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Efficiency of ECO_2-Fume fumigant and ozone against the red flour beetle

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Abstract

Wheat, a staple food in Egypt, is severely damaged by insect pests, particularly the red flour beetle, *Tribolium castaneum* (Herbst), when stored in bulk quantities. The current study was conducted to determine the efficacy of ECO_2-Fume fumigant (mixture of phosphine and carbon dioxide gases) and Ozone on *T. castaneum* different stages. The ECO_2-Fume fumigant was treated at concentrations of 30, 35, 40, and 50 g/m^3, with exposure for three days. Mortalities increased with increasing concentration and exposure period. After three days of application ECO_2-Fume gas the mortality rate was 80.06, 85.6, 76.7, and 82.2% for eggs, larvae, pupae, and adults, respectively when applied at a rate of 40 g/m^3. The mortality rate was 100% for previous stages respectively when applied at a rate of 50 g/m^3. Application of Ozone gas in this experiment were 500 ppm for at ten different exposure times of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 hours. The mortality percentage increased gradually by increasing the exposure time after treatment. The LT_{50} and LT_{90} to Ozone gas against *T. castaneum* larvae to 500 ppm were 0.07, and 1.75 days; and it was 0.19 and 6.22 days for adult. Therefore, the use of ECO_2-Fume fumigant and Ozone gas is a very important alternative method to protect stored grains and their products against *T. castaneum*.

Keywords: wheat flour, stored products, insects, fumigant, ECO_2-Fume, Ozone gas.

Introduction

Wheat flour is a major food source worldwide. Large amounts of flour are kept in open shounas and shades in several Asian and African nations, including Egypt. In these circumstances, a variety of biological agents, including insects, may target flour. One of the main crops farmed is wheat, and whole-wheat flour is an essential component used in the baking industry. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is the most common pest in stores, retail food stores, and flour mills, ranking as the fourth most important pest. It leads to quantitative losses to wheat flour, which lowers the quality of the flour (Mangang *et al.*, 2020). This pest effects on many products, such as grains, beans, cocoa, dried fruit, wheat, peas, almonds, and spices. The quantity and quality of food that is stored are directly impacted by the presence of both adults and larvae (Bakhtawar, 2013).

Control of stored products pests was one of the major tasks for conservators because the damage inflicted to foodstuff is irreversible. A few insect species pose a potential threat to a variety of stored products (Ja Hyun and Ryoo 2000). These days, a lot of fumigators use pesticide tablets or sprays, such magnesium phosphide and aluminum phosphide. Even though phosphine-resistant stored goods insects are becoming more common (Konemann *et al.*, 2017).

ECO_2Fume® fumigant gas eliminates the concern associated with deactivating unspent metal phosphide residue and disposal of the waste product (Cavasin *et al.*, 2001). ECO_2-Fume® fumigant gas is a cylinderized formulation of 2% PH_3 and 98% CO2 by weight. It is packaged in high pressure aluminum or steel cylinders with net content of 31 kg of PH3/CO2 mixture and an equivalent phosphine amount of 620 g (Tumambing *et al.*, 2012).

Ozone is a safe fumigation method, while natural compounds from plant sources offer low toxicity, rapid degradation, and local availability. Validation studies are needed to determine biological controls as replacements (Abd El-Aziz 2011). Insects can be killed by the highly oxidative toxic gas Ozone, which also breaks down quickly into oxygen. This makes it a viable substitute for the fumigant phosphine, to which insects are becoming resistant (Rizana and Barbara 2018). Concerning as, 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). Furthermore, many investigation evident the potential efficacy and suppression role of Ozone gas application against coleopterous and lepidopterous stored-product insect pests (Isikber and Oztekin 2009).

The aim of this study to evaluate the effectiveness of ECO_2-Fume fumigant and Ozone gas on larvae, pupae, and adult of the red flour beetle, *T. castaneum*.

2. Materials and Methods

Rearing of insect cultures:

The insects were reared in glass jars (approximately 1000 ml) containing about 250 g of sterilized and conditioned wheat flour for *Tribolium castaneum* (1–2 weeks old) were introduced into the jars to laying eggs under controlled conditions. Three days later, all insects were separated from the food and the jars were kept again in the rearing room. This procedure was repeated several times in

order to obtain large numbers of *T. castaneum* adults needed to carry out the tests.

ECO_2-Fume® fumigant

ECO2-Fume Formulation

Carbon Dioxide (CO2): 98%; Phosphine (PH_3): 2% (by weight); 2.6% (by volume).

-Chemical name: PH3 and CO2.

CYTEC Company, ECO_2-Fumegas cylinders produced in Canada, piles of 240 Jute bags each containing 100 kg of wheat grains, protective clothing, a silo check (PH_3 detector), sealing supplies, a weight digital scale, and plastic sheets (14x20m).

Bioassay tests of ECO_2-Fume gas

ECO_2-Fume gas has been applied in a Shona (grain store) Qalyoub, Qalyubia governorate Egypt. There were three piles of 240-jute sacks, each containing 100kg of wheat grains. Twenty five grams of wheat flour was packed in each small cloth bags (14 x 20cm). Thirty adult beetle (7-14 days old) and 30 larvae (5 days old) of *T. castaneum* were added to each bag then closed well and secured with rubber bands. For every concentration of ECO_2-Fume gas, there were a total of 24 bags—6 bags in each of the four directions (North, South, Middle, West). The filled bags were added to the group and dispersed in the four directions mentioned before. Exact and secure covering of the heaps was achieved with a 14 by 20 meter plastic sheet. Once the fumigation area has been sealed and the gas cylinder has been placed on the platform balance, determine the required concentrations. Four concentrations of ECO_2-Fume were employed: 30, 35, 40, and 50 g/m^3. The piles were aerated after three days of exposure to ECO_2-Fume gas, and the holding adults were promptly inspected for adult mortality and adjusted using Abbott's formula (Abbott, 1925). Fourteen days later, the grownups were taken out. F1 progeny bags of larvae were maintained at 30±1 °C and 65±5% relative humidity. The percentage of insects' reduction was calculated. Similar amounts of packed with insects were distributed in another mound of wheat flour using the same procedures—minus the application of ECO_2-Fumegas.

Ozone gas

Using an Ozone generator model OZ06VTTL from OZO Max Ltd. in Shefford, Quebec, Canada, Ozone gas was produced from air using purified extra-dry oxygen feed gas at the National Research Center's Food Toxicology and Contamination Laboratory. A belt pan in the monitor-controller provides for concentration control within a preset range, and the plug-in sensor on the monitor-controller is adjustable for different Ozone concentration ranges. This allowed for control over the amount of Ozone produced as described by Hawash (2020).

Bioassay tests of Ozone gas:

Small jute bags each contained 25 grams of wheat flour. Thirty adults (7–14 days old) of *T. castaneum* were added to each jute bag; they were closed well and secured with rubber bands. All bags were exposed inside five glass gare (4 liters each) as described by **Hawash (2020)**. Each consisted of a glass jar with a short neck, closed with a rubber stopper, and two holes. One hole was for the Ozone line, and the other was for tubing connected to the Ozone destruct unit. Ten different exposure times (treatment of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 hours at 500 ppm concentration), with three replicates for each treatment. After exposure, treatments were transferred carefully into glass jars (0.25kg capacity each) covered with muslin cloth and secured with rubber bands. Glass jars for each replicate were incubated at 30°C, 65±5% RH.

Statistical Analysis:

The average percent mortality of the tested insects was calculated and corrected using Abbott's formula (**Abbott, 1925**). Toxicity values (LC_{50} and LC_{99}) were calculated by Probit analysis (**Finney, 1971**) using Ldp–line software to obtain the toxicity regression lines. Simple correlations and partial regression were used for the effect of ECO_2-Fume and ozonation, which can be attributed to variance explanation ratios (EV %) as the effect of concentration and time. In the case of microwave, the combined effect of time and energy was used. Collection, Regression, and ANOVA in SAS were used to evaluate the collected data (Anonymous, 2003). The analysis of data was performed on each dependent variable using the treatments compared for significance with means, and separated using LSD test ($P>0.05$).

3. Results and Discussion

Evaluate of the efficacy of ECO_2-Fume against the larvae, pupa and adult of *T. castaneum* at test temperatures at 30°C and 65% RH.

The results in Table (1) indicate that the mortality rate of the different developmental stages and adults of *T. castaneum* **depended on** ECO_2-Fume gas concentrations and exposure periods. Insect mortality values increased as the concentration of the gas increased. Mortality values were recorded after three days of exposure to ECO_2-Fume gas. A complete mortality rate of 100% was recorded for eggs, larvae, pupae, and adults at a concentration of 50 g/m^3. The mortality percentage of the previous stages was 19.02, 33.3, 24.4, and 30%, respectively, at a concentration of 30 g/m^3. Therefore, the results indicated that higher concentrations induced higher percentages of mortality. But these values increased with an increase in ECO_2-Fume gas concentrations, ranging from 80.06, 85.6, 76.7, and 82.2% mortality for eggs, larvae, pupae, and adults at 40 g/m^3, respectively. There is a significant difference in the mortality rate eggs, larvae, pupae, and adults of *T. castaneum* at different concentrations of ECO_2-Fume gas.

The data presented in Table (2) showed the effect of ECO_2-Fume gas at various exposure times and concentrations on *T. castaneum*. Highly significant positive correlation values were observed for the egg, larvae, pupae, and adult stages of *T. castaneum* at different concentrations. The explained variance (EV %) was respectively 92.34, 91.0, 95.7, and 92.6% % on the eggs, larvae, pupae, and adults of *T. castaneum* Table (2).

Data presented in Table (3) indicated the lethal concentration of ECO_2-Fume gas on the eggs, larvae, pupae, and adults of *T. castaneum* **under a** plastic sheet. The LC_{50} for eggs, larvae, pupae, and adults were 31.7, 28.6, 31.33, and 30.33, respectively. Likewise, the LC_{90} were 48.4, 42.8, 48.2, and 45.35 for previous stages, respectively. The obtained results showed that the larvae of *T. castaneum* were more sensitive to ECO_2-Fume gas than adults. The obtained results showed that the larvae of *T. castaneum* were more sensitive to ECO_2-Fume gas than other stages.

Table (1) Mortality percentage (Mean ± SE) of the different developmental stages of *Tribolium castaneum* at different concentrations of ECO_2-Fume gas three days under plastic sheet.

Conc. (g/m)	Corrected mortality% (Mean ± SE)			
	Egg	**Larvae**	**Pupae**	**Adults**
30	19.02±0.24 d	33.3±0.21 d	24.4±0.32 d	30.1±0.22 d
35	38.2±0.52 c	56.7±0.42 c	45.6±0.16 c	46.7±0.61 c
40	80.06±0.66 b	85.6±0.71 b	76.7±0.32 b	82.2±0.21 b
50	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a
LSD at 0.05	4.78	7.89	6.78	7.89

Means followed by the same letters in the same column are not significantly different at P=0.05.

Table (2) Simple correlation and multiple regression values for the effect ECO_2-Fume® fumigant at various concentrations on *Tribolium castaneum*.

Stage	Factor	Simple correlation		Multiple regression				
		r	p	b	P	F	P	E.V.%
Egg	Con.%	0.96	0.0391	4.13	0.0391	24.11	0.0391	92.34
Larvae	Con.%	0.95	0.0460	3.32	0.0460	20.23	0.0460	91.0
Pupae	Con.%	0.97	0.0219	3.82	0.0219	44.24	0.0219	95.7
Adult	Con.%	0.96	0.0374	3.60	0.0374	25.21	0.0374	92.6

r = correlation, P= probability, b= slope, EV% = explanation variance, conc.= concentration.

Table (3) Lethal concentration values and parameters of mortality regression line of the different developmental stages of *Tribolium castaneum* at different concentrations of ECO_2-Fume gas.

Stages	**Confidence limits**		**Slope ± SD**	**R**
	LC_{50}	**LC_{90}**		
Egg	**31.7 (30.7-32.9)**	**48.4 (38.2-55.1)**	**10.7±0.84**	**0.97**
Larvae	**28.6 (24.8-33.1)**	**42.8 (37.03-49.44)**	**7.3±0.13**	**1.00**
Pupae	**31.33 (27.14-36.24)**	**48.2 (41.42-55.43)**	**6.9±0.14**	**0.99**
Adults	**30.33 (26.01-34.73)**	**45.35 (39.37-52.40)**	**7.1±0.14**	**0.99**

LC = Lethal concentrations, R = Correlation coefficient, SD = Standard deviation.

These results are in agreement with earlier findings by Mohamed and Sayed (2017) reported 100% mortality was achieved for the adult and juvenile stages of *Ephesia cautella*, *Ephesia calidella*, and *O. surinamensis* following a fumigation with ECO_2-Fume gas three days after exposure. Kengkanpanich *et al.* (2018) found that ECO2-Fume® phosphine fumigant effectively controlled mixed-age insect cultures in packed rice stacks and was 100% effective in raw material rice stacks, suggesting commercial tarp fumigation can be performed without top-up. Hawash (2020) found that *S. oryzae* and *S. granarius* adults were more susceptible to ECO_2-Fume gas than other stages, while eggs were more tolerant. *S. oryzae* was more susceptible to ECO_2-Fume than *S. granarius* at all stages, with adults and pupae recording the highest mortality rates. The study also found that *S. oryzae* had higher sensitivity than S. *granarius* at all stages. Amin *et al.* (2022) found ECO_2-Fume gas as a safe alternative to toxic phosphine fumigant for controlling *Callosobruchus maculatus* and *C. chinensis* L. in cowpea piles. The highest resistance was observed in pupae at 40 g/m^3.

Evaluate of the efficacy of Ozone gas against the larvae, pupa and adult of *T. castaneum* at test temperatures at 30°C and 65% RH.

The effect of ozone gas at 500 ppm on *T. castaneum* **different stages is presented** in Tables (4, 5, and 6). The results show that the mortality percentages of *T. castaneum* larvae, pupae, and adults increased with increasing exposure times to ozone gas as well as the days post treatment. A highly significant difference in the mortality rate of ozonation larvae, pupae, and adults at different exposure times was observed. After three days treatment, the mortality rates were 17.7, 27.7, 33.3, 36.7, 42.2, 47.8, 61.1, 70.1, 80.0 and 83.3 % for larvae; 5.6, 8.9, 18.8, 28.9, 33.3, 41.1, 50.4, 56.1, 63.3, and 73.3% for pupae; and 7.2, 15.5, 27.7, 31.1, 37.8, 44.4, 52.2, 61.1, 67.8, and 76.7% for adults at exposure time 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 hours, respectively. At the same exposure time, the mortality rates after 10 days of ozonation were 41.1, 55.1, 61.3, 75.6, 81.1, 84.4, 87.7, 92.2, 95.5, and 100.0% for adults, respectively.

Significant positive correlation values were observed for larvae, pupae, and adults of *T. castaneum* at an exposure time and time after treatment, respectively, of 0.66, 0.66, 0.61, 0.72, 0.62, and 0.69, with P-values of 0.0001, respectively, for larvae, pupae, and adults of *T. castaneum*. The explained variance (EV%) for larvae, pupae, and adults of *T. castaneum*, respectively, was 88.5, 90.7, and 87.2%. These results indicated that exposure time are effective on *T. castaneum* mortality (Table 7).

Table (4) The efficacy of 500ppm Ozone gas against *Tribolium castaneum* larvae at 30±1°C; 65 ± 5% RH.

Exposure time (hours)	Mortality % after indicated days				
	1	**3**	**5**	**7**	**10**
1	0.0 f	17.7±0.32 h	28.9±0.32 h	34.4±0.57 f	43.3±0.57 f
2	8.2±0.21 e	27.7±0.57 g	37.8±0.57 g	41.4±0.87 e	65.5±0.32 e
3	10.1±0.54 e	33.3±0.57 g	47.8±0.32 f	56.9±0.57 d	73.3±0.3 2 d
4	12.2±0.32 e	36.7±0.87 f	55.6±0.57 e	68.3±0.32 c	80.0±1.2 c
5	16.1±0.22 d	42.2±0.32 e	61.1±0.32 de	75.8±0.57 b	84.4±0.87 bc
6	20.1±0.33 d	47.8±0.57 d	65.6±0.57 d	77.9±0.87 b	88.9±0.32 b
7	33.3±0.51 c	61.1±0.32 c	75.6±1.2 c	81.9±0.57 b	95.5±1.2 a
8	38.2±0.57 b	70.1±0.57 b	83.3±0.32 b	91.1±0.87 a	96.7±01 a
9	40.5±0.41 b	80.0±10.9 b	86.7±3.30 b	93.3±6.94 a	100±0.12 a
10	45.1±0.75 a	83.3±12.2 a	95.6±6.3 a	96.7±0.12 a	100±00 a
LSD at 0.05	4.27	5.86	5.86	5.58	5.18

Means followed by the same letters in the same column are not significantly different at P=0.05.

Table (5) The efficacy of 500ppm Ozone gas against *Tribolium castaneum* Pupae at 30±1°C; 65 ±5% RH.

Exposure time (hours)	Mortality % after indicated days				
	1	**3**	**5**	**7**	**10**
1	0.0 h	5.6±0.22 g	10.4±0.32 f	17.7±0.57 g	33.3±0.57 f
2	0.0 h	8.9±0.51 fg	15.6±0.57 f	30.2±0.82 f	48.9±0.32 e
3	5.1±0.11 g	18.8±0.32 f	30.1±0.42 e	35.3±0.57 f	55.5±0.22 e
4	8.2±0.22 fg	28.9±0.57 e	40.6±0.55 d	45.4±0.32 e	66.7±1.2 d
5	12.2±0.31 ef	33.3±0.32 e	55.5±0.33 c	61.1±0.57 d	72.2±0.87 d
6	15.1±0.32 de	41.1±0.27 d	61.4±0.52 c	70.0±0.87 c	80.2±0.32 c
7	20.1±0.51 cd	50.4±0.32 cd	71.1±1.2 b	82.22±0.55 b	82.2±1.2 bc
8	24.1±0.33 c	56.1±0.57 bc	77.9±0.32 b	88.89±0.27ab	88.2±0.21 bc
9	33.2±0.57 b	63.3±1.33 b	86.7±2.99 a	91.11±3.03 a	92.2±3.98 ab
10	38.89±0.27 a	73.3±1.09 a	90.0±5.81 a	93.3±4.83 a	95.5±3.98 a
LSD at 0.05	4.74	9.15	7.89	7.88	8.60

Means followed by the same letters in the same column are not significantly different at P=0.05.

Table (6) The efficacy of 500ppm Ozone gas against *Tribolium castaneum* adult at 30±1°C; 65 ±5% RH.

Exposure time (hours)	**Adult mortality (%) after indicated period (days) ±SD**					Average no. of Emerged adults after 55 days	Red. in progeny %
	1	3	5	7	10		
1	0.0f	7.2±0.23h	12.2±0.31h	21.1±0.57h	41.1±0.27g	50.1	9.73
2	5.1±0.22e	15.5±0.2g	24.4±0.57g	38.83±0.52g	55.1±0.32f	45.5	18.01
3	8.2±0.12e	27.7±0.32f	36.3±0.45f	50.0±0.57f	61.3±0.22e	43.3	22.1
4	10.1±0.3e	31.1±0.57f	50.0±0.51e	58.9±0.32e	75.6±1.2d	40.1	27.74
5	15.1±0.5d	37.8±0.3e	58.8±0.31de	67.8±0.57d	81.1±0.87cd	33.3	40.00
6	20.1±0.3d	44.4±0.5d	66.7±0.51cd	76.7±0.87c	84.4±0.32c	23.3	58.01
7	27.1±0.8c	52.2±0.3c	75.5±1.20ab	85.5±0.53bc	87.7±1.20c	20.1	63.8
8	30.1±0.2cd	61.1±0.5b	83.3±0.3ab	90.1±0.27b	92.2±0.23bc	12.2	78.01
9	33.3±0.1b	67.8±0.19ab	86.7±7.0a	92.2±3.09a	95.5±7.43ab	10.1	81.8
10	41.1±0.2a	76.7±2.9 a	92.2±0.1a	95.5±1.02a	100.0±6.09a	6.3	88.6
Control	0	0	0	0	0	55.5	0.0
LSD 0.05	5.28	8.02	9.99	6.55	7.18		

Means followed by the same letters in the same column are not significantly different at P=0.05.

Table (7) Simple correlation and multiple regression values for the effect of Ozone gas.

Stage	Factor	Simple correlation		Multiple regression				
		r	p	b	P	F	P	EV%
Larvae	Time	0.66	0.0001	6.06	0.0001	568.9	0.0001	88.5
	Exposure	0.66	0.0001	6.53	0.0001			
Pupae	Time	0.61	0.0001	6.0	0.0001	718.1	0.0001	90.7
	Exposure	0.72	0.0001	7.64	0.0001			
Adult	Time	0.62	0.0001	5.84	0.0001	502.0	0.0001	87.2
	Exposure	0.69	0.0001	7.14	0.0001			

r = correlation, P= probability, b= slope, EV% = explanation variance.

Lethal times of Ozone gas at 500 ppm on the larvae, pupae, and adults of *T. castaneum* are presented in Tables (8, 9, and 10). The results showed that mortality percentage increased with increasing exposure time, and the larvae were more susceptible than adult and pupae stages. LT_{50} values on larvae, pupae, and adults were 10.61, 16.83, and 14.33 days, and LT_{90} values were 60.72, 62.62, and 52.12 days after one hour of exposure time, respectively**.**

These results are in agreement with earlier findings by Kells *et al*., (2001) indicated that high mortality was achieved for adults, of *Sitophilus zeamiis* and *Tribolium castaneum* exposed to 50 ppm ozone for three days. Bonjour *et al.* (2011) found that increasing ozone exposure time can decrease the survival ratios of egg, larvae, and pupa of *Plodia interpunctella* in hard red winters wheat, resulting in mortality rates of 71.66%, 68.33%, 78.33%, and 85%, respectively. Ghazawy *et al.* (2021) found that ozone technology can control caterpillar larvae, but increased mortality and degenerated muscles were observed with concentration and exposure periods.

Abdelfattah *et al.* (2021) found that ozone gas is more effective and safe than phosphine in controlling *Oryzaephilus surinamensis*, a common grain product pest causing significant damage to date crops, with adult insects showing high resistance. The reduction rate of vital insects was 100% in all stages. Abdel-Aziz *et al.* (2023) found that ozone gas concentrations and gamma radiation doses affect *Oryzaephilus surinamensis* adults in stored dates, with mortality increasing with doses and exposure time. The LC_{50} for ozone gas was 2.79, 1.06, 1.05, and 0.46 days for Bermuda and Kandella varieties, respectively.

Table (8) Lethal time values and confidence limits for the larvae of *Tribolium castaneum* at various exposure periods of Ozone.

Exposure time (hours)	LT_{50} (day)	LT_{90} (day)	Confidence limits				Slope ± SD	R
			LT_{90}		LT_{50}			
			Upper	Lower	upper	Lower		
1	10.61	60.72	6.17	18.74	34.82	105.76	1.70±0.58	0.98
2	7.21	39.20	4.10	12.60	23.22	66.19	1.75±0.57	0.87
3	5.20	23.50	3.25	8.31	14.71	37.55	1.95±0.51	0.98
4	4.08	16.75	2.61	6.38	10.71	26.19	2.09±0.47	0.99
5	3.31	14.64	2.05	5.35	9.06	23.68	1.98±0.50	0.91
6	2.57	12.43	1.53	4.30	7.33	21.21	1.84±0.54	0.91
7	1.95	8.54	1.12	2.21	4.19	14.32	2.04±0.49	0.80
8	1.25	5.15	0.66	2.37	3.22	11.34	1.84±0.52	0.89
9	0.37	5.48	0.12	1.14	1.86	16.32	1.11±0.89	0.75
10	0.07	1.75	0.01	0.44	0.304	10.01	1.08±0.95	1.00

R = Correlation coefficient of regression line, SD = Standard deviation, LT= Lethal time.

Table (9) Lethal time values and confidence limits for the pupae of *Tribolium castaneum* at various exposure periods of Ozone.

Exposure time (hours)	LT_{50} (day)	LT_{90} (day)	Confidence limits				Slope ± SD	R
			LT_{90}		LT_{50}			
			Upper	Lower	upper	Lower		
1	16.83	62.62	10.35	27.66	38.2	162.59	2.25±0.44	0.98
2	11.03	35.92	7.38	16.49	24.03	53.72	2.51±0.39	0.96
3	9.38	42.41	5.1	15.21	26.16	68.48	1.95±0.51	0.96
4	6.65	35.22	3. 97	11.13	21.03	58.92	1.77±0.56	0.92
5	4.48	26.22	2.6	7.75	15.14	45.33	1.67±0.62	0.99
6	3.43	16.52	2.11	5.74	9.1	27.23	1.88±0.53	0.96
7	2.55	14.42	1.44	4.43	8.14	25.71	1.67±0.59	0.99
8	1.83	11.31	0.978	3.43	6.04	21.22	1.62±0.61	0.99
9	1.20	8.52	0.582	2.53	4.11	17.44	1.51±0.66	0.86
10	0.52	6.66	0.189	1.44	2.39	18.27	1.16±0.86	0.86

R = Correlation coefficient of regression line, SD = Standard deviation, LT= Lethal time.

Table (10) Lethal time values and confidence limits for the Adult of *Tribolium castaneum* at various exposure periods of Ozone.

Exposure time(hours)	LT_{50} (day)	LT_{90} (day)	Confidence limits				Slope ± SD	R
			LT_{90}		LT_{50}			
			Upper	Lower	upper	Lower		
1	14.33	52.12	9.01	22.84	82.81	82.81	2.29±0.46	0.93
2	9.53	36.78	6.25	14.24	65.82	65.82	2.19±0.45	0.95
3	6.93	34.42	4.22	11.42	59.68	59.68	1.84±0.54	0.95
4	5.15	19.57	3.35	7.86	29.70	29.70	2.20±0.45	0.99
5	3.84	17.53	2.44	6.20	28.35	28.35	1.94±0.51	0.96
6	2.58	15.31	1.44	4.51	27.64	27.64	1.62±0.61	0.99
7	1.53	14.61	0.704	3.31	25.73	25.73	1.28±0.77	0.92
8	0.62	14.27	0.200	1.99	23.01	23.01	1.76±0.78	0.92
9	0.50	6.22	0.183	1.49	17.24	17.24	1.17±0.85	0.95
10	0.19	3.95	0.49	0.73	15.19	15.19	0.97±1.02	0.99

R = Correlation coefficient of regression line, SD = Standard deviation, LT= Lethal time.

Conclusion

Our study provides information about the efficacy of ECO_2-Fume fumigant and Ozone gas on the red flour beetle, *T. castaneum*. The mortality rate for eggs, larvae, pupae, and adults increased with exposure time, while the LC_{50} and LC_{90} for Ozone gas against *T. castaneum* larvae, pupae and adults increased with exposure time. The study suggests that using ECO_2-Fume fumigant and Ozone gas as an alternative method to protect stored grains and their products against *T. castaneum* is crucial for Egypt's staple food industry.

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