

Research Article

Integrated effect of gamma radiation and temperature on *Plodia interpunctella* (Lepidoptera: Pyralidae)

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Abstract: The Indian meal moth, *Plodia interpunctella* (Hubner), is one of the main economic pests of stored products, including dried figs, in Iran and the world. In this research, the effect of different doses of gamma radiation (50-1000 Gy) and temperature (20-35 ± 1 °C) was investigated on the life stages of *P. interpunctella*. The results showed that *P. interpunctella* eggs and adults exposed to gamma radiation (50 Gy) died after a short time. Therefore these stages are so sensitive to gamma radiation and were not used for further investigation on a combination of gamma radiation and temperature. In all temperatures, the survival period of larvae significantly decreased with increased radiation doses, especially at 600, 800, and 1000 Gy. Similarly, at 33 and 35 °C, low gamma radiation doses (50, 100, and 200 Gy) caused early larval mortality. In all temperatures, doses of 800 and 1000 Gy of gamma radiation resulted in 100% mortality of pupae; thereby, adult emergence declined to zero. However, in lower radiation doses of 50, 100, and 200 Gy and at 35 °C, the pupal mortality increased to 70, 70, and 83.33%, respectively. Results are promising for the integrated management of *P. interpunctella* on dried figs in storage.

Keywords: Indian meal moth, irradiation, temperature, IPM

Introduction

Fig is a valuable and nutritious food source rich in fiber and minerals such as magnesium, potassium, calcium, and phosphorus (Wendeln and Runkle, 2000). Due to difficulties in storage, the commercial importance of dry and tinned figs has increased. The advantage of dried figs is the ability to stock the fruit year-round (Farahnaky *et al.*, 2009; Vallejo *et al.*, 2012). Dried figs are one of the most popular commercial dried fruits in the world (Benalia *et*

al., 2016). The primary three producers of figs in the world are Turkey, USA, and Iran. Iran, with an annual production of more than 104,000 tons of dried figs, is one of the top five fig-producing countries in the world (Ahmadi *et al.*, 2019). In developing countries, the average losses of pests on target stored productions have been estimated to be up to 80% due to a lack of proper equipment and unsuitable warehouse conditions (Lee *et al.*, 2001; Bagheri-Zenouz, 2014).

The Indian meal moth, *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae), is a

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significant economic insect pest of stored products and is found on every continent except Antarctica (Rees, 2004). The eggs of *P. interpunctella* hatch in 7 days at 20 °C and 3-4 days at 30 °C. After hatching, the larvae can complete their development in 6 - 8 weeks at 18 to 35 °C. The duration of the pupal stage is about 15-20 days at 20 °C and 7-8 days at 30 °C. *P. interpunctella* has been reported as a pest on stored products such as cereals, nuts, and dried fruits in warehouse conditions (Na and Ryoo, 2000; Campbell *et al.*, 2002), especially dried figs (Bagheri-Zenouz, 2014). This pest is an external feeder; the larvae spin a silky texture inside and on top of the food surface, feeding within the tissue. The webbing contains larval droppings and molten debris, giving the infested commodity an unpleasant odor. The contaminated product is sometimes covered on the surface with a thick mat of silk mesh. Infestations of *P. interpunctella* can directly reduce the product's quantity and indirectly increase economic costs through pest control costs, quality losses, and consumer complaints (Phillips *et al.*, 2000). Therefore, using appropriate methods to manage this pest seems essential.

Methyl bromide and Phosphine fumigation have been found as effective methods for eliminating insect pests in stored commodities because of their fast and good dispersion in the product mass (Lee *et al.*, 2001). Methyl bromide is being phased out because of the high environmental risk due to its Ozone-depleting capabilities and disadvantages for human health (Fields and White, 2002). Also, phosphine resistance among the insect population was reported in-store (Collins *et al.*, 2005). Therefore, alternative and adequate control methods are suggested to manage stored pests (Fields, 1998). Botanical pesticides (Navarro *et al.*, 2001; Ahmadi *et al.*, 2008), biological control (Bagheri-Zenouz, 2014), gamma radiation in combination with the medicinal plant (Ahmadi *et al.*, 2013), gamma irradiation, and environmental manipulation (Asgarian Dehkordi *et al.*, 2018), cold and heat as physical methods (Fields, 1992), ionization (Lepine,

1991) and diatomaceous (Korunic and Ormisher, 1996) are effective methods to control of stored pests.

High and low temperatures are used for disinfestations of dried fruits and nuts, perishable commodities (fruits) (Beckett *et al.*, 2007; Phillips *et al.*, 2012), and grains (Beckett *et al.*, 2007) against stored pests. The body temperature of insects is influenced by environment temperature, thus, fluctuating in environment temperature can result in changes in insect physiology, and finally, they will die (Overgaard and Sorenson, 2008; Karl *et al.*, 2011).

Radiation has been used to control insects in storage. Irradiation, whether by isotopes or machine sources (e-beam or X-ray), has the same mode of action: the gamma rays, X-rays, or electrons knock electrons out of their orbits, creating ions and radicals. The free electrons collide with further electrons resulting in an electron shower. The ions and radicals cause further damage to large organic molecules such as DNA, stopping the development of irradiated organisms. Indeed, the secondary damage caused by ions and radicals produced by the electron shower may cause more damage to organic molecules than the primary radiation itself. In organisms, radiation most easily affects sites of ongoing cell division, which in the adult insect include the gonads and midgut. At minimal doses that stop the functions of these organs, insects will not reproduce and will cease feeding because the midgut cannot process food (Hallman, 2013). The low cost, readily available resources, mobile sources, high resolution, and high sensitivity and specificity are advantages of gamma radiation; therefore, it is more common than other radiations for use in storage (Eustice and Bruhn, 2006).

There are no studies on the combination of temperature and gamma radiation to control *P. interpunctella* on important dried fruits such as dried figs in Iran. Therefore, the main objectives of this research are to determine the best temperature and gamma dosage to control of different life stages of *P. interpunctella* on dried figs in warehouse conditions.

Materials and Methods

Insect rearing

A culture of *P. interpunctella* originated from dried dates in the Department of Entomology, College of Agriculture, Tehran University. They were reared at 27 ± 1 °C, $65 \pm 5\%$ RH, and a photoperiod of 16L: 8D h. The larvae were maintained in cylindrical plastic containers (20×12 cm) on yeast and dried figs, providing them with excess food throughout their development. The top of the container was covered with micro mesh for ventilation.

Irradiation

Different life stages (eggs, larvae, pupae, and adults) of *P. interpunctella* were placed in glass Petri dishes and exposed to 60-Co gamma source (PX-30) at a dose rate of 0.4 Gy/s at Nuclear Agriculture Research Institute, Karaj, Iran.

Combined effect of gamma radiation and temperature on eggs

Several pairs (male and female) of newly emerged *P. interpunctella* were kept in an incubator at 20 ± 1 °C. After mating and ovipositing, eggs were collected and used for irradiation. Ten eggs (less than 24 h old) were placed in glass Petri dishes and irradiated in a gamma cell. Radiation was applied to the eggs at three doses (50, 100, and 200 Gy). After irradiation, eggs were returned immediately to 20 ± 1 °C, $65 \pm 5\%$ RH, and photoperiod of 16 L: 8 D h) and then placed in Petri dishes with a central well made by ringing a central area (2 cm diameter) with glue. After four days, the Petri dishes were examined under a binocular, and the number of hatched eggs was counted daily until no further hatched egg was observed. Three replicate batches of eggs were used for each dose level. This experiment was also done at 23, 26, 28, 30, 33, and 35 ± 1 °C.

Combined effect of gamma radiation and temperature on larvae

One-three days old larvae were selected for experiments. These larvae were reared in an incubator at 20 ± 1 °C. Ten larvae were placed in glass Petri dishes, then irradiated with the dose of

50, 100, 200, 400, 600, 800, and 1000 Gy. Immediately after treatment, irradiated larvae were transferred to Petri dishes containing a rearing medium. The Petri dishes were transferred to laboratory conditions at 20 ± 1 °C, $65 \pm 5\%$ RH, and photoperiod of 16 L: 8 D h). The larval mortality was recorded daily. Also, the survival time (days) of larvae for each dose was recorded. Three replicates were used for each dose level. This experiment was repeated at 23, 26, 28, 30, 33, and 35 ± 1 °C.

Combined effect of gamma radiation and temperature on pupae

One-three days old pupae were selected for experiments. These pupae were reared in an incubator at 20 ± 1 °C. Ten pupae were placed in glass Petri dishes and irradiated at six doses of 50, 100, 200, 400, 600, 800, and 1000 Gy. Then the Petri dishes containing irradiated pupae were transferred to laboratory conditions like the above. The number of adults that emerged from irradiated pupae was recorded, and their percentages were calculated. Three replicates were used for each dose level. This experiment was also done at 23, 26, 28, 30, 33, and 35 ± 1 °C.

Combined effect of gamma radiation and temperature on adults

The same-aged adults were chosen for experiments; these adults were reared in an incubator at 20 ± 1 °C. 10 adults placed in glass Petri dishes in a refrigerator (about 4 °C) for 15 minutes. They were exposed to six doses of 50, 100, 200, 400, 600, 800, and 1000 Gy. Then the Petri dishes containing irradiated adults were transferred to laboratory conditions, the same above. After 48 h, adult mortality was recorded. These experiments were done with three replications for each dose level. This experiment was also done at 23, 26, 28, 30, 33, and 35 ± 1 °C.

Statistical analysis

A completely randomized design with three replications was used to statistically analyze the data on the effect of irradiation and temperature on different life stages of *P. interpunctella* longevity. Two-way ANOVA was used to determine the

interaction between two factors containing gamma radiation doses and temperatures at each life stage of *P. interpunctella*. Also, data comparison at doses per temperature was analyzed using one-way ANOVA. If significant differences were detected, means were compared by Tukey's test ($P < 0.05$). Statistical analyses were conducted using SPSS software version 18 (SPSS, 2009).

Results

Combined effect of gamma radiation and temperature on eggs

The results showed that eggs of *P. interpunctella* are susceptible to gamma radiation. At each temperature, all irradiated eggs with 50, 100, and 200 Gy died, and none of them did not hatch. Therefore, we could not investigate the interaction between gamma radiation and different temperatures in this life stage of *P. interpunctella*.

Combined effect of gamma radiation and temperature on larvae

According to analyzed data, the interaction between gamma radiation and temperature is significant in the developmental period of larvae of *P. interpunctella* (Table 1). The data obtained from experiments dealing with the irradiation of larvae are summarized in Table 2. At each temperature, the survival time of larvae of *P. interpunctella* significantly decreased with increasing radiation doses, especially at 600, 800, and 1000 Gy. All irradiated larvae with 1000 Gy died after one day at 20, 23, and 30 °C. Also, the

temperature had a significant effect on larval mortality. The survival time of larvae decreased with increasing temperature (especially at 33 and 35 °C) too. According to observation, at high temperatures such as 33 and 35 °C, larval mortality happened soon, even at low doses of gamma radiation (50, 100, and 200 Gy) (Table 2).

Table 1 Analysis of variance of gamma radiation and temperatures on survival time of larvae of *Plodia interpunctella* on dried figs under laboratory conditions.

Factor	Mean Square	df	F	P
Gamma radiation	487.72	6	35.00	< 0.0001
Temperature	739.75	7	53.09	< 0.0001
Gamma radiation × temperature	77.42	42	5.56	< 0.0001

Combined effect of gamma radiation and temperature on pupae

The statistical analysis showed that the interaction between gamma radiation and temperature is significant in the adult emergence of *P. interpunctella* from irradiated pupae (Tables 3 and 4). Pupae showed more resistance to radiation than larvae. A dose of 1000 Gy completely prevents the development of the pupae at all temperatures. The gamma radiation doses and temperatures significantly affected the percentage of adults that emerged from irradiated pupae. The maximum pupa mortality percentage (or minimum adult emergence %) was observed in low doses at 35 °C. In this temperature, adult emergence was 30, 30, and 16.67% in doses of 50, 100, and 200 Gy, respectively.

Table 2 Survival time of larvae of *Plodia interpunctella* influenced by gamma radiation and different temperatures on dried figs in laboratory conditions.

Temperature (°C)	Survival time (Mean ± SE) (days)						
	50 (Gy)	100 (Gy)	200 (Gy)	400 (Gy)	600 (Gy)	800 (Gy)	1000 (Gy)
20	20.0 ± 5.0 b	25.0 ± 0.0a	25.0 ± 0.0a	19.0 ± 3.0 b	9.0 ± 1.0c	2.0 ± 0.0d	1.0 ± 0.0d
23	21.0 ± 1.0 a	17.7 ± 1.5c	18.7 ± 0.7 b	1.0 ± 0.0d	1.0 ± 1.0d	1.0 ± 0.0d	1.0 ± 0.0d
26	12.0 ± 1.0 de	22.0 ± 1.0c	26.0 ± 1.7 b	27.0 ± 2.4a	13.0 ± 2.0d	12.0 ± 2.0de	11.0 ± 1.7e
28	22.0 ± 3.0 b	21.0 ± 2.6b	24.0 ± 1.0a	18.0 ± 2.0c	18.0 ± 1.0c	15.0 ± 1.0d	15.0 ± 1.0d
30	18.7 ± 0.3 b	23.0 ± 2.6 b	24.0 ± 1.0a	11.0 ± 1.0d	13.0 ± 0.0c	9.0 ± 1.0e	1.0 ± 0.0f
33	6.0 ± 0.0a	5.7 ± 0.3ab	5.0 ± 0.0 b	5.3 ± 0.3b	4.0 ± 0.6c	4.0 ± 0.0c	3.0 ± 0.0d
35	4.7 ± 0.3a	4.7 ± 0.3a	4.7 ± 0.3a	5.3 ± 0.3a	4.0 ± 0.0a	4.0 ± 0.0 a	4.0 ± 0.0 a

Means with the same letters in each row are not significantly different (Tukey's test, $P < 0.05$).

Table 3 Analysis of variance of gamma radiation and temperatures on adult emergence of *Plodia interpunctella* from irradiated pupae on dried figs.

Factor	Mean Square	df	F	P
Gamma radiation	393.42	6	29.18	< 0.0001
Temperature	791.80	7	58.73	< 0.0001
Gamma radiation × temperature	78.06	42	5.79	< 0.0001

Table 4 Adult emergence of irradiated pupae of *Plodia interpunctella* in dried figs.

Temperature (°C)	Adult emergence (Mean ± SE) (%)						
	1000 (Gy)	1000 (Gy)	1000 (Gy)	1000 (Gy)	1000 (Gy)	1000 (Gy)	1000 (Gy)
20	60.0 ± 10.0c	90.0 ± 5.8a	63.3 ± 12.0c	73.3 ± 14.0 b	60.0 ± 0.0c	0	0
23	36.7 ± 10.0c	56.7 ± 8.8b	56.7 ± 3.3 b	36.7 ± 12.0c	66.7 ± 3.3a	10.0 ± 5.8d	0
26	76.7 ± 3.3bc	70.0 ± 5.8d	80.0 ± 0.0a	73.3 ± 8.8 cd	50.0 ± 10.0e	3.3 ± 3.3f	0
28	80.0 ± 5.8a	70.0 ± 0.0b	73.3 ± 3.3b	0	0	0	0
30	63.3 ± 3.3 d	83.3 ± 8.8 b	93.3 ± 6.7a	76.7 ± 13.0c	40.0 ± 15.0e	0	0
33	100 ± 0a	100 ± 0a	90.0 ± 5.8 b	93.3 ± 3.3b	43.3 ± 10.0c	0	0
35	30.0 ± 10.0a	30.0 ± 5.8a	16.7 ± 3.3c	23.3 ± 6.7b	0	0	0

Means with the same letters in each row are not significantly different (Tukey's test, $P < 0.05$).

Combined effect of gamma radiation and temperature on adults

When adults were exposed to gamma radiation at the dose of 50 Gy, all of them died after 24 h. Also, higher doses had the same effects on adult mortality. Therefore, we could not investigate the interaction between gamma radiation and different temperatures in this life stage of *P. interpunctella*.

Discussion

The results show that *P. interpunctella* to irradiation varied at different life stages. Also, temperature and its interaction with gamma radiation can be essential for developing different life stages of *P. interpunctella*. In this study, a dose of 50 Gy completely sterilized the eggs of *P. interpunctella*. In previous studies, doses of 350 Gy (Ayvaz et al., 2008), 400 Gy (Hosseinzadeh et al., 2011), and 450 Gy (Ozyardimci et al., 2006) are required to gain complete sterility eggs of *P. interpunctella*. Also, Ozyardimci et al. (2006) reported that no *Ephestia kuehniella* hatched when exposed to 300 Gy. The differences between our results and others may have been related to different species of pest and the differences in egg age at the time of irradiation. Radio sensitivity varies with the stage of embryologic development (Ayvaz et al.,

2008). The eggs of *P. interpunctella* are radiosensitive for approximately half of their development time and become 25 times more radiosensitive at 2 h than at 72 h post-oviposition (Brower, 1974). They mentioned that 18, 24, and 30 h, old eggs were similarly sensitive, and 24-h eggs were selected as the suitable age for irradiation with a low dose. In the present study, high doses of gamma radiation, when combined with low temperature, caused a reduction in the survival time of larvae. Also, applying a low dose of gamma radiation at high temperatures can decrease larval survival time. Irradiation can induce specific alterations that can modify both the chemical composition and the nutritional value of foods. These changes depend on the food composition, the irradiation dose, and factors such as temperature and the presence or absence of oxygen in the irradiating environment. The sensitivity of vitamins to radiation is unpredictable, and food vitamin losses during irradiation are often substantial (Dionisia et al., 2009). Therefore, low doses of gamma radiation (50 and 100 Gy) recommend eliminating larvae on stored products at high temperatures. The study revealed that all irradiated larvae with 1000 Gy died after 1-15 days, whereas Shafqat et al. (2006) found that a dose of 1000 Gy was applied to the larvae of *P. interpunctella*, they died entirely after 21 days.

Previous research has shown that the minimum dose of gamma irradiation is 300-450 Gy (Azelmat *et al.*, 2005; Hosseinzadeh *et al.*, 2011), ultimately preventing larval development of *P. interpunctella* and the larvae did not reach pupation. Also, a dose of 250 Gy was required to give total mortality for the last-instar larvae of *E. kuehniella* (Ayvaz and Tuncbilek, 2006). The differences between the results of our study and previous studies can refer to different larval ages and insect species. The younger larvae were more sensitive to radiation than the older ones (Ayvaz *et al.*, 2008), and the dose required to prevent adult emergence was lower (200 Gy) than for mature larvae (250 Gy) of *E. kuehniella* (Ayvaz and Tuncbilek, 2006).

The highest efficiency of gamma radiation to decrease adult emergence was obtained at 35 °C. Also, when the pupae of *P. interpunctella* were irradiated, the percentage of adult emergence was reduced by increasing doses. Also, Hosseinzadeh *et al.* (2011) reported that the percentage of adult emergence decreases by doses up to 650 Gy. Mortality of pupae was 78.89% after irradiation, reaching 95.55% on the 21st day after storage irradiated at 500 Gy (Shafqat *et al.*, 2006). Irradiated *E. kuehniella* pupae with doses of 200-800 Gy caused decreased adult emergence significantly. Also, a dose of 1000 Gy prevented the emergence of both sexes (Boshra and Mikhael, 2006).

Several stored pests belonging to Lepidoptera, such as *P. interpunctella* are more resistant to radiation sterilization than other insects; therefore, they require much higher levels of ionizing radiation to obtain complete sterility (Hallman, 2000). According to the results of this study, low-level doses of gamma radiation can effectively control *P. interpunctella* if used at high temperatures (35 °C). These methods can be used as an alternative to chemical fumigants, and these results may be helpful for the integrated management of *P. interpunctella* on dried figs in storage.

Our work found a synergistic effect of high gamma radiation doses (1000 Gy) with low temperatures (20 °C). However, the long-term

effects of this combined treatment deserve further investigation.

Statement of Conflicting Interests

The authors state that there is no conflict of interest.

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تأثیر تلفیق پرتو گاما با گرما به عنوان تیمار قرنطینه‌ای شبیره هندی (*Plodia interpunctella* (Lepidoptera: Pyralidae))

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چکیده: شبیره هندی، *Plodia interpunctella* (Hubner) یکی از آفات مهم اقتصادی محصولات انباری در ایران و جهان می‌باشد. در این پژوهش، تأثیر دزهای مختلف پرتو گاما (۵۰-۱۰۰۰ گری) و دما ($1 \pm 20-35$ درجه سلسیوس) بر مراحل مختلف زندگی شبیره هندی مورد بررسی قرار گرفت. نتایج نشان داد که تخم‌ها و حشرات کاملی که در معرض دزهای پایین پرتو گاما (۵۰ گری) قرار گرفتند، پس از مدت کوتاهی از بین رفتند. با توجه به این موضوع این مراحل به پرتو گاما بسیار حساس بوده و امکان بررسی تلفیق پرتو گاما و دما در مورد این مراحل زندگی شبیره هندی امکان‌پذیر نبود. در همه دماها، زمان زنده ماندن لاروها با افزایش دزهای پرتوتابی به‌خصوص در ۶۰۰، ۸۰۰ و ۱۰۰۰ گری، به‌میزان قابل‌توجهی کاهش یافت. همچنین در دمای ۳۳ و ۳۵ درجه سلسیوس، دزهای پایین پرتو گاما (۵۰، ۱۰۰ و ۲۰۰ گری) باعث مرگ زودهنگام لاروها شدند. در بیش‌تر دماهای مورد آزمایش، دزهای ۸۰۰ و ۱۰۰۰ گری از پرتو گاما موجب مرگومیر ۱۰۰ درصدی در بین شفیره‌ها شده و هیچ حشره کاملی از این شفیره‌ها تولید نشد. حداکثر درصد مرگومیر شفیره (یا حداقل درصد ظهور حشرات کامل) در تلفیق دزهای ۵۰، ۱۰۰ و ۲۰۰ گری پرتو گاما با دمای ۳۵ درجه سلسیوس مشاهده شد. در این دما، با تلفیق دزهای ۵۰، ۱۰۰ و ۲۰۰ گری از پرتو گاما درصد ظهور حشرات کامل به‌ترتیب ۳۰، ۳۰ و ۱۶/۶۷ بود. این نتایج می‌تواند برای مدیریت تلفیقی شبیره هندی روی انجیر خشک در انبارها مفید باشد.

واژگان کلیدی: شبیره هندی، پرتو دهی، تیمار دما، مدیریت تلفیقی آفات