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Comparative effects of microwave energy and gamma radiation of the red flour beetle
Tribolium castaneum (Coleoptera: Tenebrionidae)

Reda, E. Omar¹; Refaat, A. Mohamed²; Amira, M. El-Shewy¹ and Heba, H. Baume²

¹Department of Plant Protection, Faculty of Agriculture, Benha University.

²Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

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Abstract

The rust flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is the most prevalent pest in stored food for human and animal consumption. The current study was conducted to determine the efficacy of microwave energy and gamma radiation on *T. castaneum* larvae, pupae, and adults. Microwave was applied with exposure times of 30, 60, 120, 180, 240, and 300 sec. at the powers of 180, 300, and 450 W. In addition, gamma radiation was applied at a dosage of 200, 400, 600, 800, and 1000 Gray. Mortality percentage was recorded after 1, 2, 3, 4, 7, and 10 days, and LD₅₀ was recorded after 7 days of treatment. The results indicated that the mortality of *T. castaneum* increased with increasing doses and exposure time. The mortality of *T. castaneum* increased, reaching 100% at power levels of 300 s and 450 W. The LT₅₀ and LT₉₀ decreased as power levels and exposure time increased. Moreover, the dosage of 1000 Gray caused 85.0 and 65.0% mortality after 7 days of irradiation for larvae and adult stages. The required dosage of gamma radiation to kill 50% (LD₅₀) of adults after 7 days was 580.1 Gray. Therefore, the use of microwave energy and gamma radiation is a very important alternative method to protect stored grains and their products against *T. castaneum*.

Introduction

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) commonly known as the rust flour beetle is a worldwide, found mostly in tropical and subtropical regions. Insect pests that attack stored products prey on broken grains, flour mills, etc., resulting in loss and damage. Adults have lengthy lifespans and can survive for more than one year (Campbell and Runnion, 2003 and Bakhtawar, 2013).

Chemical pesticides have been widely used to suppress insect infestations in

recent decades, but their excessive use has led to environmental problems. To minimize damage and minimize risks to the environment and human health, it is necessary to find safer alternatives. Non-classic control methods include physical, natural, and biological methods, as well as dust, plant extracts, low or high temperatures, modified atmospheres, microwaves, and gamma irradiation. These methods can be integrated into integrated pest management programs (El-Lakwah *et al.*, 2004 and El-Lakwah and Gharib, 2005).

The frequency range of microwaves, a kind of electromagnetic radiation, is 300 MHz to 3000 GHz. A distinctive feature of microwaves is that they raise the temperature of a medium as they pass through it. Thus, there are a variety of practical uses for microwaves in the food and agriculture industries (Seyedabadi, 2015). Microwave heat kills pests at different stages of growth quickly and without leaving behind harmful chemical residues in food products or the environment, making it an efficient method of pest control (Sadeghi *et al.*, 2018). Many researchers studied the effect of microwave ovens to control *T. castaneum* (Vadivambal *et al.*, 2008; Lu *et al.*, 2010; Manickavasagan *et al.*, 2013; Meenatchi *et al.*, 2015 and Abotaleb *et al.*, 2021).

Gamma irradiation is a cost-effective and efficient method for insect disinfection, with most insects requiring doses below 0.3 k Gray, while some stored-product moths may require doses as high as 1 kGy (Follett and Neven, 2006). Gamma irradiation was studied for its lethal effect, as well as for inhibiting the reproduction of many stored grain pest species (Mastrangelo and Walder, 2011).

The purpose of this study was to evaluate the effectiveness of gamma radiation and microwave energy on larvae, pupae, and adults of the red flour beetle *T. castaneum*.

Materials and methods

The experiments were conducted in the Laboratory of Stored Products Department, Plant Protection Research Institute, Agricultural Research Center, Dokii, Giza, Egypt. All experiments were conducted under $30 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH.

1. 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 *T. castaneum* (1–2 weeks old) and were introduced into the jars to lay 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 to obtain large numbers of stages of *T. castaneum* needed to carry out the tests.

2. Evaluate the efficacy of microwave energy:

For the microwave treatment, samples were irradiated with microwave energy using an Electra, Japan, model EM–280 M with a capacity of 28 liters. The cavity dimensions (21.9 x 35 x 35 mm) were used. The oven had three energy settings (Low, medium-low, and medium) with respectively 17, 44, and 66% power output (Output: 800W).

3. Preparation of the test insects for microwave energy:

To commence microwave irradiation, each Petri dish containing 30 insects and 25 g of wheat flour was placed in a kitchen microwave at 2450 MHz. Petri dishes were set in the oven on plate rotation. The experiments were conducted with exposure times of 30, 60, 120, 180, 240, and 300 sec. at the powers of 180, 300, and 450 W for each. An untreated group (Control) of 100 eggs (0-24 hrs.) and 30 individuals (Larvae, pupae, and adults) was obtained at room temperature, away from the influence of microwaves. Each developmental stage of egg, larvae, pupae, and adults was replicated five times. A Tullgren funnel was used to separate insects from each treatment, which was then stored for 24 hrs. at 40 W electric lights, and the insects were collected in Petri dishes and counted. Mortality percentages were corrected by Abbott's formula (1925). The bioassay was conducted at 30°C with a relative humidity of $65 \pm 5\%$.

4. Evaluate the efficacy of gamma irradiation of *Tribolium castaneum*:

Radiation source: At an irradiator facility, *T. castaneum* was irradiated at 200, 400, and 600 Gray (Gy.) using an Indian

gamma cell radiation unit 0.744 a (KGy/h). With 800 Gy. facilities, the Canadian gamma cell utilizes 0.425 KGy /hrs. It is located in the National Center for Radiation Research and Technology of the Atomic Energy Authority in Nasr City, Cairo, Egypt.

5. Bioassay of gamma irradiation:

Wheat flour weighed 25 g for each glass jar. Each glass jar contained 30 adult beetles (7–14 days old) and 30 *T. castaneum* larvae (5 days old). The same radiator was used to irradiate adults and larvae at doses of 200, 400, 600, 800, and 1000 Gy. Each treatment was carried out in three separate runs. As a comparative control, three duplicates of untreated wheat flour were also used. Each jar was covered with muslin cloth and fastened with a rubber band before being kept in a laboratory. Following treatment, mortality dates were recorded at 1, 2, 3, 4, 7, and 10 days, and they were adjusted using Abbott's formula (Abbott, 1925). After 14 days, the adults were removed to acquire Progeny (F1) in samples.

6. Statistical Analysis:

Percentages of adult mortality were calculated using the initial number of individuals placed in each cage. In the case of larvae, or pupae, the mean number of emerging adults in the control treatments was utilized as the initial number of individuals when calculating the mortality rate. For statistical analysis, the average percent mortality of the tested insects was calculated and corrected using Abbott's formula (Abbott, 1925). Toxicity values (LC₅₀ and LC₉₉) were calculated by Probit analysis (Finney, 1971) using Ldp-line software to obtain the toxicity regression lines. The study used simple correlations and partial regression to analyze the effects of microwave and gamma radiation, focusing on variance explanation ratios (EV%) and concentration and time. In the microwave, the combined effect of time and energy was used. Data was analyzed using Collection,

Regression, and ANOVA in SAS, with each dependent variable compared for significance and separated using the LSD test ($P > 0.05$). Obtained results were analyzed using one-way analysis of variance (ANOVA) in SAS (Anonymous, 2003).

Results and discussion

1. Evaluate the efficacy of microwave energy against the egg, larvae, pupa, and adult of *Tribolium castaneum* at test temperatures at 30°C and 65% RH.:

The results in Table (1) indicate that the mortality rate in *T. castaneum* increases in eggs, larvae, pupae, and adult stages when exposure time or power settings are increased. A significant difference in the mortality rate of the egg, larvae, pupae, and adult stages at different powers of microwave energy and different exposure times was observed. Using microwave energy at a power level of 180 W and 30 sec, the highest mortality percentages for egg, larvae, pupae, and adult stages were 45.5, 71.1, 44.4, and 64.4%, respectively. At 300 W and 300 s, the egg, larvae, pupae, and adult stages mortality rates were 71.1, 90.1, 63.3, and 88.8%, respectively. By increasing exposure times and microwave power levels, egg, larvae, pupae, and adult stages mortality of *T. castaneum* increased, reaching 100% at power levels of 300 s and 450 W. For each power, an increase in the exposure time led to increased mortality.

The data presented in Table (2) showed the effect of microwave energy at various exposure times and power levels on *T. castaneum*. Non-significant positive correlation values were observed for the larvae and adult stages of *T. castaneum* at a power level of 0.53. Whereas significant positive correlation values were observed for the egg and pupae stages at power levels of 0.67 and 0.66. Statistical analysis showed that significant positive correlation values were observed for the egg, larvae, pupae, and adult stages of *T. castaneum* at an exposure

time ranging between (0.71 - 0.82). The combined effect of exposure time and microwave energy on the egg, larvae, pupae,

and adult stages of *T. castaneum*, respectively, was 96.33, 95.99, 95.11, and 95.74%.

Table (1): Mortality percentage of eggs, larvae, pupae, and adults of *Tribolium castaneum* exposed to microwave energy at various exposure times and power levels.

Power (W)	Exposure time (sec)	Corrected mortality percentage (%)			
		Eggs	Larvae	Pupae	Adults
180	30	0.0 f	5.5 f	0.0 e	2.2 f
	60	3.3 e	16.6 e	2.4 e	8.8 e
	120	12.2 d	32.2 d	11.1 d	24.4 d
	180	27.8 c	48.8 c	26.6 c	32.2 c
	240	38.2 b	57.8 b	37.8 b	47.7 b
	300	45.5 a	71.1 a	44.4 a	64.4 a
LSD 0.05		3.02	3.79	2.88	1.94
300	30	4.20 f	23.3 f	7.8 f	8.2 f
	60	22.2 e	41.1 e	21.1 e	25.5 e
	120	33.3 d	52.2 d	31.1 d	38.2 d
	180	42.2 c	68.9 c	40.1 c	55.5 c
	240	55.5 b	80.1 b	50.1 b	75.5 b
	300	71.1 a	90.1 a	63.3 a	88.8 a
LSD 0.05		5.1	4.35	3.22	1.79
450	30	28.2 e	33.3 e	22.2 e	20.0 e
	60	48.2 d	55.5 d	45.5 d	50.1 d
	120	65.5 c	70.1 c	60.0 c	65.5 c
	180	80.1 b	88.9 b	75.5 b	85.5 b
	240	97.1 a	100 a	95.5 a	98.2 a
	300	100 a	100 a	100 a	100 a
LSD 0.05		5.71	3.08	5.74	2.37

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 of microwave.

Stage	Factor	Simple correlation		Multiple regression				
		R	P	b	P	F	P	EV%
Eggs	Time	0.71	0.0009	0.22	0.0001	197.1	0.0001	96.33
	Power	0.67	0.0021	0.18	0.0001			
Larvae	Time	0.82	0.0001	0.23	0.0001	179.3	0.0001	95.99
	Power	0.53	0.0228	0.13	0.0001			
Pupae	Time	0.71	0.0008	0.21	0.0001	145.9	0.0001	95.11
	Power	0.66	0.0028	0.17	0.0001			
Adults	Time	0.82	0.0001	0.26	0.0001	168.7	0.0001	95.74
	Power	0.53	0.0230	0.14	0.0001			

r = Correlation, P= Probability, b= Slope, EV% = Explanation variance.

The lethal time of microwave on the egg, larvae, pupae, and adult stages of *T. castaneum* under power levels of 180, 300, and 450 W and an exposure time of 30, 60, 120, 180, 240, and 300 sec. are presented in Table (3). The LT₅₀ were 321.82, 182.67, 323.53, and 242.28 sec. at power levels of 180 w for eggs, larvae, pupae, and adults, respectively. These values decreased with increased power levels. A similar result for the LT₉₀ was a decrease as power levels increased and exposure time increased. The obtained results showed that the larvae of *T. castaneum* were more sensitive to microwaves than other stages.

These results agree with earlier findings by Vadivambal *et al.* (2008), who studied the mortality of *T. castaneum* life stages in barley samples using a pilot-scale industrial microwave system. They found that complete mortality occurred at power levels of 400 W and exposure times of 56 s or 500 W for 28 s eggs were most susceptible. Lu *et al.* (2010) found that microwave radiation and conductive heating were used to kill adult *T. castaneum* in wheat flour without significantly affecting its quality. Manickavasagan *et al.* (2013) found that power and time significantly affected the mortality rates of adult and larval stages of *T.*

castaneum and *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), with the effects being more pronounced in the larvae of *T. castaneum* than in the adults. The mortality of *T. castaneum* larvae was also 100% when the power and exposure periods were 600 W and 40 sec., respectively. Meenatchi *et al.* (2015) used a domestic microwave oven to control *Sitophilus zeamais* (Motsch.) (Coleoptera:Curculionidae) and *T. castaneum*. Mortality studies showed complete mortality at 540 W for 25 and 30 sec. Moreover, Abotaleb *et al.* (2021) revealed that.

Trogoderma granarium Everts (Coleoptera: Dermestidae) adults and larvae suffered from substantial mortality rates as exposure times and microwave energy increased; these rates reached 100% at 180 and 300W power levels during 120 and 150 sec. exposure times, respectively. Draz *et al.* (2021) found that microwave irradiation and UV radiation effectively control coleopteran insects in stored wheat. Exposure periods for UV radiation were 12 hrs. for *Sitophilus oryzae* L. (Coleoptera:Curculionidae) and 24 hrs. for *T. castaneum*, resulting in progeny reduction and preventive efficacy. Exposure to 70°C for 10 minutes killed 100% of adults and completely stopped egg-laying.

Table (3): Lethal time values and parameters of mortality regression line for the eggs, larvae, pupae, and adults of *Tribolium castaneum* exposed to different microwave power.

Microwave power (W)	stages	LT ₅₀ (s)	LT ₉₀ (s)	Confidence limits(s)				Slope ± SD	R
				LT ₅₀		LT ₉₀			
				lower	Upper	lower	upper		
180	Eggs	321.82	1009.04	277.69	400.96	708.71	1815.41	2.58±0.33	0.99
	larvae	182.67	747.52	161.46	209.81	568.21	1098.32	2.96±0.35	0.99
	pupae	323.53	968.78	280.20	400.81	689.18	1703.58	2.09±0.20	0.99
	Adults	242.28	866.00	213.53	283.32	647.74	1319.66	2.31±0.23	0.99
300	Eggs	189.34	858.73	165.83	220.55	631.39	1332.79	1.95±0.18	0.98
	larvae	85.04	434.75	72.36	97.98	342.20	603.07	1.80±0.16	0.97
	pupae	223.07	2372.96	188.48	276.47	909.56	2745.76	1.06±0.17	0.98
	Adults	129.02	446.63	96.69	169.88	357.66	824.40	2.37±0.18	0.97
450	Eggs	62.5	268.38	36.67	84.74	210.11	578.20	2.05±0.18	0.94
	larvae	51.15	218.67	29.71	68.29	168.14	437.50	2.03±0.18	0.94
	pupae	70.81	240.77	42.53	96.41	191.43	475.69	2.41±0.18	0.93
	Adults	64.78	200.20	46.55	81.75	219.51	446.44	2.61±0.18	0.96

R= Correlation coefficient of the regression line, SD= Standard deviation

2. Evaluate the efficacy of gamma irradiation against the larvae, pupae, and adults of *Tribolium castaneum* at test temperatures at 30°C and 65% RH.:

The data in Tables (4–6) show that the mortality percentages of *T. castaneum* larvae, pupae, and adults increased with increasing gamma radiation doses. A highly significant difference in the mortality rate of irradiated larvae, pupae, and adults at different doses of gamma radiation and different time intervals

was observed after 7 days post-irradiation. The mortality rates were 38.8, 46.6, 61.1, 75.5, and 85.5% for larvae irradiated; 26.7, 38.5, 48.3, 57.7, and 65.5% for pupae; and 33.3, 41.6, 51.1, 63.3, and 73.3% for adults at 200, 400, 600, 800, and 1000 Gy., respectively. At the same doses, the mortality rates after 10 days of irradiation were 51.1, 61.6, 75.0, 86.8, and 95.0% for adults, respectively.

Table (4): Efficacy of gamma radiation against *Tribolium castaneum* larvae at 30±1 °C; 65 ±5%RH.

Dose (Gy.)	Corrected mortality* (%) after an indicated period (Days) ±SD				
	1	3	5	7	10
200	0.0 d	23.3±3.90 e	31.6±2.23 e	38.8±4.90 e	65.6±2.91 d
400	10.1±0.22 c	35.5±5.25 d	38.8±4.02 d	46.6±6.08 d	78.3±3.30 c
600	11.6±0.57 bc	45.5±6.95 c	51.1±5.82 c	61.1±2.99 c	86.6±2.98 b
800	16.7±0.33 ab	55.5±10.40 b	66.6±3.95 b	75.5±3.09 b	95.6±3.39 a
1000	21.6±0.12 a	65.6±3.30 a	78.8±3.01 a	85.5 ±2.85 a	100.0±1.0 a
LSD 0.05	5.75	8.46	6.64	8.13	6.64

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

Table (5): Efficacy of gamma radiation against *Tribolium castaneum* pupae at 30±1°C; 65 ±5%RH.

Dose (Gy.)	Corrected mortality (%) after indicated period (Days) ±SD				
	1	3	5	7	10
200	0.0 d	6.6±1.91 d	13.3±3.01 d	26.7±4.96 e	48.3±7.07 e
400	0.0 d	13.3±3.91 d	25.0±1.44 c	38.5±1.93 d	58.3±3.30 d
600	5.1±0.22 c	23.3±3.41 c	35.0±5.12 b	48.3±5.81 c	71.6±8.21 c
800	10.1±0.31 b	33.3±1.93 b	38.3±10.4 b	57.7±4.09 b	83.3±6.1 b
1000	13.3±0.57 a	41.67±4.06 a	48.87±4.06 a	65.5 ±2.43 a	94.2±1.02 a
LSD 0.05	2.34	7.78	7.04	6.21	4.69

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

Table (6): Efficacy of gamma radiation against *Tribolium castaneum* adult at 30±1°C; 65 ±5%RH.

Dose (Gy)	Corrected mortality (%) after indicated period (days) ±SD				
	1	3	5	7	10
200	0.0 c	16.7±3.21 e	25.5±5.29 d	33.3±2.2 e	51.1±7.07 d
400	0.0 c	25.5±1.77 d	31.6±4.02 c	41.6±4.82 d	61.6±3.30 c
600	8.33±0.02 b	31.1±2.2 c	33.3±10.44 c	51.1±2.99 c	75.0±2.32 b
800	11.67±0.33 a	38.8±10.40 b	43.3±3.12 b	63.3±4.09 b	86.8±1.39 a
1000	15.0±0.57 a	46.7±2.91 a	53.3±3.21 a	73.3 ±1.85 a	95.0±10.1 a
LSD 0.05	4.69	6.21	6.21	5.25	9.96

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

Significant positive correlation values were observed for larvae, pupae, and adults of *T. castaneum* at an exposure time of 0.82, 0.86, and 0.86 with P-values of 0.0001, respectively, while non-significant positive correlation values were observed for larvae, pupae, and adults of *T. castaneum* at a dose of respectively 0.49, 0.44, and 0.46. The explained variance (EV%) for larvae, pupae, and adults of *T. castaneum*, respectively, was

93.17, 95.3, and 97.0%. These results indicated that exposure time is more effective on *T. castaneum* than the dose of gamma radiation (Table 7). The values of LD₅₀ were 347.47, 477.13, and 580.1 Gy. for larvae, pupae, and adults after 7 days, respectively. While LD₉₀ were 1754.79, 3879.1, and 2245.24 Gy. for larvae, pupae, and adults after 7 days, respectively (Table 8).

Table (7): Simple correlation and multiple regression values for the effect of gamma radiation.

Stage	Factor	Simple correlation		Multiple regression				
		r	P	b	P	F	P	EV%
Larvae	Time	0.82	0.0001	7.36	0.0001	150.0	0.0001	93.17
	Dose	0.49	0.0122	0.04	0.0001			
Pupae	Time	0.86	0.0001	7.0	0.0001	224.2	0.0001	95.3
	Dose	0.44	0.0256	0.03	0.0001			
Adults	Time	0.86	0.0001	7.11	0.0001	355.9	0.0001	97.0
	Dose	0.46	0.0188	0.04	0.0001			

r = Correlation, P= Probability, b= Slope, EV% = Explanation variance.

Table (8): Lethal dose values and parameters of the mortality regression line of gamma radiation on *Tribolium castaneum* larvae, pupae, and adults at 30±1 °C; 65 ±5%RH. after 7 days.

Stages	Lethal doses (G/y) of gamma radiation and their 95% confidence limits after 7 days		Slope ± SD	R
	LD ₅₀	LD ₉₀		
Larvae	347.47 (219.53–549.90)	1754.79 (1108.5–2777.62)	1.840±0.543	0.89
Pupae	477.13 (267.8 – 850.12)	3879.1 (2177.13–6911.6)	1.416±0.706	0.91
Adults	580.1 (328.6 – 1024.1)	2245.24 (1554.1 – 7962.9)	1.440±.694	0.98

These results agree with earlier findings by Ayvaz *et al.* (2002) found that 200 Gy gamma radiations are sufficient for *T. castaneum* adult mortality in 30 days. Abbas *et al.* (2010), showed that gamma radiation was more effective against the adults and larvae of *O. surinamensis*. Adults exposed to the 200 Gy. dose died in full 28 days after the radiation therapy. The developmental duration increased in a dose-dependent manner. Prabhakumary *et al.* (2011), found that low doses of gamma radiation effectively prevent *T. castaneum* from reproducing on cashew seeds, decreasing survival with increasing doses. Salim *et al.* (2018) indicated that a 0.50 kGy dose of gamma radiation can kill 100% of *T. confusum* adults and larvae in 22 days. Hammad *et al.* (2019) found that low gamma irradiation doses (50 – 1200 Gy.) were effective in disinfecting insect pests in stored grains, with 650 Gy. being required for cowpea seed weevils. Abdel-Aziz *et al.* (2023) studied the impact of gamma radiation doses on *O. surinamensis* adults on two types of stored dates. They found that mortality increased with increasing doses and exposure time, with 91.11% and 98.89% mortality rates for *O. surinamensis* adults at 800 Gy. exposure.

The study investigated the effectiveness of microwave energy and gamma radiation on different stages of *T. castaneum*. Microwave exposure times and gamma radiation dosages were tested. Results showed that mortality increased with doses and exposure time, reaching 100% at

300 s and 450 W. The LT₅₀ and LT₉₀ decreased with increased power levels and exposure time. The 1000 Gy. dosage caused 85.0 and 65.0% mortality for larvae and adult stages, respectively. The required gamma radiation dosage to kill 50% of adults was 580.1 Gy. The study suggests microwave energy and gamma radiation as an effective alternative for protecting stored grains and their products against *T. castaneum*.

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