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Efficacy of gamma irradiation for the sterilization of Indian meal moth, *Plodia interpunctella*

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Abstract
Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is a cosmopolitan pest of stored grain commodities & processed food. About 30% of crop losses occur due to pests & diseases each year in Bangladesh. Due to negative effects of pesticides or the increasing resistance of the pests we adopted an alternative way i.e. Sterile Insect Technique methods, friendly to the environment. This experiment was done by implementing gamma irradiation (Cobalt-60) for sterilization of Indian meal moth to suppress the reproductive potential of the moth. Present study reveals that gamma radiation surprisingly prevented egg production, the number of eggs was zero at all the doses that we selected, when treated male were mated with treated female adults (TM x TF) in F_1 generation. Higher doses of radiation were more effective on late adults to control the population in F_1 generation than parental generation. 500 Gy dose was completely effective on late adults to prevent the hatchability for all cross schedules that we did in F_1 generation. This (TM x TF) would be sufficient to control the fecundity & hatchability of late adults, *Plodia interpunctella.* The main objective of using SIT was to prevent the losses of this stored grain pest while mitigating the risks of pesticides on the environment, non- target organisms including human health.

Keywords: Gamma irradiation, *Plodia interpunctella*, fecundity, hatchability, late adults

1. Introduction

Indian meal moth, *Plodia interpunctella* Plodia interpunctella (Hübner) was described by Hübner in 1827. The Indian meal moth 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 [5, 11, 14]. Infestation of *Plodia interpunctella* can cause direct product loss and indirect economic costs through pest control costs, quality losses and consumer complaints [15]. An analysis indicated that 19 of the 37 or 51% of the worst insect pest threats facing US agriculture are Lepidoptera species (ESA 2001). The larvae of Indian Meal Moth eat out the embryo of grains making them useless for seed purposes, and as a destroyer of nuts and dried fruits, it has no equal. The loss is not due alone to the web and excrement which the larvae leave behind wherever this goes until the material is an unsightly mess and not fit for human food. All SIT programs must settle on a target dose that is high enough to induce sterility, but that is not so high that the insect's performance is degraded [4]. Somatic damage during the irradiation process leads to poor post-irradiation performance [3, 10]. This irradiation damage can be manifested as decreased dispersal ability (flight ability), lower mating success, and shorter lifespan [8, 9, 10, 12, 13, 16,]. Bangladesh as a developing country needs to control the pest for better development. Some insecticides have negative impacts on the environment, non-target organisms and also may cause health hazards to humans. Due to the negative effects of pesticides or the increasing resistance of the pests we have to consider an alternative way i.e. Sterile Insect Technique (SIT) controls methods. The use of the Sterile Insect Technique (SIT), applied as part of an area-wide integrated pest management approach [7], provides an environmentally safe and species-specific method to suppress, and in some situations eradicate, pests of agricultural importance worldwide. The sterile male technique has been proposed as a residue- free method of controlling Lepidoptera pests of stored -food commodities [1, 2]. The SIT has been used mostly against species that are highly harmful to agriculture or public health or which elimination would have significant economic benefits. About 38 facilities are making research on SIT or sterilizing millions of insects per week for national area-wide Integrated pest control programmes (IDIDAS.2015). 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.

2. Materials and Methods

2.1 Insect colony

The initial *P. interpunctella* 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 conditions at 27 ºC 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 inches. 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 20 times development time period from egg to adult emergence for *P. interpunctella* was recorded.

2.9 Experimental Procedures

3-4 days old early 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 late adults

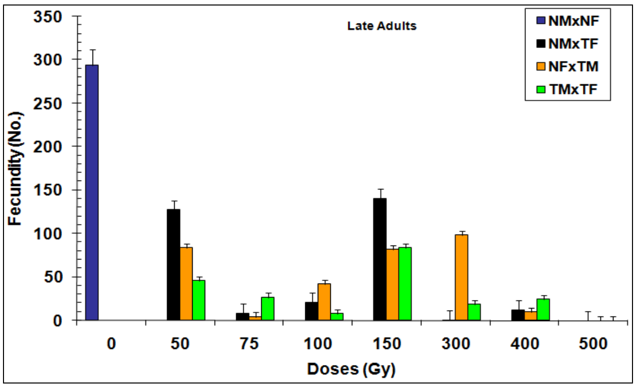

Fig 1 a: Effect of gamma radiation on the fecundity in parental generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated late adults

The effects of gamma radiation on the fecundity of the late adults are shown in Fig. 1A. The results also revealed that the highest (148) fecundity was recorded for (NM x TF) at 150Gy dose and the lowest (2) fecundity was recorded for (NM x TF) at 300Gy dose. Effect of gamma radiation on the fecundity in parental generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated late adults is shown in (Appendix Table-A) which indicates that the fecundity in parental (F=9.849, P=0.0004) were varied significantly at different radiation doses of late adults.

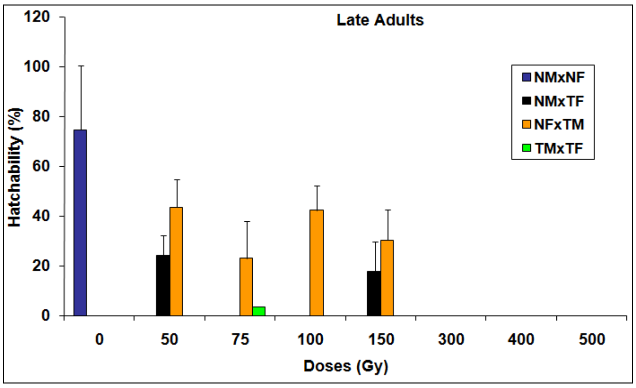

Fig 1 b: Effect of gamma radiation on the hatchability in parental generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated late adults

The effects of gamma radiation on the hatchability of the late adults are shown in Fig. 1B. The result also revealed that, the highest percentage of hatchability (44%) was recorded at 50Gy and the minimum hatchability was recorded (23%) at 75Gy dose for (NF x TM) in late adults. It was also noticed that hatchability of late adults was completely prevented by 300Gy, 400Gy & 500Gy respectively for all crosses that have done. Effect of gamma radiation on the hatchability in parental generation of Indianmeal moth, *P. interpunctella* developing from different cross schedules of irradiated late adults is shown in (Appendix Table-B) which indicates that the hatchability in parental (F=10.90, P=0.0002) were varied significantly at different radiation doses of late adults.

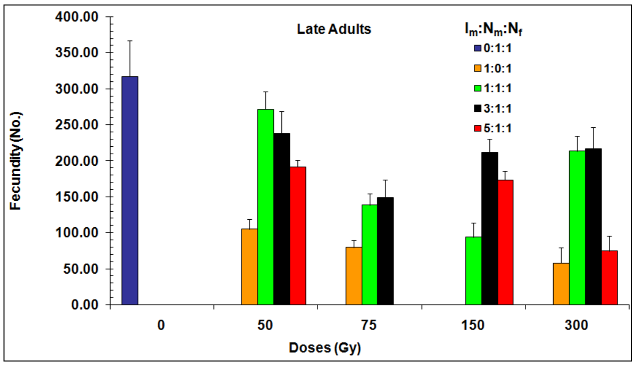

Fig 1 c: Effect of gamma radiation on the fecundity in parental generation of Indian meal moth, *P. interpunctella* developing from different ratios of cross schedules of irradiated late adults

The effect of bonding ratios of normal and irradiated adults on the fecundity in parental generation showed in Fig. 1C. This figure also showed that egg production was completely prevented, when we crossed normal & irradiated adults at the ratios of I_m:N_m:N_f (5:1:1) & (1:0:1) at 75Gy and 150Gy respectively. The maximum number of fecundity (271) occurred at I_m:N_m:N_f (1:1:1) ratio at 50Gy dose and the minimum fecundity (93) occurred at (1:1:1) ratio at 150Gy dose for late aged adults. 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 late adults is shown in (Appendix Table-C) which indicates that the fecundity in parental (F=4.06, P=0.032) were varied significantly at different radiation doses of late adults.

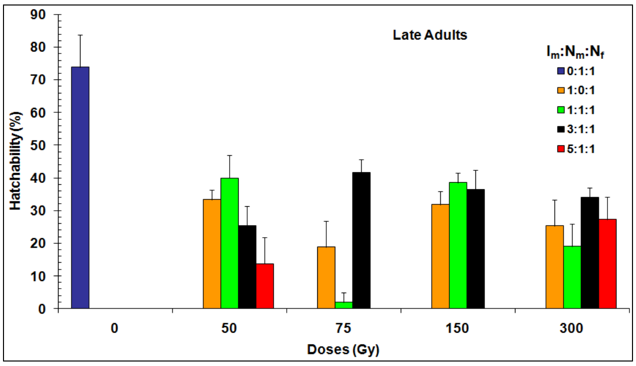

Fig 1 d: Effect of gamma radiation on the hatchability in parental generation of Indian meal moth, *P. interpunctella* developing from different ratios of cross schedules of irradiated late adults

The Fig. 1D. showed the effect of bonding ratios of normal and irradiated adults on the hatchability developing from irradiated late adults. Among all doses, the highest hatchability (45%) was observed at I_m:N_m:N_f (3:1:1) ratio at 75Gy and the lowest hatchability(5%) was observed at 75Gy at I_m:N_m:N_f (1:1:1) ratio. We also recorded that, when we crossed adults at the ratios of I_m:N_m:N_f (5:1:1) at 75Gy and 150Gy respectively, the number of hatchability was zero, i.e. it was completely prevented. Effect of gamma radiation on the hatchability in parental generation of Indianmeal moth, *P. interpunctella* developing from different ratios of cross schedules of irradiated late adults is shown in (Appendix Table-D) which indicates that the hatchability in parental (F=0.690, P=0.596) were not significantly different at different radiation doses of late adults.

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

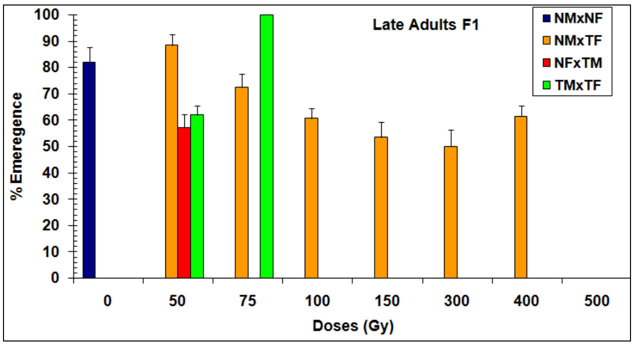

Fig 2 a: Effect of gamma radiation on the percent adult emergence in F_1 generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated late adults

The results were revealed that, the highest percentage (100%) of adult emerged at 75Gy and the lowest percentage (51%) was found at 50Gy dose but, at 100Gy, 150Gy, 300Gy, 400Gy & 500Gy adult emergence was prevented from the cross (TM x TF). Among all doses, adult emergence was completely prevented for cross (NF x TM), except at 50Gy dose (Fig.2A).

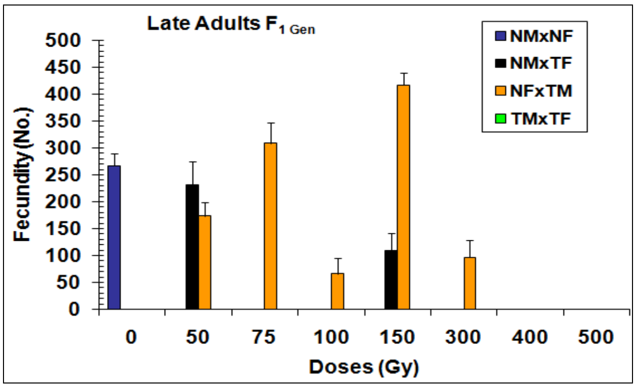

Fig 2 b: Effect of gamma radiation on the fecundity in F_1 generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated late adults

The effects of gamma radiation on the egg laying capacities in F_1 generation of the adults developing from irradiated late adults are shown in Fig. 2B. The highest number of fecundity was recorded (412) at 150 Gy dose for cross (NF x TM) & the lowest fecundity (70) was found at 100Gy dose. For the cross (TM x TF), egg production was completely prevented by all doses. The result also revealed that egg production capacity was completely prevented by 400Gy and 500Gy for all crosses. Effect of gamma radiation on the fecundity in F_1 generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated late adults is shown in (Appendix Table-E) which indicates that the fecundity in F_1 generation (F=1.616, P=0.225) were varied significantly at different radiation doses of late adults.

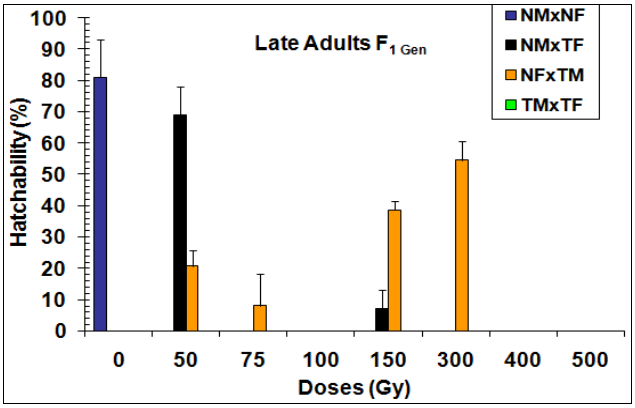

Fig 2 c: Effect of gamma radiation on the hatchability in F_1 generation of Indian meal moth, *P. interpunctella* developing from different cross schedules of irradiated late adults

The effects of gamma radiation on the hatchability in F_1 generation developed from irradiated late adults are shown in Fig. 2C. The result also revealed that, the highest percentage of hatchability (69%) was recorded at 50Gy and the lowest percentage was (8%) recorded at 150 Gy dose for cross (NM x TF) in late adults. It was also noticed that hatchability was completely prevented by 100Gy, 400Gy and 500Gy doses for all crosses. However, hatchability from the cross (TM x TF) was prevented by all doses.Effect of gamma radiation on the fecundity in F_1 generation of Indianmeal moth, *P. interpunctella* developing from different cross schedules of irradiated late adults is shown in (Appendix Table-F) which indicates that the hatchability in F_1 generation (F=1.174, P=0.380) were varied significantly at different radiation doses of late adults.

4. Discussion

The unique genetic phenomena responsible for inherited sterility in Lepidoptera, as compared with full sterility in the parental generation, provide multiple advantages for pest control. When partially sterile males of the parental generation are released & mate with virgin wild females the radiation- induced deleterious effects on the chromosomes & genes are inherited by F_1 generation. As a result, egg hatch in the F_1 generation is reduced & the resulting offspring are both highly sterile & predominantly male. Numerous studies have been carried out on inherited sterility with lepidopteran pests [6]. In this research, sterility appeared in the F_1 generation for (TM x TF). The result also revealed that when *Plodia interpunctella* females irradiated with 150Gy & mated with untreated males oviposited more eggs (142) than untreated females (oviposited 82 eggs) mated with treated males & when females irradiated with 300Gy oviposited fewer eggs (2) than untreated females (oviposited 98 eggs) mated with treated males. But, at 100Gy dose treated females when mated with untreated males oviposited fewer eggs (22) than when untreated females (oviposited 42 eggs) mated with treated males. Irradiated *E. saccharina* females mated with untreated males oviposited fewer eggs than untreated females mated with treated males and the untreated control, when treated with radiation doses of 200 to 250Gy [17].

In this research, when treated male mated with normal female, eggs produced at almost all doses except 500Gy, but the average percentage of hatchability of those eggs was less than 50 percent with complete prevention at 300Gy & 400Gy. In addition, in spite of hatching eggs at 50Gy & 150Gy doses, adults emerge only at 50Gy dose. Considering those results, it would be possible to suppress the population of Indian meal moths by releasing irradiating male moths in the wild & mating them with wild females.

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 Indianmeal moth.

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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 late adults

ANOVA			
Source of Variation	*df*	*F*	*P-value*
Between Cross	3	9.849	0.0004
Within Groups	18		
Total	21		

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 late adults

ANOVA			
Source of Variation	*df*	*F*	*P-value*
Between Cross	3	10.90	0.0002
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 late adults

ANOVA			
Source of Variation	*df*	*F*	*P-value*
Between Cross	4	4.06	0.032
Within Groups	10		
Total	14		

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 late adult

ANOVA			
Source of Variation	*df*	*F*	*P-value*
Between Cross	3	0.690	0.596
Within Groups	5		
Total	8		

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 late 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		
Total	20		

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