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Impact of gamma radiation doses on sperm competitiveness, fecundity and morphometric characters of peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephiritidae)

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ABSTRACT

Successful of Sterile Insect Techniques (SIT) depend on ability of sterile male population to compete with normal male for mating with female and lead to a reduction in pest population numbers and sufficiently effective autocidal control. So effects of radiation doses (0, 30, 50, 70 and 90 Gy) on fertility traits and wings morphometric characters for both sexes of peach fruit fly (PFF) *Bactrocera zonata* were assayed, in addition different levels of over loading irradiated males to normal population of PFF (sex ratio 1:1) were also concerned. Percents of observed and expected egg hatching and daily egg laying, then competiveness values between irradiated and normal females were estimated. Irradiated males, mated with normal females, induced them to reduce egg laying rates less than the case of irradiated females only or both sexes. However, dose of 70 Gy for males and 50 Gy for either females or both sexes will be sufficient to decrease daily egg laying of females. On the other hand, significantly reduction of egg hatching percentages was noticed with doses of 70 Gy for either females or both sexes, and 90 Gy in case of treated males only. Gamma radiation doses have significant effect on angles and wings length of males and wings width of females. Confined males irradiated with 70 or 90 Gy in numbers as four times as normal males number in PFF population caused depleting in egg hatching percents (ranged between 5.07%: 13.55%). Moreover, the last case gave egg hatching percentages close significantly to cases of irradiation both sexes or male only with 90 Gy (4.28 and 5.49%, respectively), that harboured highest competitiveness value of irradiated males.

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1. Introduction

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) is a serious polyphagous insect pest that attacks over 50 known host plants. Tropical area of Asia is a native region of it. PFF is spreading to other regions of the world including the Middle East. This pest was officially identified and recorded for the first time in Egypt in 1998 (El-Minshawy, El-Eryan, & Awad, 1999). It is well established in most Egyptian provinces and it causes severe damage to a wide range of fruits (e.g. mango, peach, guava, apricot and citrus) (El-Minshawy et al., 1999; FAO/IAEA, 2000; Khan, Ashfaq, Akram, & Lee, 2005). Although the species has not been introduced to the European Contraries, it has been included in the A1 list of pests, which the European and Mediterranean Plant Protection Organization (EPPO) recommend to be regulated as quarantine pests, detected that Annual losses of fruit crop by the peach fruit fly are estimated at 190 million € in Egypt (EPPO, 2005).

Traditional control measures using chemical insecticides experience disadvantages such as residual problems and disability of insecticides to penetrate infested fruits to kill larvae. The Sterile Insect Technique (SIT) is a biologically-based method for the management of key insect pests of agriculture (Dowell, Siddiqui, Meyer, & Spaugy, 2000). This technique (SIT) was conceived in the 1930 (Knipling, 1965) and first applied on a significant scale in the 1950 against the New World screwworm *Cochliomyia hominivorax* (Coquerel) (Baumhover et al., 1955; Knipling, 1968) and subsequently to a number of other pest species (Dyck, Hendrichs, & Robinson, 2005).

Use of SIT provides an environmental safe and species-specific method to suppress or eradicate Tephritid fruit flies of agricultural importance worldwide (Teal, Gomez-Simuta, Dueben, Holler, & Olson, 2007). This technique has been used successfully against a number of insect pests such as Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Gilmore, 1989; Penrose, 1996; Rossler, Ravins, & Gomes, 2000), melon fly, *Bactrocera cucurbitae* (Kuba et al., 1996), Queensland fly, *B. tryoni* (Froggatt) (Fisher, 1996).

The irradiation process may reduce the mating performance of sterilized males (Calcagno, Manso, & Vilardi, 2002). The successful application of the SIT requires knowledge of the target population's ecology, sex ratio, over flooding ratio and how that density changes over time (Ito & Yamamura, 2005; Knipling, 1979; Lindquist, 1969; Lindquist, Butt, & Moore, 1974). In this type of autocidal control "birth control", sequential releases of the sterilized insects in adequate to wild male over flooding ratio's lead to a reduction in pest population numbers, because of wild female insects of the pest population do not reproduce when they are inseminated by released radiation-sterilized males. Sexual sterility is induced with radiation emitted from radioisotopes such as caesium-137 and cobalt-60. The dosage of radiation applied must have no significant adverse effect on the males' longevity, searching behavior and mating ability (Hooper, 1971). The sterilization process is important in determining the quality of the released insects and their ability to compete with the wild population. Thus, optimization of the sterilization process is critical for the efficacy of SIT programs and should be given due consideration. The absorbed dose of radiation that is used to induce sterility is of critical importance to a SIT program. Insects that receive too low an absorbed dose are not sufficiently sterile and those that receive too high absorbed dose may be uncompetitive, reducing the effectiveness of the program by requiring that a greater number of sterile insects must be released (Calkins & Parker, 2005; Lance & Mclnnis, 2005; Robinson, 2002). So The effect of irradiation must also be assessed in quality control tests on egg hatchability and mating ability (Resilva, Obra, Zamora, & Gaitan, 2007) as well as effect of Gamma rays dose on adult emergence, deformed pupae, sex ratio and sterility of male and females of PFF (Draz, El-Aw, Hashem, & El-Gendy, 2008); moreover, female fecundity, pupal size and flight ability and mating competitiveness of PFF sterile males (Mahmoud & Barta, 2011); sperm precedence of irradiated and normal males when mating with normal females (Draz, 1989). On the other hand, the most important factor in release strategies for SIT is the ratio of sterile to target wild insects (Zahan, 2012). So current study aimed to investigate the effect of radiation dose level, type of sex ratio structure (over flooding ratio) of SIT males on fertility and fecundity, some morphometric characters of PFF adults, and then their impacts on observed, expected egg hatching and comparativeness between sterile and wild males collected from Kafer El-Shikh Agro-ecosystem, where PFF attacks many fruit host plants along a year with 7–8 generation, highest numbers of adults is observed in autumn (Draz, Tabikha, El-Aw, El-Gendy, & Darwish, 2016).

2. Materials and methods

2.1. Initial culture rearing

The initial culture of peach fruit fly (PFF), *B. zonata* (Saunders), has been obtained from infested guava fruits, collected from orchards at El-Dahab Island (located between N31° 12′ 30″ to N31° 12′ 4″ and from E 30° 33′ 37″ to E 30° 33′ 11″), Fowa district, in Kafer El-Shikh Governorate, during October and November, 2014. Infested guava fruits were transferred to laboratory and were kept in plastic jars, furnished by sterilized sand and preserved under semi controlled conditions at 25 ± 2 °C and 60–65% R.H. until pupation. Emerged pupae were collected daily and transferred to adult rearing woody cages (30 × 30 × 30 cm), that coated with wire screen except frontal side closed with muslin clothes sleeve for daily egg collection and investigation food supplies for adults. Caged adults were provided with food consists of sugar mixed with hydrolyzate protein (3:1w/w) and wet cotton wick as a source of drinking water.

The deposited eggs were collected daily and cleaned with tap waters, then collected eggs were transferred to plastic trays (10 × 5 × 3 cm), half-filled with larval artificial diets consisted of (500 ml distilled water, 330 gm wheat bran, 82.5 gm brewer's yeast, 82.5 gm granulated sugar, 3 gm sodium benzoite, 3 gm citric acid) and covered with thick fabric for the first three days to safe the moisture till egg hatch, then it was replaced with muslin fabrics until pupation. Apart of these pupae were transferred into the rearing cages to start a new generation, while other similar age pupae were directed to irradiation process.

2.2. *Gamma radiation treatment for pupae*

The pupae *of B. zonata* at age 8-day-old (24 h before adult elusion) gained from the laboratory culture and held in plastic vials in four groups (each group contains 600 pupae) according to the procedures in the manual of "Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies" (FAO/IAEA/USDA, 2003), and transferred to cobalt-60 gamma-irradiation unit installed in Atomic Energy Authority, Naser City, Cairo, Egypt. The gamma irradiation was carried out at a dose rate 10 Gy/18.2 s and four different doses (30, 50, 70 and 90 Gy) were used for pupae treatment in each group. Emergence of adults was determined by placing the irradiated pupae of each dose in Petri dish inside rearing cages. Emerged adults from treated pupae in each irradiation dose were collected using an aspirator, temporarily paralyzed by chilling then sexed. Same procedure was also occurred with emerged adults from normal pupae (control).

2.3. *Mating between normal and irradiated adults in designated sex ratio*

To clarify effect of gamma radiation doses on fertility and fecundity of PFF adults, Equal number of normal or/and irradiated males and females with four doses of gamma radiation were placed in plastic cups (10 cm height and 5 cm diameter) in four different treatments of mating structure with all tested doses of gamma radiation as follow:-

1) 2 Normal females (♀$_N$) confined with 2 Normal males (♂$_N$).
2) 2 Normal females (♀$_N$) confined with 2 Irradiated males (♂$_T$).
3) 2 Irradiated females (♀$_T$) confined with 2 Normal males (♂$_N$).
4) 2 Irradiated females (♂$_T$) confined with 2 Irradiated males (♀$_T$).

On the other hand, studying of over loading irradiated males to normal mating system with sex ratio 1:1 were also concerned in different doses of gamma radiation. So, three levels of over loading irradiated males [2, 4 or 8 Irradiated males (♂$_T$)] were added to [2 Normal females (♀$_N$) with 2 Normal males (♂$_N$)], to give three mating structures as follow:-

1) 2 Normal females (♀$_N$) confined with 2 Normal males (♂$_N$) + 2 Irradiated males (♂$_T$).
2) 2 Normal females (♀$_N$) confined with 2 Normal males (♂$_N$) + 4 Irradiated males (♂$_T$).
3) 2 Normal females (♀$_N$) confined with 2 Normal males (♂$_N$) + 8 Irradiated males (♂$_T$).

All treatments or mating structures were designed in six replicates.

Cups of all mentioned cases were provided with adult food consists of sugar mixed with hydrolyzate protein (3:1w/w) and wet cotton wick as a source of drinking water, then coated with muslin fabric as an ovipositing site. After reaching adults sexual maturity, the cups were provided with fruit scent as an attractive substance for ovipositing. The deposited eggs were daily collected and counted then washed with tap water and rowed on wet black cloth. After three days of incubation under 25 ± 2 °C and 60–65% R.H, the eggs were examined; the hatched and non hatched eggs were counted and recorded.

So percentage of observed egg hatching *EHo* (%), daily egg laying were used to compare among different treatment. Each of Expected egg hatchability percentage *EHe* (%) and competitiveness value (CV) were computed and estimated in cases of overloading irradiated males with different radiation doses to normal population, by applying following equation, that described by Fried (1971).

1- % Expected egg hatch *EHe* (%) = [N(Ha) + S (Hs)]/[S + N]

where Ha = % egg hatch in case of mating normal males with normal females (control).

Hs = % egg hatch in case of mating irradiated males with normal female.

N = number of normal males in over loading cases.

S = number of irradiated males in over loading cases.

Values of "1" indicate an equivalent level of competitiveness between irradiated and non-irradiated males, while values close to zero indicate superior competitiveness of the non-irradiated males (Fried, 1971).

2- Competitiveness value (CV) = % Expected egg hatch/% Observed egg hatch

2.4. *Morphometric characters for wings of irradiated adults*

Fresh specimens of males and females from a colony maintained in the laboratory (non-irradiated) and adult males and females resulted from irradiated pupae were collected in age of 30 days. Fore wings of normal or irradiated males and females with radiation doses 50, 70 or 90 Gy were separated by fine forceps and mounted on sticky transparent boards. Each sticky board contain ten pairs of wings for each treatment, were scanned by HP scanner G3110 at 1200 dpi. All obtained wings images as shown in Fig. 1 were subjected to "Image J" computer program to estimate five main angles for investigated veins, which are Apical angle, Anal angle, Humeral angle, angle A (between radius vein and r-m cross vein), and Angle B (between medium vein and m-cu cross vein) and to photoshop7 computer program for measuring width and length of wings.

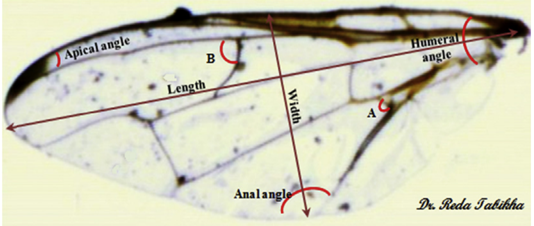


Fig. 1 – Image of fore wing for PFF adults, shown veins, tested angles and wing dimensions.

2.5. Statistical analysis

Mean and Standard Error (SE) were calculated for obtained data of daily egg laying for female, percentages of observed egg hatch *EHo* (%), and all morphometric characters of wings in different treatment then subjected to ANOVA test analysis to show effect of radiation on fertility, fecundity and measurements of wings in both sexes. The means were compared and arranged by Duncan's Multiple Range test 0.05 to detect most effective radiation dose and best level of over loading irradiated males to normal adults as quality control parameters, and studying the interacted effect of over loading level and radiation doses on previous mentioned parameters. Simple correlation analysis was also performed to declare type of relationship between radiation doses or over loading of irradiated male on them, and then regression equation could be suggested. All statistical analyses of obtained data were conducted by using COSTAT (2008) statistical software computer program.

3. Results

Effects of radiation doses and mating ratio of peach fruit fly (PFF) adults population emerged from pupae treated with one of five ascending radiation dose (0, 30, 50, 70 and 90 Gy), on laying and hatchability percentages of PFF eggs, were assayed along 10 days from adult life span. Obtained data declared that mating of normal twelve females and twelve males with sex ratio (No. female: No. males) 1:1 gave 395 eggs along ten days with mean egg laying 38.5 egg/day and highest egg hatchability 98.57%.

3.1. *Effect of gamma radiation doses on fertility and fecundity of PFF adults mated in sex ratio structures (1:1)*

The current issue aimed to declare and compare absolute effects of radiation doses on fertility of only males or only females and then fecundity, under laboratory conditions through obtained data in Table 1 and graphically illustrated in Fig. 2a and b.

In case of irradiated males with ascending treatments of gamma radiation doses (30–90 Gy) and mated with normal females in sex ratio 1:1, gave high significant difference for percentages of egg hatching among treatments ($F = 178.277^{***}$, LSD 0.05 = 9.324), that dose of 90 Gy gave lowest significant percentage of egg hatch (5.49%) comparing with untreated males populations or males treated with less doses. On the other hand, mean of daily egg laying differed significantly from treatment to another ($F = 46.810^{***}$, LSD 0.05 = 4.352). Irradiated males with radiation doses from 70 to 90 Gy induced females to deposit lowest daily egg numbers (12.0 and 14.8 egg/day). Rate of daily egg laying wasn't significantly differ according to age changes of PFF adults in all treatments.

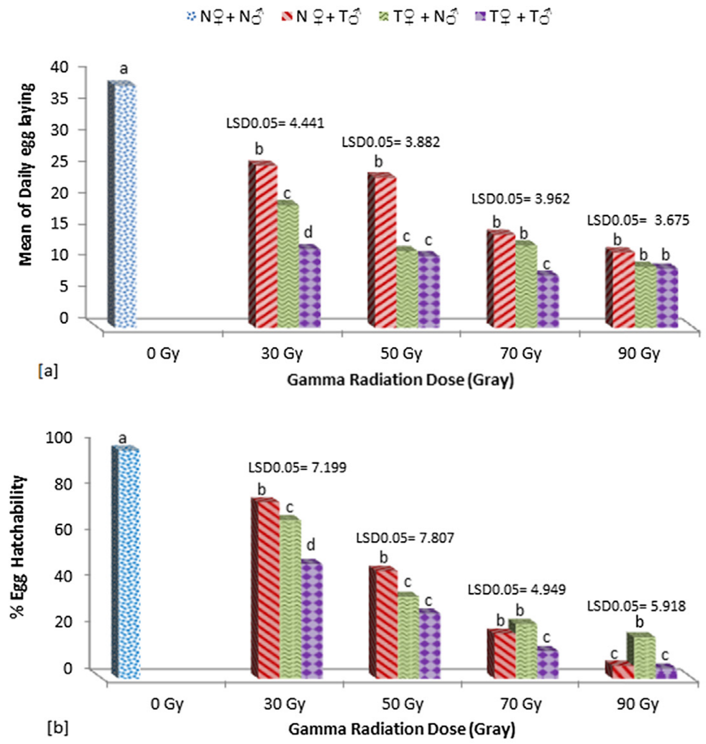
In opposite case, that females treated with later mentioned ascending treatments of gamma radiations doses and mated with untreated (normal) males in sex ratio (1:1), reflected that radiation treatment had high significant effect on each of daily egg laying ($F = 82.792^{***}$, LSD 0.05 = 3.662) and egg hatch percentage ($F = 133.229^{***}$, LSD 0.05 = 10.479) of irradiated females comparing with untreated population. Irradiated females with dose of gamma radiation ranged between 50 and 90 Gy gave significantly decreasing in daily egg laying of treated females ranged between 9.7 and 13.1 egg/day, while lowest dose of gamma radiation (30 Gy) doubled daily egg laying to 19.1 egg/day. Moreover, doses of 70 and 90 Gy were most affected egg hatch percentages (23.48 and 17.79%, respectively), while dose of 50 Gy gave moderated effect on egg hatchability (35.22%) then dose of 30 Gy (68.30%).

Complete irradiation for all individuals of both sexes that mated in commonly sex ratio (1:1) showed highly significant differences among tested radiation doses according to the used biological parameters of quality control as daily egg laying rates ($F = 93.159^{***}$, LSD 0.05 = 3.740) and % egg hatch ($F = 99.276^{***}$, LSD 0.05 = 14.417). Complete irradiation of PFF population with radiation doses 50 Gy or more harboured lowest daily egg laying (11.4, 8.3 and 9.4 egg/day, for doses of 50, 70 and 90 Gy respectively). In general, radiation treatment decreased daily egg laying to less than 50% as untreated population. On the other hand, lowest percentages of egg hatching was observed with doses 70 – 90 Gy (11.81 and 4.28%, respectively) and significantly followed by doses of 50 then 30 Gy (27.97 and 49.36%, respectively). So dose of 50 Gy will be sufficient to decrease significantly only egg deposit of females, while 70 Gy gave sufficient and significant decreasing in daily egg laying and egg hatch percentage of PFF in case of full irradiation for population.

Table 1 – Daily egg laying and percents of egg hatching for *Bactrocera zonata* adults emerged from irradiated pupae with ascending radiation doses.

Sex ratio structure No.♀ (N or T): No. ♂ (N or T)	Biological Parameters	Irradiation dose (Gray)				
		Cont. (0 Gy)	30 Gy	50 Gy	70 Gy	90 Gy
12♀$_{N}$: 12♂$_{N}$	Daily egg laying/12 Fem.	38.50 ± 1.60	–	–	–	–
	% Egg Hatching	98.57 ± 0.80	–	–	–	–
12♀$_{N}$: 12♂$_{T}$	Daily egg laying/12 Fem.	–	25.8 ± 1.66^{b}	23.9 ± 1.37^{b}	14.8 ± 1.58^{c}	12.00 ± 1.4^{c}
	% Egg Hatching	–	76.04 ± 1.96^{b}	46.36 ± 2.67^{c}	19.23 ± 2.04^{d}	5.49 ± 1.63^{e}
12♀$_{T}$: 12♂$_{N}$	Daily egg laying/12 Fem.	–	19.50 ± 1.38^{b}	12.20 ± 0.95^{c}	13.10 ± 1.20^{c}	9.70 ± 1.19^{c}
	% Egg Hatching	–	68.30 ± 2.18^{b}	35.22 ± 1.72^{c}	23.48 ± 2.05^{d}	17.79 ± 3.21^{d}
12♀$_{T}$: 12♂$_{T}$	Daily egg laying/12 Fem.	–	12.50 ± 1.54^{b}	11.40 ± 1.41^{bc}	8.30 ± 1.07^{bc}	9.40 ± 0.76^{c}
	% Egg Hatching	–	49.36 ± 3.98^{b}	27.97 ± 4.34^{c}	11.81 ± 1.67^{d}	4.28 ± 1.81^{d}

•Values of same sex ratio structure, with same letters, indicate that no significant difference among radiation doses.



- Columns of same radiation doses, with same letters, indicate that no significant difference among values of sub-treatment (matting structure).

Fig. 2 – Combined effect of Gamma radiation doses and mating system on daily egg laying (a) and % Egg hatch (b) resulting in mating between normal or/and treated adults of PFF in sex ratio 1:1.

The influence of gamma radiation doses on fertility and fecundity of both sexes of PFF adults, graphically illustrated in Fig. 2a and b) in addition ANOVA test analysis carried out among four different mating systems with sex ratio 1:1 (normal female with normal male; normal female with sterile male; sterile female with normal male; and sterile female with sterile male) with ascending doses of gamma radiation, which indicated that irradiated males with 90 Gy gave adequate reduction in daily egg laying close to either case of irradiated females only or both sex, their egg hatch percent close to case of irradiated both sex and lower than irradiated females only in mating. If males or females were treated with 70 Gy then mated with normal opposite sex, they will significantly produce higher daily egg laying and hatch percent than case of sterility of both sex. On contrary, if females were treated only with 50 Gy then mated with normal or treated males they will significantly produce daily egg laying and hatch percent lower than case of irradiated of only males. Finally, treated males with 30 Gy will produce significantly increasing in daily egg laying and hatchability comparing with treated females matted with normal or treated males. So we can conclude that dose of 50 Gy is economic and adequate for sterility of females, while dose of 90 Gy is more suitable for significant sterility of males.

So it may be recommended that irradiated pupae with 50–90 Gy and releasing irradiated adults to mate with normal females with sex ratio not increase than 1:1, that could be significantly decrease daily egg laying and egg hatchability of normal females to lowest values that close to cases of mating either irradiated females with normal males or between irradiated both sexes in doses of gamma radiation from 50 to 90 Gy, which was also confirmed by applying general ANOVA test among the twelve sub-treatments for values of daily egg laying ($F = 18.854^{***}$, LSD 0.05 = 3.697) and egg hatch percents ($F = 82.471^{***}$, LSD 0.05 = 7.273).

3.2. *Effect of overloading of irradiated males with gamma radiation doses to normal peach fruit fly adults populations*

Sterile Insect Technique (SIT) involves the suppression of sterile male population that could compete with wild male for pairing with female adults and lead to decrease offspring numbers, thus reducing the offspring of next generation. On the other hand, The most important factor in release strategies for SIT is the ratio of sterile to wild males (S:W ratio). So releasing of the sterilized males in adequate numbers to normal male in over flooding ratios will significantly lead to a reduction in pest population numbers and sufficiently effective autocidal control. Therefore current issue will concern about studying combined effect of irradiation doses and sterile males over flooding to wild fertile population with sex ratio 1:1 as shown in Table 2 and graphically illustrated in Fig. 3a and b to compare the competitiveness and then its effectiveness on population reduction through assaying daily

Table 2 – Percentages of observed and expected egg hatchability of normal *Bactrocera zonata* females mated with normal and irradiated males in various over flooding ratios, in addition to values of daily egg laying.

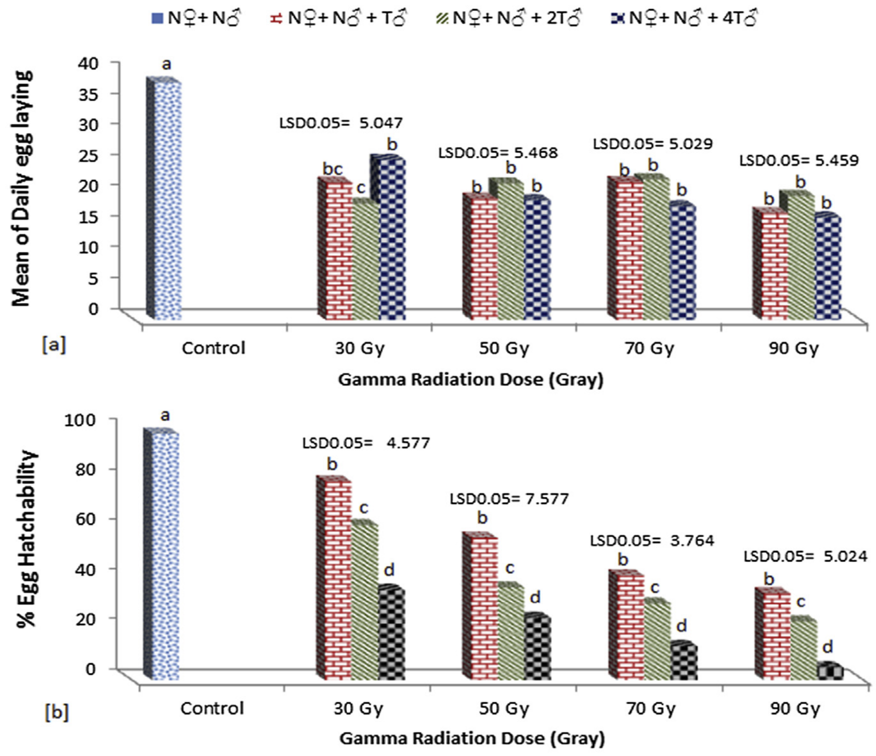
Overloading ratio ♀$_N$: ♂$_N$ + ♂$_T$	Biological Parameters	Irradiation dose (Gray)			
		30 Gy	50 Gy	70 Gy	90 Gy
1♀$_N$: 1♂$_N$ + 1♂$_T$	Daily egg laying/12 Fem.	22.20 ± 2.08^{a}	19.60 ± 2.23^{a}	22.30 ± 2.10^{a}	17.30 ± 2.36^{a}
	EHo (%)	79.36 ± 1.89^{a}	56.85 ± 4.90^{b}	41.86 ± 1.78^{c}	34.67 ± 2.87^{c}
	EHe (%)	87.31	72.47	58.90	52.03
	Competitiveness value (CV)	1.10	1.27	1.41	1.50
1♀$_N$: 1♂$_N$ + 2♂$_T$	Daily egg laying/12 Fem.	18.80 ± 10.19^{a}	22.10 ± 13.74^{a}	22.80 ± 10.15^{a}	20.10 ± 12.31^{a}
	EHo (%)	61.89 ± 1.53^{a}	37.07 ± 1.08^{b}	30.59 ± 1.27^{bc}	23.38 ± 1.30^{c}
	EHe (%)	83.55	63.76	45.68	36.52
	Competitiveness value (CV)	1.35	1.72	1.49	1.56
1♀$_N$: 1♂$_N$ + 4♂$_T$	Daily egg laying/12 Fem.	25.9 ± 1.71^{a}	19.4 ± 1.51^{b}	18.4 ± 1.67 ^{b}	16.6 ± 1.61^{b}
	EHo (%)	36.05 ± 1.87^{a}	24.79 ± 1.40^{b}	13.55 ± 1.16^{c}	5.07 ± 1.26^{d}
	EHe (%)	80.55	56.80	35.10	24.11
	Competitiveness value (CV)	2.23	2.29	2.59	4.76

•Values (Mean ± Se) of Daily egg laying or *EHo* (%) of each over loading ratio that take same letters, indicate that no significant difference among radiation doses.

***EHo* (%)**: % egg hatch observed in the experiments.

***EHe* (%)**: % expected egg hatch that calculated according to Fried (1971).

CV: Competitiveness value that calculated according to Fried (1971).



- Columns of same radiation doses, with same letters, indicate that no significant difference among values of sub-treatment (matting structure).

Fig. 3 – Effect of overloading of irradiated males with ascending doses of gamma radiation on mean daily egg laying (a) and percentage of egg hatchability (b) of PFF normal females.

egg laying of females and percentages of observed and expected egg hatching.

In case of adding irradiated males in equal numbers to normal males in PFF population with sex ratio 1:1, radiation dose of released males hasn't any significant effect on mean daily egg laying of wild females. While highly significant difference was detected for percentages of observed egg hatch in all tested irradiation treatments (F = 34.562 ***, LSD $_{0.05}$ = 10.518). The lowest observed and expected percentages of egg hatch was noticed clearly at doses 70 and 90 Gy (41.86 and 58.9%) and (34.67 and 52.03%), respectively and highest competitiveness value ofirradiated males (1.41 and 1.5

respectively). On the other hand, doses of 50 Gy gave moderated effect on egg hatch followed by dose of 30 Gy. So, irradiated males with 70 Gy will gave economical and desired results in case of adding (over loading) equal numbers of irradiated males to normal males of PFF population.

When doubled numbers of irradiated males to normal males in same population and its sex ratio structure become (1 normal female: 1 normal male: 2 irradiated male), highly significant differences ($F = 29.126^{***}$, LSD0.05 = 9.354) among treatments were also detected with percentages of egg hatch to prove that doses of 50 and 70 Gy gave nearly related values of observed egg hatch percentages (37.07 and 30.59%) and expected egg hatch percentages (63.76 and 45.68%, respectively). Treatment of 70% was also significantly close to observed egg hatch percentage of 90 Gy treatment (23.38%). In contrary, doses of gamma radiation haven't any significant effect on mean daily egg laying of females. So irradiated males with 50 Gy then mating in doubled numbers to normal males in population reduce percentage of egg hatchability for PFF to values related to treatments of 70 and 90 Gy, with highest competitiveness value of irradiated males (1.72).

If numbers of irradiated males were compounded with four times as number of normal males in PFF population with sex ratio 1:1, radiation doses will have significant effect on both of daily egg laying of females ($F = 6.209^{**}$, LSD 0.05 = 4.664) and percentage of egg hatch ($F = 18.114^{***}$, LSD 0.05 = 8.258). That irradiated males with doses from 50 to 90 Gy will significantly induce pairing females to reduce daily egg laying (16.6–19.4 eggs/day) comparing with 30 Gy treatment that gave higher value (25.9 eggs/day), while dose of 90 Gy will be more effective on observed and expected egg hatching percentages that were reduced to 5.07% and 24.11%, respectively, followed significantly by 70, 50 then 30 Gy, consequently. So in case of over loading of irradiated males with 90 Gy to four times as normal males numbers will cause a reduction egg hatching percentages and daily egg laying, with highest value of irradiated males competiveness (4.67).

To clarify the most effective sex ratio structure that contain irradiated males, both of daily egg laying and percentage of egg hatching were chosen to evaluated each level of irradiated. In addition ANOVA test analysis carried out among three different type of sex ratio structures that contain irradiated males in different levels compete with normal males to mat with normal females. It is obvious that adding irradiated males to normal population of PFF induced significantly normal females to decrease daily egg laying in all tested radiation doses. Although all tested adding levels of irradiated males hadn't any significant effect on daily egg laying except in case of adding irradiated males with 30 Gy in double numbers to normal males, that showed lowest daily egg laying comparing with the other levels. On contrary, percentages of egg hatch was varied significantly from adding level to another, that lowest egg hatch percentages were observed in case of adding irradiated males in number as four times of normal males.

Interaction effect of gamma radiation dose and level of over loading irradiated males in normal population was studied through applying general ANOVA analysis test among all tested sub-treatments, which detect that high significant difference detected among the thirteen sub-treatments ($F = 162.551^{***}$, LSD0.05 = 5.762), that presence irradiated males with 90 Gy in numbers four times as normal males numbers in PFF population, produced egg with lowest hatch percentages (5.07%) followed ascending by case of presence irradiated males with 70 Gy in same ratio (13.55%), while presence irradiated males with 90 Gy with equal numbers of normal males caused moderated egg hatchability of females (23.38%) and closed significantly with case of presence irradiated males with 50 Gy in numbers as four times as normal males number in PFF population comparing with untreated population (24.79%).

General statistical assay for all mating structure in different doses of gamma radiation treatment (25 sub-treatments) showed highly significant difference among them ($F = 114.674^{***}$, LSD0.05 = 6.495) for parameter of egg hatchability percentage, where case of irradiated males with 90 Gy then overloaded in numbers equal four time as normal males in normal population ($1♀_N$: $1♂_N + 4♂_T$) are significantly close to either cases of irradiation both sexes ($♀_T$: $♂_T$) with 90 Gy or irradiated male only ($♀_N$: $♂_T$) with 90 Gy in population with sex ratio 1:1, that harboured highest reduction level of egg hatch percentage with no significant difference among them that their percents of egg hatch ranged between 4.28: 5.49%. On the other hand, it showed also high significant difference among them ($F = 16.862^{***}$, LSD0.05 = 4.612) for daily egg laying, where either cases of irradiation of both sexes or female only with doses from 50 to 90 Gy, irradiation of both sexes with 30 Gy or irradiation of males only with 90 Gy gave closed and lower values of daily egg laying.

So irradiated males with 90 Gy then overloaded in numbers as four times as normal males in wild population, will be sufficient and recommended practically and economically in field application for decreasing egg hatchability percentages of PFF wild females.

Finally, Expected percentages of egg hatch could be calculated by applying the following regression Equation:-

$$\%\ \text{Expected egg hatchability}\ (Y) = 99.893 - 0.625\ X_1 - 10.832\ X_2$$

That shows the combined effect of gamma radiation dose and loading level of irradiated males (1,2,3 .... etc. times as wild males) in wild population with sex ratio 1:1.

where X_1: (Radiation dose).

X_2: (Irradiated males loading level).

3.3. *Effect of gamma radiation doses on some morphometric characters of peach fruit flies wings*

Current issue aimed to study the effect of Gamma radiation doses of 50, 70 and 90 Gy on seven morphometric traits of fore wings (Anal, Apical, Humeral, A and B angles, length and width of wings) for irradiated males and females of PFF. Obtained data, ANOVA test analysis and simple correlation were shown in Table 3, which revealed that gamma radiation dose had positive significant effect on both of humeral angles and width of males' wings only due to significant difference among radiation treatments. Doses of radiation ranged between 70 and 90 Gy caused highest values of humeral angle in wings of males comparing with dose 30 Gy or untreated individuals. In

Table 3 – Effect of Gamma radiation doses on some morphometric characters for wings of *Bactrocera zonata*.

Wing morphometric characters		Irradiation dose (Gray)				"F" value	Correlation coefficient (r)
		0 Gy	50 Gy	70 Gy	90 Gy		
Male	Humeral angle	55.47 ± 1.73^{bc}	54.67 ± 1.78^{c}	62.23 ± 0.82^{a}	61.15 ± 3.03^{ab}	3.666^{*}	0.478^{*}
	Anal angle	130.99 ± 0.55	134.23 ± 0.61	130.49 ± 1.14	132.07 ± 2.48	1.355^{ns}	0.056^{ns}
	Apical Angle	26.67 ± 0.81^{a}	23.10 ± 0.85^{bc}	23.95 ± 0.40^{ab}	20.39 ± 1.63^{c}	6.398^{**}	-0.676^{**}
	Angle "A"	16.35 ± 0.24	17.57 ± 0.73	18.84 ± 0.70	19.24 ± 1.58	1.909^{ns}	0.504^{*}
	Angle "B"	79.18 ± 0.90^{a}	76.80 ± 1.10^{a}	72.30 ± 1.18^{b}	67.20 ± 1.21^{c}	23.963^{***}	-0.833^{***}
	Wing length $_{(cm)}$	2.03 ± 0.014^{b}	2.12 ± 0.13^{a}	2.09 ± 0.03^{a}	2.12 ± 0.02^{a}	4.663^{*}	0.603^{**}
	Wing width $_{(cm)}$	0.80 ± 0.002^{b}	0.83 ± 0.002^{a}	0.85 ± 0.01^{a}	0.79 ± 0.01^{b}	13.106^{***}	0.137^{ns}
Female	Humeral angle	61.17 ± 0.74^{ab}	66.88 ± 3.34^{a}	49.75 ± 1.82^{c}	56.97 ± 1.54^{b}	11.995^{***}	-0.370^{ns}
	Anal angle	134.35 ± 0.76^{a}	123.79 ± 1.64^{b}	125.08 ± 1.05^{b}	133.37 ± 1.43^{a}	18.689^{***}	-0.240^{ns}
	Apical Angle	20.81 ± 0.46^{ab}	20.56 ± 2.69^{ab}	24.26 ± 0.69^{a}	16.00 ± 0.60^{b}	4.257^{*}	-0.159^{ns}
	Angle "A"	19.79 ± 0.37	19.57 ± 1.70	17.16 ± 0.54	17.45 ± 1.32	1.613^{ns}	-0.402^{ns}
	Angle "B"	83.43 ± 0.89^{a}	71.7 ± 1.28^{c}	78.12 ± 2.28^{b}	78.37 ± 0.62^{b}	11.507^{***}	-0.376^{ns}
	Wing length $_{(cm)}$	2.10 ± 0.009	2.00 ± 0.60	2.09 ± 0.01	2.09 ± 0.03	1.970^{ns}	-0.017^{ns}
	Wing width $_{(cm)}$	0.84 ± 0.004^{a}	0.82 ± 0.01^{ab}	0.81 ± 0.01^{b}	0.79 ± 0.005^{c}	7.661^{**}	-0.728^{***}

•Values (Mean ± Se) of angles, length and width of wing that take same letters, indicate that no significant difference among radiation doses.

contrary, untreated or treated females with 50 Gy showed highest value. Doses from 50 to 90 Gy caused significant increasing in wings length of males comparing with untreated males, but they hadn't any effect on length of females' wings.

Radiation doses showed negative significant effect on apical angle and angle "B" of males' wings and width of females' wings, where their lowest values were observed in treatments of 90 Gy. Significant effect of gamma radiation on angle "B" was noticed with doses over 50 Gy. On contrary, those angles didn't affect by radiation doses in opposite sex that no effects of gamma radiation doses on values of apical angle and angle "B" in females, lowest values of them were observed in treatments of 50 or 90 Gy. Wings width of males affected by doses (50–70 Gy) to achieve highest values comparing with lowest doses (0 or 90 Gy).

Anal angles of males' wings didn't affect by gamma radiation doses, while anal angles of females' wings was decreased in low or moderated doses (50–70 Gy) comparing with highest doses (90 Gy) or untreated females. Finally, although value of angle "A" of females' wings didn't also affect by gamma radiation doses, it decreased gradually consequence with increasing gamma radiation dose for males but without significant difference among treatments.

4. Discussion

As noticed, dose of 70 Gy for males and 50 Gy for females or both sexes were sufficient to decrease daily egg laying of mated females. On the other hand, significantly reduction of egg hatching percentage was clear at doses of 70 Gy for females or both sexes treatment, and 90 Gy in case of treated males only. While (Mahmoud & Barta, 2011) found that mating non-irradiated females with treated males did not affect the production of eggs, but it seriously reduced their hatchability; detected high negative relationship between dose and egg hatchability; observed that doses $\leq$ 70 Gy did not prevent egg hatch comparing with dose of 90 Gy.

In previous studies, 70–90 Gy was most effective irradiation dose range for *B. zonata* (Huque & Ahmad, 1966). Where, a dose of 60–90 Gy had the most deleterious effect on male gonads of *B. zonata* (Shehata, Younes, & Mahmoud, 2006). Generally, Within Diptera, Coleoptera, and Hemiptera, radiation doses vary widely among families and ranged from 20 to 200 Gy, while Tephritids have relatively homogeneous sensitivity to gamma irradiation, with most major pest species requiring <100 Gy to achieve suitably high levels of sterility (Bakri, Heather, Hendrichs, & Ferris, 2005). That was 90–100 Gy for *Anastrepha fraterculus* (Wiedemann) or *B. cucurbitae* (Coquillett) (Allinghi, Gramajo, Willink, & Vilardi, 2007); 67–74 Gy for *Bactrocera philippinensis* (Resilva et al., 2007). In other studies, however, a dose to induce total sterility of fruit fly males was a little lower ranging from 40 to 60 Gy, for example 40 Gy was recorded for *B. cucurbitae* (Nahar, Howlader, & Rahman, 2006), 50 Gy for *Anastrepha suspense* (Walder & Calkins, 1993) and *B. zonata* (Draz et al., 2008), or 60 Gy for *Anastrepha obliqua* (Macquart) (Toledo, 1993).

Finally, females of Ethiopian fruit fly, Dacus ciliatus were completely sterilized at 60 Gy, whereas complete sterilization of the males was observed only at 140 Gy (Rempoulakis, Castro, Nemny-Lavy, & Nestel, 2015). Irradiated males of *Bactrocera correcta* with 0, 5, 10, 15 and 30 Gy and crossed with untreated females, caused sterility levels of the males as follow 23.85, 21.78, 59.10, 72.57 and 98.34%, respectively. The percent sterility at 5 Gy was not significantly different from the control (0 Gy), while they both differed from those of 10, 15 and 30 Gy. Dose of 30 Gy was the most suitable gamma radiation dose to obtain the highest sterility of males of *B. correcta* when applied on pupae 48 h before adult emergence (Puanmanee, Wongpiyasatid, Sutantawong, & Hormchan, 2010). Dose of 60 Gy gave a high percentage of sterility in males of *B. correcta*, but caused no egg laying in females (Pransopon & Sutantawong, 2005).

Over loading sterilized males to normal populations of PFF were concerned that irradiated mature pupae of *C. capitata* with gamma irradiation at doses 60–100 Gy had no effect on the mating ability of irradiated males (Katiyar & Ramirez, 1969). Increasing the radiation dose affecting mating success of *A. suspense*, when sterile and fertile males were held in equal numbers with fertile females, an increase in egg hatch reduction was correlated with increasing radiation dose, the sterile flies appeared to competitive at all ratio, and the reduction in egg hatch correlated positively with increasing over flooding ratio (Calkins, Draz, & Smittle, 1988), Although there appears to be a general consensus that the irradiation process negatively affects the total competitiveness of males (Pereira et al., 2007). Irradiated males with 30 and 70 Gy successfully competed with non-irradiated ones. The more suitable irradiation dose was $\leq$ 70 Gy (Mahmoud, 2010). At constant number of non-treated males in mating trials, egg hatch decreased significantly when a proportion of treated males increased and values for the Fried's CV ranged from 0.21 to 0.69. While the values were nearly equivalent for both sex ratios at 70 Gy, the CV at 30 Gy was 1.85 times greater in the mating trial with a higher portion of sterile males. Irradiated males competed successfully with non-irradiated males (Mahmoud & Barta, 2011). Irradiated *B. cucurbitae* males irradiated as mature pupae with doses of 30 Gy. The competitiveness value of different ratios (1:1:1 and 3:1:1) were 0.91 and 0.74, respectively (Nahar et al., 2006). Egg hatches of irradiated male: normal male: normal female of *B. correcta* in ratios of 0:1:1, 1:0:1, 1:1:1 and 3:1:1 were 89.43, 0.66, 30.98 and 10.94%, respectively. The competitiveness value from the ratios 1:1:1 and 3:1:1 (irradiated male: normal male: normal female) were 1.45 and 2.09, respectively, indicating that irradiated males were fully competitive with normal males (Puanmanee et al., 2010).

Effects of gamma radiations doses on wings traits of treated adults were also concerned that there were no-significant differences in means of wing length between the irradiated and non-irradiated males. However, the wing width of the irradiated males was significantly reduced than that for untreated males. Wing length may lead to a good competition of the irradiated males of peach fruit fly against untreated males to mate with the normal females in the field during the application of SIT program. However, the significant decrease in the wing width may affect the flight ability of the irradiated males for location the host plant fruit for feeding, courtship and mating behaviors. Moreover, the apical and humeral angles of the irradiated males were significant increased than that untreated males, while the anal angle was decreased significantly than that of the untreated males (El-Akhdar & Afia, 2009). Male mating success in the Mediterranean fruit fly rely on male size and other morphometric traits (eye length, head width, face width, thorax length and wing length) (Rodriguero, Vilardi, Vera, Cayol, & Rial, 2002). The wing movements of courting male *C. capitata* include previously non-described twisting movements during continuous wing vibration that may cause the sexually dimorphic rear portions of the male's wings to waft pheromone toward the female (Briceno & Eberhard, 2000).

5. Conclusion

Irradiated pupae with 50–90 Gy then releasing irradiated adults to mate with normal females with sex ratio not increase than 1:1, that could be significantly decrease daily egg laying and egg hatchability of normal females to lowest values, which are close to cases of mating either irradiated females with normal males or between irradiated both sexes in same doses. Irradiated males with 90 Gy then overloaded in numbers as four times as normal males in wild population, will be sufficient and recommended practically and economically in field application for decreasing egg hatchability percentages of PFF wild females (5.07%), and harboured highest competitiveness value of irradiated males. Moreover, the last case gave egg hatching percentages close significantly to cases of irradiation both sexes or male only with 90 Gy (4.28 and 5.49%, respectively). Gamma radiation doses have significant effect on angles and wings length of males and wings width of females.

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