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Orijinal araştırma (Original article)

Assessment of the influence of magnetic fields on aspects of the biology of the adult Mediterranean flour moth *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae)

Akdeniz un güvesi *Ephestia kuehniella* Zeller 1879 (Lepidoptera: Pyralidae)'nın ergin biyolojisi üzerine manyetik alanın etkisinin belirlenmesi

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Summary

All living systems are under the influence of magnetic fields (MFs) from various sources. In this study, experiments were performed to investigate the effect of MFs from a DC power supply on the longevity of adults, fecundity and daily egg laying pattern of female *E. kuehniella.* Adults of *E. kuehniella* (24 h old) were exposed to power supply generating MFs of different levels (0, 1.5, 3, 5, 7.5 and 10 mT) at 50 Hz for 24 h exposure time. Mortality increased with increasing MFs level and complete mortality was achieved at the level of 10 mT. Adult viability was highest in control (92%). Pest longevity was significantly reduced with increasing level of MFs ($P<0.05$). There was no significant difference in the longevity of males and females ($P>0.05$). Exposing adults to increasing level of MFs significantly influenced daily egg laying patterns and fecundity of magnetized females. Larval emergence from these eggs was completely prevented at highest level of MFs. The LD_{50} and LD_{99} values of MFs against adult stages of *E. kuehniella* were measured as 5.23 mT and 12.81 mT, respectively. According to these results, 10 mT MFs for 24 h is sufficient to control adult survival and production of progeny in this pest.

Key words: Magnetic fields, *Ephestia kuehniella*, progeny, mortality, longevity

Özet

Tüm canlı sistemler çeşitli kaynaklardan gelen manyetik alanın etkisi altındadır. Bu çalışmada deneyler ergin ömür uzunluğu, yumurta açılımı ve *E. kuehniella* dişilerinin günlük yumurta verimi üzerine güç kaynağından gelen DC manyetik alanın etkisini araştırmak için yapılmıştır. *E. kuehniella* erginleri (24 saatlik) farklı seviyelerde (0, 1.5, 3, 5, 7.5 ve 10 mT) 50 Hz'de 24 saat süreyle manyetik alan üreten bir güç kaynağına maruz bırakılmıştır. Artan manyetik alanda ölüm oranları giderek artmış ve 10 mT'da erginlerin tamamı ölmüştür. Canlı ergin oranı % 92 ile en yüksek kontrolde elde edilmiştir. Zararlının ömür uzunluğu artan manyetik alanla önemli ölçüde azalmıştır ($P<0.05$). Erkek ve dişi bireyler arasında ömür uzunluğu açısından fark bulunmamaktadır ($P>0.05$). Artan manyetik alana maruz kalan dişilerin günlük yumurta verimi ve bu yumurtaların açılımları önemli ölçüde etkilenmiştir. Bu yumurtalardan larva çıkışı en yüksek dozda tamamen engellenmiştir. *E. kuehniella* erginlerine karşı manyetik alanın LD_{50} ve LD_{99} değerleri sırasıyla 5.23 mT ve 12.81 mT olarak bulunmuştur. Bu sonuçlara gore, 24 saat süreyle 10 mT manyetik alan uygulaması bu zararlının canlılığını ve yumurta verimini kontrol etmek için yeterlidir.

Anahtar sözcükler: Manyetik alan**,** *Ephestia kuehniella*, yumurta verimi, ölüm oranı, ömür uzunluğu

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Introduction

The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is a pest found in flour mills around the world. Its larvae cause quantitative and qualitative economic damage to stored products. Many pyralid moths were reported to have non-feeding adults (Cook & Gage, 1995). Research has been conducted on alternative methods to traditional chemical applications for controlling development stages of this serious pest. Among these alternative methods, the radiation technique provides environmentally safety control of pests. The advantages of irradiation of pests over using chemical insecticides are: 1) radiation provides direct control of stored product pests, 2) pest insects don't develop resistance, 3) radiation doesn't leave any residues in the treated foods and 4) radiation causes few substantial changes in the physicochemical characteristics of treated foods (Abbas et al., 2011; Azizoglu et al., 2011).

Different radiation types such as gamma radiation, ultra violet light (UV) radiation and microwave were used against different insects by many researchers (Ayvaz & Tuncbilek, 2006, Faruki et al., 2007 Ayvaz et al., 2008; Tuncbilek et al., 2009; Mansour, 2010; Azizoglu et al., 2011). Irradiation was used to keep under control stored product pests and for sterile insect release. Generally, forms of ionizing radiation, such as gamma radiation and X-rays are used to prevent the infection of stored products by insects (Sumer et al., 2007). UV radiation, which is a non-ionizing radiation form, is effectively used as an insect attractant and for surface disinfection of insect eggs (Azizoglu et al., 2011). At the same time, UV or gamma radiation can be used to kill the host egg embryos to prevent larval hatching when an egg parasitoid is reared on host eggs (Tuncbilek et al., 2009). Other than these radiation sources, magnetic fields (MFs) has attracted the attention of researchers due to their biological effects. Most of the studies about MFs' effects have focused on vertebrates and relatively fewer studies have been done on insects and their stored-product environment (Starick et al., 2005).

For detecting the biological effects of MFs, applications should be done under the same environmental conditions, with and without the same MF level. Test material used in the study must be of sufficient number and uniform quality. The most commonly used materials in MFs studies are rats, rabbits, monkeys and human beings. But it is relatively difficult to control the quality and the physical condition of these materials. At the same time, their large body volumes have caused one by one usage of the specimens in the studies. So, insect eggs may be a good alternative due to their small volume (Pan, 1996).

MFs have been shown to affect the orientation (Jones & Macfadden, 1982), oviposition and development (Ramirez et al., 1983), fecundity and behaviour (Starick et al., 2005) of a wide variety of insects. Kirschvink et al. (1997) demonstrated an ability to detect alternating (a.c.) fields of 2.2 mT peak amplitude from d.c. at frequencies up to 60 Hz by the honeybee. Nenadovic et al. (2005) investigated the effects of constant temperature and an extremely low-frequency MF (ELF-MF, 50 Hz and average induction of 20 mT) on the activity of the medial protocerebral neurosecretory neurons (A1 and A2) and *corpora allata* of *Cerambyx cerdo* L. (Coleoptera: Cerambycidae) larvae with 30 days of exposure.

A literature search showed a lack of information on the effects of exposure to MFs on stored-product insects. We therefore observed the biological effects of different levels of MF on longevity, fecundity and daily egg laying pattern, and determined the LD_{50} and LD_{99} values for adults of the Mediterranean flour moth, *E. kuehniella*.

Materials and Methods

Rearing of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

The Mediterranean flour moth, *Ephestia kuehniella*, culture was obtained from the Biological Control Research Station in Adana, Turkey.. *E. kuehniella* larvae were reared on a mixture of 1 kg wheat flour, 5% yeast, and 30 g wheat germs (Tuncbilek et al., 2009). Throughout the experiments, cultures were maintained at constant temperature (27 ± 1 ºC) relative humidity (70 ± 5%) and under a light regime

of 14 h light followed by 10 h darkness. After a period of around 2 months, emerged adults were collected from stock cultures and placed in plastic jars with screen bottoms. Eggs that fell through the screen were collected the following days and placed in petri dishes. The eggs were collected daily and were used for new cultures.

Magnetic Field System and Exposure

As it can be seen from Figure 1, MFs were obtained with a DC current supply. The strength of the MFs was stable on the specimens during the experiments. MFs of the coil center were measured by Gauss meter and plated as distance against MFs in mili Tesla (mT) (Figure 1). During the experiments, temperature and stabilization of MFs were controlled carefully. The measured MFs at the center of the coil were 0, 1.5, 3, 5, 7.5 and 10 mT. Each group of adults was put into a 1.5 cm diameter and 1.5 cm high plastic box that was tightly covered by its lid. Adults were put at the coil center. Each replicate consisted of 10 adults of *E. kuehniella* and for all levels six replicates were used.

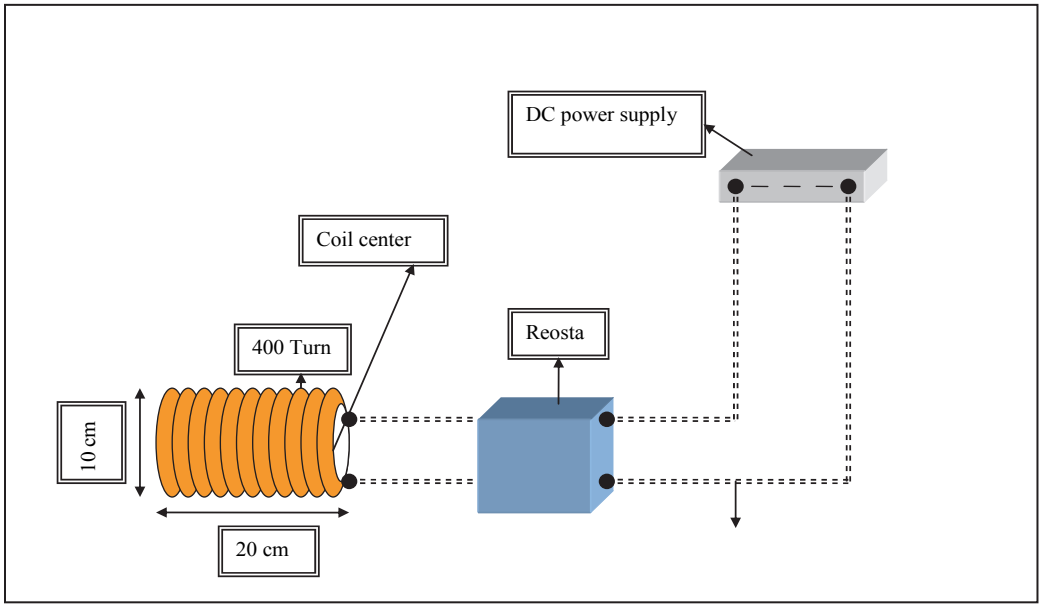

Figure 1. Schematic diagram of DC magnetic field apparatus.

The MF was applied continuously for up to 10 mT. Then specimens were taken from the field. During the 24 h of the experiment and after treatment, the temperature difference between control-and-field-exposed cultures never exceeded 0.2 ˚C and was maintained at 27 ± 1 ºC and 70 ± 5% r.h. Immediately after treatment, the adults of *E. kuehniella* were transferred to plastic jars inverted over petri dishes, their eggs were collected for a day. Collected eggs were transferred to petri plates and rearing medium was placed in the petri dish around the central well to provide food for any hatched larvae and the apparatus was placed at 27 ± 1 ˚C. The petri dishes were examined under a binocular microscope, and the number of hatched eggs was counted daily until no further egg hatch was observed. The rearing medium containing the small larvae was then transferred to 300 mL glass jars containing ca 150 g of food. The mean number of eggs that hatched per batch was computed and expressed as the hatching percentage per female for each treatment.

The total number of eggs laid per female was counted daily until each female died. Male and females were kept in separate cages to observe their longevity and the period was recorded. An unmagnetized control population was started at the same time. This methodology was adapted from Ayvaz et al., (2008) with some modifications.

Data analysis

Differences among experimental groups within the 10 day observation time period of the study were analyzed with the software program SPSS 10.0 for Windows.The significance was calculated using one-way analysis of variance (ANOVA),followed by Tukey multiple comparison procedure to calculate the significance. A $P < 0.05$ value was taken as statistically significant. Percentage mortality values for different exposure times were subjected to analysis of variance (one-way ANOVA) using the same statistical program (SPSS 2001) for probit analysis which was used to estimate the LD_{50} and LD_{99} values (Abbott, 1925). Data were transformed using arcsine $\sqrt{x}$ transformation to meet normality, which is recommended for analysis of variance (ANOVA).

Results

Fecundity and daily egg laying patterns

The fecundity and daily egg laying pattern of *E. kuehniella* females under different levels of MF treatment are shown in Figures 2 and 3.

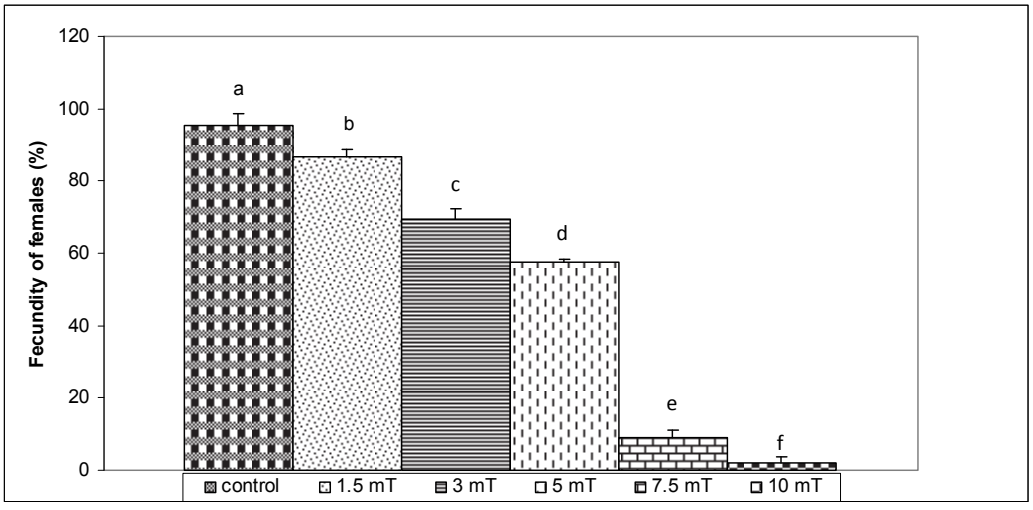

Figure 2. Percentage fecundity of females of *Ephestia kuehniella* exposed to different levels of magnetic field. Bars with different letters above them, for a difference between levels, are significantly different (Tukey's test at P< 0.05).

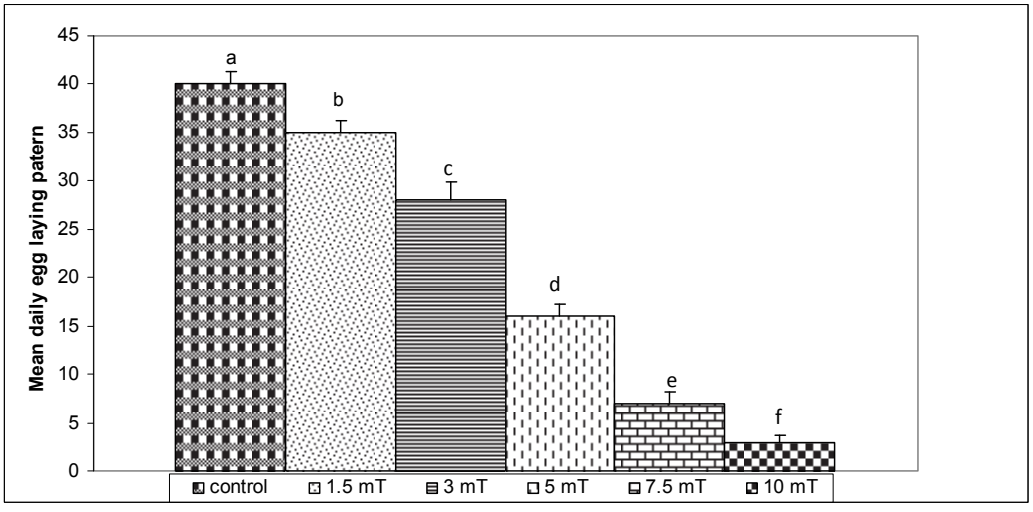

Figure 3. Mean daily egg laying pattern of *Ephestia kuehniella* females exposed to different levels of magnetic field. Bars with different letters above them, for a difference between levels, are significantly different (Tukey's test at P< 0.05).

The fecundity (percentage hatching of eggs laid) from these eggs fell to zero at the highest MF level (Figure 2) and differences between levels were statistically significant (F= 122.44 DF= 5, P < 0.05). A total of 86.66% of eggs hatched at 1.5 mT, while only 2% of eggs hatched at 10 mT.

Mean daily egg production of treated adults with MFs decreased significantly with increasing levels of MF (F= 104.8 DF= 5, P < 0.05). Daily egg production of treated adults was 3.01 ± 0.87 at the highest level (10 mT) (Figure 3).

Adult mortality

The percentages of adult mortality of *E. kuehniella* after using different levels (0, 1.5, 3, 5, 7.5 and 10 mT) at 50 Hz for 24 h are shown in Figure 4. The differences among MFs levels on adult mortality rates were statistically significant. The percentages of adult mortality of the pest were higher when the levels of MFs increased (*E. kuehniella* adult stage; F= 2580 DF= 5, P < 0.05). A total of 15% and 99% of adults died at 1.5 mT and 10 mT, respectively. According to the probit analysis, LD_{50} and LD_{99} of the MFs were at 5.23 and 12.81 mT for the adult stages of *E. kuehniella*.

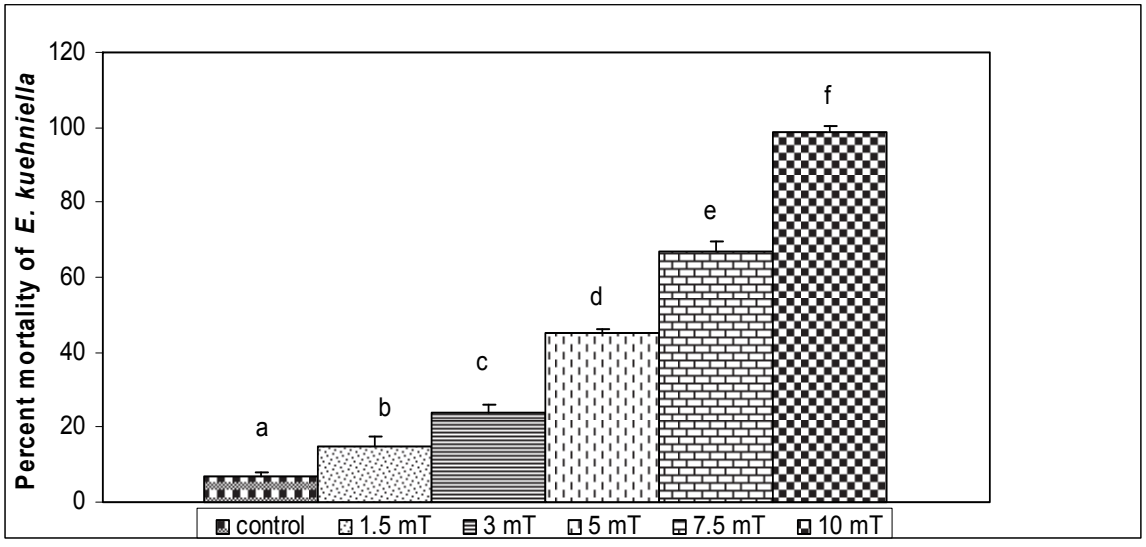

Figure 4. Percentage mortality of adult stages of *Ephestia kuehniella* exposed to different levels of magnetic field. Bars with different letters above them, for a difference between levels, are significantly different (Tukey'stest at P< 0.05).

Longevity studies

Mean longevity (days) of magnetized adults of *E. kuehniella* are shown in Figure 5. There was a dose-dependent decrease in the longevity of *E. kuehniella* adults after magnetization and differences between levels were statistically significant (F= 1.065 DF= 5, P < 0.05). There were no significant differences (P > 0.05) between the mean longevity (days) of adult males and females of *E. kuehniella* treated with increasing level of MFs. The mean longevity of *E. kuehniella* adults treated with 1.5 mT and 10 mT MFs were 5.99 ± 0.23 and 0.08 ± 0.17 days, respectively.

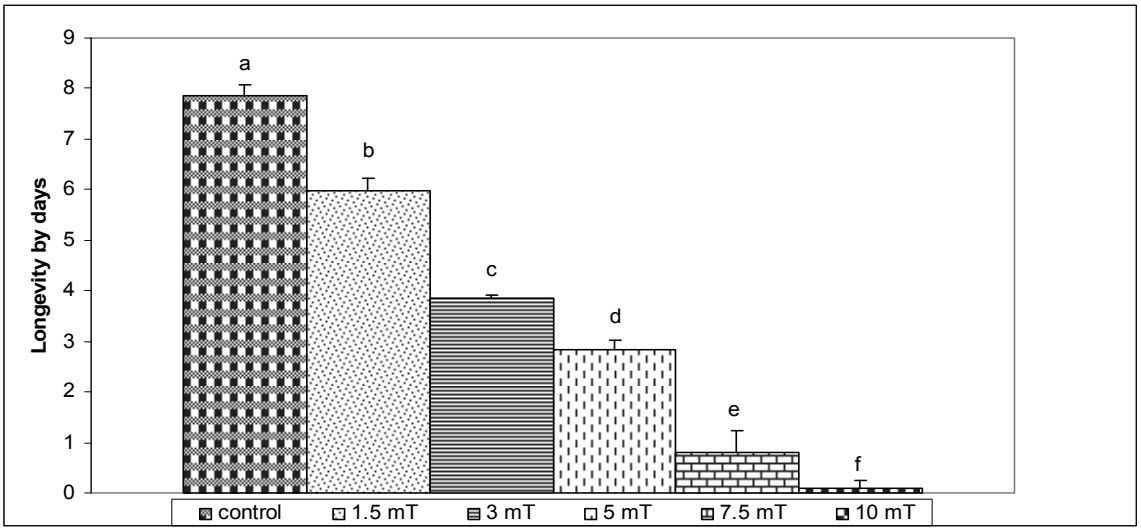

Figure 5. Mean longevity (days) of adults of *Ephestia kuehniella* exposed to different levels of magnetic field. Bars with different letters above them, for a difference between levels, are significantly different (Tukey's test at P< 0.05).

Discussion

There are increasing restrictions on the use of pesticides and on the number of chemical compounds officially registered for pest control in durable food products. Moreover, the use of methyl bromide for the fumigation of food commodities and facilities has been phased out in accordance with the Montreal Protocol due to its effect on the ozone layer (UNEP, 2006). Owing to the increasing restrictions on the methods available for the control of pests in stored products, alternative methods require investigation. One such method, the use of gamma radiation for the sterilization of pests, has proved to be a technically feasible alternative to conventional methods for controlling stored-product insects (Cornwell 1966; Watters 1968; Ayvaz et al. 2008). The development of alternative treatments for pest control is an increasing demand from the food industry. Alternatives should meet consumer demands for the reduced use or elimination of pesticides, while at the same time maintaining a high degree of control efficacy (Riudavets et al., 2010). The present study established the efficacy of using static magnetic fields (SMF) for 24 h to control stored product pests. The advantages of magnetization as a pest control method is that it does not leave undesirable residues and cause resistance development in *E. kuehniella*

Many researchers have studied the biological effects of extremely low frequency electromagnetic fields (EMF) on different organisms; however, the effects of static magnetic fields (SMF) on different organisms are less studied, significant but inconsistent (Ghodbanea et al., 2011). Although extremely low frequency (ELF, < 300 Hz) MFs exert a variety of biological effects, the MFs sensing/transduction mechanism (or mechanisms) are yet to be identified (Prato et al., 1995). Progeny production was increased when *Drosophila melanogaster* was reared in an electrical field (Levengood & Shinkle, 1960). On the other hand, eggs of *Drosophila* treated with extremely low frequency EMF and SMF showed higher mortality than the control for all tested levels (Ramirez et al., 1983). Different levels and types of MFs have been used against different insects. Ramirez et al. (1983) found that 1 mT reduced the oviposition rate and increased the immature mortality rate. SMF was classified as weak (<1 mT), moderate (1 mT to 1 T), strong (1-5 T) and ultrastrong (>5 T) types (Luciana & Luigi, 2005). We used a moderate intensity SMF (0, 1.5, 3, 5, 7.5 and 10 mT) to detect its biological effects on *E. kuehniella* adults. The longevity of adults, fecundity and daily egg production and LD_{50} and LD_{99} values of *E.*

kuehniella for different level MFs were measured. All life stages of *E. kuehniella* were relatively susceptible to increasing MF level.

Numerous earlier experiments showed that static or extremely low frequency MFs with small flux density had an effect on various living organisms (Pal, 2005). Investigations of radio-frequency (RF) and microwave energy action on insects and microorganisms have been carried out for the last 50 years in different countries (Borodin et al., 1993; Mischenko et al., 2000; Ponomaryova et al., 2010).The influence of radio-frequency electric fields of high strength on insect mortality was also reported (Ponomaryova et al., 2010). We investigated the effects of MFs on survival of a serious stored-product insect, *E. kuehniella* and at the highest level of MF (10 mT) complete mortality was obtained.

There is only one study about effects of MFs on a stored-product insect, *Tenebrio molitor* L. (Starick et al., 2005). Pan (1996) reported the biological effects of a 7 T MFs on egg hatching. The hatching of the eggs in the 7 T field was delayed and hatching rate was reduced. In our study, after treatment with MFs, *E. kuehniella* egg hatchability fell with increasing MFs strength. At the same time, exposing *E. kuehniella* adults to increasing levels of MFs influenced their daily egg production; there was a significant reduction in progeny production, indicating an adverse effect on the multiplication potential of the survivors.

The effect of hexaflumuron on the number of eggs laid, egg hatching and adult longevity through treatments of adults of *E. kuehniella* and *Spodoptera exigua* has been evaluated by Marco & Vinuela, 1994.. Longevity was also significantly reduced in both lepidopterous pests (Marco & Vinuela, 1994). *E. kuehniella* adults were stored at 10°C for 1–10 weeks. Reproductive ability and number of living adults decreased, depending on the length of the storage period. Long-term exposure to cold lengthened the life of the adults and 50% mortality was reached after 6 weeks (Ayvaz & Karaborklu, 2008). The effects of gamma radiation on the fecundity, fertility, longevity, development and level of inherited sterility in the Mediterranean flour moth *E. kuehniella* were determined. Laboratory studies revealed that radiation did not affect the longevity of irradiated male and female adults (Ayvaz et al., 2007). In this study, when adults of *E. kuehniella were* magnetized, the longevity of the adults decreased at increasing levels of MFs. At LD_{50} doses,magnetization provides an opportunity for developmental of the survivors in this pest. But LD_{99} level of MFs for 24 h is sufficient to prevent adult survival and progeny of this pest, thus achieving complete mortality.

The current study was on the effects of MFs on *E. kuehniella* adults, egg production, egg hatching and longevity. The highest level of MF caused 99% mortality in the adult stage of *E. kuehniella* in the study. Ayvaz et al (2007) recently developed a sterile insect tecnique (SIT) for the control of this pest. Therefore, our study results may assist in the development of an alternative control method for *E. kuehniella* and suggests possible effects of MFs on other stored-product insects. For instance, in an integrated control program, the sequential application of a natural enemy and MFs may be used to suppress the population density of the pest.

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