Academic Journal of Entomology 11 (3): 66-75, 2018
ISSN 1995-8994

DOI: 10.5829/idosi.aje.2018.66.75

Efficacy of Modified Atmospheres Enriched with Carbon Dioxide Against Different Life Stages of the Spider Beetle, *Gibbium psylloides* (Czen.) (Coleoptera: Ptinidae)

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Abstract: The spider beetle, *Gibbium psylloides* (Czen.), is a small insect, flightless detritus feeder sometimes found in damp, mouldy grain residues. It feeds on cereals, stored seeds, stale bread and various dried fruit. Modified atmospheres have been used for disinfesting raw or semi-processed food products, such as cereal grains and dried fruits, while still in storage. The aim of this study was to evaluate the effectiveness of modified atmospheres based on high CO_2 contents at optimum temperatures in controlling *G. psylloides*. Eggs, fourth instar larvae, pupae and adults of *G. psylloides* were exposed to three modified atmospheres (MAs) containing different concentrations of CO_2 in air under the prevailing laboratory conditions. The tested MAs were: MA_1: 20% CO_2, 16% O_2 and 64% N_2; MA_2: 40% CO_2, 12 % O_2 and 48% N_2 and MA_3: 80% CO_2, 4%O_2 and 16% N_2. The exposure period of MAs treatments was started by 3 h and was duplicated until 100% mortality occurred. Mortalities of the tested stages responding to MAs increased significantly ($P < 0.01$) with the increase of exposure period and CO_2 concentration. The three tested MAs varied in lethal effect through different developmental stages of the insect. At 27 C°, 1, 3, 5 and 10 days were adequate to kill completely larvae, adults, pupae and eggs, respectively, under all tested MAs. The larvae and adults were more sensitive to MAs than pupae and eggs.

Key words: Life Stages · *Gibbium psylloides* · Modified Atmospheres

INTRODUCTION

Chamomile (*Matricariae chamomilla L.*) is one of the most economic medicinal plants. Its flower heads are the main source of highly aromatic essential oil and belong to those drugs that experienced a wide medical application in ancient times [1]. *G. psylloides* is often a minor insect and receives little attention as stored products pest, but this insect becomes very important pests on chamomile [2-4]. Some studies were found that *G. psylloides* caused the highest weight loss of dry chamomile (35.2%) in plastic net after two years of storage [2]. Controlling spider beetles starts with a thorough inspection to find the source of the infestation. Then using non-chemical control methods have gained importance in integrated pest management, as policies aiming to minimize the application of residual chemical insecticides are being adopted by many companies and a growing market of organic produce. Governmental and international regulations and loss of synthetic insecticides such as methyl bromide [5]. Greater restrictions on the use of dichlorvos, have left few alternatives for even non-organic food processors.

The spider beetle, *G. psylloides* (Czen.), is a small insect, a flightless detritus feeder sometimes found in damp, mouldy grain residues, but also in vegetable, leather and other animal products. It is found feeding on cereals, wheat, bran, baby food, stored seeds, stale bread, dog biscuits and various dried fruit. The larvae bore holes in host to pupate and cause damage packaging or the commodities themselves. The larvae of *G. psylloides* can damage not only dried plants in herbaria collections and upholstery, but also textiles, mummies, animal mounts and insect collections [6-8]. The larvae do not wander away from the food stuff for pupation, which takes place in a spherical cocoon. Large numbers apparently in tunnels which they had excavated, occur in a desiccated fragment of organic material and there are also large

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numbers in similar material stuck to the inside of a large potsherd. *G. psylloides* also occurs in material from the Tomb of Tutankhamun [9] as well as one of the Manchester mummies [10]. The life cycle attains 22 - 42 weeks and the adult usually lives 30 - 40 weeks, at 25°C on the granaries and mills products. *G. psylloides* may be associated with similarly foul organic residues.

In many cases, the control of stored-product pests requires control of large numbers of pest individuals hidden in large amounts of product or structurally complex buildings. These can be killed with different non-chemical methods and these methods are preferred in storage as they don't damage the objects, kill all stages and don't harm the environment or health of storage staff. Physical treatments are achieved due to freezing, controlled heating [11-13] micro wave radiation or gamma radiation of the objects [14] high concentrations of zinc and manganese of insect coleopterans [15]. Methyl bromide (MB) can be controlled different stages of storage pests, because it is efficacy, rapid action and relatively low cost. But MB is scheduled for worldwide withdrawal from routine use as a fumigant in 2015 under the directive of the Montreal protocol on ozone-depleting substances [16, 17]. No single treatment method is perfect and the best method applied has to be selected depending on the financial recourses, availability and type of pests and materials [8]. Now there is an urgent need to find alternative methods for post-harvest use.

Modified atmospheres have been used for disinfesting raw or semi-processed food products, such as cereal grains and dried fruits, while still in storage. Treatments based on low oxygen atmosphere that is achieved using increase Nitrogen (N_2) or increase carbon dioxide (CO_2) contents are technically suitable alternatives for storage pests control in durable commodities [18-21]. Almost atmospheres with high contents of N_2 are less effective than atmospheres enriched with CO_2 in controlling pests [20, 22]. Data on the effects of different concentrations of CO_2 treatments and dosages on key pests are available for many species and stages of stored-product insects under particular sets of conditions [23-25]. The toxicity of CO_2 to pests is known to vary among species, developmental stages and age groups. In the majority of studies involving CO_2, much attention has been focused on determining the time required to kill insect pests [26-30]. Therefore, the aim of this study was to evaluate the effectiveness of modified atmospheres based on high CO_2 contents at 27 °C in controlling *G. psylloides*.

MATERIALS AND METHODS

Insect Culture: The insect's strain was obtained from infested chamomile flowers. Stock culture was kept in an incubator at 27±2°C and 65±5% relative humidity (r.h.) in the laboratory of stored grain insects at the Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University. The adult insects were reared on dry commercial chamomile. The chamomile was conserved at 4°C about one week before using to eliminate potential contamination with other living pests [31]. Newly emerged adults were obtained from cultures that were sieved daily.

Laboratory Preparations: The experiments were conducted in the Laboratory of Modified Atmospheres at the Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University. The experiments aimed to study the sensitivity of all developmental stages of *G. psylloides* to different concentrations of CO_2 for different exposure periods. The eggs, fourth instar larvae, pupae and adults that were required for the MA experiments were obtained from the stock culture. After exposure to MAs, the treated stages were kept under laboratory conditions of 27±2°C and 65± 5% r.h. All MAs treatments were repeated three times and three similar replicates of every stage were left untreated for control purposes.

Gas Treatment Equipment: The treatment with gas mixtures carried out inside gas-tight Dreshel flasks of 550 cm^3 capacity [32]. Each flask was tightly plugged with a special glass stopper containing an inlet and outlet valve leading to two vertical glass tubes. Each tube was long and reached near the bottom of the flask; anther was short and reached just below the stopper. The long tube worked as the gas inlet and the short one as the gas outlet. A CO_2 cylinder was applied for the CO_2 supply and was connected to the inlet tube of the flask with a short hose. Valves were opened at the beginning of the treatment and left open until the desired gas concentration inside the flask was applied, as indicated by an oxygen analyzer (Servomex 570 A) connected to the outlet tube of the Dreshel flask by a short hose [33].

Preparing Different Stages of *G. psylloides* for Gas Treatment: Eggs were carefully transferred to Petri dishes and then examined under a stereomicroscope to eliminate injured or malformed. The eggs were then carefully transferred to glass tubes at a density of 50 eggs/tube and introduced into the Dreshel flask for exposure to the

selected MA. Last instar larvae separated with sieving through a 60-mesh sieve but the pupae and newly emerged adults were separated from the medium with a camel brush. Twenty specimens for each stage were put inside a glass tube containing 2 g of dry chamomile (Except the vials containing pupae). The tubes were closed with muslin secured tightly with rubber bands and were introduced into the Dreshel flask for the selected MA.

The CO_2 Concentrations in Air and Exposure Periods Tested: Using the gas treatments equipment described above eggs, fourth instar larvae, pupae and adults of *G. psylloides* were exposed to three different concentrations of CO_2 in air under the prevailing laboratory conditions. The tested MAs were: MA_1: 20% CO_2, 16 % O_2 and 64% N_2; MA_2: 40% CO_2, 12 % O_2 and 48% N_2 and MA_3: 80% CO_2,4% O_2 and 16% N_{-2} The exposure periods of each of the three tested MA lasted for 3, 6, 12, 24, 48, 72, 96, 120 and 144 h for larvae stage. The exposure periods of eggs, pupae and adults started from one day till 11 days.

Mortality Percentages of the Developmental Stages: At the end of the tested exposure periods, each of Dreshel flasks was aerated. Treated eggs were incubated under the conditions of 27±2°C and 65±5% relative humidity (r.h.). The eggs remained inside the tubes and were examined daily to record their mortality percentages. Treated larvae and adults were examined 24 h after aeration to record their mortality. Treated pupae were left until the emergence of adult and record their mortality.

Statistical Analysis: Duncan and F tests were adopted for calculating the mortality rates of *G. psylloides* and were performed with an SPSS computing program using ANOVA, as described by Snedecor and Cochran [34]. Data on the effect of exposure periods on the mortality of the different stages of *G. psylloides* were subjected to probit analysis, as described by Finney [35]. LT_{50} and LT_{95} values were calculated using the computer program developed by Noack and Reichmuth [36].

RESULTS

Mortalities of all stages (egg, fourth instar larva, pupa and adult) of *G. psylloides* responding to MAs increased significantly ($P< 0.01$) with increasing exposure period and with increasing CO_2 concentration (Tables 1-5). Figures 1-4 show the regression lines obtained.

Eggs: Data in Table (1) show the effect of the three tested MAs on the mortality of newly-laid eggs under different exposure periods at 27°C. The mortality percentages of eggs increased with the increase of exposure period. This increase was significantly with increasing CO_2 concentrations except the mortality percentages recorded at one and five days exposure which were insignificantly within all modified atmospheres. MA_1 caused 80 % mortality after 11 days of exposure, but MA_2 and MA_3 were recorded 100% mortality after 10 and 11 days exposure, respectively.

Larvae: The data in Table (2) demonstrate the difference between mortality percentage of fourth instar larvae. The larvae were more sensitive than the eggs. At MA_1 treatment, 86.6 % mortality was recorded after 144 h. No significant difference was observed between exposure periods from 24 to 96 h. MA_2 was reached 100 % mortality after 120 h. No significant differences were recorded between mortality percentages of larvae at 24 and 48 h and also at 72 and 96 h. At MA_3 treatment, the complete mortality was recorded after 24 h and significant difference was observed between the mortality percentages at all exposure periods. High significant differences between mortality percentages were recorded in MA_3 and each of MA_1 and MA_2 at 3, 6 and 24 h while no significant difference was observed between MA_1 and MA_2 at the same exposure periods.

Pupae: Table (3) shows the corrected mortality percentages of pupae of *G. psylloides* exposed to three MAs. The mortality percentages reached to 100% after 11, 10 and 5 days exposure for MA_1, MA_2 and MA_3, respectively. MA_3 recorded the highest significant mortalities at exposure times 1, 2, 3, 4 and 5 days exposure. No significant differences were observed between mortality percentages with MAs at concentrations MA_1 and MA_2 with all exposure periods except 9 and 10 days.

Adults: The effect of three tested MAs at different exposure periods on corrected mortality percentages of newly emerged adults of *G. psylloides* at 27°C was clarified in Table (4). No mortality recorded after one and two days exposure with MA_1. The corrected mortality percentages after one day exposure recorded 48 and 68 % with MA_2 and MA_3, respectively. The complete mortality percentage was recorded at 3, 6 and 12 days exposure to MA_3, MA_2 and MA_1, respectively. However the lower MA level (MA_1) needed to long time to kill all larvae (12 days). The significant difference was clearly observed between mortality percentages at all exposure of each treatment.

Table 1: Corrected mortality percentages for eggs stage of *Gibbium psylloides* exposed to modified atmospheres (MAs) containing different concentrations of CO_2 combined with varies exposure periods.

	Modified atmosphere (MA)				
Exposure period (Day)	MA_1 (20% CO_2)	MA_2 (40% CO_2)	MA_3 (80% CO_2)	F value	P value
1	0.0±0.0d	0.0±0.0g	20.0±7.0f	0.07	4.000
2	0.0±0.0dB	0.0±0.0gB	30.0±5.0efA	108.00	<0.001
3	0.0±0.0dB	6.67±6.6gB	40.0±5.0eA	31.00	0.01
4	0.0±0.0dC	30.0±4.5fB	40.0±5.0eA	156.00	<0.001
5	0.0±0.0d	35.0±5.0ef	70.0±5.0d	441.00	<0.001
6	6.67±5.7dC	40.0±5.0eB	76.6±3.3dA	165.500	<0.001
7	25.0±5.7cC	48.0±5.7dB	80.0±5.0cdA	58.449	0.001
8	32.0±5.0cC	70.0±6.0cB	88.0±5.0bcA	73.030	0.001
9	50.0±5.0bC	75.0±5.0bcB	95.0±4.5abA	42.772	0.002
10	53.33±7.5bC	80.0±0.0bB	100.0±0.0aA	7.789	0.021
11	80.0±10.0a	100±0.0a		4.000	0.116
F values	117.872	150.110	72.290		
P values	<0.001	<0.001	<0.001		

Small letters indicate to the significant differences between exposure periods, while the capital letters indicate to the significant differences between modified atmosphere treatments (CO_2 concentrations)

Table 2: Corrected mortality percentages for larva stage of *Gibbium psylloides* exposed to modified atmospheres (MAs) containing different concentrations of CO_2 combined with varies exposure periods

	Modified atmosphere (MA)				
Exposure period (h)	MA_1 (20% CO_2)	MA_2 (40% CO_2)	MA_3 (80% CO_2)	F values	P values
3	0.00±0.0dB	0.00±0.0dB	46.67±3.33cA	196.00	<0.001
6	0.00±0.0dB	0.00±0.0dB	66.67±3.33bA	400.00	<0.001
24	36.67±3.33cB	46.67±12.0cB	100.0±0.0aA	22.357	0.002
48	40.0±5.77c	46.67±3.33c		1.000	0.374
72	43.0±3.33cB	73.33±6.67bA		16.200	0.016
96	43.0±3.33cB	83.33±3.33bA		72.000	0.001
120	70.0±5.77bB	100.0±0.0aA		27.000	0.007
144	86.6±3.33a				
F values	64.914	50.719	98.000		
P values	<0.001	<0.001	<0.001		

Small letters indicate to the significant differences between exposure periods, while the capital letters indicate to the significant differences between modified atmosphere treatments (CO_2 concentrations)

Table 3: Corrected mortality percentages for pupa stage of *Gibbium psylloides* exposed to modified atmospheres (MAs) containing different concentrations of CO_2 combined with varies exposure periods

	Modified atmosphere (MA)				
Exposure periods (Day)	MA_1 (20% CO_2)	MA_2 (40% CO_2)	MA_3 (80% CO_2)	F values	P values
1	10.00±5.0gC	33.33±3.33fB	66.67±3.33bA	19.909	0.002
2	33.33±3.33fB	36.67±3.33fB	80.00±5.77bA	36.600	<0.001
3	36.67±3.33fB	46.67±3.33eB	80.00±5.77bA	27.800	0.001
4	56.67±3.33eB	66.67±3.33dB	96.67±3.33aA	39.000	<0.001
5	63.33±3.33dC	76.67±3.33cB	100.0±0.0aA	46.500	<0.001
6	69.0±5.0cd	78.67±5.7c		2.008	0.292
7	73.33±3.33c	80.00±5.0bc		0.400	0.561
8	83.3±7.0b	86.0±5.0b		0.270	0.655
9	89.0±5.0bB	95.0±7.0aA		75.000	0.013
10	90.0±5.0bB	100.0±0.00aA		12.000	0.026
11	100.0±0.0a				
F values	108.728	90.738	10.500		
P values	<0.001	<0.001	<0.001		

Small letters indicate to the significant differences between exposure periods, while the capital letters indicate to the significant differences between modified atmosphere treatments (CO_2 concentrations).

Table 4: Corrected mortality percentages for adult stage of *Gibbium psylloides* exposed to modified atmospheres (MAs) containing different concentrations of CO_2 combined with several exposure periods

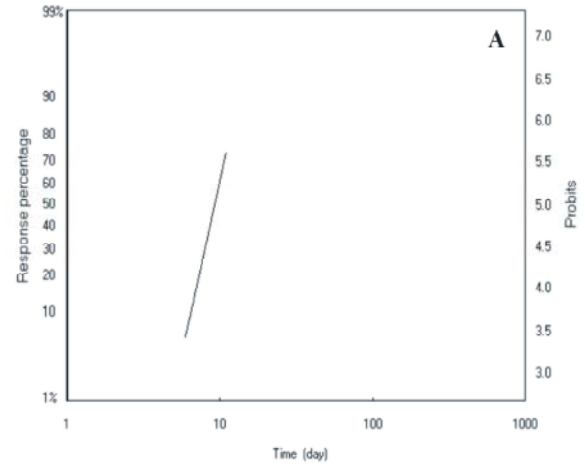
	Modified atmosphere (MA)				
Exposure period (Day)	MA_1 (20% CO_2)	MA_2 (40% CO_2)	MA_3 (80% CO_2)	F values	p values
1	0.0±0.0gB	48.0±15.9bA	68.0±3.74bA	15.225	<0.001
2	0.0±0.0gC	52.0±11.5bB	96.0±2.44aA	48.158	<0.001
3	5.8±2.0gC	60.0±5.4bB	100±0.00aA	172.67	<0.001
4	15.0±4.0fB	64.0±5.1bA		50.77	<0.001
5	38.0±5.0eB	92.0±2.0aA		57.41	<0.001
6	65.0±6.0dB	97.0±2.0aA		174.11	<0.001
7	74.0±5.7cBA	100.0±0.0aA		4.126	0.043
8	74.0±5.7cA			42.47	0.003
9	81.0±4.7bcA			275.63	<0.001
10	85.0±5.0bA			208.79	<0.001
11	95.0±2.4aA			12.50	0.008
12	100±0.0a			5.00	0.056
P values	<0.001	<0.001	<0.001		
F values	173.207	6.229	45.600		

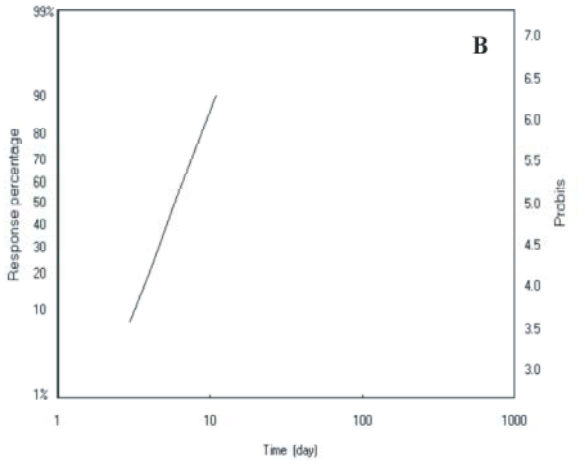
Small letters indicate to the significant differences between exposure periods, while the capital letters indicate to the significant differences between modified atmosphere treatments (CO_2 concentrations)

Table 5: LT_{50} an LT_{95} values with their confidence limits for all stages of *Gibbium psylloides* exposed to modified atmospheres (MAs) containing different concentrations of CO_2

				Confidence limits (d)					
				LT_{50} (d)		LT_{95} (d)			
Stage	MAs	LT_{50} (d)	LT_{95} (d)	Lower	Upper	Lower	Upper	Slop ± SE	Chi-square (χ^2)
Egg	MA1	9.247	14.53	7.22	11.66	13.64	18.97	8.38±0.90	4.8776
	MA2	5.874	12.86	3.92	8.567	13.773	36.865	4.83±0.34	24.90
	MA3	3.218	10.09	2.55	5.84	8.96	11.65	2.45±0.20	39.2669
larva	MA1	2.60	36.08	2.33	5.26	29.87	38.54	1.44±0.20	35.5104
	MA2	1.44	7.18	1.12	3.62	5.96	8.65	2.35±0.25	29.5878
	MA3	0.15	0.62	0.09	0.21	0.54	2.82	2.64±0.36	3.8188
Pupa	MA1	3.498	17.192	3.158	3.86	13.74	23.185	2.378±0.18	5.1998
	MA2	2.356	14.503	1.362	3.156	13.58	45.009	2.08±0.178	27.5154
	MA3	0.483	8.432	0.38	0.84	5.65	12.98	1.32±0.278	12.3836
Adult	MA1	5.868	11.58	4.162	7.118	10.87	19.64	5.57±0.354	40.0570
	MA2	1.54	5.93	0.84	2.66	5.23	7.45	1.91±0.208	43.4035
	MA3	0.793	1.821	0.622	0.916	1.585	2.292	4.56±0.745	0.3862

Comparative Lethal Times of Different Stages of *G. psylloides* Exposed to MAs: The calculated LT_{50} and LT_{95} values with their confidence limits for different stages of *G. psylloides* exposed to MAs (Table 5 and Fig 1-4). According to the LT values and toxicity lines, the MA enriched with 80% CO_2 was more effective on all stages followed by 40% and 20% CO_2. Egg stage was the most tolerant stage to MAs. LT_{50} and LT_{95} were (9.24 & 14.53 days), (5.87 & 12.86 days) and (3.21 & 10.09 days) with MA_1, MA_2 and MA_3, respectively. Larvae were more sensitive to MAs than all stages. LT_{50} and LT_{95} were (2.6 & 36.08 days), (1.44 &7.18 days) and (0.15 & 0.62 days) with MA_1, MA_2 and MA_3, respectively.





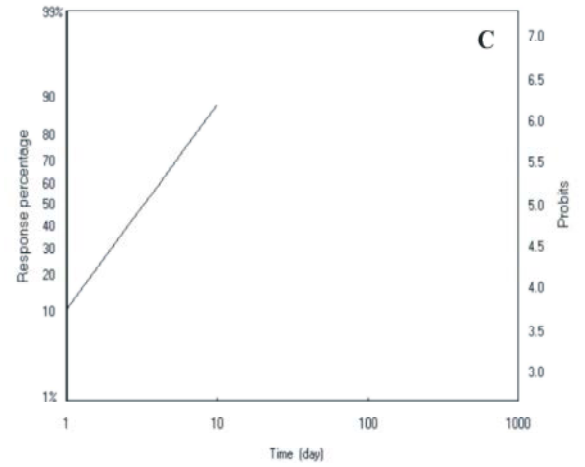
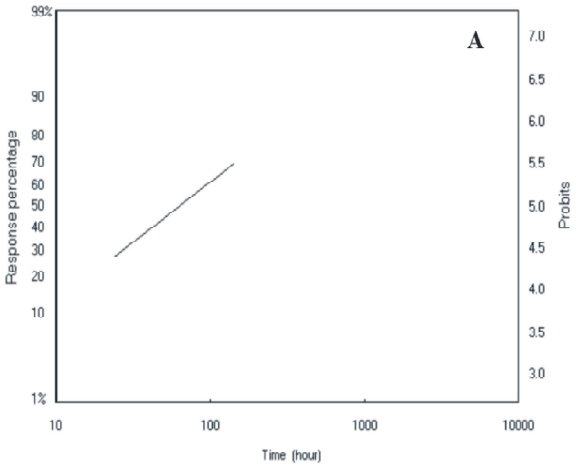
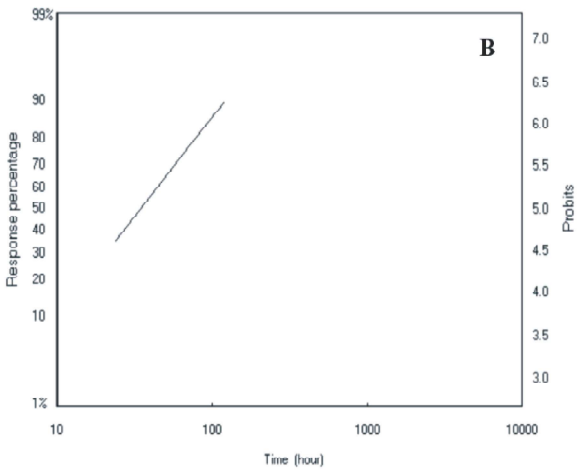


Fig. 1: Toxicity lines for *Gibbium psylloides* eggs exposed to 20% CO_2 (A), 40% CO_2 (B), 80% CO_2 (C) combined with different exposure periods at 27°C.





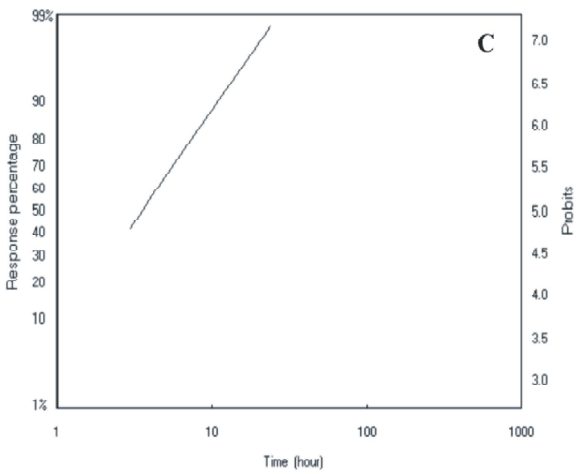


Fig. 2: Toxicity lines for *Gibbium psylloides* larvae exposed to 20% CO_2 (A), 40% CO_2(B), 80% CO_2(C) combined with different exposure periods at 27°C.

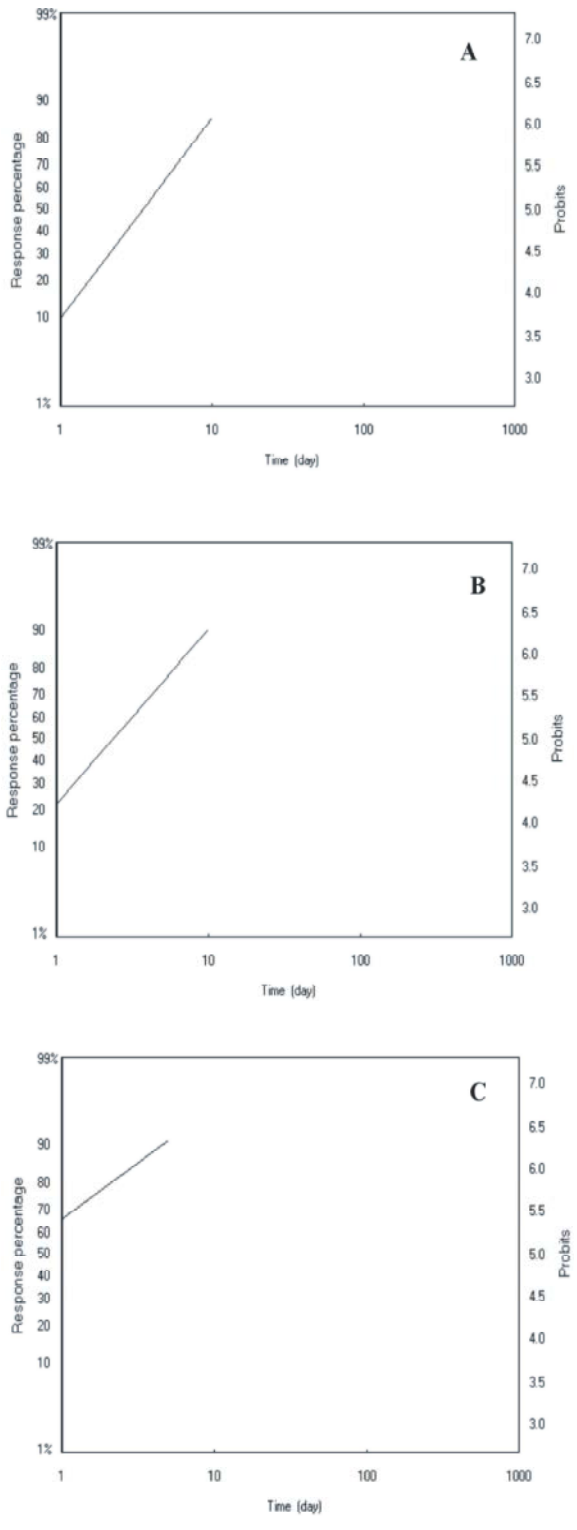


Fig. 3: Toxicity lines for *Gibbium psylloides* pupa exposed to 20% CO_2 (A), 40% CO_2 (B), 80% CO_2 (C) combined with different exposure periods at 27°C.

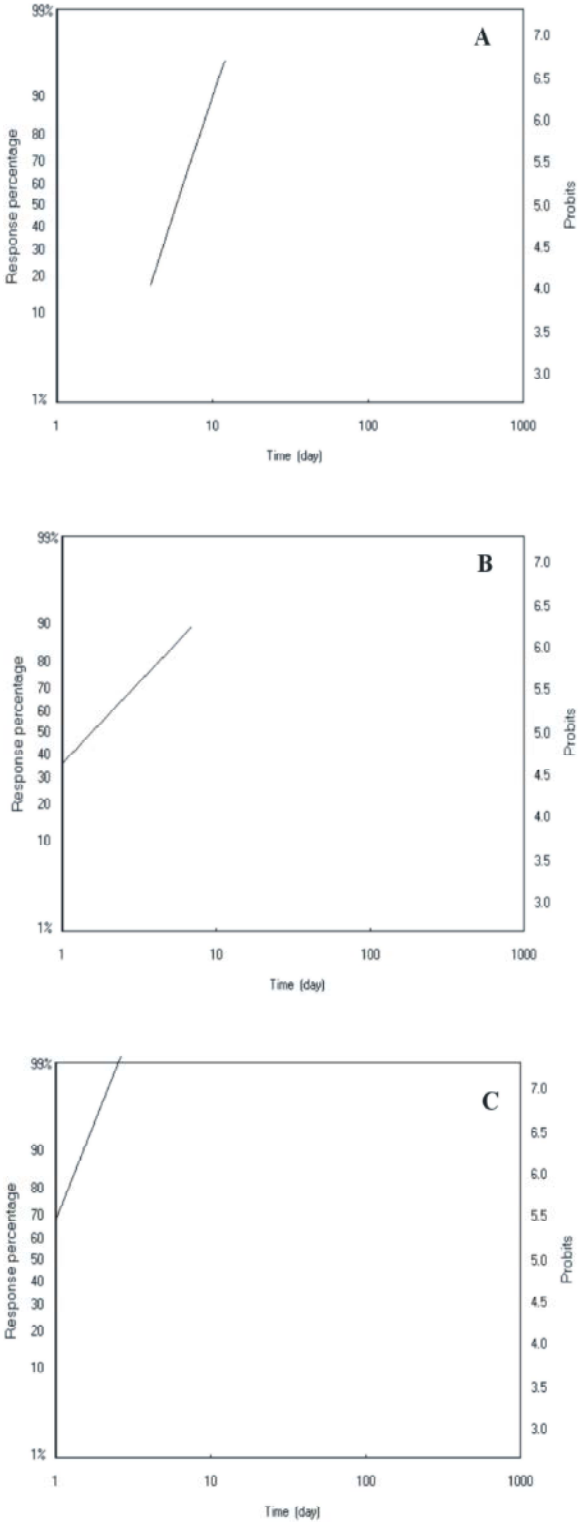


Fig. 4: Toxicity lines for *Gibbium psylloides* adults of exposed to 20% CO_2 (A), 40% CO_2 (B) and 80% CO_2 (C) combined with different exposure periods at 27°C.

DISCUSSION

In this research, different MAs containing 20%, 40% and 80% CO_2 in air were firstly tested against eggs, larvae, pupae and adults of *G. psylloides* at 27°C and 65±5% r.h. There are no previous studies using modified atmospheres (MAs) against *G. psylloides* even with experimental conditions differ from those used in this study. According to mortality percentages and LT_{50} and LT_{95} values, the sensitivity of all stages to the MAs tested here could be arranged as larva > adult > pupa> egg. The slopes of the toxicity lines were the highest in the stages of adults and larvae. While the majority of both pupae and eggs were more tolerant to different concentrations of CO_2 than larvae and adults. These results were similar to study has been observed for *Lasioderma serricorne* (L.), *Cryptolestes ferrugineus* (Stephens) and *Sitophilus oryzae* (L.) [25, 37, 38] and also for several *Liposcelis* species [39]. This finding may be attributed to fact that the larval and adult stages are active stages, while egg and pupal stages are inactive [20, 40]. Hashem *et al*. [41] treated fresh eggs of *C. cephalonica* with MAs contain high level of CO_2 at four concentrations (20, 40, 60 and 80%) at 25, 30 and 35°C. All tested modified atmospheres exhibited an ability to completely kill the eggs in four days or less.

In the present study, it was found that the *G. psylloides* larvae responded to all tested MAs earlier than other stages. There were many studies used different MAs in controlling various developmental stages of stored products insect. Most of these studies clarified that the larvae were the most tolerant stage to MAs especially coleopterous larvae. Hashem *et al.* [42] evaluated the susceptibility of different life stages of saw-toothed grain beetle *Oryzephilus surinamensis* to different MAs containing 55%, 65%, 75% and 85% CO_2 at 30 °C. They found that the larvae were more susceptible beside adults while eggs and pupae were more tolerant to CO_2. Cheng *et al.* [43] found that the gases mixture 2% O_2 + 18% CO_2 + 80% N_2 significantly affected the development and survival of all insect developmental stages of cowpea bruchid, *Callosobruchus maculates*. These findings agree with that recorded by Riudavets *et al.* [29]. They found that all stages (exceptionally, pupa) of *O. surinamensis* which treated with MAs contained 50% CO_2 were completely killed at 4 days exposure. They added that pupa was completely killed at the same period when it was treated with MAs contained 90% CO_2. Some studies detected that the larvae of lepidopterous insect were the most tolerant stage that disagreement with the present study. Ahmed and Hashem [30] studied the susceptibility of the different life stages of Indian meal moth *P. interpunctella* and almond moth *E. cautella* to MAs containing 40%, 60% and 80% CO_2 in air at 27 °C. They showed that five days were adequate to kill all eggs and pupae of the two moths under all tested MAs. Exposure time needed to be extended to 6 and 7 days at 80% CO_2 to obtain complete mortality of larvae of *E. cautella* and *P. interpunctella*, respectively. They also recorded that the order of sensitivity of *P. interpunctella* to MAs was: egg > pupa > larva, while for *E. cautella* it was pupa > egg > larva. Hashem *et al.* [44]. Investigated sensitivity of immature stages of the angoumois grain moth, *Sitotroga cerealella,* to MAs containing 30%, 45%, 65% and 75% CO_2 in air at 27 °C. They found the order of sensitivity of the three developmental stages of *S. cerealella* to MAs was eggs > pupae > larvae.

CONCLUSION

The lethal effect of MAs containing 20%, 40% and 80% CO_2 at 27°C against *G. psylloides* varied through eggs, larvae, pupae and adults. At this temperature, one, three, five and ten days were adequate to completely kill larvae, adults, pupae and eggs of *G. psylloides*, respectively, under all tested MAs. The larvae and adults stages were more sensitive to MAs than pupae and eggs stages.

The most effective MAs are those containing high level of gases such 40% and 80%. So, it is recommended to use these high levels of MAs in controlling all stages of this insect.

ACKNOWLEGMENTS

The authors would like to thank Prof. Dr. Mohamed Yousri Hashem, Professor of Economic Entomology, Faculty of Agriculture, Cairo University for his technical support.

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