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RESEARCH

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Improving quality of stored *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) by inducing diapause or quiescence

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Abstract

Background Efficacy of parasitic wasps from the genus *Trichogramma* to attack the eggs of numerous important pests in various agricultural crops makes them one of the most prevalent biocontrol agents. In *Trichogramma*, the pre-pupal stage can survive during the cold season in a dormant state (diapause or quiescence). The optimal conditions for the induction of this pause of development during mass rearing to store the parasitoid for a long time in good quality depend on the species. In the present study, four factors [the incubation periods (24 and 48 h), diapause induction temperatures (9 and 11 °C), durations of the diapause induction (from 0 to 6 weeks), and the periods of storage (from 0 to 6 months)], were experimented to force *Trichogramma evanescens* Westwood, reared on *Sitotroga cerealella,* to enter diapause or quiescence and estimating their impacts on the efficacy of stored parasitoid.

Results Results confirmed that the life parameters of *T. evanescens* as the percentage of adult emergence, female percentage, rates of wing deformation of emerged adults, and fecundity of emerged females were significantly affected by all experimented factors. The pre-storage treatments made it possible to store the parasitoid for at least 2 months at 3 °C, with no much changes in their fitness, the emergence rate of adults reached more than 80%, and the egg-laying efficacy of females reached more than 42 eggs per emerged female, when diapause induction treatments were applied for 5 weeks at 11 °C after 24 h of incubation. Furthermore, there is the possibility of storage for 6 months with an acceptable level of parasitoid's quality, when diapause induction treatments were applied for 5 weeks at 9 °C after 24 h of incubation, the emergence rate reached 70%, and the number of eggs per female was 52 eggs, while no emergence of adult insects was recorded after 3 months of cold storage without diapause induction treatments.

Conclusion Two storage programs were reached for *T. evanescens* (depending on the adult emergence rate and fecundity of emerged females). There is a long-term storage (6 months), when diapause was induced at a low temperature (9 °C) after 24 h of incubation. Short-term storage (from 2 to 4 months), when quiescence was induced under a higher temperature of 11 °C after both 24 and 48 h of incubation.

Keywords *Trichogramma evanescens*, Diapause, Quiescence, Storage period, Life parameters

Background

Egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) have been used successfully in biological control programs in various parts of the world, mostly through inundative releases (Smith 1996). Among all *Trichogramma* species, *T. evanescens* Westwood has been considered one of the most important species. In Egypt, Sherif et al. (2008) recorded about

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70% control of the rice stem borer, *Chilo agamemnon* Błeszyński (Lepidoptera: Crambidae), due to the inundative releases of *T. evanescens*. Also, *T. evanescens* was released to reduce the damage caused by the grape berry moth, *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae), to 96.8% (El-Wakeil et al. 2009). Field trials on the use of commercially available *T. evanescens* against the olive moth, *prays oleae* Bernard (Lepidoptera: Yponomeutidae), reduced the attacks of the pest by 42.9, 71, and 69.9%, and treated trees yielded significantly bigger olive fruits by 10.5 and 12.5% than untreated trees in the 2002 and 2004 olive seasons (Agamy 2010).

Inundative release requires large numbers of *Trichogramma* for each control season (Vinson et al. 2015). Given the problems associated with the availability of parasitoids throughout the year and their high production costs, research has focused on optimizing storage techniques for the production of large quantities of high-quality parasitoids at reasonable cost and to allow synchronization of field releases during pest outbreaks (Colinet and Boivin 2011).

Cold storage is the most commonly used preservation method for *Trichogramma* (Pitcher et al. 2002). However, this method not only cannot guarantee shelf life but also affects the quality of natural enemy products. Cold storage of *Trichogramma* proved possible only for short-term and prolonged periods depending on the factitious host used (Du et al. 2015). Long-term storage induces negative effects owing to reduced moisture content, dry matter, and pH in host eggs (Wu et al. 2018). For example, after 3-week storage at 10 °C, the emergence rate of *T. evanescens* sharply decreased (Shawer et al. 2021).

During harsh environmental conditions, a period of dormancy interrupts the life cycle of many insect species to protect them. Dormancy could occur in most of the parasitoids either by quiescence or diapause (Boivin 1994). Ragland et al. (2019) defined diapause as: a physiologically dynamic and hormonally controlled state of decelerated or arrested morphological development that allows insects to survive unfavorable conditions. Diapause is typically induced/terminated by environmental stimuli (e.g., photoperiod or temperature), though some diapause responses may appear functionally obligate in the field. Juliano et al. (2002) defined quiescence as: a short period of dormancy characterized by slowed metabolism and directly induced by unfavorable environmental conditions that can be quickly reversible when favorable conditions return. In 1996, Smith reported that immatures of several *Trichogramma* species can enter diapause or quiescence within host eggs and thereby tolerate long periods of subfreezing temperatures. Preferable temperatures and photoperiodic conditions control diapause in *Trichogramma* during the pre-pupal stage. *Trichogramma dendrolimi* Matsumura can enter diapause which enables storage for up to 4 months without impairing their traits (Zhou et al. 2014). So, diapause induction is a key for efficient long-term storage of mass-reared *Trichogramma* (Zhang et al. 2018).

Since different species of *Trichogramma* vary in their responses to the storage temperature as well as the period of cold storage (Voegele et al. 1988), the appropriate approaches to the induction of diapause differ among *Trichogramma* species and are not easy to control. Laboratory evaluation of individual species for such treatments becomes mandatory. In this context, the objective of the present study was to determine the treatments that lead to *T. evanescens* entering diapause and quiescence and, thus, the possibility of storing it for the longest possible period with acceptable quality.

Methods

Study site

The present study was carried out during 2021–2023 at the Biological Control Laboratory—Pests and Plant Protection Department—Agricultural and Biological Research Institute—National Research Centre (NRC), Giza Governorate, Egypt.

Cultures of Trichogramma and the host

Cultures from the parasitoid sp. *T. evanescens* and its host *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) eggs (< 24 h. old) were established and supplied by the Laboratory of Biological Control, Research Institute of Plant Protection, ARC, Giza, Egypt.

Experimental technique

Freshly *S. cerealella* eggs were glued to a self-adhesive strip (10 × 15 cm.). The strips carrying *S. cerealella* eggs were exposed to *Trichogramma* adults in plastic jars (1 L) covered with a muslin cloth, held in position by a rubber band. Parasitized egg cards were incubated at (21 ± 1 °C), relative humidity (60–70%), and a 10:14 h L/D photoperiod.

After adult emergence, 12 newly emerged and mated parasitoid females were placed in a glass test tube (1.5 × 10cm) plugged with cotton, for a total of 1960 tubes. Every glass tube contained a labeled strip which had a ½ cm disk diameter to glue approximately 120 *S. cerealella* eggs. Females were removed after 6 h, and tubes were left for 18 h to complete 24 h at 23 ± 1 °C, 8:16 h L/D photoperiod, and 60–70% RH.

After parasitism took place, the parasitized cards were divided into two groups and incubated for 24 and 48 h at 21 ± 1 °C, 8:16 h L/D, and 60–70 RH. After the incubation period (IP), every group was divided into sub-groups that were assigned to the pre-storage conditions (diapause

induction conditions), 9 ± 1 and 11 ± 1 °C, 8:16 h L/D, 60–70 RH for 0, 1, 2, 3, 4, 5, and 6 weeks.

After diapause induction (DI), tubes containing the patches of parasitized eggs from each treatment were placed for storage in full darkness at 3 ± 1 °C for 0, 1, 2, 3, 4, 5, and 6 months. During storage periods, the tubes containing the parasitized eggs were kept in zipper bags containing a piece of cotton saturated with a solution of NaCl to maintain relative humidity (Pizzol and Pintureau 2008).

After the dedicated period of storage, 10 replicates of every storage period were directly transferred to a chamber at 25 ± 1 °C, and 14:10 h L/D. Three parameters were measured after adult emergence: the emergence rate (the number of black eggs with an emergence hole divided by the total number of black eggs × 100), the wing deformation rate, the female percentage, and the fecundity (the number of black eggs during three days by a single female). The fecundity was obtained by choosing ten mated females from every storage duration. Everyone was inserted into a tube glass (1.5 × 10 cm) containing fresh eggs of *S. cerealella* glued on self-adhesive paper cards and kept until the emergence of *T. evanescens*. All designed treatments are illustrated in Fig. 1.

Pre-pupal formation

After diapause induction, three egg cards carrying 120 eggs in each for every treatment were placed in bleached and agitated solution (10% sodium hypochlorite) for 5–10 min or until the chorion sloughed (Jarjees and Merritt 2002). Immature stages were transferred to a saline solution. Then, place it on the center of a microscope slide with a drop of glycerol and phosphate buffer. They were observed by an OPTICA stereoscope with a magnification power of 10x. 4.5, and the pre-pupae pictures were shot by an iPhone 12-megapixel dual camera.

Statistical analysis

In a split plot design, General Linear Model Univariate Analysis was used to study the effects of incubation period, diapause induction temperature, diapause induction period, period of storage at 3 °C, and their interactions on the percentage of adult emergence, female percentage, deformities, and fecundity of emerged females (number of black eggs). A general linear model was also conducted on data regarding the number of parasitoids in the pre-pupal stage before storage. All analyses were performed using SPSS version 26.0 (SPSS 2019) at a confidence level of 95%.

Results

Pre-pupal formation

Pre-pupal formation was significantly affected by the incubation period ($F = 2263.996$; $df = 1, 56$; $P < 0.001$), diapause induction temperature ($F = 529.011$; $df = 1, 56$; $P < 0.001$), duration of exposure to the diapause induction temperature ($F = 6249.070$; $df = 6, 56$; $P < 0.001$), and all the possible interactions among these three factors ($P < 0.001$).

The onset of the pre-pupal stage was indicated by appearance of urate bodies scattered around the midgut (Fig. 2A), compound eyes were formed and became bright red (Fig. 2B), and the larval integument was not shed (Fig. 2C). The duration of diapause induction at 9 °C

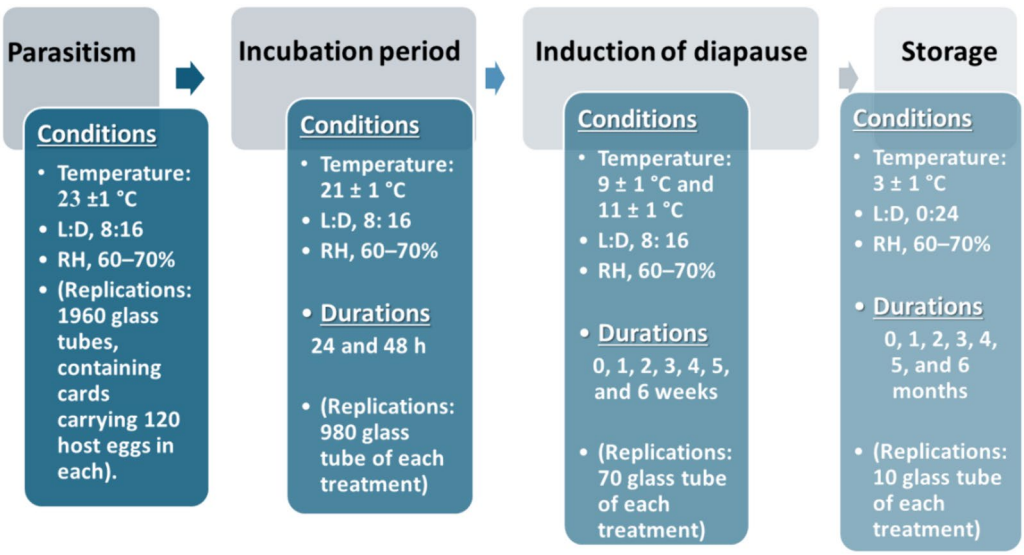

Fig. 1 Experimental treatments of the present study

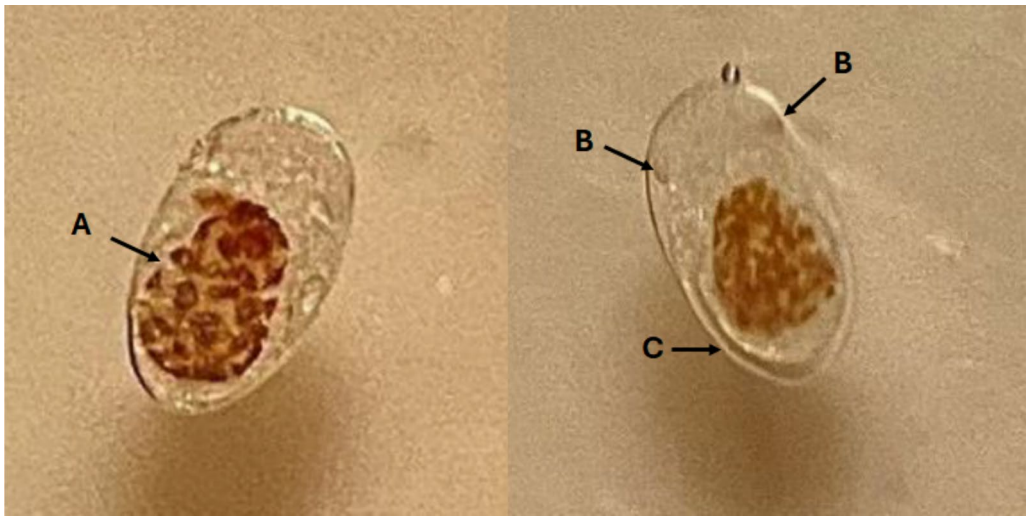

Fig. 2 *Trichogramma evanescens* pre-pupae: **A** urate bodies, **B** compound eyes, and **C** larval integument

after 24 h of incubation resulted in a significant effect on pre-pupal formation percentage. The highest rate (76.1 and 75.6%) was recorded after 5 and 6 weeks of induced diapause (Fig. 3A).

When the temperature of inducing diapause was 11 °C, after 24 h of incubation, the duration of inducing diapause significantly affected the pre-pupal formation. After 4 weeks of induced diapause, it was 70.3%. This rate increased to 80.3% after 5 weeks of diapause induction. Then, it decreased again to 75.2% after 6 weeks of diapause induction (this was due to starting pupal formation) (Fig. 3B).

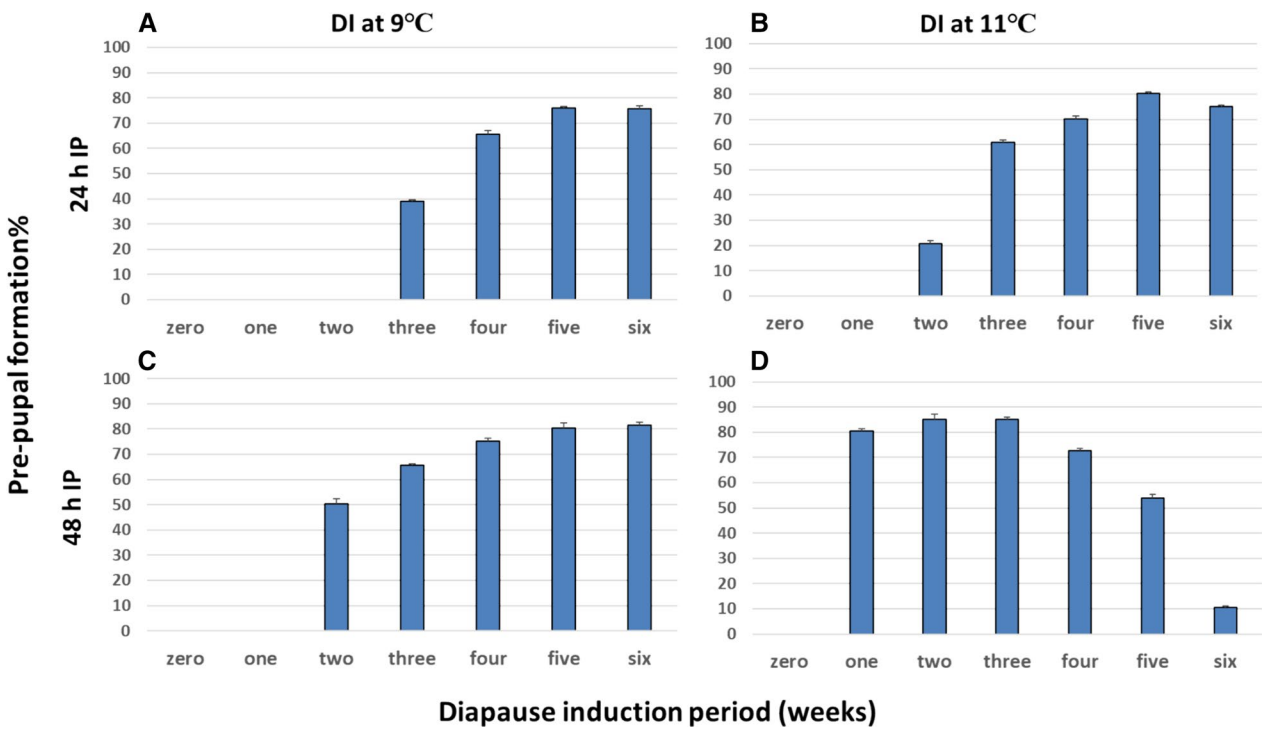

Fig. 3 Mean percentage of pre-pupal formation (± s.e.) of *T. evanescens* after 24 h of incubation at 21 ± 1 °C, followed by diapause induction periods (0, 1, 2, 3, 4, 5, and 6 weeks) of exposure to **A** 9 °C, **B** 11 °C, and after 48 h of incubation at 21 ± 1 °C, followed by diapause induction periods (1, 2, 3, 4, 5, and 6 weeks) of exposure to **C** 9 °C, and **D** 11 °C

Pre-pupal formation was significantly affected by different durations of diapause induction at a temperature of 9 °C after 48 h of incubation. The pre-pupae started formation after 2 weeks of induced diapause at a rate of 50.3%. This rate increased as the diapause induction period increased, reaching 81.5% after 6 weeks (Fig. 3C).

The duration of diapause induction at 11 °C after 48 h of incubation showed a significant effect on pre-pupal formation percentage. It was 80.6% after only 1 week of induced diapause, increased to 85.3% after 2 weeks, and 85.2% after 3 weeks. After 4 weeks of induced diapause, it decreased sharply to 72.7%; this decline continued until it reached 10.5% after 6 weeks (due to pupal formation) (Fig. 3D).

Adult emergence

The analysis showed that the percentage of adult emergence of *T. evanescens* was significantly affected by the incubation period ($F=395.912$; $df=1,1764$; $P<0.001$), diapause induction temperature ($F=2183.569$; $df=1$, 1764; $P<0.001$), duration of exposure to the diapause induction temperature ($F=3659.885$; $df=61764$; $P<0.001$), period of storage at 3 °C ($F=23,453.790$; $df=6,1764$; $P<0.001$), and all the possible interactions among these four factors (all $P<0.001$).

It was found that, after 24 h of incubation, when diapause was induced for 5 weeks under 9 °C, the percentage of emerged adults continued at a rate greater than 70% even after 6 months of storage. In the case of no inducing diapause period, the adult emergence sharply decreased after only 2 months of storage reaching 20.9%, and stopped after that completely (Fig. 4A).

After incubation period of 24 h, 5 and 6 weeks of diapause induction at 11 °C were good conditions for short- and medium-term storage (2 to 4 months). 5-week diapause induction appeared as a good condition for a short period of storage (2 months), where adult emergence was more than 80%. After 6 weeks of induced diapause, the emergence rate after 2, 3, and 4 months of storage was fairly constant at about 70%. In the case of long-term storage, the emergence of adults continued after 6 months of storage, but it sharply decreased after 5 months of storage, reaching less than 54% (Fig. 4B).

At an incubation period of 48 h and a diapause induction temperature of 9 °C, in short periods of diapause induction, the adult emergence decreased sharply after 2 months' storage. In long periods of diapause induction

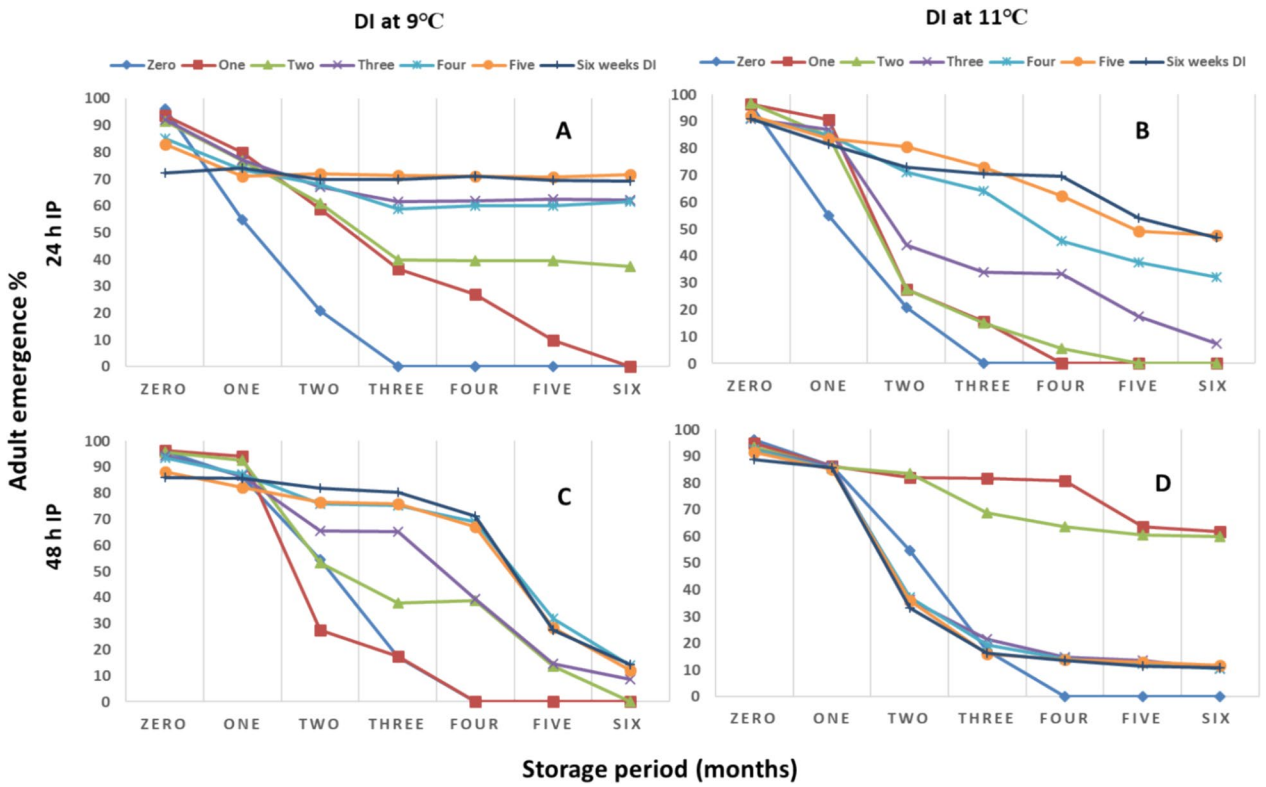

Fig. 4 Mean percentage of adult emergence of *T. evanescens* after 24 h of incubation, followed by several diapause induction periods (0, 1, 2, 3, 4, 5, and 6 weeks) of exposure to **A** 9 °C, **B** 11, and after 48 h of incubation, followed by diapause induction periods (1, 2, 3, 4, 5, and 6 weeks) of exposure to **C** 9 °C, and **D** 11 °C, followed by storage at 3 °C for 0, 1, 2, 3, 4, 5, and 6 months

(4, 5, 6 weeks), the adult emergence decreased gradually, reaching about 70% after 4 months of storage (Fig. 4C).

After 48 h of incubation and inducing diapause at 11 °C associated with increasing the storage period by more than 2 months, adult emergence decreased sharply in the absence of an induction period of diapause, as well as when this period exceeded more than 2 months. When the period of inducing diapause was for 1 and 2 weeks, the decrease in adult emergence was gradual and continued to reach about 60% after 6 months of storage (Fig. 4D).

Female percentage

The female percentage was also significantly affected by the incubation period ($F=335.403$; $df=1,1764$; $P<0.001$), the diapause induction temperature ($F=12.641$; $df=1,1764$; $P<0.001$), the duration of exposure to the diapause induction temperature ($F=3086.608$; $df=6,1764$; $P<0.001$), the period of storage at 3 °C ($F=2167.738$; $df=6,1764$; $P<0.001$), and all the possible interactions among these four factors (all $P<0.001$).

The emergence rate of females decreased by exposing the immature stages to cold temperatures. The rate of decrease was higher after a short storage duration in the case of no diapause induction period, as it reached 53.5% after 2 months of storage when the incubation period was 24 h compared to 65.5% in the control (Fig. 5A, B), and 52.3% after 48 h of incubation, followed by 3 months of storage in comparison with 65.5% in the control (Fig. 5C, D). When diapause induction was held, the rate of deficiency in most cases was about 10%, even after 6 months of storage (Fig. 5A–D).

Wing deformation

The rate of wing deformation was significantly affected by the incubation period ($F=135.616$; $df=1,1764$; $P<0.001$), the diapause induction temperature ($F=295.790$; $df=1,1764$; $P<0.001$), the duration of exposure to the diapause induction temperature ($F=343.288$; $df=6,1764$; $P<0.001$), the period of storage at 3 °C ($F=139.864$; $df=6,1764$; $P<0.001$), and all the possible interactions among these four factors ($P\leq0.001$), except the interaction between incubation period and diapause induction temperature ($F=1.049$; $df=1,1764$; $P=0.306$).

The rate of wing deformation in adults after 24 h of incubation increased with increasing exposure to low

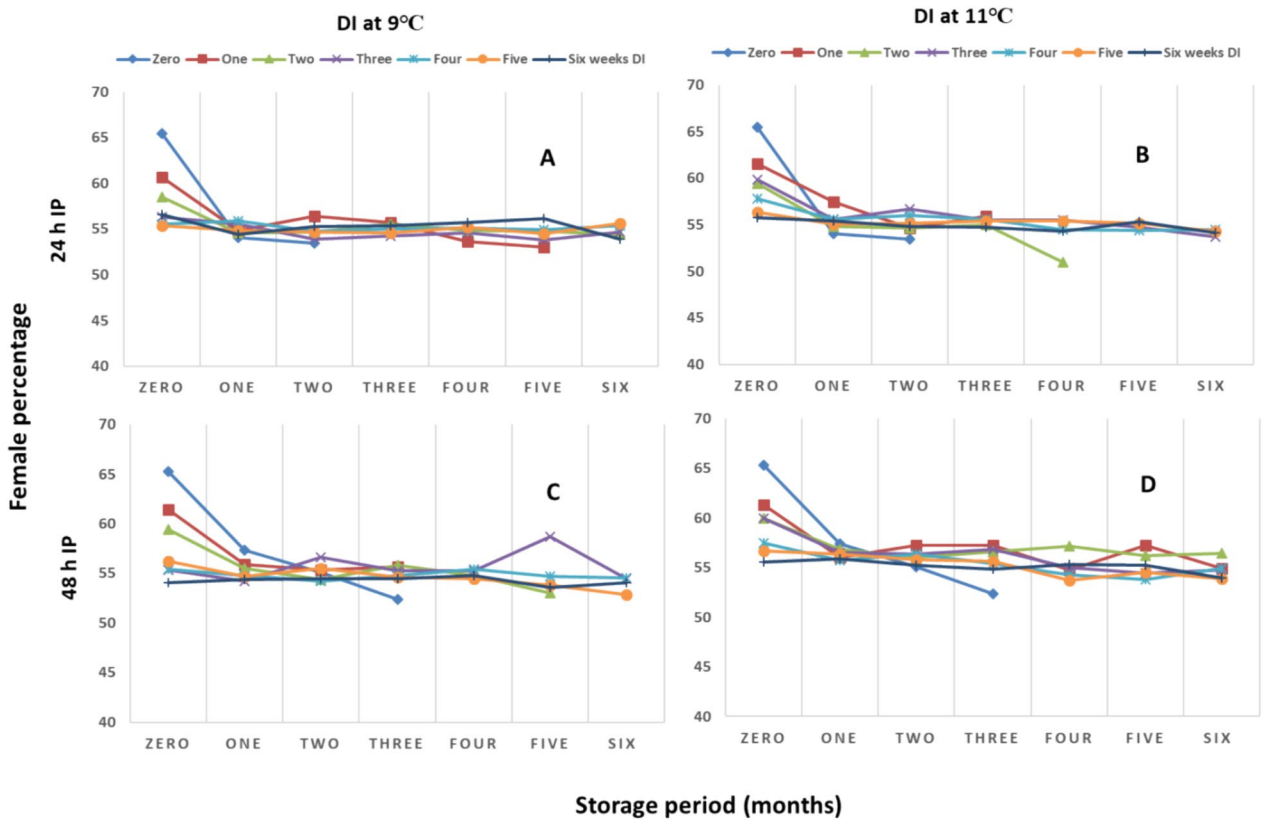

Fig. 5 Female percentage of *T. evanescens* after 24 h of incubation, followed by several diapause induction periods (0, 1, 2, 3, 4, 5, and 6 weeks) of exposure to **A** 9 °C, **B** 11, and after 48 h of incubation, followed by diapause induction periods (1, 2, 3, 4, 5, and 6 weeks) of exposure to **C** 9 °C, and **D** 11 °C, followed by storage at 3 °C for 0, 1, 2, 3, 4, 5, and 6 months

temperature reaching 14.0% after 2 months of storage in case of non-inducing diapause period, compared to 1.9% in control (without diapause induction treatments and storage). However, this increasing rate was lower after several periods of diapause induction under 9 °C, when it was 6.9% after 6 weeks of diapause inductions, followed by 6 months of storage (Fig. 6A).

In the case of 24 h of incubation and diapause induction at 11 °C, the rate of wing deformation decreased with inducing diapause for 5 and 6 weeks reaching 9.5, 10.3, and 10.7, 11.1% after 2 and 3 months of storage, respectively, compared to 14.0% after 2 months of direct storage (Fig. 6B).

The cold temperature also affected the wing deformation rate significantly after an incubation period of 48 h and a diapause induction temperature of 9 °C. It increased after 3, 4, 5, and 6 weeks of diapause induction, followed by 6 months of storage. This deformation rate was lower in cases of shorter periods of storage, where it reached 7.4, 7.8%, and 5.6, 6.8% after 2 and 3 months preceded by 5 and 6 weeks of diapause induction, compared to 20.7% after 3 months of storage in case of non-diapause induction (Fig. 6C).

After 48 h of incubation and inducing diapause at 11 °C, increasing the storage period more than 2 months after 3, 4, 5, and 6 weeks of inducing diapause led to a significant increase in the rate of wing deformation. It reached 17.3% after 6 months of storage, preceded by 6 weeks of diapause induction. But in the case of inducing diapause for only 1 and 2 weeks, the percentage of wing deformation was only 8.5 and 7.6 after 6 months of storage (Fig. 6D).

Fecundity (mean number of black eggs per emerged female)

The mean number of black eggs per emerged female was also significantly affected by the incubation period ($F=73.197$; $df=1,1764$; $P<0.001$), the diapause induction temperature ($F=350.241$; $df=1,1764$; $P<0.001$), the duration of exposure to the diapause induction temperature ($F=435.482$; $df=6,1764$; $P<0.001$), the period of storage at 3 °C ($F=695.093$; $df=6,1764$; $P<0.001$), and all the possible interactions among these four factors (all $P<001$), except the interaction between incubation period and diapause induction temperature ($F=7.958$; $df=1, 1764$; $P=0.005$).

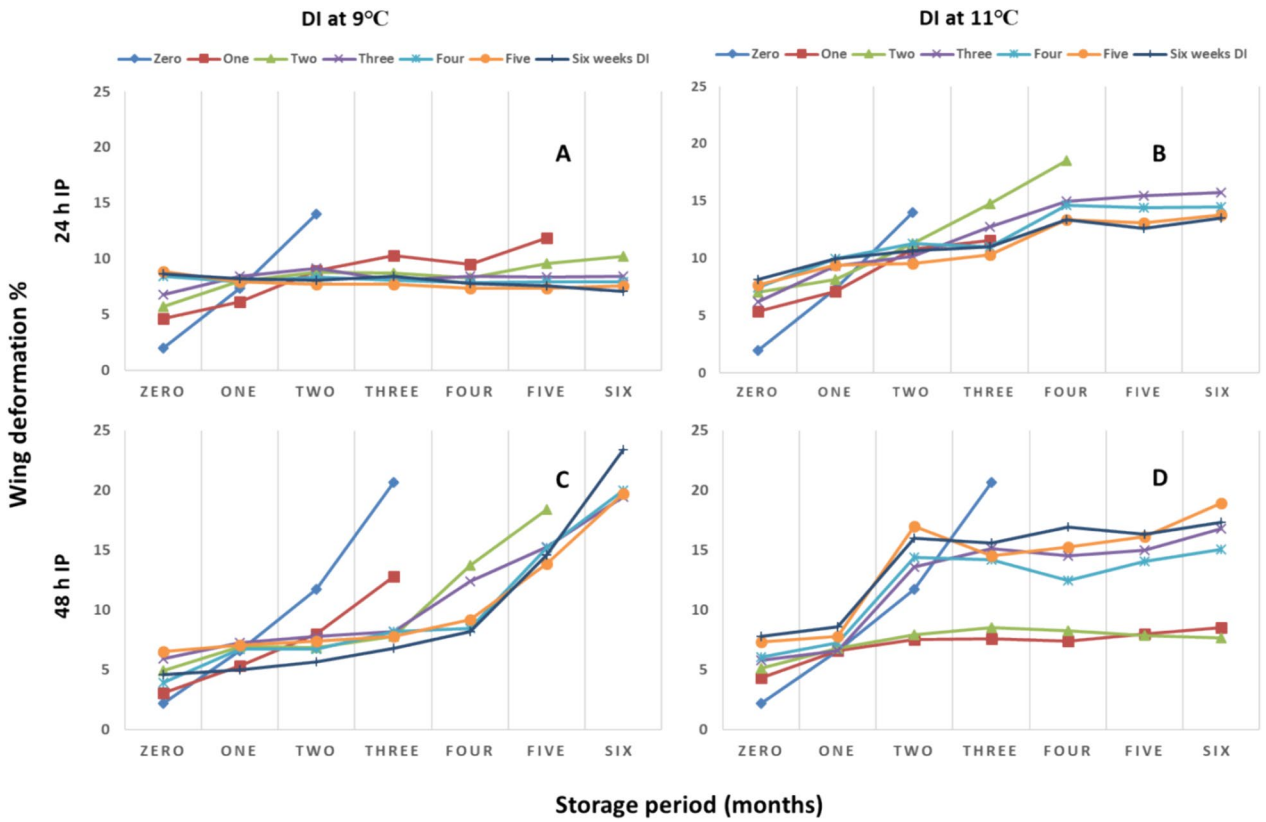

Fig. 6 Mean percentage of wing deformation of *T. evanescens* after 24 h of incubation, followed by several diapause induction periods (0, 1, 2, 3, 4, 5, and 6 weeks) of exposure to **A** 9 °C, **B** 11, and after 48 h of incubation, followed by diapause induction periods (1, 2, 3, 4, 5, and 6 weeks) of exposure to **C** 9 °C, and **D** 11 °C, followed by storage at 3 °C for 0, 1, 2, 3, 4, 5, and 6 months

In the case of 24 h of incubation and under 9 °C of inducing diapause temperature, the fecundity of females emerged after storage decreased significantly, especially with the lack of inducing diapause treatments or their short duration. But it increased again with the increase in period of diapause induction, especially after a period of 5 weeks, as the number of eggs laid by one female reached 52.6 eggs after 6 months of storage compared to 50.5 eggs per female in the case of control group (Fig. 7A).

After an incubation period of 24 h and diapause induction at 11 °C, 5- and 6-week diapause induction appeared in good conditions for short-term storage. When diapause was induced for 5 weeks, the fecundity was 42.1 eggs per female after 2 months of storage. After 6 weeks of induced diapause, the fecundity after 2, 3, and 4 months of storage was stable at more than 42 eggs per female. But in the case of long-term storage (after 6 months of storage), the egg laying decreased, reaching less than 24 eggs per female (Fig. 7B).

At an incubation period of 48 h and a diapause induction temperature of 9 °C, the fecundity of emerged females decreased with an increasing storage period. This deficiency was severe in the absence of an induction period of diapause before storage, as well as when this period was short (1 week) (Fig. 7C).

After 48 h of incubation and inducing diapause at 11 °C, the fecundity of the emerged females was fairly stable in the case of exposure to the induction of diapause for a week and ranged from 35 to 42 eggs per female (after all periods of storage), while exposure to 2 weeks of inducing diapause decreased it to about 29 eggs per female after 5 and 6 months of storage (Fig. 7D).

Discussion

Low temperature has been identified as one of the key factors in most species of *Trichogramma* responsible for diapause induction (Zaslavski and Umarova 1990). *Trichogramma* programmed pre-pupal diapause during the sensitive stage, which is fairly brief and occurs before the actual diapausing stage (from the early embryo up to the early pre-pupal stage) (Reznik et al. 2008). In the present study, the formation of urate bodies then the compound eyes without shedding the larval integument was evidence of the formation of *T. evanescens* pre-pupa. This agrees with results reported by Jarjees and Merritt (2002).

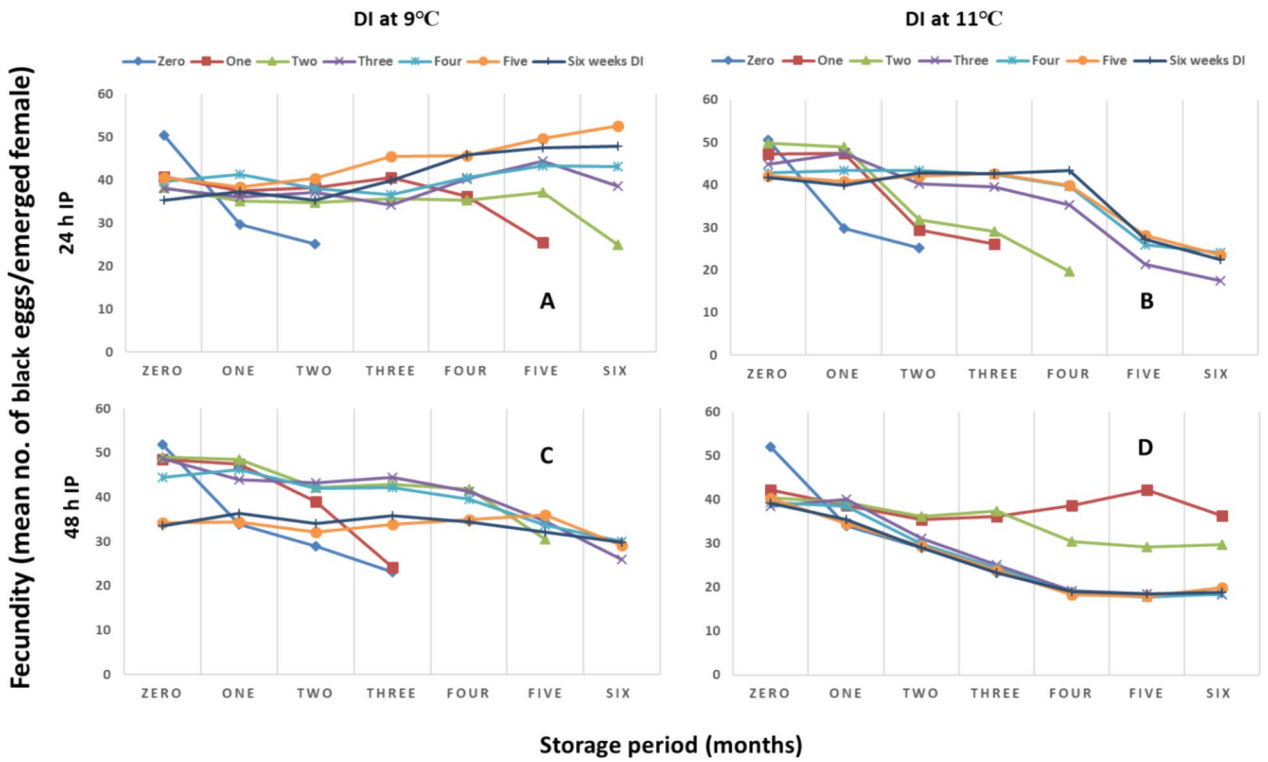

Fig. 7 Fecundity (mean number of black eggs per emerged female) of *T. evanescens* after 24 h of incubation, followed by several diapause induction periods (0, 1, 2, 3, 4, 5, and 6 weeks) of exposure to **A** 9 °C, **B** 11, and after 48 h of incubation, followed by diapause induction periods (1, 2, 3, 4, 5, and 6 weeks) of exposure to **C** 9 °C, and **D** 11 °C, followed by storage at 3 °C for 0, 1, 2, 3, 4, 5, and 6 months

It was noticed that the cold exposure at a low temperature (9 °C) of the early sensitive stage (after 24 h of incubation) was a strong stimulus needed to cause the reaction (diapause). The duration of the cold exposure causing a detectable increase in the proportion of diapausing individuals in *T. evanescens* was 5 weeks, and this was an appropriate period for the formation of the prepupa, which was in the stage that enters diapause. When these conditions existing, this species could be stored for 6 months at 3 °C with a satisfactory loss of biological potential in comparison with direct storage (without diapause induction). There was a decrease in the efficacy of the parasitoid after long-term storage compared to the control (without storage), even after interring diapause, which was expected. Prolonged diapause can be costly for individuals, which can be seen as increased mortality (Salman et al. 2019) and/or reduced performance (Matsuo 2006).

The duration needed for *Trichogramma* to enter diapause varies depending on the exposure temperature and the species. In previous studies, the duration of 30 days of cold exposure at 10 °C had a significant effect on the proportion of diapause in *T. cordopensis* Vargas & Cabello (Garcia et al. 2002) and *T. dendrolimi* (Ma and Chen 2006). In another study, Reznik et al. (2008) recorded that the duration of only one day of cold exposure for *T. dendrolimi was enough to increase the proportion of diapausing individuals, and this variation between the results of* Reznik et al. (2008) *could be related to the difference in methodology.*

Quiescence, a state of developmental arrest, was described by Smith (1996) for *Trichogramma*. Quiescence does not allow prolonged storage at 3 °C, as such storage leads to a rapid deterioration of vital traits. An increase in temperature may mostly suggest quiescence rather than diapause (Cagnotti et al. 2018). As seen in the present results, *T. evanescens* could also be stored in a quiescent state for 2 to 4 months at 3 °C, maintaining good quality. The cold exposure to a higher temperature (11 °C) of early sensitive larvae after 24 h of incubation, or the late-larvae (after 48 h of incubation), was the stimuli needed to cause the reaction of quiescence. In previous studies on the same species, Hasan et al. (2024) reported that *T. evanescens* pre-pupae exposed to a temperature of 10 °C for 7 days before storage can be stored for up to 30 days at 4 °C in a state of quiescence without much loss of performance.

The female percentages of *T. evanescens* decreased when exposed to the cold temperature. That is probably because low temperatures reduce the vitality of parasitized eggs. Obtained results agree with the findings of Chen et al. (2008) on *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae), Mohamed and El-Heneidy (2020) on *Trichogrammatoidea bactrae* Nagaraja, and Shawer et al. (2021) on the species *T. evanescens.*

The quality of the emerged adults was also affected by the low temperatures, as was observed by the number of wing deformations in individuals. The rate of wing deformation increased with increasing the duration of exposure to low temperature, especially when the exposure was after 48 h of incubation. But the rate of such an increase was lower in the case of entering *T. evanescens* into diapause or quiescence. Increases in the proportion of deformed adults with an increasing duration of cold storage could result from the exposure of parasitized eggs to extremely suboptimal temperatures (Tezze and Botto 2004).

It is obvious that the temperature is an important stimulus causing diapause or quiescence. But this depends on other factors, including the age of the sensitive stage exposed to this temperature as well as the duration of exposure. The present results indicated that the selected species received the affecting stimuli from the conditions it was exposed to in stages before storage that arrest their development at the pre-pupal stage. These result are in line with those reached by Ghosh and Ballal (2017) on *T. chilonis* Ishii.

Conclusion

Obtained results proved that 24 and 48 h of incubation followed by induction of diapause at 11 °C for 5 and 2 weeks, respectively, were the most suitable conditions for *T. evanescens* to enter quiescence and be stored for 2 to 4 months without much loss of their fitness. In addition, 24 h of incubation followed by induction of diapause at 9 °C for 5 weeks provided suitable conditions for *T. evanescens* to enter diapause and be stored for a long period (6 months) with acceptable level of parasitoids' quality.

Abbreviations

IP Incubation period
DI Diapause induction

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Author contributions

All authors were involved in the conception and design of the work. ShM performed the experiments and involved in the analysis and interpretation of the data and drafting of the paper. EA, ME-H, TE, and HE supervised and coordinated the laboratory work and results analysis, revised the paper critically, and provided constructive discussion, and EA made the final approval of the version to be published. All authors agree to be account for all parts of the work.

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Availability of data and materials

The data and material used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval and consent to participate are not required for this study.

Consent for publication
Not applicable for that section.

Competing interests
The authors declare that they have no competing interests.

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