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THE POSSIBILITY OF APPLYING INHERITED STERILITY AGAINST CODLING MOTH *CYDIA POMONELLA* L. (LEPIDOPTERA: TORTRICIDAE)

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ABSTRACT

Males of the codling moth, *Cydia pomonella* (L.), were treated with increasing doses of gamma radiation, and were mated with untreated females. The fertility, fecundity and longevity dropped slowly with increasing doses radiation. However, the doses lower than 200 Gy did not effect on the mating ability and number of mating of codling moth males. Inherited effects resulting from irradiation of males parents at nominated doses were recorded for the F_1 progeny. As the dose of radiation, increased, significant differences were appeared in fecundity, fertility, mortality, the developmental time, frequency of mating and mating ability between F_1 progeny and their irradiation parental generation P_1. Moreover, competitiveness of irradiated and normal sperm in twice-mated codling moths females was tested in P_1 and F_1. However, the sperm competitiveness effected by sterility level of male mated with feral female when apply inherited sterility technique IST against *C. pomonella*. This study provides valuable information about Syrian codling moth, for the use of IST as an effective control method against codling moth in an integrated pest management program.

Key words: Codling moth, IST, Sperm competitiveness.

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INTRODUCTION

The codling moth, *Cydia pomonella* L.(Lepidoptera: Tortricidae) is considered to be the most important pest of apple in Syria that need to be addressed due to the economic impact of this insect(Basheer *et al*., 2016). Insecticides are widely used to control this pest. However, such chemical control methods are costly, non-selective, environmentally unfriendly and effective for only a short period of time at the treated area. Moreover, *C. pomonella* has already developed resistance to wide spectrum of insecticides (Harba & Idris 2018).Therefore, new control methods, which are more reliable and environmentally safe, are required. The sterile insect technique SIT is considered as an important approach to control this insect in the wide area of apple orchards (Rondon 2010; Makee *et al*., 2012). Subsequently, the possibility of applying inherited sterility as an alternative control method to suppress codling moth population was examined in numerous of studies (Vreysen *et al*., 2016). Besides, all current SIT programs against Lepidoptera, both males and females are mass-reared, irradiated, and the released within the targeted area, because no-practical methods are available to separate the adult moths by gender (Eyidozehi *et al*., 2015). The males are partially sterile at 100 Gy of gamma radiation, whereas the female are completely sterilized at this dose. Therefore, an area wide control program, that releases fully sterile females and partially sterile male moths can take advantage of the phenomenon of inherited sterility, that can suppress the pest population more quickly than releasing fully sterile males and females (Vreysen *et al*., 2016). Although, many reports are available on IST against codling math, however, there is a lack of information about sperm competitiveness of offspring in resulted of irradiated males parents and, normal sperm in twice-mated codling moth females. Therefore, our aims are firstly to study the effects of IST in F_1 progeny correlated with the increases of doses of gamma radiation under the laboratory conditions on *C.pomonella* Syrian strain. Secondly, to determine the sperm competitiveness of (irradiated males P_1/ offspring of irradiated males F_1) and normal sperm in twice- mated female.

Materials and Methods

Insects

The codling moth insects used in this study obtained from our laboratory stock culture, Newly emerged males and females adults were crossed in Petri dishes (12 cm diameter), 5 pairs in each. As water source, a wet cotton wool was placed into each Petri dish. After 3-4 days, the dishes which had eggs, were collected and soaked with 2 % sodium hypochlorite solution for 2 min for egg sterilization. Then the dishes were washed with tap water and left to dry. Eggs were checked daily for hatching. Newly hatched larvae were placed on artificial diet composed of the following ingredients: agar-agar, maize, wheat germ, casein, yeast, Wesson salts, benzoic acid, ascorbic acid, fumidil, nipagine and vitamins (Makee *et al*., 2012). All insect stages were incubated under constant temperature of 25 ± 1˚C with 70 ± 5% RH, and a photoperiod of 16:8 h (L:D). In all the experiments, pupae were sexed and individually placed in small plastic tubes until eclosion. For oviposition, newly emerged females and males were paired in Petri dishes (12 cm diameter) provided with a wet cotton wool. “Mating incidence” in this study was used to indicate successful spermatophore transfer and/or spermatophore presence in the *bursa copulatrix* of the female. Mating number was reflected by the number of spermatophores in the *bursa copulatrix* .

Irradiation

All irradiated males in this study treated with Co60 source Issledov Gamma Irradiator, Techsnabexport Co. Ltd., Moscow, Russia, http://www.tenex.ru . The average dose rate at the time of irradiation was approximately 44.24 Gy /min.

Effects of gamma irradiation on fecundity, fertility, mating frequency, mating ability, and male longevity in the codling moth

Newly emerged males (>24 old) were exposed to gamma radiation doses (0, 50, 100, 150, 200 Gy). The experiments were conducted three times for each dose (15 moth pairs / replicate). After treatments, irradiated males were paired individually with newly emerged unirradiated females in Petri dishes (9 cm diameter). A wet cotton wool was placed in each Petri dish as drinking water source. At each dose, the mated pairs were kept together until death and longevity of males was recorded. The dishes, with eggs, were collected daily for the surface sterilization of eggs as mentioned above. Eggs were checked daily for hatching and counted to determine the total number of eggs per female (fecundity). The eggs were incubated for one week and daily observation was carried out to determine the percentage of hatched eggs (fertility). After death, females were dissected and mating success was assessed by the presence of the spermatophores in the *bursa copulatrix*, and frequency of mating was assessed by counting the number of spermatophores present in the *bursa copulatrix*.

IST in F_1 progeny of irradiated codling moth males

Newly hatched F_1 larvae were placed in dishes on artificial diet (95 larvae/dose).The dishes were keep under the same experimental conditions as mentioned above. Then, the developmental time, larval mortality and sex ratio for F_1 adults were determined. At each dose, three different crosses between treated (T) and normal (N) moths were performed: (♀T x ♂T)F_1 newly emerged females were individually paired with newly emerged F_1 males, (♀T x ♂N) F_1 newly emerged females were individually paired with newly normal males, and (♀N x ♂T) normal newly emerged females were individually paired with newly emerged F_1 males. The experiments were conducted three times for each crosses with (15 moth pairs / replicate). Males and females were kept for oviposition until death. All counted eggs were collected and allowed to hatch for determination the fecundity and fertility. To determine the number of mating and mating ability, after death, the females were dissected and examined for the presence of spermatophores in the *bursa copulatrix*.

Sperm competitiveness of (irradiated P_1/ offspring of irradiated males F_1) and normal sperm in twice-mated codling moth females

For irradiated males parents, newly emerged males (>24) h were irradiated with 150 Gy and were divided in four groups (80 males/group). For offspring of irradiated males F_1, newly hatched F_1 larvae were placed in dishes on artificial diet. The dishes were incubated under the same experimental conditions as mentioned above. Newly emerged males (>24) h were divided in four groups (80 males/group).

Newly emerged females mated twice with normal or (irradiated males P_1/ offspring of irradiated males F_1): (first ♂ S, then ♂N), (♂N, ♂S), (♂S, ♂S) and (♂N, ♂ N) as a control. Therefore, males of each group were individually paired with newly emerged females; after

24 h, the first males were replaced with new males for 24 h then the second males were removed. Females were kept for oviposition until death. All eggs were collected, counted and allowed to hatch for determine the F_1 and F_2 fecundity and fertility. After death, the females were dissected for the presence of spermatophores in in the *bursa copulatrix*, only those receiving tow spermatophores were used in the data analysis. The percentage of egg fertilized by sperm from the second male (p_2 value) was calculated according to ITOUS (1992) formula : $p_2 = 1/[\mathrm{sqrt}(kh)+1]$.

$k = (x_{N\text{-}N} - x_{S\text{-}N})/(x_{S\text{-}N} - x_{S\text{-}S})$, $h = (x_{N\text{-}S} - x_{S\text{-}S})/(x_{N\text{-}N} - x_{N\text{-}S})$.

Where $x_{N\text{-}N}$ and $x_{S\text{-}N}$ indicate the egg hatch of *N,N* mating pattern and *S,N* mating pattern ,respectively; and $x_{S\text{-}S}$ and $x_{N\text{-}S}$ indicate the egg hatch of *S,S* mating pattern and *N,S* mating pattern, respectively.

Data analysis

The statistical analysis was done using the STATISTIC program version 6 (Statsoft, Inc. 2003) at 5% significance level ($P = 0.05$). Data were subjected to the analysis of variance for determining statistical significance of differences between means, followed by Tukey HSD test. The percentages were analyzed by applying normal approximation test (analysis of proportion). The sex ratio analyzed by (chi-square test). The percentages of larvae mortality was calculated using the formula: Number of unemerged adults/total number of tested larvae. The percentage of sterility was calculated using the formula of Toppozada *et al.* (1966): % sterility = $[1 - (F_t \times F_{et} / F_c \times F_{ec})] \times 100$ where F_t is fecundity of irradiation females, F_{et} is fertility of irradiation females, F_c is fecundity of control, and F_{ec} is fertility of control.

Results and Discussion

Effects of gamma irradiation on fecundity, fertility, mating frequency, mating ability and male longevity in the males codling moth

Various doses of gamma irradiation that were applied to codling moth males showed significant dose-dependent reduction in the fecundity, fertility and longevity. However, the result indicated that the ability of males to mate was significantly reduced only when the doses above of 150 Gy. The frequency of mating decreased as well when 200 GY was applied (Table1). Therefore, doses lower than 200 Gy did not affect the mating ability and the number of mating of codling moth males.

IST in F_1 progeny of irradiated codling moth males

Table 2 shows that the mean developmental time of F_1 progeny of irradiated male parents was significantly higher than the control. The mean developmental time of F_1 progeny of 50 and 100 Gy irradiated males parents was significantly lower than F_1 progeny of male parents exposed to 150 and 200 Gy. The results revealed that the mortality of F_1 progeny was significantly higher than the control (Table 2). The mortality increased significantly with the increasing the applied to the male parents. The sex ratio of F_1 males to F_1 females of irradiated male parents was significantly higher than that of the control F_1 progeny (Table 2).

However, the sex ratio of F_1 progeny of irradiated male parents increased significantly in the favor of males only when higher doses were used. The fecundity of F_1 progeny of irradiated male parents at different crosses was significantly lower than that of the control F_1 progeny (Table 3). However, at all tested doses the fecundity of females mated to F_1

males of irradiated male parents was lower than the of F_1 females. The decline in the fecundity was significantly higher, when F_1 males of irradiated male parents mated to F_1 females. Fertility of F_1 progeny of irradiated male parents at all different crosses was lower than in both i) the control and, ii) the irradiated parents, especially when F_1 males mated to F_1 females (Table 3). The results also showed that when F_1 males and F_1females of irradiated male parents were crossed with normal females and males, respectively, their mating ability and mating frequency did not differ from those in the control and the irradiated male parents (Table 3). However, when F_1 males and F_1females mated together, their mating ability and frequency of mating reduced significantly compared to the other crosses and to the irradiated male parents (Table 3).The percentage of sterility in the F_1 progeny of irradiated male parents at different crosses was lower than that in the F_1 progeny of irradiated female parents (Table 3).

Sperm competitiveness of (irradiated males P_1 / offspring of irradiated males F_1) and normal sperm in twice-mated codling moth females

Our data concerning the sperm precedence pattern indicated that when a *C. pomonella* female mated twice with normal males or (irradiated males P_1 / offspring of irradiated males F_1) (females of N, N or S, S mating). Fertility of a *C. pomonella* female were 80.5 % and 19 %, respectively for irradiated males P_1. Opposite, for offspring of irradiated males F_1 were 84.6 % and 6.5 %. However, in cases when the first mating was with a normal male and the second mating with an irradiated male or vice versa (females of N,S or S,N mating). The corresponding for the fertility values were 0.77, 0.44, respectively for irradiated males P_1 and 70.4%, 55.2%, respectively for offspring of irradiated males F_1 (Table 4). According to ITOU's method, a complete last-male sperm precedence will obtained, if the p_2 value's equal to 1. Therefore, by applying the aforementioned method a p_2 value of 0.15 was obtained for irradiated males $P_1$and 38%, for offspring irradiated males F_1. However, this p_2 value suggests the second male mate fathered 15% and 37% of irradiated males $P_1$and offspring of irradiated males F_1, respectively and by the first male mate were 85% and 63%, respectively. The p_2 value of 0.15 for irradiated males $P_1$was increased significantly to 38% in offspring irradiated males F_1 (based on P-value comparisons, $P<0.001$).

Discussion

Recent studies demonstrated that the moth irradiation with low doses is the most appropriate for the pest control program. However, the use of radiation-induced inherited sterility with the dose of 150 Gy led to a complete sterility of codling moth females, whereas the males were partially sterile (Vreysen *et al*., 2016). Our results showed that males responded to the increasing doses of gamma radiation by decline in fecundity, fertility and longevity. Similar results were reported by many researchers for a number of economically important *lepidopteran* insects such as, *Phthorimaea opercullela* Zeller (Gelechiidae), and *Cryptophlebia leucotreta* (Meyrick) *Lobesia botrana* (Denis and Schiffermuller) (Tortricidae) (Tortricidae) (Makee & Saour 2004; Bloem *et al*., 2007; Saour 2014). In comparison with the previous studies, our data revealed 12% and 5% differences in the proportion of sterile males that were exposed to 100 and 200 Gy, respectively .Correspondingly, our data showed differences between 100 and 200 Gy on sterility of male and female in F_1 progeny compared to data of Canadian strain (Bloem *et al*., 2007). Therefore, these differences were 14% and 17 % for male and female sterility at 100 Gy, respectively. However, these differences decreased at 200 Gy dose to 5% and 11%, respectively. This apparent discrepancy in results might be caused by genetic variation and/or by different handling with the moths (Carpenter *et al*., 2010; Ismail *et al*.,2016). Moreover, Hallman (2000) attributed the slight increase in radio sensitivity of eggs from the Syrian *C. pomonella* strain in comparison with the American strain to irradiation factors such as a higher dose rate (Bloem *et al*., 2007). In agreement with Blomefield *et al.* (2011), our results revealed that the F_1 progeny of irradiated parents develop more slowly. F_1 adults began to enclose at day 37 in the control and at day 42 when male parents were treated with 100 Gy. However, this increase of F_1 progeny development was not significant in doses higher than 100 Gy. In contrast, Blomefield *et al*. (2011) reported a developmental delay of 24 h for each incremental dose of 100 Gy. In other hand, despite that the males weight had a great impact on mating ability and number of matings of *C. pomonella* (Makee *et al*., 2012), our result indicated that doses lower than 200 Gy did not affect the mating ability or mating number of *C. pomonella* males. In contrast, previous studies demonstrated that the mating success of females in *C. pomonella* and *Plodia interpunctella* Hübner (*Lepidoptera: Pyralidae*) was not affected by gamma irradiation (Steinitz *et al*., 2015; Ayvaz *et al*., 2018). Our results indicate that the majority of F_1 progeny resulted from the first mating. However, the P_2 value was found to be 15% of F_1 progeny, in contrast with other insects such as *phthormaea operculella* Zeller (Gelechiidae), witch, has the majority of F_1 progeny resulted from the second mating (Makee & Saour 2004). In addition, the P_2 value increased significantly from 15% of F_1 progeny to 38% of F_2 progeny of irradiated males. The sperms of *C. pomonella* that have been transferred during the first mating were predominantly utilized in egg fertilization in P_1 and F_1 progeny, despite the P_2 value in F_1 progeny more than P_1 progeny. In pervious study, Bloem *et al*. (2007) reported that the sterilized males transferred less sperm than wild males. In addition, the females that mated with irradiated males were more likely to re-mate than females mated with non-irradiated males (Vreysen *et al*., 2016). It has known that when IST applying against *c.pomonell*, the sterility in F_1 progeny were more than in their irradiated males parents (Vreysen *et al*., 2010). Thus, the sperm competitiveness effected by sterility level of male witch is mated with feral female when apply IST against *c.pomonell*. However, the residual fertility of eggs results in hatching larvae in the following generation can cause fruit injury (Vreysen *et al*., 2016). Therefore, it would be very useful if we could enhance the effectiveness of IST in combination with other environmental friendly techniques such as *Trichogramma sp* (Harba & Idris 2018). Hence, this study demonstrated primarily useful data about Syrian codling moth, to achieve the goal of combination between IST and biopestiside against *c. pomonella* for an integrated pest management program.

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Dose (Gy)	Mean fecundity (±SE)	Egg hatch (%)	Mean longevity (±SE)	Mating ability (%)	Mean no. of mating (±SE)
0	153.4±7.3a	73a	14.2±2.3a	90(36)a	2.66±0.22a
50	121.3±11.5b	67b	13.4±1.9a	82.5(33)a	2.73±0.27a
100	111.7±9.6b	56c	11.3±1.10b	80(32)a	2.11±0.22a
150	83.4±8.3c	43d	10.7± 0.5b	75(30)a	2.00±0.08a
200	43.5±12.3d	21e	8.6± 0.5c	62.5(25)b	1.5±0.3b

Table 1. Effect of gamma irradiation on fecundity, fertility , longevity, mating frequency and mating ability in the codling moth

Means followed by different letters (columns) are significantly different at P <0.05 (Tukey HSD test) . Percentages followed by different letters are significantly different
at P <0.05 (Analysis of proportion test). Each value represents the mean of three replicates for each dose (15 pairs / replicate).

Table 2. Developmental time, mortality and sex ratio of F_1 progeny of codling moth male parents irradiated with different doses of gamma rays

Dose(Gy)	No. of tested larvae	Mean developmental time (±SE)	mortality[a] (%)	F_1 sex ratio♂:♀
0	95	37.4±5.4a	36.8d	1:1d
50	95	39.9±3.1b	52.6c	1.8:1c
100	95	41.9±3.1c	58.9 b	1.7:1c
150	95	42.2±3.2c	63.1b	2.5:1b
200	95	41.6±3.3c	78.9a	3.1:1a

Means followed by different letters (columns) are significantly different at P<0.05 (Tukey HSD test). Percentages followed by different letters are significantly different at P <0.05 (Analysis of proportion test).[a] Number of unemerged adults/total number of tested larvae. Ratios followed by different letters are significantly different at P <0.05 (chi-square test).

Table 3. Fecundity , fertility ,mating numbers, mating ability and sterility of F_1 progeny of codling moth male parents irradiated with different doses of gamma rays (N, normal; T, treated).

Dose(Gy)	F_1crosses ♂ X ♀	Mean fecundity(±SE)	Egg hatching (%)	Mean no .of mating (±SE)	Mating ability(%)	Sterility index(%♀)
0	N X N	138.4±11.3a	76a	2.4±0.32a	92a	-
100	T X N	61.7±6.4c	25c	2.2±0.3a	80a	85.35
	N X T	98.3±9.6b	31.3b	1.9±0.21a	84a	70.75
	T X T	40.7±6.4d	14.3d	1.11±0.09b	56b	94.47
150	T X N	56.3±10.4e	18.2e	2.13±0.2a	76a	90.26
	N X T	88.2±12.6b	24.3c	1.9±0.23a	69a	79.62
	T X T	43.6±10.2f	12.1d	0.91±0.21b	44b	94.98
200	T X N	20.5±9.2h	6f	1.5±0.2b	75a	98.83
	N X T	48.5±13.6f	19.3e	1.9±0.3b	76a	91.1
	T X T	17.5±8.3g	1.5g	0.79±0.4c	36b	99.75

Means followed by different letters are significantly different at $P < 0.05$ (Tukey HSD post test). Percentages followed by different letters are significantly different at $P < 0.05$ (Analysis of proportion test).Sterility index = $[1 - (F_t \times F_{et} / F_c \times F_{ec})] \times 100$. F_t, fecundity of irradiated females; F_{et}, fertility of irradiated females; F_c, fecundity of the control; F_{ec}, fertility of the control. Each value represents the mean of three replicates for each cross (15 pairs / replicate).

	P_1		F_1		
Type of mating[a]	Twice-mated female[b] (%)	Fertility (%)	Twice-mated female[b] (%)	Mean no. of egg (SE)	Fertility (%)
N , N	20b (16)	80.5 a	25b (20)	98 ±2.31 a	84 a
N , S	22.5b (18)	44 b	27.5b(22)	96.5±3.44 a	70 b
S , N	45a (36)	77.8 a	50a (40)	99.4±2.04 a	55 c
S , S	43a (34)	19.6 b	52a (42)	95.9±3.84 a	6.5 d

Table 4. Effect of alternate mating of normal female with irradiated and normal male on fecundity and fertility of codling moth in P_1 and F_1 progeny
Percentages and means followed by different letters (columns) are significantly Different at P < 0.05 (Tukey HSD test). Type of mating[a] N, normal male; S, male irradiated with 150 Gy.Total number of tested female in P_1 and F_1 at each mating was 80.

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