cephalonica* for the management of fall armyworm. *Sci Rep* **15**: 34154. https://doi.org/10.1038/s41598-025-14656-y

30. Nordlund, D. A., Wu, Z. X. and Greenberg, S. M. 1997. In vitro rearing of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) for ten generations, with quality assessment comparisons of *in vitro* and *in vivo* reared adults. *Biol. Control* **9**: 201–207.

31. Pakyari, Hajar, and Rostislav Zemek. 2025. The effect of light cycles on the predation characteristics of *Phytoseiulus persimilis* (Acari: Phytoseiidae) feeding on *Tetranychus urticae* (Acari: Tetranychidae). *Plants* 14 (5): 687. https://doi.org/10.3390/plants14050687

394 32. Parra, J. R. P. 2010. Mass rearing of egg parasitoids for biological control programs. In:
395 Cônsoli FL, Parra JRP, Zucchis RA (eds) Egg Parasitoids in Agroecosystems with Emphasis on
396 *Trichogramma.* Springer, Netherlands, Dordrecht, pp 267–292.
397 33. Pintureau, B. 2008. Les especes europeennes de Trichogrammes, ILV ed, In Libro Veritas,
398 Cergy-Pontoise, France.
399 34. Platner, J.D., Velten, R.K., Planoutene, M. and Pinto, J.D. 1999. Slide mounting techniques
400 for Trichogramma (Trichogrammatidae) and other minute parasitic Hymenoptera. *Entomol News*
401 **110**: 56–64.
402 35. Pratissoli, D., Oliveira, H., Roberto Goncalves, J. and Zanuncio, J. 2004. Changes in
403 biological characteristics of *Trichogramma pretiosum* (Hym.: Trichogrammatidae) reared on
404 eggs of *Anagasta kuehniella* (Lep.: Pyralidae) for 23 generations. *Bio. Sci. Tech.,* **14**: 313–319.
405 36. Sadat, F., Nazari, A., Jafari, S. *et al.* How long-term mass rearing affects the quality of
406 the *Trichogramma embryophagum* (Hartig) (Hymenoptera: Trichogrammatidae) reared
407 on *Sitotroga cerealella* (Olivier) eggs. *Egypt J Biol Pest Control* **31**, 119 (2021).
408 https://doi.org/10.1186/s41938-021-00456-9
409 37. Sørensen, J. G., Addison, M. F. and Terblanche, J. S. 2012. Mass-rearing of insects for pest
410 management: Challenges, synergies and advances from evolutionary physiology. *J. Crop. Prot.,*
411 **38**: 87–94.
412 38. SPSS. 2018. SPSS for windows. Version 22.0; SPSS Institute Inc. Chicago, Il. USA.
413 39. St-Onge, M., Cormier, D., Todorova, S., & Lucas, É. 2014. Comparison of *Ephestia*
414 *kuehniella* Eggs Sterilization Methods for *Trichogramma ostriniae* Rearing. *Biol Control*, **70**:
415 73–77. https://doi.org/10.1016/j.biocontrol.2013.12.006
416 40. Sumer, F., Tuncbilek, A. S., Oztemiz, S., Pintureau, B., Rugman-Jones, P. and Stouthamer,
417 R. 2009. A molecular key to the common species of *Trichogramma* of the Mediterranean region.
418 *Biocon*, **54**: 617-624. https://doi.org/10.1007/s10526-009-9219-8
419 41. Tabebordbar, F., Shishehbor, P. and Ebrahimi, E. 2020. Suitability of different egg ages of
420 *Ephestia kuehniella* (Lep.: Pyralidae) for the development, reproduction and life table parameters
421 of *Trichogramma euproctidis* (Hym.: Trichogrammatidae). J. *Crop Prot.,* **9**: 89–99.
422 42. Tabebordbar, F., Shishehbor, P., Ebrahimi, E., Polaszek, A. and Ugine, T. A. 2022. Effect of
423 different constant temperatures on life history and life table parameters of *Trichogramma*

euproctidis (Hymenoptera: Trichogrammatidae). *J. Econ. Entomol.,* **15**: 474- 481. https://doi.org/10.1093/jee/toac007

43. Tabebordbar, F., Formisano, G., Shishehbor, P., Ebrahimi, E., Giorgini, M., Sørensen, J.G. 2023. Variation among populations of *Trichogramma euproctidis* (Hymenoptera: Trichogrammatidae) revealed by life table parameters: perspectives for biological control. *J Econ Entomol* **116**(4):1119-1127. doi: 10.1093/jee/toad075.

44. Tuncbilek, A. S., Cinar, N. and Canpolat, U. 2012. Effect of artificial diets and floral nectar on longevity and progeny production of *Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae). *Turkish J. Entomol.,* **36**: 183-191.

45. van Lenteren, J., Hale, A., Klapwijk, J. N., van Schelt, J. and Steinberg, S. 2002. Guidelines for quality control of commercially produced natural enemies. In: van Lenteren, J. (Ed) Quality control and production of biological control agents: theory and testing procedures. CAB International, Wallingford, UK, pp 265-303.

46. van Lenteren, J. C. 2003. Quality control and production of biological control agents: Theory and testing procedures. CABI.

47. Zhang, Y.-H.; Xue, J.-Z.; Tariq, T.; Li, T.-H.; Qian, H.-Y.; Cui, W.-H.; Tian, H.; Monticelli, L.S.; Desneux, N.; Zang, L.-S. 2024. Parasitism and suitability of *Trichogramma chilonis* on large eggs of two factitious hosts: *Samia cynthia ricini* and *Antheraea pernyi*. *Insects* **15**: 2. https://doi.org/10.3390/insects15010002

Table 1. Mean (± SE) number of parasitized eggs, pre-adult development time (days), emergence rate (%), and sex ratio (female %) of sequential generations of *Trichogramma euproctidis* reared on *Ephestia kuehniella* eggs.

Parameter	G1	G5	G10	G20	G30	G40	G50
Number of parasitized eggs	24.16 ± 2.39 bc	26.45 ± 3.07 abc	36.10 ± 2.50 a	31.33 ± 2.16 ab	26.11 ± 2.05 bc	28.66 ± 2.07 abc	20.92 ± 1.21 c
Female development time (d)	9.66 ± 0.27 c	10.36 ± 0.16 b	12.18 ± 0.02 a	10.30 ± 0.06 b	10.48 ± 0.09 b	10.25 ± 0.08 bc	10.33 ± 0.06 b
Male development time (d)	9.65 ± 0.26 c	10.26 ± 0.18 bc	12.06 ± 0.03 a	10.25 ± 0.07 bc	10.54 ± 0.10 b	10.39 ± 0.08 b	10.22 ± 0.08 bc
Emergence rate (Survival rate) (%)	77.66 ± 4.91 a	77.65 ± 2.71 a	87.53 ± 3.58 a	86.22 ± 1.02 a	80.80 ± 6.65 a	71.83 ± 4.97 a	77.17 ± 3.92 a
Sex ratio (% female)	76.14 ± 3.63 a	80.76 ± 1.68 a	80.67 ± 2.97 a	80.54 ± 1.60 a	75.70 ± 2.47 a	81.81 ± 1.92 a	78.17 ± 4.74 a

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

Table 2. Mean (± SE) longevity (days), daily fecundity and total fecundity of sequential generations of *Trichogramma euproctidis* reared on *Ephestia kuehniella* eggs.

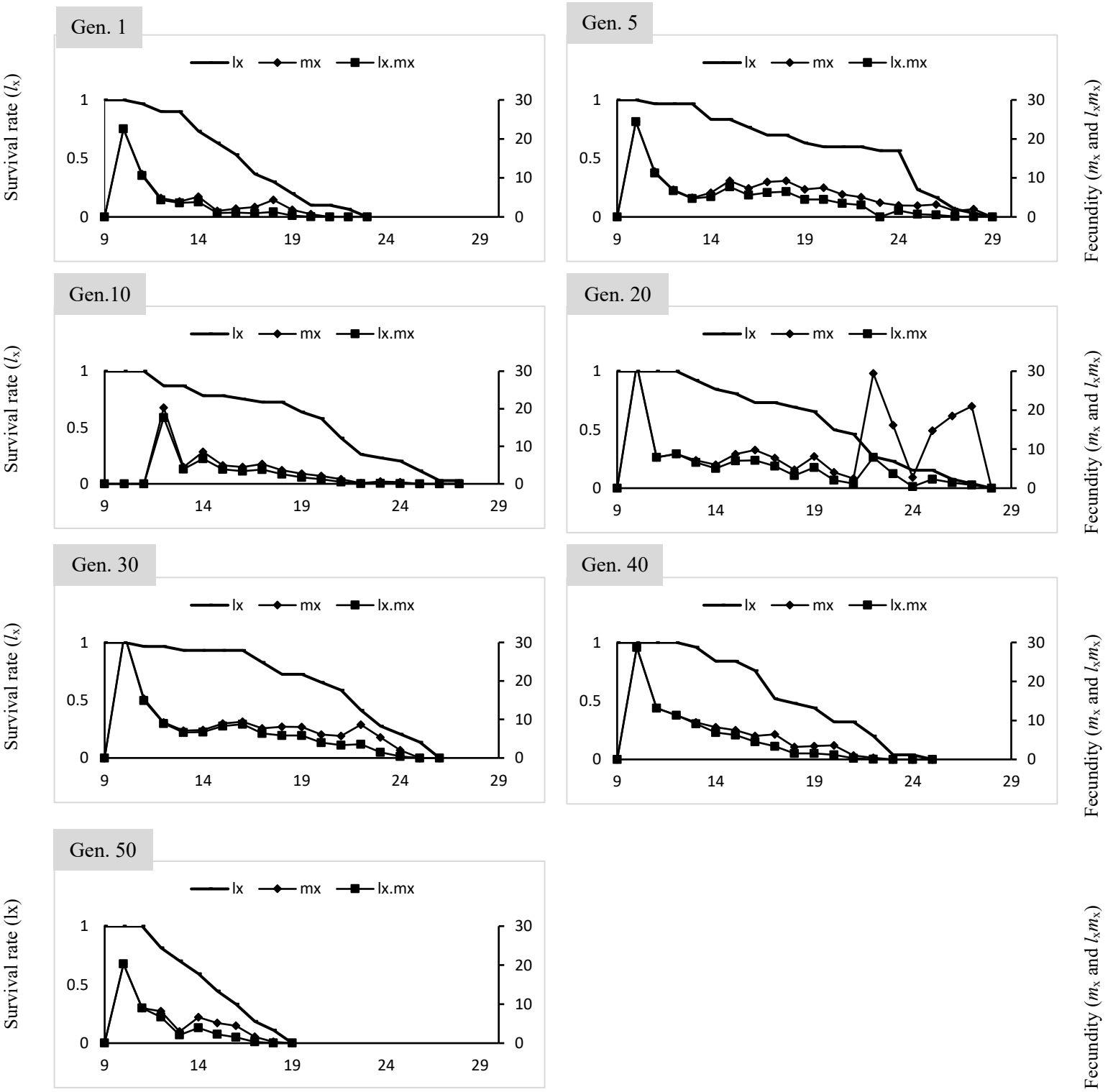
Parameter	G1	G5	G10	G20	G30	G40	G50
Female longevity	6.83 ± 0.58 cd	11.76 ± 0.97 a	8.96 ± 0.71 abc	9.63 ± 0.89 abc	10.75 ± 0.79 ab	8.54 ± 0.71 bc	5.07 ± 0.55 d
Daily fecundity	8.12 ± 0.67 bc	8.64 ± 0.56 bc	6.72 ± 0.77 c	10.75 ± 0.59 abc	13.67 ± 2.06 a	10.37 ± 0.44 abc	11.59 ± 1.27 ab
Total fecundity	48.73 ± 3.76 b	109.85 ± 8.56 a	50.96 ± 3.86 b	108.07 ± 10.90 a	121.43 ± 8.96 a	91.79 ± 7.24 a	47.50 ± 3.66 b

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

Table 3. Mean (± SE) life table parameters of sequential generations of *Trichogramma euproctidis* reared on *Ephestia kuehniella* eggs.

Parameter	G1	G5	G10	G20	G30	G40	G50
GRR (eggs/individual)	60.49 ± 0.14 e	128.67 ± 0.32 c	58.72 ± 0.17 ef	208.83 ± 1.12 a	141.14 ± 0.27 b	102.26 ± 0.26 d	58.30 ± 0.15 f
R_0 (eggs/individual)	37.08 ± 0.12 f	75.25 ± 0.26 c	44.04 ± 0.13 e	90.36 ± 0.39 b	91.57 ± 0.28 a	60.86 ± 0.23 d	34.28 ± 0.11 f
r (day^{-1})	0.326 ± 0.0004 e	0.347 ± 0.0002 d	0.278 ± 0.0002 g	0.368 ± 0.0002 b	0.373 ± 0.0002 a	0.355 ± 0.0003 c	0.319 ± 0.0002 f
λ (day^{-1})	1.385 ± 0.0006 e	1.414 ± 0.0004 d	1.320 ± 0.0002 g	1.445 ± 0.0003 b	1.452 ± 0.0003 a	1.426 ± 0.0004 c	1.377 ± 0.0003 f
T (day)	11.05 ± 0.018 f	12.45 ± 0.005 b	13.61 ± 0.007 a	12.23 ± 0.009 c	12.09 ± 0.003 d	11.56 ± 0.005 e	11.04 ± 0.004 f
DT (day)	2.12 ± 0.003 c	1.99 ± 0.005 d	2.49 ± 0.001 a	1.88 ± 0.001 f	1.85 ± 0.001 g	1.95 ± 0.001 e	2.16 ± 0.001 b

Values within each row followed by the same lowercase letter are not significantly different according to one-way ANOVA followed by Tukey's HSD test (P < 0.05) based on jackknife pseudo-values. Note that *GRR* is growth rate of reproduction, R_0 is net rate of reproduction, r is the intrinsic rate of increase, λ is finite rate of increase, T is mean generation time in days, and DT is doubling time in days.



454

455 **Figure 1.** Survival rate (l_x) and Fecundity (m_x and l_xm_x) of *Trichogramma euproctidis* reared on
456 *Ephestia kuehniella* eggs in fifty subsequent generations.