

## IMPACT OF CARBON DIOXIDE ON THE LARVAE AND PUPAE OF ONION BULB FLY, *Eumerus amoenus* LOEW (DIPTERA: SYRPHIDAE)

Ismail, I.I.; M.M. Elbolok ; E.M. Risha ; M.Y. Hashem and H.A. Elshabrawy

Department of Economic Entomology and Pesticides, Fac. of Agric., Cairo Univ., Giza , Egypt .

### ABSTRACT

The immature stages [ 2<sup>nd</sup>, 3<sup>rd</sup> larval instars , 3 and 7- day-old pupae ] of the onion bulb fly , *Eumerus amoenus* Loew were exposed to 4 gas mixtures contained different carbon dioxide concentrations (10 , 20 ,40 and 80 % CO<sub>2</sub>). Seven exposure periods were applied ( 24 - 168 hr ) for each gas mixture . The 2<sup>nd</sup> instar larvae were more susceptible than the 3<sup>rd</sup> instar larvae to the tested gas mixtures . Also , the 3 - day - old pupae were more susceptible than the 7 – day – old pupae . The pupal stage was tolerant than the larval stage to treatments . Complete mortality was obtained by using gas mixture containing 80 % CO<sub>2</sub> after 96 hr , 120 hr and 144 hr exposure period in the 3 – day – old pupae , the larval stage and the 7 – day – old pupae , respectively .

### INTRODUCTION

To control the insect pests in the stores without affecting the environment or the food material, one might use inert gases to suffocate the insects. This effect could be achieved by using relatively inexpensive gases such as carbon dioxide (CO<sub>2</sub>) and nitrogen (N<sub>2</sub>). Recent reviews in this subject were provided by Navarro and Jay (1987); Rajendran (1990); El-Lakwah *et al.* (1991); Whiting *et al.*(1992); Ofuya and Reichmuth (1993); Hashem and Reichmuth (1994) ; El-Sinary ( 1995 ) ; Mbata *et al* ( 1995 ) ; Mbata and Reichmuth (1996) ; Risha *et al.* (1996 and 1999) and Hashem and Risha (1998) .

Applying improved controlled atmospheres technology began with apples and pears more than 50 years ago, and its application is expanding to other fresh fruits and vegetables ( Dilley, 1990 ) .

The onion bulb fly, *Eumerus amoenus* Loew, is one of the most common and destructive insects to onion bulbs cultivated for seed production or stored for human consumption .

In a previous study ( Risha *et al.* ,1996 ) found that , high carbon dioxide atmosphere was effective as protectant, when onion bulbs were artificially infested with the eggs of *E . amoenus* . The present work aimed to evaluate the efficacy of certain gas mixtures containing high carbon dioxide on the larvae and pupae of this pest.

### MATERIALS AND METHODS

#### 1st-Insect rearing:

The rearing technique was as described by EL - Shabrawy (1990) . The flies were fed on a mixture of dried milk and honey to form a non sticky

crumbled texture applied to the surface of the petri-dish and / or on the side walls of the cage . The larvae were reared on onion slices .

**2nd- Experimental design :**

All treatments were carried out at  $30\pm 1^{\circ}\text{C}$  and  $65 \pm 5\%$  R.H. Four gas mixtures ( GM1, GM2, GM3, and GM4) contained 10, 20, 40 and 80 %  $\text{CO}_2$  were used in each treatments . The larvae ( 2<sup>nd</sup> and 3<sup>rd</sup> instar ) and the pupae ( 3 and 7 - day- old ) were exposed to each gas mixture for seven exposure periods (24, 48, 72, 96, 120, 144, and 168 hr ) .

The techniques used for preparing the mixtures were as described in details by Risha *et al.*, (1996 and 1999) .

**3rd- Statistics:**

Mortality results obtained were corrected for mortalities in the control experiments by using Abbott's formula (Abbott , 1925 ). The data were transferred to arcsine units and subjected to analysis of variance(Snedecor and Cochran , 1967). To find out the regression equations for time mortality response (Finney, 1971) , the computer program of Noack and Reichmuth (1978) was used .

## **RESULTS AND DISCUSSION**

**A- Effect on the larval stage:**

Table (1) shows the mortality percentages of the second instar larvae of *E. amoenus* exposed to four gas mixtures for seven exposure periods . It appears that , the percentages of mortality increased with the increase of both  $\text{CO}_2$  concentration in the gas mixture(GM) at each exposure period , and exposure period for each GM. For example , at 48 hr exposure period the percentages of mortality for the second larval instar were 45.8 , 52.6 , 72.7 , and 91.0 % using gas mixture containing 10,20,40 and 80%  $\text{CO}_2$ , respectively . The averages of percentages mortality calculated across the seven exposure periods used were 76.6 , 81.4 , 89.6 and 96.9 % using the above mentioned GM concentrations , respectively . Statistical analysis revealed significant differences in mortality percentage between GMs at all exposure periods except at 144 and 168 hr . No significant differences were found between GM1 and GM2 except at 72 and 120 hr ; between GM2 and GM3 at 120 hr and between GM3 and GM4 at 96 and 120 hr . Also, using GM1 against the 2<sup>nd</sup> larval instar resulted in 40.5 , 45.8 , 70.0 , 88.2 , 93.1 , 98.5 and 100 % mortality at 24 , 48 , 72 , 96 , 120 , 144 and 168 hr exposure period respectively . The averages of percentages mortality calculated across the four GMs used were 60.8 , 65.5 , 84.3 , 94.6 , 97.6 , 99.6 and 100 % at 24 , 48 , 72 , 96 , 120 , 144 and 168 hr , respectively . The differences were insignificant between 24 and 48 hr at each gas mixture . Complete mortality was obtained after 120 hr exposure in GM4 , after 144 hr in all GMs except GM1 and after 168 hr in all GMs .

**Table (1) : Mortality Percentages of *E. amoenus* Larvae exposed to various gas mixtures for different exposure periods .**

Exposure Period (hr)	Corrected mortality % ( Arcsine )									
	2 <sup>nd</sup> larval instar*					3 <sup>rd</sup> larval instar **				
	GM1	GM2	GM3	GM4	Avg.	GM1	GM2	GM3	GM4	Avg.
24		45.9 (42.6)	68.3 (55.9)	88.3 (65.9)	60.8	28.6 (32.3)	35.3 (36.4)	65.4 (54.0)	80.9 (64.2)	52.6
48	45.8 (42.6)	52.6 (46.5)	72.7 (58.5)	91.0 (72.7)	65.5	41.8 (40.3)	51.3 (45.7)	69.1 (56.3)	92.5 (74.2)	63.7
72	70.0 (56.9)	80.2 (63.7)	89.1 (71.0)	97.9 (83.4)	84.3	61.3 (51.6)	73.7 (59.2)	91.7 (73.8)	97.3 (80.6)	81.0
96	88.2 (70.0)	93.1 (75.0)	97.9 (83.3)	99.3 (87.2)	94.6	81.6 (64.6)	90.4 (72.2)	98.6 (84.5)	99.3 (87.3)	92.5
120	93.1 (75.0)	97.9 (82.5)	99.3 (87.3)	100 (90.0)	97.6	87.8 (69.8)	94.2 (78.7)	99.3 (87.2)	100 (90.0)	95.3
144	98.5 (85.9)	100 (90.0)	100 (90.0)	100 (90.0)	99.6	97.9 (83.3)	100 (90.0)	100 (90.0)	100 (90.0)	99.5
168	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100	98.6 (84.5)	100 (90.0)	100 (90.0)	100 (90.0)	99.7
Avg.	76.6	81.4	89.6	96.9	86.1	71.1	77.8	89.2	95.7	83.4

\*L.S.D at 0.05 level = 4.94( arcsine transformation )

\*\*L.S.D at 0.05 level = 5.28( arcsine transformation )

Values of LT<sub>50</sub> and LT<sub>95</sub> for the second instar larvae of *E. amoenus* after exposure to different GMs are presented in Table (2). At LT<sub>50</sub> level the values were 40.2 , 35.6 , 26.8 and 12.2 hr using GM1 GM2,GM3 and GM4, respectively. The corresponding LT<sub>95</sub> values were 96.7,79.2 , 60.4 and 47.7 hr .

**Table (2) : LT<sub>50</sub> and LT<sub>95</sub> values for *E. amoenus* larvae and pupae exposed to various gas mixtures for different exposure periods**

Treated stage	Gas mixture (GM)	LT <sub>50</sub> (hr)	LT <sub>95</sub> (hr)	Confidence limits at 95 %			
				LT <sub>50</sub>		LT <sub>95</sub>	
				Lower	Upper	Lower	Upper
2 <sup>nd</sup> larval instar	GM1	40.2	96.7	38.4	42.1	91.5	102.3
	GM2	35.6	79.2	34.0	37.3	75.1	83.4
	GM3	26.8	60.4	25.1	28.6	57.2	63.8
	GM4	12.2	47.7	9.9	15.2	44.1	51.6
	Avg.	28.7	71.0	26.8	30.1	67.0	75.3
3 <sup>rd</sup> larval instar	GM1	45.9	130.4	43.6	48.3	121.2	140.2
	GM2	39.7	85.2	38.1	41.4	81.1	89.5
	GM3	27.0	62.6	25.3	28.9	58.9	66.5
	GM4	17.9	46.1	15.7	20.4	42.7	49.8
	Avg.	32.6	81.1	30.7	34.7	76.0	86.5
3-day-old pupae	GM1	73.2	157.5	70.7	75.8	149.2	166.3
	GM2	57.2	125.4	55.1	59.3	118.9	132.3
	GM3	39.7	87.4	38.0	41.4	83.2	91.8
	GM4	25.5	52.5	24.0	27.1	49.3	55.8
	Avg.	48.9	105.7	46.9	50.9	100.1	111.5

7-day old pupae	GM1	69.1	185.8	66.2	72.1	171.9	200.8
	GM2	56.3	128.6	54.1	58.5	121.5	136.2
	GM3	41.2	91.6	39.5	43.1	87.1	96.4
	GM4	22.0	60.0	20.1	24.0	56.2	63.9
	Avg.	47.1	116.5	45.0	49.4	109.2	124.3

The mortality percentages of the third instar larvae of *E. amoenus* exposed to four GMs for seven exposure periods are presented in Table (1) . As in the case of the second instar larvae , the mortality percentages of the 3<sup>rd</sup> instar larvae increased with increasing CO<sub>2</sub> concentration in GMs. For instance at 24 hr of exposure, the mortality percentages were 28.6 ,35.3 , 65.4 and 80.9 % using GM1 , GM2 , GM3 and GM4 , respectively . The averages of percentages mortality calculated across the seven exposure periods tested were 71.1 , 77.8 ,89.2 and 95.7% using GM1 , GM2 , GM3 and GM4 , respectively. The differences were significant between the GMs at each exposure period except between GM1 and GM2 at 24 and 48hr; between GM2 , GM3 and GM4 at 144 and 168 hr and between GM3 and GM4 at 96 hr or more . Also the mortality percentages increased with increasing the exposure period for each GM. For example , using GM<sub>2</sub> produced mortality percentages of 35.3, 51.3 , 73.7 , 90.4 , 94.2 , 100 and 100 % at 24 , 48 , 72 , 96 , 120 , 144 and 168 hr exposure period , respectively . The averages of percentages mortality calculated across the four GMs used were 52.6 , 63.7 , 81.0 , 92.5 , 95.3 , 99.5 and 99.7 % at the seven exposure periods , respectively . Statistical analysis showed that the effect of exposure period on percentage mortality was significant for each GM except between 144 and 168 hr of exposure period for all GMs , and between 24 and 48 hr or between 96 and 120 hr using GM3 .LT<sub>50</sub> and LT<sub>95</sub> values for the third instar larvae of *E. amoenus* after exposure to different GMs are given in Table (2) . At LT<sub>50</sub> level the values were 45.9 , 39.7 , 27.0 and 17.9 hr using GM1 , GM2 , GM3 and GM4 , respectively . The corresponding LT<sub>95</sub> values were 130.4 , 85.2 , 62.6 and 46.1 hr .

From the LT<sub>50</sub> and LT<sub>95</sub> values in Table (2) it is seems that , the 2<sup>nd</sup> instar larvae of *E. amoenus* were more affected by the four gas mixtures tested than the 3<sup>rd</sup> instar larvae . At LT<sub>50</sub> level , the mean values calculated across the different GMs used were 28.7 and 32.6 hr for the 2<sup>nd</sup> and the 3<sup>rd</sup> instar , respectively . The corresponding means of LT<sub>95</sub> values were 71.0 and 81.1 hr.

The results are nearly in agreement with the findings of Navarro and Jay (1987) who found that , exposure to 60 % CO<sub>2</sub> in air for 120 hr at 27 °C was sufficient to cause 100 % mortality of all stages of *Oryzaephilus surinamensis* . El-Lakwah *et al.* (1991) reported that, CO<sub>2</sub> at 20 , 50 or 78 % had slight effects on larval and pupal mortalities of *Sitotroga cerealella* at short exposures , but at 24 - 72 hr, mortalities were 4 - 60 % at 20 and 28 °C . According to Whiting *et al.* (1992) , the order of decreasing estimated time for 99 % mortality ( LT<sub>99</sub> ) was , 5<sup>th</sup> instar larvae > 3<sup>rd</sup> instar larvae > 1<sup>st</sup> instar larvae of *Epiphyas postvittana* and 3 tortricid pests of apples in controlled atmosphere of 0.4% O<sub>2</sub> and 5 % CO<sub>2</sub> . Also , Ofuya and Reichmuth (1993)

reported that, the old larvae and pupae of *Acanthoscelides obtectus* were the most tolerant to high CO<sub>2</sub>. All developmental stages were killed in 3 and 7 days in the 88 and 70 % CO<sub>2</sub> at 32 °C, respectively.

According to El-Sinary (1995) reported that the 4<sup>th</sup> larval instar of *hthorimaea operculella* was more tolerant to inert gases than the 2<sup>nd</sup> larval instar. She added that exposure to controlled atmospheres containing CO<sub>2</sub> showed more deleterious effects on the larvae exposed to gas mixtures containing 60 % CO<sub>2</sub>.

#### **B- Effect On The Pupal Stage:**

The mortality percentages for treated 3-day-old pupae positively correlated with the concentration of CO<sub>2</sub> in GM at each exposure period (Table 3). For example at 72 hr exposure, the four GMs produced mortality percentages of 17.5, 34.7, 66.5 and 96.6 % for GM1, GM2, GM3 and GM4, respectively. The averages of mortality percentages calculated across the seven tested exposure periods were 48.9, 60.3, 76.7 and 90.2 % for the 4 GMs, respectively. The differences were insignificant between GM1 and GM2 at each exposure period except at 48, 72 and 144 hr; same between GM3 and GM4 except at 24, 144 and 168 hr exposure periods. Also, the mortality percentages of 3-day-old pupae positively correlated with the exposure period at each GM treatment. Using GM2, the mortality percentages were 17.0, 25.8, 34.7, 66.5, 79.6, 97.6 and 100 % at 24, 48, 72, 96, 120, 144 and 168 hr exposure periods, respectively. The corresponding averages of mortality summed across the four GMs used at the exposure periods were 32.2, 39.6, 53.8, 76.3, 86.3, 95.1 and 100 %. Statistical analysis revealed that, no significant differences in mortality percentages were found between 24 and 48 hr or between 144 and 168 hr.

The results in Table (2) indicated that, the LT values for the 3-day-old pupae of *E. amoenus* exposed to different GMs were reduced with increasing CO<sub>2</sub> concentration in GM. The LT<sub>50</sub> values were 73.2, 57.2, 39.7 and 25.5 hr using GM1, GM2, GM3 and GM4, respectively. The corresponding LT<sub>95</sub> values were 157.5, 125.4, 87.4 and 52.5 hr.

The mortality percentages of 7-day-old pupae of *E. amoenus* exposed to 4 GMs for 7 exposure periods are presented in Table (3). The results showed - as in the case of 3-day-old pupae treatment - that the mortality percentages were drastically affected by CO<sub>2</sub> concentration in gas mixture at each exposure period. At 72 hr exposure as example, the mortality percentages were 35.0, 45.5, 70.0 and 91.1 % using GM1, GM2, GM3 and GM4, respectively. Irrespective of the exposure periods, averages mortality obtained using GM1, GM2, GM3 and GM4 were 55.4, 62.1, 75.9 and 91.7%, respectively. Statistical analysis showed that, the differences between GM1 and GM2 were insignificant at all exposure periods except at 120 and 144 hr. Also, no significant differences were found between GM2 and GM3 at 144 and 168 hr or between GM3 and GM4 at 120, 144 and 168 hr.

On the other hand the mortality percentages increased with the increase of the exposure period. For instance, using GM1 resulted in

mortality percentages of 10.4 , 19.8 ,35.0 , 61.0 , 74.6 , 90.8 and 96.2 % at 24 , 48, 72,96,120,144 and 168 hr exposure periods , respectively. The corresponding averages of mortality percentages calculated across the four GMs used were 33.5 , 42.8 , 60.4 , 78.3 , 89.2 , 96.3 and 98.6 % . Generally , the differences were insignificant between 24 and 48 hr or between 144 and 168 hr exposure periods using each gas mixture .

**Table (3): Mortality Percentages of *E. amoenus* pupae exposed to various gas mixtures for different exposure periods.**

Exposure Period (hr)	Corrected mortality % ( Arcsine )									
	3-day-old*					7-day-old**				
	GM	GM2	GM3	GM4	Avg.	GM1	GM2	GM3	GM4	Avg.
24	8.4 (16.3)	17.0 (24.1)	44.2 (41.6)	59.1 (50.4)	32.2	10.4 (18.5)	13.9 (21.3)	33.3 (35.0)	76.3 (57.0)	33.5
48	10.2 (17.7)	25.8 (30.3)	46.6 (42.9)	75.7 (60.7)	39.6	19.8 (26.3)	23.3 (28.8)	48.3 (44.0)	79.9 (63.7)	42.8
72	17.5 (24.4)	34.7 (35.8)	66.5 (54.7)	96.6 (81.3)	53.8	35.0 (36.1)	45.5 (42.5)	70.0 (56.9)	91.1 (72.9)	60.4
96	53.5 (47.0)	66.5 (54.8)	85.3 (67.6)	100 (90.0)	76.3	61.0 (51.5)	69.7 (56.9)	85.8 (68.6)	96.5 (81.1)	78.3
120	71.1 (57.6)	79.6 (63.4)	94.4 (79.0)	100 (90.0)	86.3	74.6 (60.0)	87.7 (70.0)	96.3 (80.9)	98.1 (85.4)	89.2
144	82.2 (65.4)	97.6 (85.6)	100 (90.0)	100 (90.0)	59.1	90.8 (72.5)	96.3 (83.5)	98.0 (85.3)	100 (90.0)	96.3
168	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100	96.2 (80.9)	98.1 (85.4)	100 (90.0)	100 (90.0)	98.6
Avg.	48.9	60.3	76.7	90.2	69.0	55.4	62.1	75.9	91.7	71.3

\*L.S.D at 0.05 level = 10.52 ( arcsine transformation )

\*\*L.S.D at 0.05 level = 8.92 ( arcsine transformation )

LT<sub>50</sub> and LT<sub>95</sub> values for the 7-day-old pupae of *E. amoenus* exposed to the 4 GMs are presented in Table (2) . At LT<sub>50</sub> level the values were 69.1 , 56.3 , 41.2 and 22.0 hr using GM1 , GM2 , GM3 and GM4 , respectively . The corresponding LT<sub>95</sub> values were 185.8 , 128.6 , 91.6 and 60.0 hr .

Generally it could be reported that the young pupae ( 3-day-old ) were more susceptible than the old pupae ( 7-day-old ) to four gas mixtures of high CO<sub>2</sub> contents tested at the seven exposure periods . At LT<sub>95</sub> level the mean values calculated across the four gas mixtures used were 105.7 hr for the young pupae and 116.5 hr for the old pupae . At LT<sub>50</sub> level the means were very close ( 48.9 and 47.1 hr for young and old pupae respectively ) .

In harmony with these observations , Rajendran (1990) reported that , using of CO<sub>2</sub> against *Tribolium castanum* pupae , produced maximum of 11% mortality of pupae exposed to 10 - 70 % levels for 24 hr . Ofuya and Reichmuth (1993) stated that, the old pupae of *Acanthoscelides obtectus* were very tolerant to high CO<sub>2</sub> . Also, these findings may agree with the results of other workers who demonstrated differences in succceptibility among developmental stages of some stored product insects to modified atmospheres such as *Callosobruchus subinnotatus* (Mbata and Reichmuth, 1996); *C. maculatus* and *C. Subinnotatus* (Mbata et al., 1995) and *C. chinensis* (Hashem and Risha, 1998).

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### تأثير المعاملة بغاز ثانى اكسيد الكربون على يرقات و عذارى ذبابة البصل الكبيرة *Eumerus amoenus* Loew (Diptera: Syrphidae)

اسماعيل اسماعيل - محمد مصطفى البلك - السيد محمد ريشة - محمد يسرى  
هاشم - حمدي عبد الصمد الشبراوى .  
قسم الحشرات الاقتصادية والمبيدات - كلية الزراعة - جامعة القاهرة .

تم تعريض الأطوار غير الكاملة ( يرقات العمر الثانى والثالث والعذارى عمر 7، 3 ايام ) لذبابة البصل الكبيرة *Eumerus amoenus* Loew لاربعة مخاليط غازية تحتوى على تركيزات مختلفة من ثانى اكسيد الكربون ( 10، 20، 40، 80 % ) . وكانت فترات التعريض لكل مخلوط غازى 7 فترات ( 24-168 ساعة). اظهرت النتائج ان يرقات العمر الثانى كانت اكثر حساسية للمعاملة من يرقات العمر الثالث وكذلك كانت العذارى عمر 3 ايام اكثر حساسية من العذارى عمر 7 ايام فى جميع المعاملات . كما كانت اليرقات اكثر حساسية من العذارى بصفة عامة . وكانت العلاقة طردية بين نسبة الموت وزيادة كلا من تركيز ك أ2 فى المخلوط الغازى وفترة التعريض . وصلت نسبة الموت 100 % عند المعاملة بالمخلوط الغازى المحتوى على 80 % ك أ2 فى العذارى عمر 3 ايام بعد 96 ساعة تعريض وفى اليرقات ( عمر ثانى وثالث ) وبعد 120 ساعة تعريض . وفى العذارى الكبيرة عمر 7 ايام بعد 144 ساعة تعريض .



