Gamma irradiation for management of pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)

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THESIS

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2020

DECLARATION

I hereby declare that this thesis entitled **"Gamma irradiation for management of pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Gamma irradiation for management of pulse beetle,** ***Callosobruchus maculatus*** **(Fabricius) (Coleoptera: Chrysomelidae)"** is a bonafide record of research work done independently by **Mr. Abinsha Ashraf (2018-11-033)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

Increase in global population at an alarming rate can affect food availability and nutritional security. India is one of the most populated countries in the world, came out of the shadow of food insecurity due to the visionary steps taken in agricultural policies. Since then, the agricultural policies have aimed at the improvement of pulse production in the country as pulses are equally consumed by both affluent and deprived families of the country (Mohant and Satyasai, 2015). Pulses are considered as the beautiful gift from the nature as they play a crucial role in improving the nutritional status of humankind and improve the soil health as well. Pulses are having high dietary protein and soil ameliorative properties by fixing atmospheric nitrogen. Pulses are considered as the poor man's protein as it accounts for 20-25 per cent protein by weight, thrice the protein available in rice.

Pulses occupy a unique position in the country as India being the largest producer (25 % of global production), consumer (27 % of world consumption) and importer (14 %) of pulses in the world (Singh *et al.*, 2015). Pulses account for around 20 per cent of the area under food grains and contribute around 7-10 per cent of the total food grain production in the country. Among the pulses, Cowpea (*Vigna unguiculata* L. Walp.) is one of the most widely cultivated and nutritious grain legumes. Cowpea grains contain 23-25 per cent protein, 50-67 per cent starch, vitamins such as folic acid and essential nutrients such as iron, calcium, and zinc (Omueti and Singh, 1987).

Despite the significance of cowpea in providing nutritional security, it is one of the crop that faces many constraints including postharvest losses. Post-harvest damage by insects is an important constraint in pulse production (Khaire *et al.*, 1992). Storage insect pests can cause direct damage that result in weight loss and which in turn, lowers the market value of the pulses (Fakayode *et al.*, 2014).

During storage, bruchids can cause considerable loss to pulse grains. In India, Arora (1977) recorded 117 species of bruchids under 11 genera. Among this, *Callosobruchus maculatus* (Fabricius) and *Callosobruchus chinensis* (Linnaeus) are the most common and economically important species that attack stored pulses throughout the world and causes 5 to 10 per cent loss during storage (Lal and Verma, 2007).

Under typical storage condition, *Callosobruchus maculatus* often infest up to 100 per cent of stored grains and a single insect can cause 3-5 per cent reduction in weight of cowpea seed (Booker, 1967). Deshpande *et al*. (2011) noticed 3-27 per cent actual weight loss and 8-31 per cent apparent weight loss in different varieties of cowpea after 90 days of the release of *C. maculatus.*

Chemical control is the most common practice adopted to manage bruchid infestation in stored 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 (Bhatia, 1990; Ross, 1999). The draft gazette notification issued by Government of India on 18^{th} May 2020 proposes to ban Deltamethrin and Malathion recommended for stored product pest management in India (DACFW, 2020). The limited use or complete restriction of these chemicals necessitated the development of effective, ecofriendly and economical alternatives. Non-chemical methods such as hermetic storage, controlled atmosphere storage, irradiation, thermal disinfection and mechanical impaction strategies can serve as alternatives for pest management in storage (Donahaye, 2000).

Radiation entomology, a branch of science that deals with the effect of ionizing radiation on insects, is gaining recognition as a solution for many entomological problems that were previously considered difficult to solve. Radiation technology mainly using gamma radiation, is a promising technology for disinfection of agricultural products. It is used worldwide because of its effectiveness, fast and easy application, absence of residues, lack of development of resistance and minimal changes to the properties of the treated grain (Lapidot *et al*., 1991; Richard and Patrick, 2014). According to The Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food (JECFI), consumption of food administrated with ionizing radiation treatment under adequately controlled conditions is safe. JECFI stated that "Irradiation of any food commodity up to an overall average dose of 10

KGy causes no toxicological hazard; hence, toxicological testing of treated food is no longer required" (WHO, 1981).

With this background, the present study is proposed to explore the possibility of using gamma radiation for the management of pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)" with the following objectives.

1. To study the effect of gamma radiation on different developmental stages of *C. maculatus*
2. To study the effect of gamma radiation on reproduction of *C. maculatus*
3. To study the effect of radiation on nutritional factors of irradiated grain.

Review of Literature

2. REVIEW OF LITERATURE

Pulses are one of the essential food commodities in the world. Pulses are grown as annual or seasonal crop and stored for several months. One of the primary factor for post-harvest losses of pulses during storage is pest attack. Bruchids are the most common and severe pest infesting pulses. Among bruchids, *Callosobruchus maculatus, Callosobruchus chinensis* and *Callosobruchus analis* are predominant beetle pests attacking pulses. The beetles can infest pulses both in the field and storage as well. A wide range of control measures are available to mitigate the problem. Gamma radiation technology is a promising technology in alleviating the bruchid infestation.

Previous works carried out on gamma radiation for the management of stored grain pests is reviewed and presented in this chapter. Effect of radiation on different development stages of insects have been compiled separately and reviewed.

2.1 EFFECT OF GAMMA RADIATION ON EGGS OF STORED GRAIN PESTS

Burkholder *et al.* (1966) reported the radio sensitivity of eggs of grain mite, *Acarus siro*, to gamma radiation at different levels of treatments *viz.,* 13.2, 17.5, 25, 45, and 100 Krad along with control. The eggs were hatched in all treatments except at 100 Krad. There was no emergence of F_1 generation adults at doses above 25 Krad.

Kumagai (1969), studied the effect of gamma radiation on the eggs of *Callosobruchus chinensis*. Eight-hour-old eggs were most susceptible whereas the 5-day-old eggs were most resistant to gamma radiation.

Cogburn *et al.* (1973) studied the effect on eggs of almond moth, *Cadra cautella* after exposure to gamma radiation. Eggs (3-day-old) administrated with doses ranging from 5 Krad to 100 Krad decreased the egg hatching with increase in dose of irradiation. A significant reduction in egg hatching was there between 20 Krad and 100 Krad, and eggs remained unhatched at the highest dose of 100 Krad. Besides, less than 1 per cent of eggs treated with dose 10 Krad and above successfully developed to adults.

Brower and Tilton (1973) compared the effect of radiation on two different species of *Tribolium*, *T. madens* and *T. castaneum*. Two-day-old eggs of *T. madens* irradiated with doses between 0 to 100 Krad were more radiosensitive than 3 to 4-day-old eggs of *T. castaneum*, and there was no hatching of eggs in the former at a dose of 5 Krad or more. On the other hand, *T. castaneum* had 50 per cent or more egg hatch at all treatment levels, but hatching of eggs declined with increase in dose.

Elbadry and Ahmed (1975) evaluated the consequence of gamma radiation on eggs of *C. maculatus.* Effect of gamma radiation differed on one and five day-old eggs differently. There was a hundred per cent mortality of 1-day-old eggs at 1000 rad whereas, it took 8000 rad to cause complete mortality of 5-day-old eggs. Also, they observed that females of *C. maculatus* were more radiosensitive than males.

Seal and Tilton (1986) explained that the radiosensitivity of egg decreased with increase in embryonic development. There was complete inhibition of development of 24-h-old eggs of *Dermestes maculatus* at 150Gy. Whereas, hatching of 72-h-old and 96-h-old exposed to the same dose did not differ significantly from control.

Irradiation of eggs of *C. maculatus* at a dose of 40 Krad, resulted in complete sterility (Hekal and El-Kady, 1987). Dawes *et al.* (1987) found that eggs of sweet potato weevil, *Cylas formicarius elegantulus* were highly radiosensitive. Even a dose of 5 Krad resulted in death of eggs of different ages.

Age of eggs at the time of irradiation has a significant effect on its survival (Hussain and Imura, 1989). One-day-old eggs of *C. chinensis* were most radiosensitive than 5-day-old eggs. There was no hatching of eggs at 12 Gy. LD_{50} value of 2-day-old egg was 5Gy whereas, it was 17 Gy for 3-day-old egg. Further, the late stage of egg (four-day and five day-old) required a value above 32 Gy to cause 50 per cent mortality.

Olaifa *et al.* (1990) irradiated 2-day-old eggs of *C. maculatus* with different doses of gamma radiation ranging from 1-10 Gy and reported that they could not observe adult emergence in any of those doses.

Dongre *et al.* (1997) observed that 1 to 24-h-old eggs of *C. maculatus* could not hatch at a dose of 10 Gy. As the age of eggs increased to 3 days hatching of eggs could be observed, but further development of the insect was adversely affected.

Irradiation at 1 Krad inhibited the hatching of 1-day-old eggs of *C. maculatus*, whereas, inhibition of hatching of 3-day-old eggs occurred at a higher dose of 3 Krad (Ghogomu, 1990). Irradiation of eggs of *C. maculatus* at 2.5 Krad resulted in complete sterility of adults as reported by Gill and Pajni (1991).

Aldryhim and Adam (1999) studied the effect of gamma radiation on the eggs of *Sitophilus granarius* subjected to doses ranging from 10 to 500 Gy and compared with the control. At doses ranging from 30–500 Gy, egg mortality was high, and per cent adult emergence was zero. There was adult emergence at 10 Gy but with a significant reduction in survival compared to control. Damage to the wheat grains infested with *S. granarius* was also studied, and the weevil could produce only 4.3 per cent damage at 10 Gy, and there was no damage to wheat grains irradiated above 10Gy.

Four-day-old eggs of *C. chinensis* was subjected to radiation doses of 40, 80, 120, 160 and 180 Gy. There was a decrease in egg hatching with increased levels of irradiation. Mortality of eggs at 40 and 80 Gy was not significantly different. Similarly, irradiation at 120 Gy, 160 Gy and 180 Gy were not significantly different in inducing mortality of eggs. Besides, the age of the eggs and their mortality were not necessarily inversely proportional to the dose of radiation (Supawan *et al.*, 2005).

Hatching of 1 to 3-day-old eggs of *Ephestia*, irradiated at doses ranging from 0 to 400Gy was inversely proportional to the dose irradiated (Ayvaz and Tuncbilek, 2006). The number of eggs hatched was significantly lower for all doses except for 50 Gy and 100 Gy. Regarding adult emergence from the irradiated eggs, control and 50 Gy showed no significant difference from each other whereas, a significant reduction in adult emergence was observed for 100 Gy and above. Adult emergence was absent at a dose of 200 Gy and above.

Hosseinzadeh *et al.* (2010) studied the effect of gamma radiation on 1 to 2-day-old eggs of *Oryzaephilus surinamensis* exposed to gamma source at seven dose levels ranging from 0 to 60 Gy. Only 6.25 per cent of larvae were emerged at 50 Gy when compared to 85 per cent in control. Further, none of the eggs had hatched at a radiation dose of 60 Gy. The per cent pupation also showed a decrease with increase in dosage.

2.2 EFFECT OF GAMMA RADIATION ON LARVAE OF STORED GRAIN PESTS

Burkholder *et al.* (1966) observed complete mortality of larval stage of *A. siro* at doses 45 Krad and above. Only 5.8 per cent larvae successfully developed into the adult stage at 25 Krad.

According to Huque (1971), adult formation from first-stage larvae of *Corcyra cephalonica* was inhibited at 10 Krad and a dose of 20 Krad was required for full-grown larvae to arrest adult emergence.

Cogburn *et al.* (1973) noticed different responses of almond moth, *Cadra cautella* larvae at different radiation doses. At lower doses of 5 and 10 Krad, the larvae survived for six weeks and pupated successfully. Larvae, after irradiation with a dose of 20 to 30 Krad, survived for 9-10 weeks, but there was no pupation of survived larvae. However, complete mortality of larvae was observed when irradiated at 50 and 100 Krad within six weeks and four weeks, respectively.

Study on the sensitivity of 12-day-old *Cylas formicarius elegantulus* larvae to gamma radiation revealed that irradiation arrested larval development at doses ranging from 5 to 50 Krad (Dawes *et al.*, 1987).

Brower and Tilton (1973) compared the response of mature larvae of *T. madens* and *T. castaneum* to different levels of radiation. *Tribolium madens* larvae were more radiosensitive as any dose above 5 Krad prevented adult emergence. Contrary to this, irradiation at 10 Krad was required to prevent adult emergence in *T. castaneum.* Besides, Brower (1973c) compared the sensitivity of *Tenebrio molitor* and *Tenebrio obscurus* larval stages to gamma radiation and found that larval stages of both the species failed to develop into adults at a dose of 5 Krad although *T. molitor* showed more radiosensitivity.

Bhuiya *et al.* (1985) reported that adult emergence from fourth instar larvae of *C. chinensis* and *Callosobruchus analis* was inhibited at a dose of 0.32 and 0.28 KGy, respectively.

Kovacs *et al.* (1985) studied the mortality of *Tribolium confusum* larvae subjected to gamma radiation. Fifty per cent mortality of larvae was observed by eighth day of irradiation at a dose of 0.8 KGy. Whereas, larval mortality was delayed further by four days when irradiated at lower doses of 0.2–0.4 KGy.

Seal and Tilton (1986) disproved that resistance to gamma radiation increase with increase in age of the metamorphic stage in his study with *Dermestes maculatus* larvae (1^{st}, 4^{th}, 5^{th}, 6^{th} and 7^{th} instars) treated at different doses ranging from 0 to 200 Gy. They observed total mortality of all the larval instars, except for fourth and fifth instar, at 200 Gy.

LD_{50} values of 7-day-old and 10-day-old grubs of *C. chinensis* was worked out by Hussain and Imura (1989) by exposing them to radiation ranging from 10 Gy to 640 Gy. LD_{50} value of 7-day-old grub was 7.1 Gy, whereas, 8.4 Gy was needed to cause 50 per cent mortality in 10-day-old grubs. Further, total mortality of 7-day-old grub occurred at 40 Gy, but only 5 per cent of 10-day-old grubs were survived at 40 Gy.

According to Ghogomu (1990), young larvae (10-day-old) and older larvae (14 to 16-day-old) of *C. maculatus* exposed to gamma source of ^{60}Co and a lower dose of 6 Krad resulted in prevention of adult emergence from younger larvae. However, a higher dose of 30 Krad could prevent adult emergence from older larvae.

Olaifa *et al.* (1990) concluded that 10 Gy could cause mortality in third and fourth instar larva of *C. maculatus* while only 4 Gy was required to prevent the development of first larval instar. Further, 4 Gy could induce sterility in adults developed from irradiated third and fourth instar larva. According to Gill and Pajni (1991), *Callosobruchus maculatus* larvae exposed to 25 Gy resulted a complete sterility of emerged adults.

Yang *et al.* (1993) irradiated larvae of *C. chinensis* at different levels of radiation between 10 Krad and 70 Krad and observed 100 per cent mortality at 69 Krad.

Dongre *et al.* (1997) reported that there was complete inhibition of the growth of early and late larval stages of *C. maculatus* when irradiated at a dose of 20Gy.

Aldryhim and Adam (1999) irradiated *Sitophilus granarius* larval stages and found that 30-500 Gy were detrimental to the development of larvae as it caused complete mortality. At 10 Gy, a few larvae developed into adults, but there was a significant decrease in larval development.

Irradiation, at a dose of 100 Gy, completely prevented the development of the larval stage of *T. castaneum* (Tunçbilek *et al.*, 2003).

Mansour (2003) studied the radiosensitivity of fifth instar larvae of *Cydia pomonella.* Diapausing stages were more radiosensitive than non-diapausing stages as per cent pupation of former and later exposed to a dose of 250 Gy were 9.5 per cent and 20.5 per cent, respectively. According to them, females were more sensitive to gamma radiation than males as adults emerged from larvae exposed with 150 Gy were not females and the emergence of adult females was less than 14 per cent at 100 Gy.

Saour and Makee (2004) observed that larval mortality in potato tuber moth was directly proportional to the rate of radiation despite the age of larvae radiated. Survival of larvae decreased with increase of radiation above 100 Gy at all larval stages. However, at 75 Gy, the development of larvae into pupae were not significantly different from the control and the adult developed from the survived larvae were malformed.

Mortality of *C. chinensis* larvae (10-day-old) increased with the increase of radiation dose. The larval mortality of *C. chinensis* administrated at 0, 100, 300, 500 and 800 Gy were 0, 27.37, 83.86, 96.14 and 100 per cent, respectively (Supawan *et al.*, 2005).

Ayvaz and Tuncbilek (2006) concluded that the age of the *Ephestia kuehniella* larvae could influence the effect of radiation. Though adult emergence was not recorded in 7-day-old larvae irradiated at 200 Gy, a higher dose of 250 Gy prevented adult emergence in the last instar larvae. Further, female larvae were more radiosensitive than male larvae irrespective of the age of larvae used for irradiattion.

Hosseinzadeh *et al.* (2010) investigated the effect of radiation at doses ranging from 0 to 400 Gy on 15 day-old larvae of *O. surinamensis*. The per cent pupation was not significantly different at higher doses, *i.e.*, 7.5 per cent and 2.5 per cent at 300 Gy and 350 Gy, respectively. However, a dose of 350 Gy and more prevented adult emergence.

Irradiation of third instar larvae of *T. castaneum*, at doses ranging from 10-500 Gy, resulted in dose-dependent decrease in the formation of pupae. At a dose of 100 Gy and more, there was complete inhibition of pupae formation. Pupal period was increased with increase in dose and the per cent adult emergence and adult longevity decreased with increase in dose (Tandon *et al.*, 2009).

Hosseinzadeh *et al.* (2011) reported 100 per cent mortality of 15-day-old *Plodia interpunctella* larvae when irradiated at 400 Gy.

2.3 EFFECT OF GAMMA RADIATION ON PUPAE OF STORED GRAIN PESTS

According to Huque (1971), female pupae of *Corcyra cephalonica* were more sensitive than male pupae as there was no adult emergence from female pupae even at 5 Krad instead of 15 Krad to prevent adult emergence from male pupae.

Brower and Tilton (1973) reported that middle-aged pupae of both *T. madens* and *T. castaneum* showed resistance to gamma radiation at 5 to 50 Krad as there was 100 per cent adult emergence for *T. madens* and adult emergence was near to control for *T. castaneum.* Though there was decrease in per cent adult emergence of irradiated pupae of *Tenebrio molitor* and *Tenebrio obscurus* with increase in dose of irradiation, total mortality of *T. molitor* pupae and *T. obscurus* occurred only at 100 Krad and 50 Krad, respectively (Brower., 1973c).

Cogburn *et al.* (1973) observed that none of the adults were emerged from irradiated pupae of almond moth, *C. cautella* when irradiated at 100 Krad.

The response of pupae to gamma radiation varied with the age of the *Dermestes maculatus* pupae (Seal and Tilton, 1986). A dose of 150 Gy resulted in complete mortality of 24-h-old pupae, whereas, 100 per cent emergence was observed in 72-h-old pupae at the same dose.

According to Dawes *et al.* (1987), two-day-old pupae of *Cylas formicarius elegantulus* was more susceptible than 5-day-old pupae. Furthermore, adults emerged from 5-day-old pupae were deformed when irradiated at ≥35 Krad, and a dose of 5 Krad was enough to induce sterility in adults.

Hussain and Imura (1989) concluded that pupal stages are more resistant to irradiation than egg and larval stages. The mature pupae (5-day-old) of *C. chinensis* were killed instantly at 640 Gy, and the LD_{50} value of pupae was 56 Gy.

Pupae of *C. maculatus* exposed to irradiation at 30 Krad were emerged as adults, but, adult life span did not vary significantly with control when early stages of pupae treated below 2 Krad (Ghogomu, 1990). According to Olaifa *et al.* (1990), adult emergence in *C. maculatus* was decreased significantly after exposure of pupae to 10 Gy compared to control.

Gill and Pajni (1991) reported that 35 Gy caused complete mortality in early stage pupae of *C. chinensis*, whereas, even 80 Gy could not cause complete mortality of older pupae but could induce sterility in emerged adults. They observed complete sterility of adult beetles when they irradiated older pupae at 8 Krad.

Yang *et al.* (1993) irradiated mung bean infected with *Callosobruchus chinensis* pupae at different levels of radiation (11, 34, 46, 57 and 69 Krad) and observed 100 per cent mortality at 69 Krad.

According Dongre *et al* (1997), comparatively higher dose was required to cause complete mortality of pupae in *C. maculatus* as 150 Gy couldn't prevent adult emergence of 50 per cent population of irradiated pupae.

Aldryhim and Adam (1999) reported that *S. granarius* pupae got affected differently to different radiation ranges. Adult emergence was completely prevented at 500 Gy while adult emerged at 100 Gy died instantly after emergence.

Supawan *et al.* (2005) reported significant reduction in pupal mortality in *C. chinenesis* after exposure to gamma radiation when compared to control and reported hundred per cent mortality at 800 Gy.

Boshra and Mikhaiel (2006) studied the effect of gamma radiation on mature pupae of date moth, *Ephestia calidella* irradiated at doses of 200-1000 Gy. Male pupae were more resistant than female pupae. The per cent adult emergence of male pupae were 85.9, 68.8, 40.0 and 11.2 per cent at 200, 400, 600 and 800 Gy, respectively, while that of female pupae decreased to 79.2, 63.2, 31.2 and 6.4 per cent at 200, 400, 600 and 800 Gy, respectively. However, there was 100 per cent inhibition of adult emergence at 1000 Gy.

Hosseinzadeh *et al.* (2010) studied the effect of gamma radiation on *Oryzaephilus surinamensis* pupae. Five day-old pupae were killed completely at a dose of 700 Gy.

Hosseinzadeh *et al.* (2011) found that 650 Gy was the dose that inhibited complete development of 5-day-old pupae of Indian meal moth, *Plodia interpunctella.*

2.4 EFFECT OF GAMMA RADIATION ON ADULTS OF STORED GRAIN PESTS

Brower (1973b) observed that a sterility inducing dose of 70 Gy was sufficient for commercial irradiation of legumes infested with *C. maculatus.*

Adult males of *T. madens* and *T. castaneum* were more resistant than females to gamma radiation. There was a complete sterility and shortening of life span of adults of both species at 30 Krad and above (Brower and Tilton, 1973).

Brower (1973c) compared the response of mealworm, *Tenebrio molitor* and *Tenebrio obscurus* adults to gamma radiation. Females were more susceptible than males to sterilizing dose, and 15Krad resulted in complete sterility. Besides, the life span was shortened at 10 Krad and above for both species. In contrast, Brower (1973a) found that the depressed flour beetles, *Palorus subdepressus* were less sensitive towards sterilizing doses than any other storage pest as 30 Krad did not induce complete sterility. But the longevity and fecundity of adults were significantly reduced and none of the adults survived after three weeks.

Females of *C. maculatus* irradiated at 100 Krad laid only two eggs/female, whereas, untreated females laid 74.5 eggs/female (Ahmed *et al.*, 1980). But, none of the eggs laid by the irradiated females at doses 20, 50 and 100 Krads had been hatched. Confinement of virgin untreated females with sterilized males resulted in the production of infertile eggs.

According to Dawes *et al.* (1987) lifespan of *Cylas formicarius elegantulus*was shortened with increase in the level of gamma radiation. The survival period of sweet potato weevil was 11 days and 18 days at 27°C after an irradiation at 100 Krad and 75 Krad, respectively.

Hussain and Imura (1989) studied the mortality of one-day-old adults of *C. chinensis* irradiated using a gamma source. The LD_{50} value of females were 420 Gy, and males were 550 Gy, but without any significant difference. Also, a dose of 640 Gy caused instantaneous death in both sexes.

Irradiation at doses between 200 and 300 Gy resulted in complete sterility of 24-h-old *Dermestes maculatus* (Seal and Tilton., 1986).

Olaifa *et al.* (1990) concluded that the longevity of four-day-old *C. maculatus* had been extended more than that of the two-day-old adults when administrated with gamma radiation between 10 to 100 Gy. But, even a dose of 100 Gy couldn't cause a cent per cent mortality.

Roy and Prasad (1993) recommended 1 Krad as an effective dose for 6-month storage of mungbean and gram against *C. chinensis.* Adult beetles were killed entirely within one week at 1 Krad and two weeks at 0.5 Krad.

Adults of rice weevil, *Sitophilus oryzae* were irradiated between 0 to 180 Gy. The LD_{50} and LD_{99} values of ten-day-old adults were 38.14 Gy and 119.14 Gy, respectively (Tunçbilek, 1995).

Dongre *et al.* (1997) reported a significant reduction in longevity of *C. maculatus* adults after irradiation at a dose ranging between 500 Gy and 1500 Gy and reported mortality of adults within three days on exposure to 1000 Gy.

Aldryhim and Adam (1999) noticed an inverse relationship between age and mortality of *Sitophilus granarius* adults at different exposure levels. There was complete mortality of 3-day-old adults at 49, 42, 42, 35, 28, 14 and 7 days after exposure at 10, 30, 50, 70, 100, 300 and 500 Gy, respectively. On the contrary, the life span of 4-week-old adults were 28, 28, 28, 21, 14 and 2 days at treatments levels 10, 30, 50, 70, 100 and 300-500 Gy, respectively.

Kongratarpon *et al.* (2002) recorded LD_{50} and LD_{99} values of *Lasioderma serricorne* after 12^{th} day of irradiation as 931 and 1,746 Gy, respectively. Supawan *et al.* (2005) irradiated two-day-old adults of *C. chinensis* and observed that per cent mortality was significantly different at all treatment levels compared with control at 4 and 7 days after irradiation. On exposure to 300, 500 and 800 Gy, per cent mortality was 16, 86.5 and 97 per cent after four days, and 81, 100 and 100 per cent, after seven days respectively.

Ayvaz and Tuncbilek (2006) observed that fecundity of irradiated adults of *Ephestia kuehniella* was not significantly influenced by gamma radiation except at a dose of 550 Gy. Furthermore, the longevity of both males and females were on par with the untreated ones.

Survival of *Tribolium castaneum* decreased with an increase in radiation doses. Survival period was 35 days at 500 Gy, whereas the untreated individuals survived for more than 79 days (Tandon *et al.*, 2009).

Hosseinzadeh *et al.* (2010) reported that the more the dose applied, the less *Oryzaephilus surinamensis* adults were survived. Five-day-old adults irradiated with 300 to 600 Gy were died within 14 days after radiation while the insect survived for 28 days at 200 Gy.

2.5 EFFECT OF GAMMA RADIATION ON THE REPRODUCTIVE BEHAVIOUR OF STORED GRAIN PESTS

Burkholder *et al.* (1966) observed that production of F_1 and F_2 generation of *Acarus siro* were prevented at a dose of 25 Krad and more when both parents are irradiated.

Cent per cent sterility of *C. maculatus* male was obtained at 10 Krad and 25 Krad (Tilton and Brower, 1971). They suggested that this dose could be used commercially for the management of *C maculatus*.

Hossain *et al.* (1972) studied the development of resistance to radiation in pulse beetle, *C. maculatus*. A population of adult beetles were irradiated with sub sterilizing doses (0.5 – 4 Krad) for six successive generations and with an acute dose (16 and 50 Krad) for subsequent 7th generation. The population died out in 1st generation at 4 Krad and 6th generation at 2 Krad. The possibility of the development of resistance was minimal as the mean survival time of 7th generation significantly decreased from control.

Cogburn *et al.* (1973) reported that sterility inducing dose of *Cadra cautella* male and female were 100 Krad and 30 Krad, respectively. Besides, egg production was more adversely affected when irradiated females mated with normal males than *vice versa.*

Callosobruchus maculatus adults were subjected to sub sterilizing doses (0.5 to 4 Gy) of radiation continuously for 30 generations to study the resistance and reproductive capacity to irradiation (Brower, 1974). Reproductive capacity decreased with each successive generation. There was a positive correlation between the number of generations treated and the dose of irradiation. The sensitivity of *C. maculatus* to irradiation increased with an increase in exposure to radiation during the previous generations. Reproductive capacity decreased in each generation, and there was no tolerance to gamma radiation even after 30 generations.

Ahmed *et al.* (1977) observed that egg sterility of *C. maculatus* increased by increasing the ratio of irradiated males in the population. Egg sterility increased from 62.60 per cent at flooding ratio of 1:1:1 (irradiated males: normal males: normal females) to 96.5% at 15:1:1 ratio.

According to Hussain and Imura (1989), irradiation of *C. chinensis* male and female at 80 Gy caused sterility in both sexes. Though the competitiveness of sterile males was not affected, hatchability of eggs decreased as the ratio of sterile male increases. Hatchability was less than 10 per cent when the ratio of sterile males to normal males was 9:1 and 15:1.

Ghogomu, 1991 reported the adverse effect of gamma radiation on the reproductive behaviour of *C. maculatus* adults. Radiation at 10 Krad completely sterilized both females and males. Mean fecundity decreased from 74 eggs per female in control to 2 eggs per female at 150 Krad. Egg hatching was prevented at 7 Krad when both parents were irradiated, while egg hatching was prevented at 10 Krad when either one pair was untreated. Furthermore, the minimum dose required to prevent adult emergence was much less than that required for preventing egg hatching, *i.e.*, 6 Krad for both parents irradiated and 9 Krad for either one parent irradiated.

Boshra (1994) reported that gamma radiation adversely affected fecundity and fertility of adult stages of *C. chinensis.* The adult stages were irradiated at doses ranging from 0 to 120 Gy and crossed at different combinations. Irradiation of both pairs resulted in a severe decrease in fecundity and fertility when compared to irradiation and mating of either one of the pair. Further, the sterility inducing dose was 120 Gy, when either one pair was irradiated. Whereas, the dose decreased to 80 Gy when both the sexes were irradiated.

Makee and Saour (1997), studied the effect of gamma radiation on F_1 progeny of irradiated males of *Phthorimaea operculella* at sub sterilizing doses of 100,150 and 200 Gy. The sub sterilizing doses caused a significant effect on F_1 generation like higher mean development time, higher mortality, lower fecundity, lower mean longevity and lower fertility than control and parent population. Moreover, the sex ratio skewed towards males than females.

Pajni *et al.* (1997) examined the induction of sterility in both sexes of *C. maculatus*at different doses. In males, the minimum and maximum sterility of 40.9 and 96.9 per cent was attained at 500 and 6000 rads, respectively, whereas, minimum and maximum sterility was 51.8 and 100 per cent at an exposure of females at 500 and 4000 rads, respectively.

Supawan *et al.* (2005) observed that *C. chinensis* females were more susceptible to gamma radiation than males, as the sterility of females was 100 per cent at 80 Gy. Whereas, in males, a dose of 120 Gy induced 91.47 per cent sterility.

2.6 EFFECT OF GAMMA RADIATION ON NUTRITIONAL FACTORS OF IRRADIATED GRAINS

Al-Kaisey *et al.* (2003) concluded that gamma radiation had a remarkable effect on improving the nutritional quality of broad beans. The broad bean seeds irradiated at doses ranging between 0 to 10 KGy had not exhibited any significant difference in protein and carbohydrate content with values 29.2, 29.2, 29.0, 29.0 and 29.0 per cent for protein and 66.1, 66.1, 66.3, 66.2 and 66.4 per cent for carbohydrate at 0, 2.5, 5, 7.5 and 10 KGy, respectively.

Bhat *et al.* (2008) estimated crude protein and carbohydrate content of gamma-irradiated *Mucuna pruriens* seeds by irradiating at doses ranging from 2.5 KGy to 30 KGy. The crude protein content increased from 28.2 per cent at 2.5 kGy to 31.06 per cent at 30 KGy. But, a slight decrease in crude carbohydrate content was observed from 54.7 per cent to 54.3 per cent when irradiated at 2.5 KGy and 30 KGy, respectively.

Hassan *et al.* (2009) reported the effect of gamma radiation on protein content of maize cultivars. In Maize 75, the protein content in control and two KGy were 8.4 and 8.9 per cent, respectively. Similarly, in Maize 6616, the protein content was 7.3 and 7.4 per cent in control and two KGy, respectively.

2.7 EFFECT OF GAMMA RADIATION ON THE PHYSIOLOGY OF INSECT

Insects are in general more sensitive to radiation than lower organism like bacteria, protozoa and viruses and resistant than higher vertebrates (Whicker and Schultz, 1982). Baxter and Blair (1969) reported irradiation to shorten the lifespan of insect. Also, irradiation hasten senescence in insect (Aly *et al.*, 1996).

Exposure of male ground beetle, *Blaps polycresta* with an irradiation dose of 32 Gy and 64 Gy induced biochemical disorders and tissue damage by increasing the level of detoxifying metabolic enzyme AST, ALT, ACP, ALP and inhibition of antioxidant enzyme GSH, GST and GPx. Further, the irradiation caused inhibition of total protein and DNA content (Kheirallah and El- Samad, 2016).

Tribe and Webb (1979) observed decreased enzyme activity in *Calliphora erythrocephala* due to denaturation of protein at an irradiation dose of 80 Krad.

Gabarty (2008) reported a notable reduction in the total content of haemolymph proteins, lipids and carbohydrates of F1 progeny to the 6th instar larvae *Agrotis ipsilon* resulted from irradiation of parents at a dose of 100 Gy. Irradiation of male pupae of *Spodoptera littolaris* at 50, 100 and 150 Gy significantly reduced the protein concentration and humoral immune enzyme (phenoloxidase, prophenoloxidase, lysozyme) activities (Gabarty *et al.* 2013)

Castillon *et al.* (1971) noticed a reduction in acetylcholine esterase levels due to irradiation of 4-day-old adults of *Ceratitis capitata*at 84 Gy. Further, Salama *et al.* (2000) reported irradiation of egg stages of *Ceratitis capitata* with 2.5 Gy and 5 Gy caused a significant reduction in the level of lactate dehydrogenase enzyme activity in the pupae of *C. capitata.*

Abdel-Bakey *et al.* (1990) reported that the exposure of gamma radiation at 30 Gy, 60 Gy and 90 Gy inhibited ATPase activity in all stages of *Plodia interpunctella.* Lupa (1998) studied that gamma radiation adversely affected the melanin formation by altering the production of phenoloxidase enzyme in larvae of stored product moths. Gamma radiation adversely affected food utilization of larvae of *Ephestia cautella* due to reduced activity of gut enzymes (Boshra 2007).

Ashraf *et al.* (1971) reported that exposure of *Plodia interpunctella* larva with gamma radiation caused histological damage to the epithelial lining of the midgut. Higher doses of 25, and 50 Krad destroyed the entire epithelial cells, and lower doses of 5 and 10 Krad destroyed the regenerative cells, which replaces the epithelial cells.

Germ cells are more radiosensitive than somatic cells since cells undergoing continuous differentiation were more sensitive towards radiation (Bakri *et al*., 2005). Tilton and Bower (1983) studied that higher radiation doses could induce dominant lethal mutation and inactivate all the sperm. Also, the lower doses had a significant effect on sperm production.

Materials and Methods

3. MATERIALS AND METHODS

This chapter details the materials used and methods followed in investigation on the effectiveness of gamma radiation for the management of pulse beetle, *Callosobruchus maculatus*.

3.1 LOCATION

The study was carried out from October 2019 to June 2020 at Pesticide Residue Laboratory, Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Kerala Agricultural University, Thrissur. All the investigations were carried out at laboratory conditions of temperature 29ºC and 78 per cent relative humidity.

3.2 TEST INSECT

The nucleus culture of the test insect, *C. maculatus* (Coleoptera: Chrysomelidae) was procured from the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, KAU, Thrissur. Morphological characters such as antennae and inner carina of hind femur helped in identifying the species (Haines, 1989). Adults from the nucleus culture were mass multiplied and the stock culture was maintained throughout the study period.

The sex of adult pulse beetles was distinguished based on general body appearance; the female beetle being larger than males. Further, the most distinguishing feature, extension of the pygidium was used to confirm the sex. The pygidium of females are extended and with dark stripes on both sides. Whereas, in males, pygidium is not extended and they lack stripes (Haines, 1989).

3.2.1 Maintenance of test insect culture

The culture of pulse beetle was maintained on green gram grains procured from local market. The grains were washed thoroughly in water to remove the impurities and insecticides, if any. After washing, the grains were dried under shade. To eliminate the possibility of any insect infestation, the grains were sterilized in a hot air oven at 60°C for one hour and stored at room temperature in air tight plastic containers (1 litre volume). The adult male and female beetles obtained from nucleus culture were released into plastic containers (1 litre volume) containing 100 g of sterilized green gram grains. The containers were then covered with muslin cloth; secured with rubber bands and were kept for incubation after labelling the date of inoculation, in the insect rearing racks maintained at 29°C temperature and relative humidity of 78 per cent (Plate 1). The insects emerged from this culture was used for sub-culturing and for conducting various experiments. The rearing was continuously done on alternate days for ensuring continuous availability of uniform aged test insects throughout the experimental period.

3.3 SOURCE OF IRRADIATION

The irradiation unit used was Gamma Chamber-5000, placed at the Radio Tracer Laboratory, College of Horticulture, Vellanikkara, KAU, Thrissur. The Gamma Chamber-5000 is a Co^{60} gamma irradiator with compact shield providing an irradiation volume of 5000 cc and a minimum irradiation time of seven seconds. Samples loaded in the sample chamber located in the vertical drawer can move up and down. The vertical drawer moves down to the radiation field, which is provided with a set of Co^{60} sources. The vertical drawer having rotating mechanism for uniform exposure of the samples. The lead shield, provided in the irradiation chamber keep the external radiation field within the permissible limit.

3.4 EFFECT OF GAMMA RADIATION ON DIFFERENT LIFE STAGES OF PULSE BEETLE

To understand the effect of gamma radiation on different stages of pulse beetles sterilized grains of cowpea, *var. Kanakamony* was used. Different developmental stages of pulse beetles were transferred to plastic containers and subjected to irradiation.

Plate 1. Test insect culture

Plate 2.a: Gamma irradiation unit (GC-5000)

Plate 2.b: Sample chamber

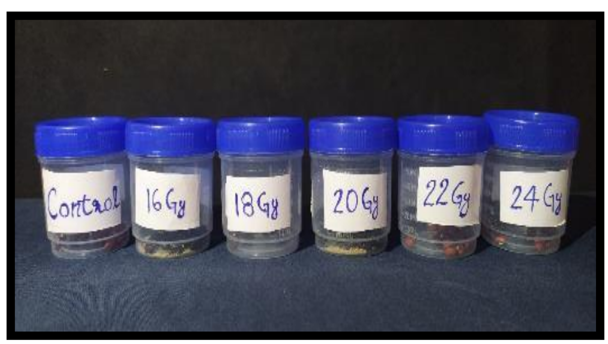


Plate 2.c: Containers for irradiation

Plate 2. Irradiation unit

3.4.1 Irradiation of eggs

Five pre-mated female beetles were released into Petri plates containing a layer of cowpea grains and allowed for egg-laying. Freshly laid eggs were oval or spindle-shaped, clear, shiny and they were firmly glued to the grain surface. Six Petri plates were maintained to assure sufficient number of grains with single egg. Grains with single egg were separated from the Petri plates into plastic containers (50 ml) on the first and third day after the release of adults. One-day and three-day-old eggs thus obtained were used for irradiation experiments.

Grains with single egg were exposed to various doses of gamma radiation *viz*. 16 Gy, 18 Gy, 20 Gy, 22 Gy, 24 Gy and compared with control. Four replications were kept for each treatment with a minimum of 10 grains with single egg in each replication. The irradiated grains with single egg were kept at room temperature. After irradiation, each grain with single egg was observed daily under a stereo microscope to record the hatchability of irradiated eggs. The irradiated eggs were observed daily to record total number of eggs hatched. Hatching was marked successful when the hatched larva was able to burrow into the grains. Once the eggs were hatched, the colour turns from clear and transparent to yellow and opaque (Plate 3). The observations were continued for eight days. The following parameters were worked out from the observations made from the experiment.

a. <u>Per cent egg hatchability</u>

The per cent egg hatchability was calculated using the formula.

$$Percent\ egg\ hatchability = \frac{Total\ number\ of\ eggs\ hatched}{Total\ number\ of\ eggs\ laid} \times 100$$

b. <u>Mean egg period</u>

The mean egg period was estimated using the formula.

$$Mean\ egg\ period = \frac{\sum[(Days\ from\ irradiation\ of\ eggs) \times Number\ of\ eggs\ hatched\ on\ that\ day]}{Total\ number\ of\ eggs\ kept\ for\ hatching}$$

c. Per cent adult emergence

The irradiated eggs were kept further to observe the number of adults emerged. Per cent adult emergence was estimated using the formula

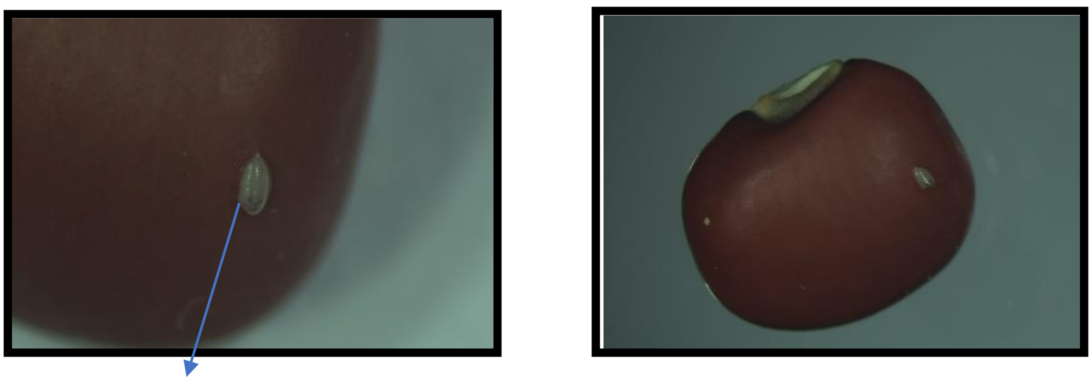
$$Per\ cent\ adult\ emergence = \frac{Total\ number\ of\ adults\ emerged}{Total\ number\ of\ eggs\ kept\ for\ emergence} \times 100$$

3.4.2 Irradiation of grubs

To study the effect of gamma radiation on grub stage and to study susceptibility of different stages of grubs, 7-day-old (mid instar) and 10-day-old (late instar) grubs were subjected to irradiation. Five pre mated adult females were released into each Petri plate having sterilized cowpea grains and allowed to lay eggs. Grains with single egg were collected from the Petri plate daily and observed under a microscope for the development of eggs until all the eggs are hatched. The grubs that were about to hatch out from the eggs exhibited some movements prior to the day of emergence and after hatching, they bored into the grains. The transparent and clear eggs upon hatching, turns yellow and then becomes opaque. Eggs hatched on the same day were kept separately to get grubs of the desired age for irradiation (Plate 4).

On 7^{th} day after egg hatching, the grains containing 7-day-old grubs were exposed to different doses of gamma radiation *viz*., 16 Gy, 18 Gy, 20 Gy, 22 Gy and 24 Gy. Similarly, 10-day-old grubs were also treated with 50 Gy, 75 Gy, 100 Gy, 125 Gy and 150 Gy. Four replications were kept for each treatment, along with an untreated control. A minimum of 10 grains with grubs were kept in each replication. After irradiation, the containers (50 ml) were kept under room temperature for further observation. The irradiated cowpea grains were observed from 11^{th} day after egg hatching as average grub period of pulse beetle is 12 days. The pupal development was confirmed by the presence of a window cut by the last instar grub. Observations were made on mortality of grubs based on number of pupae formed, mean grub period and adult emergence.

Plate 3.a: One-day-old egg



Mouth of the developing egg

Plate 3.b: Three-day-old egg

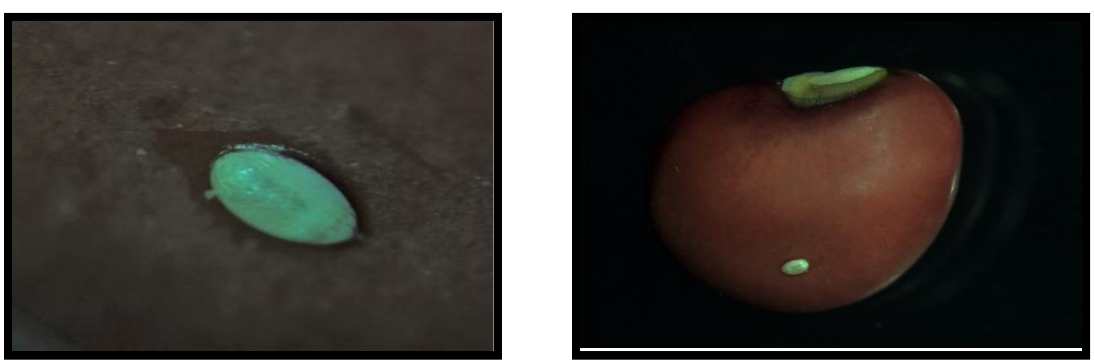


Plate 3.c: Hatched egg

Plate 3. Egg stages of *Callosobruchus maculatus*

Ten-day-old grub (left) and seven-day-old grub (right)

Plate 4. Grub stages of *Callosobruchus maculatus*

a. **<u>Percent pupation</u>**

Per cent pupation was estimated using following formula to infer the mortality of grubs.

$$Per\ cent\ pupation = \frac{Total\ number\ of\ pupae\ formed}{\substack{Total\ number\ of\ grains\ with\ grub \\ kept\ for\ observation}} \times 100$$

a. **<u>Mean grub period</u>**

The mean grub period was estimated using the formula.

$$Mean\ grub\ period = \frac{\substack{\sum[(Days\ from\ hatching\ of\ eggs\ to\ pupation) \times Number\ of \\ pupae\ formed\ on\ that\ day]}}{\substack{Total\ number\ of\ grains\ with\ grub \\ kept\ for\ observation}}$$

b. **<u>Per cent adult emergence</u>**

The number of adults emerged from the irradiated grains with grub were observed for estimating the per cent adult emergence.

$$Per\ cent\ adult\ emergence = \frac{Total\ number\ of\ adults\ emerged}{\substack{Total\ number\ of\ grains\ with\ grub \\ kept\ for\ observation}} \times 100$$

3.4.3. Irradiation of pupae

Four-day-old pupae were selected to study the effect of gamma radiation on the pupal stage (Plate 5). Usually, the last instar grub burrows to a position just underneath the grain coat before pupation and cut a window, which later become the exit hole for the adults.

The development of window was used to confirm the formation of pupa. The cowpea grains having 4-day-old pupae were treated with doses of 50Gy, 75 Gy, 100 Gy, 125 Gy, 150 Gy and were compared with an untreated control. Four replications were maintained for each dose. Each replication contained a minimum of 10 grains with the pupae. After irradiation, the grains were observed for adult emergence and the per cent adult emergence was calculated.

a. **Per cent adult emergence**

$$Per\ cent\ adult\ emergence = \frac{Total\ number\ of\ adults\ emerged}{Total\ number\ of\ grains\ with\ pupa\ kept\ for\ observation} \times 100$$

3.4.4 Irradiation of adults

One-day-old male and female beetles were irradiated separately to study the effect of gamma radiation on different sex forms (Plate 6). Green gram grains in Petri plates were inoculated with 10 to 15 pairs of adult beetles.

After five days, the released adult pairs were removed and Petri plates were incubated to obtain freshly emerged adult females and males. One-day-old adult females and males were separated out, kept in Petri plates, and were irradiated separately with five doses of gamma radiation *viz.*, 100 Gy, 500 Gy, 750 Gy, 1000 Gy and 1500 Gy. Four replications were kept for each doses, and each replication had ten adults.

Untreated adults were kept as control. Mortality of adults were recorded daily till death of the last adult beetle.

a. **Adult longevity**

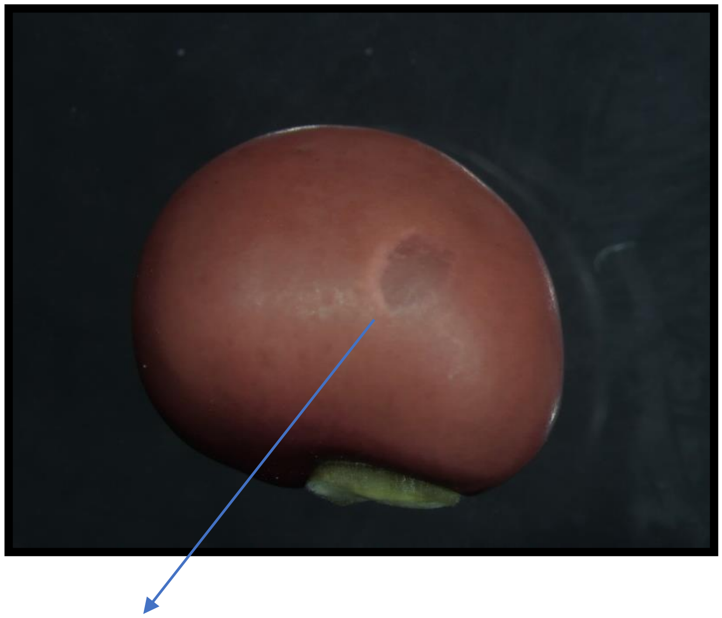
Adult longevity was calculated using the formula.

$$Adult\ longevity\ period = \frac{\sum[(Days\ from\ irradiation\ of\ parents - 1)] \times Number\ of\ dead\ adults\ on\ that\ day}{Total\ number\ of\ dead\ adults}$$

b. **Per cent adult mortality**

The per cent adult mortality was calculated.

$$Per\ cent\ adult\ mortality = \frac{Total\ number\ of\ adults\ dead\ on\ each\ day}{Total\ number\ of\ adults\ dead} \times 100$$



Circular window made by last instar grub

Plate 5.a: Cowpea grain infested with 4-day-old pupae

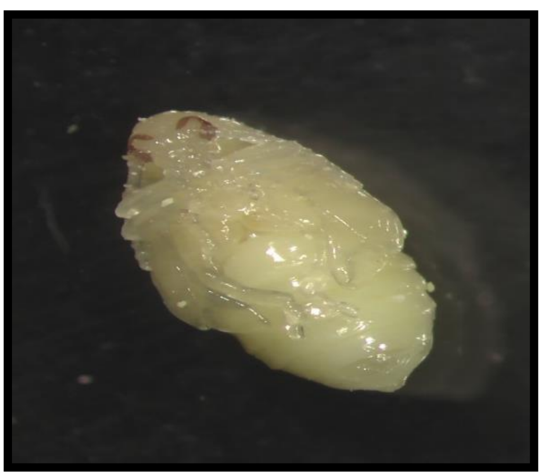


Plate 5.b: Four-day-old pupae

Plate 5.c: Cowpea grain with exit hole

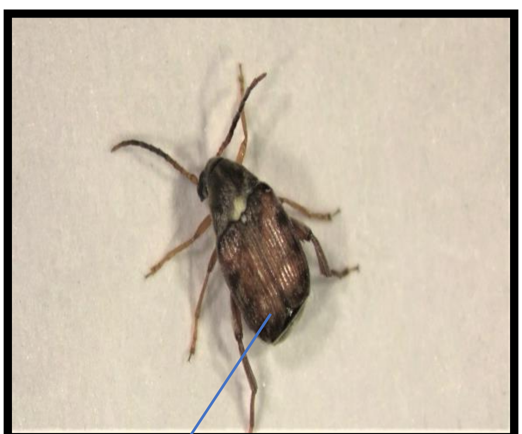
Plate 5. Pupal stage of *Callosobruchus maculatus*

Dorsal view

Lateral view

Plate 6.a: One-day-old female

Pygdium exposed



Dorsal view

Lateral view

Plate 6.b: One-day-old male

Pygdium not exposed

Plate 6. Adults of *Callosobruchus maculatus*

3.5 EFFECT OF GAMMA RADIATION ON F_1 PROGENY

Effect of gamma radiation on the biology of F_1 progeny was studied after irradiating 0-24 hour old virgin adults. To obtain virgin adults, five pre-mated females were released into a Petri plate with a single layer of green gram grains. The females were removed after egg laying. Grains with single egg were removed and placed in individual glass vials and reared to adult emergence. Virgin males and females thus obtained were irradiated separately after exposure to different doses of gamma radiation *viz.,* 16 Gy, 18 Gy, 20 Gy, 22 Gy and 24 Gy.

Five pairs of irradiated adults from each dose was released into a plastic container containing 20g of cowpea grains. The adults were 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 out and kept for observing egg hatchability, number of grubs, number of pupae, per cent adult emergence, total developmental period, adult longevity and sex ratio. Four replications were maintained for each treatment along with an untreated control. The following parameters were worked out from the observations.

a. <u>Egg hatchability</u>

The number of eggs laid and hatched were recorded for five days after irradiation of adult beetles. Grains with single egg were observed under microscope, and the total number of eggs hatched in each replication was counted. Change in the colour of eggs and boring of grubs into the grain confirms egg hatching. Egg hatchability was calculated using the formula (Nagrare and Bhatia, 2001).

$$Egg\ hatchability = \frac{Total\ number\ of\ eggs\ hatched}{Total\ number\ of\ eggs\ laid} \times 100$$

b. <u>Adult emergence</u>

After 20 days of irradiation emergence of adults was observed. Observation was continued on a daily basis until the mortality of the last adult. Per cent adult emergence was estimated Nagrare and Bhatia, (2001).

$$Per\ cent\ adult\ emergence = \frac{Total\ number\ of\ adult\ emerged}{\begin{array}{c}Total\ number\ of\ single\ egg\ grains\\ kept\ for\ emergence\end{array}} \times 100$$

c. Developmental period`

Daily emergence of adults was observed and used for estimating developmental period (Nagrare and Bhatia, 2001).

$$Mean\ developmental\ period = \frac{\begin{array}{c}\sum[(Days\ from\ irradiation\ of\ parents - 1)] \times\\ Number\ of\ adults\ emerged\ on\ that\ day\end{array}}{Total\ number\ of\ adults\ emerged}$$

d. Adult Longevity

The death of F_1 progeny was observed till the death of the last beetle. The recorded data was used for estimating the mean longevity period of F_1 progeny (Nagrare and Bhatia, 2001).

$$Mean\ longevity\ period = \frac{\begin{array}{c}\sum[(Days\ from\ irradiation\ of\ parents - 1)] \times\\ \text{Number of dead adults on that day}\end{array}}{Total\ number\ of\ dead\ adults}$$

Mean adult longevity was estimated by subtracting mean developmental period from mean longevity period.

e. Sex ratio

The sex of adults emerged was determined on the basis of morphological characters. The number of males and females emerged was counted, and the sex ratio was estimated using the following formula to know the effect of radiation on the sex.

$$Sex\ ratio = \frac{Number\ of\ male\ adults}{Number\ of\ female\ adults}$$

3.6 EFFECT OF GAMMA RADIATION ON INFESTED GRAINS

The effect of gamma radiation on cowpea grains infested with different stages of *C. maculatus* was studied after exposing cow pea grains infested with different developmental stages of pulse beetle. Twenty grains with one-day-old eggs, twenty grains with 7-day-old grubs and twenty one-day-old adult in 1:1 sex ratio were released into a 500 grams of Cowpea lot. The grain lots were incubated for ten days.

Each grain lot was irradiated separately with five different doses of gamma radiation *viz.*, 20 Gy, 40 Gy, 60 Gy, 80 Gy and 100 Gy. Four replications were maintained for each dose along with an untreated control. The released adults were removed after 10 days of irradiation and the number of adults emerged from the grain lot was observed daily till the adult emergence had stopped.

3.7 EFFECT OF IRRADIATION ON NUTRITIONAL FACTORS OF COWPEA GRAINS

The nutritional factors such as total carbohydrate and protein content of irradiated cowpea grains were analyzed after exposing grains to different doses *viz.*, 20 Gy, 40 Gy, 60 Gy, 80 Gy and 100 Gy of gamma radiation and compared with control. Total carbohydrate was estimated using anthrone reagent method (AOAC, 1980), and total protein content was estimated using Lowry's method (Sadasivam and Manickam, 1992).

3.7.1 Estimation of total carbohydrate

Anthrone reagent method was used to estimate total carbohydrate content in irradiated and non-irradiated cowpea grains. A green coloured product with an absorption maximum at 630 nm will be formed with the addition of anthrone reagent.

<u>Reagents</u>

1. Anthrone reagent – The reagent was prepared fresh before use by dissolving 200 mg anthrone in 100 ml of ice cold 95% H_2SO_4.
2. 2.5 N HCl

<u>Preparation of standard glucose stock</u>

Standard glucose stock was prepared by dissolving 100 mg of glucose in 100 ml of distilled water. From this, working standard was prepared by making up 10 ml of stock to 100 ml of distilled water to obtain a concentration of 0.1 mg of glucose/ml.

Extraction of carbohydrate from the sample

Weigh 100 mg of irradiated cowpea grains into a boiling tube. Hydrolyze it by keeping in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl and cool to room temperature. The samples were neutralized with sodium carbonate until the effervescence ceases. The volume was made up to 100 ml and centrifuged at 10,000 rpm for 5 minutes. The supernatant was collected and 1 ml aliquots were taken for analysis.

Estimation of carbohydrate

From the working standard already prepared 0, 0.2, 0.4, 0.6, 0.8 and 1 ml was taken in test tubes, where 0 serves as blank. All the tubes including sample tube were made up to 1 ml by adding distilled water. Four ml of anthrone reagent was added to all the tubes. The tubes were heated in a boiling water bath for eight minutes. It was cooled rapidly and the green colour was read at 630 nm using spectrophotometer. A standard graph was plotted with concentration on X-axis and absorbance on Y-axis. Carbohydrate content was calculated from the graph, and the carbohydrate content per 100g grain was calculated using the formula.

$$Total\ carbohydrate\ in\ 100\ mg\ of\ sample = \frac{mg\ of\ glucose}{volume\ of\ the\ sample} \times 100$$

3.7.2 Estimation of total protein

Lowry’s method of protein estimation was used to study protein content in irradiated and non-irradiated grains. Protein contains amino acids like tyrosine and tryptophan will form a blue-purple complex with Folins-ciocalteau reagent, having maximum absorption at 660 nm.

Reagents

1. Reagent A: 50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solution (0.4 g of NaOH in 100 of distilled water)
2. Reagent B: 10 ml of 1.56% copper sulphate solution mixed with 10 ml of 2.37% sodium potassium tartrate solution.
3. Reagent C: Mix 2 ml of reagent B with 100 ml of reagent A.

4. Folins- ciocalteau reagent: Dilute the commercial reagent (2N) in distilled water at 1:1 ratio.
5. Saturated lead acetate solution
6. 10% Potassium hydroxide.
7. 10% Trichloroacetic acid

Preparation of Standard BSA stock

Standard bovine serum albumin stock solution was prepared by dissolving 100 mg of bovine serum albumin in 100 ml of distilled water. A working standard with a concentration of 0.2 mg protein/ ml was prepared by making up 20 ml of stock solution to 100 ml of distilled water.

Extraction of protein

The irradiated cowpea grains were ground into a powder. The powdered sample (0.1 mg) was dissolved in 10 ml of distilled water. The sample was centrifuged, and the supernatant was used for further analysis. Equal quantities of saturated lead acetate and 10% potassium hydroxide were added drop wise to the supernatant till the white precipitate persist. The supernatant was centrifuged at 10000 rpm for 5 minutes and was decanted. Again the supernatant was centrifuged for 5 minutes at 10000 rpm after adding 10% TCA. Finally, the supernatant was decanted, and 1 ml of distilled water was added to it. Thus obtained protein extract were further used for estimation.

Estimation of protein

Protein standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard of BSA, where 0 serves as blank. Pipette out 0.1 ml of sample for estimation. All the tubes, including the sample tube, was made up to 1 ml by adding distilled water. To each test tube, 5ml of alkaline copper sulphate solution was added. Tube was mixed well and kept for 10 minutes. Folins-ciocalteau reagent (5 ml) was added to each tube and mixed well. Tubes were kept in the dark for 30 minutes until the development of blue colour. Absorbance was read at 660 nm using a spectrophotometer. Standard graph was plotted with the concentration on X-axis and absorbance on Y-axis.

$$Total\ protein\ in\ 100\ mg\ of\ sample = \frac{concentration\ of\ protein}{volume\ of\ the\ sample} \times 100$$

3.8 ANALYSIS OF DATA

The data obtained in the experiments were analyzed in completely randomized design using statistical package, SPSS 16.0 after required transformation.

Result

4. RESULT

The effectiveness of gamma radiation for the management of pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) is presented in this chapter under the following headings.

1. Effect of gamma radiation on different developmental stages of *Callosobruchus maculatus*
2. Effect of gamma radiation on reproduction of *Callosobruchus maculatus*
3. Effect of gamma radiation on cowpea grains infested with different stages of *Callosobruchus maculatus*
4. Effect of radiation on nutritional factors of irradiated food.

4.1 EFFECT OF GAMMA RADIATION ON DIFFERENT DEVELOPMENT STAGES OF *Callosobruchus maculatus*

Cowpea grains with different life stages of *C. maculatus* were irradiated to determine the susceptibility of these stages to various doses of gamma radiation.

4.1.1 Effect of gamma radiation on one-day-old eggs of *Callosobruchus maculatus*

Irradiation at 16, 18, 20, 22 and 24 Gy of gamma radiation, along with untreated control, assessed the toxicity of gamma radiation to one-day-old eggs. Mean egg period, per cent egg hatchability and per cent adult emergence was recorded (Table 1). None of the treated eggs were hatched out and hence, calculations were not made on mean egg period, per cent egg hatchability and per cent adult emergence for all the doses of gamma radiation. In control, mean egg period was 5.25 days, hatchability was 97.50 per cent, and adult emergence was 47.50 per cent.

Table 1. Effect of gamma radiation on one-day-old eggs of *Callosobruchus maculatus*

Treatments (Gy)	Mean egg period (days)	Egg hatchability (%)	Adult emergence (%)
16	0.00	0.00	0.00
18	0.00	0.00	0.00
20	0.00	0.00	0.00
22	0.00	0.00	0.00
24	0.00	0.00	0.00
Control	5.25	97.50	47.50

Table 2. Effect of gamma radiation on three-day-old eggs of *Callosobruchus maculatus*

Treatments (Gy)	Mean egg period (days)	Egg hatchability (%)	Adult emergence (%)
16	5.11[a]	47.50[a] (0.76)*	0.00
18	5.11[a]	45.00[a] (0.74)*	0.00
20	5.08[a]	32.50[a] (0.61)*	0.00
22	5.07[a]	37.50[a] (0.66)*	0.00
24	5.36[b]	35.00[a] (0.63)*	0.00
Control	5.15[a]	85.00[b] (1.17)*	52.50

*Figures in the parentheses are arc sine transformed values

In vertical columns, means followed by same alphabets do not differ significantly by DMRT (P = 0.05)

4.1.2 Effect of gamma radiation on three-day-old eggs of *Callosobruchus maculatus*

Cowpea grains with three-day-old single egg were irradiated at 16, 18, 20, 22 and 24 Gy along with an untreated control to assess the susceptibility of older eggs to gamma radiation.

Mean egg period in control was 5.15 days. Exposure to gamma radiation at the highest dose of 24 Gy resulted in significant increase in egg period over control (Table 2). Mean egg period was 5.11 days both at 16 Gy and 18 Gy. When irradiated with 20 Gy, egg period was 5.08 days, and with 22 Gy it was 5.07 days. Egg period in control was 5.15 days. All the treatments, including control, were statistically on par to each other except for 24 Gy (5.36 days).

Exposure to gamma radiation significantly decreased the egg hatchability. Egg hatchability was 85 per cent in control and it decreased to 47.50 per cent at 16 Gy and further decreased to 45, 32.50, 37.50and 35 per cent at 18, 20, 22 and 24 Gy of gamma radiation, respectively. Egg hatchability was significantly lower in all the treatments compare to control but there was no significant difference among various doses of radiation. There was no adult emergence from irradiated eggs. Whereas, the adult emergence in control was 52.50 per cent.

4.1.3 Effect of gamma radiation on seven-day-old grubs of *Callosobruchus maculatus*

Exposure of seven-day-old grubs to gamma radiation at 16, 18, 20, 22, 24 Gy and untreated control resulted in mean grub period of 12.21, 12.13, 12.00, 12.00, 12.00 and 12.03 days, respectively. Mean grub period was highest in 16 Gy, which was on par with mean grub period in control and 18 Gy. All the treatments, including control, were statistically on par with each other, except 16 Gy.

Per cent pupation of seven-day-old grubs irradiated with 24 Gy was 15 per cent which was significantly different when compared to 82.50 per cent in untreated control. Per cent pupation at radiation doses 16, 18 and 20 Gy significantly differed from

Table 3. Effect of gamma radiation on seven-day-old grubs of *Callosobruchus maculatus*

Treatments (Gy)	Mean grub period (days)	Pupation (%)	Adult emergence (%)
16	12.21[b]	35.00[b] (0.63)*	0.00
18	12.13[ab]	37.50[b] (0.66)*	0.00
20	12.00[a]	30.00[b] (0.58)*	0.00
22	12.00[a]	22.50[ab] (0.49)*	0.00
24	12.00[a]	15.00[a] (0.40)*	0.00
Control	12.03[ab]	82.50[c] (1.14)*	82.50

*Figures in the parentheses are arc sine transformed values

In vertical columns, means followed by same alphabets do not differ significantly by DMRT (P = 0.05)

all other treatments with per cent pupation of 35.00, 37.50 and 30.00 per cent, respectively. At 22 Gy, per cent pupation was 22.50 per cent which is on par with all the other treatments except control.

No adults emerged from survived pupae in any of the treatments with any of the gamma radiation doses. However, an average adult emergence of 82.50 per cent was recorded in control.

4.1.4 Effect of gamma radiation on ten-day-old grubs of *Callosobruchus maculatus*

The mean grub period of ten-day-old grub was slightly affected with an increase in gamma radiation doses. The mean grub period was 12.43 days in untreated control and was highest to all irradiation treatments. Exposure o to 100 Gy and 125 Gy reduced mean grub period to 12.00 days which is significantly different from control. But the treatment with 50 Gy, 75 Gy and 150 Gy were on par with other treatments. The mean grub period was 12.25, 12.05 and 12.31 at radiation doses 50 Gy, 75 Gy and 150 Gy, respectively.

The gamma radiation also affected further development of 10-day-old grubs into pupa. Exposure to 50 Gy and 75 Gy resulted in 47.50 per cent pupation followed by 40.00per cent, 35.00 per cent and 30.00 per cent at 100, 125 and 150 Gy, respectively. All the treatments were significantly different from untreated control, where the per cent pupation was 92.50 per cent. The percent pupa developed into adults were also significantly decreased with the increase in doses. No adults were emerged from ten-day-old grubs when irradiated at doses 50 Gy and above, which is significantly different from control where adult emergence was 90.00 per cent.

4.1.5 Effect of gamma radiation on four-day-old pupae of *Callosobruchus maculatus*

Irradiation of cowpea grains infested with four-day-old pupae at 50, 75, 100, 125 and 150 Gy significantly affected adult emergence (Table 5). Irradiation at doses 100 Gy and above caused cent per cent mortality of pupae, as there was no adult emergence.

Table 4. Effect of gamma radiation on ten-day-old grubs of *Callosobruchus maculatus*

Treatments (Gy)	Mean grub period (days)	Pupation (%)	Adult emergence (%)
50	12.25[ab]	47.50[a] (0.76)*	0.00
75	12.05[ab]	47.50[a] (0.76)*	0.00
100	12.00[a]	40.00[a] (0.68)*	0.00
125	12.00[a]	35.00[a] (0.63)*	0.00
150	12.31[ab]	30.00[a] (0.58)*	0.00
Control	12.43[b]	92.50[b] (1.29)*	90.00

*Figures in the parentheses are arc sine transformed values

In vertical columns, means followed by same alphabets do not differ significantly by DMRT (P = 0.05)

Table 5. Effect of gamma radiation on four-day-old pupae of *Callosobruchus maculatus*

Treatments (Gy)	Adult emergence (%)
50	40.00[c] (0.68)*
75	20.00[b] (0.46)*
100	0.00[a] (0.16)*
125	0.00[a] (0.16)*
150	0.00[a] (0.16)*
Control	87.50[d] (1.21)*

* Figures in the parentheses are arc sine transformed values

In vertical columns, means followed by same alphabets do not differ significantly by DMRT (P = 0.05)

Adult emergence was 20.00 per cent at 75 Gy, which was significantly lower than 40.00 per cent adult emergence at 50 Gy. Adult emergence in control was 87.50 per cent, which was significantly higher when compared to irradiation at various doses.

4.1.6 Effect of gamma radiation on one-day-old adult females of *Callosobruchus maculatus*

On day 1, there was no mortality in control, whereas, per cent mortality was 2.50, 17.50, 22.50, 12.50 and 22.50 per cent at 100, 500, 750, 1000 and 1500 Gy of doses, respectively (Table 6). Mortality at 500, 750 and 1500 Gy of doses was significantly higher as compare to control. Adult females when irradiated at 100 and 1000 Gy resulted in mortality of 2.50 and 12.50 percent, respectively, but they were statistically on par with control.

After two days of irradiation, mortality was significantly higher at 40.00 per cent at the highest dose of 1500 Gy. There was no mortality in control on day two also, but it was at par with all the other treatment doses except 1500 Gy. Mortality of one-day-old adult females when irradiated at 100 Gy, 500 Gy, 750 Gy and 1000 Gy was 2.50 per cent, 5.00 per cent, 10.00 per cent, and 7.50 per cent, respectively.

After three days of exposure of one-day-old adult females to irradiation, there was a significantly higher mortality of 42.50 per cent and 37.50 per cent at 1000 Gy and 1500 Gy, respectively, but, they were statistically on par to each other. Mortality at all the other doses, including control, was at par with each other. Mortality was 12.50 per cent, 5.00 per cent, 2.50 per cent and 2.50 per cent at 100 Gy, 500 Gy, 750 Gy, and control, respectively.

Mortality was 7.50 per cent, 2.50 per cent at 100 Gy and 500 Gy respectively, after four days of irradiation, which were statistically on par among each other. No mortality was observed in control. But significantly higher mortality of32.50 per cent and 37.50 per cent was observed at higher doses of 750 Gy and 1000 Gy, respectively.

There observed 10.00 per cent mortality in control at fifth day which was significantly lower than the mortality at 500 Gy and 750 Gy. Mortality was 20.00 per cent, 30.00 per cent and 32.50 per cent at 100 Gy, 500 Gy and 750 Gy, respectively, which were at par with each other.

After six days of irradiation, there was no significant difference between control and irradiation treatments at doses of 100 Gy and 500 Gy. Mortality was 17.50 per cent both at 100 and 500 Gy and it was 10.00 per cent in control.

There was a significantly higher mortality of 20.00 per cent and 22.50 per cent at 100 Gy and 500 Gy, respectively at seventh day and it was significantly different from control, which recorded 5.00 per cent mortality.

Mortality was 17.50 per cent after eight days of irradiation, which was significantly higher to control (5.00 per cent). All the irradiated adults died by 8th day, but adults survived upto 13 days in control. Mortality in control was 10.00, 17.50, 7.50, 15.00 and 17.50 per cent after 9, 10, 11, 12 and 13 days, respectively. The adult females survived for 13 days in control compared to 3 days in 1500 Gy. Complete mortality occurred at eight, seven, five and four days respectively in 100 Gy, 500 Gy, 750 Gy and 1000 Gy.

The results on mean adult longevity also revealed the toxicity of gamma radiation in the survival pattern of the adult female. Mean adult longevity period was highest in control (8.47 days), which is significantly superior over irradiation treatments (Table 7). There was a dose-dependent decrease in mean adult longevity with increase in the dose of irradiation. Mean adult longevity statistically differed from each other in all the treatments except at 750 Gy and 1000 Gy which were at par with each other.

4.1.7 Effect of gamma radiation on one-day-old adult males of *Callosobruchus maculatus*

Exposure of one-day-old malesto gamma radiation significantly affected the survival of adult males (Table 9).On the first day after irradiation, mortality was 7.50 per cent at 100 Gy, but it was at par with control in which no mortality was observed for two days.

Mortality was 15.00 per cent both at 500 Gy and 1000 Gy. There was 10.00 per cent mortality at 1500 Gy which was at par with 100 Gy, 500 Gy and 1000 Gy. Mortality was 22.50 per cent at an irradiation dose of 750 Gy, which was significantly higher but at par with mortality at 500 Gy.

On day two, mortality at 1500 Gy was 35.00 per cent which was significantly higher than all other treatments. Mortality at radiation doses of 100 Gy, 500 Gy, 750 Gy, 1000 Gy and control are at par with each other. Mortality was 7.50 per cent at 500 Gy, 750 Gy and 1000 Gy, 2.50 per cent at 100 Gy, which were at par with each other. Though there was no mortality in control, it was not significantly different from that in various irradiation doses, except for the highest dose of 1500 Gy.

All the adult males died by third day at the highest dose of 1500 Gy. Mortality was 10.00 per cent in control, which was at par with mortality at 100 Gy, 500 Gy and 750 Gy. At lowest two doses, 100 Gy and 500 Gy, mortality was 5.00 per cent, and it increased to 12.50 per cent at 750 Gy. At 1000 Gy, mortality was 25.00 per cent, which was significantly superior to the two lower doses, 100 Gy and 500 Gy but not differed significantly from mortality at 750 Gy.

Per cent mortality was maximum at 1000 Gy (50.00%) on day four, followed by 750 Gy (32.50%) and were on par, but differed significantly from remaining doses. Mortality at lower two doses 100 Gy (22.50%) and 500 Gy (20.00%) were on par with that of 750 Gy as well as control (12.50%). Though mortality was less (12.50%) in control, it was at par with mortality at 100 Gy and 500 Gy.

All the adults exposed to 750 Gy and 1000 Gy died by 5th day. However, mortality was 27.50 per cent, 35.00 per cent and 25.00 per cent at 100 Gy, 500 Gy and 750 Gy, respectively and were at par with each other, but significantly higher than other treatments. Mortality was only 10.00 per cent in control, and the mortality at 1000 Gy was 2.50 per cent.

On 6th day, the per cent mortality at 100 and 500 Gy was 22.50 per cent and 17.50 per cent respectively and were on par with each other, but were significantly different from the mortality of 12.50 per cent in control. All the irradiated adults died by 6th in 500 Gy.

Mortality of all the adults was observed by 7th day in all the irradiation treatments. Mortality was 12.50 per cent at 100 Gy and 7.50 per cent in control, which were not significantly different from each other. However, survival of adults after 7th day was observed in control. Per cent mortality in control on 8th, 9th, 10thand 11th day was 10.00 per cent, 17.50 per cent, 15.00 per cent and 10.00 per cent, respectively. The adult males survived for 11 days in control, whereas, the adult males survived only for three days after irradiation with the highest dose of 1500 Gy. Days of survival of adults decreased with an increase in the dose of radiation.

The mean adult longevity indicates the adverse effect of gamma radiation on the survival of adult males (Table 8). Mean adult longevity decreased significantly with increase in the dose of irradiation. The mean adult longevity was 6.25 days in control which was significantly superior to all the irradiation treatments. There was significant difference between all the doses except 750 Gy and1000 Gy, which were statistically on par in mean adult longevity. Longevity was 1.45 days at 1500 Gy, 2.33 days at 1000 Gy, 2.30 days at 750 Gy, 3.05 days at 500 Gy, 3.78 days at 100 Gy and 6.25 days in control.

4.2 EFFECT OF GAMMA RADIATION ON REPRODUCTION OF *Callosobruchus maculatus* ADULTS

Exposure of one-day-old pairs of beetles to gamma radiation doses ranging from 16 Gy to 24 Gy helped to study the effect of gamma radiation on reproductive behaviour *C. maculatus*. There was no significant difference in the fertility as the no. of eggs/ 20 grains were not significantly different among treatments and control (Table 10). The number of eggs/ 20 grains at different treatments, 16 Gy, 18 Gy, 20 Gy, 22 Gy, 24 Gy and control were 19.25, 19.25, 19, 18.75 and 19.75, respectively and were on par with each other. Whereas, egg hatchability was decreased significantly with irradiation. Egg hatchability in control was 95.00 per cent which was significantly superior to all irradiation

Table 6. Mortality of one-day-old adult females at different days after exposure to gamma radiation

Treatments (Gy)	Mortality (%)												
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**
100	2.50^{ab} (0.16)*	2.50^{a} (0.16)*	12.50^{a} (0.36)*	7.50^{a} (0.28)*	20.00^{bc} (0.46)*	17.50^{b} (0.43)*	20.00^{b} (0.46)*	17.50^{b} (0.43)*	-	-	-	-	-
500	17.50^{bc} (0.43)*	5.00^{a} (0.23)*	5.00^{a} (0.23)*	2.50^{a} (0.16)*	30.00^{c} (0.58)*	17.50^{b} (0.43)*	22.50^{b} (0.49)*	-	-	-	-	-	-
750	22.50^{c} (0.49)*	10.00^{a} (0.32)*	2.50^{a} (0.16)*	32.50^{b} (0.61)*	32.50^{c} (0.61)*	-	-	-	-	-	-	-	-
1000	12.50^{abc} (0.36)*	7.50^{a} (0.28)*	42.50^{b} (0.71)*	37.50^{b} (0.66)*	-	-	-	-	-	-	-	-	-
1500	22.50^{c} (0.49)*	40.00^{b} (0.68)*	37.50^{b} (0.66)*	-	-	-	-	-	-	-	-	-	-
Control	0.00^{a} (0.16)*	0.00^{a} (0.16)*	2.50^{a} (0.16)*	0.00^{a} (0.16)*	10.00^{ab} (0.32)*	10.00^{ab} (0.32)*	5.00^{a} (0.23)*	5.00^{a} (0.23)*	10.00^{b} (0.32)*	17.50^{b} (0.43)*	7.50^{b} (0.28)*	15.00^{b} (0.40)*	17.50^{b} (0.43)*

*Figures in the parentheses are arc sine transformed values

In vertical columns, means followed by same alphabets do not differ significantly by DMRT (P = 0.05)

Table 7. Mean adult longevity of one-day-old females of *Callosobruchus maculatus* exposed to different doses of gamma radiation

Treatments (Gy)	Mean adult longevity (days)
100	4.60^{d}
500	3.65^{c}
750	2.43^{b}
1000	2.05^{b}
1500	1.15^{a}
Control	8.47^{e}

* In vertical columns, means followed by same alphabet do not differ significantly by DMRT (P = 0.05)

Table 8. Mean adult longevity of one-day-old males of *Callosobruchus maculatus* exposed to different doses of gamma radiation

Treatments (Gy)	Mean adult longevity (days)
100	3.78^{d}
500	3.05^{c}
750	2.30^{b}
1000	2.33^{b}
1500	1.45^{a}
Control	6.25^{e}

* In vertical columns, means followed by same alphabet do not differ significantly by DMRT (P = 0.05)

Table 9. Mortality of one-day-old adult males at different days after exposure to gamma radiation

Treatments (Gy)	Mortality (%)										
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
100	7.50[ab] (0.28)*	2.50[a] (0.16)*	5.00[a] (0.23)*	22.50[bc] (0.49)*	27.50[b] (0.55)*	22.50[b] (0.49)*	12.50[a] (0.36)*	-	-	-	-
500	15.00[bc] (0.40)*	7.50[a] (0.28)*	5.00[a] (0.23)*	20.00[bc] (0.46)*	35.00[b] (0.63)*	17.50[b] (0.43)*	-	-	-	-	-
750	22.5[c] (0.49)*	7.50[a] (0.28)*	12.50[ab] (0.36)*	32.50[cd] (0.61)*	25.00[b] (0.52)*	-	-	-	-	-	-
1000	15.00[bc] (0.40)*	7.50[a] (0.28)*	25.00[b] (0.52)*	50.00[d] (0.79)*	2.50[a] (0.16)*	-	-	-	-	-	-
1500	10.00[b] (0.32)*	35.00[b] (0.63)*	55.00[c] (0.84)*	-	-	-	-	-	-	-	-
Control	0.00[a] (0.16)*	0.00[a] (0.16)*	10.00[a] (0.32)*	12.50[ab] (0.36)*	10.00[a] (0.32)*	7.50[a] (0.28)*	7.50[a] (0.28)*	10.00[a] (0.32)*	17.50[a] (0.43)*	15.00[a] (0.40)*	10.00[a] (0.32)*

*Figures in the parentheses are arc sine transformed values

In vertical columns, means followed by same letter do not differ significantly by DMRT (P = 0.05) treatments. The egg hatchability was 3.75 per cent, 2.50 per cent, 1.25 per cent, 1.25 per cent and 0.00 per cent at radiation doses of 16, 18, 20, 22 and 24, respectively. As a result of the effect of gamma radiation on egg hatchability, the number of grubs/ 20 grains was also decreased significantly in the irradiation treatments. The number of grubs/ 20 grains at 16, 18, 20, 22 and 24 were 0.75, 0.50, 0.25, 0.25, and 0.00 respectively compared to 19.00 in control.

Survived grubs failed to develop further and hence, there are no observations for the number of pupae/20 grains, adult emergence, sex ratio, developmental period and adult longevity. Whereas, in control, number of pupae/ 20 grains, per cent adult emergence, adult longevity, developmental period and sex ratio were 16.75, 81.25 per cent, 7.49 days, 23.85 days and 0.75, respectively.

4.3 EFFECT OF GAMMA RADIATION ON COWPEA GRAINS INFESTED WITH DIFFERENT STAGES OF *Callosobruchus maculatus*

Adult emergence, after 15 days of irradiation, from grain lots infested with different stages of *C. maculatus* irradiated at 20, 40, 60, 80 and 100 Gy, along with an untreated control is given in Table 11.

Results indicate that the number of beetles emerged significantly decreased with increase in dose of gamma radiation. The number of adults emerged after 15 days of radiation was 190 in control which was significantly different from all irradiation doses. Further, adult emergence at doses 80 Gy and 100 Gy were significantly lower from other irradiation treatments at 20, 40 and 60 Gy. The number of adults emerged at 20, 40, 60, 80 and 100 Gy were 42.25, 41.25, 41.25, 31.25 and 25.20 adults, respectively. After 15 days, there was no adult emergence in any of the irradiation treatments. The number of adults emerged in control 16 days and 17 days after irradiation were 218.50 and 129.00 adults, respectively.

Table 10. Effect of gamma radiation on reproduction of *Callosobruchus maculatus*

Treatments (Gy)	No of eggs/ 20 grains	Egg hatchability (%)	No of grubs/ 20 grains	No of pupae/ 20grains	Adult emergence (%)	Sex ratio (M:F)	Developmental 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

*Figures in the parentheses are arc sine transformed values

In vertical columns, means followed by same letter do not differ significantly by DMRT (P = 0.05)

Table 11. Adult emergence from cowpea grains infested with different stages of *Callosobruchus maculatus*

Treatments (Gy)	No. of adults emerged		
	15 DAI	16 DAI	17 DAI
20	42.25^{b}	0^{a}	0^{a}
40	41.25^{b}	0^{a}	0^{a}
60	41.25^{b}	0^{a}	0^{a}
80	31.25^{a}	0^{a}	0^{a}
100	25.2^{a}	0^{a}	0^{a}
Control	190^{c}	218.5^{b}	129^{b}

In vertical columns, means followed by same letter do not differ significantly by DMRT (P = 0.05)
DAI –Days after irradiation

4.4 EFFECT OF GAMMA RADIATION ON NUTRITIONAL FACTORS OF COWPEA GRAINS

Carbohydrate and protein content of irradiated cowpea grains were estimated to study the effect of gamma radiation on nutritional factors of cowpea grains.

4.4.1 Effect of gamma radiation on the carbohydrate content of cowpea grains

Cowpea grains were subjected to gamma irradiation at doses of 20, 40, 60, 80 and 100 Gy and were compared with an untreated control. The result suggested that carbohydrate content was not significantly different from that of control. Carbohydrate content in all the irradiation doses were on par with the untreated control. Carbohydrate content in control was 49.59 per cent while it was 48.46 per cent, 49.86 per cent, 49.96 per cent, 48.90 per cent and 48.46 per cent at 20 Gy, 40 Gy, 60 Gy, 80 Gy and 100 Gy, respectively.

4.4.2 Effect of gamma radiation on the protein content of cowpea grains

To determine the effect of gamma radiation on protein content, cowpea grains were irradiated with 20, 40, 60, 80 and 100 Gy of gamma radiation and were compared with an untreated control. None of the treatments caused any significant difference in protein content with respect to control. Protein content in control was 24.85 per cent which was on par with all irradiation doses. The protein content was 24.45 per cent, 23.39 per cent, 23.76 per cent, 24.93 per cent and 21.37 per cent at 20, 40, 60, 80 and 100 Gy, respectively.

Table 12. Effect of gamma radiation on the carbohydrate content of cowpea grains

Treatments (Gy)	Carbohydrate content (%)
20	48.46[a]
40	49.86[a]
60	49.96[a]
80	48.90[a]
100	48.46[a]
Control	49.59[a]

In vertical columns, means followed by same alphabet do not differ significantly by DMRT (P = 0.05)

Table 13. Effect of gamma radiation on the protein content of cowpea grains

Treatments (Gy)	Protein content (%)
20	24.45[a]
40	23.99[a]
60	23.76[a]
80	24.93[a]
100	21.37[a]
Control	24.85[a]

In vertical columns, means followed by same alphabet do not differ significantly by DMRT (P = 0.05)

Discussion

5. DISCUSSION

Different stages of pulse beetle, *Callosobruchus maculatus* exhibited variation in susceptibility to gamma radiation. Within a stage itself, there was variation in sensitivity to gamma radiation. This chapter discusses the results obtained in the current study with the already reported data under the following headings.

5.1 Variation in the susceptibility of different stages of *Callosobruchus maculatus* to gamma radiation

5.2 Effect of gamma radiation on progeny production of *Callosobruchus maculatus*

5.3 Effectiveness of gamma radiation in protecting grains infested with different stages of *Callosobruchus maculatus*

5.4 Impact of radiation on nutritional factors of treated grains

5.1 VARIATION IN THE SUSCEPTIBILITY OF DIFFERENT STAGES OF *Callosobruchus maculatus* TO GAMMA RADIATION

Susceptibility of egg, larva, pupa and adult stages to gamma radiation varied between stages and even within a stage itself (Table14).

5.1.1 Susceptibility of *Callosobruchus maculatus* eggs to gamma radiation

Mortality of one-day-old eggs was 100 per cent at doses (16 to 24 Gy) used for irradiation. In three-day-old eggs, mortality varied from 52.50 per cent at 16 Gy to 65 per cent at 24 Gy (Table 14). Mortality was 55, 67.50 and 62.50 per cent at 18, 20 and 22 Gy, respectively. In control, the mortality was only 15 per cent, which was significantly lower than all treatments. There was no significant difference in mortality among different radiation doses.

Ghogomu (1990) reported that one-day-old eggs of *C. maculatus* irradiated at 10 Gy didn’t hatch, while three-day-old eggs required a higher dose of 200 Gy to prevent

Table 14. Mortality of different stages of *Callosobruchus maculatus* at different doses of 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

*Figures in the parentheses are arc sine transformed values

In vertical columns, means followed by same alphabet do not differ significantly by DMRT (P = 0.05) hatching. Similar to the result of the current study, Elbadry and Ahmed (1975) reported that, at 10 Gy, there was complete inhibition of hatching of newly laid eggs of *C. maculatus* but 100 per cent mortality of older eggs (5-day-old) occurred at 80 Gy. Dongre *et al*. (1997) reported that zero to 24-h-old eggs of *Callosobruchus maculatus* didn't hatch at 10 Gy, whereas, hatching was normal in three-day-old eggs at 10 Gy. But Olaifa *et al*., (1990) reported hundred per cent inhibition of hatching of two-day-old eggs of *C. maculatus* at a dose of 1 Gy itself.

The results of study on gamma radiation with eggs of *C. chinesis* also matched with the present findings. At 16 Gy, there was no hatching of one-day-old eggs of *C. chinensis,* while hatching of three-day-old eggs was 51 per cent (Hussain and Imura, 1989). . But, Supawan *et al*., (2005) reported a higher dose of 180 Gy to cause total mortality of 4-day-old eggs of *C. chinensis*.

In yet another study on hatchability of eggs of *C. chinensis* irradiated at a dose ranging from 1 Gy to 16 Gy, there was no hatching of eggs at 16 Gy with one-day and two-day-old eggs. However, tolerance to radiation increased with the age of the egg, and even a dose of 1024 Gy could not completely inhibit the hatching of five-day and six-day-old eggs (Kumagai, 1969).

Hatching of eggs of *Oryzaephilus surinamensis* was 52.20 per cent at 20 Gy (Hosseinzadeh *et al.* 2010). But the eggs of another tenebrionid storage pest, *Tribolium castaneum* was more tolerant to radiation in comparison to *O. surinamensis.* Even at a dose of 500 Gy, only 48.90 per cent eggs developed into larvae (Brower and Tilton, 1973).

Eggs of the lepidopteran storage pest, almond moth, *Cadra cautella* was more resistant to gamma radiation as 64.20 per cent eggs developed into larvae at 100 Gy (Cogburn *et al.,* 1973). As in *Cadra cautella,* eggs (1 to 24 h old) of another lepidopteran storage pest, Indian meal moth, *Plodia interpunctella* required a higher dose (400 Gy) to prevent the hatching of eggs (Hosseinzadeh *et al.,* 2011). Hatchability of 1 to three-day-old eggs of *Ephestia kuehniella* was higher when compared to *C. maculatus.* At a dose of 400 Gy, hatching of *Ephestia kuehniella* eggs was 26.6 per cent (Ayvaz and Tuncbilek, 2006).

Egg of *Dermestes maculatus* was less radiosensitive in comparison to *Callosobruchus sp.* as 47.5 per cent of one-day-old eggs hatched when irradiated at 50 Gy (Seal and Tilton, 1986). Hatching of egg in depressed flour beetles, *Palorus subdepressus*, decreased with increase in radiation doses and hatchability was 13.3 per cent at 10 Krad (100 Gy) and 11.1 per cent at 20 Krad (200 Gy) (Brower, 1973a). Irradiation of eggs of sweet potato weevil, *Cylas formicarius elegantulus* at 50 Gy completely inhibited the hatching of 1, 3 and 5-day-old eggs (Dawes *et al*., 1987). Compared to insect pests, eggs of acarine storage pest, *Acarus siro* were extremely resistant to gamma radiation as 18.3 per cent eggs hatched at a dose of 1000 Gy (Burkholder *et al.*, 1966). Hence, radiosensitivity of insect eggs varies with the species of insects.

The mean egg period significantly increased with an increase in the dose of radiation. At the highest dose of 24 Gy, mean egg period significantly increased to 5.36 days over other treatments, including control. Similar to these results, Soumya (2015) also reported a significant increase in the incubation period of two-day-old eggs of *C. chinensis* with 6 days in eggs treated with a dose of 15 Gy in comparison to 4.5 days in control. Similar findings were there on the record in studies with *Plodia interpunctella* at a dose 200 Gy (Hosseinzadeh *et al.,* 2011) and *Ephestia kuehniella* at an irradiation dose of 100 Gy (Ayvaz and Tuncbilek, 2006).

As in the present study, Ghogomu (1990) also reported that treating one and three-day-old eggs of *C. maculatus* with 10 and 20 Gy resulted in zero adult emergences. According to Elbadry and Ahmed (1975) observed adult emergence from one-day-old eggs of *C. maculatus* irradiated at a dose below 7.5 Gy. While, with five-day-old eggs, there was adult emergence even at a higher dose of 10 Gy. There was no adult emergence in the present study with three-day-old eggs of *C. maculatus* as a higher dose is used in comparison to the study of Elbadry and Ahmed (1975). Irradiation of one-day-old eggs of *C. chinensis* at a much lower dose of 1 and 2 Gy resulted in 81.1% and 67.1% adult emergence (Kumagai, 1969). However, there was no adult emergence at doses16 Gy and above, which is in accordance with the present study.

Irradiation of eggs of almond moth, *Cadra cautella* at a dose of 50 Gy resulted in 24.6 per cent adult emergence (Cogburn *et al.*, 1973). Likewise, Hosseinzadeh *et al.*

(2011) reported adult emergence from one to two-day-old eggs irradiated at 300 Gy. This shows that radiosensitivity varies between different species of insects.

The results prove that increase in irradiation dose increases mortality of eggs of *C. maculatus.* However, as age of the eggs increases, susceptibility to gamma radiation decreases. Besides, the developmental period of eggs also get extended with increase in irradiation doses.

5.1.2 Susceptibility of *Callosobruchus maculatus* grubs to gamma radiation

Early instar grub (7-day-old) was more radiosensitive compared to mature grub (Table 14). Radiation doses ranging from 16 to 24 Gy resulted in significantly higher mortality of seven-day-old grubs over control. Mortality of grubs was 65 per cent at 16 Gy, 62.50 per cent at 18 Gy, 70.00 per cent at 20 Gy and 77.50 per cent at 22 Gy. Mortality was 85.00 per cent at highest dose of 24 Gy, which was significantly higher over all other doses, except 22 Gy. The mortality in control was 17.50 per cent which was significantly lower in comparison to irradiation treatments. As the age of the grub increases to 10 days, a higher dose of 50 Gy was required to cause 52.50 per cent mortality.

Mortality of ten-day-old grubs of *C.chinensis* was 27.37 per cent at 100 Gy, whereas there was 100 per cent mortality with dose 800 Gy (Supawan, 2005). In another study, with seven-day-old grubs, mortality was complete at 40 Gy, but for ten-day-old grubs, total mortality occurred with doubling of dose to 80 Gy (Hussain and Imura, 1989).

In comparison to pulse beetles, 15-day-old larvae of saw-toothed grain beetle, *Oryzaephilus surinamensis* required a higher doses to cause significant mortality. At 350 Gy, the mortality of larvae was only 85 per cent (Hosseinzadeh *et al.,* 2010). Response of 15-day-old larvae of *Plodia interpunctella* was similar to that of larvae of *O. surinamensis,* as the mortality was 81.87 per cent at 350 Gy (Hosseinzadeh *et al.,* 2011). But larvae of *Tribolium castaneum* were more susceptible to radiation as only 3.89 per cent larvae survived a dose of 40 Gy (Tunçbilek *et al.* 2003).

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.* Mortality of 1 to 1.5 days old larvae was 85.30 per cent at 150 Gy, which decreased significantly to 72.30 per cent when the age of larvae was progressed to 12 to 12.5 days. Fifteen-day-old almond moth larva, *Cadra cautella* successfully pupated at the irradiation dose 50 and 100 Gy, whereas, an increase of dose above 200 Gy resulted in complete mortality of the larvae (Cogburn *et al.*, 1973).

Hosseinzadeh *et al.* (2011) irradiated *P. interpunctella* larvae at doses ranging from 50 to 350 Gy, and there was no significant difference between larval duration at doses ranging from 0 to 100 Gy. But, with the increase in dose over 100 Gy, larval duration also increased. But, in our study, irradiation did not significantly alter larval duration, and this could be due to the lower irradiation dose chosen in the present study.

5.1.3 Susceptibility of *Callosobruchus maculatus* pupae to gamma radiation

Mortality of four-day-old pupae of *C. maculatus* was significantly higher at all the radiation doses compared to control. At 50 Gy, pupal mortality was 60.00 per cent, which was significantly higher over control but lower than all other treatment doses. Increasing the dose to 75 Gy resulted in significant increase in mortality (80.00%) over control and complete mortality of pupae occurred at a dose of ≥100 Gy (Table 14).

Reports on susceptibility of *C. maculatus* pupae to radiation varied with literature. According to Ghogomu (1990), irradiation of *C. maculatus* pupae at 10 Gy resulted in only 8.00 per cent adult emergence. But, reported complete inhibition of adult emergence at a higher dose of 300 Gy. Dongre *et al.* (1997) recorded more than 50 per cent 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 and irradiated them at their pupal stage at 100 Gy. But, only one adult hatched out after irradiation. Soumya (2015) reported complete inhibition of adult emergence at 100 Gy from the pupae of *C. chinensis*, similar to the observation in the present investigations.

Variation in the age of pupae could be the reason for the variation in susceptibility. Dawes *et al.* (1987) working with two-day and five-day-old pupae of sweet potato weevil reported higher tolerance of older pupae to irradiation. There was adult emergence at 200 Gy when five-day-old pupae were irradiated. But there was no adult emergence even at the lowest dose of 5 Gy with two-day-old pupae.

5.1.4 Susceptibility of *Callosobruchus maculatus* adults to gamma radiation

Compared to other stages, both female and male adults of *C. maculatus* were more tolerant to gamma radiation. Complete mortality of both sexes occurred at highest dose of 1500 Gy after three-days of exposure, which was significantly higher over all other treatments and control (Table 14).

On 3rd day, the mortality of female adults in control was only 2.50 per cent, whereas it was 10.00 per cent in adult males. At lowest dose of 100 Gy, mortality of adult females and males were 17.50 and 15.00 per cent, respectively. At 500 Gy, mortality was 27.50 per cent in both female and male adults. Mortality was 35.00 per cent in female and 42.50 per cent in males at 750 Gy, which increased to 62.50 per cent and 47.50 per cent in female and male adults, respectively at 1000 Gy.

After three days of irradiation, mortality did not significantly differ from control at the lowest dose of 100 Gy with both females and males. Mortality of adult females at 500 and 750 Gy were at par with mortality at 100 Gy. Mortality of adult females after irradiation at 1000 Gy significantly differed from all other treatments.

Irradiation at 500 Gy resulted in statistically similar mortality of adult males with that of mortality in 100 Gy and 750 Gy. But, the mortality at 750 Gy was significantly higher over mortality at 100 Gy. But statistical analysis revealed that there was no significant difference in the sensitivity of male and female to gamma radiation (df =1, F =0.003, P=0.958). Hussain and Imura (1989) working with male and female adults of *C. chinensis* also reported similarity in the susceptibility of males and females to gamma radiation.

As in the current study, Dongre *et al.* (1997) also reported total mortality of *C. maculatus* adults at 1500 Gy. In *C. chinensis*, mortality of two-day-old adult was 97.00 per cent at 800 Gy after four-days of exposure (Supawan *et al.,* 2005). In the present study, the mortality of one-day-old adult irradiated at 750 Gy was 67.50 per cent for females and 75.00 per cent for males. Mortality was 100.00 per cent in *C. chinensis* after seven days at both 600 and 700 Gy (Supawan *et al.,* 2005), which is similar to the present investigation. They also reported the survival of *C. chinensis* adults at the lowest dose of 300 Gy after seven days of exposure.

There is variation in the sensitivity of different species of insects to irradiation. Adults of sweet potato weevil died within seven days after exposure to 1000 Gy (Dawes *et al.*, 1987). But the mortality of adults of *Lasioderma serricorne* was 100.00 at 1500 Gy only after 12 days of irradiation (Kongratarpon *et al.,* 2002). *Tribolium castaneum* adults were most tolerant to irradiation as mortality was 100.00 per cent at 500 Gy after 35 days of irradiation (Tandon *et al.,* 2009). Similarly, 19.83 per cent *Sitophilus oryzae* adults survived after one week when irradiated at 180 Gy (Tunçbilek, 1995). Adults of *Oryzaephilus surinamensis* was also less radiosensitive as 27.50 per cent adults survived after seven days at 600 Gy of irradiation (Hosseinzadeh *et al.,* 2010).

There was a significant reduction in the longevity of both male and female adults of *Callosobruchus maculatus,* and there was a significant difference between sexes in the case of longevity (df =1, F=30.986, P =≥ 0.000). The longevity of males was lower to females in control also (Fig 1). Fox *et al.* (2003) reported greater life span for *C. maculatus* females compared to males as in the present study. Olaifa *et al.* (1990) reported that irradiation at 100 Gy didn't cause any change in the longevity of two days old adults of *C. maculatus.* But in the present study, the longevity of female and male adults of *C. maculatus* significantly decreased over control due to irradiation. Shivanna (2006) also reported a significant drop in longevity of male and female adults of *C. maculatus* due to irradiation at a dose ranging from 10 to 50 Gy.

5.1.5 Comparison of mortality of different stages of *Callosobruchus maculatus* due to gamma radiation

Comparing the dose required to cause 100.00 per cent mortality to various stages of *C. maculatus*, we could observe that radiosensitivity of different developmental stages of *C. maculatus* decreased as the insect developed from egg to adult. Eggs and early-stage grubs were most sensitive as none of these stages developed into adults at the lowest dose of 16 Gy (Fig.2). But, for late-stage grubs (10-day-old), a higher dose of 50 Gy was required to prevent adult emergence. Ten-day-old grubs were more tolerant to radiation as 47.50 per cent grubs developed into pupae at 50 Gy. But irradiation of seven-day-old grubs at 24 Gy resulted in only 15.00 per cent pupation of the grubs. Among the immature stages, pupae were most tolerant to radiation as there was no adult emergence at a dose of 100 Gy, which was double the dose required for late-stage grub. Though the reproduction of adults was adversely affected at lower dose, complete mortality of adults required a higher dose of 1500 Gy. This finding was in conformity to the work of Olaifa *et al.* (1990), Ghogomu (1990), Dongre *et al.* (1997), and Hammad *et al.* (2020) wherein they reported decrease in radiosensitivity as the insect develops from egg to adult.

There were reports on variation in sensitivity of different development stages to gamma radiation in other species also as in the present findings with *C. maculatus.* Exposure to 12 Gy caused complete mortality of eggs, 40 Gy caused complete mortality of larvae, and a higher dose of 640 Gy caused complete mortality of pupae (Hussain and Imura, 1989). Similar results were recorded with *Sitophilus granaries* (Aldryhim and Adam, 1999), *Ephestia kuehniella* (Ayvaz and Tuncbilek, 2006) and *Oryzaephilus surinamensis* (Hosseinzadeh *et al.,* 2010).

Eggs were highly susceptible to gamma radiation, as rapidly multiplying cells are more sensitive to gamma radiation (Arthur *et al.*, 2015). Susceptibility of grubs to gamma radiation can be due to the effect of radiation on midgut epithelium and the destruction of regenerative cells of midgut epithelium (Ashraf *et al.*, 1971). Riemann and Flint (1967) reported the destruction of midgut epithelium due to gamma radiation in adult boll weevil (Riemann and Flint, 1967) and Lee (1964) reported the same phenomenon in queen honey bee.

Destruction of midgut epithelium lead to reduced or abandoned feeding of grubs leading to mortality (Ashraf *et al.,* 1971). Irradiation of *Ephestia cautella* resulted in decrease in the consumption of food (Boshra, 2007). The damage to midgut epithelium may be the reason for higher susceptibility of grubs to radiation. Adults of *C. maculatus* are non-feeding, and the impact of gamma radiation through food consumption and utilization is limited, which in turn results in higher tolerance of adults.

According to the law of Bergonie and Tribondeau (2003), the sensitivity of a biological tissue is directly proportional to mitotic activity and inversely proportional to the degree of differentiation of cells. Hence the midgut epithelium, regenerative cells of midgut and germ cells are more radiosensitive. Higher sensitivity of eggs and grubs and lower sensitivity of pupae of *C. maculatus* to gamma radiation can be due to the faster cell division occurring in eggs and grubs. Induction of sterility in adults of *C. maculatus* at lower doses can be due to the damage to the rapidly multiplying germ cells of adult insect.

Mortality of insect due to irradiation might be due to the impact of gamma radiation on enzyme activities in the insect body. Radiation could lead to the production of reactive oxygen, which would cause damage to cells (Tominaga *et al.,* 2004).

Kheirallah and El- Samad, (2016) reported increased levels of detoxification enzymes such as alkaline phosphatase (ALP), acid phosphatase (ACP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). While the level of glutathione-S-transferase (GST), reduced glutathione (GSH) and glutathione peroxidase (GPx) decreased. Radiation resulted in decrease in DNA content and protein in insect, leading to its mortality

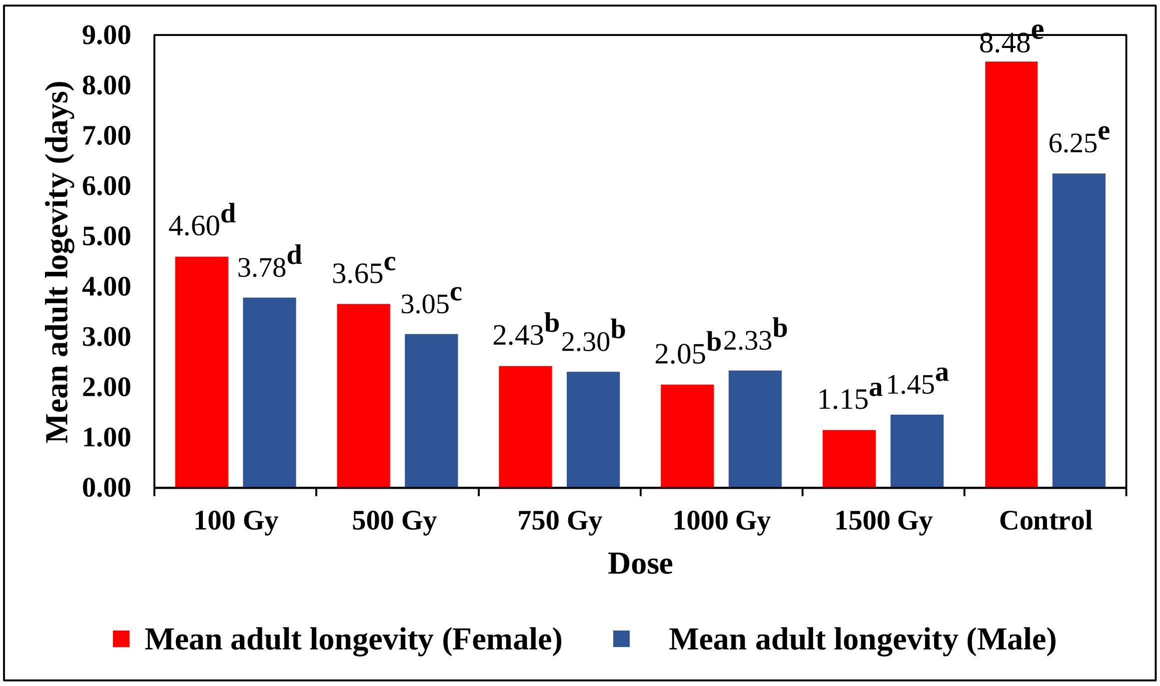


Fig 1. Effect of gamma radiation on mean adult longevity period of adults

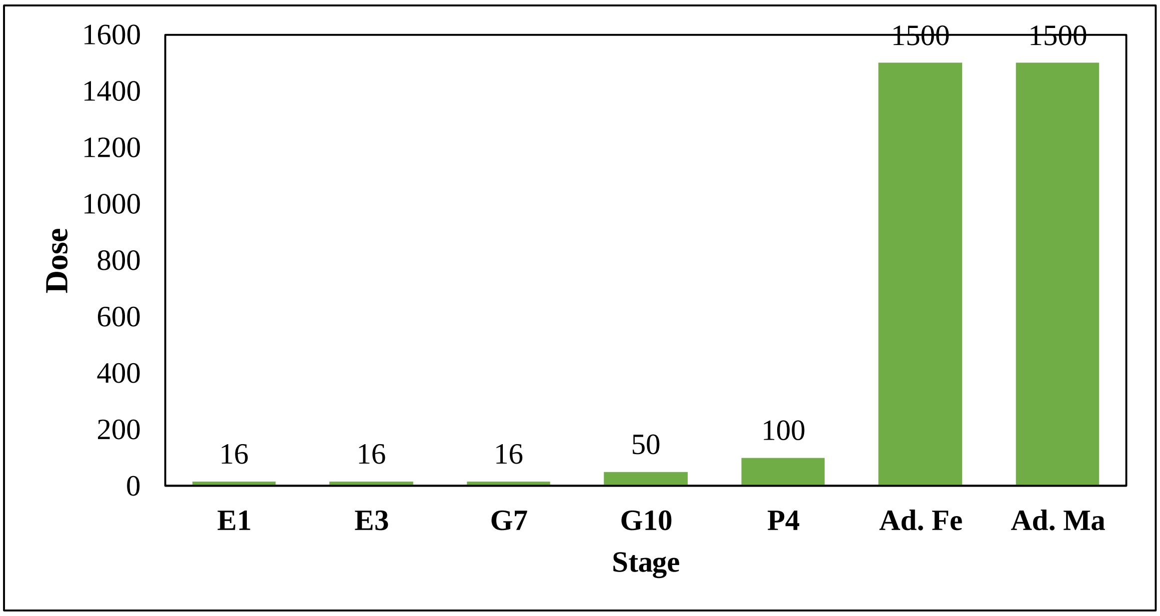


Fig 2. Doses showing maximum mortality per cent for different stages

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

5.2 EFFECT OF GAMMA RADIATION ON PROGENY PRODUCTION OF *Callosobruchus maculatus*

Exposing adults of *C. maculatus* to sublethal doses of gamma radiation (16, 18, 20, 22 and 24 Gy) did not significantly alter the fecundity. But, more than 96.00 per cent of the laid eggs were sterile in all the doses (Fig.3). While in control, 95.00 per cent eggs hatched into grubs. Brower (1973b and 1974) also reported significant decrease in progeny production after irradiation of adults of *C. maculatus.* But 100.00 per cent 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 per cent 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 per cent hatchability at a lower dose of 10 Gy. A dose of 40 Gy was sufficient to cause 100.00 per cent sterility of adult females of *C. maculatus* but adult male required 60 Gy for obtaining 96.90 per cent sterility (Pajni *et al.,* 1997).

Most of the studies consider the change of colour of eggs form clear and transparent to opaque and yellow as an indication of hatching of eggs of bruchids. But, we observed each egg under a microscope to check for the development of grub inside the egg. We counted only those eggs with a developing grub as hatched. Hatching of eggs based on the change in colour can overestimate hatchability of eggs as there was a colour change in those eggs without any developing grub.

The reproductive ability of different species subjected to irradiation varied as complete sterility of female and male adults of sweet potato weevil, *Cylas formicarius elegantulus* occurred at 200 and 300 Gy, respectively (Dawes *et al.,* 1987). A dose of 160 Gy was sufficient to cause sterilization in *Lasioderma serricorne* (Pendlebury *et al.,* 1966), a dose of 100 Gy for *Sitophilus granarius*, (Aldryhim and Adam, 1999) and 200 Gy for inducing100 per cent sterility of both sexes of *T. castaneum* (Brower and Tilton, 1973).

5.3 EFFECTIVENESS OF GAMMA RADIATION IN PROTECTING GRAINS INFESTED WITH DIFFERENT STAGES OF *Callosobruchus maculatus*

Irradiation of cowpea grain infested with different development stages of *C. maculatus* resulted in significantly lower emergence of adults compared to control after 17 days of irradiation. In control, the mean number of adults emerged was 537.50, whereas, in the highest dose of 100 Gy, only 25.2 adults emerged. The adults emerged in all the treatments were inactive and moribund. Active adults emerged from the control occupied the top portion of the culture bottle, but none of the adults emerged from treatment doses, ranging 20 to 100 Gy climbed to the top of the culture bottle.

Though gamma radiation has good penetration power, the efficiency of the treatment may vary with bed thickness of the grain lot (Mazima *et al.*, 2018). Hammad *et al.* (2020) irradiated a large number of adults of *C. maculatus* (27,754) in 100 g grain lot and reported a dose of 650 Gy to cause complete inhibition of adult emergence. For conducting experiment on cowpea grains with different stages of insect, we used 500 gram of cowpea grains per replicate in contrast to 10 grains/ replicate in the experiment with each stage separately. Slight increase in adult emergence was observed in experiment with infestation of different stages might be due to the higher quantity of grains used in this experiment.

Complete inhibition of insect infestation occurred when 2 Kg wheat and 1.5 Kg semolina was irradiated at 1000 Gy and 250 Gy, respectively (Azzeh *et al.*, 2010). Irradiation of 2 Kg commercial wheat flour bags at 250 Gy completely prevented insect infestation upto 6 months (Marathe *et al.*, 2002). Ahmed (1990) reported that a dose below 200 Gy could disinfect pulse and beans, which is similar to the result obtained in our study.

5.4 IMPACT OF RADIATION ON NUTRITIONAL FACTORS OF TREATED GRAINS

Irradiation of cowpea grains at 100 Gy did not significantly alter the protein and carbohydrate content of cowpea grains (Fig.4). Similar to the present study, Hammad *et al.* (2020) irradiated cowpea grains with 650 Gy and found no significant difference

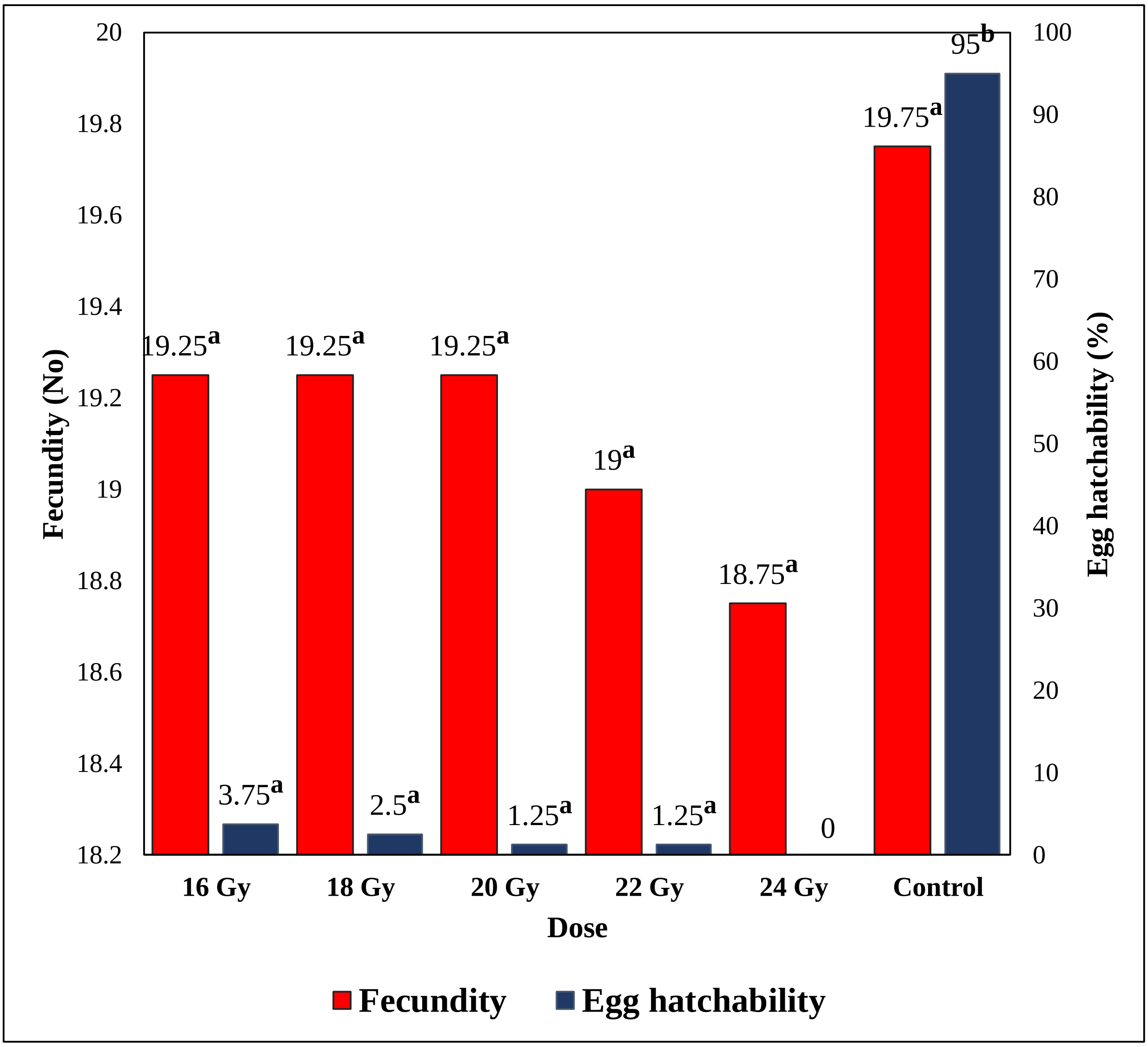


Fig 3. Effect of gamma radiation on fertility of *Callosobruchus maculatus* adult

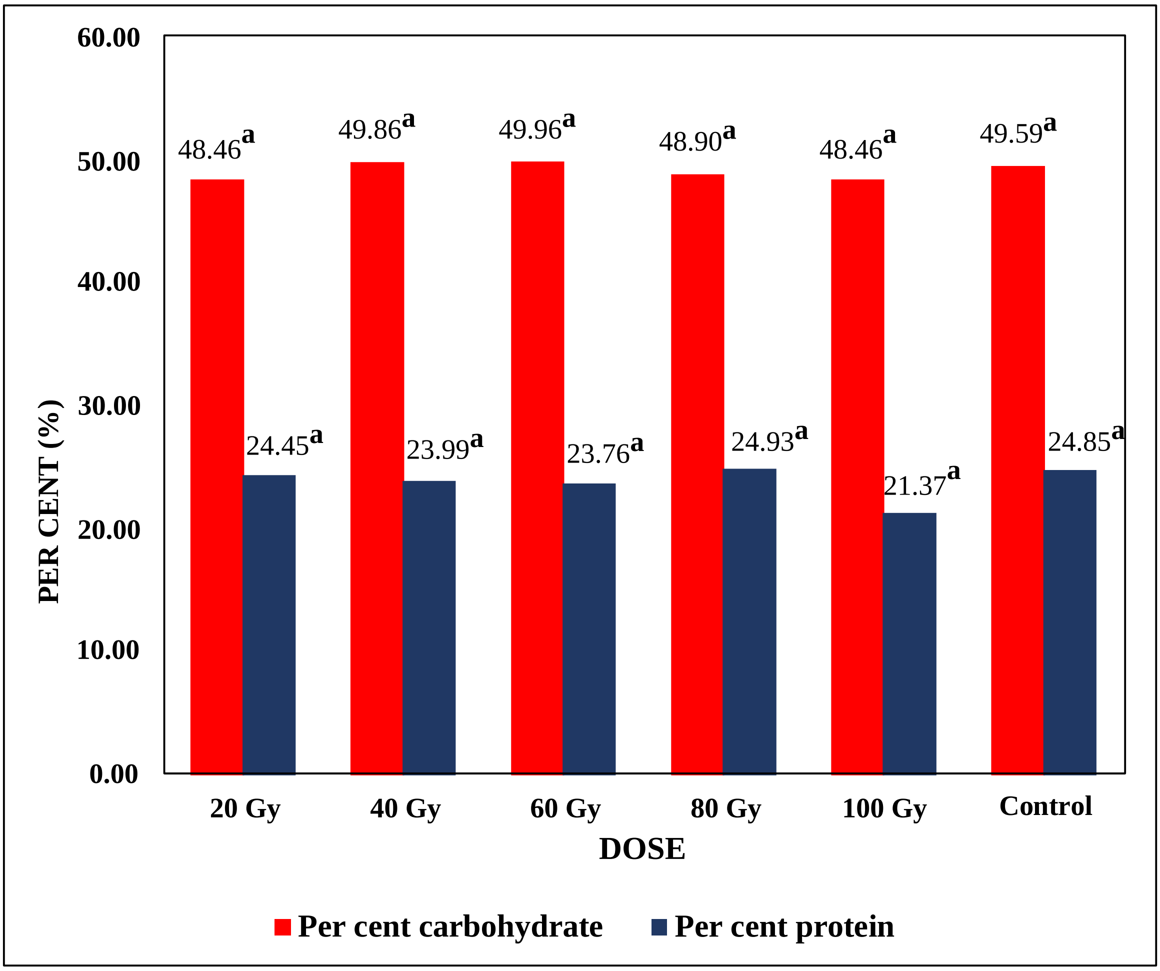


Fig 4. Effect of gamma radiation on protein and carbohydrate content of cowpea

in moisture, protein, lipid, ash and carbohydrate content of cowpea grain 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 fava)* irradiated at a dose ranging from 0 to 10 KGy.

But irradiation of wheat grains with gamma radiation decreased the crude protein content of wheat grain and total carbohydrate content of wheat flour at 1000 Gy (El-Nagger and Mikhael, 2011). Hence the effect of gamma radiation on nutritional parameters might vary among commodities. Though, our study didn't find any change in protein and carbohydrate content after irradiating cowpea grains at 100 Gy.

Summary

6. SUMMARY

Pulses occupy a distinct position in the agriculture sector of our country as India is the largest producer, consumer and importer of pulses in the world. Cowpea (*Vigna unguiculata* L. Walp.) is one of the major crops among pulses as it has tremendous economic and dietary importance. Despite its significance, it is one of the crops that faces many constrains like post-harvest loss. Many of the storage pest, especially bruchids, causes considerable loss to cowpea during storage. The bruchid, *Callosobruchus maculatus* can cause complete damage of stored pulses. The widely adopted management strategy to alleviate bruchid infestation is to disinfest using chemicals. But the recurrent use of chemicals resulted in the development of insecticide resistance and adverse effect on health and the environment. Recently, a draft gazette notification issued by the Government of India proposes to ban commonly recommended pesticides in storage. Hence, it necessitates the development of an effective and safe alternative for the management of storage pests. Keeping this in mind, the present investigation studied the effectiveness of gamma radiation for the management of storage pests.

A study was conducted to investigate the effectiveness of gamma irradiation for the management of *C. maculatus* and the effect of irradiation on the nutritional factors of cowpea grain. The results obtained are summarized hereunder.

Irradiation of cow pea grains with egg stage at doses ranging from 16 Gy to 24 Gy revealed that one-day-old egg was more sensitive to radiation than three-day-old egg. None of the irradiated one-day-old egg hatched out into grub. While, with three-day-old eggs, mortality increased significantly with increase in irradiation doses. Also, exposure to gamma radiation resulted in a significant increase in mean egg period over control at the highest dose of 24 Gy.There was no adult emergence from gamma exposed one day and three-day-old eggs.

Mortality of seven-day-old grub increased significantly with an increase in radiation dose. Exposure of seven-day-old grub to gamma radiation at doses ranging from 16 Gy to 24 Gy showed significant increase in per cent mortality from 65.00 per cent at 16 Gy to 85.00 per cent at 24 Gy. As the age of the grub advances to 10 days, a higher dose of 50 Gy resulted in 52.50 per cent mortality of the grubs, which was significantly different from control. The mean grub period of both 7-day and 10-day-old grub didn't change significantly due to gamma radiation. Irradiation of seven-day-old grubs at 16 Gy and ten-day-old grubs at 50 Gy completely inhibited adult emergence. Ten-day-old grubs were more tolerant to gamma radiation than 7-day-old grubs. Irradiation at 100 Gy resulted in complete mortality of 4-day-old pupae.

Irradiation of one-day-old male and female adults of *C. maculatus* significantly decreased the adult longevity of both sex forms. Adult mortality increased in a dose-dependent manner, while irradiation at 1500 Gy caused 100 per cent mortality within three days.

Results obtained from studies on different developmental stages of *C. maculatus* reveals that the sensitivity to gamma radiation increases with the increase in radiation dose and decreases with the increase in the age of the insect. Egg stage was most radiosensitive, and adult stage was the least radiosensitive.

Study on the reproductive behaviour of the adult showed that progeny production of adults decreased significantly with gamma radiation. Irradiation did not significantly alter fecundity of *C. maculatus*, but hatchability of eggs decreased significantly. None of the egg laid by the irradiated adults developed into pupa. Further, a dose of 24 Gy induced complete sterility in adults of *C. maculatus* as none of the eggs laid had hatched out at this dose.

Adult emergence from a 500g lot of cowpea grains, containing different developmental stages of *C. maculatus*, significantly decreased when irradiated at doses ranging from 20 to 100 Gy. Mean number of adult emergence in control after 17 days of irradiation was 537.50 adults, while in treatment with 100 Gy mean number of adult emergence decreased significantly to 25.20 adults.

Nutritional studies of both irradiated and control treatments revealed that irradiation did not significantly alter protein and carbohydrate content of cowpea grain at any of the irradiation doses

The results obtained in the study revealed that gamma radiation is effective against all stages of *C. maculatus* without affecting protein and carbohydrate content of the irradiated grains.

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Gamma irradiation for management of pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)

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ABSTRACT OF THE THESIS

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ABSTRACT

The study entitled "Gamma irradiation for management of pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)" was carried out from October 2019 to June 2020 at Pesticide Residue Laboratory, College of Horticulture, Vellanikkara. The objectives of the study were to assess the effectiveness of gamma irradiation for the management of pulse beetle, *C. maculatus* and to study the effect of gamma irradiation on the nutritional factors of cowpea grain.

Susceptibility of different developmental stages - *viz.*, egg, larva, pupa and adult – of *C. maculatus* to gamma radiation was studied by irradiating cowpea grains (variety- *Kanakamany*) with insect stages. Irradiation of one-day and three-day-old eggs at doses ranging from 16 Gray (Gy) to 24 Gy resulted in 100 per cent mortality of one-day-old eggs. While in three-day-old eggs, mortality varied from 52.50 per cent at 16 Gy to 65 per cent at 24 Gy. However, in control, the mortality was only 15 per cent. The mean egg period of three-day-old eggs significantly increased from 5.15 days in control to 5.36 days in treatment with the highest dose of 24 Gy. Although the irradiated eggs hatched out, the grubs did not develop further and reached the adult stage. One-day-old eggs were more radiosensitive than three-day-old eggs.

Gamma irradiation on different grub stages revealed that early instar grub (7-day-old) was more sensitive to radiation than late instar grub (10-day-old). Exposure of seven-day-old grubs to gamma radiation at doses ranging from 16 Gy to 24 Gy showed a significant decrease in per cent pupation from 35 per cent at 16 Gy to 15 per cent at 24 Gy and were significantly different from that of control (82.5%). As the age of grubs advanced to 10 days, a higher dose of 50 Gy and 75 Gy caused 52.50 per cent mortality followed by 60, 65 and 70 per cent, respectively at 100, 125 and 150 Gy and was significantly higher compared to control (7.50%). Gamma radiation did not significantly alter mean grub period. However, none of the irradiated grubs were developed into adults. Exposure of four-day-old pupae to gamma radiation (100 Gy and above) completely inhibited adult emergence.

One-day-old female and male adults were irradiated separately at doses of 100, 500, 750, 1000 and 1500 Gy to assess the effect of gamma radiation. Mean adult longevity of both female and male was decreased significantly with an increase in

radiation dose. Cent per cent mortality of both sexes occurred on third day at 1500 Gy whereas, in control, the adult female and male survived for 13 and 11 days, respectively.

Irradiation of one-day-old adults at doses ranging from 16 Gy to 24 Gy adversely affected progeny production of *C. maculatus*. The fecundity of irradiated adults did not differ significantly from control, but hatchability of the eggs reduced significantly. None of the eggs laid by the irradiated adults were developed into pupa. Irradiation at a dose of 24 Gy induced complete sterility in adults of *C. maculatus*.

To study the effectiveness of gamma irradiation in protecting grains infested with different developmental stages of *C. maculatus,* a lot of 500 gram of infested cowpea grains was irradiated with a range of 20 to 100 Gy and recorded the adult emergence. After 17 days, in control treatment the mean adult emergence was 537.50 while it was only 25.20 beetles in 100 Gy irradiated lot.

Nutritional studies of both irradiated and control treatments revealed that irradiation did not significantly alter the protein and carbohydrate content of cowpea grain at any of the irradiation doses. Hence, gamma radiation is a promising technology for the management of pulse beetle because of its effectiveness, fast and easy application, absence of residues, and minimal changes to the nutritional properties of the treated cowpea grain.