



Effect of cold storage on performance of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) reared on *Ephestia kuehniella* and *Ectomyelois ceratoniae*

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Abstract. Trichogrammatid wasps are the most common natural enemies around the world used for biocontrol many important lepidopteran pests. Therefore, the availability of suitable storage methods for these parasitoids is valuable in biological control. In this study, the performance of *Trichogramma brassicae* reared on two different hosts and in two different cold storage periods was investigated. Wasp pupae formed on *Ephestia kuehniella* and *Ectomyelois ceratoniae* eggs, were kept in 10 °C for 30 days and then in 4 °C for 4 and 8 weeks. The effect of the treatment combinations on wasp's emergence rate, sex ratio and the number of female and male parasitoids in parental wasps (F₀) and their progeny (F₁) were estimated. In addition, adult wasp's shape (wingless and abnormality) and parasitism rate were investigated in parental wasps (F₀) and their progeny (F₁). The results of this study showed that emergence rate of *T. brassicae* in F₀ was significantly influenced by the interaction between host and cold storage period. The highest and lowest emergence rate were respectively found on *E. ceratoniae* in control and *E. kuehniella* in 8 weeks cold storage period. Sex ratio and the number of female parasitoids in F₀ and F₁ were significantly influenced either by host or cold storage period, whereas interactive effect of these factors did not affect significantly sex ratio and the number of females in both generations. Also, wingless and abnormality of wasps in F₀ was not significantly influenced by any treatment combination. Parasitism rate of wasps reared on both hosts in F₁, significantly decreased by increasing in cold storage duration. Finally, the results indicated that the most of qualitative characteristics of wasps grown on *E. ceratoniae*, were clearly better than those of wasps grown on *E. kuehniella* both in F₀ and F₁.

Keywords: biological control, egg parasitoid, augmentative release, quality control, carob moth

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Introduction

Ectomyelois ceratoniae (Zeller) (Lepidoptera: Pyralidae), is the key pest of pomegranate orchards in Iran and many regions around the world and causes significant damage on nut and fruit products such as almonds, pistachios, pomegranates, stone and pomes fruits (Botha *et al.*, 2004; Azema & Mirabzadeh, 2005). Several control methods, including sterile insect technique (Chakroun *et al.*, 2017), mating disruption (Mamay *et al.*, 2016), mass trapping (Mamay & Dağ, 2016), biological control (Memari *et al.*, 2016; Zougari *et al.*, 2021), mechanical control like fruit bagging and removal (Mamay, 2021) and chemical control (Warner *et al.*, 1990; Mnif *et al.*, 2013), have been examined to reduce carob moth damage in pomegranate orchards. However, the use of insecticides is not

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applicable because the larvae are protected from insecticides inside the fruit (Kishani-Farahani *et al.*, 2012). Mechanical and biological control methods particularly egg parasitoids of *Trichogramma* species are mostly used in Iran to control this pest (Ebrahimi, *et al.*, 1998; Moezipour, 2006).

Trichogramma brassicae Bezdenko has been proven to be the most widespread *Trichogramma* species in Iran and is commonly mass-reared for release in rice, corn, cotton, tomato, soybean fields and pomegranate orchards to control serious lepidopteran pests (Farrokhi *et al.*, 2010; Moezipour *et al.*, 2008). The success of biological control of mentioned pest depends not only on the time and the number of *Trichogramma* wasps released, but also on their quality (i.e. emergence, longevity, fecundity and searching capacity) (Bigler, 1994; Cerutti & Bigler, 1995; Dutton *et al.*, 1996; Van Lenteren & Tommasini, 2002).

Long-term storage of *Trichogramma* species is possible; especially cold-storage methods are common (Wang & Smith, 1996; Garcia *et al.*, 2002; Ayvaz *et al.*, 2008). Two main cold storage techniques have been used in mass rearing of *Trichogramma* spp. including with and without previous diapause induction (Greenberg *et al.*, 1996). Pre-storage (representing fall conditions: acclimation period) and cold-storage (representing winter conditions), cause long-term storage in *T. brassicae* with high emergence rate of adults (Rahimi-Kalde *et al.*, 2017; Cagnotti *et al.*, 2018). Acclimation of either 30 days at 10 °C or 24 days at 13 °C allowed *T. brassicae* immatures to develop with a lower mortality than those exposed directly at 5 °C (Lessard & Boivin, 2013).

Also, duration of exposure to low temperature has considerable effect on quality of biological agents (Denlinger & Lee, 2010). For example, different storage periods of the wasp's pupae in 4 °C (1, 2, 3 and 4 weeks) had a significant effect on the adult emergence rate of *T. cacoeciae* Marchal, *T. evanescens* Westwood and *T. brassicae* (Ozder, 2004). A significant reduction in adult emergence due to the lower cold-storage temperatures and longer duration, compared with the control group, was observed in these three species and other *Trichogramma* spp. (Jalali & Singh, 1992; Pitcher *et al.*, 2002; Ozder, 2004).

On the other hand, different hosts produce parasitoid wasps with different performance and efficiency (Muli *et al.*, 2010; Farazmand, 2007). For example, when the *T. evanescens* reared on three various hosts (*Sitotroga cerealella* Olivier, *Ephestia kuehniella* Zeller and *Galleria mellonella* Linnaeus) for controlling cotton bollworm *Helicoverpa armigera* (Hübner), had different qualitative characteristics such as parasitism and emergence rates, fecundity and longevity (El-Wakeil, 2007).

Indeed, there are many studies which are separately focused on the effect of cold storage time or host's species on the performance and efficiency of *Trichogramma* wasps (Rahimi-Kaldehy *et al.*, 2017; Abbes *et al.*, 2020). However, there is little information for influence of interaction between cold storage period and host's species on the abilities of *Trichogramma* wasps. Thus, in the current study, we aimed to evaluate interactive effect of cold storage periods (control, 4 and 8 weeks) and two different hosts (*E. kuehniella* and *E. ceratoniae*) on *T. brassicae* qualitative characteristics both in parental and progeny generations.

Materials and methods

Insect culture

Ectomyelois ceratoniae larvae were collected from pomegranate orchards in Birjand (South- Khorasan Province, Iran). The larvae were transferred to container (30 × 20 × 5 cm), containing pistachio powder (with low marketability and quality; about 50 larvae per unit). Containers kept in a big cage with net walls (80 × 80 × 150 cm) in room conditions set at 25 ± 2 °C, 50 ± 5% (RH), and 16:8 (L:D) photoperiod (Ziaoddini *et al.*, 2011). After adults' emergence, the moths were allowed to fly in the cage for one day to get ready for mating. Then, moths were transferred to small cages for egg-laying. The pomegranate moths were reared for several generations in this condition.

The populations of *E. kuehniella* and *T. brassicae* were obtained from the colonies maintained at Insect Ecology and Pest Management Laboratory of Agriculture Faculty in Ferdowsi University of Mashhad. The parasitoids were reared on 24 hours old eggs of the *E. kuehniella*, in growth chamber (25 ± 1 °C, 65 ± 5% RH, 16:8 [L:D]).

Cold storage treatments

The eggs of both hosts (*E. kuehniella* and *E. ceratoniae*) were sterilized by exposing them to UV-C lamp (15 W Philips Holand) with a wavelength of 280 nm with a distance of 30 cm for 30 minutes (Nazeri *et al.*, 2015). Fifty numbers of 24-hours old eggs of *E. kuehniella* and *E. ceratoniae*, were separately sprinkled over a 20% honey solution layer on paperboard strips. Each strip was placed in glass vial (10 × 1 cm) with five pair of newly emerged parasitoid, *Trichogramma brassicae* and a droplet of honey solution for feeding parasitoids. Vials were plugged with cotton and held for one day at 25 ± 1 °C, 65-70% RH and 16:8 h (L:D) photoperiod. After 24 h, adult parasitoids were removed from glass vials. Strips containing the parasitized eggs were kept under rearing conditions as above mentioned until they reached the blackened stage (6th day of the pupal stage). The pupal stage was used because it showed better tolerance to cold storage compared to other embryonic stages in several *Trichogramma* spp. (Lopez & Morrison, 1980; Jalali & Singh, 1992; Abbes *et al.*, 2020). Then vials were kept in 10 °C in darkness for 30 days (as acclimation duration) (Rahimi-Kaldeh *et al.*, 2017)

Two different cold storage durations were examined: the glass vials were stored in the refrigerator at 4 ± 1 °C in darkness for 4 and 8 weeks, respectively (Ozder & Saglam, 2005; Ayvaz, *et al.*, 2008). Treatment combinations including two different host's eggs and two cold storage durations were replicated 20 times. At the end of each storage duration, vials were transferred into an incubator and maintained at standard rearing conditions until adult's emergence. Control groups were kept at standard rearing conditions without any cold storage duration in 20 repetitions. The effect of cold storage durations and different hosts on qualitative characteristics of parasitoids were estimated by determining the emergence rate (dividing the number of parasitized eggs presenting emergence holes by the total number of parasitized eggs as a percentage for each egg card), sex ratio, the number of female and male parasitoids, and wingless percentage for each vial.

Quality of progeny

The mated female wasps (24-h old) from each treatment combination (cold storage period and different hosts), were used to quality assessment of the wasp progeny. One female wasp was released in a glass vial with a card contains 20 eggs of *E. ceratoniae* or *E. kuehniella*. Vial was plugged with cotton and held for one day at 25 ± 1 °C, 65-70% RH and 16:8h (L:D) photoperiod. After 24 h, adult parasitoid was removed from the glass vial and strip containing the parasitized eggs was kept under standard rearing conditions until adult emergence. Then the emergence rate, sex ratio and parasitism percentage were estimated for each vial. This experiment had 20 repetitions for each treatment combination.

Statistical analyses

All data of wasps (F_0 and F_1) except wing deformity were tested for normality and the other assumptions of analysis of variance (ANOVA). The effect of cold storage periods and different hosts on qualitative characteristics of *T. brassicae* were analyzed by two-way full factorial ANOVA (cold storage period × different host). When ANOVA indicated a significant effect, means were separated using Tukey's honestly significant difference test. Wing deformity of wasps (F_0) was analyzed as binary responses using logistic regression (logit link function) with the cold storage period, different hosts and their interaction as predicted factors. All statistical analyses were performed using R project for statistical computing (R Core Team, 2020).

Results

Interactive effect of cold storage period and host on the emergence rate of adult wasps was significant in parental generation (F_0) ($F_{2, 114} = 13.8$, $P < 0.01$). The emergence rate of wasps stored on both hosts significantly decreased with an increase in cold storage period (Fig.1).

It was a significant reduction in sex ratio by increasing in cold storage period in F_0 ($F_{2, 114} = 55.7$, $P < 0.001$), (Table 1). Also, the sex ratio of wasps was significantly higher on *E. ceratoniae* than that on *E. kuehniella* in F_0 ($F_{1, 114} = 15.85$, $P < 0.001$), (Table 1). However, results showed that the interaction between host and cold storage period had no significant effect on sex ratio in F_0 ($F_{2, 114} = 0.44$, $P = 0.65$). The number of female parasitoids was significantly affected either by cold storage period ($F_{2, 114} = 491.5$, $P < 0.001$) or host ($F_{1, 114} = 36.14$, $P < 0.001$), (Table 1).

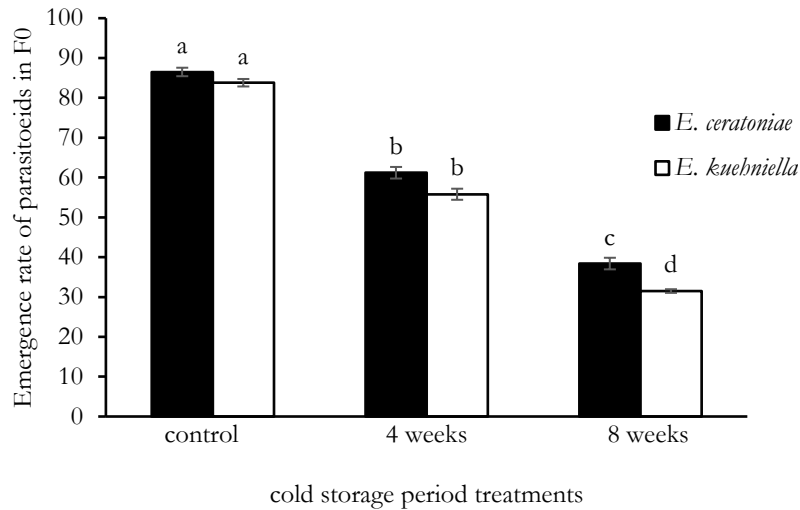


Fig 1. Emergence rate in the parental generation (F_0) of *Trichogramma brassicae* reared on *Ephestia kuehniella* and *Ectomyelois ceratoniae* in 0, 4 and 8 weeks cold storage periods. Different letters indicated a significant difference between treatment combinations ($P < 0.01$)

The number of male parasitoids was not significantly influenced neither by cold storage period ($F_{2,114} = 1.7$, $P = 0.18$) or host ($F_{1,114} = 2.14$, $P = 0.11$), (Table 1). The interaction between cold storage period and host did not affect the number of female and male parasitoids ($F_{2,114} = 1.7$, $P = 0.19$ and $F_{2,114} = 1.8$, $P = 0.16$, respectively).

Effect of cold storage period on wing deformity of parasitoid wasps in F_0 was not significant ($\chi^2 = 1.05$, $df = 2$, 116 , $P = 0.35$). Similarly, there was no statistical differences in the number of adult parasitoid wasps with wing deformity among *E. ceratoniae* and *E. kuehniella* hosts ($\chi^2 = 1.41$, $df = 1$, 116 , $P = 0.25$).

Either cold storage period or host affect significantly the emergence rate of *T. brassicae* wasps in progeny generation (F_1) ($F_{2,114} = 338.4$, $P < 0.001$ and $F_{1,114} = 51.5$, $P < 0.001$, respectively). However, the interaction between these factors did not affect this parameter ($F_{2,114} = 0.28$, $P = 0.75$). Within the same host species, the emergence rate of wasps stored for 8 weeks, was significantly lower than those of stored for 4 weeks and control (Table 2). In comparison between two hosts' species, the emergence rate of adult wasps reared on eggs of *E. ceratoniae* was significantly higher than that reared on eggs of *E. kuehniella* (Table 2). Sex ratio of adult wasps in progeny generation (F_1) not only influenced by each factor individually (cold storage period: $F_{2,114} = 87.1$, $P < 0.001$; host: $F_{1,114} = 4.7$, $P < 0.01$) but also significantly affected by the interaction of both factors ($F_{2,114} = 3.6$, $P = 0.01$). While the highest percent of sex ratio was observed from wasps kept on *E. ceratoniae* in control, the lowest percent of sex ratio was found from those stored on *E. kuehniella* in 8 weeks cold period (Table 2).

Table 1. Effect of cold storage period and host on sex ratio, number of female and male of *Trichogramma brassicae* in parental generation F_0 . Different letters in each column indicated a significant difference between treatment combinations ($P < 0.01$), $n = 20$.

cold storage period	host	sex ratio (%)	number of females	number of males
control	<i>E. ceratoniae</i>	79.4±1.6a	34.2±0.6a	9.0±0.8a
	<i>E. kuehniella</i>	70.0±3.4a	29.2±1.4b	12.7±1.6a
4 weeks	<i>E. ceratoniae</i>	65.6±1.8b	19.9±0.5c	10.6±0.7a
	<i>E. kuehniella</i>	60±2.8b	16.6±0.5d	11.3±0.9a
8 weeks	<i>E. ceratoniae</i>	53.6±2.3c	10.0±0.2e	9.1±0.7a
	<i>E. kuehniella</i>	44.5±1.7d	7.6±0.2e	9.6±0.3a

Table 2. Effect of cold storage period and host on emergence rate, sex ratio, number of female and male of *Trichogramma brassicae* in progeny generation F₁. Different letters in each column indicated a significant difference between treatment combinations ($P < 0.01$), $n = 20$.

Parental cold storage period	host	emergence rate (%)	sex ratio (%)	number of female	number of male
control	<i>E. ceratoniae</i>	67±1.3a	76±2.0a	9.1±0.3a	2.8±0.2a
	<i>E. kuehniella</i>	59±0.8b	67±2.0b	7.1±0.1b	3.5±0.2a
4 weeks	<i>E. ceratoniae</i>	42±1.7c	56±2.6b	4.8±0.2c	3.7±0.1a
	<i>E. kuehniella</i>	34±1.1d	54±2.2b	3.8±0.1d	3.1±0.2a
8 weeks	<i>E. ceratoniae</i>	28±0.8e	40±0.02c	2.2±0.1e	3.3±0.1a
	<i>E. kuehniella</i>	20±0.6f	43±2.0c	1.7±0.08e	2.3±0.1b

Interactive effect of cold storage period and host on the number of female and male parasitoids was also significant in progeny generation (F₁) ($F_{2,114} = 5.9$, $P < 0.01$ and $F_{2,114} = 7.4$, $P < 0.01$, respectively). The highest number of female parasitoids was found from wasps kept on *E. ceratoniae* in control, but the lowest ones was observed from those stored on *E. kuehniella* in 8 weeks cold period (Table 2).

The parasitism rate in progeny generation (F₁) not only was significantly influenced by cold storage period ($F_{2,114} = 148.5$, $P < 0.001$) and host ($F_{1,114} = 63.2$, $P < 0.001$) individually, but also it was significantly affected by the interaction between these two factors ($F_{2,114} = 4.1$, $P = 0.02$). The female wasps grown on the host with longer cold storage period had a significantly lower parasitism rate compare to those grown on the hosts with shorter cold storage period and control (Fig. 2). Also, the parasitism rate of female wasps grown on *E. ceratoniae* was significantly higher than those on *E. kuehniella*.

Discussion

It would be useful to store the parasitoid for some biocontrol program when synchronizing the parasitoids with pest population. However, the potential value for use of cold storage in commercial production needs to be evaluated in terms of economies. Finding methods to increase duration of storage for biological control agents is an important issue and helps producers to provide a large number of beneficial insects at the appropriate time in field. It also causes the easier acceptance of natural hosts by a reduction in the number of generations which is necessary for their mass rearing (Nazeri *et al.*, 2015; Rahimi-Kaldehy *et al.*, 2017).

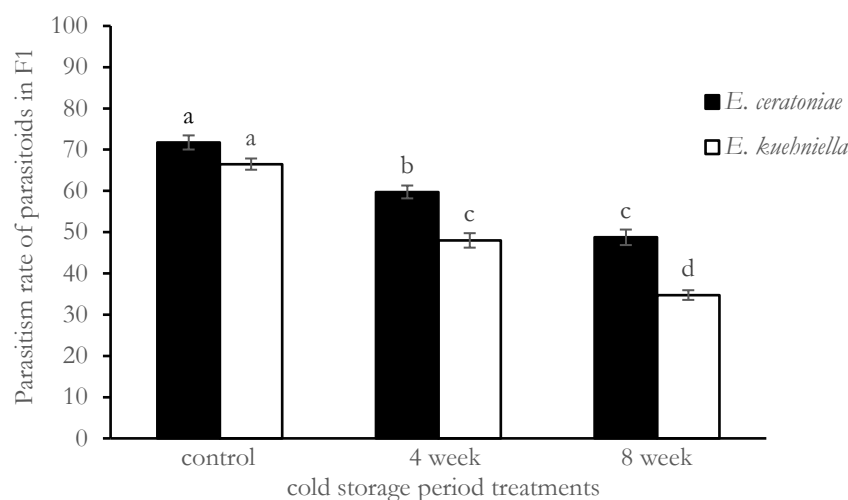


Fig 2. Parasitism rate in the progeny generation (F₁) of *Trichogramma brassicae* reared on *Ephestia kuehniella* and *Ectomyelois ceratoniae* in 0, 4 and 8 weeks cold storage periods. Different letters indicated a significant difference between treatment combinations ($P < 0.01$).

Several studies showed that temperature, photoperiod, cold storage duration, species or even strains of wasps and hosts, influence on diapause induction, post-diapause duration and quality of stored *Trichogramma* spp. (Laing & Corrigan, 1995; Rahimi-Kaldehy *et al.*, 2017). In our study, a reduction in the emergence rate of parasitoids due to the cold storage of *T. brassicae* was found and the percentage of parasitoid emergence rate in control was greater than any cold-stored ones. The decrease in the emergence rate of parasitoids primarily is due to the mortality caused by the cold storage of *T. brassicae* during the pupal stage and, due to negative physiological and physical changes that have been occurred to pupa of *T. brassicae*. Moreover, freezing and subsequent thawing could also have provoked changes in the hardness of the host eggs chorion and making it more difficult for the wasps to cut it with their mouth parts for emergency (Kivan Kilic, 2005). Unlike to the results of current study, 1 to 16 weeks cold storage of *T. bactrae* in 4 °C, had no significant effect on the emergence rate of female wasps (Mohamed & El-Heneidy, 2020), probably because of difference parasitoid species.

Here, cold storage specifically in a longer period (8 weeks) affected negatively the quality of emerged adults of *T. brassicae*; so that, the sex ratio and parasitism rate of *T. brassicae* lessened as cold storage period increased. Several studies have addressed the negative effect of cold storage period on the quality of emerged parasitoid adults (Tezze & Betto, 2004; Lessard & Bovin, 2013; Rahimi-Kaldehy *et al.*, 2017; Vaez *et al.*, 2018; Abbes *et al.*, 2020). These may be attributed to the increase in energy necessity and gradual weakness of the individual during metamorphosis in the storage period, and therefore accumulation of toxic metabolites and oxidative stress caused by a buildup of reactive oxygen (Amice *et al.*, 2008). The results also showed that the number of emerging female parasitoids significantly decreased with an increase in the cold storage period, while the number of emerging males was not affected by the period of cold storage in both the F₀ and F₁ generations. Some studies have reported a shift towards the production of male-biased parasitoids after prolonged cold exposure, indicating differential mortality of females (Farid *et al.*, 2001; Chen *et al.* 2008). The quality of the host influences the oviposition patterns of parasitoids, with female eggs typically being laid on higher-quality hosts (Van den Assem, 1971; Otto & Mackauer, 1998). Since the extended period of cold storage decreases the quality of the host (Colinet *et al.*, 2006), increasing the duration of host storage probably reduces the proportion of female offspring of parasitoids.

In some *Trichogramma* species and other parasitoids, morphological alterations occur during cold storage period; such as adult size reduction, deformation in wings, legs, antennae and sensillae structure (Pintureau and Daumal, 1995; Rundle *et al.*, 2004; Bourdais *et al.*, 2006), although our results didn't show a significant difference in adult deformation after cold storage in different host and cold storage period.

This study showed that *T. brassicae* pupae reared on *E. ceratoniae* eggs, were relatively amenable to cold storage and can be used in mass production process. The qualitative characteristics of F₀ and F₁ parasitoids reared on *E. ceratoniae*, were significantly better than those reared on *E. kuehniella*. This phenomenon may be attributed to that *E. ceratoniae* is natural host for *T. brassicae*, whereas *E. kuehniella* is a fictitious host for it.

In general, our results indicated that *T. brassicae* could be exposed to an additional 4 weeks at 4 °C after acclimation and diapause induction at 10 °C for 30 days without much decrease in fitness of parasitoids. Therefore, acclimation can protect *T. brassicae* efficiency and quality, and should be considered in storage protocol of this parasitoid. Similar results were reported by Rahimi-Kaldehy *et al.* (2017). The possible reason of acclimation during cold storage may be that the amount of cryoprotectants (e.g. glycerol and alanine), which reduce the ice formation at any temperature by increasing the total concentration of all the solutes, increases in diapausing insects during acclimation period (like autumn) and so, causes more adaptation for wasp pupa to tolerate cold storage period (Rivers *et al.*, 2000). From a practical point of view, our results revealed that the treatment with 30 days of acclimation and 4 weeks of cold store is useful to establish a cold storage protocol for *T. brassicae*. However, the decrease of *T. brassicae* performance after cold storage period should be considered in mass production, and the release percentage should be increased by the equivalent of lack % emergence. Moreover, using the other methods such as 'recurrent warming method' and 'rapid acclimation method' [a short pre-exposure (minutes or hours) to sub-lethal low temperature] which are improve the efficiency of cold stored parasitoids, should be considered (Denlinger & Lee, 2010; Gardner *et al.*, 2012). On the other hand, the influence of cold storage conditions on the field success of *T. brassicae* should be assessed because the efficacy of *Trichogramma* species is different in field from laboratory (Hached *et al.*, 2021).

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تأثیر ذخیره‌سازی در سرما بر عملکرد (*Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) پرورش یافته روی *Ectomyelois ceratoniae* و *Ephestia kuehniella*)

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چکیده

زنبورهای پارازیتوئید خانواده Trichogrammatidae، مهمترین دشمنان طبیعی در برنامه های کنترل بیولوژیک تعداد زیادی از آفات مهم بالهولکدار در سرتاسر دنیا هستند. از این رو وجود روش های مناسب نگهداری برای این پارازیتوئیدها در کنترل بیولوژیک ارزشمند است. در این تحقیق عملکرد زنبور *Trichogramma brassicae* پرورش یافته روی دو میزبان مختلف و دو دوره متفاوت ذخیره سازی در سرما بررسی شد. شفیقه زنبورهای که روی تخم بید آرد *Ephestia kuehniella* و شب پره کرم گلوگاه انار *Ectomyelois ceratoniae* پرورش یافته بودند، به مدت ۳۰ روز در دمای ۱۰ درجه سلسیوس و سپس به مدت ۴ و ۸ هفته در دمای ۴ درجه سلسیوس نگهداری شدند. اثر ترکیب تیماری روی نرخ ظهور زنبورها، نسبت جنسی و تعداد زنبورهای ماده و نر در نسل مادری (F₀) و نتاج آنها (F₁) بررسی شد. به علاوه، میزان بد شکلی زنبورها و نرخ پارازیتسم به ترتیب در نسل مادری و نتاج تحت تاثیر ترکیب تیماری اندازه گیری شد. نتایج این مطالعه نشان داد که نرخ ظهور زنبور *T. brassicae* در (F₀) به طور معنی داری تحت تاثیر برهم کنش میزبان و مدت ذخیره سازی در سرما بود. حداکثر و حداقل نرخ ظهور به ترتیب در *E. creatoniae* شاهد و تیمار ۸ هفته ذخیره سازی در *E. kuehniella* رخ داد. نسبت جنسی (ماده به نر) زنبورهای F₀ و F₁ به صورت معنی داری تحت تاثیر میزبان یا طول دوره ذخیره سازی در سرما بوده اما بر همکنش عوامل مذکور روی این ویژگی زنبورها در هر نسل معنی دار نشد. همچنین بی بالی و بد شکلی در F₀ تحت تاثیر هیچ یک از تیمارها قرار نگرفت. نرخ پارازیتسم زنبورهای F₁ پرورش یافته روی هر دو میزبان، با افزایش مدت زمان نگهداری در سرما به طور معنی داری کاهش یافت. به طور خلاصه نتایج بر این موضوع دلالت دارد که بیشتر ویژگی های کیفی زنبورهای پرورش یافته روی *E. creatoniae* به طور محسوسی نسبت به آنهایی که روی *E. kuehniella* پرورش پیدا کرده بودند، در هر دو نسل F₀ و F₁ بهتر بود.

کلمات کلیدی: کنترل بیولوژیک، پارازیتوئید تخم، رهاسازی اشیاعی، کنترل کیفیت، شب پره کرم گلوگاه انار

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