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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 F_1 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 F_1 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 (F_1). Furthermore, female biased sex ratios were observed at all storage temperatures in the parental and F_1 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

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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 a reduction 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; Lü 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 *Trichogrammatoidea* 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 (Tricho-cards) 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 stereo-microscope (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 F_1 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)[R]. Data were arcsine √x transformed before analysis to meet normality. Means were separated, using the least significant difference (LSD) at $P \leq 0.05$ 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)

Parental generation

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 $P \leq 0.05$ ($LSD_{(A)} = 8.321$, $F = 89.945$; $LSD_{(B)} = 14.412$, $F = 1.284$; and $LSD_{(A \times B)} = 24.692$, $F = 9.094$).

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 $P \leq 0.05$ ($LSD_{(A)} = 0.113$, $F = 2497.415$; $LSD_{(B)} = 0.196$, $F = 746.871$; and $LSD_{(A \times B)} = 0.339$, $F = 911.762$).

Adults' emergence percentage The effect of cold storage on the adult emergence percentage decreased significantly at all storage periods ($P = 0.000$) than the control (Table 2). The percentage increased with the increase of low temperature but decreased with increasing the cold exposure period. Subsequently, it increases the proportion of deformed adults. There were significant variations for the effect of temperature (A), storage period (B), and their interaction (A × B) at $P \leq 0.05$ ($LSD_{(A)} = 9.429$, $F = 16.908$; $LSD_{(B)} = 16.332$, $F = 1.946$; and $LSD_{(A \times B)} = 28.288$, $F = 6.048$).

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

(a) Parasitism %

Temp.	Duration (weeks) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4 °C	74.19 ± 2.65Bc	30.15 ± 1.49Cb	11.08 ± 1.38Db	3.93 ± 1.18Eb	2.40 ± 0.22Fc	1.30 ± 0.14Gc	–	–	–	–	–	–	–	–	–	–
7 °C	86.65 ± 1.02Aa	89.25 ± 1.16Aa	87.41 ± 0.90Aa	86.73 ± 0.78Aa	84.09 ± 1.17ABb	80.68 ± 1.38CDb	81.91 ± 1.61BCa	78.14 ± 1.34Db	77.64 ± 1.14Da	–	–	–	–	–	–	–
10 °C	81.91 ± 1.26BCb	84.34 ± 3.68ABa	91.55 ± 1.01Aa	90.16 ± 1.11Aa	92.08 ± 1.15Aa	89.13 ± 1.15Aa	80.3 ± 2.44Ca	83.82 ± 2.25Ba	81.85 ± 1.79BCa	91.34 ± 1.43A	91.97 ± 0.91A	92.84 ± 0.54A	92.39 ± 1.0A	89.22 ± 1.37A	85.36 ± 2.04A	87.14 ± 2.05A
Control	**88.91** ± **1.40A**															

(b) Developmental period (days)

Temp.	Duration (weeks) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4 °C	9.19 ± 0.26Da	13.51 ± 0.29Ba	13.67 ± 0.49Ba	12.75 ± 0.19Ca	13.61 ± 0.14Ba	14.30 ± 0.00Aa	–	–	–	–	–	–	–	–	–	–
7 °C	7.44 ± 0.15Gb	7.41 ± 0.13Gb	7.98 ± 0.06Fb	7.92 ± 0.08Fb	9.24 ± 0.18Eb	9.75 ± 0.15Db	10.43 ± 0.12Ca	10.91 ± 0.26Ba	11.50 ± 0.41Aa	–	–	–	–	–	–	–
10 °C	7.69 ± 0.16Fb	6.51 ± 0.19FGb	6.31 ± 0.17Gc	5.55 ± 0.14Hc	4.83 ± 0.20Ic	4.88 ± 0.20HIc	4.17 ± 0.24Jb	2.98 ± 0.14Kb	2.27 ± 0.25Lb	10.87 ± 0.27D	13.40 ± 0.21B	14.51 ± 0.15A	12.51 ± 0.15C	10.07 ± 0.22DE	9.94 ± 0.27E	9.59 ± 0.10E
Control	**7.28** ± **0.18FG**															

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

Table 2 Percentages of adults' emergences of *Trichogrammatoidea bactrae* in parental generation during storage of parasitoid larvae at different low temperatures

Adults' emergence %

Temp.	Duration (weeks)															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
4 °C	86.75 ± 1.4Bc	71.24 ± 0.95Cc	53.95 ± 6.68Ec	44.59 ± 9.54Fc	61.00 ± 5.31Dc	37.67 ± 2.91Gc	-	-	-	-	-	-	-	-	-	-
7 °C	91.55 ± 1.15Bb	86.52 ± 1.66Cb	86.38 ± 1.98Cb	78.26 ± 1.46Db	73.50 ± 2.05Eb	67.00 ± 2.01GHb	67.84 ± 1.72FGb	66.05 ± 1.18Hb	49.30 ± 2.16Ia	-	-	-	-	-	-	-
10 °C	98.20 ± 0.50Aa	98.81 ± 0.37Aa	95.51 ± 1.09Aa	91.91 ± 1.12Aba	89.67 ± 1.39Ba	87.21 ± 2.05BCa	86.67 ± 1.83BCa	73.13 ± 2.44Da	51.29 ± 3.35FGa	53.28 ± 3.37EF	44.60 ± 2.78H	47.07 ± 4.84GH	33.18 ± 2.15J	24.21 ± 2.45K	34.36 ± 4.62IJ	23.01 ± 2.33K
Control	**97.40 ± 0.85AB**															

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

F_1 progeny

Parasitism rate The percentage of parasitism in F_1 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 F_1 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 $P \leq 0.05$ ($LSD_{(A)} = 1.289$, $F = 4150.58$; $LSD_{(B)} = 2.233$, $F = 439.585$; and $LSD_{(A \times B)} = 3.868$, $F = 263.179$).

Adults' emergence percentage The emergence rate of F_1 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

Table 3 Percentages of parasitism and adult emergence rates of *Trichogrammatoidea bactrae* in F_1 progeny during storage of parasitoid larvae at different low temperatures

(a) Parasitism %

Temp.	**Duration (weeks)**															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4 °C	80.65 ± 1.16Cb	91.17 ± 0.79Ab	21.14 ± 3.03Ec	45.19 ± 3.71Dc	–	–	–	–	–	–	–	–	–	–	–	–
7 °C	95.28 ± 0.66Aa	95.31 ± 0.49Aa	97.65 ± 0.28Aa	96.78 ± 0.32Aa	93.81 ± 0.47Aa	89.21 ± 1.18ABa	88.82 ± 0.88Bb	67.93 ± 2.39Ca	38.56 ± 2.4Db	–	–	–	–	–	–	–
10 °C	82.09 ± 0.98Db	90.04 ± 1.11Ab	86.24 ± 1.30BCb	88.27 ± 0.90ABb	86.98 ± 2.48BCb	87.47 ± 0.57ABa	91.00 ± 0.38Aa	63.47 ± 0.90Fa	54.34 ± 1.39HIa	88.63 ± 1.17AB	78.59 ± 2.01E	83.39 ± 1.15D	57.04 ± 4.38G	41.53 ± 3.73J	52.99 ± 2.71I	13.06 ± 2.45K
Control	**91.36 ± 1.36AB**															

(b) Adults' emergence %

Temp.	**Duration (weeks)**															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4 °C	83.91 ± 2.86Cb	96.74 ± 0.59Aa	97.83 ± 0.49Aa	97.44 ± 0.72Aa	–	–	–	–	–	–	–	–	–	–	–	–
7 °C	91.68 ± 0.72ABa	96.94 ± 0.42Aa	93.95 ± 1.01Ab	88.76 ± 1.21Bc	94.35 ± 0.51Ab	95.16 ± 0.66Aa	93.17 ± 1.41Ab	94.20 ± 0.63Ab	88.28 ± 1.06Bb	–	–	–	–	–	–	–
10 °C	80.87 ± 1.71Fb	78.00 ± 2.58FGb	85.56 ± 1.57Dc	95.39 ± 0.37Ab	97.33 ± 0.56Aa	93.72 ± 0.73Aa	97.81 ± 0.62Aa	96.16 ± 0.39Aa	95.21 ± 0.83Aa	88.92 ± 1.38BC	81.64 ± 1.11EF	78.68 ± 1.17FG	93.43 ± 2.01AB	90.26 ± 1.27AB	92.42 ± 0.99AB	86.98 ± 2.93CD
Control	**96.79 ± 0.75AB**															

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

adult emergence. The effect of temperature (A), storage period (B), and their interaction (A × B) in adult emergence percentage was significantly different at $P \le 0.05$ ($LSD_{(A)} = 9.734$, $F = 16.95$; $LSD_{(B)} = 16.859$, $F = 4.101$; and $LSD_{(A \times B)} = 29.201$, $F = 9.503$).

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 F_1 progeny, the female percentage was similar somewhat with that of the parental generation (Table 4 (b)). There were significant ($P \le 0.05$) 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 $LSD_{(A \times B)} = 2.462$, $F = 347.235$) and the F_1 progeny ($LSD_{(A)} = 1.113$, $F = 3087.604$; $LSD_{(B)} = 1.928$, $F = 629.73$; and $LSD_{(A \times B)} = 3.339$, $F = 272.043$).

Longevity of adults The decrease in adults' longevity in the parental generation was more prominent than in F_1 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 (F_1), significant variations ($P \le 0.05$) for the effect of temperatures (A), storage periods (B), and their interactions (A × B) were noticed on longevity of fed ($LSD_{(A)} = 0.694$, $F = 36.589$; $LSD_{(B)} = 0.911$, $F = 6.017$; $LSD_{(A \times B)} = 2.081$, $F = 6.732$ and $LSD_{(A)} = 1.468$, $F = 3087.604$; $LSD_{(B)} = 1.928$, $F = 629.73$; $LSD_{(A \times B)} = 3.339$, $F = 272.043$, respectively) and of un-fed adults ($LSD_{(A)} = 0.289$, $F = 74.089$; $LSD_{(B)} = 0.379$, $F = 11.692$; $LSD_{(A \times B)} = 0.657$, $F = 10.87$ and $LSD_{(A)} = 0.24$, $F = 171.416$; $LSD_{(B)} = 0.315$, $F = 9.108$; $LSD_{(A \times B)} = 0.546$, $F = 16.107$, respectively).

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

Parental generation

Parasitism percentage When the pre-pupae 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 $P \le 0.05$.

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 F_1 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 ± 0.46Dc	73.37 ± 0.65Bb	54.5 ± 0.57EFc	50.00 ± 0.00Fc	–	–	–	–	–	–	–	–	–	–
7 °C	70.62 ± 1.05Cb	70.50 ± 0.92Cb	72.50 ± 1.29Cb	72.00 ± 0.78Cb	70.00 ± 1.31Cb	66.50 ± 1.94DEb	63.87 ± 0.72Eb	69.50 ± 1.18CDa	77.87 ± 0.29Bb	–	–	–	–	–	–	–
10 °C	82.12 ± 1.19Aa	83.37 ± 0.65Aa	80.37 ± 0.71ABa	85.00 ± 1.29Aa	80.00 ± 0.50Aba	80.00 ± 0.73ABa	77.87 ± 0.44CDa	72.00 ± 1.27Ea	80.62 ± 1.00ABa	84.62 ± 0.68A	79.00 ± 0.33BC	77.62 ± 0.91CD	79.87 ± 0.44ABC	78.00 ± 0.00C	79.50 ± 0.42ABC	76.25 ± 0.36D
Control	**84.62 ± 0.62A**															

(b) F_1 generation

Temp.	Duration (weeks)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4 °C	84.25 ± 0.56Aa	63.12 ± 3.45Bc	50.00 ± 0.00Dc	53.37 ± 0.32Cc	–	–	–	–	–	–	–	–	–	–	–	–
7 °C	70.50 ± 1.76Bb	70.62 ± 1.19Bb	72.00 ± 1.07Bb	68.50 ± 1.32Cb	70.37 ± 1.27Ba	69.00 ± 1.21BCa	71.00 ± 0.68Bb	69.50 ± 0.73BCb	81.5 ± 1.58Aa	–	–	–	–	–	–	–
10 °C	87.12 ± 1.61Aa	84.00 ± 1.67Aa	79.25 ± 0.99Ba	74.75 ± 1.01DEa	69.87 ± 1.34Fa	70.25 ± 1.41Fa	80.00 ± 1.02ABa	77.52 ± 1.03BCa	77.00 ± 2.40BCa	81.00 ± 1.57A	74.62 ± 0.86CD	80.50 ± 1.44AB	76.12 ± 1.64CD	81.25 ± 0.45A	72.87 ± 1.97E	79.75 ± 1.68AB
Control	**85.00 ± 0.73A**															

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

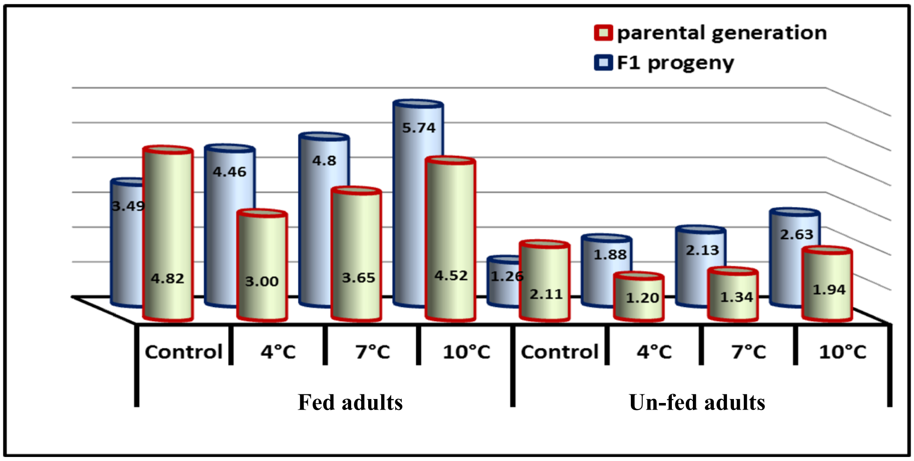

Fig. 1 Mean longevity of *Trichogrammatoidea bactrae* adults (fed and un-fed) of parental and F_1 progeny after storage of parasitoid larvae at different low temperatures

°C, and the faster one was 2.37 days at 10 °C. Statistically, the developmental period after cold storage varied significantly ($P \leq 0.05$) with temperatures ($LSD_{(A)}$ = 0.126, F = 4589.366), storage durations ($LSD_{(B)}$ = 0.219, F = 49.867), and their interactions ($LSD_{(A \times B)}$ = 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-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	**88.64 ± 0.88A**			**4.93 ± 0.15**			**95.56 ± 0.47A**		
1	90.30±0.70Aa	88.48 ± 0.83Aa	89.30 ± 1.88Aa	6.43 ± 0.15Da	5.47 ± 0.20Bb	5.13 ± 0.28Ab	97.19 ± 0.61Aa	65.49 ± 2.49Ab	98.85 ± 0.63Aa
2	91.20±0.67Aa	90.26 ± 1.25Aa	90.04 ± 1.21Aa	5.37 ± 0.16Fa	4.63 ± 0.19Bb	4.56 ± 0.19Ab	88.93 ± 1.47Bb	56.92 ± 2.71Bc	96.00 ± 0.65Aa
3	89.60 ± 1.53Aa	89.06 ± 0.36Aa	92.76 ± 1.40Aa	5.86 ± 0.14Ea	4.40 ± 0.22Bb	2.69 ± 0.15Cc	89.12 ± 1.12Bb	55.84 ± 1.08Bc	94.42 ± 0.88Aa
4	91.58 ± 1.03Aa	89.47 ± 0.88Aa	91.00 ± 1.17Aa	7.73 ± 0.42Ca	4.59 ± 0.05Bb	3.18 ± 0.25Bc	47.64 ± 2.94Cc	49.21 ± 1.97Cb	87.13 ± 1.21Ba
5	88.55 ± 1.68Aa	88.78 ± 1.29Aa	89.20 ± 1.25Aa	9.77 ± 0.11Aa	4.35 ± 0.11Bb	2.84 ± 0.12Cc	22.65 ± 2.13Dc	49.44 ± 3.79Cb	81.95 ± 2.28Ca
6	87.72 ± 2.73Aa	86.13 ± 1.15Aa	89.15 ± 1.19Aa	8.64 ± 0.11Ba	5.12 ± 0.13Bb	1.36 ± 0.17Dc	14.33 ± 1.84Ec	35.47 ± 3.63Db	68.18 ± 5.19Da
7	85.38 ± 1.11Ab	84.46 ± 1.79Ab	92.12 ± 0.87Aa	9.21 ± 0.01ABa	4.86 ± 0.03Bb	0.75 ± 0.10Ec	9.73 ± 2.60Fc	33.50 ± 1.65Db	56.12 ± 2.74EFa
8	87.37 ± 1.77Ab	85.62 ± 0.84Ab	91.59 ± 0.54Aa	9.89 ± 0.06Aa	5.26 ± 0.13Bb	0.68 ± 0.11Ec	9.76 ± 1.77Fc	26.89 ± 1.48Eb	48.37 ± 4.19Fa
9	83.58 ± 3.91Ba	85.79 ± 2.86Aa	70.04 ± 2.05Bb	5.16 ± 0.00Fb	6.56 ± 0.13Aa	0.17 ± 0.00Fc	1.76 ± 0.56Gc	14.15 ± 1.75Fa	5.93 ± 2.14Gb

Means ± SE sharing the same capital letters in the same columns, and same small letters in the rows are statistically insignificant ($P \geq 0.05$)

(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

Table 6 Percentages of parasitism and adults' emergence of *Trichogrammatoidea bactrae* in F_1 progeny during storage of parasitoid pre-pupae at different low temperatures

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**			**95.63 ± 1.24A**		
1	91.05 ± 0.43Ac	96.58 ± 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 ± 2.52Bab	94.20 ± 0.85Aa	84.71 ± 2.56Bb
3	82.13 ± 1.19Bc	95.73 ± 0.61Aa	91.21 ± 0.98Ab	96.10 ± 1.13Aab	97.36 ± 1.06Aa	93.82 ± 0.91Ab
4	80.13 ± 0.62BCc	95.57 ± 0.49Aa	87.38 ± 1.05Cb	91.67 ± 0.51Bbc	97.32 ± 0.32Aa	93.88 ± 1.30Ab
5	78.53 ± 0.52Cb	96.94 ± 0.25Aa	90.61 ± 0.51ABa	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 ± 4.55Cb	87.30 ± 1.37Bb	87.69 ± 1.03Bb
7	–	82.85 ± 1.08Ba	76.03 ± 1.74Db	–	93.37 ± 0.63Ab	96.38 ± 0.70Aa
8	–	70.09 ± 2.03Ca	34.67 ± 3.13Fb	–	86.60 ± 1.46Bb	93.24 ± 1.01Aa
9	–	60.23 ± 1.15Da	–	–	92.65 ± 0.80A	-

Means ± SE sharing the same capital letters in the same columns, and the same small letters in the rows are statistically insignificant ($P \geq 0.05$)

Table 7 Percentages of *Trichogrammatoidea bactrae* females in parental and F_1 generations during storage of parasitoid pre-pupae at different low temperatures

Storage period (weeks)	Females (%)					
	(a) Parental generation			(b) F_1 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	68.87 ± 3.57CDb	82.33 ± 1.93Aa	90.00 ± 0.71Aa	77.00 ± 1.49Bb	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 ± 2.34Cb	78.33 ± 0.78BCa	81.00 ± 1.66Da	70.00 ± 0.82Cb	87.12 ± 0.29Ca
6	81.11 ± 0.27Ab	74.00 ± 1.00Bc	85.00 ± 1.31Aa	73.00 ± 0.42Eb	70.87 ± 0.69Cc	82.25 ± 0.36Da
7	81.00 ± 0.77Aa	71.37 ± 1.15BCb	79.11 ± 0.74ABa	–	70.25 ± 1.01Cb	65.50 ± 1.43Fc
8	66.33 ± 0.26Bc	69.25 ± 1.50Cb	76.00 ± 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 ($P \geq 0.05$)

influence negatively surviving of adults in the progeny comparable to control. At 4 °C, longevity of adult parasitoids in F_1 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 ($LSD_{(A)} = 0.413$, F = 4.178; $LSD_{(B)} = 0.716$, F = 8.462; $LSD_{(A \times B)} = 1.24$, F = 7.256 and $LSD_{(A)} = 0.993$, F = 614.293; $LSD_{(B)} = 1.721$, F = 561.701; $LSD_{(A \times B)} = 2.98$, F = 428.99, respectively) and starved adults ($LSD_{(A)} = 0.188$, F = 19.828; $LSD_{(B)} = 0.325$, F = 15.349; $LSD_{(A \times B)} = 0.563$, F = 7.56 and $LSD_{(A)} = 0.232$, F = 26.533; $LSD_{(B)} = 0.402$, F = 9.296; $LSD_{(A \times B)} = 0.696$, 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 ($P \leq 0.05$) variations in parasitism percentage.

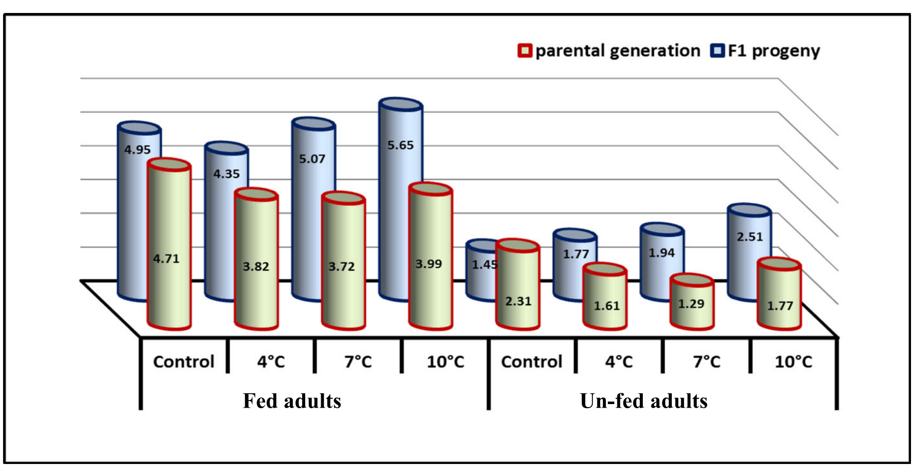

Fig. 2 Mean longevity of *Trichogrammatoidea bactrae* adults (fed and un-fed) of parental and F_1 progeny during storage of parasitoid pre-pupae at different low temperatures

Table 8 Effect of cold storage on the parasitism%, developmental period and adults' emergence % of *Trichogrammatoidea 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 ± 1.39Aab	92.24 ± 0.70Ab	94.49 ± 0.44Aa	3.78 ± 0.25Da	3.18 ± 0.28Da	3.63 ± 0.20Aa	98.17 ± 0.21Aa	91.56 ± 1.62Cb	97.67 ± 0.73Aa
2	93.36 ± 0.75Aa	91.50 ± 0.81Aa	89.62 ± 1.46Aa	3.06 ± 0.06Ea	3.00 ± 0.12Dab	2.66 ± 0.15Bb	95.18 ± 1.03Ba	76.30 ± 2.76Db	97.18 ± 0.37Aa
3	93.45 ± 0.43Aa	90.87 ± 0.90Ab	92.00 ± 1.11Aab	3.69 ± 0.17Da	3.13 ± 0.15Db	1.23 ± 0.09Cc	79.84 ± 2.41Cb	60.59 ± 1.64Ec	94.24 ± 0.66Ba
4	93.55 ± 0.41Aa	90.52 ± 0.61Ab	88.94 ± 1.03Ab	5.47 ± 0.19Ca	3.01 ± 0.12Db	1.18 ± 0.03CDc	66.65 ± 2.01Db	46.21 ± 2.80Fc	92.86 ± 0.40Ca
5	92.29 ± 0.89Aa	87.43 ± 2.17Aba	90.07 ± 1.91Aa	6.57 ± 0.18Aa	3.50 ± 0.10Cb	1.10 ± 0.22Dc	38.99 ± 3.05Eb	41.01 ± 2.78Gb	88.87 ± 1.24Da
6	90.76 ± 1.64Aab	86.95 ± 1.42Bb	90.96 ± 0.77Aa	5.78 ± 0.16BCa	3.74 ± 0.21Cb	0.87 ± 0.19Ec	22.49 ± 2.30Fc	32.14 ± 2.52Hb	90.41 ± 1.61CDa
7	91.52 ± 0.71Aa	87.96 ± 1.36ABb	90.96 ± 0.77Aab	6.14 ± 0.02ABa	4.32 ± 0.14Bb	0.81 ± 0.11Ec	9.41 ± 0.67Gc	27.76 ± 1.55Ib	90.30 ± 1.58CDa
8	91.44 ± 1.13Aa	86.23 ± 1.71Bb	91.90 ± 0.42Aa	6.52 ± 0.15Aa	4.68 ± 0.14Bb	0.68 ± 0.15Fc	8.83 ± 1.79Gc	25.94 ± 2.58 Ib	80.71 ± 2.32Ea
9	88.03 ± 1.05Aa	85.25 ± 1.20Ba	72.53 ± 1.34Bb	6.11 ± 0.03ABa	5.87 ± 0.10Aa	0.42 ± 0.05Gb	2.68 ± 0.98Hc	25.14 ± 2.98 Ia	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 ($P \geq 0.05$)

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 ($P \leq 0.05$) 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 ($P \leq 0.01$) 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 ($P \leq 0.05$) 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 ($P \leq 0.045$) 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 $P \leq 0.05$ (LSD = 1.582, F =

Table 9 Percentages of parasitism and adults' emergence of *Trichogrammatoidea 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	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	84.43 ± 1.99Cb	93.76 ± 1.16ABa	92.85 ± 0.92BCa
2	90.93 ± 0.74Bb	94.13 ± 0.39ABa	90.43 ± 0.58Cb	91.03 ± 1.07Bb	92.08 ± 1.03Bab	94.71 ± 1.15ABa
3	82.86 ± 0.64Db	91.39 ± 1.19Ca	91.28 ± 1.06Ba	90.15 ± 1.79Ba	93.78 ± 1.80ABa	93.94 ± 0.40ABa
4	87.49 ± 1.65Cb	89.87 ± 0.41Cb	92.95 ± 0.96Ba	91.71 ± 1.18Bb	96.24 ± 0.83Aa	96.02 ± 1.49Aa
5	82.14 ± 1.25Da	92.67 ± 1.16Ba	87.41 ± 0.75Db	83.64 ± 1.51Cb	97.13 ± 0.46Aa	96.17 ± 0.79Aa
6	56.48 ± 4.53Ec	73.68 ± 3.14Db	68.04 ± 2.81Eb	96.22 ± 0.54Aa	83.37 ± 2.33Dc	88.81 ± 1.77Cb
7	33.17 ± 2.03Fc	66.17 ± 3.45Ea	54.40 ± 1.51Fb	97.92 ± 0.62Aa	94.06 ± 1.05Ab	92.76 ± 0.77BCb
8	–	66.14 ± 2.93Ea	13.48 ± 0.51Gb	–	89.58 ± 1.23Ca	80.83 ± 2.36Db
9	–	49.70 ± 5.35F	–	–	96.26 ± 1.25A	–

Means ± SE sharing the same capital letters in the same columns, and same small letters in the rows are statistically insignificant ($P \geq 0.05$)

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 decreased significantly (P = 0.000) than the control (96.82). Temperature (A), storage duration (B), and their interaction (A × B) did not affect negatively the adult emergence 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; $LSD_{(A \times B)}$ = 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; $LSD_{(A \times B)}$ = 0.48, F = 6.737 and $LSD_{(A)}$ = 0.243, F = 12.72; $LSD_{(B)}$ = 0.422, F = 19.767; $LSD_{(A \times B)}$ = 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-pupae, and pupae), 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.

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 °C	7 °C	10 °C	4 °C	7 °C	10 °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 ± 0.46ABa	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 ± 0.00CDc	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 ($P \geq 0.05$)

2017; Ghosh and Ballal 2018; Lü et al. 2019; and Abbes et al. 2020). 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°, then 7 °C for a short-term in all developmental stages of the parasitoid. But, only at 10 °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 °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 °C for only 3 days and also for 10 days at 10

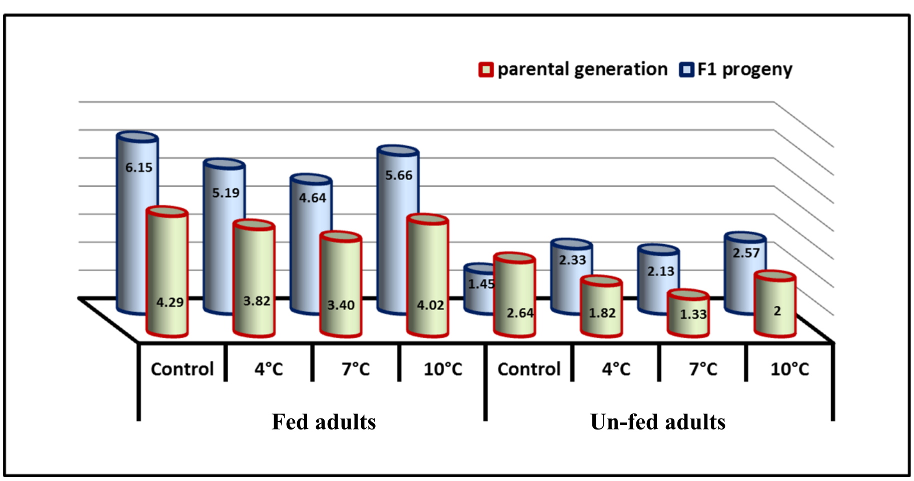

Fig. 3 Mean longevity of *Trichogrammatoidea bactrae* adults (fed and un-fed) of parental and F_1 progeny after storage of parasitoid pupae at different low temperatures

°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 F_1 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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