T.R.
ERCİYES UNIVERSITY
GRADUATE SCHOOL OF
NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY

EFFECTS OF UV RADIATION ON DIFFERENT LIFE STAGES OF MEDITERRANEAN FLOUR MOTH *Ephestia kuehniella ZELLER* (LEPIDOPTERA: PYRALIDAE)

Prepared by
ASEEL FADHIL MAHMOOD

Supervisor
Prof. Dr. Abdurrahman AYVAZ

MSc. Thesis

July 2017
KAYSERİ

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CONFORMITY TO THE SCIENTIFIC ETHICS

I declare that all information in this study is obtained in accordance with academic and ethical rules. At the same time, I also declare that I transmit and gave it as a reference all materials and results that are not at the core of this work, as required by these rules and behaviors.

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COMPLIANCE WITH GUIDELINES

This Master of Science Degree thesis entitled: **"Effects of UV Radiation on Different Life Stages of Mediterranean Flour Moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)"** has been prepared in accordance with the Thesis Proposal and with the Guidelines for Writing Theses of Erciyes University.

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ACCEPTANCE AND APPROVAL PAGE

The study entitled **"Effects of UV Radiation on Different Life Stages of Mediterranean Flour Moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)"** has been prepared by **ASEEL FADHIL MAHMOOD** supervised by **Prof. Dr. Abdurrahman AYVAZ**, is accepted as a M.Sc. thesis in Erciyes University Graduate School of Natural and Applied Science Department Of biology at the Institute of Science and Technology of Erciyes University, has been accepted as a Master of Science Degree thesis by our jury whose identifications and endorsements are given below.

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JURY:

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According to decision dated...18./07./2017.... and numbered .2017./30.-09 acceptance of this thesis is approved by Graduate School Administrative Board.

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Prof. Dr. Mehmet AKKURT
Director of the Institute

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بسم الله الرحمن الرحيم

وقل اعملوا فسيرى الله عملكم ورسوله والمؤمنون وستردون الى عالم الغيب والشهادة فينبئكم بما كنتم تعملون

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ASEEL FADHIL MAHMOOD

July 2017, KAYSERİ

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ABSTRACT

In this study, we have tested the effects of Ultra violet (UV) radiation on eggs, larvae, pupa and adults of Mediterranean flour moth, *Ephestia kuehniella*. When different age groups of *E. kuehniella* eggs (0-72 h) were exposed to shortwave UV-radiation for six different exposure time (50-600 s), mortality rates of the eggs increased with increasing exposure time. At the 400s application, complete mortality was only observed for 24 h old eggs. While 476.61s was sufficient for 24 h old eggs, 861.37s was required to prevent larval emergence from 0-2 h old eggs completely. Killing effects of longwave UV-radiation were lower than that of shortwave UV-radiation on the *E. kuehniella* eggs and no complete mortality was achieved with these exposure times. Embryonic development periods were not changed for 0-2, 24, and 48 h age groups of eggs with increasing exposure time. However, embryonic development period decreased for 72 h old eggs when compared control. On the other hand, there were no differences among age groups with respect to embryonic development when the eggs irradiated with longwave UV-radiation at all exposure times. Mortality rates increased with increasing exposure time when the larvae exposed to shortwave UV- radiation showing much higher effect than longwave radiation. Because the larvae irradiated with longwave UV-radiation there was no significant change up to 30 minutes. On the other hand, both short and longwave radiation caused a decrease in adult emergence periods from irradiated pupae. Longevity of these adults was not changed depending on the exposure times. In the experiments with the adult *E. kuehniella,* UV-radiation does not change longevity of the adults after exposure. However, egg production and hatchability of these eggs decreased with increased periods for both short and longwave UV-radiation.

Key words: *Ephestia kuehniella*, ultraviolet radiation, mortality, longevity

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ÖZET

Bu çalışmada, ultra viyole (UV) radyasyonunun Akdeniz Un Güvesi *Ephestia kuehniella*'nın yumurta, larva, pupa ve erginleri üzerindeki etkileri araştırılmıştır. Yaşları 0 ila 72 saat arasında değişen *E. kuehnella* yumurtalarına altı farklı sürelerde (50-600 s) kısa dalga boylu UV radyasyonu uygulandığında yumurta ölüm oranı artan uygulama süresine bağlı olarak artmıştır. 400s uygulamada sadece 24 saat yaşındaki yumurtaların tamamından larva çıkışı engellenmiştir. 72 saat yaşındaki yumurtalardan larva çıkışını engellemek için 476.61s'lik uygulama süresi yeterli olmuşken, 0-2 yaşındaki yumurtalar için 861.37s'lik uygulama süresine ihtiyaç olmuştur. *E. kuehniella* yumurtaları üzerinde uzun dalga UV radyasyonunun öldürücü etkileri kısa dalga UV-radyasyonununkinden daha düşük olmuş ve uygulama sürelerinin hiç birisinde %100 mortalite sağlanamamıştır. Embriyonal gelişim süresi 0-2, 24 ve 48. yaş grupları arasında değişmemiş fakat, 72 saat yaşındaki yumurtalarda azalmıştır. Ayrıca, yumurtalar uzun dalga boylu UV radyasyonuna maruz bırakıldıklarında embriyonal gelişim açısından yaş grupları arasında fark görülmemiştir. Larvalar kısa dalga UV radyasyonuna maruz kaldıklarında artan uygulama süresine bağlı olarak ölüm oranlarının arttığı ve larvaların uzun dalga boyu UV ışınına karşı daha dayanıklı oldukları belirlenmiştir. Larvalar uzun dalga UV ışını ile ışınlandığında, 30 dakikalık uygulamayla kontrol arasında önemli bir değişiklik olmamıştır. Kısa ve uzun dalga UV radyasyonuna maruz bırakılan pupalardan ergin çıkış süresi kontrole göre azalmış, fakat ömür uzunlukları değişmemiştir. *E. kuehniella* erginleri farklı sürelerde UV radyasyonuna maruz kaldıklarında ömür uzunluklarında bir değişim gözlenmemiştir. Fakat bununla birlikte hem kısa hem de uzun dalga UV radyasyonuna maruz bırakılan erginlerin yumurta verimi ve bu yumurtalardan larva çıkışı azalmıştır.

Anahtar kelimeler: *Ephestia kuehniella*, ultraviyole radyasyonu, ölüm oranı, ömür uzunluğu

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INTRODUCTION

The Mediterranean Flour Moth or mill moth (*Ephestia kuehniella*) is a moth of the family Pyralidae and is considered among the most important pests of stored products in storehouses. All of the 70 species of moth infesting stored products belong to Pyralidae, Gelechiidae, Oecophoridae, and Tineidae. Adults of *E. kuehniella* are more active at night and feeding larvae in dry store produce silk for pupation and pollute the commodities [1]. This insect was first reported in North America in 1889 and found in most of the temperate and sub-tropical parts of the world. They live in places with a temperature range of 20 -25°C [1, 5, 6]. They are likely found in any warehouse or flour or other powdered cereal products [1]. Developmental time of *E. kuehniella* from egg to adult is approximately 50 days in suitable temperature with a food source [4]. Adults do not need to be fed, so they can sustain their existence in arid conditions [1]. *E. kuehniella* shows complete metamorphosis or holometabolous development composed of egg, larvae, pupa, and adult. This feature makes it possible for these insects to survive in different environmental conditions [1, 8, 9]. Larvae of these pest's cause damage to the crop in grain stores and produce a nasty smell in the product. Several chemical and physical control methods such as insecticides, heat, and UV- radiation are used in controlling of these pests [1, 5, 6, 7]. However, using intensive insecticides cause several environmental and health problems and necessitates exploration of alternative non-toxic pest control methods. Because of the residue free advantages over chemical fumigation, irradiation becomes a more suitable control tactic in controlling stored product insects in storage conditions [8].

Many studies have been conducted to determine the effect of UV radiation on stored product pests [1, 4, 5, 7]. The wavelength of the UV-radiation reaching the Earth's surface varies between 290 and 400 nm [9]. Australia and the United States are

launched the UV alert in 2005, the goal of these countries was to increase awareness of the dangers of UV-radiation from the sun reaching the world [10]. The Ultraviolet (UV) part of the range is widely used as antiseptic and for the surface purification from insect eggs [11]. The biological activity of UV-radiation varies with the wavelength of the light. UV wavelength spectrum is divided into three types: UVA, UVB, and UVC [48**].** UV-radiation is efficient against grain pest by causing sterility and mortality in insects depending on exposure time[12]. Female insects are generally more sensitive than males against UV- radiation, this may allow modification of the radiation dose so that the treated females are completely sterile and males partially sterile [13, 14]. Sterile insect release technique (SIT) has been suggested as a means of controlling phycitine moths that attack stored agricultural products [14, 15, 16, 17]. The objective of this study is to develop a safer pest control tactic in order to reduce the use of chemical pesticides [18]. Although there are numerous studies on the effects of ionizing radiation on harmful insects, the number of studies for the effects of none- ionizing radiation such as UV radiation are relatively low [7, 8, 19]. In this research, we want to test the effects of different exposure times of UV-radiation on different life stages of *E. kuehniella* [19]. After irradiation of the eggs, larvae, pupae, and adults of *E. kuehniella* with short and longwave UV-radiation egg hatch, developmental period, longevity and progeny production were investigated.

CHAPTER 1

1. GENERAL INFORMATION

1.1 Description of insect

1.1.1 Adults

Adults are gray colored, seems long and narrow, its length is about 10-14 mm and wingspan 18-27 mm when it does not move [2]. Its body consists of three regions: head has chewing mouthparts, thorax has three pairs of legs and abdomen [20]. The adults have a distinctive feature while large wings are covering its body. Front and hind wings are linked together by a bristle so both wings move in synchrony during flight [20]. Mating and egg laying begins almost after adults emerge from the pupa. Adult *E. kuehniella* does not feed and die within 10 days [21]. Female moths can lay between 116 – 678 small eggs, they are putting them on or near the dried food source [22].

1.1.2 Eggs

Eggs are oval shape and white in color, the average weight of an egg is 0.02 mg [1]. It contains slight projection on one end [23]. The surface of the eggs before hatching turns light yellow indicating the development of the embryo inside the egg [1, 23]. The egg is secured by a hard-furrowed defensive external layer of shell, called the chorion which encloses the eggs. It is fixed with a thin covering of wax, which keeps the egg from drying out. Each egg contains various micropyles toward one side allowing sperm to enter the egg [24].

1.1.3 Larvae

The larvae are cylindrical in shape with legs on thorax and abdomen, its length is about 13 mm –16 mm, its color is white with a black head [1, 2]. The head contains a pair of short antennae with simple eyes on each side of the head, and chewing mouthparts [54]. The caterpillar are able to produce silk around them self and they prefer to feed on the stored grain [23, 24, 25,26]. The larvae are considered a major pest in stored grains [12].

1.1.4 Pupae

Immediately after passing from the larva to the pup, the color of the pupa is yellow color and then changes to dark brown in the last days of this stage. The mean size of pupae is 9 mm [1]. The pupa attaches to the surface by silk produced by the larva in the cocoon [23] and able to move by the muscles. When examined under a microscope or naked eye the wings are distinguished folded down the ventral surface with the legs and antennae [23]. To facilitate pupation corrugated cardboard is placed into the rearing jar.

1.2 Taxonomic tree

- Domain: Eukaryota
- Kingdom: Metazoa
- Phylum: Arthropoda
- Subphylum: Uniramia
- Class: Insecta
- Order: Lepidoptera
- Family: Pyralidae
- Genus: *Ephestia*
- Species: *Ephestia kuehniella*

1.3 Life cycle

E. kuehniella belong to Lepidoptera order and show complete metamorphosis consisting of four different stages such as egg, larvae, pupae, and adult (Figure 1.1). It is found in the warehouse, powder cereal, dry grain and store [1]. The life cycle of this insect

begins as small eggs laid on dry grains or flours. During the embryonic development, eggs turn from white to yellowish in four days. Immediately after egg hatching the small larvae move around to search for food. The larval stage is considered more harmful to crops and grains because it is the only stage that insect feeding in the life cycle. After a short period, the larvae begin to produce silk surrounding itself. The larva starts to pupate within 35 to 40 days after their hatch. It becomes the adult within about 10 days after pupation. Usually, Lepidoptera reproduces sexually, the number and the size of the eggs decrease with increasing age. Butterfly and moth eggs differ enormously in size [27].

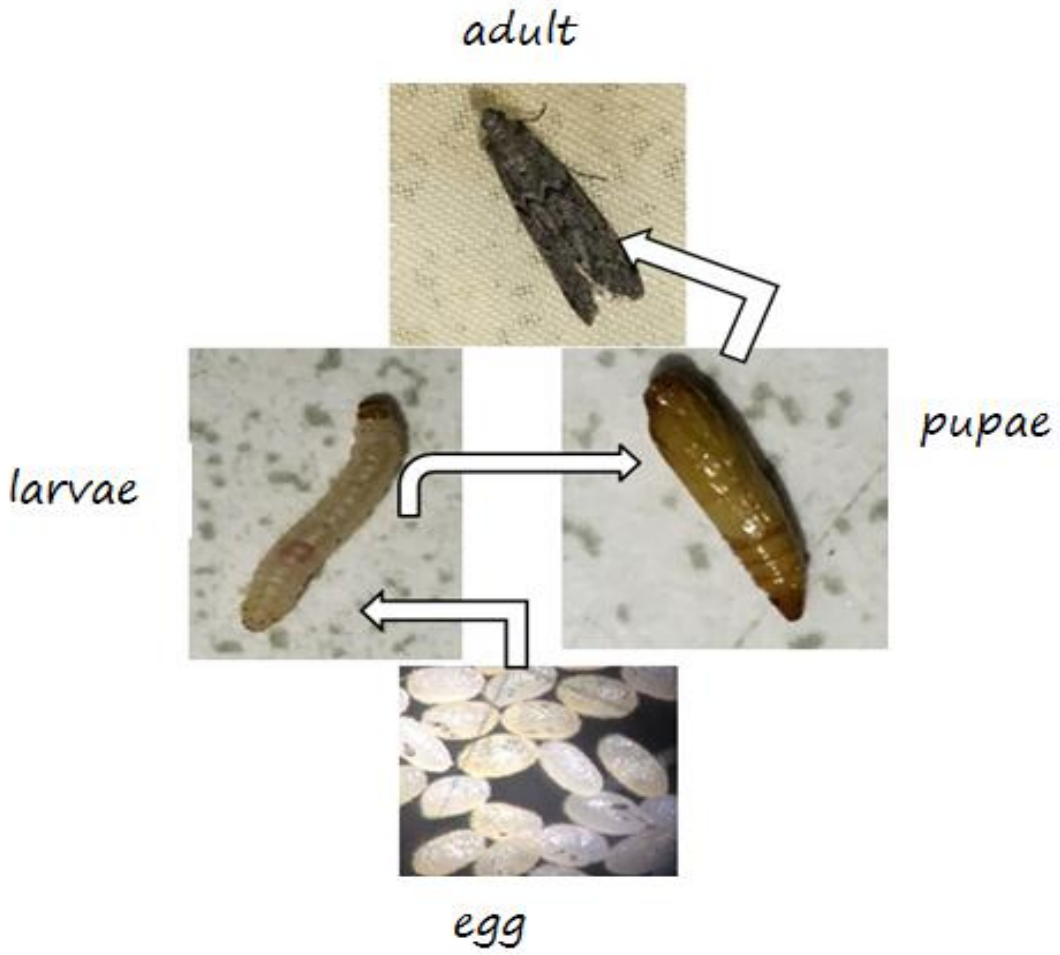


Figure 1.1 Life cycle of *E. kuhniella*

1.4 Mating behavior

Shortly after mating, the female starts laying eggs by extending its ovipositor and raising the mid-region of the abdomen. This position, which empowers her to transmit pheromones, can be taken up again a few days. The male can fertilize about 5-6 females throughout his life [23]. The age of the adult varies extremely. Usually, the female has high fecundity rate and produce approximately 100 -500 eggs during its lifetime [20].

1.5 External morphology

1.5.1 Head

The head is comparatively small and round and the face consists of 2 compound eyes found on each side of the head (Figure1. 2). The antennae are slender and multi segmented in shape and its main function is detecting odors [11, 24]. *E. kuehniella* have chewing mouthparts that is shaped a wrapped-up tube (proboscis) under the head [20]. These insects are no longer feed after adulthood.

Figure 1.2 Head of *E. kuehniella* Zeller [28].

1.5.2 Thorax

The thorax is the second part of the body and divided into three distinct segments, prothorax, mesothorax, and metathorax and each segment have a pair of legs: The prothorax has the first pair of legs and a pair of the respiratory hole (spiracles) and the second pair of spiracles are found on mesothorax. The adults also have two pairs of wings, first pairs of wings are located at mesothorax while the second pairs are at metathorax [20].

1.5.3 Abdomen

The abdomen includes 10 segments with membranes between them enable the movement. Each segment of the abdomen has a pair of spiracles except the last two segments. Auditory organs are found in the first or second abdominal segment. The reproductive organ of both male and female are complex. In the male includes a valve used to grasp the female during mating, while in females it's include ovipositors used to lay the egg. Female lays 75% of the eggs at the first two days of oviposition [24]. So, using young females will be more suitable to obtain more eggs.

1.6 What is radiation?

Radiation is a kind of energy released from the sun to the earth and moves from one place to another, the radiation includes waves, water waves, light waves, sound waves, and heat waves [28, 29]. The spectrum contains two divisions:

1.6.1 Ionizing radiation:

Because Ionizing radiation causes ionization of atoms and molecules it alters the function of biochemical molecules. The ionization of biological macromolecules indirectly leads to the formation of free radicals in and out of the cell. Because of the ionization, protein structure can be impaired , enzymes become inactive, lipids in cell membranes undergo peroxidation, structural carbohydrates dissolve, and more importantly, nucleic acids are mutated [30] .

Ionized radiation is categorized into three groups:

1.6.1.1 alpha particles

Alpha dissolution only occurs in heavy elements as thorium, radium, and uranium [31, 32, 33]. They have no penetrability of paper or skin. They contain 2 protons and 2 neutrons in the atomic nucleus. These particles have high mass and low velocity and they lose their energy quickly, so they interact more easily than beta particles or gamma rays [28].

1.6.1.2 Beta particles

Bata particles have fast moving electrons because of lightweight and higher speed. These particles may be positively or negatively charged (β- or β+) so they interact less quickly than alpha rays and can be stopped by a thick layer of aluminum [28, 34].

1.6.1.3 Gamma rays

Gamma radiation is a form of electromagnetic rays having high energy and their frequencies greater than 10^{18} cycles per second. It has wavelengths of less than 100 Pico meters (pm). They occupy the same region of the EM spectrum as X-rays. While X-rays are produced by accelerating electrons, gamma-rays are produced by atomic nuclei [28].

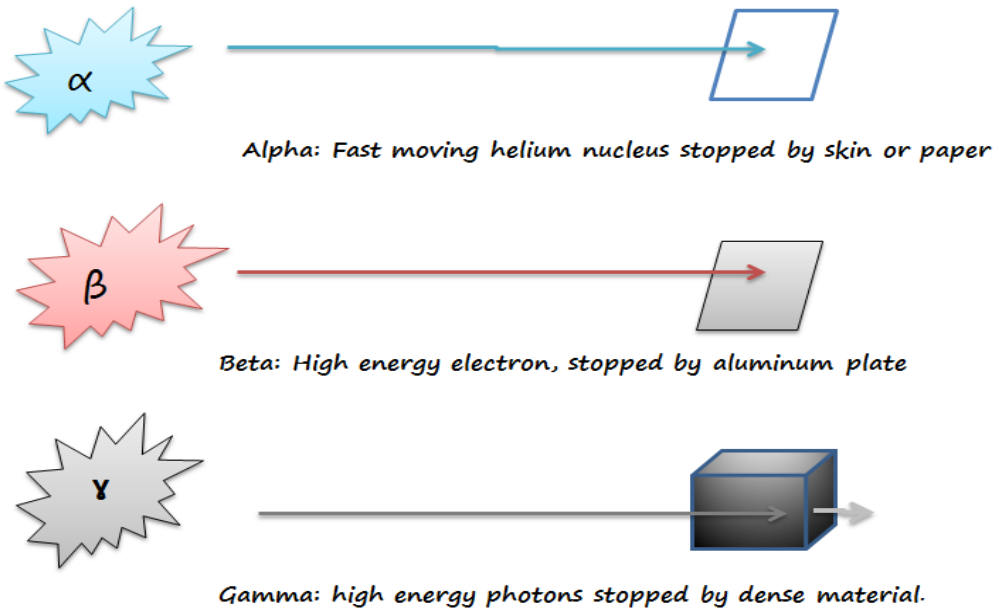


Figure 1.3 Ionizing radiations

1.6.2 Non-ionizing radiation

Non-ionizing radiations are considered part of a series of electromagnetic energy waves which does not have enough energy to ionize. It includes; Ultra Violet (UV), radio frequency, visible light, microwave, lasers and infrared radiation [28, 33].

1.7 What is a light spectrum?

The light spectrum is the many different wavelengths of energy produced by a light source. Light is measured in nanometers (nm). Each nanometer represents a wavelength

of light or band of light energy [28]. Visible light is the part of the spectrum from 380 nm to 780nm.

The line of these various colors consisted:

- Red Wavelength 625 - 740 (nm)
- Orange Wavelength 590 - 625 (nm)
- Yellow Wavelength 565 - 590 (nm)
- Green Wavelength 520 - 565 (nm)
- Cyan Wavelength 500 - 520 (nm)
- Blue Wavelength 435 – 500 (nm)
- Violet Wavelength 435 -380 (nm)

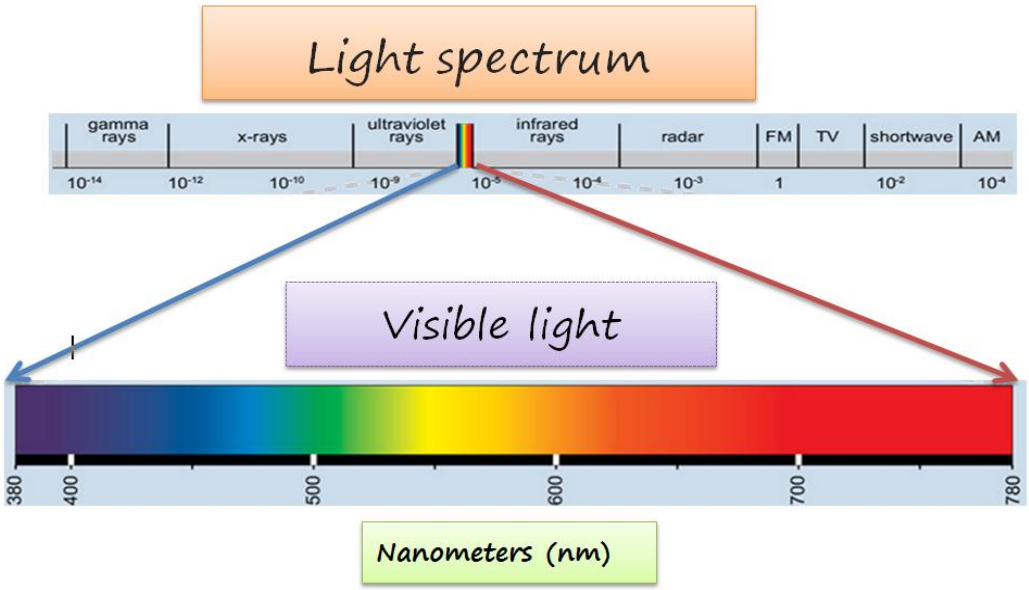


Figure 1.4 light spectrum

1.8 Ultraviolet (UV) Radiation

The sun irradiates energy in a wide ambit of wavelengths; most of the waves are invisible to human eyes. Canada was the first country introduce issue formal

predictions of UV levels [10]. UV-radiation is a part of the non-ionizing electromagnetic radiation that is produced by the sun and absorbed by particles in the earth's atmosphere. It's wavelength between 10 nm (30 PHz) to 400 nm (750 THz). The other wavelengths of this spectrum contain microwaves, radio waves, infrared radiation, X-rays and visible light. It can also be produced by electric light [9, 35]. Shorter wavelengths of the light are important in several fields, including medicine treatment, biological processes, energy source, tanning and disinfectant for germs and insects [11, 36]. UV-radiation varies in quality and quantity depending on factors scattering and absorption by air molecules in the atmosphere and Ozone Layer [9, 37]. Biological studies and effects of UV-radiation vary significantly depending on wavelength. So, the UV spectrum is then subdivided into three regions [37]:

1.8.1 UVA

This type of UV-radiation is widely from other radiation because it cannot be absorbed in the atmosphere and approximately 99% of the UV reaches the Earth's surface. UV-radiation can penetrate glass and clouds because UVA is less intensive than UVB and UVC present during all daylight throughout the years [36, 38]. UVA radiation is divided into two groups: UVA I (340-400 nm) and UVA II (320-340 nm) [38]. Although UVA breakthrough the human skin below than UVB, it causes significant biological damage as they affect the DNA strand the causing cancer [39].

1.8.2 UVB

UVB radiation has a wavelength between 290 and 320 nm, and only 1% of this radiation reaches the Earth's surface. [36]. UVB is the cause of skin sunburn, reddening and damage to the skin's superficial layers, it is rising the developments of skin cancer and (UVB) less penetrates to glass [36, 38].

1.8.3 UVC

UVC radiation have a wavelength range between 200 and 290 nm. This type of radiation is filtered out by the ozone layer so that none reaches the earth's surface. Ultraviolet C is germicidal and is also used in ultraviolet phototherapy [36].

1.9 Effects of radiation on insects

Several studies have been carried out by various researchers on the effect of radiation on insects. While there are many studies on the effect of gamma radiation on harmful insects, relatively few studies are conducted on the effects of Ultra-Violet (UV) radiation on insects [28, 40]. Some of the work done in this regard is briefly summarized below.

Ultraviolet (UV) irradiation is used to kill insects at various stages in their life cycles (Baden et al., 1996). UVC is the most effective and most biologically damaging UV source and has the shortest wavelength (100–280 nm). It is the most active and has the greatest potential to induce biological damage (Pattison & Davies, 2006) [41]. The efficacy of UVC against some beetles and mite pests in stored products is well established (Calderon & Navarro, 1971; Furaki et al., 2007), with sensitivities depending on the species and dose [6, 43].

Poushand et al (2017) have describe the effects of UV-radiation (254 nm wavelength) on the adult insect *Trialeurodes vaporariorum* at the different height (70, 80 and 90 cm) under different exposure times (0.5, 1, 2, 4, 6, 8, 10,12 minutes) at 27±2 ºC and 65±5% RH. They have indicated that UV light can be used in the elimination of insects living on stored products. When they increased the exposure periods and decreased heights of the UV source the mortality rates of adults increased [44].

Hassan et al (2017) have indicated that the gamma irradiation lowered the reproductive potential of male *Culex pipiens* and caused deformity in spermatogenesis [45].

Shetty et al. (2016) have reported that different doses of gamma radiation increased the longevity of the Progeny of the parental *Aedes aegypti* adults in the F1 generation by 10.56 and 8.66 days, respectively [40].

Witayakul et al. (2016) showed that effects of UVC-radiation and microwave on the expression of heat shock protein in *Sitophilus zeamais* at four developmental stages: egg, larva, pupa and adult, they found UVC-radiation did not affect the longevity of

adults, but determined that UVC and microwave irradiation affected the expression profile of heat shock protein (hsp) genes [12].

Sorungbe et al (2016) reported that different doses of UV-radiation type (UVC-254nm) to eggs of *Ephestia cautella* at different ages (1, 2, 3) days, leading to a lower hatching of eggs and decrease the emergence of adults from irradiated larvae [46].

Sang et al (2016) tested the effects of UVB radiation for *Tribolium castaneum* and reported that the radiation not only kills the larvae, but also reduce the developmental period, delay the pupa formation, and cause a decrease in size [47].

When Sheeja et al (2015) applied UV-radiation (type UVC-254 nm) on the eggs of *Corcyra celphalonica*, they reported that the greater the doses applied the lower the hatchability of eggs [13]. Similar results were obtained by Modarres Najafabadi et al (2014) for *Callosobruchus maculatus* with UV-radiation (type UVC-254nm). They reported that younger eggs were less affected by UV-radiation than older ones [48].

When Lah et al (2012) have exposed the eggs and adults of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* to UVC radiation under different exposure periods ranging from 5 to 60 minutes and several distances (10-55cm), they obtained complete mortality rate after an exposure from 10 cm distance for both species. They also indicated that UV-radiation caused the death of the organism because of the destructive effects of UV-radiation on DNA and RNA, and therefore the mortality rates increase as application distance and duration of application increase [42].

Tunçbilek et al (2012) studied the effect of gamma and UV-radiation on the egg parasitoids *Trichogramma euproctidis*, they found that when the age of insect increased the effect gamma and UV-radiation decreased. They also reported that the pupa and adult stages were the most resistant to both UV and gamma radiation [8].

Mahmoud and Barta (2011) have tested the effects of gamma radiation to *Bactrocera zonata* pupae, they indicated a reduction in adult emergence and hatchability of the eggs with increased exposure periods [49].

Azizoğlu et al (2011) were achieved complete mortality when irradiated the eggs of *E. kuehniella* with microwave radiation at the powers of 150, 360, 430, and 600 W for different exposure periods, they were reported that increasing power and exposure periods caused decrease larvae emergence and increased mortality [18].

Mohan and Kumar (2010) found that increasing exposure time of UV irradiation (254 nm) caused a decrease in the longevity of *Dysdercus koenigii* nymphs [50].

Faruki et al (2007) reported a decrease in hatching ability of *the Tribolium castaneum* eggs after exposed to UV-radiation, and also, they found that the younger eggs were more tolerant than older [6]. Begum et al. (2007) have studied the effect of *triflumuron* and UV-radiation on the generation of *Alphitobius diaperinus* eggs. They examined the hatching ability and found that the populations of *Alphitobius diaperinus* decreased after one week of exposure [51].

Ghanem and Shamma (2007) have studied the effect of non-ionizing UVC-radiation on the development of *Trogoderma granarium.* They found that Mortality rate of larvae increased with increasing exposed periods. They reported that the older larvae were more tolerant against UV-radiation than the younger [55].

Ayvaz et al (2006) studied the effects of gamma radiation on the different life stages of *Ephestia kuehniella* Zeller and found that the egg hatching decreased with increasing dose [52].

Faruki and Khan (2001)) have tested pathogenicity of *Bacillus thuringiensis* ssp on *Cadra cautella* larvae after exposed to gamma radiation, they found a decrease in adult longevity and fecundity [5].

Elbadry and Ahmed (1975) have studied effects of gamma radiation of the *Callosobruchus maculatus* eggs, they reported a reduction in egg hatching. They also found that males are more resistant to UV-radiation than female [53].

CHAPTER 2

2. MATERIAL AND METHODS

2.1 Insect culture

To cultivate *E. kuehniella* cultures, 300 ml glass jars with sieved caps were used for experiments (Figure 2. 1). Each jar was supplemented with 2 volumes of wheat flour and 1 volume of wheat bran (about 100 g), and the mixture was thoroughly mixed. Stock cultures were produced in 1l jars containing the same nutrient mixture. The insects could grow in a rearing condition arranged to 27 ˚C temperature, 14:10 h light: dark photoperiod and 65% relative humidity.

Figure 2.1 Insect culture

2.2 Production of egg

Many *E. kuehniella* adults growing in stock culture were withdrawn from the culture medium by water pump and taken to 1 L glass jars. The caps of the jars were removed and closed with a sieve to collect the eggs. The jars then placed into the glass Petri

dishes in an inverted position. The eggs laid in Petri dishes were taken on a white paper and the particles were removed. The cleaned eggs were collected in a small glass petri dish.

2.3 UV irradiation experiments

Eggs, larvae, pupae, and adults of *E. kuehniella* were exposed to UV radiation at different exposure times and results were recorded. Long and shortwave length of UV radiation were applied separately to each stages of *E. kuehniella* using a radiation source capable of applying long and shortwave lengths of UV radiation (UVGL-58 Handheld UV lamp (Mineralight), 254/365 nm UV, 215–250 V, 6-Watt, ~50 Hz, 0.12 Amps, Figure 2. 2).

Figure 2.2 The UV-radiation source and the cabinet used in the application

2.3.1 Irradiation of the eggs

This study was planned to determine the susceptibility of different stages of developing embryo in the egg against UV-radiation and how embryonic development is affected.

Eggs of 0-2, 24, 48 and 72 h old ages were irradiated with long and shortwave lengths of UV-radiation separately for different exposure times ranging from 50-600 s. The eggs prepared for irradiation were placed in a glass petri dish with the lid open and placed in a UV cabinet. Approximately 100 intact eggs were examined and selected for each age group and exposure time. The UV source is in the special burrow on the cabinet, and the distance between the source and the sample is 19 cm. Eggs were irradiated for different exposure periods ranging from 0-600s with UV source shown in Figure 2.2. Exposure time is determined by using a stopwatch. Immediately after treatment eggs were then stuck onto rectangular cut paper. These cards were placed in the middle of the petri dish and the food was added to the cards to feed the emerging larvae. Petri dishes were closed and moved to the rearing room to observe egg hatch. Starting from the third day of the application, embryonic development in eggs was examined under a stereomicroscope and eggs containing live and dead larvae were identified and counted. To determine the larval emergence and developmental periods inside the eggs, observations are continued until the 8th day of the study. The experiments for each age group of eggs and application period were carried out in three replicates (Figure 2.3). The same procedures were applied similarly for long and shortwave UV-radiation.

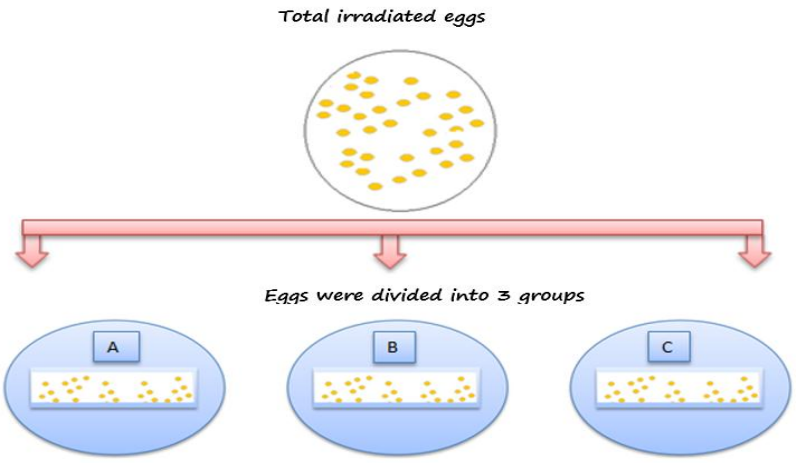


Figure 2.3 Experimental setup for egg irradiation

2.3.2 Irradiation of the larvae

To obtain 30 d old larvae, many *E. kuehniella* eggs were placed in the glass jars together with the necessary nutrients and the eggs could develop in the rearing room. At the 30 days of developmental period, the contents of the glass jars containing the larvae were discharged into plastic cuvettes (30 x 20 x10 cm) and the larvae were collected by using a clean brush and transferred to the glass Petri dishes with nutrients as in Figure 2.4. A total of 30 larvae in glass Petri dishes were placed inside the UV cabinet as in the egg irradiation and exposed to UV-radiation for different exposure times ranging 0 to 40 minutes. Immediately after irradiation, the larvae were placed in 3 separate Petri dishes with 10 larvae each. Enough nutrients were added to each petri dish to feed the larvae, and the lids of the Petri dishes were closed and taken to the rearing room. To determine the effects of UV-radiation on the larvae, pupation, adult emergence, egg production, egg hatch, and longevity were recorded. To determine egg production male and female adults are put into glass jars and the caps of the jars were removed and closed with a sieve to collect the eggs. The jars then placed into the glass Petri dishes in an inverted position. The eggs laid in Petri dishes were taken on a white paper and counted under a stereomicroscope. The same procedures were applied similarly for a long and short wave of UV-radiation.

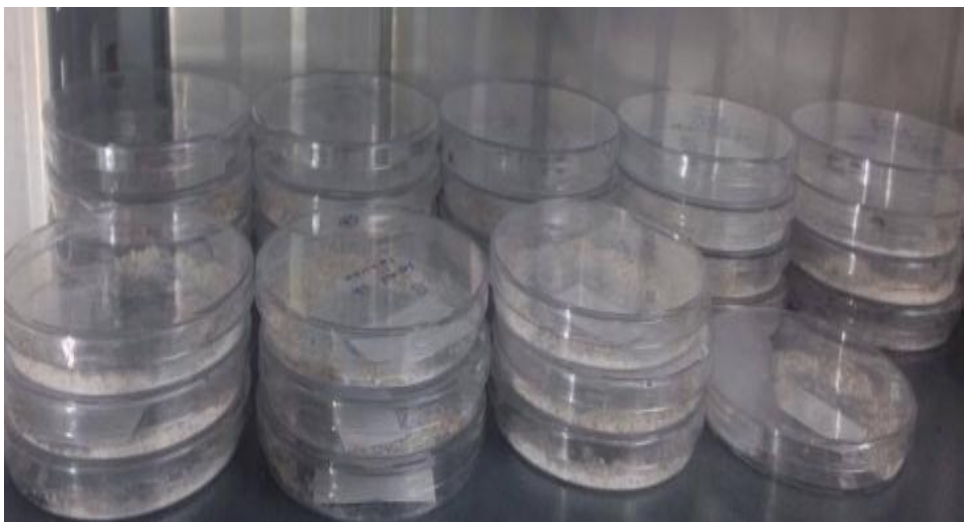


Figure 2.4 30 d- old *E. kuehniella* larvae prepared for UV- radiation

2.3.3 Irradiation of the pupae

To obtain 3 d old of pupae, many *E. kuehniella* eggs were placed in the glass jars together with the necessary nutrients and allowed to develop in the rearing room. After 30 days, the contents of the glass jars containing the larvae were discharged into plastic cuvettes (30 x 20 x10 cm) and the larvae were collected by using a clean brush and transferred to the glass Petri dishes with nutrients. A total of 40 larvae were put into glass Petri dishes and waited until pupae formation, where the incubation period was 40 days then prepared for irradiation. Glass Petri dishes containing pupae were inserted into UV cabinet with the lid open and irradiated for 0, 40, 60, 80 and 160 minutes, respectively. Approximately 40 intact pupae were selected for each group and divided into 3 groups for replication. The UV source is in the special burrow on the cabinet, and the distance between the source and the sample is 19 cm. The experiments for each age group were carried out in three replicates. The same procedures were applied similarly for long and shortwave UV-radiation. All the experiments for each irradiated stage were performed at rearing room adjusted to 27 ˚C temperature, 14:10 h light: dark photoperiod and 65% relative humidity (Figure 2.5).

Figure 2.5 The rearing room where the experiments were made.

2.3.4 Irradiation of the adult

To obtain 1- day old adults, all the *E. kuehniella* adults were pulled out of the cups from rearing jars with a vacuum pump. After the adults were removed, we waited for the emergence of new adults. These 1-day old 18 adults were gathered in small glass vials and their opening was covered with the plastic sieve and inserted into the UV cabinet for irradiation under different exposure times ranging between 0 to 300 minutes (Figure 2.6). Male and female adults were taken into the 300 ml glass jars to determine the egg production and the opening of the jars were covered with the plastic sieve, these jars were inverted into glass Petri dishes and eggs were collected and counted. This process continued for each group until all the adults die. The longevity and the sex ratio of the adults were also determined. The total number of eggs obtained for each group was divided by the number of adult females and the egg production per individual female was calculated. To determine the hatchability of the eggs from irradiated adults about 150 eggs were glued on small cartons and these cartons inserted into glass Petri dishes. This observation continued for 8 days.

Figure 2.6 E. *kuehniella* adults placed in the UV cabinet for irradiation.

2.4 Data and statistical analyses

All the data from the irradiation experiments were subjected to analysis of variance (ANOVA) by using the Turkey-Kramer HSD posttest and means were separated at the 5% significance level. LT50 and LT99 values were estimated by using probit analysis (SPSS, 2010).

CHAPTER 3

3. RESULTS

3.1 Effects of UV-radiation on the life stages of *E. kuehniella*

3.1.1 Effects of shortwave/longwave UV- radiation on the different age of eggs.

3.1.1.1 Effects of shortwave UV- radiation

In this study, different age groups of *E. kuehniella* eggs (0-72 h) were exposed to shortwave UV-radiation for six different exposure time ranging between 50-600 s, the mortality rates of the eggs increased with increasing exposure time and the results obtained are given in Table 3.1. Embryological development was negatively affected and mortality rates significantly increased with increasing exposure time *(p>0.001).*

Table 3.1 Mortality rates of the different age groups of *E. kuehniella* eggs after exposed to shortwave UV-radiation

Exposure time (s)	Shortwave			
	0-2 h	24 h	48 h	72 h
Control (0)	12.40 a*	10.50 a	19.04 a	19.04 a
50	37.04 Ab**	27.00 Bb	36.09 Ab	43.09 Ab
100	41.07 Abc	52.72 Bc	62.57 Bc	53.01 Bc
200	41.72 Abc	60.62 Bc	70.11 Bc	67.64 Bd
300	48.77 Ac	79.03 Bd	82.75 Bd	72.85 Bd
400	69.06 Ad	100.00 Ce	87.05 Bd	91.87 Be
600	97.78 Ae	100.00 Be	100.00 Be	100.00 Be
Statistics	*(F=118.264; df=6; p<0.0001).*	*(F=69.676; df=6; p<0.0001).*	*F=144.858; df=6; p<0.0001*	*F=93.229; df=6; p<0.0001)*

*Means followed by the same lowercase letter in the same column are not significantly different at the 0.05 level by ANOVA test. **Means followed by the same uppercase letter in the same row are not significantly different at the 0.05 level by ANOVA test.

When the 0-2 h old eggs were exposed to UV-radiation, mortality rate significantly increased with increasing doses *(F=118.264; df=6; p<0.0001)*. After irradiation, it was observed that 69.06% of the eggs were killed at 400s and at the highest exposure time 97.78% eggs were killed. Mortality rates of the eggs irradiated for 24 h old eggs were also significantly increased with increasing exposure time *(F=69.676; df=6; p<0.0001)*. However complete mortality was observed at 400s and above exposure periods for 24 h old eggs (Figure 3. 1). Significant reduction in egg hatch was observed in 48 and 72 h old eggs completely mortality rates achieved at (600s).

When we are compared these groups the different age groups of *E. kuehniella* eggs after exposure to shortwave UV-radiation for a duration of 50s, the mortality rate of 24 h eggs were significantly lower than that of other age groups (*F=5.572; df=3; p<0.05)* as in Table 3. 2.

But in 100, 200, 300, 400 and 600s exposures the mortality rates of the 0-2 h old eggs were significantly lower than that of other age groups (Table 3.1).

While the 0-2 h old eggs of the same 400 s exposure were found to have a mortality rate of 69.06%, in 24 h old eggs, all eggs were killed by this exposure period. Also at the highest exposure period (600 s), all the eggs were completely killed with UV-radiation in 48 h and older groups.

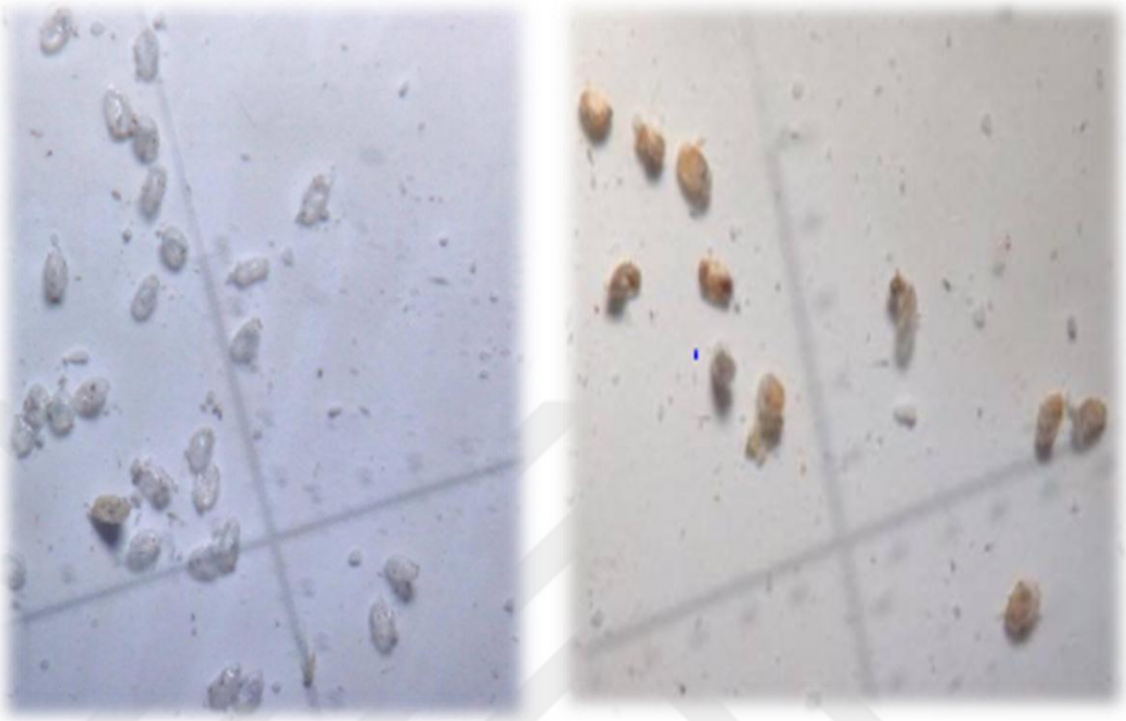
Table 3.2 Comparison of different ages groups against the same exposure time (shortwave)

Exposure time (s)	**Statistics**		
	F	*Df*	*P*
50	5.572	3	0.023
100	6.853	3	0.013
200	6.092	3	0.018
300	13.456	3	0.002
400	36.915	3	0.0001
600	3.360	3	0.032

Age groups: 0-2, 24, 48 and 72h; ANOVA results is given in Table: F value (F), Degree of Freedom (df), Significance (P value)

When Lethal Time (The time required to kill half of the population, LT_{50}) values are considered, the LT_{50} value of 0-2 h old eggs were higher than that of 24 h old eggs. As

can be understood from the results in Table 3.3, the highest LT_{50} value was estimated for 0-2 h old eggs suggesting that these eggs are more resistant to UV-radiation than other age groups.



Control eggs (600 s)

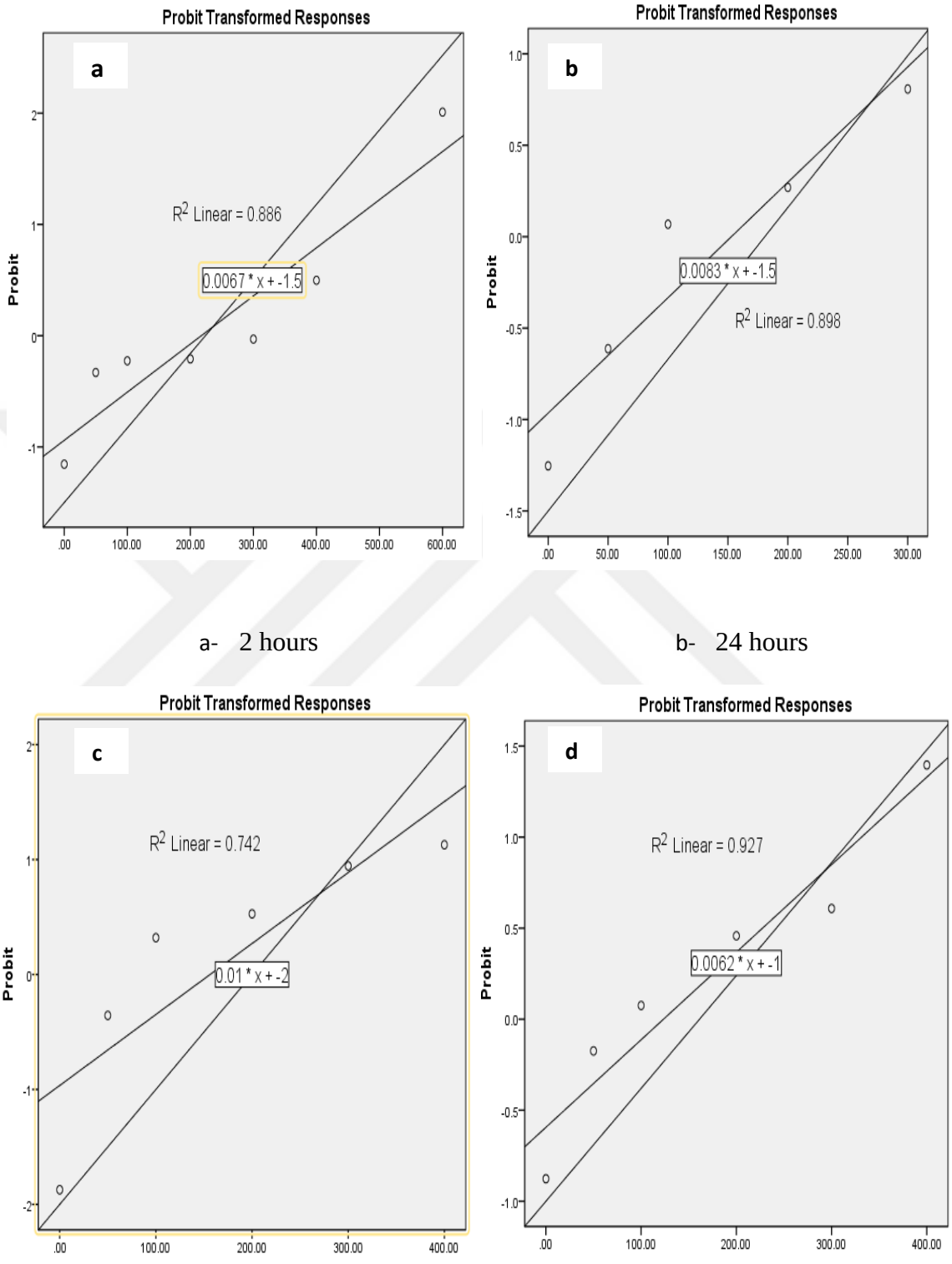
Figure 3.1 Eggs of unexposed (control) and exposed (600 s) of 24h old *E. kuehniella* eggs to shortwave UV-radiation

As can be seen in Figure 3.1, although 100% of the eggs are hatched in unexposed eggs, the larvae in the eggs exposed to 600 s of shortwave UV radiation are all killed under the examination of the stereomicroscope.

Table 3.3 LT values for the eggs after exposed to different exposure periods of shortwave UV-radiation

Age of eggs	Shortwave	
	LT_{50}	LT_{99}
0-2 Hour	227.75 (135.76-329.42)	861.37 (636.9-1496.13)
24 Hour	141.59 (92.53-189.15)	476.61 (378.1-685.7)
48 Hour	132.28 (16.10-219.2)	538.5 (383.0-1113.4)
72 Hour	120.2 (62.38-167.53)	596.018(478.85-824.97)

LT: Lethal time, LT_{50}: Time required to kill half of the population, LT_{99}: Time required to kill 99% of the population.



a- 2 hours b- 24 hours

c- 48 hours d- 72 hours

Figure 3.2 Probit transformed responses after exposed to shortwave UV-radiation.

However, when we see Table 3.4 it was observed that embryological developmental periods were not different for 0-2, 24 and 48h of eggs up to 300s. But this period decreased for 72 h of eggs depending on increasing exposure periods. On the other hands after 400s of exposure, since the larvae of the eggs at 24, 48 and 72 hours died after the 400s administration, embryonal development was observed only in 0-2 h old eggs in 600s exposure (ANOVA results for 0-2h old, *F=2.269; df=6; p<0.097*; for 24h old, *F=0.799; df=4; p=0.553;* for 48 h old, *F=0.784; df=5; p=0.581* and for 72 h old, *F=7.866; df=5; p<0.002).*

When compare the effect of 50s exposure on 0-2, 24, 48 and 72 h old eggs, we observed the embryonic development period decreased for 72 h old eggs *(F=3.931; df=3; p=0.054*). This situation showed a similar trend for periods of 100, 200 and 300s. (ANOVA results for 100s, *F=1.511; df=3; p=0.284*, for 200s, *F=1.359; df=3; p=0.323*, for 300s, *F=0.354; df=3; p=0.788* and for 400s, *F=1.904; df=2; p=0.22*).

Table 3.4 Embryonic development period of the different age groups of *E. kuehniella* eggs after exposed to shortwave UV-radiation

Exposure time (s)	**Shortwave**			
	0-2	24	48	72
Control (0)	5.22 ± 1.62 a*	5.29 ±0.83 a	5.71±1.30a	5.55±0.59 a
50	5.25±1.62 aAB**	5.54±1.40 aB	5.61±0.88 Aa	4.50±0.63 b A
100	4.75±1.28 Aa	5.66±1.17 aA	5.34±0.72 Aa	4.87±0.81bc A
200	4.94±1.13 aA	5.43±0.95aA	5.40 ±1.10 Aa	5.01±0.44 c A
300	4.82±1.14 aA	4.82±0.74aA	5.19±1.02 Aa	5.03±0.46 c A
400	5.06±1.54 aA	-	5.28±1.05 Aa	4.77±0.38 bc A
600	3.67± 1.53 b	-	-	-

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test. **Means followed by the same upper-case letter in the same row are not significantly different at the 0.05 level by ANOVA test

3.1.1.2 Effects of longwave UV- radiation

When different age groups of *E. kuehniella* eggs (2-72 h) exposed to longwave UV-radiation for six different exposure time (50-600 s) the mortality rates of the eggs increased with increasing exposure time and the results obtained are given in Table 3.5. Effects of longwave UV-radiation was lower than that of shortwave UV-radiation on the *E. kuehniella* eggs. No mortality was achieved at any exposure periods applied and these exposure times were not enough to kill all the eggs with longwave UV radiation and much higher exposure times were required.

At the highest exposure period, the lowest mortality rate was obtained for 24 h old egg (64.42%) suggesting that this age group are more resistant to longwave UV-radiation. When we are comparing these groups the different age groups of *E. kuehniella* eggs after exposed to longwave of UV-radiation, the mortality rate of 24 h old eggs were significantly higher than that of other age groups at 50, 100, 200, 300s the result is given in Table 3. 5. But for 600s exposure mortality rates of the 0-2 h and 72 h old eggs was significantly higher than that of other age groups (*F=3.637; df=3; p<0.006)* (Table 3.5 and Table 3.6).

Table 3.5 Mortality rates of the different age groups of *E. kuehniella* eggs after exposed to longwave UV-radiation

Exposure time (s)	Longwave			
	0-2 h	24 h	48 h	72 h
Control (0)	12,40 a *	15,75 a	14,79 a	19,04 a
50	22.40 ABb**	33.33 B b	17.71 Aab	20.54 ABab
100	30.95 Bc	33.38 B b	21.04 Aab	26.41ABab
200	32.36 Bc	44.21Cc	23.87 A b	29.43 ABab
300	34.14 Ac	50.79 Bcd	47.82 Bc	36.84 Ac
400	35.38 Ac	53.69 Bd	54.60 Bc	51.52 Bc
600	84.51 Bd	64.42 Ae	79.77Abd	84.13 Bd
Statistics	*F=118.264; df=6; p<0.0001*	*F=60.167; df=6 ; p<0.0001*	*F=55.638; df=6; p<0.0001*	*F=17.553; df=6; p<0.0001*

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test. **Means followed by the same upper-case letter in the same row are not significantly different at the 0.05 level by ANOVA test.

Table 3.6 Comparison of different ages groups against the same exposure time (longwave)

Exposure time (s)	**Statistics**		
	F	*DF*	*P*
50	2.332	3	0.015
100	4.057	3	0.05
200	19.396	3	0.001
300	6.590	3	0.015
400	10.256	3	0.04
600	3.637	3	0.06

Age groups: 0-2, 24, 48 and 72h; ANOVA results is given in Table: F value (F), Degree of Freedom (df), Significance (P value)

The Probit analysis after the exposure of longwave UV-radiation suggested the effect of longwave UV-radiation was lower than that of shortwave UV-radiation, these results were estimated and given in Table 3.7. For example, all 24 h old eggs were killed at 476.61s with shortwave UV, but to achieve the same result with longwave UV 1580.90s was required (Table 3.3, Table 3.7 and Figure 3.3).

Table 3.7 LT values for the eggs after exposed to different exposure periods of longwave UV-radiation

Age of eggs	**Longwave**	
	LT_{50}	LT_{99}
0-2 Hour	379.82 (267.5-627.1)	1236.80 (861.9-2636.1)
24 Hour	345.49 (256.7-487.5)	1580.90 (1156.7-2700.1)
48 Hour	352.10 (318.4-391.6)	1077.59 (955.5-1247.4)
72 Hour	347.19 (287.7-427.7)	1141.80 (925.8-1542.6)

LT: Lethal time, LT_{50}: Time required to kill half of the population, LT_{99}: Time required to kill 99% of the population,

When measuring embryo development period of the different age groups of *E. kuehniella* eggs after being exposed to longwave UV-radiation, there were no decrease up to 400s of exposure but this period decreased with 600s for these eggs (ANOVA results for 0-2h old, *F=8.177; df=6; p<0.001*). While there was no significant change in the embryonic developmental periods of 24 and 48 h old eggs up to 400s exposure, the period changed significantly for these groups of eggs with 600s exposure time (ANOVA results for 24h, *F=1.943; df=6; p=0.143* and for 48 h old, *F=6.80; df=6; p=0.669).* The 48 and 72 h of eggs showed similar trends with respect to embryonic development periods for all exposure times as shown in the Table 3.8 (ANOVA results for 72 h, *F=1.795; df=6; p=0.172)*

Table 3.8 Embryonic development period of the different age groups of *E. kuehniella eggs* after exposed to longwave UV-radiation

Exposure time (s)	Longwave			
	0-2	24	48	72
Control (0)	5.22 ± 1.62 ab*	5.03±0.81 ab	6.15±1.35 a	6.03±0.76 a
50	4.99±0.84aB**	4.96±0.70 abB	5.77 ±0.77 aA	5.40±0.63 bAB
100	5.07 ±0.70 aA	5.49±0.86 bA	5.60±0.98 aA	5.60±0.78 abA
200	5.27±0.83 aA	5.10±0.92 abA	5.14±0.85 aA	5.55±0.80 abA
300	4.86±0.88 aA	5.36±1.09 bA	5.06±0.77 aA	5.51±0.07 abA
400	5.21±0.66 aA	5.49±0.90 bA	5.08±0.76 aA	5.41±0.60 bA
600	3.11±0.72 bA	4.32±0.70 aB	5.76±0.69 aC	5.48±0.91 bC

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test. **Means followed by the same upper-case letter in the same row are not significantly different at the 0.05 level by ANOVA test

When compared the embryonic development among different age groups, only 50 and 600s of exposure caused a change, however other exposure times did not cause a change in the embryonic development periods of different age groups-When the eggs irradiated during 600s significant changes were observed among age groups with respect to embryonal development as can be seen in Table 3.8 (ANOVA results: *F=10.855; df=3; p=0.003*).

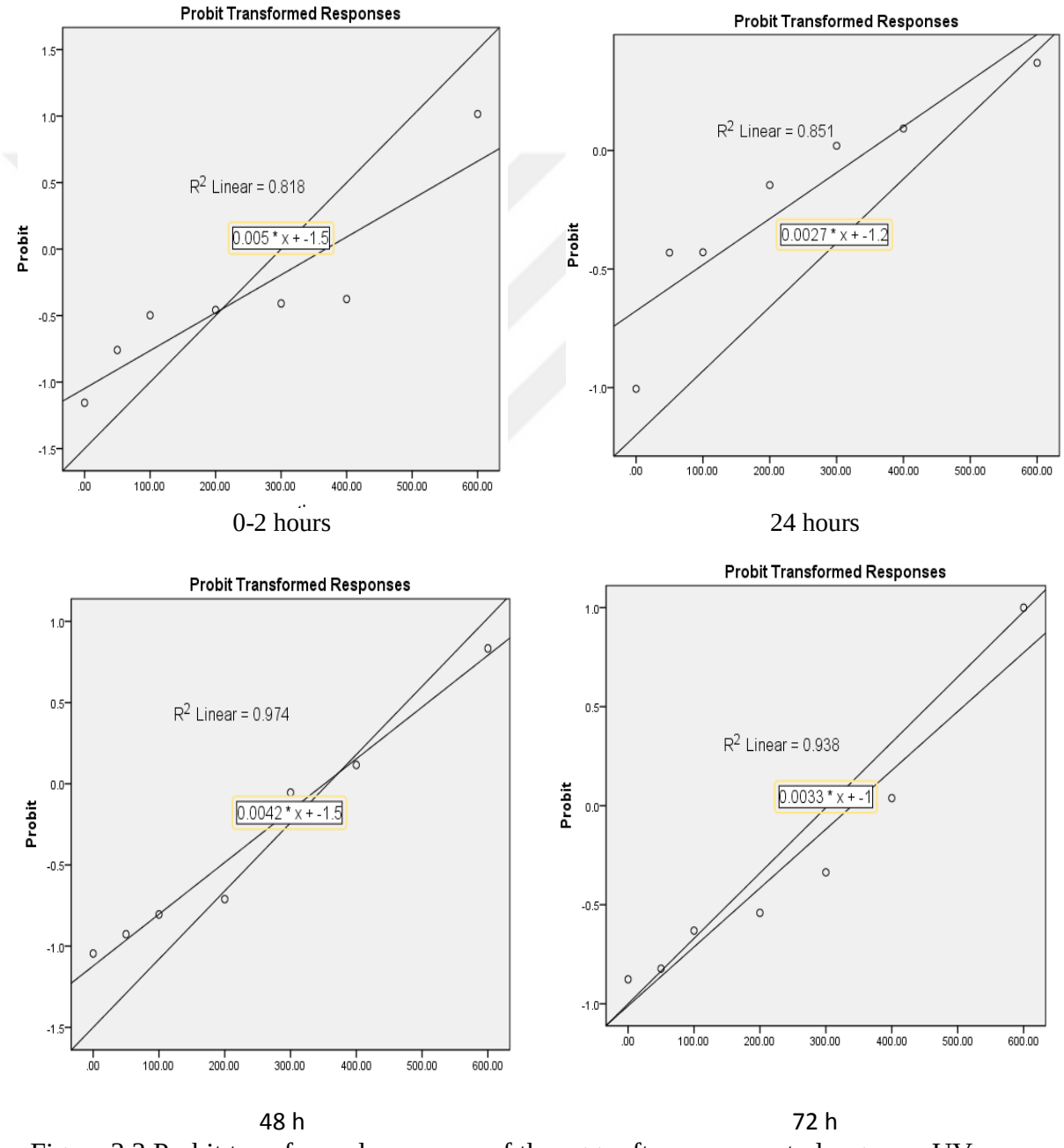


Figure 3.3 Probit transformed responses of the eggs after exposure to longwave UV-radiation

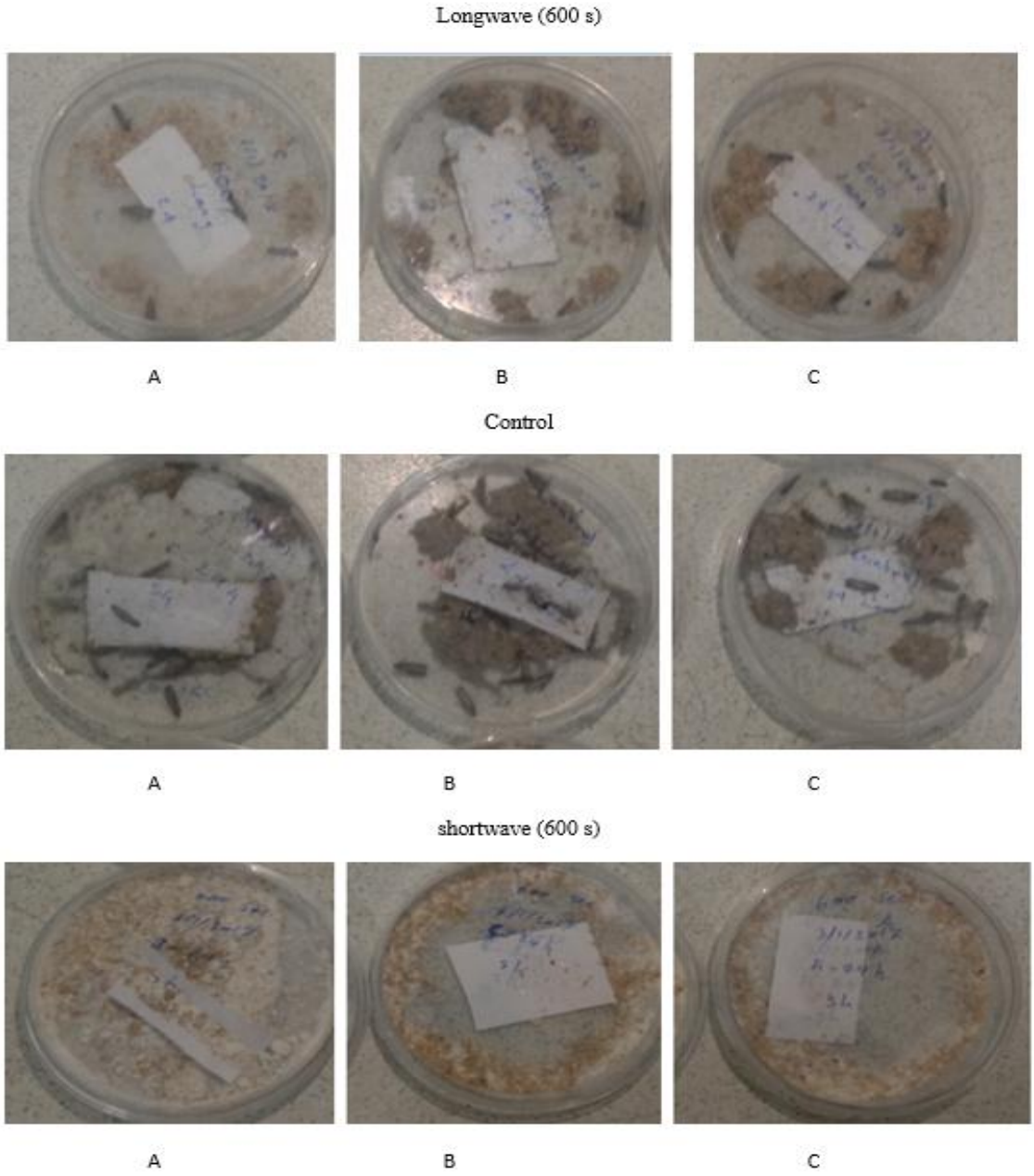


Figure 3.4 Development of *E. kuehniella* after being irradiated as eggs (24 h old) with short and longwave UV-radiation (600 s).

As can be seen in Figure 3.4, after irradiation of the eggs with long and short wavelength of UV radiation, most of unexposed control eggs developed to adults and the larvae developed from these eggs destroyed the nutrients. When eggs were irradiated with longwave UV for 600 s, although not as much as in control adult emergence was also observed, and destruction in the product decreased compared to control. However, when the eggs were irradiated with shortwave UV for 600 s, larvae and adult emergence were not observed and was no damage was found on the product (Figure 3.4).

3.1.2 Effects of UV-radiation (short/long wave) on 30 days-old *E. kuehniella* larvae

When we irradiated the 30 days old *E. kuehniella* larvae with shortwave/longwave UV-radiation at different exposure time, percent mortality rates were recorded and estimated on the 9th day of exposure and the results obtained are given in Table 3.9. The mortality rates significantly increased with the increasing exposure time when the larvae exposed to shortwave UV- radiation *(F=5.84; df=4; p<0.011*). However, there was no significant change up to 30 minute of exposure time *(p>0.05)* when the larvae irradiated with longwave UV radiation, but percent mortality rate significantly increased with 40 min exposure (*F=4.68; df=4; p<0.022).*

Table 3.9 Mortality rates of the *E. kuehniella* larvae (30day old) after exposed to UV- radiation

Exposure time (min)	**Mortality rates (%)**	
	Shortwave	Longwave
Control (0)	16.67 a*	14.39 a
10	23.33 a	25.00 a
20	53.33 ab	21.33 a
30	93.33 b	23.33 a
40	93.33 b	50.00 b

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test.

By using Probit analysis LT_{50} and LT_{99} values were also estimated and given in Table 3.10. These probit values showed that to obtain complete mortality approximately 50 minutes of shortwave UV radiation should be applied. It is necessary about 158 minutes of exposure to achieve the same results with longwave UV-radiation. Probit transformed responses are seen in Figure 3.5.

Table 3.10 LT values for *E. kuehniella* larvae (30day old) after exposed to UV-radiation

UV-radiation	LT_{50}	LT_{99}
Shortwave	16.66	48.53
Longwave	49.87	157.93

LT: Lethal time, LT_{50}: Time required to kill half of the population, LT_{99}: Time required to kill 99% of the population,

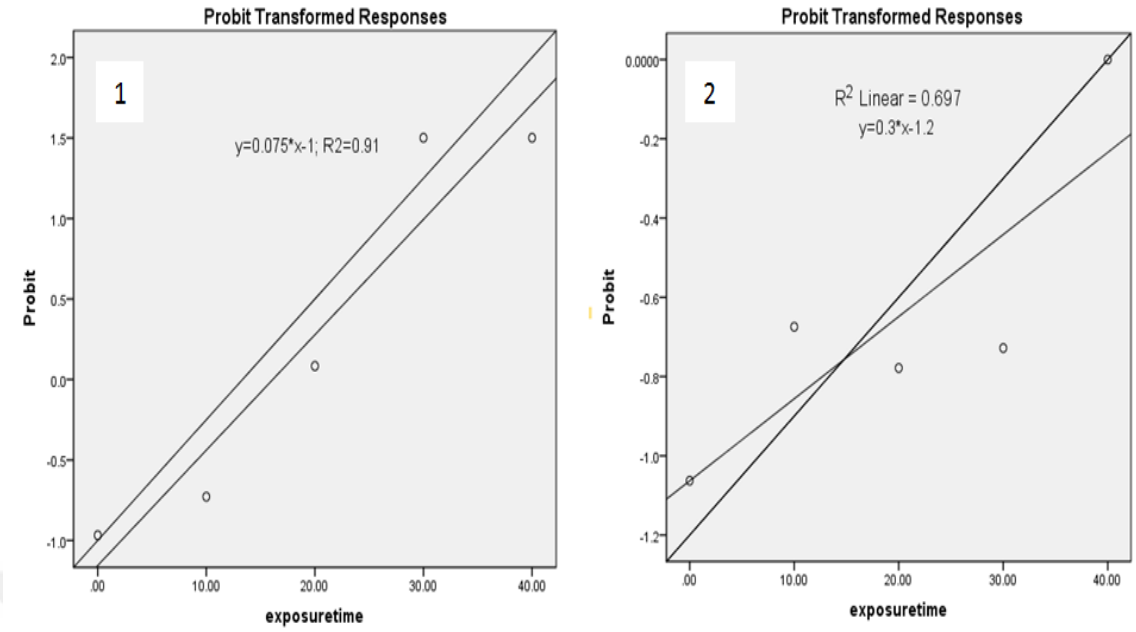
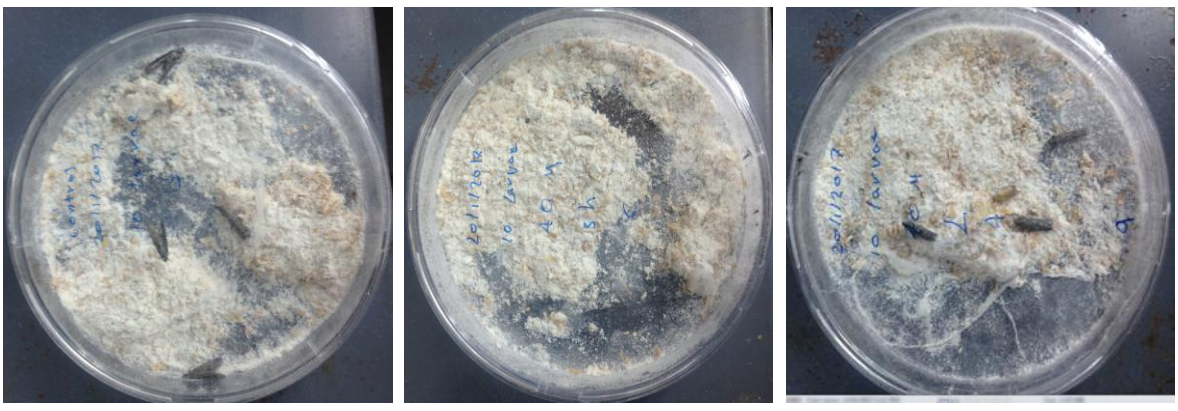


Figure 3.5 Probit transformed responses after exposed to 1. shortwave 2. longwave UV- radiation

When shortwave UV-radiation exposed to the larvae of *E. kuehniella*, both egg production and hatchability decreased with the increasing exposure periods (*F=200.981; df=3; P<0.05*) as in Table 3.11. Despite the egg production decreased for the adults developed from irradiated larvae, hatchability of these eggs was not significantly affected by longwave UV-radiation *(F=0.605; df=4; P=0.668).*



a- Control b- Shortwave (40 min.) c- Longwave (40 min.)

Figure 3.6 Adult emergence from the 30-d old *E. kuehniella* larvae exposed to short/longwave UV-radiation

Table 3.11 Egg production and hatchability of the adults from larvae after exposed to different wavelength of UV-radiation

Exposure time (min)	Shortwave		Longwave	
	Hatchability	Egg production	Hatchability	Egg production
Control (0)	92.64±1.81 a*	149.7	93.91± 2.96a	135.7
10	91.21±4.20 ab	137.4	85.7±9.03a	126.9
20	84.06±6.58 b	48.13	85.86±5.93a	94.22
30	35±00 c	42.0	85.03±9.49a	93.25
40	00	00	84.83±11.7a	91.71

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test.

When we investigate the longevity of the adults obtained from irradiated larvae, there were no significant differences with respect to adult longevity depending on different exposure periods of shortwave UV-radiation for male insects *(F=0.893; df=4; P=0.503).* The same trend was also observed for the female adults obtained from the larvae irradiated with shortwave UV radiation (*F=0.097; df=4; P=0.981).*

On the other hand, there was no significant change in the longevity of male and female adults depending on the exposure time after being irradiated with longwave UV radiation (for male: *F=0.831; df=4; P=0.535* and for female: *F=0.270; df=4; P=0.891;* Table 3.12)

Table 3.12 Longevity of adult from 30d larvae after exposed to the different exposure times of UV-radiation.

Exposure time (min)	Shortwave		Longwave	
	Male	Female	Male	Female
Control (0)	7.60 ±3.96a*	7.27 ±4.54 a	9.25 ±4.27 a	7.71 ±2.95 a
10	5.15 ±4.36 a	7.80 ±6.18 a	8.33 ±3.50 a	9.13 ±4.55 a
20	9.0 ±1.26 a	8.38 ±2.83 a	11.19 ±3.75 a	9.12 ±4.55 a
30	7.00± 00 a	7.0 ±00a	11.83 ±3.79 a	6.78 ±4.97 a
40	7.00±00 a	-	7.38±1.77 a	6.57±4.86 a

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test.

3.1.3 Effects of UV-radiation (short/longwave) on 3-day-old *E. kuehniella* pupae

When we irradiated the 3 d-old *E. kuehniella* pupae with shortwave/longwave UV-radiation at different exposure times, adult emergence periods were recorded and given

in Table 3.13. There was no significant difference among the exposure times with respect to adult emergence period for both long and shortwave UV-radiation (For shortwave: *F=0.392; df=4; P=0.810* and for longwave: *F=0.820; df=4; P=0.541).*

Table 3.13 Adult emergence periods (day) from irradiated pupae.

Exposure time (min)	**Adult emergence periods**	
	Shortwave	Longwave
Control (0)	4,61±2,73 $^{(35)}$ a*	4,61±2,73$^{(35)}$a
40	2,70±2,17 $^{(44)}$a	2,80±2,33$^{(42)}$a
80	3,05±2,25 $^{(43)}$a	3,05±0,73$^{(38)}$a
120	3,74±0,68 $^{(32)}$a	2,81±2,41$^{(40)}$a
160	3,01±2,14 $^{(35)}$a	2,57±2,56$^{(41)}$a

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test; Figures in parentheses indicate the number of pupae exposed to UV radiation.

Longevity of the adults obtained from irradiated pupae was also investigated and shown in the Table 3.14. There was no significant change in the longevity of the adults with respect to increasing exposure time for both short and longwave UV radiation (Statistics on this experiment were given in Table 3.14)

Table 3.14 Longevity of the adults from pupae after exposed to different exposure times of short/longwave UV-radiation.

Exposure time (min)	Shortwave		Longwave	
	Male	Female	Male	Female
Control (0)	5.4±2.84a*	5.42±3.11a	5.4±2.84a	5.42±3.11a
40	4.87 ± 3.49a	4.95±2.82a	6.35±3.2a	4.82±2.39a
80	4.43±3.17a	4.95±2.87a	5.17±3.49a	5.13±2.53a
120	5.82±3.87a	5.76±3.45a	5.79±3.31a	4.5±2.99a
160	4.87±2.83a	6.0±2.8a	4.13±2.23a	5.45±3.0a
Statistics	*F=0.081; df=4; P=0.986*	*F=0.0731; df=4; P=0.989*	*F=0.208; df=4; P=0.928*	*F=0.067; df=4; P=0.991*

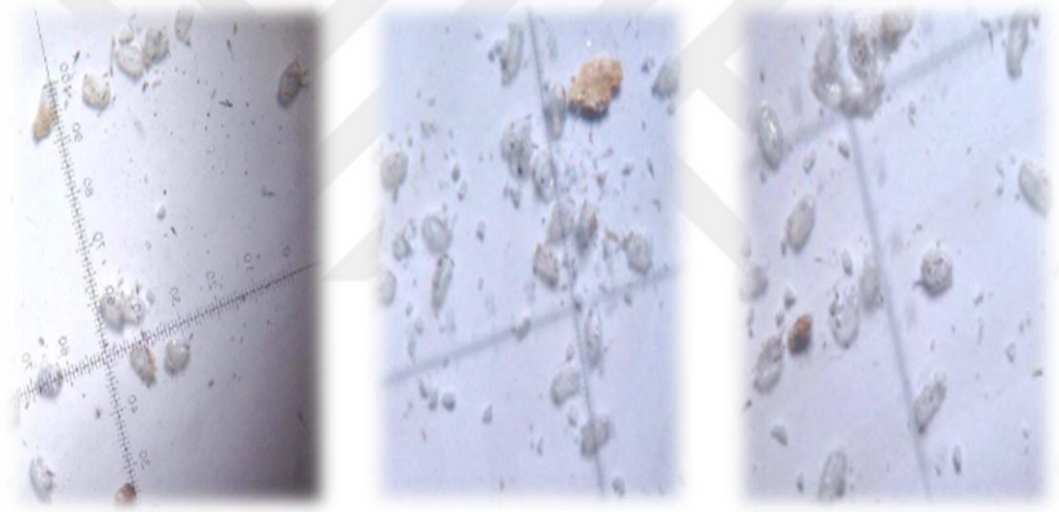
*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test.

Adults from irradiated pupae were also evaluated for egg production and hatchability of these eggs, and the findings were given in the Table 3.15. As can be seen from the table, there was no significant difference among the exposure times with respect to hatchability for both short and longwave UV radiation (Table 3.15). However, the egg production of the adults decreased when compared to control for both long and shortwave UV-radiation. (as in Figure 3.7)

Table 3.15 Egg production, hatchability and hatchability periods of the adults from pupae after exposed to different wavelength of UV-radiation.

Exposure time (min)	**Shortwave**			**Longwave**		
	Hatchability	Hatchability period	Egg Production	Hatchability	Hatchability period	Egg Production
Control	91.83±00a	4.19± 1.33a*	83.7	91.83± 00a	4.19 ±1.33a	83.7
40	91.38± 2.17 a	3.69 ± 0.79a	61.4	91.26±0.9a	3.16±0.76a	61.8
80	89.88±2.54 a	3.93 ± 1.02a	71.7	88.58±1.71a	4.08 ±1.20a	69.6
120	88.95±2.63a	3.60 ±1.08a	64.5	88.17± 0.69a	3.79±1.22a	70.0
160	89.89 ±1.15a	3.53 ± 0.74a	65.1	88.05±1.39 a	3.56 ±0.68a	61.3
Statistics	*F=1.086; df=4; P=0.414*	*F=0.191; df=4; P=0.938*		*F=0.475; df=4; P=0.754*	*F=0.430; df=4; P=0.784*	

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test.



160 min. Shortwave Control 160 min longwave

Figure 3.7 Effects of shortwave UV-radiation on the embryonic development in the eggs of the from 3-day-old *E. kuehniella* pupae

3.1.4 Effects of UV-radiation (short/longwave) on 24 h old *E. kuehniella* adults

When 1 d-old *E. kuehniella* adults irradiated with UV radiation, the longevity of the adults recorded and given in Table 3.16. As can be seen from the table, the longevity of the adults after exposed to UV-radiation was not changed significantly for both male and female and for short and longwave UV-radiation (Table 3.16). It was observed the level of egg production in females after exposure to UV-radiation decreased when compared to control *(F=9.115; df=4; p=0.002*). Furthermore, hatchability of the eggs decreased with increasing exposure time after being irradiated

with shortwave UV-radiation (Figure 3.8). On the other hand, as we increased exposure time the production of eggs in females decreased, while on the longwave of UV-radiation *(F=7.117; df=4; p=0.006)* it was observed less affected than shortwave (Table 3.17) and Probit transformed responses after exposure to longwave UV-radiation shown in Figure 3.9.

Table 3.16 Longevity of the adults (24 h old) after being exposed to different exposure periods of short/longwave UV-radiation

Exposure time (min)	Short wave		Long wave	
	Male	Female	Male	Female
Control (0)	4.57±2.51 a*	3.09±1.64 a	4.57±2.51 a	3.09±1.64 a
150	4.00±1.53 a	4.13±1.51 a	7.63±1.77 a	6.10±1.85 a
200	4.86±1.77 a	3.45±1.97 a	7.35±2.00 a	6.85±1.82 a
250	4.75±2.22 a	2.83±1.83 a	7.33±1.41 a	6.11±1.69 a
300	5.50±0.58 a	4.36±1.98 a	8.22±1.72 a	5.78±1.64 a
Statistics	*F=0.599; df=4; P=0.672)*	*F=0.418; df=4; P=0.793*	*F=1.816; df=4; P=0.202*	*F=2.467; df=4; P=0.113*

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test.

Table 3.17 Egg production and hatchability from UV-irradiated 24h adult

Exposure time (min)	Short wave		Long wave	
	Egg production per female	Hatchability of the eggs	Egg production per female	Hatchability of the eggs
Control (0)	113.66±9.20 a*	97.60± 0.92 a	113.66±9.20 a	97.60± 0.92 a
150	69.26±7.39 bc	79.30± 6.89 b	84.51±8.55 b	86.84± 7.07 a
200	78.86±15.94 bc	73.97± 4.56 bc	81.44±12.21 b	84.88± 6.82 a
250	64.84±9.56 b	70.03± 7.43 bc	112.72±7.80 a	79.29± 4.95 ab
300	88.28±11.55 c	63.84± 11.23 c	97.16±9.82 ab	78.58± 8.68 b
Statistics	*F=9.115; df=4; p=0.002*	*F=8.588; df=4; P=0.003*	*F=7.117; df=4; p=0.006*	*F=0.605; df=4; P=0.668*

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test

Table 3.18 Embryonic development periods in the eggs of 24h old adults being irradiated with short/longwave UV- radiation.

Exposure time (m)	**Embryonic development periods (day)**	
	Shortwave	Longwave
Control (0)	3.77±1.25 a*	3.79±1.23 a
150	4.85±0.76 a	3.51±0.72 a
200	4.57±0.68 a	3.50±0.76 a
250	4.91±0.67 a	3.25±0.64 a
300	4.25±0.52 a	4.30±1.04 a
	F=1.048; df=4;P=0.430	*F=0.547; df=4; P=0.705*

*Means followed by the same lower-case letter in the same column are not significantly different at the 0.05 level by ANOVA test

When 24 h old adults of *E. kuehniella* irradiated with short/longwave UV-radiation for different exposure time range between 150-300 minute, development periods of the embryos in the egg was not significantly changed for both short and longwave UV- radiation. (Table 3.18). It is understood from this that UV radiation did not change the embryonic developmental period in the female reproductive system, but it reduced the number of living larvae in the eggs depending on the increasing exposure times (Table 3.17 and 3.18).

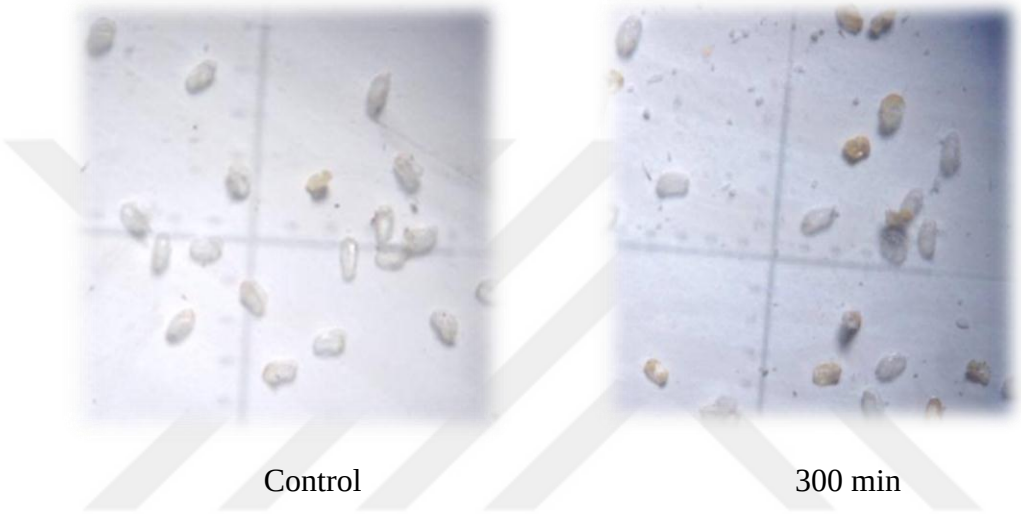


Figure 3.8 Effects of shortwave UV-radiation on the embryonic development of the eggs from 24 h old *E. kuehniella* adults.

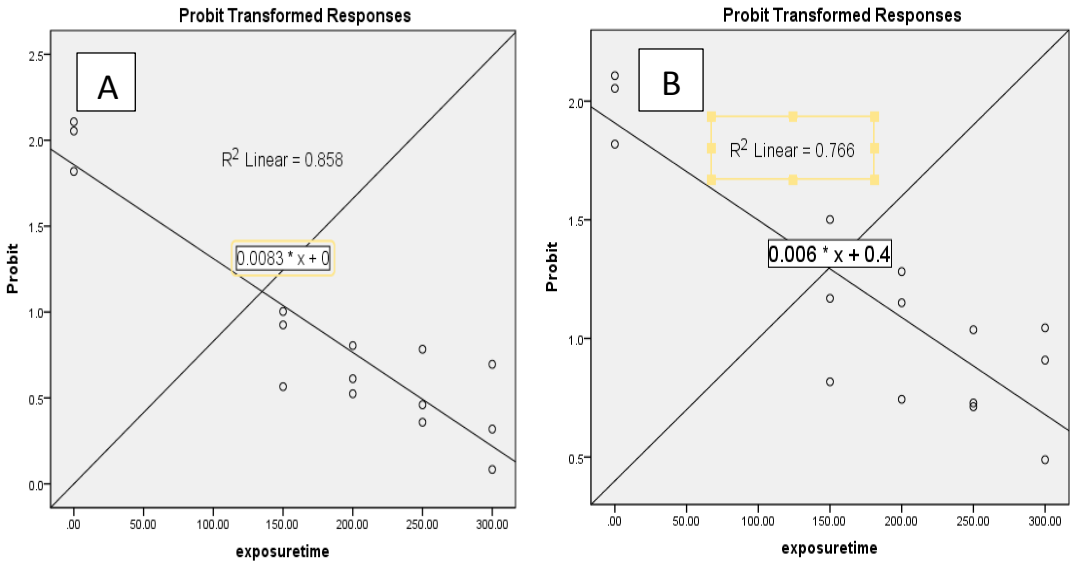


Figure 3.9 Probit transformed responses after exposure to longwave UV-radiation.
A- shortwave B- Longwave

CHAPTER 4

4. DISCUSSION

In this study, different age groups of *E. kuehniella* eggs (0-72 h) were exposed to shortwave UV-radiation for six different exposure time (50-600 s), mortality rates of the eggs increased with increasing exposure time and embryological development was negatively affected. Decreased egg hatch or larval emergence from irradiated *E. kuehniella* and *E. cautella* eggs was also reported by Hodges et al (1984). The dose dependent decrease in egg hatch was also reported in another study [17, 46].

In the present study, 0-2 d old eggs were more resistant against the UV-radiation and least lethal times were calculated for this groups of eggs. Similarly, Modarres et al. (2014) reported that older eggs were more sensitive than younger ones against UV-radiation [48], because the DNA strands are not complete yet. When they irradiated different age groups of cowpea weevil eggs, they found the percentage of hatched eggs in control treatment as 95 %, while in 1, 2 and 3-day-old eggs were 7.5, 1.67 and 0.83%, respectively. Similarly, in our study, while 88 % of the control eggs were hatched the egg hatch for 2, 24, 48 and 72 h old eggs were found as 30.04, 0.00, 12.95 and 8.13% respectively with 400s exposure. Among the eggs of different ages, the most sensitive to UV-radiation were the eggs at 24 h of age. For example, in the 400s treatment, larval emergence was inhibited by 69% from the eggs of 0-2 hour-old eggs, while emergence from 24-hour-old eggs was completely blocked and 100% mortality rate was obtained from the experiment with these eggs. When the results of the probit analysis were examined, it was found that 861.37s was required to obtain complete mortality for 0-2 h old eggs, however, 476.61s was enough for 24 h to kill eggs.

Seidel et al (1940) was indicated that the damage caused by the UV in the peripheral parts of the egg did not reach the activation center of the embryo because the embryo development was not yet completed in the young eggs [57]. They also described that when the embryonic development continued, embryonic regions began to become more specialized and the tissues growing toward the outside were more affected by the radiation [6, 57]. In another study, it was stated that the effect of UVC radiation was limited to the surface and decreased in depth of the tissue [55]. As is known, superficial segmentation is seen in insect eggs. The amount of vitellus in insect eggs covers most of the eggs. Therefore, the cytoplasm is pushed towards the peripheral part of the egg. In the newly produced egg, the zygote nucleus, known as the energid, clones itself in the beginning and splits 12 to 13 division cycles to yield about 5000 daughter nuclei [20]. These nuclei then migrate toward the periphery and from blastoderm. Therefore, they may be less affected by UV radiation as the energid are deeply involved in newly laid eggs. However, as blastoderm formation may have occurred in 24 h of age, the cell layer developing on the surface may be more affected by radiation.

When the eggs of *E. kuehniella* irradiated with different exposure times of the UV radiation, increased exposure time caused increased embryonic death for all applications. Similar findings were reported by Guerra, (1968) and Ghanem & Shamma (2007). When they examine *Trogoderma granarium* eggs structure microscopically, they have determined that egg fluids leaked out of the egg. It is understood that the UV-radiation breaks the structure of the egg and causes the decrease in chorion thickness [54, 55].

Most of the studies investigating the effects of radiation on insects showed that the most sensitive stage was eggs (Tilton & Brower, 1983; Zhao et al., 2007; Vadivambal et al., 2010; Modarres et al., 2014). It is understood that UV-radiation can be used safely and effectively for the control of stored products and that the pests in the egg stages can be controlled in a shorter exposure time. Hatchability of the eggs was decreased when eggs of different ages were exposed to UV irradiation. The results showed that older eggs of *E. kuehniella* were more sensitive to shortwave of UV-radiation than younger eggs [48, 58, 59, 60].

Mortality rates in the 30 days old *E. kuehniella* larvae significantly increased with increasing exposure time. In some study, as Güven et al (2015) reported that the UV-radiation of 254 and 365 nm wavelength increased mortality rates of 1-2 days old *E. kuehniella* larvae depending on the exposure times ranging from 15 to 60 minutes [56], and their findings support our results.

When the 30-d old *E. kuehniella* larvae were irradiated with UV light at different doses and adult emergence decreased with increasing exposure time. Similar results have been reported for *P. interpunctella*, *A. diaperinus* and *T. Casteneum* (Faruki, 2005; Faruki et al, 2007; and Beard (1972) [6, 7, 62]. At present study, 93.33% of larvae were killed with shortwave UV radiation. Boadi (2014) showed that none of the *Anopheles gambiae* (Diptera: Culicidae) larvae reached to adulthood when they were exposed to UVC radiation for 90 and 120 minutes [61].

We found that 30 days old *E. kuehniella* larvae reacted differently to long and short wavelength of UV-radiation when exposed to different periods of UV radiation. For example, in experiments with long-wave UV-radiation, there was no significant change in the egg production and larval emergence from these eggs. However, experiment with the shortwave UV-radiation showed a significant reduction in both egg production and larval emergence. Güven et al (2015) express an increase in the antioxidant enzyme activity and the formation of Malondialdehyde (MDA) after short wave UV-radiation, also indicated that the DNA damage both directly and indirectly with oxidative stress [56].

When studies related to the effects of UV-radiation on insects are examined, it is stated that UV-radiation affects different insect groups and different stages of the same insect at different levels [62, 63]. For example, in *Trogoderma granarium* and *Anopheles gambiae* it has been resulted premature adult emergence from irradiation pupae with UV-radiation. However, in our study, the duration of adult emergence from irradiated pupae did not show any significant change compared to the control for all exposure periods.

It was observed that the pupae were more resistant to UV-radiation when compared to larvae. Larvae of the stored product insects are usually untanned since they live in dark storage conditions. The melanism is expressed globally, and only in the pupal stage of the insects. After pupation, melanin begins to accumulate in the sheath of the pupae [55]. This is possibly one reason why the pupae were more resistant than larvae against UV-radiation, because increased melanin cause an increase in the tolerance of the body against UV radiation. This may be a reason why adults are also resistant to UV radiation.

On 24 h old *E. kuehniella* adults both of short and longwave UV-radiation was no significant effects in the longevity of the adults for both male and female. So, more exposures times of UV-radiation is needed to kill *E. kuehniella* in the adult stage.

When Poushand et al (2017) exposed *T. vaporariorum* adults to the UVC- radiation during different exposure times, they showed there shortwave UV-radiation have high insecticidal activity on these adults. However, in our study, we found that increasing exposure periods did not affect the longevity of the adults. On other hands egg production and hatchability of the eggs from these adults significantly decreased with increasing exposure periods for short and longwave UV-radiation.

As a result, the findings from these studies show that using UV-radiation can be used effectively in controlling an important stored product pest, *E. kuehniella* at any stage of the life cycle. However, the damage caused by this pest can be reduced. Moreover, this method would be a good alternative to harmful chemicals, which poses enormous risks for environmental and human health. In addition, *Ephestia kuehniella* is a factitious host of the biological control agents such as *Trichogramma* and is of great importance in the mass production of egg parasitoids. In this regard, when UV-radiation is applied for longer than 300 minutes, many sterile eggs can be obtained from irradiated adults suggesting that these eggs can be used effectively in the production of egg parasitoids. This study can also be used in the molecular biology by identifying the type of damage to the DNA in the exposed eggs.

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