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THE EFFECTIVENESS OF CARBON DIOXIDE AND NITROGEN ON DIFFERENT DEVELOPMENTAL STAGES OF *Cadra cautella* (LEPIDOPTERA: PYRALIDAE)

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The efficacy of CO_2 and N_2 gases alone or in a mixture was tested against eggs, larvae, and pupae of the almond moth, *Cadra cautella*, a major date fruit pest, under temperature regimes of 5°C and 25°C. Our results indicated that exposing different stages to higher concentrations of CO_2 significantly affected egg hatchability, larval mortality, and pupal mortality as compared to 100% N_2 gas, at both temperature regimes. Application of CO_2 at lower doses (below 50%) did not give 100% mortality of any developmental stage even at longer exposure timings. However, treatments at higher doses (75 and 100% CO_2) were effective at 24 h and longer treatment duration. The application of 100% N_2 did not effectively control *C. cautella*. Of all the stages, the larvae of *C. cautella* were most susceptible to the gases. Our results indicated that temperature has a significant effect on the efficacy of gases or their combinations tested after an exposure of 48 h and longer. Based on current findings, we conclude that CO_2 gas is a good candidate for successful control of *C. cautella* immature stages under storage conditions and can be a potential alternative to highly toxic methyl bromide.
Keywords: Methyl bromide, almond moth, *Cadra cautella*, modified atmosphere, packaging

INTRODUCTION

Methyl bromide and phosphine have been used extensively for decades to control various stored product pests including the almond moth, *Cadra cautella* (Walker). However, these fumigants have serious issues. For example, methyl bromide has been declared an ozone depleting chemical and has been banned in developed countries since 2005 (UNEP, 1997). It is scheduled to be banned worldwide in 2015 in accordance with the Montreal Protocol (Schneider *et al*., 2003). Phosphine (PH_3) fumigation efficacy is greater at low temperature of 15°C. Long exposure timings are required to achieve higher mortalities of khapra beetle, *Trogoderma granarium* Everts; red flour beetle, *Tribolium castaneum* (Herbst); rice weevil, *Sitophilus oryzae* (L.); maize weevil, *Sitophilus zeamais* Motschulsky, and lesser grain borer, *Rhizopertha dominica* (F.) (Hole *et al*., 1976).

Efforts are being made to develop economical, safe, and environmentally friendly alternatives for the control of stored product insect pests. Chemicals such as carbonyl sulfide, sulfuryl fluoride, ozone, cyfluthrin, iodomethane, and non-chemical treatments including modified atmospheres, high pressure, heat/cold treatments, sanitation, radio frequency, long-wave energy and irradiation have been utilized (Desmarchelier *et al*., 1998; Johnson *et al*., 2000; Fields and White, 2002; Schneider *et al*., 2003; Aksoy *et al*., 2004; Cetinkaya *et al*., 2006).

Several studies have demonstrated that applications of modified atmospheres (MA) and ozone are possible alternatives for methyl bromide against dried fruits pests including *C. cautella* (Soderstrom *et al*., 1984; Soderstrom *et al*., 1986; Navarro *et al*., 1993; Donahaye *et al*., 1994; Navarro *et al*., 1997; Donahaye *et al*., 1998; Navarro *et al*., 1998; Navarro *et al*., 2000, Husain *et al.*, 2015; Husain *et al.*, 2017). Application of MA, such as purging with CO_2 or N_2 are well-known, dependable and proven to be residue-free techniques for disinfestation of food storage products. Lethal effects of CO_2 (89%) on different stages of *S. zeamais* and *S. oryzae* was proven as a good MA fumigant in a rice processing mill (*Carvalho et al.*, 2012). Jay (1984, 1986) reported that CO_2 applications at higher concentrations with increased temperatures of 38°C or above can effectively control stored product pests like *R. dominica* and *S. zeamais* within 24-48 h. Similarly, Navarro and Donahaye (1990) reported that higher insect mortality could be obtained in comparatively short exposure times at higher CO_2 atmospheres than at lower O_2 applications.

The objective of the present study was to evaluate the efficacy of CO_2 and N_2 gases as a replacement for the current use of methyl bromide and phosphine for controlling developmental stages of *C. cautella.*

MATERIALS AND METHODS

Different developmental stages, eggs, larvae, and pupae of *C. cautella* were exposed to CO_2 and N_2 gases and in different combinations/ratios at temperature regimes of 5°C and 25°C in the laboratory of Economic Entomology Research Unit (EERU), Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia.

One day-old eggs, 13-15 day-old larvae, and 1-2 day-old pupae of *C. cautella* were obtained from a mass culture maintained in the EERU laboratory on an artificial diet. Each 1 kg diet contained 275 g broiler diet + 275 g layer diet + 250 g wheat flour +100 g dates (powder of var. "Sukri") 100 ml glycerin (Al-Azab, 2007). The insects were kept in an incubator at 25±1°C with 65±5% relative humidity (RH) and a photoperiod of 15:9 (L:D) (Bell and Bowley, 1980; Ozyardimci *et al*., 2006).

The experiments were performed using a Complete Randomized Design (CRD) with a total of six-treatments: 1 (100% CO_2), 2(75% CO_2 + 25% N_2), 3. (50% CO_2 + 50% N_2), 4 (25% CO_2 + 75% N_2), 5 (100% N_2), and 6 Control (Atmospheric/Normal air). Each treatment type was conducted in quintuplicate at two temperatures, 5 and 25±1°C, as well as four durations (5, 24, 48 and 72 h). Five replicates were used for each exposure time, for each treatment. The treatments were kept under constant conditions of 65±5% RH and a photoperiod of 15:9 h (L: D) at their respective temperatures. For each treatment, 10-eggs, 10-larvae, and 10-pupae were placed separately in petri dishes which were then transferred to 600 ml airtight rectangular containers (Lock and Lock®, China) for gas application. Holes were made on both sides of the container to fix inlet and outlet valves. An inlet valve was used to fill the containers with gases and an outlet valve was used to release the air and monitor the concentration of gases. The device (PBI- Dan Sensor, Denmark) was used to measure the gas concentration in the container. The gas was measured only at the time of application. Containers were filled with gases and transferred to incubators fixed at temperature regimes of 5 and 25°C and kept for specific exposure periods of 5, 24, 48, and 72 h. After each treatment, gases were released from the containers and their impact on each developmental stage was observed in terms of mortality (%). Eggs were observed daily until hatching for 8 d whereas larvae were observed daily until pupation (14 d) and pupae were observed daily until adult emergence (18 d) (Aldawood *et al*., 2013; Husain *et al*., 2017).

Recorded data were analyzed using the analysis of variance (ANOVA) PROC GLM procedure of SAS (SAS, 2009) and means were separated using the least significant difference at ($P \geq 0.05$).

RESULTS

***Effectiveness of CO_2 and N_2 gases against egg, larval and pupal stages of Cadra cautella after an exposure of 5 h at 5 and 25°C*:** The influence of CO_2 and N_2 gas applications on different development stages of *C. cautella* in terms of mortality (%) after an exposure of 5 h, at temperature regimes of 5°C and 25°C are presented in Table 1. The highest egg mortality of 10% was recorded using of 25% CO_2 at 5°C (ANOVA: F= 3.50; df = 5, 29; P= 0.0161). Similarly, the highest larval and pupal mortality of 50% and 20% was recorded in case of 75% CO_2 at 25 and 5°C, respectively, (ANOVA: F= 4.86; df = 5, 29; P= 0.0033). Carbon dioxide by itself or mixed with nitrogen were effective for controlling *C. cautella* causing significantly greater mortality in larvae and pupae. Eggs appeared to be the least vulnerable stage at all tested concentrations under each temperature regime. In contrast, application of nitrogen (100%) did not induce a significant difference in percent mortality among any developmental stage at any temperature regime (Table 1).

Effectiveness of CO_2 and N_2 gases against egg, larval and pupal stages of Cadra cautella after an exposure of 24 h at 5 and 25°C: The influence of CO_2 and N_2 gas applications on different development stages of *C. cautella* in terms of mortality (%) after an exposure of 24 h, at temperature regimes of 5°C and 25°C are presented in Table 2. The highest egg mortality of 34% was recorded using 50% CO_2 at 25°C (ANOVA: F= 4.55; df = 5, 29; P= 0.0047). Similarly, the highest larval and pupal mortalities of 100% and 62%,

Table 1. Mean percentage mortality of different developmental stages of *Cadra cautella* exposed to CO_2 and N_2 alone or their mixtures for a period of 5 h at 5°C and 25°C.

Temperature	Stage	100% CO_2	75% CO_2	50% CO_2	25% CO_2	100% N_2	Normal Air
5°C	Egg	0.0±0.0C	2.0±2B	0.0±0.0B	10.0±7.7B	0.0±0.0A	2.0±2.0B
	Larvae	18.0±8.6B	24.0±6.8B	34.0±7.5A	28.0±9.7AB	10.0±5.4A	18.0±9.7A
	Pupae	8.0±2.0BC	20.0±6.3B	4.0±2.4B	8.0±3.4B	2.0±2.0A	0.0±0.0B
25°C	Egg	8.0±5.8BC	4.0±4.0B	20.0±20.0AB	8.0±8.0B	26.0±19.4A	12.0±5.8AB
	Larvae	46.0±7.5A	50.0±13.0A	24.0±4.0AB	50.0±12.6A	32.0±10.7A	20.0±5.5A
	Pupae	12.0±7.4BC	18.0±9.7B	10.0±6.3AB	20.0±8.4B	20.0±15.5A	2.0±2.0B

Means followed by the same letters in the same column are not significantly different, at $P \leq 0.05$.

Table 2. Mean percentage mortality of different developmental stages of *Cadra cautella* exposed to CO_2 and N_2 alone or their mixtures for a period of 24 h at 5°C and 25°C.

Temperature	Stage	100% CO_2	75% CO_2	50% CO_2	25% CO_2	100% N_2	Normal Air
5°C	Egg	22.0±6.8D	24.0±14.6B	24.0±13.0C	16.0±15.9BC	20.0±9.3BC	6.0±3.7AB
	Larvae	82.0±0.0AB	90.0±6.0A	76.0±6.8AB	42.0±8.3B	30.0±9.7B	22.0±5.8AB
	Pupae	52.0±6.8BCD	16.0±19.8B	8.0±8.6C	12.0±14C	4.0±4.9C	0.0±4.5AB
25°C	Egg	28.0±16.9CD	28.0±21.0B	34.0±12.8C	4.0±15.5C	12.0±3.4C	0.0±0.0AB
	Larvae	100.0±0.0A	90.0±2.0A	82.0±4.0A	72.0±13.6A	70.0±11.7A	24.0±5.1A
	Pupae	62.0±4.0BC	26.0±11.1B	36.0±12.0BC	22.0±17.2BC	6.0±11.1C	4.0±6.3B

Means followed by the same letters in the same column are not significantly different, at $P \leq 0.05$.

Table 3. Mean percentage mortality of different developmental stages of *Cadra cautella* exposed to CO_2 and N_2 alone or their mixtures for a period of 48 h at 5°C and 25°C.

Temperature	Stage	100% CO_2	75% CO_2	50% CO_2	25% CO_2	100% N_2	Normal Air
5°C	Egg	36.0±6.8C	32.0±14.6C	20.0±13.0B	28.0±15.9C	16.0±9.3AB	8.0±3.7AB
	Larvae	100.0±0.0A	94.0±6.0A	86.0±6.8A	70.0±8.3AB	32.0±9.7AB	12.0±5.8AB
	Pupae	34.0±6.8C	48.0±19.8C	28.0±8.6B	26.0±14C	12.0±4.9AB	10.0±4.5AB
25°C	Egg	76.0±16.9B	50.0±21.0BC	22.0±12.8B	30.0±15.5BC	8.0±3.4B	0.0±0.0B
	Larvae	100.0±0.0A	98.0±2.0A	94.0±4.0A	84.0±13.6A	36.0±11.7A	14.0±5.1A
	Pupae	96.0±4.0AB	82.0±11.1AB	68.0±12.0A	36.0±17.2BC	18.0±11.1AB	10.0±6.3AB

Means followed by the same letters in the same column are not significantly different, at $P \leq 0.05$.

Table 4. Mean percentage mortality of different developmental stages of *Cadra cautella* exposed to CO_2 and N_2 alone or their mixtures for a period of 72 h at 5°C and 25°C.

Temperature	Stage	100% CO_2	75% CO_2	50% CO_2	25% CO_2	100% N_2	Normal Air
5°C	Egg	36.0±10.3B	28.0±18.8C	32.0±12.0C	26.0±16.0C	2.0±02.0B	4.0±4.0B
	Larvae	100.0±00.0A	88.0±08.0A	88.0±04.9A	72.0±06.6BA	20.0±11.4AB	26.0±5.1A
	Pupae	76.0±08.1A	22.0±03.7C	22.0±10.2C	28.0±10.7BC	8.0±03.7B	4.0±2.4B
25°C	Egg	72.0±13.9A	54.0±14.0BC	42.0±18.3BC	12.0±09.7C	16.0±11.2AB	0.0±0.0B
	Larvae	100.0±00.0A	100.0±00.0A	100.0±00.0A	88.0±02.0A	34.0±02.4A	4.0±2.4B
	Pupae	86.0±14.0A	84.0±11.7AB	74.0±16.9AB	60.0±15.8AB	6.0±02.4B	4.0±2.4B

Means followed by the same letters in the same column are not significantly different, at $P \leq 0.05$.

respectively, were recorded using 100% CO_2 at 25°C (ANOVA: F= 6.19; df = 5, 29; P= 0.0008). Comparison of *C. cautella* different stages mortality exposed to CO_2 and N_2 at different concentrations for 24-h, revealed that the larval stage was most susceptible to all tested concentrations of the above gases, followed by pupal and egg stages at each temperature regime. However, mortality percentage was increased when the exposure time was extended to 24 h. Higher percent mortality was observed at 25°C as compared to 5°C (Table 2).

Effectiveness of CO_2 and N_2 gases against egg, larval and pupal stages of Cadra cautella after an exposure of 48 h at 5 and 25°C: The influence of CO_2 and N_2 gas applications on different development stages of *C. cautella* in terms of mortality (%) after an exposure of 48 h, at temperature regimes of 5°C and 25°C are presented in Table 3. The highest egg mortality of 76% was recorded at 100% CO_2 at 25°C (ANOVA: F= 14.87; df = 5, 29; P= <0.0001). Similarly, the highest larval and pupal mortalities of 100 and 96%, respectively were recorded using 100% CO_2 at 25°C (ANOVA: F= 14.87; df = 5, 29; P= <0.0001). The exposure of *C. cautella* immature stages for 48 h revealed that CO_2 applications at higher concentrations (100%) caused significantly greater larval mortality (100%) as compared to the egg and pupal stages at both temperature regimes. The trend of higher mortality was apparent in all CO_2 treatments. The percentage mortality was very low at 100% N_2 applications and no significant difference was observed when compared to control treatments (Table 3).

Effectiveness of CO_2 and N_2 gases against egg, larval and pupal stages of Cadra cautella after an exposure of 72 h at 5 and 25°C: The influence of CO_2 and N_2 gases on different development stages of *C. cautella* in terms of mortality (%) after an exposure of 72 h at 5 and 25°C is presented in Table 4. The highest egg mortality of 72% was recorded using 100% CO_2 at 25°C (ANOVA: F= 6.05; df = 5, 29; P= 0.0009). The highest larval and pupal mortalities of 100 and 86%, respectively, were recorded using 100% CO_2 at 25°C (ANOVA: F= 6.05; df = 5, 29; P= 0.0009). A similar trend of higher mortality in larval stage occurred after an exposure of 5, 24, and 48 h, followed by pupae and egg stages, at both

temperature regimes. The percent mortality was low in 100% N_2 treatments and there was no significant difference as compared with control treatments (Table 4).

DISCUSSION

The main objective of this study was to examine the efficacy of CO_2 and N_2 applications against immature stages of the almond moth at two temperature regimes (5°C and 25°C) and four exposure timings (5, 24, 48, and 72 h).

Application of MA with CO_2 or N_2 is well-known and has proven to be a residue free technique for disinfestation of the stored products. The use of atmospheric gases has been practiced for centuries as an alternative control measure for disinfestation of warehouse commodities because of their toxic effect on insects and their environmentally friendly properties (Nicolas and Sillans, 1989; Gunasekaran and Rajendran, 2005). Bailey (1965) has reported that most stored-product insects are killed by <3% O_2 or >40% CO_2 and that exposure to CO_2 is required for several days for different species of insects at different life stages. In the present study, pure CO_2 and N_2 and mixtures were evaluated in combination with different temperatures and exposures for effectiveness against immature stages of *C. cautella*. Our findings suggest that CO_2 is an effective protocol for almond moth control under typical storage conditions. Our study indicates that the concentrations of CO_2 and exposure time are the most important factors influencing efficacy of the treatments.

Carbon dioxide applications at higher concentrations (50-100%) showed promising results at longer exposure periods (24 h and above). However, exposure to pure N_2 was not significantly different from control. Our data confirms that the larval stage is most susceptible to CO_2 followed by the pupal stage and then the egg stage. Some studies have confirmed the combined effects of CO_2 mixed with N_2 on stored product pest moth larvae. When larvae of Mediterranean flour moth, *Ephestia kuehniella* (Zeller) and Indian meal moth, *Plodia interpunctella* (Hübner) were exposed to 50% CO_2 (balanced with N_2 and 3% O_2) at 25ºC, 100% mortality was achieved after 4 d exposure time (Riudavets *et al*., 2009). Similar findings have been reported for *S. zeamais* with 100% CO_2, when all life stages were dead in 79 h (Noomhorm *et al*., 2013).

In present studies, treatment with 100% CO_2 resulted in 100% mortality of 5th instar *C. cautella* larvae at 25°C at an exposure of 24 h or above. There is the possibility that insect species may respond differently to CO_2 applications. Comparatively less mortality has been reported for the navel orange worm, *Amyelois transitella* when CO_2 was applied for 60 h at 27°C (Brandle *et al*., 1983; Soderstrom *et al*., 1990). However, 95% mortality was achieved after exposing mature larvae of the codling moth, *Cydia pomonella* to 60% CO_2 for 7 d at 25°C. Emekci *et al*. (2004) also has reported similar results regarding the effectiveness of CO_2 against C. *cautella* at higher concentrations. Several other studies have also advocated for the efficiency of MA applications against arthropod pests of dried fruits and as potential alternatives to methyl bromide (Soderstrom *et al*., 1984; Soderstrom *et al*., 1986; Navarro *et al*., 1993; Donahaye *et al.*, 1994; Navarro *et al*., 1997; Donahaye *et al*., 1998; Navarro *et al*., 1998; Navarro *et al*., 2000). Recently, Ahmed *et al*. (2014) reported that all eggs and larval stages of angoumois grain moth, *Sitotroga cerealella* (Olivier) were killed within 3 d and all pupae within 4 d when treated with 75% CO_2 at 34°C. Our studies indicated promising results for controlling the almond moth, with 100% CO_2 for 24 hours yielding 100% larval mortality.

***Conclusions*:** The overall findings of our study demonstrated that 1) CO_2 is a good candidate for controlling *C. cautella* under typical storage conditions, 2) use of CO_2 indicated more favorable results at higher concentrations of 75% and 100%, with an exposure of 48 h or more, 3) long exposure times (≥24 h to the CO_2 are of key importance to ensure mortality and 4) temperature did not influence the efficacy of the gases used in this study especially for lower exposure times of 5 h and 24h.

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