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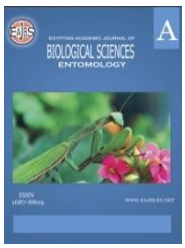

Efficiency of Carbone Dioxide and Aluminum Phosphide Gasses on *Ephestia cautella* and *Oryzaephilus surinamensis* Insects and Microbial Load on Stored Date Fruits

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ABSTRACT

This research aims to study the efficiency of carbon dioxide as a natural gas on *Ephestia cautella* and *Oryzaephilus surinamensis* insects, their stages and microbial load compared to the insecticide (aluminum phosphide gas). The semi-dry date fruits were exposed to aluminum phosphide gas with different concentrations (1/16 recommended dose, 1/8 recommended dose, 1/4 recommended dose, 1/2 recommended dose and recommended dose) for 5 days at (27 ± 2◦C and 65 ± 5% R.H.), and 100% carbon dioxide for different times) 30min ,1hr ,2hr ,4hr,6hr ,8hr ,12hr and 16hr), respectively. The finding revealed that the increasing concentration of aluminum phosphide gas increases the mortality till the recommended dose (1.170 g / m 3 / 5 days), while the suitable time with carbon dioxide gas was 16 hours which completed % mortality of eggs and lavera *E. cautella* and adult of *O. surinamensis*. The efficiency of exposure to aluminum phosphide gas at the recommended dose was reduced microbial load less than carbon dioxide. Also, the treated date fruits with carbon dioxide were reduced *E. coli* and *Saccharomyces cervisiae* than the other ones.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.), a tropical and subtropical tree in the palmae (Arecaceae) family, is one of man's oldest cultivated plants, and it has played an important role in people's daily lives in the Arabian Peninsula for over 7000 years (Ahmed *et al.*, 1995). Over the last 30 years, global dates output has expanded dramatically and continuously. Arab countries account for around 74.5 % of global output. Egypt is one of the top 10 producers of dates in the world (FAO, 2018). Egypt produces 1.562.171 tonnes of date fruits, accounting for 15.1 % of overall Egyptian fruit production (1542111 tons,). Food processing accounted for only 16.5 % of total date fruit production (Ministry of Agriculture and Land Reclamation, 2017). Microbial invasion is responsible for a significant annual loss of dates quality, and the rate of contamination is influenced by a number of critical factors including cultivation weather, ripening stage, postharvest conditions such as hygienic practices during processing, handling, storage, and transportation (Zamir *et al.*, 2018). This insect does not target dates in the Kimri,

Khalal, or Rutab stages; only fruits in the Tamar stage are affected (Yahia *et al.*, 2013). Many pests and diseases affect date palms, and their nature and severity vary depending on cultivar, regional, weather, and cultural techniques (Zaid *et al.*, 2002). At this time as a result of physiological changes in the fruit, which favour pathogen development, there was an increase in postharvest illnesses following harvest and storage. Yeasts (the most common), moulds, and bacteria can all cause microbial deterioration. Saccharomyces yeast species are more tolerant of high sugar content than other yeast species found in dates (Yahia *et al.*, 2013). After 6-7 months of storage, nearly half of the dates were lost, according to Mewtally *et al.* (2007). With an average infection rate of 16.8% (Al-Mjeni *et al.*, 1983*), E. cautella* causes significant damage to dates in storage (Al-Zadjali *et al.*, 2006). *Ephestia cautella* Walk (fig-moth) is a postharvest insect that can attack dates in the orchard, packinghouses, or stores in some growing zones (Ahmed *et al.*, 1994). Total viable count (TVC) in undervalued-date and processed dates by-products ranged from 1.7×10^3 CFU/g to 3.5×10^8 CFU/g and from 22 CFU/g to 44×10^3 CFU/g, according to Bellaouchi *et al.* (2017). Lactic acid bacteria, spore-forming bacteria (Bacillus), yeasts, and moulds were found in all samples, with varying levels of presence depending on water activity and moisture content. Phosphine gas (aluminum phosphide, PH_3) was used for disinfesting stored products especially date fruits in Egypt. Sometimes a failure occurs as a result of fumigation from mortality insects or their stages and this is due to this adjusting the concentrations of fumigation materials, the times needed to mortality insects, or the quality of some packages (Dakhil *et al.*,2012). The convenient dose of ECO2 – Fume gas (2% PH3 + 98% CO2 w/w) against all developmental stages of *E. cautella* was 500 ppm (36 g/m3) for 3 days exposure (Mohamed and Sayed,2013). This research aims the effect of carbon dioxide as natural gas on some insects that infect dates, especially semi-dry and dry varieties, and microbes, especially *E. coli* and saccharomyces compared to one of the insecticides that produce aluminum phosphides

MATERIALS AND METHODS

Date Fruits:

Date fruits (Siwi date semi-dry variety) were obtained from Al Bahreia Oasis, Giza, Egypt.

Carbon Dioxide:

Carbon dioxide was purchased from Gulf Industrial and Medical Gasses Co., Egypt, as pure gas in pressurized steel cylinders. A pressure regulator was attached to the cylinder. For the atmosphere of nearly pure CO_2 100%, the valve of each cylinder was opened for three minutes in order to fill the gas-tight Dreshel exposure flask with the gas. Carbon dioxide gas was used in the experiment with a concentration of 100% at different times (0.5hr to 16 hrs).

The efficacy of phosphine at various concentrations at recommended time (5) days and carbon dioxide at different times was investigated against stored date fruits pests at temperature ($27 \pm 2^{\circ}C$ and $65 \pm 5\%$ R.H)

Fumigation Tablets:

Aluminum Phosphide tablets 56% as commercial name (Celphos) was provided from Sumitomo India LTD. A 100-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.) for 5days.

Collection and Rearing of *E. cautella* and *O. surinamensis* Insects.

The two insect species were collected from infested date fruits and were reared on their standard food diets. Insects were cultured in an incubator at a temperature of 27±2°C and relative humidity (RH) of 65±5%. The adult insects have reared on semi-dry date fruits Siwi cultivar. The date fruits used in rearing culture were kept in the freezer for two weeks before being utilized to eliminate the possibility of pest contamination. The larvae of Lepidoptrous species; *E. cautella* and Coleopterous species; *O. surinamensis* were separately to use of experiential.

Microorganism Strains:

Escherichia coli and *Saccharomyces cervisiae* were obtained from the Egyptian Microbial Culture Collection, (EMCC), Cairo Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. All microbiological media used were obtained from Oxoid Division of Oxiod Ltd., London.

Preparing the Insect Species Samples for Bioassay Tests of Gases:

A number of 30 *E. cautella* fourth instar larvae and eggs were kept in 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 hr at 27 ± 2°C and 65±5%. After the exposure periods, the flasks were aerated for 24 hr, the insects were transferred into Petri dishes and kept at 27 ± 2°C and 65 ± 5% RH and were examined to record the mortality percentage. While in the case of aluminum phosphide 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±2°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 ± 2°C and 65 ± 5% RH and were examined to record the mortality percentage.

Preparing the Insect Species Samples for Bioassay Tests of Gases:

A number of 30 *E. cautella* fourth instar larvae and eggs were kept in 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 hr at 27 ± 2°C and 65±5%. After the exposure periods, the flasks were aerated for 24 hr, the insects were transferred into Petri dishes and kept at 27 ± 2°C and 65 ± 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±2°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 ± 2°C and 65 ± 5% RH and were examined to record the mortality percentage.

Bioassay of Gases:

The efficacy of Carbon dioxide and aluminum phosphide at various concentrations was investigated against two species of stored date fruits pests at tested

temperature (27 ± 2◦ C and 65 ± 5% R.H.) after 24 hr for the egg of *E. cautella* and adults of *O. surinamensis* and after 7 days for larvae of *E. castella*. The mortality percentages of the tested insects were calculated and corrected according to the formula of Abbott (1925) as follows: Mortality %= Mortality in treatment - Mortality in control x 100 / Mortality in control

Refreshment of *E. coli* and *Saccharomyces cervisiae:*

E. coli and *Saccharomyces cervisiae* were refreshed by suspending in nutrient broth for 24 hr at 37ºC *for E. coli* and 25ºC for *Saccharomyces cervisiae***.**

Microbiological Analysis:

Total Count and Yeasts and Molds:

Ten grams of each treated date palm fruit with carbon dioxide 100% for 16hr and with the recommended dose of aluminum phosphide gas (1.170g) were added to 90 ml saline solution (0.85%) and mixed well for 5 min. and then 1 ml of mixed sample serially diluted (up to the sixth dilution) after that, 1 ml of each dilution was poured in petri dish and followed by pouring about 15 ml of plate count agar medium and the petri dishes were incubated at 37ºC /24-48 hr. From previews, serial dilutions, 1 ml of each dilution was poured and about 15 ml of potato dextrose agar (PDA) was poured in dishes and the dishes were incubated at 25ºC /5 days as described by Busta *et al.* (1984). The results of the count of bacteria were turned into log according to Snedecor and Cochran, (1980).

***E. coli* and *Saccharomyces cervisiae*:**

Ten grams of treated date palm fruit samples were weighed and crushed in a sterile mortar pestle with 1 ml of *E. coli* (25x107 cfu) and another date sample was crushed with 1 ml of *Saccharomyces cervisiae* (204x104 cfu). After grinding well, the samples were exposed to carbon dioxide aluminum phosphide gas. Samples were added to 90 ml of saline solution (0.85%) and serial dilutions of samples were prepared in test tubes containing 9 ml saline solution up to the sixth dilution. Then 1 ml of each dilution is poured into petri plates followed by pouring of McConkey agar medium for *E. coli* and PDA for *Saccharomyces cervisiae.* After those plates were incubated at 37ºC for 24-48 hr *for E. coli* and for 25ºC /5 days for Saccharomyces cervisiae according to Scheldeman *et al.* (2005). The previous steps were repeated with *E. coli* ($25x10^7$ cfu) and *Saccharomyces cervisiae* ($204x10^4$ cfu) with selected media (without date samples).

RESULTS AND DISCUSSION

Effect of Different Concentrations of Aluminum Phosphide Gas on Both Eggs and Larvae of *E. cautelal* and Adults of *O. surinamensis* Insects:

Results in Table (1) show the effect of different concentrations of aluminum phosphide gas on both eggs and larvae of *Ephestia cautella* and *O. surinamensis* in its adult stage. From the results in Table (1) it is clear that increasing aluminum phosphide leads to an increase in effect on insects, regardless of the species or stages of the insects. The result in the same table indicates that eggs of *Ephestia cautelle* were increased mortality percentage with increasing aluminum phosphide gas till (1) tablet equal (1.170g) was 100% mortality after 7 days. Also, the larvae of *Ephestia cautella* and the adults of *O. surinamensis* were completely 100% mortality at recommended dose after 24hr. The results showed that the sensitivity of eggs is less than the larval stage of the insect *Ephistia cautella* at the same concentration of aluminum phosphide. These are in agreement with (Hubhachen *et al.*,2018) mentioned that increasing the phosphine concentrations, increased the mortality percentage of *O. surinamensis.* Hema *et al.*,

(2012) showed that the pre-adult stages of insects are generally more tolerant to fumigants, due to lower respiratory rates compared to adult insects (Chaudhry,1997).

Table 1: Effect of different concentrations of aluminum phosphide gas on both eggs and larvae of *E. cautelal* and adults of *O. surinamensis* insects.

Aluminum phosphide gas concentrations	***Ephestia cautella***		***O. surinamensis***
	Eggs	**Larvae**	
1/16 (0.073 g)	33.33	34.33	23.33
1/8 (0.146 g)	60.00	63.33	36.67
1/4 (0.292 g)	66.67	76.67	50.00
1/2 (0.585 g)	86.67	90.00	66.67
1 (1.170 g)	100.00	100.00	100.00

Effect of Different Concentrations of Carbon Dioxide Gas on Both Eggs and Larvae of *E. cautelal* and Adults of *O. surinamensis* Insects.

Results in Table (2) show the effect of exposure to carbon dioxide gas at different times from half an hour to 12 hours on the *Ephestia cautella* in the egg and larval stages and the *O. surinamensis* in the adult stage. The results showed that exposure to carbon dioxide for half an hour did not approximately affect the mortality stages of insects. The results also showed that the duration of exposure of carbon dioxide gas for 4 hours led to Mortality percentages of 50% in the eggs stage and more than 50% in larvae of *Ephestia cautella* insects, while the mortality percentages in adults of *O. surinamensis* insects did not exceed 30%. The results showed that the duration of carbon dioxide gas 16 hours was sufficient to Mortality percentages 100 % all tested insects.

Table 2: Effect of different concentrations of carbon dioxide gas on both eggs and larvae of *E. cautelal* and adults of *O. surinamensis* insects.

Exposure time (h.) of carbon dioxide	***Ephestia cautella***		***O. surinamensis***
	Eggs	**Larvae**	
1/2	0.00	6.67	0.00
1	10.00	16.67	0.00
2	30.00	36.67	10.00
4	50.00	56.67	30.00
8	70.00	76.67	60.00
12	86.67	93.33	90.00
16	100.00	100.00	100.00

Effect of Aluminum Phosphide and Carbone Dioxide on Microbial Load of Selected Media:

The best treatments were used to eliminate insects and their stages, (aluminum phosphide 1.170 g/m3 for 5days and carbon dioxide gas100% for 16 hours to study their effect on microbes probably to be 5 presents in dates or natural microbes in dates. Stability of *E. coli* and *Saccharomyces cervisiae* without date sample to phosphine and carbon dioxide was assayed after exposure to phosphine 1.170 g and carbon dioxide 100% for 16hr. Results in Table (3) represent that aluminum phosphide reduced the growth log of *E. coli* with a rate of 0.5 logs when reduced growth log of *Saccharomyces cervisiae* with a rate of 2.1 logs. Carbon dioxide reduced the growth of *E. coli* with a rate

of 2.7 logs and in *Saccharomyces cervisiae* the reduction was 0.5 log. Results in Table (3) showed the efficiency of phosphine gas and carbon dioxide on the microbial load of *E. coli* and *Saccharomyces cervisiae* in selected media (without dates).

Table 3: Effect of aluminum phosphide and Carbone dioxide on microbial load of selected media**.**

Microbial load	Initial counts			Aluminum phosphide			Carbon dioxide		
	Cfu/g	log		Cuf /g	Log		Cfu/ g	Log	
		Cfu	R		Cfu	R		Cfu	R
E. coli	$25x10^7$	8.4	0	$93x10^6$	1.9	0.5	$49x10^4$	5.7	2.7
Saccharomyces cervisiae	$204x10^4$	6.3	0	$141x10^2$	4.2	2.1	$65x10^4$	5.8	0.5

cfu: Colony form unit R: Log ratio of reduction

Effect of Aluminum Phosphide and Carbon Dioxide on Microbial Load With Date Fruits:

Table (4), data show the effectiveness of aluminum phosphide and carbon dioxide on microbial loading on date samples. The microbes were assayed as total count, yeasts and molds, *E. coli* and *Saccharomyces cervisiae*. The results showed that aluminum phosphide was no effect on the total count, in yeasts and molds the decreasing rate was 0.9 log when *E. coli* growth was decreased with a rate of 0.3 logs and *Saccharomyces cervisiae* 2.9 logs. In the carbon dioxide treatment, the decreasing rate of microbial rate was 0.5 log, nil, 1.2 log and 0.6 logs on total count, yeast and molds, *E.coli and Saccharomyces cervisiae*, respectively. The decreasing growth rates are due to aluminum phosphide gas because it is considered a strong inhibitor of respiration of mitochondria especially cytochrome c oxidase (in eukaryotic) when in anaerobic condition phosphine is produced naturally. Phosphine is toxic to aerobically respiring organisms, it also has effects on the survival and growth of some aerobic bacteria and fungi (Castro, *et al.,*2000 and Solanki *et al.*, 2019). So, the decreasing growth rate of *Saccharomyces cervisiae* was high (eukaryotic) and was low in *E. coli* (prokaryotic). These results were in agreement with Jenkins *et al.* (2000) who showed that phosphine gas was generated naturally in the anaerobic condition in the presence of mixed acid fermenter bacteria (*Salmonella gallinarum, Salmonella arizonae* and *Escherichia coli*). Ding *et al.* (2005) found that phosphine production was increasing in anaerobic sludge culture because of the fermentor bacteria. On the other hand, the effect of carbon dioxide has appeared on aerobic bacteria when the facultative anaerobic bacteria could grow in presence of CO2. The previous results were confirmed with Eklund (1984) when presented that CO2 inhibited the growth of *Bacillus sublitis* with concentration 40% when unaffected on *E. coli* until concentration 100%. Martin *et al.* (2003) studied the effect of CO2 on bacterial growth in milk and found that the total count of bacteria in raw milk was highly decreased when *E. coli* bacteria increased in presence of CO2 with a concentration of 0.4 mM. Also, Spilimbergo and Bertucco (2003) explained mechanisms of microbial inactivation as vegetative form with treated CO2 can easily diffuse through the membrane and accumulate inside the cell. Abdul Al *et al.*, (2018) reported that the decrease in the total count and total coliform bacteria were in dates packed under vacuum because the storage conditions are not suitable for the growth of bacterial cells, especially under vacuum packing antenna conditions.

Table (4): Effect of treatments on microbial load of date fruits.

Microbial load	Initial counts			Aluminum phosphide			Carbon dioxide		
	Cfu/g	log		Cuf /g	Log		Cfu/ g	Log	
		Cfu	R		Cfu	R		Cfu	R
Total counts	$150x10^1$	3.2	0	$143x10^1$	3.2	0	$49x10^1$	2.7	0.5
Yeast and mold	$45x10^2$	3.7	0	$70x10^1$	2.8	0.9	$63x10^2$	3.7	0
E. coli	$25x10^7$	8.4	0	$123x10^6$	8.1	0.3	$15x10^6$	7.2	1.2
Saccharomyces cervisiae	$204x10^4$	6.3	0	$28x10^2$	3.4	2.9	$53x10^4$	5.7	0.6

cfu: Colony form unit R: Log ratio of reduction

Conclusion:

It is clear after conducting experiments to compare aluminum phosphide gas produced from sylphos tablets (insecticide) and carbon dioxide gas (natural gas). It is recommended to use carbon dioxide in fumigating dates fruits, as it is more effective on insects, their different phases and microbes, in addition to that it is safer than aluminum phosphide gas.

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ARABIC SUMMARY

تاثير كفاءه غازثانى اكسيد الكربون وفوسفيد الالومنيوم على حشرات دودة البلح العامرى وخنفساء السورينام والحمل الميكروبى للتمور المخزنة

مصطفى طه محمدى عسوس[1]، وائل كمال محمد الشافعى[1]، لؤى لواء منصور [1]، رحاب عيدراوس محمد السيد سالم[2]

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[2]قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق

يهدف هذا البحث إلى دراسة كفاءة غاز ثاني أكسيد الكربون كغاز طبيعي على بعض الحشرات ومراحلها والحمل الميكروبي مقارنة بالمبيد الحشري (غاز فوسفيد الألومنيوم). تم تعريض ثمار التمر نصف الجافة لغاز فوسفيد الألومنيوم بتركيزات مختلفة (16/1 جرعة موصى بها، 8/1 جرعة موصى بها، 4/1 جرعة موصى بها، 2/1 جرعة موصى بها، والجرعة الموصى بها) لمدة 5 أيام عند درجة حرارة 27± 2 C° ورطوبة نسبية 65 RH ± 5٪) و ثاني أكسيد الكربون بتركيز 100%لأوقات مختلفة (30 دقيقة ، 1 ساعة ، 2 ساعة ، 4 ساعات ، 6 ساعات ، 8 ساعات ، 12 ساعة و 16 ساعة) ، على التوالي. أظهرت النتائج أن زيادة تركيز غاز فوسفيد الألومنيوم يزيد من نسبة الموت حتى الجرعة الموصى بها (1.170 جم / م ^{3}/5 أيام)، بينما الوقت المناسب بغاز ثاني أكسيد الكربون كان 16 ساعة هو المناسب لموت اليرقات والبيض لحشرة (*E. cautella*) والحشرات الكاملة (*O surinamensis*). كفاءة التعرض لغاز فوسفيد الألومنيوم بالجرعة الموصى بها انخفض الحمل الميكروبي أقل من ثاني أكسيد الكربون. كما أن ثمار التمر المعامل بثاني أكسيد الكربون اكثر انخفاضا فى الحمل الميكروبى (*E. coli and Saccharomyces cervisiae*) عن مثيلاتها الأخرى.