

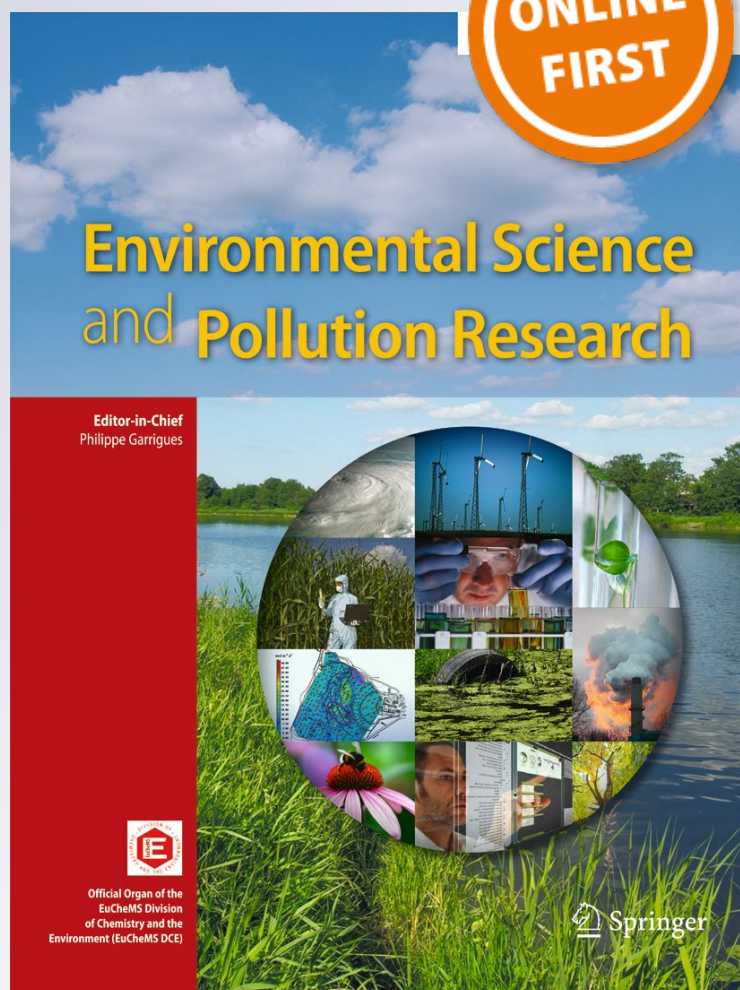
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Effectiveness of carbon dioxide against different developmental stages of *Cadra cautella* and *Tribolium castaneum*

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Abstract Methyl bromide is an excellent fumigant but has been banned because it has high potential for depleting the ozone layer which leads to many environmental and human health hazard issues. In this connection, effectiveness of carbon dioxide (CO₂, 99.9%) was studied as an alternative to methyl bromide under various exposure timings, 25 ± 1 °C, against different developmental stages of the almond moth, *Cadra cautella*, and red flour beetle, *Tribolium castaneum*. In case of *C. cautella*, the LT₉₉ against adult, pupa, and larval stages was achieved after 37.5, 78.1, and 99.9 h of CO₂ application, respectively. While for *T. castaneum*, the LT₉₉ values were obtained after exposure timings of 29.3, 153.9, and 78.4 h against adult, pupa, and larval stages, respectively. Adults were very susceptible; in contrast, pupae and larvae were more tolerant. The susceptibility order was observed as follows: *T. castaneum* adult > *C. cautella* adult > *C. cautella* pupae > *T. castaneum* larvae > *C. cautella* larvae > *T. castaneum* pupae. This study could be useful in developing the management strategies to prevent stored dates from *C. cautella* and *T. castaneum* infestation.

Keywords Carbon dioxide · Mixed infestation · Dates · Stored pests · Modified atmosphere · Saudi Arabia

Introduction

The Kingdom of Saudi Arabia is one of the major producers of fine quality dates and ranks second in the global date production. Unfortunately, date fruits are attacked by several insect species under storage conditions. Al-Zadjali et al. (2006) have reported seven species attacking date fruits and among them almond moth, *Cadra cautella* (Walker) (Lepidoptera: Pyralidae), has been recognized as the most serious pest insect. In the meantime, red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), is also a major household pest against a wide variety of cereals, nuts, and grains (Athanasios et al. 2010; Opit et al. 2012). Pest infestation of stored products has many disadvantages such as losses of product weight, nutritive contents, commercial and esthetic value as well as it may have health hazard.

Traditionally, several fumigants have been tried, from the last three decades; particularly phosphine and methyl bromide had been used extensively for the disinfection of dried fruits including dates, grains, and grain products. The Montreal Protocol has directed phasing out the use and production of methyl bromide because it is harmful to the environment and human health. It has high potential for depleting the ozone layer because of its high chemical reactivity. As a result, it has been phased out in many regions of the world (Bulathsinghala and Shaw 2013, UNEP 2014). Phosphine has the potential to be accepted as an alternative to methyl bromide but there are some limitations about its usage. It requires longer exposure time and may also develop resistance in key insect species (Pimentel et al. 2008). Molecular studies have been confirmed the role of *rph1* and *rph2* genes in

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resistance development in lesser grain borer, *Rhizopertha dominica* (F.) (Coleoptera: Bostrichidae) (Opit et al. 2012; Mau et al. 2012). Also, the resistance genes, P45S and P49S, have been identified in *T. castaneum* and in *R. dominica*, respectively (Chen et al. 2015).

It is very challenging to deal with the diverse infestation of key insect species such as *C. cautella* and *T. castaneum*. Sadeghi et al. (2011) used a mixture of nitrogen and phosphine against adults of rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae); *T. castaneum*; *R. dominica*; cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae); and 3rd instar larvae of Indian meal moth, *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae). To minimize the usage of methyl bromide and phosphine for stored food commodities fumigation, modified atmosphere like the use of CO₂ and natural products like plant extracts and vegetable oils are the most logical and environment friendly approaches (Brandl et al. 1983; Riudavets et al. 2009; Navarro, 2012; Mohapatra et al. 2015; Rajashekar et al. 2016).

Several studies have focused on the use of modified and controlled atmosphere treatments to evaluate the tolerance of different stored products pests and fruit moths (Neven and Rehfield-Ray 2006; Tilley et al. 2007; Tiwari et al. 2011; Neven et al. 2012; Son et al. 2012; Li et al. 2015; Yan et al. 2016). Carbon dioxide has been extensively studied as modified atmosphere in stored commodities such as grains, peanuts, and date fruits. Dhouibi et al. (2015) used a mixture of phosphine and CO₂ at 25 ± 1 °C, to control the date fruit pest *Ephesia kuehniella* and concluded that 16 h and a recommended dose of 3 g/m³ are required to control *E. kuehniella*. Higher CO₂ concentrations of 75.1% are requisite for an exposure period of 144 h to achieve 100% mortality of all life stages of red-legged ham beetle, *Necrobia rufipes* (De Geer) (Coleoptera: Cleridae), and ham mite, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), at a temperature of 23 °C (Hasan et al. 2016). Similarly, Emami et al. (2016) used CO₂ alone and mixed with acetone against adults of *T. castaneum* and concluded that combination of acetone and CO₂ have good additive effect to manage the adults of *T. castaneum*. It has been reported that the usage of CO₂ more than 70% concentrations kills insect quickly. Moreover, it has a microbial growth inhibitory action by disrupting the organism's respiration (Navarro 1978; Navarro and Calderon 1979; Jay 1980; Navarro et al. 2001). Carbon dioxide has the minimal influence over environment, residue free, and its application for the long time in sealed structures (Annis 1990; Tarr et al. 2001).

Carbon dioxide alone or combined with nitrogen have been tested against various stages of several insect species (Jay 1984; Tutuncu et al. 2000; Navarro et al. 2001; Alrukban 2010; Vachanth et al. 2010). We have tested 22 ppm ozone to the larval stage of *C. cautella* inside the pitted date fruits at

different temperature regimes (Husain et al. 2015). Current study reports the stimulatory effects of CO₂ application to control the mixed infestation of two stored product insect pests. The objective of the study was to assess the mandatory exposure time to achieve 100% mortality by exposing a variety of life stages to CO₂ at 25 ± 1 °C temperature at 65 ± 5% relative humidity (RH).

Materials and methods

Insects

Three developmental stages, newly emerged adults (1–2 days), pupae (1–2 days), and larvae (10–12 days), of each of almond moth, *C. cautella* (Walker) (Lepidoptera: Pyralidae) and red flour beetle, *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), were used in the experiment. All stages were obtained from the mother colony reared at the Economic Entomology Research Unit (EERU) Lab, King Saud University, Riyadh, Kingdom of Saudi Arabia. To obtain 1–2 day-old adults, pupae were collected from the colony and when they emerged into adults, they were transferred to a 50-g plastic cups. Similarly, to obtain 1–2 day-old pupae of *C. cautella* and *T. castaneum*, last instar larvae were collected from the colony and placed in separate containers and observed daily. When they pupated, they were used for the experiment. Similarly, 10–12 day-old larvae of each species were also obtained from the mother colonies. Twenty individuals of each life stage were placed in a separate 50-g plastic cup. Diet was provided according to each species developmental stage requirement. *C. cautella* adults were provided with a small piece of cotton soaked in 10% sugar solution. There was no diet provided to *C. cautella* pupae, while *C. cautella* larvae were provided with 5 g of date fruit. Similarly, *T. castaneum* adults and larvae were provided with 1 g of wheat flour.

Gas application

Airtight boxes of 1.9 l (64 oz) capacity (Lock and lock® absolutely, Vietnam) were used as the treatment chamber. For gas filling and evacuating air, on both sides of the box, holes were made then inlet and outlet valves were fixed prior to the experiment. Tested insects were kept in a separate plastic cup of 50 g volume along with aforementioned diet according to species and stage requirement. Each cup containing 20 individuals of each developmental stage was considered as one replicate. All the lids of the cups have holes. Then six cups, as we have three different developmental stages of two species, were placed randomly in a fumigation box. After that, the box was wrapped with a thin cling film plastic (12.5 µm thick). In this study, 99.9% CO₂ was used as treatment while

Table 1 Means (%) ± SE immediate mortality of different developmental stages of *Cadra cautella* and *Tribolium castaneum* exposed to CO₂ for 9–72 h at 25 °C and 65% RH

| Exposure | <i>C. cautella</i> | | | | | | <i>T. castaneum</i> | | | | | |
|----------|--------------------|----------|-----------------|-----------|-----------------|----------|---------------------|----------|-----------------|----------|-----------------|---------|
| | Adult | | Pupa | | Larva | | Adult | | Pupae | | Larva | |
| | CO ₂ | Control | CO ₂ | Control | CO ₂ | Control | CO ₂ | Control | CO ₂ | Control | CO ₂ | Control |
| 9 | 1 ± 1a | 0 ± 9a | 10 ± 0.6a | 1 ± 0.3a | 0 ± 0a | 0 ± 0a | 0 ± 0 a | 2 ± 1.2a | 0.0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 12 | 15 ± 2.7b | 2 ± 1.1a | 29 ± 2.2b | 2 ± 0.3ab | 1 ± 1a | 0 ± 1a | 6 ± 2.4a | 1 ± 1.2a | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 18 | 74 ± 2.9c | 1 ± 1.2a | 30 ± 1.8b | 2 ± 0.3ab | 6 ± 1.9a | 1 ± 1.2a | 64 ± 14.9b | 2 ± 1.2a | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 24 | 84 ± 2.4d | 2 ± 1.2a | 40 ± 1.2c | 2 ± 0.6ab | 40 ± 5.8b | 2 ± 1.2a | 99 ± 1c | 3 ± 1.2a | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 36 | 97 ± 0e | 1 ± 1.2a | 57 ± 2.3d | 2 ± 0.3ab | 54 ± 11.5b | 3 ± 1.2a | 99 ± 1c | 2 ± 1.2a | 0 ± 0a | 2 ± 1.2b | 0 ± 0a | 0 ± 0a |
| 48 | 100 ± 0.0e | 1 ± 1.2a | 80 ± 1.2e | 4 ± 0.9b | 85 ± 4.5c | 3 ± 1.2a | 100 ± 0.0c | 0 ± 0a | 0 ± 0a | 0 ± 0a | 57 ± 3.7b | 0 ± 0a |
| 72 | na | na | 96 ± 0.0f | 4 ± 0.6b | 100 ± 0.0d | 3 ± 1.2a | na | na | 0 ± 0a | 0 ± 0a | 97 ± 1c | 0 ± 0a |

Numbers in the same column followed by the same letters are not significant different at 95% confidence level

the normal air was used as a control. For the gas application, box outlet valve was opened and the gas cylinder tube was connected with box's inlet valve. The gas was discharged through the inlet valve, air came out from the outlet valve and gas concentration was measured after 10–15 s from the outlet valve with a check point (PBI-Dansensor, Denmark). When the required concentration of CO₂ was indicated by the sensor gas, introduction was stopped and both valves were tightly closed. All the treatments were replicated three times. The insects were exposed to the gas for different exposure intervals, i.e., 9, 12, 18, 24, 36, 48, 72, 96, and 120 h. After gas application, all the treatments were kept in the environmental chamber at 25 ± 1 °C temperature, 65 ± 5% RH, and 9: 15 dark and light. The control (breathing air) boxes were treated the same way as the CO₂ boxes.

Data collection

At the end of each exposure time, boxes were removed out from the incubator and were opened, gas was released and mortality counts were measured. Immediate mortality observations were made right after each relevant exposure time. The insects were gently touched with No. 0 camel hair brush to confirm their movement. The suspected developmental stage whose movement or any other signs of life or mortality were not clear, observed under microscope (Omano OM4413 dual power stereo microscope). Mortality signs were considered as no movement in all life stages, change in color, body stiffness, and body shrinkage for larvae and pupae. Delayed mortality was observed for one-month post treatment for adults; for larvae and pupae, mortality was observed until they

Table 2 Means (%) ± SE delayed mortality of different developmental stages of *Cadra cautella* and *Tribolium castaneum* exposed to CO₂ for 9–120 h at 25 °C and 65% RH

| Exposure | <i>C. cautella</i> | | | | | | <i>T. castaneum</i> | | | | | |
|----------|--------------------|----------|-----------------|-----------|-----------------|----------|---------------------|-----------|-----------------|----------|-----------------|----------|
| | Adult | | Pupa | | Larva | | Adult | | Pupae | | Larva | |
| | CO ₂ | Control | CO ₂ | Control | CO ₂ | Control | CO ₂ | Control | CO ₂ | Control | CO ₂ | Control |
| 9 | 13 ± 2a | 3 ± 1.2a | 12 ± 0.6a | 2 ± 0.6a | 3 ± 3a | 1 ± 1a | 6 ± 2.9a | 3 ± 1.2ab | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 12 | 27 ± 1.2b | 6 ± 1a | 35 ± 1.2b | 5 ± 1.2ab | 1 ± 1a | 2 ± 1.2a | 11 ± 4.3a | 6 ± 1b | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 18 | 75 ± 2.7c | 4 ± 1a | 38 ± 1.2b | 7 ± 2.7bc | 19 ± 1.9b | 3 ± 1.2a | 87 ± 4.5b | 2 ± 1.2a | 0 ± 0a | 0 ± 0a | 0 ± 0a | 0 ± 0a |
| 24 | 86 ± 1.9d | 4 ± 1a | 43 ± 0.9c | 5 ± 1.0ab | 48 ± 9c | 3 ± 1.2a | 98 ± 0c | 4 ± 1ab | 0 ± 0a | 0 ± 0a | 2 ± 1.2a | 0 ± 0a |
| 36 | 100 ± 0e | 3 ± 1.2a | 64 ± 3.5d | 4 ± 0.6ab | 65 ± 7.4d | 3 ± 1.2a | 100 ± 0c | 6 ± 1b | 0 ± 0a | 0 ± 0a | 1 ± 1a | 0 ± 0a |
| 48 | 100 ± 0e | 6 ± 2.2a | 86 ± 2.5e | 10 ± 1.2c | 90 ± 4.5e | 3 ± 1.2a | 100 ± 0c | 3 ± 1.2ab | 34 ± 2.4b | 0 ± 0a | 59 ± 5.6b | 0 ± 0a |
| 72 | na | na | 100 ± 0f | 4 ± 0.6ab | 100 ± 2e | 3 ± 1.2a | na | na | 39 ± 5.6b | 0 ± 0a | 99 ± 1c | 0 ± 0a |
| 96 | na | na | 100 ± 0f | 8 ± 1.0bc | na | na | na | na | 84 ± 1.9c | 2 ± 1.2b | 99 ± 1c | 2 ± 1.2b |
| 120 | na | na | na | na | na | na | na | na | 99 ± 1d | 3 ± 1.2b | na | na |

Numbers in the same column followed by the same letters are not significant different at 95% confidence level

Table 3 Lethal time of different developmental stages of *Cadra cautella* and *Tribolium castaneum* exposed to CO₂ at 25 °C and 65% RH

| Species | Stage | Number ^a | Slope ± SE | LT ₅₀ | 95% Fiducial limit | | LT ₉₉ | 95% Fiducial limit | | X ² |
|---------------------|-------|---------------------|-------------|------------------|--------------------|-------|------------------|--------------------|--------|----------------|
| | | | | | Upper | Lower | | Upper | Lower | |
| <i>C. cautella</i> | Adult | 60 | 2.83 ± 0.19 | 16.47 | 13.18 | 20.18 | 37.53 | 26.53 | 81.7 | 79.20 |
| | Pupa | 60 | 1.19 ± 0.08 | 25.86 | 15.86 | 30.38 | 78.16 | 55.08 | 133.17 | 19.16 |
| | Larva | 60 | 2.02 ± 0.13 | 31.65 | 23.3 | 44.4 | 99.96 | 62.13 | 135.6 | 55.25 |
| <i>T. castaneum</i> | Adult | 60 | 2.82 ± 0.31 | 16.68 | 12.71 | 21.1 | 29.34 | 23.17 | 36.2 | 17.69 |
| | Pupa | 60 | 5.49 ± 0.52 | 64.95 | 52.09 | 67.2 | 153.85 | 133.7 | 174.57 | 29.79 |
| | Larva | 60 | 4.68 ± 0.45 | 47.72 | 43.62 | 52.67 | 78.42 | 66.43 | 113.23 | 12.87 |

X² indicates the chi-square value

^aThe number of individual used in the bioassay experiments

succeeded or failed to develop into the next life stage (Valizadegan et al. 2012; Husain et al. 2015).

Experimental design and statistical analysis

A completely randomized design (CRD) was used in the experiment. Data were analyzed for differences in treatment mortality ($\alpha = 0.05$) using one-way analysis of variance (1-way ANOVA) and Fisher's least significant difference (LSD) test. Mortality data were corrected using Abbot's formula. The LT₅₀ and LT₉₉ were calculated using probit analysis. All statistical analyses were performed by SPSS (2005) version 13.0.

Results

Immediate mortality

The effect of CO₂ application on the immediate mortality of *C. cautella* and *T. castaneum* are shown in Table 1. We used these intervals of exposure until 100% mortality was achieved. Exposure time interval of CO₂ application had significant effect on tested species mortality. Species mortality

was directly proportional to CO₂ application exposure timings. Main effects of immediate mortality were significant, with exception of the *T. castaneum* pupal stage. In case of *C. cautella* adult, 100% mortality was reached after 48 h of CO₂ application ($F = 451.9$, $df = 5; 12$, $P < 0.001$). Whereas, for pupal and larval stages, mortalities were reached up to 100% after 72 h ($F = 482.25$, $df = 7, 16$, $P < 0.001$) ($F = 56.1$, $df = 6;14$, $P < 0.001$), respectively. It suggests that the adult stage was more vulnerable than larval stage to CO₂ application. A similar trend was noticed in *T. castaneum* mortality. Extreme mortality of adult stage was observed after 24 h of CO₂ application; ($F = 57.8$, $df = 5; 12$, $P < 0.001$). The *T. castaneum* pupal stage, unlike the adults, was the most resistant followed by larval stage, and there was no response to the immediate mortality until 72 h after the CO₂ application.

Delayed mortality effect of CO₂

Insects delayed mortality after CO₂ application is given in Table 2. For *C. cautella*, the most significant mortality was observed after 36 h ($F = 635.4$, $df = 8; 18$, $P < 0.001$), 96 h ($F = 335.16$, $df = 7, 16$, $P < 0.001$), and 72 h ($F = 64.4$, $df = 6; 14$, $P < 0.001$) of gas application for the adult, pupal, and

Fig. 1 The regression of delayed probit mortality of *Cadra cautella* adult caused by the application of CO₂ in different exposure time

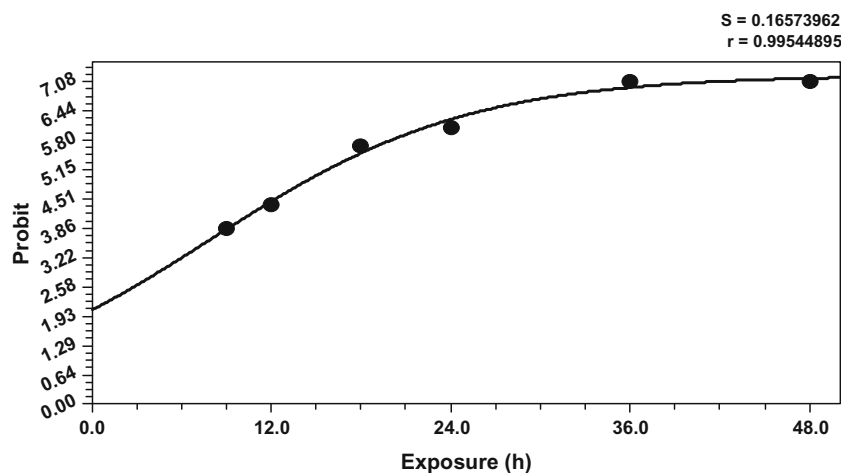
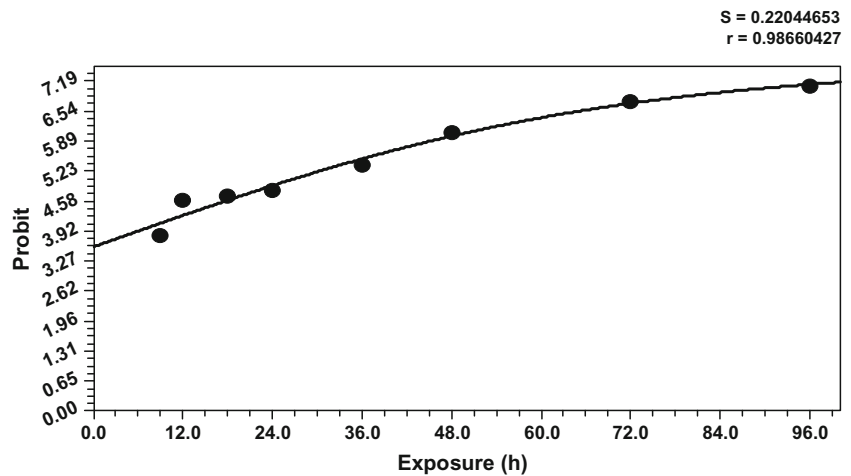


Fig. 2 The regression of delayed probit mortality of *Cadra cautella* pupa caused by the application of CO₂ in different exposure time



larval stages, respectively. While for the *T. castaneum*, a high mortality was observed when CO₂ was applied for 24 h ($F = 407.4$, $df = 5; 12$, $P < 0.001$), 120 h ($F = 332.1$, $df = 8; 18$, $P < 0.001$), and 72 h ($F = 426.7$, $df = 7; 16$, $P < 0.001$) against adult, pupal, and larval stages, respectively.

The lethal time assessment for *C. cautella* and *T. castaneum* after the CO₂ application is presented in Table 3. The results imply that the time needed for CO₂ applications to control these insects differs based on the species and the developmental stage. But the adult stage of both species were almost similar as for as LT₅₀ is concerned. The CO₂ applications were estimated to control almond moth at 99% level if applied 38, 78, and 100 h for adult, pupal, and larval stages, consecutively. Based on our results, it showed that the application of CO₂, for the *T. castaneum* pupal stage, it is the longest (154 h). It suggests that these CO₂ strategies might be applied to control both of these insects in the storage by at least 154 h of exposure timing. The regression of delayed probit mortality of each developmental stage caused by the application of CO₂ in different exposure time is presented from Figs. 1, 2, 3, 4, 5, and 6.

Discussion

In the present study, we presented that altogether, adults and larvae of stored-product pest species were greatly vulnerable to the CO₂. Our existing outcomes indicate that adult developmental stages of *C. cautella* and *T. castaneum* were tremendously prone to CO₂. In contrast, *T. castaneum* pupae were more tolerant to CO₂ than larvae or adults. In our bioassays, we found that to achieve a complete (100%) mortality of *T. castaneum* pupae; it needs about four times longer exposure to that of the adult stages of *C. cautella* and *T. castaneum*. Similar results have been reported by Hashem et al. (2014) for the *C. cautella*, and Jones (1938) for the *T. castaneum*. Concentration and exposure time recommendation is a crucial for reliable disinfestation of mixed age stored insect pest infestations. This mixed age insect pest infestations simulation is an approach to identify the most tolerant/vulnerable stage of the species. This present study clearly showed that there were differences in susceptibility to CO₂, covering stages and species. The susceptibility between stages in a single species can be very varied. Thus, it is essential to be assured that the exact stage is targeted. In this study, the

Fig. 3 The regression of delayed probit mortality of *Cadra cautella* larva caused by the application of CO₂ in different exposure time

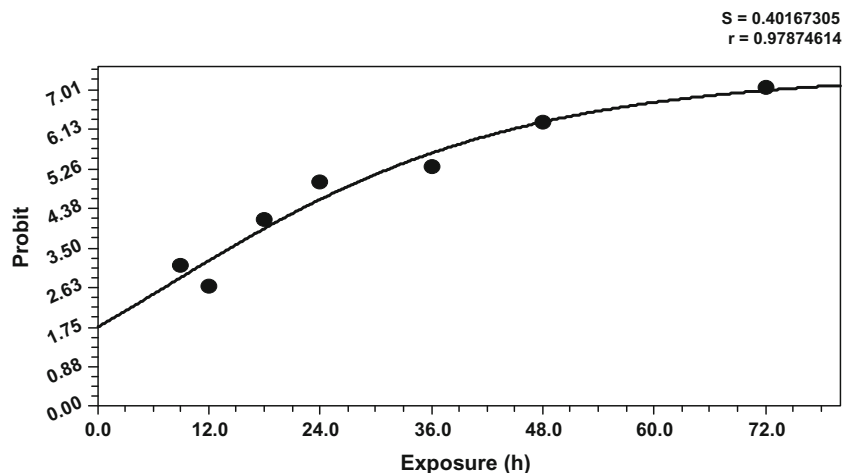
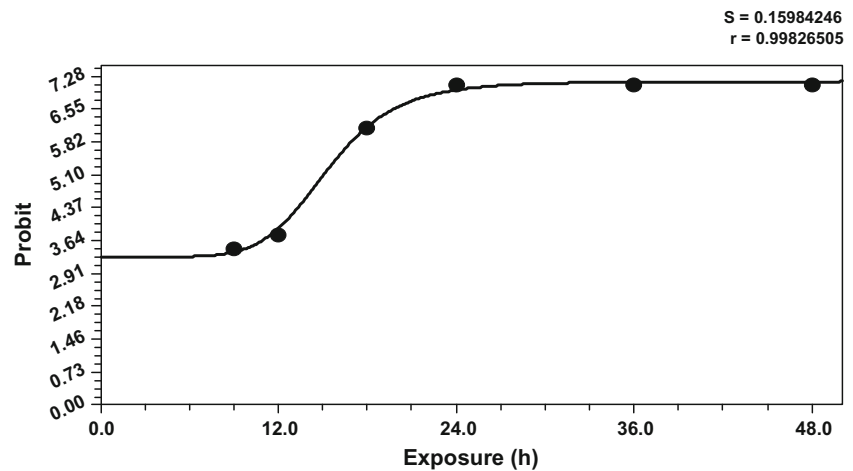


Fig. 4 The regression of delayed probit mortality of *Tribolium castaneum* adult caused by the application of CO₂ in different exposure time



susceptibility order was as follows: *T. castaneum* adult > *C. cautella* adult > *C. cautella* pupae > *T. castaneum* larvae > *C. cautella* larvae > *T. castaneum* pupae.

The exposure times and mortality relationship is bit complex when there is stage and species mixed infestation. Required time interval to get 100% mortality of the tested species may depend on the time of exposure and CO₂ concentration. Efficacy of 63% CO₂, concentration balanced with air, for 5 days exposure at 27 °C, showed 100% mortality of *C. cautella* larvae (Jay 1984; Alrukban, 2010), these findings are in highly-flavored of our results. Similarly, Vachanth et al. (2010) reported 100% efficacy of CO₂ mixture (35% CO₂, 52% N₂, and 13% O₂) and exposures (12 days) on adult stage of *T. castaneum* in stored millet. In contrast, a 100% mortality of *T. castaneum*, adults were observed when exposed to 99.9% CO₂ for 5 min, under a pressure of 20 Kg/cm² (Nakakita and Kawashima 1994), while in case of our results a minimum of 24–48 h are required to get 100% mortality of *T. castaneum* adults.

Table 2 showed that complete mortality for all exposed developmental stages are achievable. The complete mortality

of *T. castaneum* larval was achieved almost in 72 and 120 h for pupal stages, while adult mortality was achieved after 24 h of exposures. We used these intervals of exposure until 100% mortality was achieved. Our data match satisfactorily with those of Jay and Cuff (1981) who observed 100% mortality in 72 and 24 h for *T. castaneum* larvae and adults, respectively, when the insects were exposed to a gas mixture (97% CO₂, 0.5% O₂, and 2.5% N₂, at 26.7 °C and 60 ± 5% RH). Our present study indicated that 154 h are required to achieve 100% *T. castaneum* pupal mortality while they achieved after 72 h. It indicates that the gases mixture and raised temperature are needed to gain the synergistic effects on the pests.

Carbon dioxide stimulates the spiracles to unlock which results in insect death because of desiccation. It also has direct toxic effects on the nervous system. In certain circumstances, it might acidify the hemolymph which disrupts some tissues (Nicolas and Sillans 1989). Breathing activity of living animals in the storage may decrease O₂ content by 1–2%, whereas, during exhalation, the CO₂ rises from an ambient 0.035% to near 20% (White & Jays 2003). Long and persistent sub lethal CO₂ doses application can have harmful effects on the

Fig. 5 The regression of delayed probit mortality of *Tribolium castaneum* pupa caused by the application of CO₂ in different exposure time

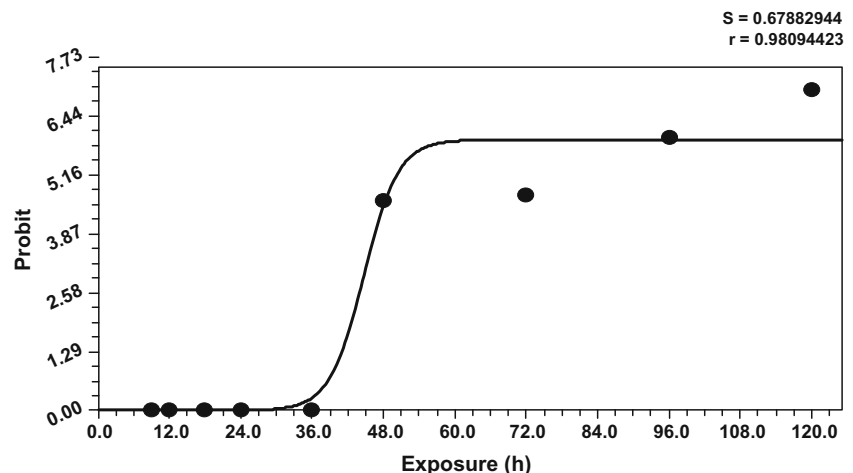
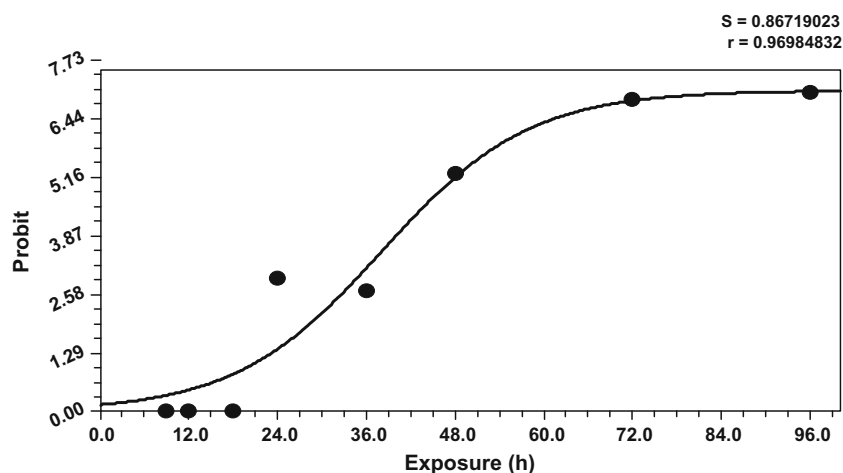


Fig. 6 The regression of delayed probit mortality of *Tribolium castaneum* larva caused by the application of CO₂ in different exposure time



insect development, growth, and reproduction (White et al. 1995; Nicolas and Sillans 1989). The application should count the overlaid of the high mortality effect on resistant stage/species in long exposure time. Thus, the exposure of the CO₂ application should be prolonged in order to engage the resistant stage/species, which is at least 154 h for these two insect species.

Differences in temperature also fluctuates the mortality; in such cases, the mortality either could be enhanced or inhibited. Brandl et al. (1983) calculated an LT₉₅ of about 58 h with 99.9% CO₂, for 25-day-old larvae of navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae) at 26.7 ± 1 °C and 60% RH, while, Ahmed and Hashem (2012) calculated an LT₉₅ of about 150.4 and 169.9 h with 80% CO₂, 4% O₂, and 16% N₂ for fourth instar larvae of *C. cautella* and Indian meal moth, *P. interpunctella* (Hubner) (Lepidoptera: Pyralidae), respectively, at 27 ± 2 °C and 60 ± 5% RH. We found, in this study, an LT₉₉ of about 99 h with CO₂, for 15-day-old larvae of *C. cautella* at 25.0 ± 1 °C and 65% RH. Sauer and Shelton (2002) reported that efficacy of 80% CO₂ and 20% N₂, for 12 h exposure, at 32.2 °C showed 100% mortality of *P. interpunctella* pupae. However, the same treatment at low temperature of 26.6 °C showed only 49.2% mortality. In our study, we used the standard ambient temperature of 25 ± 2 °C and 65 ± 5%, RH.

Soderstrom et al. (1990) treated the mature larvae of codling moth, *Cydia pomonella* (L.) with 60% CO₂ for 7 days exposure times at 25 °C and achieved 95% mortality. These findings are in strong conformity of our results. Similarly, Soderstrom et al. (1996) calculated time interval of 5.2 h for 95% mortality with 98% CO₂ for diapausing larvae of codling moth, *C. pomonella* (L.) (Lepidoptera: Tortricidae), at 43 °C. Present study deducted that at the 99.9% CO₂ concentration, after 120 h (close to 5 days) of exposure, all adults, larvae and pupae of tested developmental stages were dead. Practically, this means that CO₂ has a strong effect against tested developmental stages at ambient temperature, which stands in

accordance with previous reports for 17–20 day-old *C. cautella* larvae (Sen et al. 2009; Alrukban 2010; Husain et al. 2015). These findings may have practical importance to use this strategy for multiple insect species infestation in stored products.

Storage pest management with controlled atmospheres by Annis (1986) pointed out that carbon dioxide concentrations of 80 and 100% will eradicate adult rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemphloeidae) in 3 and 2 days, respectively, at 20–29 °C. A 99.9% CO₂ atmosphere controlled *T. castaneum* pupae in 14 days at 15.6 °C and 38% RH (Aliniyee 1972). Mortality of *T. castaneum* reached close to 100% after 48 h exposure to 2% O₂ sensitivity to the hypoxia atmosphere (Cao et al. 2010). In our study, all of *T. castaneum* adults killed within 36 h, the aforementioned studies are in strong conformity of our results.

Conclusions

The depletion of ozone layer and health hazardous effects of methyl bromide has made obligatory for the scientists to explore some environmentally friendly alternatives to methyl bromide. The present research was an idea to check the efficacy of carbon dioxide on different developmental stages of two major stored product pests from different insect orders. The results proved that carbon dioxide has the potential to be an effective management tactic to reduce the infestation of stored pest. This study has explored the track which indicated that there is a potential alternative in the form of carbon dioxide but suggesting that further studies could focus on the other parameters such as cost benefit ratio, availability of carbon dioxide, health hazardous effects, and residual effect in this study, we have started the exposure from very less timings and exposure interval continues until the death of all developmental stages. From our results, it is very clear that which developmental stage of these two major stored product pest will be

killed after how long exposure time with the tested concentration of carbon dioxide.

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