IRREVERSIBLE FERTILITY OF IRRADIATED *PHTHORIMAEA OPERCULELLA* (LEPIDOPTERA : GELECHIIDAE) FEMALES

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ABSTRACT

The fertility of *Phthorimaea operculella* females irradiated with four different gamma ray doses, 0, 100, 150 or 200 Gy, was investigated. Egg distribution and hatching during the oviposition period were monitored. The effects of delayed and repeated mating on fertility of irradiated *P. operculella* females were also studied. The egg distribution was found to be influenced by female age. A significant reduction in the number of eggs was observed when the females were 3 days - old or older. The results showed that late and early embryo death, in relation to sterility of the females, depended on gamma radiation dose. Neither delayed mating nor repeated mating influenced the viability of sterilized females. Fertility was never recovered after the irradiation of *P. operculella* females. Low-dose irradiated females can be used to collect and dispose wild males' sperm, which has an advantage in bi-sex release strategy. These findings provide important information to be considered when the partially sterilized male technique is employed as a control method against *P. operculella*.

KEY WORDS: *Phthorimaea operculella*, radiation, recovery, fertility, mating.

INTRODUCTION

The potato tuber moth *Phthorimaea operculella* Zeller (Lepidoptera : Gelechiidae) is a cosmopolitan pest. It has been monitored and reported in more than 90 countries around the world (Harsimran 2014). The total production losses range from 50 to 100 % as a result of potato tuber moth (Ahmed *et al*. 2013). Insecticides are widely used to control this pest, but this method is costly, nonselective, environmentally unfriendly and only effective for a short period of time in treated areas (Harba and Idris, 2018; Idris *et al*., 2019). Moreover, resistance to insecticides is a recorded frequent phenomenon among Lepidoptera. (Larraín, 2009). Thus, new control methods, that are more environmental friendly, are required. The sterile insect technique (SIT) is considered an important approach to control insects in large areas, and can be used as a main component of an integrated pest management system (Eyidozehi *et al*., 2015). Furthermore, the costs of using SIT are likely to be more acceptable in terms of monetary expenditures and efficacy, when they released dominant–lethal strain *Ae. aegypti* mosquitoes (Khater, 2018) . For the potato tuber moth, the inherited sterility technique has been suggested as an alternative control method (Vreysen *et al*., 2016). In all current sterilizing programs against Lepidoptera, both males and females are mass-reared, irradiated, and released into a target area. This is because no practical methods for separating adult moths by gender are available (Blomefield *et al*., 2011; Eyidozehi *et al*., 2015). Earlier reports pointed out that irradiation of moths with a 200 Gy dose resulted in partial sterility in males and full sterility in females. Inherited sterility did not occur in *P. operculella* females, and infertility of irradiated males is irreversible (Makee and Saour 2004). Therefore, it is crucial that females do not recover fertility before release to prevent any increase in pest populations (Vreysen *et al*., 2016). Thus, the aim of this study was to determine whether irradiated females recover their fertility after irradiation. For this purpose, we investigated the fertility of irradiated females in relation to egg distribution during the oviposition period, delayed and repeated mating, mating ability, and the mean number of laid eggs.

Materials and Methods

Insects and irradiation

The insects used in this study were reared on wax coated potato slices, and maintained at a constant temperature of 25±1˚C, with relative humidity of about 70±5 %, and 12 hours light-darkness cycle, as described by Makee and Saour (2004). Newly emerged *P.operculella* females (0-18 h) were irradiated with one of various doses of gamma rays: 0, 100, 150 or 200 Gy in a gamma cell supplied with a Co-60 source rounded the cylindrical (15x25cm^2) irradiation chamber (Isslcdo-vatel Gamma Irradiator, Techsnabexport Co. Ltd. USR). The average dose rate at the time of irradiation was approximately 40.12 Gy/ min with a factor of homogeny (max: min dose ratio) of about 1.05 and the absorbed dose was calibrated with Fricke solution. During this treatment, adult females were kept individually in small plastic tubes inside the irradiation source.

Eggs distribution during the oviposition period and fertility of irradiated females

Thirty newly emerged females were irradiated for each dose 0, 100, 150, 200 Gy. The irradiated females were paired individually with newly emerged, unirradiated males in a 350 mL transparent plastic boxes provided with a band of filter paper for oviposition and

10 % sucrose solution as food source. At each studied dose, the irradiated females were left with males for 24 h. The females were then kept for oviposition until death, and the eggs laid were collected daily, and counted and set aside for determining the fecundity and fertility of the irradiated females. The fertility was calculated using the following formula: fertility = number of hatched eggs / total number of eggs. After death, the females were dissected and examined for the presence of spermatophores in the bursa copulatrix to determine their status. Statistical analyses were performed using the STATISTICA program version 6 (Statsoft, Inc. 2003) at 0.05 level ($p < 0.05$). Data were subjected to analysis of variance (ANOVA) to determine the differences among groups of irradiated females. Differences between means were tested for significance using Tukey's HSD test.

The effects of delayed and repeated mating on fertility of irradiated females

Three hundred newly emerged females were irradiated for each dose 0, 100, 150, 200 Gy. Different groups of virgin irradiated females individually confined in 350 mL transparent plastic boxes for different periods of time (1-10 days) before mating with unirradiated males (0-24 h). Each group of irradiated females was divided into ten subgroups (each comprising 30 individuals). Females of the first subgroup were individually paired immediately after irradiation with unirradiated males. In the second subgroup, females were individually paired with unirradiated males after one day of irradiation and the third subgroup individually paired with unirradiated males after two days of irradiation. Females in subgroups 4-10 were individually paired with unirradiated males after 3-9 days of irradiation, respectively. The males in each subgroup were removed after 24h, and the females were set to monitor for oviposition. Eggs were collected daily, counted and allowed to hatch to determine the fecundity and fertility of females. After death, females were dissected in order to determine the presence and number of spermatophores as evidence mating. Simple linear regression analysis was done for studying the relationship of mating time and the ability of mating, fertility and fecundity in the irradiated females. Significance of liner relationship were tested by t-tests.

The effect of repeated mating on the mating ability, the mean number of eggs and fertility in P. operculella irradiated females

Fifty newly emerged females were irradiated for each dose 0, 100, 150, 200 Gy.

Groups of irradiated females, were individually paired with newly emerged males. The males were then removed after 24 h and replaced with new 1-day-old males. The same procedure was followed for 10 successive days. The females were then kept for oviposition until death. The eggs were collected, counted and allowed to hatch. After death, the females were dissected to determine the presence and number of spermatophores. The percentages analyzed by applying normal approximation test (analysis of proportions) and differences between means were tested for significance using Tukey's HSD test.

Results and Discussion

Eggs distribution during the oviposition period and fertility of irradiated females

Figure 1 presents the mean numbers of eggs laid by irradiated and unirradiated females laid per day. Daily oviposition decreased steadily with age in unirradiated females

"F=2.847, df =1, 23, P<0.0001". A reduction in the number of eggs was apparent 3 days after the oviposition period (Fig. 1). The differences in the daily number of laid eggs were significant for 100, 150, 200 Gy. Apparently, most of the eggs of unirradiated and irradiated females at 100 Gy were laid between of 2 and 4 days, while at higher doses (150 - 200 Gy), most eggs were laid in days 3-4 of the oviposition period, and no regularity in the eggs distribution was observed during the oviposition period at these high doses. The obtained results indicated that the irradiation of the females significantly affected egg distribution. There was a significant difference in the mean number of eggs between irradiated and unirradiated females in the first two days. However, there were no significant difference in the mean number of eggs on days 3 and 4 at 100 and 150 Gy (Fig. 1). Figure 2 shows that the egg hatchability of females decreased with age from the 3rd days of oviposition period "F= 27891.742, df= 1, 23, P< 0.001". For example, the percentage of hatched eggs in the first day of the oviposition period was ~ 82% (for females aged 1 day), this was significantly decreased by 38% and 18% for females aged 4 and 5 days, respectively (based on P-value comparisons, P<0.001). The percentage of hatched eggs reached to zero after the fifth day of oviposition period. The results also indicated that fertility was influenced by gamma irradiation; a decline was apparent in the ratio of hatched eggs of females irradiated with 100 Gy in the last days of oviposition period (based on P-value comparisons, P<0.001) (Fig. 2). The eggs of irradiated females with 200 Gy did not hatch, regardless of the time of oviposition. Figure 3 shows significant relationships between late embryo death (eggs that died during the late embryonic), early embryo death (eggs that died during the early embryonic) and sterility with different doses of gamma rays "R^2=0.92, P<0.001; R^2=0.92, P<0.001; R^2=0.87, P<0.001, respectively".

The effect of delayed and repeated mating on the fertility of irradiated females

Figure 4 suggests that, the mating ability of irradiated and unirradiated females decreased significantly with increased mating delay. The present results show, negative relationships between the mating ability and age of females for each applied dose "R^2 = 0.97, P<0.001; R^2 = 0.93, P<0.001; R^2 =0.95, P<0.001; R^2=0.92 , P<0.001, respectively" (Fig. 4). There were significant differences in the mating ability between unirradiated and irradiated females regardless of the mating delay, which seems correlated to the female age at 100, 150, 200 Gy "t = 7.2 , P <0.05; t = 6.7, P <0.05; t = 6.2 , P <0.05, respectively". The results also showed significant decline in the fertility of irradiated and unirradiated females due to the mating delay, especially when high doses were applied. The results also illustrated the existence of significant linear relationship between female age during the mating process and fertility at 0, 100, 150 Gy doses "R^2 = 0.88, P<0.001; R^2 =0.98, P<0.001; R2 =0.97, P<0.001, respectively" (Fig. 5). There were significant differences in fertility between unirradiated and irradiated females at 100, 150, 200 Gy "t = 6.7, P <0.001; t = 6.9, P <0.001; t = 6.4, P <0.001, respectively".

The effect of repeated mating on the mating ability, the mean number of eggs and fertility in P. operculella irradiated females

The results show that a fraction of the females (irradiated or unirradiated) did not mate in spite of repeated exposure to new males, and that this fraction was higher in groups exposed to high doses "F=31.678, df =1, 3, P<0.0001". The percentage of mating females in the control group was 98%, while it was 20% in the group of females irradiated with 200 Gy. Despite exposure to new males every 24 hours for 10 consecutive days, females (irradiated or unirradiated) were unable to mate for more than 3 times despite repeated exposure the males. The percentage of females that mated once was significantly higher than the percentage of females mating two and three times, whether the females were irradiated or not "$F=5.947$, $df=1, 11$; $P<0.0001$". The results show that irradiated females with 150 and 200 Gy were unable to mate more than twice (Table. 1). The mean number of eggs produced by unirradiated females that refused repeated mating was about 85 eggs, and was 81 and 83 eggs for females mated twice and three times, respectively "$F= 0.072$, $df =1, 2$, $P<0.930$; $F= 0.052$, $df =1, 2$, $P<0.949$; $F= 0.114$, $df =1, 2$, $P<0.893$ and; $F= 0.283$, $df =1, 2$, $P<0.755$". Similar results were obtained for females treated with different doses of gamma radiation, and for fertility of eggs of irradiated and unirradiated females (Table 1). Repeated mating had no effect on the percentage of eggs hatching regardless of the number of matings "$F= 0.118$, $df =1, 2$, $P<0.889$; $F= 0.119$, $df =1, 2$, $P<0.888$; and $F= 1.178$, $df =1, 2$, $P<0.322$".

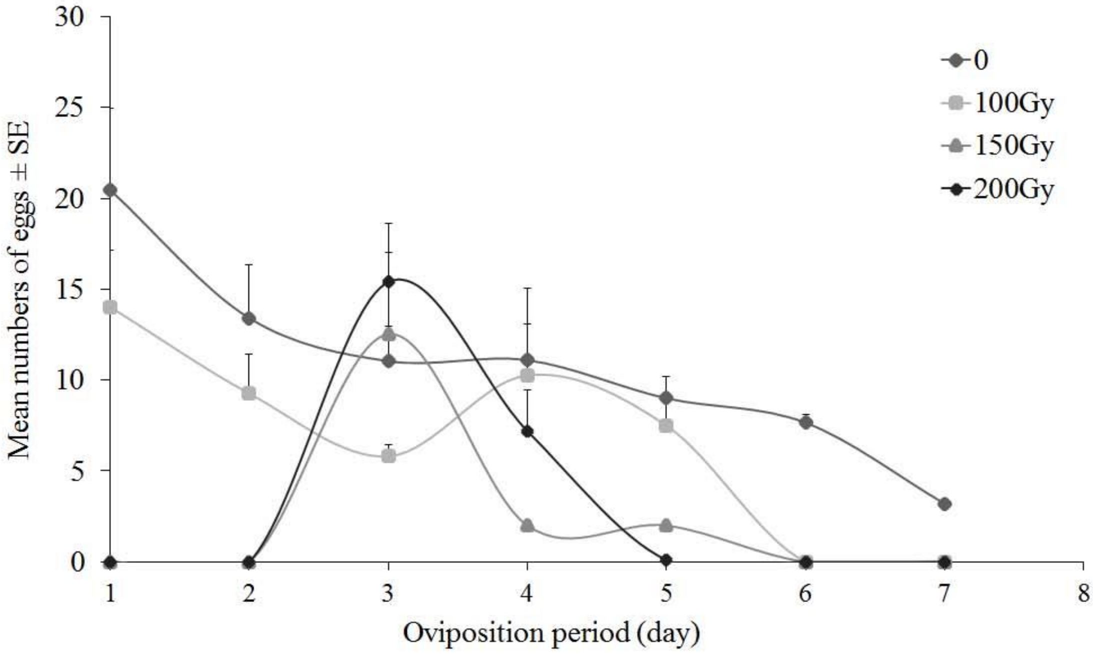


Fig 1. Mean numbers laid by eggs of irradiated *P. operculella* females per day

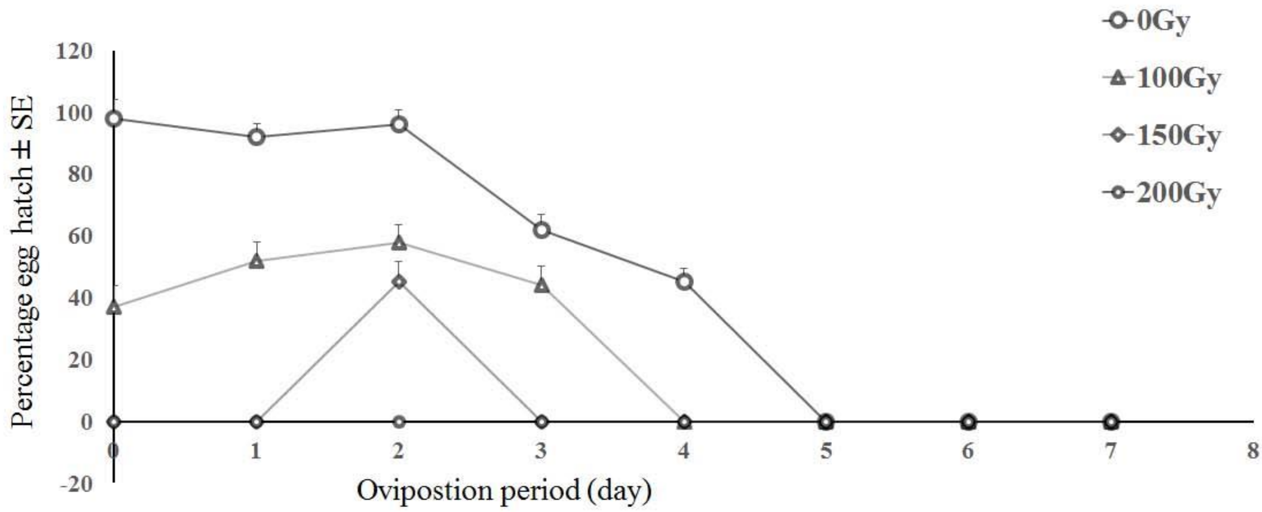


Fig 2. Percentage egg hatch of irradiated and unirradiated *P. operculella* females per day

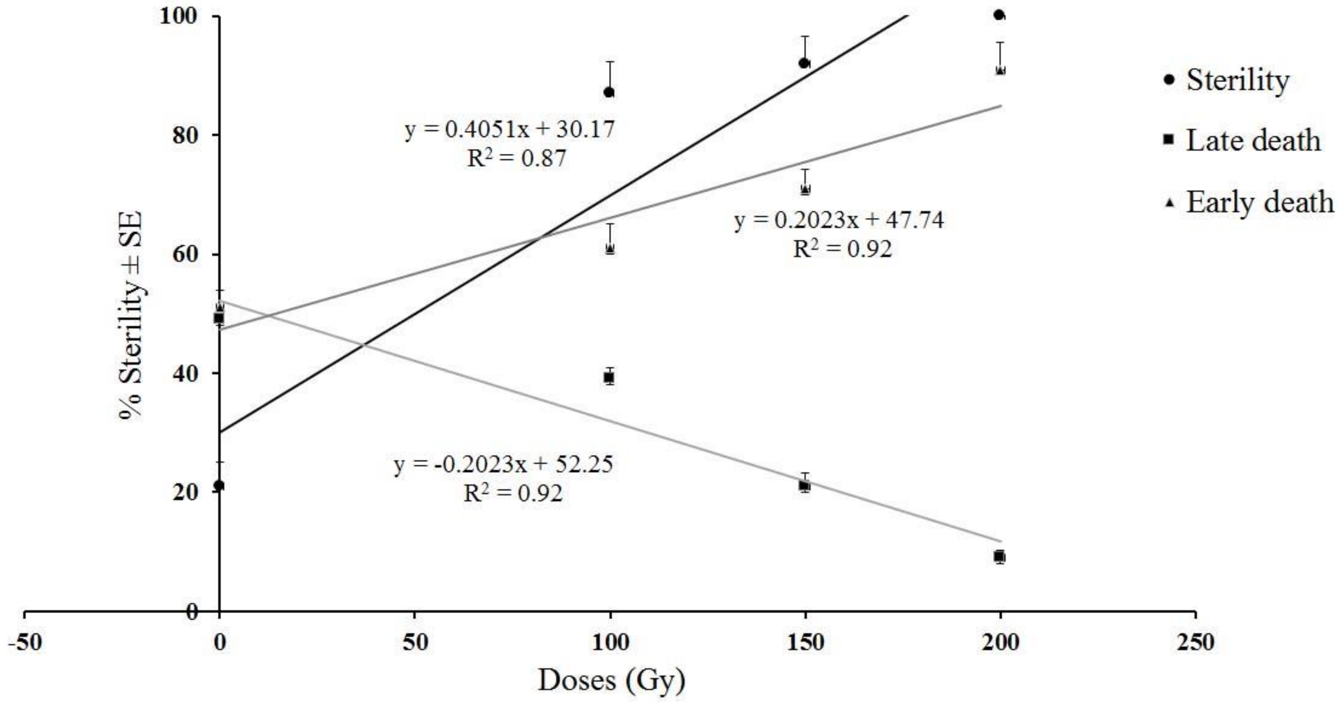


Fig 3. Relationship between the percentage of late embryo death, early embryo death and sterility of irradiated *P. operculella* females with different doses of gamma ray

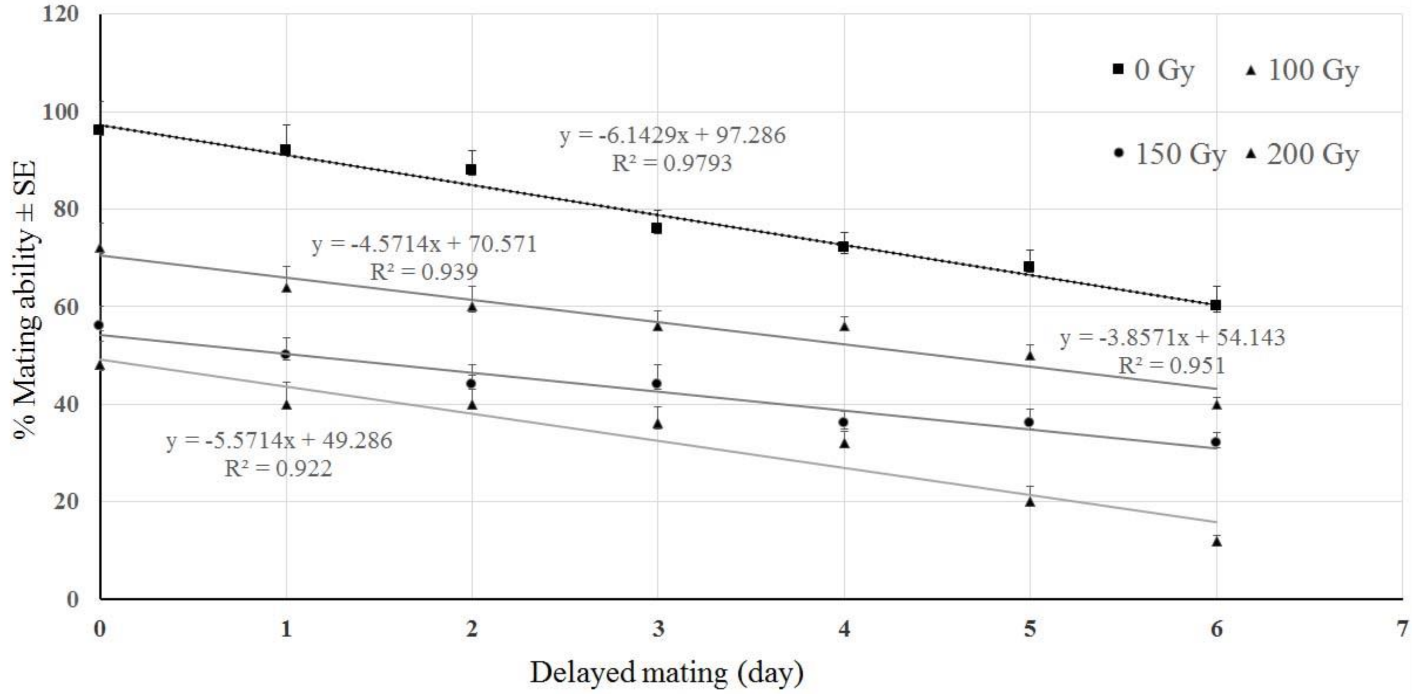
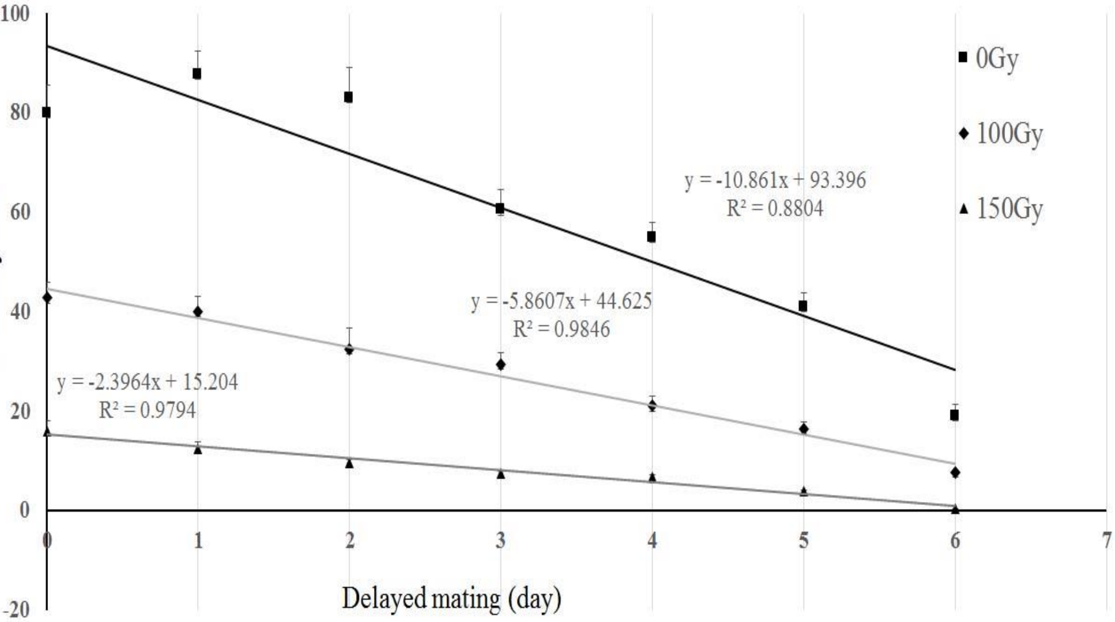


Fig 4. Effect of delayed mating on mating ability of irradiated *P. operculella* females



Effect of delayed mating on the percentage of fertility of irradiated *P. operculella* females

Table 1. Effect of repeated mating on percentage of mating ability, fecundity and fertility of irradiated and unirradiated *P. operculella* females

I

II

Doses Gy	% Mating Ability[a] ± SE	No. of Mating	%Mating Females a ± SE	Doses Gy	No. of Mating	Mean No. of Eggs/females ± SE	% Fertility ± SE
0	98± 2 a (49)	1	53± 9.7 b (26)	*0*	1	85±25a	81 ± 7.6 a
		2	30.6 ± 11.8 c (15)		2	83±29a	77 ± 10.8 a
		3	16.4 ± 13 d (8)		3	81±31a	82 ±13.5 a
100	70 ± 6.4 b (35)	1	57 ± 11 b (20)	*100*	1	46±21a	29 ± 10.1a
		2	32 ± 14 c (11)		2	49±18a	28 ± 13.5a
		3	11 ± 15 d (4)		3	50±11a	30 ± 23 a
150	50 ± 7c (25)	1	72 ± 10.5 a (18)	*150*	1	33±19a	12 ± 7.6 a
		2	28 ± 17 c (7)		2	29±21a	10 ± 11.3a
		3	0		3	34±25a	0
200	20 ± 5.3 d (10)	1	80 ± 14 a (8)	*200*	1	22±10a	0
		2	20 ± 28 c(2)		2	24±12a	0
		3	0		3	21±13a	0

(I) Percentages within a column between applied doses followed by a different letter are significantly different at P< 0.05 (Analysis of proportion).

(II) Mean and percentage within a column for each applied dose followed by the same letter are not significantly different at P< 0.05 (Analysis of proportion), (Tukey's HSD test). [a] Number of mating females. Fifty females for each applied dose.

DISCUSSION

The fertility of *P. operculella* females was affected by irradiation, even when low doses were applied. A dose of 400 Gy induced almost 90% sterility in irradiated males while, a complete sterility in *P. operculella* females was achieved by 200 Gy dose (Saour, 2016; Sachdev, 2017). Fecundity was also greatly reduced when *P. operculella* females were irradiated with doses above 150 Gy (Ayvaz *et al*., 2015). Our results showed that irradiation, particularly at high doses, negatively affected the distribution of egg laying. Fertility declined with increasing irradiation dosage. Irradiated females with low doses produced eggs daily, but those eggs did not hatch (Fig. 2). Females of *P. operculella,* as females of other Lepidoptera, typically carry small numbers of mature eggs in their ovaries. The immature eggs develop and mature during the adult phase (Wu *et al*., 2018). The decrease in the number of eggs laid daily by irradiated females may be attributed to irradiation damage to ovule- generating cells, i. e. oogonia, or a decline in mating success and numbers of matings in the irradiated *P. operculella* females (Ayvaz *et al*., 2015). Sterility in females exposed to gamma radiation may be associated with many factors; an important factor may be formation of dominant lethal mutations in sex cells (Ayvaz *et al*., 2015; Idris *et al*., 2019). However, these mutations do not hinder formation and maturity of gametes or zygotes, but prevent the completion of zygote development. Therefore, the decline in the fertility of irradiated females in our study may be associated with dominant lethal mutations carried in their eggs (Baltzegar *et al*. 2018). Lethal mutations can appear at any stage during insect oviposition and cause embryo death (Demirbas-Uzel *et al*., 2018). The results of our study suggested two types of dose-dependent embryo deaths. First, the death of post developed embryo prior to egg hatching, at low doses, and second, early death of the embryo before its development, at high doses (Fig. 3). Many researchers studied the influence of delayed of mating on the mating ability of lepidopteran insects, focusing on behavior of mating callings (Stelinski *et al*., 2014). Prior studies showed that the mating ability of *P. operculella* females aged 1 day and 2-3 days are similar (Cameron *et al*., 2005). This is consistent with our results, in which females showed a delay in mating from 1 to 3 days, with no effect on the mating ability. However, delay of mating for more than 3 days led to a significant reduction in the mating ability (Fig. 4). The difference in mating ability between irradiated and unirradiated females was highly significant, regardless of the mating day. This suggests that the decline in mating ability of females was associated with female age and radiation dose. These results are cosistent with those reported earlier for other species of insects, including *Eoreuma loftini* (Beuzelin *et a*l. 2016), *Pectinophora gossypiella* (Mori *et al*. 2013), *Plodia interpunctella, Lymantria dispar* (Scheff *et al*., 2018; Wu *et al*., 2018), and *Heliothis virescens* (Saour, 2016). Fertility and mating ability of irradiated and unirradiated females were significantly changed with the delay of mating. Thus, the fertility of irradiated females and new adults were significantly higher than, those of irradiated or aged females, regardless of the time of post irradiation mating (Fig. 5). Fertility reduction in irradiated and aged unirradiated females may be attributed to the decreased maiting capability of females. Consequently, it prevented egg maturation or absorption by females during the delayed period of mating (Leather, 2018). In addition, irradiated females, independently of mating delay, laid seemingly damaged eggs. The eggs affected by radiation may hold dominant lethal mutations and irreversible damage. Eggs produced after mating were abnormal, likely as a result of a major damage to oogonia by irradiation, especially at high doses. Consequently, the females either were rendered unable to produce eggs throughout their remaining life span, or the doses radiation directly damaged the nutritive cells at the initial stages of eggs formation (Maru *et al*., 2018). As a result, production and maturation of eggs and fertility may have been affected. Females of *P. operculella*, like other polygamous lepidopteran females, seek an additional mating if the first mating does not supply them with sufficient quantity of effective sperm, or the secretions of sexual glands are insufficient to fertilize eggs (Vreysen *et al*., 2016). Our results showed no difference in the mean numbers of laid eggs and fertility of irradiated females in relation to the numbers of matings (Table. 1). Repeated mating with unirradiated males did not restore fertility in irradiated females. This may be attributed to damage caused to *P. operculella* females by doses radiation, which prevented them from achieving the natural reproductive capacity (Rondon, 2010).

In conclusion, the present study showed that the fertility and mating activity of irradiated *P. operculella* females was not restored despite repeated or delayed mating with unirradiated males. Therefore, our results suggested that fertility of irradiated females would not be restored after radiation. Thus, sterilized females released in the field should be completely infertile even at low doses, therefore, low-dose irradiated females can be used to collect and dispose wild males' sperm, which has an advantage in bi-sex release strategy. Consequently, that would be effective components of a sterile male strategy against *P. operculella*. This study, also, provided preliminary information concerning the mating behavior of *P. operculella* and its reproductive capacity when it is exposed to doses radiation.

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