

Full Length Research Paper

Application of gamma radiation for controlling the red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

Hosseinzadeh Abbas* and Shayesteh Nouraddin

Department of Plant Protection, Mahabad Branch, Islamic Azad University, Mahabad, Iran.

Accepted 26 June, 2011

The effect of gamma irradiation on different developmental stages of *Tribolium castaneum* Herbst and the doses required to prevent each of these developmental stages was investigated. The required dose to prevent larval emergence from irradiated 1-2 days old eggs was 150 Gray (Gy) and 100 Gy was required to prevent adult emergence from 15 day-old larvae. Also the dose of radiation required preventing adult emergence from irradiated 5 day-old pupa was 700 Gy. The dose of 200 Gy caused 100% mortality irradiated adults 28 days after treatment. There was a dose-dependent increase in the developmental periods of different developmental stages exposed to gamma irradiation. The growth index of the adult was significantly decreased with increasing dose of radiation administered to the eggs, larvae and pupae. A dose of 700 Gy is recommended to control population growth of *T. castaneum* within stored products.

Key words: Irradiation, prevent dose, developmental period, growth index, *Tribolium castaneum*.

INTRODUCTION

Tribolium castaneum Herbst is major and frequently encountered pest of stored products. It attack virtually any dried material of animal or plant origin but is especially important as pests of cereals and cereal products and is major pest of mills. It also occurs in both grain stores and mills (Rees, 2008). Currently, control practices rely on scheduled fumigation with methyl bromide or hydrogen phosphide. However, methyl bromide has been classified as an ozone depleter, and its use has recently been banned in many countries (Hansen and Jensen, 2002). Owing to the increasing restrictions on the methods available for the control of pests in stored products, alternative methods require investigating. One such method, the use of gamma radiation for the sterilization of pests, has prove to be a technically feasible alternative to conventional methods

for controlling stored-product insects (Cornwell, 1966; Watters, 1968).

Several works had been done on the use of radiation to control stored-product pests (Brower and Tilton, 1983; Hasan, 1999; Azelmat et al., 2005; Boshra and Mikhael, 2006). Irradiation is an approved method for the direct control of stored-product insects in wheat and flour in many countries, and probably would be approved for all grain, grain products and other dry food commodities (Brower and Tilton, 1983). Lower doses of irradiation do not cause immediate death of adults but can prevent an increase in pest populations through lethal effects on the immature stages and sterilization of adults (Brower and Tilton, 1983). The advantages of irradiation as a pest control measure include the absence of undesirable residues in the foods treated, no resistance development by pest insects and few significant changes in the physicochemical properties or the nutritive value of the treated products (Ahmed, 2001; Lapidot et al., 1991). The present work focuses on determining the minimal effective doses that prevent commodity damage caused by eggs, larvae, pupae and adults of *T. castaneum*. The study was also designed to assess the effect of gamma irradiation on biology of different stages of *T. castaneum*.

*Corresponding author. E-mail: abas1354@yahoo.com.

Abbreviations: Gy, Gray; ANOVA, analysis of variance; LSD, least significant difference; SPSS, statistical package for social sciences.

MATERIALS AND METHODS

Insect culture

T. castaneum used in experiments were derived from a laboratory culture initially established from adults collected from infested wheat in Urmia Province, northwest Iran. Adults were reared on a rearing medium composed of wheat flour and 5% brewers yeast (19:1, w/w) over several generations (Ayvaz et al., 2002). The flour was disinfested at 60°C for 10 h to eliminate possible contaminants (Tuncbilek and Kansu, 1996). Throughout the experiments, insect cultures were maintained under controlled laboratory conditions of $27 \pm 1^\circ\text{C}$ and $65\% \pm 5\%$ RH and continuous darkness.

Irradiation of eggs

T. castaneum eggs were obtained by placing 500-1000 adult beetles in 500 g rearing medium. After 24 h the eggs laid were separated from the flour using a 90 mesh sieve. 21-to 22-day old 20 eggs were placed in glass petri dishes and irradiated in a calibrated ^{60}Co irradiator (Issledovatel type PX-30) with the activity of 4.5 kCi and dose rate of 0.65 Gray (Gy)/s at the Karaj nuclear research center for agriculture and medicine. Ferric dosimetry system was used for the facility.

Radiation was applied at seven dose levels ranging from 0 to 200 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 $27 \pm 1^\circ\text{C}$. 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 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 controls.

Irradiation of 15-day old *T. castaneum* larvae

15-day old larvae were selected for uniformity in size before irradiation. Larvae were irradiated in the same irradiator and dose ranges from 0-200 Gy. Immediately after treatment, the irradiated and the 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 brown colouration with no observable movement. The adults that developed from irradiated larvae were removed and counted daily. Four replicates (20 larvae per replicate) were used for each dose level and control.

Longevity and developmental periods of the adults from irradiated eggs, larvae and pupae

The developmental periods, from egg to adult, of *T. castaneum* that developed from irradiated eggs, larvae and pupae were recorded. The longevity of these adults was also determined. The growth index (percentage adult eclosion/total developmental period) was calculated for the irradiated eggs, larvae and pupae (Azelmate et al., 2005).

Irradiation of pupae

5-day-old pupae were placed in Petri dishes and irradiated at seven dose levels of between 0 and 700 Gy. Adults that emerged from irradiated pupae were recorded. Four replicates (20 pupae per replicate) were used for each dose level.

Irradiation of adults

5-day-old adults placed in petri dishes and irradiated at eight dose levels of between 0 and 700 Gy. Four replicates (20 adults per replicate) were used for each dose. Mortality of adults was assessed 2 days after treatment and thereafter at weekly intervals.

Statistical analysis

Data from the experiments were subjected to analysis of variance (ANOVA) using statistical package for social sciences (SPSS) for windows 10.0. Percentage data were arcsine transformed before ANOVA. Means were separated at the 5% probability level by the least significant difference (LSD) test.

RESULTS

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 egg hatch was observed that was dependent on the radiation doses ($F = 511.756$; $df = 7$; $P < 0.05$). Percentage larval emergence from irradiated eggs was 89.37% in the untreated controls but was reduced to 3.67% at a dose of 125 Gy, with all irradiated eggs completely sterile at 150 Gy (Table 1). The percentage of emergent larvae that survived to the pupa stage also decreased with increasing radiation doses ($F = 566.385$; $df = 7$; $P < 0.05$). At doses of 75 and 100 Gy, pupa development was 6.25% and 1.87% respectively. The percentage of pupation that survived to the adult stage also decreased with increasing radiation doses ($F = 494.419$; $df = 7$; $P < 0.05$). Percentage adult emergence from irradiated eggs was 73.12% in the untreated controls but was reduced to 1.25% at a dose of 75 Gy and no adult emerged at doses of 100 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 show that the percentage of irradiated larvae that survived to the pupa stage decreased with increasing radiation doses ($F = 1144.440$; $df = 7$; $P < 0.05$). The percentages of pupae developed from irradiated larvae were 2.5 % at 125 Gy, compared with 80.62 % in the control (Table 2). The percentage of pupation that survived to the adult stage also decreased with increasing radiation doses ($F = 336.047$; $df = 7$; $P < 0.05$). Statistical analysis of data indicated that irradiation

Table 1. Egg hatch, pupation, and adult emergence of the *T. castaneum* irradiated as eggs (1 to 2 day old).

Dose(Gy)	Percentage egg hatch	Percentage pupation	Percentage adult emergence
0	89.37±2.13 ^a	80.62±1.57 ^a	73.12±1.20 ^a
25	72.50±1.44 ^b	46.87±1.20 ^b	34.37±0.62 ^b
50	50.62±2.13 ^c	33.12±1.20 ^c	16.25±0.72 ^c
75	30.62±1.57 ^d	6.25±0.72 ^d	1.25±0.72 ^d
100	11.25±1.61 ^e	1.87±0.62 ^e	0.00±0.00 ^d
125	3.67±0.77 ^f	0.00±0.00 ^e	0.00±0.00 ^d
150	0.00±0.00 ^f	0.00±0.00 ^e	0.00±0.00 ^d
200	0.00±0.00 ^f	0.00±0.00 ^e	0.00±0.00 ^d

Data are means ±S.E. of four replicates. Means within each column followed by the same letter are not significantly different at 5% probability level.

Table 2. Pupation, adult emergence and mortality of the *T. castaneum* Irradiated as larvae (15 days old).

Dose (Gy)	Percentage Pupation	Percentage adult emergence	Percentage mortality
0	80.62±0.62 ^a	58.75±1.61 ^a	11.25±0.72 ^f
25	46.25±1.61 ^b	27.50±1.44 ^b	43.12±1.20 ^e
50	21.25±1.61 ^c	12.50±1.02 ^c	65.00±2.04 ^d
75	10.31±0.79 ^d	3.75±0.72 ^d	76.87±1.20 ^c
100	7.50±1.02 ^d	0.00±0.00 ^e	80.00±1.02 ^c
125	2.50±1.02 ^e	0.00±0.00 ^e	91.25±3.14 ^b
150	0.00±0.00 ^f	0.00±0.00 ^e	100 ^a
200	0.00±0.00 ^f	0.00±0.00 ^e	100 ^a

Data are means ±S.E. of four replicates. Means within each column followed by the same letter are not significantly different at 5% probability level.

significantly affected mortality of larvae ($F = 157.353$; $df = 7$; $P < 0.05$). Mortality of irradiated larvae began 4day after the treatment and continued through the time of observation (Table 2).

Effects of gamma radiation on the developmental period and longevity of the adult *T. castaneum* derived from irradiated eggs, larvae and pupae

There was a dose-dependent increase in the developmental time of *T. castaneum* adults developed from irradiated eggs ($F = 8.142$; $df = 2$; $P < 0.002$), larvae ($F = 5.428$; $df = 7$; $P < 0.001$) and pupae ($F = 8.25$; $df = 7$; $P < 0.05$) (Table 3). The mean developmental time from egg to adult was 48.00 days in the controls, which rose to 51.50 days for the development time of insects derived from eggs irradiated with a dose of 100 Gy. The development of the irradiated larvae was also extended from 25.70 days in the controls to 31.30 days for insects exposed to a dose of 500 Gy. The mean developmental time from pupa to adult was 6.50 days in the controls, which rose to 9.90 days at a dose of 500 Gy. The growth index of the adults was significantly decreased with increasing doses of radiation administered to the eggs (F

$=1141.967$; $df = 7$; $P < 0.005$), larvae ($F = 456.928$; $df = 7$; $P < 0.005$) and pupae ($F = 163.485$; $df = 7$; $P < 0.005$) (Table 3).

Effects of gamma radiation on 5-day-old pupae

Pupae showed more resistance to radiation than eggs or larvae. The adult emergence in the control was not significantly different from that at 200 Gy. A dose of 700 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 ($F = 233.396$; $df = 6$; $P < 0.05$) (Figure 1):.

Effects of gamma radiation on 5-day-old adults

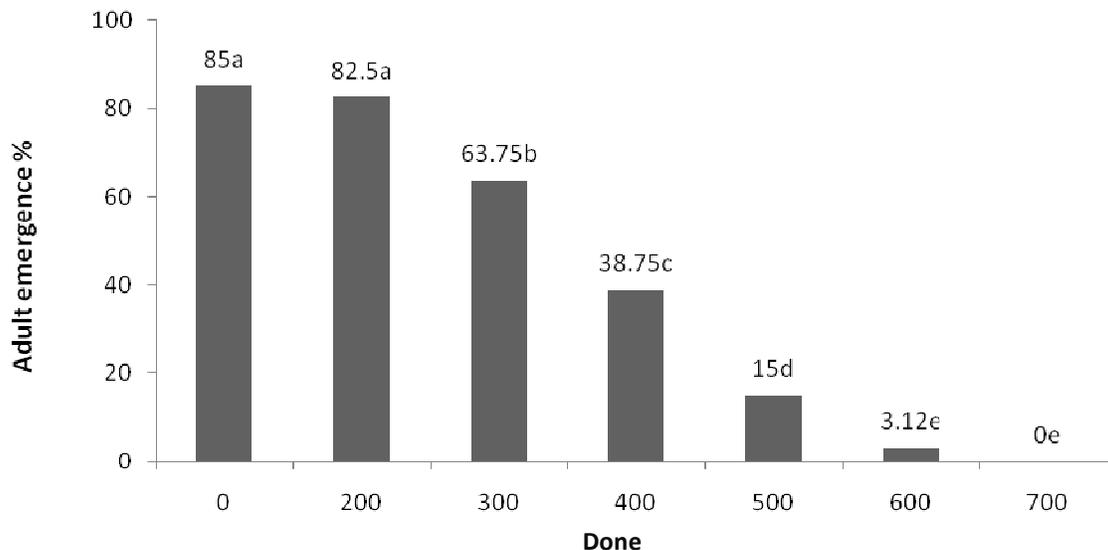
The effects of gamma radiation on irradiated adults are shown in Table 4. No mortality was recorded in the control for 21 days after treatment. Doses of 400-700 Gy caused 100% mortality within 14 days, whereas dose of 200 Gy caused 100% mortality 28 days after irradiation.

None of the dose levels were sufficient to cause

Table 3. Mean developmental period of *T. castaneum* adults irradiated as eggs, larvae and pupae ^a.

Dose (Gy)	Developmental period from eggs to adults (days)			Growth index ^b		
	Eggs treated	Larvae treated	Pupae treated	Eggs treated	Larvae treated	Pupae treated
0	45.30±0.30a	22.60±0.83a	6.50±0.34a	1.30a	3.16a	12.69a
25	45.70±0.21a	23.30±0.70ab	6.80±0.36ab	0.28b	3.06a	11.94ab
50	47.80±0.73b	24.10±0.92ab	7.20±0.47ab	0c	2.86b	11.28bc
100	49.40±0.76b	24.10±0.92ab	7.90±0.43bc	0c	2.12c	10.44c
200	-	24.90±0.77abc	8.60±0.45cd	-	0.68d	9.44d
300	-	25.30±0.77bc	9.10±0.46cd	-	0.09e	6.66e
400	-	26.80±0.53cd	9.20±0.44cd	-	0e	4.07f
500	-	28.60±1.11d	9.90±0.48d	-	0e	1.26g

Data are means ±S.E. of four replicates. ^a Means within each column followed by the same letter are not significantly different at 5% probability level. ^b Growth index: percentage adult eclosion/total developmental period.

**Figure 1.** Adult emergence of the *T. castaneum* irradiated as pupae (5-day old).

immediate mortality.

DISCUSSION

The results show that increasing radiation doses caused reduced egg viability, pupation and adult emergence when one- to two-day-old eggs were irradiated. The susceptibility of *T. castaneum* to irradiation varies as development progresses from egg to adult. In an early study, the development of adults reared on different flours from irradiated eggs and larvae was prevented by exposure to 40 and 50 Gy of gamma radiation, respectively (Tuncbilek and Kansu, 1996). It had been found that no irradiated eggs of *T. castaneum* hatched when eggs were exposed to dose of 200 Gy gamma ray (Zolfaghari et al., 2004). This difference between the present results and those from previous studies may

have been caused by differences in the age of the experimental eggs at the time of irradiation or differences in the strain of *T. castaneum* used. Radio sensitivity varies with the stage of embryologic development. Mature eggs of *T. castaneum* were more resistant to radiation than young eggs; development to adults was prevented at a dose of 0.08 kGy for one to two day old eggs but was not stopped for the three to four day old eggs at doses as high as 0.16 kGy (Guchangco, 2002). Mortality following irradiation of *Sitophilus granarius* (L.) eggs is relatively high and no adults emerged at doses of 30-500 Gy (Azemat et al., 2005). In the present study, a dose of 200 Gy completely prevented egg eclosion.

In the present study, a dose of 150 Gy completely prevented larval development and the larvae did not reach pupation. Tuncbilek and Kansu (1996) reported that larvae and adults of *T. confusum* were completely suppressed at 50 and 100 Gy, respectively. Mehta et al.

Table 4. Survivorship of irradiated 5-day old adults of *T. castaneum*.

Dose (Gy)	Percentage survival after treatment				
	2 day	7 day	14 day	21 day	28 day
0	100.00±0.00 ^a	100.00±0.00 ^a	100±0.00a	98.75±1.25 ^a	97.50±1.44 ^a
100	87.50±1.44 ^b	72.5±3.23 ^b	52.50±1.44 ^b	32.50±1.44 ^b	18.75±1.20 ^b
200	83.75±1.25 ^{bc}	58.75±2.39 ^c	17.50±1.44 ^c	8.75±2.39 ^c	0.00±0.0 ^c
300	81.25±2.39 ^c	47.5±1.44 ^d	5.00±1.02 ^d	1.25±.72 ^d	0.00±0.0 ^c
400	73.75±1.25 ^d	41.25±1.25 ^e	0.00±0.0 ^e	0.00±0.0 ^d	0.00±0.0 ^c
500	70.00±2.04 ^d	35.00±2.04 ^f	0.00±0.0 ^e	0.00±0.0 ^d	0.00±0.0 ^c
600	62.50±1.44 ^e	31.25±1.25 ^f	0.00±0.0 ^e	0.00±0.0 ^d	0.00±0.0 ^c
700	56.25±1.25 ^f	23.75±2.39 ^g	0.00±0.0 ^e	0.00±0.0 ^d	0.00±0.0 ^c

Data are means ±S.E. of four replicates. Means within each column followed by the same letter are not significantly different at 5% probability level.

(1990) found that a 6-Krad dose of gamma radiation administered to 10-day-old larvae of the stored products pest *T. castaneum* was sufficient to inhibit adult emergence completely; at 2-4 Krad prolonged the larval period and delayed pupation. Hu et al. (1985) have shown that irradiation of eggs and larvae of *T. castaneum* at doses above 41 Gy killed 100% before they reached the adult stage.

Gamma radiation doses caused extended developmental periods when immature stages of *T. castaneum* were irradiated with increasing doses. Dose-dependent developmental delay was also reported for the irradiated eggs and larvae of *T. castaneum* (Mehta et al. 1990). Increasing post-treatment longevity for irradiated *Plodia interpunctella* Hubner the false codling moth, *Cryptophlebia leucotreta* Meyrick, and the Mediterranean flour moth, *Ephestia kuehniella*, Zeller have been reported previously (Azemat et al., 2005; Johnson and Vail, 1988; Ayvaz et al., 2008). Typically, irradiated nymphs or larvae will have a prolonged nymphal or larval stage and may live longer than non-irradiated control insects (Hasan, 1999).

When the pupae of *T. castaneum* were irradiated, the percentage of adult emergence was decreased by doses up to 700 Gy, when male and female pupae of *Ephestia calidella* (Guenee) were irradiated with doses of 200-800 Gy, the percentage adult emergence decreased in accordance with gamma radiation doses (Boshra and Mikhael, 2006). Gochangco et al. (2002) reported that *T. castaneum* pupae were the most resistant stage to irradiation, and older pupae were more resistant than younger pupae. Development to adults was not prevented in both types of pupae to 0.16 kGy. The results of the present study confirmed the findings of other researchers.

In this study, the life span of irradiated adults was determined 28 days after treatment, and no adult survived at 200 Gy and above. Tuncbilek et al. (2003) reported that the longevity of the adults from most irradiated samples were significantly reduced compared with the control on day 30 and no adult survived doses of

100Gy and above. Ayvaz et al. (2002) found no *T. confusum* (Jacquelin du Val) adult living 30 days after irradiation at 140 Gy and above. Nakakita et al. (1985) reported complete mortality at 150 Gy for *T. castaneum* adults of the susceptible strain and at 200Gy for those of the resistant strain.

From the results of this study a dose of ≥200 Gy is sufficient to control the population growth of *T. castaneum* when targeting eggs, larvae and adults within stored products. However, a dose 700 Gy is needed to control the pupal stage.

REFERENCES

- Ahmed M (2001). Disinfestation of stored grain, pulses, dried fruits and nuts, and other dried foods. In: Molins R (ed.) Food Irradiation Principles and Applications, Wiley, New York, pp. 77-112.
- Ayvaz A, Albayrak S, Karaborklu S (2008). Gamma radiation sensitivity of the eggs, larvae and pupae of Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). J. Pest Manage. Sci., 64(5): 505-512.
- Ayvaz A, Ozturk F, Yaray K, Karahastioğlu E (2002). Effects of gamma radiation and Malathion on confused flour beetle, *Tribolium confusum*. Pakistan. Biol. Sci., 5(5): 560-562.
- Azemat K, Sayah F, Mouhib M, Ghailani N, Elgarrouj D (2005). Effects of gamma irradiation on forth-instar *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae). J. Stored Prod. Res., 41: 423-431.
- Boshra SA, Mikhael AA (2006). Effect of gamma radiation on pupal stage of *Ephestia calidella* (Guenee). J. Stored Prod. Res., 42: 457-467.
- Brower JH, Tilton EW (1983). The potential of irradiation as commodities. In: Moy JH (ed) Proceedings: Radiation disinfestation of food an agricultural products conference. Hawaii Institute of Tropical Agricultural and Human Research, University of Hawaii, pp. 75-86.
- Cornwell PW (1966). The Entomology of Radiation Disinfestation of Grain. Pergamon Press, Oxford, UK, p. 235.
- Guchangco MU, Sanjuan EM, Lster AO (2002). Irradiation as an alternative treatment to methyl bromide for disinfestations of *Tribolium castaneum* in stored cacao. Proceeding of a final research coordination meeting organized by the joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria, pp. 111-123.
- Hasan M (1999). Mating competitiveness of adult males of *Tribolium* spp (Coleoptera: Tenebrionidae) developing from irradiated pupae. J. Stored Prod. Res., 35: 307-316.
- Hansen LS, Jensen KMV (2002). Effect of temperature on parasitism

- and host-feeding of *Trichogramma turkestanica* (Hymenoptera: Trichogrammatidae) on *Ephesia kuhniella* (Lepidoptera: Pyralidae). *J. Econ. Entomol.*, 95: 50-56.
- Hu T, Tsai LT, Fu K, (1985). Gamma irradiation controls *Tribolium castaneum* Herbst in wheat flour. *Plant Protection Bulletin, Taiwan*, 27(4): 371-378.
- Johnson JA Vail PV (1988). Post treatment survival development and feeding of irradiated Indian meal moth and navel orangeworm larvae (Lepidoptera: Pyralidae). *J. Econ. Entomol.*, 81: 376-380.
- Lapidot M, Saveanu S, Padova R, Ross I (1991). Insect disinfestation by irradiation. *Insect Disinfestation of food and Agricultural Products by Irradiation. Proceedings IAEA, Vienna*, p. 103.
- Mehta VK, Sethi GR, Garg AK (1990). Effect of gamma radiation on the development of *Tribolium castaneum* after larval irradiation. *J. Nuclear Agric. Biol.*, 19(2): 124-127.
- Nakakita H, Hayashi T, Aoki S, Kawashima A (1985). Radiosensitivity of phosphin-resistant and susceptible strains of the red flour beetle, *Tribolium castaneum* Herbst. *J. Appl. Entomol. Zool.*, 29(3): 242-246.
- Rees D (2008). *Insects of Stored Products*. SBS publisher & distributor PVT. LTD. New Delhi, p. 181.
- SPSS (2001). *SPSS Version 10.0*. SPSS Inc, 233 S. Wacker Drive, Chicago, Illinois.
- Tuncbilek AS, Ayvaz A, Ozturk F, Kaplan B (2003). Gamma radiation sensitivity of larvae and adults of red flour beetle, *Tribolium castaneum* Herbst. *J. Pest Sci.*, 76: 129-132.
- Tuncbilek AS, Kansu IA (1996). The influence of rearing medium on the irradiation sensitivity of eggs and larvae of flour beetle, *Tribolium confusum*. *J. Stored Prod. Res.*, 32(19): 1-6.
- Watters FL (1968). An appraisal of gamma control irradiation for insect foods. *Manitoba Entomol.*, 2: 37-45.
- Zolfaghari HR, Bagheri-Zenouz E, Bayat-Asadi H, Mashayekhi Sh, Fatollahi H, Babaii M (2004). Application of Gamma Radiation for Controlling Important Store- Pests of Cereals, Pulses, and Nuts. *Iranian J. Agric. Sci.*, 35(2): 415-426.