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Orijinal araştırma (Original article)

UV radiation-induced oxidative stress and DNA damage on Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae

UV radyasyonun, Un güvesi, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), larvalarında meydana getirdiği oksidatif stres ve DNA hasarının belirlenmesi

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Summary

Exposure to ultraviolet (UV) radiation is hazardous for all organisms. UV shows numerous effects on insects. In this study, mortality effect of UV on *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) larvae was studied. The larvae were exposed to UV radiation (254 and 365 nm) at different time periods (15, 30, 45 and 60 min). After exposure, mortality rate, comet assay studies, antioxidant enzyme activities (SOD, CAT, GST and GPx), and MDA levels were investigated on the larvae. The mortality rate of *E. kuehniella* larvae increased with increasing exposure times. It was determined that 254 nm was more effective than 365 nm compared to the effects of short (254 nm) and long-wave (365 nm) radiations on mortality rate of *E. kuehniella* larvae. Likewise mortality rate, 254 nm had more potency than 365 nm on antioxidant enzyme activities and levels of MDA. According to the comet assay results, the tail DNA% and comet tail length significantly increased in all exposure times at 254 nm, but these changes were seen only 45 min and 60 min at 365 nm of UV radiation. Therefore, tail DNA% and tail lenghts of 254 nm radiation could be greater than the tail lenghts of 365 nm UV radiation. As a result, the UV radiation could be effectively as a safe pest control method and as an alternative to environmentally hazardous chemical pesticides.

Key words: *Ephestia kuehniella*, UV radiation, mortality, antioxidant enzymes, comet assay

Özet

Ultraviyole (UV) radyasyona maruz kalmak tüm canlılar için tehlikelidir. UV böcekler üzerinde birçok etki göstermektedir. Bu çalışmada, *Ephestia kuehniella* 1879 (Lepidoptera: Pyralidae) larvaları üzerine UV'nin öldürücü etkisi çalışıldı. Larvalar UV radyasyonuna (254 ve 365 nm) farklı zaman peryotlarında (15, 30, 45 ve 60 dk.) maruz bırakıldı. Çalışma sonunda larvaların ölüm oranları, komet değeleri ve antioksidan enzim aktiviteleri (SOD, CAT, GST ve GPx) ve MDA seviyeleri araştırıldı. Işınlama süresi arttıkça *E. kuehniella* larvalarının ölüm oranları artmıştır. Kısa (254 nm) ve uzun (365 nm) dalga boylarını ölüm oranları üzerindeki etkilerini karşılaştırdığımızda 254 nm dalga boyunun 365 nm dalga boyundan daha etkili olduğu belirlenmiştir. Bu durum, ölüm oranı, antioksidan enzim aktivitesi ve MDA seviyesinde de tespit edilmiştir. Komet değerlendirme sonuçlarına göre kuyruk % DNA'sı ve komet kuyruk uzunluğu 254 nm dalga boyutunun tüm uygulama zamanlarında istatistiksel olarak artarken bu değişimler 365 nm UV radyasyonun yalnızca 45. ve 60. dk. larında tespit edilmiştir. Bundan dolayı, kuyruk % DNA'sı ve kuyruk uzunluğu 254 nm dalga boyunda 365 nm dalga boyuna göre daha fazladır. Sonuç olarak, UV radyasyon depo zararlılarının kontrolünde güvenilir bir metod ve çevreye zararlı kimyasal pestisitlere bir alternatif olarak kullanılabilir.

Anahtar sözcükler: *Ephestia kuehniella*, UV radyasyon, ölüm oranı, antioksidan enzimler, komet değerlendirme

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Introduction

Cereals are the main source of food because they include lots of nutritious substances such as minerals, vitamins, carbohydrates, and proteins for people (Schöller et al., 1997). However, stored product pests cause critical losses to both the cereal's quantity and quality. *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) is a serious and common pest in cereals around the world (Lynn & Ferchovic, 2004). Thus, it can cause economic losses (Hansen and Jensen, 2002). Principally, the control of major pests is carried out with insecticides (Arthur, 1996). However, insecticides have a lot of environmental problems (Hansen & Jensen, 2002). Also, they have showed a development towards a resistance in insects causing toxicity in the mammalian and failure of the food chain (Azizoglu et al., 2011). Because of all of these undesired effects of insecticides, alternative control methods are needed (Ayvaz & Karabörklü, 2008).

Radiation technique is one of the alternative control methods for stored product pests (Ayvaz et al., 2008; Azizoglu et al., 2010; Hallman, 2013). For many years, UV radiation has been recognized as a potential stress for organisms and is known to have a wide range of hazardous effects (Mora et al., 2000). UV is a very prominent environmental toxic agent. Due to UV radiation, genes are easily damaged and mutations can subsequently occur (Gruijl et al., 2001). The major damage produced in DNA by UV consists of pyrimidine dimers (Tornaletti & Pfeifer, 1995; Myllyperkiö et al., 2000; Mone et al., 2001). UV can negatively affect phytoplankton, and the eggs and larval stages of fish and other aquatic animals (Häder et al., 2007). Also, it has numerous effects on insects (Halverson et al., 1999). There are studies which determined the efficiency of UV radiation on stored product pests. UV is used both as an insect attractant, and for the disinfection of insects' eggs (Faruki et al., 2007).

UV radiation has the ability to produce reactive oxygen species (ROS) (Gruijl et al., 2001); thus, it is proved that it generates DNA damage both directly and indirectly via oxidative stress (Ichihashi et al., 2003). ROS conduces to DNA and enzyme oxidation, changes in scavenging enzymes and lipid peroxidation (LPO) in insect tissues. Malondialdehyde (MDA) end the product of LPO, is the most studied marker in oxidative stress and can cause biomolecular damage. Detoxification and antioxidant systems play a major role in the defense of cells (Büyükgüzel et al., 2010). ROS are eliminated by a group of antioxidant enzymes in insects. The antioxidant enzymes contain superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) in insects (Büyükgüzel & Kalender, 2009). They form a protector complex against endogenously produced ROS (Büyükgüzel et al., 2010).

To determine the genotoxic effects induced by physical and chemical agents, there are several test systems and different types of cells such as bacteria, mammalian cells, and plant cells can be used (Mamur et al., 2010). Single cell gel electrophoresis (SCGE) or ''Comet'' assay is used to determine the potential genotoxicity (Rencuzogulları et al., 2004; Yılmaz et al., 2008). It is a marker of the early biological effects of carcinogen exposure (Liou et al., 2002). Comet assay has been proved to be a good indicator of DNA damage (Mpountoukas et al., 2008; Mamur et al., 2010). It is a reliable, sensitive, and rapid method for assessing DNA damage in cells (Zengin et al., 2011).

This study is conducted to develop a safer pest control method in order to decrease the use of chemical pesticides. For this, UV radiation was tested on *E. kuehniella* larvae and the effects of UV via comet assay, observing mortality rates and measuring of antioxidant enzyme (SOD, CAT, GST and GPx) activities and levels of MDA were examined.

2. Materials and Methods

2.1. Chemicals

All the chemicals used to measure the MDA level, antioxidant enzyme activities, and comet assay were obtained from Sigma–Aldrich (Germany).

2.2 *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) culture

The insect stock culture was procured from the Adana Plant Protection Research Institute. After a period of around 2 months, the adults that emerged from the culture medium were placed into new glass jars, and their eggs were collected in one day, and used for the new cultures (Azizoglu et al., 2011). The larvae were reared on a mixture comprising of 1 kg wheat flour, 55 g yeast, and 30 g germs of wheat (Tuncbilek et al., 2009). The normal rearing states were 27 ± 1 ºC and 70 ± 5 % r.h. The cultures were kept under a light regime of 14 hour light followed by 10 hour of darkness.

2.3. UV radiation treatments

1 to 2 days-old, six number of *E. kuehniella* larvae were collected from the culture and then transferred into Petri plates. The larvae were exposed to UV radiation to test the effects of both the short (254 nm) and long wave (365 nm) UV radiation at different time periods (15, 30, 45 and 60 min). 254 nm and 365 nm waves (Camag UV Lamps, 254/365 nm) were chosen according to Azizoglu et al. (2011). All the treatments were replicated six times.

2.4. Preparation of single-cell suspension

About 1.0 g of very thin slices of six 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 cycles. 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. The 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 onto precoated slides (Erel et al., 2009).

2.5. DNA comet assay

The coated slides were immersed in the 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, the electrophoresis was performed at 2 V/cm for 2 min (Erel et al., 2009). Ethidium bromide staining was employed in order to visualize the DNA. The slides were examined using a microscope (BS 200 ProP, BAB Imaging System with fluorescence, Ankara, Turkey) at a magnification 20 x 10 with a 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 tail DNA percentages and tail length.

2.6. Tissue collection

Ten larvae were used to specify the MDA content and antioxidant enzyme activities. They were frosted on ice for 5 min and sterilized with ethanol. Then, they were cut and placed into eppendorf tubes filled with a cold homogenization buffer (pH 7.4) and stored at -80°C. Before using, the tubes were kept at room temperature until the tissue began to thaw.

2.7. Sample preparation

The extracts of larvae were prepared at 4°C with a homogenizer (Ika T-18 Basic Ultra Turrax Homogenizer) and subsequent centrifugated (NUVE NF800R) for 15 min at 4°C. Then the supernatants were collected for analysis. The supernatants were centrifuged at 1000g at 4°C for SOD and CAT assays, 16,000g for GST and GPx and 2000g for MDA. The antioxidant enzyme activities and MDA levels were specified by measuring the absorbance of the samples using a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). The protein concentrations were determined according to Lowry et al. (1951).

2.7.1. Measurement of malondialdehyde (MDA)

The MDA level was assayed using the thiobarbituric acid (TBA) test as described by Ohkawa et al. (1979). The absorbance was measured at 532 nm. The level of MDA was defined as nmol/mg protein.

2.7.2. Measurement of antioxidant enzyme activities

The SOD activity was determined according to the method described by Marklund & Marklund (1974) at 440 nm. The activity was defined as U/mg protein. The CAT enzyme activity was measured according to the method described by Aebi (1984) by assaying the hydrolysis of H_2O_2. The absorbance was measured at 240 nm. The activity was defined as mmol/mg protein. The activity of the GST was analyzed by measuring the formation of the GSH (Glutathione) and the 1-chloro 2, 4-dinitrobenzene (CDNB) conjugate (Habig et al., 1974). The absorbance was recorded at 340 nm. The specific activity of the GST was expressed as µmol/mg protein. The GPx activity was measured according to the method described by Paglia & Valentine (1987). The reaction was monitored at 340 nm. The enzyme activity was expressed as nmol/mg protein.

2.8. Statistical analysis

The data were subjected to analysis of the variance (ANOVA) using SPSS (2001) for Windows, followed by Tukey's procedure for multiple comparisons. The $P < 0.05$ was considered statistically significant.

3. Results

3.1. Mortality ratio result

The *E. kuehniella* larvae were exposed to 254 and 365 nm UV radiation for time periods of 15, 30, 45 and 60 min, and the mortality ratio was observed. It was seen that depending on the increasing time periods, the mortality was increased (For 254 nm, $F= 558.25$; $df = 4$; $P < 0,05$ and for 365 nm, $F = 368.99$; $df= 4$; $P < 0,05$) (Figure 1). The increased exposure times that were dependent on the mortality ratios of *E. kuehniella* larvae were evident after 254 nm of UV radiation, e.g. 9.17 % mortality ratios at the control (untreated larvae) reached 95.5 % after the 60 min exposure. Otherwise, the mortality ratios of the larvae at 365 nm UV did not change significantly up to the 30 min exposure. With 365 nm UV, only the 83.83% mortality was determined at the longest exposure time period (Figure 1).

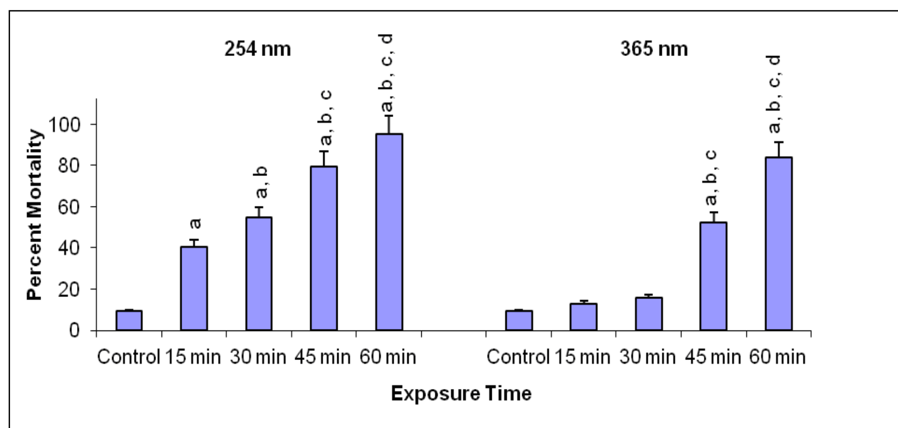

Figure 1. Effect of 254 and 365 nm UV radiation on mortality ratio of *E. kuehniella* larvae. [a]Comparison of control and other groups. [b]Comparison of 15 min and other groups. [c]Comparison of 30 min and other groups. [d]Comparison of 45 min and other groups. Data represents the means ± SD of samples. Significance at P < 0.05.

3.2. MDA level and antioxidant enzymes activities results

At a 254 nm radiation, the MDA level was significantly increased with the increasing exposure time. However, the content of the MDA at 365 nm UV radiation did not alter up to the 30 min exposure (Figure 2).

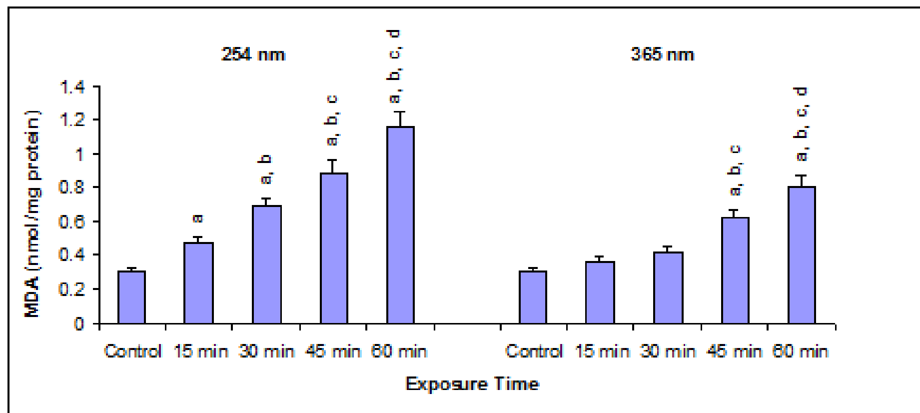

Figure 2. Effect of 254 and 365 nm UV radiation on MDA level of *E. kuehniella* larvae. [a]Comparison of control and other groups. [b]Comparison of 15 min and other groups. [c]Comparison of 30 min and other groups. [d]Comparison of 45 min and other groups. Data represents the means ± SD of samples. Significance at $P < 0.05$.

The SOD, CAT, GST and GPx activities were significantly decreased with exposure to 254 nm UV radiation depending on the increasing time periods, but a significant decrease on these antioxidant enzyme activities was seen at 45 min and 60 min exposure times at 365 nm UV radiation (Figures 3,4,5,6).

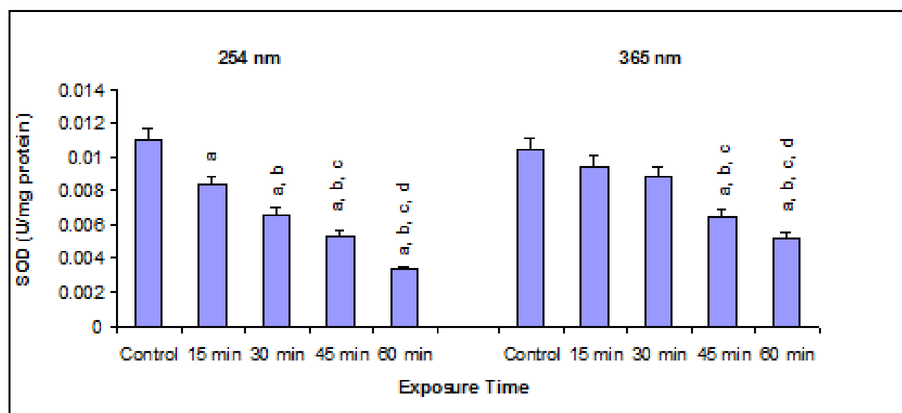

Figure 3. Effect of 254 and 365 nm UV radiation on SOD activity of *E. kuehniella* larvae. [a]Comparison of control and other groups. [b]Comparison of 15 min and other groups. [c]Comparison of 30 min and other groups. [d]Comparison of 45 min and other groups. Data represents the means ± SD of samples. Significance at $P < 0.05$.

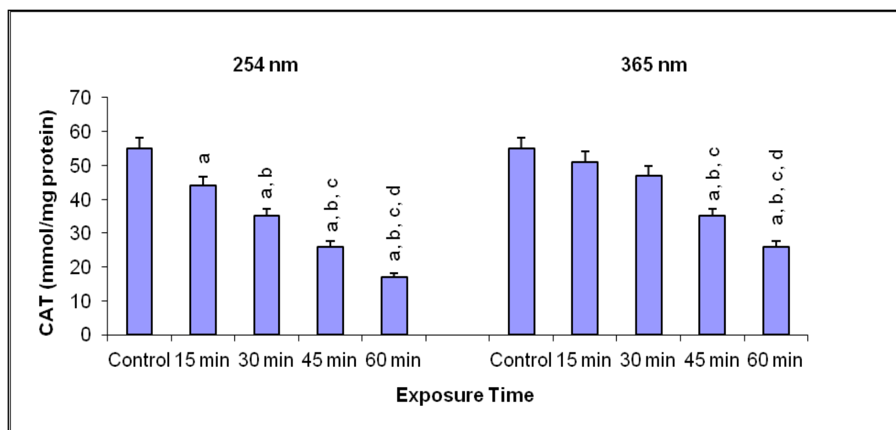

Figure 4. Effect of 254 and 365 nm UV radiation on CAT activity of *E. kuehniella* larvae. [a]Comparison of control and other groups. [b]Comparison of 15 min and other groups. [c]Comparison of 30 min and other groups. [d]Comparison of 45 min and other groups. Data represents the means ± SD of samples. Significance at $P < 0.05$.

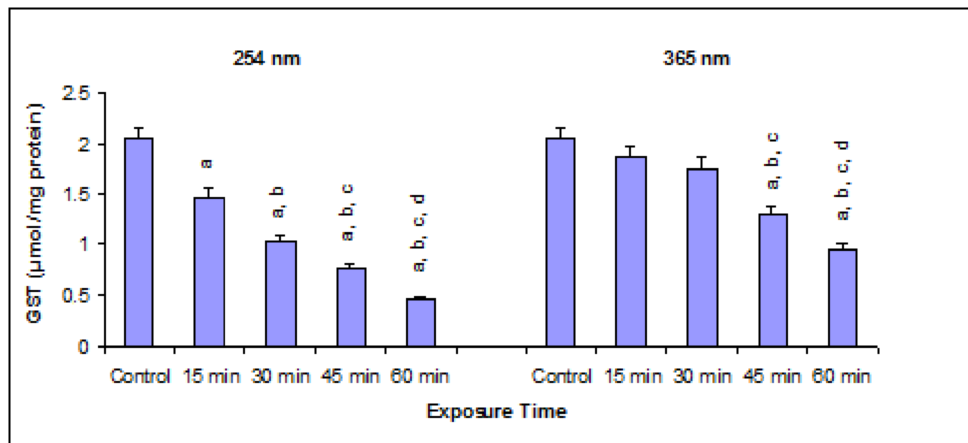

Figure 5. Effect of 254 and 365 nm UV radiation on GST activity of *E. kuehniella* larvae. [a]Comparison of control and other groups. [b]Comparison of 15 min and other groups. [c]Comparison of 30 min and other groups. [d]Comparison of 45 min and other groups. Data represents the means ± SD of samples. Significance at P < 0.05.

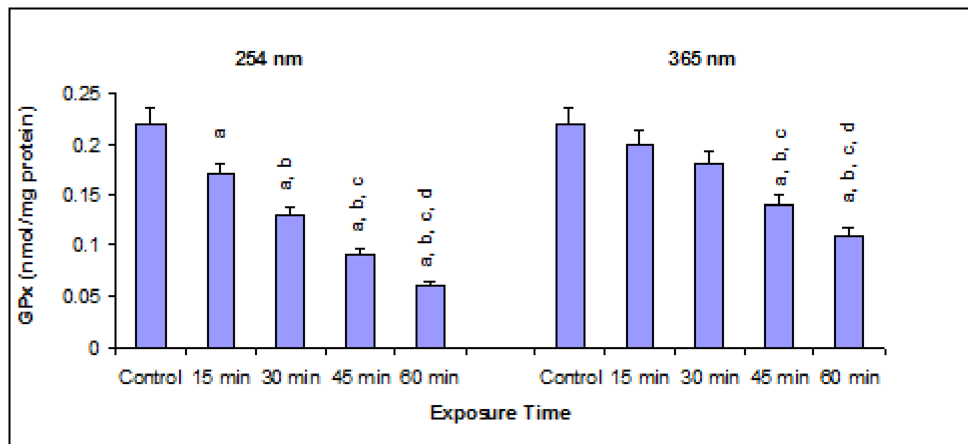

Figure 6. Effect of 254 and 365 nm UV radiation on GPx activity of *E. kuehniella* larvae. [a]Comparison of control and other groups. [b]Comparison of 15 min and other groups. [c]Comparison of 30 min and other groups. [d]Comparison of 45 min and other groups. Data represents the means ± SD of samples. Significance at P < 0.05.

3.3. Commet assay results

According to the comet assay results, the tail DNA %, mean tail length, and tail moment, significantly increased with the increasing exposure times at 254 nm UV radiation (Figure 7, Table 1). The significantly ($p < 0.05$) high tail DNA % (94.55 ± 3.28) induced by 60 min of 254 nm UV. However, the increasing tail DNA % was seen at only 45 and 60 min at 365 nm as 82.72 ± 4.01 and 93.30 ± 3.03, respectively.

When the damage was evaluated in terms of the tail length, the value was higher for the 60 min 254 and 365 nm UV-treatment compared with the other treatment groups (Figure 7, Table 1).

Table 1. Estimated mean values of tail DNA%, tail length and tail moment of comets by image analysis on different wavelenghts of UV.

Different exposure times and wavelenghts of UV		Tail DNA% Mean±SE	Tail lenght Mean±SE	Tail moment Mean±SE
Control		51.90 ± 0.28	11 ± 0.70	5.71 ± 1.17
254 nm	15 min	69.0 ± 0.38[a]	16.00 ± 1.20	11.72 ± 1.35
	30 min	80.13 ± 1.95[ab]	21.84 ± 2.35[ab]	17.5 ± 3.12[ab]
	45 min	84.91 ± 1.93[abc]	31.55 ± 1.75[abc]	26.79 ± 3.45[abc]
	60 min	94.55 ± 3.28[abcd]	91.75 ± 1.68[abcd]	86.75 ± 4.96[abcd]
365 nm	15 min	64.86 ± 1.08	11.65 ± 2.74	7.56 ± 2.03
	30 min	64.95 ± 0.75	19.61 ± 2.35	22.19 ± 2.64[ab]
	45 min	82.72 ± 4.01[abc]	26.7 ± 1.12[abc]	14.08 ± 3.75[abc]
	60 min	93.30 ± 3.03[abcd]	80.46 ± 8.05[abcd]	76.87 ± 9.09[abcd]

Superscript letters indicate significant differences among exposed to different times and wavelenghts of UV larvae stages of *E. kuehniella*. Significance at $p < 0.05$.

The highest tail length of the larvae was observed at the 60 min-treatment of 254 nm of UV radiation (Figure 7).

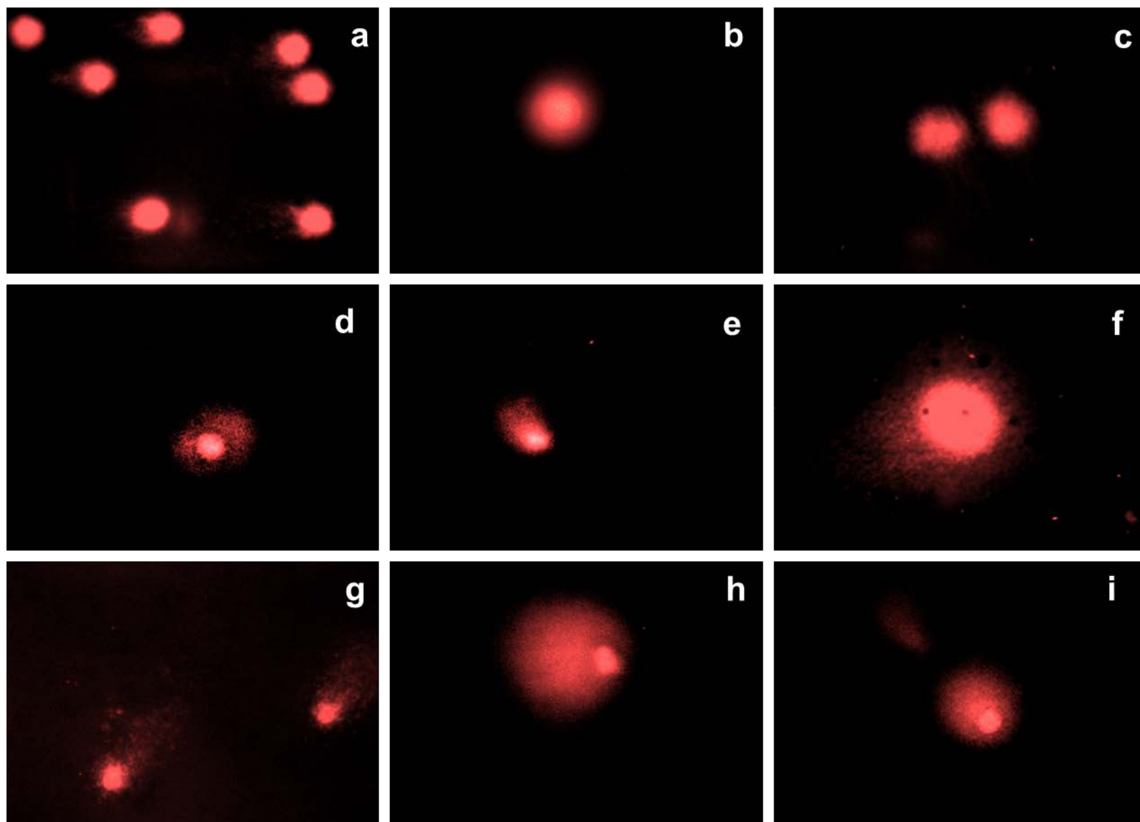

Figure 7**.** Comet appearances of the UV treated larvae and control group in *E. kuehniella* (a) control, (b-c) 15 min-, (d-e) 30 min-, (f-g) 45 min-, (h-i) 60 min-exposure for short-wave (254 nm) and long wave (365 nm) of UV in larvae DNA damage, respectively.

4. Discussion

UV radiation can cause damage to DNA indirectly via the action of photosensitizers and it also induces cytotoxic and mutagenic effects (Patton et al., 1999). There are advantages to UV radiation when used as a pest control. The advantages include the absence of unwanted residues in foods, only a little alteration in the chemical properties or the nutritive value of foods, and no resistance development by the pest insects (Zhao et al., 2007). Ghanem & Sharma (2007) showed that UV radiation causes damage to egg chorion and induces the leakage of egg fluid. Guerra et al. (1968) exposed the *Heliothis virescens* (Fabricius), 1777 and *Heliothis zea* (Boddie), 1850 (Lepidoptera: Noctuidae) eggs to 254 nm UV radiation and specified that increasing the time period increased the mortality ratio significantly. Furthermore, supporting results were procured from the eggs of *Tribolium castaneum* (Herbst), *T. confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) and *Cadra cautella* (Walker), 1863 (Lepidoptera: Pyralidae) when they were exposed to short-wave UV radiation (Faruki et al., 2007). This study showed that the mortality rate of the larvae treated at 254 nm of UV radiation for 60 min was 95.5 %, but 83.83 % at 365 nm UV radiation. Our results indicate that the mortality effect of the 254 nm UV radiation was more effective than the 365 nm UV treatment. When the UV wavelengths were shortened, the harmful effect on the insects significantly increased (Cohen et al., 1975). In addition, it was observed in this study that, depending on the increasing time periods, the mortality rates were increased. The enhanced mortality ratio in the insects with increased exposure times treated with UV radiation was also reported by Azizoglu et al. (2011).

UV radiation can produce free radicals, including oxygen-derived species, which are known to cause LPO (Patton et al., 1999) and oxidative damage to DNA bases (Gruijl et al., 2001). LPO has been suggested as a possible mechanism for the phototoxic effects (Patton et al., 1999). MDA is a main oxidation product of peroxidized polyunsaturated fatty acids. It has been used to specify both the LPO level, and as a marker of oxidative stress (Büyükgüzel et al., 2010). MDA, the quantitatively predominant aldehyde, forms Schiff bases with amines of proteins, phospholipids, and nucleic acids leading to damaged cellular biomolecules (Büyükgüzel & Kalender, 2010). In this study, enhanced oxidative stress, as evidenced by the elevated level of MDA, may be associated with a reduction in antioxidant enzyme activities. Antioxidant enzymes are important protectors of cells against oxidative damage (Messerah et al., 2012). SOD and CAT are responsible for the dismutation of superoxide radicals into oxygen and hydrogen peroxide (H_2O_2), and catalyse H_2O_2 conversion respectively, in tissues (Büyükgüzel & Kalender, 2009). The GPx enzyme can prevent oxidative damage of the cell membranes via the catalyses H_2O_2 conversion to H_2O (Messerah et al., 2012). GST is one of the main detoxification enzymes that play a major role in the obstruction of oxidative damage by detoxifying LPO products (Büyükgüzel & Kalender, 2009). There are studies that have examined the MDA level and antioxidant enzyme activities for investigating the oxidative stress degree in insect tissues (Büyükgüzel & Kalender, 2009; Büyükgüzel et al., 2010). Our study demonstrates a significant increase in the MDA level and a decrease in SOD, CAT, GST, and GPx activities in *E. kuehniella* larvae. The changes in antioxidant enzyme activities may be due to the generation of ROS, and this ROS can cause oxidative stress, and lead to uncontrolled lipid peroxidation, protein, enzyme, and DNA oxidation in the insect tissues (Ahmad, 1995; Krishnan & Kodrik, 2006). A portion of ROS is scavenged by non-enzymatic antioxidants such as ascorbate, glutathione, tocopherols, and carotenoids (Felton & Summers, 1995), but most are eliminated by a suite of antioxidant enzymes in insects. The elevated enzyme activities under increasing exposure times of MFs probably result from a decreased transcription and/or translation. Therefore, the suppressed activities of the antioxidant enzymes and simultaneous increase in lipid peroxidation in the larvae tissue were attributed to the oxidative stress arising from the insufficient neutralization of the reactive species (Modesto & Martinez, 2010).

In cells, DNA is known to be a target for UV radiation damage. During recent years, the investigation into DNA damage induced by agents such as radiation and environmental toxins has been performed via comet assay (Rapp et al., 2000). Thus, in this study, the comet assay was used to examine the DNA damage of the *E. kuehniella* larvae as being exposed to UV radiation. In this rapid and sensitive method, a damaged cell takes the shape of the appearance of a comet in head and tail regions. The tail length and density indicates the number of single-strand breaks in the DNA. Also, the percentage of DNA in the tail procures quantitive data of the damaged DNA (Patton et al., 1999). In previous studies, Myllyperkiö et al. (2000), Rapp et al. (2000) and Patton et al. (1999) showed the effects of UV radiation via commet assay. In this study, it was observed that the tail lengths were elongated with the increasing exposure time at 254 nm of UV radiation. However, at 365 nm this elongation was seen only at 45 min and 60 min. It is clear that 254 nm is more effective on *E. kuehniella* larvae tissue than 365 nm radiation.

With this study, it was shown that mortality rates, MDA levels, and comet tail lengths were increased; and SOD, CAT, GST and GPx activities were decreased depending on the exposed time of the UV radiation on the *E. kuehniella* larvae. The data presented in this study supports our hypothesis that UV radiation causes oxidative stress and induces DNA damage. As a result, it is possible to say that UV radiation (especially short-wave) has insecticidal activity on *E. kuehniella* larvae. UV radiation may be used as an alternative to chemical control. The use of UV radiation may be a more reliable and a healthy method for controlling insects.

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