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Evaluate The Efficiency of Ozone Gas To Control Adult And Larvae of The Greaterwax Moth Laboratory. Galleria Mellonella L. (Lepidoptera: Pyralidae)

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Abstract: *The effect of ozone gas was tested to control adult[1] (male and female) and larvae (primary, intermediate, advanced) of the greater wax moth, Galleria mellonella L.The gas ozone treatment in the laboratory was conducted under temperature conditions of 38±1 Celsius and at a level of relative humidity of 45 ± 5%. During the time exposures(1.0, 2.0, 3.0, 7.0 hours), it showed that ozone gas mortality rates reached 100% (LT100) as treatments had an effect on both the insect's adult and larvae stages.The adult stage (male and female) was more sensitive to the treatments than the larval stage as the 100% mortality rate was reached in 2 hours and 2 hours, respectively, while the larval stage was most resistant to the ozone treatments and the time required to achieve 100% mortality was 7 hours.*

The study results showed that advanced larvae are most resistant from the middle and primary ages,and the time required to attain 100% mortality was 3,3,and 7hours at the preliminary, intermediate, and advanced ages, respectively. The male insect was more sensitive than females to the effect of ozone gas as the mortality rate percentages in the first hour of exposure was 0.16% and 0.0% respectively.

Treatments caused rates of mortality at 50% (LT50) in insect adult stages (male, female) and larvae (ages primary, intermediate, advanced) but the results varied significantly among them. The results showed that the rates of mortality reached50% (LT50) in the male and female insects and in each of the three ages of larvae at the following times: 50, 65,130,145 and 155 minutes respectively.

Keywords: *control ,gas , moth, ozone, wax.*

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I. Introduction

The greater wax moth, *Galleria mellonella* L., is considered one of the most important and major bee pests of honeybee products owing to the destructive feeding habit of its larva,. The damage caused by the wax moth larvae is they make tunnels in the honeycomb that are covered with strong silk strings that hinder the bees' movement and can cause severe injury.The burrowing of larvae and lining of tunnels with silk within the honeycomb leads to bee migration from the hive and weakness of the whole hive [1]. Beekeepers have lost 3300 bee hives and 13,500 frames damaged a result of the lack of control in the United States, causing losses of more than $8 million [2].

To control this pest , several methods have traditionally been used,such as the burning of severely infected bee hives to remove all stages of the greater wax moth; the use of chemicals , suchuse of Fumigants that have been previously used and have been shown to be effective against wax moth include phosphine, sulphur, acetic acid, ethylene bromide, calcium cyanide, naphthalene, carbon dioxide ,methylbromide and paradichlorobenzen [3] [4] , as well as the use of Kama rays to influence the pupae and adults using GY, [5], [6] ,[7].

The biological control cusage of *Bracon hebetorApanfeles galleria,*an ectoparasitoid of many lepidopteran pestes of the family Pyralidae [8][9]. Effect Plant extracts were first used as a beneficial and safe treatment so that different concentrations of Neem plant Azadrirachtina indica, seed and Neem leaf extract and the phenyl balls and different ages of natural bees wax combs were used to determine the mortality of the greater wax moth *Galleria mellonella* L. [10] .The alcoholic extract of cinnamon to reduce a concentration of 3% weight first instar and third greater wax moth. Experiments for the treatment of wax frames using extract alcohol and oil of Eucalyptus leaves plant have been carried outand found to protect the frame from injury for three months [11] , also mentioning that frames treated with extract concentration of 2% provided protection of frames for 10 months.

The traditional methods of using chemical pesticides to control the wax moth is still the most common, despite the failure of these methods to control pests in warehouses or prevent bees from migrating, also caused health problems for those working in this area.Poisoning resulted from the gases released in the operations of steaming infected hives and stores and also created the possibility of fires[4].In addition,there is the cost of these materials and pesticides from the foreign exchanges needed to import them from producing countries.

The outlook for the development of alternative anti-wax moth controls,like those for other agricultural pests indicated [12], relies on the need formodernresearchto focusonthe uses of ozone gas technology against insects found in stored agricultural materials to see its effect on mortality.In the United States, gas has been used in storage containers against these types of insects and adopted as a modern method to control insects on grapes, potatoes and onions[13],[14] . In a study [15] to evaluate the efficiency of the use of ozone in the control of various stages of insect T. granarium where success reached 100%mortality,the laboratory used continuous exposure to ozone gas at 400mg/12cubic meters per hour without any damage to stored food(wheat).

II. Material & Method

II.I- Insect culture

The greater wax moth was collected from infected frames from apiariesin the west of Baghdad. The adult insects collected had caused injury to several farms for breeding. The laboratory used anatural diet of wax and natural honeytaken from the hive of honey bees in plastic dishes measuring25× 10 × 5cmeach and transferred to the incubator at a temperature of 38± 1 Celsius with relative humidity of 45 ± 5% in order to ensure access to all stages of the insect. Eggs of the insect were obtained from ten pairs of modern adults (1-2 days) put on the inside of a lantern bottle covered from upper by borer plastic and the bottle base was set on a glass petri dish with a depth of 1.5 cm and a diameter of 9 cm. Egg collection for the purpose of the study was prepared with several dishes for obtaining a sufficient number of eggs; dishes were examined daily for the collection of eggs and for the larvae of the insect. Newly hatched larvae were transferred from the collection of egg dishes to new dishes with the natural food mentioned previously. Subsequently, when larvae entered the pupae stage, they were moved to new dishes and then, in the same way, cultivated as the pupae turned into adults.

II.II -Device-Generator ozone:

Generating the ozone occurred due to the production of Laisen Company electronic devices, charging an electric manner (charging) pump the amount of 12 cubic meters by 400 mg /hour.

The effect of ozone gas treatment in the insect-mover stages

Each stage of the insects was exposed to ozone gas treatment: adult (male, female) and larvae ages (primary, intermediate and advanced) within the laboratory temperature of 38± 1with the level of relative humidity45 ± 5% prevailing at the time of the test. times Exposure of 1.0,2.0, 3.0, and 7.0 h respectively, within the interior dimensions of the device (height 22 cm × width 22 cm × 29 cm depth) were used to obtain different ratios to reach the total mortality of 100%. There were three replicates per treatment and ten members of each replicate. Tested in these treatments were adult stages , aged 1-3 days and the larvae (primary: two weeks , middle: four weeks and advanced age: more than four weeks).

An additional control treatment entered in to the incubator under conditions of heat and relative humidity of 38± 1and 45 ± 5 respectively. Placed separately in the device and exposed to ozone gas by the previous measurements, the lethal rates were determined after the expiration of the exposure time. Insect samples were treated progressively under the controlled settings of the incubator at ideal conditions mentioned previously and the rates of insect mortality recorded after 24 hours.

Determine LT50% for the stages of *G. mellonella L.* under effect of ozone gas.

The stages of adults (male, female) and larvae (primary, intermediate and advanced) of the greater wax moth to exposure of ozone gas treatments, included all treatment, replicates and the different time and controls which entered into the incubator under conditions with heat and relative humidity(38± 1, 45 ± 5).

The value of LT50 calculated according to the straight-line equation between log extended exposure and probability values of lethality.

The statistical analysis.

(CRD) Complete Randomized Design tests showed less significant difference at the level of 5% probability to make sure the moral differences between the various treatment and used the analysis Nested Factorial Experiments and system software SAS for the year 2001 in the analysis of the results of these studies statistically[16].

III. Results And Discussion.

Effect of ozone gas on the larvae ages of *G. mellonella L.*.

Treatment of different ages of larvae of the great wax moth with ozone gas at the time(1.0, 2.0,3.0, 7.0) h, respectively .Table (1) showed clear effect of the treatment in the mortality of primary, intermediate and advanced larvae, with proportions of the total mortality 100% in the treatment of ozone gas at exposure time 7.0 hour in three ages respectively. Treatment of ozone within the time of 1.0 hour failed to affect these different stages in this time of exposure, the results in the table show that the time of 3.0 hours was more effective in the mortality of the three ages of larval stage. When exposure to ozone gas reached total mortality of the three ages, 100, 100 and 96%, respectively, there is a direct correlation between the increased time of exposure and the percentage of mortality in the three ages of larvae. We can conclude that there are significant differences in the sensitivity of the larval ages to the effect of ozone gas and evidence that the advanced ages were less sensitive compared to the control treatment, while the treatment primary less resistant to them. Statistical analysis showed significant statistical difference in the rates mortality of three larval ages in gas treatment.

Effect ozone gas in adult for the *G. mellonella L.* .

The results of table (2) showed effect treatment of adults great wax moth (male and female) for the ozone gas at the time (1.0,2.0,3.0, 7.0) h. respectively ,The percentage of total mortality of adults (male and female) reached 100 and 100% respectively in the gas treatment in the exposure time at 7.0 hours, while the treatment of ozone gas failed with in a time of 1.0 hour, and the results showed the time 2.0 hour was the most effective in mortality of in adult (male and female). Indicates the increase in rates of mortality for male and female of the insect to increased exposure time and there is a direct correlation between the increase in time of exposure and the percentage of mortality. There is a noticeable difference between male and female sensitivity to the effect of ozone gas at the exposure time of 1.0 hour, which shows that females were less sensitive to treatment and males were more sensitive as mortality rates equaled 0.0 for females 0.16 and for males. Statistical analysis showed no significant statistical difference in male and female mortality in gas ozone treatment from 2 to 7 hours.

Table (3) determine LT50% for *G. mellonella L.* stages under effect of ozone gas.

The results table(3) showed that the effect of ozone gas on the stages of insect adult (male ,female) and larvae(preliminary, intermediate and advanced)when exposure to these stages reached a mortality rate of 50% (LT50)the rate of time was calculated .The results showed effectiveness of ozone gas treatment increased as the exposure time increased.

Table (1) the effect of ozone gas in different ages of larvae.

Hour/Time Of E Xposure				Ages	Stages
7	3	2			
%Percentages Of Mortality					
100.0	100,0	6.0	0.0	Primary	Larvae
100.0	100.0	0.0	0.0	Intermediate	
100.0	6.09	0.0	0.0	Advanced	
0.0	0.0	0.0	0.0	Primary	0.05 Control
0.0	0.0	0.0	0.0	Intermediate	
0.0	0.0	0.0	0.0	Advanced	

LSD Ages=17.04 LSD interference =29.52

LSD Exposure time=17.04

155minutes, respectively .Statistical analysis of the results shows significant difference in the exposure times of the gas treatments necessary to achieve 50% mortality rates on the various stages of the adult and larval insects.

Table (2) the effect of ozone gas in the adult of *G. mellonella L.*.

HOUR/TIME OF E XPOSURE				SEX	STAGES
7	**3**	**2**	**1**		
%PERCENTAGES OF MORTALITY					
100.0	**100.0**	**100.0**	**0.16**	**MALE**	**ADUIT**
100.0	**100.0**	**100.0**	**0.0**	**FEMALE**	

0.0	**0.0**	**0.0**	**0.0**	**MALE**	**CONTROL**
0.0	**0.0**	**0.0**	**0.0**	**FEMALE**	

LSD interference= 0.03 LSD stage=0.01
LSD Exposure time= 0.02

Table (3) determine the half mortality time (LT50) in stages of G. mellonella when exposed to ozone gas.

TRETMENT OZONE	sexual	STAGES
HALF LETHAL 50% (LT50)		
50	MALE	ADUIT
65	FEMALE	
130	PRIMARY	LARVAE
145	INTERMEDIATE	
155	ADVANCED	

LSD larvae=96.92 LSD adult = 40.86
LSD stages=48.86

IV. Discussion

This study agreed with a number of researchers in their studies about the effect of ozone gas and high temperature , either united or separate ,on the types of insects like[17]. In the field study of both adults Trbolium castaneum, Sitophilus maize and larvae Plodia interpunctella to gas concentrations of 25 and 50 ppm, researchers found that mortality rates in the three insects ranged between 92-100% in the time of exposure of three days. The same experiment showed the researchers the effectiveness of the gas in the reduction of the presence of fungi by 63% on grain in the tests, approximately 8.9 tons of weight, and three days of ozonation at 50 ppm resembles the time required to effect phosphine .

Also recorded in their research that gas and heat factors were more effective on insect mortality at different times[18]. [19] has recorded in a study to evaluate the effect of temperatures of 40,35,30,20 degrees Celsius on a pile of grain .To assess the toxicity of ozone on the insect *Sitophilus maeze*, small packets placed inside the pile of grain were pumping ozone gas at a concentration of 50 ppm for 24-48 hours per thermal degree separately. Mortality rates calculated at the end of the time found that after 24 hours of exposure the toxicity of the ozone increased with the increase of temperatures and mortality rates were highest when temperatures reached 35-40 degrees Celsius . These results agreed with another field study, [20] that doses of ozone were lethal when used in different concentrations against the insect *Ephestia Kuhniella* in all stages of life when placed inside stacks of grain, weighing 2 kg. Using varying degrees of vacuum for two hours of exposure, the researcher noted that increased time and concentration increased mortality for subjects of the experiment and that five hours of exposure was not sufficient to reach the total mortality for this insect. The results of this study agreed with a recent study carried out by [21] when he used the mortality rates measured by the effect of ozone gas tested on different storage insects and external feeding insects such as beetles *Cryptolestes ferrugineus* (Stephens), *Oryzaephilus surinamensis*(L.), *Triboliumcastaneum* (Herbst), *Triboliumconfusum* and *Stegobium paniceum* (L.) Also moths *Ephestiakuhniella* (Zell) and *Plodiainterpunctella*(Hubner) which includes weevils such as *Sitophilus spp* and internal nutrition insects, such as the micro-grain pest *Rhizopertha domenica* (F.) and moth *Sitotrogacerealella*. Research has shown that increasing the concentration and time of exposure caused an increase in mortality on storage insects. Ozone used at more than 50 ppm concentration for four days resulted in the scaling of population density of storage insects, and at concentration of 135 ppm for a time of eight days produced 100% mortality of the insects tested and proved to the researcher that ozone gas does not leave a taste or smell in the material treated and it also removes odors resulting from storage.

When interpreting the effect of ozone gas and possible damage, it has been suggested that if the respiratory tract is the main way for ozone to enter the insect's body, an increased respiratory rate with increasing temperature produces an increase in the percentage of mortality as the speed of ozone gas enters the body .An increase in the effect of ozone increases with rising temperatures, but research found there is no significant relationship between respiratory rate and sensitivity to ozone. There fore it is difficult to determine the lethal dose of ozone on insects in grain piles and the study explained the reason for this difference is due to the effectiveness of ozone and time to deteriorate, unlike insect-prone laboratory where it arrives directly and effectively high, and the larval ages (primary and intermediate and advanced) were different in the doses needed for 100% mortality. In the search[15] ,evaluation of the efficiency of the use of ozone gas with high temperatures and heat alone in combat stages animated beetle grain (Akhabra) in vitro and in interpreting the effect of ozone on insects , remember that ozone works to break down cell membranes through a process of Peroxidation process formation (H_2O_2), OH and O and change the chemical composition influenceon the nature of the fat layer phospholipids layer.

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