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Research Article

Effect of gamma irradiation on reproduction biology, inherited sterility and mating competitiveness of *Spodoptera exigua* (Lepidoptera: Noctuidae) under laboratory conditions

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Abstract: The beet armyworm, *Spodoptera exigua* Hübner, poses a significant threat affecting sugar beet and various field crops and vegetables. AS larvae can devastate entire plants, it is crucial to implement effective and safe control measures to prevent economic losses. However, traditional chemical control methods disrupt ecosystem balance and contribute to the development of insecticide resistance. This study explores the potential of the sterile insect technique (SIT) and inherited sterility to manage *S. exigua* through the irradiation of pupae with Co60 gamma rays. Key factors such as reproduction, sterility, growth biology, sex ratio, mating competitiveness, and inherited sterility in both parent (P1) and F1 generations were assessed. Notably, irradiation of pupae at 250 Gy and 400 Gy resulted in sterile P1 males and females, respectively. While female emergence significantly decreased at 400 Gy, their longevity increased with higher doses. When P1 male or female pupae were irradiated at 250 Gy or 180 Gy, respectively, the developmental period of F1 larvae extended beyond that of the control group. Additionally, F1 male emergence declined with increasing irradiation doses. Crossbreeding treated male parents with normal females resulted in a sex ratio of F1 progeny skewed in favor of males. The F1 generation exhibited greater sterility compared to the P1 generation, with F1 males demonstrating a higher level of sterility than F1 females. Laboratory assessments of mating competitiveness revealed that males irradiated at 250 Gy (IM) successfully competed against untreated males (UM) for mating with untreated females (UF), even at a 1:1: 1 ratio of IM:UM:UF.

Keywords: Beet armyworm, Co^{60} Gamma ray, F_1 sterility, Radiation biology, Sterile insect technique

Introduction

The beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), is a polyphagous insect that feeds on a variety of crops, including vegetables, field crops, and ornamental plants. This pest has become a severe pest of concern worldwide and can destroy the entire plant due to larvae's serious feeding on the leaves, which leads to a significant reduction in the harvest.

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Insecticides are extensively used to control *S. exigua*, and resistance to many insecticides has resulted in control failure. Resistance to conventional insecticides (endosulfan, organophosphates, carbamates, pyrethroids, spinosad, abamectin, emamectin benzoate, indoxacarb, lufenuron, and methoxy fenozide, etc.) has been recorded (Che *et al.*, 2013).

Therefore, more effective and environmentally friendly control tactics such as the sterile insect technique (SIT) and inherited sterility (IS), which are the autocidal pest control methods, offer great potential. The advantages of these techniques include species specificity compatibility with other integrated pest management methods such as mating disruption, biological control, cultural control methods, and biopesticides (Vreysen *et al.*, 2016). The sterile insect technique (SIT) is a biological pest control method in which sterile insects are injected in large quantities over a wide area to reduce the fertility of the field population of the same species. SIT involves rearing many target pest species, exposing them to gamma or X-ray radiation to induce sexual sterility, and then releasing them on a large scale into the target pest population. The released sterile males mate with wild females to prevent them from reproducing. The possibilities for using SIT against lepidopteran pests are different. In Lepidoptera, some dominant lethal mutations can be expressed just before hatching, but most broken chromosomes are passed on to the F_1 generation. This phenomenon is especially true when debilitating low doses cause inherited sterility. Releasing congenitally infertile males can suppress wild populations more than releasing the same number of completely infertile males (Dyck *et al.*, 2021). The 'Inherited or F_1 sterility technique' (IS), using sub-sterilized male moths, has been widely proposed as a pest control against lepidopteran pests (Angmo *et al.*, 2023a).

Availability of primary data related to irradiated insects, ideally within two years before intervention, is one of the prerequisites of SIT programs (Bourtzis and Vreysen 2021). The success of SIT in controlling some other pests was previously evaluated in Iran, like Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Ahmadi *et al.*, 2021) and old-world bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Nuctuidae) (Osouli *et al.*, 2021). The effects of sterilizing and sub-sterilizing doses of gamma rays have been investigated on *S. exigua* in the distant past (El-Badry *et al.*, 1971; Debolt and Wright, 1976), and there is various research on similar species of *Spodoptera*; the Egyptian cotton leafworm, *S. littoralis* Boisd. (Kiyoku and Tsukuda, 1970; Hosny *et al.*, 1972; Wakid *et al.*, 1972; Wakid and Hayo, 1974; Abass *et al.*, 2017; Hassan *et al.*, 2017); the cotton leafworm *S. litura* F. (Mochida and Miyahara, 1974; Seth and Sharma, 2001; Ramesh *et al.*, 2002; Seth *et al.*, 2016; Yadav *et al.*, 2017; Jiang *et al.*, 2022; Sengupta *et al.*, 2022; Angmo *et al.*, 2023 a,b); the fall armyworm *S. frugiperda* J. E. Smith (Jiang *et al.*, 2023). In this research, extensive radiobiological investigations of the developmental and reproductive biology of *S. exigua* were conducted. This research aimed to find the appropriate radio-sterilization dose for females to release with sub-sterile males to perform IS beside SIT effectively. Additionally, the mating competitive ability of irradiated males was assessed using Fried's (1971) formula, and the corresponding laboratory release rate of treated males was determined. According to our previous study (Ashouri *et al.*, 2023), the pupal period of *S. exigua* on studied diets was somehow longer in males (7.65 to 8.12 days) than in females (8.52 to 8.56 days). Therefore, the six-day-old pupa was selected for irradiation a day before the emergence of male adults.

Materials and Methods

Insect rearing

Spodoptera exigua larvae were collected from a sugar beet field, transported to the laboratory with host plant leaves, and kept individually in 50 mL plastic cups on beet leaves until pupation. Adult moths were released into egg-laying boxes. These oviposition boxes were transparent plastic containers (8.5 cm diameter, 6.5 cm height) with air-permeable caps wrapped with a white polypropylene non-woven filter cloth to facilitate egg laying. They contained cotton balls soaked with 10% sucrose solution in the lid of a bottle for adult feeding. Larvae were reared on an artificial diet as described by Ashouri *et al.* (2023) for three generations at 27 ± 1 ºC, 30 ± 5% relative humidity, and photoperiod 16:8 (L:D) h.

Irradiation

Six-day-old pupae were harvested from the rearing containers. Male and female pupae were separated, according to Bandoly and Steppuhn (2016). Thirty female and 35 male pupae were transferred to different Petri dishes (100 mm × 15 mm) and treated with 60, 120, 180, 250, and 400 Gy doses gamma radiation. These doses were selected according to preliminary studies that included a wide dose range (30, 60, 90, 120, 150, 180, 200, 250, 300, 350, and 400 Gy). The irradiation was performed at the Nuclear Agriculture School, Nuclear Science and Technology Research Institute, Karaj, Iran. Gamma rays from a Co^{60} 265 curie (Gamma cell Issledovatle PX30) at the dose rate of 160 Gy/h irradiated the pupae at ambient air temperature with the Fricke dosimeter. This process was conducted in four replications. Irradiated male and female pupae were transferred individually into separate transparent plastic containers with moistened cotton balls and 10% sucrose solution until the emergence of adult.

Adults emergence of parental generation

The percentage of emerged male and female moths was calculated after two days of radiation. Unenclosed pupae and emerged adults with unflyable wings were considered pupal mortality.

Reproductive biology of parental generation

The effects of different doses of gamma rays were evaluated on the fecundity and fertility of emerged adults. For these trials, the enclosed adults were paired (5 males, 5 females, and four replications for every dose) in oviposition boxes in the following four combinations:

Unirradiated Female (UF) × Unirradiated Male (UM)

Unirradiated Female (UF) × Irradiated Male (IM)

Irradiated Female (IF) × Unirradiated Male (UM)

Irradiated Female (IF) × Irradiated Male (IM)

The non-woven filter cloths were collected daily, and the egg batches were brushed into 50 mL plastic cups labeled with the replication number and date. The number of eggs per female and the number of hatched eggs were recorded daily for all replications. This process was continued until the last female died. Additionally, the percentage of sterility index was calculated according to equation (1) reported by Toppazada *et al.* (1966):

$$\%\ \text{Sterility} = 100 - \left(\frac{a \times b}{A \times B} \times 100\right) \quad (1)$$

Where:

a: number of eggs per female in treatment,

b: % hatching eggs in treatment,

A: number of eggs per female in control,

and B: % hatching eggs in control.

Adults longevity of parental generation

The longevity of male and female adults was recorded daily until the last moth died. These two sexes were distinguished by the shape of the last segment of the abdomen, the male's narrow abdomen versus the female's bloated abdomen.

Developmental periods of F_1 immature stages, adult emergence, and sex ratio

The hatched eggs of each trial were reared to measure the developmental periods of larval and pupal stages. For this purpose, the eggs laid by the females in each combination were divided into four replicates of 25 hatched individuals for each dose and individually fed by a piece of artificial diet daily. The day when each larva turned into a pupa, and each pupa turned into an adult were recorded. At the pupal stage, the sex ratio of the F_1 generation was determined. Finally, the percentage of adult emergence was calculated.

Reproductive biology of F_1 generation

The effects of four different sub-sterilizing doses of gamma rays (60, 120,180, and 250 Gy) were evaluated on the number of eggs (fecundity) and the percentage of hatched eggs (fertility) of F_1 adults that emerged from irradiated male parents. For this purpose, five emerged male or female F_1 adults with an equal number of normal pairs of the same age (1:1) were placed in egg-laying boxes. All collected eggs per female were counted, and the hatching percentage was calculated until the last female died. Finally, the rate of sterility index was evaluated.

Male mating competitiveness

The effect of 250 Gy gamma ray on the mating competitiveness of irradiated males was evaluated at 1:1:1, 3:1:1, 5:1:1, 10:1:1, 15:1:1 and 20:1:1 (irradiated male (IM): unirradiated male (UM): unirradiated female (UF), respectively) ratios compared with 0:1:1 and 1:0:1 ratios as controls (1 in all ratios is three individuals). For this purpose, the 6-day-old male pupae were placed in Petri dishes and irradiated with 250 Gy. The emerged male adults were combined with normal males and females according to the designated ratio. The number of eggs laid and the hatching rate were determined. The competitiveness values of the irradiated males were calculated according to Fried's (1971) formula based on this basic assumption that all factors that influence competitiveness will be reflected in egg hatching, when the normal and the treated males compete for mates. Expected egg-hatch (E) was calculated using equation (2) (Fried, 1971):

(2) $$E = \frac{N(Ha)+S(Hs)}{N+S}$$

Where:
E: Expected percentage of egg-hatch,
Ha: Percentage of hatched eggs laid by unirradiated females mated with unirradiated males,
Hs: Percentage of hatched eggs laid by unirradiated females mated with irradiated males,
N: The number of unirradiated males,
and S: The number of irradiated males.

The observed egg resulted from the mating experiments using various ratios of irradiated to normal males mated with unirradiated females. The chi-square test was used to compare the observed and the expected egg- hatch at each dose. Competitiveness value (CV) based on the data of hatched eggs was calculated using the following equation (3) developed by Fried (1971):

(3) $$CV = \frac{Ha-E}{E-Hs} \div \frac{S}{N}$$

Where:
E: Egg-hatch in competitiveness mating,
and S/N: The ratio of sterile to normal males.

Data analysis

The effects of radiation on the biological and reproductive parameters of P_1 and F_1 generations were assessed by a one-way ANOVA. A completely randomized design with four replications was used. The mean values were compared using Duncan's multiple-range test. The data were analyzed via SPSS version 22.0. (IBM ©) software. Microsoft Excel was used for creating graphs.

Results

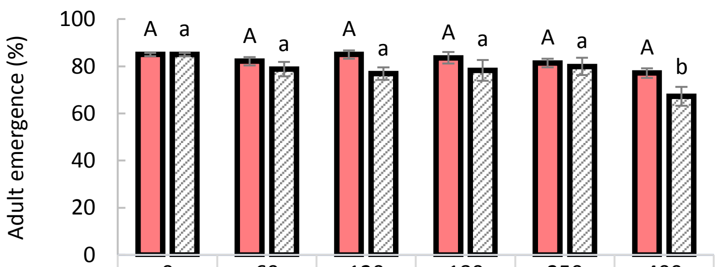
Irradiation of 6-day-old male and female pupae with different doses of gamma rays significantly reduced adults' fecundity and fertility, and increased sterility rates (Table 1). This significant reduction in the number of eggs per female was observed in all three combinations: IM × UF ($F_{(5,23)}$ = 14.189, $P < 0.01$), UM × IF ($F_{(5,23)}$ = 152.181, $P < 0.01$), and IM × IF ($F_{(5,23)}$ = 141.226, $P < 0.01$). Additionally, the percentage of hatching eggs was diminished in all three combinations: IM × UF ($F_{(5,23)}$ = 89.289, $P < 0.01$), UM × IF ($F_{(5,23)}$ = 95.602, $P < 0.01$), and IM × IF ($F_{(5,23)}$ = 161.362, $P < 0.01$). The decrease in the egg number and hatchability induced by gamma radiation were positively correlated with the dose level. These two parameters were used to calculate sterility, which was increased significantly by increasing the gamma dose in all three combinations: IM × UF ($F_{(4,19)}$ = 67.447, $P < 0.01$), UM × IF ($F_{(4,19)}$ = 44.52, $P < 0.01$), and IM × IF ($F_{(4,19)}$ = 17.239, $P < 0.01$). In P_1 generation, differences in radiosensitivity between males and females were evident. The fully sterile female and male were observed at 250 and 400 Gy treatments, respectively. However, no significant differences between 250 and 400 Gy treatments were found in all combinations and reproductive parameters.

The emergence of P_1 adults (Fig. 1) and their longevity (Fig. 2) from irradiated fully grown pupae were recorded. The pupal irradiation did not significantly affect the male adult emergence ($F_{(5,23)} = 2.558$, $P = 0.064$). However, the female adult emergence after irradiation at 400 Gy was 67.27%, different from the control rate of 85%, indicating a significant decrease ($F_{(5,23)} = 3.348$, $P = 0.026$). The male's longevity was unaffected by radiation exposure ($F_{(5,23)} = 0.118$, $P = 0.987$), whereas females longevity was prolonged by increasing radiation dose, significantly ($F_{(5,23)} = 6.213$, $P < 0.01$) (Fig. 2).

Table 1 Fecundity, fertility and sterility (Mean ± SE) of parental generation of *Spodoptera exigua* adults that emerged from pupae irradiated at different gamma-ray doses (Gy).

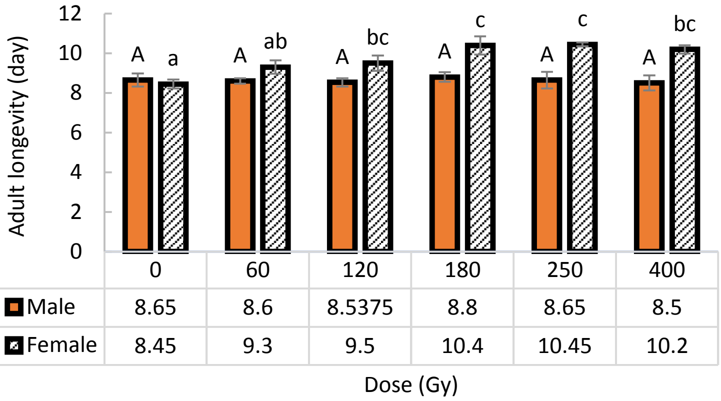
Combination	Dose (Gy)	Eggs/Female ± SE	Egg hatch (%) ± SE	Sterility (%) ± SE
IM × UF	0	517.05 ± 18.54 a	75.44 ± 5.00 a	
	60	505.30 ± 18.75 a	45.08 ± 4.09 b	41.70 ± 5.46 a
	120	355.65 ± 25.53 b	28.25 ± 3.13 c	74.39 ± 2.80 b
	180	344.80 ± 27.15 b	13.24 ± 1.56 d	88.36 ± 1.66 c
	250	344.10 ± 23.79 b	3.12 ± 1.37 e	97.26 ± 1.20 d
	400	349.55 ± 18.57 b	0.00 ± 0.00 e	100 ± 0.00 d
UM × IF	0	517.05 ± 18.54 a	75.44 ± 5.00 a	
	60	312.35 ± 9.59 b	39.13 ± 4.97 b	68.52 ± 4.44 a
	120	239.30 ± 13.77 c	19.05 ± 1.78 c	88.49 ± 0.39 b
	180	133.70 ± 12.77 d	3.70 ± 1.74 d	98.59 ± 0.77 c
	250	100.75 ± 16.85 de	0.00 ± 0.00 d	100 ± 0.00 c
	400	57.75 ± 8.33 e	0.00 ± 0.00 d	100 ± 0.00 c
IM × IF	0	517.05 ± 18.54 a	75.44 ± 5.00 a	
	60	286.80 ± 10.59 b	12.86 ± 2.62 b	90.51 ± 2.06 a
	120	223.85 ± 15.11 c	4.09 ± 1.06 c	97.56 ± 0.77 b
	180	162.25 ± 10.35 d	0.29 ± 0.16 c	99.88 ± 0.07 b
	250	110.10 ± 15.58 e	0.00 ± 0.00 c	100 ± 0.00 b
	400	95.35 ± 2.86 e	0.00 ± 0.00 c	100 ± 0.00 b

U: unirradiated, I: irradiated, M: male, and F: female.
Means followed by the different letters in each separate column are significantly different (Duncan's multiple-range test, $P < 0.01$).



	0	60	120	180	250	400
Male	84.995	82.14286	85	83.57143	81.42857	77.14286
Female	84.995	78.78788	76.89197	78.25758	79.92424	67.27273

Figure 1 Male and female adult emergence of *Spodoptera exigua* from irradiated pupae with different gamma radiation doses (Gy). Means followed by the same uppercase or lowercase letters in each separate column are not significantly different (Duncan's multiple-range test, $P < 0.05$).



	0	60	120	180	250	400
Male	8.65	8.6	8.5375	8.8	8.65	8.5
Female	8.45	9.3	9.5	10.4	10.45	10.2

Figure 2 Male and female adult longevity (day) of *Spodoptera exigua* resulting from irradiated pupae with different gamma radiation doses (Gy). Means followed by the same uppercase or lowercase letters in each separate column are not significantly different (Duncan's multiple-range test, $P < 0.05$).

The biology and sterility of male and female's progeny (F_1 generation) were investigated using sub-sterilizing doses. The progeny of male parents treated at 60, 120, 180, and 250 Gy and female parents treated at 60, 120, and 180 Gy, emerged as adults. Larval and pupal periods and adult emergence were recorded for each sex (Table 2). The developmental time of male larvae was prolonged significantly only at 250 Gy in the IM × UF combination ($F_{(4,19)} = 7.584$, $P < 0.01$), and also at 180 Gy in the UM × IF combination ($F_{(3,15)} = 6.158$, $P < 0.01$). The female larval duration was increased only at 250 Gy in the IM × UF combination ($F_{(4,19)} = 4.452$, $P < 0.05$), and no significant difference was seen in the UM × IF combination ($F_{(3,15)} = 1.4$, $P = 0.291$). The pupal duration of male and female did not change significantly in the IM × UF combination ($F_{(4,19)} = 1.08$, $P = 0.398$; $F_{(4,19)} = 1.516$, $P = 0.248$, respectively), also in UM × IF combination ($F_{(3,15)} = 0.502$, $P = 0.688$; $F_{(3,15)} = 1.688$, $P = 0.222$, respectively). The survival to adulthood decreased with the increasing dose, but in the case of females, differences were not significant in both the IM × UF and UM × IF combinations ($F_{(4,19)} = 1.592$, $P = 0.228$; $F_{(3,15)} = 2.529$, $P = 0.107$, respectively). The percentage of male adult emergence showed significant differences in both the IM × UF and UM × IF combinations ($F_{(4,19)} = 4.691$, $P < 0.05$; $F_{(3,15)} = 24.766$, $P < 0.01$, respectively).

The sex ratio (number of male pupae per total number of pupae) in these two combinations was calculated (Fig. 3). The F_1 adults sex ratio in the IM × UF combination was altered in favor of males, significantly ($F_{(4,19)} = 31.321$, $P < 0.01$). However, the sex ratio in the UM × IF combination was normal, and no significant differences were seen ($F_{(3,15)} = 0.753$, $P = 0.542$).

This research aimed to generate more competitive and sterile F_1 insects that could be used to control this pest. Male and female F_1 adults were obtained from the IM × UF combination in P_1 in which parent males had been irradiated at 60, 120, 180, and 250 Gy. These F_1 adults were mated with unirradiated pairs. Fecundity, fertility, and sterility rates of F_1 were recorded (Table 3). There was a significant reduction in the number of eggs per female in the F_1M × UF combination ($F_{(4,19)} = 28.45$, $P < 0.01$) and the UM × F_1F combination ($F_{(4,19)} = 71.79$, $P < 0.01$). Furthermore, the percentage of hatched eggs was reduced significantly in the F_1M × UF combination ($F_{(4,19)} = 111.62$, $P < 0.01$) and the UM × F_1F combination ($F_{(4,19)} = 177.09$, $P < 0.01$). The reduction in both parameters was positively correlated with the dose level. Consequently, sterility was improved significantly by increasing gamma doses in the F_1M × UF combination ($F_{(3,15)} = 12.207$, $P < 0.01$) and the UM × F_1F combination ($F_{(3,15)} = 104.287$, $P < 0.01$). No significant differences between 180 and 250 Gy treatments were found in the two combinations and three reproductive parameters of F_1 generation. Generally, F_1 sterility was higher than P_1 sterility, and irradiation impact was more pronounced in F_1 males than in F_1 females, reflecting that F_1 males inherited more sterility than F_1 females.

Table 2 The male and female larvae and pupae duration and adult emergence (Mean ± SE) of *Spodoptera exigua* F_1 progeny resulting from irradiated P_1 pupae with different sub-sterilizing gamma radiation doses (Gy).

Combination	Dose (Gy)	Larval period (d) ± SE		Pupal period (d) ± SE		Adult emergence (%) ± SE	
		♂	♀	♂	♀	♂	♀
IM × UF	0	13.15 ± 0.03 b	13.06 ± 0.12 b	8.00 ± 0.13 a	6.81 ± 0.07 a	92.42 ± 0.28 a	91.29 ± 3.41 a
	60	13.68 ± 0.12 b	13.57 ± 0.13 b	7.91 ± 0.80 a	7.05 ± 0.15 a	86.31 ± 4.08 ab	84.79 ± 4.72 a
	120	13.68 ± 0.32 b	13.21 ± 0.18 b	8.17 ± 0.33 a	6.97 ± 0.18 a	77.98 ± 1.58 bc	88.18 ± 5.66 a
	180	13.60 ± 0.08 b	13.78 ± 0.37 b	7.92 ± 0.13 a	7.00 ± 0.18 a	81.54 ± 4.78 abc	83.04 ± 6.42 a
	250	14.46 ± 0.15 a	14.86 ± 0.61 a	8.13 ± 0.16 a	7.32 ± 0.14 a	71.10 ± 5.28 c	75.30 ± 2.81 a
UM × IF	0	13.15 ± 0.03 b	13.06 ± 0.12 a	8.00 ± 0.13 a	6.81 ± 0.07 a	92.42 ± 0.28 a	91.29 ± 3.41 a
	60	13.57 ± 0.25 b	13.76 ± 0.18 a	7.98 ± 0.22 a	7.04 ± 0.16 a	91.05 ± 1.79 a	88.18 ± 6.98 a
	120	13.44 ± 0.35 b	13.55 ± 0.36 a	7.77 ± 0.09 a	6.74 ± 0.06 a	86.36 ± 4.49 a	90.83 ± 4.11 a
	180	14.55 ± 0.23 a	13.72 ± 0.35 a	8.06 ± 0.24 a	6.92 ± 0.08 a	65.00 ± 1.67 b	76.25 ± 0.16 a

U: unirradiated, I: irradiated, M: male, and F: female.
Means followed by the different letters in each separate column are significantly different (Duncan's multiple-range test, $P < 0.01$).

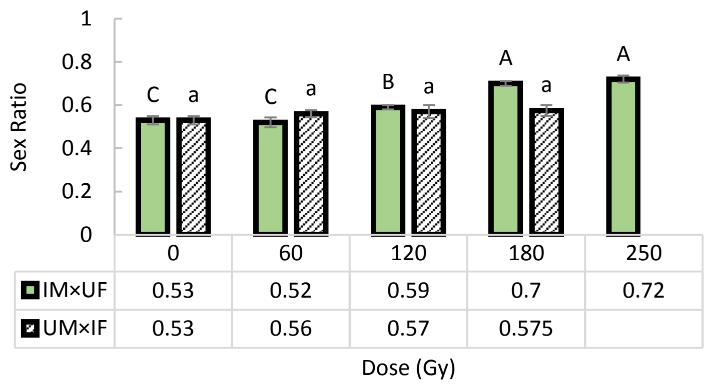


Figure 3 Sex ratio (proportion of male pupae to all pupae) of *Spodoptera exigua* F_1 progeny resulting from irradiated P_1 pupae with different gamma radiation doses (Gy). U: unirradiated, I: irradiated, M: male, and F: female. Means followed by the same uppercase or lowercase letters in each separate column are not significantly different (Duncan's multiple-range test, $P < 0.05$)

Table 3 Fecundity, fertility and sterility (Mean ± SE) of *Spodoptera exigua* F_1 adults resulting from irradiated P_1 male pupae with different sub-sterilizing gamma ray doses (Gy).

Combination	Dose (Gy)	No. of Eggs/Female ± SE	Egg hatch (%) ± SE	Sterility (%) ± SE
F_1M × UF	0	478.00 ± 19.00 a	70.15 ± 2.54 a	
	60	341.90 ± 23.85 b	30.71 ± 5.63 b	67.52 ± 8.64 b
	120	342.5 ± 16.28 b	5.06 ± 0.96 c	94.87 ± 0.96 a
	180	271.75 ± 4.13 c	2.05 ± 0.89 c	98.31 ± 0.73 a
	250	244.10 ± 15.16 c	0.00 ± 0.00 c	100.00 ± 0.00 a
UM × F_1F	0	478.00 ± 19.00 a	70.15 ± 2.54 a	
	60	346.00 ± 5.32 b	43.12 ± 2.10 b	55.55 ± 2.81 c
	120	351 ± 8.29 b	16.74 ± 2.62 c	82.58 ± 2.55 b
	180	213.1 ± 18.12 c	3.30 ± 0.71 d	97.99 ± 0.30 a
	250	216.5 ± 7.89 c	4.42 ± 1.25 d	97.17 ± 0.78 a

U: unirradiated, M: male, and F: female.
Means followed by the different letters in each separate column are significantly different (Duncan's multiple-range test, $P < 0.01$).

According to the P_1 and F_1 results, 250 Gy was determined as the appropriate dose for sterility. Therefore, the mating competitiveness of male moths emerging from gamma-irradiated pupae at 250 Gy was evaluated. Conferring Fried's CV values, treated males were completely competitive against normal males at ratios 1:1:1, 3:1:1, 10:1:1, 15:1:1, and 20:1:1 (IM: UM: UF), nevertheless the mating competitiveness of the sterile males was less than normal males at ratio 5:1:1 (Table 4). The number of eggs laid by untreated females mated with treated males in the various released ratios showed a significant reduction compared to the controls ($F_{(7,31)} = 9.918$, $P < 0.01$). The percentage of observed egg hatching significantly decreased in various ratios of the treated males compared to the control ($F_{(7,31)} = 50.425$, $P < 0.01$). According to the chi-square test, the observed values of egg hatch were significantly less than those of the expected ones at 1:1:1, and 3:1:1 ratios. However, at 5:1:1 ratio, the observed egg hatch was significantly greater than the expected one. In contrast, at ratios 10:1:1, 15:1:1, and 20:1:1 no significant differences were seen between observed and expected egg hatch percentage. Therefore 1:1:1 ratio was an appropriate ratio of treated male to unirradiated ones released in the experimental situation.

Discussion

The objective of this research was to investigate the effects of gamma radiation on developmental biology, finding appropriate male and female sterilizing doses and sub-sterilizing doses of males to effectively perform the inherited sterility technique for *S. exigua* beside SIT. Also, the best releasing ratio was evaluated in laboratory conditions.

Hassan *et al.* (2017) found that 300 Gy could be the sterile dose for *S. littoralis*. Ramesh *et al.* (2002) indicated that when male pupa of *S. litura* was treated with 70 Gy gamma-ray and paired with unirradiated female, it resulted in a 10.1% reduction in oviposition and 37.24% decline in fertility. Sengupta *et al.* (2022) noted a decrease in fecundity and fertility of *S.litura* with increasing radiation dose (100–200 Gy) compared to control. They identified 130 Gy for females and 200 Gy for males as a suitable sterilization dose. Our research found completely sterile females and males at 250 and 400 Gy, respectively.

Ramesh *et al.* (2002) found that after irradiating pupae with 70 Gy, *S. litura* matured by 75.5% compared to 83.3% in control. Hassan *et al.* (2017) stated that the sub-sterilizing doses did not affect the adult emergence of *S. littoralis*. Still, the reduction in the appearance of adults was significant at the sterilizing dose. However, in current research, only female emergence at 400 Gy treatment was significantly reduced compared to the control. Also, the longevity of female adults was extended when radiation doses increased.

Table 4 Number of eggs per female, observed and expected egg hatch (Mean ± SE) and competitiveness indexes of *Spodoptera exigua* irradiated as male pupae with 250 Gy gamma ray at different releasing ratios of irradiated to unirradiated males.

Ratio (IM: UM: UF)	Eggs/Female ± SE	Observed egg hatch (%)	Expected egg hatch (%)	χ^2 (df = 3)	CV
0:1:1	441.67 ± 16.69 a	59.25 ± 0.79 a			
1:1:1	307.83 ± 38.73 b	19.09 ± 3.53 c	33.23	28.84^{*}	3.38
3:1:1	315.75 ± 19.01 b	19.22 ± 4.16 c	20.21	10.13^{*}	1.11
5:1:1	329.00 ± 82.70 b	27.62 ± 3.57 b	15.88	46.02^{*}	0.31
10:1:1	201.33 ± 4.81 c	11.87 ± 1.46 cd	11.93	2.44^{ns}	1.01
15:1:1	159.17 ± 16.69 c	7.73 ± 1.49 d	10.45	5.95^{ns}	6.42
20:1:1	120.50 ± 13.43 c	7.90 ± 0.72 d	9.68	1.82^{ns}	3.66
1:0:1	365.50 ± 15.25 ab	7.20 ± 0.38 d			

U: unirradiated, I: irradiated, M: male, and F: female.

χ^2 values- asterisk (*) indicates a significant difference between observed and expected egg hatch at $P < 0.05$; CV: competitiveness value (Fried, 1971); Means followed by the different letters in each column are significantly different (Duncan's multiple-range test, $P < 0.01$); 1 in all ratios is three individuals.

There may be a relation between reduced fertility and longer life expectancy in females exposed to gamma irradiation. In other words, irradiated females lay fewer fertile eggs and live longer than control.

Abass *et al.* (2017) and Hassan *et al.* (2017) found that the larval and pupal periods of S.*littoralis* were significantly prolonged by gamma irradiation. Furthermore, Seth and Sharma (2001) claimed that F_1 progeny of *S. litura* developed more slowly than the control. In the current study, when P_1 male pupae were irradiated with 250 Gy, the F_1 male and female larval duration was longer than that of normal larvae, and when P_1 female pupae were irradiated with 180 Gy, only the F_1 male larval duration was longer than the normal larvae. Nevertheless, their F_1 pupal periods did not affect them significantly.

Seth and Sharma (2001) found that approximately 61-64% of the F_1 progeny of 100 Gy treated male parents of *S. litura* emerged as adults, whereas 79–82% of controls reached adulthood. However, in our study, increasing the dose reduced the appearance of female adults, but the difference was not significant. In this research, about 65-71% of the male progeny of 180 and 250 Gy-treated female and male parents, developed into adults, whereas 92.42 % of male adults emerged in the controls. They claimed the F_1 sex ratio of *S. litura* shifted in favor of males when male parent pupae were irradiated with sub-sterilizing doses. This is consistent with our results. When treated male parents at 250 Gy were mated with unirradiated females, 72% of the emerged adults were male, compared to 53% for controls. This is a desirable result, because the basis of SIT and IS is breeding sterile male insects, and the higher the number of males to females, the better results can be obtained.

We observed when the dose increased up to 250 Gy, a drastic decrease in F_1 egg hatching occurred. When F_1 males whose pupal parents were irradiated with 250 Gy were mated with untreated females, all eggs laid were sterile, and when F_1 females were mated with untreated males, the sterility rate was 97%. Seth and Sharma (2001) claimed that the effect of radiation was more pronounced in *S. litura* F_1 females than in F_1 males, reflecting F_1 males are more sensitive to sub-sterilizing doses than F_1 females. This suggests that lower gamma irradiation resulted in more inherited sterility in F_1 males. These findings are in accordance with our results on *S. exigua*, where P_1 reproductive ability was lower for irradiated females than for irradiated males, and the inheritance of deleterious effects was greater for progeny from irradiated males. In order to effectively perform SIT in the P_1 generation and IS in the F_1 generation, it is very important to find the appropriate full radiation sterilization dose for females to release with sub-sterile males. We also reported here there was no progeny in UM × P_1F at 250 Gy and these results indicate the suitability of this dose for implementing both SIT and IS methods in controlling this pest.

Radiation doses used in inherited sterility (IS) programs should not reduce the quality and competitiveness of the released males (Seth *et al.*, 2016). A Fried's CV value of 1 indicates a comparable level of competitiveness between the irradiated and non-irradiated males. Values close to zero indicate superior competitiveness of non-irradiated males (Fried, 1971). In the current study, mating competitiveness was evident in all tested ratios except in 5:1:1 ratio (CV = 0.31). Ahmadi *et al.* (2021) found in the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), Fried's CV ranged from 0.35 to 0.63 at 100 Gy male pupae radiation for various mating rates in field cage tests, which showed fully competitiveness of irradiated males with non-irradiated males. Osouli *et al.* (2023) found that the competitiveness index of irradiated P_1 and F_1 males of citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), and European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), varied in acceptable ranges and as whole F_1 males were more competitive than their irradiated male parents.

In this research, laboratory trials were carried out to control this pest using SIT and IS and the desired results were achieved. This method is expected to be effective in field cage trials and environmental conditions in the subsequent studies.

Conclusion

Gamma irradiation affected the reproductive performance of *S. exigua* in the P_1 and F_1 generations. It was concluded that a sub-sterilizing dose of 250 Gy can induce the full sterility of P_1 females, partial sterility of P_1 males, and complete sterility in male progeny (F_1). A release ratio 1:1:1 (IM: UM: UF) was sufficient for the treated males at 250 Gy to compete with the normal males. Furthermore, radiation of pupae at 250 Gy had favorable impacts on almost all examined biological parameters (P_1 adult emergence and longevity, developmental duration of F_1 progeny, F_1 sex ratio, and F_1 adult emergence). According to these results, the employing P_1 partial sterility of male moths and inherited sterility of them in F_1 progeny by the administration of 250 Gy Co^{60} gamma-ray to parent pupae and releasing them by 1:1:1 (IM: UM: UF) ratio at laboratory condition were recommended for management of *S. exigua* by sterile insect technique. Of course, future field cage experiments must be conducted to achieve this goal.

Conflict of Interests

There is no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abass, A. A., Salem, H. M., Abd El Hamid, N. A., Gabarty, A. and Embaby, D. M. 2017. Effects of gamma irradiation on the biological activity of the cotton leaf worm, *Spodoptera littoralis* (Boisd.). Journal of Nuclear Technology in Applied Science, 5(1), 19-26.

Ahmadi, M., Osouli, S., Abd-Alla, A. M. M. and Kalantarian, N. 2021. Assessment of mating competitiveness of sterile *Ceratitis capitata* in laboratory and field cage tests in northern Iran. Entomologia Experimentalis et Applicata, 169(6): 550-562.

Angmo, N., Sengupta, M., Vimal, N. and Seth, R. K. 2023a. Receptivity and remating propensity in female *Spodoptera litura* (Fabricius) after mating with an irradiated male or its F_1 male progeny. Insects, 14(7): 651.

Angmo, N., Vimal, N., Sengupta, M. and Seth, R. K. 2023b. Effect of male irradiation and its mating status on the remating propensity, insemination quality and reproductive behavior of male *Spodoptera litura* (F.). Indian Journal of Entomology, 85: 95-102.

Ashouri, S., Osouli, S. and Ahmadi, M. 2023. Comparative study of different artificial diets on the biology of beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae). Journal of Entomological Society of Iran, 42(3): 183-193.

Bandoly, M. and Steppuhn, A. 2016. Bioassays to investigate the effects of insect oviposition on a plant's resistance to herbivores. Bio-protocol, 6(11): 1-13.

Bourtzis, K. and Vreysen, M. J. 2021. Sterile insect technique (SIT) and its applications. Insects, 12(7): 638.

Che, W., Shi, T., Wu, Y. and Yang, Y. 2013. Insecticide resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) from China. Journal of Economic Entomology, 106(4): 1855-1862.

Debolt, J. W. and Wright, F. K. 1976. Beet armyworm: inherited sterility produced by irradiation of pupae or adults. Journal of Economic Entomology, 69(3): 336-338.

Dyck, V. A., Hendrichs, J. and Robinson, A. S. 2021. Sterile insect technique: principles and practice in area-wide integrated pest management. Taylor & Francis.

El-Badry, E. A., Wakid, A. M., Zaki, M. M. and Ahmed, M. Y. Y. 1971. Effects of gamma radiation on mating competitiveness and sperm activity of the lesser cotton leaf worm, *Spodoptera exigua* Hb. Zeitschrift für Angewandte Entomologie, 67(1-4): 44-48.

Fried, M. 1971. Determination of sterile-insect competitiveness. Journal of Economic Entomology, 64(4): 869-872.

Hassan, R. S., Sileem, T. M. and Sayed, W. A. A. 2017. Impact of gamma irradiation and phenol on the biological activity of the cotton leaf worm, *Spodoptera littoralis* (Boisd.). Arab Journal of Nuclear Science and Applications, 50(1): 10-17.

Hosny, M. M., Wakid, A. M., Elbadry, E. A. and Sallam, H. A. 1972. Effects of gamma radiation on the pupae of the cotton leaf worm, *Spodoptera littoralis* Boisd. Zeitschrift für Angewandte Entomologie, 72(1-4): 205-212.

Jiang, S., Fu, X. W., Jiang, S. S., Yang, X. M., Zhao, H. Y. and Wu, K. 2022. Effect of X-ray irradiation on development, flight, and reproduction of *Spodoptera litura*. Frontiers in Physiology, 13: 947848.

Jiang, S., Sun, X. T., Ge, S.S., Yang, X. M. and Wu, K. M. 2023. Mating competitiveness of male *Spodoptera frugiperda* (Smith) irradiated by X-rays. Insects, 14(2): 137.

Kiyoku, M. and Tsukuda, R. 1970. Studies on the ecology of insects sterilized artificially (gamma radiation). V. Development and reproduction of the progeny from irradiated pupae, *Spodoptera littoralis* Boisd, by gamma radiation. Scientific Reports of the Faculty of Agriculture, Okayama University, 36: 19-26.

Mochida, O. and Miyahara, Y. 1974. Effect of gamma radiation on male pupae and adults of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) following pupal exposure. Applied Entomology and Zoology, 9(1): 41-47.

Osouli, S., Ahmadi, M. and Kalantarian, N. 2021. Radiation biology and inherited sterility in *Helicoverpa armigera* Hübner (Lepidoptera: Nuctuidae). International Journal of Tropical Insect Science, 41: 2421-2429.

Osouli, S., Atapour, M. and Ahmadi, M. 2023. Effect of gamma irradiation on F_1 generation and competitive ability of *Phyllocnistis citrella* and *Ostrinia nubilalis* and evaluation of the sterile insect release ratio on plant infestation in field cages. Entomologia Experimentalis et Applicata, 171(6): 475-486.

Ramesh, K., Garg, A. K. and Seth, R. K. 2002. Interaction of substerilizing gamma radiation and thiodicarb treatment for management of the tobacco caterpillar *Spodoptera litura*. Phytoparasitica, 30: 7-17.

Sengupta, M., Vimal, N., Angmo, N. and Seth, R. K. 2022. Effect of irradiation on reproduction of female *Spodoptera litura* (Fabr.) (Lepidoptera: Noctuidae) in relation to the inherited sterility technique. Insects, 13(10): 898.

Seth, R. K. and Sharma, V. P. 2001. Inherited sterility by substerilizing radiation in *Spodoptera litura* (Lepidoptera: Noctuidae): Bioefficacy and potential for pest suppression. Florida Entomologist, 183-193.

Seth, R. K., Khan, Z., Rao, D. K. and Zarin, M. 2016. Flight activity and mating behavior of irradiated *Spodoptera litura* (Lepidoptera: Noctuidae) males and their F_1 progeny for use of inherited sterility in pest management approaches. Florida Entomologist, 99(sp1): 119-130.

Toppazada, A., Abdallah, S. and Eldefrawi, M. E. 1966. Chemosterilization of larvae and adults of the Egyptian cotton leaf worm, *Prodenia litura* by Apholate, Metepa and Tepa. Journal of Economic Entomology, 59, 1125-1128.

Wakid, A. M., Elbadry, E. A., Hosny, M. M. and Sallam, H. A. 1972. Studies on the dose-fractionation, mating competitiveness and restoration of egg viability in the gamma-irradiated populations of the cotton leaf worm, *Spodoptera littoralis* Boisd. Journal of Applied Entomology, 72(1-4): 330-335.

Wakid, A. F. M. and Hayo, J. M. 1974. Inherited sterility in progeny of irradiated male cotton leaf worm, *Spodoptera littoralis* Boisd. Journal of Applied Entomology, 76(1-4): 331-335.

Yadav, S., Thakur, K. and Seth, R. K. 2017. Effect of 200 Gy as gamma sterilizing radiation on reproductive performance and life span of male moths, *Spodoptera litura* (Fabr.). Journal of Research: The Bede Athenaeum, 8(1): 143-149.

Vreysen, M. J. B., Klassen, W. and Carpenter, J. E. 2016. Overview of technological advances toward greater efficiency and efficacy in sterile insect inherited sterility programs against moth pests. Florida Entomologist, 99(1): 1-12.

تأثیر پرتو گاما بر زیست‌شناسی، تولیدمثل، عقیمی وراثتی و رقابت در جفت‌گیری *Spodoptera exigua* (Lepidoptera: Noctuidae) در شرایط آزمایشگاهی

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چکیده: کرم برگخوار چغندرقند *Spodoptera exigua* Hübner آفت خطرناک چغندرقند و سایر محصولات زراعی، سبزی و صیفی است. با توجه به این‌که لاروهای این حشره چندخوار هستند و می‌توانند کل گیاه را از بین ببرند، کنترل مؤثر و ایمن این آفت قبل از وقوع خسارت اقتصادی ضروری است. از سوی دیگر، کنترل شیمیایی تعادل اکوسیستم را برهم زده و منجر به ایجاد مقاومت در برابر حشره‌کش‌ها می‌گردد. در این پژوهش، تأثیر روش نرعقیمی و عقیمی وراثتی در کنترل کرم برگخوار چغندرقند توسط پرتودهی شفیره با پرتو گاما Co^{60} بررسی شده است. قدرت تولیدمثل، عقیمی، بیولوژی رشدی، نسبت جنسی، رقابت در جفت‌گیری و عقیمی وراثتی در نسل‌های P_1 و یا F_1 افراد بالغ ظاهر شده مورد بررسی قرار گرفت. هنگامی‌که شفیره‌ها با ۲۵۰ و ۴۰۰ گری پرتودهی شدند، به‌ترتیب ماده‌ها و نرهای P_1 کاملاً عقیم شدند. ظهور ماده‌ها به‌طور قابل‌توجهی در ۴۰۰ گری کاهش یافت، اما طول‌عمر آن‌ها با دزهای بالاتر افزایش یافت. هنگامی که شفیره‌های نر یا ماده P_1 به‌ترتیب با ۲۵۰ یا ۱۸۰ گری پرتودهی شدند، دوره رشدی لاروهای F_1 طولانی‌تر از حد معمول بود. ظهور حشرات نر F_1 با افزایش دز کاهش یافت. هنگامی‌که والدین نر پرتودهی شده با ماده‌های طبیعی تلاقی کردند، نسبت جنسی F_1 به نفع نر تغییر کرد. نتاج F_1 عقیمی بیش‌تری نسبت به نسل P_1 و نرهای F_1 عقیمی بیش‌تری نسبت به ماده‌های F_1 به ارث بردند. آزمایش رقابت در جفت‌گیری در شرایط آزمایشگاهی نشان داد که نرهای پرتودهی شده با ۲۵۰ گری (IM) در برابر نرهای پرتودهی نشده (UM) در جفت‌گیری با ماده‌های پرتودهی نشده (UF) حتی در نسبت ۱:۱:۱ (IM:UM:UF) رقابت می‌کنند.

واژگان کلیدی: کرم برگخوار چغندرقند، پرتو گاما Co^{60}، عقیمی F_1، زیست‌شناسی پرتوی، تکنیک نرعقیمی