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Investigation of Cold Storage Possibilities in Mass Production of Adult Stages of *Nesidiocoris Tenuis* Reuter (Hemiptera: Miridae)

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HIGHLIGHTS

- This study will help prevent the irregular use of pesticides in agriculture and increase the application of biological control techniques.
- This study aims to increase the availability of *Nesidiocoris tenuis*, the natural enemy of *Bemicia tabaci* and Tuta absoluta pests, which are economically important in agriculture.

Abstract

In this study, the effect of exposing the adult stages of the predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) to cold temperatures at different times was examined. In the experiments, individuals that reached the adult stage on the same day were kept in the dark for 5, 10, and 15 days at 7, 10, and 15°C. Adults kept at low temperatures were fed three times per week with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs and honey water. Individuals of *N. tenuis* that survived after cold exposure were reared at 25°C under a 16:8 (L:D) day-night cycle. The lowest adult survival rate after cold exposure was 65.00% when held at 7°C for 15 days. The highest survival rates were 94.00% and 98.00% when stored at 7 and 10°C, respectively, for 5 days. The most extended longevity in individuals reared following cold exposure was 14.06 days at 7°C and 14.94 days at 10°C, respectively, while the shortest lifespan was 8.10 days in adults held at 15°C for 15 days. The average number of nymphs produced by adults kept at 7°C for 15 days was 322.4, while the number produced by adults kept at 15°C for 15 days was 47.6. Except for the nymphs acquired from adults held at 15°C for 15 days, the number of nymphs obtained from adults stored at other temperatures and durations did not differ statistically from the control group. The study concludes that those who intend to mass-produce *N. tenuis* adults should store them at temperatures between 7 and 10°C for up to 10 days.

Keywords: *Nesidiocoris tenuis*, Biological Control, Mass Production, Refrigerated Storage

1. Introduction

The primary objective in the management of numerous pests that cause product loss in terms of quality and quantity in agriculture is to produce a high yield per unit area and to cultivate crops that are suitable for sustainable agriculture techniques sensitive to the environment, human, and animal health (Uygun et al. 2010). The intensive use of pesticides increases the development of resistance in pests. Besides, it poses a threat to human and animal health, causes environmental pollution, and deteriorates the natural balance. However, the

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high cost of pesticides indicates that alternatives to chemical control should be developed. Therefore, biological control agents have been commercialized, mass-produced, and released for the control of pests.

Biological control is one of the most promising methods that have been widely used and demonstrated to be environmentally friendly (Abdel-Baky et al. 2015). In recent years, it has been utilized extensively, particularly in ecologically based integrated pest management programs.

It is widely acknowledged that mass production is a vital instrument of biological control programs (Kui et al. 2014). It focuses on the mass-rearing of insects, known to be effective in biological control, in climate-controlled rooms where optimal circumstances may be modified, in sufficient numbers to meet demand (Sürücü 2009). In terms of application, the cold storage of these laboratory-grown natural enemies is crucial for the advancement of biological control (Pitcher et al. 2002; Ayvaz et al. 2008).

Cold storage refers to the practice of storing beneficial insects or their hosts at low temperatures for a set period. This method enables natural enemies to be stored in the freezer to extend their shelf life. Simultaneously, it allows for the collection or stockpiling of natural enemies in acceptable climatic conditions, enabling the synchronized release of sufficient quantities of natural enemies to production regions during critical occasions such as epidemics (Sürücü 2009).

Several parameters of natural enemies, such as morphology, behavior, physiology, development, egg production, fecundity, lifespan, nutritional effects, adult-larvae emergence, and sex ratio, should not degrade after cold storage (Leopold 1998). Accordingly, an effective storage temperature and duration significantly contribute to the sustainability of natural enemy populations. As a result, numerous experiments have been conducted to preserve natural enemies employed in biological control. For example, JeongHwan et al. (2009) indicated that *Orius laevigatus* (Fieber), Bueno et al. (2014) demonstrated the same for *Orius insidiosus* (Say), Yanık and Ünlü (2015) for *Anthocoris minki* (Dohrn), Yaz and Özder (2016) for *Ephestia kuehniella* eggs parasitized by *Trichogramma pintoi* (Voegele), and Zhang et al. (2020) for *Tamarixia radiata* (Waterston), all of which can be stored in cold conditions.

Predator *N. tenuis* is a significant zoophytophagous species that is commonly and commercially used in both greenhouses and open vegetable areas as part of biological control programs. Whiteflies (*Bemisia tabaci* (Hem.: Aleyrodidae)) and tomato moths (*Tuta absoluta* (Meyrick) (Lep.: Gelechiidae)) can be controlled by releasing this predator (Sanchez 2009; Sohrabi and Hosseini 2015). In addition to these two essential pests, *Nesidiocoris tenuis* helps to control thrips, mites, leaf gallery moths, and other lepidopteran pests in greenhouses (Urbaneja-Bernat et al. 2013).

No research is found on the storage of the commercially released predator *N. tenuis* at low temperatures in the literature. The aim of this study is to investigate the effects of low temperature storage on post-storage survival rates, life spans and egg production in *N. tenuis* adults. The two most important factors for natural enemy cold storage are the level of cold storage and the duration of cold exposure, therefore these parameters were studied in this work. This study thus attempts to expand the usability of *N. tenuis* efficiently by achieving the best combination of low temperature and storage time.

2. Materials and Methods

The eggs of the predator bug *N. tenuis* and the laboratory host *E. kuehniella* were the primary study materials. Climate chambers with adjustable temperatures were employed in the experiment. In addition, green bean pods, binoculars, various glass and plastic containers were used. *Ephestia kuehniella* was grown using a mixture of flour and bran.

2.1.Rearing of Nesidiocoris tenuis

Adult *N. tenuis* was obtained from Biopheropoint Biological Control Systems Agriculture Chemical Industry Company. The stock culture was reared in climate rooms with 25±1°C temperature, 65±10% relative humidity, and 16:8 hours light-dark conditions in 12 cm diameter and 13 cm high plastic containers with ventilation holes in the laboratory. As food, *E. kuehniella* eggs and green bean pods were placed in the containers during experiments. Simultaneously, cottons soaked with 10% honey water were placed in plastic containers to suit the water needs of *N. tenuis* nymphs and adults (Urbaneja-Bernat et al. 2013). Bean pods containing *N. tenuis* eggs were removed every two day and transferred to another container in the controls, and new fresh pods were placed into the container for oviposition. Adult *N. tenuis* individuals ranging in age from 0 to 24 years old were collected from the laboratory colony and used in the experiments.

2.2.The effect of cold storage on the biological properties of Nesidiocoris tenuis

The post-storage survival rates, life spans and nymph numbers of *N. tenuis* were determined after storage at 65±5% relative humidity at 7, 10 and 15±1ºC for 5, 10 and 15 days, respectively. The studies were conducted in plastic containers with a diameter of 12 cm and a height of 13 cm with ventilation holes on them, 10 males and 10 females from the stock culture, with 10 replications for each distinct holding time of each temperature. The control group consisted of 10 males and 10 females produced immediately at 25±1ºC without being kept cold. During the controls, *E. kuehniella* eggs were added to the containers every two days, and bean pods containing *N. tenuis* eggs were removed and replaced with new pods. Dried cottons were re-moistened with honey water.

After storing *N. tenuis* at various temperatures and times, the viability rates were evaluated by recording the dead individuals in each container subsequent to the controls.

The adult samples that died in the containers were counted in the controls every two days, and their life span was computed at 25±1ºC following storage. At the same time, the bean pods with eggs were placed in separate containers and stored until the nymphal emergence was complete, then the number of nymphs emerging was recorded. Because *N. tenuis* lays its eggs in plant tissue, which is difficult to observe even with binoculars, the number of nymphs was determined rather than egg count in both application and control dishes.

2.3.Statistical analysis

Analysis of variance to determine whether there is a difference between the averages of survival rates, life spans and nymphal numbers of *N. tenuis*. Since the insects used at each storage temperature and time are different, the measurements obtained here are not dependent on each other. Due to nymph numbers do not show normal distribution and are obtained by counting, square root (√X) transformation was applied to these data (Düzgüneş et al. 1987). The Randomized Plot 3x3 Factorial Experimental Desing (ANOVA) was used in the study. Tukey's multiple comparison test was applied to determine the different overall and interaction averages.

3. Results and Discussion

3.1.The effect of cold storage on the life span of Nesidiocoris tenuis

The effect of storing adult individuals of *N. tenuis* for 5, 10 and 15 days at 7, 10 and 15°C on the life span is given in Table 1.

Table 1. The effect of cold storage on the life span of *Nesidiocoris tenuis* (day)

Storage period (day)	**Temperature**			
	7°C	**10°C**	**15°C**	**Control**
5	13.83±0.73^{ABC}	12.53±0.87^{ABC}	11.42±0.83^{BCD}	15.83±1.30^{A}
10	14.06±0.42^{AB}	14.94±0.49^{AB}	9.83±0.70^{CD}	15.83±1.30^{A}
15	12.52±0.24^{ABC}	13.09±0.47^{ABC}	8.10±0.61^{D}	15.83±1.30^{A}

[1] The difference between the averages shown with the same uppercase letters in the same row and column is not statistically significant (A-D: p<0.01).

In table 1, the values in the inner part of the table show the averages of the interactions. In the study, the interaction effect between cold storage times and degrees was found to be significant. The most extended longevity of *N. tenuis* following storage was 14.06 and 14.94 days at 7 and 10°C in adults kept for 10 days, respectively. The difference between the life spans of the adult samples kept at these two temperatures was not statistically significant (P<0.01). Adults kept for 15 days had the shortest life duration of 8.10 days at 15°C, and this difference was statistically significant (P<0.01) (Table 1). The difference in the samples' storage lifespans at 7 and 10°C for 5 and 15 days was determined to be statistically negligible (P<0.01) (Table 1).

Different cold storage studies were carried out with *Orius* spp. and *Anthocoris* spp., which are in the same subfamily with *N. tenuis*. According to Bueno et al. (2014), the most extended longevity for *O. insidiosus* was 14.1 days when kept at 8°C for up to 10 days. The study showed a similar result found in the literature, as the adult life span of *N. tenuis* kept for 10 days at 10°C was 14.9 days. Kim et al. (2009), reported that after 20 and 40 days of storage at 10°C, the female lifetime of *O. laevigatus* was 19.8 and 23.7 days at 25°C, respectively. Female *O. majusculus* was stored for 30 and 50 days at 9°C, followed by 25.9 and 19.8 days at 22°C, according to Rudolf et al. (1993). According to Yanık and Ünlü (2015), the longest life span of *A. minki* for females was 33.30 days when held at 11°C for 20 days, and 29.75 days for males when stored at 15°C for 20 days. Furthermore, they indicated that female samples kept in the cold had a shorter life duration (54.66 days) than the control group reared at 25°C. Similar to earlier experiments, this study discovered that an increase in the waiting period lowered the life span of *N. tenuis* adults.

3.2.The effect of cold storage on the survival rate of Nesidiocoris tenuis

The survival rates of *N. tenuis* adults after storage at 7, 10 and 15°C for certain periods are given in Table 2.

Table 2. The effect of cold storage on the survival rate of *Nesidiocoris tenuis* (%)

Storage period (day)	**Temperature**		
	7°C	**10°C**	**15°C**
5	94.00±4.00^{A}	98.00±3.54^{A}	89.00±4.47^{AB}
10	90.00±1.22^{AB}	89.00±3.67^{AB}	86.00±3.40^{AB}
15	65.00±2.45^{C}	77.00±2.45^{BC}	88.00±2.00^{AB}

[1] The difference between the averages shown with the same uppercase letters in the same row and column is not statistically significant (A-C: p<0.01).

In Table 2, the values in the inner part of the table show the averages of the interactions. According to the experimental results, the interaction effect was found to be significant. The highest survival rate of *N. tenuis* was observed to be 94.00% and 98.00%, respectively, at 7 and 10°C in adults held for 5 days, according to the experimental data. Adults kept at 7°C for 15 days had the lowest viability rate of 65.00%, which was statistically significant (P<0.01) (Table 2).

According to Colinet and Boivin (2011), storage time is an essential factor in cold storage research. Several studies on cold storage at various holding times have found that natural enemy survival rates decrease inversely as storage time increases (Abd El-Gawad et al. 2010; Tunca et al. 2014; Yanık and Ünlü 2015). Similarly, in this study, the average survival rates of *N. tenuis* were 93.67% after 5 days of storage, 88.33% after 10 days of storage, and 76.67% after 15 days of storage, indicating that survival rates decrease while cold storage period increases.

When adults of *O. laevigatus* were kept at 6, 8, 10, and 12°C, Kim et al. (2009) reported a survival rate of 70% after 36 days at 10°C. According to Rudolf et al. (1993), adults of *O. majusculus* had a 50% survival rate after 42 days of low-temperature storage, while adults of *O. laevigatus* had a 75-80% survival rate after 40 days of storage at 9°C. In this study, survival rates of 77% were achieved in individuals of *N. tenuis* held at 10°C for 15 days, which is consistent with the literature. Bueno et al. (2014) found that adult *O. insidiosus* survival rates following storage at 8, 10, and 12°C were generally greater than 70% for both females and males. According to the study's findings for *N. tenuis*, a viability rate of more than 70% was recorded at all low temperatures and waiting times. According to Yanik and Ünlü (2015), the lowest survival rate (7.33%) was recorded in the 1-3 nymphal stages when held at 7°C for 40 days, while the maximum survival rate (90.0-92.0%) was recorded in the 1-3 nymphs and adults during the period when they were kept at 11°C for 10-30 days.

3.3. The effect of cold storage on the number of nymphs of Nesidiocoris tenuis

The effect of storage at 7, 10 and 15°C for 5, 10 and 15 days on the number of nymphs of *N. tenuis* was investigated and the results are presented in Table 3.

Table 3. The effect of cold storage on the number of nymphs of *Nesidiocoris tenuis*

Storage period (day)	**Temperature**			
	7°C	**10°C**	**15°C**	**Control**
5	188.0±14.98^{B}	181.6±15.12^{B}	185.6±44.27^{B}	250.2±13.60^{AB}
10	194.6±6.40^{B}	216.4±23.10^{B}	172.0±15.25^{B}	250.2±13.60^{AB}
15	322.4±14.82^{A}	166.6±9.37^{B}	47.6±8.05^{C}	250.2±13.60^{AB}

[1] The difference between the means shown with the same capital letters in the same row and column is not statistically significant (A-C: p<0.01).

In Table 3, the values in the inner part of the table represent the averages of the interactions. The interaction effect was found to be significant. The average number of nymphs produced by *N. tenuis* adults kept at 7°C for 15 days was 322.4, while it was 47.6 for adults kept at 15°C for 15 days. According to the statistical analysis, this difference is significant (P<0.01). The study observed no difference in the nymph numbers of the other groups compared to the control group, except for the adults kept at 15°C for 15 days (P<0.01) (Table 3).

Kim et al. (2009) found that after 20 and 40 days of storage at 10°C, females of *O. laevigatus* deposited 109.2 and 69.2 eggs, respectively, at 25°C. In contrast, the control group laid 224.5 eggs. According to Rudolf et al. (1993), females of *O. laevigatus* laid 145 and 72 eggs, respectively, at 22°C after 20 and 50 days of storage at 9°C, while the control group laid 190 eggs. When kept at 11°C, adults of *A. minki* laid 155.85 eggs, whereas the control group laid 207.51 eggs, according to Yanik and Ünlü (2015). In this study, the number of *N. tenuis* nymphs decreased with increasing waiting time, as determined by the researchers' previous studies (Table 3).Cold storage is critical in the large production of beneficial insects. The ideal storage conditions for predators should be found to maximize nymph productivity, life span, and viability. When we examined the results of survival rates after cold storage for *N. tenuis* (Table 2), we see that the normal life expectancy in the control group is 15 days. Therefore, 15 days of storage at different low temperatures will not be suitable for adults, but 5 and 10 days of storage will be more beneficial. The effect of cold storage on life span indicates that it is established that adults stored at 15°C have the shortest life span, making storage at this temperature unsuitable for mass production (Table 1). Besides, when we evaluated the effect of cold storage on the number of nymphs, we detected no difference between the nymph numbers of the other groups and the control group, except for the nymph numbers of the adults stored at 15°C for 15 days (Table 3). The viability rate after storage, life span, and nymph yield showed that *N. tenuis* could be preserved at 7 and 10°C for up to 10 days.

4. Conclusions

Cold storage has proven to be a valuable method for preserving predator *N. tenuis*, extending its shelf life, and enabling synchronized releases of sufficient numbers of natural enemies to production areas under favorable weather conditions, especially during critical moments such as epidemics and during off-season periods when demand is low. Furthermore, cold storage allows for the aggregation or stockpiling of natural enemies, which benefits mass manufacturing in a cost-effective manner. This study aimed to increase the efficiency of *N. tenuis* in biological control by determining the best combination of low temperature and storage period.

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