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Potential use of sterile insect technique (SIT) against the Indian meal moth, *Plodia interpunctella*

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Abstract
Indian meal moth, *Plodia interpunctella* (Hübner) is one of the most important stored products pests in the world. In Bangladesh, annual losses to different stored commodities occurs more than 20%. In this research, an environmentally safe & species specific method i.e. Sterile Insect Technique (SIT) was applied as a part of integrated pest management approach. This experiment was done by implementing gamma irradiation (Cobalt-60) for sterilization of Indian meal moth to suppress the reproductive potential of the moth. The effects were more significant in F_1 early progeny than parental generation because it was possible to suppress the egg production, hatchability & finally adult emergence successfully in F_1 generation. The dose of radiation required to prevent fecundity of *Plodia interpunctella* from irradiated adults was 400 Gy in parental generation (P), whereas it was 300Gy & 400Gy in F_1 generation in the present study. The hatchability would be prevented by the cross schedule of treated male with treated female (TM x TF). 400 Gy dose would be sufficient to control the hatchability of Indian meal moth both in P & F_1 generation. Higher doses of radiation had a significant effect on adult emergence in F_1 early progeny of the moth. Surprisingly, the number was zero for the cross schedule of normal female with treated male (NF x TM) & treated male with treated female (TM x TF) at all the selected doses except 50Gy in F_1 early progeny in the present study. The 300Gy & 400Gy doses used in the present experiment would be sufficient to control the population of Indian meal moth.

Keywords: Sterile Insect technique, *plodia interpunctella*, fecundity, hatchability, early adults

1. Introduction

The Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is a common insect pest of stored, milled and processed cereals and its product, oilseeds, dried fruits, dried vegetables, nuts, animal feed and garlic seeds and manufactured products [11, 27, 30]. *Plodia interpunctella* (Hϋbner) was described by Hϋbner in 1827 and in 1858, Asa Fitch described the moth and called it *Tinea zeae* Fitch and gave it the common name in use today, Indianmeal moth [14]. It is also found in farms, food processing plants, houses, retail stores, granaries and warehouses. Indianmeal moth, *P. interpunctella* has been found to be distributed across the continent of the world and ranked as one of the most important storage pests in warm temperate and subtropical climates [31]. This moth species has a very strong economic impact because it uses a wide range of products in its diet [1, 9, 26, 31, 34, 36]. Management of *P. interpunctella* and other stored product pests is undergoing a rapid change from an insecticide-based system to a more integrated approach [3, 39]. Strains of *P. interpunctella* have developed resistance to the organophosphate malathion and several other organophosphates [3]. The use of insecticides in Bangladesh was stated to date back in 1957/58. Methyl bromide (MB) is a widely used fumigation for control of insect infestation in many agricultural commodities. 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 [15]. Cold disinfestation often requires long exposure periods, measured in days, and are of more use for preservation of products than as a means of disinfestations [13]. Other studies have shown that heat treatment for 3 hours at 45 °C completely controlled *P. interpunctella* pupae [2]. But heat treatment can affect the quality of host commodities. There is board international consensus that the management of these key pests is ideally based on the concept of area-wide integrated pest management (AW- IPM) [17, 20, 24], and control tactics should be integrated based on their suitability for a given pest species and local ecological characteristics, and the control tactics should target an entire insect population (total population control) [21, 23, 37]. In view of the above made considerations on the use of insecticides, control tactics that are effective but more friendly to the environment such as the Sterile Insect Technique (SIT) offer great potential and should be increasingly adopted [12]. The use of the Sterile Insect Technique (SIT), applied as part of an area-wide integrated pest management approach [17], provides an environmentally safe and species-specific method to suppress, and in some situations eradicate, pests of agricultural importance worldwide. So, it is very important to apply SIT for the prevention of the losses of stored grain products due to the *P. interpunctella*, mitigating the risks of pesticides on the environment, non-target organisms including human health. The SIT involves the use of ionizing radiation to sterilize male insects, which are then released into a target area where they will mate and prevent the reproduction of wild females [22]. The objective was to study the implementation of gamma irradiation for sterilization of Indian meal moth to suppress the reproductive potential of the moth.

2. Materials and Methods

2.1 Insect colony

The initial *P. interpuntella* culture was obtained from the post-harvest Entomology laboratory, Department of Zoology, University of Rajshahi, Bangladesh. They were originally collected from the local food facilities located at Rajshahi Metropolitan City. They were reared on maize flour under laboratory condition at 27ºC and 70-75% RH at the Post Harvest Entomology laboratory, Department of Zoology, University of Rajshahi, Bangladesh.

2.2 Culture box

The size of the culture box was L-14.0, W-11.2 cm and H-8.50 cm. The box was covered with a lid having a small mesh net prior to allow easy gaseous exchange. The gap between lid and plastic box were sealed carefully so that the larva cannot escape from the culture box.

2.3 Adult release

Fifty pairs of adult *P. interpunctella* were released in each mass culture box. All the adults were fresh and newly emerged. Tissue paper was used for the laying of eggs. Each female usually laid eggs for 3 days. Male dies soon after mating and the female dies after completing their egg laying. The dead adults were removed carefully from the culture box.

2.4 Cellulae

Newly emerged virgin males (age less than one hour) were paired with 1–3 days old virgin females (treatments 4–6). Each treatment was replicated 10 times by introducing the moths in glass jars (10×5 cm). After pairing insects, jars were covered with small pieces of muslin cloth. The glass jars were checked daily by sieving for counting eggs laid by females. The longevity of adults was recorded until all adults were dead.

2.5 Egg collection

Collected eggs were placed daily in Petri dishes of size 25×10 mm along with rearing diet. Eggs of the Indian meal moth appear grayish white and range in length from 0.3 to 0.5 mm. Eggs are oviposited singly or in clusters, and are generally laid directly on the larval food source.

2.6 Larval collection

There are five to seven larval instars. Their color is usually off-white, but has been observed to be pink, brown or almost greenish, depending on the food source. They have five pairs of well-developed prologs that help them move considerable distances to pupate. After 25 days of releasing adults in the mass culture box mature larva was collected from the food and their weight was measured for calculating total larva number statistically. The size of each spot was 2 inch. For measuring the weight of the mature larva we took 10 larvae randomly from the mass culture box. This was repeated 20 times.

2.7 Pupae

The larvae pupate either in a silken cocoon or unprotected. The pupae are 1/4 to 2/5 inch long (6 to 11 mm) and are pale brown in color. Pupation takes place away from the infested material. In fact, late instar larvae can travel such distances that they are often mistaken for clothing pests. Within the pantry, small larvae often climb to other shelves before pupating. This misleads people trying to find the source of the infestation.

2.8 Adult collection

Adults are about 1/2 inch (12.7 mm) long with a wing span of about 5/8 inch (16 to 20 mm). The forewings of this moth are reddish brown with a copper sheen on the outer two thirds and gray on the inner third. At rest, the wings are held roof-like over the body. The head and thorax of the moth appears gray and the posterior brown, with a coppery sheen. When the adults emerged then they were collected. Their weight was measured. Ten adults were weighed each time. This was 20 times development time period from egg to adult emergence for *P. interpunctella* was recorded.

2.9 Experimental Procedures

1-2days old adults of *P. interpunctella* were selected for sterilization with gamma radiation. Seven doses of gamma radiation *viz*. 0 (control), 50, 75, 100, 150, 300, 400 and 500 Gy were selected for sterilization.

Adults were irradiated from the Atomic Energy Research Institute, Ganokbari, Savar, Dhaka, Bangladesh. Immediately after treatment, irradiated adults and the control adults were transferred to plastic trays containing food medium and then were subsequently transferred to the rearing conditions. After radiation, the dead adults were counted and separated from living adults. Male and female adults were counted and removed from the plastic trays. Then, these adults were allowed to mate.

The irradiated adults were mated as cross and ratio wise. In cross schedule, adults were mated in three combinations: a) Normal Male by Treated Female (NM x TF), b) Normal Female by Treated Male (NF x TM) and c) Treated Male by Treated Female (TM x TF) followed by Normal Male by Normal Female (NM x NF) as control. On the other hand, in ratio mating – these adults were mated in Irradiated male is to Normal male is to Normal female (Imale: Nmale: Nfemale) combinations with different numbers of male and female. These were a) 1:0:1 (Imale: Nmale: Nfemale), b) 1:1:1 (Imale: Nmale: Nfemale), c) 3:1:1 (Imale: Nmale: Nfemale) and d) 5:1:1 (Imale: Nmale: Nfemale).

Three replications each with sub-replications were used for each step of each dose level and control. The total number and viability (hatching) of the eggs laid by the irradiated adults were recorded for both P and F_1 generations. The sex ratio of the emerged adults from irradiated parental adults was also recorded. The data were subjected to analysis of variance.

2.10 Data analysis

The data for each dose and cross were analyzed by using one way analysis of variance (ANOVA) with the help of computer package MSTAT-C to determine the effect on the reproductive potential of the irradiated adults.

3. Results

3.1 Effect of gamma radiation in parental generation developing from irradiated early adults

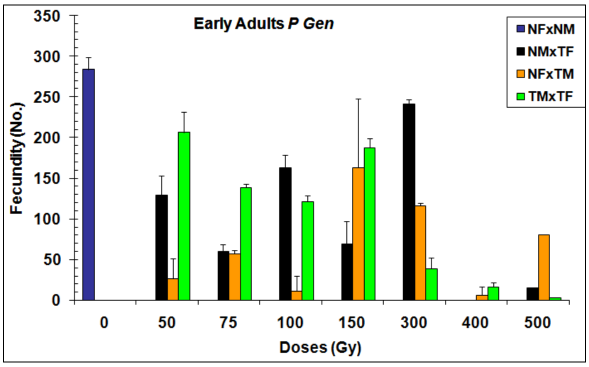

Fig 1 a: Effect of gamma radiation on the fecundity in parental generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated early adults.

Effects of gamma radiation on the fecundity in parental generation developed from irradiated early adults are shown in Fig. 1A. The result revealed that, when normal adult males were crossed with treated adult females (NM x TF) the highest number of fecundity was recorded (283) at 300 Gy dose and the lowest fecundity was recorded (24) at 500 Gy dose. However, egg production for the cross (NM x TF) was completely prevented by 400 Gy dose. For the cross (TM x TF), eggs were produced at all doses but at 500 Gy dose, the quantity of this egg production was very low. Effect of gamma radiation on the fecundity in parental generation of Indianmeal moth, *P. interpunctella* developing from different cross schedules of irradiated early adults is shown in (Appendix Table-A) which indicates that the fecundity in parental (F=2.582, P=0.087) were not significantly different at different radiation doses of early adults.

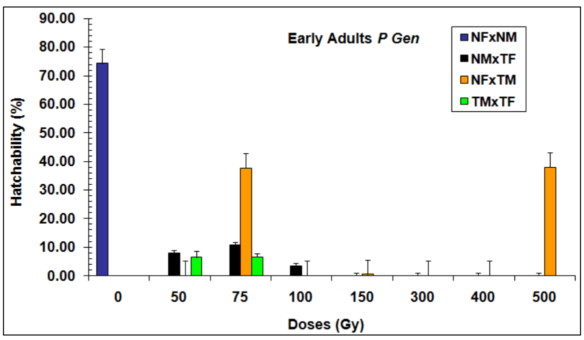

Fig 1 b: Effect of gamma radiation on the hatchability in parental generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated early adults.

The radiation effects on the hatchability in parental generation developing from irradiated early adults are shown in Fig. 1B. The result showed that the same hatchability (39%) was recorded for cross (NF x TM) both at 500 Gy dose and at 75 Gy dose in early adults and that showed the highest hatchability. It was noticed that the hatchability was completely prevented by 150Gy, 300Gy and 400Gy doses for all crosses. Effect of gamma radiation on the hatchability in parental generation of Indianmeal moth, *P. interpunctella* developing from different cross schedules of irradiated early adults is shown in (Appendix Table-B) which indicates that the hatchability in parental (F=13.25, P=0.00008) were varied significantly at different radiation doses of early adults.

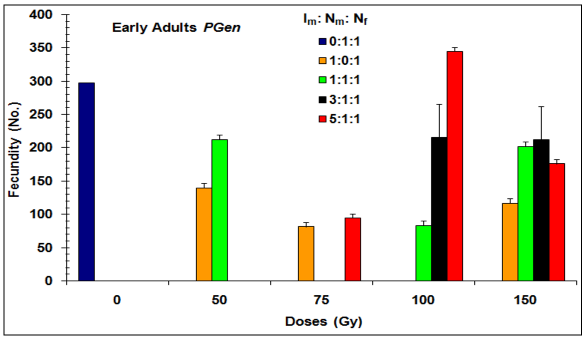

Fig 1 c: Effect of gamma radiation on the fecundity in parental generation of Indian meal moth, *P. interpunctella* developing from different ratios of cross schedules of irradiated early adults.

As Fig. 1C showed the effect of bonding ratios of normal and irradiated adults on the fecundity in parental generation. In early adults, the maximum number of fecundity (350) obtained at I_m:N_m:N_f (5:1:1) ratio at 100 Gy dose and the minimum number of fecundity (90) obtained at (1:0:1) ratio at 75 Gy dose. It was noticed that the fecundity was zero, when we crossed I_m:N_m:N_f at the ratio of (3:1:1) at 75Gy & 50Gy doses respectively. Effect of gamma radiation on the fecundity in parental generation of Indianmeal moth, *P. interpunctella* developing from different ratios of cross schedules of irradiated early adults is shown in (Appendix Table-C) which indicates that the fecundity in parental (F=1.263, P=0.368) were not significantly different at different radiation doses of early adults.

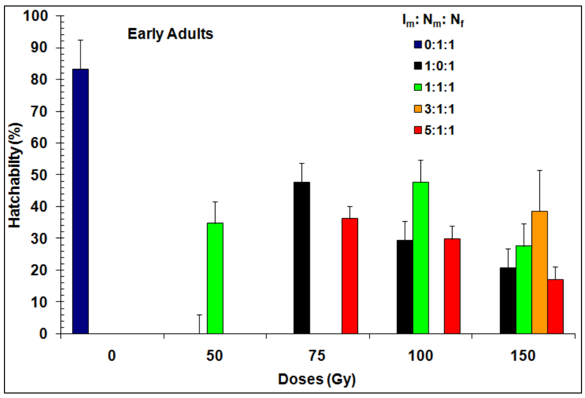

Fig 1 d: Effect of gamma radiation on the hatchability in parental generation of Indian meal moth, *P. interpunctella* developing from different ratios of cross schedules of irradiated early adults.

The Fig. 1D showed the effect of bonding ratios of normal and irradiated adults on the hatchability developing from irradiated early adults. This figure also showed that, the percentage of hatchability was observed same (48%), both at

I_m:N_m:N_f (1:0:1) & (1:1:1) ratios at 75Gy & 100Gy respectively and that was the highest percentage of hatchability. The lowest hatchability (less than 20%), was observed at 150Gy for I_m:N_m:N_f (5:1:1) ratio. Effect of gamma radiation on the hatchability in parental generation of Indian meal moth, *P. interpunctella* developing from different ratios of cross schedules of irradiated early adults is shown in (Appendix Table-D) which indicates that the hatchability in parental (F=3.27, P=0.082) were not significantly different at different radiation doses of early adults.

3.2 Effect of gamma radiation in F_1 generation developing from irradiated early adults

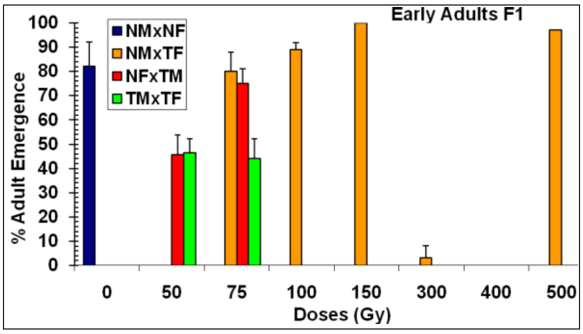

Fig 2 a: Effect of gamma radiation on the percent adult emergence in F_1 generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated early adults.

The result surprisingly revealed that 100% adults emerged at 150Gy dose for (NM x TF) in early adults which was the highest percentage of adult emergence among all doses including the control. The lowest emergence was (7%) recorded at 300Gy dose for (NM x TF) but, at 50Gy dose adult emergence was completely prevented for that cross. We also noticed that adult emergence was completely prevented when we crossed (NF x TM) and (TM x TF) at each doses of 100Gy, 150Gy, 300Gy, 400Gy & 500Gy in early adults (Fig. 2A). Effect of gamma radiation on the percent adult emergence in F_1 generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated early adults is shown in (Appendix Table-E) which indicates that the percent adult emergence in F_1 generation (F = 4.315, P = 0.027) were varied significantly at different radiation doses of early adults.

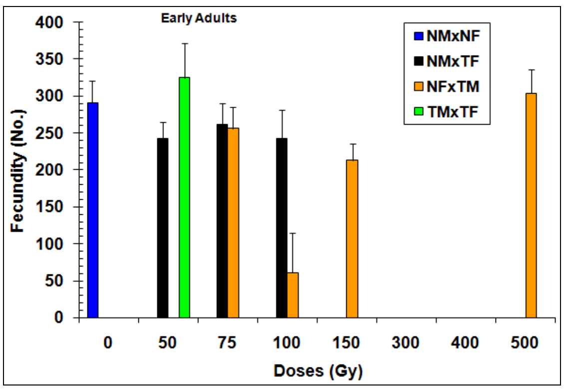

Fig 2 b: Effect of gamma radiation on the fecundity in F_1 generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated early adults.

The effects of gamma radiation on the egg laying capacities in F_1 generation of the adults developing from irradiated early adults are shown in Fig. 2B. The highest number of fecundity was recorded (325) at 50Gy dose for cross (TM x TF), but at other doses for cross (TM x TF) was not produced any egg i.e. egg production was prevented. The result also revealed that egg production capacity was completely prevented by 300Gy and 400Gy for all crosses that we did in our work. Effect of gamma radiation on the fecundity in F_1 generation of Indian meal moth *P. interpunctella* developing from different cross schedules of irradiated early adults shown in (Appendix Table-F) which indicates that the fecundity in F_1 generation (F = 1.616, P = 0.225) were not significantly different for between cross and (F = 4.536, P = 0.034) were varied significantly for within groups at different radiation doses of early adults.

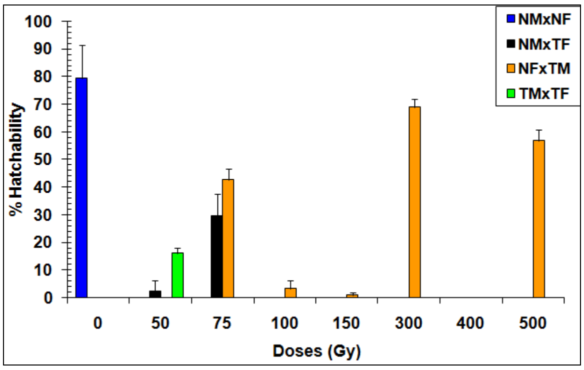

Fig 2 c: Effect of gamma radiation on the hatchability in F_1 generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated early adults.

The effects of gamma radiation on the hatchability in F_1 generation developed from irradiated early adults are shown in Fig. 2C. The result also revealed that the highest percentage of hatchability (69%) was recorded at 300Gy & the lowest percentage was (2.2%) recorded at 150Gy dose for cross (NF x TM) in early adults. It was also noticed that hatchability was completely prevented by 400Gy for all crosses that we did in our work. Among all doses, hatchability of cross (TM x TF) was prevented except at 50Gy dose shown in figure. Effect of gamma radiation on the hatchability in F_1 generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated early adults shown in (Appendix Table-G) which indicates that the hatchability in F_1 generation (F = 1.174, P = 0.380) were not significantly different for between cross and (F = 1.51, P = 0.258) were not significantly different for within groups at different radiation doses of early adults.

4. Discussion

The sterile insect technique is used to eradicate pests from regions or maintain low pest population levels [12]. This study was designed to assess the effect of gamma radiation on the sterilization in *Plodia interpunctella* irradiated as adults. The effects of gamma radiation on fecundity, hatchability & adult emergence from irradiated parental early adults had a great impact. The study represents information that helps to understand the potential use of SIT by comparing the results from Parental & F_1 generation of *Plodia interpunctella* on the basis of fecundity, hatchability & adult emergence.

Irradiated *E. saccharina* females mated with untreated males oviposited fewer eggs than untreated females mated with treated males & the untreated control, when treated with radiation doses of 200 to 250Gy [38] In our study, the result revealed that, when *P. interpunctella* females irradiated with 150Gy & mated with untreated males oviposited fewer eggs (70) than untreated females (oviposited 162 eggs) mated with treated males & when females irradiated with 300Gy oviposited more eggs (245) than untreated females (oviposited 115 eggs) mated with treated males.

It was reported that 1% of eggs laid by Indian meal moth adults irradiated with a target absorbed dose of 400Gy hatched [16], and 350Gy sterilized the adult Indian meal moths [40]. The present study revealed that the percentage of hatchability was recorded around 69% & 60% at 300Gy & 500Gy respectively for (NF x TM), however, the percentage was zero at 400Gy not only for (NF x TM) but also for all the crosses that we did in our work. Egg hatch when males were mated to non-irradiated females average 1.0% [19].

Reproduction in Indian meal moth was stopped with 450Gy but not 400Gy when a total of 20 irradiated 1-day-old adult females were paired with non-irradiated males [5, 6]. We noticed that adult emergence was completely prevented when adult females were paired with non-irradiated male (NM x TF) at 400Gy in F_1 generation (Fig. 2A).

A linear decline in fertility with increasing radiation doses has been observed for males of the Indian meal moth, *P. interpunctella* Hübner (Lepidoptera: pyralidae) [4, 7], the pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) [18, 25], the sugarcane borer, *D. saccharalis* [32, 33], the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera; Crambidae) [28], and the common cutworm, *Spodoptera litura* F. (Lepidoptera; Noctuidae) [35]. These studies showed that females were more sensitive than males to increasing doses of radiation. This is common in Lepidoptera, where fertility is a better indicator of radiation sensitivity than fecundity [8, 29]. In the present study, *P. interpunctella* responded similarly, in that fertility was significantly reduced with increasing radiation dose in all parental crosses. It was also observed that female *P. interpunctella* was much more sensitive to radiation than males. The higher radiation sensitivity of *P. interpunctella* female is an advantage for a potential SIT program over the above species in view of mating behavior of *P. interpunctella*. For the control purposes, incomplete male sterility should not be considered a disadvantage; within the treated product, irradiated males presumably will mate with irradiated, sterile females. Treated males could produce progeny only by mating with females invading the product after irradiation. These invading females probably would be mated already, so the presence of partially sterile males would not contribute significantly to a post treatment infestation. Although the presence of partially sterile males in the treated product should not present a threat of re-infestation, these males may actually inhibit post treatment infestation in the manner of a sterile insect release [10]. It is concluded that the selected radiation doses used in the present experiment would be sufficient to control Indian meal moth. Moreover, the results from this study should provide a foundation of knowledge to advance the development of Sterile Insect Technique (SIT) strategies for controlling Indian meal moth as well as other Lepidopteran pests.

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Appendices

Appendix Table A: ANOVA for the effect of gamma radiation on the fecundity in parental generation of *Plodia interpunctella* developing from different cross schedules of irradiated early adults

ANOVA			
Source of Variation	df	F	P-value
Between Cross	3	2.582	0.087
Within Groups	17		
Total	20		

Appendix Table B: ANOVA for the effect of gamma radiation on the hatchability in parental generation of *Plodia interpunctella* developing from different cross schedules of irradiated early adults

ANOVA			
Source of Variation	df	F	P-value
Between Cross	3	13.25	0.00008
Within Groups	18		
Total	21		

Appendix Table C: ANOVA for the effect of gamma radiation on the fecundity in parental generation of *Plodia interpunctella* developing from different ratios of cross schedules of irradiated early adults

ANOVA			
Source of Variation	*df*	*F*	*P-value*
Between Cross	4	1.263	0.368
Within Groups	7		
Total	11		

Appendix Table D: ANOVA for the effect of gamma radiation on the hatchability in parental generation of *Plodia interpunctella* developing from different ratios of cross schedules of irradiated early adults

ANOVA			
Source of Variation	*df*	*F*	*P-value*
Between Cross	4	3.27	0.082
Within Groups	7		
Total	11		

Appendix Table E: ANOVA for the effect of gamma radiation on the fecundity in F_1 generation of *Plodia interpunctella* developing from different cross schedules of irradiated early adults

ANOVA			
Source of Variation	*df*	*F*	*P-value*
Between Cross	4	4.315	0.027
Within Groups	10		
Total	14		

Appendix Table F: ANOVA for the effect of gamma radiation on the fecundity in F_1 generation of *Plodia interpunctella* developing from different cross schedules of irradiated early adults

ANOVA			
Source of Variation	df	F	P-value
Between Cross	6	1.616	0.225
Within Groups	2	4.536	0.034
Error	12		

Appendix Table G: ANOVA for the effect of gamma radiation on the hatchability in F_1 generation of *Plodia interpunctella* developing from different cross schedules of irradiated early adults

ANOVA			
Source of Variation	df	F	P-value
Between Cross	6	1.174	0.380
Within Groups	2	1.51	0.258
Error	12		
Total	20		