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Orijinal araştırma (Original article)

Storage studies of different stages of *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae) under low temperatures

Düşük sıcaklıklarda *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae)'nin farklı dönemlerinin depolanması

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Summary

The goal of this study was to evaluate the biological parameters of the predator *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae) over various periods of growth at low temperatures. Storage studies were conducted for the following three stages: 1) 1-3 stage nymphs, 2) 4-5 stage nymphs, and 3) adult stages. All stages of the predator were stored at 7, 11, and 15 ± 1°C for 10, 20, 30, and 40 days under continuous scotophase. During storage, food eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were added once a week. Following storage, the predator was transferred to long day periods (16:8 [L:D] h, 25 ±1 °C). A lower survival rate (7.33%) was determined for the 1-3 nymph stage following storage at 7°C for 40 days. The highest survival percentage (90.0-92.0%) was determined for 1-3 stage nymphs and adult stage stored at 11°C for 10-30 days. The largest quantity of eggs was obtained when *A. minki* was stored at 11°C. Overall, our results indicated that *A. minki* can be stored for up to 40 days at 11°C.

Keywords: *Anthocoris minki,* biological control, low temperature, mass rearing, storage

Özet

Bu çalışma ile farklı düşük sıcaklıkların ve farklı sürelerin predatör *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae)'nin biyolojik özelliklerinin belirlenmesi amaçlanmıştır. Denemelerde *A. minki*'nin 1-3. nimf, 4-5. nimf ve ergin dönemleri kullanılmıştır. Her gruptaki bireyler 7, 11 ve 15±1°C sıcaklıklarda ve 10, 20, 30 ile 40 gün sürekli karanlıkta tutulmuştur. Düşük sıcaklıkta depolanan predatörlere haftada bir besin olarak *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) yumurtaları sunulmuştur. Depolama sonrası canlı kalan *A. minki* bireyleri uzun gün koşullarında (16:8 [A:K] h, 25±1°C) yetiştirilmeye alınmıştır. Depolama süresince en düşük canlı kalma oranı (%7.33), 7°C'de 1-3. nimf döneminde 40 gün depolandığında, en yüksek canlı kalma oranı (%90.0-92.0) ise 11°C'de 1-3. dönem nimf ve ergin döneminde 10-30 gün depolandığında gerçekleşmiştir. Depolama sonrası yetiştirilen bireylerde en fazla yumurta 11°C'de depolandığında elde edilmiştir. Sonuç olarak, *A. minki* 11°C'de 40 güne kadar depolanabilir.

Anahtar sözcükler: *Anthocoris minki*, biyolojik mücadele, düşük sıcaklık, depolama, kitle üretimi

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Introduction

Pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) is an important pest that causes yield loss in pistachio trees (Mart et al., 1995; Mehrnejad, 2001; Souliotis et al., 2002). Due to increased resistance to insecticides, *A. pistaciae* is difficult to control (Mehrnejad, 2001). As a result of insecticide failure, biological controls are gaining prominence for controlling pistachio psylla. In Turkey, *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae) has an important role as a natural enemy of pistachio psylla (Çelik, 1981; Mart et al., 1995; Yanik & Unlu, 2010). Based on data obtained from insect release studies in pistachio orchards, Yanık et al. (2007) reported that *A. minki* was effective for controlling pistachio psylla and was responsible for decreasing the pest population at the economic injury level. Detailed studies regarding the biology, ecology, and release of *A. minki* for controlling pistachio psylla are available in the scientific literature (Yanik & Unlu, 2010; 2011a, b; Yanık et al., 2007; 2009; 2011a, b; 2012).

In regards to the mass rearing of commercially produced biocontrol agents, low temperature storage is an important component. Synchronization, flexibility, and effectiveness is achieved in mass rearing operations when release demand has the highest biological control. Thanks to low temperature storage, standard stocks can be provided for long term ecologic, physiologic, or genetic research purposes (Leopold, 1998). However, parameters such as emergence rate, longevity, number of eggs laying and reproductive success can impact the number of beneficial insects that can be stored at low temperatures (Leopold, 1998). Therefore, knowledge of an effective storage period and the temperature required for the mass rearing of natural enemies significantly contributes to the sustainability of biocontrol agent production.

The anthocoridae family includes species than can be commercially reared for use as biocontrol agents (Anonymous, 2014a, b, c). Several studies regarding storage at low temperatures have been performed for Anthocorid species. Rudolf et al. (1993) stored *Orius majusculus* (Reuter) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) adults at 13 and 9°C. The eggs of *Orius sauteri* (Poppius) were stored in the laboratory at 7.5°C and 12.5°C by Murai et al. (2001). Kim et al. (2009) stored *O. laevigatus* at 10°C and Bueno et al. (2014) reported that *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) can be stored at 8°C for 10 days without a loss of quality. A large body of scientific research is also available on the low temperature storage of other natural enemies (e.g. Osman & Selman, 1993; Abdel-Salam & Abdel-Baky, 2000; Uçkan & Gülel, 2001; Bayram et al., 2005; Lo´pez & Botto, 2005; Coudron et al., 2007; Larentzaki et al., 2007; Luczynski et al., 2008; Tunca et al., 2014).

Based on the studies provided above, cold storage has been determined to be an important method in the mass production of biological control agents. However, to our knowledge, no studies regarding the storage of the Anthocorid predator *A. minki* at low temperatures are currently available in the scientific literature. The goal of this study was to determine the effects of cold storage on the survival rate, the potential reproductive rate, the longevity, and the fecundity of *A. minki*. Since cold storage temperature and the duration of temperature exposure are the two most important factors when defining a cold storage system for a natural enemy, these parameters were investigated in this study.

Materials and Methods

Insect stock cultures

The adult *A. minki* colony used for our study was collected from pistachio trees located in Şanlıurfa province in Turkey. Transparent plastic containers with a diameter of 12 cm and a height of 13 cm, covered with nylon tulle on two sides with a ventilation hole were used to rear adults and nymphs. Frozen *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (0.1 gr), attached to black cardboard (5x5 cm) using water, were supplied as a food source. A green bean pod (*Phaseolus vulgaris* L.) was supplied as an egg laying site for predators and as a water source. *Ephestia kuehniella* eggs and green bean pods were replaced every two or three days. New cultures were formed by moving green bean pods containing *A. minki* eggs into separate plastic containers. To allow predator insects to hide and wander, the bottom of rearing containers was lined with paper towels. Adults and nymphs of *A. minki* were reared in a climate chamber at 25 ± 1^{o}C at a relative

humidity of 65 ± 5% for 16:8 hours of light:dark. *Ephestia kuehniella* was reared using methods described previously (Bulut & Kılınçer, 1987). *Anthocoris minki* used for tests were obtained from the stock culture.

Effects of cold storage on survival rate, longevity and fecundity of *Anthocoris minki*

The three biological stages of *A. minki* (1-3 stage nymphs, 4-5 stage nymphs, and the adult stage) were stored at constant temperatures of 7, 11, and 15 ± 1ºC for 10, 20, 30, and 40 days under constant scotophase. To enable nymphal stages to develop and to survive for a longer period, a food source was provided once per week. Fifty individuals of the same age from the *A. minki* population were randomly selected from the stock culture for each biological stage, each low temperature, and each storage period. Individuals were placed in plastic containers (12 cm in diameter x 13 cm in height) in three separate replicates (n = 3, a total of 150 individuals for each storage period; a total of 1,800 individuals for the four different storage periods at each temperature; and a total of 5,400 individuals in all combinations for the three temperatures examined). *Anthocoris minki* individuals were placed with *E. kuehniella* eggs. A creased paper towel was used so individuals could hide. No initial sex determination was performed for the adult stage individuals stored although Yanik & Unlu (2011a) reported that the sex ratio of *A. minki* was 1:1. Tests were performed in plastic containers having a diameter of 10 cm and a height of 10 cm with a ventilation hole, the lateral sides of which were covered with tulle. Following storage at low temperature for the stated periods, dead individuals in each container (replication) were recorded and the post-storage survival percentage was determined. Surviving individuals for each storage period were moved to an environment with a temperature of 25 ± 1ºC, a relative humidity of 65 ± 5%, and 16:8 hr light:dark. The study was continued by providing 0.2 grams of *E. kuehniella* eggs daily within the containers as a food source and by placing green bean pods in containers as egg laying material. In controls performed three times a week, male and female longevities were determined by performing a sex determination on dead individuals. The number of eggs lain was also recorded. The number of eggs laying, male and female longevity, and the pre-oviposition period starting date were determined, beginning from the day when adults surviving the various temperature and storage periods began to be reared at 25 ± 1ºC. The food source and green beans were replaced during these controls. For the control group, 50 *A. minki* individuals within the 0-24 hour population were collected from the stock culture and reared at a temperature of 25 ± 1ºC in three replicates. Male and female longevity and fecundity were determined. The number of eggs laying per female was determined by dividing the total number of eggs by the number of females contained in each replicate.

Determining ovarian development

Using the same method employed to determine survival rate and fecundity, *Anthocoris minki* were reared in combinations formed using various temperatures and storage times. *A. minki* that survived each low temperature and storage replicate were then reared at 25 ± 1ºC. Rearing at 25 ± 1ºC lasted for 10 days for previously cold-stored adult stages; 14 days for 4-5 stage previously cold-stored nymphs, and 18 days for 1-3 stage previously cold-stored nymphs in order to provide egg development in ovarioles. The rearing periods were several times longer than the normal pre-oviposition period required for the fecundity study. At the end of the reared periods, we recorded whether an oocyte was present in each female by dissecting *A. minki* individuals under a stereo-binocular microscope. For the procedure, female Anthocorids were placed into a drop of water on a dissection slide. Abdomen sclerites were removed using an insect pin and we carefully separated the ovaries from other internal material using a needle. If cold-stored individuals from the nymph stage were not able to reach the adult stage by the end of the storage periods, they continued to be reared at 25 ± 1ºC until they reached the adult stage. Individuals that were stored as 4-5 stage nymphs were reared for 14 days, while those that were stored as 1-3. stage nymphs were reared for 18 days. Adults were dissected at the end of storage. For the control group, new *A. minki* adults aged 0-24 hours obtained from the stock culture were dissected after being reared at 25 ± 1ºC for 10 days. Tests to determine the reproductive rate were performed using three replicates for each combination so that fifty randomly selected individuals of the desired stage were obtained from the stock culture. The percentage of females with developed ovaries was calculated. Thus, the potential reproductive rate was determined.

Statistical analysis

A two-way ANOVA analysis was used to evaluate the impact of various low temperatures and storage periods on male and female longevity, preoviposition periods, and the number of eggs laying. Tukey's multiple comparison test was used to determine the difference between averages.

Results and Discussion

The work presented is the first study on the effects of storage at low temperature on the biological parameters of *A. minki*. Significant differences were determined between the survival capacities of insects and mite species in cold storage. Therefore, it is not possible to generalize the tolerance of taxa, families, or even genera to cold storage (Leopold, 1998). Supplying food during the cold storage of insects is generally beneficial (Leopold, 1998). For example, Abdel-Salam & Abdel-Baky (2000) reported that feeding *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) adults prior to cold storage significantly affected the survival percentage, the longevity, the fecundity, and prey consumption rates. In our study, food was supplied during cold storage. Food was especially necessary during the storage of nymph stages of *A. minki* so that development could be completed.

Table 1. The effects of cold storage on the survival rate of *Anthocoris minki* (%)

Temperature (°C)	Storage period (day)	1-3.stage nymph	4-5.stage nymph	Adult
7	10	28.66	58.00	84.00
	20	24.00	55.33	84.00
	30	21.33	35.33	84.66
	40	07.33	14.00	42.00
11	10	90.00	78.66	92.00
	20	91.33	75.33	92.00
	30	86.00	77.33	90.00
	40	80.66	64.66	88.33
15	10	84.00	82.00	87.33
	20	84.66	80.66	83.33
	30	89.33	84.00	86.00
	40	78.00	78.66	80.66

Our results indicated that the lowest survival rate of *A. minki* (7.33%) occurred for the 1-3 nymph stage at a temperature of 7ºC when stored for 40 days (Table 1). The highest survival rate (92.0-92.0%) was observed for the adult stage with a storage time of 10-20 days at 11ºC. The highest survival rate (88.33%) for a 40 day storage period occurred for the adult stage at 11ºC. Kim et al. (2009) reported that when stored at temperatures of 6, 8, 10, and 12°C, 10°C was the most suitable storage temperature for adults of *O. laevigatus*. These authors also reported a survival rate of 70% at this temperature at the end of 36 days. Rudolf et al. (1993) reported that *O. majusculus* adults had a survival rate of 50% for a storage time of 42 days, while *O. laevigatus* adults had a survival rate of 75-80% for a storage time of 40 days when stored at 9°C. Bueno et al. (2014) reported that the survival rate of *O. insidiosus* following storage at 10°C for 20 days was 61.4% for females and 50.4% for males. Based on the scientific literature, it is understood that the survival rate of different Anthocoridae species following storage at low temperatures is lower than the survival rates determined during our study. Such findings result indicates that the genera used for other studies have an adult longevity that is shorter than that of *A. minki*.

Since the post-storage death rate of *A. minki* stored at 7°C for the 1-3 and 4-5 nymph stages during various storage periods was very high, the potential reproductive rate could not be determined. Apart from these biological stages, the lowest potential reproductive rate (80.96%) was observed for storage at 15°C for 40 days for adult stages. The highest potential reproductive rate (98.22%) was observed for storage at 15°C for 40 days for the 1-3 nymph stage (Table 2). While *A. minki* had a potential reproductive rate of 95.91% for the control group, the potential reproductive rate after storage at 11°C was above 90% (90.33 - 95.84%) for all storage periods and biological stages.

Table 2. The effects of cold storage on the potential reproductive rate of *Anthocoris minki* (%)

Temperature (°C)	Storage period (day)	1-3.stage nymph	4-5.stage nymph	Adult
7*	10	0	0	88.24
	20	0	0	95.66
	30	0	0	90.90
	40	0	0	93.75
11	10	90.33	93.11	90.63
	20	92.31	90.91	92.18
	30	95.24	92.43	92.31
	40	92.59	95.84	91.38
15	10	92.10	91.30	97.30
	20	93.11	92.85	95.32
	30	94.65	92.46	87.50
	40	98.22	96.88	80.96
25 Control**	0	95.91		

* Since the post-storage death rate of *A. minki* stored at 7°C during the 1-3 and 4-5 nymph stages was very high, the potential reproductive rate could not be determined.
** Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

The post-storage female longevity for cold-stored adults and 1-3 stage nymphs of *A. minki* was significantly affected by temperature (adult, F=7.72, df=2, P=0.0026; 1-3 nymph, F=3.96, df=2, P=0.0327). However, the storage period and the interaction between the temperature-storage period was not statistically significant during all of the biological stages ($p>0.05$). The 1-3 stage nymphs stored at 7°C for 40 days displayed the longest post-storage female longevity (60.33 days) (Table 3). When storage periods were not taken into account, post-storage female longevity was statistically longer for the storage of 1-3 stage nymphs at 7 and 11°C, and for the storage of adults at 11 and 15°C as compared to other temperature values. However, no statistical difference between temperatures in terms of female longevity was determined for the 4-5 stage nymphs. Bueno et al., (2014) reported that female longevity of *O. insidiosus* at 25°C following storage at 10°C for 10 and 20 days lasted for 7.8 and 3.8 days, respectively. Kim et al. (2009) reported that female longevity for *O. laevigatus* lasted for 19.8 and 23.7 days at 25°C following storage at 10°C for 20 and 40 days, respectively. In our study, female *A. minki* stored at low temperatures at various stages displayed shorter longevity as compared to the control group (54.66 days) reared at 25°C without cold storage, suggesting that storage has a negative impact on adult longevity. Rudolf et al. (1993) reported that female *O. majusculus* lived for 25.9 and 19.8 days at 22°C following a 30 and 50 day storage, respectively, at 9°C, while Bahşi & Tunç (2012) reported that female *O. majusculus* lived for 45.0 days in *E. kuehniella* at 26°C without cold storage.

Table 3. Female longevity of various stage of *Anthocoris minki* (under 25°C, 16:8 L:D) after various storage periods at various low temperatures (day)

Temperature (°C)	Storage period (day)	1-3.stage nymph	Mean	4-5.stage nymph	Mean	Adult	Mean
7	10	41.91ab*	45.69a	25.34a	31.67a	17.74a	21.26b
	20	39.93ab		30.25a		22.51a	
	30	40.61ab		45.75a		24.14a	
	40	60.33a		25.33a		20.68a	
11	10	43.66ab	42.95ab	30.01a	30.66a	32.17a	30.81a
	20	44.47ab		31.83a		33.30a	
	30	45.66ab		29.32a		31.27a	
	40	38.01ab		31.47a		26.53a	
15	10	36.48ab	35.71b	30.17a	29.25a	28.40a	27.52a
	20	37.65ab		29.05a		25.92a	
	30	38.78ab		29.13a		28.09a	
	40	29.94b		28.63a		27.68a	
25 Control**	0	54.66					

*Means in the same column followed by a different letter are significantly different (Tukey test, P<0.05).
* *Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

Temperature, storage period duration, and temperature-storage period interaction for all biological stages stored did not have a statistically significant impact on post-storage male longevity (p>0.05). When storage periods are not taken into account, male longevity was found to be significantly longer for the storage of 1-3 stage nymphs at 11°C and for storage of adults at 15°C as compared to other temperature values (p<0.05). However, no statistically significant difference was determined between temperatures for the storage of 4-5 stage nymphs (p>0.05) (Table 4). Male longevity was shorter than the control group (64.54 days) for all temperature and storage periods.

Table 4. Male longevity of various stage of *Anthocoris minki* (under 25°C, 16:8 L:D) after various storage periods at various low temperatures (day)

Temperature (°C)	Storage period (day)	1-3.stage nymph	Mean	4-5.stage nymph	Mean	Adult	Mean
7	10	24.32a*	37.29b	27.59a	29.35a	20.80a	20.41b
	20	32.91a		28.94a		18.47a	
	30	43.66a		33.60a		22.06a	
	40	48.25a		26.88a		20.32a	
11	10	43.69a	44.50a	24.71a	26.25a	22.70a	23.99ab
	20	45.56a		26.65a		23.52a	
	30	45.50a		28.25a		24.32a	
	40	43.21a		29.25a		27.32a	
15	10	33.38 a	36.55b	27.43 a	27.89a	28.97 a	26.52a
	20	35.23 a		30.51 a		29.75 a	
	30	39.86a		28.26 a		24.79 a	
	40	37.75a		25.38a		22.58a	
25 Control**	0	64.54					

*Means in the same column followed by a different letter are significantly different (Tukey test, P<0.05).
** Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

The storage temperature was determined to have a significant impact on the pre-oviposition period for all cold-stored biological stages of *A. minki* (1-3 nymph, *F*=10.89, df=2, *P*=0.0004; 4-5 nymph, *F*=10.75, df=2, *P*=0.0005; adult, *F*=44.44, df=2, *P*=0.0001).

The interaction of the temperature-storage period was statistically significant for the storage of 1-3 stage nymphs and adult stages (1-3 nymph, *F*=2.70, df=6, *P*=0.0379; adult, *F*=7.11, df=6, *P*=0.0002), while storage periods were determined to be statistically significant only during the pre-oviposition period for stored adult stages (*F*=7.56, df=3, *P*=0.0010). Shortness of the pre-oviposition period during mass rearing is important for economic production. In our study, we determined that the post-storage, pre-oviposition period was shorter than that of control group that underwent no storage, excluding the storage of 1-3 and 4-5 stage nymphs of *A. minki* at 7°C (Table 5). Based on our results, cold storage has a positive impact on mass rearing in terms of shortening the pre-oviposition period of *A. minki*. The fact that the post-storage, pre-oviposition period of *A. minki* adults was shorter than the other stored stages could have resulted from mating during storage.

Table 5. Preoviposition period of various stage of *Anthocoris minki* (under 25°C, 16:8 L:D) after various storage periods at various low temperatures (day)

Temperature (°C)	Storage Period (day)	1-3.stage nymph	Mean	4-5.stage nymph	Mean	Adult	Mean
7	10	8.66ab*	11.08a	10.00ab	10.16a	2.66a	2.16a
	20	11.33ab		12.00ab		2.00a	
	30	16.33b		13.66b		2.00a	
	40	8.00ab		5.00ab		2.00a	
11	10	4.33a	4.66b	3.00a	5.08b	0.00b	0.50b
	20	4.66a		6.00ab		0.00b	
	30	2.66a		5.33ab		0.00b	
	40	7.00ab		6.00ab		2.00a	
15	10	8.66ab	6.75b	5.66ab	4.83b	3.33a	2.16a
	20	9.00ab		7.00ab		1.33ab	
	30	5.00a		3.66a		2.00a	
	40	4.33a		3.00a		2.00a	
25 Control**	0			10.76			

*Means in the same column followed by a different letter are significantly different (Tukey test, P<0.05).
** Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

The storage temperature was found to have a significant influence on the number of eggs laid per female for cold-stored 4-5 stage nymphs and adults of *A. minki* (4-5 nymph, *F*=7.81, df=2, *P*=0.0024; adult, *F*=16.44, df=2, *P*=0.0001). On the other hand, the storage period during all of the stored biological stages and the interaction between the temperature-storage period had no statistically significant impact on number of eggs lain per *A. minki* female (p>0.05). When storage periods were not taken into account, the storage of 4-5 stage nymphs (55.95 pcs/female) and adults (77.23 pcs/female) at 7°C yielded the lowest mean number of eggs laid per female (Table 6).

Considering all of the biological stages of *A. minki* tested, the mean number of eggs laid per female following storage at 11°C was closer to the control group values when compared to other temperature values. On the other hand, we determined that the 1-3 stage nymphs of *A. minki* stored at 15°C for 10 days laid quite a similar numbers of eggs as those of the control group that was not cold-stored. The fact that the mean number of eggs laid per *A. minki* female following cold storage was lower than those of the control group indicates a negative effect for cold storage in terms of the number of eggs laid. Kim et al. (2009) reported that following storage at 10°C for 20 and 40 days, females of *O. laevigatus* laid 109.2 and 69.2 eggs at 25°C, respectively, while the control group that was not stored cold laid 224.5 eggs. Rudolf

et al. (1993) reported that following storage at 9°C for 20 and 50 days, females of *O. laevigatus* laid 145 and 72 eggs, respectively, at 22°C, while the control group laid 190 eggs; females of *O. majusculus* laid 75 and 34 eggs while the control group laid 122 eggs. For mass rearing, the creation of a rearing environment that produces a high number of eggs is necessary to achieve economic viability. Considering that cold storage is an indispensable part of mass rearing, determining the most suitable storage condition for egg growth is important.

Table 6. Fecundity of various stage of *Anthocoris minki* (under 25°C, 16:8 L:D) after various storage periods at various low temperatures (number/female)

Temperature (°C)	Storage period (day)	1-3.stage nymph	Mean	4-5.stage nymph	Mean	Adult	Mean
7	10	131.91a*	121.03a	39.31a	55.95b	54.90b	77.23c
	20	114.81a		47.88a		69.59bc	
	30	121.68a		81.04a		96.82abc	
	40	108.83a		55.55a		87.61abc	
11	10	174.95a	155.43a	164.49a	148.83a	155.85a	141.54a
	20	168.02a		152.01a		144.55ac	
	30	157.16a		143.82a		141.22ac	
	40	121.56a		135.02a		124.54abc	
15	10	194.99a	163.02a	133.26a	120.55a	112.13abc	106.47b
	20	175.04a		132.72a		109.98abc	
	30	155.64a		118.26a		101.24abc	
	40	126.43a		98.10a		102.53abc	
25 Control**	0			207.51			

*Means in the same column followed by a different letter are significantly different (Tukey test, P<0.05).
** Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

Based on our experiments on *A. minki*, performed at various low temperatures and storage periods we suggest that this predator can be stored for up to 40 days at 11°C. Cold storage for the predator *A. minki* may be a valuable tool for insectaries, allowing them to store insects for prolonged periods rather than continuously rearing colonies during the off-season when demands for them are low. Additionally, cold-storage has the added benefit of making standardized cultures available for research and provides flexibility and efficiency for mass production, therefore, synchronizing the desired stage of development for crop release as well as facilitating the availability of insects to users. Determining low cost and effective storage methods for insect rearing is especially important for obtaining a high quality of biological control agents. Furthermore, understanding suitable storage conditions for obtaining higher numbers of natural enemy insects ready for crop release during intensive release periods is of great importance for mass rearing.

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References

Anonymous 2014a. (web page: http://www.koppert.com/products/products-pests-diseases/) (Accessed date: 22.12.2014)

Anonymous 2014b. (web page: http://www.biobest.be/productenalg/2/3/) (Accessed date: 22.12.2014)

Anonymous 2014c. (web page: http://bioplanet.it/en/) (Accessed date: 22.12.2014)

Abdel-Salam, A. H. & N. F. Abdel-Baky, 2000. Possible storage of *Coccinella undecimpunctata* (Col., Coccinellidae) under low temperature and its effect on some biological characteristics. Journal of Applied Entomology**,** 124: 169-176.

Bahşi, Ş. U. & I. Tunc, 2012. The response of a southern strain of *Orius majusculus* (Reuter) (Hemiptera: Anthocoridae) to photoperiod and light intensity: biological effects and diapause induction. Biological Control, 63: 157–163.

Bayram, A., H. Ozcan & S. Kornosor, 2005. Effect of cold storage on the performance of *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae), an egg parasitoid of *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae). Biological Control, 35: 68-77.

Bueno, V. H. P., L. V. Carvalho & J. C. Van Lenteren, 2014. Performance of *Orius insidiosus* after storage, exposure to dispersal material, handling and shipment processes. Bulletin of Insectology, 67 (2): 175-183.

Bulut, H. & N. Kılınçer, 1987. "Yumurta paraziti *Trichogramma* spp. (Hym.: Trichogrammatidae)'nin un güvesi (*Ephestia kuehniella* Zell.) (Lep.: Pyralidae) yumurtalarında üretimi ve konukçu-parazit ilişkileri, 13-16". Türkiye I. Entomoloji Kongresi, İzmir.

Coudron, T. A., M. R. Ellersieck & K. S. Shelby, 2007. Influence of diet on long-term cold storage of the predator *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). Biological Control, 42: 186–195.

Çelik, M. Y., 1981. Gaziantep ve Çevresinde Antep Fıstıklarında Psylloidea'ya Bağlı Önemli Zararlı Türlerin Tanınmaları, Yayılışları, Konukçuları, Kısa Biyolojileri ve Doğal Düşmanları Üzerinde Araştırmalar. T.C. Tarım ve Orman Bakanlığı, Araştırma Eserleri Serisi, No: 51, Ankara.

Kim, J. H., H. Y. Kim, M. Y. Han, M. Y. Choi, S. J. Hwang & M. S. Lee, 2009. Cold storage effect on the biological characteristics of *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). Korean Journal Applied Entomology, 48: 361-368.

Larentzaki, E., G. Powell & J. W. Mike Copland, 2007. Effect of cold storage on survival, reproduction and development of adults and eggs of *Franklinothrips vespiformis* (Crawford). Biological Control, 43: 265–270.

Leopold, R. A., 1998. "Cold storage of insects for integrated pest management, 253-267". In: Temperature Sensitivity in Insects and Application in Integrated Pest Management (Eds: G. J. Hallman & D. L. Denlinger). Westview Press, Boulder.

Lo´pez, S. N. & E. Botto, 2005. Effect of cold storage on some biological parameters of *Eretmocerus corni* and *Encarsia formosa* (Hymenoptera: Aphelinidae). Biological Control, 33: 123-130.

Luczynski, A., J. P. Nyrop & A. Shi, 2008. Pattern of female reproductive age classes in mass-reared populations of *Phytoseiulus persimilis* (Acari: Phytoseiidae) and its influence on population characteristics and quality of predators following cold storage. Biological Control, 47: 159–166.

Murai, T., Y. Narai & N. Sugiura, 2001. Utilization of germinated broad bean seeds as an oviposition substrate in mass rearing of the predatory bug, *Orius sauteri* (Poppius) (Hemiptera: Anthocoridae). Applied Entomology and Zoology, 36(4): 489-494.

Mart, C., N. Uygun, M. Altin, L. Erkiliç & H. Bolu, 1995. General review on the injurious and beneficial species and pest control methods used in pistachio orchards of Turkey. Acta Horticulturae, 419: 379–385.

Mehrnejad, M. R., 2001. The current status of pistachio pests in Iran. Cahiers Options Méditerranéennes, 56: 315–322.

Osman, M. Z. & B. J. Selman, 1993. Storage of *Chrysoperla carnea* Steph (Neuroptera, Chrysopidae) eggs and pupae. Journal of Applied Entomology, 115: 420-424.

Rudolf, E., J. C. Malausa, P. Millot & R. Pralavorio, 1993. Influence of cold temperature on biological characteristics of *Orius laevigatus* and *Orius majusculus* (Het.: Anthocoridae). (Influence des basses temperatures sur lespotentialites biologiques d'*Oriuslaevigatus* et d'*Oriusmajusculus* (Het.: Anthocoridae)). Entomophaga, 38(3): 317-325.

Souliotis. C., D. Markoyiannaki-Printziou & F. Lefkaditis, 2002. The problems and prospects of integrated control of *Agonoscena pistaciae* Burck. and Laut. (Hom., Sternorrhyncha) in Greece. Journal of Applied Entomology, 126: 384–388.

Tunca, H., A. N. Yeşil & T. F. Çalışkan, 2014. Cold storage possibilities of a larval parasitoid, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). Türkiye Entomoloji Dergisi, 38(1): 19-29.

Uçkan, F. & A. Gülel, 2001. The Effects of cold storage on the adult longevity, fecundity and sex ratio of *Apanteles galleriae* Wilkinson (Hym.: Braconidae). Turkish Journal of Zoology, 25: 187-191.

Yanık, E., L. Ünlü & A. Yücel, 2007. "Antepfıstığında *Agonoscena pistaciae* Burck. and Laut. (Hom.: Psyllidae)'nın biyolojik mücadelesinde *Anthocoris minki* Dohrn (Het.: Anthocoridae)'nin kitle üretimi ve salımı, 10". Türkiye II. Bitki Koruma Kongresi (27-29 Ağustos, Isparta) Bildirileri.

Yanik, E., L. Unlu & A. Yucel, 2009. Determination of insect predators species found on almond and wild almond tree adjacent of pistachio orchard. Acta Horticulturae (ISHS): V. International Symposium on Pistachio and Almonds, 06-10 October, Sanliurfa, Turkey, 912: 743-750.

Yanik, E. & L. Unlu, 2010. The effects of different temperatures and relative humidity on the development, mortality and nymphal predation of *Anthocoris minki*. Phytoparasitica, 38(4): 327-335.

Yanik, E. & L. Unlu, 2011a. Influences of temperature and humidity on the life history parameters and prey consumption of *Anthocoris minki* Dohrn (Heteroptera: Anthocoridae). Applied Entomology and Zoology, 46(2): 177-184.

Yanik, E. & L. Unlu, 2011b. Biological traits and prey consumption of *Anthocoris minki* fed on *Agonoscena pistaciae* and *Brachycaudus* (*Thuleaphis*) *amygdalinus*. Phytoparasitica, 39: 333-342.

Yanik, E., L. Unlu & A. Yucel. 2011a. Phenology of emergence from artificial overwintering sites by predatory arthropods in pistachio orchards and adjacent habitats. Ekoloji, 20(78): 1-6.

Yanık, E., L. Ünlü & A. Yücel, 2011b. Antepfıstığında zararlı *Agonoscena pistaciae* Burckhardt and Lauterer (Homoptera: Psyllidae)'nın doğal düşmanı *Anthocoris minki* Dohrn (Heteroptera: Anthocoridae)'nin üretimi ve biyolojik mücadelede kullanılma olanakları. Sonuç Raporu, Mart 2011, Şanlıurfa, TÜBİTAK-TOVAG-1070734.

Yanık, E., L. Ünlü & A. Yücel, 2012. Avcı *Anthocoris minki*'nin antepfıstığı bahçelerinde bulunan farklı predatör türler, *Campylomma lindbergi, Deraeocoris pallens* ve *Oenopia conglobata* ile birlik içi avcılığı. Bitki Koruma Bülteni, 52(2): 123-133.