EFFICACY OF GAMMA IRRADIATION FOR THE CONTROL OF COWPEA BRUCHID, (*Callosbruchus maculatus* F.)

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Abstract

Cowpea, *Vigna unguiculata* (L.) is a major source of protein in Nigeria. Cowpea bruchid, *Callosobruchus maculatus* F. is a major storage pest of cowpea. Experiments were conducted at the Crop Protection Research Centre, St Xavier's College (Autonomous), Palayamkottai, India under laboratory conditions (28±2°C, 70 – 75 % RH and 11 : 13h photoperiod) to determine the effect of gamma irradiations on the postharvest control of Cowpea bruchid, *C. maculatus*. Five pairs of 2 day old *C. maculatus* collected from stock culture were kept in a Petri-dish containing 50 wholesome seeds (Ife brown variety) and exposed to gamma rays (Cobalt 60 source) at 500, 600, 700, 800, 900 and 1000 Grays (Gy). The experiment was conducted using Completely Randomized Design (CRD) replicated five times. Results showed that gamma irradiation at 1000 Gy gave 97.78 % mortality at 96 hours after exposure which was not significantly different from mortality obtained at 800 Gy and 900 Gy but were significantly different and higher than all other treatments. Similarly, these treatments 800, 900 and 1000 Gy gave the lowest percentages of cowpea seed perforated and best WPI. There was reduction in the total body protein and total genomic DNA of insects exposed to gamma irradiation when compared with control. However, a reduction in seed viability with increase in dosage of gamma irradiation was observed though not significantly different from the control at ($P \leq 0.05$). Hence, gamma irradiation could be in corporated into the Integrated Pest Management Strategy of *C. maculatus*.

Keywords : Efficacy, Gamma Irradiation, Cowpea Bruchid Control.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is an important grain legume grown mostly in the dry Savannah of the tropics in an estimated area of 12.5 million hectares with annual production of about 3.3 million tons (FAO, 2005). Nigeria is the world's largest producer with 2.1 million tons (IITA, 2003). It is a source of income for many smallholder farmers and contributes to the sustainability of cropping systems and soil fertility improvement in marginal lands through provision of ground cover and plant residue, nitrogen fixation and suppression of weeds (Sanginga *et al*., 2003; Allotey *et al*., 2012).

However, production of this important crop has been constrained by insect pests among other factors (Adedire, 2001). Cowpea infestation by Cowpea bruchids starts in the field and continues in storage. Bruchids are minor pests in the field which assume a major pest status during storage (Ofuya and Bamigbola, 1991). It is a cosmopolitan field – to- store pest of cowpea which causes substantial quantitative and qualitative losses manifested by seed perforation and reduction in weight, market value and germinability of seeds (Ogunwolu and Odunlami, 1996; Adeduntan and Ofuya, 1998). A substantial loss of about 30 – 80 percent of the total annual production of cowpea valued at over 30 million US dollars is lost annually in Nigeria alone to this bruchid (IITA, 2003).

In the recent past, the control of insects and other storage pests was basically through the use of chemical methods comprising fumigation of stored commodity with carbon disulphide, phosphine or dusting with malathion, carbaryl, pirimiphos-methyl or permethrin. These chemicals have been reported to be effective against *C. maculatus* and other insect pests (Akinkurolere *et al*., 2006). However, the problems of many synthetic insecticides which include: high persistence, poor knowledge of application by resource-poor-farmers, high cost, non availability, genetic resistance and hazards to environment and human health have necessitated the search for relatively cheap, environmentally safe and sustainable control measures (Akinkurolere *et al*., 2006). As part of the quest for an alternative to chemical insecticides, research efforts are currently being focussed on eco-friendly control measures such as irradiation, heat treatments, biopesticides, integrated pest management, use of insect hormones (Follett *et al*., 2007; Begum *et al*., 2007).

Irradiation is used for insect disinfestations in grains, inhibition of sprouting in tubers and bulbs, alteration of post harvest ripening and senescence of fruits, inactivation of protozoa or helminths in meat and fish, elimination of spoilage microorganisms from fruits and vegetables, pasteurization of dry spices and vegetables (Thayer, 1984). Ionization irradiations like X-rays and gamma rays (Islam and Laz, 2001; Follett *et al*., 2007; Tandon *et al*., 2009) and non ionizing irradiations like Ultraviolet (UV) rays (Faruki, *et al*., 2007) and microwave irradiation have been used to limit reproduction and survival of a variety of insect pest species. Interest in the use of irradiation as a phytosanitory treatment for agricultural commodities is growing worldwide particularly since the publication of the International plant protection convention (IPPC) standard that endorses and facilitates trade based on this disinfestations methods (Follett *et al*., 2007; Gasemzadeh *et al*., 2010). However, there is dearth of information on the use of gamma irradiation in the control of cowpea bruchid, *Callosobruchus maculatus* F.

The objectives of this research were to ascertain the effect of gamma irradiation on the mortality of *C. maculatus*; viability of cowpea seeds and total genomic DNA and body protein of *C. maculatus*

Materials and methods

Insect culture

Stock culture of *Callosobruchus maculatus* was established from infested cowpea seeds purchased from World Bank Market, New Owerri, Imo State. Insects were reared on susceptible wholesome seeds (Ife Brown) kept in plastic containers covered with perforated muslin cloth held in place with tight rubber bands. The culture was maintained under laboratory conditions (28 ± 2 ^{o}C, 70 – 75% RH and 11:13h photoperiod) at crop protection Research Centre, St Xavier's College, Palayamkottai, Tamil Nadu, India. Normal, healthy 1-2 day old male and female *C. maculatus* were used for the experiment. Sexing was done following the methods of Halstead (1963).

Gamma Irradiation Bioassay

Five pairs of 1- 2 day old *C. maculatus* collected from stock culture were kept into Petri-dish containing 50 healthy cowpea seeds (Ife Brown) and exposed to gamma rays (cobalt 60 source) at 500 Grays (Gy), 600 Gy, 700 Gy, 800 Gy, 900 Gy and 1000 Gy. The experiment was conducted using Completely Randomized Design (CRD) replicated five times. Mortality was recorded at 24, 48, 72 and 96 hours after treatments. Mortality data were corrected using the method of Abbot (1925). LD_{50} values were estimated by subjecting mortality data to the maximum likelihood program of probit analysis (Robertson *et al*., 2007) using SPSS software. Weevil perforation index (WPI) was determined using the procedure of (Fatope *et al.*, 1995)

Effect of gamma irradiation on the viability of cowpea seeds

Germination Test

This was carried out in the screen house using seedling Trays. Two seeds from each treatments were randomly selected and planted in each hole and watered daily. The experimental design was Completely Randomized Design (CRD) replicated ten times.

Data were collected on seedling emergence at 4, 5, 6 and 7 days after planting. Percentage emergence was determined using the formular: $\frac{\text{Number of emerged seeds x 100}}{\text{Number of seeds planted}}$

Effect of irradiations on the total body protein of *C. maculatus*

After irradiations, dead insects were collected and preserved using 70% ethanol. Twenty dead insects from each treatment were randomly selected and homogenised with 1.0 Ml of Phosphate Buffer, crushed using pestle and mortar and centrifuged at 12,000 rpm for six minutes (Sahayaraj and Auxellia, 2012). The supernatant was used for the estimation of total body protein using the procedure of Bradford (1976) as follows:

Preparation of Bradford reagent:

100 mg of Coomassie Brillient Blue G250 was dissolved in 50ml of 95% ethanol. The solution was mixed with 100 ml of 85% phosphoric acid and made up to 1 litre with distilled water and filtered with whatman no. 1 filter paper and stored in amber bottle at room temperature.

Protein standard: Bovine serum albumin at a concentration of 1mg / ml in distilled water was used as a stock solution.

Procedure :

Protein standard 0.1ml of 1ml was pipetted into a series of test tubes which contain 10 to 100µg of protein and made up to1ml using distilled water.

1ml of distilled water was used as the reagent blank

0.1ml of unknown sample was taken and made up to 1ml using distilled water.

5ml of Bradford reagent was added to each test tube and mixed well by inversion.

UV visible spectrophotometer was used to measure the Absorbance at 595nm of the samples between 2 minutes and 1 hour after mixing.

Effect of irradiations on the total genomic DNA of *C. maculatus*

Genomic DNA was extracted from 20 dead insects from each treatments using modification of a general procedure for extraction with phenol (Sambrook *et al.*, 1989). The insects were crushed and incubated at 4^{0C} in 0.6mg / ml Proteinase K and 300µl TNES Buffer (50Mm Tris – Hcl, P^{H} 7.5, 0.4 M Nacl, 20Mm EDTA, 0.5% SDS) for 4 hours. The DNA was then purified by washing with organic solvents :

Once with Chloroform : Isoamyl alcohol (24 :1 v/v)

Once with Chloroform : Phenol (1 : 1 v/v)

Once with Chloroform only.

DNA was then precipitated with absolute ethanol and quantified using UV visible spectrophotometer at 580 nanometer (nm),

Data Analysis. Data collected were subjected to Analysis of Variance and significant means separated using Students – Newman – Keuls (SNK) ($P < 0.05$)

Results and Discussion

Bioassay of Gamma Irradiation: Effect of gamma irradiation on the percentage mortality of *C. maculatus* is shown in Table 1. Results showed that gamma irradiation was very effective against *C. maculatus*. At 24 hours after treatments, all the treatments significantly reduced the population of *C. maculatus* when compared with control. Gamma irradiation at 900 gray recorded the highest mortality value (94.00 ± 4.39) while control had the least value (0.00 ± 0.00). However, there was no significant difference among gamma irradiation at 800, 900 and 1000 Gy but they significantly ($P \leq 0.05$) differed from other treatments.

Similar trend was recorded at 48 hours after treatments. Gamma irradiation at 900 Gy had the highest value (94.00 ± 4.39) while control had the least value (0.00 ± 0.00). There was no significant difference among gamma irradiation at 800, 900 and 1000 Gy. Similarly, there were no significant

difference between gamma irradiation at 700 Gy and 600 Gy but they differed significantly from gamma irradiation at 500 Gy and control.

At 72 hours after irradiation, gamma irradiation at 1000 Gy had the highest mortality value (95.28 ± 2.90) while control had the least value. All the treatments significantly differed from control.

Similar observation was recorded at 96 hours after exposure where gamma irradiation at 1000 Gy had the highest value of 97.78 ± 4.97 while control recorded the least value (0.00). There was no significant difference among irradiation at 800, 900 and 1000 Gy. Also, there was no significant difference among gamma irradiations at 600 and 700 Gy, but they significantly differed from 500 Gy and control.

The mortality of *C. maculatus* increased with increased doses of gamma rays indicating that the efficacy of the treatment was dosage – dependent. This is probably due to higher penetration of gamma rays into the insect tissues which inflicted greater damage and thus caused greater mortality. Ionizing irradiation like gamma rays affect mortality of insects through cell cycle disruption following damage to DNA (Ayvaz and Tuncbilek, 2006).

Lethal Dose (LD_{50}): Results on the computation of lethal dose (LD_{50}) of gamma irradiations are shown in Table 2. Results showed that 579.5 Gray is required to kill 50% of *C. maculatus* after 96 hours of exposure. Similarly, 611.9 Gray is required 50% of *C. maculatus* after 24 hours of gamma irradiation.. The result showed that gamma irradiation is dose dependent. Similar observation was made by *Zhao et al*., (2007) who worked on the a thermal lethal model of rice weevils subjected to microwave irradiation and reported that power level (dosages) and treatment time affect efficacy of irradiation.

Table 1. Effect of gamma irradiation on the percentage mortality of *C. maculatus*

Treatments Gamma irradiation (in Gy)	24 hours	48 hours	72 hours	96 hours
Control	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00
500	20.89^{c}±4.76	28.50^{c}±7.31	32.50^{c}±5.65	32.50^{c}±5.65
600	58.00^{b}±10.06	62.33^{b}±8.20	62.33^{b}±8.20	62.33^{b}±8.20
700	60.00^{b}±5.73	60.00^{b}±5.73	60.00^{b}±5.73	60.00^{b}±5.73
800	79.56^{a}±8.38	82.33^{a}±5.40	85.83^{a}±7.14	87.50^{a}±7.91
900	94.00^{a}±4.39	94.00^{a}±4.39	94.00^{a}±4.39	94.00^{a}±4.39
1000	92.00^{a}±3.74	93.56^{a}±4.39	95.28^{a}±2.90	97.78^{a}±4.97

Means with the same letters within the same column are not significantly different (P≥0.05)

Table 2. Lethal Dose (LD_{50}) in *C. maculatus* adults exposed to gamma irradiations

Time (hrs)	LD_{50} (Gray)
24	611.9 ± 16.10
48	591.2 ± 13.80
72	590.4 ± 18.80
96	579.5 ± 9.80

Damage Assessment. Efficacy of gamma irradiation on the percentage perforation of cowpea seeds, number of cowpea seeds perforated and Weevil Perforation Index is shown in Table 3. Results showed that control had the highest value of percentage seed perforation while gamma irradiation at 1000 Gray (Gy) had the lease value (28.40±3.54). Similarly, gamma irradiation at 1000 Gy had the least value of number of seeds perforated (14.20±1.77) while control had the highest of seeds perforated (40.60±1.21).

The table also showed the efficacy of gamma irradiation on the Weevil Perforation Index. Gamma irradiation at 500 Gy had WPI (67.0), irradiation at 600 Gy had WPI (61.6), irradiation at 700 Gy had WPI (54.2), irradiation at 800 GY had WPI (50.2), irradiation at 900 Gy had WPI (41.4) while irradiation at 1000 Gy had WPI (35.0).

Low value of Weevil Perforation Index and percentage seed perforation observed when cowpea weevils were exposed to gamma irradiation showed that the treatments were effective in protecting cowpea seeds from the insects after three months of storage. This could be due to the ability of gamma irradiation to penetrate tissues of insects and causing their mortality through cell cycle disruption following damage to DNA (Ayvaz and Tuncbilek, 2006). It could also be due to reduction in the moisture contents of cowpea seeds thereby increasing the hardness of the seed coats which makes it difficult for the insects to perforate the seeds.

Germination Test. Results showed that gamma irradiation did not significantly affect the viability of cowpea seeds (Table 4.). However, at 4 days after planting, gamma radiation at 500 Gy recorded the highest emergence (70.00 ± 11.06) while gamma irradiation at 500 Gy and control had the least value (20.00).

At 5 days after planting, all the treatments except 500 Gy did not differ significantly from control which had the least value (20.00 ± 8.17) while gamma irradiation at 500 Gy had the highest value (75.00 ± 11.18). There was no significant difference among other treatments except gamma irradiation at 1000 Gy.

At 6 days after planting, there were no significant differences among all treatments. However, gamma radiation at 500 Gy had the highest value (75.00 ± 11.18) while gamma irradiation at 1000 Gy had the least value (25.00 ± 13.44). At 7 days after planting, there were no significant differences among

treatments. Gamma irradiation at 500 Gy performed better than other treatments (80.00 ± 11.06) while gamma irradiation at 1000 Gy had the least emergence value (25.00 ± 13.44).
Unlike microwave irradiation which significantly reduced the germination of cowpea seeds as observed in this experiments which corroborate the findings of (Vadivambal, *et al.*, (2010); Blanco, *et al.*, (1977); Campana *et al.*, (1993)), gamma irradiation did not significantly ($P \geq 0.05$) affect the viability of cowpea seeds. However, germination decreased with increase in the dosage of gamma rays.

Table 3. Damage assessment of cowpea seeds by *C. maculatus* exposed to gamma irradiation

Treatments Gamma irradiation (in Gy)	Perforated seeds	Unperforated seeds	% seed perforation	WPI
Control	40.60^{a}±1.21	9.40^{e}±1.21	81.20^{a}±2.42	-
500	27.20^{b}±1.86	22.8^{d}±1.86	54.4^{b}±3.71	67.0
600	25.00^{bc}±1.27	25.00^{cd}±1.27	50.00^{b}±2.53	61.6
700	22.00^{bcd}±1.27	28.00^{bcd}±1.27	44.00^{bc}±2.53	54.2
800	20.40^{cd}±1.33	29.60^{bc}±1.33	40.80^{c}±2.65	50.2
900	16.80^{de}±1.96	33.20^{ab}±1.96	33.60^{cd}±3.92	41.4
1000	14.20^{e}±1.77	35.80^{a}±1.77	28.40^{d}±3.54	35.0

Table 4: Effect of gamma irradiation on the percentage germination of cowpea seeds

Treatments Gamma irradiation (in Gy)	4 DAP	5 DAP	6 DAP	7 DAP
Control	20.00^{b} ± 8.17	20.00^{b} ± 18.17	40.00^{ab} ± 14.53	40.00^{ab} ± 14.53
500	70.00^{a} ± 11.06	75.00^{a} ± 11.18	75.00^{a} ± 11.18	80.00^{a} ± 11.06
600	50.00^{ab} ± 14.91	55.00^{ab} ± 15.72	55.00^{ab} ± 15.72	55.00^{ab} ± 15.72
700	40.00^{ab} ± 12.47	40.00^{ab} ± 12.47	40.00^{ab} ± 12.47	40.00^{ab} ± 12.47
800	35.00^{ab} ± 10.67	40.00^{ab} ± 12.47	40.00^{ab} ± 12.47	40.00^{ab} ± 12.47
900	40.00^{ab} ± 12.47	40.00^{ab} ± 12.47	40.00^{ab} ± 12.47	40.00^{ab} ± 12.47
1000	20.00^{b} ± 13.33	25.00^{b} ± 13.44	25.00^{b} ± 13.44	25.00^{b} ± 13.44

Effect of gamma irradiation on the total body protein and Genomic DNA of *C. maculatus*

Results obtained (Table 5) showed that gamma irradiation significantly affected the total body protein of *C. maculatus*. Control significantly differed from other treatments. Control recorded the highest value (0.723 µg / mg) while gamma irradiation at 1000 Gy had the least value (0.11 µg / mg). Protein is necessary for the maintenance of body growth, reproduction and other biological and biochemical activities of insect pests. Depletion in protein content of insect pests could lead to mortality and deformation of insect pests. Similar observation was made by *Bakr et al.*, 2004 who worked on the effect of different insect growth regulators on the main metabolites in the haemolymph of desert locust, *Schistocerca gregaria* reported depleted protein content in the insect after treatment with Chlorfluazuron. *Bakr et al.*, (1991) also recorded an inbitory effect of Diflubenzuron, Triflumuron and Methoprene on the total protein content of *Musa domestica*.

The effect of gamma irradiation on the Genomic DNA of *C. maculatus* is presented in Table 5. Results showed that control significantly ($P \leq 0.05$) differed from other treatments. However, there were no significant differences ($P \geq 0.05$) among different gamma dosages used. Control had the highest value (4.65±0.33) while gamma irradiation at 1000Gy had the least value (3.60±0.01). DNA and protein are essential in controlling biological and chemical reactions in the cell metabolism (Hassan, 2002). Similar observation was made by Sahayaraj and Auxelia, (2012) that UV irradiation adversely affected total body protein and genomic DNA of Red Cotton Bug.

Table 5. Effect of gamma irradiation on the total body protein and Genomic DNA of *C. maculatus*

Treatments Gamma irradiation (in Gy)	Total Body Protein (µg / mg)	DNA (µg / mg)
Control	0.72^{a} ± 0.00	4.65^{a}±0.33
500	0.24^{e} ± 0.00	4.00^{b}±0.02
600	0.27^{b} ± 0.00	4.00^{b}±0.02
700	0.26^{c} ± 0.00	4.00^{b}±0.01
800	0.26^{c} ± 0.00	3.81^{b}±0.07
900	0.15^{e} ± 0.00	3.76^{b}±0.06

1000	$0.11^{f} \pm 0.00$	$3.60^{b} \pm 0.01$

Conclusion: Gamma irradiations at 800, 900 and 1000Gy were effective in causing mortality of cowpea bruchid and protecting cowpea seeds against *C. maculatus*. This radiation method could be incorporated into the integrated management strategy of *C. maculatus* on stored cowpea.

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