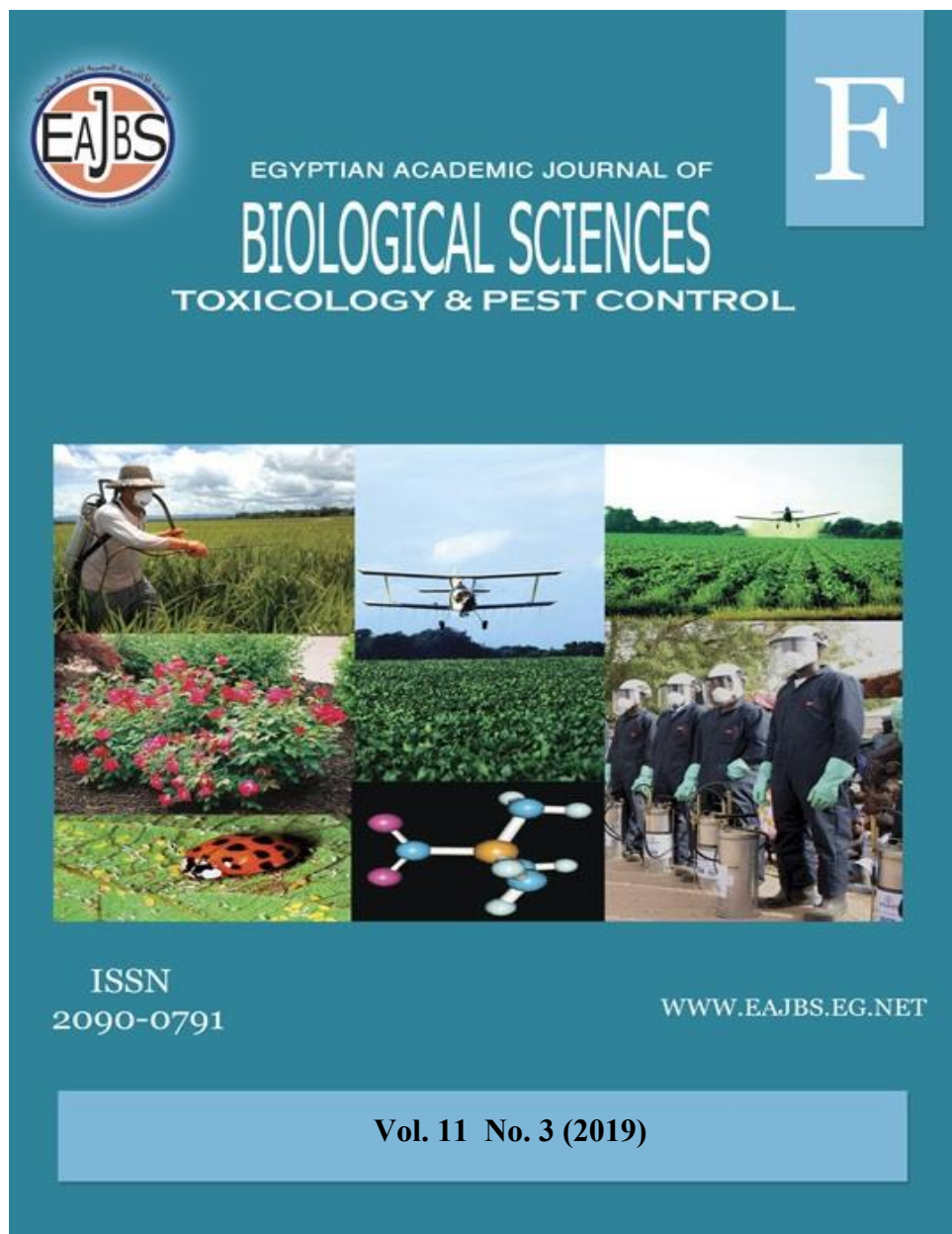


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Control of One of the Vital Stored Date Insects, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), by Using Ozone Gas.

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ABSTRACT

This study aimed to evaluate ozone (O₃) technology as management tools to control all life stages of Indian meal moth, *Plodia interpunctella* (Hübner) (eggs, larvae, and pupae) infesting stored date and its effect on some larval enzymes activity and the quality of date fruits. Three concentration of ozone gas 100, 300 and 500 (ppm) has been tested against *P. interpunctella* life stages (eggs, larvae, and pupae) at various exposure times, which were ranged from 15 to 150 minutes. Results revealed that the mortality of tested life stages increased by increasing the exposure time in each ozone concentration. The required exposure time to reach 100 % mortality of the insect stages decreased by increasing the ozone con. from 100 to 500 (ppm). Corrected egg mortality reached 100% after 150 min. from exposure to 500-ppm ozone. The corrected second instar larval mortality recorded 100 % after 150 and 90 min. from exposure to 300 and 500-ppm ozone con. respectively. While the corrected mortality of 4th instar larvae reached 100 % after 150 and 120 min. of exposure to 300 and 500-ppm ozone con. respectively. On the other hand, pupal corrected mortality was 100 % just when its exposure to 500 ppm for 150 min. Results showed that the egg was the most tolerant stage to the ozone gas while the 2nd larval instar was the most susceptible one. On the other hands, 4th larval instar was more susceptible than the pupa. Also, data showed that ozone seemed to have no effect on tested chemical contents of dates fruits. In addition, results revealed that the larval rate of respiration was affected by ozone gas.

INTRODUCTION

The date palm in Egypt is considered a strategic crop for the past, present, and future. Egypt occupies the first place in date production at the global level, followed by Iran and Saudi Arabia, where annual production is estimated at 1.68 million tons, which is produced from 14.95 million palm trees which equivalent to 17% of the world production estimated at 7.5 million tons (Egyptian Ministry of Agriculture, 2015).

Dates palm trees face many agricultural problems due to several factors, the most important of which is the infestation of dates with insect pests, which leads to the reduction of their production in terms of quantity and quality, Ali *et al.*, (2001/2002)

stated that date fruits pests cause 20-73.3% loss of tamr annually, consequently shortage production. Mewtally *et al.*, (2007) reported that approximately 50% of stored dates were lost after elapsing 6-7 months of storage.

The Indian meal moth, *P. interpunctella* (Lepidoptera: Pyralidae), is a serious insect pest of stored products (Mohandass *et al.*, 2007). It is a cosmopolitan pest attacking a wide range of stored products including dried vegetable and fruits commodities. It can infest a variety of products and is perhaps the most economically important insect pest of processed food (Perez-Mendoza and Aguilera-Pena, 2004; Mohandass *et al.*, 2006).

Ozone (O₃) could become an alternative pest control method for stored products. Ozone is able to penetrate large masses of grain, is highly oxidative, unstable and decomposes rapidly to oxygen without leaving residues. Ozone is widely used in many fields, such as drinking water treatment and disinfection of medical appliances, and to eliminate odors, colors, pesticides, inorganic, and organic compounds (Qin Zhanggui *et al.*, 2003). Applications in agriculture include storage and preservation of vegetables and fruits, surface decontamination of perishable foods, and disinfection of manufacturing equipment, water and packaging materials (Mendez *et al.*, 2003). The US Food and Drug Administration (FDA) classified ozone for treating bottled water as “generally recognized as safe” (GRAS) and has also approved its use as a direct additive for food treatment, storage, and processing (FDA, 2001). Thus, these experiments aimed to evaluate the effect of ozone gas in controlling *P. interpunctella* life stages and its effect on both the enzymatic activity of the insect and the chemical properties of treated date fruits.

MATERIALS AND METHODS

Experiments were conducted at the central laboratory of date palm, ARC, Giza-Egypt, using laboratory strain of *P. interpunctella* which was reared at the same laboratory.

Insect Cultures:

Cultures of Indian meal moth, *P. interpunctella* were reared at 25 ±2 °C and 65 ±5 R.H.

Production of Ozone Gas:

Ozone gas was a product from the air using an ozone generator Model OZO 6 VTTL OZO Max Ltd, Shefford, Quebec Canada (OZO Max Ltd, Shefford, Quebec, Canada) from purified extra dry oxygen feed gas at the laboratory of Food Toxicology & Contaminants, National Research Center. The amount of ozone output was controlled by a monitor- controller having a plug-in sensor onboard which is changed for different ranges of ozone concentration and a belt pan in the monitor-controller allows controlling the concentration in a selected range.

Efficacy of Ozone Application on *P. interpunctella*:

Frihi date cultivar used in this study because it was the most preferable for this pest. A number of 100 *P. interpunctella* eggs were kept into small cloth bags (4×8 cm) filled with about 50 g of date and closed with rubber bands. 30 larvae from both the second instar and the fourth instar larvae each separately, were put also with 50 g of date and pupae were introduced into organza bags (4×8 cm) then fixed with rubber band. Three replicates of each concentration and exposure times were prepared, then the bags were directly closed well and exposed to their concentrations of ozone (100,300 and 500 ppm) at different exposure periods (15, 30, 60, 90,120 and 150 min.). Untreated bags were kept as control for each stage. Jut bags for each replicate were observed after 24 hours to count numbers of alive and dead larvae and calculate mortality percent corrected according to

Abbott's formula (1925). The mortality of the egg was recorded according to the number of larvae resulted from the treated eggs. Also the mortality of pupa recorded according to the number of moth resulted from the treated pupa.

Quality Analysis of Date:

1. Determination of Reducing Sugar and Soluble Sugars:

The alkaline potassium ferricyanide colorimetric method of Shales 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 Mc.Grath (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).

4. Determination of Total Indols:

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

Anylasis For Larvae:

1. Acetyl Cholinesterase Determination:

AchE (acetylcholinesterase) activity was measured according to the method described by Simpson *et al.* (1964)

2. Oxidase Activity (MFO):

P-nitroanisole a-demthylation was assayed to determine the mixed-function oxidase activity according to the method of Hansen and Hodgson (1971)

3. Lactate Dehydrogenase Catalyzes (LDH):

The method described here is derived from the formulation recommended by the German Society for clinical chemistry (DGKC, 1972).

Statistical Analysis:

Data on the effect of ozone concentration and exposure periods on the tested insects were subjected to probit analysis, as described by Finney (1971). LT₅₀ and LT₉₉ values were calculated using the computer program developed by Noack and Reichmuth (1978).data of Quality analysis of date& anylasis for larvae were analyzed using Proc., ANOVA in SAS (SAS Institute 2006).

RESULTS AND DISCUSSION

Effect of Ozone Gas Application on A Different Stage of *P. interpunctella*:

Results concerning the evaluation efficacy of ozonation on a different stage of *P. interpunctella*, are shown in Table (1). The corrected mortality of different stages increased with the increase of both concentration and exposure periods. At the same time, complete corrected mortality (100%) was noticed only at 500 ppm in egg and pupa at 150 min, while in the case of larvae it showed the 100% mortality at 90 min for the second instar and 120 min for fourth instar larvae. These results show that egg stages were more tolerant of ozone gas than the two other stages. Our results are in agreement with those of Abd El-Aziz *et al.*, (2017) mentioned that the larvae and pupae of *Sitotroga cerealella* recorded higher sensitivity to ozone when compared to eggs. Also, Keivanloo *et. al.*, (2013) reported that, by increasing the concentration and exposure time, the rate of mortality increased for all tested stages.

The lethal concentration of ozone gas to eggs, larvae, and pupae of *P. interpunctella* at 120 min presented in Table (2) showed that the ozone was more effective in larvae than eggs and pupae stages. LC₉₉ values on the egg, second instar larvae, fourth instar and pupae of *P. interpunctella* were 921.89, 502.59, 556.31 and 786.74 ppm respectively. Our

results in a harmony with Hassan (2014) who reported that by increasing the ozone concentration the rate of mortality increased.

Table 1: Effect of ozone concentration and exposure time on mortality (mean \pm SE) of *P. interpunctella* life stages.

Life stage	Exposure time (min)	Corrected mortality% \pm SE		
		Ozone concentration (ppm)		
		100	300	500
Egg	15	0.00 \pm 0.0 ^c	5 \pm 0.0 ^b	12.50 \pm 1.6 ^a
	30	2.50 \pm 1.6 ^c	14.17 \pm 1.6 ^b	26.67 \pm 2.36 ^a
	60	9.17 \pm 0.0 ^c	27.50 \pm 1.3 ^b	38.33 \pm 0.6 ^a
	90	25.83 \pm 0.8 ^c	37.50 \pm 2.5 ^b	50.83 \pm 2.1 ^a
	120	41.67 \pm 0.0 ^b	62.50 \pm 1.2 ^a	66.67 \pm 1.3 ^a
	150	54.17 \pm 0.3 ^c	75 \pm 2.8 ^b	100 \pm 0.0 ^a
Second instar larvae	15	5.83 \pm 0.4 ^c	18.33 \pm 0.9 ^b	30.83 \pm 1.6 ^a
	30	13.33 \pm 1.3 ^c	43.33 \pm 1.9 ^b	60 \pm 2.3 ^a
	60	29.17 \pm 0.8 ^c	57.50 \pm 2.6 ^b	90 \pm 1.3 ^a
	90	50 \pm 1.2 ^c	78.33 \pm 1.6 ^b	100 \pm 0.0 ^a
	120	71.67 \pm 3.1 ^{bc}	85 \pm 2.1 ^b	100 \pm 0.0 ^a
	150	80 \pm 2.4 ^b	100 \pm 0.0 ^a	100 \pm 0.0 ^a
fourth instar larvae	15	3.33 \pm 1.3 ^b	15.00 \pm 2.1 ^a	19.17 \pm 2.1 ^a
	30	6.67 \pm 1.3 ^c	30.83 \pm 1.6 ^b	51.67 \pm 0.9 ^a
	60	12.50 \pm 2.1 ^c	47.50 \pm 2.1 ^b	70.83 \pm 2.7 ^a
	90	34.17 \pm 0.0 ^c	67.50 \pm 0.8 ^b	81.67 \pm 0.9 ^a
	120	50.00 \pm 0.3 ^c	82.50 \pm 2.1 ^b	100 \pm 0.0 ^a
	150	73.33 \pm 0.7 ^b	100 \pm 0.0 ^a	100 \pm 0.0 ^a
Pupae	15	1.67 \pm 0.9 ^b	10.00 \pm 1.3 ^a	13.33 \pm 0.0 ^a
	30	4.17 \pm 1.3 ^c	20.83 \pm 0.8 ^b	33.33 \pm 1.3 ^a
	60	11.67 \pm 0.9 ^c	37.50 \pm 2.5 ^b	52.50 \pm 1.1 ^a
	90	32.50 \pm 0.8 ^c	46.67 \pm 1.0 ^b	60.00 \pm 3.3 ^a
	120	45.00 \pm 2.1 ^b	65.00 \pm 0.6 ^a	67.50 \pm 0.8 ^a
	150	58.33 \pm 1.2 ^c	79.17 \pm 0.0 ^b	100 \pm 0.0 ^a

Table 2: LC50 and LC99 values with their confidence limits for different stages of *P. interpunctella* exposed to three concentrations of ozone at an exposure period of 120 min

stages	LC ₅₀ (ppm)	LC ₉₉ (ppm)	Confidence limits(hrs)				Slope \pm SE	Chi-squareX ²
			LC ₅₀		LC ₉₉			
			Lower	Upper	Lower	Upper		
Egg	159.44	921.89	81.80	226.84	668.03	1447.54	0.9 \pm 0.25	0.32
2 nd instar larvae	45.78	502.59	39.23	117.90	412.09	801.54	1.60 \pm 0.29	4.26
4 th instar larvae	102.60	556.31	75.88	126.39	430.85	823.27	2.24 \pm 0.28	0.98
Pupa	131.81	786.74	49.36	196.82	461.49	1092.80	0.86 \pm 0.25	0.47

Some Chemical Characteristics Of Frihi Date Fruits Treated By Ozone Gas:

Data of some chemical contents of date palm fruits (*Phoenix dactylifera* L. cv. Frihi) under ozone gas treatment recorded in Table (3) shows that total sugars, reducing and non- reducing sugars recorded (0.815, 0.767 and 0.048 mg/g f.w.) in treatment and (0.816, 0.765 and 0.052 mg/g f.w.) in control with no significant differences. The same trend could be applied for total proteins, which was 53.437 mg/g f.w. under ozone treatment and 54.563 mg/g f.w. under control. On the contrary amino, acids exhibited significant differences between ozone treatment (3.833 mg/g f.w.) and control (3.100 mg/g f.w.). Also, total phenols which affected by any stress, recorded significant differences between ozone treatment (316.400 mg/g f.w.) and control (193.200 mg/g f.w.). In addition, total Indoles showed significant differences between ozone treatment (0.011 mg/g f.w.) and control (0.11 mg/g f.w.).

Our results in a harmony with Hassan (2014) who reported that no clear correlation was found between the tested chemical composition and ozone gas treatment to Frihi date. Shaghaghian, *et al.*, (2014) mentioned that the proposed ozone treatment is a promising approach replacing the application of MB for disinfestations of examined date fruits, as no remarkable changes were observed on pH of the date fruits and its chemical compositions. Niakousari *et al.*, (2010) evaluated the effect of the ozonation process on the sugar content of dates and the results revealed that ozone did not have any influence on the sugar content of Kabkab dates fruits.

Table (3): Effect of ozone gas on biochemical contents of treated date fruits.

Biochemical contents	Total sugar (mg/g f.w.)	Reducing sugar (mg/g f.w.)	Non reducing sugar (mg/g f.w.)	Total protein (mg/g f.w.)	Amino acides (mg/g f.w.)	Phenols (mg/g f.w.)	Indoles (mg/g f.w.)
Control	0.816±0.0 ^a	0.765±0.0 ^a	0.052±0.00 ^a	54.56±1.25 ^a	3.10±0.12 ^b	193.2±2.3 ^b	0.11±0.01 ^a
Ozone (500 ppm)	0.815±0.0 ^a	0.766±0.0 ^a	0.053±0.02 ^a	53.43±1.20 ^a	3.83±0.15 ^a	316.4±2.4 ^a	0.01±0.00 ^b
P. value	0.5498	0.8985	0.9197	0.5440	0.0168	<.0001	<.0001
L.S.D	0.0053	0.0374	0.0479	4.7238	0.5153	9.0961	0.0161

Effect of Ozone Gas on The Larval Enzyme's Activity of *P. interpunctella*:

The effect of ozone gas treatment in the activity of some enzyme in larvae of *P. interpunctella* presented in Table (4). In the case of AchE there was insignificantly increased in the activity of treated larvae while an insignificant decrease of LDH enzyme was recorded. On the other hand, a significant decrease in the activity of MFO under ozone treatment. According to this result, we can see the ozone influence on the respiration phase in an insect. Our data are in agreement with Hetz and Bradley (2005) proposed that insects breathe discontinuously to minimize oxidative damage. Ozone acts as a toxic chemical that can cause oxidative damage to tissues even at low concentrations (Liu *et al.*, 2007). Therefore, changes in the concentration of ozone have the potential to affect the rate of respiration. Baoqian *et al.*, (2009) showed that there were decreases in the rate of respiration in *Rhyzopertha dominica* and *Tribolium castaneum* with an increase in ozone concentration. Also, Nathan *et al.*, (2006) study the effect of bacterial toxins (*Bacillus thuringiensis*) and botanical insecticides (*Azadirachta indica* and *Vitex negundo*) on lactate dehydrogenase (LDH) activity in *Cnaphalocrocis medinalis* and found that was a decrease in enzyme activity over controls at all concentrations tested. El-Shafei (2015) ; Zinhoum (2015) and Abd El-Raheman (2011) found that the activity of acetylcholinesterase enzyme increased in treated larvae of *Ephestia cautella*, *Ephestia kuehniella*, and *Sitotroga cerealella* respectively, with modified atmospheres.

Table 4: Effect of ozone gas treatment on the certain enzymes activity in the larval insect's body.

Enzymes Treatment	AchE (ug Ach Br/min/g.b.wt)	LDH (U/g.wt)	MFO (n mol sub. Oxidized/min /g.b.wt)
control	113.3±0.9 ^a	37.53±1.2 ^a	96.43±2.9 ^a
Ozone (500 ppm)	118.3±1.2 ^a	33.86±0.7 ^a	81.83±2.2 ^b
P. value	0.3641	0.0650	0.0159
L.S.D	13.57	4.032	10.086

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ARABIC SAMMARY

مكافحة واحدة من حشرات التمور المخزنة الاساسية فراشة جريش الذرة الهندية
Plodia interpunctella (Hübner)
باستخدام غاز الأوزون.

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¹: معهد بحوث وقاية النباتات – مركز البحوث الزراعية – مصر.

²: قسم افات وامراض النخيل – المعمل المركزى للنخيل – مركز البحوث الزراعية – مصر

تهدف هذه الدراسة إلى تقييم تكنولوجيا الأوزون (O_3) كأداة لإدارة ومكافحة لكل اطوار فراشة جريش الذرة الهندية (بيض، يرقات و عذارى) التي تصيب التمر المخزن وتأثيره على نشاط انزيمات اليرقات وجودة ثمار البلح المعامل. تم اختبار ثلاث تركيزات من غاز الأوزون 100 , 300 و 500 جزر في المليون ضد اطوار حشرة جريش الذرة الهندية (بيض، يرقات و عذارى) على اوقات تعريض مختلفى تراوحت بين 15 الى 150 دقيقة. اظهرت النتائج ان نسبة الموت لجميع اطوار الحشرة المختبرة تزيد بزيادة وقت التعريض فى كلتركيز منتركيزات الأوزون. الزمن اللازم للقضاء على 100 % من اطوار الحشرة قل بزيادة التركيز من 100 حتى وصل الى 500 جزء بالمليون. وصلت نسبة الموت المصححة للبيض الى 100% بعد التعرض لمدته 15 دقيقة لغاز الأوزون تركيز 500 جزء بالمليون، سجلت نسبة الموت المصححة ليرقات العمر الثانى 100 % بعد 150 و 90 دقيقة من التعريض لتركيزى غاز الأوزون 300 و 500 جزء بالمليون على التوالى . بينما وصلت نسبة الموت المصححة للعمر اليرقى الرابع 100 % بعد 150 و 120 دقيقة منالتعرض لغاز الأوزون بتركيزى 300 و 500 على التوالى. من ناحية اخرى، كانت نسبة الموت المصححة للعذارى 100% فقط عند التعرض لغاز الأوزون بتركيز 500 % لمدة 150 دقيقة . اظهرت النتائج ان البيض كان اكثر الاطوار مقاومة لغاز الأوزون بينما كان العمر اليرقى الثانى اكثرها حساسية. ومن ناحية اخرى كان العمر اليرقى الرابع أكثر حساسية من العذارى. كما أظهرت البيانات أن الأوزون لم يكن له أي تأثير على الصفات الكيميائية المختبرة لثمار التمور المعاملة. بالإضافة إلى ذلك ، كشفت النتائج أن معدل التنفس لليرقات يتأثر بغاز الأوزون .