



Gamma Irradiation effect on red flour Beetle of Stored Grain

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Abstract: Red flour beetle is a serious pest. Gamma irradiation effect on different stages of red flour beetle, their preventive doses and adult sterility were investigated. Eggs being radiosensitive 24 hours old eggs failed to hatch at 10 Gy. In 7 days old radiated larvae 100Gy showed more mortality and lower pupae formation while adult emergence prevented at 80Gy and 60 Gy caused F1 sterility in adults. The treated pupae of 7 days showed adult emergence in all treatment but 100 Gy showed minimum adults and F1 sterility in males achieved at 80 Gy while female were sterile at 80 60. In treated adults, irradiated male and irradiated female were sterile at 100Gy while irradiated male and female together showed sterility at 80 Gy. Developmental stages longevity increased as the doses increased and the growth index decreased with increment of doses. A dose of 100 Gy is recommended to control all stages in stored products.

Keywords: Red flour beetle, gamma irradiation, egg hatchability, larval development, mortality

1. INTRODUCTION

Red flour beetle *Tribolium castaneum* is a serious stored products pest which found in warm areas of the world. Insect pests causes 10-20% of postharvest losses of stored products (Phillips and Throne, 2010). In Pakistan, stored products losses range 4-10% (Huque *et al.*, 1969). Stored pests reduce quantity and quality of products (Weaver and Subramanyam, 2000). This pest can attack any dried material get from plants or animals and damage is caused by larvae and adults (Rajendran, 1990 and Rees, 2008). Commonly stored pest are controlling by fumigants or synthetic insecticides which are developing resistance in pests (Zettler and Cuperus, 1990; White, 1995; Ribeiro *et al.*, 2003 and Saeed *et al.*, 2012). Chemicals such as methyl bromide or hydrogen phosphide use on stored products have adverse effect on human and environment due to residues but safely storage of stored products is major task (Risk *et al.*, 2001 and Haq *et al.*, 2005). Methyl bromide is known as ozone deplete and many countries have banned its use (Hansen and Jensen, 2002). Due to increasing restrictions on chemicals use, investigations are required on alternative methods. One alternative method to conventional methods is the application of gamma

irradiations on pests which is technically feasible for controlling stored product pests, irradiation have no residual effect on treated commodities without any resistance in insect pests (Cornwell, 1966; Watters, 1968; Lapidot, *et al.*, 1991; Ahmed, 2001). Irradiation application is approved method on stored and dried products (Brower and Tilton, 1983). By the application of lower doses, immature stages can be prevented via lethal effect but do not cause immediate death of adults and sterilization in adults can be noticed (Brower and Tilton, 1983). Brower and Tilton (1985) recommended the use of irradiation as a phytosanitary treatment against pest control. Present study was designed to investigate the effect of gamma irradiations particularly lower doses on eggs, larvae, pupae and adult stages of *Tribolium castaneum*.

2. MATERIALS AND METHODS

Red flour beetle live adults were collected from local market and brought to plant protection laboratory at NIFA, Peshawar. The adults were colonized on wheat flour and yeast (95:5 by weight) (Muhammad *et al.*, 2013) in 1 kg volume of plastic jar and maintained at temperature 30°C ±2 and R.H 65% ±5. Different

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developmental stages of homogenous age (eggs, larvae, pupae and adult) were collected from rearing medium by sieving through 50mm mesh size and exposed to gamma irradiation source Co60 at the Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Pakistan. Eggs were given relatively lower doses 0, 10, 20, 30 and 40 Gy while larvae, pupae and adults were given 0, 40, 60, 80 and 100 Gy. All stages were replicate thrice except adults had seven replications and experimental design used was completely randomized design (CRD). Post irradiation samples were kept at temperature 30°C \pm 2 and R.H 65% \pm 5. Post irradiation parameters like development to the next stage, prolongation of life stages, any mortality after irradiation exposure and sterility in adults were studied. Data was recorded after every 2 days till the adults emergence. Sterility tests were done as virgin emerged adults from larvae and pupae reciprocally crossed with virgin opposite sex as treated male crossed with untreated female and treated female crossed with untreated males. Statistical analysis factor factorial was done on each experiment with the help of Statistix 8.1 by using Fisher's Analysis of Variance (ANOVA) technique and least significant difference (LSD) test at 5% probability level to compare the treatment means (Steel *et al.*, 1997).

3. RESULTS AND DISCUSSIONS

Effect of radiations on eggs

The data of eggs hatching after gamma radiation treatments showed significantly that control showed 94.43% eggs hatch while other treatments showed no signs of hatch.

Table 1. Red flour beetle 24 hours old eggs hatch (%) after gamma radiation treatments

Treatments(Gy)	Eggs hatch(%)
0	94.43 \pm 0.33 ^a
10	0.0 \pm 0.0 ^b
20	0.0 \pm 0.0 ^b
30	0.0 \pm 0.0 ^b
40	0.0 \pm 0.0 ^b

Where, means carrying same letters in a column not significantly different, the values were mean of three replicates \pm standard error

Effect of radiations on larvae

Effect of radiation on larvae showed in table 2 revealed significant differences in larval mortality, pupae formation and adult emergence due to gamma radiation treatments on larvae. The highest larval mortality was recorded in 100 Gy (91.1%) and the lowest mortality showed by (0 Gy) 10.0% meanwhile other mortalities were followed by 80, 60 and 40 Gy as 83.3%, 71.6% and 65.5%) respectively. The highest pupae formed from treated larvae were as 90.0% by 0

Gy) and the lowest pupae formation was noted in 100 Gy (8.90%) while other treatments chased as 40 Gy (34.5%), 60 Gy (28.9%) and 80 Gy (16.7%). The highest percentage of adult emergence from larvae recorded as 81.1% by 0 Gy and no emergence was recorded in 100 Gy and 80 Gy while 40 and 60 Gy showed 13.3 and 6.70% respectively.

Table 2: 7 days old larvae of red flour beetle mortality^a, pupae formation^b and adult emergence^c (%) after gamma irradiation treatments

Treatments (Gy)	Mortality (%)	Pupae formation (%)	Adult emergence (%)
0	10.0 \pm 0.00 ^c	90.0 \pm 0.00 ^a	81.1 \pm 0.33 ^a
40	65.5 \pm 0.67 ^d	34.5 \pm 0.67 ^b	13.3 \pm 0.58 ^b
60	71.1 \pm 0.33 ^c	28.9 \pm 0.33 ^c	6.70 \pm 0.00 ^c
80	83.3 \pm 0.00 ^b	16.7 \pm 0.00 ^d	0
100	91.1 \pm 0.33 ^a	8.90 \pm 0.33 ^e	0

Where, means carrying different letters in a column varied significantly, the values were mean of three replicates \pm standard error, larval mortality^a, pupae formation^b and adults emergence^c were statistically analyzed separately.

F₁ sterility of adults emerged from 7 days old radiated larvae

Effect of gamma irradiation on sterility of adult emerged from treated larvae in table 3 exhibited significant differences in eggs laying and hatching. F₁ adults of radiated larvae showed the highest number of eggs laid by 0 Gy (67.67) and the lowest in treatment 80 and 100 Gy (0.0) and other treatments were chased as 40 Gy (33.33) and 60 Gy (12.67) while the highest percentage of hatching was also recorded in 0 Gy (87.05%) and the lowest was (0.0%) in 60 Gy while 40 Gy showed (42.91%) and 100 and 80 Gy did not laid any eggs.

Table 3:F₁ sterility of adults emerged from 7 days old radiated larvae

Treatments (Gy)	Radiated larvae	
	F ₁ adults eggs	
	Laid (n)	Hatched (%)
0	67.67 \pm 1.45 ^a	87.05 \pm 3.46 ^a
40	33.33 \pm 1.86 ^b	42.91 \pm 1.76 ^b
60	12.67 \pm 1.20 ^c	0.0 ^c
80	0.0 ^d	0.0 ^c
100	0.0 ^d	0.0 ^c

Where, means carrying different letters in a column varied significantly, the values were mean of three replicates \pm standard error, n is denoted by number

Table 4: **7 days old larvae of red flour beetle mortality^a, pupae formation^b and adult emergence^c (%) after gamma irradiation treatments**

Treatments (Gy)	Mortality (%)	Adult emergence (%)
0	10.0±0.00 ^d	90.0±0.58 ^a
40	26.7±0.58 ^c	38.9±0.33 ^b
60	26.7±0.58 ^c	34.4±0.33 ^c
80	38.9±0.33 ^b	21.1±0.33 ^d
100	48.9±0.33 ^a	17.8±0.33 ^d

Where, means carrying different letters in a column varied significantly, the values were mean of three replicates ± standard error, pupal mortality^a and adults emergence^b were statistically analyzed separately

Effect of radiations on pupae

Effect of radiations on pupae in table 4 revealed significant differences in pupae mortality and adult emergence. The highest pupae mortality was recorded by 100 Gy (48.9%) and the lowest was noted by 0 Gy as 10.0% while other treatments chased as 80 Gy (38.9%) however 40 and 60 Gy shared common value (26.67%). In adults emergence, the highest shown by 0 Gy (90.0%) and the lowest adults were noted by

100 Gy as 17.8% while other treatments shadowed as 40 Gy (38.9%), 60 Gy (34.4%) and 80 Gy (21.1%).

F₁ sterility of adults emerged from 7 days old radiated pupae

F₁ adults of treated pupae exhibited in table 5 that F₁ males, the highest numbers of eggs laying was recorded in 0 Gy (69.33) and the lowest numbers were showed (0.0) by 100 Gy while other treatments were shadowed as 32.33, 15.67 and 6.67 by 40, 60 and 80 Gy respectively. In the percentage of eggs hatching from F₁ male of radiated pupae showed maximum hatching of 86.63% by 0 Gy and lower values given (0.0%) by 80 Gy and other values were 66.20%, 44.27% by 40 and 60 Gy respectively. Other values in F₁ females were shown as, the highest numbers of eggs laying was noted in 0 Gy (69.33) and lowest were in 100 and 80 Gy (0.0) while other values were as follows 27.00 and 10.0 by 40 and 60 Gy respectively. Similarly, in eggs hatching values, the highest was in 0 Gy (86.63%) and no emergence was noticed in 60, 80 and 100 Gy while 40 Gy (38.30%).

Table 5: **F₁ sterility of adults emerged from 7 days old radiated pupae**

Treatments (Gy)	Radiated pupae			
	F ₁ male adults eggs		F ₁ female adults eggs	
	Laid (n)	Hatched (%)	Laid (n)	Hatched (%)
0	69.33±0.88 ^a	86.63±2.08 ^a	69.33±0.88 ^a	86.63±2.08 ^a
40	32.33±1.45 ^b	66.20±0.88 ^b	27.00±1.53 ^b	38.30±0.88 ^b
60	15.67±0.88 ^c	44.27±0.33 ^c	10.0±0.58 ^c	0.0 ^c
80	6.67±0.33 ^d	0.0 ^d	0.0 ^d	0.0 ^c
100	0.0 ^e	0.0 ^d	0.0 ^d	0.0 ^c

Effect of gamma radiations on longevity of developmental stages

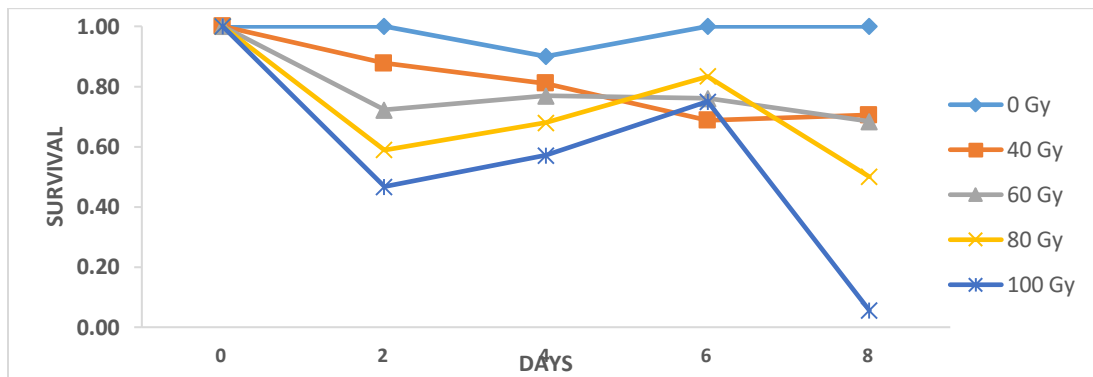
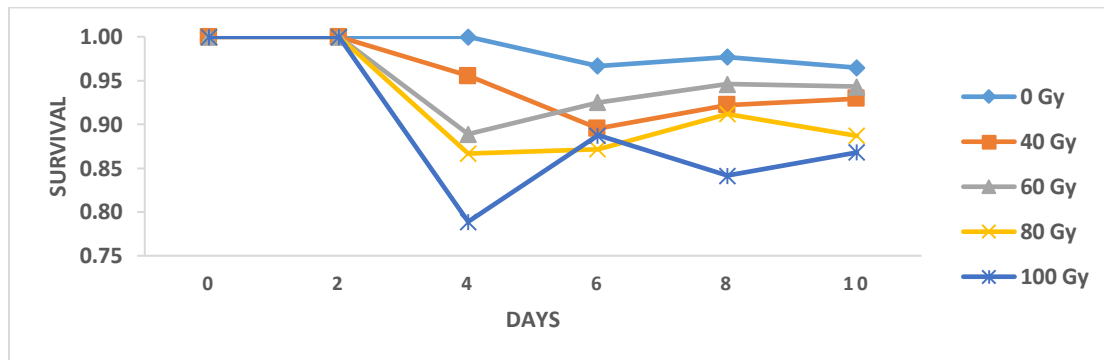
The data in (Table 6, Fig. 1 and 2) showed that gamma radiation treatments caused significant delay in developmental periods from treated larvae to adult and treated pupae to adult emergence. Developmental period increased by dose range increment resulted the highest days period taken from treated larvae to adult by 60 Gy (25.0) and the lowest by 0 Gy (18.3) while 40 Gy (23.0). Meanwhile, growth index was decreased by increasing dose range as the highest growth index showed by 0 Gy (4.43) and the lowest by 60 Gy (0.30) while 40 Gy as (0.57). Similarly, developmental days

period taken from treated pupae to adult, the highest as 17.3 by 100 Gy and the lowest as 3.33 by 0 Gy while other treatments followed as 13.3 (80 Gy), 8.00 (60 Gy) and 7.67 (40 Gy). Growth index from treated pupae to adult unveiled the highest by 0 Gy (29.7) and the lowest by 100 Gy (1.00) and others treatments shadowed as 40 Gy (5.43), 60 Gy (4.33) and 80 Gy (1.60). Longevity and survival from treated larvae and treated pupae showed in (Fig 1 and 2) where 100 Gy showed more mortality and less survival while 0 Gy showed less mortality and more survival of radiated pupae and radiated larvae were unable to convert into adults when treated by 80 and 100 Gy.

Table 6: Red flour beetle adult developmental period after gamma irradiation treatment to larvae and pupae^a

Treatments (Gy)	Developmental DAT to adults		Index ^b	
	Treated larvae	Treated pupae	Treated larvae	Treated pupae
0	18.3±0.67 ^c	3.33±0.67 ^d	4.43±0.07 ^a	29.7±6.81 ^a
40	23.0±0.00 ^b	7.67±0.33 ^c	0.57±0.08 ^b	5.43±0.65 ^b
60	25.0±0.00 ^a	8.00±0.00 ^c	0.30±0.01 ^b	4.33±0.14 ^b
80	-	13.3±0.67 ^b	-	1.60±0.08 ^c
100	-	17.3±0.67 ^a	-	1.00±0.05 ^c

Where, means carrying different column varied significantly, the values were mean of three replicates ± standard error, treated larvae and adults emergence^a, percent adults emerged/total developmental period (growth index^b) were statistically analyzed separately

**Fig. 1: K. M survival days graph of red flour beetle 7 days old larvae after gamma radiation treatment (DAT)****Fig. 2: K. M survival days graph of red flour beetle 7 days old pupae after gamma radiation treatment**

Effect of radiations on adults

Gamma irradiation effect on adults in table 7 showed significant differences in eggs laying (n) and hatching (%) of radiated male, female and male + female. Radiated adults (males) uncovered that the highest eggs laid by 0 Gy (60.43) and the lowest in 100 Gy (0.0) while other treatments showed 40 Gy (34.14), 60 Gy (21.71) and 80 Gy (9.14) and the highest hatching recorded in 0 Gy (92.67) and lowest in 80 Gy (34.33) and other values chased as 40 Gy (62.14) and 60 Gy (50.30).

However, radiated female adults unveiled the highest eggs laid in 0 Gy (60.43) and the lowest (0.0) by

100 Gy shadowed by 40 (27.71), 60 (16.71) and 80 Gy (8.43) respectively. While, hatched eggs, maximum showed by 0 Gy (92.67%) minimum value was (13.10%) by 80 Gy and other values were followed 61.63% and 44.63% by 40 and 60 Gy respectively.

In radiated male and female adults the highest eggs were laid by 0 Gy (60.43) and the lowest by 80 and 100 Gy (0.0) and other values were (22.14) 40 Gy and (9.00) 60 Gy. However, 0 Gy (92.67%) showed the highest eggs hatching and 60 Gy (30.99%) revealed the lowest values and 40 (60.96%) while 100 and 80 Gy were unable to lay eggs after radiation treatments.

Table 7: Red flour beetle eggs laid (n) and hatched (%) from virgin adults reciprocal crosses after gamma radiation treatments

Treatments (Gy)	Male irradiated (eggs)		Female irradiated (eggs)		Male and female irradiated (eggs)	
	Laid (n)	Hatched (%)	Laid (n)	Hatched (%)	Laid (n)	Hatched (%)
0	60.43±1.11 ^a	92.67±1.70 ^a	60.43±1.11 ^a	92.67±1.70 ^a	60.43±1.11 ^a	92.67±1.70 ^a
40	34.14±0.74 ^b	62.14±0.42 ^b	27.71±1.11 ^b	61.63±0.51 ^b	22.14±1.40 ^b	60.96±0.49 ^b
60	21.71±0.71 ^c	50.30±0.57 ^b	16.71±0.61 ^c	44.63±0.26 ^b	9.00±0.31 ^c	30.99±0.00 ^c
80	9.14±0.51 ^d	34.33±0.00 ^c	8.43±0.48 ^d	13.10±0.0 ^c	0.0 ^d	0.0 ^d
100	0.0 ^e	0.0 ^d	0.0 ^e	0.0 ^d	0.0 ^d	0.0 ^d

Where, means carrying different letters in a column varied significantly, the values were mean of three replicates (±SE), n is denoted by number

4. **DISCUSSION**

Our current results indicated that radiation treatments on red flour beetle (*Tribolium castaneum*) for management at different stages level control effectively. Eggs were very sensitive to radiations as they showed no emergence even at lower dose of 10 Gy the possible reason may be the younger age of eggs at 24 hours were less resistant and 100 Gy was effective dose for mortality of larvae, pupae formation and adults emergence when larvae was treated and prolongation was also noted in larval stage and adults emergence was less compared with normal conditions. Similarly pupa was treated and pupa showed some resistance and mortality percentage was lesser compared with larvae and a delay in pupal stage to adults was noticed as in control it was on time according to temperature and 100 Gy was on top among all treatments. Moreover, adults were also treated with gamma radiations to check any sterility induced by radiations among adults and different doses showed different eggs laying and hatching ability even in radiated male, female alone or male and female jointly. Males were on little resistant side while female and pair of both showed infertility of eggs, another finding was the ability of eggs laying reduced with gamma radiation applications as the doses rates were increased eggs laying reduced also, more was the hatchability reduced and 100 Gy was sufficient for male and female reduced hatchability to zero while both male and female pair jointly reduced at 80 Gy.

In F₁ adults emerged from radiated larvae and pupae had very lower ability to lay eggs and most of them were infertile for hatching. For that 60 Gy showed good results in larvae to adults while from pupae male showed infertility at 80 Gy and female were at 60 Gy as well. These immediate results are in agreement with (Abbas and Nouraddin 2011) finding where they found that at 100 Gy was sufficient for 15 days red flour beetle larvae to complete their cycle to adults and here in our case 5 days old larvae was unable to produce adults even at 80 Gy, eggs were not hatched at 10 Gy while

they tested till 150 and larvae were not emerged which agreed with their result. They recommended that 700 Gy was sufficient to control red flour beetle effectively but here in recent results 100 Gy was best to produce sterility at all stages but mortality was less which is agreed with above workers.

Another group of scientists Zolfaghari et al. (2004) resulted 200 Gy was effective to prevent eggs hatchability of *T. castaneum* but in contrast in current findings 10 Gy was enough to prevent eggs hatching but an agreement can made with ability of radiations to prevent eggs hatchability. Recent results are in agreement with (Tuncbilek and Kansu 1996) where they resulted that 40 and 50 Gy gamma radiation on eggs and larvae were enough to develop adults which showed the effectiveness of gamma radiation for *Tribolium castaneum* managements. The present and recent results regarding eggs difference by possible reasons that age of eggs at the time of radiation applications. Sensitivity to radiation varies with embryonic stage. *Tribolium castaneum* mature eggs showed resistance compared with newly eggs and development to adults prevented at 80 Gy for 2 days eggs but not enough to eggs of more age required 160 Gy (Guchangco, 2002) which can make an agreement with low doses application for eggs to stop hatching.

Another group of investigators Azelmat et al. (2005) tested 30-500 Gy on eggs of *Sitophilus granaries* to prevent adult emergence which is in agreement with recent findings where 10 Gy was enough to prevent eggs hatchability. Immediate results are consent with Tuncbilek and Kansu (1996) where they reported that *Tribolium spp.* adults and larvae completely suppressed by 100 and 50 Gy respectively here in present case 80 to 100 Gy stood best. The low doses results in recent findings are agreed with (Mehta et al. 1990) they found low dose of 6 Krad was enough to inhibit *T. castaneum* 15 days old larvae to adults emergence and 2-4 Krad delayed larval period to pupate. A delay period

in larval and pupae stage development due to increasing radiation doses were noticed in recent studies which are agreed with Hu *et al.* (1985) finding where also reported that 41 Gy killed *Tribolium* spp. adults 100% and prolonged development period.

Another accord of immediate research on prolongation of development stages is made with Mehta *et al.* (1990) where he suggested that *Tribolium castaneum* larvae and eggs stage delayed to develop to next stage. The results of recent studies regarding pupae treatment, mortality was less in all doses ranges but increased with doses range increases and prolongation was noticed but showed resistance and adults were emerged successfully which made an agreement with (Gochangco *et al.* 2002) who told the pupae of *Tribolium castaneum* showed more resistance to gamma radiations and suggested 160 Gy to suppress this stage. Current findings on adults were that mortality was not noticed but eggs laying and hatchability was decreased to zero in 100 Gy which made an agreement with (Tuncbilek *et al.* 2003) who suggested that longevity in adults was reduced at 100 Gy and above which showed that 80-100 Gy would be sufficient to produce infertility in eggs. Results on sterility of larvae, pupae adults and adult alone direct application showed that 60-80 Gy was sufficient to induce sterility in *Tribolium castaneum* all stages which made an agreement with (Shishir *et al.* 2009) who reported that 70 Gy is sufficient to produce sterile adults of *Tribolium castaneum*. These results showed that lower doses are enough to produce sterility effectively in insect pests of stored pests in place of direct mortality by radiation application.

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