Shiraz University

Iran Agricultural Research (2022) 40(2) 17-23

Research Article

Effects of cold storage on some biological characteristics of a predatory coccinellid, *Oenopia conglobata contaminata* reared on non-prey food

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DOI: 10.22099/IAR.2021.40292.1432

ARTICLE INFO

***Article history*:**

Received **09 April 2021**
Accepted **20 December 2021**
Available online

***Keywords*:**

Biological control
Biological parameter
Cold storage
Oenopia conglobata contaminata
Predatory adult

ABSTRACT- The most important coccinellid predator in pistachio orchards, feeding on aphids and psyllids, is *Oenopia conglobata contaminata* L. (Coleoptera: Coccinellidae). The aim of the present research was to evaluate the effects of a range of cold storage temperatures (4, 8 and 12ºC) in a seven-day cold storage period on some biological parameters of the newly emerged adults of *O. conglobata contaminata* reared on non-prey food. The replicates were 100, 32 and 30 one-day old adults for 4, 8 and 12ºC, respectively. Each couple of the coccinellids was put into an experimental unit and incubated at 26±1, 60±5% RH and a photoperiod of 16L:8D till death and some biological parameters of the beetle were estimated. The results elucidated that the percent vitality and longevity of the adults markedly increased at 12 and 8°C, respectively. In addition, the longest oviposition and post-oviposition periods were observed at 8°C. The total fecundity and fertility of females significantly had the maximum values at 8°C. Moreover, the number of egg clusters and days in which the oviposition happened significantly elevated at 8°C. The survival rate of the adult coccinellids declined after a seven-day cold storage at 4 and 12°C compared to that obtained at 8°C. The results highlighted the positive effects of the suitable cold temperature (8°C) on the storage of the efficient predator to improve long distance shipment. It could help to enhance the cold storage efficacy and quality of the predator reared to release for biological control programs.

INTRODUCTION

The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) is known as an important pest of pistachio trees in Iran and accordingly, to control the pest, several chemical insecticides have been applied (Bemani et al., 2016). An alternative method to suppress insect pests is applying biological control programs that are safe for the environment and non-target organisms. It has been reported that the effective agents of biological control like predatory lady beetles have been reared and released in different ecosystems (Mehrnejad and Jalali, 2004; Jacas and Urbaneja, 2010).

The most common and important coccinellid predator, feeding on aphids and psyllids, especially, the common pistachio psylla, *A. pistaciae*, in pistachio orchards in Iran as well as some other countries such as Russia, Germany and Turkey was *Oenopia conglobata contaminata* L. (Coleoptera: Coccinellidae) (Hodek et al., 2012; Kabiri Raeis Abbad and Amiri Besheli, 2012; Salehi Pourani et al., 2019). Nevertheless, having knowledge on the ecology of the predatory beetle (Lumbierres et al., 2018) and the effects of some biotic or abiotic factors on the lady beetle are rare compared to other known predators.

Since mass production was an essential phase in biological control methods (Salehi Pourani et al., 2019), cold storage could construct favorable conditions in which a sufficient number of biological control agents were produced (Liu et al., 2014) and then stored until the time they are needed (Ayvaz et al. 2008).

Salehi Pourani et al. (2019) showed that the adults of *O. conglobata contaminata* were resistant to the temperatures below zero while their eggs were vulnerable to this thermal range. Therefore, it was shown that the maximum survivorship of the adults was 42.50% for 24 h at - 4 ºC (Salehi Pourani et al., 2019).

In another study, Koch et al. (2004) determined the supercooling points for the different developmental stages of the predatory beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and also proved that temperatures below the supercooling point resulted in increased mortality. Maes et al. (2012) presented that the mirid predator *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) adapted to low temperatures might be tolerant to freezing climate when the bug was released for biological control.

Additionally, there were several pieces of evidence for other natural enemies like parasitoids. For example, the impact of cold storage on the efficiency of *Tetrastichus brontispae* (Ferriere) (Hymenoptera: Eulophidae) parasitizing *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) was tested and exhibited that the optimum temperature for the storage of parasitized pupae was 10 °C for 10 days (Liu et al., 2014). Moreover, a previous study demonstrated that cold temperature (4 ºC) had adverse effects on the female behavior and fecundity of the egg parasitoid, *Anaphes victus* Huber (Hymenoptera: Mymaridae) (van Baaren et al., 2005). Tunca et al. (2014) also demonstrated that the development time of the larval parasitoid *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) prolonged at low storage temperature and the eclosion (emerging from the pupal case) rate conversely declined with the exception of 10 °C that was reported as an appropriate temperature.

There has been limited knowledge and information on the effects of cold storage on survivorship, longevity and the reproductive performance of the predatory lady beetle, *O. conglobata contaminata* fed on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) mixed with artificial diet. The only cold hardiness investigation of this predator was previously conducted by Salehi Pourani et al. (2019) at 0, -3, -5 and -7 ºC. Therefore, the present study aimed to evaluate the effects of a range of cold storage temperatures (4, 8 and 12ºC) on some biological parameters of the predatory coccinellid reared on non-prey food under laboratory conditions and to discover the optimal temperature for cold storage. The obtained information is novel and will be applicable for this predator mass rearing prior to release.

MATERIALS AND METHODS

Rearing of *O. conglobata contaminata*

The initial population of the predator was collected from the pistachio orchard of the Shahid Bahonar University of Kerman (Kerman province, Iran). Afterwards, the coccinellid adults were identified and then transferred to the laboratory. Accordingly, they were reared on a mixture of *E. kuehniella* eggs and lyophilized diet (ad libitum) including ground beef (100 g), hen liver (100 g), egg yolk (10 g) and sucrose solution 5% (12 ml) (De Clercq et al., 1998) and bee pollen (Hassani et al., 2019) for one generation. Subsequently, *E. kuehniella* was derived from a culture maintained at Shahid Bahonar University of Kerman. The Mediterranean flour moth was then reared on a mixture of flour and bran (2:1) diet under laboratory conditions (27±1°C, 60±5% RH, 16L: 8D). Thereafter, 30 mg of *E. kuehniella* eggs were dispersed into 1.5 kg of the previously-prepared mixture. The adults were collected using an aspirator and then transferred to plastic egg-laying containers with white papers at the bottom. Eggs were also taken from these containers. The collected eggs were stored for feeding *O. conglobata contaminata* at -18 ºC (Mamay and Mutlu, 2019).

The predators were kept on a filter paper in a plastic container (40 × 10 × 20 cm). As well, a rectangular hole (10 × 5 cm) was created on the one side of the plastic container covered with a fine mesh net for the ventilation process (26 ± 1 ºC, 60 ± 5 % RH and a photoperiod of 16L: 8D). To prepare the optimum conditions for the adult oviposition, some strips of tissue papers attached to net were suspended from the container lid.

The egg clusters laid in the containers were transferred to Petri dishes (9 cm in diameter) covered with filter papers until the eggs hatched (26 ± 1 ºC, 60 ± 5 % RH and a photoperiod of 16L: 8D). Next, a hole (4 cm in diameter) was made on each Petri dish lid covered with a fine mesh net for the ventilation process. The larvae were provisioned with the diet described above along with a cotton ball soaked into a three % sucrose solution (Bonte et al., 2010). Afterward, the emerged adults were transferred to the plastic containers described above for mass rearing.

Effect of Cold Storage on Some Biological Characteristics of *O. conglobata contaminata*

To conduct the experiment, new emerged (1-day old) adults were used. A Petri dish (9 cm in diameter) was applied as an experimental unit lined with a filter paper and a hole (4 cm in diameter) on its lid covered with a fine mesh net for ventilation.

Before storage, newly emerged adults were acclimated for one day at 12 °C according to the study by Sun et al. (2019) with some modifications. Thereafter, one hundred 1-day old adults (male and female) were separately put in each Petri dish and then exposed to 4 ºC for seven days (i.e., each adult was put in each Petri dish). Likewise, the experiment was carried out at 8 ºC (32 1-day old adults) and 12 ºC (30 1-day old adults) (Tian et al., 2020). After seven days, the dead beetles were recorded and the alive adults were transferred into the plastic container described above for mating and finally provisioned with the above-mentioned diet.

Subsequently, each couple of insects was put into an experimental unit and then incubated at 26 ± 1 ºC, 60 ± 5 % RH and a photoperiod of 16L: 8D until death. A piece of tissue paper was used as a shelter for the predator in each Petri dish. The couples were provisioned with the above-mentioned diet. Each Petri dish was daily checked and some biological parameters of the predatory lady beetle, *O. conglobata contaminata* including longevity, pre-oviposition period (from the beetle eclosion until the occurrence of the first oviposition event), oviposition period, fecundity and survival rate were estimated. Moreover, the number of egg clusters, the number of eggs in each cluster and the percent hatching of the coccinellid eggs were calculated.

Statistical Analysis

The data were computed using the analysis of variance (ANOVA) (SAS, 1989) and the means were compared using Tukey's test at a significant level of 5 %. The percentages of adult vitality and hatched eggs were arcsine square-root transferred before performing the statistical analysis. The biological parameters were calculated using the following formulas:

$L_x = N_x/N_t$ (1) (Sun et al., 2019).

where Lx was survival rate; Nx and Nt were the number of individuals in a certain time and the total number of individuals in the first day, respectively.

$V = N_t/N_0 \times 100$ (2)

where V was percent vitality; Nt and N0 were the number of alive individuals after cold storage treatment and the total number of individuals before the cold storage treatment, respectively.

$N = n_t/d$ (3)

where N was the number of eggs laid during the oviposition period; nt and d were the total number of eggs laid and the days in which the oviposition happened, respectively.

$H = n_h/n_t \times 100$ (4) (Sun et al., 2019)

where H was percent egg hatching; nh and nt were the number of hatched eggs and the total number of laid eggs, respectively.

RESULTS

The impacts of cold storage on some biological properties of the predatory lady beetle, *O. conglobata contaminata* were examined at some temperatures including 4, 8 and 12 °C. Correspondingly, the findings showed that the different temperatures of cold storage had significant effects on the percent vitality ($F_{2,\ 36}$=3.04, *P*<0.05) and longevity ($F_{2,\ 112}$=24.25, *P*<0.05) of the adult predator (Table 1). Therefore, the percent vitality and longevity of the adults significantly increased at 12 and 8 °C, respectively.

According to Table 2, the reproductive parameters of the predatory coccinellid, *O. conglobata contaminata* including the pre-oviposition, oviposition, post-oviposition period and oviposition rate were found to be significantly affected by different temperatures of cold storage. The pre-oviposition period was prolonged at 12 °C and significantly differed from 4 °C ($F_{2,\ 46}$=5.12, *P*<0.05). However, no significant difference was found in the pre-oviposition period between 8 and 12 °C as well as between the 4 and 8 °C.

The longest oviposition and post-oviposition periods were observed at 8 °C which noticeably differed from those of 4 and 12 °C ($F_{2,\ 46}$=6.73, *P*<0.05). The maximum oviposition rate of *O. conglobata contaminata* was significantly higher at 4 °C compared to the rates at other temperatures ($F_{2,\ 49}$=7.72, *P*<0.05).

The total fecundity ($F_{2,\ 49}$=11.32, *P*<0.05) and fertility of females ($F_{2,\ 49}$=5.81, *P*<0.05) had the maximum significant values when the predatory adults were exposed to 8 °C and the highest daily fertility was observed at 4 °C compared to other temperatures (Table 3). Nevertheless, there was no considerable variation for the mean eggs laid during the oviposition period per day between different tested temperatures (*P*>0.05) (Table 3).

The number of egg clusters ($F_{2,\ 48}$=27.38, *P*<0.05) and days in which the oviposition happened ($F_{2,\ 48}$=15.49, *P*<0.05) significantly elevated at 8 °C compared to other temperatures (Table 4). However, the number of eggs in each cluster was significantly increased at lower temperature compared to 8 and 12 °C ($F_{2,\ 49}$=8.57, *P*<0.05). Besides, the percent egg hatching was not differentially modified at various tested temperatures for storage (Table 4).

The results presented in Fig. 1 showed that the survival rate of the adult coccinellids declined after seven-day cold storage at 4 and 12 °C compared to the rate obtained at 8 °C. The changes in survival rate were similar at both 4 and 12 °C until day 30 after cold storage period. Thereafter, the survival rate of the predatory beetle significantly decreased at 4 °C compared to the rate at 12 °C.

Table 1. Effect of cold storage on the percent vitality and longevity of *Oenopia conglobata contaminata* after a 7-day storage period

Temperature (°C)	Percent vitality (%)	Adult longevity (day)		
		♀	♂	♂+♀
4	71.11 ± 6.36 b	41.08 ± 3.15 b	33.33 ± 2.65 b	36.93 ± 1.97 c
8	66.67 ± 9.81 b	75.50 ± 8.20 a	63.90 ± 8.23 a	69.42 ± 5.81 a
12	100.00 ± 0.00 a	37.00 ± 4.15 b	62.11 ± 5.71 a	48.84 ± 3.88 b

Means followed by different letter in the same column are significantly different (Tukey's test, *P*< 0.05)

Table 2. Effect of cold storage on the pre-oviposition, oviposition, post-oviposition periods and oviposition rate of *Oenopia conglobata contaminata* after a 7-day storage period

Temperature (°C)	Pre-oviposition period (day)	Oviposition period (day)	Post-oviposition period (day)	Oviposition rate (egg/♀/day)
4	8.65 ± 0.74 b	25.81 ± 2.35 b	7.93 ± 1.13 b	10.25 ± 0.63 a
8	10.70 ± 1.17 ab	48.20 ± 6.89 a	15.50 ± 3.38 a	7.35 ± 0.42 b
12	13.12 ± 0.39 a	27.89 ± 5.77 b	3.57 ± 0.72 b	6.35 ± 0.70 b

Means followed by different letter in the same column are significantly different (Tukey's test, *P*< 0.05)

Table 3. Effect of cold storage on the total fecundity, daily eggs laid during oviposition days, total fertility and daily fertility of *Oenopia conglobata contaminate* after a 7-day storage period

Temperature (°C)	Total fecundity (egg/♀)	Daily eggs laid (egg /♀/day)	Total fertility (larva/♀)	Daily fertility (larva/♀/day)
4	115.97 ± 10.47 b	5.61 ± 0.61 a	89.93 ± 8.63 b	7.78 ± 0.47 a
8	207.60 ± 18.32 a	4.35 ± 0.41 a	147.20 ± 22.11a	4.97 ± 0.51 b
12	100.12 ± 15.58 b	4.79 ± 0.98 a	69.75 ± 17.44 b	4.53 ± 0.73 b

Means followed by different letter in the same column are significantly different (Tukey's test, $P< 0.05$)

Table 4. Effect of cold storage on the number of egg clusters, the number of eggs in each cluster, the oviposition days and percent egg hatching of *Oenopia conglobata contaminata* after a 7-day storage period

Temperature (°C)	No. of egg cluster	No. of egg per cluster	Oviposition days	Percent hatching
4	15.16 ± 0.85 b	8.49 ± 0.46 a	11.65 ± 1.03 b	67.39 ± 4.44 a
8	35.20 ± 4.04 a	5.81 ± 0.33 b	27.40 ± 4.06 a	66.71 ± 4.49 a
12	20.12 ± 2.69 b	5.62 ± 0.71 b	17.00 ± 2.39 b	69.62 ± 5.65 a

Means followed by different letter in the same column are significantly different (Tukey's test, $P< 0.05$)

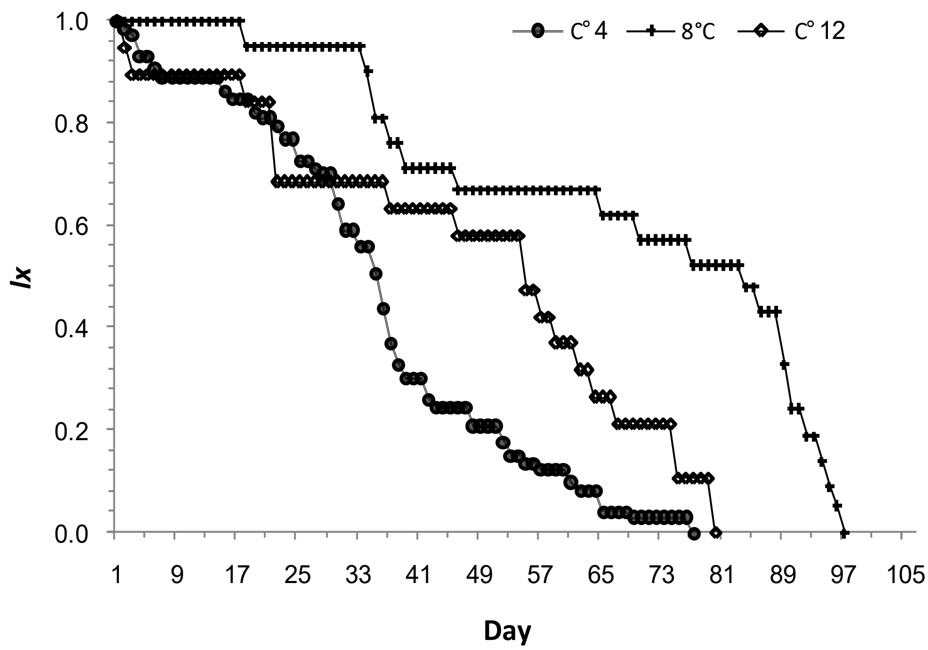

Fig. 1. The survival rate of predatory adult of *Oenopia conglobata contaminata* affected by a seven-day storage period at 4, 8 and 12 °C, Day: days after cold storage period.

DISCUSSIONS

It has been shown that cold temperature adversely affects the stored insects (Ghazy et al., 2012). Therefore, finding the most suitable storage temperature is essential in order to promote reproduction and other biological properties of natural enemies during their performance against target pests. In addition, the increasing temperature could affect the food consumption of a predator (Ziaei Madbouni and Hosseini, 2017). In the present research, by investigating the effects of cold storage on some biological parameters of the predatory adults of *O. conglobata contaminata*, it was shown that the adult longevity and oviposition period (especially oviposition days) were the most important factors affecting the total fecundity and fertility of the predator.

The values of the two aforementioned parameters were affected by the different temperatures of storage and increased at 8 °C, while the highest value of percent vitality and both oviposition rate and daily fertility significantly occurred at 12 and 4 °C, respectively. By comparison, it was discovered that the adults of *H. axyridis* were able to tolerate -11.19 ± 0.53 °C without increasing the mortality (Koch et al., 2004).

Similarly, a previous study demonstrated that the adulthood stage of *O. conglobata contaminata* was cold tolerant, especially when the beetles fed on the Mediterranean flour moth eggs (Salehi Pourani et al., 2019). Moreover, other researchers found that feeding the predatory insects before cold storage led to decreased susceptibility to cold conditions (Sun et al., 2019). Nonetheless, it was demonstrated that the complete food digestion in the adult gut of *H. axyridis*, immediately before cold exposure, could increase their cold resistance (Iperti and Bértand, 2001). In the current study, the newly born adults of *O. conglobata contaminata* were used for the cold storage experiments. Certainly, it could be stated that pre-storage nutrition of the predatory beetles would help them to improve their biological properties at a lower temperature (i.e., 4 °C).

Likewise, it has been reported that the high-quality diet (host prey) played an important role in elevating the cold resistance of *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae) compared to factitious prey (Maes et al., 2015). Therefore, it seems that the eggs of *E. kuehniella* should be substituted with natural prey to enhance the cold tolerance of *O. conglobata contaminata*.

Tian et al. (2020) investigated the influences of three different temperatures including 4, 8 and 12 °C on eggs of *Menochilus sexmaculatus* (Fabricius) (Coleoptera: Coccinellidae) for three days. Contrary to the results of the current study, they declared that the survival rates of the first larval instars, oviposition period and oviposition of the lady beetles along with the adult longevity increased at 12 °C. In addition, the pre-oviposition period of the beetles decreased at 12 °C which was different from the results of this study. Therefore, they suggested the use 12 °C as a favorable storing temperature for the eggs of *M. sexmaculatus*. While the results of the current study clarified that the suitable temperature of cold storage for the adults of *O. conglobata contaminata* was 8 °C. The difference between the results of the two studies may possibly be raised from the variation of the lady beetle species, storage period and especially the life stage of predator exposed to the cold storage.

The findings of this study showed that the higher survival rate of the tested adult ladybird beetles happened at 8 °C compared to the rates at 4 and 12 °C. However, Salehi Pourani et al. (2019) revealed that the percent survival of the adults of *O. conglobata contaminata* decreased when the temperature reduced from 0 to -7 °C. By investigating the effect of chilling temperature on the green lacewing, *Chrysopa formosa* Brauer (Neuroptera: Chrysopidae), it was indicated that the highest survival rate of the diapausing adults was at 5 °C compared to 0 °C (Li et al., 2018), and this was similar to the results of this study at 4 °C versus 8 °C.

Overall, the present outputs of the current study declared that the highest values of adult longevity, oviposition period, total fecundity and fertility and oviposition days without any significant difference in percent hatching among various treatments was observed at 8 °C. Mojib Hagh Ghadam et al. (2002) elucidated that increasing temperature caused the enhanced fecundity, fertility and oviposition days of *O. conglobata contaminata*. In addition, in the current study, it was shown that the predatory coccinellids had the highest survival rate and the most days in which the oviposition happened at 8 °C. Therefore, it can be concluded that the adults of *O. conglobata contaminata* could be stored for seven days at 8 °C with high survival rates, development and appropriate reproductive characteristics.

Likewise, some studies previously showed that the minimum developmental threshold of the immature stage of *O. conglobata contaminata* fed on the aphids *Myzus persicae* (sulzer) (Hemiptera: Aphididae) and *A. punicae* Passerini (Hemiptera: Aphididae) were happened at 8.48 °C (Mokhtari and Samih, 2014) and 9.34 °C (Rounagh et al., 2014), respectively.

CONCLUSIONS

As it was mentioned before, there was little information about the influences of cold storage on the performance of *O. conglobata contaminata*. The results of this study highlighted the positive effects of the suitable cold temperature (i.e., 8 °C) on storage of the efficient predator to improve long distance shipment. These findings need to be completed by evaluating the other biological parameters of this predatory beetle at different temperatures and cold storage durations. The results of the current study help to enhance the cold storage efficacy and quality of *O. conglobata contaminata* reared to release in biological control programs.

ACKNOWLEDGEMENTS

We gratefully thank Mr. M. Mihanyar and Miss A. Vahmani for their kind collaborations for rearing insect cultures.

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تحقیقات کشاورزی ایران (۱۴۰۰) ۴۰(۲) ۲۳-۱۷

مقاله علمی- پژوهشی

اثرات ذخیره‌سازی در دمای سرد روی برخی آماره‌های زیستی کفشدوزک شکارگر *Oenopia conglobata contaminata* پرورش‌یافته روی غذای غیر طعمه

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اطلاعات مقاله

تاریخچه مقاله:

تاریخ دریافت: ۱۴۰۰/۰۵/۰۲

تاریخ پذیرش: ۱۴۰۰/۰۶/۰۷

تاریخ دسترسی: ۱۴۰۰/۰۷/۰۳

واژه‌های کلیدی:

آماره‌زیستی

حشرات کامل شکارگر

ذخیره‌سازی در دمای سرد

کنترل بیولوژیک

Oenopia conglobata contaminata

چکیده – مهم ترین کفشدوزک شکارگر در باغات پسته که از شته و پسیل تغذیه مـی کنـد، *Oenopia conglobate contaminata* L. است. هدف از پـژوهش حاضـر ارزیـابی تـأثیرات دمای سرد (۴ ، ۸ و ۱۲ درجه سلسیوس)، در یک دورهٔ هفـت روزه ذخیـره سـازی، بـر افـراد کامل تازه ظهور یافته و پرورش یافته *O. conglobate contaminata* روی غذای غیر طعمه بود. تعداد تکرارها ۱۰۰، ۳۲ و ۳۰ حشره کامل یک روزه به ترتیب بـرای دماهـای ۴، ۸ و ۱۲ درجه سلسیوس بود. یک جفت نر و مـاده کفشـدوزک در یـک واحـد آزمایشـی و دردمـای ۱±۲۶ سلسیوس، ۵±۶۰ درصد رطوبت نسبی و ۱۶ ساعت روشنایی و ۸ ساعت تـاریکی قـرار گرفتند و تا زمان مرگ، برخی از پارامترهای بیولوژیکی سوسک محاسبه شدند. نتـایج نشـان داد که درصد زنده‌مانی و طول عمر افراد کامل به ترتیب در ۱۲ و ۸ درجه سلسیوس به طـور قابل توجهی افزایش یافت. همچنین، طولانی‌ترین دوره تخم‌گذاری و پس از تخم‌گذاری در ۸ درجه سلسیوس بود .حداکثر مقادیر بارآوری و زادآوری کل به طور معنـی داری در ۸ درجـه سلسیوس بود. علاوه بر این، تعداد دسته تخم و روزهایی که تخمگذاری در آن اتفاق افتاد در ۸ درجه سلسیوس به طور قابل توجهی افزایش یافت. میزان بقای کفشدوزک‌های کامل، پس از هفت روز نگهداری در دمای ۴ و ۱۲ درجه سلسیوس در مقایسه با دمای ۸ درجه سلسیوس کاهش یافت. نتایج نشان‌دهنده تأثیرات مثبت دمای سـرد مناسـب (۸ درجـه سلسـیوس) در ذخیره سازی این شکارگر کارآمد برای بهبود حمل و نقل راه دور بود که می تواند به افـزایش اثر ذخیره سازی و کیفیت شکارگر برای رها سازی در برنامه هـای کنتـرل بیولوژیـک کمـک کند.