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Effect of X-Ray Irradiation on Pupae of *Bactrocera zonata* and *Bactrocera dorsalis* and Subsequent Adult Development under Laboratory Conditions

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Authors' Contribution
ZUAA performed the experiments, analyzed the data and wrote the manuscript. NB supervised the study. RMM conceived the study. NHK designed the experiments. IS assisted in experiments.

Key words
***Bactrocera* spp., X-ray irradiation, Natural and artificial diets, Pupal development, Control of pupae, SIT**

ABSTRACT

The studies were performed to check the effect of X-ray doses such as 20, 40, 60, 80 and 100 Gy on the *Bactrocera zonata* (Saunders) and *Bactrocera dorsalis* (Hendel) pupae reared on different diets. The laboratory strains of the peach fruit fly and oriental fruit fly reared on guava, mango and artificial diets. Results showed a significantly ($P<0.05$) greater number of deformed adults (males and females) of *B. zonata* at 100 Gy dose of X-ray irradiation followed by *B. dorsalis* obtained from artificial diets. Less deformity in adults of both fruit fly species was found at the same dosage of irradiation when maggots tended on mango. Similarly, a reduced number of normal adults of both fruit flies were recorded at 100 Gy dose of X-rays reared on an artificial diet. Reduced pupal emergence was recorded on an artificial diet. From the above results, it is determined that *B. zonata* is more tolerant to different irradiation doses. Results of the present investigation would be useful to control or target fruit fly pupae in fruits of trade commodities as these pests are universally regulated as quarantine pests.

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INTRODUCTION

The fruit flies of the family Tephritidae belonging to the order Diptera includes over 4,000 species of which about 700 belong to genus *Bactrocera* (Fletcher, 1987). More than 250 fruit fly species are of commercial significance and are dispersed widely worldwide (Christensen and Richard, 1960). Many agricultural and horticultural crops, fruits and vegetables such as mango, guava, peach, papaya, bitter gourd, sunflower, etc., are directly infested by fruit flies (Allwood and Drew, 1997). The fruit flies of the genus *Bactrocera* are the most destructive pests of mango and guava fruits widely distributed throughout Pakistan (Mahmood, 2006). Peach, oranges, guava and mango are heavily attacked by *B. zonata* and *B. dorsalis* (Guillen and Sanchez, 2007). An estimated 20-30% direct losses on fruit production have been recorded by these fruit flies (Hendrichs *et al*., 2009). These both *Bactrocera* flies are known as key pests of mangoes due to their economic impact. Usually female flies oviposit in the fruitlets leading to the development of maggots in the pulp inside the fruits hence reduce the market value of fruits (Naqvi, 2005). Frequent use of pesticides, toxicant mixture against fruit flies destroyed the natural fauna. Moreover, recurrent application of insecticides have not only resulted colossal losses to human health, environment, soil, water and wildlife but also have potential health risks, destruction of beneficial insect fauna and development of insect pest resistance (Wong *et al*., 1991).

For the management of *B. zonata* and *B. dorsalis* major fruit flies, suppression strategies are already established around the world. Several fruit flies controlling eco-friendly techniques such as bait-applications, male annihilation technique (MAT), sterile insect technique (SIT), bio-control and other traditional control measures have been applied (Cancino *et al*., 2008; Lux *et al*., 2003a).

Production of biological control agents takes time and is expensive. To avoid the expenditures of rearing biocontrol agents and there supplement hosts the X-rays and gamma rays are used to handle such costs (Liquido *et al*., 1990).

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Irradiation also encourage reproduction rate of some insects when applied at low dose. Moreover, radiation may enhance the endurance of hosts (Ovruski *et al*., 2003).

Supply of irradiated hosts and target hosts in the field during introduction offer maximum flexibility for growth of natural enemy population consequently the desired stage of host would be available to keep pest population below economic injury level (Van and Ovruski, 2012).

Providing irradiated hosts to larval-pupal and pupal parasitoids successively conveyed an alternative scheme that prolonged the fitness of hosts to maximize the parasitization in the mass-production of fruit flies parasitoids (Cancino *et al*., 2009).

Irradiation also avoided the development of fruit flies in un-parasitized pupae. Egg, larval and pupal parasitoids of fruit flies magnificently developed in radiated hosts by reducing other time and labour taking expensive procedures of isolating fruit flies and wasps during entire period of rearing (Cancino *et al*., 2012). The RS 2400 irradiator used to observe effect of X-rays on maturation of *Ceratitis capitata* and *Anastrepha fraterculus*. Above 90 % infertility were observed on both species. Besides, maximum number of *C. capitata* (84%) survived when exposed to X-rays followed *A. fraterculus* (74%). Furthermore, similar survival of females of both fruit flies were observed when mated with radiated and un-radiated males (Mastrangelo *et al*., 2010).

The valuation of different irradiation doses 15, 30, 60, 90 and 120 Gy along with control (0) one, two and three day old pupae of *C. capitata* and *A. fraterculus* were observed for productive quality control of sterilized tephritid fruit flies. The irradiation dose above 120 Gy is sufficient for the sterilization and would be applied in consignments as quarantine security. The results on fertilization potency of both species on different dosages were also obtained and maximum fecundity were obtained by individuals provided with 15 and 30 Gy (FAO/IAEA/USDA, 2003).

An irradiation dose of 200 Gy for *Bactrocera* flies 250 Gy for all other fruit flies suggested based on outcomes by APHIS, USDA. Who officially permitted irradiation dosages 120, 150 and 200 for *Bactrocera cucurbitae*, *Bactrocera dorsalis* and *Ceratitis capitata* for exportation of fruits and vegetables from Hawaiian Islands. Whereas, according to Federal Register for all fruit flies 150 Gy and all other insect pests 300 Gy irradiation doses recommended (Seo *et al*., 1973).

The purpose of this work was to define the efficacy of X-ray radiations under laboratory conditions for the possible management of fruit flies in consignments or in trade commodities. Very little is known about the effect of X-rays on fruit flies in Pakistan. The present work is an effort for the development of bio-control using x-ray radiations.

MATERIALS AND METHODS

Pupae collection

About 10,000 adult *B. zonata* and *B. dorsalis* were maintained in Fruit Flies Laboratories, NIA, Tando Jam and provided with protein based artificial diets for mass rearing purpose.

Eggs were collected through egg laying receptacles, washed with distilled water and detached from the egg laying containers using camel hair brush very gently. About 600 eggs were seeded on natural diet (mango and guava fruits) above 1.5 to 2 cm thick saw dust and artificial diet (wheat shorts, 1000 g, sugar 500 g, Troula yeast 200 g), Methyl. P. Hydroxy Benzoate 3 g), Sodium Benzoate 6 g, HCl 10 ml, and tap water 3 L) to develop from egg stage to pupation, after completion of process, pupae of fruit flies were sieved from saw dust placed in Petri dishes and shifted to X-rays facility for irradiation doses.

X-ray irradiation of pupae and observations on subsequent development

The X rays were generated by RS-2400 irradiator at X-rays Unit NIA, Tando Jam, operated at 150 keV and 45 mA giving a rate of 14.1 0.7 Gy min^{1} at the irradiation position. A small plastic bag with the pupae was positioned in the center of the canisters (178 mm in diameter by 167 mm in length) that were suspended and revolved around the horizontal X ray tube. Further details of the RS-2400 can be found in Wagner *et al*. (2009).

Pupae of *Bactrocera* spp. reared on natural and artificial diets were kept in Petri dishes (11 × 11.2 cm) separately for X-ray irradiation treatments. The pupae were exposed to different X-ray doses 20, 40, 60, 80, 100 Gy by using an electron accelerator.

Data on partial pupal emergence, deformed emergence, normal emergence (male and female) and unemerged pupae were taken after different X-ray doses. This part of experiment replicated 5 times having 6 treatments including control, where 0 is considered as control.

Statistical analyses

Statistix® Version 8.1, Analytical Software, Inc. and Tallahassee, FL, USA were used for statistical analyses.

Two-ways ANOVA for different parameters were performed followed by Fisher's (LSD) Test to check the significance of data.

RESULTS AND DISCUSSION

In present investigation the laboratory strains of the *Bactrocera zonata* and *Bactrocera dorsalis* were reared

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on natural diet (guava and mango) and artificial diet and pupae were exposed to different X-ray doses (20, 40, 60, 80, 100 Gy) to examine the adult emergence. Results of present studies shown that ($P<0.05$) significantly more deformed adults (males and females) of *B. zonata* (27.20±1.43, 29.20±1.80, respectively) were recorded from un-irradiated pupae reared on artificial diet followed by *B. dorsalis* (22.60±0.51, 28.20±0.86, respectively). While minimum number of deformed adults (males and females) of both fruit fly species were recorded when reared on mango and provided with 100 Gy X-ray dose (Tables I and III).

Significant ($P<0.05$) more normal adult (males and females) emergence of both *Bactrocera* spp. (22.80±0.86, 21.40±0.68 and 34.60±1.33, 29.20±2.20, respectively) were observed in non-radiated pupae reared on mango. Reduced number of normal emerged adults (males and females) were recorded when maggots of both fruit fly species were provided with artificial diet and pupae irradiated with 100 Gy X-ray dose.

Furthermore, ($P<0.05$) significantly higher partial pupal emergence of *B. zonata* and *B. dorsalis* (20.60±1.21, 19.60±0.51, respectively) were recorded when larvae of fruit flies were reared on artificial diet of un-irradiated medium.

Interestingly greater number of pupae unemerged when larvae of both fruit fly species were reared on artificial diet and pupae supplied with 100 Gy dose of X-rays and lower number of un-emergence were observed in control of artificial diets (Tables II and III).

However, deformity and un-emergence in both *Bactrocera* species speckled when pupae were exposed to different x-rays doses.

In our studies, Peach fruit fly observed as more lenient towards the irradiation as compared to Oriental fruit fly because maximum number of pupae of *B. zonata* survived on the different doses of X-rays our findings are inlined with those of (Peter and John, 2004) who observed the three *Bactrocera* spp. to irradiation and found *Bactrocera cucurbitae* more tolerant to irradiation followed by

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Table I. Effects of X-ray irradiation on pupal development (Mean ± SE) of *Bactrocera zonata* reared on different diets.

Diet/Dose (Gy)	Normal adults		Deformed adults	
	Males	Females	Males	Females
Artificial diet				
0	9.60±1.44a	15.40±1.44a	27.20±1.43a	29.20±1.80a
20	9.40±1.72a	14.40±1.63a	24.80±1.69a	26.20±1.71ab
40	8.60±1.81a	14.20±1.77ab	23.80±1.74a	25.20±1.80ab
60	8.60±1.81a	14.20±1.59ab	19.00±0.71b	22.40±1.96bc
80	8.60±0.60a	14.00±1.05ab	16.40±1.08bc	19.00±2.43cd
100	8.20±2.01a	10.00±1.41b	12.80±1.16c	13.80±1.16d
Guava				
0	22.40±0.75a	33.00±1.58a	15.40±0.75a	17.20±0.86a
20	20.60±0.24abc	31.60±1.91ab	14.80±0.73a	16.80±1.11a
40	21.60±1.21ab	31.40±1.47ab	14.20±0.58a	15.60±1.33ab
60	21.00±0.45abc	31.00±1.45ab	12.40±0.24b	14.60±1.33abc
80	20.00±0.63bc	29.80±1.39ab	10.60±0.51c	13.40±1.12bc
100	19.20±0.58c	27.80±1.32b	9.20±0.58c	12.00±1.00c
Mango				
0	22.80±0.86a	34.60±1.33a	12.60±0.51a	14.00±0.55a
20	21.80±0.58a	34.00±0.45ab	12.60±0.51a	14.20±0.58a
40	20.60±0.68ab	32.00±0.45bc	11.40±0.51a	12.60±0.68a
60	19.40±0.93bc	31.40±0.51c	11.00±1.14a	12.20±1.02a
80	18.00±0.95c	28.20±0.86d	6.20±1.16b	8.00±0.71b
100	15.60±0.81d	26.80±0.97d	4.80±1.39b	7.60±0.81b

Values with the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at ($P < 0.05$).

Table II. Effects of X-ray irradiation on pupal development (Mean ± SE) of *Bactrocera dorsalis* reared on different diets.

Diet/ Dose (Gy)	Normal adults		Deformed adults	
	Males	Females	Males	Females
Artificial diet				
0	9.80±0.37a	12.00±0.45a	22.60±0.51a	28.20±0.86a
20	9.60±0.51a	11.40±0.51ab	21.00±0.32b	25.20±1.20b
40	8.60±0.40ab	10.40±0.40bc	19.00±0.55c	22.00±0.71c
60	7.80±0.66b	9.40±0.40c	16.00±0.45d	20.00±0.00cd
80	6.20±0.49c	7.60±0.68d	13.60±0.51e	18.40±0.51d
100	4.40±0.40d	5.40±0.51e	9.80±0.66f	15.20±0.20e
Guava				
0	16.00±1.34a	22.60±0.68a	19.00±0.71a	23.60±0.81a
20	15.00±0.95ab	21.20±0.49ab	18.00±0.55a	22.00±0.63ab
40	13.80±0.73ab	20.00±0.55b	16.20±0.58b	20.00±0.55bc
60	12.60±0.68b	17.20±0.58c	15.00±0.00b	18.00±1.00c
80	10.00±0.32c	13.80±0.73d	11.40±0.51c	14.80±0.49d
100	8.60±0.51c	10.40±0.75e	9.40±0.51d	10.40±0.51e
Mango				
0	21.40±0.68a	29.20±2.20a	16.00±0.71a	18.20±1.07a
20	20.20±0.58ab	28.40±1.89a	14.20±0.73ab	16.60±1.08ab
40	19.20±0.37b	27.40±1.57a	12.60±0.68bc	15.00±0.95bc
60	18.80±0.37b	26.40±1.69a	10.80±0.58c	13.00±0.95cd
80	16.80±0.66c	24.60±1.44a	9.40±0.51cd	11.60±1.03de
100	12.00±0.45d	17.80±0.86b	7.00±0.71e	9.20±0.58e

Values with the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at ($P < 0.05$).

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Bactrocera dorsalis and *Ceratitis capitata*. An irradiation dose of 100 Gy resulted in minimum survival of *B. zonata* and *B. dorsalis* pupae indicating that this dose is sufficient for the quarantine security (Couey and Chew, 1986; Robertson *et al*., 1994) also determined that lowering the X-ray dose than 150 Gy would reduce the survival of fruit flies and increase the capacity for treatment our outcomes are in slight agreement with them.

Furthermore, Alpen (1998) described laboratory strains of *B. dorsalis* and *B. cucurbitae* when reared on natural diets and pupae were irradiated found more tolerant as compared with artificial diet survival ratio on contrary we detected maximum emergence of fruit flies on different doses of X-ray when larvae reared on artificial diet. However, natural diet (fruit) would be used as rearing medium because it most closely simulates real quarantine treatment conditions (Mansour and Franz, 1996; Bustos *et al*., 2004) testified minimum adult emergence of Mediterranean fruit fly at 30-50 Gy in fruit whereas we have recorded reduced adult emergence at 60-100 Gy when maggots reared on natural diet. More tests to determine the efficacy of irradiation on wild and laboratory strains of *B. zonata* and *B. dorsalis* are needed whether their radio tolerance is comparable.

CONCLUSION

It was concluded that emergence of adults from irradiated pupae decreased with increasing X-ray dose (Gy) for both species of fruit flies. Moreover, maximum number of pupae unemerged with increased X-ray dose that might show the effect of irradiation on physiological parameters of the *Bactrocera* flies. However, increased partial pupal emergence and adult (male and female) deformity were found on all provided X-ray doses applied on pupae of both fruit fly species reared on artificial diet. That might because artificial diet do not possess the required ingredients for the sustenance of maggots in comparison to natural diet. X-ray irradiation, used to sterilize males of fruit flies for SIT program. Nonetheless we irradiated fruit flies pupae

on different doses of X-ray. It is recommended that an irradiation dose of 100 is adequate for the infant stages of fruit flies. All the *Bactrocera* flies registered as quarantine pests by many countries in the world. It is proposed from our studies that X-ray dose of 100 Gy to above is considered as quarantine treatment against *B. zonata* and *B. dorsalis*. Conclusively *B. zonata* was observed more tolerant to the X-ray irradiation than *B. dorsalis*.

Table III. Effects (Mean ± SE) of X-ray irradiation on pupal development of *Bactrocera zonata* and *Bactrocera dorsalis* reared on different diets.

Diet/ Dose (Gy)	Un-emerged pupae	Partial pupal emergence
B. zonata		
Artificial diet		
0	3.60±1.21e	20.60±1.21a
20	7.00±1.55de	19.40±0.87ab
40	10.80±0.97d	18.20±0.80ab
60	18.00±1.92c	17.00±0.63bc
80	24.60±2.68b	15.00±0.71c
100	40.20±2.87a	10.80±0.66d
Guava		
0	2.80±0.73c	10.80±0.86a
20	6.00±0.71c	10.80±0.73a
40	6.40±1.17c	8.40±0.24b
60	13.60±2.77b	8.20±0.20bc
80	19.60±2.01a	7.20±0.37bc
100	24.80±2.58a	6.80±0.37c
Mango		
0	3.60±1.21e	10.00±0.71a
20	7.00±1.55de	10.20±0.86a
40	10.80±0.97d	9.80±0.73a
60	18.00±1.92c	9.00±0.71a
80	24.60±2.68b	6.00±0.71b
100	40.20±2.87a	2.60±0.51c
B. dorsalis		
Artificial diet		
0	7.80±0.80a	19.60±0.51a
20	15.00±1.14b	17.80±0.86a
40	24.60±0.68c	15.40±0.93b
60	33.20±1.16d	13.60±0.81bc
80	41.80±1.16e	12.40±0.75c
100	57.20±1.74f	8.00±0.71d
Guava		
0	4.40±0.40a	14.40±1.03a
20	11.00±0.45b	12.80±0.66ab
40	18.60±0.51c	11.40±0.60bc
60	27.20±1.07d	10.00±0.32cd
80	41.80±0.86e	8.20±0.37d
100	55.60±1.21f	5.60±0.68e
Mango		
0	7.80±0.80a	19.60±0.51a
20	15.00±1.14b	17.80±0.86a
40	24.60±0.68c	15.40±0.93b
60	33.20±1.16d	13.60±0.81bc
80	41.80±1.16e	12.40±0.75c
100	57.20±1.74f	8.00±0.71d

Values with the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at ($P < 0.05$).

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Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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