

Research Article

Generation-dependent fitness of the parasitoid wasp *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) in laboratory and insectarium condition

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Abstract: Deep understanding of biological and behavioral characteristics of trichogrammatid wasps, helps us for better and more efficient rearing of these parasitoids. In the current study, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) was reared on Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) for several generations and the effect of long-term rearing of the parasitoid on some of its attributes like parasitism capability, emergence rate, sex ratio, and wingless rate of adults was determined in both laboratory and insectarium conditions. The laboratory investigation showed that long-term rearing decreased fitness of the parasitoid, so that, parasitism rate and adult emergence rate decreased. By contrast, sex ratio and number of wingless individuals increased with increasing generations. Our results in insectarium were a little different. However, adult emergence rate had significant difference and trichocards quality decreased with increasing generations. The sex ratio in different periods had no significant difference. Furthermore, there was no significant difference in wingless rate in different rearing time periods. Accordingly, the parasitoid's fitness was affected by number of generations in both conditions. Although, mass rearing under laboratory condition provided wasps with more desired traits than those reared in insectary, both decreased fitness of *T. brassicae* when number of generations increased.

Keywords: Angoumois grain moth, long-term rearing, parasitoid fitness, *Sitotroga cerealella*

Introduction

Trichogramma is a well-known genus of minute polyphagous wasps that are endoparasitoids of insect eggs. *Trichogramma* is one of around 80 genera in the family Trichogrammatidae, with over 200 species worldwide (Querino *et al.*,

2010; Goettig and Herz, 2016). In particular, over 400 species of Lepidoptera family including several economically important agricultural pests are attacked by different species of *Trichogramma* (Newton, 1993). *Trichogramma* wasps are the most widely studied and used agents for biological control of lepidopteran pests worldwide (de Freitas Bueno *et al.*, 2009). According to outdated estimates, different species of *Trichogramma* are annually released in over 32 million ha of agricultural fields and forests for control of about 28

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lepidopteran pest species in 30 countries. The targeted host plants include corn, rice, sugarcane, cotton, cabbage, tomato, soybean, fruit trees, pine and spruce (Li *et al.*, 1994; Smith, 1996). *Trichogramma* wasps have a number of advantages when used as an insect biocontrol agent. For example, most trichogrammatids are polyphagous, meaning that they attack and control different pest species within the same habitat, thus they have higher chance of survival when the target host is rare (Smith, 1996). In addition to this benefit, *Trichogramma* wasps are highly aggressive in parasitizing host eggs and kill the target pest before appearance of the damaging larval stage (Hassan *et al.*, 1998).

The first rearing and augmentative release of *Trichogramma* wasps was conducted on a limited scale by Russian researchers for control of the codling moth, *Cydia pomonella* (Lep.: Tortricidae) (Flanders, 1930). The use of biological control agents decreased with the rise of chemical insecticides, but research on different aspects of *Trichogramma* wasps (ecology, systematics, mass rearing, and application) was revitalized in the 1960s (Smith, 1996). Although several biological control programs by *Trichogramma* wasps have been conducted in the former USSR and China before the 1950s, the mass rearing and release of these wasps in North America and Europe did not start until the early 1970s (Hassan *et al.*, 1998; Smith, 1996).

In Iran, applied research on *Trichogramma* wasps began in 1974 by importing two species from the former USSR and Germany and releasing in rice fields of the northern regions for control of the striped rice stem borer, *Chilo suppressalis* (Walker) (Lep.: Crambidae). Mass rearing of *Trichogramma* wasps was initiated between 1975 and 1977 and a national project on mass rearing of these wasps was performed by the Iranian Research Organization for Science and Technology (IROST) in 1984 (Ebrahimi *et al.*, 1998). Despite numerous studies made on species diversity, mass rearing and application of *Trichogramma* in Iran during the last four decades, *Trichogramma* currently

plays a minor role in biological control of agricultural pests in Iran (Nazeri *et al.*, 2015). This is particularly related to poor knowledge on commercial production and quality control of *Trichogramma* wasps, which may negatively affect its capability to suppress target pests efficiently.

A prerequisite for the mass rearing of natural enemies for use in biological control programs is that they can be reared at low costs on factitious hosts in laboratory (Takada *et al.*, 2001). Fortunately, *Trichogramma* wasps are easily reared under laboratory conditions on alternative hosts such as the Mediterranean flour moth, *Ephesia kuehniella* Zeller (Lep.: Pyralidae) and the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lep.: Gelechiidae) (Altoe *et al.*, 2012). Another important issue in a successful biological control program is to maintain the quality of produced agents for successive generations in laboratory or insectarium. Certain guidelines have been developed by the International Organization for Biological and Integrated Control (IOBC), through which the quality of commercially produced natural enemies can be evaluated. For example, the quality of the trichogrammatid parasitoids reared on laboratory hosts can be checked using a variety of biological parameters such as emergence rate, fecundity, life span, sex ratio, and parasitism rate (Bigler *et al.*, 1987).

In this study, we evaluated the fitness and quality parameters of a native population of the parasitoid, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) when reared for successive generations on the factitious host, *S. cerealella* under both laboratory and insectary conditions.

Materials and Methods

Insects

This study was performed in two separate phases (laboratory and insectary experiments) to evaluate and compare the quality parameters of reared *Trichogramma* wasps under these conditions for successive generations. A line of

Trichogramma was collected from tomato fields located at the Imam Khomeini Higher Education Centre (Karaj, Iran). For this purpose, pieces of white papers (21 × 11cm) containing *S. cerealella* eggs were placed in different parts of the field and collected after 24 hours. The papers were incubated in a growth chamber at 25 ± 2 °C, 60 ± 5% RH and 16L:8D h. Microscopic slides from antennae and genitalia of the emerged adult *Trichogramma* wasps were prepared and the specimens were identified as *T. brassicae* by the Iranian Research Institute of Plant Protection (IRIPP) (Tehran, Iran). Stock colonies of *T. brassicae* were established in both laboratory and insectary conditions and reared for three generations, on *S. cerealella* eggs as host. Adult wasps emerged as the fourth generation were used to evaluate quality parameters of eight successive generations of the laboratory colony (F 4-11) and those of 10 successive generations of the insectary colony (F 4-13).

Laboratory assays

This experiment was performed with 30 replicates in a growth chamber with controlled conditions (25 ± 2 °C, 60 ± 5% RH, 16L:8D h). Four quality parameters, including parasitism rate, adult emergence rate, sex ratio and rate of wing deformity, were calculated for 8 successive generations (F 4-11). To evaluate the parasitism rate of laboratory reared parasitoids, a total of 100 *S. cerealella* eggs were uniformly glued to rectangular pieces of white cards (6 × 1.5cm) using water-honey solution (10%). The cards were placed individually inside glass tubes (length 7 cm, diameter 2 cm) and one mated 24-h-old female of *T. brassicae* was released in each tube. After 36 h, adult wasps were removed and parasitism rate was determined by counting the number of parasitized (blackened) eggs on the third day of the experiment.

To estimate adult emergence rate, the cards containing *T. brassicae* broods were incubated in the growth chamber at the above mentioned condition until adult emergence. The rate of adult emergence was then estimated by dividing

the number of emerged adults by the number of parasitized eggs. The sex ratio (% males) of emerged adults was determined by dividing the number of adult males by the total number of emerged adults in each tube. Adult males are easily recognized by their long hairy antennae, when compared to shorter hairless antennae of females. Finally, the occurrence of individuals with wing deformity was evaluated by counting the number of brachypterous and wingless wasps under stereomicroscope (Olympus SZ40, Japan).

Insectary assays

The insectary assays were conducted in the Imam Khomeini Higher Education Centre insectarium (Karaj, Iran). To do so, insectary was set as a dark chamber (3 × 2 × 2 m) equipped with a light source at one side to attract ovipositing females. The white papers containing parasitized *S. cerealella* eggs were placed in vicinity of fresh eggs at the side of light source. After oviposition of the newly emerged adults, the papers were transferred to rearing chamber and incubated under controlled condition (25 ± 2 °C, 70 ± 5% RH, 16L:8D h) for pre-adult development.

In this experiment, the quality parameters of *T. brassicae* reared from *S. cerealella* in the abovementioned insectary were evaluated for 10 successive generations (F 4-13). To estimate the rate of adult emergence, pieces of white papers containing an average number of 500-700 *S. cerealella* eggs were exposed to ovipositing females of each generation (see above) for 24 h. The papers were then transferred to rearing chamber, where they were incubated under controlled conditions (see above) until adult emergence. The number of parasitized eggs and the number of adult emergence was recorded and used to calculate the rate of adult emergence. The sex ratio of emerged adults as well as the occurrence of wing deformity in the population was calculated as described for the laboratory study. The parasitism rate of *T. brassicae* was not calculated for insectary population, due to unknown number of ovipositing females.

Data analysis

The normality of data was checked by kurtosis and skewness tests in SPSS prior to analysis. One way analysis of variance (ANOVA) was used to test significant differences between treatments and then means were separated by least significant difference (LSD) test. Statistical analysis was performed by SAS version 9.1.3.

Results and Discussion

Biological control agents are routinely reared on insects rather than other target hosts in order to reduce the cost and increase the efficiency of mass production in laboratory (Hoffmann *et al.*, 2001). The quality of parasitoids may be decreased when they are reared for many generations under uniform conditions and on alternative hosts (Smith, 1996). Therefore, it is crucial to monitor and maintain parasitoid quality in order to avoid decreased efficiency of wasps reared for successive generations. In this study, we evaluated some quality parameters of a native population of *T. brassicae*, reared for successive generations on the factitious host, *S. cerealella* under both laboratory and insectary conditions. Results of the laboratory and insectary assays for quality parameters of *T. brassicae* have been summarized in Tables 1 and 2, respectively.

An initial experiment was conducted to determine host acceptance and parasitism rate of the established colony of *T. brassicae* on *S. cerealella* over successive generations. Despite the wasps accepting *S. cerealella* eggs as host, the rate of egg parasitism was relatively low (< 40%). There was significant difference in parasitism rate of *T. brassicae* among generations ($F_{7,232} = 7.96$, $P < 0.001$). The maximum egg parasitism rate was observed in the third generation (39.1%), followed by a descending pattern, such that the lowest egg parasitism rate (26.2%) occurred in generation 8 (Table 1). The parasitism rate was not estimated for insectary population, because the number of females involved in oviposition was

undefined. In line with these results, Soares *et al.* (2012) showed that recently collected populations of *T. pretiosum* showed a decreased parasitism rate during initial generations, probably because they were in an adaptation process to laboratory condition and to the factitious host used. Similarly, Pratisoli *et al.* (2004) recorded lower rates of *E. kuehniella* egg parasitism by *T. pretiosum* during the first four generations, when the wasps were reared for 39 generations under laboratory condition. By contrast, Hoffmann *et al.* (2001) found no evidence of decreased parasitism rate in *T. minutum* reared for many generations under laboratory conditions and on factitious hosts.

The emergence rate of adult wasps was statistically different among successive generations under both laboratory ($F_{7,232} = 3.94$, $P < 0.001$) and insectary ($F_{9,90} = 5.51$, $P < 0.001$) conditions (Table 1 and 2). In the laboratory, the highest rate of adult emergence was recorded for the fifth generation (96.3%) followed by the first generation (95.49%). By contrast, the lowest rate of adult emergence was recorded for wasps in the second generation (93.05%) (Table 1). In the insectary, the highest rates of adult emergence were recorded for the first three generations (95, 94.5, and 92.6%, respectively), while the five last generations showed significant decrease in the rate of adult emergence (Table 2). The wasps in laboratory experienced slightly higher rate and more stable pattern of adult emergence than those in insectary, probably due to more strict control of climatic conditions (temperature and humidity) in the laboratory. The emergence rate of different species of *Trichogramma*, reared on laboratory hosts, are reported to range from 92.5% to 100%. However, the emergence rate may be much less when the wasps are reared on target hosts (Goncalves *et al.*, 2005). Although, the arte of adult emergence in laboratory population was within this range, the decreased emergence rate of insectary-reared wasps may highlight the unsuitability of the rearing method, factitious host, or/and climatic condition. According to Soares *et al.* (2012), the emergence rate of *Trichogramma* wasps

may depend on the size and quality of the host egg, the number of parasitoids that develop per egg, and temperature. For example, younger

eggs (as provided in laboratory assay) may supply the parasitoid with better and greater availability of the food reserves.

Table 1 Generation-dependent fitness of the parasitoid wasp *Trichogramma brassicae* in laboratory condition.

Generation	Parasitism (%)	Adult emergence (%)	Adult males in population (%)	Wingless males (%)	Wingless females (%)	Wingless adults (%)
1	36.5 ± 1.83 ^a	95.5 ± 0.36 ^a	17.3 ± 1.21 ^b	7.8 ± 1.79 ^a	9.52 ± 1.13 ^a	9.35 ± 0.97 ^a
2	38.9 ± 2.24 ^a	93.1 ± 1.16 ^c	10.5 ± 1.32 ^d	11.9 ± 2.80 ^a	8.09 ± 0.83 ^a	8.61 ± 0.91 ^a
3	39.1 ± 2.04 ^a	94.4 ± 0.62 ^a	13.2 ± 0.89 ^c	11.5 ± 2.45 ^a	5.46 ± 0.53 ^b	6.34 ± 0.53 ^b
4	37.8 ± 1.81 ^a	93.4 ± 0.41 ^b	9.8 ± 0.58 ^e	16.4 ± 4.15 ^a	5.38 ± 0.83 ^b	6.37 ± 0.84 ^b
5	33.6 ± 1.67 ^a	96.3 ± 0.33 ^a	15.9 ± 0.96 ^b	12.6 ± 2.45 ^a	5.91 ± 0.86 ^b	7.25 ± 0.80 ^a
6	29.1 ± 1.27 ^b	94.3 ± 0.43 ^a	22.8 ± 1.24 ^a	13.9 ± 2.06 ^a	7.34 ± 0.75 ^a	8.95 ± 0.80 ^a
7	30.3 ± 1.71 ^b	93.5 ± 0.45 ^b	22.3 ± 1.03 ^a	15.6 ± 2.11 ^a	7.98 ± 0.68 ^a	10.50 ± 0.81 ^a
8	26.2 ± 1.14 ^c	93.5 ± 0.38 ^b	24.2 ± 1.26 ^a	14.1 ± 2.07 ^a	8.11 ± 0.94 ^a	10.50 ± 1.11 ^a
F _{7,232}	7.961	3.949	27.944	1.090	3.175	3.865
P	< 0.0001	< 0.0001	< 0.0001	0.370	0.003	0.001

Means followed by the same letters in each column are not significantly different (LSD test, P < 0.05).

Table 2 Generation-dependent fitness of the parasitoid wasp *Trichogramma brassicae* in insectary condition.

Generation	Adult emergence (%)	Adult males in population (%)	Wingless males (%)	Wingless females (%)	Wingless adults (%)
1	95.0 ± 0.58 ^a	24.9 ± 1.22 ^a	10.3 ± 0.59 ^a	10.1 ± 0.52 ^a	10.1 ± 0.46 ^a
2	94.5 ± 0.59 ^a	26.3 ± 2.11 ^a	11.9 ± 0.81 ^a	11.1 ± 0.87 ^a	11.3 ± 0.73 ^a
3	92.6 ± 0.79 ^a	27.4 ± 2.42 ^a	10.4 ± 0.91 ^a	11.3 ± 1.68 ^a	10.7 ± 0.87 ^a
4	91.3 ± 0.85 ^b	25.4 ± 1.65 ^a	11.7 ± 0.85 ^a	11.9 ± 2.06 ^a	11.5 ± 1.32 ^a
5	92.5 ± 0.71 ^a	27.8 ± 1.78 ^a	10.8 ± 0.65 ^a	11.2 ± 1.41 ^a	10.8 ± 0.98 ^a
6	90.2 ± 0.76 ^b	30.1 ± 3.4 ^a	10.3 ± 0.61 ^a	13.4 ± 2.59 ^a	11.5 ± 1.18 ^a
7	91.1 ± 0.68 ^b	28.2 ± 1.96 ^a	11.1 ± 0.86 ^a	12.9 ± 2.69 ^a	11.9 ± 1.72 ^a
8	89.9 ± 0.82 ^b	30.1 ± 0.76 ^a	11.8 ± 0.98 ^a	11.1 ± 0.96 ^a	11.3 ± 0.81 ^a
9	90.7 ± 0.69 ^b	29.4 ± 1.54 ^a	12.1 ± 0.51 ^a	12.4 ± 1.37 ^a	12.2 ± 0.99 ^a
10	91.1 ± 0.73 ^b	31.8 ± 1.97 ^a	11.8 ± 0.56 ^a	13.2 ± 1.35 ^a	12.5 ± 0.95 ^a
F _{9,90}	5.514	1.489	0.876	0.432	0.448
P	< 0.0001	0.164	0.550	0.920	0.905

Means followed by the same letters in each column are not significantly different (LSD test, P < 0.05).

We found also significant difference in the ratio of adult males among generations in laboratory assay (F_{7,232} = 27.94; P < 0.001). The laboratory population produced significantly higher number of males in the three last generations, while the lowest number of males was observed in descending order in generations 4, 2, and 3, respectively (Table 1). Although, the ratio of adult males in the population tended to increase generation after generation in the insectary, the difference in sex

ratio was not statistically significant among treatments (F_{9,90} = 1.48, P = 0.164) (Table 2). For all generations, the insectary population produced higher ratio of males than laboratory ones. In haplodiploid Hymenoptera (including *Trichogramma*), females are developed from fertilized diploid eggs, while males arise from unfertilized haploid eggs. The female wasps of many species are able to selectively fertilize their eggs to produce diploid female offspring or deposit unfertilized eggs to produce male

offspring (Godfray, 1994; Morrill *et al.*, 2000). The proportion of females tends to increase under favorable climatic conditions as well as host quality, age, and density (Morrill *et al.*, 2000). Therefore, a male-biased sex ratio, as observed in our insectary population, is typically interpreted as poor rearing conditions (Martel, 2003).

Under laboratory condition, the percentage of male wing deformity (aptery and brachyptery) was not statistically different among generations ($F_{7,232} = 1.09$, $P = 0.370$). By contrast, significant difference was found in wing deformity of adult females among generations ($F_{7,232} = 3.15$, $P < 0.01$), with significantly lower rates of deformity in generations 4 (5.38%), 3 (5.46%), and 5 (5.91%) (Table 1). By considering both male and female wasps, there was significant difference in the occurrence of wing deformity among treatments ($F_{7,232} = 3.86$, $P < 0.001$), with lower rates of wing deformity in generations 3 (6.34%) and 4 (6.37%) (Table 1). The higher percentage of wing deformity in the first generation may be attributed to the unfamiliarity of *T. brassicae* to the factitious host. After adaptation to this host, however, the proportion of adults with malformed wings decreased. In continue and by increasing the number of generation reared under the same condition, proportion of the wing deformity increased again (Table 1).

No significant difference was found in the rate of wing deformity of either males ($F_{9,90} = 0.87$, $P = 0.550$), females ($F_{9,90} = 0.43$, $P = 0.920$), or the whole population ($F_{9,90} = 0.44$, $P = 0.905$) among generations under insectary condition. However, adults of the later generations tended to have higher rate of wing deformity, when compared to those of the earlier generations (Table 2).

Flight capacity is an important characteristic of parasitoid wasps involving dispersion and searching capability of both male and female parasitoids (Soares *et al.*, 2012). Under laboratory or insectary production systems, the percentage of adults with deformed wings and lower flight capacity may increase due to

unfavorable rearing conditions (Prezotti *et al.*, 2002). An increase in the proportion of adult wasps with malformed wings is expected to significantly affect the parasitism rate and control efficiency due to decreased dispersion and searching ability (Knutson, 1998). In our study, the proportion of adults with malformed wings in the insectary was nearly always higher than the laboratory, which may again emphasize on the unfavorable rearing conditions of the insectary (Tables 1 & 2). Nazeri *et al.* (2015) showed that host quality has an important effect on the occurrence of wing deformity in laboratory-reared *T. brassicae*.

The *Trichogramma* wasps are released in the field after several generations of mass rearing in the laboratory or insectary (Smith, 1996). A large number of studies have argued that laboratory rearing for many generations can negatively affect some quality parameters of biological control agents and this can further result in control failure (Bigler *et al.*, 1987; Pratissoli *et al.*, 2004). These negative effects result from a variety of factors, including rearing multiple generations on unnatural host, the absence of plants, crowding and interference, rapid generation time, and failure to rejuvenate genetic stock (Bigler *et al.*, 1987). The loss of preference for the laboratory host over generations is considered as an important reason why the quality of *Trichogramma* wasps decreases under continuous rearing systems. However, studies on different *Trichogramma* species and different laboratory hosts have provided conflicting results (Smith, 1996). The current approaches to counter these negative effects include limiting the maximum number of generations that the biological control agent can be reared in the laboratory and periodical renewal of the colony by field collected wasps as well as periodical switching of the parasitoids to other factitious hosts (Smith, 1996; Knutson, 1998). Further studies may evaluate the efficiency of laboratory- and insectary-reared wasps for control of a target pest in field or semi-field condition.

As mentioned before, this research was aimed to study potential and quality of

parasitoids reared in the insectarium of Imam Khomeini Higher Education Centre. We revealed that there were some discrepancies in the criteria measured in the present study compared with those gained in insectariums with respect to the quality control principles and demonstrated some problems exist in mass rearing of parasitoids in this insectarium which should be readjusted in the future. These problems can be categorized as human and non-human errors. We strongly suggest calibration of the tools and protocols used in rearing of natural enemies according to those suggested by van Lenteren (2003).

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بررسی ویژگی‌های زیستی در نسل‌های متوالی زنبور *Trichogramma brassicae* پرورش یافته در شرایط آزمایشگاهی و انسکتاریوم

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چکیده: درک بیش‌تر و بهتر ویژگی‌های زیست‌شناختی و رفتاری زنبورهای تریکوگراما، به ما در تولید بهتر و بهینه‌تر این زنبور پارازیتوئید کمک می‌کند. بدین‌منظور در این پژوهش اثر پرورش‌های طولانی مدت زنبور پارازیتوئید *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) روی میزبان بید غلات *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) مورد بررسی قرار گرفت و در دو بخش مطالعات آزمایشگاهی و ارزیابی‌های انسکتاریومی درصد پارازیتیسیم، نرخ خروج، نسبت جنسی، نسبت افراد بی‌بال نر و ماده بررسی شد. نتایج در بخش مطالعات آزمایشگاهی به‌خوبی نشان داد که پرورش طولانی مدت روی یک میزبان از توانمندی‌های زنبور می‌کاهد به‌گونه‌ای که درصد پارازیتیسیم و نرخ خروج با افزایش نسل کاهش یافت و نسبت جنسی نر و افراد بی‌بال در جمعیت افزایش یافت. با این حال در ارزیابی‌های انسکتاریومی نتایج به گونه دیگری رقم خورد. در این بخش اگرچه نرخ خروج بالغین دارای تفاوت معنی‌داری بود و از کیفیت تریکوکارته‌ها با افزایش تعداد نسل در انسکتاریوم کاسته شد اما در نسبت جنسی این زنبور در دوره‌های مختلف تفاوت معنی‌داری مشاهده نشد. در بررسی نتایج حاصل از شمارش افراد بی‌بال در دوره‌های مختلف نیز تفاوت معنی‌داری وجود نداشت. محدود نمودن تعداد دفعات پرورش، معرفی متناوب جمعیت‌های مزرعه‌ای زنبور پارازیتوئید به جمعیت در حال پرورش و هم‌چنین معرفی دیگر میزبان‌های زنبور *T. brassicae* به جمعیت آزمایشگاهی می‌تواند در کاهش صفات نامطلوب پرورش متوالی این زنبور مؤثر باشد.

واژگان کلیدی: بید غلات، توانمندی پارازیتوئید، پرورش طولانی مدت، *Trichogramma brassicae*