KSÜ Tarım ve Doğa Derg 25 (1): 150-157, 2022
KSU J. Agric Nat 25 (1): 150-157, 2022
https://doi.org/10.18016/ksutarimdoga.vi.872440

# The Influence of Age and Exposure Time on the Susceptibility of *Carpophilus hemipterus* Pupa to High Carbon Dioxide with Low Oxygen Treatment

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## ABSTRACT

Modified/controlled atmosphere applications, as an alternative to the chemical treatments, are an effective technique in controlling pest of stored products, especially dried fruits. In gas tight units, it is applied with the principle of preventing the insect respiration by changing the oxygen ($O_2$), carbon dioxide ($CO_2$) and nitrogen ($N_2$) gas levels of the atmosphere. In the study, 1-, 2- and 3- d-old pupae of dried fruit beetle, *Carpophilus hemipterus* (L.), which is an important dried fruit pest, were exposed to the modified / controlled atmosphere. The modified/controlled atmosphere consisting of 2.1% $O_2$ + 90% $CO_2$ + 7.9% $N_2$ gas mixture was applied for 48, 72, 96 and 120 h at 20°C and 75 ± 5% relative humidity. One, two and three d old pupae responded similarly to modified/controlled atmosphere application. Mortality rates remained close in each exposure time and no significant difference was found between age groups. Unlike the age factor, the exposure time was found to be statistically significant and the mortality rates increased as the exposure time increased in each age group. The highest mortality rates were found as 38, 60 and 47% in 1-, 2- and 3-d old pupae after 120 h of application, respectively.

**Research Article**

**Article History**
Received : 01.02.2021
Accepted : 22.04.2021

**Keywords**
Nitidulidae
Modified Atmosphere
Dried Fruit
Sap Beetle
Mortality

# *Carpophilus hemipterus* (L.) Pupasının Düşük Oksijenli Yüksek Karbondioksit Uygulamasına Duyarlılığı Üzerinde Yaşın ve Uygulama Süresinin Etkisi

## ÖZET

Kuru meyveler başta olmak üzere depolanmış ürün zararlılarıyla mücadelede, kimyasal kullanımına alternatif olarak, değiştirilmiş / kontrollü atmosfer uygulamaları etkili bir savaşım tekniğidir. Gaz geçirmez ünitelerde, atmosferin oksijen ($O_2$), karbondioksit ($CO_2$) ve nitrojen ($N_2$) gazı seviyelerinin değiştirilmesi sayesinde solunumun engellenmesi prensibiyle uygulanır. Araştırmada, önemli bir kuru meyve zararlısı olan ekşilik böceği, *Carpophilus hemipterus* (L.)'un 1, 2 ve 3 günlük pupaları değiştirilmiş / kontrollü atmosfere maruz bırakılmışlardır. Gaz karışımı %2.1 $O_2$ + %90 $CO_2$ + %7.9 $N_2$' den oluşan değiştirilmiş / kontrollü atmosfer 48, 72, 96 ve 120 saat süreyle 20°C sıcaklık ve 75 ± 5% orantılı nem koşullarında uygulanmıştır. Bir, iki ve üç günlük pupalar değiştirilmiş / kontrollü atmosfer uygulamasına benzer tepki vermişlerdir. Ölüm oranları değiştirilmiş / kontrollü atmosfer uygulamasının her bir uygulama süresinde yakın seyretmiş ve yaş grupları arasında önemli bir fark tespit edilmemiştir. Yaş faktörünün aksine, uygulama süresi istatistiki anlamda önemli bulunmuş ve her bir yaş grubunda uygulama süresi arttıkça ölüm oranları artmıştır. En yüksek ölüm oranları 120 saatlik uygulama sonucu 1, 2 ve 3 günlük pupalarda sırasıyla %38, 60 ve 47 olarak tespit edilmiştir.

**Araştırma Makalesi**

**Makale Tarihçesi**
Geliş Tarihi : 01.02.2021
Kabul Tarihi : 22.04.2021

**Anahtar Kelimeler**
Nitidulidae
Değiştirilmiş Atmosfer
Kuru Meyve
Ekşilik Böceği
Mutlak Ölüm

**To Cite:** Tütüncü Ş 2022. The Influence of Age and Exposure Time on the Susceptibility of *Carpophilus hemipterus* Pupa to High Carbon Dioxide with Low Oxygen Treatment. KSU J. Agric Nat 25 (1): 150-157. https://doi.org/10.18016/ksutarimdoga.vi.872440.

## INTRODUCTION

Sap beetles cause economic losses by feeding on many stored products, especially in the postharvest or ripening period of fruits and grains (Emekci and Moore, 2015). In addition, they play a role in the occurrence of serious health problems due to toxin formation (Öksüztepe and Erkan, 2016) by contaminating harmful microorganisms (Rodriguez-Del-Bosque et al., 1998; Emekci and Moore, 2015). Dried fruit beetle, *Carpophilus hemipterus* (Linnaeus, 1758) (Coleoptera: Nitidulidae) is commonly found in figs and dates (Turanlı, 2003; Burks et al., 2015; Emekci and Moore, 2015; Rosi et al., 2019), which are economically important dried fruits for world trade. According to the data of 2019, approx. 479 (1000 US$) of the world dried fruit export value of 1.461 (1000 US$) is provided by dried fig alone. In addition, dates that can be consumed in fresh and dried form have an important world export value (2.001.634 US$) (Anonymous, 2020). The common preferred method of disinfestation of stored product pests is the use of synthetic chemicals. However, the long-term implementation of synthetic chemicals can cause negative effects on the practitioners, consumers and the environment due to their residue. In addition, pests can develop resistance to the insecticides (Collins, 2006; Jagadeesan et al., 2012) as another negative effect. Therefore, it is necessary to turn to alternative pest management techniques in order to reduce the negative effects on risk groups. Non-chemical control methods have been tried and developed to remove or reduce pest populations from stored products, especially dried fruits (Emekci and Ferizli, 2000; Bagci et al., 2006; Finkelman et al., 2006; Kavallieratos et al., 2012; Abo-El-Saad and El-Shafie, 2013; Burks et al., 2015; Navarro and Navarro, 2015; Karakoç et al., 2018; Rosi et al., 2019; Tutuncu and Emekci, 2019; Yılmaz et al., 2020). Among these alternative methods, modified / controlled atmosphere treatment is applied by changing the $CO_2$ and / or $O_2$ levels of the normal atmosphere to the levels at which insects cannot perform normal respiration and metabolic activity, in a gas-tight environment, by giving $N_2$ or $CO_2$ gas or by using an exothermic gas generator (Navarro, 2012). The important factors that determine the mortality time in low $O_2$ or high $CO_2$ atmospheres are specified as the level of gas concentration, the ambient temperature and humidity at which the application takes place, the species of the insect, its biological stage and the age of the stage (Navarro, 2012). The egg stage, which is the embryological development period, and the pupal stage, which is the metamorphosis period, are relatively more resistant to modified / controlled atmosphere application compared to other active biological stages (Gbaye and Odeyemi, 2005; Riudavets et al., 2009; Wong-Corral et al., 2013; Tutuncu and Emekci, 2019). In these stages where metabolic activity is low, a U-shaped curve of oxygen consumption indicates that oxygen demand changes with development / age (Fink, 1925; Odell, 1998). Therefore, 1-, 2- and 3- d-old pupae were tested in present study. There are very few studies of modified / controlled atmosphere applications related to control of *C. hemipterus* (Donahaye et al., 1994; Emekci et al., 2003; Gbaye and Odeyemi, 2005). In these studies, the effects of the atmosphere consisting of relatively high $CO_2$ with low $O_2$ (1, 2 and 3% $O_2$ +14, 13.3 and 12% $CO_2$, respectively, balance $N_2$) and high $CO_2$ with low $O_2$ (0 - 1.2% $O_2$ + 95-100% $CO_2$, balance $N_2$) on different biological stages of *C. hemipterus* under high temperature (26-35°C) were investigated. Under these conditions, exposure times required for the death of half or all of the population are reported as 137 h (50% mortality) at 2% $O_2$, 5 d (100% mortality) at approx. 1% $O_2$ and 39.8 h (50% mortality) at 0% $O_2$, respectively.

This study was carried out on 1-, 2- and 3-d-old pupae of *C. hemipterus* for 48-120 h at low temperature (20°C) and high $CO_2$ with low $O_2$ atmosphere (2.1% $O_2$ + 90% $CO_2$ + 7.9% $N_2$). The objective of the study, rather than determining the complete mortality time, was to determine whether the lethal effect would make a difference according to age and also how effective the exposure times up to 5 d at 20°C in controlling the pupal stage.

## MATERIAL and METHOD

### Insect Culture and Pupal Stage

*Carpophilus hemipterus* was grown on artificial feed consisting of water (1 liter), corn flour (125 g), glucose (90 g), sugar (44 g), yeast (50 g), agar agar (18 g), propionic acid (3.1 ml) and methyl 4 – hydroxybenzoate (1 g) (Donahaye and Navarro, 1989) at 25 ± 5°C and 75 ± 5% relative humidity (r.h) under a dark condition. Food preparation and obtaining pupae are as described in the previous study (Tutuncu and Emekci, 2014). Briefly, food slices (7x7x1cm) prepared by cooking were placed in sterile jars (1 liter) and 100 adults of mixed sex were transferred on the food. The jars were closed by sterile American cotton cloth cut and folded in half instead of the jar lid. Eggs left between American cotton cloth by adults were taken after 24 h and were transferred to new jars containing food and their age (0-24 h old) and date were recorded. By following the biological stages, 12-d-old (from egg hatching) mature larvae were obtained from these jars. Then, the new jars containing these mature larvae were monitored daily and 1-d-old (0-24-h-old) pupae were obtained. Pupae aged 2 (24-48-h-old) and 3 (48-72-h-old) d were obtained by keeping 1-d-old pupae in acclimatized laboratory conditions. Pupae aged 1, 2 and 3 d were

used in the experiments.

### Experimental Equipment and Setup

Gas cylinders containing 2.1% $O_2$ + 90% $CO_2$ + 7.9% $N_2$ gas composition were used in the experiments (Linde Gas, Ankara). The experimental setup and gas washing procedure were as mentioned in Tutuncu and Emekci (2017). Briefly, Plexiglas test tube (10 ml) containing test pupae, and humidity solution tube (50 ml KOH) were placed in gas washing bottles (8 cm diameter and 25 cm height) with a capacity of 550 ml. After inserting the tubes, the lid of the gas washing bottle was closed. Gas was supplied from the gas cylinder connected to the gas flushing valve of the gas washing bottle at a flow rate of 100 ml $min^{-1}$ in 15 min. At the end of the period, the desired gas value (2.1% $O_2$) was measured with the oxygen meter (OxyCheq Expedition O2 Analyzer, OA-01-01, OxyCheck, Marianna, FL, USA) connected to the outlet valve of the gas washing bottle. After the measurement, the gas inlet and outlet valves were closed and the gas flushing process was completed. Afterwards the gas washing bottles were put into incubators at a temperature of 20 ± 0.5°C and 75 ± 5% r.h, and measurements were made with a temperature / humidity meter (Hobo® UX100-003, Onset Computer Corporation, MA, USA) inside the gas washing bottles during the exposure period. The control groups were prepared in the same way and placed in the incubator under normal atmospheric conditions, leaving the valves of the gas washing bottles open. The experiments were carried out with 3 repetitions (3 parallels x 3 replicates) using 30 pupae in each. Dead/live pupae were counted at 0 (control), 48, 72, 96 and 120 h of exposure time.

### Post-treatment Evaluation

At the end of the exposure period, the gas washing bottles were removed from the incubator and the Plexiglas test tube containing the test pupae were removed and aerated. In addition, 1 g of food was added to the test tubes in order to prevent the adult cannibalism. The test tubes were kept in the acclimatized insect rearing room at 25 ± 5°C and 75 ± 5% r.h until the end of the observation after the experiment. After daily count, pupae become adult were removed from the Plexiglas test tubes and recorded as alive. The observations were continued until the pupae did not have any signs of vitality (i.e., they dried up and darkened). The numbers of alive and dead in the control groups were performed using the same method as the test groups.

### Statistical Analysis

Since the mortality rate in the control group was less than 5%, no correction was applied to the control. Factorial ANOVA was used for the statistical analysis of mean mortality rates. The mean differences between exposure times and pupal ages was made according to the Duncan's multiple range test ($P < 0.05$). Statistical calculations were made using the Statistica 7.0 (StatSoft, 2004) program.

## RESULTS

Mortality rates of *C. hemipterus* pupae, aged 1, 2 and 3 d, exposed to gas composition of high $CO_2$ with low $O_2$ at 20°C and 75 ± 5% r.h were shown in Figure 1.

According to the statistical values of "exposure time" ($F(4; 14) = 28.195$, $P$=0.0001), "age" ($F(2; 14) = 0.800$, $P$=0.4684) and "exposure time x age" interaction ($F(8; 14) = 1.626$, $P$=0.2036), only the exposure time was found to have significant effect on mortality rates of *C. hemipterus* pupae. The mortality rate increased with the increase in exposure time in each pupal age group. However, the statistically difference was generally seen between 120 h and other exposure times (Table 1). For 1-d-old pupa, although ≥96 h of exposure time resulted in significantly higher pupal mortality compared to the control group, 120 h of exposure was not sufficient to achieve complete pupal mortality, and 38% mortality was obtained. In the comparison of 1-, 2- and 3-d-old pupae, there was no statistically significant difference between ages. However, at 72 and 96 h of exposure times, low mortality rates were observed in 2- and 3-d-old pupae, while the mortality rate for 1-d-old pupae increased up to 33%, was remarkable. The opposite situation was observed at 120 h of exposure. The highest mortality rate was observed at approx. 60% for 2-d old pupae. This was followed by 3-d-old pupae and 1-d-old pupae (Table 1 and Figure 1).

## DISCUSSION and CONCLUSION

The highest mortality (60%) was achieved in 2-d-old pupae of *C. hemipterus* for 5 d of exposure time under high $CO_2$ with low $O_2$ atmosphere. This highest mortality rate (60%) detected at 20°C is supported by the result of Donahaye et al. (1994), carried out at 26°C. Researchers determined the lethal time 50 ($LT_{50}$) values of the 1-and 2-d-old *C. hemipterus* pupa in a low $O_2$ atmosphere (2% $O_2$ with 12% $CO_2$) at 26, 30 and 35°C as 137, 43 and 36 h, respectively (Donahaye et al., 1994). Due to the low $O_2$ level (<1% $O_2$ + 97% $CO_2$) and the high temperature (average 27.97°C), the mortality rate obtained in the mixed culture of *C. hemipterus* is higher than that found in the current study. In the study, 100% mortality was achieved after 5 d of modified atmosphere application (Emekci et al., 2003). As in the current study, low mortality rates were also observed in 2-4-d-old pupae of *Carpophilus dimidiatus* (Fabricius, 1972) (Coleoptera: Nitidulidae) after 8 h hours of modified atmosphere (pure $CO_2$ + ≤1% $O_2$) application at 29.5°C, and 33.3% mortality was obtained (Odeyemi

et al., 2004). However, complete mortality was achieved by applying pure (100%) $CO_2$ to *C. hemipterus* pupae (2-4-d-old) for 8 h at 29.0 ± 2°C and 90 ± 5 r.h (Gbaye and Odeyemi, 2005). Although both studies were performed at nearly the same temperature, gas concentration and exposure time, the high mortality rate in *C. hemipterus* for such a short exposure time, is probably due to the difference in species. Also, complete mortality for *C. hemipterus* pupae achieved for 8 h of exposure time (Gbaye and Odeyemi 2005) is not similar to the result of the current study. This could be explained by the difference in temperature and gas concentration among the parameters explained below. As the temperature increases, the critical $O_2$ level

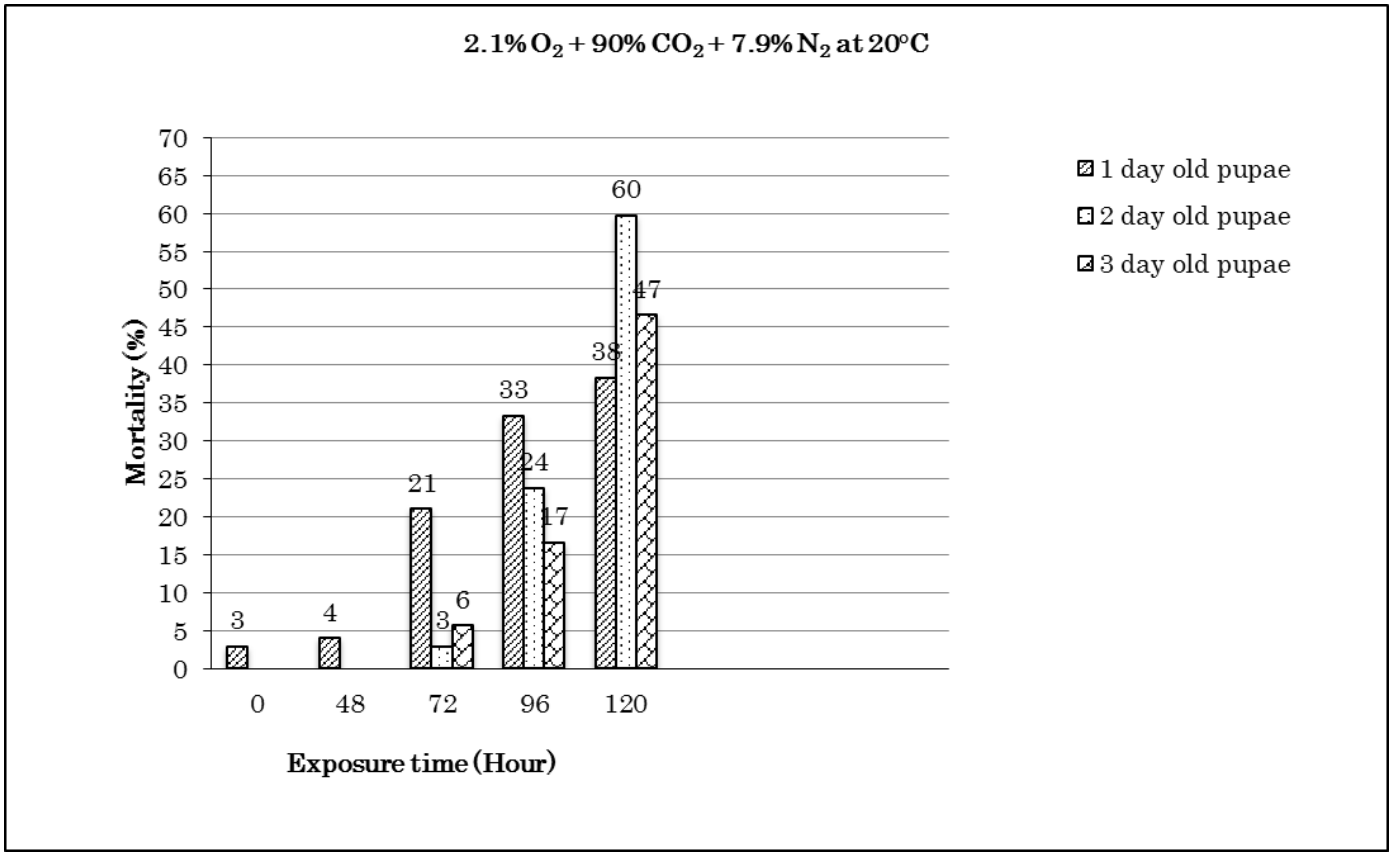


Figure 1. Mortality rates of different aged pupae of *Carpophilus hemipterus*, exposed to high carbon dioxide with low oxygen (2.1% $O_2$ + 90% $CO_2$ + 7.9% $N_2$) treatment for different periods at 20°C

*Şekil 1. Düşük oksijenli yüksek karbondioksit atmosfer uygulamasına farklı uygulama sürelerinde ve 20°C sıcaklıkta maruz bırakılan değişik yaşlı Carpophilus hemipterus pupalarının ölüm oranları*

Table 1. Mean ± SE mortality (%) of different aged pupae of *Carpophilus hemipterus* exposed to high carbon dioxide with low oxygen (2.1% $O_2$ + 90% $CO_2$ + 7.9% $N_2$) treatment for 48, 72, 96 and 120 hours at 20 °C

*Çizelge 1. Düşük oksijenli yüksek karbondioksit (%2.1 $O_2$ + %90 $CO_2$ + %7.9 $N_2$) uygulamasına 20 °C sıcaklıkta 48, 72, 96 ve 120 saat süreyle maruz kalan Carpophilus hemipterus 'un değişik yaşlı pupalarına ait ortalama ± SH ölüm oranı (%)*

| Exposure time (hours) | Biological stage | | | | | |
|---|---|---|---|---|---|---|
| | n | 1-day-old pupae | n | 2-day-old pupae | n | 3-day-old pupae |
| 0 (control) | 360 | 2.94 ± 9.32 aA | 360 | 0.00 ± 6.59 aA | 360 | 0.00 ± 6.59 aA |
| 48 | 270 | 4.0 ± 9.32 aA | 270 | 0.00 ± 9.32 aA | 270 | 0.00 ± 9.32 aA |
| 72 | 270 | 21.05 ± 9.32 abA | 270 | 2.94 ± 9.32 aA | 270 | 5.71 ± 9.32 aA |
| 96 | 270 | 33.33 ± 5.38 bA | 270 | 23.79 ± 5.28 aA | 270 | 16.66 ± 5.38 aA |
| 120 | 270 | 38.33 ± 5.38 bA | 270 | 59.72 ± 5.38 bA | 270 | 46.66 ± 5.38 bA |

*In each column, the same lowercase letters and in each row, the same uppercase letters mean that the difference between the means for exposure times and pupal age is not significant (Duncan's) ($P$>0.05)

*Her bir kolona ait aynı küçük harfleri ve her bir satıra ait aynı büyük harfleri içeren ortalamalar arasındaki fark Duncan testine göre istatistiki olarak önemli değildir ($P$ >0.05)*

needed by the insect to survive increases (Zhou et al., 2000), and also insect metabolism accelerates and the effect of $CO_2$ increases (Navarro, 2012). Both the fact indicate that the increase in temperature shortens the mortality time or mortality rate in modified / controlled atmosphere applications. Sen et al. (2010) showed that the application of low $O_2$ (1% $O_2$ + 12% $CO_2$) in a high temperature (41°C) may be sufficient to provide complete mortality for a short exposure time. Sixteen hours of application in the study conducted at 41°C, was sufficient to complete mortality in the mixture culture of *Carpophilus* spp. At similar gas level tested by Sen et al. (2010) the $LT_{95}$ value was found as 60 h for the pupae of *C. hemipterus* at 35°C (Donahaye et al., 1994). It can be commended that the main factor affecting the difference between complete mortality times in these two studies conducted at similar gas concentrations, is temperature. In addition, the effect of temperature on the exposure time in modified atmosphere applications is also emphasized by Donahaye et al. (1994). In these two studies (Donahaye et al., 1994; Sen et al., 2010), the mortality rates determined in short exposure times are considerably higher than the mortality rate of 60% after 120 h of exposure in this study. The parameters affecting this difference are seen as temperature and $O_2$ / $CO_2$ gas levels. Increase in mortality rate and the shortening of the complete mortality time due to the increase in $CO_2$ levels have been reported in previous studies (Navarro et al., 2002; Hashem et al., 2012; Wong-Corral et al., 2013). Also, in another study complete mortality could not be achieved in some species even with 12 d of exposure at 50% $CO_2$ (with 3% $O_2$) concentration, while was achieved in 12 d with 90% $CO_2$ (with 3% $O_2$) (Riudavets et al., 2009). In addition, relationship between $O_2$ level increase and mortality time increase or mortality rate decrease, was observed in different stage of *C. hemipterus*, *Urophorus humeralis* (Fabricius, 1798) (Coleoptera: Nitidulidae), *Cadra cautella* (Walker, 1863) (Lepidoptera: Pyralidae), *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) and partly *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Curculionoidea) (Navarro, 1978; Donahaye et al., 1994). Humidity level, such as temperature and gas concentration, is a physical parameter that affects the mortality rate / complete mortality time in modified /controlled atmosphere applications. While it is stated that low humidity conditions increase the mortality rate in modified / controlled atmosphere applications (Ofuya and Reichmuth, 2002), it is seen that this effect should be evaluated on the basis of species (Soderstrom et al., 1986). Although various relative humidity levels were not studied in present study, it is not thought that the humidity level of the study has a decreasing or increasing effect on mortality. Because, the humidity level in which this study was conducted is optimal for the survival, reproduction and development of *C. hemipterus* (James and Vogele, 2000; Kumkum, 2017). In addition, mortality rates were below 5% in the control group exposed to the normal atmosphere under the same temperature and humidity conditions as the experimental groups.

According to the present results of the study, the mortality was increased by the increasing exposure time. However, the significant difference started from 96 h of exposure in 1-d-old pupae, while it was between 120 h of exposure and shorter exposure times for 2- and 3- d-old pupae. Similarly, the positive correlation between exposure time and increase of mortality in 2-4-d-old pupae of *C. hemipterus* exposed to 100% $CO_2$ (Gbaye and Odeyemi, 2005) supports the results obtained from present study. In the study increase in mortality (from approx. 10% to 100%) with increased exposure from 6 to 8 h was found to be significant. In *C. dimidiatus* pupae, the mortality rate increased with the increase in the exposure time, but this increase was not found to be statistically significant (Odeyemi et al., 2004). Such differences between species belonging to the same genus have also been seen in previous studies (Lindgren and Vincent, 1970; Conyers and Bell, 2007). As the exposure time increases, the mortality rate increases in other stored product pest species were also observed. In a study involving many species *Lasioderma serricorne* (Fabricius,1792) (Coleoptera: Anobiidae), *Cryptolestes ferrugineus* (Stephens, 1831) (Coleoptera: Cucujoidea), *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae), *Tribolium confusum* (Jaqcquelin du Val, 1868) (Coleoptera: Tenebrionidae), *S. oryzae* and *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae) of the order Coleoptera, *Plodia interpunctella* (Hübner,1813) (Lepidoptera: Pyralidae) and *Ephestia kuehniella* (Zeller,1879 ) (Lepidoptera: Pyralidae) of the Lepidoptera, a psocid and mite *Liposcelis bostrychophila* (Badonnel, 1831) (Psocoptera: Liposcelididae), *Tyrophagus putrescentiae* (Schrank) (Astigmata: Acaridae), this situation was generally observed in all species (Riudavets et al., 2009). Similarly, as the exposure time increased, an increase in mortality rate was observed in the modified atmosphere application performed in 3-96 h interval in 3-d-old pupae of *O. surinamensis* (Hashem et al., 2012). In another study performed at 50-90% $CO_2$ (in air) concentration between 3-9 d, this correlation was demonstrated in the pupae of *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae), *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae) and *Zabrotes subfasciatus* (Bohemann, 1833) (Coleoptera:

Chrysomelidae) (Wong-Corral et al., 2013). In addition, are other studies Locatelli and Daolio (1993 and Husain et al. (2017) reported that the mortality rate increases significantly with the increase in exposure time in Coleopteran and Lepidopteran species. The effect of modified / controlled atmosphere application on the development of pupae at different ages has been shown in *Sarcophaga crassipalpis* (Macquart, 1839) (Diptera: Sarcophagidae) (Kukal et al., 1991). In the study, while 1-5-d-old pupae exposed to low $O_2$ atmosphere for 4 d could not complete their development, 5 to 8-d-old pupae were able to complete their development and eclose to adult stage. In another study, at 90% $CO_2$, 1-d-old *C. cautella* pupae were found to be more resistant, while at 96% $CO_2$ 1- and 3-d-old pupae were more resistant than 2-d-old pupae (Tutuncu and Emekci, 2019). Similarly, in Storey's (1975) study, it was observed that resistance changes in pupae of *P. interpunctella* and *C. cautella* depending on their age. However, according to the results of this study, mortality rates in 1, 2 and 3-d-old pupae of *C. hemipterus* were found to be similar in all exposure times and no significant difference was detected between 1-, 2- and 3-d-old pupae.

In this study, the effect of high $CO_2$ with low $O_2$ atmosphere treatment for up to 5 d of exposure time on different aged pupae of *C. hemipterus* has been demonstrated at low temperature. These results are thought to contribute to the preference of modified / controlled atmosphere application and determination of application parameters for the control of *C. hemipterus*.

## Statement Contribution of the Authors

Authors declares the contribution of the authors is equal.

## Statement of Conflict of Interest

Authors have declared no conflict of interest.

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