Effect of cold storage temperature on quality of the parasitoid, *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae)

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**Abstract**

This study was designed to find out the optimum cold storage temperature (4, 7, and 10 °C) and storage period (1–16 weeks) of 3 different immature developmental stages (2, 4, 6 days post parasitism) of the egg parasitoid, *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) to produce high-quality individuals to be utilized. Also, the effects of cold storage on parasitoids’ fitness in terms of parasitism percentage, developmental period, adults’ emergence percentage, female percentage, and longevity (fed and un-fed) of parents and F1 progeny were investigated. The obtained results revealed that *T. bactrae* larvae (2-day post parasitism) could be stored for at least 7 weeks at 10 °C, with least changes in their fitness in both the parents and F1 progeny, followed by 7 °C, whereas storage at 4 °C was the fatal temperature in this early stage of parasitoid with a maximum mortality rate that extended to the next generation (F1). Furthermore, female biased sex ratios were observed at all storage temperatures in the parental and F1 generations but with different values. After a cold exposure of pre- and pupal stages (4, 6 days post parasitism) of the parasitoid at 7 °C, adults’ emergence percentage in the parents was low, but the biological performance in progeny was great. In addition, these late stages could be stored for a short period up to 4 weeks at 10 °C with highest performances in both generations, followed by 7 °C. Ten degrees Celsius proved to be the most suitable storage temperature at different parasitoid ages (larvae, pre-pupae, and pupae). Only the larval stage could continue up to 16 weeks of cold storage, and hence, it can be recommended for a short- and/or a long-term storage period.

**Keywords:** *Trichogrammatoidea bactrae*, Cold storage, Parasitism percentage, Developmental period, Adults’ emergence percentage, Sex ratio, Longevity

**Background**

Biological control is a worldwide major component used in pest management for the regulation of pest populations, using natural enemies (DeBach and Rosen 1991; and Bale et al. 2008). Parasitic wasps of the genera, *Trichogramma* and *Trichogrammatoidea*, are among the most common and effective bio-control agents because of their capability to attack eggs of many economical pests in different agricultural crops (Hutchison et al. 1990). The egg-parasitoid, *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae), was described first from lepidopterous pest specimens collected from rice (*Oryza sativa* L.), cabbage (*Brassica oleracea* Var.), and corn (*Zea mays* L.) (Nagaraja 1978). The main problem of releasing the parasitic wasps as biological control agents is field requirements and high cost of their mass production for augmentative releases at the suitable time whenever required (Tezze and Botto 2004; Ayvaz et al. 2008; and Lü et al. 2019). To overcome...
these problems, improving an effective cold storage technique is an important tool for increasing and mass producing high-quality individuals when needed (Tezze and Botto 2004). Prolonged cold storage periods of the parasitoids during their immature stages can cause physiological malfunctions (Colinet and Boivin 2011), depletion of energy reserves (Chen et al. 2008), and morphological alterations as deformations of reproductive organs (Denlinger and Lee 1998), a reduction in body size (Rundle et al. 2004), wing’s distortion (Dutton and Bigler 1995; and Tezze and Botto 2004), and alteration on antennal structure (Pintureau and Daumal 1995). Otherwise, low temperature exposure can negatively affect the fitness of produced individuals and offspring such as causing a reduction in adults’ emergence (Özder and Sağlam 2004; Yilmaz et al. 2007; and Nadeem et al. 2010), a decrease in longevity (Rundle et al. 2004; Yilmaz et al. 2007; and Gharbi 2014), fecundity (Colinet and Boivin 2011 and Gharbi 2014), more males production (Chen et al. 2008), and alteration in parasitoid’s mobility (Tezze and Botto 2004 and Ayvaz et al. 2008). Worldwide, several researches on cold storage of different Trichogramma species have been studied extensively (Rundle et al. 2004; Özder and Sağlam 2004; Kim et al. 2009; Nadeem et al. 2010; Gharbi 2014; Bhargavi and Naik 2015; Rahimi-Kaldeh et al. 2017; Vigneswaran et al. 2017; Ghosh and Ballal 2018; Liu et al. 2019; and Abbes et al. 2020).

Based on the authors’ knowledge, few instances were pointed on the cold tolerance of different developmental stages of the Trichogrammatatoidea species. Meanwhile, this study aimed to evaluate the effects of short- and long-term cold storages on the fitness of the produced T. bactrae population for mass rearing programs.

Materials and methods

Host rearing

The Angoumois grain moth, Sitotroga cerealella (Oliver) (Lepidoptera: Gelechiidae) stock culture, was reared on wheat grains, Triticum aestivum L. (Sedes1), that obtained from the laboratory colonies at the mass rearing unit of Trichogramma, Plant Protection Research Institute, Agricultural Research Center (ARC), in Assiut. S. cerealella eggs were used as a factious host for the studied parasitoid.

Parasitoid rearing

T. bactrae, used in this study, was imported for the first time from Australia in 1992 as an egg-parasitoid for control of the pink bollworm (Lepidoptera: Gelechiidae) by Dr. A. H. El-Heneidy (ARC, Egypt) through a collaborative project with American Universities. This parasitoid species was established under Egyptian environmental conditions and was mass reared at the Center of Bio-organic Agricultural Services (CBAS), in Aswan. Freshly laid eggs of S. cerealella, glued onto a piece of white cardboard by a fine gum film, were introduced into plastic jars (1L capacity) harboring newly emerged T. bactrae wasps for 24 h to avoid super-parasitism. The parasitism was performed under standard rearing conditions of 25 ± 1 °C and > 60% RH with 14L: 10D cycle (Nadeem et al. 2010). Parasitoid adults were provided by sugar solution (10%) for nutrition. After 24 h of exposure to the parasitoid, parasitized host egg cards (Trichocards) were collected and maintained in clean jars.

Experimental design for cold storage

Once the Tricho-cards reached 2, 4, and 6 days post parasitism, thus providing 3 different developmental stages of the parasitoid (larvae, pre-pupae, and old-pupae). Samples from each age consisted of a piece of cardboard strip holding approximately 350 ± 50 parasitized eggs were placed in 250-ml plastic jars, then maintained at 3 different incubators, and stored at 3 low temperatures (4, 7, and 10 ± 1 °C; > 60% RH and full darkness) for various storage durations (1–16 weeks) until parasitoid adult emergence percentage reached a very low level ≤ 10%. For each treatment (storage period), 12 replicates were used. After each storage duration, parasitoid strips were removed from the storage incubator to the standard rearing room conditions as mentioned above. Control (un-stored card) was also kept at the standard rearing room conditions for comparison.

Effect of cold storage on the parasitoids’ fitness

Effect of low temperature exposure for different periods on the fitness of the egg-parasitoid, T. bactrae (the parents), was assessed by measuring the following biological variables at each immature developmental stage compared to the control: parasitism percentage (no. of parasitized eggs (blackened eggs)/ total no. of egg’s exposed); developmental time (the time till adult emergence for control), for treated groups as (the time between the end of cold storage till adult emergence); adult emergence rate (no. of emerged adults/ no. of parasitized eggs); and females’ ratio was determined by examining dead adults under a stereomicroscope (no. of emerged adult females/ total individuals). Longevity of adults was recorded daily from the time of adult emerged till mortality (for fed and un-fed adults). For the parasitoid nutrition, few droplets of sugar solution (10%) were provided daily until the wasps died.

The F1 progeny obtained from the cold stored parental generation was also counted at the aforementioned rearing conditions. Each sample consisted of a piece of a cardboard strip, with approximately 250 ± 50 eggs of S. cerealella exposed for 24 h to the produced parasitoid from cold storage. Parasitism percentage, adult emergence rate, female ratio, and longevity of fed and un-fed adults were calculated as the same way as described above for the parental generation.
Statistical analysis
Obtained data were subjected to one- and two-way analysis of variance (ANOVA) by the Advanced Statistical Analysis Package (ASAP). Data were arcsine √x transformed before analysis to meet normality. Means were separated, using the least significant difference (LSD) at P ≤ 0.05 level. All calculations and graphs were used by the Microsoft Excel software according to Fowler et al. (1998).

Results and discussion
Cold storage of parasitoid larvae (2-day post parasitism)

Parasitism percentage Cold storage at 4 °C reduced drastically the parasitism rates compared with the other temperatures (7 and 10 °C) and control (Table 1 (a)). The parasitism stopped at 4 °C after 6 weeks of storage only, while at 7 °C, it continued for 9 weeks. In case of 10 °C, it developed and extended to 16 weeks. The 2-way ANOVA revealed that there were significant differences for the effect of temperature (A), storage period (B), and the effect of storage period-temperature interaction (A × B) at P ≤ 0.05 (LSD(A) = 8.321, F = 2497.415; LSD(B) = 0.196, F = 746.871; and LSD(A × B) = 0.339, F = 911.762).

Developmental period Developmental period of the parasitoid increased significantly (P < 0.0001) with extending the storage period at 4 °C than in the control and the two other temperatures (Table 1 (b)). No difference between storage at 7 °C and control up to 4 weeks; then, the duration increased significantly (P = 0.000). On contrary, at 10 °C, only the first 2 weeks of storage was almost similar to control, and afterwards the period decreased significantly (P = 0.000). There were significant variations for the main effects of temperature (A), storage period (B), and their interaction (A × B) at P ≤ 0.05 (LSD(A) = 0.113, F = 2497.415; LSD(B) = 0.196, F = 746.871; and LSD(A × B) = 0.339, F = 911.762).

Table 1 Percentages of parasitism and developmental period of Trichogrammaidea bactrae in parental generation during storage of parasitoid larvae at different low temperatures

(a) Parasitism %

| Temp. °C | Duration (weeks) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|----------|-----------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|
| 4        |                 | 74.19 ± 0.15 | 30.15 ± 1.18 | 11.08 ± 0.01 | 3.93 ± 0.46 | 2.40 ± 0.22 | 1.30 ± 0.14 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 7        |                 | 86.65 ± 0.25 | 89.25 ± 0.78 | 87.41 ± 0.72 | 86.73 ± 0.94 | 84.09 ± 1.18 | 80.68 ± 1.61 | 78.14 ± 1.14 | 77.64 ± 1.14 | 76.0 ± 0.00 | 74.19 ± 0.00 | 72.0 ± 0.00 | 70.0 ± 0.00 | 68.0 ± 0.00 | 66.0 ± 0.00 | 64.0 ± 0.00 | 62.0 ± 0.00 |
| 10       |                 | 81.91 ± 0.41 | 84.34 ± 0.66 | 91.55 ± 0.24 | 90.16 ± 0.15 | 92.08 ± 0.26 | 89.13 ± 0.01 | 83.82 ± 0.91 | 81.85 ± 0.91 | 80.8 ± 0.00 | 80.3 ± 0.00 | 79.3 ± 0.00 | 78.3 ± 0.00 | 77.3 ± 0.00 | 76.3 ± 0.00 | 75.3 ± 0.00 | 74.3 ± 0.00 |
| Control  |                 | 88.91 ± 0.40 |                |               |               |               |               |               |               |               |               |               |               |               |               |               |               |

(b) Developmental period (days)

| Temp. °C | Duration (weeks) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|----------|-----------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|
| 4        |                 | 9.19 ± 0.26 | 13.51 ± 0.49 | 13.67 ± 0.49 | 12.75 ± 0.14 | 13.61 ± 0.01 | 14.30 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 7        |                 | 7.44 ± 0.15 | 7.41 ± 0.13 | 7.41 ± 0.06 | 7.98 ± 0.18 | 9.24 ± 0.12 | 9.75 ± 0.12 | 10.43 ± 0.14 | 10.91 ± 0.04 | 11.50 ± 0.00 | 11.16 ± 0.00 | 10.82 ± 0.00 | 10.58 ± 0.00 | 10.34 ± 0.00 | 10.10 ± 0.00 | 9.86 ± 0.00 | 9.62 ± 0.00 |
| 10       |                 | 7.69 ± 0.16 | 6.51 ± 0.17 | 6.31 ± 0.17 | 5.55 ± 0.14 | 4.82 ± 0.14 | 4.88 ± 0.14 | 4.17 ± 0.24 | 2.98 ± 0.14 | 2.27 ± 0.12 | 1.58 ± 0.14 | 1.07 ± 0.12 | 0.58 ± 0.14 | 0.27 ± 0.12 | 0.20 ± 0.12 | 0.20 ± 0.12 | 0.20 ± 0.12 |
| Control  |                 | 7.28 ± 0.18 |                |               |               |               |               |               |               |               |               |               |               |               |               |               |               |

Means ± SE sharing the same capital letters in the same row, and same small letters in the columns are statistically insignificant (P ≥ 0.05)
Table 2 Percentages of adults’ emergences of Trichogrammatoidea bactrae in parental generation during storage of parasitoid larvae at different low temperatures

| Temp. °C | Duration (weeks) | F1 progeny | Adults emergence % |
|---------|-----------------|------------|---------------------|
| 4 °C    | 1 2 3 4 5 6     | 86.75 ± 1.4Bc | 97.40 ± 0.85AB     |
| 7 °C    | 7 8 9 10 11 12 13 14 15 16 | 91.55 ± 1.15Bb | 95.28 ± 0.50Aa |
| 10 °C   | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 | 98.20 ± 0.50Aa | 97.44 ± 0.37Aa |

Means ± SE sharing the same capital letters in the same row, and same small letters in the columns are statistically insignificant (P ≥ 0.05)

Table 3 Percentages of parasitism and adult emergence rates of Trichogrammatoidea bactrae in F1 progeny during storage of parasitoid larvae at different low temperatures

(a) Parasitism %

| Temp. °C | Duration (weeks) | Parasitism % | Adult emergence % |
|---------|-----------------|--------------|------------------|
| 4 °C    | 1 2 3 4 5 6     | 86.75 ± 1.4Bc | 86.59 ± 1.09Ba |
| 7 °C    | 7 8 9 10 11 12 13 14 15 16 | 91.55 ± 1.15Bb | 84.08 ± 1.12Ab |
| 10 °C   | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 | 98.20 ± 0.50Aa | 67.93 ± 1.39Hi |

(b) Adults’ emergence %

| Temp. °C | Duration (weeks) | Parasitism % | Adult emergence % |
|---------|-----------------|--------------|------------------|
| 4 °C    | 1 2 3 4 5 6     | 86.75 ± 1.4Bc | 97.40 ± 0.85AB |
| 7 °C    | 7 8 9 10 11 12 13 14 15 16 | 91.55 ± 1.15Bb | 97.44 ± 0.37Aa |
| 10 °C   | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 | 98.20 ± 0.50Aa | 97.44 ± 0.37Aa |

Means ± SE sharing the same capital letters in the same row, and same small letters in the columns are statistically insignificant (P ≥ 0.05)

Parasitism rate The percentage of parasitism in F1 progeny emerged from cold-exposed, did not change for 7 weeks, when the parasitoid was held at 7 °C (88.82%) and 10 °C (91.00%), the latter percentage was close to that in the control group (92.36%) (Table 3 (a)). Although the adult emergence percentage in the parental generation after storage at 7 °C was low, the parasitism was higher in F1 than at the 2 other temperatures. However, temperature of 4 °C did not provide storage more than 2 weeks. The effect of temperature (A), storage period (B), and the interactions (A × B) was significantly different at P ≤ 0.05 (LSD (A) = 1.289, F = 4150.58; LSD (B) = 2.233, F = 439.585; and LSD (A × B) = 3.868, F = 263.179).

Adults’ emergence percentage The emergence rate of F1 progeny was not significantly changed in all the tested storage temperatures compared with the control (Table 3 (b)). High numbers of emergency were observed during all storage periods. At 7 °C, the percentage of emerged adults was not different from the control up to 9 weeks of storage. Furthermore, when the parasitoid was held at 10 °C, great rates of emergences were noticed and continued for 16 weeks. Regardless to storage periods, there was insignificant difference between 7 and 10 °C in the percentage of parasitoid larvae.
adult emergence. The effect of temperature (A), storage period (B), and their interaction (A × B) in adult emergence percentage was significantly different at P ≤ 0.05 (LSD(A) = 9.734, F = 16.95; LSD(B) = 16.859, F = 4.101; and LSD(A × B) = 29.201, F = 9.503).

Percentages of female adults The majority of adults’ emergence in the generation subjected to storage at low temperature was female-biased in all treatments but with different values (Table 4 (a)). The percentage of female adults emerged at 10 °C was significantly (P = 0.000) higher, followed by 7 °C, while it was the lowest one at 4 °C. In F1 progeny, the female percentage was similar somewhat with that of the parental generation (Table 4 (b)). There were significant (P ≤ 0.05) variations for the effect of temperature (A), storage periods (B), and storage-period-temperature interaction (A × B) in the parental generation (LSD(A) = 0.821, F = 3021.527; LSD(B) = 1.421, F = 949.085; and LSD(A × B) = 2.462, F = 347.235) and the F1 progeny (LSD(A) = 1.113, F = 3087.604; LSD(B) = 1.928, F = 629.73; and LSD(A × B) = 3.339, F = 272.043).

Longevity of adults The decrease in adults’ longevity in the parental generation was more prominent than in F1 progeny (Fig. 1). Fed adult parasitoids lived significantly (P < 0.001) longer than un-fed ones at all the tested temperatures in both generations. Moreover, insignificant effect (P = 0.143) was observed in the longevity of adults at 4 and 7 °C in the parental generation. At 10 °C storage temperature with different durations, adults’ longevity was better for short- and long-term storages without detrimental effects to adults, regardless to whether supplied with food or starved in both generations. In the parental and the following generations (F1), significant variations (P ≤ 0.05) for the effect of temperatures (A), storage periods (B), and their interactions (A × B) were noticed on longevity of fed (LSD(A) = 0.694, F = 36.589; LSD(B) = 0.911, F = 6.017; LSD(A × B) = 2.081, F = 6.732 and LSD(A) = 1.468, F = 3087.604; LSD(B) = 1.928, F = 629.73; LSD(A × B) = 3.339, F = 272.043, respectively) and of un-fed adults (LSD(A) = 0.289, F = 74.089; LSD(B) = 0.379, F = 11.692; LSD(A × B) = 0.657, F = 10.87 and LSD(A) = 0.24, F = 171.416; LSD(B) = 0.315, F = 9.108; LSD(A × B) = 0.546, F = 16.107, respectively).

Storage of parasitoid pre-pupae (4 days after parasitism) Parental generation

Parasitism percentage When the pre-pupa of *T. bactrae* were subjected to storage at the 3 temperatures (4, 7, and 10 °C), parasitism percentage was not affected with the control (Table 5 (a)). The 2-way ANOVA revealed that the effect of temperature (A), storage period (B), and storage period-temperature interaction (A × B) had insignificant variations at P ≤ 0.05.

Developmental period The length of cold exposure on the developmental period of the parasitoid, significantly (P = 0.000) increased in case of 4 °C than in the control and the 2 other temperatures (Table 5 (b)). It required the longest time at 4 °C to develop. The mean developmental period was 7.28 days, followed by 5.03 days at 7

| Table 4 | Percentages of *Trichogrammatoidea bactrae* females in parental and F1 generations during storage of parasitoid larvae at different low temperatures |
|---------|--------------------------------------------------------------------------------------------------|
| **(a) Parental generation** | **Temp.** | **Duration (weeks)** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| 4 °C | 81.00 ± 0.33Aa | 67.5 ± 0.78Cc | 62.00 ± 0.46Cc | 73.37 ± 0.65Bb | 54.5 ± 0.57Fc | 50.00 ± 0.00Ff | – | – | – | – | – | – | – | – | – | – | – |
| 7 °C | 70.62 ± 0.36Ba | 70.50 ± 0.09Cc | 72.50 ± 0.91Cb | 72.00 ± 1.29Cb | 70.00 ± 0.78Cb | 66.50 ± 1.31Cb | 63.87 ± 1.94Db | 69.50 ± 0.72Db | 77.87 ± 1.18Cd | 77.12 ± 0.26Da | – | – | – | – | – | – | – |
| 10 °C | 82.12 ± 0.11Aa | 83.37 ± 0.65Aa | 80.37 ± 0.71Aa | 85.00 ± 1.29Aa | 80.00 ± 0.50Aa | 77.87 ± 0.73Aa | 72.00 ± 0.44Cd | 80.62 ± 1.27Ea | 84.62 ± 0.68Aa | 79.00 ± 0.33Bc | 77.62 ± 0.91Cd | 79.97 ± 0.44Bc | 78.00 ± 0.00Cc | 79.50 ± 0.62Ab | 76.25 ± 0.36D | – | – |
| **Control** | 84.62 ± 0.62Aa | | | | | | | | | | | | | | | | | |

| **(b) F1 generation** | **Temp.** | **Duration (weeks)** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| 4 °C | 84.25 ± 0.36Ba | 63.12 ± 0.34Bc | 50.00 ± 0.00Cc | 53.37 ± 0.32Cc | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 °C | 70.50 ± 1.76Ba | 70.62 ± 1.19Bb | 72.00 ± 1.07Bb | 68.50 ± 1.32Cb | 70.37 ± 1.27Ba | 69.00 ± 1.21Ba | 71.00 ± 0.68Bb | 69.50 ± 0.73Bc | 81.5 ± 1.58Aa | – | – | – | – | – | – | – | – |
| 10 °C | 87.12 ± 1.61Aa | 84.00 ± 1.67Aa | 79.25 ± 0.99Ba | 74.75 ± 1.01Da | 69.87 ± 1.34Fa | 70.25 ± 1.41Fa | 80.00 ± 1.02Aa | 77.52 ± 1.03Bc | 81.00 ± 2.40Bc | 74.62 ± 1.57Aa | 80.50 ± 0.86Cd | 76.12 ± 1.44Ab | 81.25 ± 1.64Cd | 72.87 ± 0.45Aa | 79.75 ± 1.68AB | – | – |
| **Control** | 85.00 ± 0.73Aa | | | | | | | | | | | | | | | | | |

Means ± SE sharing the same capital letters in the same row, and same small letters in the columns are statistically insignificant (P ≥ 0.05)
°C, and the faster one was 2.37 days at 10 °C. Statistically, the developmental period after cold storage varied significantly (P ≤ 0.05) with temperatures (LSD (A) = 0.126, F = 4589.366), storage durations (LSD (B) = 0.219, F = 49.867), and their interactions (LSD (A × B) = 0.288, F = 300.014).

Adults’ emergence percentage The ratio of parasitoid’s emergence was significantly (P = 0.000) decreased as the period of cold exposure was prolonged according to different temperatures (Table 5 (c)). Compared to the control group (95.56%), the emergence increased slightly (97.19%) only after 1 week of storage at 4 °C, then it declined sharply (P = 0.002). At 7 °C, the ratio of emerged adults was significantly decreased (P = 0.000) till the 9th week of storage (14.15%). Furthermore, when the parasitoid was stored at 10 °C, it emerged in high numbers and did not change significantly up to the 3rd week. There were significant variations for the effect of temperature (A), storage period (B), and the interaction

**Table 5** Percentages of parasitism, developmental period and adults’ emergence of *Trichogrammatoidea bactrae* in parental generation during storage of parasitoid pre-pupa at different low temperatures

| Storage period (weeks) | (a) Parasitism % | (b) Developmental period | (c) Adults’ emergence % |
|------------------------|------------------|--------------------------|------------------------|
| Control                | 88.64 ± 0.88A    | 4.93 ± 0.15              | 95.56 ± 0.47A          |
| 1                      | 90.30 ± 0.70Aa   | 88.48 ± 0.83Aa           | 97.19 ± 0.61Aa         |
| 2                      | 90.26 ± 0.70Aa   | 90.04 ± 1.25Aa           | 88.93 ± 5.92Ab         |
| 3                      | 89.06 ± 0.70Aa   | 92.76 ± 1.40Aa           | 89.12 ± 5.84Ab         |
| 4                      | 91.58 ± 0.70Aa   | 91.00 ± 7.73 ± 0.42Ca    | 47.64 ± 49.21Bb        |
| 5                      | 88.55 ± 0.70Aa   | 89.20 ± 7.97 ± 0.11Aa    | 22.65 ± 49.44Bb        |
| 6                      | 87.72 ± 0.70Aa   | 89.15 ± 8.64 ± 0.11Ba    | 14.33 ± 35.47Bb        |
| 7                      | 85.38 ± 0.70Aa   | 92.12 ± 9.21 ± 0.13Ba    | 18.4 ± 36.33Ba         |
| 8                      | 87.37 ± 0.70Aa   | 91.59 ± 9.89 ± 0.01Ba    | 9.73 ± 26.06Bb         |
| 9                      | 83.58 ± 0.70Aa   | 70.04 ± 5.16 ± 0.05Bb    | 1.76 ± 5.66Bb          |

Means ± SE sharing the same capital letters in the same columns, and same small letters in the rows are statistically insignificant (P ≥ 0.05)
(A × B) on the adults’ emergence percentage at $P \leq 0.05$ (LSD$_{(A)} = 8.463$, $F = 37.25$; LSD$_{(B)} = 14.659$, $F = 9.004$; and LSD$_{(A \times B)} = 25.39$, $F = 4.845$).

F$_1$ progeny

Parasitism rate Prolonged storage of the parental generation declined parasitism performance in progeny in all the storage temperatures (Table 6 (a)). The parasitism percentage emerged from 4 °C during the first 2 weeks was similar to control (92.02%); then, it decreased significantly ($P = 0.000$). The parasitoid adults failed to parasitize any eggs in the progeny from the 7th week of storage. Although the adult emergence percentage in the parental generation after storage at 7 °C was low, the parasitism rate was high in F$_1$ and increased significantly ($P < 0.001$) than the control and the other temperatures up to 5 weeks. Moreover, in case of 10 °C, the parasitism in F$_1$ was influenced by cold exposure and the percentage decreased slightly. After 9 weeks of storage in the parental generation, the females failed to parasitize any eggs. The effect of temperature (A), storage period (B), and the interaction (A × B) in F$_1$ progeny was significantly ($P \leq 0.05$) varied (LSD$_{(A)} = 1.221$, $F = 1285.299$; LSD$_{(B)} = 2.116$, $F = 782.419$; and LSD$_{(A \times B)} = 3.664$, $F = 221.383$).

Adults’ emergence percentage The effect of cold storage in the parental generation on the adults’ emergence of progeny had not changed significantly at 7 and 10 °C. High emergency rates were observed (≥ 86%) during all storage periods throughout different temperatures (Table 6 (b)). Statistically, the different temperatures (A), storage duration (B), and their interaction (A × B) had significant effects on the adult emergence percentage in F$_1$ progeny at $P \leq 0.05$ (LSD$_{(A)} = 10.136$, $F = 10.02$; LSD$_{(B)} = 17.556$, $F = 4.091$; and LSD$_{(A \times B)} = 30.408$, $F = 3.319$).

Percentages of female adults The majority of adult emergency in the generation subjected to storage at low temperature was strongly female-biased in all treatments with different values (Table 7 (a)). The females percentage emerged from storage at 4 and 10 °C were not significantly ($P = 0.613$) affected by the length of storage duration, but 7 °C was the lowest one. Although the ratio of females emerged from 7 °C was significantly different ($P = 0.000$) than the control but had not change in relation to storage period for 9 weeks. In the following generation (F$_1$), the overall sex ratio of emerged adults was female biased, but it was significantly different ($P = 0.000$) from control in all tested temperatures and storage durations (Table 7 (b)). The percentage of female in progeny increased but not significantly ($P \geq 0.2$) differed than the parental generation. There were significant variations ($P \leq 0.05$) of temperature (A), storage period (B), and storage period-temperature interaction (A × B) on females percentage in the parental generation (LSD$_{(A)} = 1.320$, $F = 380.428$; LSD$_{(B)} = 2.286$, $F = 39.152$; and LSD$_{(A \times B)} = 3.959$, $F = 12.347$) and also in the progeny (LSD$_{(A)} = 0.993$, $F = 614.293$; LSD$_{(B)} = 1.721$, $F = 561.701$; and LSD$_{(A \times B)} = 2.98$, $F = 428.99$).

Longevity of adults Fed adult parasitoids significantly ($P = 0.000$) lived much longer than the starved ones at all tested temperatures and storage durations in both generations (parental and F$_1$ progeny) (Fig. 2). For the longevity of parasitoid stored at 10 °C, adults lived non-significantly ($P > 0.5$) longer than that maintained at 4 and 7 °C, regardless to whether supplied with food or starved. The length of cold exposure at the 3 low temperatures occurred in the parental generation did not

| Storage period (weeks) | (a) Parasitism % | (b) Adults’ emergence % |
|------------------------|-----------------|-------------------------|
|                        | 4 °C            | 7 °C                    | 10 °C                   | 4 °C          | 7 °C          | 10 °C          |
| Control                | 92.02 ± 0.92A   | 96.58 ± 0.52A           | 93.18 ± 0.40A          | 87.28 ± 0.78Bc| 98.32 ± 0.70Aa| 95.49 ± 0.30Ab|
| 1                      | 91.05 ± 0.43Ac  | 95.68 ± 0.52Aa          | 93.18 ± 0.40Ab         | 87.28 ± 0.78Bc| 98.32 ± 0.70Aa| 95.49 ± 0.30Ab|
| 2                      | 92.26 ± 0.36Ab  | 96.58 ± 0.56Aa          | 88.68 ± 1.26Bcc        | 88.84 ± 2.52Bb| 94.20 ± 0.85Aa| 84.71 ± 2.56Bb|
| 3                      | 82.13 ± 1.19Bc  | 95.73 ± 0.61Aa          | 91.21 ± 0.98Ab         | 95.10 ± 1.13Aab| 97.36 ± 1.06Aa| 93.82 ± 0.91Ab|
| 4                      | 80.13 ± 0.62Bcc | 95.57 ± 0.49Aa          | 87.38 ± 1.05Bc         | 91.67 ± 0.51Bbc| 97.32 ± 0.32Aa| 93.88 ± 1.30Ab|
| 5                      | 78.53 ± 0.52Cb  | 96.94 ± 0.25Aa          | 90.61 ± 0.51AAb        | 94.52 ± 0.45Aa| 93.71 ± 0.80Aa| 96.19 ± 0.64Aa|
| 6                      | 16.44 ± 4.09Dc  | 81.21 ± 0.94Bb          | 72.19 ± 1.15Ec         | 81.74 ± 4.55Bc| 87.30 ± 1.37Bb| 87.69 ± 1.03Bb|
| 7                      | –               | 82.85 ± 1.08Bb          | 76.03 ± 1.74Db         | –             | 93.37 ± 0.63Ab| 96.38 ± 0.70Aa|
| 8                      | –               | 70.09 ± 2.03Ca          | 34.63 ± 3.13Fb         | –             | 86.60 ± 1.46Bb| 93.24 ± 1.01Aa|
| 9                      | –               | 60.23 ± 1.15Da          | –                       | –             | 92.65 ± 0.80Aa| –             |

Means ± SE sharing the same capital letters in the same columns, and the same small letters in the rows are statistically insignificant ($P \geq 0.05$).
influence negatively surviving of adults in the progeny comparable to control. At 4 °C, longevity of adult parasitoids in F1 progeny had non-significantly changed \( (P = 0.706, 0.685) \) than the parental generation, but it prolonged significantly at 7 °C \( (P = 0.001, 0.004) \) in both fed and un-fed adults, respectively. On the other hand, the longevity of parasitoid in progeny after parental storage at 10 °C, the adult survived longer but non-significantly \( (P = 0.485, 0.082) \) than the control and parental generation in case of fed but significantly increased \( (P = 0.003, 0.040) \) for un-fed adults, respectively. Statistically, all the main effects and their interactions proved to be significant \( (P < 0.05) \) on longevity of adults in the parental and the progeny in case of fed \( (\text{LSD(A)} = 0.413, F = 4.178; \text{LSD(B)} = 0.716, F = 8.462; \text{LSD(A × B)} = 1.24, F = 7.256 \) and LSD(A) = 0.993, F = 614.293; LSD(B) = 1.721, F = 561.701; LSD(A × B) = 2.98, F = 428.99, respectively) and starved adults \( (\text{LSD(A)} = 0.188, F = 19.828; \text{LSD(B)} = 0.325, F = 15.349; \text{LSD(A × B)} = 0.563, F = 7.56 \) and LSD(A) = 0.232, F = 26.533; LSD(B) = 0.402, F = 9.296; \( \text{LSD(A × B)} = 0.696, F = 14.058 \), respectively).

**Storage of parasitoid pupae (6 days after parasitism)**

**Parental generation**

**Parasitism percentage** When the pupae of *T. bactrae* were maintained at the 3 storage temperatures, the mean percentage of parasitism was not influenced negatively than the control during all storage periods (Table 8 (a)). The effect of temperature \( (A) \), storage period \( (B) \), and storage period-temperature interaction \( (A × B) \) had insignificant \( (P ≤ 0.05) \) variations in parasitism percentage.

### Table 7 Percentages of *Trichogrammatoidea bactrae* females in parental and F1 generations during storage of parasitoid pre-pupae at different low temperatures

| Storage period (weeks) | Females (%) | | | |
|-------------------------|-------------|-----------------|-----------------|-----------------|
|                         | (a) Parental generation | (b) F1 progeny |
|                         | 4 °C | 7 °C | 10 °C | 4 °C | 7 °C | 10 °C |
| Control                 | 82.78 ± 1.52A | 91.12 ± 1.65A |
| 1                       | 81.55 ± 1.07Aa | 48.87 ± 3.57CDB | 82.33 ± 1.93Aa | 90.00 ± 0.71Aa | 77.00 ± 1.49Ba | 91.00 ± 1.49ABa |
| 2                       | 82.55 ± 0.82Aa | 67.37 ± 1.63Db | 83.22 ± 2.01Aa | 89.25 ± 0.49ABb | 70.12 ± 1.57Cc | 91.12 ± 0.48Aa |
| 3                       | 82.44 ± 1.60Aa | 70.87 ± 0.35Cb | 81.89 ± 1.52Aa | 88.00 ± 0.57Ba | 71.00 ± 0.96Cb | 88.00 ± 0.33BCa |
| 4                       | 83.89 ± 0.48Aa | 71.37 ± 1.40Bcc | 81.00 ± 0.63Ab | 84.12 ± 1.01Ca | 70.75 ± 0.96Cb | 87.25 ± 0.41Ca |
| 5                       | 78.89 ± 0.12Aa | 70.50 ± 2.34Cbc | 78.33 ± 0.78BCa | 81.00 ± 1.66Da | 70.00 ± 0.02Ca | 87.12 ± 0.29Ca |
| 6                       | 81.11 ± 0.27Ab | 74.00 ± 1.00Bbc | 85.00 ± 1.31Aa | 73.00 ± 0.42Eb | 70.87 ± 0.69Cc | 82.25 ± 0.36Da |
| 7                       | 81.00 ± 0.77Aa | 71.37 ± 1.15BCbc | 79.11 ± 0.74ABa | – | 70.25 ± 1.01Cb | 65.50 ± 1.43Fc |
| 8                       | 66.33 ± 0.26Bc | 69.25 ± 1.50Cbc | 70.00 ± 1.07Ca | – | 70.50 ± 1.02Cb | 71.12 ± 1.87Eb |
| 9                       | 50.00 ± 0.00Cc | 63.87 ± 0.54Ea | 51.75 ± 0.41Db | – | 63.25 ± 0.56D | – |

Means ± SE sharing the same capital letters in the same columns, and same small letters in the rows are statistically insignificant \( (P ≥ 0.05) \).
Developmental period  The effect of cold exposure on the total developmental period of the parasitoid was significantly ($P = 0.000$) prolonged in case of 4 °C than the control and the 2 other temperatures (Table 8 (b)). Occasionally, from the pupae stored at 10 °C, adult emergence occurred during the period of storage before being transferred to the standard condition of 25 °C, usually when storage was ≥ 7 weeks. The mean developmental period after storage of pupae at 4 °C lasted about (5.23 days), followed by 7 °C with (3.83 days), while at 10 °C, it was faster (1.40 days) than the control (3.79 days). The developmental period varied significantly ($P ≤ 0.05$) with temperatures (LSD = 0.083, $F = 3084.431$), storage periods (LSD = 0.144, $F = 66.264$), and their interactions (LSD = 0.25, $F = 217.805$).

Adults’ emergence percentage  Throughout the 3 storage temperatures, the emergence rate decreased ($P = 0.000$) with increasing the cold exposure period but with different values in all treatments (Table 8 (c)). Compared to the control group (97.07%), the emergence percentage was similar during the first 2 weeks of storage at 4 °C; then, it declined sharply ($P = 0.0001$). At 7 °C, the percentage of emerged adults was significantly decreased ($P ≤ 0.01$) up to the 9th week (25.13%) of storage. Furthermore, when the parasitoid pupae were stored at 10 °C, they continued to emerge with high numbers up to the 8th week (> 80%). Afterwards, the majority of emerged adults (> 85%) was deformed. There were significant differences ($P ≤ 0.05$) for the effect of temperatures, storage periods, and also storage period-temperature interactions in adult emergence percentage (LSD = 8.697, $F = 14.473$; LSD = 15.064, $F = 12.223$; and LSD = 26.092, $F = 3.281$, respectively).

$F_1$ progeny

Parasitism rate  With prolonged storage period of parental generation, the parasitism rate in the following progeny declined significantly ($P = 0.000$) in all tested temperatures (Table 9 (a)). The parasitism percentage occurred at female’s parasitoid emerged from the storage at 4 °C during the 1st week (92.95%) was similar to the control (92.88%); then, it began to decrease significantly ($P ≤ 0.045$) till 7 weeks of storage. Afterwards, the T. bactrae adult failed to parasitize any eggs. Although the adult parasitoids emerged after cold exposure at 7 °C was low, the parasitism rate in $F_1$ progeny was great and increased significantly ($P = 0.001$) during 2 weeks of storage; afterward, it was almost similar to the control up to 5 weeks. In case of 10 °C, the parasitism rate was not influenced negatively ($P = 0.716$) by cold exposure for 4 weeks; then, the percent declined significantly ($P = 0.0000$) till the 8th week. After 9 weeks of parents’ storage, the performances of adult females were affected and completely failed to parasitize eggs. The effects of temperature, storage period, and storage period-temperature interaction in percentage of parasitized eggs were significantly different at $P ≤ 0.05$ (LSD = 1.582, $F = 12.223$).

Table 8  Effect of cold storage on the parasitism%, developmental period and adults’ emergence % of Trichogrammaidea bactrae in parental generation during storage of parasitoid pupae at different low temperatures

| Storage period (weeks) | (a) Parasitism % | (b) Developmental period | (c) Adults’ emergence % |
|------------------------|------------------|--------------------------|-------------------------|
|                        | 4 °C             | 7 °C                     | 10 °C                   | 4 °C           | 7 °C           | 10 °C         | 4 °C          | 7 °C       | 10 °C       |
| Control                |                   |                          |                         |               |               |               |               |           |             |
|                        | 92.67 ± 0.94A     | 3.79 ± 0.17              | 97.07 ± 0.55AB          |               |               |               |               |           |             |
| 1                      | 92.68 ± 1.39AaAb  | 92.24 ± 0.40Aa          | 94.49 ± 0.25a           | 3.78 ± 0.25Da | 3.18 ± 0.28Da | 3.63 ± 0.20a  | 98.17 ± 0.21a | 91.56 ± 0.21a | 97.67 ± 0.20a |
| 2                      | 93.36 ± 0.75Aa    | 91.50 ± 0.81Aa          | 89.62 ± 0.14Aa          | 3.05 ± 0.12Db | 3.00 ± 0.15Db | 2.66 ± 0.15Bb | 95.18 ± 1.03Bb | 76.30 ± 1.03Bb | 97.18 ± 0.70Aa |
| 3                      | 93.45 ± 0.43Aa    | 90.87 ± 0.90Ab          | 92.00 ± 0.17Da          | 3.69 ± 0.17Da | 3.13 ± 0.15Db | 1.23 ± 0.15Bb | 79.84 ± 2.41Cb | 60.59 ± 1.64Ec | 94.24 ± 0.66Ba |
| 4                      | 93.55 ± 0.41Aa    | 90.52 ± 0.61Ab          | 88.94 ± 1.11Aab         | 5.47 ± 0.19Ca | 3.01 ± 0.12Db | 1.18 ± 0.03Cd | 66.65 ± 2.81Db | 46.21 ± 2.80Fc | 92.86 ± 0.40Ca |
| 5                      | 92.29 ± 0.89Aa    | 87.43 ± 2.17Ab          | 90.07 ± 1.91Aa          | 6.57 ± 0.18Aa | 3.50 ± 0.22Dc | 1.10 ± 0.22Dc | 38.99 ± 3.05Eb | 41.01 ± 2.78Gb | 88.87 ± 1.24Da |
| 6                      | 90.76 ± 1.64Aab   | 86.95 ± 1.42Ab          | 90.96 ± 0.77Aa          | 5.78 ± 0.16Bc | 3.74 ± 0.21Cb | 0.87 ± 0.19Ec | 22.49 ± 2.30Fb | 32.14 ± 2.52Hb | 90.41 ± 1.61CaD |
| 7                      | 91.52 ± 0.71Aa    | 87.96 ± 1.36Ab          | 90.96 ± 0.77Aa          | 6.14 ± 0.02Aba | 4.32 ± 0.14Bb | 0.81 ± 0.11Ec | 94.1 ± 0.66Gc | 27.76 ± 1.55Hb | 90.30 ± 1.58CDa |
| 8                      | 91.44 ± 1.13Aa    | 86.23 ± 1.71Ab          | 91.90 ± 0.42Aa          | 6.52 ± 0.15Aa | 4.68 ± 0.14Bb | 0.68 ± 0.15Fc | 88.3 ± 1.79Gc | 25.94 ± 1.61Hb | 80.71 ± 2.32Ea |
| 9                      | 88.03 ± 1.05Aa    | 85.25 ± 1.34Ab          | 72.53 ± 0.03Aba         | 6.11 ± 0.03Aba | 5.87 ± 0.10Aa | 0.42 ± 0.05Gb | 2.68 ± 0.98Hc | 25.14 ± 2.18Jb | 17.07 ± 2.18Fb |

Means ± SE sharing the same capital letters in the same columns, and same small letters in the rows are statistically insignificant ($P ≥ 0.05$).
201.705; LSD = 2.74, \( F = 560.328 \); and LSD = 4.746, \( F = 61.161 \), respectively).

**Adults’ emergence percentage** High numbers of emerged adults in \( F_1 \) were observed during all storage periods on different temperatures and did not change significantly in 7 and 10 °C, reaching the highest value (> 96%) in the 5th week. At 4 °C, the mean emergence of parasitoid was 88.19% throughout the first 5 weeks and significantly in 7 and 10 °C, reaching the highest value percentage of parasitoid in \( F_1 \) at \( P \leq 0.05 \).

**Percentage of female adults** The majority of adults’ emergency in the parental generation was strongly female-biased in all treatments (Table 10 (a)). The mean percentage of females emerged from storage at 4 and 10 °C was non-significantly different (\( P = 0.624 \)), while a significant difference (\( P = 0.000 \)) was recorded between 7° and the 2 other temperatures. In addition, the percentage of females in progeny was significantly different (\( P = 0.000 \)) from control in all storage temperatures and exposure periods (Table 10 (b)). The general mean of female percentage in \( F_1 \) was non-significantly (\( P \geq 0.50 \)) changed than the parents at all tested temperatures. Generally, it was noticeable that 10 °C gave the highest percentage of females, followed by 7 °C, while 4 °C had the lowest one. Statistically, there were significant variations (\( P \leq 0.05 \)) in the effect of temperatures, storage periods, and their interactions on females percentage in parent (LSD = 1.248, \( F = 537.36 \); LSD = 2.162, \( F = 51.922 \); and LSD = 3.745, \( F = 11.299 \), respectively) and \( F_1 \) progeny (LSD = 1.112, \( F = 244.265 \); LSD = 1.927, \( F = 248.712 \); and LSD = 3.337, \( F = 232.102 \), respectively).

**Longevity of adults** Fed adult parasitoids significantly (\( P = 0.000 \)) lived much longer than un-fed ones at all tested temperatures and cold storage periods in both generations (parent and \( F_1 \) progeny) (Fig. 3). Cold storage at 4 and 10 °C occurred in parental generation did not influence negatively (\( P > 0.5 \)) on the mean of adults survival in \( F_1 \) in both fed and un-fed adults. However, in case of 7 °C, the parasitoid longevity in \( F_1 \) increased significantly (\( P = 0.001, 0.000 \)) in both fed and starved ones as compared with parents, respectively. Moreover, the highest longevity was verified when parasitoids were stored at 10 °C, regardless to whether supplied with food or not, followed by 4 and then 7 °C. The effect of temperature (A), storage duration (B), and their interaction (A × B) had significant variation on longevity of fed adults in the parental generation only at \( P \leq 0.05 \) (LSD(A) = 0.465, \( F = 4.996 \); LSD(B) = 0.805, \( F = 7.833 \); LSD(A × B) = 1.395, \( F = 4.035 \)). For un-fed adults, all the main effects and their interactions proved to be significant in the parental generation and the subsequent one at \( P \leq 0.05 \) (LSD(A) = 0.16, \( F = 36.999 \); LSD(B) = 0.277, \( F = 15.846 \); LSD(A × B) = 0.48, \( F = 6.737 \); and LSD(A × B) = 0.243, \( F = 12.72 \); LSD(B) = 0.422, \( F = 19.767 \); LSD(A × B) = 0.73, \( F = 9.737 \), respectively).

In essence, when the parasitoid was subjected to low temperature (4, 7, and 10 °C) during its immature developmental stages (larvae, pre-pupa, and pupa), the percentage of parasitism, adult emergence, and longevity of emerged adults (whether fed or un-fed) increased with the increase of storage temperature but decreased as the exposure period prolonged. These results are somewhat similar with the results reported by other researchers on different *Trichogramma* species (Tezze and Botto 2004; Rundle et al. 2004; Özder and Sağlam 2004; Yilmaz et al. 2007; Nadeem et al. 2010; Gharbi 2014; Bhargavi and Naik 2015; Rahimi-Kaldeh et al. 2017; Vigneswaran et al. 2019).

### Table 9 Percentages of parasitism and adults’ emergence of *Trichogrammaidea bactrae* in \( F_1 \) progeny during storage of parasitoid pupae at different low temperatures

| Storage period (weeks) | (a) Parasitism % | (b) Adults’ emergence % |
|------------------------|------------------|------------------------|
|                        | 4 °C             | 7 °C                   | 10 °C                  |
| Control                | 92.88 ± 0.45ABC  | 96.82 ± 0.72A          |
| 1                      | 92.95 ± 0.58Ab   | 96.76 ± 0.79Aa         | 95.83 ± 0.38Aa         |
| 2                      | 90.93 ± 0.74Bb   | 94.13 ± 0.39Aa         | 90.43 ± 0.58Bb         |
| 3                      | 82.86 ± 0.64Db   | 91.39 ± 1.19Ca         | 91.28 ± 1.06Bb         |
| 4                      | 87.49 ± 1.65Cb   | 89.87 ± 0.41Cb         | 92.95 ± 0.96Bb         |
| 5                      | 82.14 ± 1.25Da   | 92.67 ± 1.16Ba         | 87.41 ± 0.75Db         |
| 6                      | 56.48 ± 4.53Ec   | 73.68 ± 3.14Db         | 68.04 ± 2.81Eb         |
| 7                      | 33.17 ± 2.03Fc   | 66.17 ± 3.45Ea         | 54.40 ± 1.51Fb         |
| 8                      | –                | 66.14 ± 2.93Ea         | 13.48 ± 0.51Gb         |
| 9                      | –                | 49.70 ± 5.35F          | –                      |

Means ± SE sharing the same capital letters in the same columns, and same small letters in the rows are statistically insignificant (\( P \geq 0.05 \)).
In addition, the majority of adult emergency in the generation exposed to storage and the subsequent one \( (F_1) \) was strongly female-biased in all treatments but with different values. This finding is important and vital in mass production for releasing the parasitoid in the field during the insect control programs, and it proved that low temperature exposure did not affect the fertility and vigorous of females parasitoid. Contrary to the obtained observations, in some cases, a shift towards producing higher proportion of males has been reported after cold exposure, suggesting a differential mortality of females (Riddick 2001; Farid et al. 2001; Chen et al. 2008; and Abd El-Gawad et al. 2010).

It is obvious that the most suitable temperature for storage of \( T. \) bactrae was 10\(^\circ\)C, then 7 \(^\circ\)C for a short-term in all developmental stages of the parasitoid. But, only at 10 \(^\circ\)C, larval stage could be stored for a long-term (up to 2.5 months) without much loss of performance in both parental and \( F_1 \) progeny based on the achieved results. Obtained findings are consistent with the observations recorded previously in other \( Trichogramma \) species (Rundle et al. 2004; Ma and Chen 2006; Yılmaz et al. 2007; Kim et al. 2009; Abd El-Gawad et al. 2010; Nadeem et al. 2010; Xi et al. 2013; Vigneswaran et al. 2017; and Lü et al. 2019). All of them recommended 10 \(^\circ\)C as a preferable storage temperature, regardless to the developmental stage of the parasitoid subjected to the cold exposure and the storage period. In contrast, Krishnamoorthy and Mani (1999) reported that the adult parasitoid of \( T. \) bactrae failed to emerge from pupae stored at 7 \(^\circ\)C for only 3 days and also for 10 days at 10 \(^\circ\)C.

![Fig. 3 Mean longevity of Trichogrammatoidea bactrae adults (fed and un-fed) of parental and F1 progeny after storage of parasitoid pupae at different low temperatures](image-url)

**Table 10** Percentages of \( Trichogrammatoidea bactrae \) females in parental and \( F_1 \) generations after storage of parasitoid pupae at different low temperatures

| Storage period (weeks) | Females (%) | (a) Parental generation | (b) \( F_1 \) progeny |
|------------------------|-------------|-------------------------|----------------------|
|                        | 4 \(^\circ\)C | 7 \(^\circ\)C | 10 \(^\circ\)C | 4 \(^\circ\)C | 7 \(^\circ\)C | 10 \(^\circ\)C |
| Control                | 86.89 ± 0.84A | 89.37 ± 0.68A |
| 1                      | 84.00 ± 1.37Aa | 71.12 ± 1.37Cb | 85.89 ± 1.00Aa | 89.25 ± 1.89Aa | 67.5 ± 0.96Db | 90.12 ± 1.37Aa |
| 2                      | 82.44 ± 0.72Ba | 70.00 ± 2.64Cb | 83.44 ± 0.466Ba | 89.62 ± 1.02Aa | 71.75 ± 1.61Bcb | 88.87 ± 1.17Aa |
| 3                      | 81.22 ± 0.75Cab | 78.12 ± 1.52Bb | 82.89 ± 0.95Ba | 89.50 ± 0.80Aa | 74.00 ± 0.33Bc | 86.00 ± 0.27Bb |
| 4                      | 76.67 ± 1.12Db | 66.75 ± 1.47Dc | 79.11 ± 0.46CDa | 81.50 ± 1.03Ba | 69.62 ± 1.31Cc | 76.37 ± 0.92Db |
| 5                      | 81.33 ± 0.23Ca | 71.25 ± 0.36Cc | 79.00 ± 1.11CDb | 64.00 ± 2.72Cc | 71.00 ± 0.65Cb | 79.37 ± 0.62Ca |
| 6                      | 75.44 ± 0.29Eb | 70.00 ± 0.00Cc | 80.00 ± 0.41Ca | 58.75 ± 1.42Db | 71.87 ± 0.85BCa | 76.62 ± 2.07Da |
| 7                      | 81.33 ± 0.32Ca | 66.75 ± 3.25Dc | 80.11 ± 0.41Cb | 51.50 ± 0.42Ec | 71.25 ± 1.30Cb | 80.25 ± 1.36Ca |
| 8                      | 78.33 ± 1.23Da | 65.12 ± 3.31Deb | 77.89 ± 0.88Da | – | 71.37 ± 0.62Cb | 86.87 ± 0.29Ba |
| 9                      | 58.89 ± 0.22Fa | 65.00 ± 0.50Ea | 63.25 ± 0.16Eb | – | 57.87 ± 1.90E | – |

Means ± SE sharing the same capital letters in the same columns, and same small letters in the rows are statistically insignificant \((P \geq 0.05)\).
°C (Ghosh and Ballal 2018). Moreover, Geng et al. (2005) considered 10 °C was unfavorable temperature for long-term storage of T. dendrolimi. On the other hand, they noted the most appropriate stages for long-term storage were larval and pupal stages at 4 °C, but egg and pupal stages at 7 °C. However, storage parasitoid during its larval stage at 4 °C in the present study did not provide a suitable storage period more than 2 weeks only, and this may be due to the sub-lethal effect of this low temperature, and the high mortality rate was noticed. As mentioned previously by many researchers on cold storage of different Trichogramma species regardless the immature stages, Garcia et al. (2002), Özder (2008), Xi et al. (2013), Singhamuni et al. (2015), and Lü et al. (2019) showed that ≤ 5 °C seemed to be the least suitable storage temperatures. Finally, optimizing low storage temperature for this parasitic wasp is very critical for a successful mass production and field releases.

Conclusion
Obtained results proved that T. bactrae larvae were the most suitable immature stage to be stored for a short period (7 weeks) and for a long term up to (2.5 months) at 10 °C without much losses of their fitness in both the parental and F1 progeny, followed by 7 °C for a short period only (4 weeks). However, cold exposure to 4 °C did not provide a suitable storage period more than 2 weeks. In addition, pre-pupae and pupae could be stored for a short period up to 4 weeks only in the all tested temperatures.

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Authors’ contributions
HOM participated in the design of this study, conducted the experiments, prepared the manuscript, and performed the statistical analysis. AHE contributed to the design of the study and revised the manuscript. Both authors read and approved the final manuscript.

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