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# EFFECT OF CARBON DIOXIDE ON CERTAIN INSECTS INFESTED STORED DRY DATE FRUITS

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## ABSTRACT

Different concentrations of $CO_2$ were tested as curative control method against the the saw-toothed beetle, *Oryzaephilus saurinamensis* and the warehouse moths, *Ephestia Spp.* which attack the dry date fruits during storage. The mortality percentages positively correlated with $CO_2$ concentration and the exposure period. Treatment with 20% $CO_2$ (lowest concentration) for 1 day exposure gave 35.3% mortality of *O. saurinamensis* but only 16.9 and 26.6% mortality of *Ephestia Spp.*larvae and pupae, respectively. 98.9% mortality of *O. saurinamensis* was obtained with 50% $CO_2$ (highest concentration) for 7 days, while the same treatment gave 93.3% and 90.3%. mortality of *Ephestia Spp.*larvae and pupae, respectively.

## INTRODUCTION

The main insect pests attack the dry date fruits during storage the saw-toothed beetle,*Oryzaephilus saurinamensis* L.( Cucujidae, Coleoptera) and the warehouse moths, *Ephestia Spp.* (Pyralidae, Lepidoptera). In most cases dry date fruits stored for human consumption without applying control measures, the losses due to insect infestations may reach high economic levels. There is a positive correlation between insect infestation and reduction in total and reducing sugars, proteins and phenolics during storage (Abu-Goukh *et al.*, 2002). The use of conventional insecticides for controlling stored product pests is discouraged because of hazards caused by the toxic residues left on the treated products and resistance developed in insect pests exposed to insecticide ( Banks and Annis,1990 ).

Many investigators had focused on the possibility of using the inert gases ($CO_2$ and/or $N_2$) as an alternative of chemical fumigants to control of stored product pests. This method of treatment is commonly termed modified atmosphere (MA) or controlled atmosphere (CA) (Reichmuth, 1986; Jay *et al.*,1990; El-Lakwah *et al*., 1991; El-Sinary, 1995; Hashem *et al.* 1995; Omer *et al.,* 1995; Al- Redhaiman ,2002; Ahmed, 2007 and Conyers and Bell 2007).

With this outlook, the present study was conducted to investigate the suitability of high carbon dioxide atmospheres for the disinfestations of stored dry date fruits infested with the saw-toothed beetle,*O.saurinamensis* and the warehouse moths, *Ephestia Spp.* Different concentrations of $CO_2$ were tested as curative control method against these insects.

## MATERIALS AND METHODS

A stock culture of the tested insects in the present study started by using infested date fruits collected from stores. All treatments were carried out at 25±1˚C and 60 ±5% R.H.

**Preparation of insects to treatment:**

The experimental unit (replicate) was 50 date fruits put in glass jar with 100 adults (beetles) of *O. saurinamensis* and 20 4th instar larvae of warehouse moths, *Ephestia Spp*. Jar was covered by cloth piece, fitted by rubber bands. 10 pupae of warehouse moths were put in a wire cage (4.5 cm length and 1.5 cm diameter).

**Treatment with carbon dioxide:**

The tested insects were exposed to different modified atmospheres containing 20, 30, 40 and 50 % $CO_2$ for 7 exposure periods (1, 2, 3, 4, 5, 6 and 7 days).

The following four modified atmospheres (MAs) were tested :

MA1 contains 16 % $O_2$ , 64 % $N_2$ and 20 % $CO_2$.

MA 2 contain 14 % $O_2$ , 56 % $N_2$ and 30 % $CO_2$.

MA 3 contains 12 % $O_2$ , 48 % $N_2$ and 40 % $CO_2$.

MA4 contains 10 % $O_2$ , 40 % $N_2$ and 50 % $CO_2$.

Plastic containers were used as exposure chambers contained the glass jars. The containers were closed with plastic covers, each fitted with two tubes, one extending to near the bottom and the other to the upper quarter of the chamber. The short tube was used as gas exit and the long one as gas inlet for interval determination of the gas concentrations. The technique of treatment with $CO_2$ was as described by Hashem (1990). Control treatments were those containers untreated with carbon dioxide.

At the end of each exposure period, the jars were transferred outside the container and were examined to account and record the died insect ones. All treatments were replicated three times including the untreated ones. Mortality percentages corrected by Abbott's formula (Abbott, 1925) and subjected to analysis of variance (Snedecor and Cochran, 1967). The data were subjected to probit analysis (Finney, 1971) to find out the regression equations for time mortality response . The computer program of Noack and Reichmuth (1978) was used .

## RESULTS AND DISCUSSION

**Effect on the saw-toothed beetle, *O. saurinamensis* :**

The effect of different concentrations of $CO_2$ in on the saw-toothed beetle, *O. saurinamensis* are present in Table (1). The results indicate that, the mortality percentages positively correlated with $CO_2$ concentration and the exposure period. When the tested insects were exposed for on day to the four tested $CO_2$ concentrations, the mortality percentages were 35.3, 36.2, 52.4 and 61.6%, respectively. The corresponding mortality percentages at 7 days exposure (longest exposure period) were 84.2, 87.1, 98.9 and 98.9%, respectively. The average mortality percentages for the 7 exposure periods were 64.1, 72.2, 79.5 and 83.4% for 20, 30, 40 and 50% $CO_2$ concentration, respectively. The differences were significant between 20 and 30% $CO_2$ concentration at all exposure periods except at 1 and 7 days exposure period while, the differences were significant between 40 and 50% $CO_2$ concentration at 1 and 2 days exposure period. The 7 exposure periods for

20% $CO_2$ concentration gave mortality percentages of 35.3, 42.6, 61.5, 69.3, 75.3 80.3 and 84.2%. The corresponding mortality percentages of 50% $CO_2$ concentration were 61.6, 70.4, 81.3, 87.2, 90.3, 94.4 and 98.9%. The differences were significant between exposure periods at each $CO_2$ concentration except for long periods.

**Table (1): Mortality percentage of saw-toothed beetle, *O. saurinamensis* exposed to different $CO_2$ concentrations for different exposure periods.**

| $CO_2$ % | Corrected mortality percentage after indicated periods (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
| 20 | 35.3 | 42.6 | 61.5 | 69.3 | 75.3 | 80.3 | 84.2 | 64.1 |
| 30 | 36.2 | 55.3 | 73.6 | 81.9 | 84.3 | 87.2 | 87.1 | 72.2 |
| 40 | 52.4 | 63.6 | 79.3 | 84.4 | 87.2 | 90.8 | 98.9 | 79.5 |
| 50 | 61.6 | 70.4 | 81.3 | 87.2 | 90.3 | 94.4 | 98.9 | 83.4 |
| Average | 46.4 | 57.9 | 73.9 | 80.7 | 84.2 | 88.2 | 92.3 | 74.8 |

**L.S.D.** $_{0.05\%}$ **level = 4.43**

Data as shown in Table (2) indicate that, the LT values decreased with increasing $CO_2$ concentration. At $LT_{50}$ level, the values were 40.2, 32.7, 24.3 and 20.1 hr when using 20, 30,40 and 50% $CO_2$ concentration, respectively. The respective $LT_{95}$ values were 124.2, 77.8, 58.9 and 48.9 hr, respectively.

**Table (2): $LT_{50}$ and $LT_{95}$ values of saw-toothed beetle, *O. saurinamens*is exposed to different $CO_2$ concentrations for different exposure periods.**

| $CO_2$ % | $LT_{50}$ (hr) | $LT_{95}$ (hr) | Confidence limits at 95% | | | |
|---|---|---|---|---|---|---|
| | | | $LT_{50}$ | | $LT_{95}$ | |
| | | | Lower | Upper | Lower | Upper |
| 20 | 40.2 | 124.2 | 37.8 | 42.7 | 114.7 | 134.5 |
| 30 | 32.7 | 77.8 | 30.9 | 34.6 | 73.1 | 82.7 |
| 40 | 24.3 | 58.9 | 22.5 | 26.3 | 55.1 | 62.9 |
| 50 | 20.1 | 48.9 | 18.1 | 22.3 | 45.2 | 51.9 |
| Average | 29.3 | 77.3 | 27.3 | 33.7 | 72.0 | 83.0 |

Similar to these findings, Jay *et al.* (1990) found that $CO_2$ concentrations of about 60% gave over 95% kill for most of the stored grain insects after 4 days of exposure at 27˚C or higher. Reichmuth (1986) stated that, the lethal exposure period was 6 days to achieve 95% mortality with adults of *Sitophilus granaries* at 3% $O_2$ (85% $CO_2$).

Omar *et al.*(1995) reported that, the lethal $CO_2$ concentration of the adult stage of *S. oryzae* was 65%. Hashem *et al.*(1995) found that, 100% mortality for adults of *Callosobruchus maculates* was produced from the gas mixture containing 55% $CO_2$. Conyers and Bell (2007) stated that, the population growth of *O. saurinamensis* was only 4% under modified atmospheres contain 10- 20% $CO_2$ .

**Effect on the warehouse moths, *Ephestia Spp*.:**

The 4$^{th}$ instar larvae and pupae of the warehouse moths, *Ephestia Spp*.were exposed to four different concentrations of $CO_2$.

**Effect on the larval stage:**

The toxic action of different concentrations of $CO_2$ against 4th instar larvae of the warehouse moths, *Ephestia Spp*. are present in Table (3). In general, the percentages of larval mortality were increased with the increase of $CO_2$ concentration at each exposure period. For example, using different concentration of $CO_2$ for one day produced 16.9, 21.5, 27.9 and 42.9% mortality when using 20, 30, 40 and 50% $CO_2$ concentration, respectively. The percentages of larval mortality averaged across the exposure periods 50.7, 59.3, 67.0 and 75.4% at 20,30 40 and 50% $CO_2$ concentration, respectively. The differences were significant between $CO_2$ concentrations at all exposure periods except at 1 and 7 days for 20 and 30%, while no significant differences between 40 and 50% concentration except at 1, 2 and 4 days exposure period.

**Table (3): Mortality percentage of warehouse moths, *Ephestia Spp.* larvae exposed to different $CO_2$ concentrations for different exposure periods.**

| $CO_2$ % | Corrected mortality percentage after indicated periods (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
| 20 | 16.9 | 24.7 | 42.6 | 54.2 | 64.5 | 72.1 | 79.8 | 50.7 |
| 40 | 21.5 | 36.3 | 56.8 | 64.2 | 72.2 | 80.7 | 83.7 | 59.3 |
| 40 | 27.9 | 45.4 | 59.3 | 74.8 | 85.6 | 85.3 | 90.5 | 67.0 |
| 50 | 42.9 | 55.8 | 66.6 | 85.6 | 90.8 | 92.8 | 93.3 | 75.4 |
| Average | 27.3 | 40.5 | 56.3 | 69.7 | 78.3 | 82.7 | 86.8 | 63.1 |

**L.S.D. $_{0.05\%}$ level = 6.66**

Also, exposure period affected the mortality percentages of each concentration. For example, at 1 day exposure period, 20% $CO_2$ caused 16.9% mortality while using the same concentration gave 24.7, 42.6, 54.2, 64.5, 72.1 and 79.8% mortality at 2, 3, 4, 5, 6 and 7 days exposure periods, respectively. The averages of mortality percentages calculated across the four $CO_2$ concentrations were 27.3, 40.5, 56.3, 69.7, 78.3, 82.7 and 86.8% at 1, 2, 3, 4, 5, 6 and 7 days, respectively. The exposure periods significantly affected mortality percentages for each concentration except at long periods as a result of high mortality percentages.

The probit analysis (Table 4) indicated that, $LT_{50}$ values were 67.6, 51.4, 42.1 and 32.3 hr for 20, 30, 40 and 50% $CO_2$, respectively. The corresponding values for $LT_{95}$ were 176.9, 122.5, 85.4 and 64.1hr.

**Table (4): $LT_{50}$ and $LT_{95}$ values of warehouse moths, *Ephestia Spp.* larvae exposed to different $CO_2$ concentrations for different exposure periods.**

| $CO_2$ % | $LT_{50}$ (hr) | $LT_{95}$ (hr) | Confidence limits at 95% | | | |
|---|---|---|---|---|---|---|
| | | | $LT_{50}$ | | $LT_{95}$ | |
| | | | Lower | Upper | Lower | Upper |
| 20 | 67.6 | 176.9 | 64.8 | 70.5 | 164.5 | 190.3 |
| 30 | 51.4 | 122.5 | 49.2 | 53.6 | 115.7 | 129.6 |
| 40 | 42.1 | 85.4 | 40.5 | 43.8 | 81.5 | 89.6 |
| 50 | 32.3 | 64.1 | 30.9 | 33.8 | 61.0 | 67.3 |
| Average | 48.3 | 112.2 | 46.3 | 50.4 | 105.7 | 119.2 |

Similar to these findings, Elsinary (1995) found that, exposure to controlled atmospheres containing $CO_2$ showed more deleterious effects on the *Phthorimaea. operculella* larvae exposed to gas mixture containing 60% $CO_2$.

Ahmed (2007) found that, when the 4th larval instar of *Sitotroga cerealella* exposed to modified atmospheres contain 30, 45, 65 and 75% $CO_2$ the reduction percentage in adult emergence reached 100% at 27 $^oC$ after 264 hr.

**Effect on the pupal stage:**

Table (5) shows the toxic action of $CO_2$ concentration on the pupal stage of the warehouse moths, *Ephestia Spp*. The obtained results indicate that, mortality percentages were positively affected by both $CO_2$ concentration and the exposure period. For instance, using 20% concentration for 1 day of exposure produced 26.6% mortality increased gradually until it reached 61.4% by using 50% concentration at the same exposure period. The mortality percentages averaged across the exposure periods were 47.1, 50.7, 57.9 and 78.2% for 20, 30, 40 and 50% $CO_2$ concentration, respectively. Exposure for one day to 30% $CO_2$ concentration gave 36.4% mortality increased gradually until it reached 81.9% for 7 days exposure period by using the same concentration. The mortality percentages averaged across the $CO_2$ concentrations were 42.4, 49.2, 54.1, 57.5, 59.8, 66.8 and 82.0% for the 7 exposure periods, respectively. The mortality percentages significantly affected by $CO_2$ concentration at the same exposure period except at the long periods. Also, the differences were significant between exposure periods at the same concentration except 50% concentration for more than 4 days exposure period.

**Table (5): Mortality percentage of warehouse moths, *Ephestia Spp.* pupae exposed to different $CO_2$ concentrations for different exposure periods.**

| $CO_2$ % | Corrected mortality percentage after indicated periods (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
| 20 | 26.6 | 36.3 | 42.2 | 44.2 | 49.1 | 58.9 | 72.6 | 47.1 |
| 30 | 36.4 | 40.9 | 42.3 | 45.2 | 48.7 | 59.3 | 81.9 | 50.7 |
| 40 | 45.1 | 48.2 | 55.5 | 55.5 | 56.2 | 61.6 | 83.2 | 57.9 |
| 50 | 61.4 | 71.4 | 76.2 | 85.1 | 85.1 | 87.2 | 90.3 | 78.2 |
| Average | 42.4 | 49.2 | 54.1 | 57.5 | 59.8 | 66.8 | 82.0 | 58.5 |

**L.S.D. $_{0.05\%}$ level = 5.19**

The probit analysis (Table, 6) show that, at $LT_{50}$ level, the values were 72.2, 56.6, 35.3 and 17.9 hr for 20, 30, 40 and 50% $CO_2$ concentration, respectively. The respective $LT_{95}$ values were 412.2, 339.3, 283.0 and 55.8 hr, respectively.

The results are in agreement with, El-Lakwah *et al.* (1991) who reported that, modified atmospheres containing 20, 50 or 78% $CO_2$ had negligible effects on the larval and pupal mortalities of *S. cerealella* at short exposures. Elsinary (1995) stated that, the highest average mortality for 3-days-old pupae of *Ph. operculella* (95.6%) when treated with a gas mixture

containing 75% $CO_2$. Ahmed (2007) found that, when 3-days-old- pupae of *S. cerealella* were exposed to modified atmospheres the reduction percent in adult emergence increased gradually with increasing $CO_2$ concentration or exposure period and reached 100% after 216 hr at 75% $CO_2$ concentration.

**Table (6): $LT_{50}$ and $LT_{95}$ values of warehouse moths, *Ephestia Spp.* pupae exposed to different $CO_2$ concentrations for different exposure periods.**

| $CO_2$ % | $LT_{50}$ (hr) | $LT_{95}$ (hr) | Confidence limits at 95% | | | |
|---|---|---|---|---|---|---|
| | | | $LT_{50}$ | | $LT_{95}$ | |
| | | | Lower | Upper | Lower | Upper |
| 20 | 72.2 | 412.2 | 67.6 | 77.1 | 340.1 | 499.6 |
| 30 | 56.6 | 339.3 | 52.6 | 60.9 | 282.8 | 407.1 |
| 40 | 35.3 | 283.0 | 31.2 | 39.8 | 232.8 | 344.1 |
| 50 | 17.9 | 55.8 | 15.6 | 20.5 | 51.8 | 60.2 |
| Average | 45.5 | 272.6 | 41.7 | 49.6 | 226.9 | 327.7 |

Therefore, it could be concluded that, treatment of stored dry date fruits with carbon dioxide is an effective and safe measure control against the insect infestations. Also, storage of this food product under modified atmospheres containing high concentrations of carbon dioxide prevent any pest infestations. In this respect, Al- Redhaiman (2002) found that, modified atmospheres containing 10 and 20% $CO_2$ extended storage period of date fruits to 119 and 182 days, respectively compared to control (49 days) with keeping the quality .

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## تـأثير ثـانى أكسـيد الكربـون علـى بعض الحشـرات التـى تصـيب ثمـار الـبلح الجـاف المخزون

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تم اختبار تأثير المعاملة بأربع تركيزات من ثانى أكسيد الكربون (٢٠، ٣٠ ، ٤٠ ،٥٠%) على بعض الحشرات التى تصيب ثمار البلح الجاف أثناء التخزين ، وهى خنفساء السورينام (طور كامل) ويرقات (عمر رابع) وعذارى ديدان البلح من جنس *Ephestia*.

أظهرت النتائج أن نسبة الموت زادت طردياً بزيادة تركيز غاز ثانى أكسيد الكربون أو بزيادة فترة التعريض . كانت خنفساء السورينام أكثر حساسية لفعل الغاز من يرقات وعذارى ديدان البلح حيث كانت نسبة الموت أعلى فى خنفساء السورينام فى جميع المعاملات عنها فى ديدان البلح . أدت المعاملة بتركيز ٢٠% من ثانى أكسيد الكربون (أقل تركيز) لمدة ٧ أيام تعريض الى نسبة موت ٨٤,٢% فى خنفساء السورينام بينما حققت نفس المعاملة ٧٩,٨% فى اليرقات و ٧٢,٦% فى عذارى ديدان البلح . أما عند المعاملة بأعلى تركيز من الغاز (٥٠%) لمدة سبعة أيام كانت نسبة الموت ٩٨,٨% فى خنفساء السورينام و ٩٣,٣% فى اليرقات و٩٠,٣% فى عذارى ديدان البلح.