GAMMA RADIATION SENSITIVITY OF DIFFERENT STAGES OF SAW-TOOTHED GRAIN BEETLE *ORYZAEPHILUS SURINAMENSIS* L. (COLEOPTERA: SILVANIDAE)

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Abstract: The effect of gamma irradiation on different developmental stages of *Oryzaephilus surinamensis* L., was investigated. Results showed that a required dose to prevent larval emergence from irradiated 1–2-days-old eggs was 60 Gray (Gy), and 350 Gy was required to prevent adult emergence from 15-days-old larvae. Also the required dose of radiation to prevent adult emergence from irradiated 5-days-old pupa was 700 Gy. The dose of 200 Gy caused 100% mortality of irradiated adults 28 days after treatment. In addition, the effect of gamma rays was studied on the developmental stage period of each irradiated stage till adult emergence. The results revealed that there was a dose-dependent increase of the developmental periods. The growth index of adults was significantly decreased with increasing dose of radiation administered to eggs, larvae and pupae. It is recommended that doses between 600 and 700 Gy should be used to control population growth of *O. surinamensis* when targeting pupae and adults present in stored products.

Key words: irradiation, preventing dose, developmental period, growth index, *Oryzaephilus surinamensis* L.

INTRODUCTION

Oryzaephilus surinamensis L., the saw-toothed grain beetle, is an important pest of stored cereals, particularly milled and processed products. It also occurs on a very wide range of other commodities including dried fruits, nuts and oilseeds (Rees 2008). Currently, control practices rely on scheduled fumigation with methyl bromide or hydrogen phosphide. However, methyl bromide was classified as an ozone depleter, and its use was recently 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 should be investigated. One such method, the use of gamma radiation for sterilization of pests, proved to be a technically feasible alternative to conventional methods for controlling stored-product insects (Cornwell 1966; Watters 1968).

Much research was conducted on the use of radiation to control storedproduct pests (Brower and Tilton 1983; Hasan and Khan 1998; Azelmate *et al*. 2005; Boshra and Mikaiel 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 (Brown and Tilton 1983). Lower doses of irradiation do not cause immediate death of adults but can prevent increase in pest populations through lethal effects on the immature stages and sterilization of adults (Brown and Tilton 1983). The advantages of irradiation as a pest control measure include the absence of undesirable residues in food products treated, no resistance development in pest insects and few significant changes in the physicochemical properties or a nutritive value of the treated products (Lapidot *et al*. 1991; Ahmed 2001). The present work focuses on determining minimal effective doses that prevent commodity damage caused by eggs, larvae and pupae of *O. surinamensis* L. The study was also designed to assess the effect of gamma irradiation on different biological stages of *O. surinamensis*.

MATERIALS AND METHODS

Insects

O. surinamensis used in all experiments were derived from a laboratory culture initially established from adults collected from infested rice in Urmia Province, Northwest IRAN. Adults were reared on a rearing medium composed of wheat flour and 5% brewers yeast (by weight) over several generations. Throughout the experiments, insect cultures were maintained under controlled laboratory conditions (27±1°C and 65±5% RH and continuous darkness).

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Irradiation of eggs

Eggs from *O. surinamensis* were obtained by placing 500–1 000 adult beetles in 500 g rearing medium. After 24 h eggs and adults were separated from flour by sieving through US standard sieves of 710 and 212 µm, respectively. Twenty eggs (1–2-days-old) 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 of 0.65 Gy/s at the Iran Nuclear Research center for Agriculture and Medicine. Ferric dosimetry system was used facility.

Radiation was applied at seven dose levels ranging from 0 to 60 Gy to the eggs. Control eggs were subjected to the same conditions as the irradiated eggs but 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 (2 cm diameter) with glue and allowing the glue to dry. Rearing medium was placed in a Petri dish around the central well to provide food for any hatched larvae, and the apparatus was placed in 27±1°C and 65±5% RH. 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 small larvae was then transferred to Petri dishes as food. Pupation and adult emergence of larvae were recorded. Four replicate batches of eggs were used for each dose level and the control.

Irradiation of 15-days-old *O. surinamensis* larvae

15-days-old larvae were selected for uniformity in size before irradiation. Larvae were irradiated in the same irradiator and dose ranges from 0–400 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 replicates (20 larvae for each replicate) were used for each dose and the control.

Developmental periods and longevity of *O. surinamensis* adults derived from irradiated eggs, larvae and pupae

The developmental periods from egg to adult of *O. surinamensis* that developed from irradiated eggs, larvae and pupae were recorded. The longevity of adults was also determined. The growth index (percentage adult emergence/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, 200, 300, 400, 500, 600 and 700 Gy. The number of adults that emerged from irradiated pupae were recorded. Four replicates (20 pupae in each) were used for each dose level.

Irradiation of adults

Five-day-old adults were placed in Petri dishes and irradiated at six dose levels of 0, 100, 200, 300, 400, 500 and 600 Gy. Four replications (20 individuals each) 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 SPSS for windows 11.5. Data were transformed using arcsine $\sqrt{x}$ before ANOVA. Means were separated at the 5% significance level (LSD).

RESULTS

Effects of gamma radiation on eggs

The data obtained from experiments dealing with irradiation of eggs are summarized in table 1. A significant reduction in egg hatch was observed and it was correlated with the radiation doses ($F = 103.765$; $df = 6$; $p < 0.05$). Percentage of larval emergence from irradiated eggs was 85% in the untreated control but was reduced to 6.25% at the dose of 50 Gy, and all irradiated eggs were completely sterile at 60 Gy (Table 1). The percentage of emerged larvae that survived to the pupa stage were also decreased with increasing radiation doses ($F = 114.066$; $df = 6$; $p < 0.05$). At doses of 40 and 50 Gy, pupal development was 15% and 2.5% respectively.

Table 1. Egg hatch, pupation, and adult emergence of the *O. surinamensis* irradiated as 1–2-days-old eggs

Dose [Gy]	Egg hatch [%] ±SE	Pupation [%] ±SE	Adult emergence [%] ±SE
0	85±2.89 a	72.50±1.44 a	62.50±1.44 a
10	70±3.54 b	57.50±4.33 b	51.25±2.39 b
20	52.5±1.44 c	43.75±2.39 c	37.50±2.50 c
30	27.5±3.23 d	18.75±2.39 d	13.75±1.25 d
40	22.50±1.44 d	15±2.04 d	10±2.04 d
50	6.25±2.39 e	2.5±1.44 e	0 e
60	0 e	0 e	0 e

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

The percentage of pupation that survived to the adult stage was also decreased with increasing radiation doses ($F = 307.153$; $df = 6$; $p < 0.05$). Percentage of adult emergence from irradiated eggs was 62.5% in the untreated controls but was reduced to 10% at the dose of 40 Gy and no adult emerged at the doses of 50 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. Recorded data showed that the percentage of irradiated larvae that survived to the pupal stage decreased with increasing radiation doses ($F = 178.914$; $df = 7$; $p < 0.05$). The percentages of pupae developed from irradiated larvae were 7.5% at 300 Gy, compared with 88.75% in the control (Table 2). The percentage of pupae that survived to the adult stage also decreased with increasing radiation doses ($F = 180.571$; $df = 7$; $p < 0.05$). Statistical analysis of data indicated that irradiation significantly affected mortality percentages of larvae ($F = 149.535$; $df = 7$; $p < 0.05$). Mortality of irradiated larvae began 4 days after treatment and continued through the time of observation (Table 2). Irradiation at 400 Gy caused 100% mortality after 45 days.

Effects of gamma radiation on the developmental period and longevity of adults of *O. surinamensis* derived from irradiated eggs, larvae and pupae

There was a dose-dependent increase in the developmental time of *O. surinamensis* adults developed from irradiated eggs ($F = 2.996$; $df = 2$; $p < 0.043$), larvae ($F = 4.00$; $df = 7$; $p < 0.001$) and pupae ($F = 8.127$; $df = 7$; $p < 0.05$) (Table 3). The mean developmental time from egg to adult was 48.00 days in the control, which rose to 48.50 days for insects derived from eggs irradiated with the dose of 50 Gy. The development of irradiated larvae was also extended from 25.70 days in the control to 31.30 days for insects exposed to the dose of 500 Gy. A mean development time from pupa to adult was 1.60 days in the control, which rose to 4.90 days at the dose of 500 Gy. The growth index of adults was significantly decreased with increasing doses of radiation administered to eggs ($F = 1141.967$; $df = 7$; $p < 0.005$), larvae ($F = 456.928$; $df = 7$; $p < 0.005$) and pupae ($F = 334.860$; $df = 7$ 4; $p < 0.005$) (Table 3).

Effects of gamma radiation on five-days-old pupae

Pupae showed more resistance to radiation than eggs or larvae. The adult emergence in the control was

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

Dose [Gy]	Pupation [%] ±SE	Adult emergence [%] ±SE	Mortality [%] ±SE
0	88.75±2.39 a	81.25±2.39 a	6.25±2.39 a
100	66.25±2.39 b	57.50±2.50 b	22.5±3.23 b
150	56.25±2.39 c	37.50±1.44 c	33.75±2.39 c
200	42.50±1.44 d	18.75±2.39 d	47.5±1.44 d
250	17.50±1.44 e	7.50±1.44 e	68.75±1.25 e
300	7.50±1.44 f	2.50±1.44 e	75.00±2.04 f
350	2.50±1.44 f	0 e	85.00±2.04 g
400	0 f	0 e	100 h

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

Table 3. Mean (±SE) developmental period of *O. surinamensis* 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	48.00±0.36 a	25.70±0.87 a	1.60±0.31 a	1.30 a	3.16 a	51.56 a
25	48.50±0.27 a	26.10±0.98 a	2.10±0.23 ab	0.28 b	3.06 a	38.69 b
50	49.30±0.62 ab	26.70±1.04 a	2.40±0.37 abc	0 c	2.86 b	33.85 c
100	–	27.10±0.92 ab	2.90±0.43 bcd	–	2.12 c	28.45 d
200	–	27.50±0.99 ab	3.60±0.45 cde	–	0.68 d	22.57 e
300	–	28.10±0.79 ab	4.10±0.46 def	–	0.09 e	14.63 f
400	–	29.90±0.87 bc	4.20±0.44 ef	–	0 e	8.92 g
500	–	31.30±1.15 c	4.90±0.48 f	–	0 e	2.55 h

[a] means followed by the same letter in a column within each dose are not significantly different ($p > 0.05$)
[b] growth index: percentage adult emergence/total developmental period

not significantly different from that at 200 Gy. A dose of 700 Gy completely prevented the development of pupae. The percentage of adults that emerged from irradiated pupae was significantly affected by the gamma radiation doses administered 5 days after pupation (F = 124.179; df = 6; $p < 0.05$) (Fig. 1).

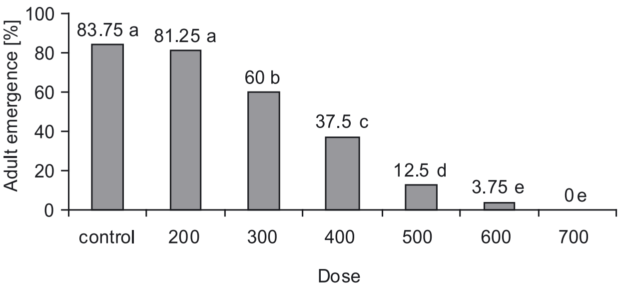

Fig. 1. Adult emergence of the *O. surinamensis* irradiated as 5-days-old pupae

Effects of gamma radiation on five-days-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 300–600 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 immediate mortality.

DISCUSSION

The results showed that increasing radiation doses caused reduced egg viability, pupation and adult emergence when 1-2-days-old eggs were irradiated. The susceptibility of *O. surinamensis* to irradiation varies as development progresses from egg to adult. Eggs and larvae were most susceptible to irradiation and no irradiated eggs and larvae reached the adult stage at applied doses of 0.1, 0.3, 0.5, 0.7 and 1.0 kGy; whereas, pupae and young adults were most tolerant (Aldrihim and Adam 1998). It had been found previously that no irradiated eggs of *O. surinamensis* hatched when eggs were exposed to the dose of 0.05 kGy gamma ray (Zolfagharieh 2004). 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. Ratio of sensitivity varies with the stage of embryologic development. Five-days-old eggs were more tolerant to irradiation than 1 and 4-days-old eggs. A dose of gamma irradiation of 8 krad (= 8 Gy) of *O. surinamensis* eggs revented the development of adults (Younes and Ahmed 2007). Mortality retarded following irradiation of *Sitophilus granarius* L. eggs is relatively high and no adults emerged at the doses of 30–500 Gy (Aldrihim and Adam 1999). In the present study, a dose of 60 Gy completely sterilized the eggs.

Table 4. Survival of irradiated five-day-old adults of *O. surinamensis* (mean ±SE)

Dose [Gy]	% survival after treatment				
	2 d	7 d	14 d	21 d	28 d
0	100±0.00 a	100.00±0.00 a	100±0.00 a	100±0.00 a	97.50±2.50 a
100	85.00±2.89 b	65.00±2.89 b	42.50±1.44 b	25.00±2.89 b	17.50±1.02 b
200	80.00±2.04 bc	55.00±2.89 c	7.50±1.44 c	2.50±1.44 c	0.0 c
300	77.50±1.44 cd	42.50±1.44 d	0.0 d	0.0 c	0.0 c
400	72.50±1.44 d	37.50±1.44 d	0.0 d	0.0 c	0.0 c
500	66.25±2.39 e	30.00±2.04 e	0.0 d	0.0 c	0.0 c
600	60.00±2.04 f	27.50±1.44 e	0.0 d	0.0 c	0.0 c

Means within each column followed by the same letter are not significantly different ($p > 0.05$); d – days

When the effects of five gamma radiation dosages, ranging from 100 to 1 000 Gy, were investigated for all life stages of *O. surinamensis*, eggs and larvae were unable to develop to adults at any dose, but pupae were able to reach adult stage at the doses of 0.1–1.0 kGy (Aldrihim and Adam 1998). In the present study, a dose of 400 Gy completely prevented larval development and the larvae did not reach pupation. Mortality was 100% for *O. surinamensis* larvae seven days after irradiation with a dose of 0.09 kGy (Zolfagharieh 2002). The minimum dose required to prevent adult emergence of *Plodia iterpunctella* from larvae was 300 Gy, while doses required to cause death were 450 Gy and above (Azelmat *et al.* 2005). A dose of 250 Gy was sufficient to cause complete mortality for last-instar larvae of *Ephestia kuehniella* Zeller (Ayvaz and Tuncbilek 2006). No codling moth larvae reached adulthood when larvae were irradiated with 200 Gy (Mansour 2003). Our results showed that, while the prevention of adult emergence is used as a standard for measuring the effectiveness of radiation against *O. surinamensis* larvae, a dose of 350 Gy is required. It was also found that the developed adults from *O. surinamensis* larvae were completely dead after 28 days, when they were exposed to 150 Gy (Zolfagharieh 2002). Tuncbilek and Kansu (1996) reported that larvae and adults of *Tribolium confusum* were completely suppressed at 50 and 100 Gy, respectively. Mehta *et al.* (1990) found that a 6-krad dose of gamma radiation administered to 10-days-old larvae of the stored product pest *T. castaneum*, was sufficient to inhibit adult emergence completely, and at 2-4 krad larval period delayed pupation when prolonged.

Gamma radiation doses caused extended developmental periods when immature stages of *O. surinamensis* 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 *P. interpunctella*, the false codling moth, *Cryptophlebia leucotreta* Meyrick, and the Mediterranean flour moth, *Ephestia kuehniella*, have been reported previously (Johnson and Vail 1988; Bloem *et al.* 2003; Azelmat *et al.* 2005; Ayvaz and Tuncbilek 2006). Typically, irradiated nymphs or larvae would have a prolonged nymphal or larval stage and might live longer than non-irradiated control insects (Hasan and Khan 1998).

When the pupae of *O. surinamensis* were irradiated, the percentage of adult emergence was decreased at doses up to 600 Gy. When male and female pupae of *Ephestia calidella* (Guenee) were irradiated with doses of 200–800 Gy, the percentage adult emergence was decreased in accordance with increasing gamma radiation doses (Boshra and Mikhaiel 2006). This work also showed that a 1 000 Gy dose prevented the emergence of both sexes.

No dosage used in this study was high enough to cause immediate 100% mortality of the adults of *O. surinamensis.* Similar results were obtained by Aldryhim and Adam (1998). Tuncbilek (1997) reported that, there were no beetles left alive and no progeny was produced when adults of *O. surinamensis* were exposed to 125 Gy, LD_{50} and $LD_{99.9}$ values were 55.6 and 216.4 Gy, respectively.

A dose of 60 Gy is required in order to prevent hatching the eggs and 350 Gy is required to prevent the larvae searching adulthood. It is recommended that doses of between 600 and 700 Gy should be used to control population growth of *O. surinamensis* when targeting pupae and adults within stored products. Delayed developmental periods were observed after irradiation of the eggs, larvae and pupae.

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POLISH SUMMARY

WRAŻLIWOŚĆ RÓŻNYCH STADIÓW ROZWOJOWYCH CHRZĄSZCZA *ORYZAEPHILUS SURINAMENSIS* L. (COLEOPTERA: SILVANIDAE) NA NAŚWIETLANIE PROMIENIAMI GAMMA

Badano efekt naświetlania promieniami gamma różnych stadiów rozwojowych *Oryzaephilus surinamensis* L. Wyniki wykazały, że dawką promieniowania wystarczającą do powstrzymania wylęgu larw z 1–2-dniowych jaj było 60 gray (Gy), a 350 Gy było potrzebne do zapobieżenia wylęgu formy dorosłej z 15-dniowych larw. Również wymagana dawka promieniowania do zapobieżenia wylęgu form dorosłych z naświetlanych 5-dniowych poczwarek wynosiła 700 Gy. Dawka 200 Gy powodowała 100% śmiertelności naświetlanych osobników dorosłych, w 28 dni po zabiegu. Dodatkowo badano działanie promieni gamma na czas trwania każdego ze stadiów, aż do ukazania się osobników dorosłych. Wyniki wykazały, że czas rozwoju tych form był zależny od dawek promieniowania. Wskaźnik wzrostu osobników dorosłych znacznie zmniejszał się wraz ze wzrostem dawki promieniowania zastosowanej na jaja, larwy i poczwarki. Zalecane zabiegi przeciwko wzrostowi populacji *O. surinamensis* obecnej w przechowywanych produktach powinny uwzględniać dawki pomiędzy 600 i 700 Gy.