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Research Article
Carbon Dioxide-Enriched Atmosphere to Control *Oryzaephilus surinamensis* L. On Stored Saqie Date Fruits

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Abstract

Background and Objective: Control of the stored date insects by an eco-friendly method is essential to maintain the fruit for prolonged periods, especially that dates are eaten fresh. The search for a safe method, as an alternative to commonly used chemical pesticides, is needed. The objective is to investigate CO_2 enriched atmosphere on the Mortality Percentage (MP) of the Saw-toothed grain beetle, *O. surinamensis*, life stages with special emphasis on reducing damage to stored dates. **Materials and Methods:** The effect of high levels of CO_2 as an alternative control method against the Saw-toothed grain beetle, *Oryzaephilus surinamensis* L., at different life stages was studied on infested 'Saqie' dates. Four CO_2 pressures (25, 50, 75 and 90 kPa, balance is nitrogen) were tested for 6, 12, 18, 24, 48, 72 and 96 hrs intervals. The response of different life stages of *O. surinamensis* to the different treatments varied according to CO_2 level, developmental stage and exposure period. **Results:** Mortality (%) was higher during the larval stage, followed by adults, pupae and eggs, in descending order. The larvae and adult stages were more sensitive to CO_2 treatment than the pupal and egg. Exposure time was more effective on eggs, larval and adult MP than the CO_2 atmosphere level. Mortality% at 96 hrs exposure time was almost 100% with CO_2 atmospheres of 50, 75 and 90 kPa. Mathematical equations were developed to model the relationship between mortality% and CO_2 treatments using multiple regression analyses for each life stage. **Conclusion:** The results confirmed that CO_2 could be applied to final food products during packaging to control the residual occurrence of insect pests after storage and before the packaging process to prevent further infestation in the final packages.

Key words: Modified atmosphere, saw-toothed grain beetle, *Oryzaephilus surinamensis*, mortality percentage, date-palm fruits, CO_2, storage insect pests, eco-friendly control

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Date palm cultivation has expanded very rapidly in the world, especially in the Middle East and North Africa including the Kingdom of Saudi Arabia (KSA) during the last two decades. KSA is the third-largest date producer in the world, with 1.122 million tons in 2016, which represents around 14% of the total world production[1]. By 2030, date production in KSA is expected to exceed two million tons. The future strategy for the date palm producers is to export dates to foreign markets, which would require effective technologies to maintain high-quality fruit, free from insect damage during storage and handling phases to meet the international standards[2]. The use of elevated CO_2 atmospheres during storage, transport and packages for preserving fruit quality and delaying fruit deterioration has been widely reported and used[2-4]. Elevated CO_2 atmospheres inhibit decay, disease infections and insect infestations, as well as retard fruit softening[5]. Modified atmospheres (usually with elevated CO_2 and reduced O_2 atmospheres) have been commercially used for preserving diverse food commodities, including disinfecting raw and semi-processed food products such as stored cereal grains and dried fruits[4]. There are increasing restrictions on the use of chemical pesticides and therefore, modified atmosphere application is a potentially appropriate safe and secure alternative to some applications of chemical pesticides such as methyl bromide[5,6]. Pest control treatments should maintain a high degree of efficacy[5]. Treatments based on reduced oxygen (O_2) and high carbon dioxide (CO_2) contents are technically suitable alternatives for arthropod pest control in durable commodities[5,7]. Atmospheres rich in CO_2, especially more than 40 kPa in air, are more effective at controlling pests than those with low levels of O_2 (high contents of N_2)[8]. Data on the effects of different CO_2 treatments and dosages on key insect pests are available for several species and stages of stored-product pests under specific conditions[9, 10]. Depending on the temperature, CO_2 treatments may take from a few hrs to several weeks to be effective in controlling different insect species and stages[11,12]. The toxicity of CO_2 to insects is known to vary among species and developmental stages. Parameters of the physical environment, such as CO_2 levels in storage, also influence the toxicity. In the majority of studies involving CO_2 atmosphere manipulation, attention has been focused on determining the time required to kill the insect pests[13,14].

The use of modified atmospheres with depleted O_2 and/or elevated CO_2 is an environmentally friendly alternative for the control of stored grain insect pests[15]. The modified atmospheres techniques with high levels of CO_2 (hypercapnia) in airtight storage by withholding the O_2 required for insect pest development and respiration, prevent food damage by insect pests in stored products[8]. Hermetic storage is the change of the composition of the storage atmosphere by commodity respiration, replacing O_2 with CO_2, thereby producing low oxygen, high carbon dioxide environment. Such storage is a cost-effective and environmentally friendly alternative measure to chemical fumigation[13].

Over the last few decades, considerable research has been conducted on the control effectiveness of modified atmospheres using different gas compositions for various stored pests, especially well-known cosmopolitan insect pests such as the Saw-toothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae)[4,11]. This beetle is an important and widespread pest of different food commodities. It is usually found as a secondary pest on grain damaged by other insect pests, attacking previously damaged storage products including dates[7,16].

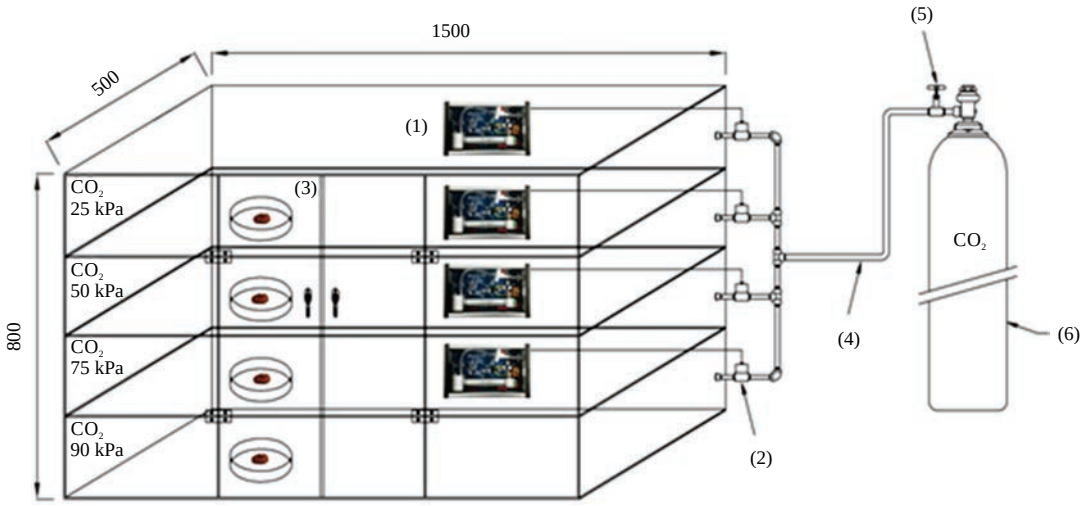
The objective of this study was to investigate the effect of CO_2 enriched atmosphere on the Mortality Percentage (MP) of different life stages of the Saw-toothed grain beetle, *O. surinamensis* L., with special emphasis on reducing damage to stored dates.

MATERIALS AND METHODS

Study area: This research was carried out at the Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia, from October, 2018-September, 2019.

Plant materials: Date palm fruit (*Phoenix dactylifera*, cultivar Saqie) were harvested from palms grown at the experimental research station, College of Agriculture and Veterinary Medicine (CAVM), Qassim University, Saudi Arabia. All palm trees were approximately the same age and uniform in size and shape. The sampled palms were in good physical condition, free from insect pest damage and diseases and were subjected to the same management operations.

Fruit samples were harvested in mid-August. Date fruit was harvested according to skin colour using Hunter Lab instrument (CIELAB) (L*: 25.1, a*: 6.5 and b*: 5.6) Reston, Virginia and soluble solids contents (30%) using compact METTLER TOLEDO EasyPlus (Easy R 40- ,FastStart™ technology) digital Refractometer (METTLER TOLEDO Switzerland)[17,18]. Immediately after harvest, the freshly collected fruits were transported to the laboratory (less than 2 km). Fruit similar in shape, colour and degree of development were divided into four groups.



Dimensions (mm)	
1. GASCARD NG CO_2 sensor	2. Electronic valve
3. Samples	4. Connecting hose
5. Main valve	6. CO_2 source

Fig. 1: Exposure set for testing the effect of elevated CO_2 atmospheres on the mortality of different stages of *Oryzaephilus surinamensis*

***Oryzaephilus surinamensis* colonies:** The Saw-toothed grain beetle, O. *surinamensis*, life stages were reared and maintained on 'Saqie' date fruits at the Entomology Laboratory, Department of Plant Production and Protection, CAVM, Qassim University, Saudi Arabia and were used for all experiments. Adults of *O. surinamensis* were collected from storage bins of date fruit that were previously infested and then reared on Al-Saqie dates in 2000 mL wide-mouth glass bottles at 21±2°C, 32±2% RH and a photoperiod of 12:12 hrs (L:D). Newly emerged adults (2 days old) were introduced in glass boxes (Fig. 1) with approximately 2 kg of 'Saqie' dates for fresh infestation. Adults were allowed to mate and lay eggs on newly prepared dates to obtain an age-synchronized culture. Date fruits were changed daily by removing the exposed fruit and introducing new ones. Although eggs on dates were easily discernible under the stereo microscope (KOPPACE 3.5X-90X) (KOPPACE Technology "SHENZHEN" CO., LTD.), larvae were hidden inside the dates. A successful egg hatch was indicated by the change of the egg colours. To obtain precise larval developmental information, dates infested as above were broken open daily to trace larval development. This insect has a complete metamorphosis development (Holometabolous), which its' life cycle passes in four different stages (egg, larva, pupae and adult)[3,19]. The larval development consists of series of stages in which each stage is separated from the next by a moult (Ecdysis).

Bioassay studies: To determine the effects of the modified atmosphere treatments on mortality of eggs, larvae, pupae and adult stages, bioassay studies were established for each *O. surinamensis* life stage. Four CO_2 pressures (25, 50, 75 and 90 kPa) and a control treatment using ambient air under standard pressure were applied at seven exposure times (6, 12, 18, 24, 48, 72 and 96 hrs) to the four life stages. All exposures were at laboratory conditions (constant temperature 21±2°C, 32±2% RH). Eggs of early (4-6 hrs old), 2nd instar larvae, three-day-old pupae and adults were separately collected. Each stage was tested under each pressure and duration treatment in a sealed 1 L glass box equipped with a ventilation tube (Fig. 1). Specifically, 50 eggs/container on 'Saqie' dates, as well as 50 larvae, pupae and adults, each, were separately kept in the experimental chamber. Each CO_2 atmospheric pressure was replicated five times for each *O. surinamensis* life stage. Besides, the same numbers of each life stage were tested under ambient air (0 kPa CO_2, to serve as the control) at the same temperature and relative humidity.

CO_2-exposed and control (exposed to air) insect pests were examined twice a day for counting and recording mortality of each stage. For eggs, those whose eggshells remained transparent or appeared shrivelled or wrinkled were considered dead, whereas those turned cream white were considered alive[7]. For larvae and pupae, individuals that failed to emerge were considered dead. Adults were considered dead if they were immobile after being stimulated by turning

the container several times. Each CO_2 treatment and the control were replicated five times and mortality was calculated for each developmental stage. The value of mortality percentage (MP) was corrected using an Abbott equation[20], as shown in the statistical analysis part.

CO_2 exposure apparatu: An exposure apparatus was designed to contain the experimental chambers. The designed unit (Fig. 1) was an airtight glass box with dimensions of 1500×500×800 mm. This unit was connected to a source of carbon dioxide gas or air. The experimental apparatus was divided into four equal-sized sections, dimensions 1500x500x200 mm for each. Each of the four sections contained date samples infested with different life stages of *O. surinamensis*. GASCARD NG CO_2 sensor (NG model, Edinburgh Instruments Ltd, England) connected to an electronic valve controlled the CO_2 pressure for each section.

The CO_2 atmospheres used were achieved by injecting CO_2 gas at different pressures (kPa) into the chambers. Required pressures were controlled through sensors inside the sealed chambers. The oxygen levels inside the chambers were measured using a GAP-100 gas analyzer probe (manufactured by CO_2 meters). The four levels of O_2 pressures corresponding to the four CO_2 pressures were 15.75, 10.50, 5.25 and 2.00 kPa for 25, 50, 75 and 90 kPa, respectively.

Predicting the exposure time to achieve the highest mortality percentage: To predict the exposure time to achieve the highest mortality percentage, the following steps were followed:

- **Derive the statistical relationships between the exposure times and mortality percentage:** Regression analysis of the resulting relationships between exposure times and mortality percentage at different life stages was studied
- **Solving the regression relationships in step (a):** Solving the regression relationships in step (a) to predict the exposure time (Te)

Statistical analysis: Statistical analysis of all data was performed using MSTACT software as a Randomized Complete Block Design (RCBD). Treatment means, Standard Deviations (SDs) and significant differences were analyzed. Means were statistically analyzed and compared according to the Least Significant Difference (LSD) at 5%. *In vitro,* to achieve the correct mortality percentage in a biological effectiveness test, the number of living individuals is compared before CO_2 application, against the number of living insects after CO_2 application, in proper time interval according to the CO_2 pressure. It is also necessary to have a check treatment (control) in which no CO_2 was applied, the mortality correction was performed according to Abbott equation 1[20]:

$$CM = \frac{Nc - Nt}{Nt} \times 100 \quad (1)$$

Where:
CM = Corrected mortality percentage (%)
Nc = Live individuals in the control after the treatment
Nt = Live individuals in the treatment after the treatment

The Average General Mortality Percentage (AGMR) is the average of mortality values of the test during application periods of a single gas concentration, except the mortality value of the 1st time of treatment. The AGMR was calculated from Eq. 2[21] as follows:

$$AGMR = \frac{Stm}{Net + Ikt} \quad (2)$$

Where:
AGMR = Average of the general mortality (%)
Stm = Sum of the total corrected mortality of each exposure time (%)
Net = Numbers of exposure times
Ikt = Initial kill time

Mathematical equations were developed to model the relationships between mortality percentage and CO_2 treatments using multiple regression analyses for each life stage. The optimum exposure time was determined by differentiating the resulting equations.

RESULTS

***Oryzaephilus surinamensis* eggs:** Both CO_2 pressure and exposure time had a significant effect on the MP of *O. surinamensis* eggs (Table 1). After 6 hrs, MP progressively increased as the CO_2 pressure increased (Table 1). Generally, this trend was observed in all exposure times, where after 12 hrs the MP increased from 5.2-36.4% as the CO_2 pressure increased from 25-90 kPa. After 18 hrs, the MP increased from 5.4-42.0% as the CO_2 pressure increased from 25-90 kPa. After 24 hrs treatment, the MP increased from 6.8-50.6% as the CO_2 pressure increased from 25-90 kPa. After 48 hrs, the MP increased from 7.6-58.6% as the CO_2 pressure increased from

25-90 kPa. The same trend continued for 72 and 96 hrs exposure times (Table 1). The exposure time was more effective on the egg MP than the CO_2 pressure. The interactive effects of both CO_2 pressure and exposure time were quite clear on the mortality percentage of *O. surinamensis* eggs.

The following formula shows the mathematical prediction model for egg mortality percentage if the same conditions were present. The multiple regression analysis resulted in a polynomial Eq. 3 as follows:

$$MP = 0.653\ CO_2 + 0.248\ Te - 20.857 \quad R^2 = 0.895 \tag{3}$$

Where:
MP = Mortality percentage (%)
CO_2 = CO_2 pressure (kPa)
Te = Exposure time (hr)

This polynomial regression could be used to predict the CO_2 pressure and exposure time needed to achieve a certain desired mortality percentage.

***Oryzaephilus surinamensis* 2nd instar larvae:** After 6 hrs of treatment, larval MP were 20.6, 35.00, 43.4, and 48.0% at CO_2 pressures of 25, 50, 75, and 90 kPa, respectively, while the control (at normal ambient air) MP was 3.2% (Table 2). The MP increased as the CO_2 pressure increased, and this incremental trend was constant for all exposure times. After 12 hrs of exposure, MP increased from 38-74.8% when the CO_2 pressure increased from 25-90 kPa, respectively. After 18 hrs, MP increased from 55.0-78.6% as the CO_2 pressure increased from 25-90 kPa. Twenty-four hrs after treatment, MP increased from 61.2-84.2% as the CO_2 pressure increased from 25-90 kPa, respectively.

After 48 hrs of treatment, MP increased from 84.4-95.6% as the CO_2 pressure increased from 25-90 kPa, respectively. The highest (100%) MP was achieved at CO_2 pressures of 50 kPa or higher for 96 hrs, or at CO_2 pressures of 90 kPa for 72 hrs or higher (Table 2). The duration of exposure time was more effective on larval stage MP than the increase in CO_2 pressures.

The following formula shows the mathematical prediction model for MP of the 2nd instar larvae if the same conditions were present. The multiple regression equation for the larvae MP was as follow (Eq. 4):

$$MP = 0.285\ CO_2 + 0.595\ Te - 34.872 \quad R^2 = 0.772 \tag{4}$$

Where:
MP = Mortality percentage (%)
CO_2 = CO_2 pressure (kPa)
Te = Exposure time (hr)

This polynomial regression could be used to predict the CO_2 pressure and exposure time needed to achieve a certain desired mortality percentage.

***Oryzaephilus surinamensis* thee-day-old pupae:** The three-day-old pupae MP (Table 3) showed the same trend as the egg MP. After 6 hrs of treatment, pupal MP were 8.4, 11.40, 14.6 and 26.6% at CO_2 pressures of 25, 50, 75 and 90 kPa, respectively, while the control (at normal ambient air) MP was 4.4%. Mortality increased as the CO_2 pressures increased. Generally, this incremental trend was consistent for all exposure times. After 12 hrs of exposure, MP increased from 12.8-36% when the CO_2 pressures increased from 25-90 kPa, respectively. After 18 hrs of exposure, MP increased from 24.8-50.8% as the CO_2 pressures increased from 25-90 kPa, respectively. After 24 hrs of treatment increased MP from 27.2-51.6% as CO_2 pressures increased from 25-90 kPa, respectively. After 48 hrs of exposure, MP increased from 52.8-66% as the CO_2 pressures increased from 25-90 kPa, respectively. The same trend continued for 72 and 96 hrs exposure times and 100% MP was achieved at 90 kPa CO_2 for 96 hrs (Table 3). The exposure time was more effective on pupae mortality than the CO_2 pressure. The combined effects of both CO_2 pressure and exposure time were significantly important in causing pupal mortality.

The following formula shows the mathematical prediction model for mortality percentage in pupae if the same conditions were present. The multiple regression analysis gave a polynomial Eq. 5 as follows:

$$MP = 0.299\ CO_2 + 0.719\ Te - 0.210 \quad R^2 = 0.943 \tag{5}$$

Where:
MP = Mortality percentage (%)
CO_2 = CO_2 pressure (kPa)
Te = Exposure time (hr)

This polynomial regression could be used to predict the CO_2 pressure and the exposure time needed to achieve a certain desired mortality percentage.

Adult stage of *Oryzaephilus surinamensis*: Values of adult MP, at 6 hrs of CO_2 exposure, were 17.2, 33.6, 41.6 and 42.0% at CO_2 pressures of 25, 50, 75 and 90 kPa, respectively, while control (at ambient room temperature) adult mortality percentage was only 3.2% (Table 4).

Adult MP increased as the CO_2 pressures increased and this incremental trend was constant for all exposure times. Adult MP increased from 38.4-72% for exposures for 12 hrs as the CO_2 pressure increased from 25-90 kPa, respectively. After

Table 1: Eggs mortality percentage (MP) of *Oryzaaephilus surinamensis* at different CO_2 pressures and exposure times

CO_2 pressures	Exposure time (hr)								Residual effect	Total activities
(kPa)	6	12	18	24	48	72	96	LSD[3]	(%)	(AGMR)[4] (%)
25	3.6±0.40	5.2±0.37	5.4±0.93	6.8±0.58	7.6±0.51	12.00±0.55	14.80±1.02	2.08**	7.91±0.68	6.93±1.53
50	13.40±0.81	15.40±0.51	17.40±0.68	19.40±0.51	18.60±0.93	21.40±0.75	22.60±0.93	2.13**	18.31±0.57	16.03±1.22
75	22.80±0.58	23.00±5.29	24.20±0.66	35.20±0.86	40.00±0.71	45.00±0.77	53.40±1.03	6.51**	34.80±2.05	30.45±4.56
90	32.20±0.97	36.40±1.12	42.00±1.05	50.60±1.50	58.60±1.33	64.00±0.71	74.80±0.86	3.26**	51.23±2.49	44.83±5.86
Control	2.40±0.40	2.40±0.40	2.40±0.40	2.40±0.40	2.40±0.40	2.40±0.40	2.40±0.40	N.S	2.40±0.14	2.10±0.00
Average	14.88±2.34	16.72±2.57	18.04±3.06	22.88±3.68	25.44±4.30	28.96±4.60	33.60±5.44	-	22.93±1.51	20.07±2.60
LSD[1]	2.07**	1.67**	7.42**	2.71**	2.61**	2.05**	2.00**	1.48**	1.26**	-
LSD[2]	3.32**									

[1]LSD (within CO_2 pressure) at 0.05 level, [2]LSD (between CO_2 pressures and exposure times) at 0.05 level, [3]LSD (within exposure times) at 0.05 level, [4]AGMR: Average of the general mortality,**Highly significant

Table 2: Second instar larvae mortality percentage of *Oryzaaephilus surinamensis* under different CO_2 pressures and exposure times

CO_2 pressures	Exposure time (hr)								Residual effect	Total activities
(kPa)	6	12	18	24	48	72	96	LSD[3]	(%)	(AGMR)[4] (%)
25	20.60±0.68	38.00±0.71	55.00±0.71	61.20±1.02	84.40±1.83	92.00±2.12	98.20±0.80	3.79**	64.20±4.61	56.18±10.93
50	35.00±0.63	55.80±1.24	70.20±0.66	70.80±0.86	91.60±0.98	98.00±0.89	100.00±0.00	2.16**	74.49±3.81	65.18±9.05
75	43.40±0.75	69.80±0.66	75.20±0.86	75.60±0.81	96.40±0.98	99.00±0.77	100.00±0.00	2.19**	79.91±3.26	69.93±7.74
90	48.00±0.71	74.80±0.58	78.60±0.60	84.20±0.37	95.60±1.03	100.00±0.00	100.00±0.00	1.82**	83.03±2.94	72.65±6.99
Control	3.20±0.80	3.20±0.80	3.20±0.80	3.20±0.80	3.20±0.80	3.20±0.80	3.20±0.80	N.S	3.20±0.27	2.80±0.00
Average	30.04±3.35	48.32±5.30	56.44±5.69	59.00±5.90	74.24±7.32	78.44±7.71	80.28±7.87	-	60.97±2.68	53.35±6.89
LSD[1]	1.88**	2.60**	2.19**	2.56**	3.75**	3.22**	1.35**	1.06**	0.90**	-
LSD[2]	2.37**									

[1]LSD (within CO_2 pressure) at 0.05 level, [2]LSD (between CO_2 pressures and exposure times) at 0.05 level, [3]LSD (within exposure times) at 0.05 level, [4]AGMR = Average of the general mortality,**Highly significant

Table 3: Thee-day-old pupae mortality percentage of *Oryzaaephilus surinamensis* at different CO_2 pressures and exposure times

CO_2 pressures	Exposure time (hr)								Residual effect	Total activities
(kPa)	6	12	18	24	48	72	96	LSD[3]	(%)	(AGMR)[4] (%)
25	8.40±0.51	12.80±0.49	24.80±1.02	27.20±1.50	52.80±1.02	57.20±2.33	78.00±2.10	3.67**	37.31±4.12	32.65±9.74
50	11.40±0.75	17.60±0.89	33.20±1.02	35.60±0.75	57.20±2.06	61.20±1.36	78.40±1.60	3.70**	42.09±3.90	36.83±9.23
75	14.60±0.68	28.40±0.75	40.40±0.75	40.40±0.40	60.40±0.75	68.80±0.49	90.80±3.77	4.71**	49.11±4.15	42.98±9.79
90	26.60±1.08	36.00±0.63	50.80±1.20	51.60±0.75	66.00±1.41	70.80±1.02	100.0±0.00	2.95**	57.40±3.88	50.23±9.20
Control	4.40±0.75	4.40±0.75	4.40±0.75	4.40±0.75	4.40±0.75	4.40±0.75	4.40±0.75	N.S	4.40±0.26	3.85±0.00
Average	13.08±1.57	19.84±2.31	30.88±3.26	31.68±3.22	48.16±4.58	52.48±5.04	70.32±6.99	-	38.06±2.10	33.31±7.56
LSD[1]	2.38**	2.45**	2.73**	2.80**	3.71**	4.32**	5.65**	1.56**	1.32**	-
LSD[2]	3.48**									

[1]LSD (within CO_2 pressure) at 0.05 level, [2]LSD (between CO_2 pressures and exposure times) at 0.05 level, [3]LSD (within exposure times) at 0.05 level, [4]AGMR: Average of the general mortality,**Highly significant

Table 4: Adult mortality percentage of *Oryzaaephilus surinamensis* at different CO_2 pressures and exposure times

CO_2 pressures	Exposure time (hr)								Residual effect	Total activities
(kPa)	6	12	18	24	48	72	96	LSD[3]	(%)	(AGMR)[4] (%)
25	17.20±1.02	38.40±0.75	53.20±1.02	61.20±1.02	84.40±1.83	91.20±2.33	98.00±0.89	4.22**	63.37±4.73	55.45±11.21
50	33.60±1.17	53.60±1.83	65.20±1.36	68.80±1.36	90.40±0.75	96.80±1.02	100.0±0.00	3.48**	72.63±3.91	63.55±9.26
75	41.60±0.75	68.40±0.40	73.60±1.94	74.00±1.10	95.60±1.33	97.20±1.20	100.0±0.00	3.32**	78.63±3.34	68.80±7.89
90	42.00±0.89	72.00±0.63	77.60±0.75	81.60±0.75	86.00±6.03	100.00±0.00	100.0±0.00	6.99**	79.89±3.25	69.90±7.48
Control	3.20±0.80	3.20±0.80	3.20±0.80	3.20±0.80	3.20±0.80	3.20±0.80	3.20±0.80	N.S	3.20±0.27	2.80±0.00
Average	27.52±3.11	47.12±5.11	55.28±5.60	57.04±5.70	71.92±7.16	77.68±7.64	80.24±7.87	-	59.54±2.66	52.10±7.10
LSD[1]	2.96**	3.33**	4.12**	3.24**	8.96**	3.54**	1.43**	1.84**	1.55**	-
LSD[2]	4.11**									

[1]LSD (within CO_2 pressure) at 0.05 level, [2]LSD (between CO_2 pressures and exposure times) at 0.05 level, [3]LSD (within exposure times) at 0.05 level, [4]AGMR: Average of the general mortality,**Highly significant

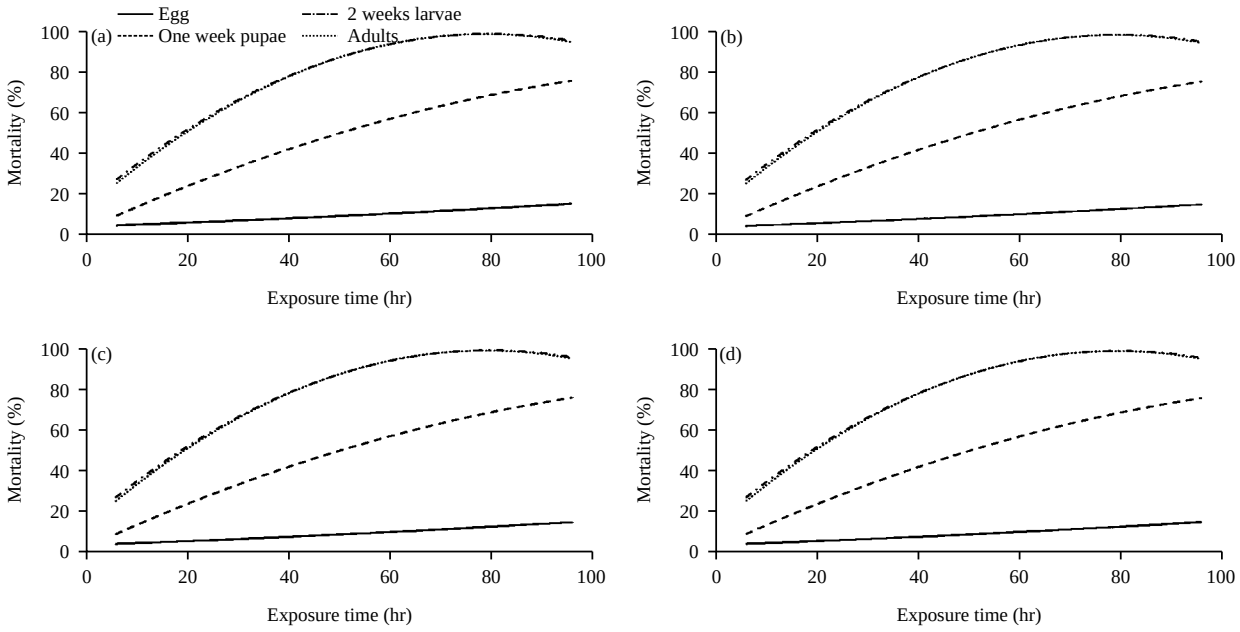


Fig. 2(a-d): Effect of exposure time on the mortality percentage under several CO_2 partial pressures on four life stages of *O. surinamensis*
(a) 25 kPa CO_2, (b) 50 kPa CO_2, (c) 75 kPa CO_2 and (d) 90 kPa CO_2

18 hrs of treatment, adult MP increased from 53.2-77.6% as the CO_2 pressures increased from 25-90 kPa, respectively. After 24 hrs of treatment, MP increased from 61.2-81.6% as the CO_2 pressures increased from 25-90 kPa, respectively. After 48 hrs, adult MP increased from 84.4-86% as the CO_2 pressures increased from 25-90 kPa, respectively. The 72 and 96 hrs exposure times had the highest MP for almost all the CO_2 pressures tested and 100% MP was achieved with exposure to 50 kPa CO_2 or higher for 96 hrs (Table 4). The exposure time was also more effective on adult mortality than the CO_2 pressure.

The following formula shows the mathematical prediction model for mortality percentage in adults if the same conditions prevailed. Multiple regression analysis gave a polynomial Eq. 6 as follows:

$$MP = 0.258\ CO_2 + 0.618\ Te - 33.783 \quad R^2 = 0.777 \qquad (6)$$

Where:
MP = Mortality percentage (%)
CO_2 = CO_2 pressure (kPa)
Te = Exposure time (hr)

This polynomial regression could be used to predict the CO_2 pressure and exposure time needed to achieve a certain desired mortality percentage.

Effect of the CO_2 treatments on the different stages of *O. surinamensis*: The result of Fig. 2(a-d) summarizes the effects of CO_2 pressures and the exposure times on the different developmental stages of *O. surinamensis*. The 2nd instar larvae and adult stages were more affected by the CO_2 treatments than the pupal and the egg stages. It is also clear that higher CO_2 pressures increased the MP for all the life stages tested. Eggs were the least affected stage by the treatments. The treatment effects on pupae started at nearly the same level as the eggs at the minimum exposure time, although pupal MP increased faster than the MP of the egg stage for all the tested CO_2 pressures (Fig. 2). Both the 2nd instar larvae and the adult stages had similar trends and almost the same slope in mortality percentage (Fig. 2).

Predicting the exposure time to achieve the highest mortality percentage: To predict the exposure time to achieve the highest mortality percentage, the following steps were followed:

- **Derive the statistical relationships between the exposure times and mortality percentages:** Regression analysis of the resulting relationships between exposure times and mortality percentages were: Regression

Table 5: Regression values of mortality percentage of different life stages of *O. surinamensis* and CO_2 pressures

O. surinamensis life stages	CO_2 pressure (kPa)	Statistical relationship	R^{2*}
Eggs	25	MP = 0.0003 Te^2+0.0873 Te+3.7082	0.975
	50	MP = -0.0009 Te^2+0.1758 Te+13.672	0.859
	75	MP = -0.0017 Te^2+0.5133 Te+18.867	0.948
	90	MP = -0.0032 Te^2+0.7616 Te+29.288	0.969
2nd instar Larvae	25	MP = -0.0131 Te^2+2.0908 Te+14.981	0.972
	50	MP = -0.0122 Te^2+1.8466 Te+32.679	0.95
	75	MP = -0.0112 Te^2+1.6381 Te+43.871	0.913
	90	MP = -0.0107 Te^2+1.5177 Te+50.404	0.879
3-day-old pupae	25	MP = -0.004 Te^2+1.142 Te+2.377	0.976
	50	MP = -0.0059 Te^2+1.2819 Te+6.488	0.967
	75	MP = -0.0036 Te^2+1.1002 Te+14.784	0.962
	90	MP = -0.001 Te^2+0.7936 Te+28.726	0.928
Adults	25	MP = -0.0137 Te^2+2.1687 Te+12.681	0.966
	50	MP = -0.0118 Te^2+1.8374 Te+30.229	0.966
	75	MP = -0.011 Te^2+1.6295 Te+42.366	0.91
	90	MP = -0.0091 Te^2+1.3971 Te+48.001	0.82

*R^2: Coefficient of determination, Te: Exposure time, MP: Mortality percentage

Table 6: Predicted exposure time and mortality percentage for different stages of *O. surinamensis* under different CO_2 pressures

O. surinamensis stages	CO_2 pressure (kPa)	Exposure time (hr)	Morality (%)
Eggs	25	439.5	100
	50	96.5	22.6
	75	149.75	57.6
	90	117.75	74
2nd instar larvae	25	79.75	98.41
	50	61.25	100
	75	55.00	100
	90	51.25	100
3-day-old pupae	25	137.75	80.4
	50	107.5	76.11
	75	151.75	98.84
	90	103.25	100
Adults	25	79	98.51
	50	65.75	100
	75	58.5	100
	90	63.5	100

relationships between the exposure time (Te) and mortality percentage (MP) for *O. surinamensis* life stages at four CO_2 pressures were derived (Table 5)

- **Solving the equations in Table 5:** Solving the equations resulting from Table 5 to determine the exposure time that achieves the highest mortality percentages taking into account the physical limitations of the predicted equations (Table 6)

DISCUSSION

The mortality (%) of *O. surinamensis* was higher during the larval stage, followed by adults, pupae and eggs, in descending order. The larvae and adult stages of *O. surinamensis* were more sensitive to CO_2 treatment than the pupal and egg. Moreover, the exposure time was more effective on the egg, larval and adult MP than the CO_2 atmosphere level. Despite Modified Atmosphere (MA) have been used for more than 30 years in pest control and maybe playing an effective role in storage pest management, its mechanisms in controlling insects and its effects (adaptation to low O_2 (hypoxia) and high carbon CO_2 (hypercapnia)) still not completely understood[22,23]. Modified atmospheres, especially atmospheres rich in CO_2, are considered an economic-effective method to eradicate the target insect pests and also protect our stored food products[23]. High carbon dioxide (CO_2) content offers an alternative to classical control measures for date palm storage and insect pests control. As it is known that the date fruits are eaten directly and accordingly, the classical control strategies like pesticides affect the characteristics of the fruit, leaving harmful residual substances to humans and it makes the fruits unacceptable commercially.

Therefore, the present study aimed to establish the efficacy of using CO_2 as a Modified Atmosphere during Packaging (MAP) to control the life stages of the Saw-toothed

grain beetle, *O. surinamensis* that affect date fruits during storage and commercialization. This research shows the effect of four pressures of CO_2 during storage of date fruit to prevent damage by *O. surinamensis*. Under normal respiration conditions, the respiratory system of the insect takes O_2 faster than it diffuses CO_2 out and the accumulations of CO_2 under high pressures (MAs) become more harmful for the insect tissues[22,24]. The increase in CO_2 results in an increase of carbonic acid, which modifies the pH[22,24]. This was discussed in the study of Neven and Hansen[25] on the effects of CA on ATP in codling moth at different temperatures. This study clearly showed significant effects on the mortality of the different life stages of *O. surinamensis* in response to CO_2 pressures and exposure times. High CO_2 levels are thought to inhibit respiratory enzymes in insects, especially at pressures higher than 20 kPa[22]. The effects of CO_2 pressures and exposure times varied significantly according to the different life stages of *O. surinamensis* (eggs, larvae, pupae and adults). For each *O. surinamensis* developmental stage, the higher the CO_2 pressure used, the higher mortality percentages achieved. The same trend was observed regarding the relationship between CO_2 exposure time and MP among *O. surinamensis* life stages.

When *O. surinamensis* were exposed to 25, 50, 75 and 90 kPa (O_2 pressures 15.75, 10.50, 5.25, respectively), eggs were able to complete development and successfully enter the next developmental stage. The Same trend was recorded with the pupal stage, which obtained a slightly higher MP compared with the eggs (Table 1 and 3). In contrast, the same CO_2 pressures were able to kill larval and adults. Severe CO_2 treatments, i.e., 25, 50, 75 and 90 kPa of CO_2 (O_2 pressures 15.75, 10.50, 5.25, respectively), led to a cessation of development of all stages. Effects on the 2nd instar larvae and adults were most dramatic, sensitive and susceptible to CO_2 treatments they could not withstand 2-3 days' exposure (100% mortality, Table 2 and 4). Further, eggs and pupae at early exposure times (6 hrs) and later stages (96 hrs) were tolerable and least sensitive and could survive compared to other insect life stages (Table 1 and 3). *O. surinamensis* life stages may be having a tolerable level to decrease O_2 level and increase CO_2 (hypoxia and hypercapnia) due to decreased metabolisms, decrease NADPH enzyme activity (nicotinamide adenine dinucleotide phosphate oxidase) and others[26].

This effect could be due to the role of CO_2 in inhibiting the respiration processes in the insect at the different stages of development[24]. Although high CO_2 pressures (50, 75 and 90 kPa) were necessary to reach the highest levels of mortality for the different *O. surinamensis* life stages, the time of exposure was the crucial factor to eliminate the insect pests' different stages. All *O. surinamensis* life stages tested had low levels of MP at 25 kPa CO_2 for 6 hrs, which indicates that neither CO_2 pressure at 25 kPa nor 6 hrs of exposure time was enough to affect the respiration process effectively. On the contrary, treatment with CO_2 at 90 kPa for 92 hrs achieved total mortality percentages of all tested stages of *O. surinamensis*.

Our results indicated that some developmental stages of *O. surinamensis* exhibited lower levels of sensitivity to CO_2 treatment especially at a lower exposure time (6 hrs) at the lower pressures (25 or 50 kPa). This effect was quite clear for eggs, more than in any other stage of development. This effect of CO_2 at the lower exposure time on eggs could be related to the low metabolic rate of the eggs compared with the other stages, which results in lower respiration rate and subsequently lower sensitivity to CO_2.

It is also notable that the higher the metabolic levels of the different life stages of *O. surinamensis,* the higher their mortality when exposed to the same CO_2 pressure and exposure times[3,27,28]. High-CO_2 stress suppresses the production of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and subsequently, glutathione, which is involved in the protection against the toxic effects of the reactive oxygen species[29]. In the bean weevil (*Callosobruchus chinensis* Linnaeus), Cui *et al.*[30] reported that the levels of carbohydrates, amino acids and organic acids increased, whereas those of free fatty acids decreased in response to hypoxia.

High levels of carbon dioxide are low oxygen levels (High 75 and 90 kPa CO_2 pressures meet 5.25 and 2.00 kPa O_2 pressures, respectively). So, under very low atmospheric O_2 partial pressure (ATP production is limited, which results in reduced rates of feeding, digestion, absorption and protein synthesis[10]. In *S. panicum* and *Lasioderma serricorne* F., carboxylesterase activity increased compared to that in the normal condition after exposure to a CO_2-enriched atmosphere[31].

In the summary, the modified atmospheres treatments with medium and higher concentrations (50, 75 and 90 kPa) of carbon dioxide (CO_2) can provide a cost-effective IPM method to eliminate the target insect pests of stored date palm fruits and protect stored products. Complete reduction of the insect was achieved with relatively short periods of CO_2 atmosphere exposure. It was observed that the relationship between the initial CO_2 pressures and time was important to achieve higher percentages of mortality for the larval stage, adult, pupae, respectively. It is therefore important to determine the

candidate developmental stages that could be controlled to select the best CO_2 pressure and most effective time with which to obtain the most effective level of insect mortality.

CONCLUSION

This study indicates that the higher the CO_2 pressure and the longer the exposure time, the higher the mortality (%) of the tested life stages of *O. surinamensis*. Exposing all the tested life stages of *O. surinamensis* to CO_2 at 90 kPa for 96 hrs achieved a 100% mortality. Moreover, a mathematical model for predicting the relationship for MP is presented using the multiple regression analysis as a function of both CO_2 pressures and exposure time for each stage of insect development to guide further investigation into the use of CO_2 enriched atmosphere for the control of *O. surinamensis* and other pests of stored dates. Finally, *O. surinamensis* larvae and adults were easier to kill by CO_2 than eggs and pupae.

SIGNIFICANCE STATEMENT

In this study, we found that MAs provide some highly safe effective non-chemical control procedures for stored-date palm fruit pests. The results confirmed that CO_2 could be applied to storage date fruits or/and during packaging to control the residual occurrence of insect pests during the storage process and before the packaging process to prevent further infestation. The effectiveness of MAs on *O. surinamensis* life stages, that infest date palm fruits, can be reasonably improved through combination with other abiotic stresses, or by using suitable facilities and techniques or other measures.

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