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Effect of gamma radiation on pulse beetle, *Callasobruchus chinensis* (L.)

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Abstract: Studies on effects of gamma radiation on different life stages of *Callasobruchus chinensis* (L.) were carried out in a growth chamber at temperature of 30ºC and relative humidity of 70 per cent. Two days old eggs, grubs (seven and twelve days old) , mature pupae (one day before adult eclosion) along with pigeonpea seeds and four days old were exposed to gamma radiation in range of 0- 20 Gy, 0- 40 Gy, 0- 100 Gy and 0- 200 Gy respectively. Complete mortality of two days old eggs was obtained by exposure to 20 Gy, a dose of 40 Gy and 100 Gy caused 100 per cent mortality in seven, 12 days old grubs and pupae respectively. The fecundity and fertility of adults decreased with the increase of irradiation dose. No eggs were hatched after irradiated with 50 Gy, hence 50 Gy was found to be optimum dose to induce sterility. Females irradiated at 0 Gy, 10 Gy and 50 Gy were capable to lay eggs upto eight days. Whereas, egg laying of females were restricted to four days at 150 Gy and 200 Gy.

Key words: Gamma radiation, Pigeonpea, Pulse beetle, Sterility

Introduction

Pulses have been an integral part of sustainable agriculture since ages on account of their vital role in nutritional security and soil ameliorative properties. Pigeonpea (*Cajanus cajan* L. Millsp) is one of the important pulse crops in India and is the major source of dietary protein for most of the population of our country. India is the primary centre of origin of pigeonpea. Major constraints that this crop is confronting in northern Karnataka, are inadequate rainfall, pod borer complex in field and pulse beetles in storage.

Amongst the various pests of pigeonpea, the pulse beetles are of major importance since they infest the grains, both in field and store-houses where they multiply rapidly causing heavy losses. Generally, infestation starts in the field but population builds up more in storage. The pests generate exceedingly high levels of infestation even when they pass only one or two generations on the host. The grubs of the beetle feed on the pulse seed contents reducing their degree of usefulness making them unfit either for planting or for human consumption (Ali *et al.,* 2004).

Pigeonpea seeds are attacked by numerous species of beetle beetles, namely*, Callasobruchus maculatus* (Fabricius), *Callasobruchus chinensis* (Linnaeus), and *Callasobruchus analis* (Fabricius). All of them feed by residing inside the seeds and feed primarily on endosperm, thus making the seeds unfit for food and seed purpose. However, the most severe losses to the extent of 30-55 per cent during storage of pigeonpea interms of both qualitative and quantitative constituents, result primarlily due to *C. chinensis.*

It is important to note at this juncture that there has been growing awareness world- wide about adverse impact of conventional chemical methods adopted for management of insect pests both in field and storage, on environment and human health at large. Management of insect pests population is being attempted through alternative means such as strategies targeting fecundity traits. This kind of strategy is mainly focusing on influencing the reproduction ability of the insect pests there by bringing down the population of storage pests below the threshold level leading to reduction in storage losses. Such an information on induction of sterility in the storage pests influencing the fecundity traits will be of paramount significance in the present context. Irradiation can be effectively employed for inducing sterility phenomenon in storage pests. Gamma irradiation is looked upon as one of the irradiation strategies for induction of sterility in storage insect pests. However, information on efficacy of gamma irradiation on fecundity and population dynamics of storage pests of pulses such as beetle species is very scanty and hence, the related studies are more appropriate in the current era of global safety and human health. The aims of the present study were to investigate the effects of gamma radiation on various developmental stages and reproduction of *C. chinensis.*

Material and methods

Present investigations on Effect of gamma radiation on pulse beetle, *Callasobruchus chinensis* (L.) (Coleoptera: Chrysomelida) were carried out during 2014-2015 at Agricultural Research Station, Kalaburagi and College of Agriculture Raichur, University of Agricultural Sciences, Raichur, Karnataka, India

Kalaburagi is situated in North eastern dry zone of Karnataka between 16º 16' latitude and 77 º 20' longitudes and at 389 meters above mean sea level. The rainfall is confined to the monsoon period from June to November with occasional showers in pre-monsoon months of April and May with an average rainfall of 760 mm. Mean maximum tem01perature is more than 30°C throughout the year except in December, 47 relative humidity values are uniformly high during monsoon months from July to September and uniformly low during the summer months from March to May.

Rearing of *C. chinensis*

The culture was started with eggs of *Callosobruchus chinensis* (obtained from Seed unit, UAS Raichur) on pigeonpea seeds kept at room temperature of 30°C and relative humidity of 70 per cent. After about 35 to 40 days adults started emerging from the culture and were utilized for the maintenance of subcultures. This included the rearing of grubs, pupae as well as the adults in pigeonpea seeds. All life stages were used in various experiments.

Irradiation of eggs, grubs and pupae

All the stages were exposed to gamma radiation in Gamma chamber -5000 having a source strength of 5000 Ci and dose rate of 9 KGy/ hr with different dosages.

Seeds containing two days old eggs were exposed to different dosages *viz.,* 0 Gy, 5 Gy, 10 Gy, 15 Gy, and 20 Gy. After irradiation the glass vials (2.5 dia and 7 cm height) containing irradiated eggs along with seeds were kept in growth chamber. Four replications were maintained for each dose level. The eggs were observed for hatching under microscope on daily basis.

The pigeonpea seeds having the beetle eggs were kept separately to get different aged grub *viz.*, mid instar (7 days old) and late instar grub (12 days old). Later they were exposed separately to different dosages of gamma radiation *viz.,* 0 Gy, 5 Gy, 10 Gy, 20 Gy, 30Gy and 40Gy and were compared with untreated control treatment. Four replications were maintained for each dose level. The pigeonopea seeds were dissected for mortality count after 24 hr of gamma irradiation and further.

The pigeonpea seeds having late aged pupae (4th day old) were exposed to different doses of radioactive Cobalt-60 material *viz.,* 0Gy, 10 Gy, 20 Gy, 40 Gy 60 Gy 80 Gy and 100 Gy as explained above and were compared with control treatment. Three replications were maintained for each dose level.

Fecundity and fertility

To obtain virgin adults of the same age, seeds with eggs were kept in small glass vials until adult emergence in growth chamber. Later, fourth day old adult male and females were exposed to gamma radiation at different dosages *viz.*, 0 Gy, 10 Gy, 50 Gy, 150 Gy and 200 Gy. Later the test insects were paired immediately after irradiation and released in the plastic box (12 cm dia and 13 cm height) containing 100 g seeds to observe for advancement of stages further. The dose at which males and females were made sterile was observed for its effect on reproduction.

Results and discussion

Effects of irradiation on two days old eggs

The data obtained from experiments dealing with the irradiation of eggs are summarized in Table 1.Incubation period was prolonged significantly with increased radiation dose. Incubation period ranged from 4.5 to six days in the unirradiated treatment and at 15 Gy respectively. The egg hatch in unirradiated treatment was about 95 per cent and it was significantly lower in all the irradiated treatments. The experiment showed that the eggs of *C. chinensis* were killed completely at 20 Gy which was in agreement with the results of study in Vigna sinensis Sayi (Anon,1968). However, similar level of mortality was repored by Supawan *et al.,* 2005, Molin (2001) at higher dose of 160 Gy and 200 Gy in *C. maculatus* eggs respectively. This indicated the differential response of species of the genus *Callasobruchus* to different doses of irradiation.

Per cent grub formation, pupae formation and adult emergence from the irradiated eggs reduced significantly as the radiation dose increased. Formation of grubs and pupae from the irradiated eggs completely inhibited at 15 Gy accounting to 10 per cent and 5 per cent respectively which was significantly lower than unirradiated treatment (93.33% grub formation and 88.33% pupae formation). Adults ecolosed from the irradiated eggs showed greater degree of variations with respect to

Table 1. Effect of gamma radiation on developmental stages of *C. chinensis* when two days old eggs irradiated

Irradiation doses (Gy)	@Number of eggs laid by two females	Incubation period (days)	Number of eggs hatched	Egg hatching (%)	Number of grubs formed	Grub formation (%)	Number of pupae formed	Pupation (%)	Number of adults emerged	Adult emergence (%)
0	21.33	4.50 (3.12)#	20.33 (5.50)#	95.00 (77.08)##	18.67 (5.32)#	93.33 (75.24)##	17.67 (5.20)#	88.33 (70.11)##	16.33 (5.04)#	80.00 (63.43)##
5	20.33	5.00 (3.23)	10.00 (4.16)	49.00 (44.43)	7.67 (3.76)	40.00 (39.23)	5.67 (3.38)	28.33 (32.14)	1.67 (2.29)	8.33 (16.59)
10	20.67	5.50 (3.34)	3.00 (2.73)	14.52 (22.40)	2.00 (2.41)	10.00 (18.43)	1.00 (2.00)	5.00 (12.75)	0.00 (1.00)	0.00 (0.00)
15	20.67	6.00 (2.44)	1.00 (2.00)	4.84 (12.71)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)
20	20.33	0.00 (1.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)
S.Em ±		0.269	0.149	0.275	0.211	0.821	0.211	0.810	0.211	0.821
C.D. at 1%		1.205	0.668	1.234	0.945	3.678	0.945	3.630	0.945	3.681
CV (%)		9.187	3.760	1.522	6.444	5.348	5.234	6.099	7.042	0.679

Figures in the parentheses are $\sqrt{x+1}$ transformed values @ The data on number of eggs laid by two females is provided for comparision

Figures in the parentheses are arc sine transformed values,

increased radiation doses, no adults emerged from the eggs irradiated at 10 Gy and it was significantly lower than unirradiated treatment (80%).

Effects of irradiation on seven and 12 days old grubs

The effects of gamma radiation on irradiated seven days old and 12 days old grubs are shown in Table 2 and Table 3. The data showed that the percentage of irradiated grubs that survived to the pupal stage were decreased with increasing radiation doses. Sensitivity of grubs gamma radiation varied within their instars wherein, 12 days old grubs were more resistant to gamma radiation than seven days old grubs.

In the experiment conducted, per cent pupation and per cent adult emergence from seven days old irradiated grubs were completely inhibited at 30 Gy which was compared with unirradiated treatment (88.33% pupation and 81.67% adult emergence), whereas, 12 days old grubs required 40 Gy to prevent per cent pupation and adult emergence from those grubs were prevented at 30 Gy which was significantly lower than unirradiated treatment (91.67% pupation and 85% adult emergence). Mortality of seven and 12 days old grubs were found to be vary directly with the increased radiation dose and it was found that cent per cent when grubs were irradiated at 30 Gy which was compared with unirradiated treatment (18.34% mortality for seven days old grubs and 14.97% for 12 days old grubs) . Since 95 per cent and 91.67 per cent mortality obtained at 20 Gy for seven and 12 days old grubs respectively, estimated weight loss of pigeonpea seeds at 20 Gy accounting to 4.89 per cent (seven days old grubs) and 5.73 per cent (12 days old grubs) which was significantly low when compared with unirradiated treatment (16.67% for seven days old grubs and 18.33% for 12 days old grubs), zero weight loss of pigeonpea seeds occurred at 30 Gy which was considerably superior to other treatments.

Abbas *et al.* (2011) at Urmia University, Northwest Iran, reported that percentage of irradiated larvae of *Plodia* interpunctella that survived to the pupal stage were decreased with increasing radiation doses. The percentages of pupae that developed from irradiated larvae were 5 per cent at 350 Gy when compared with 85.62 per cent in the control. Irradiation at 400 Gy caused 100 cent mortality. Aye *et al.* (2006) at Department of Entomology, Yezin Agricultural University, Myanmar reported that pupation did not occur when fifth-instar larvae were irradiated at 0.25 kGy and higher doses, but an ED_{95} value of 0.31 kGy was calculated when three days old fifth instar larvae were irradiated.

Effect of irradiation on four days old pupae

Pupae showed more resistance to radiation than eggs or grubs. The percentage of adults that emerged from irradiated pupae was significantly affected with increased dose of gamma irradiation (Table 4). A dose of 100 Gy completely prevented the development of the pupae into adults which was compared with unirradiated treatment (91.67% adult emergence). 80 Gy also considered to cause significant mortality in pupae accouting to 95 per cent. Since irradiated pupae were failed to

Table 2. Effect of gamma radiation on developmental stages of *C. chinensis* when seven and twelve days old grubs were irradiated

Irradiation doses(Gy)	Number of pupae formed per 20 seeds		Pupation(%)		Number of adults emerged per 20 seeds		Number of adults		Number of adults		Weight loss of seeds (%)	
	seven day old grubs	twelve day old grub	seven day old grubs	twelve day old grub	seven day old grubs	twelve day old grub	seven day old grubs	twelve day old grub	seven day old grubs	twelve day old grub	seven day old grubs	twelve day old grub
0	17.67	18.33	88.33	91.67	16.33	16.67	81.67	85.00	18.34	14.97	16.67	18.33
	(5.20)*	(5.28)*	(70.10)**	(73.38)**	(5.04)*	(5.08)*	(64.69)**	(67.21)**	(25.35)**	(22.75)**	(24.09)**	(25.34)8**
5	10.00	11.00	50.00	55.00	8.67	9.67	43.33	48.33	56.59	51.61	13.07	15.25
	(4.16)	(4.31)	(45.00)	(47.87)	(3.94)	(4.10)	(41.16)	(44.04)	(48.78)	(45.95)	(21.19)	(22.99)
10	8.00	8.33	40.00	40.00	6.00	7.00	30.00	35.00	69.83	65.00	10.39	12.87
	(3.82)	(3.88)	(39.23)	(39.23)	(3.44)	(3.64)	(33.21)	(36.27)	(56.68)	(53.73)	(18.80)	(21.02)
20	2.00	4.00	10.00	20.00	1.00	1.67	5.00	8.33	95.00	91.67	4.89	5.73
	(2.41)	(3.00)	(18.43)	(26.56)	(2.00)	(2.29)	(12.92)	(16.59)	(77.11)	(73.24)	(14.04)	(16.13)
30	0.00	1.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(1.00)	(2.00)	(0.00)	(12.92)	(1.00)	(1.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(1.00)	(1.00)	(0.00)	(0.00)	(1.00)	(1.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
S.Em±	0.136	0.192	0.589	0.753	0.192	0.236	0.648	0.845	0.358	0.310	0.068	0.064
C.D. at 1%	0.588	0.831	2.546	3.251	0.831	1.018	2.801	3.652	1.548	1.337	0.294	0.278
CV (%)	3.755	4.687	3.545	3.911	6.250	6.999	4.433	5.354	1.792	1.644	0.906	0.783

* Figures in the parentheses are $\sqrt{x+1}$ transformed values

** Figures in the parentheses are arc sine transformed values

Table 3. Effect of gamma radiation on developmental stages of *C. chinensis* when four days old pupae irradiated

Irradiation doses(Gy)	Number of adults emerged per 20 seeds	Adult emergence(%)	Mortality(%)	Weight loss of seeds(%)
0	18.33(5.28)*	91.67(73.40)**	8.34(16.78)**	20.21 (26.72)**
10	13.00(4.60)	65.00(53.72)	35.03(36.29)	17.36(24.62)
20	11.33(4.36)	56.67(48.83)	43.33(41.16)	14.25(22.17)
40	7.33(3.70)	35.00(36.27)	64.67(53.52)	10.41(18.82)
60	5.00(3.23)	25.00(30.00)	75.00(60.00)	7.82(16.24)
80	1.00(2.00)	5.00(12.92)	95.00(77.78)	5.61(13.70)
100	0.00(1.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
S.Em ±	0.218	0.784	0.294	0.051
C.D. at 1%	0.919	3.302	1.238	0.239
CV (%)	4.725	3.728	1.249	0.563

* Figures in the parentheses are $\sqrt{x+1}$ transformed values

** Figures in the parentheses are arc sine transformed values

Table 4. Effect of gamma radiation on the reproductive behaviour of *C. chinensis* when four days old adults irradiated

Irradiation doses(Gy)	Number of eggs recorded/ 20 seeds	Egg hatching (%)	Number of grubs obtained from 20 seeds	Grub formation (%)	Number of pupae formed from 20 seeds	Pupation (%)	Number of adults per 100 g of seeds	Adult emergence (%)
0	18.33 (5.28)*	91.67 (73.41)**	17.67 (5.20)*	88.33 (71.38)**	16.67 (5.08)*	83.33 (66.81)**	487.67 (23.08)*	85.35 (67.18)**
10	11.33 (4.36)	56.67 (48.84)	7.00 (3.64)	35.00 (37.26)	5.00 (3.23)	25.00 (30.00)	158.33 (13.58)	27.98 (31.68)
50	9.00 (4.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)
150	5.00 (3.23)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)
200	3.00 (2.73)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)
S.Em±	0.211	0.929	0.149	0.449	0.149	0.180	0.745	0.025
C.D. at 1%	0.945	4.162	0.668	2.013	0.668	0.808	3.341	0.114
CV (%)	3.912	3.703	5.234	3.581	5.958	1.614	0.992	0.222

** Figures in the parentheses are arc sine transformed values

* Figures in the parentheses are $\sqrt{x+1}$ transformed value

Table 5. Effect of gamma radiation on the fecundity of four days adults of *C. chinensis*

	Number of eggs laid by ten pairs at different days after irradiation			
Irradiation doses (Gy)	Two	Four	Six	Eight
0	278.67(17.36)#	115.33(11.73)#	101.33(11.06)#	76.67(9.75)#
10	262.33(17.19)	105.33(11.26)	92.67(10.62)	64.67(9.04)
50	217.67(15.75)	85.67(10.25)	81.67(10.03)	49.00(8.00)
150	197.33(15.04)	12.67(4.55)	0.00(1.00)	0.00(1.00)
200	186.00(14.63)	7.67(3.76)	0.00(1.00)	0.00(1.00)
S.Em (±)	0.298	0.333	0.258	0.211
C.D. at 1%	1.336	1.494	1.157	0.945
CV (%)	0.226	0.884	0.811	0.959

\# Figures in the parentheses are $\sqrt{x+1}$ transformed value

emerge as adults at 100 Gy, weight loss of pigeonpea seeds accounted to zero per cent compared to unirradiated treatment (20.21%).

This study also agreed with Bhuiya *et al.* (1985) who found 100 per cent pupal mortality of *C. chinensis* at 800 Gy. When male and female pupae of *Ephestia calidel-la* (Guenee) were irradiated with doses of 200–800 Gy, the percentage adult emergence was decreased in accor-dance with increasing gamma radiation doses (Boshra and Mikhaiel 2006).

Effect on fecundity and fertility

Results revealed that at various doses of gamma radiation, the fecundity of treated females decreased accounting to increased per cent sterility.When four days old males and females of *C. Chinensis* were exposed to gamma radiation doses ranging from 0- 200 Gy, egg laying was observed to be the highest (18.33) in the unirradiated treatment accounting to 91.67 per cent egg hatch and significantly low (3.00) at 200 Gy. No eggs were hatched after irradiated with 50 Gy, hence 50 Gy was found to be optimum dose to induce sterility (Table 5). Present study is in accordance with the reports of Brower and Tilton (1985) who showed the doses required to sterilize stored-product insects to vary widely from 70 Gy for the cowpea weevil, *C. chinensis.* Adult irradiation reduces fertility and the reduction was correlated with the dose. The sterilizing dose for males or females paired with the untreated unmated opposite sex was I20 Gray, while, it decreased to 80 Gray when both sexes were irradiated and mated together (Salwa, 1994).

The fecundity on successive days of oviposition varied significantly with the increased irradiation dose. It was observed that females irradiated at 0 Gy, 10 Gy and 50 Gy were capable to lay eggs upto eight days. Whereas, egg laying of females were restricted to four days at 150 Gy and 200 Gy (Table 5). This indicated the age of the adult females as the days passed in combination with the irradiation dose played crucial role in the fecundity level.

Conclusion

The results revealed that there was a dose-dependent increase in the developmental periods, and the growth index and reproductive ability of the adults were significantly decreased with increasing dose of radiation administered to the eggs, larvae and pupae too. It is concluded that irradiation can be used as a safe method to control stored pests. All the approaches, use of fumigants has been adopted on large scale for the management of bruchids which, of late, is considered to have hazardous impact both on environment and human health. This awareness led the researchers to look for the alternative, safe and cost effective novel strategy. In this direction conspicuous approaches include irradiation of insects using gamma rays, which targets the reproductive ability of the pests there by influencing the population build up of the storage pests. The present investigation has generated bench mark data for further research and paves path for pilot scale study for developing feasible irradiation technology for its practical application in warehouses in real time basis.

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