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Original article

The impact of cold storage durations on *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae) during their pupal stage

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ABSTRACT

This experimental study was done at the Biological Control Laboratory, Sakha Agricultural Research Station, Sakha, Kafrelsheikh, Egypt. We aimed to estimate the impact of different cold (10 °C) storage durations [0 (non-cold-stored parasitized eggs), 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days], on *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae) during the pupal stage using the eggs of *Sitotroga cerealella* after exposing to *T. evanescens*. The emergence percentage of non-cold-stored eggs of *S. cerealella* was higher than all cold-stored durations. Also, the female's percentages of *T. evanescens* in the cold storage durations were lower than the non-cold storage one, and they were influenced by extended cold storage durations. There were non-significant differences in the female's longevity of *T. evanescens* obtained from 0, 3, and 6 days cold-stored parasitized eggs of *S. cerealella* at 10 °C, but it began to decrease from those produced after 9 days of cold-stored eggs. In addition, the emergence percentage in F1 progeny of *T. evanescens* was greater than 50% until 21 days of cold storage. It could be concluded that cold storage reduced the % emergence, % females, female's longevity, and emergence percentage in F1 progeny of *T. evanescens*. For a successful biological control program, the decrease of *T. evanescens* performance after cold storage durations should be considered in mass production, and the release percentage should be increased by the equivalent of a lack of % emergence. Also, the economic importance of using cold storage periods in commercial mass rearing should be assessed in the biological control program.

1. Introduction

Biological control can play an essential role in reducing pest numbers in field crops, fruit orchards, and forests. *Trichogramma* spp. are true egg parasitoids, and they are considered the most natural enemies widely used in the world, partially due to their ease of mass production and their ability to attack a variety of serious insect crop pests (Li, 1994), especially lepidopterous ones in their early stages (their eggs) (Smith, 1996).

Storage of natural enemies is extremely useful to be available when needed. Developing storage strategies for biocontrol agents is essential to provide efficiency and flexibility in mass rearing, synchronise a desired stage of development and have enough stocks for a maximum release (Leopold, 1998; Gosh and Ballal, 2017). Developing storage ways for parasitoids without affecting their fitness is very important in the mass rearing process as indicated by (Leopold, 1998). In the last few years, many studies on the storage of *Trichogramma* spp. have been conducted due to its importance in the biological control field (Huang et al., 2017).

Immatures of many *Trichogramma* species have the ability to enter quiescence or diapauses within their host eggs, allowing them to tolerate long times of cold temperatures (Smith, 1996;

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Abbes et al., 2020). Quiescence is a fast reaction to unsuitable environmental conditions (e.g., low temperature) and may involve physiological changes and causes development to be stopped or slowed, then comes back to grow when appropriate conditions return. Diapauses in several species of *Trichogramma*, make it easy to store them for long durations (Bonnemaison, 1972). Many previous studies have evaluated the impacts of cold storage periods at 2–10 °C on essential biological parameters of many *Trichogramma* spp. Ayvaz et al. (2008); Aydin et al. (2009); Rodrigues and Sampaio (2011) have indicated the short term of cold storage periods (5–25 days) at 8–10 °C have no negative impact. Whereas, Jalali and Singh (1992); Ahmad et al. (2011) and Lessard and Boivin (2013) have found negative effects on F1 progeny after long-term cold storage (45–100 days) at 2–6 °C. According to Iacob and Iacob (1972), the emergence rate of egg parasitoid was significantly lower after cold storage at 9–12 °C. Abd El-Gawad et al. (2010) indicated that the emergence percentage was higher at 4 °C than at 10 °C. Furthermore, the decline in the emergence percentage becomes significant after 21 days of cold storage at 4 °C for *T. brassicae* and *T. evanescens* (Ozder and Saglam, 2004). The emergence rate of *T. carverae* decreased significantly after 3 weeks of cold storage (10 °C) (Bradley et al., 2004). Also, cold storage durations had a clear negative impact on the emergence rate with *T. nerudai* (Tezze and Botto, 2004), and *T. cordubensis* (Ventura Garcia et al., 2002). The reduction in the percentage of adult females increased by extended cold storage times (EL Khayat et al. 2001; Mohamed and El-Heneidy, 2020). Moreover, the longevity of adult females of *T. evanescence* decreased as the storage durations extended at 4 °C (Karabörklü and Ayvaz, 2007).

Sitotroga cerealella Olivier (Lepidoptera: Gelechiidae) is a commercially important host of *Trichogramma* parasitoids under laboratory conditions (Hassan, 1995). Also, the efficiency of *Trichogramma* parasitoids may be affected by the cold storage of the host eggs that will be exposed to the parasitoid after being stored for various periods (Rundle et al., 2004). The survival of the parasitoids produced as a result of prolonged cold storage may be harmed, with a significant reduction in the productivity of produced females (Ozder, 2004).

The main aim of this study was to estimate whether *T. evanescens* could be stored during the pupal stage at 10 °C for various durations without losing efficiency. This estimation will lead to calculate the right release percentages when the cold storage parasitized eggs were used in a biological control program.

2. Material and methods

2.1. Study site

This study was conducted at the Biological Control Laboratory, Rice Research and Training Center, Sakha Agricultural Research Station, Sakha, Kafrelsheikh, Egypt in 2019 to assess the effect of various colds (10 °C) storage durations for *Trichogramma evanecsens* during the pupal stage.

2.2. Mass rearing of T. Evanecsens and S. Cerealella

The mass rearing of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) was done as described by Hassan (1995). The rearing of *T. evanescens* was done according to the method of Abd and Hafez (1995) by adding the new eggs (<24 hrs old) of *S. cerealella* on the paper cards (1 × 1 cm) with a very thin layer of glue. The cards were exposed to *T. evanecsens* in glass jars with some drops of sugar honey for feeding the newly emerged *T. evanecsens* adults. The jars were covered with cloth-wrapped cotton. The cards were changed every 24 hrs to keep away the used cards from the superparasitism.

2.3. Storage of T. Evanecsens pupal stage for 30 days

The parasitized eggs on the paper cards were kept under 25 ± 1 °C and LD 16:8 photoperiods until they became at the pupal stage (blackened level). Following that, the cards were stored at different durations: 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days in an incubator at 10 °C. Then, three parasitized cards were taken out from the incubator after the end of each storage duration and inserted in a tube of glass (3 × 7 cm) under laboratory conations (25 ± 1 °C and LD 16:8 photoperiods) until the emergence of *T. evanecsens*. They were examined under a binocular microscope to estimate some biological parameters such as total parasitized egg, the emergence percentages, the parasitoids longevity, and the sex ratio. The emergence percentage was calculated using the following equation:

$$Emergence\ percentage = \frac{No.emerged\ parasitoid}{Total\ egg\ number} \times 100$$

The longevity (days) was determined from the emergence day to the day of death. The female's percentage was estimated using the following equation:

$$Female's\ percentages = \frac{No.females}{Total\ individual\ number} \times 100$$

Males and females of *T. evanecsens* were determined according to the variations in antenna types.

The F1 progeny was obtained by choosing twenty individuals after each storage period. Ten males and ten females were randomly selected from the same storage duration and inserted into a tube glass (3 × 7 cm), and keeping them for 24 hrs for mating. Ten females were transferred to a new tube glass (3 × 7 cm) containing fresh eggs of *S. cerealella* glued on paper cards (1 × 1 cm) and kept until the emergence of *T. evanecsens*. A binocular microscope was used to examine the samples to estimate the emergence percentage of F1.

2.4. Statistical analysis

Data of this experiment were analyzed using SPSS software (SPSS, 2006). ANOVA, a statistical analysis of variance, was used to find the significant variations between treatments and to determine the effect of cold storage duration on the efficacy of *T. evanescens*, and Tukey's HSD Post-hoc test was used to detect the differences between means. Additionally, the correlations between cold-stored periods and emergence percentage, female's percentage, female longevity, the number of emergence F1 progeny, and emergence percentage of F1 progeny were estimated statistically by the Pearson correlation coefficient.

3. Results and discussion

3.1. Emergence percentages of T. Evanescens

The results of this study indicated the emergence percentages declined gradually as the storage periods extended at 10 °C. The percentages of emergence on control (non-cold-stored parasitized eggs of *S. cerealella*) were greater than any cold-stored ones (Table 1). Cold storage of parasitized host eggs (*S. cerealella*) had a significant impact on the emergence percentages of *T. evanescens* as determined by one-way ANOVA ($F(10, 44) = 1597.78$, $P < 0.05$). Considering 30 days stored parasitized eggs of *S. cerealella*, the proportion of emergence of *T. evanescens* declined from 79.13 to 31.60%. In a previous study, a higher emergence percentage

Table 1
Impact of using cold-stored parasitized eggs of *S. cerealella* on the effectiveness of *T. evanescens.*

Storage duration (days)	No. parasitized eggs of *S. cerealella*	No. emerging *T. evanescens*	% emergence	% female	Longevity (days)
0	281.80 ± 9.14	223.00 ± 7.35^{a}	79.13 ± 0.38^{a}	70.04 ± 0.26^{a}	4.25 ± 0.10^{a}
3	264.40 ± 4.31	202.00 ± 2.55^{b}	76.42 ± 0.41^{b}	67.23 ± 0.25^{b}	4.08 ± 0.08^{a}
6	284.00 ± 1.82	184.80 ± 2.13^{c}	65.06 ± 0.42^{c}	64.28 ± 0.19^{c}	3.95 ± 0.06^{a}
9	289.00 ± 3.11	183.00 ± 2.00^{c}	63.32 ± 0.18 ^{cd}	60.75 ± 0.27^{d}	3.54 ± 0.09^{b}
12	282.20 ± 1.43	175.40 ± 0.68 ^{cd}	62.15 ± 0.28^{d}	57.01 ± 0.21^{e}	3.29 ± 0.07^{bc}
15	280.40 ± 1.40	167.80 ± 1.16^{de}	59.84 ± 0.20^{e}	52.43 ± 0.37^{f}	3.12 ± 0.06 ^{cd}
18	277.00 ± 2.68	156.60 ± 1.89^{ef}	56.52 ± 0.20^{f}	52.23 ± 0.63^{f}	2.79 ± 0.10^{de}
21	277.40 ± 2.54	146.60 ± 1.89^{f}	52.84 ± 0.25 ^{g}	50.34 ± 0.34 ^{g}	2.58 ± 0.05^{e}
24	278.40 ± 1.29	132.00 ± 1.38 ^{g}	47.40 ± 0.29 ^{h}	50.29 ± 0.21 ^{g}	2.04 ± 0.07^{f}
27	273.80 ± 2.15	95.00 ± 1.84 ^{h}	34.68 ± 0.40^{i}	49.05 ± 0.48^{gh}	1.41 ± 0.07 ^{g}
30	271.80 ± 3.95	86.00 ± 3.15^{i}	31.60 ± 0.73^{j}	48.37 ± 0.57 ^{h}	0.66 ± 0.07 ^{h}
Sig.	^{NS}	**	**	**	**

Values are the mean ± standard deviation. The means of each column followed by the different letters are significantly different at the 0.01 level. ** indicate P < 0.01, ^{NS} indicates P > 0.05.

(78.30%) of *T. bourarachae* had been obtained when stored at 4 °C for 60 days (Abbes et al., 2020). These results agreed with Abd El-Gawad et al. (2010) they found the least negative effect on the emergence rate at 4 °C, followed by 10 °C. In comparison with the non-cold-stored parasitized eggs, the emergence percentage declined by 4.01, 7.78, 9.98, 21.46, 24.38, 28.57, 33.22, 40.10, 56.17, and 60.07% in cold storage parasitized eggs for 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days, respectively (Table 2). It means that the amount of parasitized host eggs should be increased to compensate the loss of emergence percentage due to storage.

This research indicated that the pupae of *T. evanescens* could tolerate cold storage. However, a long cold storage period can negatively affect the emergence rate. Statistical analysis of our data indicated that *T. evanescence* could store at 10 °C for up to 21 days with a small loss of emergence rate. Data are given in Table 4 indicated that there were strong negative correlations between storage periods and emergence percentage (r = -0.97, P < 0.01). Our findings agreed with Bradley et al. (2004), the emergence rate of *T. carverae* was negatively affected by lower temperatures (10 °C) especially after 3 weeks of storage. Ozder and Saglam (2004) have noted a reduction in the emergence rate for *T. brassicae* and *T. evanescens* after 3 weeks of storage at 4 °C. A decrease in the emergence percentage due to the cold storage periods was also reportedly with *T. nerudai* (Tezze and Botto, 2004), and *T. cordubensis* (Ventura Garcia et al., 2002). The decrease in emergence percentage may be due to Trichogramma pupae being exposed to low temperatures (10 °C) without first acclimating. In addition, detrimental physiological and physical changes have occurred in both *S. ceralella* eggs and diapaused pupae of *Trichogramma* spp. after long-term cold storage.

3.2. Female's percentage of T. Evanescens

The percentages of the female of *T. evanescens* were influenced by the cold storage durations (F (10, 44) = 426.57, P < 0.05). The percentage of females was 70.04% in non-cold-stored parasitized eggs, while it became 67.23% for the 3 days of cold storage at 10 °C. The female's percentages of *T. evanescens* in the other cold storage durations (6, 9, 12, 15, 18, 21, 24, 27, and 30 days) were 64.28, 60.75, 57.01, 52.43, 52.23, 50.34, 50.29, 49.05, and 48.37%, respectively (Table 1). Moreover, there were strong negative correlations between storage periods female's percentage (r = -0.95, P < 0.01) (Table 4). This may be because of the cold temperatures, which decrease the quality and vitality of parasitized eggs. Our results were in harmony with the findings of EL Khayat et al. (2001), Farid et al. (2001), Chen et al. (2008) and Mohamed and El-Heneidy (2020) they found that the adult female's percentages were significantly influenced by the cold storage durations. In comparison with the non-cold-stored parasitized eggs, the female's percentage declined by 4.01, 8.22, 13.26, 18.60, 25.14, 25.43, 28.13, 28.20, 29.97, and 30.94% in cold storage parasitized eggs for 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days, respectively (Table 2).

3.3. Female longevity of T. Evanescens

Female longevity decreased dramatically as the time of cold storage of the host eggs increased (F (10, 44) = 193.70, P < 0.05). Females obtained from 3 or 6 days cold-stored parasitized eggs of *S. cerealella* at 10 °C lived almost as long as the non-cold-stored parasitized eggs, but they began to vary from those produced from 9 days of cold-stored eggs. The female longevity from the cold-stored host eggs for 18, 21, 24, 27, and 30 days was found to be shorter life with the means of 2.79, 2.58, 2.04, 1.41, and 0.66 days, respectively (Table 1). Compared to the non-cold-stored parasitized eggs, the female longevity declined by 4.00, 7.06, 16.71, 22.59, 26.59, 34.35, 39.29, 52.00, 66.82, and 99.84% in cold storage parasitized eggs for 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days, respectively (Table 2). Also, there were negative correlations between storage periods and female longevity (r = -0.96, P < 0.01) (Table 4). The decrease in longevity is due to the cold stor-

Table 2
The reduction percentages of % emergence, % female, and longevity of *T. evanescens* based on the non-cold-stored parasitized eggs.

Storage duration (days)	% emergence	% female	Longevity	% emergence of F1 progeny
3	4.01	4.01	4.00	4.84
6	17.78	8.22	7.06	8.29
9	19.98	13.26	16.71	14.97
12	21.46	18.60	22.59	20.17
15	24.38	25.14	26.59	23.96
18	28.57	25.43	34.35	27.15
21	33.22	28.13	39.29	32.10
24	40.10	28.20	52.00	37.61
27	56.17	29.97	66.82	46.81
30	60.07	30.94	99.84	60.07

age period induced poor health caused by inadequate nutritional content at immature stage of their development (Boivin, 2010). The longevity of *T. evanescens* emerged from cold stored conditions influenced by extended storage duration. These results agreed with the findings of Jalali and Singh (1992), who found that longevity of *T. japonicum* Ashmead, *T. chilonis* Ishii, and *T. Achaea* Nagaraja and Nagarakati decreased after 2 weeks of cold storage for at 2–5 °C. Also, Karabörklü and Ayvaz (2007) found the longevity of adults of *T. evanescence* emerged from stored eggs at 4 °C decreased as the storage periods increased. Longevity was significantly reduced, maybe due to sensitivity of immature stages to cold exposure without previous adaptation. A previous study indicated that acclimatization of the maternal generation in advance to 30 days at 10 °C increased the longevity in F1 progeny of *T. brassicae* compared to those without an acclimatization time when the immature stages were stored directly at 5 °C (Lessard and Boivin, 2013).

3.4. Impacts of various cold storage times on F1 progeny of T. Evanescens

As shown in Table 3, the emerged F1 progeny of *T. evanescens* was reduced due to the cold storage duration during the pupal stage. Significant differences were detected between non-cold-stored parasitized eggs and all of the cold storage durations as determined by one-way ANOVA ($F(10, 44) = 91.24$, $p = P < 0.05$). Additionally, the emergence of *T. evanescens* decreased from 89.20 to be 35.60 individuals after 30 days of cold storage. The various durations of cold-stored parasitized eggs of *S. cerealella* had significant differences on the emergence percentages of F1 progeny ($F(10, 44) = 488.91$, $p = P < 0.05$). Based on the non-cold-stored parasitized eggs, the emergence percentages of F1 progeny declined by 4.84, 8.29, 14.97, 20.17, 23.96, 27.15, 32.10, 37.61, 46.81, and 60.07% in cold storage eggs for 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days, respectively (Table 2). Also, data in Table 4 showed that there were strong negative correlations between storage periods and average number of emergence F1 progeny, and emergence percentage of F1 progeny ($r = -0.95$ and $-.98 P < 0.01$). A significant negative impact increased due to extended cold storage periods in tested performance of *T. evanescen*, the average number of emergence F1 progeny and emergence percentage of F1 progeny. A reduction in the emergence percentage of F1 progeny due to the cold storage in various *Trichogramma* spp. have been found (Jalali and Singh, 1992; Ozder, 2004). The decrease in the emergence rate of F1 progeny primarily due to the mortality caused by the cold storage durations of *Trichogramma* spp. during the pupal stage and also due to negative physiological and physical changes that have occurred to pupae of *T. evanescens* and their host eggs *S. cerealella*. Furthermore, exposing pupae of *T. evanescens* to 10 °C without the maternal generation's earlier adaptation to cold temperatures. Our results indicated that the emergence percentage in F1 progeny was more than 50% of *T. evanescens* until 21 days of cold storage.

Table 3
Effect of using cold-stored parasitized eggs of *S. cerealella* on the emergence of F1 progeny of *T. evanescens*.

Storage duration (days)	No. exposed eggs of *S. cerealella*	No. emerging *T. evanescens*	% emergence of F1 progeny
0	111.60 ± 2.66	89.20 ± 2.08^{a}	80.01 ± 1.83^{a}
3	112.40 ± 2.50	85.60 ± 2.06^{a}	76.14 ± 0.21^{b}
6	115.00 ± 3.54	84.40 ± 2.69^{ab}	73.38 ± 0.19^{b}
9	112.60 ± 3.50	76.60 ± 2.34^{bc}	68.03 ± 0.17^{c}
12	111.80 ± 2.15	71.40 ± 1.36 ^{cd}	63.87 ± 0.13^{d}
15	113.40 ± 1.40	69.00 ± 1.00 ^{cd}	60.84 ± 0.26^{de}
18	112.20 ± 1.02	65.40 ± 0.51^{de}	58.29 ± 0.21^{e}
21	118.20 ± 3.93	64.20 ± 2.01f^{de}	54.33 ± 0.12^{f}
24	115.80 ± 1.43	57.80 ± 0.66^{e}	49.92 ± 0.21 ^{g}
27	114.20 ± 1.11	48.60 ± 0.60^{f}	42.56 ± 0.28 ^{h}
30	111.20 ± 1.77	35.60 ± 1.69 ^{g}	31.95 ± 1.05^{i}
Significance	^{NS}	**	**

Values are the mean ± standard deviation. The means of each column followed by the different letters are significantly different at the 0.01 level. ** indicate $P < 0.01$, ^{NS} indicates $P > 0.05$.

Table 4
Pearson correlation coefficients for cold-stored periods and % emergence, % female, female longevity, No. emergence of F1 progeny, and % emergence of F1 progeny.

	Parameters	1	2	3	4	5	6
1	Storage durations						
2	% emergence	−0.97**					
3	% females	−0.95**	0.89**				
4	Female longevity	−0.96**	0.97**	0.86**			
5	No. emergence of F1 progeny	−0.95**	0.95**	0.87**	0.97**		
6	% emergence of F1 progeny	−0.98**	0.98**	0.90**	0.98**	0.98**	

** Correlation is significant at the $P < 0.05$. (2-tailed).

4. Conclusion

This study revealed that cold storage reduced the % emergence, % females, and emergence percentage in F1 progeny of *T. evanescens*. The differences in female longevity of *T. evanescens* among the non-cold-stored parasitized eggs and 3 or 6 days were insignificant, then it had a significant decrease from that stored at10°C for 9 days. For a successful biological control program, the decline of *T. evanescens* performance after cold storage durations should be considered in mass production, and the release percentage should be increased to compensate the loss of emergence percentage due to storage. Also, the economic importance of using cold storage periods in commercial mass rearing must be assessed in the biological control program.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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