Microwave Radiation as an Eco-friendly Tool for Controlling the Red Flour Beetle *Tribolium castaneum* (Hbst.) (Coleoptera:Tenebrionidae)

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ABSTRACT: The current investigation aimed to assay a non-traditional method to control the red flour beetle *Tribolium castaneum* without any changes on contents and quality of wheat grains they infest. Therefore, a household microwave system was used to assay the effect of microwave radiation on different developmental stages (larvae, male pupae, female pupae and unsexed adults) of the red flour beetle *Tribolium castaneum* (Hbst.), where all experiments were conducted at an exposure range of times of 3-20 s at power level of 800 W. Meanwhile, the effect of microwave radiation on treated wheat grain characters and germination was also investigated. The results showed that as the microwave exposure time increase, the mortality of different developmental stages of *T. castaneum* increased. The mortality of treated larvae of all ages (1-7, 7-14 and 14-21 days), male pupae, female pupae and adults were 100% at the power and exposure time of 800W and 20 s, respectively. The median lethal time (LT_{50}) of exposure to microwave irradiation indicated that the adult stage (LT_{50}= 8.951±0.114 s) was more tolerant to microwave irradiation than pupae (LT_{50}= 4.015±0.0.230 s). The calculated percentage means of germination for the exposed grains to microwave radiation at 20, 15 and 10, decreased from 94.17% in the untreated control up to 40.83, 63.33 and 70.83 %, respectively. High degradation and aggregation of DNA of the red flour adult beetle were detected after the microwave treatment of 400W for 20 s. The results showed that there were 8 bands of esterases while the control pattern showed only 4 bands which prove that the adults increasing their enzymes activity as a way of protecting themselves from the produced radiation stress from the microwave. It could be concluded that the microwave (800 W)-treated grains or flour for 20 s can be stored free of different developmental stages of the red flour beetle and can be used only for consumption purposes. Because the germination of the treated grains was affected and rather reduced, while the composition of the grains (carbohydrates, fat, protein, ash, fiber and water content) was not significantly affected.

Keywords: Control, Microwave radiation, *Tribolium castaneum*, LT_{50}, Larvae, Pupae, Adults, Wheat germination and components, DNA, Isozymes activity.

NTRODUCTION

Generally, postharvest food losses are estimated to range from 8 to 10% in industrialized countries (Ciepielewska and Kordan, 2001; Brader *et al*., 2002). Insects are a problem in stored grain throughout the world because they reduce the quantity and quality of grain (Sinha and Watters, 1985; Madrid *et al*., 1990; Rajendran, 2002; Warchalewski and Gralik, 2010). The red flour beetle *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae) is a secondary insect- pest species that attacks stored food products in cereal mills, food processing plants, grocery stores, households and ships. The red flour beetle is reddish brown to blackish in color with antennae that ends as a prominent three-segmented club (Bousquet, 1990). It has a length of 2.3-4.4 mm and can fly when conditions are hot and humid. It is considered to be a serious pest species that attacks a very wide range of foodstuffs, especially oilseed cake, groundnuts, cereals,

milled cereal products, beans and other dried food products. The female lays tiny white eggs (5-15 eggs/day) that hatch after about 9 days. The larvae are creamy to light brown with two dark projections on the last body segment. Larvae remain feeding outside of the grain (Kranz *et al*., 1977). The larval stage duration ranges from about 22 to over 100 days depending on temperature. Adults emerge from the pupa after about 8 days and they live for three years feeding on the host (Walter, 1990). *T. castaneum* is considered to be as a major important insect-pest infesting wheat, barley, millet, rice and maize (Champ and Dyte, 1976) and it is a common internal feeder.

Control of the stored grain insects around the world depends primarily upon applications of organophosphorus, pyrethroid insecticides and the fumigants (i.e. methyl bromide and phosphine). Contact insecticides and fumigants have been used for a long time to protect stored products from insect pests (Daglish, 2006). In response to a growing market demand for food stuffs that are free of pesticide residues, and because stored-product insects are developing resistance to insecticides, such chemicals have been remained under increasingly restrictive policies over the past years (Kljajic and Peric, 2005; Collins, 2006). New trends and safe means in the field of stored grain insects management are needed.

One of the safe and effective control methods is the usage of microwave energy as a physical control method (Johnson *et al.,* 2003). Microwave is an eco-friendly method and doesn't cause any hazardous impacts toward humans (Vadivambal *et al.,* 2010). Exposure to microwave energy could reduce reproduction rates and cause physical injuries and malformation in surviving insects (Nelson, 1996). Microwave radiation, with good penetrability, can kill pests existing inside or outside grain kernels (Halverson *et al*., 1996). Significant reduction of stored-grain damage by insect pests can be achieved with the use of microwaves (Nawrot *et al*., 2006).

Wheat grains and baking quality of flour are also affected by microwave radiation (Que, 2013; Warchalewski *et al.,* 2011). Qu *et al.* (2017) found that gluten, farinograph properties and viscosity of the flour of microwave treated wheat were influenced to a small extent at the exposure time of 20 seconds at700W. The physiological processes of insects are also negatively affected by microwave radiation which leads to a considerable reduction in their reproduction and survival (Webber *et al*., 1980; Wang *et al*., 2003; Vadivambal *et al*., 2008; Valizadegan *et al*., 2009&2011). Microwave radiation affected DNA and biochemical characteristics of *T. castaneum* adults and other stored product insects (Lu *et al*., 2010&2011; Sang *et al*., 2012, Pandir and Guven, 2014; Tungjitwitayakul *et al.*, 2016).

The microwave has potential applications in pest management and it has been proven to be rapid, nondestructive targeting the incipient insect infestations and would be of benefit to both the producers and consumers of packaged foods (Abdelaal and El-Dafrawy, 2014). Therefore, the present investigation was conducted to assay the efficacy of microwave radiation against different developmental stages of the red flour beetle *Tribolium castaneum* and the impact of microwave power on wheat grains components and germination. Moreover, the study also investigated the effect of microwave treatment on DNA and isozymes of the treated adults of *T. castaneum*.

MATERILS AND METHODS

1. Culturing of the tested insect *Tribolium castaneum*

The culture medium used in the present work was a mixture of wheat middling: whole meal wheat flour: dried yeast in the ratio 18: 17: 1 (w/w). The medium and filter papers were sterilized by heating at 70° C for two hours. About 300 mixed adults *T. castaneum* from a previous culture were introduced into a sterilized 2.5 liters glass jar containing approximately 450 g culture medium. The jars were sealed with filter paper and maintained at 25° C and 70% R.H.

2. Collection of larvae with different ages, pupae and adults for bioassay

After one week, the mated adults were sieved out of the medium by a 25 mesh sieve and the flour containing eggs and first instar larvae was collected. The larvae of the 1-7 days age were transferred to 3 Petri dishes; each containing 20 larvae with 10 grams of media. After 2 and three weeks the same manner was performed to collect larvae with different ages of 7-14 and 14-21 days age to be exposed to microwave. The rest of flour containing larvae were resealed in a new jar together with some fresh medium. Culture jars were stored on trays containing 50 ml of liquid paraffin to prevent mite infestation. When the pupae were formed they were collected and sexed (Photo1). The emerged adults were used for bioassay tests as sex mixed populations of 2 - 3 weeks old. The elapsed time from culture inoculation till use of adults for bioassay test averaged 8 – 9 weeks.

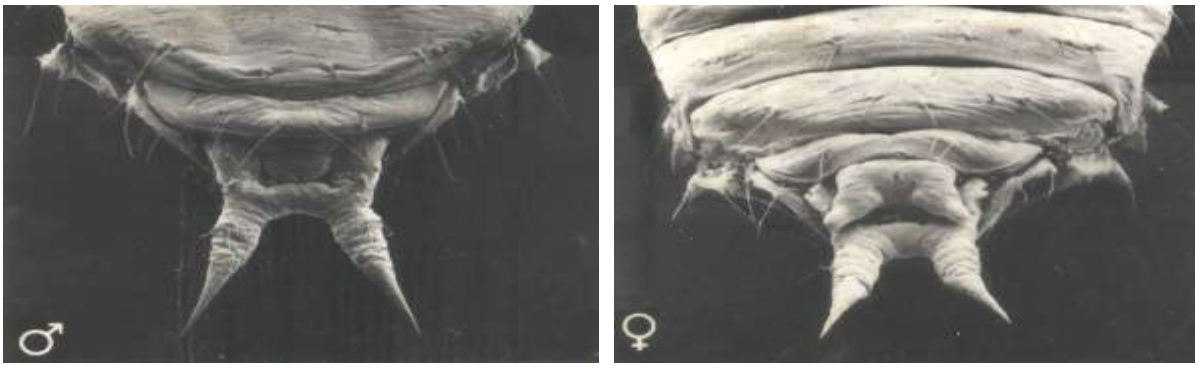

Photo (1). Terminal view of male (♂) and female (♀) *Tribolium* pupae showing the sexual characters and the difference between them.

3. Microwave treatments

Batches of 10 pairs of 1-7, 7-14 and 14-21 days old larvae were used. Also sexed males and/or females pupae and two weeks old unsexed adults of *T. castaneum* were introduced in Petri dishes (9cm in diameter). These Petri dishes with insects and about 10 grams of flour media were used for assessing the effect of microwave exposure periods. The microwave oven that has been used in the present investigation was ELECTRN® (800W output power) model No. EM-280M (220-240v 50-70 Hz with a capacity of 28 liters (21.9 X 35 X 35 cm, Electra Ltd- Japan. It had three levels of output power; the lower (136W), the median (400W) and high level (800W).The larvae, pupae and adult insects were exposed to microwave radiation (800 W) for different periods of 3, 5, 8, 10, 15 and 20

seconds. Thereafter, they were left for one hour and the mortality percentages were calculated as compared with the untreated check individuals (control).

4. Effect of microwave radiation on wheat grain characteristics

4.1. Chemical analyses of wheat samples

The induced changes in the nutritional characteristics of exposed grains to microwave radiation compared with the untreated ones were determined (crude protein, crude fat, crude fiber, ash content and carbohydrates) according to the methods of AOAC (1990). The chemical analysis of the grains of the tested wheat cultivar (Giza 168) was done only for the untreated and treated grains that have been exposed for 20 s inside the microwave. The chemical analysis was carried out at the Central Laboratory, Faculty of Agriculture, Alex. Univ., Egypt.

4.2. Determination of moisture content (MC %) of wheat grains

Moister content of the grain before and after microwave treatment was determined to investigate the correlation between the moisture content of the grain and the effect of radiation. The determination was done by oven method (Horwitz, 1970). Samples of 20 g of ground wheat were weighed, put in open aluminum dishes and placed in the oven at 130 ± 3°C for 1 hour together with their covers. Then the dishes kept inside the oven were covered, transferred to a desiccator, cooled up to room temperature and weighed. The difference in weight was expressed as a percentage of the tricely initial sample weight. Assessment of moisture content was performed for replicated wheat samples.

4.3. Grains germination as affected by microwave radiation

Microwave treated wheat grains were subjected to germination test according to Qi and Burkholder (1981) with slight modification. Three replicates each of 20 grams microwave treated wheat grains were subjected to germination in Petri dishes embedded with soaked cotton layer with tap water and covered with wet tissue paper. Germination percentages of treated and untreated wheat grains were recorded after 2 and 4 days.

4.4. DNA and isozymes electrophoresis

4.4.1. DNA Extraction

DNA damage was measured by the cetyl-trimethyl ammonium bromide (CTAB) method (Taylor and Powell, 1982; Fang *et al*., 1992), using phenol–chloroform extraction. Adult beetles were weighed (0.5 g); placed in a small and clean glass mortar; 0.9 ml extraction buffer (2% cetyl-trimethylammonium bromide [CTAB], 1.4 M NaCl, 20 mM EDTA, 100 mM Tris–HCl pH 8, 0.2% polyvinyl-polypyrrolidone [PVPP], 0.01% sodium dodecyl sulfate [SDS], 0.2% a-mercaptoethanol) was added to the mortar and the beetles fully ground to a paste, which was transferred to a 2-ml centrifuge tube with the tip cut off and replaced with a 0.5-cm blue suction head. After 45 min of extraction at room temperature, samples were twicely washed with 0.6 ml chloroform-isoamyl alcohol (24:1, v/v) followed by centrifugation for 10 min (12000 rpm). DNA was precipitated with a half volume of isopropanol for 20 min at 20°C and pelleted by 20 min of centrifugation at 4°C. The pellet was washed with 70% cold ethanol, vacuum dried and dissolved in 0.3 ml TE buffer (pH 8). After 30 min of RNase (0.02 mg) treatment at 37°C, samples were stored at 20°C (Doyle and Doyle, 1987).

4.4.2. Isozymes electrophoresis

The esterase enzyme activity of *Tribolium castaneum* adults was assayed as follow:

a- Esterase analysis: Agar-starch-polyvinyl pyrolidine (PVP) gel electrophoresis was carried out according to the procedures described by Shaw and Kaen (1967) and Andrews (l981). Soon after insects were killed, the extracts were made by grinding 15 unsexed adults in a mortar with 10 µl of electrode buffer and centrifuged for15 seconds. The gel buffer used was 0.07 M Tris, 0.007 M citric acid with pH= 8.3. Finally, the staining process was done according to Youssef *et al*. (1989). As conventional symbols in electrophoretic analysis, a pattern was first described in terms of Anodal (A) or Cathodal (C) zones according to their direction of mobility in the electrophoretic field. Each zone was assigned for a locus coding for an esterase isozyme. The locus with the least migration is designated the first; the next one is the second, and so on. Allelic variants, however, were designated according to their relative electrophoretic mobility within a locus; the allele for slow migrating band was specified by (S) and the allele for the fast one by (F). In case where no isozyme is expressed at a certain locus, the presence of a third allele, (N) (for null) was assumed.

b- Electrode buffer composition: This buffer was prepared by dissolving 92.75 g of 0.3M boric acid and 12 g sodium hydroxide in 5 liters of distilled water, then the solution was adjusted to pH= 8.3.

c- Gel buffer composition: The used gel buffer was 0.07 M Tris 0.007 M citric acid (pH=8.3). One liter of the gel buffer was prepared by dissolving 9.21 g Tris, l.05 g citric acid in distilled water, and kept in a refrigerator until experimental use. Agar-starch-polyvinyl pyrolidine (P.V.P) gel was prepared by dissolving 1.0g, 0.5g P.V.P. and 0.5g of hydrolyzed starch with 10 ml electrode buffer and 90 ml distilled water. The mixture was shaken vigorously and cooked in a boiling water bath until the solution become transparent. The hot liquid gel was poured on glass plates (20 X 30 cm) to produce a smooth surface layer with thickness of 0.8-0.9 mm and they were kept at 4°C until usage (El-Metainy *et al*., 1977).

d- Running conditions: Electrophoresis experiments were conducted in an incubator refrigerator adjusted at 4°C using a 250 volts AC electrical current, with constant voltage throughout the 90 minutes of the running period.

e- Staining procedure: Phosphate buffer of 0.l M at pH=7.0 was used as a staining buffer by adding 39 ml of 0.l M solution of monobasic sodium phosphate to 61 ml of 0.1 M solution of sodium dibasic phosphate and completed to a final volume of 200 ml with distilled water. Each gel was incubated in l00 ml phosphate buffer (pH=7.0) containing 20 mg α-naphthyl acetate (α-NA), 20 mg β-naphthyl acetate (B-NA) dissolved in l ml acetone and completed to 5 ml by distilled water. Fifty milligrams of fast blue RR salt was dissolved in 5 ml distilled water and added after three minutes of the addition of α- and β-NA. Incubation was extended for thirty minutes at room temperature and complete darkness. Plates were then distained in distilled water until a clear background of gel plate was appeared (Youssef *et al*., 1989).

5- Statistical analysis

Mortality/ time regression analysis for the treatments of female pupae, male pupae and adults was done by a program adapted as a BASIC computer program to calculate LT_{50} and $LC_{95,s}$ and the associated parameter (slope [b]) (Finney, 1971). To determine the significant differences among treatments mean values at 0.05 probability level, all data were subjected to one-way analysis of variance (ANOVA) followed by Duncan multiple range test (Duncan, 1955). Moreover, to determine the significant differences among two means, *t*-test was applied.

RESULTS AND DISCUSSION

1- Effect of microwave radiation on the immature and adult stages of *T. casatneum*

In case of exposing the different developmental stages of the red flour beetle *T. castaneum* to microwave radiation (800W), the results listed in Table (1) revealed that the short exposure period of 5 sec gave 73.0, 60.0, 53.0, 60.0, 70.0 and 15.0 % mortality for the exposed 1-7, 7-14 and 14-21 days-old larvae, female and male pupae and unsexed adults, in respect. Complete mortality of all tested stages of *T. castaneum* achieved at a power level of 800 W and an exposure time of 20 sec. Among the tested stages of *T. castaneum*, 1-7 days old larvae and 1day-old male pupae were the most susceptible to microwave energy while adults were the least susceptible. It could be noticed that there was an increase in the mortality with an increase in exposure time. The present results are in agreement with those reported by Vadivambal *et al.* (2008) and Vadivambal *et al.* (2009) who reported that complete mortality of all life stages of *T. castaneum* can be achieved at a power level of 400 W and an exposure time of 56 s or at 500 W for 28 s. They also added that eggs were the most susceptible to microwave energy and adults were the least susceptible. It could be also noticed that as the microwave exposure time increase, the mortality of different developmental stages of *T. castaneum* increased. The entire or complete mortality of *T. castaneum* larvae (100%) was recorded when the power and exposure time were 800W and 20 s, respectively and these results also are in agreement with those arrived at by Manickavasagan *et al*. (2013) who found that complete mortality of *T. castaneum* larvae was achieved at the power of 600W and exposure time of 40 s.

Table (1). Effect of microwave radiation on different developmental stages of *T. casatneum*

Exposure time (Seconds)	Mean No. of responded individuals after exposure to microwave irradiation (800W) (Mortality %)					
	Larval age (days)			1 day-old Pupae		Unsexed adults (2 weeks old)
	1-7	7-14	14-21	♀	♂	
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
5	14.6 (73.0)	12.0 (60.0)	10.6 (53.0)	12.0 (60.0)	14.0 (70.0)	3.0 (15.0)
10	20.0 (100.0)	20.0 (100)	20.0 (100.0)	20.0 (100.0)	20.0 (100.0)	5.0 (25.0)
15	20.0 (100.0)	20.0 (100.0)	20.0 (100.0)	20.0 (100.0)	20.0 (100.0)	16.3 (81.5)
20	20.0 (100.0)	20.0 (100.0)	20.0 (100.0)	20.0 (100.0)	20.0 (100.0)	20.0 (100.0)

To calculate LT_{50} of pupae and adults, the periodic range of exposure intervals was expanded. Table (2) shows the calculated LT_{50}, LT_{95} and the associated parameters (slop[b] and regression coefficient [r^2]). The median lethal time (LT_{50}) of exposure to microwave irradiation indicated that the adult stage was more tolerant (LT_{50}= 8.951±0.114 sec) to microwave irradiation than pupal stage (LT_{50}= 4.015±0.0.230 sec). In this respect, Barbosa *et al*. (2017) found that the lethal microwave exposure periods to the cowpea weevil *C. maculatus* larvae were 120 and 150 s at the low power level of microwave of 240W. These results assured that as the power of the microwave decrease the lethal time increase. Pandir and Guven (2014) reported that 1-2 day old larvae of the stored product pest *E. kuehniella* (the tropical warehouse moth) were exposed to microwave radiation at the powers of 70, 150, 300 and 600 W for different exposure times (1-50 s). Mortality ratio in larvae increased significantly with increasing exposure time at all powers of microwave radiation.

Table (2). Calculated LT_{50} of microwave radiation on *T. castaneum* pupae and adults

Exposure time (s)	Average No. of responded unsexed individuals		Average mortality (%)	
	Pupae	Adults	Pupae	Adults
0	0.00	0.00	0.00	0.00
3	7.33	5.33	36.65	26.65
5	15.00	3.00	75.00	15.00
8	15.33	10.33	76.65	51.65
10	20.00	5.00	100.00	25.00
12	20.00	15.33	100.00	76.65
14	20.00	12.00	100.00	60.00
15	20.00	16.33	100.00	81.65
18	20.00	16.00	100.00	80.00
20	20.00	20.00	100.00	100.00
	LT_{50}= 4.015 ± 0.230 (**LT_{95}**= 9.600 ± 0.530) **b** =0.053 ± 0.08	**LT_{50}**= 8.951 ± 0.114 (**LT_{95}**= 17.873 ± 0.246) **b** =0.033± 0.019		

2. Effect of microwave radiation on wheat composition

When applying microwave energy to reduce grain damage by insects it should always be considered how this treatment will affect wheat grain properties**.** The results listed in Table (**3**) show that carbohydrate, fat and water content (moisture %) of the microwave treated wheat grains were slightly decreased, versus a slight increase in protein and ash in comparison with the untreated wheat grains. Nevertheless, the statistical analysis using *t*-test showed that there were no significant differences between the calculated mean values of each analyzed component of wheat composition in the treated and untreated wheat grain samples. Variance analysis showed that exposing wheat to microwave (800 W) for 20 s did not significantly affect the composition of the treated wheat. Microwave treatment has the advantage of maintaining the quality of grains during production (Nair *et al*., 2011). Microwave treatment of wheat grain might be a positive process of changing the physico-chemical properties that impacts the baking quality of flour. The presented results are in agreement with those reported by Que (2013) who found that the moisture content of grain samples after microwave heating is decreased. Also, Mohamed *et al.* (2011) found that there was no significant difference in the chemical composition, crude protein, oil and total carbohydrate contents between treated and untreated faba bean seeds exposed to microwave energy. Moreover, Diraman (2010) reported that the total protein content of wheat grain samples was not affected significantly by heating with microwave treatment. His results of the flour samples indicated that the physicochemically important parameters of baking quality were improved by certain microwave treatment times (120–180 s).

Table (3). The changes of chemical properties of wheat grain composition (Giza 168) due to microwave radiation (800W for 20 s) as compared with control

Wheat grain	Carbohydrate (%)	Fat (%)	Protein (%)	Ash (%)	Fiber (%)	Moisture (%)
Untreated	72.75* ±0.98	0.92±0.06	12.32±2.31	1.36±0.38	0.73±0.20	11.92±0.78
Treated	71.66 ±0.76	0.87±0.07	12.64±2.99	1.52±0.26	1.55±0.32	11.76±1.11
Significance (*t*-test)	N.S	N.S	N.S	N.S	N.S	N.S

* ± SE (Standard Error)

3. The effect of different microwave powers on wheat germination

The effect of microwave radiation on wheat grain germination after 2 and 4 days post-treatment is shown in Table (4). The recorded results showed significant differences between all treatments except the treatment of the exposure period of 5 s as it was not significantly differed from control. The germination due to the microwave exposure for 10, 15 and 20 s decreased from 94.17% (in control) (the mean of germination percentage post 2 and 4 days from treatment) to 40.83, 63.33 and 70.83 % for the exposure periods of 20, 15 and 10 s, respectively. The presented results agreed with the results of Halverson *et al*. (1996) who found that the percentages of germination were decreased as the exposure period to microwave radiation increased. Also, Vadivambal *et al*. (2010) mentioned that the germination of corn was decreased with the increase of microwave power or exposure time or both. The presented results are also in agreement with those of Vadivambal *et al*. (2008) who reported that germination of rye was lowered after treatment with microwave

energy. It was also noticed that there was a temporary increase in the grain temperature with the increase of microwave radiation power lasted only for limited time to move or release extent reflected on the viability of grain embryo and grain gemination.

4. Effect of microwave on the insect DNA

Data in Figure 1 illustrate the electrophoretic pattern of *T. castaneum* DNA. The mobility of the main band of DNA from control beetles was consistent with a molecular size ~1000 bp. The effect of microwave heat (400W for 20 seconds) was to shift the main electrophoresis band downward in the gel as seen in treated samples where DNA aggregated at the end of the columns. Data showed downward shifts, indicating that the effect of the microwave treatment on DNA was both aggregation and degradation. Microwave treatment caused DNA damage and there is a high degradation was observed after treatment comparing with control and standard DNA. DNA damage is an indicator of physiological stress due to microwave radiation and that has been shown via the greater migration of DNA fragments on the gel. Pandir and Guven (2014) found that there was no significant DNA damage detected at the power level of 70, 150 and 300 W for 50 s when compared with control group.

Table (4). Effect of microwave radiation (800W) on grains temperature and germination of exposed wheat grains for different times

Exposure period (s)	Grains temperature °C	Germination %		
		Days after treatment		Average
		2	4	
0	27	93.33 (±5.77)**	95.00 (±10.00)	94.17 a*
5	38	90.00 (±10.00)	90.00 (±10.00)	90.00 a
10	52	70.83 (±17.56)	70.83 (±17.56)	70.83 b
15	59	63.33 (±5.77)	63.33 (±5.77)	63.33 c
20	68	40.00 (±10.00)	41.67 (±5.77)	40.83 d

*Means followed by the same letter (s) in a column are not significantly different at 0.05 level of probability.
**Standard deviation (±S.D).

However, 50 s of 600 W power showed effects on tail length and tail intensity indicating the incidence of DNA damage for the larvae of the stored product pest *E. kuehniella*.

The present results are in agreement with those of Marín-Huachaca *et al*. (2005) who reported that the irradiated beef meat cells showed an increased extension of the DNA from the nucleus towards the anode, whereas unirradiated cells appeared nearly circular or with only slight tails.

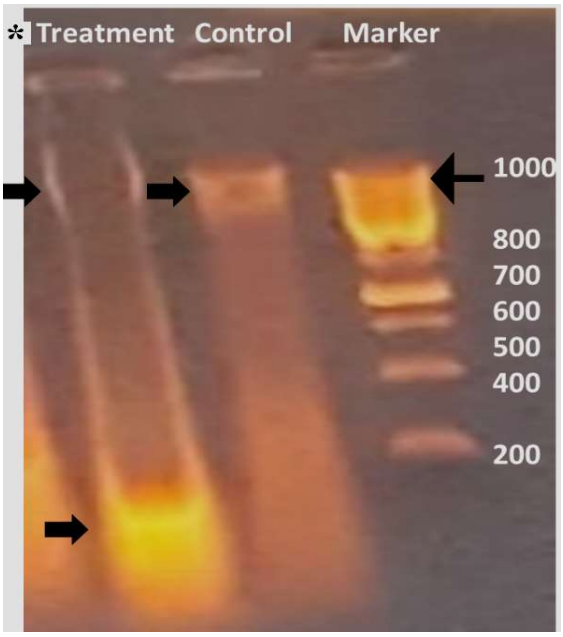

Fig. (1). Electrophoresis of *Tribolium castaneum* DNA after exposing the adults to 400W inside a microwave for 20 seconds.
(*Marker = standard DNA, Control = untreated adults and Treatment =treated adults).

5. Effect of microwave on the insect esterase enzymes activity

Eight different enzyme loci were observed in Figure (2) for esterase activity of the red flour beetle *T. castaneum*. Three loci were found at positive charge or Anode that were Est.1, 2 and 3, while five negative or cathodal bands were recorded Est.1C to Est.5C. Data showed high increase in enzyme activity with the increase in treatment exposure time (50 second), where it showed eight bands compared with four bands only in the untreated control. There were two common bands observed (Est. 2C and Est. 3C) for control and treatments. Generally, it could be concluded that the microwave radiated *T. castaneum* beetles were trying to increase their enzyme activity as they were physiologically stressed by microwave radiation or as a way to protect themselves from death under the high exposure time of microwave. The present results are in agreement with those mentioned in the work of Mohamed *et al*. (2011) who found that the maximum number of bands (14) appeared with 5 minutes of microwave exposure time at the level power of 17% of 800W (136 W) but when exposure time decreased to 3 and 4 minutes, it showed the minimum number of bands (10) when they performed SDS-PAGE in soluble faba bean seed protein (as the host of the insect) exposed to microwave radiation. Herein, it could be said that also the bands of faba bean seeds as the insect host were also affected and increased as the time of exposure increased. The activity of more enzymes in seeds might be useful for improving the quality of seeds for processing purposes. Moreover, the results of Pandir and Guven (2014) suggested that antioxidant enzymes activity of larvae of the stored product pest *E. kuehniella* increased significantly with an increase in both the power and the exposure times.

To date, a great deal of research has been conducted on the use of microwave irradiation to control pests in stored products (Vadivambal *et al*., 2008; Valizadegan *et al*., 2009; Lu *et al*., 2010). The results of the present study indicate that high powers of microwave radiation treatments cause either death or some physiological effects on the stored product insect-pest *T. castaneum*. There has been an increasing trend of microwave use not only in food industry but also in household kitchen as well.

Therefore, the use of microwave heating has the advantage of saving time and energy and did not affecting the composition of treated wheat grain especially carbohydrates and protein as well as causing significant reduction of stored-grain damage by such insect- pest. The microwave (800 W)-treated wheat grains for 20 s can be stored free of different developmental stages of the red flour beetle *T. castaneum* and can be used for consumption purposes not for plantation because the germination of these grains was affected and reduced, while the composition of grains was not affected. The treatment of infested grain by microwave appears to be a reliable alternative to conventional post-harvest insect control methods in the near future, either with stationary or mobile applicators on the farm or quarantine purposes during the loading process (pre- or post-shipment) before grain storage.

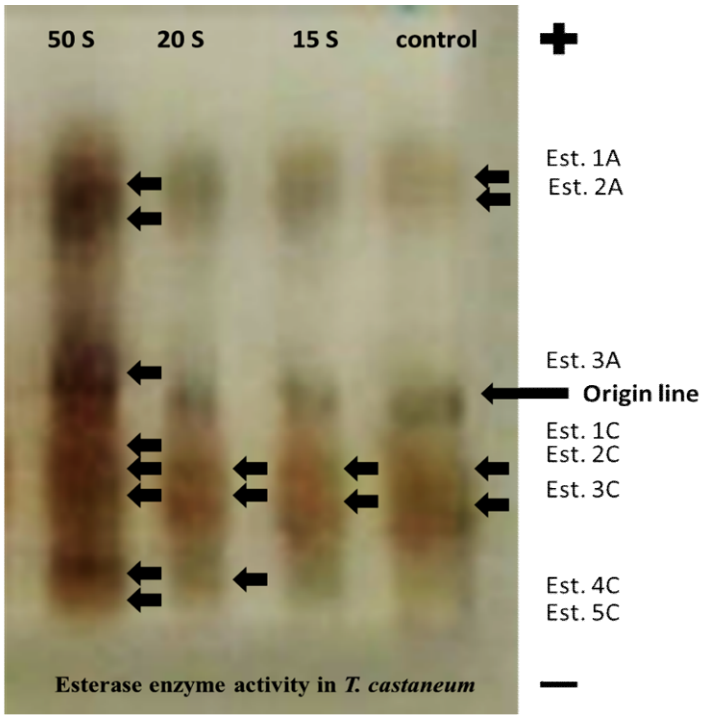

Fig. (2). Pattern of esterase enzymes activity of *T. castaneum* after different exposure periods (15, 20 and 50 s) to microwave heat radiation (400W) compared with unexposed beetles (control)

The major problem in the use of microwave energy for the eradication of pests and pathogens in seeds or/and grains that it has its adverse effect on their germination and this problem might be taken in consideration by reducing both the power level of microwave radiation and/or exposure time.

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الملخص العربي

إشعاع الميكروويف كأداة آمنة بيئياً لمكافحة خنفساء الدقيق الحمراء

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استهدفت الدراسة الحالية تقييم طريقة غير تقليدية لمكافحة خنفساء الدقيق الحمراء وبدون حدوث أي تغيرات في محتوي وجودة حبوب القمح التي تصيبها هذه الحشرة.ولهذا فقد تم إستخدام ميكروويف منزلي لتقييم الإشعاع الميكروويفي علي مراحل تطور هذه الحشرة المختلفة (يرقات ، عذاري ذكور ، عذاري إناث ، حشرات كاملة) حيث أُجريت التجارب بتعريض هذه المراحل المختلفة إلي مستوي ٨٠٠ وات بإستخدام مدي وقتي لتعرضها يتراوح من ٣–٢٠ ثانية. كما تم في نفس الوقت دراسة تأثير إشعاع الميكروويف علي خصائص ومكونات ونسبة الإنبات في حبوب القمح المعامل. وقد بينت النتائج أنه بزيادة الوقت المعرض للإشعاع تزيد نسبة الموت في الأفراد المختبرة من مراحل التطور المختلفة لخنفساء الدقيق حيث كانت نسبة موت اليرقات بجميع أعمارها والعذاري الذكور والإناث والحشرات الكاملة ١٠٠% عند ٨٠٠ وات وزمن تعرض ٢٠ ثانية.

وبحساب الزمن اللازم لقتل ٥٠% من الأفراد المختبرة (LT_{50}) وجد أن الحشرات الكاملة أكثر تحملاً للإشعاع بالمقارنة بالعذاري حيث كانت قيمة الـ LT_{50} في حالة الحشرات الكاملة أكبر (٨,٩٥١ ثانية) من تلك المحسوبة للعذاري (٤,٠١٥ ثانية). وقد أنخفضت نسبة الإنبات للحبوب المختبرة عند ٨٠٠ وات في فترات تعرض مختلفة حيث أنخفضت من ٩٤,١٧ % (كما في القمح غير المعامل) إلي ٤٠,٨٣ ، ٦٣,٣٣ و ٧٠,٨٣ % عند التعرض لمدة ٢٠ ، ١٥، ١٠ ثواني علي التوالي.

وقد أدت معاملة الحشرات الكاملة عند ٤٠٠ وات لمدة ٢٠ ثانية إلي تحطم وتجمع الـ DNA مع وجود ثمانية حزم من الإستيريزات بينما كانت هذه الحزم هي ٤ فقط في الحشرات غيرالمعاملة وعلي هذا فإن زيادة النشاط الإنزيمي قد يكون وسيلة من ضمن وسائل الحشرة للحماية ضد الإشعاع الميكروويفي. ويمكن اجمال القول بان الحبوب المعاملة بالميكروويف (٨٠٠ وات) لمدة ٢٠ ثانية سوف تكون خالية من المراحل التطورية المختلفة لخنفساء الدقيق الحمراء ويمكن إستخدامها بغرض الغذاء والإستهلاك وليس بغرض الإستخدام للزراعة حيث تأثرت نسبة إنبات حبوب القمح (صنف جيزة ١٦٨) معنوياً نتيجة المعاملة بينما لم تتأثر مكونات هذه الحبوب (الكربوهيدرات ، الدهن ، البروتين ، الرماد ، الألياف ، المحتوي الرطوبي).