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Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) Immature-Stage Predisposition Post ^{60}Co Ray Treatment

Omar, Y. M.
Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, EGYPT
Corresponding author: Youssef M. Omar (youssf@yahoo.com)

ABSTRACT

The effect of gamma irradiation on the different developmental stages of the *Plodia interpunctella* was studied. Eggs less than 24 hr. old, larvae 2 week-old and fully grown pupae were irradiated with 60, 80 and 100 Gy. Hatchability drastically decreased in the treatments compared with control, the incubation period slightly increased by increasing the radiation dose from 60 to 100 Gy. About seven percent of treated eggs were pupated but did not emerge to adult. While, increasing dose to 80 Gy caused death for all individuals. Larval and pupal duration prolonged by increasing the dosage. In general, irradiation reduced the life span of the resulting adults. Sex ratio seemed to be affected in favor of males at larvae. Fully grown pupae showed the same trend. However, sex ratio shifted in favor of females. Malformed adults increased by dose increasing. It might be summarized that the susceptibility of *P. interpunctella* was radiation-dose dependent.

Keywords: Indian meal moth, pest control, stored product, ionizing radiation

INTRODUCTION

The Indian meal moth is one of the most crucial moth attacking dried fruits in warehousing and dwellings. Larvae are general eaters and they can be found in grain product, seeds and dried fruits (Simmons & Nelson, 1975; Arbogast, 1981 and Johnson *et al*., 1995). Damage in case of *P. interpunctella* occurs when larvae spin massive amount of silk that accumulate fecal pellets, cost skins and egg shells in foodstuffs. The damage to stored products due to this taint phenomenon, which exceeds the amount of food eaten by insect (Zakladnoi & Ratanova, 1987 and Hill, 1990).

Ionizing radiations can be used to accomplish sterility for population management and eradication programs, as a phytosanitary treatment, and for control of insect plague (Bakri *et al*., 2005). Research on irradiation of grains is being directed primarily to the practical objective of controlling infestation by killing insects or by inhibiting their reproduction and thus preventing storage losses and extending storage life. Such factors as age and life cycle have a considerable effect on sensitivity to radiation. Some important effects of radiation are not immediately apparent but shown up later as death or injure in succeeding stages or even in progeny (Golumbic & Davis, 1966; Hasan & Khan, 1998 and Hallman *et al*., 2010). The detailed and complete summary on the effects of ionizing radiation on several species of stored-product insects was prepared by Hilchey (1957). The radiation affected mortality, arrested development, inhibited or prevented reproduction, reduced or enhanced longevity and affected physiological processes (Hasan & Khan, 1998 and Hallman & Phillips, 2008). Varying susceptibilities to radiation according to insect age and stage were demonstrated by Peredel'skii *et al.*, (1957) and factors affecting the susceptibility were mentioned by Watters (1968) and Hasan & Khan (1998) and studied by Hallman *et al*., (2010). Numerous contributions to the knowledge of the effects of radiation on the Indian meal moth, *P. interpunctella*, studied by many authors e.g. Cogburn *et al.* (1966); Bagheri (1974); Brower (1980); Johnson & Vail (1987, 1988 & 1989); Azelmat *et al*. (2005); Ozyardimci *et al*. (2006); Aye *et al*. (2008); Ayvaz *et al*. (2008); Hallman & Phillips (2008) and Abbas *et al*. (2011).

Therefore, this investigation was conducted to evaluate the susceptibility of eggs, larvae and pupae to gamma radiation to find out an appropriate radiation dose which can help in preventing damage and loss of stored grain.

MATERIALS AND METHODS

Rearing technique. The insects were collected from the infested maize grains stored in the main store of the Faculty of Agriculture, Assiut University. Moths of both sexes were confined and allowed to mate in 2-liter glass jars containing crushed sorghum. Cultures were maintained in the laboratory at 27+2°C and 50+5% R.H. for several generations to ensure complete adaptation of stock.

Irradiation technique. Eggs, larvae and pupae of *P. interpunctella* were exposed to gamma rays using a 60C gamma cell unite installed in Radiotherapy and Nuclear Medicine Department, Assiut University, with dose rate of 132 rad / Sec during the experimental period.

Treatments.

Egg stage. Eggs, less than twenty four hr. old, were collected in batches of 50 eggs each in small plastic tube (0.5 x 4 cm). Four batches were irradiated with 60, 80 and 100 Gy. Eggs were left for ten days for hatching to determine the incubation period, larval, pupal duration, longevity and percentages of hatchability, adult moth emergence, malformation and sex ratio.

Larval stage. Emerged larvae were placed in glass tube (4 x 10 cm) (50 larvae per tube) with food (crushed sorghum) and transferred to an incubator for two week at 25+1°C and 60+5% R.H. Two week-old larvae were radiated with 60, 80 and 100 Gy. The treated larvae were followed up until pupation and emergence of adult moths. Larval, pupal duration, percentage of adult moth emergence, longevity, percentages of malformation and sex ratio were recorded during the course of this study.

Pupal stage. Newly pupated individuals were isolated from the stock culture and were placed in glass tube (1.5 x 5 cm). About 80 one-week old pupae in 4 replicates were irradiated with 60, 80 and 100 Gy. After treatment, pupae were followed up until adult emergence. Pupal duration, percentage of emergence, longevity, percentage of malformation and percentage of sex ratio were recorded.

In all cases untreated equal number of all stages were left as control.

Data analysis. Data were analyzed using analyses of variance by Mstat-C, 1988 software package and means

were separated using the least significant differences method only in case of significant "F".

RESULTS AND DISCUSSION

Effect of gamma radiation on different stages of *P. interpunctella*

Egg stage

Data presented in Table (1) show the effect of gamma radiation on newly laid eggs (less than 24 hours) of *P. interpunctella*. The incubation periods slightly increased by dose increasing. It increased from 3.0 days in the control to be 3.08 days in the eggs treated with 100 Gy. The percentage of hatching decreased gradually as the dose increased. One-day-old eggs treated with 60, 80 and 100 Gy resulted in 33.33, 27.00 and 16.25%, respectively; while in the checks it was 99%. Larval duration prolonged to 31.50 days at 60 Gy. compared with 23.53 days in the control treatment, while 80 Gy dose level was lethal to hatched larvae from one-day-old eggs. The percentage of pupation heavily decreased from 57.64 in the control to 7.22 in those resulted from eggs treated with 60 Gy. Also, these pupae could not complete their life cycle. So, 80 Gy dose level could be considered the dose which prevented the pupation for all larvae resulted from one-day-old eggs. The larvae produced from the control eggs normally completed their life cycle.

Table 1. Effect of gamma irradiation on some biological traits of *P. interpunctella* exposed as less than 24 hrs-old-age eggs.

Dose (Gy)	Incubation period (days)	% hatch-ability	Larval duration(days)	% pupation	Pupal duration(days)	% emergence	Longevity (days)	% mal-formation	% of sex ♂	% of sex ♀
0*	3.00 ± 0.25 a	99	23.53 ±1.90 b	57.64	10.69 ± 2.20	59.19	9.42 ± 3.50	0.0	59.00	41.00
60	3.04 ± 0.25 a	33.33	31.5 ± 2.38a	7.22	0.0	0.0	0.0	0.0	0.0	0.0
80	3.07 ± 0.20a	27.00	0.0 ± 0 c	0.0	0.0	0.0	0.0	0.0	0.0	0.0
100	3.08 ± 0.27a	16.25	0.0 ± 0 c	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*** Control**

- Incubation period, larval, pupal duration and longevity expressed as means ± standard deviations.

- Insignificant means have the same letter in the same column (Significance ≤ 0.05).

In general, the early developmental stage of a creature is an uttermost radiosensitive period and insects are one of those (Tilton & Brower, 1983). However, the particular phase of embryo influences the radiosensitivity effects. Many investigations were done on unlaid eggs, agamogenetic embryos and fertilized embryos (Hussain *et al*., 1994; Ahmed *et al*., 1976; Ghomomu, 1989). Hasan & Khan (1998) could summarize the results in the aforementioned studies as follows: the susceptibility of unlaid eggs, which is subjected to gametogenesis, depend on the meiotic stage; laid eggs, whether growing agamogenetically or after fecundation, are more radiosensitive than other growing stages and 3, embryo radiosensitivity relies on the development of the stage.

The results in this work are in confirmatory with the aforesaid information and in parallel with the coming studies by many investigators, e.g. Brower (1974) stated that the eggs of *P. interpunctella* are rather susceptible to radiation for about ½ of their growing time, and become 25 folds more radio resistant at 72 h than at 2 h after oviposition. Also, Hasaballa *et al*. (1985) irradiated one-day-old-egg of *Ephestia cautella* (Walk.) with 2.5, 5, ….till 15 krad and found that irradiation has a negative effect on the percent of hatchability, pupation and adult emergence; the effect becomes more severe as increasing the dosage. Abdel-Baky *et al*. (1990) found that *Plodia interpunctella* eggs were more sensitive to gamma rays than larvae and rays also significantly reduced the hatchability, pupation and adult emergence at higher dose of 90 gray at 4-day-old-egg. The rays also shortened the longevity and increased drastically the malformation of adults. Also, Hallman (2004) proposed that more than 0.4 kGy was enough to prevent egg hatch. The rate of egg hatch of *P. interpunctella* was 7.6% at 0.3 kGy and completely stopped at 0.45kGy, where with almond moth, *Ephestia cautella* (Walker) egg hatchability was stoped at 0.3 kGy dose. This is probably because of *P. interpunctella* eggs more resistive to gamma irradiation than those of *E. cautella* (Ozyardimci *et al*., 2006). In addition, *P. interpunctella* hatching eggs was almost entirely stopped by irradiation at 0.5 kGy and higher doses (Aye *et al*., 2008), as well as when 1 to 2 days old eggs were irradiated, egg viability, pupation and adult emergence decreased with radiation doses increase. Also before, it was found that when one–to three-day-old eggs of *P. interpunctella* were exposed to 350 Gy, no eggs hatched (Ayvaz *et al*., 2008). The deviations between the present results and the previous ones might have been correlated to the egg age differences at the time of irradiation, where radiosensitivity differs with the stage of embryologic processes (Ayvaz *et al*., 2008) or because of the radiosensitivity of *P. interpunctella* eggs differ according to the developmental processes from egg to adult (Abbas *et al*. 2011). At the end, egg age drastically affected the hatchability when eggs were irradiated; resistance to irradiation was increased with egg-age increase.

Larval stage

Results obtained in Table (2) show the effect of gamma irradiation on the successive stage of 2-week-old larvae. The increase of radiation dose causes a moderate prolongation in the larval duration from 18.00 days at the control to 23.39 days at 100 Gy. The larval duration affected by different irradiation dose. The pupation percentage of irradiated larvae decreased as the dose increased. The doses 60, 80 and 100 Gy decreased the percentage of pupation to 59.35, 45.88 and 29.35 respectively, compared with 63.27 in the control. The pupal duration was less affected at all doses used. It was 10.82 days at the control, while it was 11.83 days at 100 Gy. The percentage of adult emergence was 59.19 at the control, while this value decreased to 51.52, 18.21 and 16.67 at 60, 80 and 100 Gy, respectively. When 2-week-old larvae were irradiated the longevity was 9.86, 7.70 and 7.40 days at 60, 80 and 100 Gy, respectively. The longevity of those in the control was longer than that of treated ones (10.35 days). In general, Irradiation of 2-week-old larvae reduced the life span of the resulting adults. The results show that the

percent of malformed adults that developed from irradiated larvae increased by increasing the radiation dose. The higher doses 80 and 100 Gy caused highly percentages of malformation (61.54 and 66.67), respectively, where it was 31.58 % at 60 Gy. The males seemed to be more successful in tolerance than females with all dosages used. The sex ratio (male : female) of adults emerged from larvae exposed to irradiation doses 60, 80 and 100 Gy were in favor of males.

Many studies had been done before on the sensitivity of the larval stages of miscellaneous storage pests (e.g. Nair & Subramanyam, 1963; Yang & Ducoff, 1971; Allotey, 1985; Johnson & Patrick, 1988 and Hasan *et al.,* 1989…). Their results could be summarized as following: 1, larval stage are more radioresistant than egg stage; 2, larval stage radiosensitivity varies from one another; 3, irradiant lengthened larval period and postponed pupation; 4, a high percentage of mortality can happen due to radiation to the larvae in a stage of moulting or pupating. In the present study, a dose of 100 Gy prevents about 83% of larval population from development and emergence to adult. Results in the present study were in the same line with the results mentioned above and with the results of Abbas *et al*. (2011) who reported that larval growth was entirely stopped by a dose of 400 Gy and larvae failed to pupate. Also, Last-instar larvae of *E. kuehniella* Zeller was entirely dead by a dosage of 250Gy, while a dose of 200 Gy applied to the young larvae of *E. kuehniella* entirely precluded female adult emergence, all emerged moths were males (Ayvaz & Tuncbilek, 2006).

Table 2. Effect of gamma irradiation on some biological traits of *P. interpunctella* larvae exposed as two-week-old days.

Dose (Gy)	Larval duration (days)	% pupation	Pupal duration (days)	% emergence	Longevity (days)	% mal-formation	% of sex ♂	% of sex ♀
0*	18.00 ± 2.00 b	63.27	10.82 ± 1.54 a	59.19	10.35 ± 2.97 a	0.0	60.53	39.47
60	19.94 ± 3.68 b	59.35	11.03 ± 4.22 a	51.52	9.86 ± 4.99 a	31.58	61.76	38.24
80	22.60 ± 4.34 a	45.88	11.10 ±3.41 a	18.21	7.70 ± 3.80 a	61.54	83.33	16.67
100	23.39 ± 4.22 a	29.35	11.83 ± 3.97 a	16.67	7.40 ± 2.90 a	66.67	92.31	7.69

*** Control**

- Larval, pupal duration and longevity expressed as means ± standard deviations.

- Insignificant means have the same letter in the same column (Significance ≤ 0.05).

In addition, 300 Gy was the minimum dosage foreclosed adult emergence of *P. interpunctella*, while 450 Gy and above was demanded to cause death (Azelmat *et al*., 2005). The same trend, Johnson & Vail (1988) reported that 11-day-old *P. interpunctella* larvae could not be developed to adult when radiated by 149Gy. Likewise, Mansour (2002) also found that a dosage of 200 Gy was enough to stop adult emergence of irradiated mature larvae of codling moth *Cydia pomonella* (L.). Besides, Cogburn *et al.* (1973) reported that the larvae of *Cadra cautella* Walker irradiated with 20 krad (200 Gy), no adult could emerge. The effects of sublethal doses of gamma rays on the biology and behavior of *S. cerealella* were studied by treating the early and late first larval instars with dose of 2-20 k.rad and third and last larval instars with doses of 5-25 krad; results showed that earlier stages were more sensitive than the old one, more males than females emerged when early larvae were irradiated and structural deformities included twisted wings, fusion of tarsal segments, fusion of antennal segments, incomplete emergence and tumor formation in the abdominal region as well as the adult longevity was not significantly different from the control. Also, the emergence of *Sitotroga cerealella* adults from irradiated fully grown larvae occurred at 18 k.rad, but all emerged adults were malformed (Qureshi, 1966; Qureshi *et al*., 1970 and Ahmed *et al*., 1983). Furthermore, Tilton & Brower (1983) pointed out that irradiation of the actively growing stages of an insect can have several effects, depending on the dose and age or physiological condition of the insect. Unless massive doses are employed, death is not the first effect to be observed.

Pupal stage

Pupae were found to be the most resistant metamorphic stage to radiation. As shown in Table (3) some individuals from all dosages levels were able to complete their development and became adults. The pupal duration ranged from 1.57 days in untreated pupae to 1.80 days, when irradiated with 100 Gy. Emergence from untreated pupae average 100.00 %, where it was 57.14 % at 100 Gy. It is clear from the results that mortality rates for adults greatly increased by increasing the dose levels. Irradiation of fully-grown pupae marginally shortens the lives of the emerging moths. While the normal moths lived 4.37 days, meanwhile those who resulted from irradiated pupae with 60, 80 and 100 Gy, lived 4.00, 3.79 and 3.42 days, respectively. The obtained results revealed that difference in adult emergence decreased from 71.43 to 57.14 % by increasing dose from 60 to 100 Gy, respectively. When adults emerged from irradiated fully grown pupae, malformation of moths were more pronounced. It was 53.33, 56.25 and 75.00 % at doses of 60, 80 and 100 Gy, respectively, whereas there was no malformation in the control treatment. Generally, as dose increased, the sex ratio shifted in favor of the females.

Table 3. Effect of gamma irradiation on some biological traits of *P. interpunctella* exposed as fully grown pupae.

Dose (Gy)	Pupal duration (days)	% Emergence	Longevity (days)	% malformation	% of sex ♂	% of sex ♀
0*	1.57 ± 0.79 a	100	4.37 ± 1.06 a	0.0	62.00	38.00
60	1.67 ± 0.78 a	71.43	4.00 ± 1.73 a	53.33	41.67	58.33
80	1.75 ± 0.77 a	71.43	3.79 ± 1.92 a	56.25	40.00	60.00
100	1.80 ± 0.79 a	57.14	3.42 ± 1.38 a	75.00	37.50	62.50

*** Control**

- Pupal duration and longevity expressed as means ± standard deviations.

- Insignificant means have the same letter in the same column (Significance ≤ 0.05).

The studies on the radio-susceptibility of pupae have been aroused mainly to produce a sterile but normal insects to be used in pest control (Cornwell, 1964). In different ways the pupal stage is correspondent to an embryotic stage when intense cellular division and distinction take place. Therefore, it is not exceptional that all forms of radiosensitivity of pupae are alike to the forms shown by developing eggs (Tilton & Brower, 1983). The accuracy of most pupal radiosensitivity studies is not well enough or at least not related to the developmental alteration occurring to reflect the fine details in the form of radiosensitivity because pupae can be segregated by the time elapsed after pupation or by visual changes as an evident for internal development . Irradiation can have many obvious effects on pupae, e.g. pupal mortality, adult malformations and delayed adult emergency. The mortality in pupae is hard to ascertain and the emergence failure considers a death for pupae.

In this study pupal mortality, % malformation and sex changes in favor of females increased by dose increasing. The results here are more or less likewise with the following studies. Abbas *et al*. (2011) reported that the percentage of adult emergence of *P. interpunctella* pupae decreased by doses up to 650 Gy. When *Ephestia calidella* (Guenee) pupae were exposed to doses of 200 to 800 Gy.; the percent of adult emergence was decreased with the dose increase (Boshra & Mikhaiel, 2006). In addition, the life span of adults emerged from treated pupae, was shortened depending upon; the dose (highly dose, highly shortened longevity); the species of insect (Cogburn *et al.,* 1966 and Hasaballa, 1994). Cogburn *et al*. (1971) during the treatment of mature pupae of *Sitotroga cerealella*, noted that radiation reduced insect emergence by 33.7 % when treated by about 10 krad. As well as, Cogburn *et al*. (1966) also found that the lifespan of insects when treated as pupae was not greatly shortened by the treatments. Moreover, the accurate reason(s) of mortality due to irradiation still ambiguous because the nuclear and cytoplasmic changes are hard to distinguish. They thought that mortality probably due to chemical changes in the cytoplasm during pupal differentiation to produce adult structures and radiation caused alteration in the chemistry of the pigmentation, which could develop anti-metabolites. Ultimately, irradiation of the pupae might disrupt other biochemical mechanisms which have uninterrupted from the immature stages to the adult and such interruption could produce contrary effects on metamorphosis and on the lifespan of the adult (Erdman, 1968).

Sex ratio in the present study was in the favor of males in the larval stage, whereas it was a contrary in the pupal stage. However, in both stages sex ratio alteration increased with a dose increase. Sex ratio distortion in Lepidoptera happened due to the chromosomal rearrangements which may disturb the mechanism that determines sex, perhaps by changing the production of hormones or enzymes that successively cause greater female/male mortality (Proshold & Bartell, 1970 and La Chance & Richard, 1973) or as a consequence of recessive deadly mutations caused in the Z sex chromosomes (Marec *et al*., 1999).

The literature mostly supports the results here even though the sensitivity of insects to radiation depends on many internal and external factors such as low oxygen, pest stage, host, dose rate, temperature, insect's state of hydration, or moisture content, Diurnal rhythms, genetic differences related to geographical diversity within a species and different kinetic organization of chromosomes (Cornwell *et. al*., 1957; Cornwell, 1966; Gassner & Klemetson, 1974; Fisher, 1997; Hallman, 2003 and Hallman *et al.*, 2010). Ultimately, Tolerance of stored-product insect in response to gamma rays obviously differs from one another (Selman & Hasan, 1995), as well as in the same genus (Cornwell *et al*., 1957) and also in a single stage (Laundani *et al*., 1965).

The results of the present study claims that a dose of 100 Gy is demanded to prevent adult emergence in irradiated eggs, stopped 83 and 43% from reaching adulthood in the irradiated larvae and pupae, respectively, and caused 75% malformation in the emerged adult from treated pupae. It is recommended that a dose more than 100 Gy should be applied to manage the population growth of *P. interpunctella* when targeting pupae. Irradiation disinfestation is a very safe and clean method of pest control and food preservation comparing to conventional methods. Much of the food spoiling through premature sprouting or by decay organisms or insect infestation will be fruitfully prevented through irradiation. In recent years, there has been a tremendous development in radiation research and it is hoped that newer technologies will be discovered which will help in minimizing the cost of irradiation facilities.

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حساسية الأطوار غير الكاملة لفراشة الذرة الهندية بعد المعاملة بالكوبالت المشع

يوسف محمد عمر

قسم وقاية النبات – كلية الزراعة – جامعة أسيوط – أسيوط – مصر

تم دراسة تأثير أشعة جاما على المراحل التنموية المختلفة للبلوديا إنتيربونكتيلا. وتم تعريض البيض ذات عمر أقل من 24 ساعة ، اليرقات عمر اسبوعين و العذاري كاملة النمو لجرعات 60 و 80 و 100 جراي .أظهرت النتائج إنخفاض قابلية البيض للفقس بشكل كبير في المعاملات مقارنة مع المقارنة، زيادة فترة الحضانة بمقدار طفيف بزيادة جرعة الإشعاع من 60 إلى 100 جراي. و تعذر حوالي 7 ٪ من البيض المعامل ولكن لم يصل الي طور الحشرة الكاملة، في حين أن زيادة الجرعة إلى 80 جراي تسبب بموت جميع الأفراد. استمرت اليرقات و العذاري لفترات أطول نتيجة زيادة الجرعة. بشكل عام، خفض الاشعاع عمر الحشرات الكاملة. النسبة الجنسية تأثر لصالح الذكور عند معاملة اليرقات .أظهرت العذاري نفس الاتجاه في النتائج. ومع ذلك، تحولت النسبة الجنسية لصالح الإناث. ازداد عدد الافراد المشوهة بزيادة الجرعة .ويمكن تلخيص أن حساسية فراشة الذرة الهندية يعتمد علي مقدار الجرعة الإشعاعية.