Evaluation of fast neutron irradiation as a new control method against the Indian meal moth, *Plodia interpunctella* (Hübner)

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**ABSTRACT**

Ionizing radiation is considered a promising treatment for stored product pest management. The dose levels 0, 64, 128, 192, and 256 Gy of Fast Neutron Irradiation (FNI) were tested to determine the lethal dose for preventing the reproduction of *Plodia interpunctella*. The dose needed to induce sterility in both sexes of adult moths is also evaluated. The results of immature stages irradiation demonstrated that the developmental periods and adult longevity were significantly shorter than the control treatments. Moreover, the reductions of fecundity, fertility, and growth rate were based on the dose of FNI, insect sex, and sage. The results revealed that inherited sterility in F₁ generation was more prevalent in irradiated male pupae than irradiated female pupae and also full grown larvae. The results indicated that the 256 Gy was prevented the development of all immature, thus we could considered it as a lethal dose that use in disinfestation treatment. While, full grown pupae irradiation with 128 Gy was an effective dose that induce adequate sterility of *Plodia interpunctella* that can use in the sterile insect technique program.

**KEYWORDS**

Stored product; disinfestation; lethal doses; sterile insect technique; inherited sterility

### 1. Introduction

The Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) is one of the important stored product pests in many countries around the world. It is a general feeder causing significant losses in meal, seeds, processed foods, and dried fruit and vegetables (Madrid & Sinha, 1982; Mbata, 1987; Shoukry, Khalaf, Hussein, & Khater, 2003). In recent years, *P. interpunctella* has developed resistance against the chemical treatments such as methyl bromide and phosphine fumigants that are extensive use to control stored product pests in many countries (Mullen & Dow, 2001; Rajendran & Sriranjini, 2008), these chemical treatments have a negative impact on the environment (ozone layer) and human health, thus alternative methods are required to manage this pest. The extreme temperatures have been suggested as successful methods for postharvest disinfection treatments of stored product pests (Arbogast, 1981; Chaudhr, 1997; Heather & Hallman, 2008). However, heat disinfection has a negative impact on the quality of host commodities, while the disadvantage of cold treatment is requiring a long exposure periods (Donahaye, Navarro, Rindner, & Azrieli, 1996; Gasemzadeh, Pourmirza, Safarlizadeh, & Mustafa, 2010). Many researches have been carried out into the use of ionizing radiation as disinfection treatment against stored-product pests where it is able to deeply penetrate pallet loads of food (Burditt, 1994; El-Naggar & Mikhail, 2011). The disinfestation treatment aims to kill or inhibit the development of immature stages using the lethal dose of ionizing radiation (Hallman, 2013; Mohandass, Arthur, Zhu, & Throne, 2007). It has been reported that ionizing radiation treatment is providing the absence of physiochemical effect and residues in the treated foods; moreover, it is considered as an environment-friendly technique, which makes it one of the most commonly used methods to control stored product pests in many regions around the world (Ayyaz & Yilmaz, 2015; FAO, 2003). In general, gamma and X-rays have wide use in disinfestation treatment and SIT programs in many countries, as they are cheaper than other methods of ionizing radiation. However, another form of ionizing radiation, fast neutron irradiation (FNI) is the most effective one for killing and sterilizing insects (Hallman, 2013; Snieder & Veld, 1975). Speaking, the FNI are about four times effective as compared to gamma ray in producing the same level of sterility of tsetse fly (Offori & Czock, 1971). Also, the FNI is about twice as effective as X-rays in producing lethality of *Tribolium confusum* (Glenn & Ducoff, 1976), in this respect, studying the effect of FNI on disinfestation of stored product pest and also to induce sterility in Lepidopteran insects is requiring.

Stored product insects were controlled directly with irradiation treatment in wheat, flour, grain products, and other dry food commodities in many...
countries. While, the lower doses of irradiation could prevent an increase in pest populations through induce sterilization of adult moths rather than cause immediately died (Hallman, 2000; Follett & Griffin, 2006). Sterile insect technique (SIT) is widely used against insect pests, this technique requires mass rearing of the targeted insect, sterilizing insect adults and release of the sterile adults into the field for mating with wild types in order to eradicate or suppress the population (Barnes, Hofmeyr, Groenewald, Conlong, & Wolfarter, 2015; Caceres et al., 2007; Calkins & Parker, 2005; Knipling, 1955). Inherited sterility (IS) offers significant advantage over classical sterile release method for Lepidopteran pests. Substerilizing doses of ionizing radiation induce deleterious effects that are inherited by the F₁ generation (Carpenter, 1985; Rizk, 2008). A review of the literature indicates that no risk associated with any potential escape of P. interpunctella where the F₁ sterility of irradiated moths could produce sterile progeny; therefore, no reproducing insect could be established, also no interactions between induced sterility and the ability to survive on different hosts/diets and in different environmental conditions (Hofmeyr, Hofmeyr, Carpenter, Bloem, & Slabbert, 2016); in this way, the protocol that would allow for release partial sterile moths (F₁ sterility) might be acceptable for P. interpunctella management. The current investigation highlights on the lethal dose of FNI, which could be used in disinfection treatment against the developmental stages of P. interpunctella. Also, this study aims to assess the FNI doses that induce sterility of the F₁ generation in the both sexes of P. interpunctella for using in SIT program.

2. Materials and methods

2.1. Test insect

The test insects were obtained from laboratory culture and maintained at 27 ± 2°C and 65% R.H. Newly emerged adults in Plexiglas cylinders were allowed to mate and oviposit in screen bottoms. Eggs that fell through the wire mesh were collected daily in open Petri dishes, the hatched larvae were placed in 2.0 l jars half filled with media suggested by Brower (1976). Full-grown larvae were collected daily, sexed, and kept separately in Petri dishes, each provided with corrugated cardboard as pupation site.

2.2. Fast neutron treatment

The different developmental stages, eggs (1–2 days old), full-grown larva (17 days old), and full-grown pupae (7–8 days old) of P. interpunctella were exposed directly to 14 MeV (mega electron volt) Neutron using a MP320 Neutron Generator. This source is located at the Nuclear Research Centre, Atomic Energy Authority, Cairo, Egypt. The five dose levels 0, 64, 128, 192, and 256 Gy (Gray) which equal five exposure times 0, 10, 20, 30, and 40 min, respectively, were tested. The dose rate of the source was 6.41 Gy/min.

2.3. Bioassay studies

2.3.1. Effect of FNI on egg stage

Newly eggs (1–2 days old) were placed in glass vials and exposed directly to fast neutron irradiation (FNI) source. Unirradiated eggs (control treatment) were subjected to the same conditions as the irradiated eggs that they were transferred to the FNI source and subsequently removed without exposure to the radiation source. After irradiation, eggs were placed in glass petri dishes until hatching, the egg hatch were daily counted under a binocular microscope. The newly hatched larvae were transferred to 300 ml glass jars containing 150 g of the rearing diet. Pupation, adult emergence, and longevity and sex ratio were recorded in each dose.

2.3.2. Effect of FNI on larvae stage

Full grown larvae (17 days old) were sexed and irradiated with the tested dose levels. Five replicates (25 larvae for each gender) were used for each dose level. Irradiated larvae were transferred to 300 ml glass jars containing 150 g of the artificial rearing diet and subsequently transferred to the rearing laboratory conditions. The pupae that developed from irradiated larvae were removed and counted daily. The emerged adult moths were sexed, recorded, and kept separately in order to setup the mating combination treatments.

2.3.3. Effect of FNI on pupal stage

Male and female full grown pupae (5 days old) previously sexed in larval stage were irradiated in the tested dose levels in glass petri dishes. The adult moths that emerged from irradiated pupae were sexed, counted, and kept separately. The developmental periods from egg to adult that developed from irradiated eggs larve and pupae were determined. Also, the longevities of these adults were recorded.

2.3.4. Sterility studies of FNI doses

The male and female moths that developed from irradiated larvae and pupae were crossed with the opposite sex of unirradiated adults in three combinations as follow, irradiated males with unirradiated females (U♀ × I♂), unirradiated males with irradiated females (U♀ × I♀), and unirradiated males with un-irradiated females (U♀ × U♂). Five replicates with five pairs per replicate were used for each dose level. The total numbers of eggs (fecundity) and their hatching (fertility) were
recorded. Also, the sex ratio of the emerged adults from irradiated immature stages was determined. In addition, the sterility, growth index, and reduction of F\textsubscript{1} progeny were measured according to the following equations (Kubo, Klocke, & Asano, 1981).

Sterility percentage = \( 1 - \frac{(F_t \times F_e_t)}{(F_c \times F_e_c)} \times 100 \)

Growth index = Percentage of adult emergence/total period.

Percentage of reduction in F\textsubscript{1} progeny = No. of adults emerged in control – No. of adults in irradiated treatment/No. of adults in control.

2.4. Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) technique and the means were analyzed using Duncan’s multiple range test \((P = 0.05)\) (Steel & Torrie, 1960). The data on the fertility, pupation, adult emergence, and mortality percentages were arcsine transformed, while, reported means and standard errors are of the original data. Fixed factor was the dose of fast neutron irradiation and the random effect of error was done.

3. Results

3.1. Effect of FNI on egg stage

When the egg stage (1–2 days old) was irradiated with FNI dose levels (64, 128, 192, and 256 Gy) the percentages of egg hatch \((F = 213.9; \text{df} = 15; P < 0.05)\), pupation \((F = 2511.6; \text{df} = 15; P < 0.05)\), and adult emergence \((F = 3457.6; \text{df} = 15; P < 0.05)\) were significantly reduced in the all doses applied as compared to the control treatments. The highest reduction of egg hatch was recorded at 256 Gy (0%), while it was (2%) at 192 Gy, also both pupation and adult emergence were reached 0.0% at 192 Gy (Figure 1).

The sex ratio was significantly increased in favor of males at the two doses of 64 and 128 Gy. The longevity of male moths didn’t reduce significantly as compared to the control treatment at 64 Gy, while the reduction was significant at 128 Gy; moreover, the longevity of female moths was reduced significantly at the two doses 64 and 128 Gy. (Table 1).

3.2. Effect of FNI on larval stage

The mortality percentages of the male and female larvae at the minimum dose 64 Gy were high significant (37 and 30%), respectively, as compared to (4 and 3%) in the control treatments, while, the maximum dose of 256 Gy caused highest mortality percentage (95 and 97%) of the male and female larvae (Figure 2). The same trend was observed in the pupal mortality where the mortality was increased gradually by increasing the dose levels applied.

The developmental periods of irradiated male and female larvae were significantly decreased by increasing the irradiation doses, also, a significant reduction was observed in the growth index among the different doses (Table 2).

![Figure 1. Percentages of egg hatch, pupation and adult emergence of P. interpunctella irradiated as eggs with FNI doses.](image)

| FNI doses Gy | (Av.) developmental period from egg to adult (days) ± SE | Growth index ± SE | (Av.) sex ratio | (Av.) adult longevity (days) ± SE |
|--------------|--------------------------------------------------------|-------------------|----------------|-------------------------------|
| 0            | 39.1 ± 0.57 a                                          | 2.5 ± 0.29 a      | 0.9 a          | 7.3 ± 0.33 a                  |
| 64           | 35.2 ± 0.33 b                                          | 1.3 ± 0.09 b      | 1.9 b          | 6.7 ± 0.33 b                  |
| 128          | 35.5 ± 0.33 b                                          | 0.7 ± 0.18 c      | 2.5 c          | 6.3 ± 0.33 b                  |
| 192          | 0.0                                                     | 0.0               | 0.0            | 0.0                           |

Means designated with the same letter in the same column are not significantly different \((P \leq 0.05)\)
The longevities of male and female moths were significantly decreased by increasing the FNI doses from 64 to 192 Gy as compared to the control treatment.

### 3.3. Effect of FNI on pupal stage

When full grown pupae were irradiated with dose levels of FNI, a significant reduction was observed in the pupal mortality in both males ($F = 43.8; \text{df} = 11; P < 0.05$) and females ($F = 75.5; \text{df} = 11; P < 0.05$) as compared to the control treatments and the mortality decreased gradually by increasing FNI doses to reach 100% at the dose of 192 Gy (Figure 3).

In the same treatment the average longevity in the male moths was insignificantly lowered (6.3 days) at 128 Gy than that recorded in the dose of 64 Gy (7.0 days) and the control treatment (7.3 days) While, in the female moths, the longevity averages in 64 and 128 Gy were significantly lower (5.5 and 5.7 days), respectively than the control treatment (7.7 days) (Figure 4).

### 3.4. F₁ sterility of the fast neutron irradiated larvae

The averages of fecundity were reduced gradually as the dose levels increased, this reduction were significant in the all doses applied in the two combination treatments $I^\oplus \times U^\ominus$ and $U^\oplus \times I^\ominus$ as compared to the control treatment $U^\oplus \times U^\ominus$, while no significant reduction was recorded within the two combination treatments of 64 and 192 Gy, while it was reduced significantly within the two combination treatments of 128 Gy (Table 3). The data in the same table

![Figure 2](image2.png)

**Figure 2.** Percentages of both larval and pupal mortality of *P. interpunctella* irradiated as full grown larvae with FNI doses.

| FNI doses Gy | (Av.) developmental period from egg to adults (days) ± SE | Growth index ± SE | (Av.) longevity (days) ± SE | (Av.) developmental period to adults (days)± SE | Growth index ± S.E | (Av.) longevity (days) ± SE |
|--------------|----------------------------------------------------------|------------------|-----------------------------|-----------------------------------------------|-------------------|-----------------------------|
| 0            | 34.3 ± 0.63 a                                           | 2.8 ± 0.14 a     | 7.6 ± 0.33 a                | 33.0 ± 0.56 a                                | 2.9 ± 0.06 a     | 7.0 ± 0.57 a                |
| 64           | 33.2 ± 0.07 ab                                          | 1.8 ± 0.14 b     | 6.3 ± 0.33 b                | 30.9 ± 0.05 b                                | 2.3 ± 0.06 b     | 5.7 ± 0.33 b                |
| 128          | 32.4 ± 0.91 b                                           | 1.1 ± 0.12 c     | 5.7 ± 0.33 b                | 31.2 ± 0.02 b                                | 0.85 ± 0.0 c     | 5.3 ± 0.33 b                |
| 192          | 30.5 ± 0.03 c                                           | 0.5 ± 0.07 d     | 4.7 ± 0.33 c                | 28.4 ± 0.03 c                                | 0.92 ± 0.0 c     | 3.7 ± 0.31 c                |
| 256          | 0.0 d                                                   | 0.0 e            | 0.0 e                       | 0.0 d                                        | 0.0 e            | 0.0 d                       |

Means designated with the same letter in the same column are not significantly different ($P \leq 0.05$)

![Figure 3](image3.png)

**Figure 3.** Mortality percentages of both male and female pupae of *P. interpunctella* irradiated as full grown with FNI doses.
demonstrated that the percentages of fertility were reduced significantly in the all doses at the two combination treatments as compared to the control treatment. Moreover, the mortality of immature stages (larvae and pupae) were significantly increased by increasing the FNI dose levels in the two combination treatments to reach 100% in 192 Gy. Similarly, the data of F₁ progeny revealed that the dose of 192 was more effective for using in F₁ sterility than those recorded in 64 and 128 Gy.

### 3.5. F₁ sterility of the fast neutron irradiated pupae

Table 4 shows that the average of fecundity and the percentages of fertility were significantly decreased by increasing the doses of FNI, also the fecundity and fertility in the combination I♂ × U♀ were lower than those recorded in the combination treatment U♂ × I♀ in each dose level; this lowering was significant. The data of sterility percentages indicate that at the FNI doses the sterility of irradiate male moths were higher than the female moths at the 64 Gy FNI dose. In the same table, the percentages of mortality in the immature stages and the reduction in F₁ progeny were (100%) at the dose of 128 Gy.

### 4. Discussion

Fast Neutron is another form of ionizing radiation that could be used successfully to manage the stored product pests by disinfestation treatment and induced sterility. It is recommended that the default dose of 400 Gy of X-ray or gamma ray was recently approved as a disinfestation treatment for stored product insects other than lepidopteran species, the lethal dose in lepidopteran species might be more than 400 Gy (FAO, 2003; Hallman, 2013), the Indian meal moth _P. interpunctella_ is one of the most radio tolerant insect known, which need a dose more than 600 Gy of gamma ray to prevent its reproduction (Hallman, 2000, 2013). It was reported that the FNI was more effective in producing lethality and sterility than gamma and X-rays (Offori & Czock, 1971; Glenn & Ducoff, 1976; Robinson & Van Heemert, 1981). Regarding a high penetration of FNI, it might be more effective for using in the disinfestation treatment and SIT much more than other form of irradiations.

### Figure 4

Longevity averages of male and female moths of _P. interpunctella_ irradiated as full grown pupae with FNI doses.

### Table 3. Averages of fecundity and sterility percentages of _P. interpunctella_ adults irradiated as full grown larvae with FNI and the lethal effect on the F₁ progeny.

| FNI doses Gy | Mating combinations (Av.) | Fertility (%) | Sterility (%) | Mortality of immature (%) | Reduction in F₁ progeny |
|--------------|---------------------------|---------------|---------------|---------------------------|-------------------------|
| 0            | U♂ × U♀                  | 178.3 ± 1.6 a | 97.6 ± 0.1 a  | 0.0                       | 2.03 ± 0.12 a           |
| 64           | T♂ × U♀                  | 150.0 ± 5.8 b | 68.1 ± 2.8 b  | 41.3                      | 59.03 ± 3.0 b           |
| 128          | T♂ × T♀                  | 141.7 ± 4.0 b | 49.5 ± 3.7 c  | 59.7                      | 75.9 ± 0.5 c            |
| 192          | T♂ × U♀                  | 113.0 ± 0.5 c | 26.2 ± 1.5 d  | 83.0                      | 92.8 ± 1.7 d            |

Means designated with the same letter in the same column are not significantly different ($P \leq 0.05$)

### Table 4. Averages of fecundity and sterility percentages of _P. interpunctella_ adults irradiated as full grown pupae with FNI and the lethal effect on the F₁ progeny.

| FNI doses Gy | Mating combination (Av.) | Fertility (%) | Sterility (%) | Mortality of immature (%) | Reduction in F₁ progeny |
|--------------|---------------------------|---------------|---------------|---------------------------|-------------------------|
| 0            | U♂ × U♀                  | 181.7 ± 4.4 a | 98.5 ± 0.4 a  | 0.0                       | 4.5 ± 1.4 a             |
| 64           | T♂ × U♀                  | 100.0 ± 2.9 b | 26.6 ± 2.8 b  | 85.0                      | 84.7 ± 1.0 b            |
| 128          | T♂ × T♀                  | 120.0 ± 2.9 c | 31.9 ± 0.8 c  | 78.5                      | 90.7 ± 1.0 c            |
| 192          | T♂ × U♀                  | 85.0 ± 5.7 d  | 13.6 ± 1.1 d  | 83.5                      | 100 ± 0.0 d             |

Means designated with the same letter in the same column are not significantly different ($P \leq 0.05$)
The present results indicate that the lethal dose for irradiating immature stages with FNI was varied with different stages, irradiating eggs at 192 Gy prevented the egg hatching, while, 256 Gy prevented the pupation in the case of irradiating the full grown larvae; however, irradiating full grown pupae with 192 Gy prevented adult emergence. These data of lethality are different from those recorded by other researches when gamma irradiation applied on *P. interpunctella*, the gamma irradiation dose 450 Gy was prevented the egg hatch of *P. interpunctella* (Aye et al., 2008; Ozyardimciz, Cetinkaya, Denli, Ic, & Alabay, 2006), while 650 Gy prevented the irradiated pupae to reach pupae (Abbas et al., 2011). The present results coincide with the results of Saeed, Kwon, and Kausar (2006) who noticed that the 500 Gy caused 100% mortality in the both pupae and adults of *P. interpunctella*, while the larvae were more resistant to the same dose of gamma irradiation. On the other hand, the present results indicate that the sublethal doses of FNI reduced the developmental periods at all doses applied, the mortality of immature stages with the sublethal doses is belonging to the lethal mutations induced by irradiation which may exert lethality to any developmental stage (Bloem, Carpenter, & Hofmyer, 2003; Saour & Makee, 1997). The data also revealed that the growth index was drastically decreased by increasing the dose of irradiating all immature stages. In addition, the obtained data showed that the longevity were shorter in all irradiation doses than that at the control treatment. These effective doses that induce lethality are much less than those obtained by Saeed et al. (2006), Ayvaz and Tuncbilek (2006) and Ayvaz, Albayrakl, and Karaborklu (2008) when they were using gamma irradiation technique on *P. interpunctella*.

5. Conclusion

The adult moths resulted from irradiated pupae were more sterile than the adult moths resulted from irradiated larvae at the same substerilizing dose, also, the dose of 192 Gy was recorded as a sterilizing dose of irradiated larvae, while 128 Gy caused full sterility of the irradiated pupae. Furthermore, irradiating full-grown pupae with 256 Gy of FNI is an effective dose for using in the disinfestation treatment, while the dose of 128 Gy could be used in SIT/IS program for *P. interpunctella* management.

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