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A COMPARATIVE STUDY ON RADIATION SUSCEPTIBILITY OF FOUR *BACTROCERA* SPECIES (DIPTERA: TEPHRITIDAE) AT 3.5 KR UNDER LABORATORY CONDITION

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ABSTRACT

Insect's life is seriously influenced by various abiotic stress factors including radiation. The tephritid fruit flies under *Bactrocera* genus are a group of severe agricultural pest, attack wide range of fruits and vegetables in tropical and subtropical regions of the world causing direct and indirect economic lose. Study has been carried out to evaluate the comparative influence of the irradiation on four *Bactrocera* species under laboratory condition for their fruitful application in Sterile Insect Technique (SIT). Six days old pupae of *Bactrocera cucurbitae*, *B. tau*, *B. zonata* and *B. dorsalis* were exposed to ^{60}Co gamma radiation at 3.5 Kr and observe the susceptibility of radiation dose on pupal period, adult emergence rate, male-female percentages, sex ratio and longevity upto 45 days. Pupal period of *B. cucurbitae* ($F_{1,8}$ = 12.00, P = 0.009) and *B. dorsalis* ($F_{1,8}$ = 5.33, P = 0.050) significantly affected by radiation. Adult emergence percentages were 90%, 85%, 75% and 76% for *B. cucurbitae*, *B. tau, B. zonata* and *B. dorsalis* respectively where equal percentage of male emerged from radiated and control group in *B. cucurbitae*. Sex ratio was not remarkably affected by radiation on the four *Bactrocera* species. About equal percentages of male and female of *B. cucurbitae* (92%) and *B. dorsalis* (72%) lived upto six weeks. Longevity of female flies was not significantly affected by radiation in case of *B. cucurbitae*, *B. tau* and *B. dorsalis* but in *B. zonata* the result was significant. Present study made a comparison of radiation effect on different *Bactrocera* species that will helpful to apply them in sterile insect technique application.

KEYWORDS

Insect pests, *Bactrocera*, Gamma radiation, Sterile insect technique and Irradiation.

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INTRODUCTON

Insect pests are one of the big barriers for the improvement and advancement of the agricultural sector that are continuously damaging food and their commodities[1]. More than 200 economic important insect species from eight taxonomic orders have been irradiated for radiobiological studies with relation to integrated pest management

(IPM) program and/or phytosanitary purpose. Eighty percent of them under three orders- Diptera, Lepidoptera and Coleoptera[2]. Insects are subjected to 25-30% lose of the world's food production each year, though they are an integral part of the ecological system[3]. The distribution and abundance of tephritid flies depend on different abiotic factors (temperature, relative humidity, rainfall) and some biotic factors (host plants, natural enemies)[4]. Tephritid (Family- Tephritidae) fruit flies under order Diptera are serious insect pests and causes direct and indirect economic injury by attacking various fruits and vegetables all over the world[5,6]. More than 325 species of tephritid fruit flies are documented in Indian subcontinent[7] and 15 species of these flies are infested in Bangladesh[8].

The genus *Bactrocera* Macquart (Diptera: Tephritidae) has a worldwide recognition for its massive destruction of agriculture. It damages a wide range of host species of cucurbit vegetables and certain fleshy fruits[9-11]. Since the beginning of the last century *Bactrocera* species has turn into significant pests of agricultural crops in Asia[12,13]. Often more than 50 percent of the vegetables are being unfit for human consumption by damaging them either partially or totally[9,14]. The pest may totally damage the crop production in uncontrolled situation[15]. In Bangladesh the fruit flies under *Bactrocera* genus infest seriously the fruits of melon (*Cucumis melo*), sweet gourd (*Cucurbita maxima*), ivy gourd (*Coccinia grandis*), snake gourd (*Trichosanthes cucumerina, Benincasa hispida*), white-flowered gourd (*Lagenaria siceraria*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus, Cucumis trigonus*), luffa (*Luffa aegyptiaca*), bitter gourd (*Momordica charantia*), balsam-apple (*Omordica balsamina*)[16,17]. The annual loses of agricultural production due to fruit fly infestation estimates about 10 to 30%[22]. The melon fly, *Bactrocera cucurbitae* (Coquellett), the peach fruit fly, *B. zonata* (Saunders) and the oriental fruit fly, *B. dorsalis* (Hendel) are regarded as severe polyphagous pests that attack more than 125, 50 and 270 species of cultivated and wild fruits and vegetables (Family- Cucurbitaceae and Solanaceae), respectively throughout the world[18-20], where the pumpkin fruit fly, *Bactrocera tau* (Walker) is considered as moderate serious pest in Bangladesh[8,21]. Therefore, the management and control of *Bactrocera* pests are socio-economically important.

Traditionally, application of chemical insecticides to control pest experiences inconvenience for residual problems and incompetence of insecticides to penetrate infested fruits and vegetables to kill the maggots. Moreover, the increasing of public demand for insecticide-free fresh fruits is stimulating the application of environment friendly pest control techniques. Sterile insect technique (SIT) is such an eco-friendly autocidal method for control or minimizes the pest population that is becoming a key element of integrated pest management for fruit fly control[22]. Gamma irradiation is now a common technique applied to sterilize mass reared males for SIT[2] and the effectiveness of SIT largely depends on the production of quality sterile males released into the target wild population. The absorbed dose of radiation induced in sterility is crucial to SIT because insects that receive too low absorbed dose do not sterile completely and those receive too high absorbed dose stay uncompetitive. It diminish the effectiveness of SIT where releasing a large number of sterile insects is required[23,24].

The objective of the current study is to assess the comparative effect of 3.5 Kr radiation dose on pupal period, adult emergence rate, male-female percentage, sex ratio and longevity of *B. cucurbitae*, *B. tau, B. dorsalis,* and *B. zonata* in laboratory condition.

MATERIAL AND METHODS

Experiments were conducted in the Fruit Fly Laboratory of the Radiation Entomology and Acarology Division during November 2016 to January 2017 in the institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka to observe Gamma radiation effect on biological quality parameters (pupal period, adult emergence rate, male-female percentages, sex ratio and longevity) of four *Bactrocera* species (*B. cucurbitae*, *B. tau, B. dorsalis,* and *B. zonata*) at 3.5 Kr dose.

Study Insects

Laboratory colonies of four *Bactrocera* species (*B. cucurbitae*, *B. tau*, *B. zonata* and *B. dorsalis*) has been reared in the fruit fly laboratory. Adult flies have been maintained in steel frame cages (12×10×8cm) covered with mosquito net at 25±2°C and 65-70% relative humidity (RH) with 10:14 Light/Dark photoperiod. Adults were provided a mixture of sugar and yeast extract (3:1 ratio by weight) and water as soaked cotton in 10 ml conical flaxes.

Biological Material and Irradiation

Randomly selected one hundred fifty pupae of the four *Bactrocera* spp. (6 days old/one day before adult emergence) in five replications (thirty pupae in each replication) from the lab culture were irradiated in a ^{60}Co Gamma irradiator established at the Institute of Food and Radiation Biology (IFRB), AERE, Savar, Dhaka. The dose was 3.5 Kr. Equal numbers of pupae in each replication were kept as control group.

Data Collection

After radiation treatment, radiated pupae and the control groups were maintained in the separate rearing cages for farther development. Emergence of adults was checked daily and the number of emerged flies, male and female flies was recorded. Percentages of adults, percentage of male and female flies were calculated based on the number of adults, male and female flies emerge from the pupal samples. Sex ration was calculated by the equation (male/total emerge adult) according to Draz *et al*.[25] and Mahmoud and Barta[26]. Longevity of male and female flies was recorded upto six weeks and the percentage was calculated by the number of flies (male and female) alive in this period.

Statistical Analysis

Data were analysed using SPSS 24.0 (IBM Company, USA). Data across all replications in two groups (irradiated and control) were subjected to one-way analyses of variance (ANOVA), and the descriptives analysis of mean difference were calculated by Tukey's Least Significant Deference (LSD) test where significant F-values were obtained at 95% confidence level ($p< 0.05$).

RESULTS

Pupal Period

Effects of gamma radiation on pupal period of four *Bactrocera* species are shown in Table No.1. The results indicated that pupal period of *B. cucurbitae* was highly significant between irradiated and control groups ($F_{1,8}$ = 12.00, P = 0.009) following the result of *B. dorsalis* ($F_{1,8}$ = 5.33, P = 0.050). In case of *B. tau* and *B. zonata* the relation between irradiated and control groups were insignificant ($F_{1,8}$ = 4.55, P = 0.066) and ($F_{1,8}$ = 0.40, P = 0.545), respectively. In irradiated groups, maximum pupal duration of *B. cucurbitae*, *B. tau* and *B. dorsalis* was 5 days and minimum was 4 days. The maximum pupation period of *B. zonata* was 4 days and minimum was 3 days. In control groups, pupae took minimum 3 days to emerge of the fours species of *Bactrocera* and the maximum pupal period of *B. cucurbitae* and *B. dorsalis* was 4 days where *B. tau* and *B. zonata* was 5 days and 4 days respectively.

Adult Emergence

Gamma radiation effect on adult emergence was shown in Table No.1. The results showed that radiation had significant effect on the adult emergence rate of *B. tau*, *B. zonata* and *B. dorsalis* ($F_{1,8}$ = 8.82, P = 0.018; $F_{1,8}$ = 20.95, P = 0.002; $F_{1,8}$ = 12.03, P = 0.008). Adult emergence was not significantly ($F_{1,8}$ = 0.11, P = 0.740) affected by radiation in *B. cucurbitae* where more than 90% of adult emerged both in irradiated and control groups (Figure No.1). In case of irradiated group, adult emergence percentages were 85%, 75% and 76% for *B. tau, B. zonata* and *B. dorsalis*, respectively whereas the percentages were 95%, 90% and 89% in the respective control group (Figure No.1).

Male-Female Emergence

Emergence of male and female fly significantly affected by gamma radiation for *B. zonata* ($F_{1,8}$ = 6.32, P = 0.036) and *B. dorsalis* ($F_{1,8}$ = 6.88, P = 0.031). Insignificant result was found in case of *B. cucurbitae* ($F_{1,8}$ = 0.00, P = 1.00) and *B. tau* ($F_{1,8}$ = 3.41, P = 0.12) (Table No.1). Equal percentage of male emerged from irradiated and control group in *B. cucurbitae* (46.67% and 46.67%), *B. zonata* and *B. dorsalis* (36.67% and 46.00% respectively) (Figure No.1). In *B.* tau, male emergence percentage was 38% in irradiated group and 45.33% in control group. In case of female flies, the

percentages were higher in control groups of the four *Bactrocera* species and the difference was minor in *B. cucurbitae* and *B. tau* (Figure No.1). 6.67% more female flies were emerged from control group than those of irradiated group in *B. zonata* and *B. dorsalis* (Figure No.1).

Sex Ratio

The sex ratio (males/total emergence) was affected by gamma radiation; though the effect was not prominent. 3.5 Kr dose had no significant effect on sex ratio of four *Bactrocera* species ($F_{1,8} = 0.05$, P = 0.827 in *B. cucurbitae*, $F_{1,8} = 0.36$, P = 0.566 in *B. tau*, $F_{1,8} = 0.22$, P = 0.651 in *B. zonata* and $F_{1,8} = 0.00$, P = 0.966 in *B. dorsalis*) (Table No.1). The difference of sex ratio was high in *B. tau* following to *B. zonata* while *B. cucurbitae* and *B. dorsalis* shared the same difference (Figure No.2).

Longevity

Longevity of males and females was not significantly affected by radiation with the exception of *B. zonata* female flies ($F_{1,8} = 4.84$, P = 0.059). The significance levels of male flies of four *Bactrocera* species were ($F_{1,8} = 0.00$, P = 0.971 in *B. cucurbitae*; $F_{1,8} = 0.00$, P = 1.00 in *B. tau*; $F_{1,8} = 0.83$, P = 0.390 in *B. zonata* and $F_{1,8} = 4.65$, P = 0.063 in *B. dorsalis*) (Table No.1). Significant values of female flies were $F_{1,8} = 0.19$, P = 0.671; $F_{1,8} = 0.92$, P = 0.366 and $F_{1,8} = 1.64$, P = 0.236 in *B. cucurbitae*, *B. tau* and *B. dorsalis*, respectively. Almost equal percentages of male and female of *B. cucurbitae* and *B. dorsalis* lived upto six weeks (Figure No.3). In case of *B. tau,* 76.19% male flies existed in both groups and 87.98% irradiated and 92.62% control female flies lived upto six weeks. When radiation imposed on the peach fruit fly, *B. zonata* 82.34% male and 80.93% female flies lived and in control group 87.09% and 89.98% (male and female respectively) flies stayed alive in six weeks period (Figure No.3).

DISCUSSION

Fruit flies are the most common group of Arthropods where sterilization and other biological parameters have been investigated with ionizing radiation. So radiation effects must be evaluated in quality control test on adult emergence, male-female percentage and sex ratio of released insect species[26].

As noticed, radiation dose 3.5 Kr has no significant effect on pupal period of *B. tau*. Alim *et al*,[27] worked on pupal radio sensitivity and male sterility of pumpkin fruit fly, *B. tau* where they showed that at 40 Gy and 50 Gy, 5 days old pupae took 9 days where 6 days old pupae took 8 days for adult emergence and in control group pupation period was 8 days. For another tephritid species *Anastrepha suspensa* (Loew), Calkins *et al*,[28] reported that at 3.0 Kr dose, adult eclosion was 84%, sex ratio (male : female) was 40:60, male% was 76 and female% was 84 while at 5.0 Kr adult eclosion was 81%, sex ratio (male : female) was 36:64, male% and female% was 71 and 76 respectively. It supported the results of present findings (90% adult in *B. cucurbitae*, 85.33% adult in *B. tau* and 76% adult in *B. dorsalis* emerged from irradiated groups).

Uddin *et al*,[29] studied radiation effects on different biological parameters in the peach fruit fly, *B. zonata*. The result of adult emergence rate (male 49% and female 41%) of peach fruit fly was fully consistence with the present findings. Mahmoud and Barta[26] imposed radiation on *B. zonata* pupae (two days before emergence) at different dose and found significant effect of radiation on adult emergence. They found that at 30 Gy and 50 Gy adult emergence percentages were 80.00% and 74.70% reflecting the findings of the present study, where we found 74.67% adult emerge in irradiated groups and 90.67% adult emerge in control groups. Resilva *et al*,[30] showed 97.40% adult emergence at 40 Gy for *B. philippinensis* pupae when irradiated at two days before adult emergence. Alim *et al*,[27] observed that 71% adult were emerged from 5 days old pupae of *B. tau* and 88% adult were emerged from 6 days old pupae at 40 Gy, where we found 85% adult in irradiated group and 95% adult in control group at 3.5 Kr. Draz *et al*,[25] reported that 6 days old pupae of *B. zonata* were irradiated at 30 Gy and 50 Gy. After irradiation, the adult emergence rate was 87.23% and 85.20% respectively, compared with 94.50% for unirradiated control group. Zahran *et al*,[31] found 81.70% adult at 30 Gy and 76.70% adult at 50 Gy on *B. zonata*'s 5 days old pupae. Hamza[32] showed *Eumerus anoneus* (Syrphidae: Diptera) adult emergence was reduced as gamma radiation

imposed on their pupae. Hallman and Worley[33] found that the older pupae of Mexican and West Indian fruit fly pupae were more radiation resistant than the younger pupae.

Draz *et al*,[25] observed variation in sex ratios for *B. zonata*. In their study, highest sex ratio was recorded at a dose of 30 Gy (0.60), followed by 0.56 and 0.47 for flies irradiated with 50 and 10 Gy, respectively. Mahmoud and Barta[26] found minor gamma radiation effect on sex ration in peach fruit fly, *B. zonata*. At 30 Gy the sex ratio was 0.45 and at 50 Gy, it was 0.46 whereas the ratio was 0.50 in control groups comparing the present study at 3.5 Kr, we found 0.49 sex ratio in irradiated groups and 0.51 in control groups of *B. zonata*.

Table No.1: Mean value and standard deviation of different biological parameters of four *Bactrocera* species at radiation dose 3.5 Kr

Name of species	Pupal period		Adult emergence		Male emergence		Female emergence		Sex ratio		Longevity			
											Male		Female	
	Radiated group	Control group	Radiated group	Control group	Radiated group	Control group	Radiated group	Control group	Radiated group	Control group	Radiated group	Control group	Radiated group	Control group
	Mean ±SD	Mean ±SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean ±SD	Mean ±SD	Mean±SD	Mean± SD	Mean± SD	Mean± SD
Bactrocera cucurbitae	4.60± 0.55a	3.40± 0.55a	27.00± 1.87a	27.40± 1.82b	14.00± 2.00a	14.00± 1.00b	13.00± 1.23a	13.40± 1.34b	0.52± 0.02a	0.51±0 .01b	92.80±1 0.47a	92.60± 5.32b	92.40± 9.39a	90.20± 6.01b
Bactrocera tau	4.60± 0.55b	3.60± 0.89c	25.60± 1.67b	28.60± 1.52b	11.40± 2.41b	13.60± 1.14c	14.20± 3.03b	15.00± 1.87c	0.45± 0.04b	0.48±0 .02c	76.19±1 0.07b	76.19± 10.07c	87.98± 8.13a	92.62± 7.17c
Bactrocera zonata	3.40± 0.55c	3.20± 0.45d	22.40± 0.89c	27.20± 2.17c	11.00± 1.58c	13.80± 1.92c	11.40± 1.14c	13.40± 2.07d	0.49± 0.02a	0.51±0 .02c	82.34±7. 16c	87.09± 9.22d	80.93± 6.18b	89.04± 5.46b
Bactrocera dorsalis	4.40± 0.55a	3.60± 0.55a	22.80± 1.30d	26.60± 2.07d	11.00± 1.58d	13.80± 1.79d	11.80± 1.79d	13.80± 2.05e	0.48± 0.03c	0.49±0 .02d	72.73±5. 17d	80.85± 6.64e	72.76± 8.94a	80.56± 10.27e

Mean values express by the same letter within the row had significantly different in two groups (TUKEY LSD test, P=0.05)

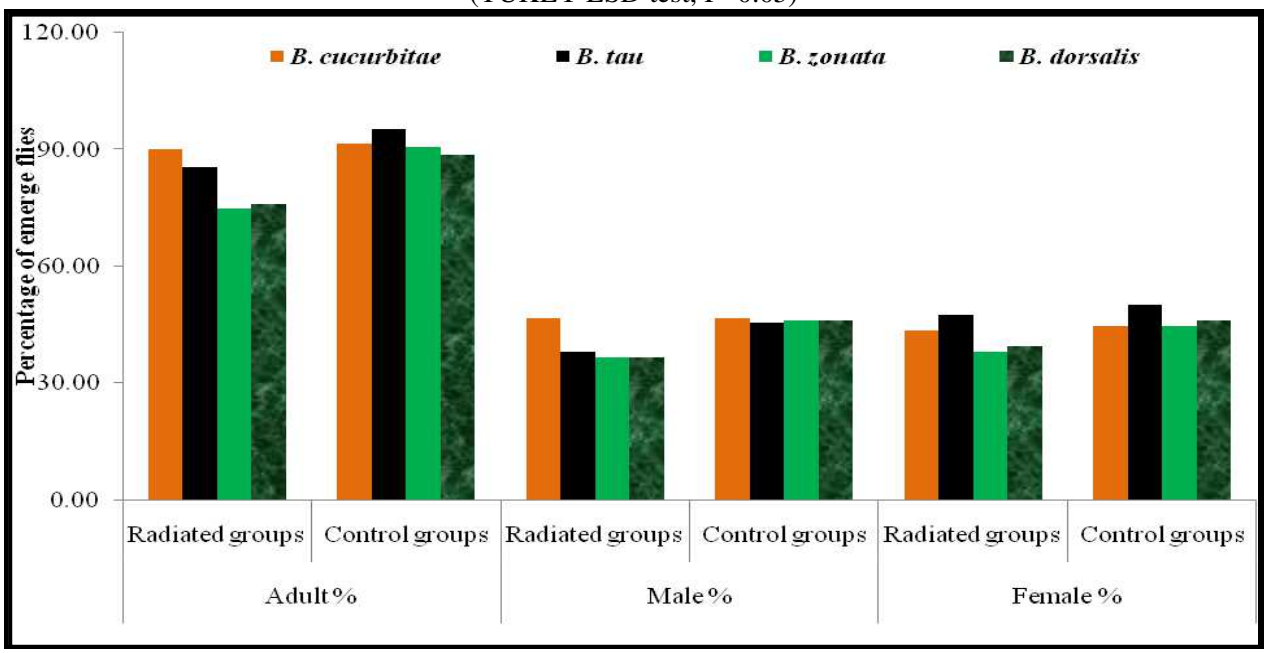

Figure No.1: Percentage of adult, male and female emergence of four *Bactrocera* spp. at radiation dose at 3.5 Kr

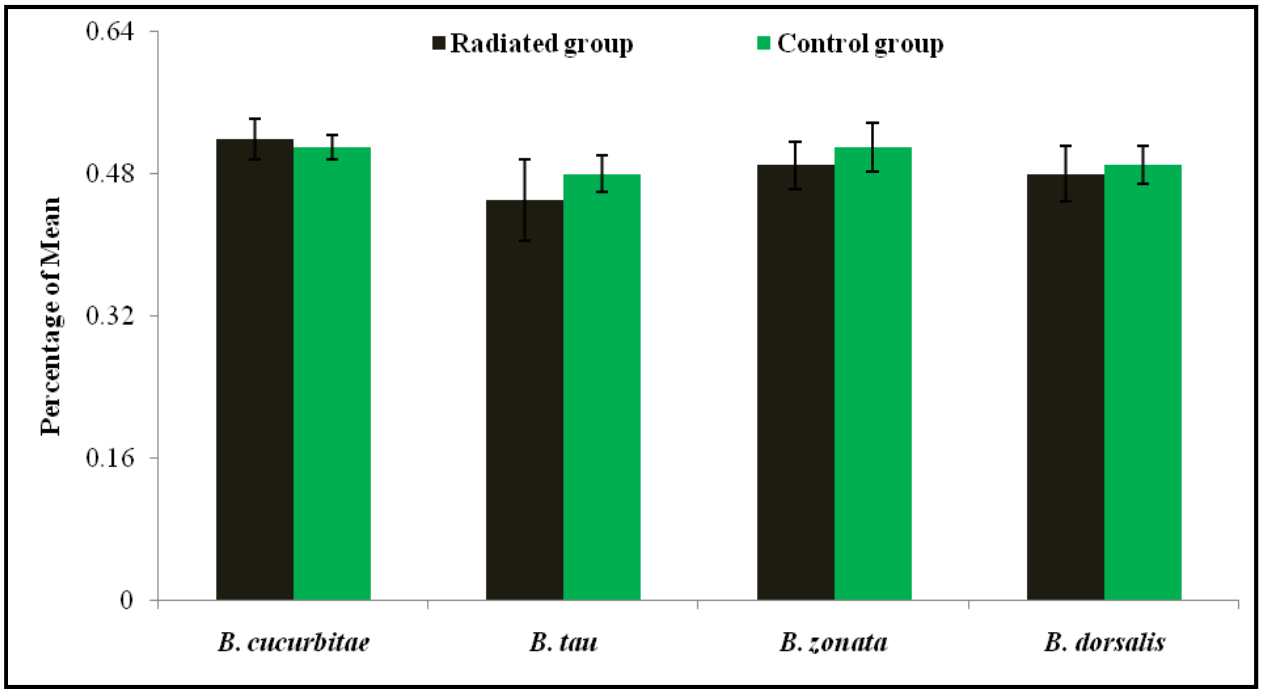

Figure No.2: Sex ration of different *Bactrocera* species at radiation dose 3.5 Kr

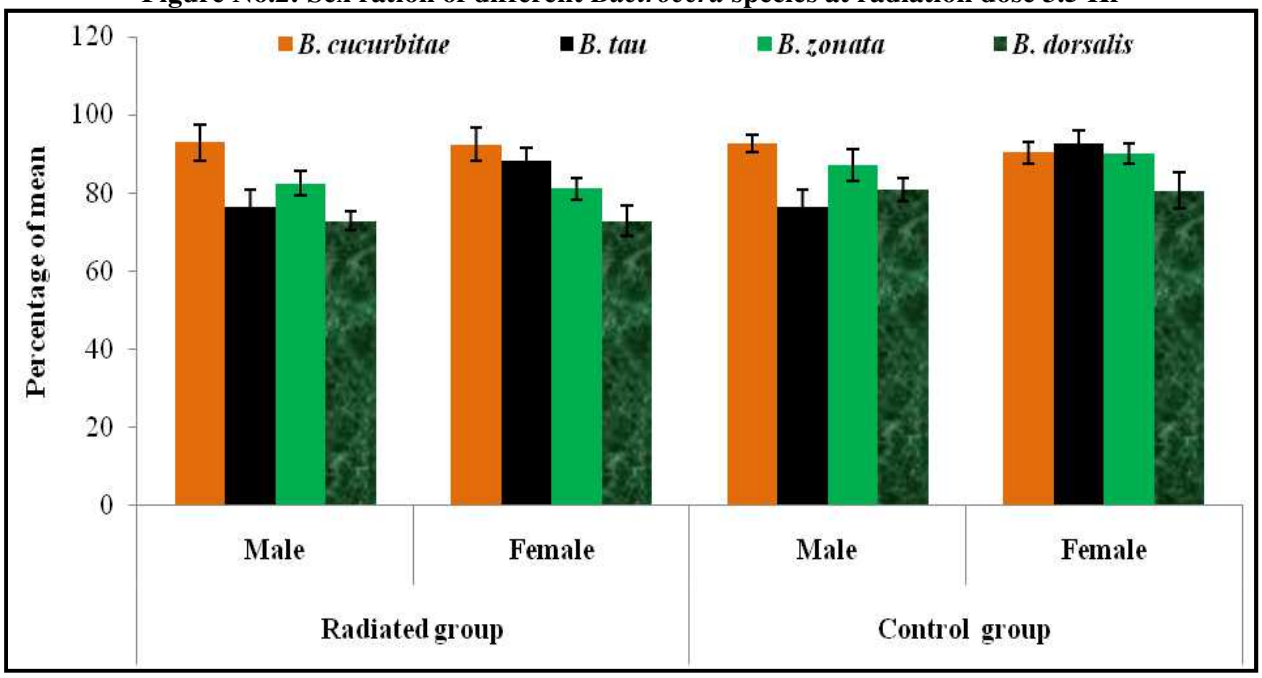

Figure No.3: Percentage of longevity up to six week of four *Bactrocera* species at radiation dose 3.5 Kr

CONCLUSION

Radiation susceptibility of Tephritids flies is an important concern in sterile insect technique application. Result of the present study evident that observed biological parameters (pupal period, adult emergence rate, male-female percentage, sex ratio and longevity) of the four *Bactrocera* spp. was not significantly affected at 3.5 Kr. The study could assist researchers to compare the effect of radiation on different biological parameters of different *Bactrocera* species in SIT program where a large number of flies needed to rear in the laboratory and then release in the field for controlling the specific pest species. Further study is continuing in the laboratory of IFRB for optimizing the appropriate radiation dose of different fruit fly species.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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