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Comparative efficacy of controlled atmospheres against two stored product insects

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Abstract: Effect of controlled atmospheres (CAs) at various concentrations of CO_2, N_2 and O_2 on the lethal times of *Tribolium castaneum* and *Trogoderma granarium* was investigated at 20 and 30 ºC. Experiments were performed using a recirculatory multi-flask apparatus. The results revealed that, the shortest times (0.1, 0.3 and 0.9 day for adults, larvae and pupae, respectively) required to obtain 50% mortality of *T. castaneum* stages were at 100% CO_2 followed by 75% CO_2, 50% CO_2, 99% N_2 + 1% O_2 and 25% CO_2, at higher tested temperature (30 °C). Adults were more sensitive to the different treated CAs than larvae, while pupae were the most tolerant stages. Diapausing larvae of *T. granarium* were the most tolerant to all treated CAs at tested temperatures. The effectiveness of CAs to decrease its LT_{50} values were 100% CO_2 followed by 99% N_2 + 1% O_2 and 98% N_2 + 2% O_2 at 30°C. It may be concluded that diapausing larvae are more difficult to control with CAs than normal larvae. A treatment with N_2 relying on the absence of O_2 will take a longer treatment time to control the diapausing larvae and in late winter, exposure times needed for control may be even longer. If CAs were to be applied under such circumstances, a high content of CO_2 would be the best option to achieve control in a comparatively short time.

Keywords: Controlled atmosphere; *Tribolium castaneum*; *Trogoderma granarium*; Recirculatory multi-flask apparatus

Introduction

Stored-product insects can cause postharvest losses, estimated from up to 9% in developed countries to 20% or more in developing countries (Pimentel, 1991). There is much interest in alternatives to conventional insecticides for controlling stored-product insects because of insecticide loss due to regulatory action and insect resistance, and because of increasing consumer demand for product that is free of insects and insecticide residues (Phillips and Throne, 2010). More recently, the worldwide phased out and ban of the fumigant insecticide methyl bromide, an effective compound for killing postharvest insects, under the international agreement of the Montreal Protocol has motivated research into various alternatives to replace methyl bromide (Fields and White, 2002). Controlled Atmosphere (CA) and Modified Atmosphere (MA) pose little danger to man and animals as well as presenting no residue problems in treated food commodities. The use of this technique, to control insects, involves the alteration of the proportions of the normal atmospheric concentrations of mainly nitrogen, oxygen, carbon dioxide and other rare gases, which make up 78%, 21%, 1.1% and 0.03%

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respectively of the normal atmosphere to create an atmosphere lethal to insects (Navarro and Jay, 1987). Low levels of oxygen and high levels of carbon dioxide impose metabolic stress on insects by hindering the oxidative breakdown of a metabolic intermediate product (pyruvate) required for energy release and also causing the accumulation of a toxic product (lactic acid) (Chapman, 1971; Friedlander, 1983; Zhou *et al.*, 2000; Emekci *et al.*, 2001; Mbata and Phillips, 2001; Mitcham *et al.*, 2006; Boardman *et al*., 2012).

Most of the research works carried out using CA for insect control are based on achieving an increase in the carbon dioxide content of the storage environment thus producing hypercarbia atmosphere or reducing the oxygen content obtained usually by flushing with nitrogen or mixture of nitrogen and carbon-dioxide thereby producing hypoxia or anoxia atmosphere (McGaughey and Akins, 1989). In CA treatment, the lethal atmosphere must be maintained for an adequate length of time for effectiveness and thus an enclosure, which is reliably gas tight for the retention of the lethal atmosphere is required. The set up can be a continuous gas flow system (Soderstrom *et al.*, 1990; Nilson *et al.*, 2006) or a static test system (Lindgren and Vincent, 1970; Leong and Ho, 1995). Also time of exposure or the treatment period is a critical factor (Navarro and Jay, 1987; Leong and Ho, 1995). However, the effectiveness of CA can be enhanced by varying other parameters such as temperature when the exposure period is reduced (Soderstrom *et al.*, 1992; Mbata and Reichmuth, 1996; Ofuya and Reichmuth, 2002; Navarro, 2012).

The heat treatments under controlled atmospheres have a dramatic effect on insect metabolism, low O_2 prevents ATP synthesis, and high CO_2 prevents use of ATP (Hochachka, 1986 and 1991; Donahaye and Navarro, 2000; Neven and Hansen, 2010). Heat combined with controlled atmospheres of nitrogen or carbon dioxide can significantly reduce treatment time for control of *Tribolium castaneum* (Soderstrom *et al.*, 1992; Buscarlet 1993; Locatelli and Daolio 1993; Adler, 1995). Susceptibility of stored product insects to CA varies, both between adult species and also between the various developmental stages of each insect species (Press *et al.*, 1967; Navarro and Jay, 1987; Ofuya and Reichmuth, 1998; Athie *et al.*, 1998; Mann *et al.*, 1999; Adler, 2001; Badre *et al*., 2005). The rust flour beetle, *Tribolium castaneum* (Herbst.) is a worldwide spread, especially in tropic and sub tropic- areas, stored product insect pest that attacks flour mills, broken grains, etc causing loss and damage. Adults are long-lived and may live for more than one year (Stoyanova and Shikrenow, 1976; Omar *et al*., 1995). Also Khapra beetle, *Trogoderma granarium* (Everts) is one of the world's most destructive insect pests of grain products and seeds. Infestations caused by grubs of khapra beetles are difficult to control because they crawl into cracks and crevices, remaining there for long periods of time. Young larvae feed on damaged grains, while older larvae are able to feed on whole grains. Severe infestation may cause unfavorable changes in chemical composition. *T. granarium* can also damage dry commodities of animal origin (ISPM, 2012). Presence of larval moulted skin and seata may cause dermatitis and allergic reactions (Ellis and Hodges, 2007).

The objective of this research was to investigate the effect of various controlled atmospheres on the lethal times of *T. castaneum* adult and its immature stages as well as *T. granarium* larvae under two different temperature degrees.

Materials and Methods

Insects

Laboratory strains of the rust flour beetle *Tribolium castaneum* (Herbst) and khapera beetle *Trogoderma granarium* (Everts) obtained from Plant Protection Research Institute, Doki, Egypt were used in these studies.

Insect cultures

The insects were reared in jars of 1000 ml capacity containing about 250 g of sterilized and conditioned wheat kernels for *T. granarium* and crushed wheat grains for *T. castaneum*. Wheat grains and crushed wheat were well treated by freezing at -18 °C for two weeks before application to eliminate any possible infestation by any other species (El-Lakwah *et al*., 2004). Insect cultures were kept under controlled conditions of 30 ± 1 °C and 65 ± 5% R. H. at the rearing room of the laboratory of the plant protection Dep., Faculty of Agric., Moshtohor, Benha University. The moisture content of the grain was around 14%. About 300 adults (1-2 weeks old) were introduced into the jars for egg laying. Three days later, all insects were separated from the food and the jars were covered tightly using rubber band and were kept again in the rearing room. This procedure was repeated several times in order to obtain large numbers of *T. castaneum* adults needed to carry out the tests. In case of *T. granarium* about 200 adults (2-5 days old) were used to develop homogenous culture of khapra beetle.

Adults and the developmental stages used

4th instar larvae, pupal stage and adults (7 -14 day-old) of *T. castaneum* were used in bioassays. In case of *T. granarium*, 3rd and 4th larval instar active and diapausing larvae were used in experiments, the diapausing (quiescent) larvae were collected from roll of paper, which had been placed on the top of the culture media (Bell *et al*., 1984).

Preparation of the test – insects

Batches of 30 active and diapausing larvae of *T. granarium* and 30 adults, 30 larvae, 30 pupae of *T. castaneum* were placed in wire gauze cages (14 mm diam. and 45 mm long), filled with about 10 g wheat grains for *T. granarium* and 10 g crushed wheat for *T. castaneum* and the cages were closed with rubber stoppers. The cages were then introduced into the 0.55 L gastight Dreshel exposure flasks. Insects in the flasks were treated for different exposure periods (24, 48, 72 and 96 hours) at 30 ± 1 ºC and 20 ± 1 °C, 65 ± 5% R. H. After the desired exposure periods, the flasks were aerated and all stages of insect species were transferred into Petri dishes and kept at the above mentioned conditions prior to mortality assessment.

Gases used

Carbon dioxide (CO_2) and nitrogen (N_2) were provided as pure gases in pressurized steel cylinders. Each cylinder was connected with a pressure regulator. The dilution method was used to achieve the required CO_2 concentration. For the atmosphere of nearly pure N_2, the valve of the N_2 cylinder was opened for two minutes in order to fill the Dreshel exposure flasks with the gas. CAs of 25, 50, 75% CO_2 (in air), 100% CO_2 and various mixtures of oxygen, nitrogen and carbon dioxide concentrations were also prepared (Fig 1).

Figure 1 Recirculatory multi-flask apparatus.

Determination of the concentrations of gases

Carbon dioxide was monitored using gas Analyzer model 200-600 (Gow-Mac-Instrument CO, USA). Nitrogen concentration was determined inside the flaks using Oxygen Analyzer 572, Servmex, England.

Bioassay tests

Tested insect samples were exposed to various lengths of time. After the desired exposure period, mortality assessment was made. Mortalities of *T. granarium* larvae and *T. castaneum* adults were determined after 24, 48, 72 and 96 hours of exposure periods. Mortality percentages were corrected by Abbott's formula 1925. The mortality of larvae and pupae of *T. castaneum* was recorded as reduction rate of the progeny which was inspected after 75 days from treatment using following formula.

$$\textbf{\%Reduction} = \frac{N_c - N_t}{N_c} \times 100$$

N_c = No. of emerged adults in control

N_t = No. of emerged adults in treatment

Statistical Analysis.
A probit computer program of Noack and Reichmuth (1978) and Finney (1971) was used to determine the lethal times for the gases.

Results

Efficacy of controlled atmosphere (CA) against *T. castaneum*

Data of the efficacy of various concentrations of CA against *T. castaneum* stages are given in Table 1. The results showed that, the adult stage was more sensitive to the different treated CAs than the larva stage whereas pupa was the most tolerant stage. The shortest times (0.1, 0.3 and 0.9 day for adults, larvae and pupae, respectively) needed to obtain 50% mortality of *T. castaneum* were at 100% CO_2 followed by 75% CO_2, 50% CO_2, 99% N_2 + 1% O_2 and 25% CO_2, at higher tested temperature (30 °C). The LT_{50} values of adults, larvae and pupae ranged between 0.1-1.5, 0.3-2.5 and 0.9-5.0 days, respectively. Also the shortest times needed to obtain 90% mortality of *T. castaneum* stages were recorded at 100% CO_2 and 30 °C, the LT_{90} values of adults, larvae and pupae were 0.6, 2.6 and 6.6 days, respectively. While the longest time required to obtain 90% mortality of the pupal stage (the tolerant stage) was 26.7 days at 25% CO_2 and 20 °C.

Table 1 Lethal times values and parameters of mortality regression line for *T. castaneum* exposed to CAs of 25, 50, 75% CO_2 (in air), 100% CO_2 and mixture of 99% nitrogen and 1% oxygen at two tested temperatures and 65 ± 5% R. H.

CA[a] (%)		Stage	Temperature: 30 °C, Lethal times (days)[b] LT 50	LT 90	Slope ± SE[c]	R[d]	20 °C, Lethal times (days)[b] LT 50	LT 90	Slope ± SE[c]	R[d]
CO_2	100	Adult	0.1 (0.1-1.2)	0.6 (0.13-1.6)	1.9±0.7	0.996	0.3 (0.1-2.4)	2.4 (1.3-3.0)	2.0±0.08	0.990
		Larva	0.3 (0.1-2.1)	2.6 (1.4-4.9)	1.6±0.03	0.923	0.4 (0.1-3.2)	5.8 (1.4-12.2)	1.7±0.3	0.919
		Pupa	0.9 (0.4-2.0)	6.6 (2.2-9.7)	1.7±0.37	0.970	1.5 (0.92-5.9)	11.1 (3-12.5)	1.9±0.01	0.947
	75	Adult	0.4 (0.1-1.2)	2.9 (1.5-5.4)	1.6±0.71	0.959	0.5 (0.1-3.6)	8.7 (5.2-12.5)	1.6±0.01	0.977
		Larva	0.5 (0.1-2.0)	4.3 (1.8-10.3)	2.5±0.08	0.985	0.6 (0.1-2.1)	9.2 (5.0-14.6)	2.8±0.16	0.954
		Pupa	1.0 (0.34-2.9)	12.1 (11.3-21.1)	1.8±0.06	0.972	2.2 (1.1-4.3)	16.8 (6.8-24.8)	2.0±0.21	0.946
	50	Adult	0.5 (0.2-1.5)	3.1 (1.2-5.0)	2.1± 0.02	0.967	0.5 (0.6-3.7)	7.0 (5.1-13.7)	2.0± 0.8	0.962
		Larva	0.7 (1.0-5.5)	5.9 (1.6-12.8)	2.5± 0.04	0.980	1.4 (0.8-5.2)	12.9 (9.8-25.7)	2.3± 0.1	0.921
		Pupa	2.4 (0.6-3.7)	14.3 (12.3-25.5)	2.3± 0.3	0.972	4.0 (2.3-6.6)	20.4 (13.6-27.2)	1.9±0.2	0.549
	25	Adult	1.1 (0.4-2.9)	4.2 (2.7-6.0)	1.9±0.01	0.990	1.5 (0.9-3.5)	11.0 (5.0-12.7)	1.3±0.1	0.999
		Larva	1.2 (0.8-5.7)	6.7 (4.4-13.0)	1.6±0.13	0.943	2.5 (1.2-8.8)	15.9 (10.7-27.3)	2.0±0.32	0.977
		Pupa	4.0 (1.33-8.3)	20.6 (13.2-42.2)	1.8±0.6	0.947	5.0 (1.2-10.1)	26.7 (16.3-50.7)	1.7±0.01	0.993
99 N_2+ 1 O_2		Adult	0.5 (0.4-1.4)	2.1 (1.7-2.9)	2.3±0.04	0.962	1.1 (0.6-1.8)	4.6 (2.41-6.9)	1.7±0.09	0.993
		Larva	1.1 (0.7-1.9)	3.6 (2.3-5.8)	2.5±0.71	0.913	1.4 (0.8-2.5)	11.5 (10.2-15.2)	2.0±0.03	0.924
		Pupa	1.5 (0.71-2.5)	12.4 (2.5-15.4)	1.9± 0.72	0.900	2.0 (1.3-2.9)	14.3 (3.5-17.9)	2.1±0.16	0.959

[a] Controlled atmosphere
[b] Lethal times (days) and their 95% confidence limits
[c] Standard error of the mortality regression line
[d] Correlation coefficient of regression line

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Efficacy of controlled atmosphere (CA) against *T. granarium* larvae

Results of the efficacy of various concentrations of CA against *T. granarium* larvae are given in Table 2. The obtained results indicated clearly that the active larvae were more sensitive to all treated CAs than diapausing larvae at tested temperature degrees. Where the LT_{50} values of the active larvae ranged between 0.4-6.1 days at 30 and 20 °C, respectively. The shortest time needed to obtain 50% mortality of *T. granarium* (active larvae) was 0.4 days at 100% CO_2 or 75% CO_2 or 86% N_2 + 4% O_2 + 10% CO_2, this time increased to 0.5, 2.0, 2.5, 3.2 and 5.3 days at 50% CO_2 or 25% CO_2, 99% N_2 + 1% O_2, 98% N_2 + 2% O_2, 96% N_2 + 4% O_2 and 91% N_2 + 4% O_2 + 5% CO_2 at higher tested temperature (30 °C), respectively. The shortest time needed to obtain 90% mortality of *T. granarium* (active larvae) was 2.5 days at 100% CO_2 and 30 °C. While the longest required to obtain 90% mortality of the active larvae was 18.0 days at 91% N_2 + 4% O_2 + 5% CO_2 and 20 °C.

Table 2 Lethal times values and parameters of mortality regression line for *T. granarium* exposed to CAs of 25, 50, 75% CO_2 (in air), 100% CO_2 and mixtures at various concentrations of oxygen, nitrogen and carbon dioxide at two tested temperatures and 65 ± 5% R.H.

CA[a] (%)		Stage	Temperature 30 °C: Lethal times (days)[b] LT_{50}	LT_{90}	Slope ± SE[c]	R[d]	Temperature 20 °C: Lethal times (days)[b] LT_{50}	LT_{90}	Slope ± SE[c]	R[d]
CO_2	100	A[e]	0.4 (0.2-2.0)	2.5 (1.4-4.7)	1.5 ± 0.12	0.924	0.8 (0.32-1.9)	5.2 (2.1-13.2)	2.5 ± 0.7	0.988
		D[f]	2.1 (1.3-3.7)	25.5 (12.0-32.0)	1.76 ± 0.2	0.915	4.6 (1.5-17.9)	29.2 (14.9-30.7)	2.1 ± 0.02	0.916
	75	A[e]	0.4 (0.2-2.6)	4.2 (1.4-5.5)	1.55 ± 0.3	0.922	0.9 (0.3-2.0)	5.6 (2.2-12.3)	1.9 ± 0.32	0.919
		D[f]	5.4 (1.5-7.6)	32.7 (25.0-76.2)	2.1 ± 0.13	0.947	8.9 (2.3-7.7)	38.6 (31.0-44.3)	1.7 ± 0.08	0.989
	50	A[e]	0.5 (0.3-3.7)	10.0 (5.5-36.2)	2.0 ± 0.03	0.919	0.9 (0.4-2.2)	12.0 (2.2-10.5)	1.6 ± 0.1	0.997
		D[f]	6.0 (1.70-8.6)	34.2 (20.0-36.5)	2.5 ± 0.06	0.974	9.0 (3.4-13.5)	47.3 (37.4-55.5)	2.8 ± 0.2	0.932
	25	A[e]	0.5 (0.1-3.0)	12.0 (4.7-14.8)	2.6 ± 1.8	0.980	2.0 (0.9-2.7)	15.0 (5.7-20.3)	1.9 ± 0.02	0.932
		D[f]	6.7 (1.9-13.3)	52.4 (21.2-91.0)	2.6 ± 0.04	0.994	10.0 (5.7-15.0)	58.6 (45.0-77.9)	1.7 ± 0.3	0.919
99 N_2 + 1 O_2		A[e]	2.0 (2.3–2.8)	6.5 (6.9–10.3)	3.1 ± 1.5	0.964	3.4 (3.1- 3.8)	9.7 (7.7–12.6)	2.6 ± 0.15	0.976
		D[f]	3.9 (3.3–8.2)	12.7 (9.2–17.2)	2.8 ± 0.62	0.951	8.2 (6.3-10.6)	19.6 (12.1–33.0)	2.2 ± 0.23	0.959
98 N_2 + 2 O_2		A[e]	2.5 (2.1–3.6)	8.4 (6.9–10.3)	2.5 ± 0.19	0.973	4.0 (3.61–4.6)	11.8 (9.0–15.3)	2.6 ± 0.16	0.976
		D[f]	4.3 (3.8–4.8)	13.1 (9.7–17.8)	2.5 ± 0.31	0.982	8.1 (4.62–10.7)	22.3 (13.9–36.9)	2.6 ± 0.11	0.984
96 N_2 + 4 O_2		A[e]	3.2 (2.9–3.6)	12.3 (10.0-16.1)	3.0 ± 1.13	0.090	6.1 (5.0-6.9)	17.0 (12.0-25.3)	2.5 ± 0.35	0.952
		D[f]	6.6 (5.3-8.1)	17.2 (12.1-33.0)	2.9 ± 0.7	0.963	8.2 (3.0–10.0)	24.6 (13.4-45.6)	2.11 ± 0.6	0.907
91 N2 + 4 O2 + 5 CO2		A[e]	5.3 (5.5-6.9)	16.8 (11.7-24.1)	2.7 ± 0.52	0.933	6.1(5.2-7.1)	18.0 (12.5-26.2)	2.0 ± 0.25	0.948
		D[f]	6.2 (5.2-7.4)	30.9 (20.8-40.6)	2.6 ± 0.69	0.915	9.7 (7.6-13.3)	40.0 (19.0-84.1)	2.2 ± 0.32	0.930
86 N_2 + 4 O_2 + 10 CO_2		A[e]	0.4 (0.2-2.6)	4.2 (1.4-5.5)	1.55 ± 0.3	0.922	0.9 (0.3-2.0)	5.6 (2.2-12.3)	1.9 ± 0.32	0.919
		D[f]	5.4 (1.5-7.6)	32.7 (25.0-76.2)	2.1 ± 0.13	0.947	8.9 (2.3-7.7)	38.6 (31.0-44.3)	1.7 ± 0.08	0.989

[a] Controlled atmosphere
[b] Lethal times (days) and their 95%confidence limits
[c] Standard error of the mortality regression line
[d] Correlation coefficient of regression line
[e] Active larvae (A)
[f] Diapausing larvae (D)

In case of diapausing larvae the LT_{50} values of the diapausing larvae ranged between 2.1 – 10.0 days at 30 and 20 °C, respectively. Results showed also that the shortest time needed to obtain 50% kill was 2.1 days at 100% CO_2 followed by 3.9, 4.3, 5.4, 6.0, 6.2, 6.6, 6.7 days at 99% N_2 + 1% O_2, 98% N_2 + 2% O_2, 75% CO_2 or 86% N_2 + 4% O_2 + 10% CO_2, 50% CO_2, 91% N_2 + 4% O_2 + 5% CO_2, 96% N_2 + 4% O_2, 25% CO_2 at higher tested temperature (30 °C). The shortest recorded time needed to obtain 90% mortality of *T. granarium* (diapausing larvae) was 12.7 days at 99% N_2 + 1% O_2 and 30 °C. While the longest time required to obtain 90% mortality of the diapausing larvae was 58.6 days at 25% CO_2 and 20 °C.

Discussion

Storage insects are aerobic organisms requiring oxygen for their survival. Therefore, they respond to altered atmospheric gas compositions containing low O_2 or high CO_2. The applications for which hermetic technology has been most widely accepted are for long-term storage of cereal grains, primarily rice, corn, barley, and wheat; for long-term storage of a variety of seeds to preserve germination potential and vigor, and for quality preservation of high-value commodities, such as dried fruits (Navarro, 2012).

In *T. castaneum*, there were greatest variations in lethal exposure times for each gas mixture. Also, adult stage was more sensitive to the different treated CAs than larval and pupal stages. Variations in lethal exposure times seem to decrease with increasing contents of CO_2 or decreasing O_2 contents. The data suggest that the increase in the temperature from 20 to 30 °C also increased the effectiveness to gas mixtures tested (Table 1).

In diapausing larvae of *T. granarium*, greatest variations in exposure times, needed to control diapausing larvae, were found in treatments with each gas mixture. The effectiveness of CAs to decrease the LT_{50} values of diapausing larvae in a descending order were 100% CO_2, 99% N_2 + 1% O_2, 98% N_2 + 2% O_2, 75% CO_2 or 86% N_2 + 4% O_2 + 10% CO_2, 50% CO_2, 91% N_2 + 4% O_2 + 5% CO_2, 96% N_2 + 4% O_2 and 25% CO_2 at 30 °C. If one compares the efficacy of gas mixture tested against active and diapausing larvae, it is quite striking that all tested mixtures of gases were more effective in controlling active larvae, but were less effective against young diapausing larvae (Table 2). This indicates some rather drastic changes in insect metabolism when the larva enters diapause, possibly the partial replacement of body water by glycerol that could reduce the detrimental effects of CO_2.

If the lethal exposure times of the diapausing larvae of *T. granarium* are compared to those obtained with other stages of *T. castaneum* and active larvae of *T. granarium* at the two tested temperatures, it becomes clear that the diapausing larvae may be the most tolerant stage.

Considering the effects of CAs on stored-product pests, one may still be surprised by the complexity and the variation of response of different species, developmental stages and strains. Post-treatment or end-point mortality seems to be another topic that needs closer attention in studies on hypoxic and hypercarbic atmospheres because of the post-treatment mortality of diapausing larvae noticed in this study and because of similar findings with adults of S. *oryzae* (Adler, 2001).

The effects on insect metabolism are different, depending on the levels of O_2 present in the controlled atmosphere. It seems that at percentages of O_2 lower than 3% (which is known as the ‘‘anaerobic compensation point’’), insects must adopt anaerobic metabolism (Mitcham *et al.*, 2006). In fact, under these conditions, the different stages of *Tribolium castaneum* (Herbst) decrease CO_2 production, which indicates the shutting down of oxidative pathways (Emekci *et al.*, 2001). Anaerobic metabolism, being much less efficient, demands an elevated consumption of reserves in order to obtain the same amounts of available energy (ATP). With the passing of time, ATP production becomes insufficient to guarantee the functioning of the ionic

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membrane pumps, with a resulting depolarization and consequent degeneration of tissues (Hochachka, 1986). In order to combat this situation, insects which Hochachka (1991) defined as ‘‘conformers’’ lower their metabolism almost to a complete stop, thus limiting their energy needs. At the same time, anaerobic metabolism, as it does not complete oxidation, leads to the accumulation of toxic products (Mitcham *et al.*, 2006). Therefore, a metabolism level that is too low, combined with the accumulation of toxic end products, is a cause of stress for the insect that eventually leads to its death (Donahaye and Navarro, 2000; Ofuya and Reichmuth, 2002; Neven and Hansen, 2010). At an O_2 range between 3 and 5% (which is defined as the ‘‘critical concentration point’’), there is not enough O_2 to produce the ATP necessary to maintain a normal metabolic level, so insects usually lower their metabolic level to reduce their energy demand (Mitcham *et al.*, 2006). Therefore, at O_2 concentrations between the anaerobic compensation point and the critical concentration point, oxidative respiration, even if reduced, is sufficient to satisfy the energy demand, which is also reduced. At O_2 levels lower than normal, but over 5% in air, insects increase their respiratory frequency so as to absorb the same amounts of O_2 and maintain their metabolism at normal levels. Zhou *et al.*, (2000) assumed that this increase of ventilation could lead to possible loss of water, keeping their spiracles open for a longer time than usual makes insects more susceptible to dehydration (Mbata and Phillips, 2001; Boardman *et al.*, 2012). High CO_2 concentrations may decrease pH which can be detrimental to membranes and cellular function. A decrease in pH will also denature enzymes, including antioxidant enzymes needed for low temperature tolerance, especially if there are no additional heat shock proteins (HSPs) to act as chaperones. In addition, high CO_2 causes a decrease in NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) enzyme and a subsequent decrease in glutathione production (Friedlander, 1983). NADPH and the antioxidant glutathione are involved in protecting against the toxicity of ROS, while NADPH also contributes to lipid synthesis, cholesterol synthesis, and fatty acid chain elongation. Secondly, high concentrations of CO_2 are commonly used as an anesthetic for insect handling. Identical to low O_2, CO_2 anesthesia blocked RCH in *D. melanogaster* after 1 h of exposure, but had no effect at shorter times (Nilson *et al.*, 2006). Badre *et al.*, (2005) investigated the mechanism underlying this response and found that in *D. melanogaster* larvae, with intact spiracles, high CO_2 caused their hearts to stop, and blocked synaptic transmission at the neuromuscular junction by decreasing the number of glutamate receptors. Further investigations showed that these effects were not due to hypoxia, low pH, or action of the central nervous system.

The obtained results coincide with the work of Adler (1995), Buscarlet (1993) and Locatelli and Daolio (1993) on the effect of temperature and the exposure to carbon dioxide or nitrogen on the developmental stages of *Sitophilus granarius*, *Tribolium confusm*, *Rhyzopertha dominca*, *Sitophilus oryzae*, *Oryzaephilus surinamensis* and *Plodia interpunctella.* Also El-Lakwah *et al.*, 2004 reported that the times required to 99% mortalities (LT_{99}s) of CAs and the variation among the developmental stages of *Tribolium castaneum* and between active or diapausing larvae of *Trogoderma granarium* was reduced with increasing temperature or concentration of CO_2.

Reduced oxygen and elevated carbon dioxide atmospheres can have an additive effect in some cases, depending on the concentrations used. The effect of these atmospheres on insects depends also on temperature, species and life stage (Mitcham *et al.*, 2006).

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مقایسه تأثیر آتمسفر کنترل شده علیه دو گونه حشره آفت محصولات انباری

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چکیده: اثر غلظت‌های مختلف گازهای دی اکسید کربن، ازت و اکسیژن روی مدت زمان لازم برای مرگ و میر شپشه گندم و لمبه گندم در دمای ۲۰ و ۳۰ درجه سلسیوس مورد مطالعه قرار گرفت. آزمایش‌ها در دستگاه چند محفظه‌ای با جریان هوای مداوم انجام شد. نتایج نشان داد که کوتاه‌ترین زمان لازم برای مرگ و میر ۵۰٪ از جمعیت لمبه گندم با غلظت دی اکسید کربن ۱۰۰٪، ۷۵٪ و ۵۰٪ ، ۹۹٪ گاز ازت + ۱٪ اکسیژن و ۲۵٪ گاز دی اکسید کربن در دمای ۳۰ درجه سلسیوس (۰/۱، ۰/۳ و ۰/۹ روز به‌ترتیب برای حشرات کامل، لارو و شفیره) بود. حشرات کامل بسیار حساس‌تر از لاروها بودند اما متحمل‌ترین مرحله شفیره‌ها بودند. لاروهای دیاپوزی لمبه گندم به تمام تیمارها در دماهای آزمایش شده متحمل‌تر بودند. مقادیر LT_{50} در تیمار مربوط به گاز دی اکسید کربن ۱۰۰٪ و ۹۹٪ ازت + ۱٪ اکسیژن و همچنین ۹۸٪ ازت + ۲٪ اکسیژن در دمای ۳۰ درجه سلسیوس کاهش یافت. می‌توان این-طور نتیجه گرفت که کنترل لاروهای دیاپوزی بسیار مشکل‌تر از لاروهای غیردیاپوزی می‌باشد. در صورتی‌که گاز ازت در غیاب اکسیژن تیمار شود مدت زمان لازم برای کنترل لاروهای دیاپوزی (تولید شده در آخر زمستان) را افزایش می‌دهد و بنابراین لازم است مدت زمان گازدهی را افزایش داد. بنابراین درصورت کنترل آفت در این شرایط استفاده از غلظت بالای گاز دی اکسید کربن بهترین گزینه برای کوتاه‌تر نمودن طول دوره گازدهی محسوب می‌شود.

واژگان کلیدی: هوای کنترل شده، *Tribolium castaneum*; *Trogoderma granarium* ، دستگاه چند محفظه‌ای با جریان مداوم

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