

Orijinal araştırma (Original article)

Toxicity of carbon dioxide to different developmental stages of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Trichogramma embryophagum* Hartig (Hymenoptera: Trichogrammatidae)

Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) ve *Trichogramma embryophagum* Hartig (Hymenoptera: Trichogrammatidae)'un farklı gelişme evrelerine karşı karbon dioksitin toksik etkisi

Dilek PANDIR^{1*}

Fahriye SÜMER ERCAN¹

Hatice BAŞ¹

Summary

The use of carbon dioxide (CO₂) is a very effective method for controlling insect pests. The effects of CO₂ on developmental stages of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Trichogramma embryophagum* Hartig (Hymenoptera: Trichogrammatidae) were determined in this study. We aimed to establish a technique that can be used for controlling the stored-product pest, *E. kuehniella* with a natural enemy, *T. embryophagum*. Egg, larva, pupa and adult stages of *E. kuehniella* and *T. embryophagum* were exposed to 0, 10 and 20% CO₂ gases in a CO₂ incubator at 27 °C and atmospheric pressure for different exposure times. The adult stage of *E. kuehniella* was more sensitive than its egg, larva and pupa in either percentage of CO₂ or increasing exposure time. But the adult of *T. embryophagum* was the most sensitive stage at only highest dose of CO₂. On the other hand the larvae of *T. embryophagum* were the most resistant stage at 10% CO₂. Consequently, the developmental stages of two insect species had different susceptibility to increasing percentage of CO₂. These results suggest that a low percentage of CO₂ may be used with *T. embryophagum* for controlling the stored-product pest, *E. kuehniella*.

Key words: Carbon dioxide, *Ephestia kuehniella*, *Trichogramma embryophagum*, developmental stages

Özet

Zararlı böceklerin kontrolünde karbon dioksit (CO₂) kullanımı oldukça etkili bir yöntemdir. Bu çalışmada *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) ve *Trichogramma embryophagum* Hartig (Hymenoptera: Trichogrammatidae)'un gelişim evreleri üzerine CO₂'in etkisi belirlenmiştir. Depolanmış ürün zararlısı *E. kuehniella*'nın kontrolü için doğal düşman *T. embryophagum* ile birlikte kullanılacak bir yöntem geliştirilmesi amaçlanmıştır. *E. kuehniella* ve *T. embryophagum*'un yumurta, larva pupa ve ergin evreleri CO₂ inkübatörü içerisinde 27 °C ve atmosferik basınç altında değişik uygulama sürelerinde % 0, 10 ve 20'lik CO₂ gazına maruz bırakılmıştır. Artan uygulama süresinde ve her iki CO₂ konsantrasyonunda *E. kuehniella*'nın ergin evresi yumurta, larva ve pupadan daha duyarlıdır. Fakat *T. embryophagum* için sadece en yüksek CO₂ dozunda en duyarlı evre ergin evredir. Diğer taraftan %10 CO₂ uygulamasında *T. embryophagum*'un larva evresi en dirençli evredir. Sonuç olarak, artan CO₂ konsantrasyonuna karşı farklı iki böcek türünün gelişim evreleri farklı duyarlılığa sahiptir. Bu sonuçlar depolanmış ürün zararlısı *E. kuehniella*'nın kontrolü için düşük CO₂ dozunun *T. embryophagum*'la birlikte kullanılabilceğini göstermektedir.

Anahtar sözcükler: Karbon dioksit, *Ephestia kuehniella*, *Trichogramma embryophagum*, gelişim evreleri

¹ Bozok University, Faculty of Arts and Science, Department of Biology, 66100 Divanliyolu/Yozgat, Turkey

* Sorumlu yazar (Corresponding author) e-mail: durak77@gmail.com

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Introduction

Food industries preserve stored dried and desiccated fruit, ingredients of many confectionery against foodstuff pests (Locatelli et al., 1999). *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is the most ruinous pest in stored products. Control of serious pests by using chemical insecticides such as malathion, dichlorvos and methyl bromide have disadvantages because of their destructive environmental effects, including depletion of atmospheric ozone, development of resistance in insects and disruption of the food chain (Tuncbilek et al., 2009, Azizoglu et al., 2011). Therefore, many studies on stored product pests have focused on improving biological control strategies (Hodges, 1999). The egg parasitoid, *Trichogramma* genus, is used worldwide in biological control against insect pests (Schöller & Fields, 2002). At the same time, environmentally friendly techniques such as aromatic plant extracts and carbon dioxide (CO₂) treatments that do not leave any pesticide residues in stored products have been used against pests as an alternative method (AliNiazee & Lindgren, 1970; Wang et al., 2009; Bachrouh et al., 2010; Razavi, 2012). For the successful control of insect pests, the usage of a combination of effective and safe methods is preferred in an integrated pest management program.

CO₂ has toxic effects on different developmental stages and age-groups of insects. Many researchers have determined the toxic effects of CO₂ according to exposure time to kill the insects (Adler, 1999; Gunasekaran & Rajendran, 2005). Furthermore, CO₂ was used as an anesthetic against insects in many studies although the precise control mechanism was not known (Valles & Koehler, 1994). CO₂ has been known as an environmentally friendly technique for control of stored-product insects with no residue in food for a long time (Jayas et al., 1991).

In *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *T. confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *Plodia interpunctella* Hubner (Lepidoptera: Phycitidae), a high CO₂ concentration caused the reduction of egg production and hatchability (AliNiazee & Lindgren, 1970; Lum & Flaherty, 1972). In another study, a complete kill of *P. interpunctella* eggs was obtained at 25 °C and 20 bar CO₂ (Reichmuth & Wohlgemuth, 1994). *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) adults were the most sensitive stage for CO₂ treatments at different high pressures (bar) and exposure times (Noomhorm et al., 2009). Prozell & Reichmuth (2001) reported 100% mortality for *S. granarius* (Linnaeus) (Coleoptera: Dryophthoridae) and *Stegobium paniceum* (Linnaeus) (Coleoptera: Anobiidae) at 15-20 bar CO₂ up to 15 °C. Furthermore, Wang et al. (2009) stated that the application of different levels of CO₂ is expensive in a long treatment period but 10-30% CO₂ combination with another potential fumigants has been researched in several laboratories in the world for disinfestation technology.

Some researchers have evaluated the combination of different high pressures and chemicals with CO₂ against all life stages of many insects (Prozell & Reichmuth, 1990; Nakakita & Kawashima, 1994; Prozell & Reichmuth, 2001). The objective of present study was to determine the efficiency of CO₂ against *E. kuehniella*, a serious pest of many stored products, and its effects on the non-target egg parasitoid, *T. embryophagum*. For that purpose, different developmental stages of *E. kuehniella* and *T. embryophagum* were exposed to different concentrations of CO₂ in a CO₂ incubator at atmospheric pressure at different times.

Materials and Methods

Test insects

Tests were applied to different life stages of the flour moth, *E. kuehniella* and its egg parasitoid, *T. embryophagum*, which were obtained from the Biological Control Research Station, Adana. All stages of *E. kuehniella* were obtained from cultures reared at 27 ± 1 °C and 70 ± 5 % r.h. under a light regime of

14 h light, followed by 10 h darkness (14 h L:10 h D), and a diet of one kg wheat flour and 5% yeast, using standard culture techniques (Donahaye, 1990). Larvae [15-day-old young larval instars (third instar)], pupae (1-day-old) and adults (24-h-old) were collected from prepared cultures. Eggs were obtained daily from the plastic jars with a 250- μ m mesh sieve. Eggs, larvae, pupae and adults of *E. kuehniella* were treated with 0, 10 and 20% CO₂ doses for 24, 48, 72 and 96 h at atmospheric pressure. Each replicate consisted of 10 adults, larvae or pupae, or 100 eggs, for each dose and exposure time of CO₂ and each treatment was replicated six times.

For tests with *T. embryophagum*, 50 eggs of *E. kuehniella* were used as hosts. Twenty hours old *T. embryophagum* adults were used for parasitization. Tests were carried out under laboratory conditions which were set at 24 \pm 1 °C and 70 \pm 5% r.h. and under a light regime of 14 h L:10 h D. Eggs (1 day after parasitization), larvae (3 days after parasitization), pupae (6 days after parasitization) (Knutson, 1998) and adults (24 h old) were collected from prepared cultures. Immediately after they were obtained, the eggs, larvae, pupae and adult stages of *T. embryophagum* were exposed to doses of 0, 10 or 20% CO₂, according to their different life stages. Each replicate consisted of 10 adults, or 50 eggs, larvae or pupae for each dose and exposure time of CO₂ and each treatment was replicated six times.

Fumigation procedure

The CO₂ incubator (Hera Cell 150) was used for fumigation with different doses of CO₂. After fumigation, the exposed adults, larvae and pupae of *E. kuehniella* were immediately transferred into 50 mL glass jars, and maintained at 27 \pm 1 °C and 70 \pm 5% r.h. Wheat flour and yeast were added as food into the glass jar containing exposed larvae. Eggs of *E. kuehniella* were transferred to test tubes and incubated under the same conditions. In order to determine the mortalities for each dose and exposure time, each stage of *E. kuehniella* was evaluated for failure to progress to the following stage (larva to pupa, pupa to adult and egg hatchability). For *T. embryophagum*, ten adults exposed to 0, 10 or 20% CO₂ for each exposure time (24, 48, 72 or 96 h) were transferred to test tubes and *E. kuehniella* eggs were provided for parasitization. The control groups were under the same conditions but without CO₂ exposure. Moribund adults were recorded as dead. Eggs of *T. embryophagum* were exposed to 0, 10 and 20% CO₂ for 24 h and then adult emergence was recorded. Fifty larvae of *T. embryophagum* were treated with 0, 10 or 20% CO₂ for 24, 48 or 72 h. For pupae of *T. embryophagum*, the same concentrations of CO₂ were used for different exposure times (24, 48, 72 and 96 h).

Data analysis

One-way analysis of variance (ANOVA) ($p < 0.05$) in the SPSS 10.0 software package was used to compare the mortality values for different life stages of insects, followed by Tukey's procedure for multiple comparisons (SPSS, 2001).

Results

The percentage mortality of different stages of *E. kuehniella* and *T. embryophagum* exposed to CO₂ at different doses and exposure times are shown in Figs 1-2 and Tables 1-2; 94% mortality of *E. kuehniella* adults was obtained at 20% CO₂ for 96 h, while 95.83% mortality of *T. embryophagum* adults was achieved at 20% CO₂ for 96 h. On the other hand, there are not significant differences between 24-96 h exposure times at 10% CO₂ for adults of *T. embryophagum*.

Mortality significantly increased with increasing exposure time for the adult stage of *E. kuehniella* (for 10% CO₂; $F = 67.9$ $df = 4$ $p < 0.05$; for 20% CO₂; $F = 100.3$ $df = 4$ $p < 0.05$) and *T. embryophagum* (only for 20% CO₂; $F = 2805.9$ $df = 4$ $p < 0.05$).

Table 1. Mean percentage mortality of different stages of *Trichogramma embryophagum* exposed to 10% CO₂. Different letters above numbers indicate significant differences between exposure times. Numbers with the same letter are not significantly different. The ages (hour and days) of different stages were as follows: eggs, 1 day after parasitization; larvae, 3 days after parasitization; pupae, 6 days after parasitization; adult 24 h old.

Exposure Time (h)	Stage			
	Egg	Larvae	Pupae	Adult
Control	3.5±1.52	4.67±1.21 ^a	4.83±0.98 ^a	3.83±1.33 ^a
24	15.67±2.58	5.33±1.51 ^a	5.33±1.21 ^a	7±0.89 ^b
48	-	5.83±1.17 ^a	15.17±2.48 ^b	7.67±0.82 ^b
72	-	6.17±1.60 ^a	49.33±3.56 ^c	8±1.41 ^b
96	-	-	80.17±3.71 ^d	8.5±2.07 ^b

Table 2. Mean percentage mortality of different stages of *Trichogramma embryophagum* exposed to 20% CO₂. Different letters above numbers indicate significant differences between exposure times. Numbers with the same letter are not significantly different. The ages (hour and days) of different stages were as follows: eggs, 1 day after parasitization; larvae, 3 days after parasitization; pupae, 6 days after parasitization; adult 24h old.

Exposure Time (h)	Stage			
	Egg	Larvae	Pupae	Adult
Control	1.83±0.75	2.67±0.82 ^a	4.67±1.21 ^a	4.17±0.75 ^a
24	28.33±3.88	3.5±1.049 ^a	25.33±2.50 ^b	4.5±1.05 ^a
48	-	4.16±1.17 ^a	30.33±4.50 ^b	79.17±3.06 ^b
72	-	37.5±2.95 ^b	69.5±3.78 ^c	93±2.37 ^c
96	-	-	95±3.22 ^d	95.83±2.64 ^c

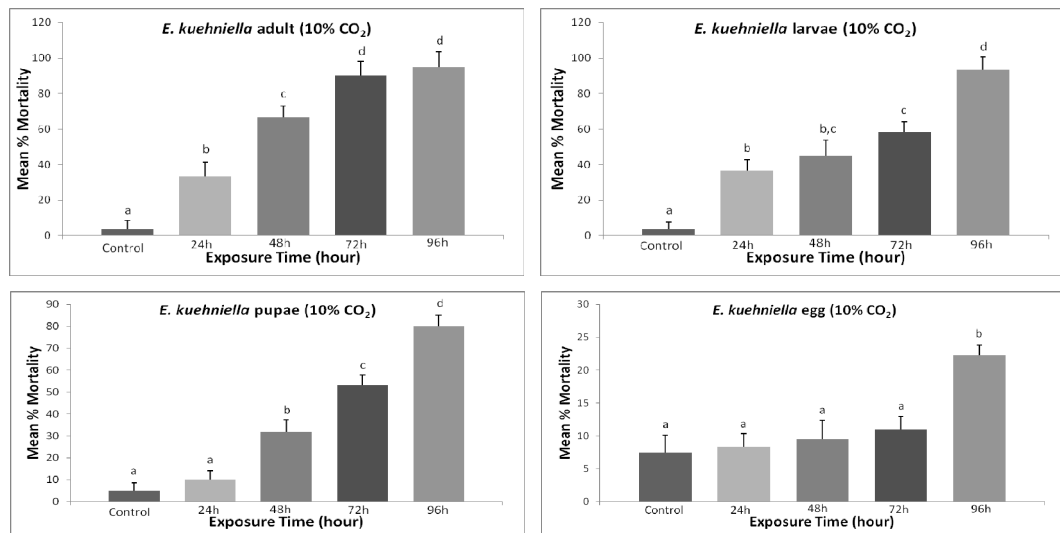


Figure 1. Mean percentage mortality of different stages of *Ephestia kuehniella* exposed to 10% CO₂. Different letters above bars indicate significant differences between exposure times; bars with the same letter are not significantly different. Error bars indicate SD of means.

The toxicity of CO₂ to the egg stage of *E. kuehniella* is shown in Figs 1-2. Twenty three percent and 59% mortality were obtained at 10 and 20% CO₂ for 96 h, respectively. Compared with the control, *E. kuehniella* eggs treated with 10% CO₂ only for 96 h had significantly reduced egg hatching. There were also significant differences between exposure times for 10% and 20% CO₂ (for 10% CO₂; F= 25.6 df= 4

$p < 0.05$; for 20% CO₂; $F = 352.8$ $df = 4$ $p < 0.05$). The egg of *E. kuehniella* was the most tolerant stage for each dose of CO₂ but *T. embryophagum* eggs reached the larval stage after 24 h exposed each dose of CO₂ (Tables 1-2).

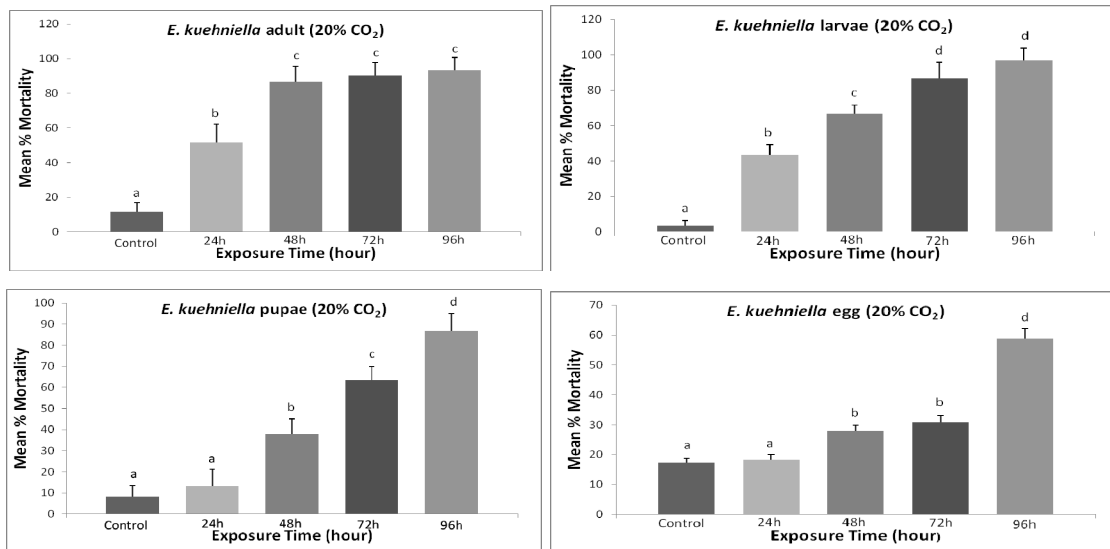


Figure 2. Mean percentage mortality of different stages of *Ephestia kuehniella* exposed to 20% CO₂. Letters above bars indicate significant differences between exposure times; bars with the same letter are not significantly different. Error bars indicate SD of means.

Ephestia kuehniella larvae were killed at 10% and 20% CO₂ for 96 h but in *T. embryophagum* larvae, there were no significant differences between exposure times at 10% CO₂ treatment ($F = 1.333$ $df = 3$ $p \leq 0.292$). The larvae of *T. embryophagum* were the most resistant stage at this concentration.

Pupae of *E. kuehniella* were more tolerant than larvae and adults at 10 and 20% CO₂. At 10% and 20% CO₂, there was a significant difference between 24-96 h exposure, and the control group was not significantly different from the 24 h application groups at 10 and 20% CO₂ (for 10% CO₂; $F = 52.7$ $df = 4$ $p < 0.05$; for 20% CO₂; $F = 39.8$ $df = 4$ $p < 0.05$). *T. embryophagum* pupae did not reach the adult stage at 20% CO₂ for 96 h.

Discussion

Carbon dioxide is one of the gases in the atmosphere, being uniformly distributed over the earth's surface at a concentration of about 0.033% or 330 ppm. It plays an important role in plant and animal metabolic processes, such as photosynthesis and respiration. Also, this gas was used as an alternative control method for disinfestation of stored-products because of its toxic effect on insects and environmental safety properties (Nicolas & Sillans, 1989). Bailey (1965) found that most stored-product insects were killed by <3% O₂ or >40% CO₂ and that CO₂ required several days exposure for different species of insects at different growth stages. In another study, development of most tested insect eggs was prevented by using high pressure (20 bar) CO₂. In the same study, adults of *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) and *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) were all killed at 20 bar pressure CO₂ treatment (Riudavets et al., 2010). Sabio et al. (2001) applied methyl bromide in combination with 0, 10 and 20% CO₂ to control *E. cautella* Walker (Lepidoptera: Pyralidae) and they revealed that this application technique decreases the methyl bromide amount and also decreases its emission that is harmful to the environment. In the present study, the same concentrations of CO₂ were used to treat developmental stages of stored-product pest *E. kuehniella* and its egg parasitoid, *T. embryophagum*.

The response of different stored-grain pests to CO₂ differs. The most tolerant stored-product insect to a CO₂ rich atmosphere (>40% CO₂) was *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) (Annis, 1986). In another study, 100% mortality was obtained for *S. zeamais* in a shorter time than that for *S. oryzae* (Annis & Morton, 1997). Variations in CO₂ tolerance of different insects may result from usage of different techniques (Noomhorm et al., 2009). Also, the time required to kill insect pests is varies for the species and its life stages. In the present study, different CO₂ concentrations (0, 10 and 20% CO₂) and exposure times (24, 48, 72 and 96 h at atmospheric pressure) were applied to all developmental stages of *E. kuehniella* and *T. embryophagum*. Lum & Flaherty (1972) reported that high CO₂ concentration reduced the egg production and hatchability of *Plodia interpunctella* (Lepidoptera: Phycitidae). In the present study, egg hatchability of *E. kuehniella* was started reducing at 10% CO₂ for 96 h exposure and 20% CO₂ for 48 h exposure. For 24 h exposure, egg hatchability was slightly reduced when compared with the control for each CO₂ concentration.

Carbon dioxide has efficacy against ethological, biological, physiological and metabolic properties of insects (Nicolas & Sillans, 1989) and all living creatures showed different resistance to high CO₂ (Annis, 1986). However, there are many unknown mechanisms of the action of CO₂ against insects. CO₂ causes a lethal decrease in the pH of hemolymph (Stahl et al., 1985; Gerard et al., 1988; Reichmuth, 1991) and destruction of cell membranes and organs due to its expansion in gas bubbles (Ulrichs & Reichmuth, 1997). This physiological action of CO₂ may vary according to the different developmental stages of the insects. *E. kuehniella* was reported to be more sensitive than most of other pest species tested at 20 bar high pressure CO₂ for 15 min (Riudavets et al., 2010). This study showed that adults of both *E. kuehniella* and *T. embryophagum* were more sensitive than their immature stages at 20% CO₂.

Bell (1976) showed that the egg was the most tolerant developmental stage of 4 species, *E. elutella* Hübner (Lepidoptera: Pyralidae), *E. kuehniella*, *E. cautella* Walker (Lepidoptera: Pyralidae) and *P. interpunctella* tested against phosphine. Similarly, in the present study, the egg stage of *E. kuehniella* was the most resistant stage against CO₂ for all exposure times. For *T. embryophagum* adults, 8.5% mortality was observed at 10% CO₂ for 96 h exposure in present study. But after 10% CO₂ treatment we look at the parasitization capacity of survivor *T. embryophagum* adults, parasitization was not observed for them. The highest mortality of *T. embryophagum* adults was obtained at 20% CO₂ for 72 and 96 h.

Use of chemical insecticides effectively reduces pest populations but leaves environmental residues. Many studies showed that CO₂ gas is a potentially efficacious insecticide in stored products and with no pesticides residues (Jayas et al., 1991; White & Jayas, 1991; Wang et al., 2009). At the same time, different insect species and developmental stages have different susceptibilities to increasing CO₂ concentration. Determination of the required exposure time and concentration of CO₂ and its effects on natural enemies is very important for any control program.

In conclusion, it may be possible to effectively use CO₂ as a part of an integrated pest management (IPM) program after the release of *Trichogramma* or alternatively a low percentage of CO₂ may be used with the egg parasitoid for controlling *E. kuehniella*. Therefore, it may also be worthwhile to investigate the use of CO₂ gas with the egg parasitoid for the control of other stored-product pests. This study showed that fumigant toxicity of CO₂ varied with different developmental stages of tested insects.

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