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Successive Rearing on *Sitotroga cerealella* Affects Quality of the Parasitoid Wasp *Trichogramma embryophagum* (Hymenoptera: Trichogrammatidae)

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ABSTRACT

Demographic parameters of the parasitoid wasp *Trichogramma embryophagum* Hartig reared on *Sitotroga cerealella* (Olivier) were determined for 40 generations (G5-G40). The experiments were done in a growth chamber at 26±2°C, 65±5% RH and photoperiod of 16:8 h (L:D). The results showed that the female longevity decreased significantly during successive production, ranging from 10.68 (in G5) to 9.64 days (in G40). On the other hand, the male longevity decreased significantly in the 20th generation and no significant difference was found from G20 to G40. The oviposition days and mean total fecundity of *T. embryophagum* decreased as the number of generations increased. Generally, the wasps in earlier generations had longer adult longevity, longer life span, and higher fecundity than later generations. Moreover, sex ratio of *T. embryophagum* was not significantly different in successive generations. Results of paired bootstrap test indicated that all population growth parameters of *T. embryophagum* reared on *S. cereallela* were significantly different in successive generations. The highest and lowest values of the intrinsic rate of increase (r), finite rate of increase (λ), net Reproductive rate (R_0), Gross Reproductive Rate (*GRR*) and mean generation Time (T) of *T. embryophagum* were observed in G40 and G5, respectively. These results suggest that *T. embryophagum* wasps reared in sequential generations can be used successfully in biological control programs until the 20th generation without any loss of quality or performance; after that, regular rejuvenation of laboratory population by occasional importing of field-collected parasitoids should be done.

Keywords: Different generations, Life table, Population growth parameters, Quality control, Rejuvenating population, *Trichogramma*.

INTRODUCTION

Many species of *Trichogramma* egg parasitoids (Hymenoptera: Trichogrammatidae) are among the most widely distributed biocontrol agents, which have long been utilized successfully in biological control programs against several lepidopteran pests in agricultural and forest areas (Smith, 1996; Razinger *et al.,* 2016). Adult females attack the egg stage of their host and kill the target pest before crop damage by larval feeding start. The augmentative release of these parasitoids has reduced pest damage by 77-92% in some crops such as sugarcane, wheat, corn, and cabbage in several countries (Li, 1994; Parra, 2010). In Iran, several *Trichogramma* species have been recorded (Ebrahimi *et al.,* 1998; Moghaddassi *et al.,* 2019) and are being mass-reared and released (Chamaani and Poorjavad, 2020). Among them, *T. embryophagum* have been noted to parasitize some important pests including *Ectomyelois ceratoniae* (Zell.) and *Cydia pomonella* L. (Poorjavad *et al.,* 2011) and have potential to

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be inexpensively produced in large numbers and easily released in fields (Haghani and Fathipour, 2003). *Trichogramma* parasitoids are usually produced on factitious hosts (Li *et al.*, 2019), among them, Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) is considered as a common host of *Trichogramma*, due to its easy and affordable rearing in laboratories (Smith, 1996).

Studying the basic demography of the mass reared biological control agents and determining their efficiency is the first step to conduct successful biological control programs (Ghaemmaghami *et al.*, 2021). However, long-term mass rearing can affect biological attributes, production efficiency and the quality of the mass-reared agents (Sørensen *et al.*, 2012; Khanamani *et al.*, 2017). Thus, an important step in effective mass rearing of natural enemies is to determine the best duration of their production without any unfavorable effect on the biological characteristics (Sørensen *et al.*, 2012). Both inbreeding depression and random genetic drift, which occurred during long-term rearing of natural enemies, led to their lower genetic variability and poor quality, and then resulted in failure of biological control programs (Cônsoli *et al.*, 2010). Because of these, the quality control is a crucial issue in mass rearing programs (Pratissoli *et al.*, 2004; Parra, 2010).

Assessment of the quality of artificially reared natural enemies as suggested by the current quality control guidelines are usually done in the laboratory with easily measured life table parameters, such as longevity, fecundity, viability, and sex ratio (Cascone *et al.*, 2015). Life Table studies are effective and comprehensive tools that provide the most detailed information about insect's population dynamics (Khanamani *et al.*, 2017).

A significant number of scientific papers is related to the effects of rearing parasitoids on artificial or natural host eggs only in a single generation (e.g., Grenier and De Clercq, 2003; Cônsoli *et al.*, 2010); only few studies have investigated the effects of continuous production for several generations (Nordlund *et al.*, 1997; Pratissoli *et al.*, 2004; Lü *et al.*, 2015, 2017). In a previous study, adult longevity of *Trichogramma pretiosum* Riley reared on *Anagasta kuehniella* (Zeller) for 23 generations reduced significantly in the last generations (Pratissoli *et al.*, 2004). Also, Ghaemmaghami *et al.* (2021) showed that mass rearing of *Trichogramma brassicae* (Bezdenko) for 45 generations on *S. cereallela* led to no loss of quality or performance until the 15th generation, but laboratory mass reared colonies declined in quality after G15. The importance of quality control of long-term mass reared natural enemies have been studied in several research works (Bellutti, 2011; Khanamani *et al.*, 2017).

Despite being a species with high economic importance, the biology of *T. embryophagum* is poorly studied (Haghani and Fathipour, 2003; Poorjavad *et al.*, 2011), and no studies have been carried out in the past to determinate its quality control in long-term mass rearing. Thus, the aim of this study was to determine biological characteristic and population parameters of *T. embryophagum* successively reared on *S. cereallela* eggs for 40 generations, in order to understand the effect of sequential mass rearing on its quality.

MATERIALS AND METHODS

Parasitoids

Trichogramma embryophagum was originally collected from the apple orchards located at the suburbs of Karaj (Alborz Province, Iran) by egg traps (pieces of white papers [21×11 cm] containing *S. cereallella* eggs). Egg traps were placed in different parts of the orchards and recollected after 24 hours. Then, the papers were incubated in a growth chamber at 26±2°C, 65±5% RH and 16:8 h (L:D) photoperiod. Stock colonies of *T. embryophagum* were reared on eggs of *S. cerealella* as a factitious host, in the laboratory under the same conditions mentioned above.

Host

A colony of Angoumois grain moth (*S. cereallella*) was established on barley grains under the same conditions mentioned above. When the flight of the first adults began, they were collected and transferred to funnels (diameter 200 mm) covered with a fine mesh net over the mouth. After mating, the eggs were collected on papers placed under the open side of the funnel.

Assessment of Biological Parameters

Approximately 80 one-day-old parasitized eggs of *S. cereallella* were selected randomly from the colony and kept in a glass tube (10×1.6 cm) until the adult wasps emerged. Pairs of newly emerged male and female *T. embryophagum* were moved into single glass tubes (10×1.6 cm). About 150 eggs of *S. cereallella* stuck to a white paper tape (1×7 cm) were supplied to each tube. Diluted honey (30%) was smeared on the tube wall to feed the parasitoid. If a male died, it was replaced by a newly emerged one (< 24 hours old) from the stock colony. These individuals were omitted from the analyses. When the adults emerged, mortality, survivorship, adult longevity, and the number of parasitized eggs were recorded daily, until the death of the last individual. These procedures were repeated for generations 5, 10, 15, 20, 25, 30, 35, and 40 of the *T. embryophagum* colony. All observations were done in a growth chamber under the same conditions mentioned above.

Statistical Analysis

The life histories data were analyzed by age-stage, two-sex life table theory (Chi and Liu, 1985) and the method described by Chi (1988). All developmental stages and reproductive attributes of different generations of *T. embryophagum* were subjected to normality testing (Kolmogorov–Smirnov test) and were compared using one-way Analysis Of Variance (ANOVA) and Tukey's test at $P < 0.05$. Statistical analyses were conducted using SAS (v. 9.2) software. The TWOSEX-MSChart program (Chi, 2020) was used to calculate the population parameters including the intrinsic rate of increase (r), net Reproductive rate (R_0), finite rate of increase (λ), Gross Reproductive Rate (GRR), and mean generation Time (T). Bootstrap procedure was used for estimating the standard errors of population parameters with 100,000 resampling (Huang and Chi, 2012). Comparison of bootstrap values of all parameters among different generations of *T. embryophagum* was done using paired bootstrap test procedure (Riahi *et al.*, 2017; Bahari *et al.*, 2018). The sex ratios in different generations were compared based on Chi-square test by SPSS (v. 22). Figures were drawn by Microsoft Excel 2016.

RESULTS

Parasitoid Life Stage Duration, Reproductive Characteristics, and Sex Ratio

The mean duration of different life stages of *T. embryophagum* are shown in Table 1. The egg, larval, and pupal stages occurred inside the host's eggs and all were grouped as "preadult." As the results show, preadult period took approximately 9.95-10 days to be completed, and no significant difference was found among generations. The female longevity varied significantly among sequential generations. The longest female longevity was recorded in G5 and G10; and rearing *T. embryophagum* on *S. cereallella* for G35 and G40 led to the shortest female longevity. On the other hand, the male longevity did not differ significantly between G5, G10, and G15, but hereinafter it decreased significantly and no significant difference was found from G20 to G40. Significant differences were found in the total life span among different generations of *T. embryophagum* (Table 1). The longest total life span was observed for G5, and during the sequential generations, it was gradually decreased and the lowest total life span was obtained for G40.

The oviposition days of *T. embryophagum* decreased as the number of generations increased, such that the longest oviposition days was recorded in G5 and G10 and the shortest oviposition days was obtained in G40. The mean total fecundity was highest and lowest in G5 and G40, respectively. Generally, the wasps in earlier generations had longer adult longevity, longer total life span, and higher fecundity than later generations.

Sex ratio (number of females/number of females and males) of *T. embryophagum* was not significantly different among different generations tested (Figure 1).

Table 1. Comparative duration of different developmental stages and reproductive attributes of sequential generations of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs.[A]

Generation	Number of individuals	Preadult (Day)	Adult longevity (Female) (Day)	Adult longevity (Male) (Day)	Total life span (Day)	Oviposition days (Day)	Fecundity (Eggs)
5	77	9.95 ± 0.03 a	11.68 ± 0.12 a	11.21 ± 0.15 a	19.64 ± 0.49 a	10.29 ± 0.16 a	81.51 ± 2.00 a
10	78	9.98 ± 0.02 a	11.49 ± 0.13 ab	11.09 ± 0.17 a	19.13 ± 0.52 a	10.05 ± 0.16 ab	76.18 ± 1.59 b
15	76	9.97 ± 0.02 a	11.15 ± 0.14 b	11.05 ± 0.15 a	18.89 ± 0.52 abc	9.77 ± 0.15 b	72.92 ± 1.80 b
20	78	9.95 ± 0.02 a	10.64 ± 0.03 c	10.32 ± 0.03 b	18.13 ± 0.49 abcd	9.13 ± 0.16 c	65.00 ± 1.54 c
25	78	10.00 ± 0.02 a	10.24 ± 0.12 d	9.95 ± 0.10 b	17.67 ± 0.50 bcd	8.65 ± 0.14 d	61.65 ± 1.42 cd
30	78	10.00 ± 0.02 a	10.03 ± 0.11 d	9.95 ± 0.12 b	17.56 ± 0.49 cd	8.43 ± 0.12 d	59.30 ± 1.57 de
35	78	10.00 ± 0.02 a	9.94 ± 0.12 de	9.70 ± 0.12 b	17.49 ± 0.49 cd	8.2 ± 0.15 d	56.61 ± 1.26 e
40	78	10.00 ± 0.02 a	9.64 ± 0.10 e	9.68 ± 0.15 b	16.81 ± 0.51 d	7.78 ± 0.14 e	48.03 ± 1.18 f
df		7, 481	7, 305	7, 175	7, 620	7, 305	7, 305
F		1.35	38.66	21.90	3.73	38.16	49.95
P		0.226	< 0.0001	< 0.0001	0.0006	< 0.0001	< 0.0001

[A] The means followed by the same letter in each column are not significantly different (Mean±SE) (P < 0.05, Tukey's test).

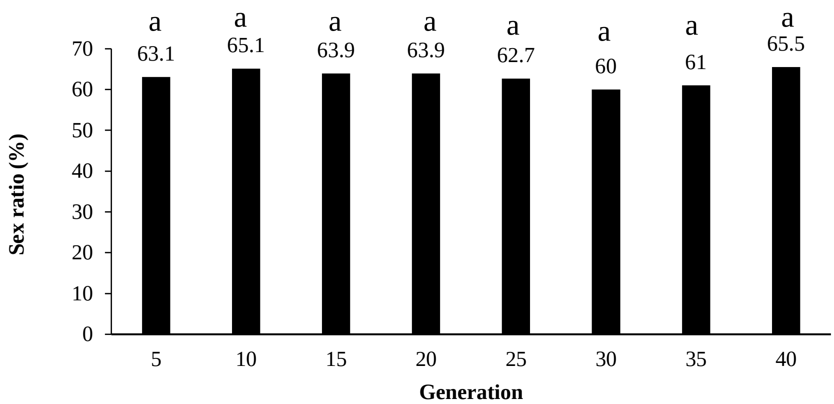

Figure 1. Sex ratio (females/males+females) of sequential generations of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs. The sex ratio in generations with the same letter are not significantly different (P< 0.05, Chi-square test).

For *T. embryophagum*, the age-stage-specific survival rate (s_{xj}), which is the probability of a newborn surviving to age x and stage j, was found to overlap among stages, demonstrating the variable developmental rates among individuals (Figure 2). The age-specific survivorship (l_x), age-stage-specific fecundity of female (f_{xj}), age-specific fecundity of population (m_x), and maternity ($l_x m_x$) of sequential generations of

T. embryophagum reared on *S. cerealella* eggs are plotted in Figure 3. The age-specific survivorship (l_x) of newly emerged *T. embryophagum* in G5-G40 were obtained as 0.96, 0.97, 0.80, 0.78, 0.75, 0.76, 0.76, and 0.71, respectively. The obtained results showed that f_{xj} of *T. embryophagum* at the first day of oviposition was 21.5, 21, 24, 21.15, 19.76, 19.76, 19.25, and 31.17 eggs, respectively. In addition, the highest value of m_x of *T. embryophagum* in G5-G40 were 14.46, 14.29, 14.11, 13.52, 12.39, 12.39, 11.75, and 11.33 eggs, respectively. According to these graphs, the highest fecundity was obtained in the first two days after the emergence of females.

The age-stage reproductive value of (v_{xj}) of *T. embryophagum* indicates the contribution of each individual at age x and stage j to population growth. The results revealed that female made the highest contribution to the population growth in days 9, 9, 9, 10, 10, 10, 10 and 10, respectively, as 58.48, 56.77, 58.99, 43.76, 41.81, 40.93, 39.60, and 34.60 (Figure 4).

The value of age-specific life expectancy (e_{xj}) of *T. embryophagum* on *S. cereallela* in successive generations is plotted in Figure 5. Life expectancy of newly emerged females in G5-G40 were 12.63, 12.46, 12.13, 10.59, 10.24, 10.03, 9.94, and 9.64 days, respectively. Also, it was estimated as 12.17, 11.09, 12.00, 11.05, 9.95, 9.95, 9.72, and 9.68 days for adult males.

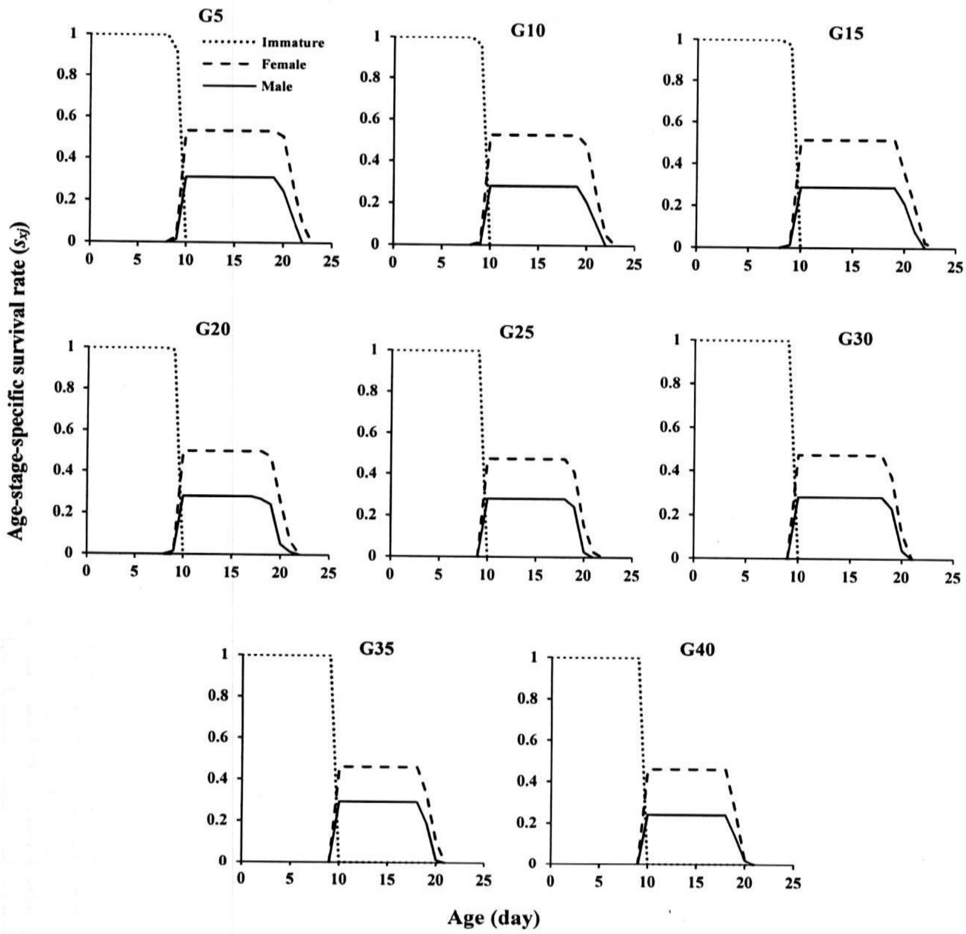

Figure 2. Age-stage-specific survival rate (s_{xj}) of sequential generations (G5-G40) of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs.

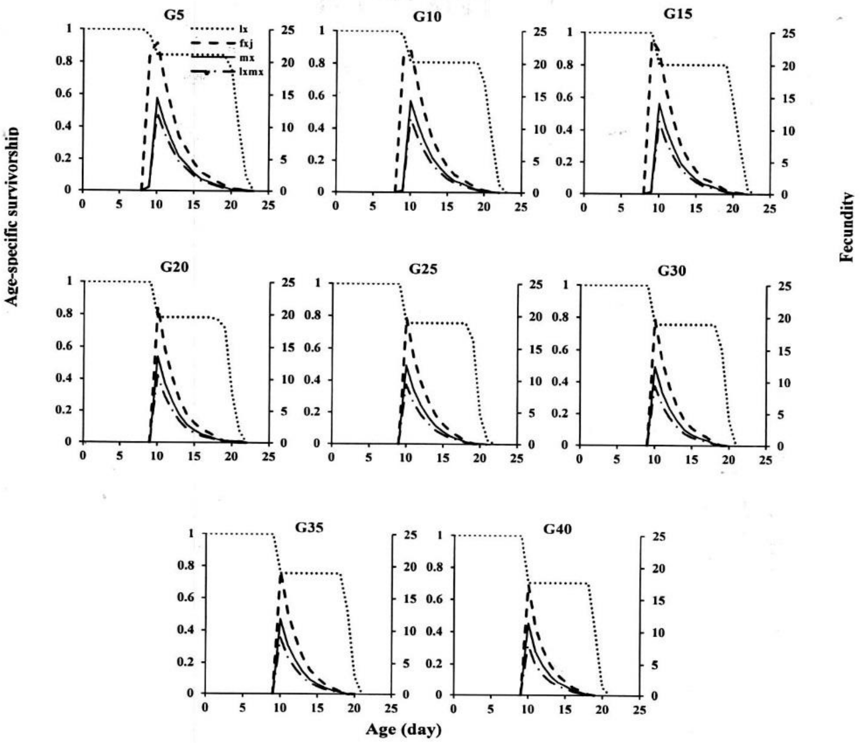

Figure 3. Age-specific survivorship (l_x), age-stage specific fecundity of female (f_{xj}) (eggs), age-specific fecundity (m_x), and age-specific maternity (l_xm_x) of sequential generations (G5-G40) of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs.

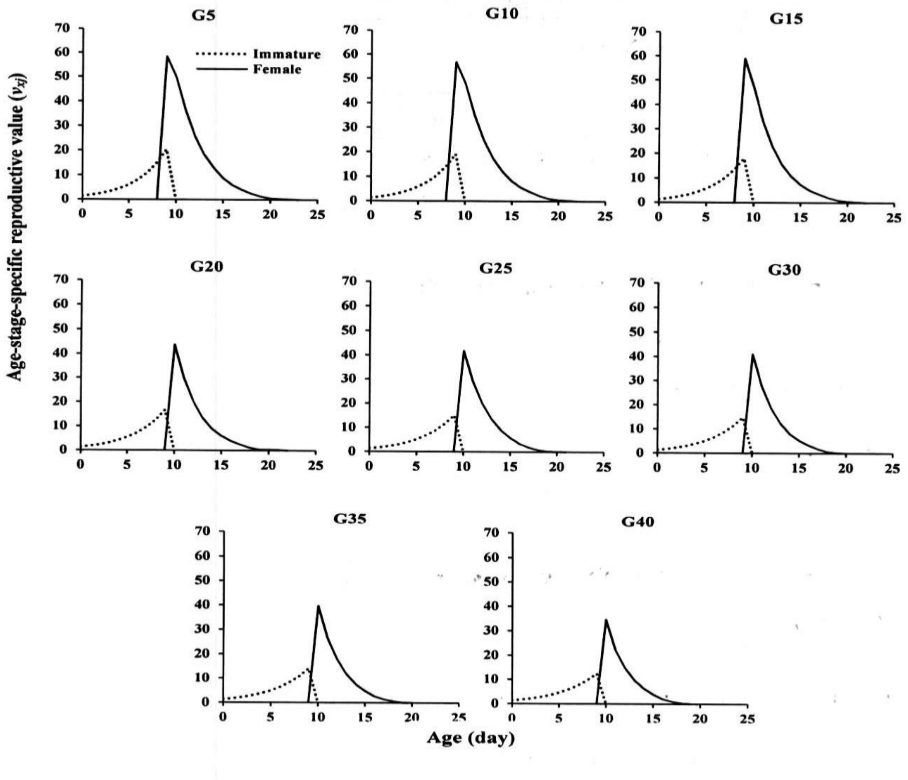

Figure 4. Age-stage-specific reproductive value (v_{xj}) of sequential generations (G5-G40) of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs.

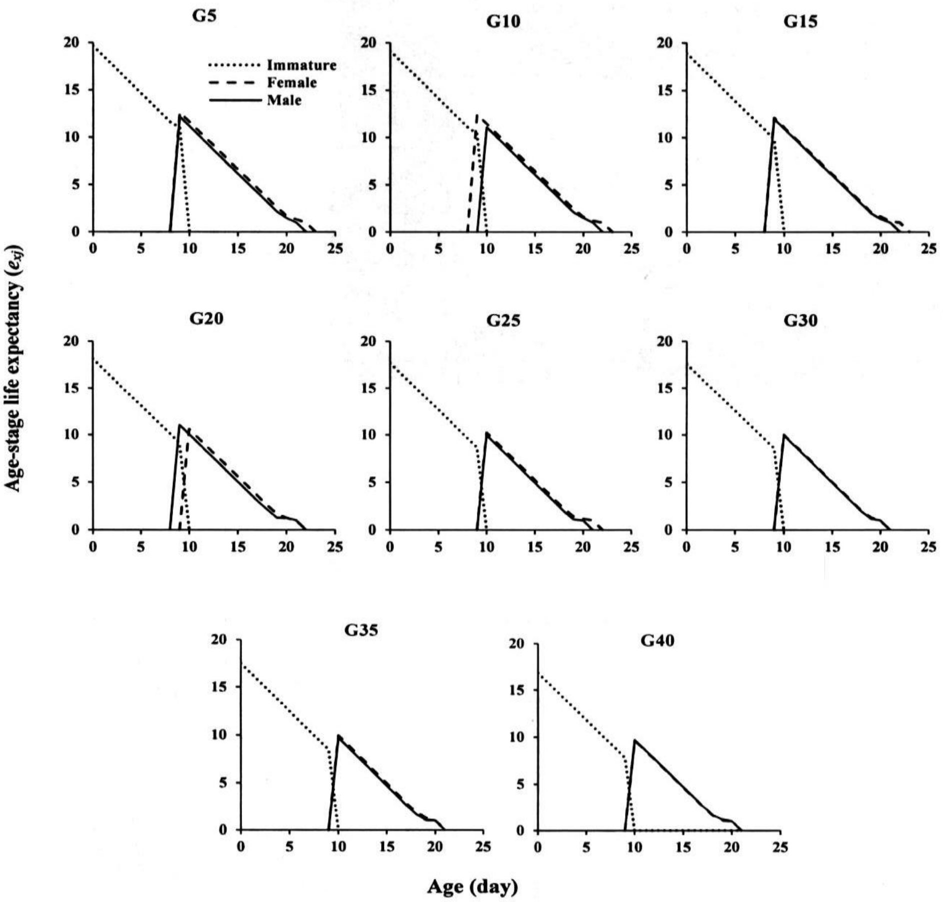

Figure 5. Age-stage life expectancy (e_{xj}) of sequential generations (G5-G40) of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs.

Population Growth Parameters

Results of paired bootstrap test indicated that all population growth parameters of *T. embryophagum* reared on *S. cereallela* were significantly different in successive generations. The highest and lowest values of the Gross Reproductive Rate (*GRR*), net Reproductive rate (R_0), intrinsic rate of increase (r), and finite rate of increase (λ) of *T. embryophagum* were observed in G5 and G40, respectively (Table 2).

Table 2. Life table parameters of sequential generations of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs.[A]

Generation	*GRR* (Eggs/Individual)	R_0 (Eggs/Individual)	r (d^{-1})	λ (d^{-1})	T (Day)
5	51.60 ± 5.056 a	43.402 ± 4.77 a	0.301 ± 0.009 a	1.351 ± 0.012 a	12.530 ± 0.055 a
10	50.08 ± 4.720 ab	40.397 ± 4.393 ab	0.295 ± 0.009 ab	1.343 ± 0.012 ab	12.546 ± 0.043 ab
15	46.67 ± 4.643 ab	37.420 ± 4.289 abc	0.291 ± 0.009 abc	1.337 ± 0.013 abc	12.468 ± 0.053 abc
20	41.88 ± 4.128 abc	32.500 ± 3.753 abc	0.280 ± 0.009 abc	1.324 ± 0.013 abc	12.411 ± 0.032 bc
25	38.73 ± 3.992 bcd	29.244 ± 3.553 bcd	0.272 ± 0.010 bcd	1.312 ± 0.013 bcd	12.429 ± 0.033 abc
30	37.20 ± 3.87 bcd	28.128 ± 3.429 bcd	0.269 ± 0.010 bcd	1.309 ± 0.013 bcd	12.374 ± 0.039 bcd
35	34.56 ± 3.684 cd	26.128 ± 3.245 cd	0.264 ± 0.010 cd	1.302 ± 0.014 cd	12.352 ± 0.038 cd
40	31.44 ± 3.173 d	22.167 ± 2.762 cd	0.252 ± 0.010 d	1.286 ± 0.013 cd	12.302 ± 0.035 d

[A] The means followed by the same letter in each column are not significantly different (Mean±SE) (P< 0.05, Paired bootstrap test.

DISCUSSION

Production of biocontrol agents has received much attention in recent years (van Lenteren, 2012; Khanamani *et al.,* 2021). One of the most common natural enemies of agricultural pests are egg parasitoids in the genus *Trichogramma*, which are produced and used in large numbers each year (Cônsoli *et al*., 2010; Moghaddassi *et al.,* 2019). *T. embryophagum* as one of the most important species of *Trichogramma* has been collected from several regions and reared in several insectaries (Mirkarimi, 2000). In insectaries, long-term mass rearing of parasitoid wasps may decrease their quality, which leads to declining efficiency of integrated pest management programs. Accordingly, frequently assessing the quality of biocontrol agents under successive rearing is vital (Khanamani *et al.,* 2017). In this study, the effect of rearing *T. embryophagum* on *S. cerealella* eggs over 40 sequential generations on its quality were determined using biological characteristics and life table parameters. According to our findings, biological attributes and population parameters of *T. embryophagum* differed significantly among successive generations.

Several factors influence parasitoids' longevity, including species of parasitoid and factitious hosts, population genetic patterns, and rearing conditions. Rearing T. embryophagum over 40 generations on S. cereallella eggs led to a significant decrease in the male and female longevity. This result is consistent with results obtained by other researchers in different species of *Trichogramma* wasps. For instance, Pratissoli *et al.* (2004) stated that male and female longevity of *T. pretiosum* decreased significantly over 23 generations. Female longevity of *T. pretiosum* reared on *A. kuehniella* was almost constant in the first 10 generations and then decreased in subsequent generations, up to the 100^{th} generation (Freitas Bueno *et al.,* 2006). This decreasing trend in male and female longevity after a certain number of generations in mass rearing was also observed in the study of Lü *et al.* (2017), and Ghaemmaghami *et al.* (2021).

One of the most important issues in mass rearing of natural enemies is the number of sequential generations that a biological control agent can be mass-reared without a significant reduction in its quality and efficiency (van Lenteren *et al.*, 2002). In the present study, the early generations of *T. embryophagum* reared on *S. cereallela* eggs had the longest female and male longevity, total life span, the number of oviposition days, and fecundity. This shows that parasitoids may demand more energy to complete their life cycle after a certain number of generations without obtaining major benefits (Nordlund *et al.,* 1997; Pratissoli *et al.*, 2004; Lü *et al.*, 2017).

The sex ratio is an economically important trait in assessing the quality of parasitoid wasps (Cerutti and Bigler, 1995; Hassan and Zhang, 2001), which plays an essential role in the dynamics of the insect population, affects the performance of the reared population, and also has a significant effect on the financial profitability of mass rearing (Pipoly *et al.,* 2015). In this research, no significant difference was observed between sex ratio of *T. embryophagum* on *S. cereallella* eggs in different generations (P= 0.99). This result differed from the findings of Pratissoli *et al. (*2004), Lü *et al.* (2017), and Ghaemmaghami *et al.* (2021) who showed a fluctuation in the sex ratio of other *Trichogramma* species.

As shown in Figures 2 and 3, the survival of *T. embryophagum* decreased over 40^{th} generations, which is consistent with those results reported in other *Trichogramma* species (Nordlund *et al.*, 1997; Pratissoli *et al.*, 2004; Lü *et al.*, 2015).

The mean generation Time (*T*) is the period of time it takes for a population to increase to R_0-folds of its current size at the stable age-stage distribution. Natural logarithm of R_0 divided by *r* yields this parameter and, therefore, its value is expected to increase with decreasing *r*. Contrary to expectations, our results showed that *T* value decreased by decreasing *r*, which is attributed to the proportion of R_0 and *r* in successive generations, such that the value of R_0 decreased more than *r*. This might be considered one of the study's main findings because it can lead to a larger population size in less time.

The obtained results showed that population parameters of *T. embryophagum* were affected by long-term mass rearing and the highest values of intrinsic rate of increase (*r*), finite rate of increase (λ), net Reproductive rate (R_0), Gross Reproductive Rate (*GRR*), and mean generation Time (*T*) were found in the first generations (up to 20th generation), and all the life table parameters decreased after the 25th generation. The *r* value is one of the most important criteria used to evaluate the efficiency of natural enemies (Fathipour and Maleknia, 2016). The highest *r* value was found for parasitoids of G5–G20, indicating their better fitness compared to the previous generations. Similar to the results of this study, a decrease in the quality of natural enemies has been reported by Lü *et al*. (2017) in *Trichogramma dendrolimi* Matsumura and Ghaemmaghami *et al.* (2021) in *T. brassicae.*

Inbreeding, which is the most common form of non-random mating, should be among the main reasons for this decline in fitness over sequential generations (Kazmer and Luck, 1991; van Lenteren and Bigler, 2010). Using small egg cards in the rearing process can increase opportunities for inbreeding, and might gradually decrease the genetic quality of reared parasitoids (Paspati *et al.*, 2019). Contradictory reports have been made about the occurrence of inbreeding in *Trichogramma* wasps. In spite of Sorati *et al.* (1996) who described lack of this phenomenon in the mass rearing of *T. brassicae*, Antolin (1999) reported the negative effect of inbreeding on reproduction and sexual ratio of *T. pretiosum*. According to these contradictory reports, confirmation of the occurrence of inbreeding in log-term mass rearing of *T. embryophagum* requires more detailed studies. Moreover, deformed adults that remained in the colony during the continuous rearing could lead to the reduction of parasitoids' quality (Suzuki and Hiehata, 1985).

Based on the obtained results, long-term mass rearing on *S. cereallella* eggs up to the 20th generation has no adverse effect on biological characteristics and population parameters of *T. embryophagum,* and the wasps reared in sequential generations up to this generation can be used successfully in biological control programs without any significant loss of quality or performance. Indeed, continuous long-term mass rearing of natural enemies in insectariums may cause the biological control program to fail, and it is necessary to consider strategies to improve the quality of the produced parasitoids. Therefore, to keep the performance fixed, after the 20th generation, the laboratory population should be rejuvenated every few generations by adding field-collected individuals.

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پرورش متوالی روی بید غلات، کیفیت زنبور پارازیتوئید *Trichogramma embryophagum* (Hymenoptera: Trichogrammatidae) را تحت تاثیر قرار می دهد

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چکیده

پارامترهای دموگرافی زنبور پارازیتوئید *Trichogramma embryophagum* Hartig پرورش‌یافته روی بید غلات، *Sitotroga cerealella* (Olivier) به مدت ۴۰ نسل مورد بررسی قرار گرفت. آزمایش‌ها در اتاقک رشد با شرایط دمایی ۲±۲۶ درجه سلسیوس، رطوبت نسبی ۵±۶۵ درصد و دوره نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی انجام شد. نتایج نشان داد که طول عمر زنبورهای ماده طی پرورش پیاپی به طور معنی‌داری کاهش می یابد و از ۱۰/۶۸ روز (در نسل پنجم) تا ۹/۶۴ روز (در نسل چهلم) متغیر بود. از سوی دیگر، طول عمر زنبورهای نر در نسل بیستم به طور معنی‌داری کاهش یافت و هیچ تفاوت معنی‌داری بین طول عمر زنبورهای نر در نسل بیستم تا نسل چهلم مشاهده نشد. طول دوره تخمریزی و میانگین کل باروری زنبور *T. embryophagum* با افزایش تعداد نسل‌ها کاهش یافت. عموماً، زنبورهای نسل‌های ابتدایی داری طول عمر، طول دوره زندگی و باروری بیشتری نسبت به نسل‌های بعدی بودند. علاوه بر این، نسبت جنسی *T. embryophagum* در نسل‌های متوالی اختلاف معنی داری نداشت. نتایج آزمون بوت‌استرپ جفت‌شده نشان داد که تمام پارامترهای رشد جمعیت زنبور *T. embryophagum* پرورش‌یافته روی بید غلات در نسل‌های متوالی به طور معنی‌داری متفاوت از یکدیگر بودند. بیشترین و کمترین مقادیر نرخ ذاتی افزایش جمعیت (r)، نرخ متناهی افزایش جمعیت (λ)، نرخ خالص تولیدمثل(R_0)، نرخ ناخالص تولیدمثل (GRR) و میانگین طول یک نسل (T) زنبور *T. embryophagum* به‌ترتیب در نسل چهلم و پنجم مشاهده شد. این نتایج نشان می‌دهد که زنبورهای *T. embryophagum* پرورش‌یافته در نسل‌های متوالی می‌توانند تا نسل بیستم با موفقیت در برنامه‌های کنترل بیولوژیک، بدون هیچ‌گونه افت کیفیت و عملکرد مورد استفاده قرار گیرند و پس از آن، باید جوانسازی منظم جمعیت آزمایشگاهی، با وارد کردن گاه‌به‌گاه پارازیتوئیدهای جمع‌آوری‌شده از مزرعه صورت گیرد.