

Comparison between using phosphine and/or carbon dioxide for controlling *Plodia interpunctella* and *Oryzaephilus surinamensis* in stored date fruits

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Received: 15 Sept. 2020 / Accepted 20 Oct. 2020 / Publication date: 30 Oct. 2020

ABSTRACT

This experiment aimed to evaluate the use of CO₂ and phosphine gas, each alone and mixed together, in controlling *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) and *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae) which infesting stored date fruits. The study also aimed to evaluate the effect of using these gases on the chemical properties and quality of the treated date fruits. Three concentrations of CO₂ (50, 75 and 100%) were tested to control the two insects for different exposure times ranged from 1 to 36 hrs. The obtained results showed that the corrected mortality percentage of the two tested insects increased by increasing the exposure time in each of the three CO₂ concentrations, and or increasing of the concentration. The corrected mortality percentages of *P. interpunctella* and *O. surinamensis* reached 100% after exposure to CO₂ (50, 75 & 100%) for exposure periods (36, 24 & 16 hrs.) respectively. For phosphine gas five concentrations were tested (0.073, 0.146, 0.292, 0.585 and 1.170 g.) of magnesium phosphide. The results of phosphine showed that the corrected mortality percentage of the two tested insects increased by increasing the concentration of phosphine, Where the corrected mortality percentages recorded (36.67, 53.33, 66.67, 83.33 and 100%) for *P. interpunctella* larvae, and (23.33, 36.67, 50.00, 66.67 and 100%) for *O. surinamensis* adults when exposed to the mentioned phosphine concentrations. When LC50 of CO₂ was mixed with LC50 of phosphine, the mortality percentages of the two tested insects increased and reached the maximum 100% by using the mixture containing (CO₂ + phosphine) Such level of mortality could be never obtained when CO₂ or phosphine was used each alone at these concentrations. Thus, we reduced the amount of phosphine gas used and increased its efficiency. Data showed clearly that *P. interpunctella* larvae was more susceptible to the two tested gases alone or mixed than the adults of *O. surinamensis*. The results showed that the gases used in the experiment had no effect on the chemical properties of the treated date fruits compared to the control, especially the mixture of gases, except in the case of treatment with carbon dioxide and phosphine gas, both of them separately, which only increased the total phenols in the fruits.

Keywords: date palm fruits, Carbon dioxide, Phosphine, *Plodia interpunctella* and *Oryzaephilus surinamensis*

Introduction

There are in excess of 2,500 palm tree species which can deliver more than 1000 products (Tisserat, 1987). This is the reason of considered as one of the most significant trees everywhere on the world. Palm trees assume an essential part in the horticultural economy of the vast majority of the nation's in the Center East as they are the top biggest date fruits producer on the world (Mallah *et al.*, 2016).

Date palms are attacked by many pests and diseases and their nature and severity vary with cultivar, location, weather and cultural practices (Zaid *et al.*, 2002). Coleoptera and Lepidoptera are main two orders that contain 23 species of insect pests inflicting damage on date fruits during harvest and storage (El-Shafie, 2012). The stored dry and semi-dry fruits of date palm face a critical problem of insects infestation mostly from saw toothed grain beetle, *O. surinamensis* reducing the dates quality as well as quantity and weight loss (Aldryhim and Adam, 1998). The strong chewing mouth parts of *O. surinamensis* allow them to access food which is stored inside boxes. They try to enter inside the material as they are attracted to stored material due to its smell and odor. Therefore, they will make every attempt to find their way inside (Mallah *et al.*, 2016).

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The Indian meal moth, *P. interpunctella*, is a cosmopolitan pest whose larvae can infest a variety of stored grains, nuts, pulses, meals, dates, dried fruits and processed foods. Larvae cause serious losses both in quantity and quality of stored products (Ayvaz *et al.*, 2008).

Currently, control practices rely on scheduled fumigations with methyl bromide or hydrogen phosphide. However, methyl bromide has been classified as an ozone depleter, and its use has recently been banned in many countries (Hansen and Jensen, 2002). Owing to the increasing restrictions on the methods available for the control of pests in stored products, alternative methods require investigating. It is well documented that phosphine resistance in different species may develop independently and that the levels of resistance in different populations and different species may vary (Collins *et al.*, 2003; Opit *et al.*, 2012; Konemann *et al.*, 2017). The current experiment aims to compare the effect of phosphine or carbon dioxide each alone and mixed together in controlling *P. interpunctella* and *O. surinamensis* in stored date fruits and to evaluate the effect of using these gases on the chemical properties and quality of the treated date fruits.

Materials and Methods

Experiments were conducted at date pests and diseases dept. Central Laboratory of Date Palm, Agricultural Research Center, Giza, Egypt.

1. Rearing of test insects Culture:

The two insect species were collected from infested date fruits and were reared on their standard food diets. Insects culture was kept in an incubator at $27\pm 2^{\circ}\text{C}$ and $65\pm 5\%$ relative humidity (RH). The adult insects have reared on semi dry date fruits Siwi cultivar. The date fruits which used in rearing culture were conserved at the freezer for two weeks before using to kill potential contamination with other pests. The larvae of Lepidoptrous species; *P. interpunctell* were separately evaluated while in the case of Coleopterous species; *O. surinamensis* adults were tested.

2. Gases Used.

Carbon dioxide was provided as pure gas in pressurized steel cylinders. The cylinder was connected to a pressure regulator. The dilution method was used to achieve the required CO_2 concentration. For the atmosphere of nearly pure CO_2 100%, the valve of each cylinder was opened for three minutes in order to fill the gastight Dreshel exposure flask with the gas. Modified Atmosphere (MA) of CO_2 concentrations 50 and 75 % in air were prepared using Gas Distribution device. Determination of the concentrations of CO_2 was monitored using a gas Analyser model 2(10-600 Gow-Mac-Instruments Company U.S.A.).

A 130-liter sealed plastic drum is equipped to perform the phosphine treatment in five concentrations were tested (1/16 recommended dose, 1/8 recommended dose, 1/4 recommended dose, 1/2 recommended dose and recommended dose) which is equivalent to (0.073, 0.146, 0.292, 0.585 and 1.170 g.) of magnesium phosphide which was made in Germany for recommended duration which is 4 days at $27 \pm 2^{\circ}\text{C}$, $65 \pm 5\%$ RH.

3. Preparing the insect species samples for bioassay tests of the two gases:

A number of 30 *P. interpunctella* fourth instar larvae were kept into small cloth bags (4×8 cm) filled with about 25 g artificial diet and closed with rubber bands. In the case of *O. surinamensis*, 30 adults were put into small cloth bags (4×8 cm) filled with about 25 g artificial diet and closed with rubber bands. For Carbon dioxide the cloth bags of the two tested insects were taken and introduced into the gastight Dreshel-flasks of 0.55L volume. The two tested insects in the gastight flasks were treated with the mentioned concentration and different exposure periods ranged from 1 to 36 hrs. at $27 \pm 2^{\circ}\text{C}$. and $65\pm 5\%$. After the exposure periods, the flasks were aerated for 24 hrs., the insects were transferred into Petri dishes and kept at $27 \pm 2^{\circ}\text{C}$ and $65 \pm 5\%$ RH and were examined to record the mortality percentage . While in the case of phosphine gas cloth bags of the two tested insects were taken, introduced into the plastic drums at mentioned concentration and recommended exposure time which is four days at $27\pm 2^{\circ}\text{C}$.Untreated insects, kept at the same temperatures for the same periods, were served as control. After the exposure periods, the drums were aerated for 24 hrs. and the insect stages

were transferred into Petri dishes and kept at $27 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH and were examined to record the mortality percentage.

4. Bioassay Tests of the two gases:

The efficacy of Carbon dioxide and/or phosphine at various concentrations was investigated against two species of stored date fruits pests at tested temperature ($27 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ R.H.). The insect stages were used for the bioassay to study their sensitivity to CO_2 , phosphine concentrations & (CO_2 + phosphine). The mortality percentages of the tested insects was calculated and corrected according to the formula of Abbott (1925) as following: -

$$\text{Corrected mortality \%} = \frac{\% \text{Mortality in treatment} - \% \text{Mortality in control}}{100 - \% \text{Mortality in control}} \times 100$$

5. Biochemical Studies:

Chemicals & quality analysis of treated date fruits:

1. Determination of Reducing Sugar and Soluble Sugars:

The alkaline potassium ferricyanide colorimetric method of Schales and Schales (1945) was used in determination reducing sugars and total soluble sugars.

2. Estimation of Total Amino Acid:

The total amino acid was estimated using ninhydrin as described by McGrath (1972)

3. Determination of Total Phenol Contents:

Estimation of total phenol contents was done by Folin Ciocalteu's method according to Elizabeth and Kelly (2007) and Patel *et al.* (2010).

4. Determination of Total Indols:

The total indols were determined according to Selim *et al.* (1978).

7. Statistical Analysis:

Data on the effect of Carbon dioxide and phosphine at various concentrations were subjected to probit analysis, as described by Finney (1971). LC50 and LC90 values were calculated using the computer program developed by Noack and Reichmuth (1978). Data of Biochemical analysis of date fruits were analyzed using Proc., ANOVA in SAS (SAS Institute 2006).

Results and Discussion

1. The Efficacy of CO_2 gas various concentrations (50, 75 & 100%) on the mortality percentages of *P. interpunctella* and *O. surinamensis*

The efficacy of the concentration 50% CO_2 against *P. interpunctella* and *O. surinamensis* at $27 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH and different exposure periods is tabulated in Table (1). The results showed that the corrected mortality percentages of the two tested insects increased by the increasing of exposure time as it was recorded (13.33, 33.33, 50.00, 60.00, 73.33, 86.67 and 100.00 %) after exposure to (2, 4, 8, 12, 16, 24 and 36 hrs.) respectively for *P. interpunctella*. While the recorded corrected mortality for *O. surinamensis* were (13.33, 40.00, 53.33, 63.33, 76.67 and 100 %) after exposure to (4, 8, 12, 16, 24 and 36 hrs.) respectively.

The efficacy of concentration 75% CO_2 against *P. interpunctella* and *O. surinamensis* at $27 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH and different exposure periods is tabulated in Table (1). The results showed that the corrected mortality percentages of the two tested insects increased by the increasing of exposure time as it was recorded (3.33, 23.33, 40.00, 56.67, 76.67, 86.67 and 100.00 %) after exposure to (1, 2, 4, 8, 12, 16 and 24 hrs.) respectively for *P. interpunctella*. While the recorded mortality for *O. surinamensis* were (6.67, 23.33, 53.33, 70.00, 80.00 and 100 %) after exposure to (2, 4, 8, 12, 16 and 24 hrs.) respectively.

The efficacy of concentration 100% CO₂ against *P. interpunctella* and *O. surinamensis* at 27 ± 2°C, 65 ± 5% RH and different exposure periods is tabulated in Table (1). The results showed that the corrected mortality percentages of the two tested insects increased by the increasing of exposure time as it was recorded (10.00, 30.00,50.00,70.00, 96.67 and 100.00 %) after exposure to (1, 2, 4, 8, 12 and 16 hrs.) respectively for *P. interpunctella*. while the recorded mortality percentages for *O. surinamensis* were (10.00 ,30.00, 60.00,90.00 and 100 %) after exposure to (2 ,4, 8, 12 and 16 hrs.) respectively.

The obtained results in Table (1) revealed that increasing of the exposure periods and or CO₂ concentration resulted in higher efficacy against *P. interpunctella* and *O. surinamensis*.

Lethal concentration values and parameters of mortality regression line for the two tested insects, *P. interpunctella* and *O. surinamensis* exposed to the three concentrations of CO₂ (50, 75, 100%) are presented in Table (3). The results showed that the CO₂ concentration required to obtain 50% mortality of *P. interpunctella* larvae was 45.57 %, while it was 49.16 % for the adults of *O. surinamensis* . The results showed clearly that *P. interpunctella* larvae was more susceptible than the adults of *O. surinamensis*. The same trend was recorded at the LC₉₀ level for the two tested insects. The obtained correlation coefficient values of regression lines of the two tested insects indicated high significant correlation between the CO₂ concentrations and mortality percentages.

The obtained results are in harmony with the findings of several investigators (Navarro *et al.*, 2003; Emekci *et al.*, 2004; El-Lakwah *et al.*, 2010; Pandir *et al.*, 2013; Hashem *et al.*, 2014 and El-Shafei *et al.*, 2019) they reported that the efficacy of the CO₂ was obviously higher at higher CO₂ concentration than at lower one. Meanwhile, the efficacy of CO₂ of various CO₂ concentrations increased with increasing the exposure period.

Table 1: Effect of CO₂ concentrations and exposure time on the mortality percentages of *P. interpunctella* and *O. surinamensis*

Tested insects	Exposure time (hrs.)	Corrected Mortality %		
		CO ₂ concentrations %		
		50	75	100
<i>Plodia interpunctella</i>	1 hrs.	0.00	3.33	10.00
	2 hrs.	13.33	23.33	30.00
	4 hrs.	33.33	40.00	50.00
	8hrs.	50.00	56.67	70.00
	12 hrs.	60.00	76.67	96.67
	16 hrs	73.33	86.67	100.00
	24 hrs.	86.67	100.00	100.00
	36 hrs.	100.00	100.00	100.00
<i>Oryzaephilus surinamensis</i>	1 hrs.	0.00	0.00	0.00
	2 hrs.	0.00	6.67	10.00
	4 hrs.	13.33	23.33	30.00
	8hrs.	40.00	53.33	60.00
	12 hrs.	53.33	70.00	90.00
	16 hrs	63.33	80.00	100.00
	24 hrs.	76.67	100.00	100.00
	36 hrs.	100.00	100.00	100.00

2. The Efficacy of phosphine gas at various concentrations on the mortality percentages of *P. interpunctella* and *O. surinamensis*

In this experiment, *P. interpunctella* larvae and *O. surinamensis* adults were exposed to various fixed concentration of phosphine (0.073, 0.146, 0.292, 0.585 and 1.170 g.) of magnesium phosphide for recommended period. Untreated insects, kept at the same temperatures for the same periods, were served as control. The obtained results are tabulated in Table (2). The present results indicated that, applying phosphine with increasing concentration resulted in increasing the corrected mortality percentages of the two tested insects as it recorded (36.67 ,53.33, 66.67, 83.33 ,100.00 %) after exposure to concentrations (0.073, 0.146, 0.292, 0.585 and 1.170 g.) of magnesium phosphide respectively for *P. interpunctella* larvae. While the corrected mortality percentages were (23.33, 36.67, 50.00, 66.67

and 100 %) respectively, after exposure to the same concentrations of phosphine for *O. surinamensis* adults.

Data in Table (2) indicated that the increasing of the phosphine concentrations increased the gas efficacy against *P. interpunctella* and *O. surinamensis*.

Lethal concentration values and parameters of mortality regression line for the two tested insects, *P. interpunctella* and *O. surinamensis* exposed to different concentrations of phosphine gas are presented in Table (4). The results showed that the phosphine concentration required to obtain 50% mortality for the *P. interpunctella* larvae was 0.129g., while it was 0.154g. for the adults of *O. surinamensis*. Results showed clearly that *P. interpunctella* larvae was more susceptible to phosphine gas than the adults of *O. surinamensis*. The same trend was recorded at the LC₉₀ level for two insects. The obtained correlation coefficient values of regression lines of the two tested insect indicated high significant correlation between the phosphine concentrations and mortality percentages. These data are in agreement with Hubhachen *et al.* (2018), who mentioned that increasing of the phosphine concentrations increased the mortality percentage of *O. surinamensis*.

Table 2: Effect of Phosphine concentrations on the mortality percentages of *P. interpunctella* and *O. surinamensis*

Phosphine concentrations (g.)	Corrected Mortality %	
	<i>P. interpunctella</i>	<i>O. surinamensis</i>
0.073 g	36.67	23.33
0.146 g	53.33	36.67
0.292 g	66.67	50.00
0.585 g	83.33	66.67
1.170 g	100.00	100.00

3. The efficacy of using phosphine mixed with CO₂ for controlling of *P. interpunctella* and *O. surinamensis*

In this experiment, after calculating of the LC₅₀s of CO₂ and Phosphine gases, the *P. interpunctella* larvae and *O. surinamensis* adults were exposed to the LC 50 of the two tested gasses (CO₂ & phosphine) which were (45.57 & 49.16 %) for *P. interpunctella* larvae and *O. surinamensis* respectively for CO₂ while, they were (0.129 & 0.154 g.) for *P. interpunctella* larvae and *O. surinamensis* adults respectively for phosphine gas. The obtained results indicated that, applying LC₅₀ of phosphine mixed with LC₅₀ of CO₂ was more effective in controlling the tested insects than CO₂ or phosphine alone. At the case of *P. interpunctella* the corrected mortality percentage was 100% when it exposed to (45.57 % CO₂ + 0.129 g. phosphine). The same trend for *O. surinamensis*, the corrected mortality % recorded 100% after exposed to (49.16% CO₂ + 0.154 g. phosphine).

Table 3: LC₅₀ and LC₉₀ values with their confidence limits for *P. interpunctella* and *O. surinamensis* exposed to three concentrations of CO₂ gas (50, 75 & 100 %) for 12 hrs.

Tested insects	LC ₅₀ (hrs.)	LC ₉₀ (hrs.)	Confidence limits(hrs.)				Slope± SD	r
			LC ₅₀ (hrs.)		LC ₉₀ (hrs.)			
			Lower	Upper	Lower	Upper		
<i>P. interpunctella</i>	45.57	88.94	25.97	55.14	74.68	142.80	4.413±0.151	0.948
<i>O. surinamensis</i>	49.16	109.26	25.89	59.87	86.56	259.73	3.695±0.176	0.969

- r : Correlation coefficient of regression line
- SD : Standard deviation of the mortality regression line.

It is worthy to state that mixture containing both phosphine and CO₂ accelerated the death of the two tested insects than CO₂ or phosphine each alone. The mortality reached its maximum (100%) only by using mixture containing CO₂ + phosphine. Such level of mortality could be never obtained when CO₂ or phosphine was used each alone at these concentrations (Tables 1&2). Thus, the mixture

containing (49.16% CO₂ + 0.154 g. phosphine) could be recommended for controlling *P. interpunctella* larvae and *O. surinamensis* adults.

In light of results in Tables (1) and (2) we could have concluded that CO₂ increased efficacy of phosphine gas in controlling *P. interpunctella* larvae and *O. surinamensis* adults. These results are in agreement with those obtained by Sadeghi *et al.* (2011); Valizadegan *et al.* (2012) ; Mohamed and Sayed (2013) and El-Shafei (2015) in this concern they repeated that addition of varying concentrations of CO₂ to fixed phosphine concentration (PH₃) induced significantly higher insects mortality than that by PH₃ alone.

Table 4: LC50 and LC90 values with their confidence limits for *P. interpunctella* and *O. surinamensis* exposed to different concentrations of phosphine gas (g.)

Tested insects	LC ₅₀ (g.)	LC ₉₀ (g.)	Confidence limits (g.)				Slope± SD	r
			LC ₅₀ (g.)		LC ₉₀ (g.)			
			Lower	Upper	Lower	Upper		
<i>P. interpunctella</i>	0.129	1.061	0.067	0.191	0.547	7.089	4.413±0.143	0.996
<i>O. surinamensis</i>	0.154	1.589	0.079	0.239	0.700	23.751	3.695±0.167	0.997

- r: Correlation coefficient of regression line
- SD: Standard deviation of the mortality regression line.

4. Effect of CO₂, phosphine & its combination on biochemical contents and quality of treated Siwi date fruits.

The effect of CO₂, phosphine & its combination on biochemical contents and quality of treated Siwi date fruits is tabulated in Table (5). The obtained data showed that total sugars, reducing and non-reducing sugars recorded (0.744, 0.745, 0.742), (0.697, 0.697, 0.694) & (0.047, 0.048, 0.048) mg/g f.w. after treated with CO₂, phosphine & its combination respectively compared to control (0.742, 0.695, 0.048) mg/g f.w. with no significant differences. The same trend could be applied for amino acids and total indoles which were (0.282, 0.278, 0.277) & (0.068, 0.071, 0.067) mg/g f.w. after treated with CO₂, phosphine & its combination respectively compared to control (0.280 & 0.068) mg/g f.w.. On the contrary total phenols which affected by any stress exhibited significant differences between CO₂ and phosphine treatments (1.656 & 1.660) mg/g f.w. compared to control 1.093 mg/g f.w. while there was no significant difference between the combination treatment (CO₂ + phosphine) 1.093 mg/g f.w. and the control 1.093 mg/g f.w.

Table 5: Effect of CO₂, phosphine & its combination on biochemical contents and quality of treated date fruits cultivar Siwi.

Biochemical contents	Total sugar (mg/g f.w.)	Reducing sugar (mg/g f.w.)	Non reducing sugar (mg/g f.w.)	Amino acids (mg/g f.w.)	Indoles (mg/g f.w.)	Phenols (mg/g f.w.)
Control	0.742±0.0025 ^a	0.695±0.0025 ^a	0.048±0.00 ^a	0.280±0.0065 ^a	0.068±0.025 ^a	1.093±0.084 ^b
CO ₂ (100%)	0.744±0.0015 ^a	0.697±0.0025 ^a	0.047±0.00 ^a	0.282±0.0025 ^a	0.068±0.0015 ^a	1.656±0.055 ^a
Phosphine (1.170 g.)	0.745±0.0015 ^a	0.697±0.0015 ^a	0.048±0.00 ^a	0.278±0.0030 ^a	0.071±0.0015 ^a	1.660±0.030 ^a
CO ₂ + Phosphine	0.742±0.0015 ^a	0.694±0.0015 ^a	0.048±0.00 ^a	0.277±0.0021 ^a	0.067±0.0015 ^a	1.093±0.040 ^b
P. value	0.225	0.140	0.045	0.5738	0.225	<.0001
L.S.D.	0.0034	0.0039	0.0011	0.0074	0.0034	0.0759

Our results in a harmony with El-Shafei (2015) who reported that the date palm fruit chemical contents under different controlling treatments of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) by CO₂, Phosphine and CO₂ + Phosphine insignificant differences were found in the chemical fruit content as total proteins, total sugars, reducing sugars and non-reducing sugars and control. While date palm fruit phenol contents showed significant variance between different controlling treatments of *E.cautella*, and significant increased under the treatment by CO₂ compared to control. Also our results

is similar to these by Mouzal *et al.* (2017) & Loay (2018) they revealed that the decrease of phenolic compounds comparing with control based on decrease of whole metabolism of plant cells.

Conclusion

It is worthy to state that mixture containing both phosphine and CO₂ accelerated the mortality percentage of the two tested insects than CO₂ or phosphine each alone without any effect on the chemical properties and quality of the treated date fruits. The mortality percentages reached its maximum (100%) only by using mixture containing CO₂ + phosphine. Such level of mortality could be never obtained when CO₂ or phosphine was used each alone at these concentrations. Thus, the mixture containing (49.16% CO₂ + 0.154 g. phosphine) could be recommended for controlling *P. interpunctella* larvae and *O. surinamensis* adults in stored date fruits. In light of obtained results in this experiment we could conclude that CO₂ increased efficacy of phosphine gas in controlling *P. interpunctella* larvae and *O. surinamensis* adults.

Acknowledgements

The author is grateful to Dr. Loay Lewaa Mansour, Dept. of Date Palm Pests and Diseases, Central Lab. for Date Palm, Agricultural Research Center, for his grateful help in biochemical analysis.

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