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Efficacy of Ozone Gas against Two Stored Product Insects

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Abstract

Efficacy of ozone gas against two of stored product insects, larvae and eggs of almond moth *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) and larvae and adults of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Cucujidae) at 30 ± 1°C was tested. The results showed that mortality increased gradually by increasing exposure time of ozone gas. Mortality percent for larvae of *E. cautella* was 10% at 0.5 h. exposure and reach to 100% at 4 hrs. after 7day of exposure period. Reduction of egg numbers was 7.65% at 0.5 hrs. exposure period and increased to 81.25% at 4 hrs. These results showed that eggs more tolerant to ozone gas than the larvae. Mortality percent of *O. surinamensis* adults was 5.2% at 1h. exposure period and increased to 90.4% at 4 hrs. after 7 days of exposure. Mortality of larvae of *O. surinamensis* was 6.4% at 0.5 hrs. exposure and increased to 92% at 4 hrs. after 7 days of exposure period.

Key words: insects, ozone, *Ephestia cautella* , *Oryzaephilus surinamensis*

Introduction

Almond moth, *Ephestia cautella* is a major world-wide insect pest of stored foods; it occurs in both tropical and temperate regions and it attacks grain, nuts, dried fruits 0061nd great varieties of other stored products. The date crop of Khargeh Oasis suffers annually to a very considerable extent from the ravages of almond moth larvae, feeding on the dates from interior reducing the value of the fruit **Gough (1917**). This horticultural crop plays a great economic role in the people's life in many communities and larvae of *E. cautella* attacks fruits either in pre-harvest or in storehouse **Abdel -Salam and El-Saeady (1983)**.

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) is a common insect pest of stored grain and its products and date fruits in many regions of the world. It is usually found as secondary pest on grain and date fruits damaged by other insects, such as *Sitophilus spp.* and *Ephestia spp.*

Semi-dry dates were the most injurious by *E. calidella, E. cautella* and *O. surinamensis* through storage.

Fumigation by methyl bromide is a halogenated compound, ozone depletory and it suspected to be carcinogenic, **Taylor (1994**). The use of methyl bromide is now being stopped in Egypt since 2015. Fumigation with phosphine is widely used to control stored product pests, has high toxicity and most effective without deleterious effects on the viability of dormant grain. However, the continuous and indiscriminate use of phosphine has resulted in the evolution of resistant populations of targeted pests, **Lorini *et al.* (2007) and Pimentel *et al.* (2007).**

Recently, worldwide attention is focus on screening and developing less hazardous and cheap materials as alternative pest control method. **Graham (1997)** reported that ozone has been used for decades in Europe and recently, the Generally Recognized as Safe (GRAS) Status of this gas has been reaffirmed in the United States by the FDA. Moreover, it was in 1997 when the FDA approved ozonation for use in the U.S food processing and fresh produced industries.

Ozone application is currently attracting attention because of its inherent advantages, ozone as a fumigant is reported to kill many of stored-grain insects, **Sousa *et al.* (2008).** Also, **Fields and White (2002)** reported the possible application of ozone in food grain preservation would address the growing concern over the use of harmful pesticides to kill storage pests. Ozone acts as a toxic chemical that can cause anti-oxidative damage of tissues even at low concentrations, **Liu *et al.* (2007)**.

The objective of this work is to study the efficacy of ozone gas against the larvae ,egg of *E. cautella* and the larvae ,adult of *O. surinamensis* at 30 ± 1°C and 65 ± 5% RH.

Materials and Methods

1- The test insects:

Almond moth, *Ephestia cautella* (Lepidoptera: Pyralidae) was reared on artificial diet consists of 250g wheat ground, 25g sugar ground, 25g dry yeast and 37.5ml glycerol under 27±1 ºC and 65±5 % RH and photoperiod of 12:12 (L: D) **El-Badawy *et al.* (2013).** The emerged adults were collected daily and placed in glass cages with screen bottom to obtain eggs. The eggs also collected daily in petri dish and transferred into plastic tubes to obtain new hatched larvae.

The original cultures of saw-toothed grain, *Oryzaephilus surinamensis* adults and larva were obtained from stored grain pests Department, Plant Protection Research Institute, Egypt. The adults were

introduced to Frihi date cultivar in glass jars (1kg. capacity each). These jars were incubated at 27± 1ºC and 65±5 % RH for two weeks, then the adults were removed and the dates were kept in the jars under the previous conditions till adult emergence.

2 - Production of ozone gas:

Ozone gas was produced from 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, Egypt. The amount of ozone output was controlled by a monitor-controller having a plug-in sensor on board 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.

3 - Ozone gas application on *E. cautella* larvae:

Small jute bags each jute bag contained 50 g of artificial diet. Twenty five larvae (25 day old), 100 egg (0-24 hrs. old) of *E. cautella* and twenty five adult beetle (7-14 days old), twenty five larvae (5 day old) of *O. surinamensis* were added to each jute bag, then closed well and secured with rubber bands.

All bags were exposed inside five glass container (4 liters capacity each) as described by **Omar (1983), Zewar and Omar (1991), Ismail *et al*. (1995) and Omar *et al*. (1995).** Each container consisted of a glass jar with a short neck, closed with rubber stopper with 2 holes; one hole was for the ozone line and the other hole for tubing connected to the ozone destruct unit. Five different exposure times (treatment) of 0.5, 1, 2, 3 and 4 hours at 300ppm concentration .Three replicates for each treatment. After exposure, treatments were transferred carefully into glass jars (0.5 kg capacity each) covered with muslin cloth and secured with rubber bands. Glass jars for each replicate were observed daily for 1, 3, 5, 7 and 10 successive days to count numbers of alive larvae of *E. cautella* and larvae of *O. surinamensis* but in case of *O. surinamensis* beetle glass jars for each replicate were observed daily for 1, 3, 5, 7, 10 and 12 successive days to count numbers of alive larvae and adult then, calculate mortality percent corrected by **Abbott's formula (1925**). Untreated (control) treatment is conduct as previously mentioned, but without ozone exposure. While in case of *E. cautella* egg after 35 day, jars were examined daily to record moth emergence until the emergence of moth stopped. Mortality was estimated as reduction rate in the progeny according the following equation.

Inhibition (%) = [(No of emerged adults in control – No. of emerged adults in treatment) / No. of emerged adults in control] × 100

4 - Statistical analysis of the obtained data:

The average percent mortality of the tested insect was calculated and corrected using Abbott's formula Abbott (1925). The corrected percentages of mortalities were statistically computed according to the method of Finney (1971). Computed percentage of mortality was plotted versus the corresponding concentrations using LDP line software program to obtain the toxicity regression lines. The lethal times LT_{50} and LT_{90} were determined.

Results And Discussion

1- Effect of ozone gas on *Ephestia cautella*:

1-1- Larvae:

Table (1) shows the efficacy of ozone gas at 300 ppm against the larvae of *E. cautella* at 30 ±1°C and 65±5% RH. Data revealed that the corrected percent mortalities increased gradually by increasing the exposure time as well as the period after treatment. The results cleared that mortality of larvae was 10.4% at 0.5h exposure period after 1 days post treatment and the mortality increased after 10 days post treatment to 49.2%, while the mortality after 4 hrs exposure period was 36% and the mortality increased after 7days post treatment to 100%.

Table 1. The efficacy of 300ppm ozone gas against *E. cautella* larvae at 30±1 °C; 65 ±5%RH.

Exposure time (hours)	Mortality % after indicated days				
	1	3	5	7	10
0.5	10.4±0.32	17.2±0.32	24.0±0.57	32.0±0.57	49.2±0.32
1	18.4±0.32	30.4±0.32	40.0±0.57	53.3±0.87	65.2±0.32
2	24.0±0.57	44.0±0.57	58.4±0.87	74.4±0.32	81.2±0.87
3	29.3±0.32	48.0±0.00	64.0±0.57	82.64±1.2	90.4±0.66
4	36.0±0.57	54.64±0.32	74.4±0.66	100.0±0.0	100.0±0.0

Eggs:

Table (2) shows the efficacy of ozone gas at 300 ppm against the egg of *E. cautella* at 30 ±1°C and 65±5% RH. Obtained data showed that the reduction rate in number of emerged adults was 7.65% at 0.5 h. exposure periods and increased to 81.25% at 4 hrs. exposure period. It was noticed that the percent of emerged moth produced from each stage decreased with increasing the exposure period of ozone. These results show that egg was more tolerant to ozone gas than the larvae.

Table 2. The efficacy of 300ppm ozone gas against *E. cautella* eggs at 30±1°C and 65±5%RH.

Exposure time (hours)	No. of emerged moths after indicated days					Reduction (%)
	35	38	40	47	55	
0.5	4.000	12.00	26.33	39.66	44.33	7.650
1	3.000	8.660	23.66	35.66	37.66	21.54
2	2.330	8.000	18.66	24.66	27.66	42.38
3	2.330	5.000	10.33	14.00	16.00	66.67
4	1.000	3.660	6.660	7.660	9.000	81.25
Control	4.330	13.66	29.00	43.00	48.00	

Lethal time values and parameters of mortality of larvae of *E. cautella* exposed to ozone 300 ppm at 30°C are presented in **Table (3)**. Results showed that the LT_{50} for the larvae was 14.30 day at 0.5 h exposure periods and 1.96 day at 4 hrs exposure periods. The findings of LT_{90} were 99.14 and 17.02 day respectively.

Table 3. Lethal time values and confidence limits for the larva of *E. cautella* at various exposure periods of ozone.

Exposure time (hours)	LT_{50} (day)	LT_{90} (day)	Confidence limits				Slope ± SD	R
			LT_{50}		LT_{90}			
			lower	upper	lower	upper		
0.5	14.30	99.14	7.09	29.08	48.96	200.73	1.52±0.65	0.999
1	6.91	89.56	3.35	14.25	43.45	184.58	1.15±0.86	0.971
2	3.24	24.59	1.81	5.81	13.74	44.01	1.46±0.68	0.972
3	2.48	14.44	1.47	4.20	8.55	24.37	1.68±0.59	0.951
4	1.96	17.02	0.90	4.28	7.82	37.67	1.37±0.72	0.903

Lethal time values and parameters of mortality regression line for the eggs of *E. cautella* exposed to ozone gas .Results showed that the LT_{50} and LT_{90} were 2.08 and 6.42 hrs, respectively. The results showed clearly that the eggs were tolerant than larvae.

2-Effect of ozone gas on *Oryzaephilus surinamensis:*

2-1- Adult:

Table (4) shows the efficacy of ozone gas at 300 ppm against the adults of *O. surinamensis* at 30±1°C and 65±5% RH. The results showed that adult mortality was 5.2% at 1h exposure periods after 1 days post treatment and the mortality increased after 12 days post treatment to 36%, while that adult mortality was 30.4% at 4hrs exposure periods and the mortality increased after 12 days post treatment to 90.4%.

Table 4. The efficacy of 300 ppm ozone gas against *O . surinamensis* adults at 30 °C and 65 ±5% RH.

Exposure time (hours)	Mortalitay % after indicated days				
	1	3	5	7	12
0.5	0	0	6.64±0.32	8±0.57	12±00
1	5.2±0.32	9.2±0.32	12±0.57	16±0.57	36±0.57
2	10.4±0.32	17.2±0.87	22.4±0.66	30.4±0.32	56±0.57
3	21.2±0.32	34.4±0.87	45.2±0.32	58.4±0.32	76±0.57
4	30.4±0.32	46.4±1.2	65.2±0.32	84±0.57	90.4±0.32

2-2- Larvae:

Table (5) shows the efficacy of ozone gas at 300ppm against the larvae of *O. surinamensis* at 30±1°C and 65±5% RH . The resulted of larvae mortality was 6.4% at 0.5h exposure periods and increased to 38.4% after 10 days post treatment but at 4 hrs exposure periods was 33.2% and increased to 92% after 10 days post treatment.

Table 5. The efficacy of 300 ppm ozone gas against *O . surinamensis* larvae at 30 °C and 65 ±5%RH.

Exposure time (hours)	Mortality % after indicated days				
	1	3	5	7	10
0.5	6.4±0.320	10.6±0.32	16±0.570	24±0.570	38.4±0.32
1	14.4±0.32	26.4±0.32	34.4±0.32	45.2±0.32	58.4±0.66
2	22.4±0.32	38.4±0.32	53.2±0.32	65.2±0.32	73.2±0.87
3	28±0.570	42.4±0.32	62.4±0.32	76.0±0.00	82.4±0.32
4	33.2±0.32	50.4±0.32	68±0.570	85.2±0.32	92±0.570

Table (6) shows lethal time values and parameters of mortality regression line for the adults of *O. surinamensis* exposed to ozone gas. The presented results showed that the LT_{50} for the adult were 22.27 and 2.43 day at 1 and 4 hrs, respectively. While LT_{90} values were 108.20 and 14.18 day at 1 and 4 hrs, respectively.

Table 6. Lethal time values and confidence limits for the adult of *O. surinamensis* at various exposure periods of ozone.

Exposure time (hours)	LT_{50} (day)	LT_{90} (day)	Confidence limits				Slope ± SD	R
			LT_{50}		LT_{90}			
			lower	upper	lower	upper		
1	22.27	108.20	11.75	42.18	57.12	204.94	1.86±0.535	0.987
2	12.62	74.38	7.09	22.46	41.79	132.40	1.67±0.598	0.957
3	4.95	47.52	2.61	9.36	25.12	89.89	1.31±0.762	0.958
4	2.43	14.18	1.44	4.11	8.39	23.95	1.68±0.593	0.935

In **Table (7)** the presented results showed that the LT_{50} values of *O. surinamensis* larvae were 21.39 and 2.18 day at 0.5 and 4 hrs, respectively, while LT_{90} values were 146.47 and 12.65 day at 0.5 and 4 hrs, respectively. The results showed clearly that the adults were the most tolerant than larvae.

Table 7. Lethal time values and confidence limits for the larva *O. surinamensis* at various exposure periods of ozone.

Exposure time (hours)	LT_{50} (day)	LT_{90} (day)	Confidence limits				Slope ± SD	R
			LT_{50}		LT_{90}			
			Lower	Upper	Lower	upper		
0.5	21.39	146.47	10.22	44.76	70.00	306.49	1.53±0.65	0.991
1	9.77	135.56	4.59	20.77	63.76	288.23	1.12±0.89	0.915
2	4.25	43.99	2.20	8.22	22.75	85.05	1.26±0.79	0.977
3	2.93	23.65	1.61	5.35	12.98	43.08	1.68±0.59	0.943
4	2.18	12.65	1.28	3.70	7.44	21.48	1.68±0.59	0.943

The obtained results are in agreement with those of **Osman (2009**) who studied the effect of ozone on *E. kuehniella* at 1 g.5h /m^3 for different exposure periods of 0.5, 1, 2, 3, 4 and 5 hrs. He found that larvae required not less than 6 days after ozone exposure to reveal the full effect in the mortality rate. Also, **Isikber and Oztekin (2009)** mentioned that *E. kuehniella* is more susceptible to gaseous ozone than larvae of *Tribolium confusum*. **Abo-El-Saad *et al.* (2011)** showed that ozonation with 2.0 ppm ozone generated with various time 4, 8 and 12 hrs gave 28, 73 and 83% mortality against *E. cautella* adults, while it gave 10, 20 and 27% mortality in case of larvae, respectively. **Lu *et al.* (2009)** observed that effect of this gas on respiration had two distinct phases, a lower respiration of the tested adult and increased the respiration of *Sitophilus oryzae, Rhyzopertha dominica* and *T. castaneum* adults when ozone degraded to oxygen. **Hansen *et al.* (2012)** reported that the freely exposed stages of the eleven species from insect species and mainly adult insects for ozone gas, full control can generally be obtained with 35 ppm for 6 days. However, for control of internal stages of *Sitophilus* spp. and *R. dominica*, full mortality requires approximately 135 ppm for 8 days. **Hussain (2014)** found that the mortalities increased gradually by increasing each of exposure time to ozone gas and period after treatment. Mortality percent was 2.76 % at 1 h. exposure, followed significant by 48.60, 83.67 and 97.22 % at 2, 3, 4 hrs, respectively. and reach 100 % at 5 hrs. exposure periods after 24 hours, as

regard to 7 days after exposure to ozone gas, the data noticed that the mortality percent was 38.92 % at 1 hour exposure, followed significantly by 66.55, 88.37, 98.55 and 100 % at 2, 3, 4 and 5 hrs. exposure periods, respectively.

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فاعلية غاز الأوزون ضد حشرتين من حشرات المواد المخزونه

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**قسم افات الحبوب والمنتجات المخزونه-معهد بحوث وقاية النباتات -مركز البحوث الزراعية-الدقي -جيزة- مصر

تهدف هذه الدراسه الي تقيم فاعلية غاز الأوزون بتركيز ppm300 وعلي فترات تعريض 0.5,1,2,3,4 ساعه ضد يرقات وبيض دودة البلح العامري ويرقات والحشرات الكامله لخنفساء السورينام واجريت هذه المعاملات بالمركز القومى للبحوث وذلك علي درجة حراره 30 ±1م° ورطوبه نسبيه 65±5% . وتم تقدير الوقت اللازم لقتل 50, 90 %من الحشرات وهذا لليرقات والحشرات الكامله اما بالنسبه للبيض فتم تقدير نسبة الانخفاض فى عدد البيض . واشارت النتائج الي ان غاز الأوزون اكثر فاعلية علي يرقات دودة البلح العامري منها على البيض اما خنفساء السورينام كانت اليرقات هى الاكثر حساسيه من الحشرات الكامله وتوصلت هذه الدراسه الي ان استخدام غاز الأوزون لمكافحة دودة البلح العامري وخنفساء السورينام في المواد المخزونه هي طريقه فعاله وآمنه.