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RESPONSE OF THE DIFFERENT STAGES OF *Callosobruchus maculatus* (F.) AND *Tribolium castaneum* HERBST TO PRESSURIZED CO_2, N_2 AND AIR UNDER TWO CONDITIONS OF TEMPERATURE

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ABSTRACT

The present work aimed to study the efficiency of pressurized CO_2, N_2 and air against the different developmental stages of the cowpea beetle, *Callosobruchus maculatus* (F.) and the rust red flour beetle, *Tribolium castaneum* Herbst under two conditions of temptrature and 5 levels of gas pressure. The stages of both insect species were reared, chosen in a same age and exposed to the pressurized gases in a metal gas chamber for a period of two days. The results concerning *C. maculatus* showed that pressurized CO_2 was the most effective than the other two tested gases whatever the conditions of temperature. Mortality of insects increased gradually as the level of gases pressure increased. Adult stage was the most susceptible recording the highest average of mortality while pupal stage was the least susceptible. These results were recorded for both conditions of testing temperature. A similar trend was also recorded for the other tested insect species (*T. castaneum*) with some exception for this insect where it was more sensitive to the pressurized CO_2 and N_2 at the lower conditions of temperature (averaging 22 °C) than the cowpea beetle, while at the higher conditions of temperature (averaging 33 °C) the mortality of both insect species was very close. The results of this work show generally that using of CO_2 at 5 bar pressure and exposure period of 2 days can be useful and a promising method to control these two insect species if the storage facilities are suitable (from the airtight point of view) to conduct these procedures.

Key words: Pressurized inert gases, *Callosobruchus maculatus* , *Tribolium castaneum*, temptrature .

INTRODUCTION

Control of stored product pests are achieved mainly by chemical protectants and toxic gases (fumigants). Due to problems arising from these conventional methods of control (residues of pesticides in treated products and resistance of pests to pesticides), inert gases (modified atmospheres) were extensively used through the last few decades as a substitute to these methods. Modified atmospheres are considered one of the most safe methods for controlling the stored product pests although it acts slowly and needs some time (days or weeks) to induce a complete kill of insects (Reichmuth and Wohlgemuth, 1994; Riudavetes *et al.*, 2010). Modified atmospheres can be applied at high pressure. When seeking a high degree of control efficacy, high-pressure treatments are much faster acting than the normal atmospheric pressure applications and offer the most rapid option among current commercial applications. However, the application of high-pressure treatments also calls for high-pressure equipment, which requires major capital investment. When CO_2 is used at high pressure, its control efficacy varies according to pest species, pressure, exposure time and temperature. With *Sitophilus oryzae* L., all stages required 45 min., at a pressure of 20 bar to achieve complete control at 25 °C (Locatelli *et al.*, 1999). According to Nakakita and Kawashima (1994), only 5 min., of exposure were necessary to control adults, larvae and pupae of *S. zeamais* Motschulsky at a pressure of 20 bar. Comparing the mortality of 7 coleopterous and 5

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lepidoperous pest species in stored products when treated with CO_2 in a 3 m^3 pressure chamber, Prozell *et al.* (1997) found that a 180-min., treatment at 20 bar and a temperature of 20°C produced 100% mortality amongst all developmental stages. However, the use of inert gases under pressure requires an approved pressure chamber, and this, together with the consumption of the gases, will make the total price escalate fast for treatment at increasing pressures (Reichmuth and Wohlgemuth, 1994). The present work aimed to investigate the effect of CO_2, N_2 and air at different degrees of relatively high pressure (1 – 5 bar) on mortality of the different developmental stages of two of the most dangerous insect species of stored products (*C. maculatus* and *T. castaneum*).

MATERIALS AND METHODS

Tested Insects

Two known species of stored product insects were chosen for the present study. These insect species were cowpea beetle, *C. maculatus* and rust red flour beetle, *T. castaneum*. The original cultures of these insects were started by batches of adults initially collected from the infested products stored in warehouses at Zagazig district and reared in the laboratory, according to the methods described by Aamir (1975) and Miller *et al.* (1969) for the first and second insect species respectively.

Rearing of the Different Developmental Stages of *C. maculatus*

Egg stage

After one day of confining newly emerged adults (0-24 hours old) with fresh cowpea seeds the adults were removed and transferred again to new jars, while seeds bearing eggs were separated and the eggs were counted. Four replicates of 100 eggs each (of the seeds bearing eggs) were prepared and put separately in wire gauze cages to be ready for exposure to gases. One and two days old eggs were used for testing.

Larval and pupal stages

Large numbers of cowpea seeds bearing eggs of 0 – 24 hours old were divided into groups of about 500 eggs each. Each group was put in a separate glass jar covered with muslin cloth and kept in an incubator of insect rearing adjusted at 30°C and 60 ± 5% R.H. After 10 days of egg laying, beginning of the 3rd larval instar (Aamir, 1975), the seeds of the first group were divided into 4 replicates of 100 hatched eggs each and put separately in wire gauze cages to be ready for the subsequent tests. After 17 days of egg laying, the middle time of pupal duration (Helaly, 1973), the seeds of the second group were divided into 4 replicates of 100 hatched eggs each and put separately in 4 cages as mentioned before to be ready for exposure to gases.

Adult stage

Newly emerged adults of 1 – 2 days old of both sexes were collected randomly from the jars of stock cultures. The collected adults were divided into 4 batches of 100 adults each (of both sexes). Each batch was confined with a 20 g of cowpea seeds in a cage of wire gauze and used for testing.

Rearing of the Different Developmental Stages of *T. castaneum*

Stock cultures

The diet used for rearing the insect was prepared according to the method described by Miller *et al.* (1969). The medium consisted of the following food components: white wheat flour 930 g, white cornmeal 930 g and dried brewer's yeast 140 g. White wheat flour and white cornmeal were sieved through a sieve of 20 mesh per inch to eliminate any insect stages, then kept at – 13 °C in a deep freezer for at least one week to kill the remaining insects and mites. The components of the medium were mixed together using a blender, then the mixture was kept in plastic bags tied with a thread and used, whenever necessary in the experiments. One kilogram glass jars were used for maintaining the cultures. The jars were filled up to nearly one third with the abovementioned medium (about 200 g/jar) and some corrugated paper stripes being also laid on the surface of the medium. Two hundreds of the collected adults (7-14 days old) were released in each rearing jar. After one week of oviposition, the adults were sifted out and transferred to fresh rearing jars for another period (one week), then the adults were

discarded. These jars contained eggs – infested medium were covered with muslin cloth, secured with rubber bands and kept in an incubator at 27 ± 1 °C and 65 ± 5% R.H. When the adults of the new generation started to emerge, they were collected from the medium with a No. 20 sieve and introduced into new glass jars prepared in the same way.

Egg stage

White wheat flour was used as an oviposition medium, therefore, it was sifted through a 52 mesh/ inch sieve until all quantity of flour finely ground and the residues were separated. Measured volumes of the sifted white flour weighing about 200 g each were put in a glass jar (1 kg) and some corrugated paper stripes were placed also on the surface of flour in each jar. Batches of 200 adults of *T. castaneum*, 1 – 2 weeks old, were released separately in each prepared jar. After one day of oviposition, the adults were removed by using a No. 20 sieve, then the medium was sifted again using a No. 52 sieve to separate the eggs. Four replicates of 50 eggs each were counted by using a stereomicroscope binocular and gently transferred separately into four bags of dense cloth containing 25 g of rearing medium and thoroughly mixed. These bags were used for the subsequent experiments.

Larval and pupal stages

Large number of the collected eggs was divided into two groups of about 1000 eggs each. Each group was put in about 200 g of rearing medium in a 1 kg glass jar and mixed thoroughly. The jars were kept in an incubator at 27 °C. After 15 days of egg deposition, the hatched eggs in both jars had become larvae in the beginning of the third larval instar (Cichy, 1971). The larvae of one of both groups were used for testing while the other group was left more until the end of the 3rd week of egg-deposition where the larvae were developed to pupae (Aamir, 1981). Pupae of 2 days old were used. For testing both stages they were introduced separately into dense cloth bags each containing 25 g of rearing medium before exposure to the tested gases.

Adult stage

Batches of 100 newly emerged adults each of about one week old were sifted out from the maintained cultures and confined into the suitable vials to be ready for exposure to gases.

Exposure of the Different Developmental Stages of Both Insect Species to Pressurized Gases

Cylindrical 37 liters capacity metal chamber was used for exposure the tested insects to gases under a high pressure. The chamber had a wide open at its top provided with a metal disc (cover), rubber gas kit, and 4 bolts. The chamber had also 2 taps at both sides, one of them attached at the upper part of one side and the other at the lower one of the other side. Vials containing then prepared insect stages were introduced inside the exposure chamber and the chamber was tightly closed. Four replicates of 50 individuals each of each tested stages of both insects were used. One of the 2 taps (upper) was connected to the source of gas (gas cylinder or air compressor in case of compressed air) using suitable hose well belted. The chamber is provided also with a manometer mounting at the upper part of chamber to measure the pressure. The other tap (lower) left opened at the beginning of treatment to get out the air from the inside of chamber then this tap was closed while the gas flow up continuously inside chamber until the pressure reach to the respective level. One to five levels of pressure were tested (1 – 5 bar). The exposure period was two days for all experiments.

At the end of the exposure period the chamber was opened and the dead or alive individuals of adults and larvae were immediately counted and recorded while the other two stages (eggs and pupae) were maintained in an incubator until the egg hatch or adults emergence from pupae. The results were statistically analyzed according to the complete randomized plots design. While the method used for testing the data was the analysis of variance (factorial experiments). In the experiments where the data were expressed as percentages, the application of the angular transformation was used according to Bliss (1967).

RESULTS AND DISCUSSION

The Cowpea Beetle, *C. maculatus*

Data presented in Table 1 show generally that pressurized CO_2 was significantly the most effective than pressurized N_2 or air. The averages of insect mortality percentages were 75.92, 63.66 and 44.18% for CO_2, N_2 and air, respectively.

It was noticed also from the results of Table 1 that mortality of insects increased gradually as the level of gases pressure was increased. The lowest percentage of insect mortality (41.52% as a general average) was recorded for the lowest level of gas pressure (1 bar). Mortality of insects increased significantly as the gas pressure increased to reach the highest percentage of insect mortality (82.64%) for the highest level of gas pressure (5 bar). The mortality percentages of insects of the other three levels of gas pressure recorded intermediate values.

As for the susceptibility of the different insect stages to the tested gases it was very clear that adult stage was the most susceptible stage recording the highest average of mortality (69.35%) as a general average irrespective of the type of gases and pressure levels. Larval stage comes in the second position of susceptibility order recording 65.11% mortality, the other two stages (eggs and pupae) were less susceptible and very close from their susceptibility point of view recording 55.33% and 55.22% mortality for eggs and pupae, respectively (Table1).

Regarding the interaction effect of CO_2 x insect stage on the insect mortality it seems from the results that adult and larval stages were approximately similar in their sensitivity to CO_2 recording approximately the same averages of mortality indicating 81.99% and 81.33%, successively. On the other hand, egg and pupal stages were less susceptible and recorded very close averages of mortality (70.33% for eggs and 70.00% for pupae).

The interaction effects of pressurized N_2 and air x insect stages on the mortality of insects, the results show a similar trend as shown before for CO_2.

In conclusion, adult and larval stages were the most sensitives stages to the pressurized tested gases, while eggs and pupae were less sensitive. The sensitivity of both two stages was very close to any of the tested gases (Table 1).

Similarly, the three tested pressurized gases were more effective as the pressure level was increased, whereas the least mean percentages of insect mortality were recorded at the first level of pressure (1 bar) while the highest ones were recorded for the highest tested level of pressure (5 bar).

The results of Table 1 show generally that there was a significant effect for gases x stages x levels of pressure on the insect mortality. The highest levels of mortality were recorded (in most cases) for the adult stage when treated with any of the three tested gases at any level of pressure. Egg and pupal stages exhibited the lowest level of sensitivity to any of the tested gas and any level of pressure.

It could be concluded that using of CO_2 at a high pressure (5 bar) induced a high percentage of *C. maculatus* mortality after two days of exposure.

The results in Table 2 show generally that mortality of insects under the tested factors obviously increased at the higher temperature (33 °C) than at the lower one (22 °C). A complete mortality of all insect stages was detected at a pressure level of 4 bar CO_2. Pressurized N_2 induced a complete mortality of all stages at 5 bar. High percentages of mortality were also recorded for all stages at the highest level of pressurized air (5 bar). It was noticed generally from the results of Table 2 that mortality of insects increased gradually as the level of gases pressure was increased. The lowest percentage of insect mortality (60.27% as a general average) was recorded for the lowest level of gas pressure (1bar). Mortality of insects increased significantly as the gas pressure increased to reach the highest percentage of insect mortality (97.36%) for the highest level of gas pressure (5 bar).

Sensitivity of insect stages to the pressurized gases (in most cases) was also differed from one stage to another. A similar trend was also recorded at this degree of temperature, whereas adult was the most sensitive stage followed in a descending order by larvae, pupae and eggs (Table 2).

Table 1. Mean mortality percentages of *C. maculatus* different stages exposed to five levels of pressurized CO_2, N_2 and air for a period of two days at room temperature (averaging 22°C)

Gases	Insect stages Pressure levels (bar)	Eggs	Larvae	Pupae	Adults	Averages of pressure levels irrespective of stages	Averages of gases irrespective of stages and pressure levels
CO_2	1	53.33	68.33	55.00	66.66	60.83	
	2	61.66	73.33	58.33	76.66	67. 50	
	3	68.33	80.00	63.33	81.66	73.33	75.92
	4	80.00	90.00	83.33	88.33	85.42	
	5	88.33	95.00	90.00	96.66	92.50	
Averages		70.33	81.33	70.00	81.99		
N_2	1	38.33	51.66	36.66	53.33	45.00	
	2	46.66	60.00	43.33	65.00	53.75	
	3	58.33	66.66	55.00	71.66	62.91	63.66
	4	68.33	75.00	70.00	80.00	73.33	
	5	80.00	83.33	78.33	91.66	83.33	
Averages		58.33	67.33	56.66	72.33		
Air	1	11.60	20.00	13.33	30.00	18.73	
	2	25.00	31.66	26.66	40.00	30.83	
	3	38.33	43.33	36.66	51.66	42.50	44.18
	4	50.00	61.66	51.66	63.66	56.74	
	5	61.66	76.66	66.66	83.33	72.08	
Averages		37.32	46.66	38.99	53.73		
Averages of stages irrespect of gases and pressure levels		55.33	65.11	55.22	69.35		
Averages of pressure levels	1	2	3	4	5		
	41.52	50.69	59.58	71.83	82.64		

1. $LSD_{0.05}$ level for gases = 0.11%.
2. $LSD_{0.05}$ level for pressure levels = 0.18%.
3. $LSD_{0.05}$ level for stages = 0.15%.
4. $LSD_{0.05}$ level for gases x stages = 0.44%.
5. $LSD_{0.05}$ level for gases x pressure levels = 0.55%.
6. $LSD_{0.05}$ level for gasses x pressure levels x stages = 2.17%.

Table 2. Mean mortality percentages of *C. maculatus* different stages exposed to five levels of pressurized CO_2, N_2 and air for two days at room temperature (averaging 33 °C)

Gases	Insect stages Pressure levels (bar)	Eggs	Larvae	Pupae	Adults	Averages of pressure levels irrespective of stages	Averages of gases irrespective of stages and pressure levels
CO_2	1	71.66	90.00	80.00	91.66	83.33	
	2	86.66	95.00	88.33	96.66	91.66	
	3	96.66	100.00	95.00	100.00	97.92	94.58
	4	100.00	100.00	100.00	100.00	100.00	
	5	100.00	100.00	100.00	100.00	100.00	
Averages		91.00	97.00	92.67	97.66		
N_2	1	51.66	43.33	73.33	76.66	61.24	
	2	76.66	81.66	85.00	91.66	83.74	
	3	90.00	88.33	91.66	100.00	92.50	86.75
	4	93.33	95.00	96.66	100.00	96.25	
	5	100.00	100.00	100.00	100.00	100.00	
Averages		82.33	81.66	89.33	93.66		
Air	1	28.33	35.00	30.00	51.66	36.25	
	2	31.66	46.66	35.00	65.00	44.58	
	3	50.00	63.33	48.33	81.66	60.83	62.75
	4	71.66	83.33	70.00	95.00	80.00	
	5	88.33	95.00	85.00	100.00	92.08	
Averages		54.00	64.66	53.67	78.66		
Averages of stages irrespect of gases and pressure levels		75.78	81.11	78.56	89.99		
Averages of pressure levels	1	2	3	4	5		
	60.27	73.33	83.75	92.08	97.36		

1. $LSD_{0.05}$ level for gases = 0.11%.
2. $LSD_{0.05}$ level for pressure levels = 0.18%.
3. $LSD_{0.05}$ level for stages = 0.15%.
4. $LSD_{0.05}$ level for gases x stages = 0.44%.
5. $LSD_{0.05}$ level for gases x pressure levels = 0.55%.
6. $LSD_{0.05}$ level for gasses x pressure levels x stages = 2.17%.

As for the interaction effects of gases x insect stages on the insect mortality, it seems from the results that adult and larval stages were the most sensitive recording approximately similar values for their sensitivity to CO_2 followed by pupae and eggs at this degree of temperature.

Different trend of mortality was recorded for N_2 x insect stages whereas adult stage remained the most susceptible followed by pupae, eggs and larvae. At pressurized air adult stage still the most susceptible followed by larvae, eggs and pupae, the last two stages were nearly similar in their sensitivity.

Data presented in Table 2 reveal generally that there was a significant effect for gases x stages x levels of pressure on the insect mortality. The highest levels of mortality were recorded (in most cases) for the adult stage when treated with any of the three tested gases at any level of pressure.

Egg stage and pupal stage generally recorded the lowest levels of sensitivity to any of the tested gases and any level of gas pressure.

In conclusion, at both tested degress of temperature pressurized CO_2 was the most effective comparing with pressurized N_2 and air. The general averages of mortality percentages at 33°C were 94.58, 86.75 and 62.75% for CO_2, N_2 and air, respectively. An obvious trend for insect stages sensitivity was recorded, adult and larval stages were the most sensitive stages to the pressurized-tested gases while pupae and eggs were the least sensitive.

At this degree of temperature, the efficacy of the three tested pressurized gases were increased as the level of pressure increased, whereas the least percentages of insect mortality were recorded for the lowest level of pressure (1 bar) while the highest percentages of insect mortality were recorded for the highest tested level of pressure (5 bar).

The Rust Red Flour Beetle, *T. castaneum*

The results concerning the rust red flour beetle, *T. castaneum* (Tables 3 and 4) show approximately similar trends as observed before for *C. maculatus*. CO_2 gas was more effective than the other two tested gases whatever at both conditions of temperature (22°C and 33°C). *T. castaneum* at 22°C was more sensitive to pressurized CO_2 and N_2 than *C. maculatus*, while at 33°C the mortality of both insect species was very close. Mortality of all stages of the tested insect species was increased as the temperature of testing conditions increased. The mortality percentages of *T. castaneum* due to exposure to pressurized gases at 22°C were 84.42, 72.91 and 42.00% for CO_2, N_2 and air, respectively. When the exposure of insects to gases was carried out at the condition of high temperature (33°C), the mortality percentages obviously increased recording 93.00, 83.33 and 60.66% for the same gases, successively.

It seems clearly from the results of Tables 3 and 4 that adult stage was the most sensitive stage at the five tested levels of pressure of any tested gas. In general, adult stage recorded the highest percentage of mortality (77.44 and 90.22%) at the two tested conditions of temperature (averaging of 22°C and 33°C), respectively. Larval stage comes in the second position of sensitivity order recording 68.89 and 81.89% mortality for the two conditions of temperature exposure, consecutively. Egg stage was the least sensitive to the pressurized gases recording the lowest percentages of mortality (53.89 and 63.11%) at both conditions of temperature exposure.

In conclusion, adult stage of both insects of this work exhibited the highest degree of response to the tested gases followed desendingly by larvae, pupae and eggs.

As regards to the interaction effect of gases x insect stages on the mortality of *T. castaneum*, the results of Tables 3 and 4 show that adult stage was the most susceptible for the three tested gases at both conditions of temperature recording the highest percentages of mortality for CO_2 followed by N_2 then air. Similar trend was also recorded for the larval stage recording the highest mortality with CO_2 followed by N_2 then air. Sensitivity of pupal stage to the pressurized gases was slightly less than that of larval stage with the same order to the three tested gases. Egg stage was the least susceptible to the gases at both conditions of temperature. CO_2 was also the most effective against eggs followed by N_2 then air.

Table 3. Mean mortality percentages of *T. castaneum* different stages exposed to five levels of pressurized CO_2, N_2 and air for two days at room temperature (averaging 22°C)

Gases	Insect stage Pressure levels (bar)	Eggs	Larvae	Pupae	Adults	Averages of pressure levels irrespective of stages	Averages of gases irrespective of stages and pressure levels
CO_2	1	41.66	76.66	71.66	80.00	67.50	
	2	45.00	78.33	75.00	85.00	70.83	
	3	75.00	90.00	83.33	91.66	85.00	84.42
	4	96.66	100.00	98.33	100.00	98.75	
	5	100.00	100.00	100.00	100.00	100.00	
Averages		71.66	89.00	85.66	91.33		
N_2	1	30.00	51.66	46.66	63.33	47.91	
	2	38.33	65.00	53.33	70.00	56.66	
	3	50.00	73.33	70.00	81.66	68.75	72.91
	4	83.33	90.00	91.66	100.00	91.25	
	5	100.00	100.00	100.00	100.00	100.00	
Averages		60.33	76.00	72.33	83.00		
Air	1	10.00	21.66	18.33	38.33	22.08	
	2	15.00	28.33	25.00	41.66	27.50	
	3	30.00	36.66	33.33	55.00	38.75	42.00
	4	40.00	50.00	48.33	70.00	52.08	
	5	53.33	71.66	68.33	85.00	69.58	
Averages		29.67	41.66	38.66	58.00		
Averages of stages irrespect of gases and pressure levels		53.89	68.89	65.55	77.44		
Averages of pressure levels	1	2	3	4	5		
	45.83	51.66	64.17	80.69	89.86		

1. $LSD_{0.05}$ for gases = 0.08.
2. $LSD_{0.05}$ for pressure levels = 0.13
3. $LSD_{0.05}$ for stages = 0.11.
4. $LSD_{0.05}$ for gases x stages = 0.32
5. $LSD_{0.05}$ for gases x pressure levels = 0.40.
6. $LSD_{0.05}$ levels for gases x pressure levels x stages = 1.58.

Table 4. Mean mortality percentages of *T. castaneum* different stages exposed to five levels of pressurized CO_2, N_2 and air for two days at room temperature (averaging 33°C)

Gases	Insect stages Pressure levels (bar)	Eggs	Larvae	Pupae	Adults	Averages of pressure levels irrespective of stages	Averages of gases irrespective of stages and pressure levels
CO_2	1	60.00	91.66	90.00	95.00	84.16	
	2	68.33	96.66	93.33	96.66	88.74	
	3	78.33	100.00	98.33	100.00	94.16	93.00
	4	91.66	100.00	100.00	100.00	97.91	
	5	100.00	100.00	100.00	100.00	100.00	
Averages		79.66	97.66	96.33	98.33		
N_2	1	50.00	66.66	70.00	80.00	66.66	
	2	55.00	75.00	71.66	88.33	72.50	
	3	70.00	88.33	83.33	95.00	84.16	83.33
	4	85.00	95.00	93.33	100.00	93.33	
	5	100.00	100.00	100.00	100.00	100.00	
Averages		72.00	85.00	83.66	92.67		
Air	1	20.00	40.00	38.33	60.00	39.58	
	2	23.33	53.33	50.00	71.66	49.58	
	3	36.66	60.00	61.66	78.33	59.16	60.66
	4	48.33	76.66	80.00	88.33	73.33	
	5	60.00	85.00	81.66	100.00	81.66	
Averages		37.66	63.00	62.33	79.66		
Averages of stages irrespect of gases and pressure levels		63.11	81.89	80.77	90.22		
Averages of pressure levels	1	2	3	4	5		
	63.47	70.27	79.16	88.19	93.89		

1. $LSD_{0.05}$ for gases = 0.08.
2. $LSD_{0.05}$ for pressure levels = 0.13
3. $LSD_{0.05}$ for stages = 0.11.
4. $LSD_{0.05}$ for gases x stages = 0.32
5. $LSD_{0.05}$ for gases x pressure levels = 0.40.
6. $LSD_{0.05}$ levels for gases x pressure levels x stages =1.58.

Regarding the interaction effect of gases x levels of pressure on the insect mortality, a general trend was found in the results (Tables 3 and 4) that, for all the tested gases and at both conditions of exposure temperature, the increase of pressure level was accompanied with the increase of mortality percentages.

However, this observation was also recorded for any stage of the tested insect. For example, 100% mortality was occurred for adult and larval stages at 4 bar only of CO_2 but at 5 bar of CO_2 and N_2 with the other two stages. This result did not occur with the pressurized air (Tables 3 and 4).

According to the results of both tested insect species it can be concluded generally that :

- Pressurized CO_2 gas was the most effective against all stages of both insect species at the two tested conditions of temperature.
- Mortality of insects increased as the level of gas pressure increased at the same period of exposure.
- Adult stage was the most sensitive to the pressurized tested gases followed descendingly by larvae, pupae and eggs at both conditions of temperature.
- Temperature of area at which the gases were used (treatment in summer or in winter) had a significant effect on the efficiency of gases. Mortality of both insects increased at the higher condition of temperature (averaging 33°C) than at the lower one (averaging 22°C).
- If the storage facilities are suitable (from the airtight point of view) to conduct these procedures, using of CO_2 gas at 5 bar pressure can be useful and a promising method for controlling these two insect species.

Use of inert gases under different conditions of temperature or pressure to control the pests of stored products is an alternative safe method instead of the traditional pest control methods that leave hazard residues in food products. The present results go in line with the findings of Annis (1987) and Riudavetes *et al.* (2010) who reported that the development of alternative treatments for pest control is an increasing demand from the food industry and had been promoted by governments through legislation and the funding of research projects. Alternatives should meet consumer demands for the reduced use or elimination of pesticides while at the same time maintaining a high degree of control efficacy. For many decades, an atmosphere with a high content of carbon dioxide has been known to be toxic to insects, and the method has a long history in the area of control of stored product pests (Annis, 1987; Reichmuth, 1987). At the same time, one of the limitations of such treatment has been the requirements of long exposure times in terms of days or weeks. Two common methods are known to increase the efficacy of treatment with carbon dioxide. The raising of temperature or pressure will have a pronounced effect on the required time for control. The eggs of *S. granaries* (L.) which are very tolerant, are killed at a normal air pressure within 35 days at 10C in an atmosphere with 90% CO_2, but in only 3 days at 30°C (Alder, 1994). In comparison, the required time for 100% mortality at the pressure of 20 bar, 10 °C and an atmosphere with 99% CO_2 is 3 hours (Prozell and Reichmuth, 1991). The present investigation deals with the use of some inert gases at moderate pressures. Inert gases can be applied at high pressure when seeking a high degree of control efficacy, high pressure treatments are much faster acting than atmospheric pressure applications and offer the most rapid option among current commercial applications. However, when CO_2 is used at high pressure, it's control efficacy varies according to pest species, pressure, exposure time and temperature. With *S. oryzae*, all stages required 45 min., at a pressure of 20 bar to achieve complete control at 25°C (Locatelli *et al.*, 1999).

The symptoms of carbon dioxide poisoning in insects initially include a narcotic effect leading to a knockdown, *i.e.*, immobilization of the insects under carbon dioxide –enriched atmospheres (Aliniazee, 1971). There is no decrease in oxygen consumption in insects anesthetized by carbon dioxide and it seems that the main result of anesthesia is to induce the spiracle's permanent opening (Wigglesworth, 1983). When a pure N_2 atmosphere is maintained, oxidization of NADH occurs by conversion of pyruvate to lactate through an aerobiosis. In this case of anaerobic carbohydrate metabolism, glycerophosphate

dehydrogenase is involved in the oxidization of NADH instead of lactate-dehydrogenase (Gade, 1985). The toxic action of inert gases under increased pressure was first described by Johnson and Quastel (1953) and Carpenter (1954). They mentioned narcotic effects after treatment with these gases. Insect death presumably occurs during treatment under high pressure as a consequence of prolonged intense narcosis. Destruction of cell membranes during decompression also causes severe damage (Ulrichs, 1994). Prozell *et al.* (1997) stated that the speed of distribution of CO_2 under pressure seems to depend on the type and density of the treated product. The death occurred after treatment under high pressure following prolonged and intense narcosis. The toxic action of carbon dioxide under high pressure is not yet clear. Possibly it acts by increasing the respiration and solving in intestinal liquids (Stahl and Rau, 1985; Stahl *et al.*, 1985) and destroying cell membranes during rapid decompression.

REFERENCES

Aamir, M.M.I. (1975). Toxicological studies on the cowpea beetle, *Callosobruchus maculatus* (F.). M. Sc. Thesis, Fac. Agric., Zagazig Univ., Egypt.

Aamir, M.M.I. (1981). Toxicological studies on the strains of *Tribolium confusum* Duv. resistant to contact insecticides. Ph.D. Thesis, Fac. Agric., N. Balcescu, Bucharest, Romania.

Alder, C.S. (1994). A comparison of the efficacy of CO_2-rich and N_2-rich atmospheres against the granany weevil *Sitophilus granaries* L. (Coleoptera: Curculionidae). Proc. of the 6th Int. Working Conf. on Stored-Product Protection, Canberra, Australia: 11-15.

Aliniazee, M.T. (1971). Effect of carbon dioxide gas on respiration of confused flour beetle. J. Econ. Entomol., 64: 1304-1308.

Annis, P.C. (1987). Towards rational controlled atmosphere dosage schedules: A review of current knowledge. Proc. of the 4th Int. Work. Conf. on Stored Product Protection, Ed. by E. Donahaye and S. Navarro, Tel Aviv, Israel., 128-148.

Bliss, C.I. (1967). Statistics in Biology. McGraw-Hill, New York, St. Louis, San Franicsco, Toronto, London, Sydney.

Carpenter, F.G. (1954). Anaesthetic action of inert and inreactive gases on intact animals and isolated tissues. Am. J. Physiol., 178 : 505-509.

Cichy, D. (1971). The role of some ecological factors in the development of pesticide resistance factors in the development of pesticide resistance in *Sitophilus oryzae* L. and *Tribolium castaneum* Herbst. Ekol. Pol., 19: 563-616.

Gade, G. (1985). Anaerobic energy metabolism. In: "Environmental Physiology and Biochemistry of Insects", Hoffmann. K.H., (Ed.), Springer- Verlog, Vienna. Cited from Flerat-lesserd, F. (1990): Effect of modified atmospheres on insects and mites ifesting stored products. In: "Food Preservation by Modified Atmospheres" Calderon, M. and Raraki-Golon, R. (Eds.), CRC Press, Boca Raton, FL, USA: 21-38.

Helaly, M.M.Y. (1973). Studies on the population dynamics of the cowpea weevil, *Callosobruchus maculatus* (Fabricius). M.Sc. Thesis, Fac. Agric., Al Azhar Univ., Egypt.

Johnson, W.J. and J.H. Quastel (1953). Narcotics and biological acetylations. Nature, 171: 602-603.

Locatelli, D.P., L. Suss and M. Frati (1999). The use of high pressure carbon dioxide (20 bar) to control some insects of foodstuffs. Proc. of the 7th Int. Working Conf. on Stroed Product Protection, Beijing, Sichuon Publishing House of Science of Technology, Chengdu, China, 1: 671-675.

Miller, A., R. Philips and H.P.D. Cline (1969). Rearing manual for stored product insects. Research and Development Laboratory, Savannah, Georgia.

Nakakita, H. and K. Kawashima (1994). A new method to control stored-product insects using carbon dioxide with high-pressure followed by sudden pressure loss. Proc. of the 6th Int. Working Conf. on Stored Product Protection, Canberra, Australia, 17-23 April 1994, CAB Int., Wallingford, Oxon, Uk., 1: 126-129.

Prozell, S. and C. Reichmuth (1991). Response of the granary weevil *Sitophilus granaries* L.

(Col.: Curculionidae) to controlled atmospheres under high pressure. Proc. of the 5th Int. Work. Conf. on Stored Product Protection. BrodeauX, France: 911-918.

Prozell, S., C. Reichmuth, G. Ziegleder, B. Schartmann, R. Matissek, J. Kraus, D. Gerard and S. Rogg (1997). Control of pests and quality aspects in cocoa beans and hazelnuts and diffusion experiments in compressed tobacco with carbon dioxide under high pressure. Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products, 21-26 April 1996, Printco Ltd., Nicosia, Cyprus: 325-333.

Reichmuth, C. (1987). Low oxygen content to control stored product insects. Proc. of the 4th Int. Work. Conf. on Stored Product Protection, Ed. by E. Donahaye and S. Navarro, Tel Aviv, Israel: 194-207.

Reichmuth, C. and R. Wohlgemuth (1994). Carbon dioxide under high pressure of 15 bar and 20 bar to control the eggs of the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) as the most tolerant stage at 25 °C. Proc. of the 6th Int. Work. Conf. on Stored-Product Protection, Ed. by E. Highley; E.J. Wright; H. J. Banks; B.R. Champ., Canberra, Australia, 163-172.

Riudavetes, J., C. Castane, O. Almoar, M.J. Pons and R. Gabarra (2010). The use of carbon dioxide at high pressure to control nine stored product pests. J. Stored Prod. Res., 46: 228-233.

Stahl, E. and G. Rau (1985). Neves verfahren Zur Entwesung [A new method for disinfestations]. Anzeiger für schädlingskunde, Pflanzenschutz und Umweltschutz, 85: 133-136.

Stahl, E., G. Rau and H. Adolphi (1985). Entwesung von drogen durch kohlendioxide druckbehandlung (PEX-verafhren) [Disinfestation of drugs with carbon dioxide treatment under pressure (PEX- method). Pharm. Ind., 47: 528-530.

Ulrichs, C. (1994). Effect of different speed of build up and decrease of pressure with carbon dioxide on the adults of the tobacco beetle *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). Proc. of the 6th Int. Work. Conf. on Stored Product Protection. Canberra, Australia, 17-23 April 1994, CAB international, Wallingford, Oxon, UK, 1: 214-216.

Wigglesworth, V.B. (1983). The physiology of insect tracheoles. Adv. Insect Physiol., 17: 85-92.

استجابة الأطوار المختلفة لحشرتي خنفساء اللوبيا وخنفساء الدقيق الصدئية لثاني أكسيد الكربون، النيتروجين والهواء المضغوطين تحت درجتي حرارة مختلفتين

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يهدف البحث إلي دراسة فعالية غازي ثاني أكسيد الكربون والنيتروجين المضغوطين بمستويات مختلفة مقارنة بالهواء المضغوط أيضاً ضد الأطوار المختلفة لحشرتي خنفساء اللوبيا وخنفساء الدقيق الصدئية وذلك عند ظروف حرارية مختلفة و٥ مستويات من الغاز المضغوط، تم تعريض أطوار كلا الحشرتين في أعمار محددة فى اسطوانة معدنية ولمدة يومين إلي هذه الغازات المضغوطة، أوضحت النتائج أن غاز ثاني أكسيد الكربون كان أكثر الغازات المستخدمة فعالية تحت نفس الظروف، تزداد نسبة الموت تدريجياً بزيادة مستوي الضغط وكانت فعالية الغازات المختبرة أعلى عند درجة الحرارة العالية مقارنة بتلك المنخفضة، تبين أن طور الحشرة الكاملة هو أكثر الأطوار حساسية حيث سُجلت أعلي نسبة موت بينما كان طور العذراء أقل الأطوار حساسية، وذلك تحت ظروف حرارية مختلفة، أظهرت النتائج تشابه كلا الحشرتين في استجابتهما للعوامل المختبرة باستثناء أن حشرة خنفساء الدقيق الصدئية كانت أكثر حساسية لغازي ثاني أكسيد الكربون والنيتروجين المضغوطين تحت درجة الحرارة المنخفضة (٢٢°م) من خنفساء اللوبيا بينما عند درجة الحرارة المرتفعة (٣٣°م) فكانت الاستجابة لكلتا الحشرتين متقاربة جداً، أظهرت النتائج أنه يمكن استخدام غاز ثاني أكسيد الكربون عند مستوي ضغط ٥ بار ولمدة يومين فى مكافحة هذين النوعين من الحشرات إذا ما سمحت ظروف المخزن بإجراء مثل هذه الطريقة.

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