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ORIGINAL RESEARCH PAPER

Influence of ozone gas on the khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae) in stored wheat

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Abstract

Khapra beetle, *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) is one of the most notorious pests of stored grains. This study was aimed to evaluate the efficacy of ozone against 2nd and 5th instars and adults of *T. granarium* in stored wheat kernels under laboratory conditions. Four ozone concentrations of 300, 600, 900, and 1200 parts per million by volume (ppmv) were used. The results revealed that the LC_{50} values were 249.76 ppmv for adult, 446.75 ppmv for 5th instar, and 275.30 ppmv for 2nd instar of *T. granarium* after 2 h exposure to ozone. No adults emerged when the ozone concentration was 1200 ppmv, while emergence was high in controls (91.20%) after a 1-h exposure period. The highest ozone concentration of 1200 ppmv resulted in strong grain protection against adult and 2nd and 5th instars, with a 0.63%, 0.73%, and 1.16% loss in grain weight, respectively. Chemical analysis of treated wheat kernels at a concentration of 1200 ppmv ozone showed no significant differences ($p > 0.05$) in fat, moisture, ash, carbohydrate, fiber, and protein content of ozone-treated wheat kernels compared to untreated grain. Thus, ozone can be effectively used to control *T. granarium* and provides sufficient protection for stored wheat.

Keywords Khapra beetle · Ozone · Mortality · Adult emergence · Weight loss

Introduction

Cereals are the main source of calories in many countries. Wheat (*Triticum aestivum* L.) is the most important cereal crop because, it provides about 20 percent of total food calories for the human race (Rathore 2001). Stored grains require continuous protection to avoid deterioration of quality and weight especially caused by insects (Padin et al. 2013). Insect infestation leads to 10–20% cereal loss supply in worldwide, human health risks (malnutrition) beside the loss of millions of dollars annually (Nagpal and Kumar 2012; Pedigo and Rice 2014).

The khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae), is one of the most destructive internal-feeding insect pests of stored grain. It feeds on a wide range of cereals such as wheat, rice, oats, barley, and rye (Kavallieratos et al. 2019; Papanikolaou et al. 2019). It is ranked as one of the 100 worst invasive species worldwide (Usman et al. 2018). This beetle is widespread in regions with high temperatures and low humidity, and is especially prevalent in some areas of the Middle East, South America, Africa, Asia, and Europe (Eliopoulos 2013; Ghanem and Shamma 2007; Harris 2009). The most destructive stage is the larvae, which feed inside the grain, leading to quantitative and qualitative losses during storage (Athanassiou et al. 2019; Kavallieratos et al. 2017; Omar et al. 2012). Infected grain attracts external-feeding insect pests and fungi, consequently leading to the deterioration of the grain and loses its market value, reduces the nutritional value of the grain, and becomes unfit for human use (Arain et al. 2006; Madkour et al. 2012).

Control of insects in stored products depends primarily on the application of insecticides, where injudicious use of insecticides and fumigants cause serious problems such as toxic residues in food grains (Wasala et al. 2015). Additionally, there are worries about the environment and human health, rising pesticide costs, the emergence of insect pests

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with resistance, unfavourable effects on organisms other than targets, and insect pests (Islam and Ahmed 2016; Meyer-Baron et al. 2015; Pourya et al. 2018). In recent years, a lot of study has concentrated on alternatives to insecticides for controlling stored materials, such as heating, radiation, biological control using bacteria and fungi as well as plant extracts (Ibrahim and Al-Nasser 2009; Mohammed et al. 2019). Ozone is an effective, as well as promising, alternative to chemical fumigants as a viable green insecticide against a range of stored-grain insects, which prevents the need to store and dispose of hazardous chemicals; there is no need for aeration to remove the gas after application (Isikber and Athanassiou 2015). Ozone has been used in the food industry for more than 100 years, and it was first used as the food preservative of frozen meat in 1910 (Carletti et al. 2013). However, growing emphasis has been placed on the positive influence of ozone application in agriculture, such as the utilization of ozone in food processing for inactivation of pathogens, reduction of storage pests and degradation of various mycotoxins (Bonjour et al. 2011). Application of ozone in stored-products is effective in controlling insects and microorganisms such as fungi and bacteria, and degrading pesticide residues (Tiwari et al. 2010). Ozone was found to be an effective environment friendly alternative against *T. granarium*, since it is a highly oxidative toxic gas, which is a potent insect killer and degrades rapidly to oxygen (Mahroof and Amoah 2018). It is efficient and economical, and does not leave a residue on stored processed products (Mendez et al. 2003; Tiwari et al. 2010). Ozone has a different efficacy against insects inside or outside the grain, mainly because grains can increase the decomposition of ozone (Işikber and Öztekin 2009). Many researchers have applied ozone to various stored commodities and it is effective against a wide range of stored insect pests such as *Sitotroga cerealella* (Olivier), *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), *Callosobruchus maculatus* (F.), *Callosobruchus chinensis* (L.), *Ephestia cautella* (Walk.), *Plodia interpunctella* (Hübner), *T. granarium* and *Rhyzopertha dominica* (F.) (Abd El-Ghaffar et al. 2016, 2017; Amoah and Mahroof 2019; Gad et al. 2021a; Ghazawy et al. 2021; Işikber and Öztekin 2009; Mishra et al. 2019; Pandiselvam et al. 2019; Sabeat and Sabr 2015; Subramanyam et al. 2017; Xinyi et al. 2019).

Therefore, preliminary laboratory studies were carried out to evaluate the efficacy of ozone gas on the 2nd and 5th instars and adults of *T. granarium*, as one of the major economic stored grain pests in Egypt. The effects of various ozone concentrations on insect mortality and adult emergence, as well as the harm caused to wheat kernels, were studied.

Materials and methods

Insect rearing

The beetles used in this study were obtained from the Plant Protection Institute, Ministry of Agriculture, Giza, Egypt. Adults of *T. granarium* were cultured in an incubator at a constant temperature of 30 °C ± 2 °C, 65 ± 5% relative humidity and photoperiod 12:12 h (light:dark) in the laboratory at Plant Protection Department, Faculty of Agriculture, Al-Azhar University, Cairo Governorate. Before being utilized as a substrate for insect rearing, the experiment's wheat variety (Sakha 95) was deinfested in an oven at 60 °C for one hour. Stock culture was set up by introducing one hundred adults pairs and confined for 7 days for oviposition into glass jars of one liter capacity each containing 500 gm wheat kernels and covered with muslin held in place by rubber bands. The culture was reared to obtain homogeneous stock of 2nd and 5th instars and adults in separate jars. The homogeneous stock was maintained for further experiments.

Production of ozone gas

Ozone gas was produced using an ozone generator cylindrical dielectric barrier discharge (DBD) at the Laboratory of the Center of Plasma Technology, Al-Azhar University, Nasr City, Cairo, Egypt.

In the gas generation process, oxygen was used as the input, passing through a DBD reactor. The discharge is produced by applying a discharge voltage between two coaxial electrodes, with a glass dielectric between them and a free space where the oxygen flows. In this free space, a filament discharge is produced, generating electrons with enough energy to break down the oxygen molecules, forming ozone (O_3). The DBD cell was fed with oxygen gas. The concentration of the generated ozone was controlled by the discharge current, and the oxygen gas flow rate was adjusted to 0.5 l/min. Ozone was applied directly into jars containing the *T. granarium* larvae and adults with the grains. The ozone gas is constantly renewed inside the exposure fumigation chamber. This renewal and the continuous flow of ozone gas during the exposure period of insects works to avoid the half-life time of the ozone, which ranges from 20 to 30 min. This continuous flow makes the ozone gas pass constantly through the fumigation chamber, which contains the insects inside and constantly renewed. The ozone concentration inside the jars was measured using an ozone analyzer (Model H1-AFX-Instrumentation, USA). The input voltage of the AC test set (specifically, a variable high voltage transformer) was 220 V at 50 Hz. A voltage transformer was connected to two outer and inner electrodes, separated by a gap. Voltages used to generate ozone were controlled through a transformer control box (Variac Variable AC Power Transformer Regulator) as shown in Fig. 1.

Characterization of electrical properties

The current–voltage oscillogram of the coaxial DBD reactor system, recorded at atmospheric pressure and room temperature, is presented in Fig. 2. The filamentary modes discharge current in DBD when the applied voltage exceeds the breakdown voltage.

In Fig. 2, waveforms of the applied voltage to coaxial DBD reactor and associated current measured for ozone at oxygen flow rate 0.5 (l/min) at different ozone concentrations of 300, 600, 900, and 1200 ppmv is 3.4, 4.0, 5.6, and 6.8 kV, respectively.

Power measurement method

The power is analyzed following the original work of Manley (1943), who has utilized voltage–charge Lissajous figures to characterize the average consumed power through the discharge (Ballinger et al. 2005; Biganzoli et al. 2014; Janeco et al. 2011; Manley 1943). The charge–voltage characteristic plot is revealed in Fig. 3. The two values effective discharge capacitance is indicated by obtaining two distinct slopes of the Q–V plot. The reactor (coaxial dielectric barrier discharge) power formula was shown in equations by Manley (1943), where the total power (*Pel*) is related to the operating frequency (f) and the peak voltage V_{max}. From this so-called Lissajous Figure, the minimum external voltage V_{min} at which the ignition occurs, the electric energy

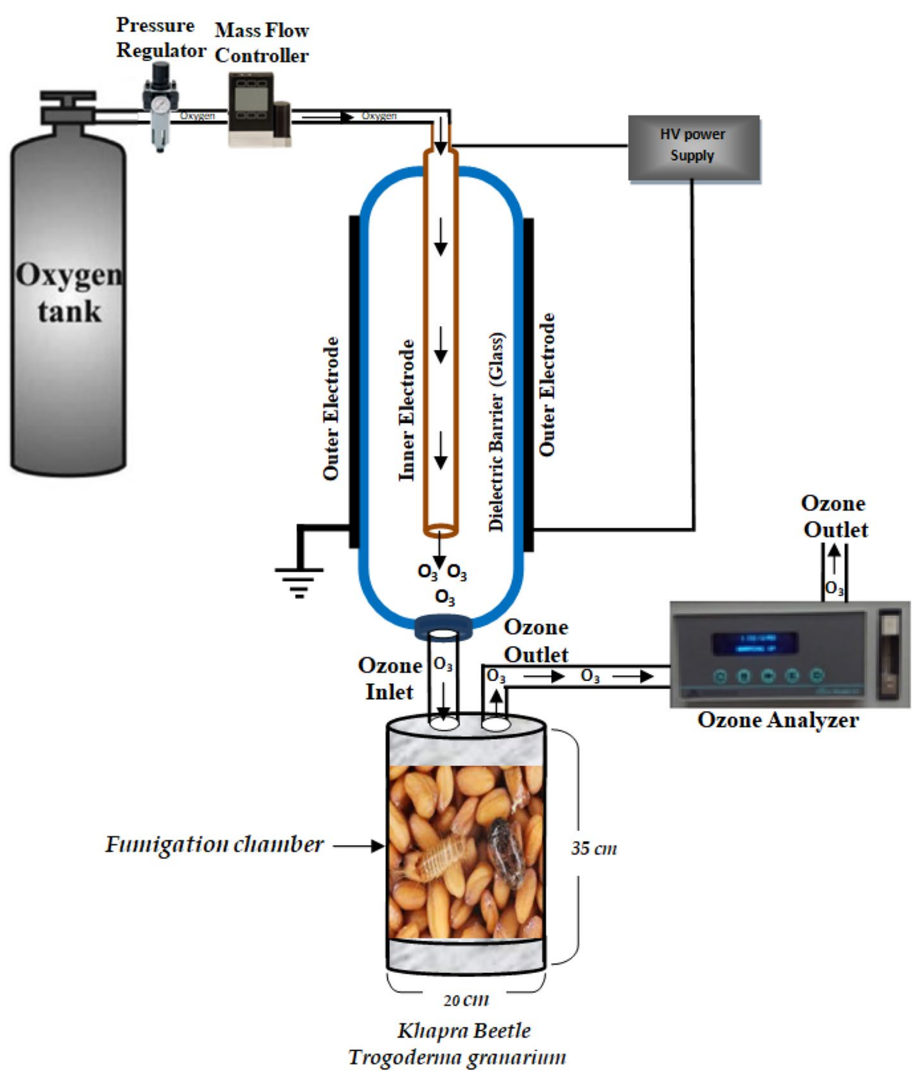


Fig. 1 Schematic diagram of the ozone generation and fumigation setup

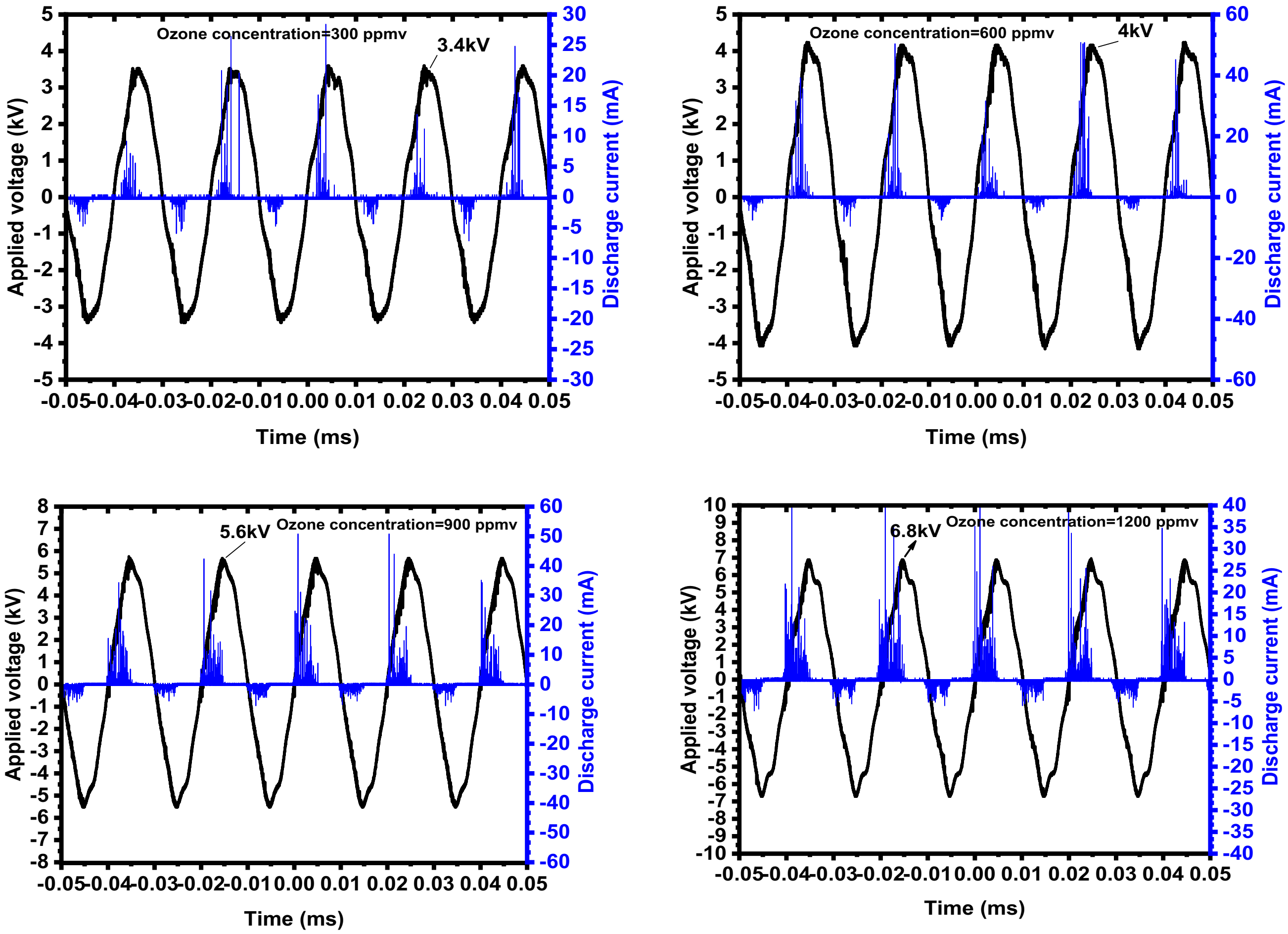
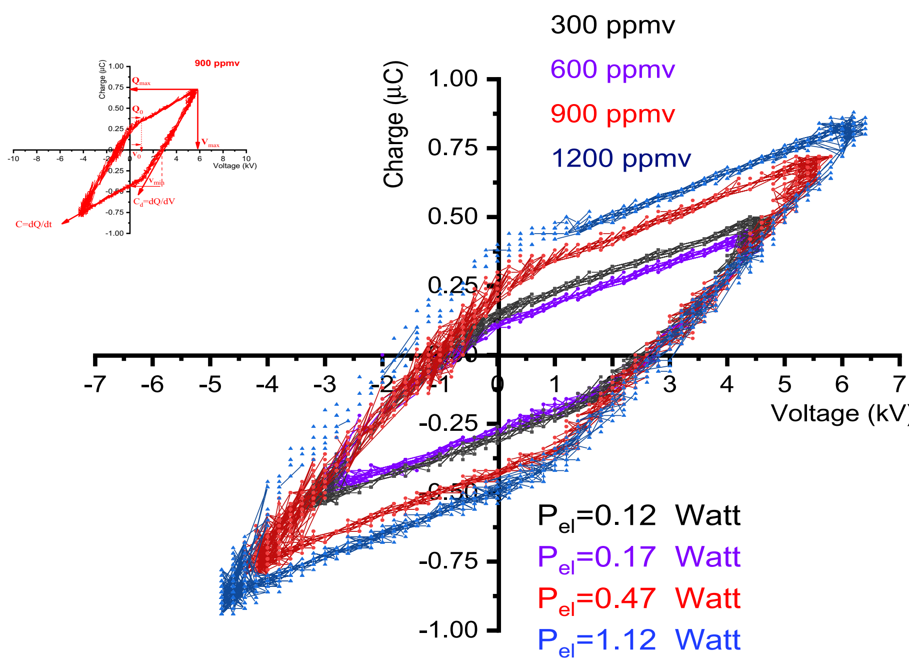


Fig. 2 Waveforms of the applied voltage to reactor and associated current measured for ozone at oxygen flow rate 0.5 (l/min)

Fig. 3 Lissajous diagrams measured for ozone at flow rate of 0.1 (l/min)



consumed per voltage cycle E_{el} and the electric power (P_{el}) can be estimated by the following relations (Ballinger et al. 2005):

$$E_{el} = 2(V_{\max}Q_0 - Q_{\max}V_0) \equiv \text{area of } (Q\text{–}V) \text{ diagram}$$

$$P_{el} = \frac{E_{el}}{T} = fE_{el} \tag{1}$$

In this study, the consumed power was determined to be 0.12 W at applied voltage of 3.4 kV, 0.17 W at 4.0 kV, 0.47 W at 5.6 kV and 6.8 kv at 1.12 watts. Treatments were applied in case of ozone concentration where f is frequency of 50 Hz, as shown in Fig. 2 and 3.

Effect of ozone gas application on the khapra beetle

Each muslin cloth bag containing 50 g of wheat kernels received 16 T. *granarium* larvae in their 2nd or 5th instars or eight pairs of adults (1–3 days old) separately. Rubber bands were used to tightly shut the bag. The bags were exposed to direct ozone application in a fumigation chamber. Four ozone concentrations were used (300, 600, 900, and 1200 ppmv) with exposure of 1 h or 2 h. Three replicates were made for each treatment (sixteen larvae or eight pairs of adults per replicate). After exposure, the treated groups were transferred (insects and wheat kernels) carefully into glass jars (250 ml capacity each) that were covered with a muslin cloth, and secured with rubber bands. The control treatment was the same but without ozone exposure. Seven days after exposure to treatments, the mortality of *T. granarium* larvae and adults was counted, and all adults were removed. Then, all jars were retained under the same conditions. The mortality percentages were corrected using Abbott's formula (1925). Data on pupal and adult formation were recorded until the emergence of F1 adults. The percent reduction of pupal and adult emergence was calculated according to Silassie and Getu (2009) using the formula:

Reduction rate (%) = Cn − Tn/Cn × 100;where Cn is the number of newly emerged insects in the control group, and Tn is the number of newly emerged insects in a treatment group.

Sixty days after treatment, the number of F1 adults that emerged was counted and recorded. Also, the percent of adult emergence was calculated using the following formula (Howe 1971):

Adult emergence (%) = Number of adult emerged/Number of pupae × 100.

The percent weight loss of the grains was estimated after 60 days following the method given by Jackai and Asante (2003):

Weight loss (%) = IW – FW/IW × 100; where IW is the initial weight and FW is the final weight.

Effect of ozone gas on chemical constituents of wheat kernels

Chemical analysis was conducted using a near-infrared spectroscopy (model DA1650 e FOSS Corporation, Denmark) to determine the effect of ozone on the ash, carbohydrate, fat, fiber, moisture, and protein levels in a 50 g sample of infested wheat kernels untreated and treated with ozone at a concentration of 1200 ppmv for 1 h (Taha et al. 2016). Carbohydrate content was calculated according to AOAC (2010): % carbohydrate content = [100 − (% protein + % moisture + % ash + % lipids + % fiber)].

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System at a 5% significance level. The mean differences were separated using the Least Significant Difference (LSD) and are shown as means ± SE. Probit analysis was conducted to reckon LC_{50} and confidence limits using SPSS 16.0 software (Chicago, IL, USA).

Results

Effect of ozone gas on mortality of larvae and adults of *T. granarium*

The probit estimates from the mortality response of 2nd and 5th instars and adults of *T. granarium* exposed to different ozone concentrations are summarized in Table 1. The results revealed that the LC_{50} values were 266.08 ppmv for adult, 280.29 ppmv for 2nd instar and 691.0 ppmv for 5th instar of *T. granarium* after 1 h exposure to ozone while, the effect of ozone after 2 h exposure with LC_{50} values 275.30, 446.75 and 249.76 ppmv was recorded for the 2nd and 5th instars and adults of *T. granarium*, respectively. The results showed a negative relationship between exposure time and LC_{50} values. The results showed that, regardless of exposure time, the toxicity of ozone was adult ˃ 2nd instar ˃ 5th instar. The slope values ranged from 1.71 to 2.44, indicating a homogenous effect across the insects in their susceptibility to ozone.

Effect of ozone gas on adult emergence

The data in Table 2 shows the effect of ozone gas concentrations on *T. granarium* adult emergence after 1 h of treatment. The results show that the mean number of adults emerged from untreated grains was 37.33. Ozone was most effective at 900 and 1200 ppmv, since no adult beetles emerged, while

Table 1 Toxicity of ozone gas against *T. granarium* 2nd and 5th instars and adults after exposure periods of 1 and 2 h

Exposure time (hrs)	Stage	Mean ± SE							
		LC_{50}[a] (ppmv)	95% Confidence limits (ppmv)		Slope[b]	Intercept[c]	(χ^2)[d]	df[e]	p[f]
			Lower	Upper					
1	2nd instar	280.29 ± 199.08	164.46 ± 163.98	445.42 ± 234.38	2.26 ± 0.36	−6.32 ± 1.04	0.2	2	0.9
	5th instar	691.0 ± 319.30	383.95 ± 313.87	26,713.24 ± 25,291.5	1.71 ± 0.01	−5.11 ± 0.11	0.34	2	0.84
	Adult	266.08 ± 45.68	10.53 ± 10.53	448.74 ± 51.18	1.76 ± 0.09	−4.24 ± 0.27	1.82	2	0.45
2	2nd instar	275.30 ± 39.34	67.48 ± 40.55	413.59 ± 36.47	2.44 ± 0.13	−5.96 ± 0.47	1.31	2	0.65
	5th instar	446.75 ± 28.42	205.73 ± 36.99	621.03 ± 38.88	2.36 ± 0.14	−6.25 ± 0.39	2.55	2	0.3
	Adult	249.76 ± 42.07	34.05 ± 33.36	407.35 ± 37.72	2.07 ± 0.16	−4.96 ± 0.52	1.18	2	0.58

[a]The concentration causing 50% mortality

[b]Slope of the concentration-mortality regression line

[c]Intercept of the regression line

[d]Chi square value

[e]Freedom degree

[f]Probability value

Table 2 Effect of ozone gas on adult emergence of *T. granarium* after an exposure of 1 h

Concentration (ppmv)	Mean ± SE		
	No. of adult emergence	Adult emergence (%)	Reduction (%)
0	37.33 ± 0.88[a]	91.20 ± 1.82[a]	–
300	3.66 ± 0.33[b]	65.71 ± 7.19[ab]	90.12 ± 1.09
600	1.66 ± 0.33[c]	55.55 ± 5.53[b]	95.53 ± 0.88
900	0.33 ± 0.33[cd]	16.66 ± 16.68[c]	99.07 ± 0.92
1200	0.00 ± 0.00[d]	0.00 ± 0.00[c]	100.00 ± 0.00
F	1170	18.9	

Means in the same column, followed by the same letter are not significantly different at 0.05 level of probability (df = 4)

the mean number of adult emergence was 3.66 adults at 300 ppmv. The percent reduction of adult emergence was 99.07% and 100% at 900 and 1200 ppmv, respectively.

Effect of ozone gas on pupation and adult emergence of 2nd and 5th instars

Table 3 indicates the effect of ozone gas on pupation and adult emergence of 2nd and 5th instars after a 1-h exposure. For the 2nd instar, the pupation reduction of *T. granarium* were 58.49 and 96.29%, while adult emergence reduction were 78.63 and 100%, at 300 and 1200 ppmv, respectively. For the 5th instar, the pupation reduction were 81.64 and 100%, while adult emergence reduction were 93.63 and 100%, at 300 and 1200 ppmv, respectively.

Effect of ozone gas on weight loss

Table 4 shows mean percentage of wheat kernels weight loss when treated with ozone gas at four concentrations compared with untreated. The mean percentage weight loss of the ozone-untreated grains were 5.06%, 5.06%, and 3.56% for the 2nd and 5th instars and adults, respectively. The

Table 3 Effect of ozone gas on pupation and adult emergence of 2nd and 5th instars of *T. granarium* after a 1-h exposure period

Concentration (ppmv)	Mean reduction (%) ± SE			
	2nd instar		5th instar	
	Pupation	Adult emergence	Pupation	Adult emergence
300	58.49 ± 8.17[b]	78.63 ± 6.30[b]	81.64 ± 4.74[c]	93.63 ± 3.19[a]
600	77.52 ± 7.11[ab]	93.33 ± 6.67[ab]	87.45 ± 2.93[bc]	96.96 ± 3.33[a]
900	93.51 ± 3.34[a]	96.66 ± 3.33[a]	96.96 ± 3.03[ab]	100.00 ± 0.00[a]
1200	96.29 ± 3.71[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
F	8.52	3.72	7.14	1.76

Means in the same column, followed by the same letter are not significantly different at 0.05 level of probability (df = 3)

Table 4 Effect of ozone gas on loss in grain weight caused by *T. granarium* 2nd and 5th instars and adults after a 1-h exposure period

Concentration (ppmv)	Mean weight loss (%) ± SE		
	2nd instar	5th instar	Adult
0	5.06 ± 0.24^{a}	5.06 ± 0.24^{a}	3.56 ± 0.24^{a}
300	3.63 ± 0.27^{b}	3.46 ± 0.57^{b}	2.82 ± 0.03^{b}
600	3.06 ± 0.54^{b}	2.86 ± 0.06^{bc}	1.90 ± 0.05^{c}
900	2.7 ± 0.36^{b}	2.20 ± 0.37^{c}	1.30 ± 0.34^{d}
1200	0.73 ± 0.12^{c}	1.16 ± 0.06^{d}	0.63 ± 0.08^{e}
F	21.1	19.6	40.5

Means in the same column, followed by the same letter are not significantly different at 0.05 level of probability (df = 4)

ozone gas concentration of 300 ppmv resulted in grain protection against 2nd and 5th instars and adults with a 3.63%, 3.46%, and 2.82% loss in grain weight, respectively. Moreover, the highest ozone concentration of 1200 ppmv resulted in strong wheat kernels protection against adult and 2nd and 5th instars, with a 0.63%, 0.73%, and 1.16% loss in grain weight, respectively. Generally, all treatments with ozone gas to control *T. granarium* significantly reduced the loss in grain weight compared to untreated.

Effect of ozone gas on wheat kernels quality

Figure 4 shows the percentages of fat, moisture, ash, carbohydrate, fiber, and protein in ozone-treated and untreated wheat kernels. The fat percentage in ozone-treated grains were 1.85%, 1.44%, and 1.89%, while in untreated grains were 2.08%, 1.63%, and 2.03%, for 2nd and 5th instars and adults, respectively (Fig. 4A). The moisture percentage in ozone-treated grains were 8.67%, 8.14%, and 9.01%, while in untreated grains were 9.64%, 9.64%, and 9.36% for 2nd and 5th instars and adults, respectively (Fig. 4B). The ash percentage in ozone-treated grains were 0.41%, 0.79%, and 0.81%, while in untreated grains were 0.35%, 0.99%, and 0.65% for 2nd and 5th instars and adults, respectively (Fig. 4C). The carbohydrate percentage in ozone-treated grains were 72.47%, 72.26%, and 71.72%, while in untreated grains were 72.56%, 71.60%, and 71.90% for 2nd and 5th instars and adults, respectively (Fig. 4D). The fiber percentage in ozone-treated grains were 2.49%, 2.83%, and 2.50%, while in untreated grains were 2.09%, 2.99%, and 2.25%, for 2nd and 5th instars and adults, respectively (Fig. 4E). The protein percentage in ozone-treated grains were 14.11%, 14.54%, and 14.07%, while in untreated grains were 13.24%, 13.15%, and 13.81%, for 2nd and 5th instars and adults, respectively (Fig. 4F).

Discussion

The study indicated that ozone is effective against *T. granarium*, and the toxicology data show that mortality happens quickly and gets worse with continued ozone exposure. These observations are in agreement with Abo-El-Saad et al. (2011) who demonstrated that ozonation at 2.0 ppmv caused 83% and 27% mortality against *E. cautella* adults and larvae after 12 h respectively. Bonjour et al. (2011) reported that 50 ppmv ozone for 4 d caused 100% mortality of immature *P. interpunctella*. According to McDonough et al. (2011), adults of *P. interpunctella* died completely after being exposed to 500 ppmv of ozone for 60 min. According to Hansen et al. (2012), the successful implementation of ozone technology necessitates an enough concentration of ozone for a suitable period of time, which changes depending on the environmental factors. Hussain (2014) reported that mortality percentages gradually increased by increasing the exposure period to ozone gas, when *E. cautella* larvae were exposed to 80 ppmv ozone, the mortality rate was 38.92% for a 1-h exposure period. Subramanyam et al. (2014) examined the toxicity of ozone at two concentrations (0.43 and 0.86 g/m^3) against adults of *R. dominica* and demonstrated that the insect was highly susceptible to ozone treatment; the toxicity of ozone after 5 d was higher than that after 1 d of treatment. According to Jemni et al. (2015), exposing *Ectomyelois ceratoniae* (Zeller) larvae to 170.7 ppmv of ozone resulted in 82% mortality in just 80 min. According to Sabeat and Sabr (2015), the application of ozone to suppress different *T. granarium* stages resulted in 100% mortality. Silva et al. (2016) evaluated the toxicity of ozone (750.8 ppmv) against *R. dominica* adults on wheat kernels and found that ozone exposure from 8.69 to 13.08 h and from 11.28 to 18.11 h caused mortality rates of 50% and 95%, respectively. Subramanyam et al. (2017) also recommended that higher ozone doses (200 and 400 ppmv) are required to control *R. dominica* adults and reported that adult mortality after one day were 67% and 42%, respectively. Gad et al. (2021b) demonstrated that treatment with 600 ppmv (1.2 g/m^3) of ozone caused complete mortality of *C. maculatus* adults at all exposure times (0.5–5 h) after 5 d. Similar results obtained as larval mortality of *E. cautella, P. interpunctella, T. granarium* and *T. castaneum* increased as concentration and exposure periods increased. Complete mortality was observed after 8 h. The LT50–99 values of ozone gas against the larvae decreased as concentration increased. Caterpillars were more sensitive to O_3 than grubs. Data also showed that the effective effect of ozonation towards the four larval species indicated that not all insects had the same sensitivity to ozone gas (Ghazawy et al. 2021).

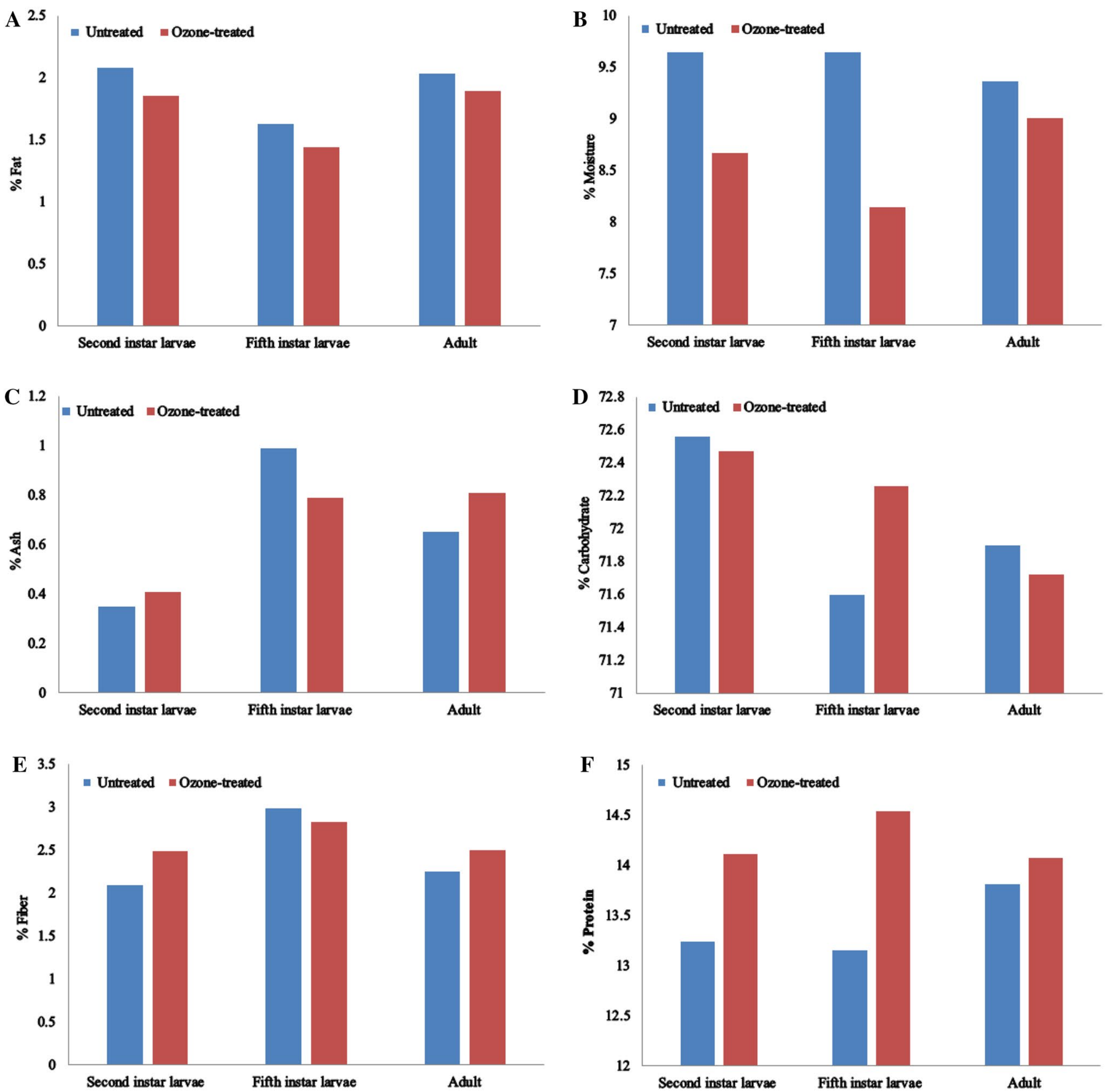


Fig. 4 Effect of ozone gas on the quality of wheat kernels. **A** fat; **B** moisture; **C** ash; **D** carbohydrate; **E** fiber; and **F** protein

Adult emergence of *T. granarium* was decreased in treated wheat kernels with all tested ozone concentrations after 60 days of treatment. The results revealed 100% reduction in Fl adult emergence of *T. granarium* at the highest concentration of ozone (1200 ppmv). Similar findings have been recorded by Bonjour et al. (2011) where a 70 ppmv ozone treatment for 4 d caused a progeny reduction of 100% of *Oryzaphilus surinamensis* (L.) and 98% of *T. castaneum*. Moreover, Abd El-Ghaffar et al. (2017) found that increasing the gas concentration decreased the adult emergence percentages of *T. granarium* adults. Xinyi et al. (2019) found that treatment with ozone at 0.42 g/m^3 suppressed adult progeny production of *R. dominica* in wheat. Gad et al. (2021a) mentioned that the number of progeny of *C. maculatus* and *C. chinensis* was significantly decreased in all ozone concentrations after 45 d of treatment.

This study also demonstrated that the wheat kernels were protected for 60 days from weight loss brought on by *T. granarium* by ozone treatment at the highest concentration of 1200 ppmv. Our results are in line with Pleijel and Uddling (2012) who observed that ozone lessens the typical weight loss of wheat kernels. Gad et al. (2021a) reported similar findings, stating that all ozone treatments decreased the weight loss of treated cowpea seeds in comparison to untreated seeds. According to Dong et al. (2022), 1440 min of ozone exposure at 700 ppmv completely controlled all life stages of *R. dominica* and *T. castaneum* insects in barley without having a negative effect on the weight of the grain.

Chemical analysis of infested wheat kernels treated at a concentration of 1200 ppmv ozone observed no significant changes in fat, moisture, ash, carbohydrate, fiber, and protein content compared with infested wheat kernels that were not treated with ozone. Changes in the nutrient contents of treated wheat kernels with ozone were supported by the results of Wang et al. (2008), who found that the protein content of ozone-treated corn is lower than that of untreated corn; this finding indicated that protein was destroyed by ozonation, thereby influencing the nutritional value of the corn. Gozé et al. (2016) observed no significant changes in wheat starch when it was treated with ozone gas at a flow rate of 33.34 L min^{-1} for up to 60 min. Our results are similar to those of Trombete et al. (2016), who reported the effect of ozone on the chemical properties of wheat at a concentration of 60 mg L^{-1} and found no significant alteration due to treatment. Mishra et al. (2019) reported that the moisture and protein content of wheat treated with 2.5 g m^{-3} ozone was lower as compared to infested wheat.

Conclusion

The results indicated that ozone gas could be one of the important alternatives to pesticides used in controlling *T. granarium*, as ozone could be used as a component of the integrated management of stored insect pests. This study demonstrated that the adults exposed to ozone for 2 h resulted in the maximum damage. Additionally, after a one-hour exposure period, no adults emerged from the areas with the greatest ozone concentration, and grains were well protected from larvae and adults. In addition, the impact of ozone on the chemical makeup of treated versus untreated wheat kernels content was minimal. The exposure to (effective concentration) O_3 for 2 h was suitable for controlling larvae and adults in a short time so it is safe to use in control pests. However, further studies are required to test effect of ozone gas on quality parameters of the tested commodity.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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