Egypt. Acad. J. Biolog. Sci., 6(2): 61 – 71 (2013) A. Entomology
Email: egyptianacademic@yahoo.com ISSN: 1687–8809
Received: 25 / 4 /2013 www.eajbs.eg.net

Using Low Oxygen Atmospheres to Control Two Insects pests Attacking Historic objects in Egypt

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ABSTRACT

The efficacy of controlled atmospheres of high nitrogen concentration (99.9%) and various carbon dioxide concentrations (25, 50,75and 99.9%) was investigated against adults and larvae of two insects attacking historic objects in Egypt, the black carpet beetle and the cigarette beetle. Also, the effect of temperature and exposures time on the mortality of insects was examined. Results revealed that the efficiency of CO_2 and N_2 gases was increased with increasing gas concentration, temperature and exposure time. Carbon dioxide gas was more toxic to both insects than nitrogen gas at the same conditions. Larvae of both species were more susceptible than adults to both gases.

Keywords: black carpet beetle- cigarette beetle- low oxygen atmospheres- temperature

INTRODUCTION

The black carpet beetle *Attagenus fasciatus* is considered to be a pest in the museums where it attacks organic articles, such as furs, hides, insect specimens, wool articles and oil seeds (Back and cotton, 1936). Carpet beetle larvae cause damage by feeding on a wide variety of materials including fur, feathers, wool and silk cloth, wool felt, hair and skins. There are many species of carpet beetles, but one of the most commonly found in museums is the black carpet beetle *A. unicolor* which is the most abundant and destructive of the carpet beetles (National Park Service, 1998). Cigarette beetle *Lasioderma serricorne* is a pest of stored tobacco, but is also a serious pest of flax, spices, crude drugs, seeds, and most importantly for museums books and dried plants (Rust *et al*. 1996).

In the past, chemical applications were widely used in museum to eradicate pests. Such application may be harmful to museum articles (Dawson, 1988). Studies have shown that fumigation with traditional chemicals can cause harm to museum operators, destroy the environment, and may damage rare antiquities and artifacts (Florian, 1988). Recent changes in public attitude and government regulations have increased the pressure to minimize the use of pesticides and have encouraged the use of preventative measures and less toxic materials and methods.

Use of modified atmospheres for pest control in museums has received an increasing amount of interest during the last decade (Kigawa *et al*. 2001).

In fact, since 1989 nitrogen atmospheres have been utilized to control bio-deterioration in Egyptian mummies (Valentin, 1989), library collections, furniture and many other historic objects (Gilberg, 1991; Maekawa, 1996 and Selwitz, 1998).

MATERIALS AND METHODS

1- Rearing Technique of Stock Cultures:

The black carpet beetles were reared according to the technique described by Ali (2010). Larvae were reared in child milk contains the following nutritive 100g: 11.9 g protein, 27.7 g fat, 55.4 g carbohydrate plus pure wool textiles. Adults were fed on tiny drops of honey placed on a sheet of paper. The cigarette beetles were reared on wheat flour containing 10% dry brewer's yeast. Insect cultures were kept under controlled conditions of 27-30 ºC and 55 ± 5 % R.H in the incubator of the pest control laboratory, Center of Research and Conservation of Antiquities, Supreme Council of Antiquities. Adults of each insect species were introduced into the jars to laying eggs under controlled conditions. After hatching, all adults were separated from the jars and introduced to new jars and kept again in the incubator. This procedure was repeated several times in order to obtain large numbers of the larvae and adults needed to carry out the experiments.

2. Exposure Procedure:

A circulatory multi flask apparatus was established to provide an exposure room suitable for gas concentrations applied (Fig. 3). The dreshel flasks with a volume of 0.55 litres were connected to each other with Polyvinyl chloride (PVC) tubing and joints were greased.

3. Gases Used:

Carbon dioxide (CO_2) and Nitrogen (N_2) were provided as pure gases of about 99.9% in pressure steel cylinders. Each cylinder was connected to a pressure regulator. The dilution method was used to achieve the required CO_2 concentrations in the flasks of the apparatus by using a gas tight pump. For the atmospheres of nearly pure N_2, the valve of the N_2 cylinder was opened for two minutes in order to fill the dreshel exposure flasks with the gas after filling , the flasks were directly closed tightly by using two metal clips and glass rods. Concentrations of 25, 50, 75 and 99.9% CO_2 and mixture from various concentrations of oxygen, nitrogen and carbon dioxide were prepared.

4. Determination of Gases Concentrations:

CO_2 was monitored using gas analyzer model 200 - 600 (Gow-Mac-Instrument CO, USA) Nitrogen concentration was determined inside the dreshel flasks using Oxygen Analyzer 572, Servomex, England .

5. Preparation of Insects for Gas Treatment:

Batches of 25 of each 3rd and 5th instars larvae and 25 of newly emerged adults of *A. fasciatus* and *L. serricorne* were put in 4 replicates and placed in wire gauze cages (Fig. 5) (14 mm diam. and 45 mm long), filled with about 10 gm of child milk powder for *A. fasciatus* and 10gm wheat flour with dry brewer's yeast for *L. serricorne* and the cages were closed with rubber stoppers. The cages were then introduced into the 0.55-L gas tight dreshel exposure flasks. Insects in the flasks were treated for different exposure periods (24, 48,72,96 and120 hours) for C.A of high N_2 content and (48 &120 hours) for C.A of various concentrations of CO_2 at 30±1°C and 20± 1°C and 65 ± 5 % R.H. After the desired exposure periods, the flasks were aerated and the insects were transferred into Petri dishes and kept at the above mentioned conditions prior to mortality assessment.

6. Bioassay Tests of Gases:

After the desired exposure period mortality assessment was performed. Both larva and adult mortalities of *A. fasciatus* and *L. serricorne* were determined after 24,

48, 72, 96 and 120 hours for C.A of 99.9% N_2 and (48 &120 hours) exposure periods for C.A of various concentrations of CO_2.

7. Statistical Analysis of the Data:

Lethal time values were determined by probit analysis using a computer program of Noack and Reichmuth (1978).

RESULTS

Efficiency of controlled atmosphere of carbon dioxide and nitrogen against larvae and adults of *Attagenus fasciatus* and *Lasioderma serricorne* was tested.

1-Susceptibility of *A. fasciatus* to Controlled Atmosphere (CA) of Carbon dioxide (CO_2).

The efficacy of different concentrations of CO_2 against 3rd and 5th instars larvae and adults of *A. fasciatus* was tested after 48 and 120 hours at two different temperatures (20±1ºC and 30±1 ºC) and 65 ± 5% RH. The results are shown in tables (Tables 1 to 6) and illustrated graphically in figures (Figs 1 to 12).

Data in Tables 1 &2 and Figs 1 & 2 shown that, larvae of *A. fasciatus* were more susceptible to all tested gas concentrations than adults. At 20±1ºC and 65 ± 5% RH, the LC_{50} values after 48 and 120 hours were 53.893 and 37.623 % for 3rd larval instars, respectively. The LC_{50} values of CO_2 for 5th larval instars after 48 and 120 hours were 62.144 and 45.458 %, respectively. Also, the results indicated that, LC_{50} values of CO_2 for adults after 48 and 120 hours were 64.656 and 48.382 %, respectively.

The efficacy of CA of 100%, 75%, 50% and 25% CO_2 against larvae and adults of *A. fasciatus* at 30±1ºC and 65 ± 5% RH is presented in Tables 1& 2 and shown graphically in Figs 1 to 4. The results illustrated that, the LC_{50} values of CO_2 for 3rd and 5th larval instars and adults after 48 were 52.739, 60.004 and 61.909 %, respectively.

After 120 hours the LC_{50} values were 35.310, 43.957 and 47.295 for 3rd and 5th larval instars and adults, respectively.

As shown in tables (Tables 1& 2) and figures (Figs. 1 & 4) the mortality of *A. fasciatus* increased as the gas concentration increased. According to LC_{50} values for *A. fasciatus*, the concentrations of CO_2 were more effective at higher temperatures than lower temperatures and the adults were more tolerant to CA contain CO2 than larval stages. Also the mortality of *A. fasciatus* increased as the exposure time increased.

Table 1: Susceptibility of *A. fasciatus* to (C.A) of CO_2 after 48 hours at 20 and 30±1ºC and 65 ± 5% RH.

Concentration %	Mortality % at indicated temperatures					
	20±1ºC			30±1ºC		
	3rd larval instar	5th larval instar	Adult	3rd larval instar	5th larval instar	Adult
25	19	10	6	20	12	11
50	30	23	22	31	24	23
75	59	50	45	61	54	50
99.8~100	93	92	89	95	94	91
LC50	53.893	62.144	64.656	52.739	60.004	61.909
Slope	3.723	4.508	4.888	3.686	4.182	4.319

Table 2: Susceptibility of *A. fasciatus* to (C.A) of CO_2 after 120 hours at 20 and 30±1ºC and 65 ± 5% RH

Concentration %	Mortality % at indicated temperatures					
	20±1ºC			30±1ºC		
	3rd larval instar	5th larval instar	Adult	3rd larval instar	5th larval instar	Adult
25	39	28	21	41	31	23
50	47	41	40	50	44	42
75	78	67	65	81	69	68
99.8~100	98	96	95	100	100	100
LC50	37.623	45.458	48.382	35.310	43.957	47.295
Slope	3.021	3.303	3.770	2.975	3.206	3.718

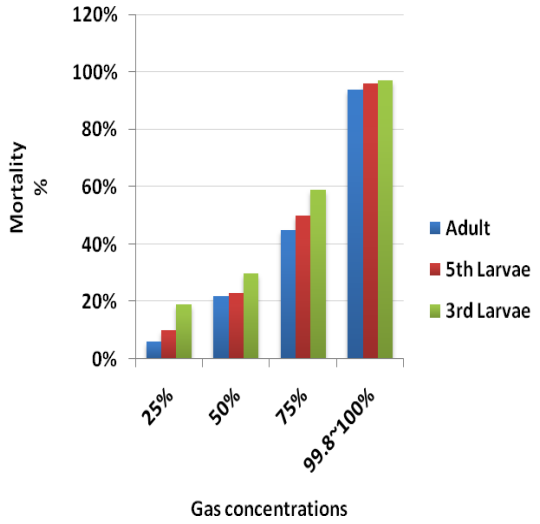

Fig. 1: Susceptibility of *A. fasciatus* to (C.A) of CO_2 after 48 hours at 30±1ºC and 65 ±5% RH.

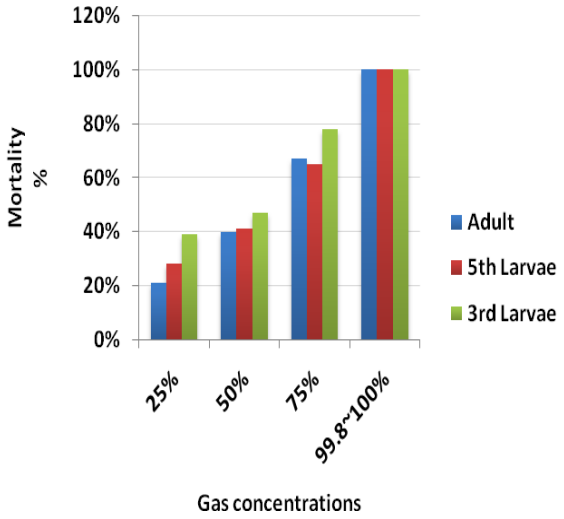

Fig. 2: Susceptibility of *A. fasciatus* to (C.A) of CO_2 after 120 hours at 20±1ºC and 65 ± 5% RH.

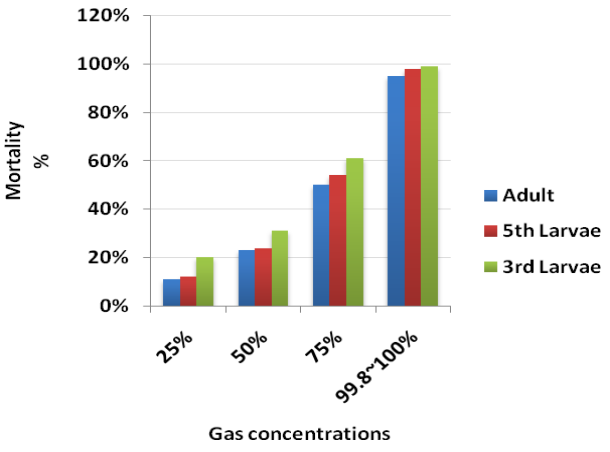

Fig. 3: Susceptibility of *A. fasciatus* to (C.A) of CO_2 after 48 hours at 30±1ºC and 65 ±5% RH.

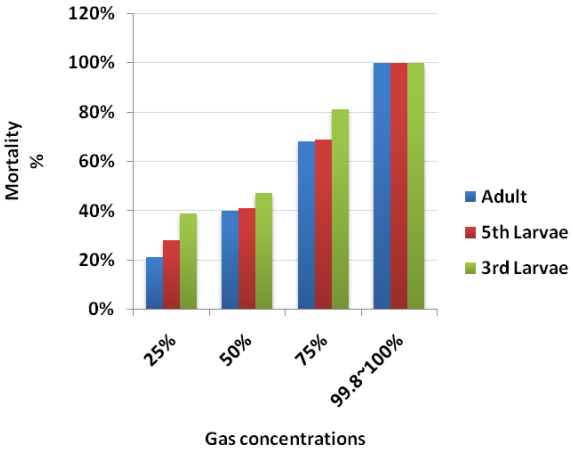

Fig. 4: Susceptibility of *A. fasciatus* to (C.A) of CO_2 after 120 hours at 30±1ºC and 65 ± 5% RH.

2- Susceptibility of *L. serricorne* to Controlled Atmosphere (CA) of Carbon dioxide (CO_2).

The efficacy of different concentrations of CO_2 against 3rd and 5th instars larvae and adults of *L. serricorne* was tested after 48 and 120 hours at two different

temperatures (20±1ºC and 30±1 ºC) and 65 ± 5% RH. The results are shown in tables (Tables 3 & 4) and illustrated graphically in figures (Figs. 5 to 8).

Table (3): Susceptibility of *L. serricorne* to (C.A) of CO_2 after 48 hours at 20 and 30±1ºC and 65 ± 5% RH.

Concentration %	Mortality % at indicated temperatures					
	20±1ºC			30±1ºC		
	3rd larval instar	5th larval instar	Adult	3rd larval instar	5th larval instar	Adult
25	18	11	9	17	9	5
50	30	25	23	29	22	21
75	60	53	49	58	49	43
99.8~100	94	90	85	90	85	82
LC50	53.999	60.964	62.962	55.440	63.769	65.692
Slope	3.831	4.341	4.361	3.822	4.499	4.897

Table 4: Susceptibility of *L. serricorne* to (C.A) of CO_2 after 120 hours at 20 and 30±1ºC and 65 ± 5% RH.

Concentration %	Mortality % at indicated temperatures					
	20±1ºC			30±1ºC		
	3rd larval instar	5th larval instar	Adult	3rd larval instar	5th larval instar	Adult
25	37	26	20	41	29	23
50	45	40	39	50	42	41
75	77	65	63	80	68	66
99.8~100	95	93	88	100	100	100
LC50	39.059	45.926	49.445	35.847	44.810	47.379
Slope	3.092	3.278	3.761	2.990	3.239	3.624

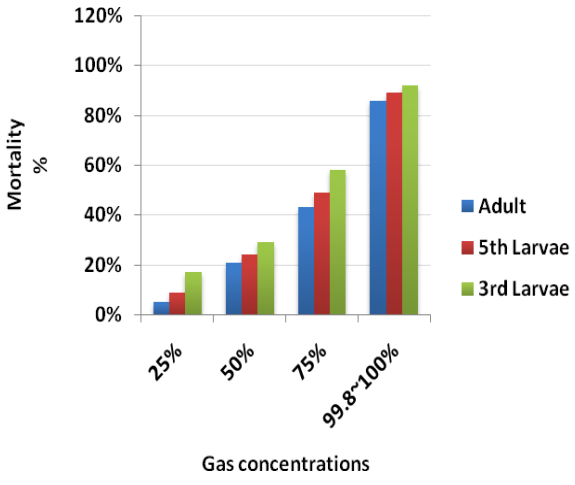

Fig. 5: Mortality % of *L. serricorne* exposed to (C.A) of CO_2 after 48 hours at 20±1ºC and 65±5%RH.

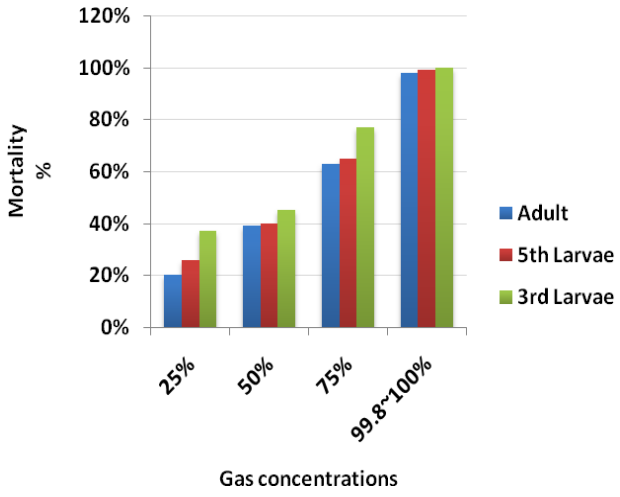

Fig. 6: Mortality % of *L. serricorne* exposed to (C.A) of CO_2 gas after 120 hours at 20±1ºC and 65±5%RH.

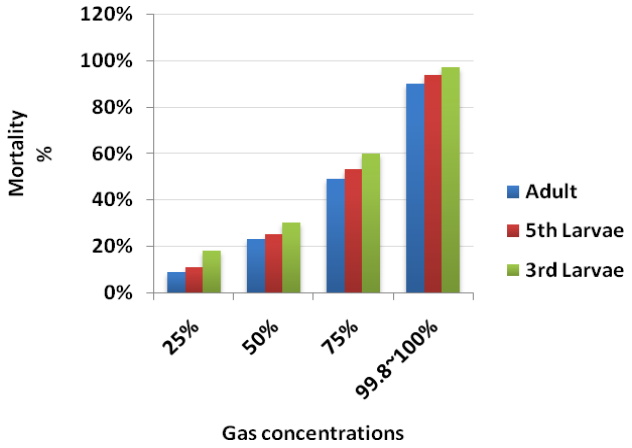

Fig. 7: Mortality % of *L. serricorne* exposed to (C.A) of CO_2 after 48 hours at 30±1ºC and 65±5%RH.

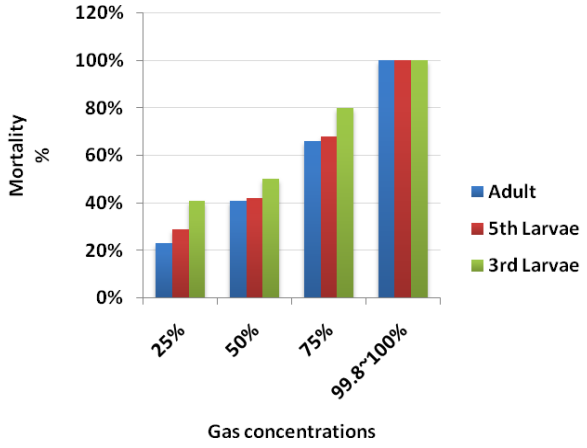

Fig. 8: Mortality % of *L. serricorne* exposed to (C.A) of CO_2 after 120 hours at 30±1ºC and 65±5%RH.

Presented data revealed that, at 20±1ºC, larvae of *L. serricorne* were more susceptible than adults to all tested gas concentrations. The LC_{50} values of CO_2 for 3rd and 5th larval instars and adults after 48 were 55.440, 63.769 and 65.692 %, respectively (Table 3).

After 120 hours, the LC_{50} values were 39.059, 45.926 and 49.445 for 3rd and 5th larval instars and adults, respectively (Table 4).

The efficacy of CA of 100%, 75%, 50% and 25% CO_2 against adults and larvae of *L. serricorne* at 30±1ºC and 65 ± 5% RH is presented in Tables 5& 7 and shown in and Figs 5& 7. The results illustrated that, the LC_{50} values of CO_2 for 3rd and 5th larval instars and adults after 48 were 53.999, 60.964 and 62.962 %, respectively (Table 3).

After 120 hours, the LC_{50} values were 35.847, 44.810 and 47.379 for 3rd and 5th larval instars and adults, respectively (Table 4).

According to LC_{50} values for *L. serricorne*, the concentrations of CO_2 were more effective at higher temperatures than lower temperatures and the adults were more tolerant to CO_2 than larval stages. Also the mortality of *L. serricorne* increased as the exposure time increased.

3- Susceptibility of *A. fasciatus* to Controlled Atmosphere (C.A) Of High Nitrogen (N_2) Content.

The efficacy of CA of 99.9% N_2 against *A. fasciatus* at 20±1ºC and 65 ± 5% RH is shown in Table 5 and Fig. 9. The results indicated that, the time needed to obtain 50% mortality of *A. fasciatus* was 3.155, 3.510 and 3.894 hours for 3rd, 5th larval instars and adults, respectively. Also, the results indicated that, the time required to obtain 95% mortality of *A. fasciatus* was 40.529, 47.320 and 79.168 hours for 3rd, 5th larval instars and adults, respectively.

The efficacy of CA of 99.9% N_2 against *A. fasciatus* at 30±1ºC and 65 ± 5% RH is presented in Table 5 and Fig. 10. The results illustrated that, LT_{50} and LT_{95} of 3rd larval instars were 0.681 and 12.731 hours, respectively.

Data in Table 5 showed that LT_{50} and LT_{95} values of 5th larval instars of *A. fasciatus* were 1.970 and 18.871 hours, respectively.

As illustrated in Table 5 and Fig. 9 the time required to obtain 50% mortality of *A. fasciatus* adults was 4.184 hours and the time required to obtain 95% mortality was 55.900 hours.

As shown in Table 5 the mortality of *A. fasciatus* increased as the exposure time increased. Also the mortality increased as the temperature increased.

According to LT_{50} and LT_{95} values for *A. fasciatus* the concentration of 99.9% N_2 was more effective at higher temperatures than lower temperatures and the adults were more tolerant to CA than larvae stages.

Table 5: Susceptibility of *A. fasciatus* to (C.A) of 99.9% N_2 at 20 and 30±1ºC and 65 ± 5% RH.

Exposure time (hours)	Mortality % at indicated temperatures					
	20±1ºC			30±1ºC		
	3rd larval instar	5th larval instar	Adult	3rd larval instar	5th larval instar	Adult
24	88	86	83	92	90	89
48	95	94	90	98	97	91
72	97	96	93	98	98	95
96	99	98	96	99	99	98
120	100	100	98	100	100	100
LT50	3.155	3.510	3.894	0.681	1.9706	4.184
LT95	40.529	47.320	79.168	12.731	18.871	55.900
Slope	1.6168	1.4561	1.1753	1.293	1.484	1.461

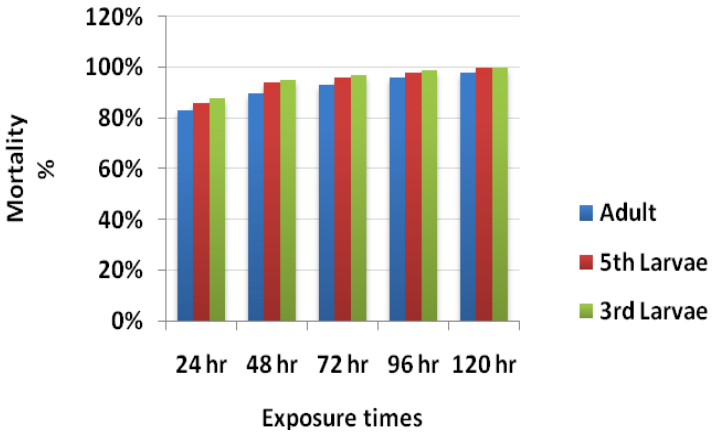

Fig. 9: Mortality % of *A. fasciatus* exposed to (C.A) of 99.9% N_2 at 20±1ºC and 65±5%RH.

4- Susceptibility of *L. serricorne* to Controlled Atmosphere (CA) Of Nitrogen (N_2).

The efficacy of CA of 99.9% N_2 against *L. serricorne* at 20±1ºC and 65 ± 5% RH is given in Table 6 and shown graphically in the Fig 10. The results indicated that, LT_{50} of *L. serricorne* were 3.360, 3.510 and 19.280 hours for 3rd, 5th larval instars and adults, respectively. Also LT_{95} values were 47.320, 55.005 and 114.118 hours for 3rd, 5th larval instars and adults, respectively.

The efficacy of CA of 99.9% N_2 against *L. serricorne* at 30±1ºC and 65 ± 5% RH is shown in Table 6 and Fig 11. The results illustrated that, LT_{50} and LT_{95} values of 3rd larval instars were 2.416 and 16.000 hours, respectively. LT_{50} and LT_{95} values of 5th larval instars were 3.067 and 27.860 hours, respectively.

The results indicated that, LT_{50} and LT_{95} values of adults were 3.394 and 70.444 hours, respectively.

Table 6: Susceptibility of *L. serricorne* to (C.A) of 99.9% N_2 at 20 and 30±1ºC and 65 ± 5% RH.

Exposure time (hours)	Mortality % at indicated temperatures					
	20±1ºC			30±1ºC		
	3rd larval instar	5th larval instar	Adult	3rd larval instar	5th larval instar	Adult
24	86	88	81	90	90	85
48	94	96	90	98	97	91
72	96	98	92	99	98	93
96	98	99	94	99	99	96
120	100	100	96	100	100	98
LT50	3.360	3.510	19.280	2.416	3.067	3.394
LT95	47.320	55.005	114.118	16.000	27.860	70.444
Slope	1.456	1.354	0.928	2.003	1.716	1.2488

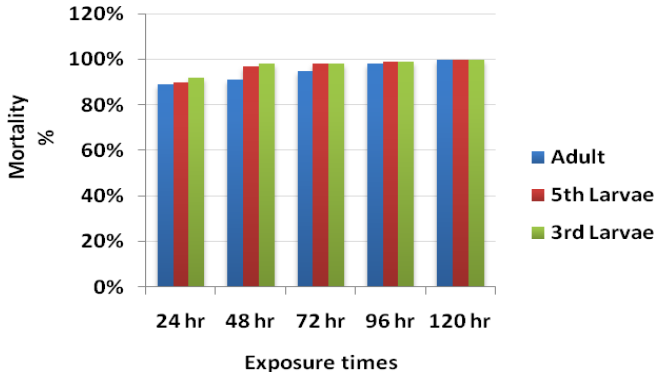

Fig. 10: Mortality % of *A. fasciatus* exposed to (C.A) of 99.9% N_2 at 30±1ºC and 65±5%RH.

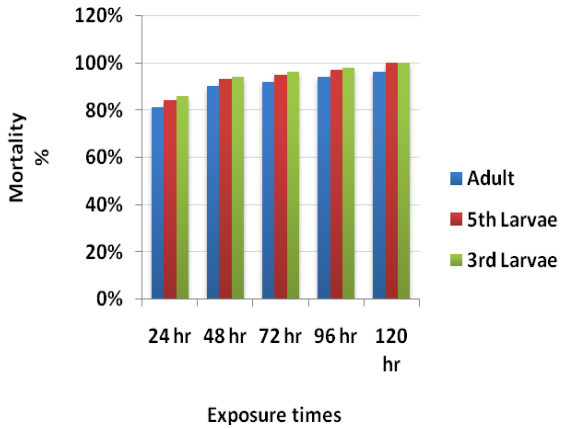

Fig. 11: Mortality % of *L. serricorne* exposed to (C.A) of 99.9% N_2 at 20±1ºC and 65 ± 5% RH.

As shown in Table 6 and Figs 11&12 the mortality of *L. serricorne* increased as the exposure time increased, also the mortality increased as the temperature increased.

According to LT_{50} and LT_{95} values for *L. serricorne* the concentration of 99.9% N_2 was more effective at higher temperatures than lower temperatures and the adults were more tolerant to CA than larvae stages.

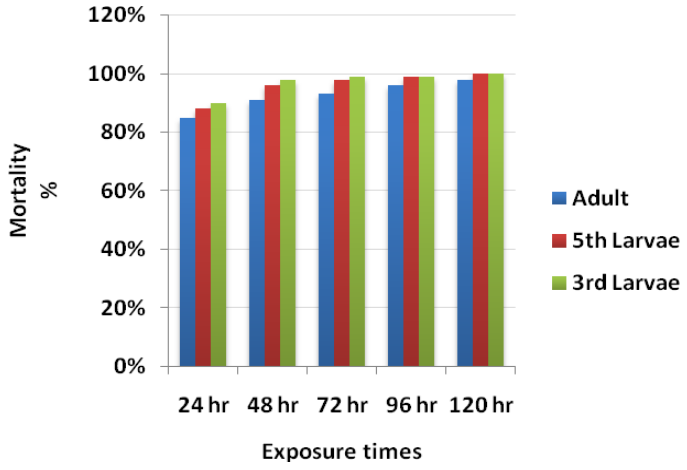

Fig. 12: Mortality % of *L. serricorne* exposed to (C.A) of 99.9% N_2 at 30º±1ºC and 65 ± 5% RH.

Data in tables (Tables 5&6) illustrated that larvae and adults of *A. fasciatus* were more sensitive to N_2 gas than Larvae and adults of *L. serricorne*. Also, the presented results show that N_2 gas was generally more effective against larvae and adults of *A. fasciatus* and *L. serricorne* than CO_2 gas.

DISCUSSION

The efficacy of CA of 100%, 75%, 50% and 25% CO_2 and (99.9%) N_2 against *A. fasciatus* and *L. serricorne* were tested at 20 and 30±1ºC and 65 ± 5% RH. According to LC_{50} values of CO_2 and LT_{50} values of N_2 for *A. fasciatus* and *L. serricorne*, results showed that, adult stage was more tolerant to CO_2 and N_2 than larval stages. Also the mortality of *A. fasciatus* and *L. serricorne* increased as the exposure time and gas concentration increased. The presented results show that N_2 gas was generally more effective against larvae and adults of *A. fasciatus* and *L. serricorne* than CO_2 gas. These findings agree with El-Lakwah *et al.* (1998) who found that controlled atmosphere of around 99.5%N_2 was found to be effective than controlled atmosphere of 96% CO_2.

The exact physiological mechanism of nitrogen anoxia is not completely understood. The sensitivity to low oxygen concentrations varied with insect species, developmental stage and the environmental conditions to which the insects were exposed before as well as during the anoxia treatment.

Most insects are capable of restoring all body function after an exposure to low-oxygen atmospheres for several hours, sometimes even for days, but die if the exposure time is extended. Researchers agree that desiccation is an important mechanism influencing insect mortality during exposure to low-oxygen atmosphere. Jay and Cuff (1981) demonstrated that weight loss of different stages of *Tribolium castaneum* (H.) was 1.6 to 4.8 times higher after exposure to low-oxygen atmosphere (99% nitrogen) than after exposure to air under identical humidity and temperature conditions. They therefore suggested that water loss was the major cause of death. The process of water loss is closely linked to respiration, the exchange of oxygen and carbon dioxide. An insect's respiratory system (tracheal system) has openings to the atmosphere (spiracles) that can be partially or completely closed to prevent water loss (Blum, 1985) this feature allows insect to maintain a sufficient water reserve in environments with low relative humidity. However, air containing low oxygen levels about 2%, or sufficient concentrations of carbon dioxide above 10% cause the spiracles of a well hydrated insect to open fully, resulting in a higher rate of water expiration (Bursell, 1970). After critically low moisture content is reached, the insect tends to reduce its oxygen consumption to minimize further water loss. Of course, reduce oxygen consumption could enable the insect to survive anoxic conditions for an extended period (Gilberg, 1989). Also, (Ali, 1972) referred the death of insects to insect's consequent accumulation of abnormal quantities of various metabolic end products. More recent study by (Adler, 1994) confirm that the accumulation of lactate in insects during exposure to a nitrogen atmosphere lead to acid imbalance in an insects body. This seriously inhibits the anaerobic metabolic pathway.

In the present study the two gases were more effective at higher temperatures than lower temperatures. These results may be illustrated as insects generally show increased metabolic rates and consume more oxygen when the temperature rises. When a certain critical temperature is reached, the insect might use evaporative cooling by opening its spiracles to control body temperature promoting increased water loss the same mechanism was illustrated by (Bursell, 1970), these two

phenomena may explain the greatly increased mortality rate of insects when anoxia treatments are carried out at higher temperatures.

Also, several studies by (Ali, 1972); Lindgren and Vincent (1970); Soderstrom *et al.* (1986) and Rust *et al.* (1996), showed that raising the temperature from 15ºC to 35ºC can reduce the required exposure time for death by 25% to 65% for each 5ºC increase.

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ARABIC ABSTRACT

استخدام أجواء محكمه تحتوي علي تركيز منخفض من غاز الأكسجين لمكافحة حشرتين من الآفات التي تصيب المقتنيات الأثرية في مصر.

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تم تقييم فعالية جو محكم يحتوي علي تركيز عالي من غاز النيتروجين (99.9٪) وتركيزات مختلفة من غاز ثاني أكسيد الكربون (25، 50،75 و 99.9٪) ضد الطور البالغ واليرقات لحشرتين من الآفات التي تصيب المقتنيات الاثريه في مصر ، خنفساء السجاد السوداء وخنفساء السجائر . أيضا، تم دراسة تأثير درجة الحرارة ووقت التعرض للغاز على نسبه الاماته لكلتا الحشرتين.

أظهرت النتائج أن كفاءة كلا من غاز ثاني أكسيد الكربون و النيتروجين تزاد بزيادة تركيز الغاز ودرجة الحرارة ووقت التعرض للغاز. وأوضحت النتائج أن غاز ثاني أكسيد الكربون كان أكثر سمية لكلتا الحشرتين من غاز النيتروجين في الظروف نفسها. وكانت يرقات كلتا الحشرتين أكثر عرضة للاماته من البالغين لكلا من الغازين.