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RESEARCH PAPER

Sensitivity of developmental stages of pulse beetle *Callosobruchus maculatus* (F.) to gamma rays

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Abstract: Sensitivity studies on effects of gamma radiation on different life stages of *Callasobruchus maculatus* (F.) were carried out in a growth chamber at temperature of 30ºC and relative humidity of 70 per cent. Two days old eggs, grubs (four and seventeen days old) and three days old pupae along with green gram seeds were exposed to gamma radiation in range of 0-50 Gy, 0-60 Gy, 0-100 Gy and 0-100 Gy, respectively. The radiation doses like 15 Gy, 40 Gy, 100 Gy and 100 Gy were effectively prevented adult formation from treated pre-imaginal stages mentioned above. For adult beetles response was assessed by selecting five pairs of four days old adult male and females along with 30 g greengram seeds kept as a food and were exposed to gamma radiation in range of 0-140 Gy. The fecundity of adults decreased with the increase of irradiation dose. There was 54.02, 61.59, 69.07, 87.80, 94.36 and 97.45 per cent reduction in egg laying in adults exposed to 20, 40, 60, 80, 100 and 120 Gy, respectively. The adults exposed to a radiation dose of 80 Gy and above were capable of egg laying but such eggs were not developed to form adult . Therefore, from this study we concluded that all stages of *C. maculatus* can be killed at 120 to 150 Gy expose dosage of gamma radiation.

Key words: *Callosobruchus maculatus,* Gamma radiation, Greengram

Introduction

India is leading in the production and consumption of pulses in the world. The pulses are being playing a vital role in the nutritional security, improvement in soil health and reduction in greenhouse gas emission. Over 200 species of insects have been recorded infesting various pulses (CAB International, 2007). Of many insect pests, the pulses are badly damaged by the pulse beetles of the genus *Callosobruchus* (Coleoptera: Chrysomilidae) during storage throughout the world and this discourages the poor farmers from large-scale production and storage of pulses.

Greengram (*Vigna radiata*) is an excellent source of high-quality protein providing vegetarian nutritional security and being extensively grown leguminous crop of India. Despite the significance of greengram in providing nutritional security, it is one of the crops that faces many constraints, including post-harvest losses. Post-harvest damage by insects is an important constraint in pulse production (Khaire *et al*., 1992) including greengram. Legume grains during pre and post harvest are infested by pulse beetles. Among the pulse beetles, *Callosobruchus maculatus* (F.) and *C. chinensis* (L.) are the most common and economically important species that attack stored pulses throughout the world and causes 5 to10 per cent loss during storage. Seed weight and protein content losses of about 55-60 per cent and 45-66 per cent, respectively found in the pulses (Gujar and Yadav, 1978).

It is an internal feeder larva is a damaging stage that consumes several times more food (endosperm) than its own body weight, leading to moderate to heavy infestations as the insects pass a major portion of their life-cycles inside the seeds (Quraishi and Metin, 1963; Bousquet, 1990; Ramzan *et al*., 1990). The insect spends its entire immature life in individual legumes seeds, where they cause weight loss, decrease germination potential and diminish the trade as well as nutritional value of the commodity (Ali *et al*., 2004). Bruchid infestation begins in the field eggs glued maturing pods by females and such stored grains in storage may be almost completely hollowed out by feeding activities of the larvae, and characteristic emergence holes are evident after the adults leave the seeds (Giga and Smith, 1983). Due to the economic importance and wide spread distribution of these pulse beetles, there is a dire need to develop suitable and effective control measures against these stored product pest species.

Radiation technology is an effective disinfestation treatment for killing stored-product insect pests. Ionizing radiation (Gamma radiation) has been suggested as a useful alternative to methyl bromide, as there is no development of insect resistance, an absence of residue in treated food and no significant loss of nutrient in commodities (Lapidot *et al.,* 1991). The information on efficacy of gamma irradiation on stored insect pests especially protection from pulse beetle in India is scanty. Considering the importance, the present study investigates "Sensitivity of developmental stages of pulse beetle *C. maculatus* to gamma rays".

Material and methods

The present investigation on Sensitivity of developmental stages of pulse beetle *C. maculatus* to gamma rays was carried out during 2021-22 at Zonal Agricultural Research Station, Kalaburagi, University of Agricultural Sciences, Raichur.

Rearing of the test insect

Pulse beetle *C. maculatus* culture was collected from ZARS, Kalaburagi store and reared on greengram seeds of variety 'BGS-9' in plastic boxes covered with muslin cloth by using the technique developed by Cred land and Wright (1989). Cleaned, washed and dried (Moisture level >12%) and irradiated green gram seeds were used for culturing of pulse beetle. Seeds were dried by exposing in hot air dryer at 60ºC for 1 hour.

Adults collected from stock culture were separated male and female based on morphological characters and 25 male and 25 female adults were introduced into plastic container contain 500g of green gram seeds and covered with muslin cloth kept at room temperature of 28°C to 33°C and relative humidity of 68 to75 per cent in the stored grain laboratory. After 35-40 days adults started emerging from the culture and were utilized for study and maintenance of sub cultures. The stock culture was cleaned at timely interval to prevent the parasitoid infestation and fresh uninfected grains were provided to prevent microbial contamination. To obtain virgin adults of the same age, seeds with eggs were kept in small glass vials until adult emergence in growth chamber having 30°C and 70 per cent relative humidity.

Effect of gamma radiation on mortality of various pre-imaginal stages of *C. Maculatus.*

Irradiation of egg

About 20 seeds attached with single egg (12 to 24 hr aged) were exposed to gamma radiation in Gamma chamber -5000 having a radioactive source of Cobalt-60 with strength of 5000 Ci and dose rate of 4.58 kGy/hr with different dosages *viz*., 0 Gy,7 Gy,10 Gy,15 Gy,20 Gy and 50 Gy. After irradiation the plastic container containing irradiated eggs along with seeds were kept in growth chamber and maintained at a temperature of 30ºC and relative humidity of 70 per cent. Three replications were maintained for each dose level. Observation on adult emergence was recorded.

Irradiation of larva

The grub period was observed to be eighteen to twenty-two days, hence early instar (4th day old) grub and last instar grub (17th day old) were selected for their relative sensitivity studies. Later they were exposed separately to different dosages of gamma radiation *viz*., 0 Gy, 7 Gy, 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy and 0 Gy, 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy, 70 Gy and 100 Gy, respectively and compared with untreated control treatment. Three replications were maintained for each dose level. Observations on adult emergence was recorded.

Irradiation of pupa

The greengram seeds having late aged pupae (three-day old pupa) were exposed to different doses of radioactive Cobalt-60 material *viz*., 0 Gy, 10 Gy, 20 Gy, 30Gy, 40Gy, 50Gy, 60Gy, 70Gy and 100Gy as explained above and were compared with control treatment. Three replications were maintained for each dose level. Observations on adult emergence was recorded.

Irradiation of adults

To obtain virgin adults of the same age, seeds with eggs were kept in small plastic vials until adult emergence in growth chamber having 30ºC and 70 per cent RH. Later, five pairs of four-day old adult male and females were released in each plastic container containing 30 g of greengram seeds and they were exposed to gamma radiation at different dosages *viz*., 0Gy, 20Gy, 40Gy, 60Gy, 80Gy, 100Gy, 120Gy and 140Gy. Observations were also recorded on number of eggs laid and number of adults emerged per 30g seeds.

Statistical analysis

The percentage of mortality for each expose dosage of radiation along with the control were computed and corrected percent mortality was worked out by Abbot's formula (Abbot 1925). Further data was subjected to ANOVA.

Results and discussion

Effect of gamma radiation on mortality of various pre-imaginal stages of *C. maculatus*

Effect of gamma radiation on eggs

The number of adults that emerged from irradiated eggs was significantly affected by the gamma radiation doses. No adults emerged when eggs were irradiated at 15 Gy and above (20 Gy and 50 Gy). All eggs taken for study were developed and emerged as adults under zero gray irradiation which was followed by 4.75 and 2.75 adult emergence per 20 seeds documented when eggs were irradiated at 7 Gy and 10 Gy, respectively (Table 1). Further, it was calculated as per cent adult emergence and found that 23.75 and 13.75 per cent at 7 Gy and 10 Gy, respectively.

The per cent reduction in egg laying was recorded when two days old eggs were exposed to gamma radiation ranged from 0-50 Gy (Figure 1). Cent per cent reduction in adult emergence was recorded when eggs were exposed to 15 Gy and above. The per cent reduction in adult emergence of 76.25 and 86.25 was recorded at 7 Gy and 10 Gy of exposure.

Effect of gamma radiation on first instar grubs

When the four day old larvae were irradiated, the highest number of adults emergence was 18.66 found in unirradiated treatment (0 Gy) which was followed by 18.33, 16.66, 15.66 and 14.33 adult emergence per 20 seeds documented when four days old grubs were irradiated at 7 Gy, 10 Gy, 20 Gy and 30 Gy, respectively and zero Gy was at par with 7 Gy. 10, 20 and 30 Gy were differed significantly with each other and also superior to zero and 7 Gy. First instar grubs treated at 40 Gy and above dose recorded zero adult emergence. Further, it was calculated as per cent adult emergence and found that the highest per

Table 1. Effect of gamma radiation on eggs of *Callosobruchus maculatus*

Gamma rays exposed dose (Gy)	Irradiated egg stage	
	Number of adults emerged/ 20 seeds	Per cent adult emergence
0	20.00(4.58)^{d}	100.00(90.00)^{d}
7	4.75(2.40)^{c}	23.75(29.14)^{c}
10	2.75(1.93)^{b}	13.75(21.70)^{b}
15	0.00(1.00)^{a}	0.00(0.00)^{a}
20	0.00(1.00)^{a}	0.00(0.00)^{a}
50	0.00(1.00)^{a}	0.00(0.00)^{a}
S.Em ±	0.04	0.58
C.D @ 1%	0.15	2.43
C.V (%)	3.63	4.97

Values in parenthesis in column 2 and 4 are $\sqrt{x+1}$, values in parenthesis in column 3 and 5 are arc sine transformed.

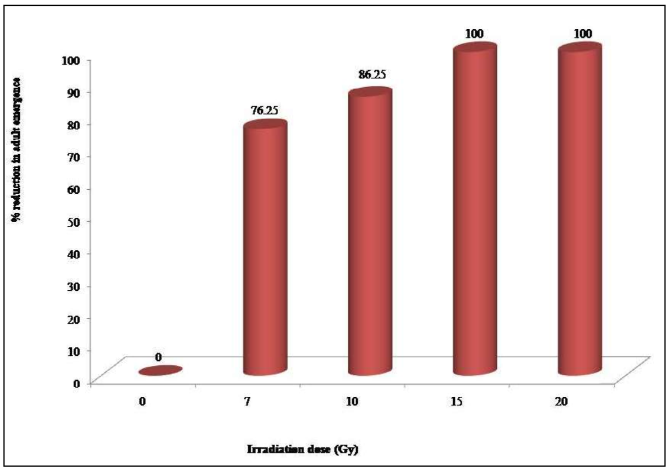

Fig. 01. Effect of gamma radiation on per cent reduction in adult emergence when two days old eggs irradiated

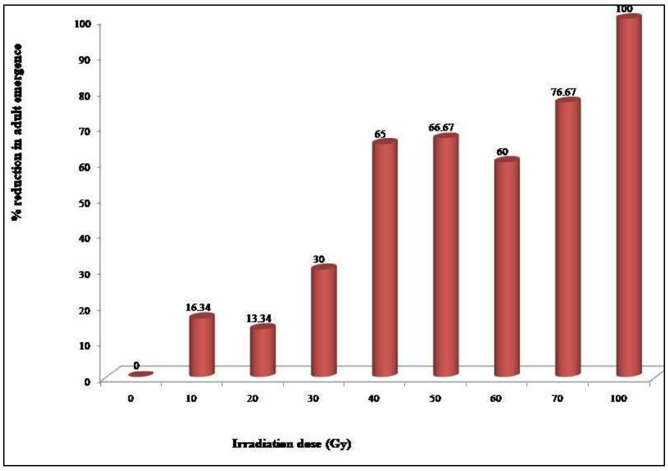

Fig. 2. Effect of gamma radiation on per cent reduction in adult emergence when threedays old pupae irradiated

cent adult emergence was 93.33 per cent found in unirradiated treatment which was followed by 7 Gy, 10 Gy, 20 Gy and 30 Gy recorded 91.66, 83.33, 78.33 and 70.00 per cent adult emergence, respectively. The most effective dose for L_1 grub stage was 40 Gy and above which recorded 100 per cent mortality of developmental stage (Table 2).

Table 2. Effect of gamma radiation on I instar grubs of *Callosobruchus maculatus*

Gamma rays exposed dose (Gy)	Irradiated larval stage (L_1)	
	Number of adults emerged/ 20 seeds	Per cent adult emergence
0	18.66(4.43)[e]	93.33(75.24)[d]
7	18.33(4.40)[e]	91.66(73.40)[d]
10	16.66(4.20)[d]	83.33(65.95)[c]
20	15.66(4.08)[c]	78.33(62.29)[c]
30	14.33(3.87)[b]	70.00(56.78)[b]
40	0.00(1.00)[a]	0.00(0.00)[a]
50	0.00(1.00)[a]	0.00(0.00)[a]
60	0.00(1.00)[a]	0.00(0.00)[a]
S.Em. ±	0.03	1.08
C.D @ 1%	0.11	4.53
C.V (%)	1.60	4.47

Values in parenthesis in column 2 and 4 are √ x+1, values in parenthesis in column 3 and 5 are arc sine transformed.

Effect of gamma radiation on last instar grubs

All last instar grubs taken for study were developed and emerged as adults and were significantly highest (20.00) under zero gray radiation which was at par with (10 Gy) 18.33 and (20 Gy) 17.33. Further the effect of gamma radiation was gradually increased with the doses and found 13.00, 9.00, 8.33, 6.33 and 4.33 adult emergence per 20 seeds when last instar grubs were irradiated at 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy and 70 Gy respectively. Further, it was calculated as per cent adult emergence and found that 1.70 per cent adult emergence was recorded at 100 Gy exposure which was significantly lowest among treatments. Other studied dosages recorded 90.00, 86.66, 65, 41.66, 45, 31.66 and 21.66 per cent adult emergence at 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy and 70 Gy, respectively (Table 3) and it shows that adult emergence was universally proportional to radiation doses.

Effect of gamma radiation on pupae

The number of adults that emerged from irradiated pupae was significantly affected by the gamma radiation doses. Zero adult emergence was found when three days old pupae were exposed to a dose of 100 Gy (Table 3). All the pupae taken for study developed and emerged as adults under zero gray

Table 3. Effect of gamma radiation on last larval (L_4) and pupal stage of *Callosobruchus maculatus*

Gamma rays exposed dose (Gy)	Irradiated larval stage (L_4)		Irradiated pupal stage	
	Number of adults emerged/ 20 seeds	Per cent adult emergence	Number of adults emerged/ 20 seeds	Percent adult emergence
0	20.00(4.58)[f]	100.00(90.00)[h]	20.00(4.58)[e]	100.00(90.00)[f]
10	18.33(4.40)[f]	90.00(71.57)[g]	16.66(4.20)[e]	83.66(65.95)[e]
20	17.33(4.28)[f]	86.66(68.86)[f]	17.33(4.28)[e]	86.66(68.66)[e]
30	13.00(3.74)[e]	65.00(53.76)[e]	14.00(3.87)[d]	70.00(56.84)[d]
40	9.00(3.16)[d]	45.00(42.13)[d]	7.00(2.82)[c]	35.00(36.24)[c]
50	8.33(3.05)[d]	41.66(40.20)[d]	6.66(2.77)[c]	33.33(35.25)[c]
60	6.33(2.71)[c]	31.66(34.23)[c]	5.90(14.05)[c]	29.50(32.89)[c]
70	4.33(2.31)[b]	21.66(27.71)[b]	4.66(2.38)[b]	23.33(28.86)[b]
100	0.34(1.16)[a]	1.70(7.49)[a]	0.00(1.00)[a]	0.00(0.00)[a]
S.Em. ±	0.05	1.14	0.05	1.17
C.D @ 1%	0.21	4.70	0.24	4.83
C.V (%)	2.35	4.02	2.79	4.33

Values in parenthesis in column 2 and 4 are √ x+1, values in parenthesis in column 3 and 5 are arc sine transformed.

irradiation with 17.33, 16.66, 14.00, 7.00, 6.66, 5.90 and 4.66 adult emergence per 20 seeds documented when three days old pupae were irradiated at 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy, and 70 Gy, respectively. Further, it was calculated as per cent adult emergence and found that 86.66, 83.66, 70, 35, 33.33, 29.50 and 23.33 per cent adult emergence was documented at 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy and 70 Gy, respectively. Cent per cent adult emergence inhibition was noticed at 100 Gy exposure.

The per cent reduction in adult emergence when three days old pupae were exposed to gamma radiation doses of 0-100 Gy (Figure 2). The cent per cent reduction in adult emergence was recorded when three days old pupae were exposed to 100 Gy and significantly lowest per cent reduction in adult emergence (0 %) was recorded in control. The per cent reduction in adult emergence of 16.34, 13.34, 30.00, 65.00, 66.67, 60.00 and 76.67 when three days old pupae were exposed to 10, 20, 30, 40, 50, 60 and 70 Gy, respectively.

Effect of gamma radiation on fecundity of *C. maculatus* adults

This study was carried out by exposing five pairs of one day old adults along with 30 g greengram seeds and observed for fecundity by counting number of eggs laid and further number of adults emerged from seeds with eggs were counted and presented in Table 4. The number of eggs laid by five pairs of adults was significantly affected by gamma radiation doses. The lowest number of egg count of 11.33 was recorded at 140 Gy treatment and the highest number of egg count was found at unirradiated treatment. The number of eggs recorded in other treatments 20 Gy, 40 Gy, 60 Gy, 80 Gy, 100 Gy and 120 Gy were 205.00, 171.33, 138.00, 54.33, 35.66 and 25.00, respectively. Further, per cent reduction in egg laying was calculated and found that 97.45, 94.39, 92.01, 87.80, 69.07, 61.59 and 54.02 per cent reduction in eggs laying from 140, 120, 100, 80, 60, 40 and 20 Gy, respectively.

Effect of gamma radiation on F1 population

The number of F1 adult emergence was significantly affected by gamma radiation doses (Table 4). The number of adults emergence was highest in unirradiated treatment (444.66) whereas it was nil for treatment 80 Gy and above. Adult emergence was 86.00, 12.00 and 6.33 per cent when irradiated at 20 Gy, 40 Gy and 60 Gy, respectively. The per cent reduction in F1 adult emergence from irradiated parental adults was significantly affected by the gamma radiation doses. The 100 per cent reduction in F1 adult emergence was recorded at 80 Gy and above (100 Gy, 120 Gy and 140 Gy) and 0 per cent in unirradiated treatment. The per cent reduction in F1 adult emergence recorded was 80.65, 97.30 and 98.57 per cent at 20 Gy, 40 Gy and 60 Gy, respectively.

The results show that increasing radiation doses caused reduced egg viability, adult emergence when 12hr to 24hrs aged eggs were exposed and found 100% inhibition of adult emergence at ≥15 Gy. Similar studies were conducted by Sutanta wong (1991) who reported that the *C.maculatus* eggs irradiated at 40 Gy were unable to form adults and also Soumya *et al.*(2017) reported on *C. chinensis* that no eggs were hatched after irradiated with 50 Gy. This difference between the present results and those from previous reports might be due to the differences in egg age at the time of irradiation. Radio sensitivity varies with the stage of embryologic development. The eggs of *P. interpunctella* are quite radiosensitive for approximately half of their development time, and become 25 times more radio resistant at 72 h than at 2 h post-oviposition (Brower 1974). Zero adult emergence from irradiated 12 hrs aged eggs of *C. maculatus* at 10 Gy was reported by Seth *et al.* (2020) and similarly Diop *et al*. (1997) found at 20 Gy which are in accordance with the present findings.

In the present studies 40 Gy and 100 Gy exposed dose were sufficient to bring cent per cent mortality in L1 (4 days aged) and L4 (17 days aged) respectively. The present study suggested that the degree of radio-susceptibility decreased as the ontogenic stages grew older. Hussain and Imura (1989) reported the similar pattern of age-dependency of radio-sensitivity during the developmental stages. Seth *et al.* (2020) reported that no adults were emerged when L1 larval stage and L4 larval stage of *C. maculatus*, *C. chinensis* and *C. analis*were irradiated at 50 Gy and 100 Gy respectively. Ashraf *et al.* (2021) reported that 7 days old grubs and 10 days old grubs of *C. maculatus* showed 85% and 70% mortality when exposed at 24 Gy and 150 Gy, respectively and a dose of 150 Gy caused 86.1%

Table 4. Effect of gamma radiation on adult stage of *Callosobruchus maculatus*

Gamma rays exposed dose (Gy)	Fecundity⁺ (per five pairs)	Per cent reduction in fecundity	Formation of F1: Number of adults emerged/ 30 g seeds	Formation of F1: Percent reduction in adult emergence
T1: 0 Gy	446.33(21.15)[g]	0.00(0.00)[h]	444.66(21.11)[e]	0.00(0.00)[e]
T2: 20 Gy	205.00(14.35)[f]	54.02(47.31)[g]	86.00(9.31)[d]	80.65(63.91)[d]
T3: 40 Gy	171.33(13.13)[e]	61.59(51.71)[f]	12.00(3.60)[c]	97.30(80.54)[c]
T4: 60 Gy	138.00(11.79)[d]	69.07(56.21)[e]	6.33(2.70)[b]	98.57(83.18)[b]
T5: 80 Gy	54.33(7.43)[c]	87.80(69.59)[d]	0.00(1.00)[a]	100.00(90.00)[a]
T6: 100 Gy	35.66(6.05)[c]	92.01(73.60)[c]	0.00(1.00)[a]	100.00(90.00)[a]
T7: 120 Gy	25.00(5.10)[b]	94.39(76.31)[b]	0.00(1.00)[a]	100.00(90.00)[a]
T8: 140 Gy	11.33(3.51)[a]	97.45(80.83)[a]	0.00(1.00)[a]	100.00(90.00)[a]
S.Em ±	0.16	0.50	0.10	0.18
C. D @ 1%	0.66	2.10	0.42	0.73
C. V (%)	2.65	1.52	3.71	0.41

Values in parenthes is in column 2 and 4 are √ x+1 transformed, values in parenthesis in column 3 and 5 are arc sine transformed.

and 100% inhibition in adult emergence from treated pupae of *C. chinensis* and *C. analis* respectively.

Diop *et al.* (1997) who reported that the pupal development was arrested at 150Gy and with Bhuiya *et al.* (1985) who reported that 100% pupal mortality of *C. chinensis* were achieved at higher dose of 800Gy. Supawan *et al.* (2005) reported that there were no adults formed when 10 days old larvae of *C. chinensis* were exposed to 600Gy

The effective gamma doses causing the metamorphic disruption for the most radioresistant preimaginal stage (pupal stage), as a more crucial parameter. Cent per cent mortality at 100 Gy irradiation of *C. maculatus* pupae (3 days old) was found in the present study. However only 79.4% inhibition in adult emergence when pupal stage was irradiated at 100 Gy (Seth *et al.* 2020) as they studied and reported combindly for all the three species and C. *Chinensis and C. analis* were competitively radio resistant than the *C. maculatus* might be the cause for variation. Effect of gamma radiation on fecundity of C. maculatus adults was studied and found that 97.45, 94.39, 92.01, 87.80, 69.07, 61.59 and 54.02 per cent reduction in eggs laying from 140, 120, 100, 80, 60, 40 and 20 Gy, exposure respectively. Prevention of F1 generation adults formation after giving radiation phytosanitary treatment for most stored grain pest *i.e.* prevention of oviposition by irradiated females (Hallman, 2016). Based on these studies a generic PI dose for Curculionidae of 150 Gy was suggested by Heather and Hallman (2008) as a posible approach. Similarly Seth *et al.* (2020) also found that the F1 adult emergence in *Callosobruchus* species was completely inhibited at 100 Gy when treated adults in different age-groups (0–1 day, 4–5 day and 6–7 day). As suggested by Molin (2001), low range radiation doses can cause insect sterilization or genetically deformed gametes, whereas the higher range doses are required to induce insect death.

Similar studies were conducted by Chauhan *et al*. (2015) reported that the complete sterility (100% sterility) of *C. maculatus* was found even at the lowest dose of 25 Gy and the eggs laid by the treated adult beetles did not develop into the adults of next generation and also Boshra (1994) reported that the dose required to disinfest or sterilize *C. chinensis* adults was 120 Gy.

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