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Orijinal araştırma (Original article)

Effect of microwave radiation on stored product pest *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae

Depo ürün zararlısı *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvaları üzerine mikrodalga radyasyonunun etkisi

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Summary

This study was designed to examine the effects of microwave radiation exposure on *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) larvae. One to two day old larvae 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. Completele mortality was achieved at the power of 70 W and the longest exposure time (50 s) Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) in the larvae tissue were evaluated.. SOD, CAT, GPx activities decreased and MDA level increased in the microwave radiation-treated larvae tissue compared to control group at 300 and 600 W ($P < 0.05$). There was no significant DNA damage detectable at 70, 150 and 300 W for 50 s when compared with control group. However, 50 s of 600 W powers was showed effects on tail length and tail intensity indicating DNA damage.These results indicate that high powers of microwave radiation treatments cause some effects on the stored product pest *E. kuehniella* larvae.

Key words: Oxidative stress, *E. kuehniella* larvae, DNA damage, microwave.

Özet

Bu çalışma *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) larvaları üzerine mikrodalga radyasyonunun etkilerini araştırmak için düzenlenmiştir. Bir iki günlük larvalar 70, 150, 300 ve 600 W lık mikrodalga radyasyonuna farklı uygulama sürelerinde (1-50 sn) maruz bırakılmıştır. Larvaların ölüm oranı mikrodalga radyasyonun tüm uygulama gücünde artan sürelerle birlikte istatistiksel olarak artmıştır. Tam ölüm 70 W dozunda 50 sn'ye kadar sürerken 600 W'ta ölümlerin başladığı en kısa süre 5 sn'dir. Larva dokularında superoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPx)'ı ve malondialdehit (MDA) 50 sn için tüm mikrodalga radyasyon gücü için değerlendirilmiştir. 300 ve 600 W gücündeki mikrodalga radyasyonuna maruz kalan larva dokuları ile kontrol grubu karşılaştırıldığında SOD, CAT, GPx aktiviteleri azalırken MDA seviyeleri artmıştır ($P < 0.05$). 50 sn lik uygulama sonunda 70, 150 ve 300 W ta DNA hasarı bulunmamaktadır. Diğer taraftan DNA hasarını gösteren kuyruk uzunluğu ve kuyruk yoğunluğu kontrol ve uygulama grupları ile karşılaştırıldığında 600 W ta 50 sn deki etkileri gösterilmiştir. Bu sonuçlar gösteriyor ki yüksek mikrodalga radyasyon gücü uygulaması depo ürün zararlısı *E. kuehniella* larvaları için toksisiteye sebep olmaktadır.

Anahtar sözcükler: Oksidatif stres, *Ephestia kuehniella* larvaları, DNA hasarı, mikrodalga.

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Introduction

Stored product pests cause serious damage both in quantity and quality of crops. Mediterranean flour moth, *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) is one of the most important stored product pests in flour mills and storehouses (Azizoglu et al., 2011). The use of synthetic insecticides in agriculture may involve serious health hazards for mammals. These insecticides are often associated with residues that are dangerous for the consumer and the environment (Lamiri et al., 2001; Aslan et al., 2005).

Microwaves are electromagnetic waves which lie between infrared radiation and radio waves in the electromagnetic spectrum. Their frequency ranges from 300 MHz (Megahertz) to 300 GHz (Gigahertz) which corresponds to the wave length 1–1000 mm (Šuhajda, 2006). Microwave radiation is generated by the transformation of the electricity to microwave energy within a generator, which consists of high-voltage tubes. Microwave radiation is partially absorbed, reflected and part is permeated. The absorbed microwave radiation is then converted into to heat (Šuhajda, 2006; Novotny et al., 2013). Microwave radiation acts on living organisms by principle in two ways. These are the non-thermal effects – or reversible, and the thermal – so called non-refundable. The interface between these two factors is called the threshold of the microwave radiation, or power density. Thermal effects arise on the basis of dielectric loss in the body. Local overheating of the organism can occur because of the movement of the molecules mutual friction and collisions.. This may lead to the weakening and killing of the organism (the removal of biological organisms is based on this principle) (Novotny et al., 2013).

Flour and flat grain storage beetles could be economically controlled with continuous microwave irradiation (Langlinais, 1989; Shayesteh & Barthakur, 1996). Thermal treatments for insect control using RF (radio frequency) and microwave systems leave no chemical residues on products, have acceptable quality and have minimal impacts on the environment (Wang et al., 2003).

Oxidative stress is caused by free radicals such as reactive oxygen species (ROS), which includes superoxide ($O_2^{\cdot-}$), peroxyl, alkoxyl, hydroxyl and nitric oxide (Gaikwad et al., 2010). Antioxidants are important to all organisms because they serve to prevent the oxidation of compounds that have the potential to cause oxidative damage (Halliwell et al., 2000). Oxidative stress occurs when the level of ROS overcomes the antioxidant supply of the host organism (Bulger & Helton, 1998). The depletion of antioxidants can thereby serve as an effective biological marker of oxidative stress (Halliwell & Gutteridge, 1990).

Oxidative DNA damage is a biomarker of oxidative stress due to radiation and other environmental mutagens. There has always been a need to develop fast and sensitive methods for monitoring DNA damage. DNA Comet Assay induces DNA fragmentation. Advantages are its simplicity, low cost and speed of measurement (Cerda et al., 1993, 1997; Delincée, 1998). If the test is carried out under neutral conditions, mainly DNA doublestrand breaks are observed, and on electrophoresis of single cells the DNA fragments migrate out of the cells forming a tail in the direction of the anode giving the damaged cells the appearance of a comet. The head of the comet is formed by the remaining nucleus, whereas the tail is dominated by the fragments. The extension of the tail is closely related to the damage intensity (Fairbairn et al., 1995; McKelvey-Martin et al., 1993). Östling and Johanson (1984) observed that fragment migration was a function of radiation dose. With increasing radiation dose more DNA fragmentation occurs and these fragments migrate further during the electrophoresis. Thus, irradiated cells will show an increased extension of the DNA from the nucleus towards the anode, whereas unirradiated cells will appear nearly circular or with only slight tails (Marín-Huachaca et al., 2005).

E. kuehniella larvae reduce the quality of crops because of webbing and feeding (Johnson et al., 1997; Ayvaz et al., 2010). *E. kuehniella is* an important target pest for stored products. Several methods based on techniques such as essential oil of different plants, gas of CO_2, magnetic fields, UV radiation were tested to control *E. kuehniella* (Ercan et al., 2013; Pandır et al., 2013a, b; Guven et al., 2014). However, there is a need for the effective control method to solve the problem radically (Ercan et al., 2013). It was shown that microwave radiation had insecticidal effects on *E. kuehniella* (Azizoglu et al., 2011). Therefore, the aim of this study was to investigate the different exposure times and powers of microwave radiation on the *E. kuehniella* larvae toxicity exhibiting with mortality ratio, oxidative stress and DNA damage.

2. Materials and Methods

2.1. Insect material

Insect culture was obtained from the Biological Control Research Station in Adana. Insects were kept in controlled environmental chambers under the following conditions: 70% RH, 27 ± 1 ºC, L14:D10. Larvae were maintained on a mixture of wheat flour, 55 g yeast, and 30 g wheat germ as previously described (Azizoglu et al., 2011) before the start of the trials.

2.2. Microwave oven and Irradiation of samples with microwave

For the microwave treatment, samples were irradiated with microwave energy using an MD 1500 BEKO microwave oven. The effective length, width and height of the oven were 300, 290 and 196 mm, respectively (700 W capacity; frequency 2.45 GHz). The power output of the generator was adjustable from 0 to 700 W.

1 to 2 days old 10 larvae were exposed to different exposure times and increasing powers of microwaves for determination of mortality, oxidative stress and DNA damage, then larvae were placed to petri dish each containing food media and incubated in a conditioned chamber.

2.3. Insect mortality

After irradiation similar experiments were done with the microwave generator being turned off and replicated six times for each treatment to determine control mortality,. Insects were inspected visually in the insect chamber for their activity and if they failed to respond to a gentle touch with a forefinger after 10 min, they were considered dead.

2.4. Biochemical Assay

Irradiated larvae were used for determining the content of the lipid peroxidation product MDA and antioxidant enzymes activities. Enzyme extraction in whole body of larvae was made according to methods of İcen et al. (2005) and Hyršl et al. (2007). All larvae were in the same chronological age. The larvae were chilled on ice for 5 min and surface sterilized in 95% ethanol.

The whole larvae were prepared at 4 °C by an ultrasonic homogenizer (Ika Ultra-Turrax) at 50 W, 40–50 s in homogenisation buffer (w/v 1.15% KCL, 25 mM K_2HPO4, 5 mM ethylen-diaminetetraacetic acid EDTA, 2 mM phenylmethylsulphonil fluoride PMSF, 2 mM dithiotreitol DDT, pH 7.0) and subsequent centrifugation at 10.000*g* for 10 min. The resulting cell-free extracts were collected for biochemical analysis. Assays were replicated six times each with ten larvae (Hyršl et al., 2007). Protein concentration

was estimated by considering Lowry's method (Lowry et al., 1951) by using bovine serum albumin (BSA) as a quantitative standard.

2.4.1. Lipid peroxidation

Lipid peroxidation was measured by quantifying malondialdehyde (MDA) level in larval homogenates on the basis of reaction with thiobarbituric acid to form a pink colored complex. MDA produced was measured at 532 nm. Specific activity was presented as nmol/mg protein. MDA contents were assayed according to Ohkawa et al. (1979).

2.4.2. Assay of antioxidant enzymes

Antioxidant enzymes were assayed in irradiated larvae spectrophotometrically. Superoxide dismutase (SOD) activity was detected by assaying the autooxidation and illumination of pyrogallol at 440 nm (Marklund & Marklund, 1974). One unit total SOD activity was calculated as the amount of protein causing 50% inhibition of pyrogallol autooxidation. Data were expressed as U (unit) SOD/mg protein. Catalase activity (CAT) was determined by the kinetic assay adapted from Aebi (1984), in which the disappearance of peroxide is monitored spectrophotmetrically at 240 nm. One unit of catalase is equivalent to µmol of H_2O_2 decomposed per minute per mg of protein. Glutathione peroxidase (GPx) was determined with 1 ml reaction mixture, containing 1 ml 50 mM phosphate buffer, pH 7.2, 1 mM EDTA, 0.05% bovine serum albumin, 10 mM oxidized glutathione, and 10 mM NADPH. The rate of change of absorbance was measured at 340 nm. One unit of enzyme activity was expressed as µmol of NADPH oxidized per minute per mg of protein (Paglia & Valentine, 1987).

2.5. Preparation of single-cell suspension

About 1.0 g of very thin slices of larvae were cut with a scalpel from the total body, transferred to a small beaker with 5 mL of ice-cold phosphate-buffered saline (PBS) and stirred for 5 min at about 500 min^{-1}. The suspensions were filtered first through 500 µm and then through 200 µm cloth sieves, and left to settle on ice for about 5 min. The supernatant was used as a cell suspension. Cell suspension (100 µL) was mixed with 1 mL of low-melting agarose (0.8% in PBS). A 100 µL of this mixture was spread on precoated slides (Erel et al., 2009).

2.6. DNA comet assay

The coated slides were immersed in lysis buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 2–9 min. Using the same buffer but devoid of SDS, electrophoresis was performed at 2V/cm for 2 min (Erel et al., 2009). Ethidium bromide staining was employed to visualize DNA. Slides were examined using a microscope (BS 200 ProP, BAB Imaging System with flourescence, Ankara, Turkey) at magnification 20 x 10 by digital color video camera.

Approximately 100 cells per slide were selected randomly and examined using an image analysis system (200 ProP with software). The parameters selected for the quantification of DNA damage were mean tail length and mean tail intensity. The significance was calculated using one-way analysis of variance (ANOVA) and followed by Tukey multiple comparison procedure to calculate the significance. $P < 0.05$ value was taken as statistically significant.

2.7. Data Analysis

Data were subjected to statistical analysis using analysis of variance (ANOVA) and means were separated using the Tukey multiple comparison test. A value of $p < 0.05$ was considered statistically significant. Probit analysis was used to estimate the LT_{50} and LT_{99} values (Abbott, 1925).

3. Results

3.1. Mortality ratio of *E. kuehniella* larvae

The results indicate that larvae mortality was caused by increasing powers of irradiation in the increasing exposure times (Figure 1). One to 2 days larvae were significantly affected when 600 W was applied at all exposure times and completely mortality was obtained at the first 5 s (for 1 to 2 days larvae: $F = 3.093$; df = 6; $p < 0.05$) (Table 1). The mortality rate of larvae of *E. kuehniella* was 99% at the 600 W when exposed from 5 to 50 s. The difference was not significant between 5, 10, 20, 40 and 50 s at 600 W ($p > 0.05$).

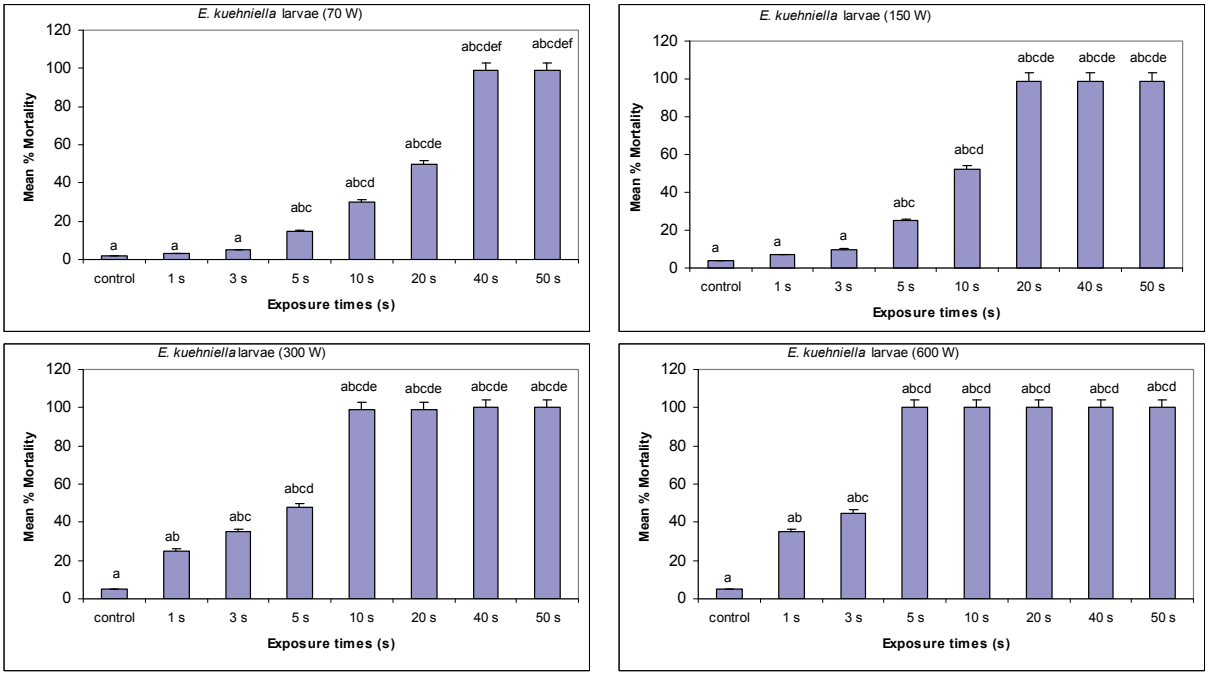

Figure 1. Mean percentage mortality of larvae stages of *Ephestia kuehniella* exposed to different powers of microwave. Letters above bars indicate significant differences exposure times. Bars with the same letter are not significantly different. Error bars indicate SD of means.

Table 1. LT_{50} and LT_{99} values of microwave powers on the larvae of *E. kuehniella*

Microwave power (W)	LT_{50} (s)	LT_{99} (s)	df	N
70 W	18.568	32.216	6	10
150 W	8.144	17.244	6	10
300 W	4.835	9.654	6	10
600 W	2.304	4.62	6	10

N: Number of the tested stages

LT_{50} and LT_{99} values of the microwave irradiation tested are given in Table 1. For 70 W probit analyses showed that LT_{50} and LT_{99} values of this microwave on *E. kuehniella* larvae were 18.568 s and 32.216 s, respectively (Table 1). For 600 W probit analyses showed that LT_{50} and LT_{99} values were 2.304 and 4.62 s, respectively.

3.2. MDA level and antioxidant enzymes activity of *E. kuehniella* larvae tissue

Markers of oxidative damage were assessed following exposure to microwave radiation. Relative to control, no significant difference in MDA content was observed when the larvae were subjected to 70 and 150 W of microwave irradiation (Figure 2). However, the significant rise of MDA level in the larvae tissue following exposure to microwave for 300 and 600 W ($p < 0.05$) at the end of 50 s in comparison with the control were observed (Figure 2).

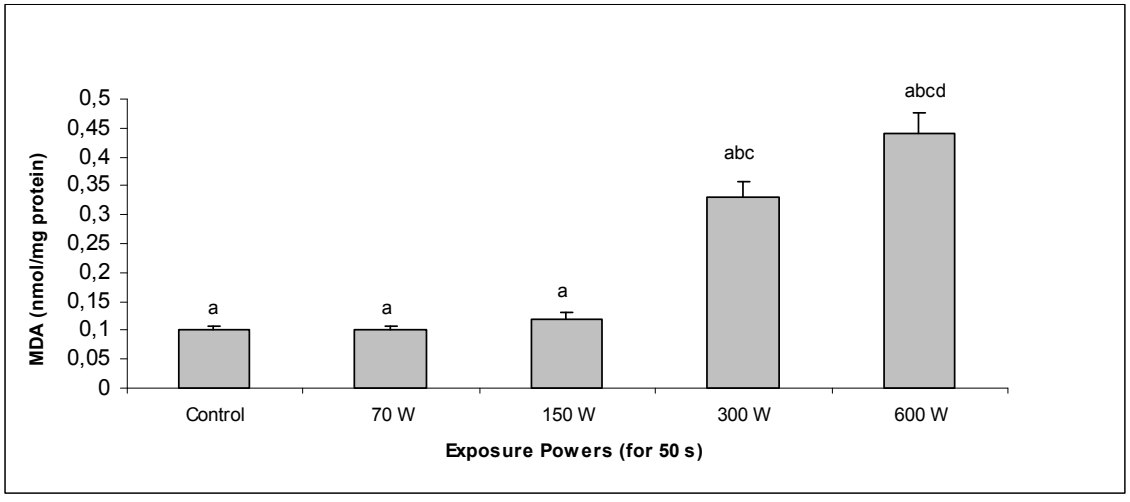

Figure 2. MDA levels in larvae of *E. kuehniella* of control and experimental groups. Letters above bars indicate significant differences between levels of larvae stages of *E. kuehniella* exposed to different powers of microwave. Error bars indicate SD of means. Significance at $p < 0.05$.

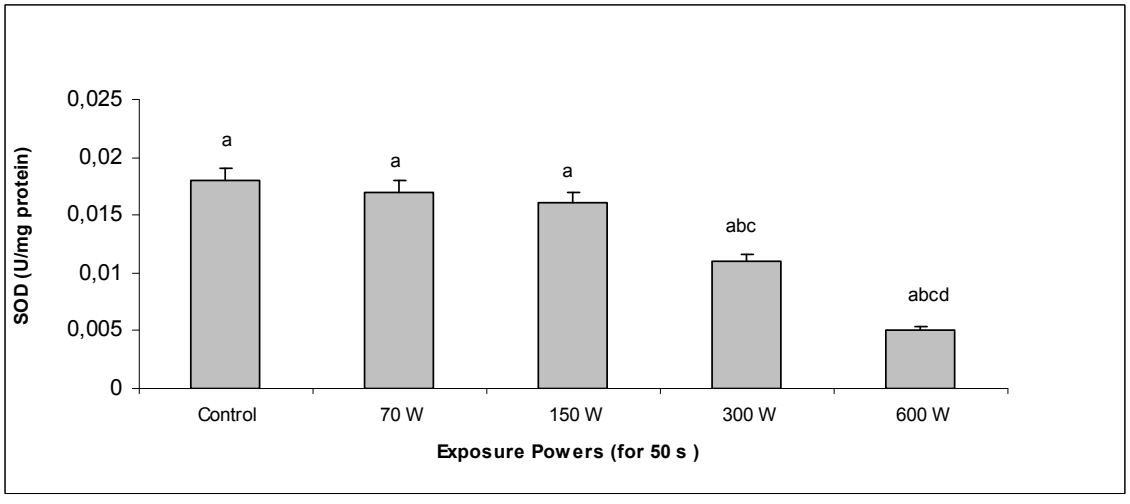

Figure 3. SOD activity in larvae of *E. kuehniella* of control and experimental groups. Letters above bars indicate significant differences between levels of larvae stages of *E. kuehniella* exposed to different powers of microwave. Error bars indicate SD of means. Significance at $p < 0.05$

A marked ($p < 0.01$) elevation of SOD activity in *E. kuehniella* larvae tissue was recorded when insects were exposed to microwave power for 300 and 600 W. However, at 70 and 150 W exposure the SOD activity was the same with the control (Figure 3).

CAT activity was significantly ($p < 0.05$) reduced in *E. kuehniella* larvae tissue following exposure to microwave irradiation at high powers for 50 s at 300 and 600 W in comparison with the control (Figure 4). At the lower exposure powers (70 and 150 W), the activity declined minor than in the control.

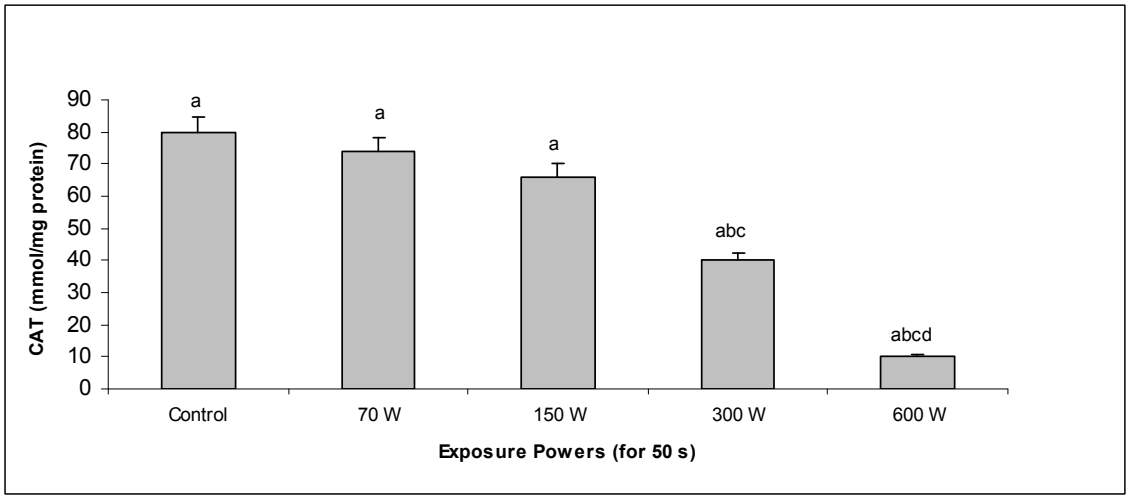

Figure 4. CAT activity in larvae of *E. kuehniella* of control and experimental groups. Letters above bars indicate significant differences between levels of larvae stages of *E. kuehniella* exposed to different powers of microwave. Error bars indicate SD of means. Significance at $p < 0.05$.

A significant ($p < 0.05$) decrease of GPx activity in *E. kuehniella* larvae tissue was recorded when insects were exposed to microwave irradiation (300 and 600 W) for 50 s. However, exposure to microwave radiation for 70 and 150 W resulted in a decline in the enzyme activity in comparison with the control (Figure 5).

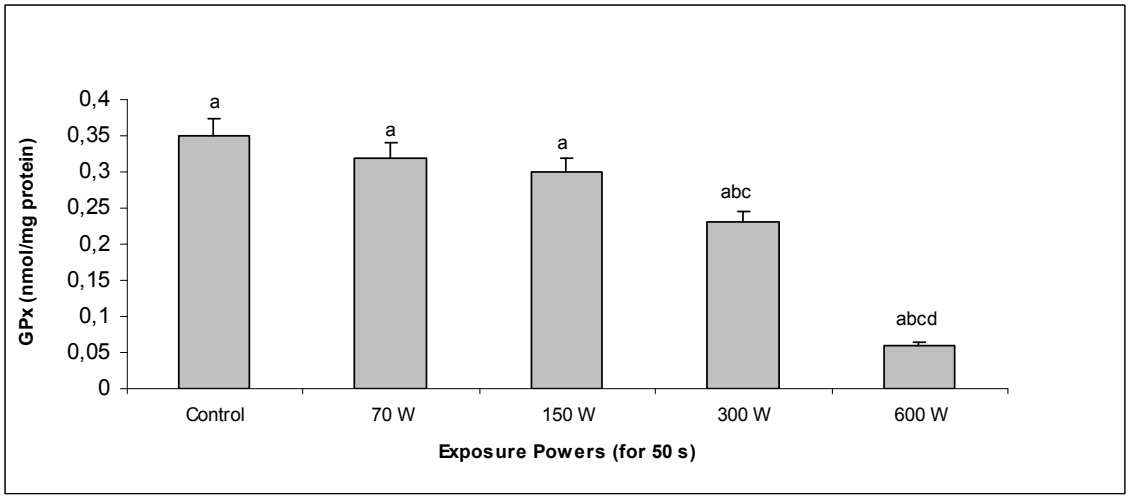

Figure 5. GPx activity in larvae of *E. kuehniella* of control and experimental groups. Letters above bars indicate significant differences between levels of larvae stages of *E. kuehniella* exposed to different powers of microwave. Error bars indicate SD of means. Significance at $p < 0.05$.

3.2. DNA damage analysis with comet assay

Results indicated DNA damage in *E. kuehniella* larvae by microwave radiation exposure.. Exposure to radiation in 1 to 2-day-old larvae caused a significant increase in DNA damage at the 600 W for 50 s intervals as indicated by a greater migration of DNA fragments on the agarose gel (Figures 6A-F). A power response effect was also observed between the intensity of radiation and DNA damage, indicating that the only 600 W of microwave radiation resulted in a significant variation in comet length for larvae tissue at 50 s after irradiation (Figures 6E-F, Table 2). The results of the comet assay showed a 13.84 ± 1.03 and 95.13 ± 8.17 µm increase in the mean tail lenght at powers of 70 and 600 W for 50 s after irradiation, respectively, when compared with a control group (Table 2). The results also showed that the mean tail lenght and mean tail intensity did not change in larvae tissue at increasing exposure powers after 70, 150 and 300 W irradiation when compared with control groups (Table 2).

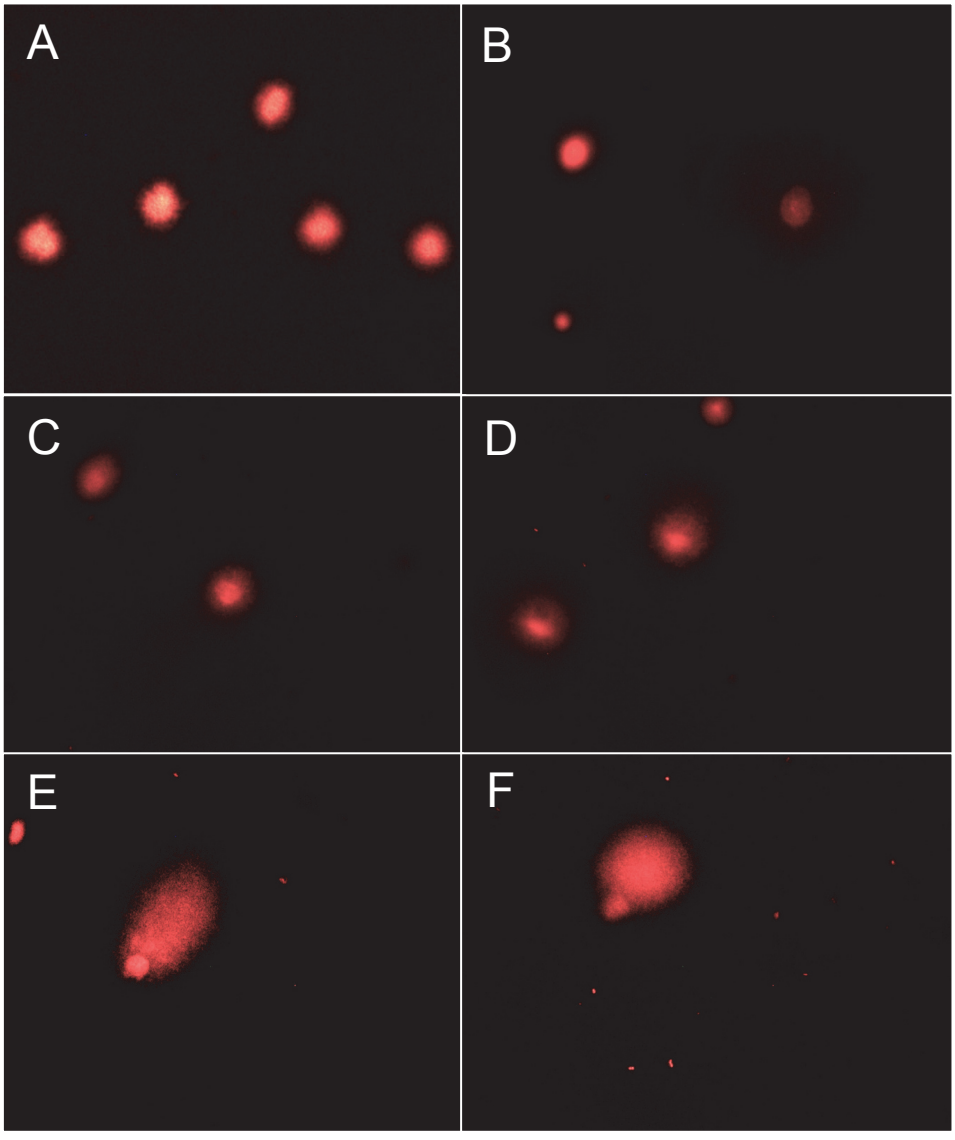

Figure 6. Effect of microwave power (A–Control, B-70 W, C-150 W, D-300 W, E, F-600 W) on DNA damage (mean ± SEM, tail intensity, %) in larvae tissue of *E. kuehniella*.

Table 2. DNA damaging activity of microwave power in larvae tissue of *E. kuehniella*

Microwave power (W)	Mean tail lenght (µm)	Mean tail intensity (%)
Control	8.70±0.97	10.23±2.15
70 W	13.84±1.03	18.70±1.38
150 W	14.56±1.37	16.78±1.65
300 W	16.06±1.72	15.29±2.99
600 W	95.13±8.17*	35.95±4.62*

100 comets were scored for each treatment. * Significant from the control $p < 0.05$ (t test)

4. Discussion

Use of high-intensity microwaves has a clear insecticidal effect, but it also affects the food quality (Lu et al., 2010). Low-intensity microwave radiation (LIMR) is thougth to have little effect on food quality (Zhao et al., 2007, Vadivambal et al., 2007). There is considerable interest in this method of controlling stored product pests. In the other study, insecticidal activity of microwave and ultraviolet radiation (UV) on *E. kuehniella* eggs were investigated by Azizoglu et al. (2011). Eggs (≤24 h) were exposed to microwave and UV radiation at different time periods. Microwave radiation was applied at the powers of 150, 360, 430, and 600 W for different exposure times (10-300 s). It was evident that increasing power and exposure times caused increasing mortality on the eggs. Shayesteh and Barthakur (1996) were investigated that mortality of the stored-product insects *Tribolium confisum* and *Ploadia interpunctella that were* exposed either intermittently or continuously to microwave radiation (2450 MHz). Intermittent exposures at 1 or 5 min intervals were generally more effective in killing insects of both species than continuous irradiation. In this study, we used different powers of microwave radiation (70, 150, 300 and 600 W) from 1 to 50 s on stored products pest of *E. kuehniella* larvae that is very highest dangerous stage than egg and adult stage of insect due to nutrition on stored products.

Although the target theory are used successfully to explain dose-response relationships for ionizing radiation, there is no inherent assumption in it to prevent its use for non-ionizing radiation, e.g. microwaves. Since the insects were randomly distributed in the medium, thermal death could be attributed partly to the heat transfer from the medium and partly to direct absorption of microwaves. The observed movements towards the surface indicated avoidance behaviour to high heat, but this also increased the probability of direct microwave absorption. The direct absorption of microwaves would also be highly effective in killing because of the heat generated due to the high frequency oscillation of the dielectric molecules such as water in the body fluid of the insects (Shayesteh & Barthakur, 1996).

In the combined treatments, the samples of wheat grains and flour were exposed to 1 KGy gamma radiation followed by microwave treatments producing two temperatures (45 and 50 ^{o}C) with an exposure time of 30 s. These treatments resulted in 50% or more mortality of most of the test species and stages (El-Naggar & Mikhaiel, 2011). Radiation affects insects at the cellular level. Cell division and tissue differentiation occur during embryonic and larval development, but much less in adults. The dividing cells are very sensitive to radiation so that newly-hatched larvae are highly susceptible to radiation whereas the adult stage is more resistant (Ahmed, 2001). Larvae stage were the most sensitive to treatments than adults of *E. kuehniella.* The result showed that the percentage mortality of the irradiated larvae of *E. kuehniella* five seconds treatment with the lowest level of radiation (70 W) was 15% whereas a power of 600 W caused 100% mortality in five seconds after treatment.

To defend against the ROS formed, animal cells use three enzymes, superoxide dismutase, catalase and glutathione peroxidase. Superoxide dismutase converts superoxide anion to oxygen and hydrogen peroxide. In biological tissues superoxide can also be converted nonenzymically into the nonradical species hydrogen peroxide and singlet oxygen (Steinbeck et al., 1993). Catalase reduces hydrogen peroxide to water and oxygen (Fridovich, 1978). Glutathione peroxidase neutralizes hydrogen peroxide by taking hydrogens from two glutathione molecules resulting in two H_2O and one molecule of an oxidized form of glutathione. The results suggested that antioxidant enzymes activity of larvae increased significantly with an increase in both the power and the exposure times. Application of microwave radiation at high (300 and 600 W) powers induced changes in antioxidant enzymes activity and MDA level of larvae tissue for 50 s. A very slight decrease was observed in SOD, CAT, GPx activities and MDA levels at 70 and 150 W for 50 s.

Radiation-induced changes in DNA could be used as the basis for detecting irradiation treatment in foods and insects (Delincee, 1996, Imamura et al., 2004a; Cerda et al., 1997). The comet assay is also used to observe DNA repair in irradiated cells (Ptacek et al., 2001, Trzeciak et al., 2008; Olive & Durand, 2005). Kameya et al. (2012) were searched the use of the DNA comet (single-cell gel electrophoresis) assay as a validity assessment for identifying the irradiation treatment history of pests (FAO, 2008; FAO, 2009) using the cigarette beetle, *Lasioderma serricorne* (Fabricius), when exposure time was increased to 50 s. ROS are also responsible for peroxidation of membrane lipids and micromolecules such as proteins and DNA (Ahmad & Pardini, 1990; George & Gatehouse, 2013). In this study, comet assay method was used as the most rapid, sensitive and useful assays to detect the potential genotoxicity of microwave radiation on larvae of *E. kuehniella*. After the treatment with microwave, a significant increase in mean comet tail length at only 600 W for 50 s in comparison to control and other treatment groups were observed. It also did not induce DNA damage of *E. kuehniella* larvae tissue at 70, 150 and 300 W.

There is an urgent need to develop safe, effective, economic, and convenient alternative methods that have the potential to replace toxic fumigants (Ayvaz et al., 2008). Therefore, alternative methods are needed to improve methods for pest control (Tuncbilek et al., 2009). The effects of irradiation on insects are many and varied, depending primarily on the species, stage and dose (El-Naggar & Mikhaiel, 2011). There is limited study on the effects of microwave applications on insects. The effects of microwave radiation on *E. kuehniella* larvae of mortality ratio, DNA and antioxidant enzymes system activity are largely unknown, and there is no evidence of different powers of toxic effects of microwave radiation exposure in different times. In this study, microwave radiation significantly decreased SOD, CAT, GPx activities, increased MDA level, mortality ratio and tail length of DNA of larvae tissue. Therefore, the different powers of microwave radiation could be used for controlling *E. kuehniella* larvae according to our study results.

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