UTILIZATION OF A MODIFIED ATMOSPHERE [MA] FOR CONTROLLING SOME COLEOPTEROUS STORED PRODUCT INSECTS

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Abstract

Efficacy of a modified atmosphere of different pressures of CO_2, N_2 and air gases at 28 ± 2 °C, 60 ± 5% R.H. against different stages of each of *S.oryzae, R. dominica and T. castaneum* in addition to active and diapausing *T-granarium* larvae inside gas-tight steel cylinders was studied. The results indicated that, Co_2 was the best for controlling the tested insects when applied at 20 bar pressure for one hour- exposuring. The results showed also that, efficacy of gas increased with increasing the period of exposure or gas pressure. It could be concluded that, an exposure period at least 60 min. and a high pressure [20 bar] of Co_2 are necessary to achieve a complete extinction of populations of these insects.

INTRODUCTION

Pest control in stored grains and their products are achieved generally by methyl bromide [MB]. Due to the fact that MB causes about 5-10 % of total ozone depletion in the earth's stratosphere, increases the level of UV radiation, which has been linked to skin cancer, eye cataracts and degradation of immune system, the montreal protocol (1997) declared the phase-out of MB in industrialized countries by the year 2005. A need for effective control method was obligatory and it was the modified atmospheres [MAs] or the controlled atmospheres. This new method was investigated by many workers as *stahl* and *Rau* (1985), studied the developed method [Known as pressure Expansion or PEX] of using carbon dioxide as a fumigant under pressure to free plant material from insect infestation is described and the apparatus used is illustrated. The advantages of carbon dioxide over conventional fumigants include lower risk to the people applying it, low cost and unlimited availability. The gas is applied at pressures of 10-50 bar for 10-20 min followed by rapid expansion. In tests with eggs and adults of red flour beetle *(Tribolium castaneum)*, adults of granary weevil *(Sitophilus granarius)*. Larvae of dark flour beetle (T. destructor) and cigarette beetle *(Lasioderma serricorne)* and eggs and larvae of flour moth *(Ephestia kuehniella)*, all the test insects were killed by the treatment.

Srivastava et al. (1985) studied the effectiveness of increasing the atmospheric concentration of carbon dioxide for controlling adults of *Sitophilus oryzae,* *Rhizopertha dominica* and *Tribolium castaneum* and larvae of *Trogoderma granarium* in wheat grain in specially-fabricated mild-steel cylindrical columns in the laboratory in India-when Co_2 was purged into the columns, in which the oxygen level was 1-1.5 %, 100% mortality of all the insects occurred within 10 days.

Calderon. M and Leesch- JG (1983) studied the effect of combining methyl bromide fumigation with reduced pressure or 20% Co_2 or both on the susceptibility of *T. castaneum* and *S. oryzae.* The combination of Co_2 and reduced pressure, without methyl bromide, produced 60% mortality of *S. oryzae* El-Lakwah et al [2002] investigated the effectiveness of certain methyl bromide alternatives controlled atmospheres [CA] of various Co_2 concentration, and very high nitrogen content against some stored product insects inside gastight steel bins. The results of the efficacy of [CA] of 99 % N_2 at grain temperature of 15 $\pm$ 2 °C and 26 $\pm$ 2°C against the adults and immature stages of *S.oryzae R.dominica* and *T.castaneum* as well as the active and diapausing larvae of T. granarium indicated that the efficacy of the CA of 99% N_2 was temperature and exposure period-dependent. Insect mortality increased with the increase of the period of exposure and it was also, greater at the higher grain temperature than at lower one. Susceptibility of the insect varied according to insect species and stage of development pupae of the various tested insects were also more tolerent to the CA of 99% N_2 than the other stages. Also the diapausing larvae of *T.granarium* were less susceptible to the CA of 99% N_2 than active one.

Treatment of 1000 tons of wheat (grain temperature of 21 $\pm$ 1°C) inside a silo with carbon dioxide at 70% concentration resulted in total mortality at all stages for *S.oryzae, S.granarium, R.dominica, T.castaneum* and *Plodia interpunctella* after 15 days. Exposure period *(Suss et al. 1991).*

EL-Lakwah, et al. (2005) studied the efficacy of MA of 99% N_2 at grain temperatures of 18 $\pm$ 2°C and 28 $\pm$ 2°C against the adults and immature stages of *S.oryzae, R.daminica* and *T.castaneum* as well as the active and diapausing larvae *T.granarium* inside gas-tight steel bins. The lethal times required to achieve population extinction of the different insect stages at 18$\pm$ 2°C were 10,35, 28 and 35 days for the adults, eggs, larvae and pupae of *S.oryzae* respectively. For *R.dominicae* was 14, 35, 28 and 35 days for the same stages of *S.oryzae*. For *T.castaneum* these values were 14, 21, 10 and 14 days for adults, eggs, larvae and pupae, respectively. As for the active and diapaused larvae of *T.granarium,* these values were amounted 28 and 35 days, respectively. Ingeneral, rsults revealed that the pupae and eggs of the different insects were highly tolerant to this MA of 99% N_2 than the other stages. Also, MA of high nitrogen content was more effective at high grain temperature. The objective of this work was to investigate the effectiveness of MA of high pressure from Co_2, N_2 and air against some stored product insects for determining the time needed for their population extinction inside gas-tight steal cylinder (Air Liquide Egypt Co.).

MATERIALS AND METHODS

1. Insect cultures

To obtain the desired stages of certain coleopterous insects, the rice weevil, *Sitophilus oryzae* (L.) (Curculionidae), the lesser grain borer, *Rhizopertha dominica* (F.) (Bostrychidae), the red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae) in addition to active and diapausing larvae of the khapra beetle *Trogoderma granarium* (Everts) (Dermestidae) the insects were maintained at the Stored Product Pests Laboratory, Plant Protection Research Institute (ARC) Ministry of Agriculture. The rearing was done in 250 ml glass jars each contained about 200 gm wheat kernels exept *T.castaneum* was reared on wheat flour. The jars were covered with muslin cloth, fixed with rubber bands and kept under controlled conditions of 28 $\pm$ 2°C and 60 $\pm$ 5% RH. The wheat grains or flour (about 14 % moisture content) were kept before using at-18°C for two weeks to eliminate any insect infestation. 1000 adults (1-2 week-old) of each of the first three mentioned insects and about 600 adults (0-24 hr) of the last one were separately introduced onto the food in the jars. A roll of paper was placed on the grain [11% moisture] to serve as a suitable diapausing larvae of *T. granarium*

2. The developmental stages

Eggs aged 24-48 hr, larvae aged 10-11 days old and 24 day old pupae of each of *S. oryzae* and *R. dominica* were needed in this work. To obtain the desired ages, group of 1000 adults 1-2 weeks was placed in a glass jar contained 500 gm food and left for 2 days to lay eggs then these adults were removed by using a sieve (2 mm) and the food was thoroughly mixed for uniformity, in case of *R. dominica* , the fine food which contained their eggs was returned to the jars for culture .

For obtaining larvae, similar adults were used gain eggs, as before and kept for two weeks under the controlled rearing conditions. Laid eggs were also maintained for 24 days to obtain the desired pupae .

In case of *T.castaneum*, 24-48 hr-old eggs, 4th larval instars and 2-3 day-old pupae were needed for the experiments. These developmental stages are easy recognizable in the media.

The adult stages [7-14 day-old] of *S.oryzae, R.dominica* and *T.castaneum* were used in the tests.

Active (3rd and 4th larval instars) and diapausing *T.granarium* larvae each weighed 1.3 ± 0.2 and 3.1 ± 0.2 mg, respectively were chosen for the tests, the diapausing larvae were collected from the deposited rolls according to *Desmarchelier 1984 and Bell et al.,(1985).*

3. The treatments

Bactches of 30 adults insects / or active and diapausing *T.granarium* larvae were confined in a wire gauze cage [14 mm diameter and 45 mm height] containing 5g of the food.

Immature stages of the first two insects, were five grams of the cultures contained eggs, larvae or pupae were separately inserted in similar wire cages. For *T.castaneum* 20 eggs, 30 larvae and 30 pupae also were separately put on 5g wheat flour in each wire cage. The cage was covered with a rubber stopper as before and three replicates were conducted for each stage in the various treatments.

4. Expossure to gases

Carbon dioxide, Nitrogen and air gases were available at 99.9% purity in pressure stell cylinders (70 bar). The cylinder was connected with a pressure regulater. Airtight steel cylinders (50 kg each) from AIR LIQUIDE company, were filled with about 50 kg wheat grains and situated in 6 October city factory. Insect wire cages were inserted in the grains just before treatment, then the cylinder was closed tightly. After the desired exposure time [30 and 60 min] the cylinders were opened and aerated. Insect samples were then taken and transferred to the laboratory for mortality assessment. For achieving different pressures [5.10, 15 and 20 bar] of each of Co_2, N_2 and air inside the treatment cylinders, the pressure regulator was used. A cylinder filled with grains without gasess was also used as a control.

5. Bioassay tests

After the desired exposure period, mortality assessment was performed and corrected by *Abbot's formula (1925).* Adult mortalities of *S.oryzae, R.dominica* and *T.castaneum* as well as active and diapausing larvae of *T.granarium* were determined after 48 hr from the aeration. Reduction rate of the F_1- progeny inspected after 45 days of treatment, for the first three insects, was recorded. The inhibition rate of progeny was calculated as follows.

$$\% \text{ mortality} = \frac{\text{No. of emerged adults in control - No. of emerged adults in treatment}}{\text{No. of emerged adults in control}} \times 100$$

6. Kernel germination

Four replicates, 25 wheat kernels each, were selected for each treatment of the tested three gases Co_2, N_2 and air at the same four pressures 5, 10, 15 and 20 bar for the two exposure times 30 and 60 min. In the same time, 100 wheat kernels [four equal replicates] were kept untreated as a control. Kernels of each replicate were deposited on a moistend filter paper in a petri-dish for 7-10days at room temperature (25-28°C). Numbers of normal sprouts were counted, then the percentages of germination were calculated *(Anonymous 1959)*.

RESULTS AND DISCUSSION

Effect of the three tested gases, at MA of high pressure, on the four considered species are shown in the following results:

1. Effect of Co_2

Results obtained from the tested insects exposured, for different periods and pressures, to Co_2 gas are listed in table [1]. The data revealed that, mortalities % in general raised clearly with increasing the pressure and exposure period. Mortalities of *S.orgzae* exposured for 30 min to 20 bar pressure were 80 $\pm$ 6.1, 48 $\pm$ 4.1, 68 $\pm$ 5.3 and 47 1.6 for the adults, eggs, larvae and pupae, respectively. These values, regardless of insect stage, increased to 100% with elongation the exposure period to 60 min under the same pressure. In case of *R.dominica,* adult mortality % at 20 bar pressure was 62 $\pm$ 7.1 % after 30 min-expsuring increased to 100% with 60 min. Egg mortality recorded 52$\pm$ 6.3 % after 30 min exposure, reached 100% after 60 min exposure to the highest pressure. Also, while 63 $\pm$ 4.2% of larvae were killed after 30 min exposure, all larvae were killed with 60 min and 20 bar.

Mortality percentages, in case of *T.castaneum* were about 38 $\pm$ 6.1, 22 $\pm$ 4.1, 40 $\pm$ 4.5 and 31 $\pm$ 3.1 % for the adults, eggs, larvae and pupae, respectively when exposured for 30 min to 20 pressure. Lengthinning the period to 60 min, a complete mortality [100%] was recorded for all stages.

As for *T.granarium,* the recorded mortalities, after 30 min and at 20 bar, were nearly similar for active and diapausing larvae, 22 $\pm$ 1.1 and 20 $\pm$ 3.2, respectively, the complete mortality was recorded 60 min post- treatment. In the same time, no effect was noticed on the larvae exposrued for 30 min to a pressure less than 10 bars, table [1]. These results showed clearly that, an exposure period of 60 min at least is necessary to achieve a total extinction of the population of the four tested insects by using MA of Co_2 under 20 bar pressure inside the gas tight steel cylinder.

2. Effect of N_2

Results obtained from the different stages of the tested insects after 30 min exposuring to 20 bar N2 pressure are given in table [2]. Mortalities of *S.oryzae* stages averaged 4.3 $\pm$ 2.2, 2.7 $\pm$1.7, 4.3 $\pm$ 1.6 and 1.3 $\pm$ 0.6 % for the adults, eggs, larvae and pupae, respectively. As noticed in the previous gas, these values increased with increasing the exposure time, 6.7 ± 1.6, 3.3 ± 1.3, 6.3 ± 0.7 and 4.3 ± 2.1 % for the same stages, respectively.

In case of *R.dominica,* mortality % after 30 min exposure ranged 1.3 ± 0.3-7.0 ± 2.3 % under 20 bar pressure . These percentages increased in case of 60 min to 4.3 ± 1.6-8.6 ± 2.7 %. *T.castaneum* mortalities were 6.7 ± 3.1 and, 18.3 ± 4.1 % for adults, 4.3 ± 1.2 and 8.7 ± 2.1 % for eggs, 6.7± 2.2 and 17.0 ± 5.3 % for larvae and 1.0 ± 0.3 and 9.3 ± 2.1 % for pupae with the two exposuring times (30 and 60 min), respectively.

Data in the same table showed that, *T.granarium* larvae (active or diapaused) neerly not cleared any responses to N_2 gas under the treatment conditions.

3. Effect of Air

Data illustrated in table (3) revealed that, effect of the air on the four insects was, in general, the lowest comparing with the previous gases. Mortalities percentages were about 0.3–3.7 for *S.oryzae,* 0.3 -3.1 for *R.dominica*, 0.3 -1.3 for *T.castareum* and 0.3– 8.7 for *T.granarium.* Response of *T.castaneum* stages to the air pressure was relatively clearer than that of the other insects. On the other hand, the *T.granarium* larvae showed, as before, the lowest response. In the same time, exposuring the four insects to the air gas for the longer period [60 min] gave higher mortality percentages about 0.3 – 13.0 % than those of the shorter period 0.3 - 6.3 %.

A general glance to all previous results revealed that, Co_2 and N_2 gases had more toxic effect against the four tested insects than air gas. The strongest toxicant gas was Co_2, 100 % mortality after 60 min-exposure under 20 bar pressure. N_2 gas occupied the second rank, 2.3-18.3% mortality followed with the air represented the weakest gas.

4. Wheat grain germination

Table [4] showed that, the MA of Co_2, N_2 and air at different pressures of 5,10,15 and 20 bar had a slight effect on the germination of wheat grains. This effect, in certain cases, increased with raising the pressure and elongating the exposure period. The germination percentages were 86 ± 3.3 and 87 ± 4.3, 86.3 ± 1.3 and 92.7 ± 2.7 and 95.1 ± 1 and 88.7 ± 3.3 for exposuring the grains for 30 and 60 min to 20 bar pressure of Co_2, N_2 and Air gases, respectively, percent germination reached 96.3–98.1 % in the control.

Results of using a modified atmosphere [MA] of a high pressure of Co_2, N_2 and air for the treatment of grains, stored inside a gas tight steel cylinder for controlling the various stages of the tested insects under study revealed that the exposure times are necessary for an effective control, these results are in agreement with the findings of other studies *(Stahl and Rau, (1985) and El-Lakwah et al., 1997, 2002 and 2005).*

Table 1. Response on different stages of tested insects, exposed to different CO_2 MA pressures for 30 and 60 minutes under 28± 2°C and 60 ± 5% RH.

Insect	Stage	30 min				60 min			
		5	10	15	20 bar	5	10	15	20 bar
S.oryzae	Adult	20± 2	27± 3	68± 2.3	80± 6.1	32± 2.2	47± 3.1	86± 6.3	100
	Egg	7± 0.1	12± 2	37± 3.2	48± 4.1	18± 3.2	23± 2.3	78± 5.1	100
	Larva	17± 0.6	23± 1.1	52± 4.6	68± 5.3	30± 1.6	48± 1.7	87± 8.1	100
	Pupa	3± 0.3	10± 1.3	33± 7.3	47± 1.6	22± 1.6	37± 1.3	72± 3.3	100
R.domin-ica	Adult	7± 1.1	18± 1.7	46± 4.1	62± 7.1	20± 2.3	38± 6.4	86± 4.1	100
	Egg	14± 2.3	26± 4.3	40± 5	52± 6.3	23± 3.2	42± 1.1	88± 7.1	100
	Larva	17± 3.2	38± 6.1	47± 6.1	63± 4.2	32± 1.6	54± 2.4	91± 2.3	100
	Pupa	12± 2.7	22± 2.3	39± 2.2	60± 1.6	27± 1.3	49± 4.3	89± 1.1	100
-T.castaneum	Adult	6± 1.4	10± 1.1	17± 1.6	38± 6.1	37± 2.7	73± 6.2	96± 1.1	100
	Egg	2± 0.3	4± 0.3	12± 1.3	22± 4.1	42± 3.6	57± 4.1	67± 3.4	100
	Larva	7± 1.6	12± 1.3	27± 4.1	40± 4.5	53± 4.1	63± 3.6	92± 1.6	100
	Pupa	3± 0.3	14± 2.3	16± 3.1	31± 3.1	38± 3.1	72± 4.5	90± 1.7	100
T.grana-rium	Active	0.0	0.0	17± 1.3	22± 1.1	18± 1.7	62± 3.2	81± 5.6	100
	Diapause	0.0	0.0	13± 2.2	20± 3.2	16± 2.3	60± 4.2	72± 6.3	100

± SE = standard error

(No moralities were recorded in the control).

Table 2. Response on different stages of tested insects, exposed to different N_2 MA pressures for 30 and 60 minutes under 28± 2°C and 60 ± 5% RH.

Insect	Stage	30 min				60 min			
		5	10	15	20 bar	5	10	15	20 bar
S.oryzae	Adult	0.0	1.3± 0.3	2.6± 0.1	4.3± 2.2	0.0	33± 1.3	5.3± 0.3	6.7± 1.6
	Egg	0.0	0.0	1.3± 0.3	2.7± 1.7	0.0	1.3± 0.3	2.7± 1.1	3.3± 1.3
	Larva	0.0	2.1± 1.1	3.3± 1.3	4.3± 1.6	0.0	2.0± 0.6	4.0± 1.1	6.3± 0.7
	Pupa	0.0	1.0± 1.3	1.0± 0.3	1.3± 0.6	0.0	0.0	3.3± 0.2	4.3± 2.1
R.domin-ica	Adult	0.0	0.0	0.0	7± 2.3	0.0	3.7± 1.6	4.6± 1.1	7.3± 3.3
	Egg	0.0	2± 0.3	3± 0.3	4± 1.3	0.0	3.1± 1.3	5.3± 1.3	5.7± 2.5
	Larva	0.0	3± 1.7	4± 1.7	6± 2.1	0.0	4.2± 0.3	6.7± 1.2	8.6± 2.7
	Pupa	0.0	0.0	0.0	1.3± 0.3	0.0	1.3± 1.1	3.3± 1.1	4.3± 1.6
-T.castaneum	Adult	1.0± 0.6	1.7± 0.6	2.3± 1.3	6.7± 3.1	0.0	6.6± 2.3	11.7± 2.3	18.3± 4.1
	Egg	0.3± 0.3	2.3± 0.7	1.6± 0.3	4.3± 1.2	0.0	4.3± 1.2	6.3± 1.7	8.7± 2.1
	Larva	0.0	4.1± 3.3	5.3± 2.2	6.7± 2.2	0.0	6.7± 3.1	12.3± 3.7	17± 5.3
	Pupa	0.0	0.0	0.0	1.0± 0.3	0.0	4.2± 1.1	6.3± 1.6	9.3± 2.1
T.grana-rium	Active	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3± 0.3
	Diapause	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

± SE = standard error

(No moralities were recorded in the control).

Table 3. Response on different stages of tested insects, exposed to different air pressures for 30 and 60 minutes under 28± 2°C and 60 ± 5% RH.

Insect	Stage	30 min				60 min			
		5	10	15	20 bar	5	10	15	20 bar
S.oryzae	Adult	0.0	0.0	0.0	1.3± 0.2	0.0	0.0	3.3± 1.1	3.7± 1.2
	Egg	0.0	0.0	0.0	0.3± 0.1	0.0	0.0	0.6± 0.3	1.3± 1.1
	Larva	0.0	0.0	0.0	0.0	0.0	0.0	0.7± 0.3	1.3± 1.1
	Pupa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R.domi-nica	Adult	0.0	0.0	0.0	0.0	0.3± 0.1	0.0	0.6± 0.3	1.3± 0.3
	Egg	0.0	0.0	0.6± 0.3	1.6± 1.3	0.3± 0.3	0.7± 0.2	1.6± 1.3	2.1± 1.1
	Larva	0.0	0.3± 0.2	0.7± 0.2	1.7± 1.1	1.1± 0.3	1.6± 1.1	2.3± 1.1	3.1± 0.6
	Pupa	0.0	0.0	0.0	0.0	0.0	0.3± 0.1	1.3± 0.6	1.3± 0.3
T.castan-eum	Adult	0.0	0.0	0.0	0.0	0.3 ± 0.3	1.3± 0.3	2.6± 1.3	4.1± 2.3
	Egg	0.0	1.3± 0.6	2.3± 1.1	4.1± 2.3	1.3 ± 1.1	2.7± 1.1	4.1± 1.6	6.3± 1.3
	Larva	1.1 ± 0.3	1.6± 0.7	2.7± 2.1	6.3± 1.6	2.7 ± 1.3	6.7± 1.6	10.1± 3.1	13± 3.1
	Pupa	0.0	2.3 ± 1.1	3.6 ± 1.3	4.3± 1.3	2.3 ± 0.3	4.1± 2.1	6.3± 2.3	8.3± 1.3
T.grana-rium	Active	0.0	0.0	0.0	0.3± 0.2	0.0	2.3± 1.1	5.6± 2.2	8.7± 1.6
	Diapause	0.0	0.0	0.0	0.0	0.0	0.0	3.3± 1.3	4.1± 1.3

± SE = standard error

(No moralities were recorded in the control).

Table 4. Different pressures of gases in relation to percentages of wheat grain germination after 30 and 60 min exposure periods.

Gas pressure (bar)		30 min	60 min
Co_2	5	88± 4.1	87± 2.3
	10	92± 3.1	88± 4.7
	15	93± 1.1	92± 2.1
	20	86± 3.3	87± 4.3
N_2	5	94± 1.3	96± 2.3
	10	93± 2.3	89± 1.7
	15	90.7± 1.7	91.3± 2.3
	20	86.3± 1.3	92.7± 2.7
Air	5	94.1± 3.3	96.3± 3.3
	10	97.1± 1.7	93.7± 2.1
	15	93.3 ± 2	98.1± 1.1
	20	95.1± 1	88.7± 3.3
	Control	98.1± 0.3	96.3± 1.3

+ SE = standard error

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استخدام الجو المعدل لمقاومة بعض حشرات المواد المخزونة غمدية الأجنحة

رفعت عبد الشافى محمد

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – وزارة الزراعة – مصر

تم اختبار فعالية جو معدل من ثلاث غازات ضد حشرات سوسة الأرز وثاقبة الحبوب الصغرى وخنفساء الدقيق الكستنائية وكذلك ضد يرقات نشطه وساكنة لخنفساء الصعيد وذلك عند درجة حرارة ٢٨ + ٢ °م ورطوبة نسبية ٦٠ + ٥% أجريت التجارب بشركة إير ليكيد بمدينة ٦ أكتوبر–القاهرة داخل اسطوانات من الصلب محكمة الغلق باستعمال ضغوط مختلفة (٥،١٠،١٥،٢٠، بار) من كل من غاز ثاني أكسيد الكربون والنتروجين والهواء لفترتي تعريض ٣٠، ٦٠ دقيقة.

أوضحت النتائج المتحصل عليها أن تأثير الغاز يتوقف على فترة التعريض ومقدار الضغط كما أن النسبة المئوية للموت قد تباينت تبعاً لنوع وطور الحشرة.

كان غاز ثاني أكسيد الكربون أفضل الغازات المستخدمة لمقاومة الحشرات المختبرة عند ضغط ٢٠ بار لفترة تعريض ساعة واحدة (١٠٠% موت).