Original article (Orijinal araştırma)

Effects of cold storage on the developmental biology of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) at different larval stages

Soğukta depolamanın farklı dönemlerdeki *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvalarının gelişimsel biyolojisine etkileri

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Abstract

Being able to store insects at low temperature is important in the mass breeding of insects for commercial purposes. The aim of this study is to investigate the effect of cold storage on pupal and adult weight, adult emergence time and proportion and adult deformation proportion of different weight *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvae. This study was conducted between 2018-2019 in the Biology Laboratory of Science Teaching Department, Sinop University. Trials were performed at 27 ± 2 and 4ºC, 65 ± 5% RH and continuous darkness. After the larvae began to form, they were weighed and divided into three groups according to their weight. They were exposed to 4ºC for 5, 10, 20, 30 and 60 d in separate Petri dishes. With increased cold storage time, the proportion of deformation increased and the proportion of adult emergence decreased. Adult weight, pupal weight and longevity depended on both larval weight and cold exposure time. In conclusion, it is recommended that mass producers or researchers pay attention to the size of the larvae and the cold exposure times to obtain the best quality product and high production efficiency.

Keywords: Cold exposure, deformation rate, insect, insect development, temperature, *Tenebrio molitor*

Öz

Böcekleri düşük sıcaklıklarda depolamak, böceklerin ticari amaçla kitlesel olarak yetiştirilmesinde önemli rol oynamaktadır. Bu çalışmadaki amaç düşük sıcaklıkta depolamanın farklı ağırlıktaki *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvalarının pupa ve ergin ağırlığı, ergin çıkış süresi ve yüzdesi ve ergin deformasyon yüzdelerine etkisini araştırmaktır. Bu çalışma 2018-2019 yılları arasında Sinop Üniversitesi, Fen Bilgisi Eğitimi, Biyoloji Laboratuvarında yapılmıştır. Denemeler 27 ± 2 ve 4ºC, %65 ± 5 bağıl nem ve devamlı karanlık şartlarında yapılmıştır. Larvalar oluşmaya başladıktan sonra tartılarak ağırlıklarına göre 3 gruba ayrılmıştır. Gruplara ayrılan larvalar ayrı petrilerde 5, 10, 20, 30 ve 60 gün olmak üzere 4ºC'ye soğuğa maruz bırakılmıştır. Sonuç olarak, soğukta depolama süresi arttıkça deformasyon yüzdesinin arttığı ve ergin çıkış yüzdesinin azaldığı görülmüştür. Ergin ağırlığı, pupa ağırlığı ve ömür uzunluğu ise larva ağırlıklarına ve soğuğa maruz kalma süresine bağlı olarak değişmiştir. Kitlesel üretim yapacak üreticiler veya araştırıcıların en iyi kaliteyi ve verimi alabilmek için larvaların büyüklüklerine dikkat etmeleri önerilmektedir.

Anahtar sözcükler: Soğuğa maruz bırakma, deformasyon oranı, böcek, böcek gelişimi, sıcaklık, *Tenebrio molitor*

Introduction

Temperature is an important factor affecting the developmental biology of insects, as in all organisms (Sinclair et al., 2003; Arbab, 2019). Large larvae and pupae of insects are more resistant to adverse climatic conditions than eggs or young larvae (Gagneâ & Coderre, 2001; Khaliq et al., 2014; Arbab, 2019). As temperature decreases, insects lose some of their ability to feed, move and fly. Prolongation of exposure to cold may cause irreversible loss of these activities (Rathee & Ram, 2018). Costa et al. (2016) found in a study of eggs and nymphs of *Podisus nigrispinus* Say, 1832 (Hemiptera: Pentatomidae) that most embryos died at 5ºC storage. They suggested that eggs can be stored at 15ºC for a maximum of 15 d.

Being able to store insects at low temperature is important in commercial breeding of insects or biological control methods (Kim et al., 2015). Insect embryos get the energy required to maintain their development directly from vitellogenic reserves (Denlinger & Lee, 2010; Chapman, 2013; Klowden, 2013). When exposed to low temperature, they slow down their metabolic activities and they can survive longer by means of the reserves they previously stored. Conversely, developmental times are prolonged. However, if exposure to the cold is prolonged, these reserves are exhausted and eventually they die (Chown & Nicolson, 2004; Kostal et al., 2004; Colinet et al., 2015). Many studies have found that cold storage can have negative effects on insect biology. For example, when *Trissolcus basalis* Wollaston, 1858 (Hymenoptera: Platygastridae) and *Telenomus podisi* Ashmead, 1893 (Hymenoptera: Scelionidae) pupae were stored at different temperatures and at different periods (12 and 15ºC, 4-7 months), and no adult emergence was observed at 12ºC (Foerster & Doetzer, 2006). In another study, Liu and Tian (1987) found that when *Encarsia formosa* was stored between 3 and 12ºC, the adult growth rate increased with the storage temperature.

The larvae of some insects such as *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae), a species that damage stored products, are used as livestock feed and even as human food in some countries. However, the last instar of this insect larvae is also used as feed in aquaculture and poultry breeding because they contain high quality protein and fat (Li et al., 2013; Shockley & Dossey, 2014; Çalışlar, 2017; Arbab, 2019; Selaledi et al., 2020). The biology of the *T. molitor* has been well studied and its optimal conditions are 27ºC at >60% RH (Li et al., 2013). According to Li et al. (2014), the egg period lasts 3-9 d, the larval stage 26-76 d, and the pupal stage 5-17 d. Before emergence, most of the larvae go through typically 15 to 20 instars (Park et al., 2014). However, developmental rate and size of insects is affected by factors such as temperature, humidity, light, food and density of samples in breeding tanks (Koo et al., 2013).

Tenebrio molitor stands out as a promising species suitable for mass production among insects. They are in high demand for use as natural feed sources for poultry and fish. For this reason, producers keep the *T. molitor* in the refrigerator until supplied to costumers because cold storage extends the shelf life of the farmed insects (Levie et al., 2005; Koo et al., 2013; Bovera et al., 2015; Sihyeon et al., 2016). However, this can cause deformed adults with low egg quality or nutritional value to reach the customers depending on the length of the waiting period. Also, when the culture is very intense in the laboratory, the researchers keep the *T. molitor* larvae or pupae in the refrigerator to study later. This also affects the parameters of the adults emerging from these larvae or pupae, such as deformed, low quality eggs and longevity. The use of these adults in other studies may also cause false results. Also, prolonged exposure time to low temperatures may have negative effects on the life cycle of insects. Especially, it can negatively affect many parameters such as developmental time, pupal emergence rate, adult emergence time and deformation percentages (Liu et al., 2014). In order to prevent these negative effects, the biology of the insect must be well known.

Previously Sönmez & Koç (2019) determined that as exposure of *T. molitor* pupae to low temperature increases, the pupal period and deformation rates increase. It is known that resistance to low temperatures is lower for larvae than for pupae (Punzo & Mutchmor, 1980). Studies on the effects of low temperature

development of larvae and pupae on rate of pupation, rate of adult emergence and deformation rate of adult are increasing (Arbab, 2019). How larvae in different larval stages respond to low temperature has become increasingly important in recent years. In this study, *T. molitor* larvae of different weight (different larval instars) were exposed to cold for different periods. The hypothesis tested was that increasing exposure time to low temperature differentially affects the development of larvae of different weight. Due to reduced lipid and protein contents, small larvae were predicted to be affected more than medium and large larvae in terms of pupal period, adult emergence time, proportion of adult emergence and proportion of adult deformation.

Materials and Methods

Tenebrio molitor cultures were grown at the Sinop University, Biology Laboratory of Science Teaching Department for five generations were used. Trials were performed at 27 ± 2 and 4ºC, 65 ± 5% RH. Flour and whole meal flour were used as food in 1:1 ratio. The insects were reared in plastic containers (30 × 20 × 5 cm). Sawdust was added in the containers to ease movement on the foodstuff. Small pieces (2 for each container, 4 × 4 × 6 cm) were cut from egg boxes for providing convenience for adults to mate and lay eggs. The containers were covered to prevent entrance of other invertebrates, but small holes were opened on the top side to enable gas exchange. Potato was used for humidity (3 × 3 × 3 cm), wrapped into aluminum foil to prevent contact, moisturizing and decay of the food. The potatoes were changed every 3 d for the food layer not to get moldy. The food layer was adjusted to 4-5 cm thickness. The food was renewed with intervals of 10 d. The larvae in the old food were separated by using a sifter and they were transferred on the new food. The containers were checked every day (Figure 1).

Figure 1. *Tenebrio molitor* rearing containers, experimental study establishment and deformed individuals.

Tenebrio molitor larvae show plasticity in the larval stage. Larval instar number may vary depending on environmental factors such as nutrients, temperature and humidity (Graham et al., 2000; Morales-Ramos, 2010; Park et al., 2014). Larval stages are therefore very difficult to distinguish. When selecting larvae, control groups were used and the methods of Graham et al. (2000) and Park et al. (2014) were used. While selecting the larvae and determining the instar difference (age), their weights were checked and the photographs of Park et al. (2014) were used. In the control group, larvae pupated after ~210 mg. Therefore, larvae larger than 210 mg were not included in the study. The larvae of 180-210 mg were

included in the study as the last larval instar. Larval weights were limited both because it was difficult to distinguish larval stages and larvae less than 100 mg were likely to be damaged during transfer (Graham et al., 2000). Therefore, while small larvae were selected, the larvae under 40 mg were not included in the trials (Graham et al., 2000; Morales-Ramos et al., 2012, 2015; Park et al., 2014). In order to set a standard, those lighter than 100 mg were divided into small larvae (7-11th larval instar), and those heavier than 180 mg were divided into large groups (17-20th last larval instar). Larvae of 100-180 mg (12-16th instar) were grouped as medium larvae (Graham et al., 2000; Park et al., 2014).

Larvae were randomly selected and weighed (Radwag AS 220.R2) and divided into three groups according to their weight. Each of the larvae separated into was exposed to the cold at +4ºC for 5, 10, 20, 30 and 60 d in a separate Petri dish with added food (9 × 1.5 cm) (for each cold group: 10 larvae × 10 Petri dishes × 3 trial groups). The groups whose cold exposure ended were brought to the laboratory where further experiments were performed (27 ± 2ºC and 65 ± 5% RH). The nutritional conditions given above were provided until the larvae pupated. The Petri dishes were checked every day. When larvae pupated, they were weighed. Date was recorded on the day they pupated. Pupae were checked every day and the date of the adult was recorded. The days between the day they become pupa and the day of adult emergence were calculated as the adult emergence time. Adult insects were weighed, those with deformation were identified and placed in separate Petri dishes (with food added) for longevity trials. The day insects died was recorded. The days between the day of became adult and the day until death was calculated as the longevity. Deformation was defined as adults without wings, with curved wings or with deformations of elytra. The deformation rates were determined by dividing the deformed individuals first by the number of pupae placed in Petri dish. The nutritional conditions as given above were provided. Trials were repeated three times for each group of larvae (<100, 100-180 and >180 mg). A total of 30 (10 × 3) larvae were used for each weight of larva groups. For example, for <100 mg cold application of 5 d, a total of 30 larvae were used, 10 larvae in three replicates. There are six cold groups together with the control groups. A total of 540 larvae were used for the whole study. In the trials, no adult emergence was observed in the <100 mg larva group that was stored in cold for 60 d only, the all pupae were dead. Therefore, adult weight, adult emergence time, adult emergence and deformation rate and longevity were not shown in the tables.

Data analysis

All the data were analyzed with the SPSS 21 statistical package program. Descriptive statistics of the data were given first in the study. Secondly, the normality assumption of the data was checked by the Kolmogorov-Smirnov test and it was observed that the data were distributed normally (p > 0.05). Analysis of groups and their interactions was done using generalized linear models (two-way ANOVA). Analysis of different groups was also investigated by Tukey HSD test. Tukey HSD test was used to determine the differences among the small (<100 mg), medium (100-180 mg) and large larvae (>180 mg) and their relationship to each other. Since no adult emergence was observed after pupation in 60-day groups less than 100 mg, the data in this group were not evaluated, and only five groups were compared and control groups. Independent t-test was performed for paired comparisons according to larval weights. For statistical analysis, average data of three trials (10 insects in each trial with three repeats giving 30 insects in total) for each cold application were obtained.

Results

The differences between the weights of the pupae ($F_{4,524}$ = 2.45, p < 0.001; $F_{2,524}$ = 115, p < 0.001; $F_{8,524}$ = 12.5, p < 0.001) and adults ($F_{4,499}$ = 11.8, p < 0.001; $F_{2,499}$ = 32.4, p < 0.001; $F_{8,499}$ = 8.16, p < 0.001) (including control groups) for small, medium and large larvae stored in the cold for 5-60 d were statistically significant (Table 1). No adults developed from small larvae stored in the cold for 60 d.

Table 1. Two-way ANOVA-Tukey HSD test results of the pupal and adult weight obtained from *Tenebrio molitor* larvae stored in cold for 5-60 d

Larval group	Periods of cold storage (d)					
	5	10	20	30	60	Control
Pupae (mg, mean ± SD)						
<100 mg	144 ± 23.2 Aa*	126 ± 20.4 Ba	128 ± 27.8 ABa	123 ± 17.5 Ba	126 ± 23.5 Ba	152 ± 19.6 Ca
100-180 mg	119 ± 18.8 Ab	141 ± 16.7 Bb	124 ± 17.3 Ba	131 ± 22.8 ABa	132 ± 19.6 ABa	158 ± 17.1 Ca
>180 mg	163 ± 26.0 Ac	164 ± 26.7 Ac	165 ± 8.8 Ab	151 ± 15.8 Ab	164 ± 24.9 Ab	156 ± 18.3 Ba
Adults (mg, mean ± SD)						
<100 mg	122 ± 16.9 Aa	112 ± 17.6 Aa	119 ± 17.8 Aa	118 ± 21.0 Aa	—	130 ± 14.3 Ba
100-180 mg	100 ± 18.3 Ab	117 ± 23.0 Ba	109 ± 15.6 ABb	113 ± 23.2 ABb	116 ± 16.7 Bb	129 ± 15.6 Ca
>180 mg	104 ± 12.6 Ab	143 ± 19.7 Bb	140 ± 8.4 Bc	123 ± 26.1 Ca	133 ± 16.5 BCa	128 ± 13.2 Da

* Means followed by the same uppercases letter within rows or lowercase letter with columns are not statistically significant (p > 0.05).

There was a difference between the emergence times of adults between all larval groups exposed to cold for 5-60 d ($F_{4,534}$ =5.90, $p < 0.001$; $F_{2,526} = 3.84$, $p = 0.022$; $F_{8,526} = 8.16$, $p < 0.001$) (Table 2). There were statistical differences between the proportion of adult emergence compared to the control ($F_{4,46} = 14.7$, $p < 0.001$; $F_{2,46} = 10.3$, $p < 0.001$; $F_{8,46} = 1.71$, $p = 0.014$) (Table 2).

Table 2. Two-way ANOVA-Tukey HSD test results of the adult emergence time and proportion of adult emergence obtained from *Tenebrio molitor* larvae stored in cold for 5-60 d

Larval group	Periods of cold storage (d)					
	5	10	20	30	60	Control
Adult emergence time (d, mean ± SD)						
<100 mg	7.3 ± 0.9 Aa*	6.4 ± 1.5 Aa	7.4 ± 2.5 Aa	7.5 ± 0.8 Aa	—	5.9 ± 0.6 Ba
100-180 mg	6.8 ± 0.9 Aa	7.5 ± 1.5 Ab	7.2 ± 0.8 Aa	7.3 ± 0.7 Aa	9.0 ± 1.5 Bb	5.6 ± 1.6 Ca
>180 mg	7.9 ± 0.8 Aa	7.5 ± 1.1 Ab	6.9 ± 1.1 Aa	7.6 ± 0.7 Aa	7.0 ± 0.9 Aa	5.0 ± 1.2 Ba
Proportion of adult emergence (%, mean ± SD)						
<100 mg	53.3 ± 15.2 Aa	40.0 ± 13.2 Aa	35.0 ± 12.9 Ba	25.0 ± 11.7 Ca	—	94.1 ± 12.4 Da
100-180 mg	41.6 ± 7.6 Aa	45.0 ± 13.5 Aa	38.3 ± 12.3 Aa	40.0 ± 18.0 Aa	8.0 ± 5.0 Ba	95.2 ± 9.8 Ca
>180 mg	68.3 ± 12.5 Ab	62.6 ± 6.3 Ab	60.6 ± 7.5 Ab	56.3 ± 10.4 Ab	10.6 ± 1.5 Bb	98.5 ± 13.5 Ca

* Means followed by the same uppercases letter within rows or lowercase letter with columns are not statistically significant (p > 0.05).

The differences between the longevity ($F_{4,524} = 2.76$, $p = 0.027$; $F_{2,524} = 3.33$, $p = 0.037$; $F_{8,524} = 1.54$, $p < 0.001$) and proportion of deformation ($F_{4,44} = 148$, $p < 0.001$; $F_{2,44} = 9.53$, $p < 0.001$; $F_{8,44} = 112$, $p < 0.001$) between all larval groups were statistically significant (Table 3).

The proportion of deformation increased in direct relationship as the period of exposure to low temperature. The proportion of adult deformation of adults developing from larvae exposed to cold increased up to 90% especially for 30 and 60 d (in medium and large larvae).

Table 3. Two-way ANOVA-Tukey HSD test results of the adult longevity (d, mean ± SD) and proportion of adult deformation (%, mean ± SD) obtained from *Tenebrio molitor* larvae stored in cold for 5-60 d

Larval group	Periods of cold storage (d)					
	5	10	20	30	60	Control
Longevity (d, mean ± SD)						
<100 mg	19.2 ± 5.0 Aa*	16.1 ± 4.7 ABa	14.0 ± 6.5 Ba	14.1 ± 3.8 Ba	—	25.1 ± 6.8 Ca
100-180 mg	15.2 ± 4.8 Ab	14.6 ± 6.9 Aa	14.2 ± 3.0 Aa	14.6 ± 4.6 Aa	15.1 ± 4.6 Aa	27.8 ± 3.2 Ba
>180 mg	16.1 ± 1.7 Ab	15.6 ± 7.4 Aa	15.7 ± 5.6 Aa	16.2 ± 7.3 Aa	16.6 ± 4.4 Aa	28.2 ± 7.7 Ba
Proportion of deformation (%, mean ± SD)						
<100 mg	32.6 ± 5.0 Aa	45.3 ± 2.5 Ba	48.3 ± 3.5 Ba	62.3 ± 5.5 Ca	—	9.7 ± 3.1 Da
100-180 mg	10.6 ± 2.5 Ab	40.3 ± 5.0 Bbc	42.3 ± 5.0 Bbc	48.3 ± 2.0 Bb	90.6 ± 4.3 Ca	8.9 ± 4.3 Da
>180 mg	12.1 ± 2.0 Ab	30.3 ± 4.3 Bc	33.1 ± 1.5 Bc	45.6 ± 3.7 Cb	92.3 ± 2.5 Da	7.3 ± 3.7 Ea

* Means followed by the same uppercases letter within rows or lowercase letter with columns are not statistically significant (p > 0.05).

Discussion

This study showed that as duration of cold exposure increased, especially in adults the proportion of deformation increased. In addition, the weights of pupa and adult obtained from the larvae in cold storage changed as the storage period increased. Although, no change was observed in the adult emergence time groups except for the 60-d-cold group and the control. The proportion of adult emergence was clearly lower in the 60-d-cold group. In terms of longevity, only the small larva group was affected and all groups were found to be significantly different from the control. Therefore, the initial hypothesis was confirmed that proportion of deformation increases with cold storage time. Although food was placed in Petri dishes during the cold storage process, the larvae remained inactive and either stopped feeding completely or fed only a little. However, the larvae were returned to laboratory conditions after cold storage recommenced feeding. For this reason, the survival of these larvae during and after cold storage depends on the proteins and lipids they can store for surviving the cold storage period. The data obtained and the increase in the deformation rate in parallel with the cold storage time show that long-term storage affects these reserves considerably (Graham et al., 2000; Kalyoncu et al., 2005; Geng et al., 2019).

In the study of Sönmez & Koç (2019), it was determined that the adult emergence times were prolonged as the time of cold exposure of the pupae increased. The proportion of deformation in emerged adults increased with duration of cold exposure. Adult weights did not change, while adult emergence rate decreased. The proportion of adult emergence of adults decreased and the deformation rate of emerging adults increased with the exposure to low temperature. However, as the exposure time to cold increased, there was a tendency was for no change in adult emergence time except when compared to control. Therefore, from a mass production point of view, larvae are more tolerant to low temperature exposure than pupae (Ludwig, 1956; Punzo & Mutchmor, 1980; Riberio, 2017). In other words, it is evident from this study that larvae kept in cold are more resistant than those kept in the pupal stage (Paul et al., 2017). According to Sönmez & Koç (2019), these differences in the proportion of adult emergence and adult emergence time could indicate a positive correlation between the lipid content stored by the larvae in their bodies and their tolerance to low temperatures (Chown & Gaston, 2010; Mitchella et al., 2017). Especially the proportion of adult emergence of the large larvae was higher than others (for example, when 5 d cold storage, small, medium and large larvae were compared). Therefore, large larvae have more stored lipids and use it for pupa formation, and have a greater tolerance to low temperatures (Packer & Corbet, 1989; Smith, 2002; Beukeboom, 2018). Since smaller larvae store less lipids, it has been reported that the generations from these may cause a decrease in fecundity and longevity, and delays in development (Mirth & Riddiford, 2007).

Acclimation is an adaptation to temperature changes and can cause deformations in some insects. Insects develop different adaptations to survive at low temperature. One of these is water loss, which reduces changes in biology at low temperatures (Punzo & Rosen, 1984; Taşkın & Ergin, 2013; Mitchella et al., 2017). In the present study, it was determined that as the exposure time of the larvae to low temperature increases, the proportion of adult deformation increases in direct proportion. Especially in medium and large larvae, the proportion of deformation in larvae exposed cold for 60 d has increased up to 90% (Table 3). It was determined that all pupae obtained from the small larvae that were exposed to cold for 60 d died and no adult emergence. It can be argued that the group most resistant to cold in terms of the resulting proportion of adult emerging was the large larvae. However, the deformation proportion of the emerging adults also increased as the duration of exposure to cold increased. The high resistance of medium and large larvae to low temperature is probably enabled by the lipids and proteins stored by the insect during embryological development, and allows the larval stage to reach adult. However, the rate of deformation of adults may be due to their inability to resist dehydration due to the period of time at low temperature (Punzo & Rosen, 1984; Selaledi et al., 2020). Large larvae appear to be more tolerant of low temperatures because they contain more lipids and proteins, but the emerging adult will not be able to have a healthy life because of higher deformation rates.

Kalyoncu et al. (2005) exposed the pupae of *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) to cold and found that the adults obtained from these pupae were small and some of them had curved wings. Pupae hatching percentage decreased depending on application time. Smith (2002) found that *Nicirophorus investigator* (Zetterstedt, 1824) (Coleoptera: Silphidae) adults that spent the winter as larvae were larger than those that spent the winter as adults and had higher survival rates. Geng et al. (2019) found that when *Phyllonorycter ringoniella* Matsumura, 1931 (Lepidoptera: Gracillariidae) egg, larva and pupa were cold stored (4ºC, 0-105 d), the highest deformation rate was observed in those in the pupal stage. The deformation rate increased in direct proportion to the duration of exposure to low temperature. Similar to the present study, in a study conducted with *T. molitor*, Graham et al. (2000) separated the larvae by their weight: 11-13, 100-120 and t>190 mg. They found that exposed the larvae to 4ºC for 4 weeks, thermal hysteresis proteins increased regardless of the larval size, but growth and development continued. In addition, they found that in the pupal stage, these proteins decreased twentyfold, and development and growth stopped. For this reason, it may be more appropriate for *T. molitor* to keep larvae of >180 mg rather than pupae. Also, adult deformation rates should be taken into account and larvae should not be kept for more than 20 d in the cold. Temperature changes can also affect the longevity of insects. In a study they conducted on *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae), Karabörklü & Ayvaz (2007) stored egg, larvae and pupae at 4ºC for 10, 20, 30 and 40 d. As the exposure time increased, the number and longevity of adults obtained from all stages significantly decreased. In the present study, the proportion of adults and longevity decreased with storage period, especially for small and medium larvae. Ayvaz & Karabörklü (2008) found the mortality close to 50% after 6 weeks and 100% after 10 weeks in *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) exposed to 10ºC for 1-10 weeks. In the present study, small larvae, in particular, could not tolerate 60 d of cold storage and there was no adult emergence. Adults obtained from medium and large larvae exposed to cold for only 5 d had shorter survival than small larvae. While the longevity of small larvae decreased as cold exposure time increased, no change was observed between medium and large larvae (Mirth & Riddiford, 2007). Gülel (1982) found that the lifespan of *Dibrachys boarmiae* Walker, 1863 (Hymenoptera: Pteromalidae) adults kept at 4ºC for 15 d increased, whereas *Apanteles galleria* Wilkinson, 1932 (Hymenoptera: Braconidae) adults died at 6ºC. In contrast, adults of *Panolis flammea* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae) emerging from the pupa exposed to 2°C for 5-20 d had decreasing longevity with increased exposure (Leather, 1990).

Tunca et al. (2014) studying *Venturia canescens* Gravenhorst, 1829 (Hymenoptera: Ichneumonidae) found that the decrease in temperature and extended storage time (5, 10 and 15ºC for 1, 3, 5, 7 and 15 d) did not affect longevity or adult weight. A study conducted by Tunca et al. (2014) gave similar results in terms of longevity and adult weight. Low temperature exposure of larvae can directly affect the size of pupae and adults (Mirth & Riddiford, 2007; Arbab, 2019). Insects consume lipids when kept at low temperatures, which can result in weight loss (Nurullahoğlu & Kalyoncu, 2000). In the present study, it was determined that the weight of pupae obtained from small larvae decreased compared to the control group. It was found that adults decreased compared to the control group, but no change was observed between the cold treatment groups. Temperature changes affect the life cycles of insects and vary according to their developmental stages (Ludwig, 1956; Bowler, 1967; Mirth & Riddiford, 2007; Costa et al., 2016; Mitchella et al., 2017; Arbab, 2019). This may be due to an increase in weight with the increase in tolerance to low temperature, as well as an increase in lipid storage (Mitchella et al., 2017; Paul et al., 2017; Beukeboom, 2018).

Conclusions

Tenebrio molitor larvae or pupae are typically cold stored during laboratory studies or during commercial mass production when the population has reached the desired density. However, low storage temperature and long exposure times will change the biology of this insect. Such exposure will particularly increase the number of deformed insects. Given that the amount of protein and lipid stored in larvae will decrease during cold storage, it may not be appropriate to use cold-stored insects in biological studies or as a commercial live-feed product. In this study, it was determined that as the storage period increases, the proportion of deformation particularly increases, and the weight of pupae and adults is decreases particularly for small larvae. Consequently, producers or researchers need to pay attention to the size of the larvae in order to obtained the best quality product and highest yield. Therefore, it is recommended that larvae are not stored for more than 20 d. Future studies should also investigate to what extent eggs of *T. molitor* are affected by low temperature exposure.

Acknowledgments

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