



## Efficacy of Ozone Gas against All Stages of Red Flour Beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) at Different Temperatures and Exposure Periods

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**Abstract:** Ozone efficacy (approximately of 600 Mg.hr<sup>-1</sup>) was evaluated against Red Flour Beetle's (RFB, *Tribolium castaneum* (Herbst)) eggs, larvae, pupae and adults at different temperatures (35, 40 and 45 °C) and exposure times (1, 2, 4, 6, 8, 10 hours) under laboratory. The control treatments included above thermal levels without ozone at the mentioned exposure periods. The results indicated ozone effectively suppressed hatching with increasing temperature. The hatch egg rates was reduced to 0 % in the treated treatments after 10 h at 45° C. However, mortality rates of RFB were increased with maximizing of the exposure time to ozone. After 10-h exposure to ozone at the lowest temperature 35° C, complete mortality (100%) or few survivals of RFB were recorded in the susceptible stages (larvae and adults). Also, the complete mortality of adults and larvae resulted after an exposure to ozone at 40 ° C for 10 hours, compared than the high survival rates at the pest's stages at 30 and 40° C, even after 10 hours of monitoring time. However the exposure time which is required to 100% mortality was decreased to 4 hours at 45 ° C, comparing to the thermal treatment only (control) which needed more time (6 h) for 100% of mortality. In conclusion, ozone application showed the efficacy on the mortality at all stages of RFB and the temperature was a potential factor enhancing the application of ozone for RFB control.

**Keywords:** Ozone gas, *Tribolium castaneum*, Non chemical control, survival.

## Introduction

Wheat is one of the first grains known to people and is currently one of the most important food staples for humanity, which is the main source of carbohydrates and about 20% of the total dietary calories worldwide (Shiferaw *et al.*, 2013; Juárez. *et al.*, 2021). Stored product insect pests are mainly

responsible for heavy losses of grain through their feeding behavior, gradual gathering of the heat and moisture, reducing grain quality and germination, as well as increasing microbial contamination (Tefera *et al.*, 2011; Kumar & Kalita, 2017). Red Flour Beetle (RFB) *Tribolium castaneum* is one of most important stored product insect pests

worldwide. It is a primary pest of flour and other grain products, and a secondary pest of stored wheat, causing severe damage to these grains and their food products (Iram *et al.*, 2013; Aboelhadid & Youssef, 2021). Both larvae and adults feed on flours and broken grains (Karunakaran *et al.*, 2004; Rees, 2004; Mariadoss & Umamaheswari, 2020).

Controlling the stored product insect pests has become enormously difficult, due to many reasons such as developing of the pesticide resistance in some insects. Whalon *et al.* (2008) reported that the rusty flour beetle showed a high level of resistance to the action of pesticides up to 200 folds. To reduce acute and chronic risks from the use of insecticides, it has prompted radical changes in pest management strategies and the substitution of pesticides with effective but less dangerous substances (Pretty & Bharucha, 2015). Ozonation is one of the promising alternatives against wide range of insects of stored products such as *T. confusum* and *Oryzaephilus surinamensis*, *Sitophilus zeamais*, *Rhyzopertha dominica* (Kells *et al.*, 2001; Mason *et al.*, 2006, Pimentel *et al.*, 2007; Sousa *et al.* 2008; Tiwari *et al.*, 2010; Sabeat, 2017; Kopacki *et al.*, 2021; Seyedabadi *et al.*, 2021). Ozone (O<sub>3</sub>) is extremely oxidative and environmentally friendly because of its decomposing rapidly to oxygen without leaving any residue side effects on the treated grain (Mendez *et al.*, 2003; Khadre *et al.*, 2001). It is used in many fields such as water purification, bleaching and sterilization of medical devices, as well as for removing odors, colors, pesticides, inorganic materials, and organic compounds (Forney *et al.*, 2007; Wei *et al.*, 2007). Moreover, it is easy to generate directly, that eliminates the need to store or dispose of dangerous chemicals (Isikber & Öztekin, 2009).

The objective of this study was to evaluate the ozonation against Red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) at different temperatures and exposure time.

## Materials & Methods

### *Tribolium castaneum* colony

Red flour beetle *T. castaneum* was obtained from the General Company for Grain Trading, Branch of Basrah; 30 pairs of sex-segregated adults (♀♂) were used to maintain the culture of RFB. The colony was maintained in sterilized 500 ml glass jars filled with wheat flour and 5% yeast powder as a source of vitamin B1 (Sial *et al.*, 2017) at  $28 \pm 2^\circ \text{C}$  and  $65 \pm 5\%$  relative humidity in a growth chamber (Binder, Germany) Several pairs of adults were reared to get eggs by removing the parent beetles after one day from ovipositing. The eggs were monitored daily to obtain the first instar-larvae. Pupae were separated daily from the colony and sex segregated. Females and males were distinguished by examining the end of the abdomen of virgin beetles under a microscope; they have a pair of abdominal appendages which are larger in females than in males (Fedina & Lewis 2006; Beeman *et al.*, 2009). The colony of offspring was maintained under the same previous conditions. The insect was identified at Research Center and Natural History Museum/University of Baghdad.

### Bioassays

The treatments included exposure and non-exposure to ozone at different temperatures (35, 40 and 45°C) and different exposure times (1, 2, 4, 6, 8 and 10 hours). All stages of *T. castaneum*, eggs, pupae (One day age), larvae (1st, 3rd and 5th instar) and adults (female and male) were exposed to ozone by using ozone generator (ROHS Model GL-3189A, China) with the capacity of 600 Mg.hr<sup>-1</sup>. The

treatments of none ozone exposure were designated to be under similar experimental conditions at the same duration, but without the ozone treatment. The bioassay of each RFB stages was conducted in glass container (5 cm diameter and 6 cm height) which covered with mesh. A drying and heating chamber (BINDER, Germany) with dimensions of (40 cm width, 38 cm height, 32 cm depth) was used as an insect exposure device<sup>20</sup> which providing temperature fixed temperature as required in each bioassay. All openings of the exposure device was blocked with an exception of that connected to the ozone generator as well as the exit opening of the gas. Each treatment was carried out in three replications and at a rate of ten individuals for each replicate, pupae and whole adults were placed separately in plastic containers of 200 ml containing the aforementioned food medium, covered with Bored cloth type fabric and tightly tied with rubber bands. As for eggs, glass dishes with a diameter of 5 cm were used for this purpose. Ten individuals were used in each container at triplicates for each treatment. After the exposure to ozone, the freely exposed RFB's stages were incubated under the same colony conditions. The percentage of killing was calculated after 24 hours of incubation of the treatments in the incubator under the ideal temperature and humidity after each experiment. As for the eggs and pupae, the percentage of hatching is calculated after the time required for hatching has passed, as a maximum of 12 and 8 days for emergence pupae.

### Statistical analysis

The hatchability and mortality ratios of the exposed RFB stages were transformed into an Arcsine transform to normalize changes in heterogeneous values, then corrected for

control of mortality using the Schneider-Orelli formula (Püntener, 1981). The experiments were conducted with complete randomized design and the means were tested using ANOVA. The means were compared with the lowest LSD significant difference with 5% probability level using the SPSS statistical program (IBM SPSS Statistics 24).

### Results

Results of the RFB bioassay (Tables 1-4) for the ozonation showed different responses according to the temperatures and the exposure times to the ozone.

### Eggs

The results of the survival depend on hatching found of RFB eggs (Table 1) showed that the hatching rate of eggs were significantly different at different temperatures and exposure times to ozone ( $F = 1298.57$ ;  $df = 72$ ;  $p < 0.0001$ ). The inhibition of hatching egg rates due to exposure to ozone were increased with the increase of the temperature. At the temperature of 30° C, the high hatching egg rates was 96.7% after 1 h of the exposure to ozone and reduced up to 50% after 10 h of the exposure to the gas, compared to the 100 and 90 of hatching egg rates after 1 and 10 h at 30° C in the untreated treatment, respectively. The hatching rate in exposed eggs to ozone for 1 h was 93.3% at 40°C, while the rate was reduced to 36.7% after 10 h of the exposure to ozone at 40°C; compared to untreated hatching rates, which did not decrease significantly with increased exposure to ozone at the same temperature (40 °C). The highest hatching rate reduction (0 %) was examined at the treated treatments after 10 h at 45° C, compared to 53.3% of hatching rates in the treated treatments after 1 h at the same tested temperature.

**Table (1): Hatchability percentages for *T. castaneum* eggs exposed to ozone gas 600 Mg.hr<sup>-1</sup> at different temperatures and exposure times.**

Hatch rates of exposed eggs to ozone at different temperatures ± SE						
Exposure time (h)	35° C		40° C		45° C	
	Not exposed	Exposed	Not exposed	Exposed	Not exposed	Exposed
1	100±0.0 a	96.7±3.3 ab	100.0±0.0 a	93.3±3.3abc	70.0±5.8 g	53.3±6.8 ij
2	100±0.0 aa	90±0.0 de	96.7±3.3 ab	86.7±3.3 cd	53.3±3.3 ij	33.3±8.8 kl
4	96.7±3.3 abab	83.3±3.3 de	96.7±3.3 ab	80±0.0 def	36.7±3.3 k	26.7±8.8 l
6	93.3±3.3 abc	70±0.0 g	93.3±3.3 abc	66.7±3.3 gh	30.0±0.0 kl	6.7±3.3 n
8	93.3±3.3 abc	63.3±3.3 gh	90.0±0.0 de	60±5.8 hi	16.7±3.3 m	3.3±3.3 n
10	90.0±0.0 d	50±5.8 j	83.3±3.3 de	36.7±3.3 k	3.3±3.3 n	0.0±0.0 n
mean	95.6	75.6	93.3	70.6	35	20.56

The means followed by different letters have significant differences. As for the means followed by one of the same letters, they are not significant. Value of LSD (0.05) = 6.96

### Larvae

In RFB larvae, mortalities due to ozone also increased with the increase of temperatures and exposure times to ozone ( $F = 4092.99$ ;  $df = 216$ ;  $p < 0001$ ; Table 2). The complete mortality of 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> larval instars were reported after 10 h of exposure to ozone at 35° C compared with untreated treatment (0%). The larvae showed more susceptibility to ozone and the exposure time was reduced with increasing the temperatures; the mortality rates increased to 100% after 8 and 10 h of exposure to ozone at 40° C in 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> larval instars respectively compared to the untreated larval instars (13.3, 10 and 0% mortality rates).

The exposure time was decreased more at 45° C and led to a complete mortality after 2 h for the 1<sup>st</sup> and 3<sup>rd</sup>, and 4 h for the 5<sup>th</sup> instar compared to the mortality rates on larvae (80, 73.3 and 83.3% respectively) at the same temperature and duration with no exposure to ozone.

### Pupae

The results also indicated that the exposure to ozone at temperatures of 30 and 40° C caused a significant ozone-dependent mortality in the pupae of RFB ( $F = 575.62$ ;  $df = 72$ ;  $p < 0001$ ;

Table (2): Mortality percentages of *T. castaneum* larvae, exposed to ozone gas 600 Mg.hr<sup>-1</sup>at different temperatures and exposure times.

Tem.	Treatment	Instars	Mortality percentages (Means ± SE)						means
			Exposure time (h)						
			1	2	4	6	8	10	
35° C	Not exposed	1 <sup>st</sup>	0.0±0.0u	0.0±0.0u	0.0±0.0u	0.0±0.0u	0.0±0.0u	0.0±0.0u	0
		3 <sup>rd</sup>	0.0±0.0u	0.0±0.0u	0.0±0.0u	0.0±0.0u	0.0±0.0u	0.0±0.0u	0
		5 <sup>th</sup>	0.0±0.0u	0.0±0.0u	0.0±0.0u	0.0±0.0u	0.0±0.0u	0.0±0.0u	0
	Exposed	1 <sup>st</sup>	6.7±3.3 st	13.3±3.3r	36.7±3.3n	73.3±3.3gh	93.3±3.3bc	100±0.0a	53.89
		3 <sup>rd</sup>	0.0±0.0 u	10±0.0 rs	26.7±3.3p	63.3±3.3jk	90±0.0 cd	100±0.0 a	48.33
		5 <sup>th</sup>	0.0±0.0 u	3.3±3.3 t	20.0±0.0q	53.3±3.3l	80±5.8 ef	100±0.0a	42.78
40° C	Not exposed	1 <sup>st</sup>	0.0±0.0 u	0.0±0.0 u	0.0±0.0 u	3.3±3.3 t	6.7±3.3 st	13.7±3.3r	3.89
		3 <sup>rd</sup>	0.0±0.0 u	0.0±0.0 u	0.0±0.0 u	0.0±0.0 u	13.3±3.3r	10.0±0.0rs	2.22
		5 <sup>th</sup>	0.0±0.0 u	0.0±0.0 u	0.0±0.0 u	0.0±0.0 u	0.0±0.0 u	0.0±0.0 u	0
	Exposed	1 <sup>st</sup>	13.3±3.3r	30.0±5.8op	50.0±5.8l	80.0±0.0ef	100±0.0a	100±0.0a	62.22
		3 <sup>rd</sup>	6.7±3.3 st	43.3±3.3m	13.3±3.3r	80.0±0.0ef	100±0.0a	100±0.0a	58.89
		5 <sup>th</sup>	0.0±0.0 u	13.3±3.3r	36.7±3.3n	70.0±5.8hi	96.7±3.3ab	100±0.0a	52.78
45° C	Not exposed	1 <sup>st</sup>	66.7±3.3ij	80.0±5.8ef	96.7±3.3ab	100±0.0a	100±0.0a	100±0.0a	90.56
		3 <sup>rd</sup>	60.0±5.8k	73.3±3.3gh	90±0.0 cd	100±0.0a	100±0.0a	100±0.0a	87.22
		5 <sup>th</sup>	53.3±3.3l	66.7±3.3ij	83.3±3.3e	96.7±3.3ab	100±0.0a	100±0.0a	83.33
	Exposed	1 <sup>st</sup>	80±0.0 ef	100±0.0a	100±0.0a	100±0.0a	100±0.0a	100±0.0a	96.67
		3 <sup>rd</sup>	76.7±3.3fg	100±0.0a	100±0.0a	100±0.0a	100±0.0a	100±0.0a	96.12
		5 <sup>th</sup>	73.3±3.3gh	96.7±3.3ab	100±0.0a	100±0.0a	100±0.0a	100±0.0a	95

The means followed by different letters have significant differences. As for the means followed by one of the same letters, they are not significant. Value of LSD (0.05) = 6.56.

**Table (3): Mortality percentages of *T. castaneum* pupae exposed to ozone gas 600 Mg.hr<sup>-1</sup> at different temperatures and exposure times.**

Mortality percentages (Means± SE)						
Exposure time (h)	35° C		40° C		45° C	
	Not exposed	Exposed	Not exposed	Exposed	Not exposed	Exposed
1	0.0±0.0 k	0.0±0.0 k	0.0±0.0 k	0.0±0.0 k	3.3±5.8 k	60.0±0.0 d
2	0.0±0.0 k	0.0±0.0 k	0.0±0.0 k	6.7±3.3 jk	30.0±10.0 g	80.0±5.8 c
4	0.0±0.0 k	6.7±3.3 jk	0.0±0.0 k	13.3±3.3 ij	80.0±10.0 c	96.7±3.3 ab
6	0.0±0.0 k	20±5.8 hi	3.3±5.8 k	26.7±3.3 gh	100±0.0 a	100±0.0 a
8	0.0±0.0 k	40±5.8 f	13.3±5.8 ij	50±5.8 e	100±0.0 a	100±0.0 a
10	0.0±0.0 k	60±5.8 d	16.7±5.8 i	76.7±8.8 c	100±0.0 a	100±0.0 a
Mean	0.0	21.1	5.6	28.9	68.9	89.4

The means followed by different letters have significant differences. As for the means followed by one of the same letters, they are not significant. Value of LSD (0.05) = 8.85

**Table (4): Mortality percentages of females and males, *T. castaneum*, exposed to ozone at a concentration of 600 Mg.hr<sup>-1</sup>, at different temperatures and exposure times.**

Tem.	Treat.	Sex	Mortality percentages (Means±SE)						mean
			Exposure time (h)						
			1	2	4	6	8	10	
35° C	Not exposed	♀	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	0
		♂	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	0
	Exposed	♀	0.0±0.0 r	3.3±5.8 r	13.3±5.8 pq	36.7±5.8 kl	70.0±0.0 ef	90.0±10.0 bc	35.55
		♂	3.3±3.3 r	20.0±5.8 no	30.0±0.0 lm	46.7±3.3 ij	76.7±3.3 de	96.7±3.3 ab	45.57
40° C	Not exposed	♀	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	6.7±3.3 qr	16.7±3.3 op	3.9
		♂	0.0±0.0 r	0.0±0.0 r	0.0±0.0 r	6.7±3.3 qr	13.3±3.3 pq	30.0±7.8 lm	8.33
	Exposed	♀	6.7±3.3 qr	26.7±3.3 mn	36.7±3.3 kl	60.0±0.0 gh	73.3±3.3 e	100±0.0 a	50.57
		♂	13.3±3.3 pq	26.7±6.7 mn	43.3±3.3 jk	63.3±3.3 fg	83.3±3.3 cd	100±0.0 a	54.98
45° C	Not exposed	♀	53.3±3.3 hi	76.7±3.3 de	93.3±3.3 ab	100±0.0 a	100±0.0 a	100±0.0 a	87.22
		♂	63.3±3.3 fg	83.4±3.3 cd	96.7±3.3 ab	100±0.0 a	100±0.0 a	100±0.0 a	90.57
	Exposed	♀	73.3±3.3 e	93.3±3.3 ab	100±0.0 a	100±0.0 a	100±0.0 a	100±0.0 a	94.43
		♂	83.3±3.3 cd	100±0.0 a	100±0.0 a	100±0.0 a	100±0.0 a	100±0.0 a	97.22

The means followed by different letters have significant differences. As for the means followed by one of the same letters, they are not significant. Value of LSD (0.05) =7.76

Table 3). The mortalities were 60 and 76.7 after 10 h of exposure to ozone, respectively in comparison with to the untreated treatments (0 and 16.7% respectively). Whereas, the mortality rate increased 96.7% and 100% after 4 and 6 h of exposure to ozone at 45° C, compared to mortalities of 80 and 100% respectively in the untreated treatment at the same duration of exposure to the gas and temperature.

### Adults

There was a significant ozone effect on overall mortalities of females and males of *T. castaneum* ( $F = 1868.3$ ;  $df = 144$ ;  $p < 0001$ ). Mortalities increased with the increase of temperatures and exposure times to ozone ( $F = 334.9$ ;  $df = 5$ ;  $p < 0001$ ) (Table 4). The mortality rate of RFB females and males increased significantly and reached the highest rates of 90 and 96.7% after 10 h of exposure to ozone at 35° C, respectively compared to the untreated treatments, whereas the mortality rates of females and males were 0% after 10 h at 35° C. Females and males of *T. castaneum* became more susceptible to ozone with the increase of the temperatures; the mortality rates of adults increased to 100% compared to the untreated (16.7 % of ♀ and 30.0% of ♂) after 10 h at 40° C. Moreover, the exposure time was decreased with increasing the temperature; exposed adults to ozone at the temperature of 45° C caused the complete mortality after 2 h for the males and 4 h for the females compared to the untreated adults (96.7 % of ♀ and 83.4% of ♂) at the same temperature and duration with no exposure to the gas.

### Discussion

The mortality and hatch egg rates of RFB *T. castaneum* infesting wheat were linked to the ozonation (approximately of 600 mg.hour<sup>-1</sup>); the rates were increased with increasing of the

temperature and the exposure time to ozone. When ozone was applied at the lowest examined temperature (35° C) for 10 h, complete mortality or few survivals of RFB were recorded in the susceptible stages (larvae and adults) compared to zero mortalities in control treatments; whereas, a 50% or more survivals were reported in less susceptible stages (eggs and pupae). Additionally, the ozone treatment at 40° C for 10 h caused complete mortality at all stages with an exception of eggs and pupae. Subsequently, the full mortality of adults, pupae and larvae stages were recorded at 4, 6, and 4 h exposure of ozone at 45° respectively, but the exposure for 10 h led to entire mortalities at all stages of RFB. A few other studies have recorded the effect of ozonation with heating on the stored grain insects; Sabeat (2017) revealed the effectiveness of using ozone gas (400 mg.h<sup>-1</sup>) with increasing of temperature levels and exposure times against larvae and adults of *T. castaneum*. Sabit & Sabr (2015) indicated that applying ozone gas with heat increased the mortality of *Trogoderma granarium* stages.

Insects respond to ozone as a toxic chemical and as a reaction they breathe intermittently to reduce their damage, however it may cause oxidative tissue damage even at low concentrations (Hetz & Bradley, 2005). The effect of ozone on the respiration of insects, *Sitophilus oryzae*, *Rhizopertha dominica* and *T. castaneum*, occurs in two stages. The first includes less respiration when under the influence of ozone and in the second stage it rises with continuing treatment, the ozone gas enters directly into the insect cavity through the respiratory system and then diffuses to the rest of the tissues through the air tubes that bifurcate throughout the body to deliver oxygen mainly to the mitochondria (Lu *et al.*, 2009; Harrison *et al.*, 2012). The circulatory system can also participate in gas exchange by



ventilating the bronchial system (Wasserthal, 1996; Miller, 1997). Because carbon dioxide can diffuse more easily through tissues compared to oxygen (Schmitz & Perry, 1999), therefore, an imbalance occurs in the gases within the insect's tissues between oxygen and carbon dioxide, which leads to an increase in breathing as well as the heart rate. The ozone penetrates the cell membranes, heading directly to the mitochondria, where it oxidizes energy compounds, causing starvation of the insect with a high drainage of ATP energy compounds, where signs of stopping movement and vital activities that end in the insect's death (Harak *et al.*, 1999). Ozone and its degradation products into free radicals (reactive oxygen species) interact directly with proteins, DNA, and polyunsaturated fatty acid double bonds and destroy biomolecules such as DNA (Hermes-Lima, 2004; Korsloot *et al.*, 2004).

The optimization of the efficiency of ozonation procedure with rising temperature have been recorded in this study, that may be attributed to ozone degradation which is accelerated at high temperature (Kim, 1998; Itoh *et al.*, 2020). Overall, the results of the ozonation application showed the efficacy of ozone on RFB mortality and the potential of temperature as a vital factor for application of ozonation for RFB control. Due to the difference in the thickness and nature of the body wall between the different roles of the same type of insect, it leads to the difference in the killing rates when exposed to high heat, and the difference in sensitivity between the larval stages may be due to the same reason, as the high killing rates are explained in the different roles of the insect in the treatment (ozone + heat and with shorter periods of time than treating heat alone to two main factors, the mechanism of opening the insect's respiratory stomata due to lack of oxygen and

thus rapidly losing water from its body as a result of the other factor, heat (Wigglesworth, 1972). The high temperature affects the protoplasm and the insects die when the phospholipids layer in the cell membranes becomes more liquid, in addition to the structure of proteins and thus the enzymes are adversely affected by the high temperature, and the high temperature also affects the pH, which negatively affects the medium in which these enzymes work. Water stress is a critical factor between 35 and 43 °C (Papanikolaou *et al.*, 2018). As for eggs and pupae, they were more tolerant to ozone than larvae and adults, and this was due to the barriers that prevent ozone from reaching the target sites, and ozone needs to cross the outer shell of the egg to come into contact with the insect's embryo, as well as the reduced respiration rate and metabolism of insect eggs and pupae (McDonough *et al.*, 2011).

Holmstrup *et al.* (2011) showed that ozone causes an overall decrease in metabolism (including transcription of genes to detoxify reactive oxygen species due to respiratory vents being closed for longer than normal in response to the presence of ozone). As for Sabat & Sabr (2015), they showed that ozone breaks down cell membranes through the process of oxidation and forms (H<sub>2</sub>O<sub>2</sub>), OH and O<sub>2</sub> and changes their chemical composition by affecting the nature of the phospholipid layer.

### Conclusions

The results indicated that ozone gas could be one of the important alternatives to the pesticides used in controlling the RFB, especially fumigants, and that the heat could be used as a synergistic agent with ozone, as ozone could be used as a component of the integrated management of stored insect pests. The results also showed that the temperature

was inversely proportional to the time required to kill all the roles of the insect, while both the temperature and time were directly proportional to the killing rates, and despite the sensitivity of the red flour beetle adults to ozone gas in general, the males were more sensitive than the females, while the eggs and the pupae required more time. To reach the percentage of 100% perdition of the remaining roles.

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## Conflicts of interest

The authors declare that they have no conflict of interests.

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## فعالية الأوزون ضد خنفساء الطحين الحمراء (*Tribolium castaneum* (Herbst)

### (Coleoptera: Tenebrionidae) عند مستويات حرارة وأوقات تعرض مختلفة

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**المستخلص:** أجري البحث مختبريا لمعرفة فاعلية غاز الأوزون برفقة الحرارة او الحرارة المنفردة عند (35،40،45) °م لكلا المعاملتين في نسبة قتل أطوار حشرة *Tribolium castaneum* خلال المدد الزمنية المنتخبة (ساعة، ساعتين، 4، 6، 8، 10) ساعات فكانت النتائج حصول نسبة قتل 100% للبالغات ولكلا الجنسين في معاملة الحرارة المنفردة 45 °م بعد ستة ساعات اما في المعاملة المزدوجة (اوزون+حرارة) فتم الوصول الى نفس النسبة ولكن عند درجة حرارة 40 °م وبعد 10 ساعات وفي درجة 45 °م فتطلب الامر ساعتين فقط للذكور و4 ساعات للإناث. وفي البيوض وصلت نسبة الفقس الى 0،0% في معاملة (اوزون+حرارة) عند 45 °م بعد 10 ساعات. اما العذارى حيث بلغت النسبة 100% في كلتا المعاملتين عند 45 °م مع وجود فروق معنوية بين بقية مدد التعريض. اما الطور اليرقي فقد أظهرت الأعمار المبكرة حساسية أكبر من المتقدمة بالعمر فبرغم الحصول على نسبة قتل 100% لجميع الاعمار قيد الدراسة في معاملة (اوزون+حرارة) عند 35 °م بعد 10 ساعات الا ان الفروق المعنوية كانت واضحة بين لأعمار المختلفة وفي المدد المختلفة فقد تم الحصول على 100% قتل عند 40 °م بعد ثمان ساعات للعمرين الأول والثالث اما الخامس فكانت بعد 10 ساعات وفي درجة حرارة 45 °م تم الحصول على نفس النسبة من القتل للعمرين الأول والثالث بعد ساعتين اما الخامس فكانت بعد اربعة ساعات. وأشارت النتائج الى ان الوقت اللازم للموت يتناسب عكسيا مع ارتفاع درجات الحرارة وكلاهما يتناسب طرديا مع نسبة الموت وان استخدام غاز الأوزون كان له الأثر الكبير في تقليص الوقت والحرارة اللازمين للموت.

الكلمات المفتاحية: الأوزون، *Tribolium castaneum*، خنفساء الدقيق الصدفية، مكافحة غير كيميائية.