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EFFECT OF PRESSURIZED CO_2, N_2 AND AIR GASES ON DIFFERENT STAGES OF *Oryzaephilus surinamensis* (L.) AND *Stegobium paniceum* (L.)

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ABSTRACT: This study aimed to evaluate the effect of pressurized CO_2, N_2 and atmospheric air against the immature stages and adults of two secondary insect species of stored products [*Oryzaephilus surinamensis* (L.) and *Stegobium paniceum* (L.)]. The insects were exposed to 5 levels (1-5 bars) of the pressurized gases in a metal gas cylinder for two exposure periods (one day and two days) at average of 20°C. The results showed generally that, Co_2 was the most effective as it induced the highest mortality percentages followed by N_2 then atmospheric air. Sensitivity of the two tested insect species to CO_2 and N_2 was approximately the same, while *S. paniceum* was more sensitive to the pressurized air than *O. surinamensis*. Egg stage of the two insect species was the more tolerant stage, while adult stage exhibits the highest level of sensitivity to all tested gases for any level of pressure and the both periods of exposure. Larvae of *S. paniceum* as exceptional case were more tolerant to the pressurized CO_2 than the other stages. It was noticed also that mortality of all insect stages of both species was significantly increased as the exposure period to the tested gases increased. Mortality of insects increased as the level of gas pressure increased recording the highest percentages of insect mortality at the highest level of gas pressure (5 kg/cm^2).

Key words: Pressurized inert gases, *S. paniceum, O. surinamensis,* exposure period, insect mortality.

INTRODUCTION

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) and the drugstore beetle, *Stegobium paniceum* (L.) are two insect species of the most known dangerous pests of stored dried fruits.

The chemical control of stored product pests by insecticides causes many problems such as: handling hazards, residues, development of resistance, resurgence and environmental pollution (Shazali *et al*., 2003).

Use of inert gases as an alternative treatment for pest control in food commodities is an increasing demand for the food industry which should meet consumer demands for the reduced use or elimination of pesticides and environmentally friend (Adler *et al*., 2000; Navarro, 2006).

Modified atmosphere treatments when used at atmospheric pressure may take from a few days to several weeks to be successful in controlling all the developmental stages of pests depending on gas concentrations and temperature (Banks and Annis, 1990; White *et al*., 1995; Riudavets *et al*., 2009). To solve the slow action of modified atmosphere, some researchers have suggested application of high pressure to cause a high degree of control efficacy, much faster acting than atmospheric pressure application and offer the most rapid option among current commercial application such as in quarantine (Navarro, 2006). When CO_2 is used at high pressure, its control efficacy varies according to pest species, stage of insect, pressure, exposure time and temperature (Locatelli *et al*., 1999).

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The high pressure of carbon dioxede (CO_2) treatment combines most of the advantage of CA technology and at the same time addresses its most serious drawback that is it requires extremely short duration for lethal exposure within the range of hours or days. This method is highly promising for quarantine treatment and rapid disinfestations of valuable products.

The present study aimed to establish the efficacy of CO_2 and N_2 in comparing with the air at 5 levels of high pressure (1-5 bars) to control different developmental stages of the two insect species *O. surinamensis* and *S. paniceum* that infest dried fruits.

MATERIALS AND METHODS

Tested Insects

Two known species of stored product insects were chosen for the present study. These insect species were: the saw-toothed grain beetle, *O. surinaminsis* and the drugstore beetle, *S. paniceum*. The collected insects were maintained at the Stored Product Pests Laboratory, Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt.

Rearing technique of *O. surinaminsis* stock culture

The diet used for rearing the insect cultures was prepared according to the method used by Miller *et al*. (1969). The medium consisted of the following food components: white flour 930 g, white corn meal 930 g and brewer's yeast 140 g. White flour and corn meal were sieved separately through a sieve of 20 mesh per inch^2 to eliminate any insect stages, then kept at -13˚C in a deep freezer for at least one week to kill remaining insects and mites. The medium components were mixed together using a blender then the mixture was put in plastic bags tied well with a thread and kept in the deep freezer to be ready for using in experiments when ever necessary. One kilogram glass jars were used for maintaining the cultures. The jars were filled up to nearly one third with the above-mentioned medium (about 300 g per jar), and some corrugated paper stripes being also laid on the surface of the medium. Two hundreds of the collected adults were released in each rearing jar. After one week of oviposition the adults were sifted out and transferred to fresh rearing jar for another period (one week) then the adults were discarded. The jars were covered with muslin cloth, secured with rubber bands and kept in an incubator adjusted at 27±2°C and 65± 5% RH. When the adults of the new generation started to emerge, they were collected from the medium with a no. 20 sieve and introduced to new glass jars prepared by the same way as mentioned before.

Rearing of the developmental stages of *O. surinamiensis* for testing

Egg stage

White flour was used as an oviposition medium, therefore, it was sifted through a 52 mesh/inch^2, inch sieve until all quantity of flour was finely ground and the residues were separated. Measured volumes of the sifted white flour weighing about 200 g each were put in glass jars (500 g) and some corrugated paper stripes were placed also on the surface of flour in each jar. Batches of about 200 adults each of the insect, 10 days old were released separately in each prepared jar. After one day of egg laying the adults and the deposited eggs were sifted out from white flour using a no. 52 sieve. The eggs were collected and counted for testing and the adults were introduced into other new jars. Replicates of 50 eggs each were counted by using a binocular and gently transferred into wire gauze cages of 3x8 cm containing 25 g rearing medium and thoroughly mixed. These cages 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 on about 500 g of rearing medium in a 1 kg glass jar and mixed thoroughly. The jars were kept in an incubator adjusted at 27±2ºC and 65±5% RH. After 20 days, the hatched eggs in both jars had become larvae in the beginning of the last instar larvae (15 days old). The larvae of one of both groups was kept until the 25th day, whereas the larvae were developed to pupae (Finkelman *et al*., 2003). Pupae of (0-1 day old) were used for testing.

Adult stage

Batches of 50 newly emerged adults each of about one week old sifted out from the maintained culture and confined with a quantity of rearing medium into the suitable vials to be ready for testing.

Rearing technique of *S. paniceum* stock culture

The rearing medium comprised of whole-wheat flour 95% and 5% brewer's yeast (Childs and Overby, 1983). The same procedures mentioned above for rearing of *O. surinamensis* were also used for rearing the stock culture of *S. paniceum*.

Rearing of the developmental stages of *S. paniceam*

The same steps which were carried out for rearing the developmental stages of *O. surinamensis* were also used for rearing the eggs, larvae and pupae of *S. paniceum*, with taking in consideration the developmental period of each stage.

Adult stage

Batches of newly emerged adults (2-3 days old) were sifted out from the maintained cultures and confined with a quantity of rearing medium in suitable vials to be ready for testing.

Exposure Period of Different Developmental Stages of *O. surinamensis* and *S. paniceum* to Pressurized Gases

Cylindrical (37 liters capacity) metal chamber was used for insect exposure to the gases. The chamber had a wide open at its top provided with a metal disc (cover), rubber gas kit, and 6 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 part of the other side. The vials containing the insect stage which prepared before, were introduced inside the exposure chamber and the chamber was tightly closed. Three replicates of 50 individuals of each tested stage of both insects were used. The tap of the upper part of the chamber was connected to the source of the tested gas (gas cylinder or air compressor) using a suitable hose and well belted. The chamber was provided also with a manometer mounting at the upper part 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 the chamber until the pressure reach to the respective level. Five levels of pressure were tested (1-5 bars). The exposure period was one or two days for all insect stages of both insects. At the end of the exposure period, the chamber was carefully and gradually opened and the dead or alive larvae and adults of both insect species were immediately counted and recorded while the other two stages (eggs and pupae) were maintained in an incubator until eggs hatching or adults emergence.

The results were statistically analyzed using the analysis of variance (factorial experiments) according to Snedecor (1970). The significant differences between means of treatments were tested using the least significant difference method.

RESULTS AND DISCUSSION

Mortality percentages of eggs, larvae, pupae and adults of both tested insect species (*O. surinamensis* and *S. paniceum*) exposed to five levels of pressurized air, CO_2 and N_2 for periods of one and two days at temperature 20ºC are presented and discussed as follows:

Pressurized Atmospheric Air

Results in Tables 1 and 2 show that *S. paniceum* was more significantly sensitive to the pressurized air than *O. surinamensis*. It was noticed generally that egg stage of both insects was significantly the most tolerant than the other three stages, while the adult stage was the most sensitive recording the highest values of mortality at any of the two exposure periods. The mortality average of the insect stages at both exposure periods were 46.65, 65.55. 51.33 and 67.29 for eggs, larvae, pupae and adults of *O. surinamensis*, respectively. These values were 51.78, 59.11, 61.11 and 74.34% for eggs, larvae, pupae and adults of *S. paniceum*, respectively (Tables 1 and 2).

Exposure period to pressurized air also affected significantly the mortality percentage of insect stages of both species. The longest the exposure period, the highest percentages of

Table 1. Mortality percentages of *O. surinamensis* exposed to five levels of pressurized air for one and two days exposure at an average of 20ºC

Insect stage	Exposure period	Level of gas pressure (in bars)					Mortality mean of exposure periods	Mortality mean of insect stages	Mortality mean of insect species
		1 bar	2 bars	3 bars	4 bars	5 bars			
		Mortality percentage							
Eggs	**1 day**	8.66	19.99	31.11	46.66	68.88	35.06	46.65	
	2 days	25.66	42.22	53.33	73.33	96.66	58.24		
Larvae	**1 day**	26.66	35.55	53.33	67.77	79.99	52.66	65.55	57.71
	2 days	48.88	65.55	82.21	95.55	100	78.44		
Pupae	**1 day**	6.66	13.33	38.88	54.44	65.55	35.77	51.33	
	2 days	32.21	41.11	76.66	85.55	98.88	66.88		
Adults	**1 day**	23.33	37.77	48.88	71.10	88.88	53.99	67.29	
	2 days	53.33	66.27	84.44	98.88	100	80.58		
Mean of pressure levels		28.17	40.22	58.60	74.16	87.36			

Table 2. Mortality percentages of *S. paniceum* exposed to five levels of pressurized air for one and two days exposure at an average of 20ºC

Insect stage	Exposure period	Level of gas pressure (in bars)					Mortality mean of exposure periods	Mortality mean of insect stages	Mortality mean of insect species
		1 bar	2 bars	3 bars	4 bars	5 bars			
		Mortality percentage							
Eggs	**1 day**	13.33	21.11	33.33	52.21	75.55	39.11	51.78	
	2 days	39.99	45.55	61.11	81.11	94.44	64.44		
Larvae	**1 day**	9.99	16.66	45.55	67.77	94.44	46.88	59.11	61.59
	2 days	33.33	58.88	75.55	89.99	98.88	71.33		
Pupae	**1 day**	17.77	34.44	49.99	63.33	86.66	50.44	61.11	
	2 days	41.11	55.55	72.22	89.99	100	71.77		
Adults	**1 day**	32.22	46.66	66.77	81.11	100	65.35	74.34	
	2 days	57.77	71.10	89.99	97.77	100	83.33		
Mean of pressure levels		30.69	43.74	61.81	77.91	93.75			

Statistical analysis of mortality percentages of both tested insects (*O. surinamensis* and *S. paniceum*) under pressurized air (Tables, 1 and 2):

$LSD_{0.05}$ level for insect stages (Ist) = 1.73.

$LSD_{0.05}$ level for pressure levels (Pl) = 19.75.

$LSD_{0.05}$ level for insect species (Isp) = 0.80.

$LSD_{0.05}$ level for exposure periods (Ep) = 12.49.

$LSD_{0.05}$ level for Isp x Ist = 2.45.

mortality of all insect stages. Mortality percentage of any of the insect stages was clearly increased as the exposure period increased from one day to two days. This observation was very true for all stages of both insect species, whereas the mortality percentages were sharply increased when the exposure period increased from one day to two days (Tables 1 and 2). It was noticed also that mortality of all insect stages increased gradually as the level of pressure increased recording the highest value of mortality at the highest level of pressure (5 bars). However, a complete mortality was occurred for adult stage at the highest level of pressure (5 bars) whether the exposure period was one day or two days.

The interaction effect of (insect species x insect stage) had a significant effect on the mortality percentages of all stages of both insect species (Tables, 1 and 2). Egg stage of *O. surinamensis* was the most tolerant stage as it recorded the lowest percentage of mortality while the adult stage of *S. paniceum* was the most susceptible stage as it recorded the highest percentage of mortality.

Pressurized CO_2 Gas

The results recorded in Tables 3 and 4 show generally that, mortality of both insect species was clearly increased when exposed to pressurized CO_2 than when exposed to pressurized air. However, mortality of both insect species did not differ significantly from each other when treated with pressurized CO_2. In other words, total mortality of both tested species with all different developmental stages and adults did not differ significantly from each other (Tables 3 and 4).

Adult stage of both species of insects was the most sensitive stage recording the highest percentages of mortality comparing with the other three stages. Egg stage of *O. surinamensis* was also the most tolerant stage to CO_2 similar to that recorded before for the pressurized air, while the larval stage of *S. paniceum* was the most tolerant stage to CO_2 however, mortality of the developmental stages of any of the tested insect species were differed significantly from each other (Tables 3 and 4).

It was found also that mortality of all insect stages of both species was increased significantly as the exposure period to CO_2 gas increased recording the highest percentages of mortality for the exposure period of two days comparing with that of one day (Tables 3 and 4).

Results in Tables 3 and 4 show also that the level of pressure was also affected significantly the mortality percentages of all stages of both species. Mortality of insect stages was increased gradually and significantly as the pressure level was increased recording 100% mortality for all stages at the pressure levels of 4 and 5 bars mostly.

All interaction effects between the tested factors on the mortality of insects exhibited a significant effect as shown in Tables 3 and 4.

Pressurized N_2 Gas

The effect of pressurized N_2 gas on the mortality percentages of all stages of both insects took a moderate position between pressurized atmospheric air and pressurized CO_2 (Tables 5 and 6).

The total mortality of all stages as a whole of both insect species exhibited approximately the same response without a significant difference between them recording 74.44 and 74.11% for *O. surinamensis* and *S. paniceum*, respectively. The different insect stages of both insect species exposed to N_2 gas was differed significantly from each other; egg stage of both species was the most tolerant to the pressurized N_2 gas than the other stages. Also, adult stage of both species was the most susceptible stage recording the highest percentage of mortality (Tables 5 and 6).

Mortality of insects clearly and significantly increased as the exposure period increased from one day to two days. This observation was very obvious for both insect species.

The results show also that, mortality of insects (both species and all stages of each species) obviously increased as the pressure increased recording the highest percentages of mortality for any insect stage at the highest level of pressure (5 bars).

A complete mortality of larvae, pupae and adults of both species was noticed at the pressure levels of 4 and 5 bars when the exposure period was extended for two days (Tables 5 and 6).

Table 3. Mortality percentage of *O. surinamensis* exposed to five levels of pressurized CO_2 for one and two days exposure at an average of 20ºC

Insect stage	Exposure period	Level of gas pressure (in bars)					Mortality mean of exposure periods	Mortality mean of insect stages	Mortality mean of insect species
		1 bar	2 bars	3 bars	4 bars	5 bars			
		Mortality percentage							
Eggs	**1 day**	39.99	54.44	70.88	93.33	100	71.73	80.31	
	2 days	63.33	85.55	95.55	100	100	88.89		
Larvae	**1 day**	58.88	68.88	93.33	100	100	84.22	88.89	
	2 days	76.66	98.88	100	100	100	95.11		85.64
Pupae	**1 day**	36.66	57.77	79.88	98.88	100	74.64	83.27	
	2 days	69.99	88.88	100	100	100	91.77		
Adults	**1 day**	62.21	75.00	97.77	100	100	87.00	89.36	
	2 days	58.55	100	100	100	100	91.71		
Mean of pressure levels		58.28	78.68	92.18	99.03	100			

Table 4. Mortality percentage of *S. paniceum* exposed to five levels of pressurized CO_2 for one and two days exposure at an average of 20ºC

Insect stage	Exposure period	Level of gas pressure (in bars)					Mortality mean of exposure periods	Mortality mean of insect stages	Mortality mean of insect species
		1 bar	2 bars	3 bars	4 bars	5 bars			
		Mortality percentage							
Eggs	**1 day**	43.33	66.66	83.33	97.77	100	78.22	85.22	
	2 days	71.10	89.99	100	100	100	92.22		
Larvae	**1 day**	35.55	45.55	58.88	81.10	100	64.22	75.56	
	2 days	63.33	77.77	93.33	100	100	86.89		86.29
Pupae	**1 day**	57.77	61.10	85.88	98.88	100	80.73	88.37	
	2 days	85.55	94.44	100	100	100	96.00		
Adults	**1 day**	78.88	87.77	100	100	100	93.33	96.00	
	2 days	93.33	100	100	100	100	98.67		
Mean of pressure levels		66.11	77.91	90.18	97.22	100			

Statistical analysis of mortality percentages of both tested insects (*O. surinamensis* and *S. paniceum*) under pressurized CO_2 (Tables 3 and 4):

$LSD_{0.05}$ level for insect stages (Ist) = 1.51.
$LSD_{0.05}$ level for exposure periods (Ep) = 0.45
$LSD_{0.05}$ level for pressure level (Pl) = 0.70.
$LSD_{0.05}$ level for (Isp × Ist)= 2.13.
$LSD_{0.05}$ level for (Isp × Pl)= 1.00.
$LSD_{0.05}$ level for (Ist × Ep)= 0.89.
$LSD_{0.05}$ level for (Ist × Pl)= 1.41.
$LSD_{0.05}$ level for (Ep × Pl) = 1.00.
$LSD_{0.05}$ level for (Isp × Ist × Ep)= 1.26.
$LSD_{0.05}$ level for (Isp × Ist × Pl)= 2.00.

Table 5. Mortality percentage of *O. Surinamensis* exposed to five levels of pressurized N_2 for one and two days exposure at an average of 20ºC

Insect stage	Exposure period	Level of gas pressure (in bars)					Mortality mean of exposure periods	Mortality mean of insect stages	Mortality mean of insect species
		1 bar	2 bars	3 bars	4 bars	5 bars			
		Mortality percentages							
Eggs	**1 day**	28.88	38.88	47.77	63.33	88.88	53.55	66.22	
	2 days	51.10	65.55	81.11	96.66	100	78.88		
Larvae	**1 day**	35.55	49.99	65.55	81.11	98.88	66.22	77.11	
	2 days	63.33	81.11	95.55	100	100	88.00		74.44
Pupae	**1 day**	33.33	41.11	58.88	75.55	91.10	59.99	71.77	
	2 days	56.66	73.33	87.77	100	100	83.55		
Adults	**1 day**	45.55	57.77	73.33	93.33	100	74.00	82.67	
	2 days	71.11	87.77	97.77	`100	100	91.33		
Mean of pressure levels		48.19	61.94	75.97	88.75	97.36			

Table 6. Mortality percentage of *S. paniceum* exposed to five levels of pressurized N_2 for one and two days exposure at an average of 20ºC

Insect stage	Exposure period	Level of gas pressure (in bars)					Mortality mean of exposure periods	Mortality mean of insect stages	Mortality mean of insect species
		1 bar	2 bars	3 bars	4 bars	5 bars			
		Mortality percentage							
Eggs	**1 day**	24.44	34.44	51.11	68.88	85.55	52.88	65.77	
	2 days	46.66	67.77	79.99	98.88	100	78.66		
Larvae	**1 day**	19.99	25.55	43.33	75.55	85.55	49.99	66.55	74.11
	2 days	58.88	73.33	83.33	100	100	83.11		
Pupae	**1 day**	35.55	47.77	65.55	83.33	78.88	62.22	74.44	
	2 days	62.21	78.88	92.22	100	100	86.66		
Adults	**1 day**	58.88	72.21	89.99	96.66	100	83.55	89.67	
	2 days	83.33	95.55	100	100	100	95.78		
Mean of pressure levels		48.74	61.94	75.69	90.41	93.75			

Statistical analysis of mortality percentages of both tested insects (*O. surinamensis* and *S. paniceum*) under pressurized N_2 (Tables 5 and 6):

$LSD_{0.05}$ level for insect stage (Ist) = 1.55.
$LSD_{0.05}$ level for Exposure period (Ep) = 4.67.
$LSD_{0.05}$ level for pressure level (Pl) = 7.39.
$LSD_{0.05}$ level for (Isp × Ist)= 2.19.
$LSD_{0.05}$ level for (Ep × Pl)= 13.2.
$LSD_{0.05}$ level for (Isp × Ist × Ep)= 20.92.

The interactions between insect species × insect stage, exposure period × level of pressure and insect species × insect stage × exposure period had significant effects on the mortality percentages of both insects. For example, the highest percentage of mortality was recorded for the adult stage of *S. paniceum* (Tables 5 and 6).

The results of the present study showed clearly that efficacy of the pressurized CO_2, N_2 and air on the mortality of the tested insects was differed significantly according to the insect species, the stage of insects, the type of gases and the level of gas pressure. The effect of the inert gases (CO_2 and N_2) on insect mortality, susceptibility of insect species and insect stages to inert gases, level of gas pressure and exposure period on insect mortality could be discussed as follows:

Annis (1987) and Riudavets *et al*. (2010), reported that the development of alternative treatment for pest control is an increasing demand from the food industry and had been promoted by government through legislation and the funding of research projects. Alternatives should use or elimination of pesticides while at the same time maintainting 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 along history in the area of control of stored product pests (Annis, 1987; Reichmuth, 1987). At the same time, one of the limitations is a long exposure time 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 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 treatment is much faster acting than atmospheric pressure applications and offers the most rapid option among current commercial applications.

However, when CO_2 is used at high pressure; its control efficacy varies according to pest species, pressure, exposure time and temperature. With *Sitophilus oryzae*, all stages required 45 min., at a pressure of 20 bars to achieve complete mortality 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 the 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 Quostel (1953) and Carpenter (1954). They mentioned narcotic effect 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; Ulrichs *et al.*, 1997). Prozell *et al*. (1997) stated that the speed of CO_2 effect 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. Wei *et al.* (1998) showed two reasons by which the pressurized CO_2 can kill the insect: 1). Cause a severe damage to the wall of their body resulting in their death in large numbers. 2). High pressure, large quantity of CO_2 could quickly penetrate the cell walls of insects which would cause toxicities and quick death to insects.

The great difference in the effects of CO_2 and N_2 treatment against the insects may be explained by the different solubility of the gases

in the haemolymph of insects. Additionally, CO_2 is known to inhibit respiration (Nielsen, 2001). This is may be due to low levels of oxygen and high levels of CO_2 (Kutbay *et al*., 2011). The lethal action of CO_2 is related to the increased solubility of CO_2 in the insect body fluids under high pressure, causing a subsequent decrease in pH. It has also been reported that a dramatic increase in the uptake of CO_2 under high pressure causes rapid expansion and rapid evaporation from the liquid when the pressure is reduced, resulting in lesions in the cell membrane of insects. The integument of insects exposed to the treatment was severely damaged due to the expansion of internally dissolved CO_2 in the body when the gas pressure was rapidly reduced to atmospheric pressure. Riudavets *et al*. (2010) mentioned that exposure times needed to achieve 100% control were still much shorter at the higher pressure of CO_2 than those needed when the same species were treated with CO_2 at atmospheric pressure since the efficacy of CO_2 is linked to the speed of solution in the body of insects and this depends on the partial pressure of CO_2 in the atmosphere.

The present findings regarding to the stability of insect species and insect stages to inert gases go on line with the findings of many authors such as Gerald *et al*. (1988). They reported that egg stage of *Plodia interpunctella* was the most tolerant stage, which supports the hypothesis: eggs have low water content and very few cell membranes and are most stable as spheres. Nakakita and Kawashima (1994) reported that susceptibility of different species of stored product insects and their development stages to pressurized CO_2 was differed form one species to another. They added that egg stage was the most resistant stage and mentioned also that there is a physical action of high pressure gas on tissues of the insects causing expansion. Nakakita *et al*. (2001) determined lethal effects of CO_2 under high pressure on the development stages of some species of stored grain insects including *Tribolium castaneum, Lasioderma serricorne*, *Oryzaephilus surinamensis*, *P. interpunctella* and *Corcyra cephalonica* and found that the egg stage was the most resistant in all tested species, it required almost the longest exposure periods. The authors added that *S. zeamais* was the most susceptible to high pressure CO_2 than the other tested species of *Sitophilus*. They added that egg stage was the most tolerant stage to high pressure of CO_2 and the tolerance levels are progressively lower for larvae, adults and pupae. In *S. oryzae* the sensitivity of the adults was very high. Riudavets *et al.* (2010) mentioned that eggs were the most tolerant stage to high pressure of CO_2 treatment. This was observed also in the case of *S. granarius* (Prozell and Reichmuth, 1991), *S. zeamai*s (Nakakita and Kawashima, 1994) and *L. serricorne* (Ulrichs *et al*., 1997) when CO_2 is applied at atmospheric pressure, it is generally accepted that metabolically active stages are more sensitive to hypoxia and hypercarbia than inactive stages (Adler, 1994; Hoback and Stanley, 2001; Navarro, 2006). Riudavets *et al.* (2010) added that the eggs of *Liposcelis bostrychophile* and *L. serricorne* were the highest tolerant stage of all species/ stages and survived the most extreme conditions tested. The beetle *O. surinamensis*, the moth *Ephestia kuehniella* and the mite *Tyrophagus putrescentia* were easier to kill than the other species tested. El-Bana *et al.* (2015) exposed the different stages of *Callasobruhus maculatus* and *T. costaneum* to five levels of pressurized CO_2, N_2 and air for two days under two conditions of temperature. The authors reported that, at the low temperature *T. castaneum* was more sensitive to the pressurized gases than *C. maculatus.* Adult stage was the most susceptible to the pressurized gases while pupae were the least susceptible. Mortality of insects increased gradually as the level of gas pressure increased.

Jay (1984) applied a modified atmosphere under high pressure (25 bars) and found that, it was effective in shortening the exposure period required for complete insect mortality. It was reported also that CO_2 treatment under high pressure provided a rapid control of insects (Rajendran, 2001).

Caliboso *et al.* (1994) found that increasing of the gas pressure had a pronounced effect on insect mortality than did duration of exposure as evidence of this, doubling the pressure resulted in 50% or more reduction in survival, the same degree of increase in exposure proved failed to produce a corresponding quantum response in insect survival or mortality. Similar results were obtained by Gerald *et al.* (1988) on *P. interpubnctella, T. confusum* and *S. paniceum.*

Shazali *et al.* (2003) investigated the use of CO_2 against different insect pests of cereal grains and reported that the exposure period can be reduced regardless of the species or the developmental stage if the CO_2 was used at a high pressure

Riudavets *et al.* (2010) added that different insect species and/or developmental stages show different levels of sensitivity to high pressure of CO_2. It is therefore important to develop which pest species and developmental stages could be present during the control procedures.

However, results confirmed that the use of high pressure of CO_2 offers an effective and fast way to control most stored product pests that affect food commodities.

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تأثير غازات ثانى أكسيد الكربون، النيتروجين والهواء المضغوط على الأطوار المختلفة لخنفساء السورينام وخنفساء العقاقير والتوابل

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أُجريت هذه الدراسة بهدف تقييم فعالية غازات ثاني أكسيد الكربون والنيتروجين والهواء الجوي تحت ضغط ضد جميع الأطوار الكاملة وغير الكاملة لحشرتي خنفساء السورينام وخنفساء العقاقير والتوابل، حيث تم تعريض الحشرات لخمسة مستويات من ضغوط هذه الغازات (١-٥ بار) داخل اسطوانة معدنية خاصة ولمدة تعريض يوم ويومين على درجة حرارة المعمل بمتوسط ٢٥°م، وقد أظهرت النتائج أن غاز ثاني أكسيد الكربون كان الأكثر فعالية يليه النيتروجين ثم الهواء الجوي، وكانت النتائج المتحصل عليها متشابهة في الحساسية لحشرتي الاختبار لغازي ثاني أكسيد الكربون والنيتروجين بينما كانت خنفساء العقاقير والتوابل أكثر حساسية للهواء الجوى المضغوط مقارنة بخنفساء السورينام، وكان طور البيضة في كلا الحشرتين هو الأكثر تحملاً للغازات عن باقي الأطوار بينما كان طور الحشرة الكاملة هو الأكثر حساسية على الإطلاق لكل مستويات الضغط وعلى كل من فترتي التعريض، كانت يرقات حشرة خنفساء العقاقير والتوابل بشكل خاص هي الأكثر تحملاً لغاز ثاني أكسيد الكربون عن باقي الأطوار، وقد أوضحت النتائج أيضاً أن نسب موت جميع الأطوار الحشرية قد زادت كلما طالت فترة التعريض للغاز ولقد تبين أيضاً أن نسب موت الحشرات ارتفعت كلما زاد مستوى ضغط الغاز مسجلا أعلى نسب موت للحشرات عند أعلى مستوى لضغط الغازات (٥ بار).

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