



Effect of gamma irradiation on quality parameters, sterility and mating competitiveness of melon fly, *Bactrocera cucurbitae* (Coquillett)

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Abstract

Radiation doses up to 30 Gy had not much adverse effects on adult emergence, flying ability and deformation, while, exposure of pupae to 40 and 50 Gy, adult emergence (58.67 and 41.67%) and adult fliers (54.18 and 37.12%) significantly decreased. Deformation was also significantly high at 40 (10.67%) and 50 Gy (16.0%), compare to 5 Gy (2.67%) and 30 Gy (6.0%). Adult longevity was not largely affected up to 30 Gy exposures. At 40 and 50 Gy exposure, life span declined to 21.0 and 18.3 days, respectively. More than fifty per cent of males irradiated at 30 Gy remained alive after 48 h of food stress, compare to un-irradiated males. Fecundity was least affected by irradiation up to 30 Gy. The sterility at 5 Gy was at par with control. The sterility increased with increase of radiation doses as irradiation of mature pupae at 30, 40 and 50 Gy resulted in male sterility of 81.47, 90.73 and 95.99%. Mating competitiveness of sterile males with normal males was estimated at cross ratios of 1:1:1, 1.5:1:1, 2:1:1, 2.5:1:1 and 3:1:1 both under lab and field cage conditions. Fecundity and egg hatchability significantly decreased with increase in the ratio of sterile males to normal males. Sterile males were more competitive compared to normal males for normal females at release ratio of 3:1:1 with competitiveness value of 1.42 under lab conditions and 1.06 in field cage conditions. Therefore, 30 Gy was considered as optimum dose to obtain sterile males of *B. cucurbitae* without deterioration of insect quality and mating vigour.

Keywords Gamma radiation · Sterile Insect Technique · Quality parameters · Sterility · Mating competitiveness

The family Tephritidae (Diptera; true fruit flies) contains some of the world's most economically important horticultural and quarantine insect pests, in terms of international trade. India ranks second in fruits and vegetables production in the world. The area under cultivation of fruits and vegetables were 6.40 and 9.57 Mha with annual production of 91.44 and 166.08 MT, respectively (Annual Report-NHB 2015–16).

The melon fly, *Bactrocera cucurbitae* (Tephritidae:Diptera) is of serious concern inflicting heavy losses to the horticultural industry. It attacks 61 plant species belonging to 19 different families, twenty-eight of them are cucurbits and 33

others non-cucurbit hosts (De Meyer et al. 2015). These are subjected to damage by melon fly right from the primordial stages of the crop up to harvest. Damage includes not only direct loss of yield and increase in management cost but also the loss of export markets. In India, the area under cultivation of cucurbits increased from 0.29 Mha in 2014–15 to 0.37 Mha in 2015–16 with production of 5.85 MT in 2015–16 compared to 5.0 MT in 2014–15 (Annual Report-NHB 2015–16). The flies are usually managed by broad-spectrum insecticides, causing major problems with pest resurgence, pesticide resistance and pesticide residues. This leads to increased demand for pest control methods that are efficient and eco-friendly. On economic and biological grounds, the sterile insect technique (SIT) for fruit flies is compelling (Vreysen et al. 2006).

SIT is a biologically based pest control method which involves the rearing, sterilization, and release of insects into a target area to induce sterility (Reddy and Rashmi 2016). Gamma radiation from isotopic sources (cobalt-60 or caesium-137) is most often used for inducing sexual sterility in target pest species due to its convenient

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and consistent method that maintains a reasonable degree of competitiveness in treated insects (Bakri et al. 2005; Parker and Mehta 2007).

Insects that receive a too low absorbed dose are not sufficiently sterile and those that receive too high absorbed dose may not be competitive. Cent per cent sterility may not be the most favourable condition for a programme⁵. An accurate estimate of adult emergence and number of fliers from a batch of sterile pupae assists in planning releases to achieve a desired cross ratio. Sterile flies must be capable to disperse from the point of release to locate shelter, food, water and their mates. The ability of flies to survive under food stress is important which reflects the availability of food reserves to the insect. The longevity of treated flies should be enough to become sexually mature and seek mates.

The optimization of sterilization process is necessary in identifying the sterilizing dose of gamma radiation which can be used for minimizing somatic damage and maximizing male competitiveness (Sayed 2013). Hence, the effect of irradiation on quality control parameters of laboratory-reared and sterilised males must be assessed for use in sterile male release technique (Draz et al. 2008; Mahmoud and Barta 2011).

The current study was carried out to investigate the effect of gamma radiation doses on adult emergence, adult flying ability, deformed adults, adult longevity, and survival under nutritional stress, fecundity and sterility. Mating competitiveness studies were also conducted in both laboratory and field cage (semi-natural) conditions to determine the sex ratio for release of sterile males in the target area to induce reproductive sterility in wild females.

Materials and methods

The study on effect of gamma radiation on insect quality parameters and mating competitiveness of melon fly, *Bactrocera cucurbitae* was conducted at Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi.

Rearing

The culture of melon fly was obtained from infested cucurbit fruits collected from the fields of IARI, New Delhi. Infested fruits were kept in glass jars having 5 cm thickness of sterilized sand for pupation and maintained in controlled conditions at 25–27°C and 65–70% R.H. Pupae were collected daily and transferred to adult rearing wooden cages (30×30×30 cm) provided with mixture of sugar and yeast hydrolysate (3:1) and water soaked cotton swabs as well.

Gamma radiation treatment

The insects required for sterilization were raised on liquid larval diet at insect nutrition studies laboratory, Division of Entomology, IARI, New Delhi (Panduranga et al. 2018). The pupae before adult eclosion (48 h before) were exposed to gamma radiation (cobalt-60 source) at the doses of 0, 5, 10, 15, 30, 40 and 50 Gy at discharge rate of 1.147 kGy/hr. The irradiation was carried out using gamma irradiator (GC-5000, BRIT, Mumbai) installed at the Nuclear Research Laboratory, IARI, New Delhi. Gamma radiation dosage was standardized according to the procedure in the manual of Product Quality Control for Sterile Mass-Reared and Released Tephritid Fruit Flies (FAO/IAEA/USDA 2014).

Optimization of radiation doses

Quality control tests

Irradiated pupae from each dose were taken in a glass petri dish. A black PVC (Polyvinyl chloride) pipe (10 cm in length and 8.5 cm in diameter) coated with talcum powder was placed over the dish and entire set up was kept inside an adult rearing cage (30×30×30 cm). Quality parameter such as adult emergence, adult flying ability and deformation (viz., including malformed pupae, adults and partially emerged flies) were recorded both for irradiated and un-irradiated pupae.

Adult emergence was calculated based on the number of adults emerging from the pupae and adult flying ability was recorded on the basis of number of flies escaping from the PVC pipe. All non-flying, partially emerged and deformed flies were observed as deformed adults. The adult longevity was studied by confining freshly emerged flies to adult rearing cage, provided with mixture of sugar and yeast hydrolysate (3:1) with water ad libitum. Cages were observed daily for fly mortality. Freshly emerged flies were confined in an adult rearing cage without food and water. After 48 h, both dead and live flies were recorded and expressed as survival under feeding stress.

Fecundity and sterility tests

Male adults obtained from irradiated pupae from different doses were confined with an equal number of normal females of same age into ovipositional cages with diet, separately. Eggng device was provided to the flies at the age of 10 days. Petridishes containing semi-solid fruit substrate covered with paraffin membrane was the eggng device, provided to each cage for 24 h for collection of eggs. On alternate days for 2 weeks the eggng device was kept in each cage. Fecundity

was expressed as number of eggs/female/day. Eggs from each cage were inoculated onto moistened black cotton cloth in petri dishes (90 mm) held at temperature of 25–27°C and 65–70% RH. After 4-days, numbers of unhatched eggs were counted and expressed as per cent sterility. Similar procedure was followed for un-irradiated males and normal females.

Mating competitiveness

The mating competitiveness index is the degree of sterility produced by sterile males in the wild population and is measured as reduction in egg hatch. In order to assess the relative mating competitiveness of sterilized males and to measure the effect of increased sterile male ratios on egg hatching, bioassays were carried out under both laboratory and field cage conditions.

Mating competitiveness in laboratory rearing cage

Sterile males, normal males and females of same age were maintained in a separate adult rearing cage (30×30×30 cm), provided with diet and water. After 12 days sterile males, normal males and normal females were confined in to oviposition cages in ratios given in the Table 1.

In order to record fecundity, each cage was provided with eggng devices for 24 h. on alternate days for 2 weeks. Eggs obtained from different treatments were transferred to moistened black cotton cloth held at controlled conditions of 25–27 °C and 65–70% RH for 4-days. The egg hatch was expressed as per cent observed egg hatch (%EH_o). Expected egg hatch (%EHe) and mating competitiveness of sterile males was calculated according to Fried's equations (Shelly et al. 2000).

$$\%Expected\ egg\ hatch\ (\%EHe) = \frac{N(Ha) + S(Hs)}{S + N}$$

$$Competitiveness\ Value\ (CV) = \frac{\%Expected\ egg\ hatch(EHe)}{\%Observed\ egg\ hatch(EHo)}$$

Table 1 Cross ratio structure for mating competitiveness studies

Treatment	Cross ratio (SM:NM:NF)	Number of flies
T1	0:1:1	50NM×50NF
T2	1:0:1	50SM×50NF
T3	1:1:1	33SM×33 NM×33NF
T4	1.5:1:1	42SM×29 NM×29NF
T5	2:1:1	50SM×25 NM×25NF
T6	2.5:1:1	56SM×22 NM×22NF
T7	3:1:1	60SM×20 NM×20NF

SM sterile males, NM normal males, NF normal females

where,

$$Ha = \%egg\ hatch\ of\ normal\ males \times normal\ females$$

$$Hs = \%egg\ hatch\ of\ sterile\ males \times normal\ females$$

$$N = number\ of\ normal\ males$$

$$S = number\ of\ sterile\ males$$

Values of “1” indicate an equivalent level of competitiveness between irradiated and non-irradiated males. Values more than 1 indicate superior competitiveness of irradiated males than non-irradiated males, while values close to zero indicate superior competitiveness of non-irradiated males (Shelly et al. 2000).

Mating competitiveness in field cage

The study was carried out at field of Entomology Division, IARI, New Delhi on mating competitiveness of sterile males in caged conditions. Thirty days old bitter guard seedlings (var.-Pusa Vishesha) were transplanted to the main field. Each plot (1.5 m×1.5 m) consisting of 6 rooted bitter guard plants was enclosed with mosquito-proof net (1 m tall×3 m diameter) provided with zipper from one side for release of flies and providing of eggng devices inside the cage. Experiment was laid out in Randomized Block Design (RBD) design with seven treatments as per the details as given in Table 1.

Release of flies

Required number of flies for each ratio was collected from the cages maintained in the laboratory for releasing into field cages. Before release of flies, plants were lightly pruned and flies were released in the early hours of the day (7.00 AM), Males were released before females to disperse and establish territories.

Ovipositioning devices were provided for 48 h and are brought to the lab for computing fecundity and egg hatchability. Percentages of Observed (%EH_o) and expected egg hatch (%EHe) for each field cage was worked out as described in case of laboratory study.

The mating competitiveness value (C) was computed using the following formula (Shelly et al. 2000).

$$CV = \frac{W/S \times Hw - Hc}{Hc - Hs}$$

where;

W: Number of normal males in competitiveness cage.

S: Number of sterile males in competitiveness cage

H_w : %Egg hatch from normal males $\times$ normal females

H_s : %Egg hatch from sterile males $\times$ normal females

H_c : %Egg hatch from sterile males $\times$ normal males $\times$ normal females

Chi-square values for competitive cages in both laboratory and field cage conditions were calculated by using the following formulae;

$$\chi^2 = \frac{\sum(O_i - E_i)^2}{E_i}$$

where;

O_i : observed value.

E_i : expected value.

Statistical analysis

Data obtained from the experiments were subjected to analysis of variance (ANOVA) with the honestly significant difference (HSD) value calculated as Tukey's statistic at $\alpha=0.05$ using SAS, version. 9.4 (ICAR-IASRI, New Delhi, India). The chi-square test was used for the null hypothesis to know significance in differences between the expected (EH_e) and observed (EH_o) egg hatches at different cross ratios in the mating competitiveness.

Results

Effect of gamma irradiation on quality control parameters

Effect of gamma radiation doses on quality control parameters of melon fly such as adult emergence, adult flying ability, deformed adults, adult longevity and survival of adults under food stress, fecundity and sterility is presented in the Table 2.

The percentage of adult emergences decreased with increase in gamma radiation doses, from the pupae irradiated at 5, 10, 15, and 30 Gy and was recorded as 77.0, 75.0, 73.67 and 72.0%, respectively, which were not significantly different from control (80.67%). Further increase in radiation doses (40 and 50 Gy), the emergence of adults decreased to 58.67 and 41.67%, respectively. The radiation doses ranging from 5 to 30 Gy has shown insignificant effect on reduction of adult fliers as it ranged between 76.87–68.39%, as compare to control (82.87%).

A drastic reduction in flight ability of adults (37.12%) was observed at 50 Gy. The highest percentage of deformation (16.0) was recorded at 50 Gy. Irradiation doses up to 30 Gy have not shown any significant effect on formation of deformed adults ranging from 2.67% (5 Gy) to 6.0% (30 Gy), when compare to the control (1.67%). The longevity of irradiated males did not vary among the irradiation doses up to 30 Gy ranging from 32.3 to 28.0 days.

Table 2 Effect of gamma radiation on quality control parameters of melon fly, *Bactrocera cucurbitae*

Radiation dose (Gy)	Adult Emergence (%)	Adult flying ability (%)	Deformed adults (%)	Adult longevity (Days)	Survival under food stress (%)	Fecundity (eggs/female/day)	Sterility (%)
0 (control)	80.67a (54.22 $\pm$ 4.38)	82.87a (56.03 $\pm$ 1.92)	1.67d (0.95 $\pm$ 0.19)	39.3a (39.3 $\pm$ 1.20)	42.33a (25.05 $\pm$ 1.52)	30.05a (1.47 $\pm$ 0.02)	21.20e (12.24 $\pm$ 1.18)
5	77.00a (50.45 $\pm$ 2.35)	76.87ab (50.27 $\pm$ 1.61)	2.67 cd (1.53 $\pm$ 0.38)	32.3ab (32.3 $\pm$ 1.20)	36.33ab (21.30 $\pm$ 0.89)	25.48ab (1.40 $\pm$ 0.01)	36.52de (21.41 $\pm$ 0.36)
10	75.00ab (48.94 $\pm$ 4.25)	73.70ab (47.56 $\pm$ 2.35)	4.00 cd (2.29 $\pm$ 0.33)	31.6ab (32.6 $\pm$ 1.76)	33.00b (19.26 $\pm$ 0.92)	23.55b (1.37 $\pm$ 0.01)	44.69 cd (26.55 $\pm$ 1.51)
15	73.67ab (47.47 $\pm$ 1.55)	72.51b (46.50 $\pm$ 1.51)	4.33 cd (2.48 $\pm$ 0.20)	31.0b (31.0 $\pm$ 1.53)	31.67b (18.46 $\pm$ 0.87)	22.12b (1.34 $\pm$ 0.01)	58.20c (35.71 $\pm$ 3.29)
30	72.00ab (46.12 $\pm$ 2.21)	68.39b (43.16 $\pm$ 1.34)	6.00c (3.43 $\pm$ 0.33)	28.0bc (28.0 $\pm$ 2.52)	22.67c (13.09 $\pm$ 0.85)	21.35bc (1.32 $\pm$ 0.01)	81.47b (54.60 $\pm$ 1.65)
40	58.67bc (35.98 $\pm$ 2.35)	54.18c (32.85 $\pm$ 2.27)	10.67b (6.12 $\pm$ 0.38)	21.0 cd (21.0 $\pm$ 1.53)	11.00d (6.31 $\pm$ 0.66)	17.0d (1.22 $\pm$ 0.03)	90.73ab (65.31 $\pm$ 2.34)
50	41.67c (24.63 $\pm$ 1.47)	37.12d (21.80 $\pm$ 1.63)	16.00a (9.20 $\pm$ 0.88)	18.3d (18.3 $\pm$ 1.86)	7.67d (4.39 $\pm$ 1.06)	14.78d (1.16 $\pm$ 0.04)	95.99a (74.43 $\pm$ 3.45)

Within a column, means followed by the same letter are not significantly different at 5% level ($\alpha=0.05$; Tukey HSD test, PROC ANOVA). Figures in parentheses are Arc Sine transformed values

An exposure of pupae to 40 and 50 Gy of gamma radiation doses significantly decreased the life span in the parental males to 21.0 and 18.3, compared to 39.3 days in control. Survival of irradiated males after 48 h under food stress was inversely related to increase in radiation doses. More than 50% of adults survived at 30 Gy, compare to control. No significant difference in survival of adults at doses ranging from 5 Gy (36.33%) to 15 Gy (31.67%) was noted. Increase in radiation dose from 30 to 40 Gy resulted in 10 percent reduction in survival of adults and least number survived at 50 Gy.

It was found that irradiation doses, above 30 Gy had negative effects on quality control parameters. Longevity and survival of male flies were also severely affected at 40 and 50 Gy.

Fecundity and male sterility

The average numbers of eggs laid by females mated with males irradiated at 5 Gy (25.48 eggs/female) were not significantly different from the control (30.05 eggs/female). However, Doses of 10, 15, and 30 Gy showed a slight decrease in fecundity (23.55, 22.12 and 21.35 eggs/female/day, respectively). Doses 40 and 50 Gy have significantly impaired egg production as fecundity recorded was 17.0 and 14.78 eggs/female/day, respectively. The viability of eggs laid by normal females mated with irradiated males at 1:1 ratio, decreased with increase in radiation dose. The percentage of unhatched eggs from the females mated with irradiated males at 5 Gy (36.52) was not significantly different from control (21.20). Further increase in radiation doses (10 and 15 Gy), sterility

increased to 44.69% and 58.20%, respectively. The egg hatch continued to decline with increase in dose. Irradiation at 30 Gy resulted in male sterility of 81.47%. Highest percentages of sterility, 90.73 and 95.99 were induced when fertile females were crossed with males treated at 40 and 50 Gy, respectively.

Mating competitiveness

Mating competitiveness of sterile males in laboratory rearing cage

Influence of different mating sex ratios of sterile males to normal males with normal females on fecundity and egg hatching, in addition to mating competitiveness of sterile males under laboratory conditions is presented in Table 3.

Egg laying and egg hatching declined sharply with increase in ratio of sterile males. Daily egg laying by normal females crossed with sterile males in 1:1 ratio (T2) was significantly reduced (18.1 eggs/female), when compare to control (26.9 eggs/female). However the fecundity of females increased by 5.52%, when sterile males were confined with equal number of normal males (1:1:1 ratio, T3), compare to T2. In different competitive ratios, fecundity significantly decreased with increase in ratio of sterile males to normal males from 1:1 (19.20 eggs/female) to 3:1 (13.5 eggs/female).

Observed egg hatch varied significantly depending on a proportion of sterile males in mating sex ratios. Percentages of egg hatch observed for the females mated with normal males in the absence of sterile males (T1, control) was

Table 3 Mating competitiveness of melon fly at different ratios of sterile (30 Gy) and normal males under laboratory conditions

Cross ratio (SM×NM×NF)	Fecundity (Eggs/female)	%Observed egg hatch(EHo)	%Expected egg hatch (EHe)	Competitive- ness Value	χ ² -Statistics
T1 (0:1:1) (control)	26.9a (1.43±0.01)	72.17a (46.25±2.03)	-	-	-
T2 (1:0:1)	18.1b (1.26±0.03)	12.36e (7.09±0.77)	-	-	-
T3 (1:1:1)	19.2b (1.30±0.02)	50.04b (30.03±1.43)	42.26	0.84	3.94
T4 (1.5:1:1)	17.8b (1.25±0.02)	43.21bc (25.60±1.50)	36.78	0.85	1.13
T5 (2:1:1)	16.7bc (1.22±0.02)	37.83 cd (22.23±1.28)	32.29	0.85	10.20*
T6 (2.5:1:1)	15.3bc (1.18±0.04)	30.31d (17.63±0.64)	29.22	0.96	15.10**
T7 (3:1:1)	13.5c (1.12±0.02)	19.22e (11.07±0.60)	27.31	1.42	20.03**

Within a column, means followed by the same letter are not significantly different at 5% level ($\alpha=0.05$; Tukey HSD test, PROC ANOVA). Figures in parentheses are Arc Sine transformed values

SM sterile males, NM normal males, NF normal females

*indicates significant differences between EHo and EHe at 5% level (df=4; P=0.05); **indicates significant differences between EHo and EHe at 1% level (df=4; P=0.01)

significantly higher (72.17) than all other mating sex ratios. Fertility of females increased by 25%, when the females were confined with equal number of sterile and normal males (T3) as compared T2. When the ratio of sterile males was double to normal males (T5), significant difference was recorded between the percentages of observed and expected egg hatch. It was noticed that there was a significant difference between the observed and expected egg hatches for 2.5:1:1 and 3:1:1 at 1% level of significance ($P=0.01$) with mating competitiveness (CV) of 0.96 and 1.42, respectively. While CV values were nearly equivalent (0.84–0.85) for 1:1:1, 1.5:1:1 and 2:1:1 sex ratios.

Mating competitiveness of sterile males under field cage conditions

The egg laying ability of females decreased with increase in ratio of sterile males to normal males in field cage. The fecundity of females confined with equal number of sterile and normal males (T3) was 15.87 eggs/female/day. Further increase in ratio of sterile males to normal males from 1.5:1 to 3:1, gradually decreased the fecundity of females from 15.26 to 10.68 eggs/female, respectively. Release of sterile males to normal males at the ratio of 3:1 decreased the fecundity by $>50\%$ compare to control (T1) (Table 4).

The percentage of egg hatch in all the competitive sex ratios ranged from 39.45 (T3) to 26.51% (T7). Observed egg hatching in all the field cages decreased gradually with increase in the ratio of sterile males to normal males. Percentages of observed egg hatch in all the competitive field cages were significantly lower than control field cage (T1) (53.55%). A significant difference between observed

and expected egg hatch was found in T7 with mating competitiveness of 1.06. The mating competitiveness values for sterile males in released sex ratios (SM: NM: NF) of 1:1:1, 1.5:1:1, 2:1:1 and 2.5:1:1 were 0.66, 0.67, 0.68 and 0.77, respectively (Table 4).

Discussion

The determination of optimal irradiation dose for sterilization of particular species is of concern with tephritid SIT program. Effect of different gamma radiation doses was studied in order to identify a threshold dose that can induce optimum sexual sterility without any deleterious effects on quality of sterile males.

Exposure of mature pupae (48 h before adult emergence) to gamma radiation doses ranging from 5–50 Gy had significant impact on sterility and quality of biological parameters. The percentage of adult emergence and adult fliers decreased with increase in radiation dose while deformation increased. Irradiation at 40 and 50 Gy significantly reduced the emergence of active fliers with increased number of deformed adults. The adult emergence and active fliers at 30 Gy was not significantly different from control.

The similar results were reported by Mahmoud and Barta (2011) for *Bactrocera zonata* (Saunders) that adult emergence was decreased significantly with increasing gamma radiation from 10 to 90 Gy. Zahan et al. (2015) irradiated the pupae of *Bactrocera dorsalis* (Hendel) at 30, 40, 50 and 60 Gy and found that increase in radiation dose significantly decreased emergence. Kuyulu and Genc (2016) while working with *Tuta absoluta* (Meyrick) reported that with increase

Table 4 Mating competitiveness of melon fly at different ratios of irradiated (30 Gy) and non-irradiated males under field cage conditions

Cross ratio (SM×NM×NF)	Fecundity (Eggs/female)	%Observed egg hatch (EHo)	%Expected egg hatch (EHe)	Competitive- ness Value	χ^2 - Statistics
T1 (0:1:1) (control)	21.06a (1.32±0.02)	53.55a (32.47±3.08)	-	-	-
T2 (1:0:1)	12.29 cd (1.08±0.02)	18.10c (10.43±2.07)	-	-	-
T3 (1:1:1)	15.87b (1.19±0.02)	39.45b (23.22±0.83)	35.82	0.66	0.02
T4 (1.5:1:1)	15.26bc (1.18±0.01)	35.98b (21.09±1.66)	32.57	0.67	8.64
T5 (2:1:1)	13.55bc (1.13±0.02)	33.01b (19.27±1.15)	29.91	0.68	7.64
T6 (2.5:1:1)	12.52 cd (1.09±0.02)	29.99bc (17.44±0.45)	28.09	0.77	8.15
T7 (3:1:1)	10.68d (1.02±0.03)	26.51bc (15.37±0.84)	26.96	1.06	12.40*

Within a column, means followed by the same letter are not significantly different at 5% level ($\alpha=0.05$; Tukey HSD test, PROC ANOVA). Figures in parentheses are Arc Sine transformed values

SM sterile males, NM normal males, NF normal females

*indicates significant differences between EHo and EHe at 5% level (df=4; $P=0.05$)

in gamma radiation dose adult emergence decreased with increased malformation. Effect of gamma radiation on *B. zonata* was studied by El-Gendy et al. (2013) and found that flight ability decreased with increase in radiation dose and further they concluded that normal capability of flight was retained when pupae of 48 h before adult emergence were irradiated at 30 Gy.

Longevity of adult males also decreased with increase in radiation dose. Males irradiated at 30 Gy lived up to 28 days. Duration of 12–28 days is required for mating activity of melon fly under natural conditions (Mir et al. 2014). The similar results were reported by Puanmanee et al. (2010) that irradiated males of guava fruit fly, *Bactrocera correcta* (Bezzi) at gamma radiation dose of 30 Gy survived up to 30.60 days. Longevity of irradiated males in *Aedes aegypti* L. declined at 50 Gy by 69.9% (Shetty et al. 2016). The ability of a laboratory reared and sterilized fly to survive to sexual maturity is very important for the application of the SIT (Dyck et al. 2005). In present study, more than 50% of males irradiated at 30 Gy were alive after 48 h of food stress, when compare to normal flies provided with food and water (control). Collins et al. (2009) also found the similar results that, Queensland fruit fly, *Bactrocera tryoni* (Froggatt) irradiated at higher dose rates suffered higher mortality under food stress. Recently, Bloomfield et al. (2017) also reported that mortality of *B. tryoni* males under food stress increased with increase in doses. The ability of treated flies to survive without food or water is important in an SIT programme because released sterile insects must survive long enough to seek mates (FAO/IAEA/USDA 2014).

Doses ranging from 5 to 30 Gy did not show any significant reduction in fecundity and doses above that decreased fecundity. Fecundity of females declined by > 50% when mated with males irradiated at 50 Gy. These results were substantiated with the findings of Shetty et al. (2016) that fecundity was not affected up to 25 Gy and doses ranging from 30 to 50 Gy brought about a significant decline in fecundity of *A. aegypti*.

Irradiation of males at 15, 30, 40 and 50 Gy increased the percentage of unhatched eggs by 2.74–3.84-, 4.28-, and 4.52-folds higher than non-irradiated males. However, the percentage of unhatched eggs among the radiation doses; 30, 40 and 50 Gy were not different. From the present study it was observed that a radiation dose of 30 Gy was optimum as it induced sterility of 81.47% without much negative impacts on quality parameters of flies such as adult emergence, adult flying ability, deformation and adult longevity.

These results were conformity with the findings of Toledo et al. (2004) who exposed the pupae of *Anastrepha oblique* (Macquart) to radiation doses ranging from 0 to 100 Gy and found high sterility (99.9%) at radiation doses between 50 and 75 Gy with decreasing trend in adult flight ability and increasing trend in adult mortality. While, sterility of 98.2%

was induced at 25 Gy without posing negative effects on fly quality. Rull et al. (2005) while working in Mexican fruit fly eradication campaign, they concluded that lowering of irradiation doses (30 Gy) results in an increase in sterility induction in wild populations *Anastrepha ludens* (Loew). Radiation dose of 30 Gy was also optimised to induced sterility levels of 98.60 and 98.34% in *B. zonata* and *B. correcta*, respectively (Draz et al. 2008; Puanmanee et al. 2010). In Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) high percentage of sterility was obtained at radiation dose 30 Gy on wards (Sayed 2013; Bloomfield et al. 2017) proposed that the target irradiation dose for *B. tryoni* should be lowered (< 40 Gy) for improved fly quality with minimal decrease in reproductive sterility.

Mating competitiveness

The term "competitiveness" is applied to the ability of the released males to compete with males of the target population and this can be quantified by means of competitive mating tests. The index of mating competitiveness is the degree of sterility produced by sterile males in the wild population and is measured as a reduction in egg hatch (Shelly et al. 2000). Higher irradiation doses decreased male fly quality in *B. tryoni* (Collins et al. 2009; Dominiak et al. 2014), *A. ludens* (Toledo et al. 2004), and *C. capitata* (Guerfali et al. 2011) which may result in decrease in competitiveness with wild males (Bakri et al. 2005; Collins et al. 2009). However, lowering the irradiation doses leads to increased risk of residual fertility of *B. tryoni* (Collins et al. 2009; Dominiak et al. 2014) possibly exacerbating fruit fly outbreaks. Thus, optimizing the irradiation dose requires a trade-off between the objectives of producing competitive adult males as well as reduction in residual fertility of flies being released in the environment. In this connection, a radiation dose, 30 Gy was found optimum for assessment of mating competitiveness of sterile males under laboratory and field cage conditions.

Number of eggs and their hatchability was reduced significantly in laboratory and field cages with sterile males and normal females compare to those with normal males and normal females. Fecundity and observed egg hatches decreased with increase in ratio of sterile males to normal males both under laboratory and field cage condition. Observed (EHo) and expected egg hatches (EHe) were significantly different for sex ratio of 2:1:1, 2.5:1:1, and 3:1:1 under laboratory conditions. While in field cage conditions, significant differences between EHo and EHe was observed only at 3:1:1. The mating competitiveness value for sterile males at release ratio of 3:1:1 (sterile male: normal males: normal females) under laboratory and field cage conditions was 1.42 and 1.01, respectively. It indicates that

males sterilized at 30 Gy were fully competitive than normal males, when allowed to mate at release ratio of 3:1:1.

These results are in agreement with the findings of Toledo et al. (2004) that sterility induction in *A. obliqua* was two times greater for males irradiated at low doses (<40 Gy) than for males irradiated at high doses (>80 Gy) at 3:1:1 ratio (sterile male to fertile male to fertile female). Similarly, Mahmoud and Barta (2011) found that mating competitiveness of *B. zonata* (peach fruit fly) males irradiated at 30 Gy was 1.85 times greater than the males irradiated at 70 Gy. Pereira et al. (2007) also studied the mating competitiveness in *B. zonata* and identified that males irradiated at both 30 and 70 Gy were successfully competed with non-irradiated males. Parker and Mehta (2007) also reported that lower irradiation doses yield better results in terms of the combination of competitiveness and sterility. Rull et al. (2014) determined that sterile males of Mexican fruit fly, *A. ludens* irradiated at 20 and 40 Gy were found to exhibit greater mating propensity in field cages than males irradiated at 60 and 80 Gy. A competitiveness study for lesser pumpkin fly (*Dacus ciliatus* Loew) was conducted by Nemny-Lavy et al. (2016) also observed that overall mating competitiveness (0.32) was reduced due to high dose of gamma radiation. Sexual competitiveness of *Anopheles coluzzii* Coetzee & Wilkerson males sterilized at 90 Gy has been studied by Maiga et al. (2014) at three different ratios (1:1, 3:1 and 5:1) of sterile to non-irradiated males in large cages and found that the sterilized males were about half (competitiveness index: 0.53) as competitive as untreated males. Davila et al. (2015) estimated that a dose of 60 Gy induced > 99% sterility in wild females, but a dose less than 40 Gy causes less damage on mating performance of sterile males of *A. ludens*.

From the present study, it is concluded that irradiation of mature pupae (48 h before adult emergence) of melon fly at a gamma radiation (Co^{60} source) dose of 30 Gy induced maximum sterility (81.47%) without compromising quality of sterile male flies. Males sterilized at 30 Gy successfully competed with normal males for normal females at a release ratio of 3:1:1 (sterile male: normal male: normal female) under both laboratory and field cage conditions. A radiation dose of 30 Gy can be suggested to be optimum dose of gamma radiation in terms of combinations of sterility and mating competitiveness of sterile males in melon fly.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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