

Full Length Research Paper

# Effect of gamma radiation on different stages of Indian meal moth *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae)

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Indian meal moth *Plodia interpunctella* Hübner is one of the most important stored products pests in the world. In this research, the effect of gamma irradiation was studied on different developmental stages of this pest and the doses required to prevent each of these developmental stages was investigated. From the results, required dose to prevent larval emergence from irradiated 1 to 24 h eggs was 400 Gray (Gy), and 400 Gy was required to prevent pupae from 15 days old larvae. Also, the dose of radiation required to prevent adult emergence from irradiated 5 days old pupa was 650 Gy. According to the results, dose of 650 Gy is adequate to control all immature stages of this pest. In addition, the effect of gamma ray was studied on developmental stage period of each irradiated existence stage till adult eclosion. The results revealed that there was a dose-dependent increase in the developmental periods, and the growth index of the adults was significantly decreased with increasing dose of radiation administered to the eggs, larvae and pupae too. It is concluded that irradiation can be used as a safe method to control stored pests.

**Key words:** Gamma irradiation, prevention dose, developmental period, growth index, *Plodia interpunctella*.

## INTRODUCTION

Iran is the largest producer of pistachios and dates in the world and according to the Food and Agriculture Organization (FAO), approximately 10% of stored products in Iran are lost due to insect infestation (Zolfagharieh, 2002). The Indian meal moth *Plodia interpunctella* Hübner is a cosmopolitan pest whose larvae can infest a variety of stored grain, nuts, pulses, meals, dried fruits and processed foods (Simmons and Nelson, 1975). Methyl bromide (MB) is a widely used fumigant for the control of insect infestation in many agricultural commo-

dities. As methyl bromide has the potential to destroy the ozone layer in the environment, alternative methods had to be sought to replace its use in insect control (Gochangco et al., 2002). It has been reported that irradiation could offer an important and attractive alternative for the export commodity fumigation uses of MB and for eliminating the problem of chemical residues (Ahmed, 1991; Marcotte, 1993). Brower and Tilton (1983) reported that irradiation is an approved method of direct control for stored product insect in wheat and wheat flour in many countries, and indications were that it would soon be approved for all grain, grain products and other dry food commodities. Commodities are exposed to gamma rays from the radioactive isotopes cesium 137 or cobalt 60, electron beams, or X rays converted from an electron beam generator. The mode of action of ionizing irradiation as a quarantine treatment involves breaking

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**Abbreviations:** MB, Methyl bromide; PTM, potato tuber moth.

chemical bonds. When this happens to DNA, normal development or reproduction of the organism may be prevented. Decades of research conducted worldwide on radiation disinfestation of food and agricultural products have shown that this method is effective. The advantages of irradiation processing include no undesirable residues in the foods treated, no resistance developed by pest insects and few significant changes in the physico-chemical properties or the nutritive value of the treated products (Ahmed, 2001; Lapidot et al., 1991; Boshra and Mikhael, 2006). Unlike other disinfestations techniques, irradiation does not need to kill the pest immediately to provide quarantine security; therefore, live (but nonviable or sterile) insects may occur with the exported commodity (Follett and Griffin, 2006).

In the present work, we have tested gamma radiation sensitivity of different life stages of *P. interpunctella* Hübner. The study was also designed to assess the effect of gamma irradiation on different stages of *P. interpunctella*.

## MATERIALS AND METHODS

### Insect culture

*P. interpunctella* Hübner used in all experiments were derived from a laboratory culture initially established for larvae collection from infested pistachios in Urmia Province, Northwest Iran. The larvae were reared on artificial diet containing bran (800 g), dry yeast powder (160 g), honey (300 g) and glycerol (200 ml / 1000 g) dry ingredient. The diet was decontaminated at 60°C for 6 h. Throughout the experiments, insect cultures were maintained under controlled laboratory conditions of  $28 \pm 1^\circ\text{C}$  and  $65\% \pm 5\%$  relative humidity (RH) with a 14:10 h light : dark photoperiod. Larvae were placed in 500 ml glass containers half full of rearing medium. Fifteen virgin pairs of males and females (0 to 24 h old) were selected for the experiments. These adults were placed in funnels capped with screen lids. Funnels were placed over radiology film for 24 h and oviposited eggs on the film were collected every 12 h.

### Irradiation of eggs

Twenty eggs (1 to 2 days old) were placed in glass Petri dishes and irradiated in a calibrated  $^{60}\text{Co}$  irradiator (Issledovatel type PX-30) with the activity of 4.5 kCi and dose rate of 0.65 Gy/s at the Iran Nuclear Research Center for Agriculture and Medicine. Ferric dosimetry system was used for the facility.

Radiation was applied at nine dose levels ranging from 0 to 400 Gy to the eggs. Controls were subjected to the same conditions as the irradiated eggs in that, they were transferred to the irradiator and subsequently removed without exposure to radiation. After irradiation, eggs were returned immediately to the laboratory and then placed in Petri dishes that had a central well made by ringing a central area (2 cm diameter) with glue and allowing the glue to dry. 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  $28 \pm 1^\circ\text{C}$  and  $65 \pm 5\%$  RH. The Petri dishes were examined under a binocular microscope, and the number of hatched eggs was counted daily until no further hatched egg was observed. The rearing medium containing the small larvae was then transferred to Petri dishes containing food. Pupation and adult emergence of these larvae were recorded. Four replicate batches

of eggs were used for each dose level and the control.

### Irradiation of 15 days old larvae

Fifteen days old larvae were selected for uniformity in size before irradiation. Larvae were irradiated in the same irradiator and dose ranges from 0 to 400 Gy. Immediately after treatment, irradiated and control larvae were transferred to Petri dishes containing rearing medium. The Petri dishes were subsequently transferred to the rearing conditions. Pupation, adult emergence and mortality of these larvae were recorded every 2 days. Mortality of larvae was determined by the brown color with no observable movement. The adults that developed from irradiated larvae were removed and counted daily. Four replicate (20 larvae for each replicate) were used for each dose level and control.

### Developmental periods and longevity of the adults derived from irradiated eggs, larvae and pupae

The developmental periods, from egg to adult of *P. interpunctella* Hübner that developed from irradiated eggs, larvae and pupae were recorded. The longevity of the adults was also determined. The growth index (percentage adult eclosion/total developmental period) was calculated for the irradiated eggs, larvae and pupae.

### Irradiation of pupae

Five-day old pupae were placed in Petri dishes and irradiated at six dose levels of 0, 150, 250, 350, 450, 550 and 650 Gy. The number of adults that emerged from irradiated pupae was recorded. Four replicates (20 pupae in each) were used for each dose level.

### Statistical analysis

Data from the experiments were subjected to analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) for windows 11.5. Data were transformed using arcsine  $\sqrt{x}$  before ANOVA. Means were separated at the 5% significance level by the Tukey test.

## RESULTS AND DISCUSSION

### Effects of gamma radiation on eggs

The data obtained from experiments dealing with the irradiation of eggs are summarized in Table 1. A significant reduction in hatched eggs was observed and it was correlated with the radiation doses ( $F = 596.522$ ;  $df = 8$ ;  $P < 0.05$ ). Percentage larval emergence from irradiated eggs was 93.75% in the untreated controls but was reduced to 2.81% at a dose of 350 Gy, and all irradiated eggs were completely sterile at 400 Gy. The percentage of emergent larvae that survived to the pupa stage also decreased with increasing radiation doses ( $F = 769.212$ ;  $df = 8$ ;  $P < 0.05$ ). At doses of 250 and 300 Gy, pupal development was 10.31 and 4.37%, respectively.

The percentage of pupation that survived to the adult stage was also decreased with increasing radiation doses ( $F = 300.892$ ;  $df = 8$ ;  $P < 0.05$ ). Percentage adult

**Table 1.** Egg hatch, pupation, and adult emergence of the *P. interpunctella* irradiated as eggs (1 to 2 days old).

Dose (Gy)	Egg hatch (%) ± SE	Pupation (%) ± SE	Adult emergence (%) ±SE
0	93.75±1.61 <sup>a</sup>	75.00±1.02 <sup>a</sup>	61.25±0.72 <sup>a</sup>
50	78.12±1.20 <sup>b</sup>	65.00±1.02 <sup>b</sup>	60.00±1.02 <sup>a</sup>
100	62.5±1.02 <sup>c</sup>	52.50±1.44 <sup>c</sup>	48.75±1.25 <sup>b</sup>
150	43.13±1.20 <sup>d</sup>	35.00±1.02 <sup>d</sup>	31.87±1.20 <sup>c</sup>
200	31.87±1.20 <sup>e</sup>	20.62±1.20 <sup>e</sup>	16.25±0.72 <sup>d</sup>
250	21.25±0.72 <sup>f</sup>	10.31±0.79 <sup>f</sup>	8.75±.72 <sup>e</sup>
300	11.87±1.20 <sup>g</sup>	4.37±1.20 <sup>g</sup>	1.87±1.20 <sup>f</sup>
350	2.81±0.79 <sup>h</sup>	0 <sup>h</sup>	0 <sup>f</sup>
400	0 <sup>i</sup>	0 <sup>h</sup>	0 <sup>f</sup>

Means within each column followed by the same letter are not significantly different (P > 0.05).

**Table 2.** Pupation, adult emergence and mortality (%) of the *P. interpunctella* irradiated as larvae (15 days old).

Dose (Gy)	Pupation(%)±SE	Adult emergence (%) ±SE	Mortality(%) ±SE
0	85.62±1.57 <sup>a</sup>	60.00±1.77 <sup>a</sup>	11.87±1.20 <sup>g</sup>
150	76.25±0.72 <sup>b</sup>	45.94±0.60 <sup>b</sup>	17.81±1.07 <sup>f</sup>
200	51.87±1.20 <sup>c</sup>	21.25±0.72 <sup>c</sup>	35.31±1.07 <sup>e</sup>
250	37.50±1.77 <sup>d</sup>	8.12±1.20 <sup>d</sup>	51.87±1.20 <sup>d</sup>
300	20.31±1.02 <sup>e</sup>	0 <sup>e</sup>	61.87±1.20 <sup>c</sup>
350	5.00±1.02 <sup>f</sup>	0 <sup>e</sup>	81.87±1.20 <sup>b</sup>
400	0 <sup>g</sup>	0 <sup>e</sup>	100 <sup>a</sup>

Means within each column followed by the same letter are not significantly different (P > 0.05).

emergence from irradiated eggs was 61.25% in the untreated controls but was reduced to 1.87% at a dose of 300 Gy and no adult emerged at doses of 350 Gy and above.

### Effects of gamma radiation on 15 days old larvae

The effects of gamma radiation on irradiated larvae are shown in Table 2. The data showed that the percentage of irradiated larvae that survived to the pupal stage were decreased with increasing radiation doses (F = 1077.006; df = 6; P < 0.05). The percentages of pupae that developed from irradiated larvae were 5% at 350 Gy when compared with 85.62% in the control. The percentage of pupation that survived to the adult stage were also decreased with increasing radiation doses (F = 998.931; df = 6; P < 0.05). Statistical analysis of data indicated that irradiation significantly affected mortality percentages of larvae (F = 1038.098; df = 6; P < 0.05). Mortality of irradiated larvae began 4 days after the treatment and continued through the time of observation. Irradiation at

400 Gy caused 100% mortality.

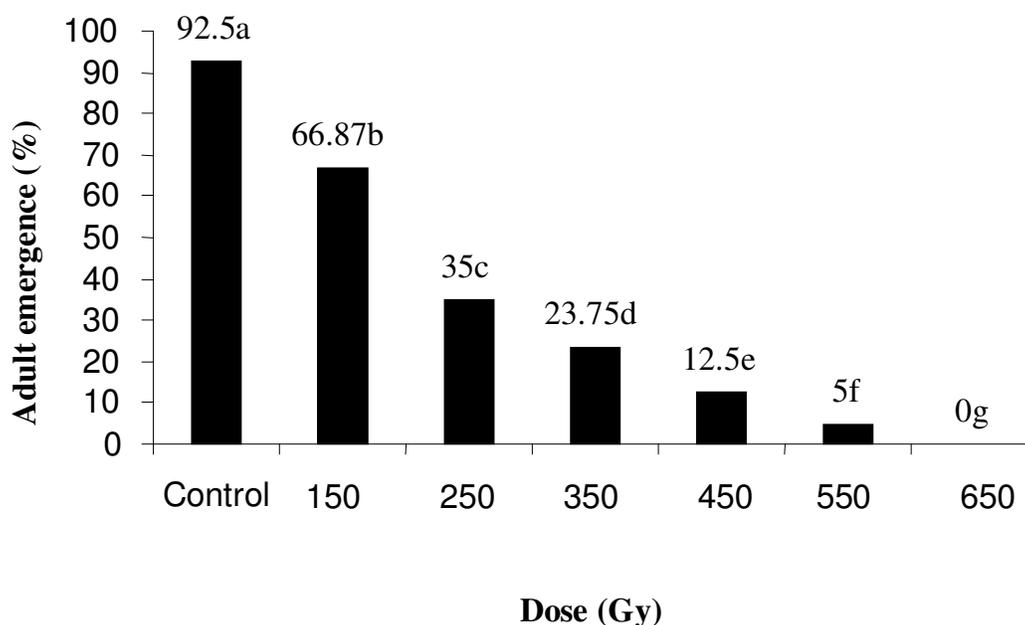
### Effects of gamma radiation on the developmental period and longevity of the adults derived from irradiated eggs, larvae and pupae

There was a dose-dependent increase in the developmental time of *P. interpunctella* Hübner adults developed from irradiated eggs (F = 21.876; df = 7; P < 0.05), larvae (F = 42.931; df = 7; P < 0.01) and pupae (F = 21.650; df = 7; P < 0.05) (Table 3). The mean developmental time from egg to adult was 44.20 days in the controls, which rose to 52.50 days for insects derived from eggs irradiated with a dose of 350 Gy. The development of the irradiated larvae was also extended from 23.40 days in the controls to 36.40 days for insects exposed to a dose of 350 Gy. The mean developmental time from pupa to adult was 3.70 days in the controls, which rose to 6.90 days at a dose of 350 Gy. The growth index of the adults was significantly decreased with increasing doses of radiation administered to the eggs (F

**Table 3.** Mean ( $\pm$  SE) developmental period of *P. interpunctella* adults irradiated as eggs, larvae and pupae<sup>a</sup>.

Dose (Gy)	Developmental period from eggs to adults (days)			Growth index <sup>b</sup>		
	Eggs treated	Larvae treated	Pupae treated	Eggs treated	Larvae treated	Pupae treated
0	44.20 $\pm$ 0.63 <sup>e</sup>	23.40 $\pm$ 0.65 <sup>c</sup>	3.70 $\pm$ 0.15 <sup>d</sup>	1.38 <sup>a</sup>	2.56 <sup>a</sup>	25.00 <sup>a</sup>
50	45.20 $\pm$ 0.44 <sup>de</sup>	24.20 $\pm$ 0.74 <sup>c</sup>	3.80 $\pm$ 0.20 <sup>d</sup>	1.32 <sup>a</sup>	2.12 <sup>b</sup>	23.87 <sup>b</sup>
100	45.60 $\pm$ 0.70 <sup>de</sup>	25.80 $\pm$ 0.49 <sup>bc</sup>	4.50 $\pm$ 0.17 <sup>cd</sup>	1.05 <sup>b</sup>	1.86 <sup>c</sup>	19.46 <sup>c</sup>
150	46.40 $\pm$ 0.65 <sup>cde</sup>	27.30 $\pm$ 0.67 <sup>b</sup>	5.00 $\pm$ 0.30 <sup>c</sup>	0.67 <sup>c</sup>	1.68 <sup>c</sup>	17.59 <sup>d</sup>
200	47.40 $\pm$ 0.65 <sup>bcd</sup>	30.50 $\pm$ 0.72 <sup>a</sup>	5.50 $\pm$ 0.28 <sup>bc</sup>	0.33 <sup>d</sup>	0.70 <sup>d</sup>	11.68 <sup>e</sup>
250	48.20 $\pm$ 0.61 <sup>bc</sup>	32.00 $\pm$ 0.80 <sup>a</sup>	6.10 $\pm$ 0.28 <sup>ab</sup>	0.17 <sup>e</sup>	0.25 <sup>e</sup>	7.77 <sup>f</sup>
300	49.40 $\pm$ 0.37 <sup>b</sup>	-	6.90 $\pm$ 0.23 <sup>a</sup>	0.03 <sup>f</sup>	-	5.37 <sup>g</sup>
350	52.50 $\pm$ 0.40 <sup>a</sup>	-	-	-	-	5.16 <sup>g</sup>

<sup>a</sup> Means followed by the same letter in a column within each dose are not significantly different ( $P > 0.05$ ); <sup>b</sup> growth index: percentage adult eclosion/total developmental period.

**Figure 1.** Adult emergence of the *P. interpunctella* irradiated as pupae (5 days old).

= 1141.967; df = 7;  $P < 0.05$ ), larvae ( $F = 456.928$ ; df = 7;  $P < 0.05$ ) and pupae ( $F = 334.860$ ; df = 7;  $P < 0.05$ ) (Table 3).

### Effects of gamma radiation on five-days-old pupae

Pupae showed more resistance to radiation than eggs or larvae. A dose of 650 Gy completely prevented the development of the pupae. The percentage of adults that emerged from irradiated pupae was significantly affected by the gamma radiation doses administered 5 days after pupation ( $F = 597.306$ ; df = 6;  $P < 0.05$ ) (Figure 1).

The results showed that increase in radiation doses caused reduced egg viability, pupation and adult emergence when 1 to 2 days old eggs were irradiated. The

susceptibility of *P. interpunctella* to irradiation varies as development progresses from egg to adult. Previously, it had been found that no irradiated eggs of *P. interpunctella* hatched when one–to three-day-old eggs were exposed to 350 Gy (Ayvaz et al., 2008). The differences between the present results and those from previous studies may have been related to 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 quite radiosensitive for approximately half of their development time, and become 25 times more radio resistant at 72 h than at 2 h post-oviposition (Brower, 1974). In the present study, a dose of 400 Gy completely sterilized the eggs, while other researchers have reported that doses of between 300 and 450 Gy are required to

obtain complete sterility for *Ephestia cautella* (Walker) and *P. interpunctella* eggs respectively (Ozyardimci et al., 2006). In our study, a dose of 350 Gy completely prevented adult emergence from irradiated eggs. Ayvaz and Tuncbilek (2006) found that no *Ephestia kuehniella* Zeller late-instar larvae received an irradiation dose of 250 Gy as emerged adults.

When the effects of six gamma radiation doses ranging from 50 to 1000 Gy were investigated against all life stages of *E. cautella*, the development of adults from treated eggs and larvae was prevented by 300 and 200 Gy, respectively (Cogburn et al., 1973). In the present study, a dose of 400 Gy completely prevented larval development and the larvae did not reach pupation. The minimum dose required to prevent adult emergence from *P. interpunctella* exposed as larvae was 300 Gy, while doses required to cause death were 450 Gy and above (Azemat et al., 2005). A dose of 250 Gy was sufficient to give complete mortality for last-instar larvae of *E. kuehniella* Zeller (Ayvaz and Tuncbilek, 2006). Our results showed that, when the prevention of adult emergence is used as a standard for measuring the effectiveness of radiation against *P. interpunctella* larvae, a dose of 300 Gy is required. It was also found that the ultimate development of adults from 11-days-old *P. interpunctella* larvae was completely prevented when these larvae were exposed to 149 Gy (Johnson and Vail, 1988). Ayvaz et al. (2006) reported that younger larvae were more sensitive to radiation than the older ones, and that the dose required to prevent adult emergence was lower (200 Gy) than for mature larvae (250 Gy). Cogburn et al. (1973) reported that from the larvae of *Cadra cautella* Walker treated with 20 krad (200 Gy), no adults were produced. Mansour (2002) also reported that 200 Gy doses were enough to prevent adult emergence from irradiated mature larvae of codling moth *Cydia pomonella* (L.). Ayvaz and Tuncbilek (2006) found that a dose of 200 Gy applied to the young larvae of *E. kuehniella* completely prevented female adult emergence (all emerged moths were males).

There was a dose-dependent delay in the developmental period of the *P. interpunctella* eggs, larvae and pupae. The longevity of the treated larvae increased when compared with controls that continued development to adult emergence. Hallman (2000) noted that insects may live for considerable periods of time after irradiation. Makee and Saour (2003) assumed that unbalanced hormonal system led to prolonged developmental time in the  $F_1$  larvae of the potato tuber moth (PTM), *Phthorimaea operculella* Zeller. Hosseinzadeh et al. (2010) found that there was a dose-dependent increase in the developmental time of *Oryzaephilus surinamensis* L. adults developed from irradiated eggs, larvae and pupae. Increasing post-treatment longevity for irradiated *P. interpunctella*, the false codling moth, *Cryptophlebia leucotreta* Meyrick, the Mediterranean flour moth, *E. kuehniella*, and the red flour beetle *Tribolium castaneum*

Herbst have been reported previously (Azemat et al., 2005; Johnson and Vail, 1988; Bloem et al., 2003; Mehta et al., 1990; Ayvaz and Tuncbilek, 2006). Typically, irradiated nymphs or larvae will have a prolonged nymphal or larval stage and may live longer than non-irradiated control insects (Hasan and Khan, 1998).

When the pupae of *P. interpunctella* were irradiated, the percentage of adult emergence was decreased by doses up to 650 Gy. When male and female pupae of *Ephestia calidella* (Guenee) were irradiated with doses of 200 to 800 Gy, the percentage adult emergence was decreased in accordance with increasing gamma radiation doses (Boshra and Mikhael, 2006). This work also showed that a 1000 Gy dose prevented the emergence of both sexes.

Hallman (2000) reported that some stored-product moths, especially the Angoumois grain moth, *Sitotraga cerealella* (Oliver) and the Indian meal moth *P. interpunctella* (Hübner), were among the most tolerant arthropods to radiation. In comparison with dipteran species, Lepidoptera require much higher levels of ionizing radiation to obtain full sterility (LaChance and Graham, 1984). The main cause of this difference is thought to be as a result of the different kinetic organization of chromosomes in these two groups of insects. The Diptera possess typical monocentric chromosomes with kinetic activity restricted to the centromere, whereas lepidopteran chromosomes are holokinetic (Gassner and Klemetson, 1974).

The results of this study support the assumption that a dose of 400 Gy is required in order to prevent hatching of eggs and 300 Gy is required to prevent the larvae from reaching adulthood. It is recommended that dose of 650 Gy should be used to control population growth of *P. interpunctella* when targeting pupae are within stored products. Delayed developmental periods were observed after irradiation of the eggs, larvae and pupae. Irradiation could be used as a disinfestation treatment in stored products as an alternative to chemical fumigants. Phos-toxin and methyl bromide are the most common fumigants for insect disinfestation in storage. Fumigants leave residues in treated products, and due to the ozone-depleting properties, methyl bromide was banned in many countries. Therefore, irradiation is a safe alternative to fumigation.

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