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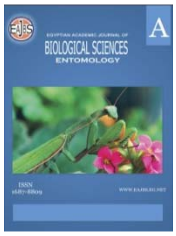


# Effect of Ozone Gas on the Red Flour Beetle, *Tribolium castaneum* (Herbst)

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**ABSTRACT**

Red flour beetle, *T. castaneum* (Herbst) is worldwide and most destructive pest of stored products and is cosmopolitan in distribution. It is the most common pest of wheat flour. It also causes serious damage upon dried fruits, pulses and prepared cereal foods. Larvae and adults of this pest were treated with ozone as a gas at three concentrations (1, 3, and 5 g/m$^3$) for six different periods (0.5, 1, 2, 3, 4 and 5 h.) compared with untreated insects. The results indicated that increasing the concentration and exposure period led to increasing the rate of mortality for both tested stages moreover to latent effect of ozone on pupation and adult emergence of this insect pest.

## INTRODUCTION

The red flour beetle, *T. castaneum* (Herbst) is worldwide and most destructive pest of stored products and is cosmopolitan in distribution. It is the most common pest of wheat flour. It also causes serious damage upon dried fruits, pulses and prepared cereal foods, such as cornflake, pasta, biscuit, beans, nuts, etc. It is an often the most common species in the pest complex attacking stored wheat although its pest status is considered to be secondary, requiring prior infestation by an internal feeder, it can readily infest with or other grains damaged in the harvesting operation. Both larvae and adults feed on grain dust and broken grain, but not the undamaged whole grains and spend its entire life cycle outside the grain kernels (Karunakaran *et al.*, 2004). In severe infestation, the flour turn grayish and has a pungent, disagreeable odour- making it unfit for human consumption. This insect causes substantial loss in storage because of its high reproductive potential (Prakash *et al.,* 1987). Alternative methods for effective post-harvest pest control have been required due to the restriction of chemical fumigants such as methyl bromide which has been a major stored-product and quarantine treatment but it has harmful effects on human health and the environment inducing significant ozone depleting substance (Ross, 1999).Currently, bulk commodities are often fumigated but the number of fumigants registered around the world is extremely limited and very few of the new treatments are acceptable for all applications. Additionally, the growing demand for organic grains has generated a need for control strategies for this niche market.

Ozone, a powerful oxidant, has numerous beneficial applications and is very familiar to the food processing industry.

It has been long been used in food processing as a water treatment to disinfect, eliminate odours, taste and colour (Kim *et al.,* 1999; EPA, 1999). Ozone ($O_3$) is an allotrope of oxygen, which can be generated by UV-light and electrical discharges in air (corona-discharge). Ozone generation by electrical discharge is most common and has several advantages, including greater sustainability of the unit and higher ozone production. Ozone has a half-life of 20-50 min, rapidly decomposing to diatomic oxygen, a natural component in the atmosphere. Because ozone can be easily generated at the treatment site using only electricity and air, it offers several safety advantages over conventional post-harvest pesticides. First, there are no stores of toxic chemicals, chemical mixing hazards, or disposal of left over insecticides or containers (Law and Kiss, 1991). Second, with a short half-life, it reverts back to naturally occurring oxygen leaving no residue on the stored products. Researchers have been recently focused on the application of ozone as a fumigant to control stored-grain insects and microorganisms and to reduce mycotoxins on grain (McDonough *et al.,* 2011; Mylona *et al.,* 2014; Savi *et al.,* 2015) and this purpose of this study is to know the effect ozone gas on *T. castaneum.*

## MATERIALS AND METHODS

The stock culture of the red flour beetle, *Tribolium castaneum* was obtained from the plant protection institute, Ministry of Agriculture. Before the experiments were started *T. castaneum* adults were maintained on sterilized wheat flours, after two days, the adults were screened out from the flours using 60 meshes to the inch sieve. The adults were discarded and the mediums, containing eggs were kept in the rearing room.

**Ozone generation techniques:**

The DBD cell consists of two cylindrical coaxial electrodes separated by a gap distance and dielectric barrier (glass). AC (50 Hz) high voltage (2-5 kV) was applied on the DBD cell to generate filamentary discharge. The DBD cell is fed by oxygen gas. The basic mechanism of ozone generation simply consists of dissociation of oxygen molecules by the discharge electrons that are formed in the discharge filaments inside the discharge gape. The atomic oxygen, which is produced due to the dissociation, reacts with the oxygen molecules to form ozone. The concentration of the generated ozone was controlled by the discharge current and the gas flow rate was adjusted to 5 L/min. The concentration of ozone formed inside the DBD system was measured using ozone detector (Model H1-AFX-Instrumentation, USA) (Garamoon *et al*., 2009). Ozone was applied directly into the tubes containing the various insect stages under investigation.

**Exposure of larvae and adults of *T. castaneum* to ozone:**

larvae 33 day old (50 larva) and adults 1 day old (50 adult) of *T. castaneum* exposed by different treatments of ozone gas viz. 1.0, 3.0, and 5.0 g/m$^3$ for 0.5, 1, 2, 3, 4 and 5 h. compared with untreated insects, the experiments were carried out in three replicates. By the end of the tested exposure periods, the treated different stages were taken out and incubated under the optimum constant conditions of 25± 2°C and 65 ± 5% r. h. Treated larvae was transferred to glass jars (5 cm in diameter and 15 cm in depth) covered with muslin cloth for incubation under the same optimum conditions and were examined daily until the pupation and adult emergence and treated adult was examined daily to record percentage of mortality which were corrected according to Abbott's formula (Abbott, 1925). The data were statistically evaluated by analysis of variance (F) followed by Duncan's multiple range test 1955 to examine the significant differences between treatments. The 5% level of probability was used in all statistical tests. The statistical software program Cost at was used for all analyses.

## RESULTS AND DISCUSSION

### Effect of ozone treatment on *T. castaneum* larvae:

While the data in Table (1) clearly show that both exposure period to ozone and the gas concentrations have obvious influence on percentage of pupation and adult emergence of *T. castaneum*. The lowest percentages of pupation (53.33± 3.3, 30.00±1.8 and 0.00±0.0%) were recorded at 5h. exposure period when concentration of ozone was 1, 3 and 5g/$m^3$comparing with control (97.20±3.0%), respectively. The same trend was obtained in respect to adult emergence. The lowest percentages of adult emergence (74.43±7.3, 0.00±0.0 and 0.00±0.0%) were recorded at 5h. exposure period when concentration of ozone was 1, 3 and 5g/$m^3$comparing with control (100.0±0.0%). Similar results were obtained by Kells *et al*. (2001) found that a higher mortality rate for the larval stage compared with other stages of *P. interpunctella* exposed to 50 ppm of ozone for 3 days. Osman (2009) studied the effect of ozone on *E. kuehniella* at 1 g /$m^3$ for different exposure periods of 0.5, 1, 2, 3, 4 and 5 h. and found that larvae required not less than 6 days after ozone exposure to reveal the full effect in the mortality rate. Niakousari *et al.,* (2010) have proved that exposing samples to higher than 2000 mg/$L^1$ $O_3$ for 120 min resulted in complete mortality of *P. interpunctella* larvae in Kabkab dates. Hussain (2014) showed that corrected mortalities percentage increased gradually by increasing in exposure period to ozone gas and period after treatment when *E. cautella* larvae treated by 80 ppm., and noticed that the mortality percent was 38.92 % at 1 h. exposure period, followed significantly by 66.55, 88.37, 98.55 and 100 % at 2, 3, 4 and 5 h. exposure periods, respectively. Obtained results from the present study demonstrate that the latent effect of ozone gas on biological aspects from treated larva, where affected with increasing in exposure period or in gas concentration resulted decreasing in pupation and adult emergence percent of tested insect.

Table 1: Effect of $O_3$ gas (g/$m^3$) on pupation and adult emergence percent of *T. castaneum* treated as full grown larvae

| Exposure Period (h) | Pupation % | | | Adult emergence % | | |
|---|---|---|---|---|---|---|
| | 1 g/$m^3$ | 3 g/$m^3$ | 5 g/$m^3$ | 1 g/$m^3$ | 3 g/$m^3$ | 5 g/$m^3$ |
| 0 | 97.20±3.0a | 97.20±3.0a | 97.20±3.0a | 100.0±0.0a | 100.0±0.0a | 100.0±0.0a |
| 0.5 | 90.00±2.9b | 86.53±2.6b | 46.00±1.1b | 92.60±3.7ab | 78.16±2.8b | 40.00±1.8b |
| 1 | 88.37± 2.5b | 79.03±2.2c | 40.00±1.2b | 90.00±1.7abc | 60.20±2.3c | 41.66±4.2b |
| 2 | 88.23±2.5b | 75.73±3.1cd | 34.63±0.7bc | 89.70±2.0abc | 60.00±2.2c | 33.33±3.0b |
| 3 | 84.60±3.1bc | 70.70±4.2de | 16.67±1.7cd | 84.80±3.0bcd | 58.93±3.9c | 19.43±5.3c |
| 4 | 80.00±1.7c | 66.70±2.1e | 15.70±2.7cd | 79.53±0.5cd | 40.00±5.7d | 4.16±4.1d |
| 5 | 53.33±3.3d | 30.00±1.8f | 0.00±0.0d | 74.43±7.3d | 0.00±0.0e | 0.00±0.0d |
| L.S.D | 5.93 | 7.08 | 18.16 | 10.26 | 6.09 | 13.47 |

Means followed by the same letter in the same column are not significantly different at P>0.05.

### Effect of ozone treatment on *T. Castaneum* adults:

The data in Table (2) show that adult mortality percent of *T. castaneum* was slightly increased by increasing exposure periods to ozone, reaching to 36.66 ±3.3%

after 7 days at 5h. Exposure period at 1g/$m^3$and reached to 54.56±2.4 and 78.42±2.5% after 1 and 7days at 5h. Exposure period, at 3 g/$m^3$, while at 5 g/$m^3$ reached to full mortality (100.0±0.0%) after1day at 5h. exposure period and 91.66±8.3% after 7 days at 4h. exposure period respectively.

Table 2: Effect of $O_3$ gas (g/$m^3$) on mortality percentage of *T. castaneum* treated as one day old adults

| Exposure Period (h) | Adult mortality % | | | | | |
|---|---|---|---|---|---|---|
| | After 1day | | | After 7day | | |
| | 1 g/$m^3$ | 3 g/$m^3$ | 5 g/$m^3$ | 1 g/$m^3$ | 3 g/$m^3$ | 5 g/$m^3$ |
| 0 | 0.00±0.0 | 0.00±0.0f | 0.00±0.0e | 0.00 ±0.0e | 0.00±0.0e | 0.00±0.0d |
| 0.5 | 0.00±0.0 | 13.30±1.5e | 16.66±4.2d | 15.55±2.2d | 24.45±2.2d | 25.00±2.9c |
| 1 | 0.00±0.0 | 26.70±1.9d | 48.20±5.7c | 20.00±2.9cd | 37.77±3.6c | 56.55±3.6b |
| 2 | 0.00±0.0 | 37.76±2.2c | 51.87±1.9c | 24.52±2.5bcd | 57.77±4.4b | 60.19±2.3b |
| 3 | 0.00±0.0 | 46.70±2.0b | 72.23±2.8b | 27.85±1.5abc | 57.77±4.2b | 88.43±6.4a |
| 4 | 0.00±0.0 | 54.03±1.3a | 77.37±4.3b | 33.33±6.7ab | 63.65±1.9b | 91.66±8.3a |
| 5 | 0.00±0.0 | 54.56±2.4a | 100.0±0.0a | 36.66±3.3a | 78.42±2.5a | ----------- |
| L.S.D | | 4.03 | 10.24 | 9.52 | 8.84 | 14.3 |

Means followed by the same letter in the same column are not significantly different at $P>0.05$.

Similar results were obtained by Kells *et al.,* (2001) indicated that high mortality was achieved for adults of *Sitophilus zeamais* and *T. castaneum* exposed to 50 ppm ozone for 3 days. Zakladnoy *et al.,* (2003) found that ozone application in concentration of 1.35 g/$m^3$ caused 100% mortality after 1and 3 days post treatment for adult of *S. oryzae* and *S. ganarius,* respectively. Sousa *et al.*, (2008) tested ozone on phosphine-resistant insects in the laboratory, and found them to be susceptible to ozone at a concentration of 0.321 g/$m^3$. Subramanyam *et al.*, (2014) exposed adults of *R. dominica* (F.) to ozone concentration of 0.43 or 0.86 g/$m^3$ for 15-36 h. or 4-30 h. The authors found that the toxicity of ozone to *R. dominica* adults was delayed with greater mortalities occurring five days after exposure compared to one day after exposure, and found that Phosphine-resistant adults of *T. castaneum* and *R. dominica* were highly susceptible to ozone concentrations of 0.43 or 0.86 g/$m^3$ after a 24 h. exposure period. Our results from the present study demonstrate that increasing in exposure period or in gas concentration of ozone resulted increasing in adult mortality percent of tested insect reaching to full mortality in treatment 5h. of 5g/$m^3$.

The higher mortality for adults may be indicated that during the degradation of ozone to diatomic oxygen, free radicals may be formed from reactive oxygen species. In addition, $O_3$ may cause the per oxidation of polyunsaturated fatty acids, resulting in the destruction of critical molecules, such as DNA and proteins. Thus, these effects, either alone or together, may result in cell damage and the death of the insects that are exposed to ozone gas thus reducing the instantaneous growth rate of insect pests (Holmstrup *et al.,* 2011).

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## ARABIC SUMMARY

### تاثير غاز الاوزون على خنفساء الدقيق الصدئية

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تعتبرخنفساء الدقيق الصدئية من اهم الافات المنتشرة فى العالم التى تصيب عدد من المنتجات المخزونة من الدقيق وغيره ولذلك تم معاملة الاطوار الضارة ( اليرقات والحشرات الكاملة ) من هذة الافة بثلاث تركيزات من غاز الاوزون (١,٣,٥جم/م$^{٣}$) وستة مدد تعريض مختلفة تتراوح من نصف ساعة الى ٥ ساعات مقارنة بالمعاملة القياسية .اشارت النتائج الى ان زيادة كلا من التركيز او مدة التعريض للاوزون تؤدى الى زيادة معدل الموت لكلا الطورين .اوضحت النتائج ايضا ان غاز الاوزون اثر على نسبة التعذير و الخروج لهذة الافة عند معاملة اليرقات .