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STERILIZATION AND MALE RATIO OPTIMIZATION OF PEACH FRUIT FLY, *BACTROCERA ZONATA* (SAUNDERS) FOR FIELD APPLICATION IN STERILE INSECT TECHNIQUE

M.R. ISLAM[1], M. MOMEN[2], M.A. HOSSAIN[3*], S.A. KHAN[4] AND N.P. KHANOM[5]

[1]M.Sc. Student; [5]Professor, Department of Zoology, Dhaka College, Dhaka, Bangladesh; [2]Senior Scientific Officer; [3]Principal Scientific Officer, [4]Chief Scientific Officer, Insect Biotechnology Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Ganakbari, Savar, Dhaka 1349, Bangladesh.

*Corresponding author & address: Md. Aftab Hossain, E-mail: mdaftabh@yahoo.com
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ABSTRACT

Islam MR, Momen M, Hossain MA, Khan SA, Khanom NP (2019) Sterilization and male ratio optimization of peach fruit fly, *Bactrocera zonata* (Saunders) for field application in sterile insect technique. *Int. J. Expt. Agric.* 9(1), 1-5.

The experiment was conducted for the sterilization and sterile male ratio optimization of peach fruit fly, *Bactrocera zonata* (Saunders) for field application in Sterile Insect Technique (SIT). To determine the male sterility dose 6 days old pupae of *B. zonata* were irradiated with doses of 10, 30, 40, 50 and 60 Gy. It was noted that percent of adult emergence and egg hatch decreased significantly with increasing radiation dose ($p<0.01$). The sterility percentage by a percent of egg hatch of *B. zonata* increased as the dose increased. Cent percent male sterility was achieved at 60 Gy. Fixed numbers (150 females) of virgin females were allowed to mate with normal and sterile (60 Gy) males at 1:1, 1:2, 1:5 and 1:9 ratios. Comparing with control group, a sharp decrease of pupal recovery was observed when females were allowed to mate with different ratios of sterile males. Minimum pupal recovery (42.10 fold less) was obtained at 1:9 ratio. Percent of normal adult emergence was decreased significanly ($p<0.01$) as the sterile male ratios were increased from 1:2 to 1:9. Deformed adult emergence, partial adult emergence and un-emerged percentage of peach fruit flies were increased significantly as the ratios of sterile males were increased.

Key words: *sterility dose, irradiation, ratio optimization, Bactrocera zonata*

INTRODUCTION

Tephritid (Diptera: Tephritidae) fruit flies are a group of dangerous insects, attack fruits of fruit trees and certain vegetable fruits in all over the world causing direct and indirect economic injury (White and Elson-Harris, 1992; Drew and Romig, 2013; Elnagar *et al.* 2018). The genus *Bactrocera* has a wide host range and the invasive power of fruit and vegetables devastation (Clarke *et al*. 2005). Peach fruit fly is known as the most serious pest of tropical and subtropical fruits (Fletcher 1987). It was recorded that more than 50 cultivated and wild plant species, mainly those with fleshy fruits including guava, mangoes, peach, apricots, figs and citrus (White and Elson-Harris, 1992; Kapoor 1993). It is originated in South and South-East Asia (Agarwal *et al.* 1999; Leblanc *et al.* 2013, 2014), and spread to other parts of the world. Female peach fruit flies lay their eggs in the fruits while the maggots devour the pulp. Subsequently, secondary infections with bacterial and fungal diseases are frequent and infested fruits drop down (White and Elson-Harris, 1992). Also, it is considered as one of the most destructive fruit pests in temperate, tropical and subtropical countries due to the losses caused by fruit larvae as they feed and live in the fruits of host plants (Agarwal *et al.* 1999; Hashem *et al*. 2001). In India, the pest status of *B. zonata* is considered equal to or greater than that of the Oriental fruit fly (*Bactrocera dorsalis*) and the melon fly (*B. cucurbitae*), and they may overlap in the same crop (Kapoor 1993). In Bangladesh, The peach fruit fly is a serious pest of a variety of fruits especially in mango (*Mangifera indica* L.), Sapodilla (*Manilkara zapota* L.) and guava (*Psidium guajava* L.) (Kabir *et al.* 1991; Hossain *et al.* 2017). Considering the economic and quarantine importance control of this pest is frequently uttering most of the countries of the world (Heather and Hallman, 2008).

Traditional control measures using chemical insecticides experience disadvantages such as residual problems and inability of insecticides to penetrate infested fruits to kill larvae. Moreover, the public demand for insecticide-free fresh fruit is encouraging the use of environment-friendly methods of pest control. The Sterile Insect Technique (SIT) is an environment-friendly method of insect pest control has practically applied for several key pests of agricultural importance. It is a species specific, environmentally safe process with potentials of suppression of the target pest population from a defined area. SIT is usually applied as a component of area-wide integrated pest management (Klassen 2005). SIT generally imposes birth control effects on the target population to further reduce its numbers. The SIT involves rearing of large number of the target species, exposing them to gamma rays to induce sexual sterility and then releasing them into the target area. The released sterile males will mate with wild females to prevent them from reproducing. So far, there are limited scientific data available for the suppression of *B. zonata* population using sterile insect technique in Bangladesh. Therefore, a study was conducted to study the sterilization and male ratios optimization of sterile and normal males for possible field applications of SIT.

MATERIALS AND METHOD

Stock culture: Laboratory cultures of *B. zonata* were maintained in the fruit fly laboratory, Insect Biotechnology Division, Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka. Larvae were maintained on artificial diet. Adult peach flies were stocked in a wooden frame cage (60×50×45 cm) covered with nylon net. The culture was usually supplied with an artificial diet (sugar:yeast extract:casein/1:2:1) and water soaked cotton. In general, 1500-2000 adult fruit flies were maintained in a stock cage. Temperature and relative humidity of the rearing room were maintained at 28±2^{o}C and 70-80% respectively.

Pupae collection and irradiation: The larvae of *B. zonata* in the artificial larval diets were kept in plastic bowls with saw dusts at the bottom for pupation. The saw dusts were sieved after pupation for the collection of dust free pupae. Same aged (6 days old) 100 pupae were transferred into Perti dishes and irradiated in a Co^{60} gamma irradiator of IFRB, AERE, Savar, Dhaka. Radiation doses ranging from 10 to 60 Gy were applied with a dose rate of 10 Gy/minute. Each dose group had 4 replications and was repeated 5 times. One batch of control was also maintained with equal number of replications. Adult emergence percentage, sex ratio, fecundity data were collected and noted.

Sterility dose determination: Fifty male flies were collected from each of 10, 30, 40, 50 and 60 Gy treated 6 day old pupae and fifty females were also isolated from the control batch and allowed to mate with individual dose treated males in laboratory rearing cages. This experiment had four replications. After two days of mating, eggs were collected in egging device inside rubbed with banana paste. Collected eggs were poured on a tissue paper and placed on larval diet. Hatching data were collected after 48 hours.

Male ratio optimization: The six days old pupae of *B. zonata* were exposed to 60 Gy gamma irradiation. The adults emerged from the pupae were sexed and males and females were sorted out. The sterile and normal males were allowed to mate with 150 virgin females at 1:1 (75 normal and 75 sterile males), 1:2 (50 normal and 100 sterile males), 1:5 (25 normal and 125 sterile males) and 1:9 (15 normal and 135 sterile males) ratios in separate adult cages. Oviposition media (piece of banana) was placed in each of the cage after 2nd days of mating. The piece of banana was removed after 24 hrs from the adult cage and placed it on artificial diet. The total numbers of pupae and adult emergence were counted and recorded. One hundred and fifty laboratory reared males and same number of females were used as control. The raw data were analyzed by statistical software (Minitab version 13.1, Microsoft Excel). For Analysis of Variance (ANOVA) of percentage data, percentage values were transformed into Arcsine values.

RESULTS AND DISCUSSION

Effects of gamma radiation on adult's emergence percentage, sex ratio, female fecundity and egg hatching of *B. zonata* are shown in Table 1. The results indicated that adult's emergence decreased significantly with increasing radiation dose at constant dose rate (p<0.01). The adult emergence percentage at 10, 30, 40, 50 and 60 Gy radiation doses were recorded 93.20, 90.52, 89.12, 88.95 and 86.17 percent respectively whereas 92.66 percent in control group (Table 1). It was observed that even high doses do not have strong deleterious effects on the pupal viability.

Table 1. Effects of different doses of gamma radiation on adult emergence (mean±SE), sex ratio, female fecundity and egg hatching percentage of peach fruit fly

Radiation dose (Gy)	**0 (control)**	**10**	**30**	**40**	**50**	**60**
Adult emergence (%)	92.66a±2.12	93.20a±5.94	90.52b±4.66	89.12bc±6.12	88.95c±5.36	86.17d±4.74
Sex ratio (male/total)	0.05	0.05	0.47	0.46	0.46	0.45
Total number of eggs/female	413a±14.4	415a±43.8	414a±39.2	407a±29.9	401a±30.8	398a±29.5
Egg hatching (%)	91.88a±0.56	52.54b±0.78	39.77c±0.50	20.27d±0.48	5.43e±0.41	0f

Means followed by same letter do not differ significantly by Tukey's pairwise comparison test

The sex ratio (males/total) was also affected with gamma radiation; however, the effect was not prominent. The sex ratio at 10, 30, 40, 50 and 60 Gy radiation doses were observed as 0.50, 0.47, 0.46, 0.46 and 0.45 respectively (Table 1). In the control group, the sex ratio was 0.50.

Male sterility of *B. zonata* was evaluated after treatment with different doses of gamma radiation and determined here by a percent egg hatch. The present results showed that mating of normal females with treated males (males developed from irradiated pupae) did not affect the production of eggs (p>0.05), but it seriously reduced their hatching capacity (p<0.01) (Table 1). The percent of sterility attained in F_1 generation was 42.82, 56.72, 77.93, 94.09 and 100 at 10, 30, 40, 50 and 60 Gy dose treatment of adult male respectively (Fig. 1). Sterility rate was increased gradually with the increment of radiation doses. Complete sterility of male peach fruit fly was observed at 60 Gy. There was a perfect negative correlation (r=–0.89) between radiation dose and egg hatchability.

Many researchers (Balock *et al.* 1963; Anwar *et al.* 1978; Mangan 2005; Shehata *et al.* 2006; Mahmoud and Barta, 2011) determined the sterility dose by irradiating pupae 2-3 days before adult emergence which is almost similar to that of the present experiments. Shehata *et al.* (2006) reported that adult emergence percentage and sex ratio of *B. zonata* decreased with increased radiation dose. The sterility percentage of *B. zonata* increased with increased radiation dose (Shehata *et al.* 2006; Draz *et al.* 2008). Mahmoud and Barta (2011) studied the effect of different doses of gamma radiation on adult emergence, sex ratio, female fecundity and egg hatching of

B. zonata and observed that adult emergence percentage, sex ratio and egg hatching percentage decreased as radiation dose increased. Hossain *et al.* (2017) observed the percent of sterility attained in F_1 generation was 30.80, 61.29, 83.37, 93.20, 98.36, 100 and 100 at 10, 20, 30, 40, 50, 60 and 70 Gy respectively and finalized 60 Gy as male sterility dose for *B. zonata*. The result of the present findings is more or less similar to that of Shehata *et al.* (2006); Draz *et al.* (2008); Mahmoud and Barta (2011) and Hossain *et al.* (2017). In general, the sterility dose of males seems to differ from laboratory to laboratory. These differences may be due to a type of irradiator cells, methodology of assay, genus of flies, age of irradiated pupae, as well as fitness of laboratory strains tested.

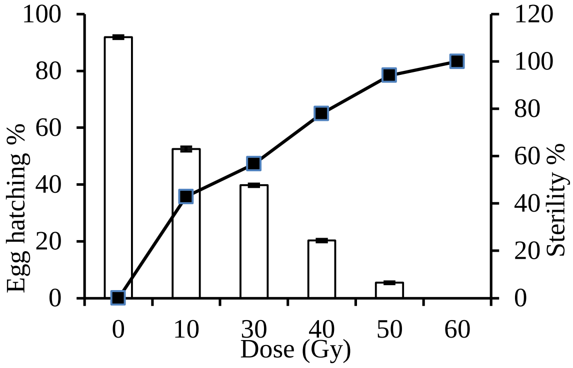

Fig. 1. Mean (±SE) egg hatching percentage and sterility percentage of peach fruit fly at different doses of gamma irradiation

The mean pupal recovery, percent of normal adult emergence, percent of deformed emergence, percent of partial emergence, percent of un-emerged fruit flies resulted from the mating of different ratios of normal male and sterile male (NM: SM) with normal female of *B. zonata* have been presented in Table 2. Pupal recoveries varied significantly from 1:2 to 1:9 male ratios (normal to sterile male ratio ($p<0.01$). On an average, 533.17±16.71, 233.21±6.41, 117.59±6.32 and 84.57±5.15 pupae were recovered from 1:1, 1:2, 1:5, 1:9 ratios respectively. On the other hand the pupal recovery from control group was 3560.53±104.41. The emergence percent of normal, deformed, partial and un-emerged flies are presented in Table 2. From 1:1 to 1:9 ratios the normal adult emergence of fruit flies were varied from about 54.66-84.00% compared to 95.00% in the control. Percentage of normal adult emergence is negatively correlated with the higher ratio of sterile male. Emergence of deformed flies (%) at 1:1 ratio was found as 3.66 which reached to 14.00 at 1:9 ratios while in the control it was only 1.66% (Table 2). From 1:1 to 1:9 ratios the partial adult emergence of fruit flies were varied from 2.66-7.66% compared to 1.00% in the control. Emergence of un-emerged flies (%) at 1:1 ratio was found as 9.00 which reached to 24.33 from 1:9 ratios (Table 2). Percentage of deformed, partial and un-emerged peach fruit flies were positively correlated with the ratio having higher number of sterile males.

Table 2. The mean (±SE) pupal recovery, normal adult emergence (%), deformed emergence (%), partial emergence (%), unemerged (%) resulted from the different mating ratios of normal and sterile male of *B. zonata*

Ratio	Mean (±SE) pupal recovery	Normal adult emergence (%)	Deformed flies (%)	Partial emergence (%)	Un-emerged flies (%)
Control	3560.53a±104.41	95.00a±0.57	1.33e±0.20	1.00d±0.01	2.66e±0.13
1:1	533.17b±16.71	84.00b±0.56	3.66d±0.48	2.66d±0.11	9.00d±1.12
1:2	233.21c±6.41	77.33c±1.20	7.66c±1.19	3.33c±0.10	11.66c±2.28
1:5	117.59d±6.32	66.33d±1.20	10.66b±1.27	5.33b±0.21	17.66b±1.29
1:9	84.57e±5.15	54.66e±2.18	14.00a±2.20	7.66a±1.24	24.33a±2.13

Means followed by same letter do not differ significantly by Tukey's pairwise comparison test

Yasmin *et al.* (2010) optimized the sterile versus normal males (1:2, 1:3, 1:5 and 1:9) of *B. cucurbitae* and obtained 43 fold less pupal recovery at 1:9 ratio as compared to control. Islam *et al.* (2012) optimized the sterile male ratio of *B. tau* and obtained 42 fold less pupal recovery at 1:9 ratio as compared to control. For field application Zahan *et al.* (2015) optimized the sterile male ratios (1:1, 1:2, 1:5 and 1:9) of *B. dorsalis* in the laboratory and reported that minimum pupal recovery was obtained in 1:9 ratios. Normal adult emergence, partial adult emergence, deformed adult emergence and un-emerged percentage of fruit flies directly depend on the sterile male ratios (Yasmin *et al.* 2010; Islam *et al.* 2012; Zahan *et al.* 2015). These findings are almost similar to that of the present observations.

The classic over flooding ratio of 9 sterile male to 1 fertile male as proposed by Knipling (1979) was worked very well against screwworm fly, *Cochliomyia hominivorax* (Coquillett). Cavalloro (1983) found in Laboratory experiment and in pilot tests in the field that the best ratio of wholly sterile males of olive fruit fly, *B. olea* per normal couple is 8:1. It has been reported that egg hatchability of the fruit fly, *Anastrepha suspensa* Loew. reduced to 46, 26, 20 and 8.5 when 1:1:1, 1:3:1, 1:6:1 and 1:9:1 (Female x Sterile male x Fertile male) were used (Calkins and Draz, 1987). The over flooding ratio of 9 sterile male to 1 fertile male was worked very well against the melon fly *B. cucurbitae* (Yasmin *et al*. 2010), the pumpkin fruit fly, *B. tau* (Islam *et al.* 2012) and the oriental fruit fly *B. dorsalis* (Zahan *et al.* 2015). They also reported that as compared to control batch minimum pupal recovery was observed in 1:9 ratio. In the present investigation, 1:9 ratio worked very well against peach fruit fly and the pupal recovery of this fly varied significantly from 1:1 to 1:9 ratio with the control batch. The result agrees with that of Knipling (1979), Yasmin *et al*. (2010); Islam *et al.* (2012) and Zahan *et al.* (2015) but they worked on other fruit fly species.

All the above parameters suggest that 1:9 ratio of normal vs sterile males of *B. zonata* is apparently the effective ratio for releasing sterilized male peach fruit fly in nature for possible field application of SIT.

CONCLUSION

Data generated from this experiment on sterility dose, pupal duration and on the ratios of irradiated males of *Bactrocera zonata* clearly revealed that 60 Gy could be used as a sterilizing dose for this species and a 9 fold ratio of irradiated male fly to unirradiated one could produce significant suppression of peach fruit fly. However, further works with larger volume will be required to generate a data more suitable for field applications of peach fruit fly SIT.

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