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EFFICACY OF GAMMA RADIATION AGAINST PULSE BEETLE *CALLOSOBRUCHUS MACULATUS* (F.)

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ABSTRACT

Susceptibility of the developmental stages of *Callosbruchus maculatus* (F.) to gamma radiation was studied by irradiating infested cowpea grains. Irradiation of one-day-old eggs at doses ranging from 16 Gy to 24 Gy resulted in 100% mortality; and mortality was 65.0 and 85.0% in three-day-old eggs and seven-day-old grubs, respectively, at the dose of 24 Gy. A dose of 150 Gy was required to cause 70.0% mortality in ten-day-old grubs. Exposure of four-day-old pupae to gamma radiation (100 Gy and above) completely inhibited adult emergence. 100% mortality of both sexes occurred by the third day at 1500 Gy. However, irradiation at a dose of 24 Gy induced complete sterility in adults of *C. maculatus*. Adult emergence from infested grain was significantly reduced due to gamma irradiation. Gamma radiation did not alter the protein and carbohydrate contents of irradiated cowpea grains.

Key words: *Callosobruchus maculatus*, cowpea, gamma radiation, 16- 24 Gy, eggs, hatchability grubs, mortality, adult emergence, grains, protein, carbohydrate, sterility

Pulses occupy a unique position in the country as India is the largest producer (25% of global production), consumer (27% of world consumption) and importer (14%) of pulses in the world (Singh et al., 2015). Among the pulses, cowpea (*Vigna unguiculata* L. Walp.) is one of the most widely cultivated and nutritious grain legumes. Despite the significance of cowpea in providing nutritional security, it is one of the crops that faces many constraints, including postharvest losses. Postharvest damage by insects is an important constraint in pulse production (Khaire et al., 1992). During storage, bruchids cause considerable loss to pulse grains. Among this, *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 to 10% loss during storage (Lal and Verma, 2007).

Chemical control is the most common practice adopted to manage bruchid infestation in pulses. But, harmful residues of chemicals, development of insecticide resistance and adverse impact on the environment has resulted in the phasing out of most of the chemicals currently used for management of storage pests (Ross, 1999). The use of deltamethrin and malathion, the chemicals recommended for management of stored grain pests in India, is under review (DACFW, 2020). The limited use or complete restriction of these chemicals necessitates the development of effective, ecofriendly and economical alternatives for storage pest management. Radiation technology- mainly using gamma radiation, is a promising technology for disinfection of agricultural products. The present study intended to explore the possibility of using gamma radiation for the management of pulse beetle, *C. maculatus.*

MATERIALS AND METHODS

The study was carried out from October 2019 to June 2020 at Pesticide Residue Laboratory, Department of Agricultural Entomology, College of Horticulture, Vellanikkara. The nucleus culture of the test insect, *C. maculatus* was maintained on green gram grains. Five pre-mated female beetles were released into Petri plates containing a layer of cowpea grains (Variety: Kanakamany) to collect eggs of uniform age. Grains with single egg were separated on the first and third day after the release of adults. Eggs, seven-day-old grubs and ten-day-old grubs were exposed to various doses of gamma radiation viz. 16, 18, 20, 22, 24 Gy, and compared with control. Egg mortality was recorded based on the hatchability of the eggs and grub mortality on the basis of pupation of the grubs.

Cowpea grains with single eggs were incubated to obtain pupae. Formation of a window on the grain served as an indication of pupation. The cowpea grains having 4-day-old pupae were treated with doses of 50, 75, 100, 1, 150 Gy and compared with an untreated control. Mortality of pupae were recorded based on the adult emergence. One-day-old adult females and males were irradiated separately with five doses of gamma radiation viz., 100, 500, 750, 1000 and 1500 Gy. Mortality of adults were recorded on the third day after irradiation. All the experiments had four replications with ten insects/developmental stage/ replicate. Virgin males and females were irradiated separately after exposure to different doses of gamma radiation viz., 16, 18, 20, 22 and 24 Gy. Five pairs of irradiated adults from each dose was released into cowpea grains (20 g) and removed after 5 days. Twenty grains with eggs were selected randomly from the grain lot to estimate the number of eggs per 20 grains. Further, 20 grains with single egg were separated and kept for observing egg hatchability, number of grubs, number of pupae, per cent adult emergence, total developmental period, adult longevity and sex ratio.

Twenty grains each with one-day-old eggs, 7-day-old grubs and one-day-old adults in 1:1 sex ratio were released into cowpea (500 g) of and incubated for ten days. Each grain lot was irradiated with five different doses of gamma radiation viz., 20, 40, 60, 80 and 100 Gy. The released adults were removed after ten days of irradiation, and the number of adults emerged from the grain lot was observed daily till the adult emergence stopped. Total carbohydrate was estimated using anthrone reagent method (AOAC, 1980), and total protein content using Lowry's method (Sadasivam and Manickam, 1992) after exposing cowpea grains to different doses of gamma radiation viz., 20, 40, 60, 80 and 100 Gy. The irradiation unit used was Gamma Chamber-5000. The data obtained in the experiments were analyzed in completely randomized design using statistical package, SPSS 16.0 after required transformation.

RESULTS AND DISCUSSION

Mortality of one-day-old eggs was 100% at all the doses (16 to 24 Gy) used for irradiation. In three-day-old eggs, mortality varied from 52.50% at 16 Gy to 65.0% at 24 Gy (Table 1). In control, the mortality was only 15.0%, which was significantly lower than all other treatments. Similar to these findings, one-day-old eggs of *C. maculatus* irradiated at 10 Gy did not hatch, while three-day-old eggs required a higher dose of 200 Gy to prevent hatching (Ghogomu, 1990). But Olaifa et al. (1990) reported 100% inhibition of hatching of two-day-old eggs of *C. maculatus* at a dose of 1 Gy itself.

Mortality of seven-day-old grubs were significantly higher over control (17.50%) when irradiated at doses ranging from 16 to 24 Gy. Mortality was maximum (85.0%) at the highest dose of 24 Gy, which was significantly more over all other doses, except 22 Gy. As the age of the grub increased to 10 days, a higher dose of 50 Gy caused only 52.50% mortality (Table 1). Mortality at all the doses of irradiation was significantly higher over mortality in control (7.50%). In comparison to *C. maculatus*, complete mortality of ten-day-old grubs of *C. chinensis* occurred at a higher dose of 800 Gy (Supawan, 2005). In conformity to the present study, Saour and Makee (2004) reported a decrease in radiosensitivity with increase in age of larvae of potato tuber moth *Phthorimaea operculella* (Zeller). Mortality of 1 to 1.5 days old larvae was 85.30% at 150 Gy, which decreased significantly to 72.30% when the age of larvae progressed to 12 to 12.5 days.

Mortality of irradated four-day-old pupae were significantly higher over control. Total mortality of pupae occurred at a dose of 100 Gy and above (Table 1). According to Ghogomu (1990), irradiation of *C. maculatus* pupae at 10 Gy resulted in only 8.0% adult emergence and complete inhibition of adult emergence occurred at a higher dose of 300 Gy. Contrary to these results, Dongre et al. (1997) observed 50% adult emergence after irradiating pupae at 150 Gy. A higher dose of 650 Gy was required to completely inhibit adult emergence after irradiation (Hammad et al., 2020). Diop et al. (1996) maintained 214 eggs of *C. maculatus* and irradiated them at their pupal stage at 100 Gy, but, only one adult emerged out after irradiation, which is similar to our study.

Complete mortality of both sexes of *C. maculatus* occurred at the highest dose of 1500 Gy after three-days of exposure, which was significantly more over all other treatments and control (Table 1). On the 3rd day of irradiation, the mortality of female and male adults in control (2.50% and 10.0%, respectively) was not significantly different from the mortality at the lowest dose of 100 Gy (17.50% and 15.0%, respectively). Dongre et al. (1997) also reported similar results after irradiating eggs, and adults of *C. maculatus*. Twenty four-hour old eggs did not hatch at a dose below 10 Gy, whereas, hatching was normal in three-day-old eggs. While there was total mortality of adults at 1500 Gy. In *C. chinensis*, mortality of two-day-old adults was 97.0% at 800 Gy after four days of exposure (Supawan et al., 2005).

Table 1. Mortality of life stages of *C. maculatus* with gamma radiation

Dose (Gy)	Mortality (%)						
	E1	E3	G7	G10	P4	Ad. Fe	Ad. Ma
16	100.00^{b} (1.41)*	52.50^{b} (0.81)*	65.00^{b} (0.94)*	-	-	-	-
18	100.00^{b} (1.41)*	55.00^{b} (0.84)*	62.50^{b} (0.91)*	-	-	-	-
20	100.00^{b} (1.41)*	67.50^{b} (0.96)*	70.00^{b} (0.99)*	-	-	-	-
22	100.00^{b} (1.41)*	62.50^{b} (0.91)*	77.50^{bc} (1.08)*	-	-	-	-
24	100.00^{b} (1.41)*	65.00^{b} (0.94)*	85.00^{c} (1.17)*	-	-	-	-
50	-	-	-	52.50^{b} (0.81)*	60.00^{b} (0.89)*	-	-
75	-	-	-	52.50^{b} (0.81)*	80.00^{c} (1.11)*	-	-
100	-	-	-	60.00^{b} (0.89)*	100.00^{d} (1.41)*	17.50^{ab} (0.43)*	15.00^{ab} (0.40)*
125	-	-	-	65.00^{b} (0.94)*	100.00^{d} (1.41)*	-	-
150	-	-	-	70.00^{b} (0.99)*	100.00^{d} (1.41)*	-	-
500	-	-	-	-	-	27.50^{b} (0.55)*	27.50^{bc} (0.55)*
750	-	-	-	-	-	35.00^{b} (0.63)*	42.50^{cd} (0.71)*
1000	-	-	-	-	-	62.50^{c} (0.91)*	47.50^{d} (0.76)*
1500	-	-	-	-	-	100.00^{d} (1.41)*	100.00^{e} (1.41)*
Control	2.50^{a} (0.16)*	15.00^{a} (0.40)*	17.50^{a} (0.43)*	7.50^{a} (0.28)*	12.50^{a} (0.36)*	2.50^{a} (0.16)*	10.00^{a} (0.32)*

E1- 1-day-old egg; E3- 3-day-old egg; G7- 7-day-old grub; G10- 10-day-old grub; P4- 4-day-old pupa; Ad.Fe- 1-day-old female; Ad.Ma – 1-day-old male; * In vertical columns, means followed by same letter do not differ significantly by DMRT, p =0.05; Figures in parentheses arc sine transformed values

Irradiation of adults significantly impaired the reproduction of *C. maculatus.* The number of eggs/ 20 grains at different treatments did not differ significantly from control. Whereas, egg hatchability decreased significantly with irradiation. Egg hatchability in control was 95.0% which was significantly superior to all irradiation treatments. There was complete sterility of irradiated adults at 24 Gy. The number of grubs/ 20 grains after irradiation were also significantly lower compared to control (19.0). Survived grubs in different treatments failed to develop further and hence; there are no observations for the number of pupae/ 20 grains, adult emergence, developmental period and adult longevity, whereas, in control, it was 16.75, 81.25%, 7.49 days, 23.85 days and 7.49 days, respectively (Table 2). Brower (1973) also reported a significant decrease in progeny production after irradiation of adults of *C. maculatus.* But 100.0% sterility of adults occurred at a higher dose of 60 Gy in comparison to the present study. Olaifa et al. (1990) reported sterility in adults after irradiating larvae and pupae of *C. maculatus* at 4 Gy. Shivanna (2006) working with *C. maculatus* observed a hatchability of 62.52% after irradiating adults at 25 Gy, which is higher compared to the results obtained in the present study. But, Soumya (2015) reported only 56.67% hatchability of eggs of *C. chinensis* at a lower dose of 10 Gy. A dose of 40 Gy was sufficient to cause 100.00% sterility of adult females of *C. maculatus* but adult males required 60 Gy for obtaining 96.90% sterility (Pajni et al*.,* 1997).

Table 2. Effect of gamma radiation on reproduction of *C. maculatus*

Treatments (Gy)	No of eggs/ 20 grains	Egg hatch-ability (%)	No of grubs/ 20 grains	No of pupae/ 20 grains	Adult emergence (%)	Sex ratio (M:F)	Develop-mental period (days)	Adult longevity (days)
16	19.25[a]	3.75[a] (0.19)*	0.75[a]	0.00	0.00	0.00	0.00	0.00
18	19.25[a]	2.50[a] (0.16)*	0.50[a]	0.00	0.00	0.00	0.00	0.00
20	19.25[a]	1.25[a] (0.11)*	0.25[a]	0.00	0.00	0.00	0.00	0.00
22	19.00[a]	1.25[a] (0.11)*	0.25[a]	0.00	0.00	0.00	0.00	0.00
24	18.75[a]	0.00[a] (0.16)*	0.00[a]	0.00	0.00	0.00	0.00	0.00
Control	19.75[a]	95.00[b] (1.35)*	19.00[b]	16.75	81.25	0.71	23.85	7.49

* In vertical columns, means followed by same letter do not differ significantly (DMRT, p=0.05); Figures in parentheses arc sine transformed values

Irradiation of cowpea grains infested with development stages of *C. maculatus* resulted in significantly lower emergence of adults. Results indicate that the number of beetles emerged significantly decreased with increase in the dose. The number of adults emerged after 17 days of radiation ranged from 25.20 at 100 Gy to 45.25 at 20 Gy (Fig. 1). Hammad et al. (2020) irradiated adults of *C. maculatus* (27,754 nos.) in 100-gram cowpea grain lot and reported a dose of 650 Gy to cause complete inhibition of adult emergence. So, for complete inhibition of adult emergence, a dose higher than 100 Gy may be required.

Gamma radiation did not alter both protein and carbohydrate content of cowpea grains. Carbohydrate content in different treatments as well as control varied from 48.46% to 49.96%. Protein content varied from 21.37% to 24.93%. Hammad et al. (2020) irradiated cowpea grains with 650 Gy and found no significant difference in moisture, protein, lipid, ash and carbohydrate content of cowpea grains immediately after irradiation and also after three months of irradiation. Likewise, Al-Kaisey et al. (2003) reported that there was no significant difference in the protein and carbohydrate content of broad bean (*Vicia faba)* irradiated at a dose ranging from 0 to 10 KGy.

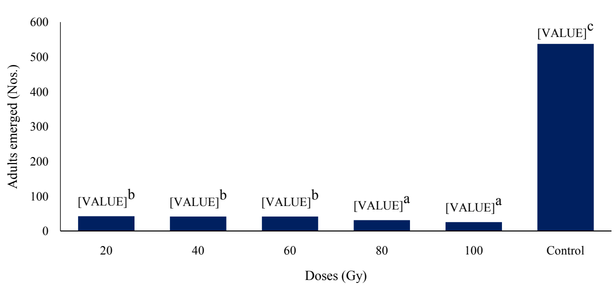

Fig. 1. Adult emergence from cowpea grains infested with lifestages of *C. maculatus*, after 17 days of irradiation

The results obtained in the study revealed that gamma radiation was effective against all stages of *C. maculatus* and can serve as an alternative to insecticides. The sensitivity to gamma radiation increases with radiation dose. But, radiosensitivity decreases with an increase in the age of the insect. Hence, the egg stage of *C. maculatus* is the most radiosensitive and adult the least.

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