IRRADIATION AND SEED COATING FOR ENHANCING STORAGE LIFE OF GRAIN COWPEA (*Vigna unguiculata* (L.) Walp.)

by

JAYASHRI S

(2019-11-037)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2021

DECLARATION

I, hereby declare that this thesis entitled "**IRRADIATION AND SEED COATING FOR ENHANCING STORAGE LIFE OF GRAIN COWPEA (*Vigna unguiculata* (L.) Walp.)**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani

Date: 8-11-21

JAYASHRI S

(2019-11-037)

CERTIFICATE

Certified that this thesis entitled **"IRRADIATION AND SEED COATING FOR ENHANCING STORAGE LIFE OF GRAIN COWPEA (*Vigna unguiculata* (L.) Walp.)"**is a record of research work done independently by Ms. Jayashri S 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 her.

Jayalekshmy.V.G

Vellayani,
Date: 8-11-21

Dr. Jayalekshmy V.G.
(Major advisor)
Professor and Head
Department of Seed Science and Technology
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Jayashri S a candidate for the degree of **Master of Science in Agriculture** with major in Seed Science and Technology, agree that the thesis entitled **"IRRADIATION AND SEED COATING FOR ENHANCING STORAGE LIFE OF GRAIN COWPEA (*Vigna unguiculata* (L.) Walp.)"** may be submitted by Ms. Jayashri S, in partial fulfilment of the requirement for the degree.

Dr. Jayalekshmy V.G.

Professor and Head
Department of Seed Science and Technology
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

Dr. Beena R

Assistant Professor
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

Dr. Sheeja K Raj

Assistant Professor
Department of Agronomy
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

Dr. Shanas S

Assistant Professor
Department of Agricultural Entomology
Integrated Farming Systems Research Station, Karamana
Thiruvananthapuram-695 002

ACKNOWLEDGEMENT

First and foremost, praises and thanks to the Almighty, for everything that happens to me...

It is my great pleasure to place a record with deepest sense of gratitude and indebtedness to my major advisor ***Dr. Jayalekshmy V.G.*** *Professor and Head, Department of Seed Science and Technology for the constructive guidance, constant inspiration, abundant encouragement, kind treatment, critical scrutiny of the manuscript and valuable suggestions which render me to accomplish the research work successfully. I extend my sincere gratitude for providing a stress free situation by the open minded approach and for the care and affection bestowed on me throughout the study period.*

I convey my heartfelt gratitude to ***Dr. Beena, R.*** *Assistant Professor, Department of Plant Physiology for the unceasing encouragement, valuable advices and whole hearted approach right from the beginning, who guided me till the completion of the thesis.*

I extend my sincere gratefulness to ***Dr. Sheeja K. Raj*** *Assistant Professor, Department of Agronomy for the valuable suggestions, technical advices and incessant motivation throughout the research work.*

I am extremely thankful to ***Dr. Shanas S.*** *Assistant Professor, Department of Entomology, IFSRS, Karamana, for the unstinting support, suggestions and passionate approach rendered during the period of research work*

I am deeply grateful to ***Reji chechi, Jisha chechi*** *of Seed Science and Technology,* ***Dr. Sajitha Rani,*** *Department of Agronomy for generous support, continues and timely advice, constructive criticisms and constant encouragement rendered to me during the course of my study and research work.*

I owe my deepest gratitude to my batch mates ***Diya Amreen, Srutha Keerthi*** *whose cooperation, love, support and affection helped me through many hardships. Without their persistent help and care, this would not have been possible.*

I express my sincere thanks to my seniors ***Pravalika, Arun, Shahiba, Arun Chacko*** *and* ***Anand*** *and lovely juniors* ***Dinesh, Rohitha*** *for their help and support.*

*Words are inadequate to express my thanks to my friends **Rajeswari, Gayathri, Shiva, Shanmuga priya, Athira, Shabana, Lakshmi, Jeena Paul** and **Karthik** for their unbound love and bighearted support*

*I am beholden beyond words to express my indebtedness to my **Appa, Amma,** and **sister** for their unconditional love, sacrifices and support bestowed on me during my hard periods.*

Once again I am thanking each and every person who helped me during my research programme.

Jayashri S

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

et al	And others
@	At the rate of
cm	Centimeter
Cs	Caesium
Co	Cobalt
CRD	Completely Randomized Block Design
CD	Critical Difference
cm^3	Cubic centimeter
^0C	Degree Celsius
^0N	Degree north
^0E	Degree east
dia	Diameter
Fig.	Figure
G	Gram
Gy	Gray
HAT	Hours after treatment
Ht	Height
kDa	Kilo Dalton
kGy	Kilo gray
kg	Kilogram
LD	Lethal dose

MSL	Mean Sea Level
µg	Microgram
mL	Milli litre
mm	Milli metre
viz	Namely
g^{-1}	Per gram
kg^{-1}	Per kilogram
l^{-1}	Per litre
%	Percentage
RBD	Randomized Block Design
SE	Standard crror
i.e.	That is
w/v	Weight per volume

INTRODUCTION

1. INTRODUCTION

Seed is the fertilized, matured ovule and a carrier of genetic potential for sustainable crop production. It is the basic and crucial input of agriculture around which all other input acts. Good quality seeds form the foundation of successful agriculture. Seeds are the first determinant of further plant development and yield potential. Therefore, food security is dependent on seed security of farming communities. Cowpea [*Vigna unguiculata* (L.) Walp.] also called black-eyed pea, lobia, barbati is a widely grown leguminous crop (2n=22), a native crop of West Africa.

Cowpea is grown throughout the year and grain cowpea is widely cultivated in Kerala in the summer rice fallows. The importance of seed storage has been recognized by humans ever since they began to domesticate plants. In India, 80 per cent of the certified seeds produced is required for one cropping season and 20 per cent of seed is stored for subsequent sowing (Bal, 1976). But, when the storage facilities and infrastructure develops, certain amount of seeds can be stored for two to three seasons as protection against low quality seeds production and crop loss.

The need of high quality seed is essential to achieve optimum plant stand and its yield. However, maintenance of seed viability in storage is highly difficult as it deteriorates like any other biological material. Seed deterioration is an irreversible and inexorable process that depends on physical, physiological and chemical composition of seeds. Seeds undergoes several biochemical processes that results in free radical production and peroxidation of lipids leading to seed deterioration. Deterioration of stored seeds is the major reason for reduction in yield and non-availability of high vigour seeds at the time of sowing. Some seeds are generally short lived and deteriorate at faster rate. Among them, pulses exhibit rapid seed deterioration due to stored pest infestation. Pulse beetle (Bruchids), *Callosobruchus chinensis* is the most significant storage pest as they multiply rapidly and cause heavy loss both in field conditions and storage (Ahmed *et al.*, 2003). Seeds are infested by bruchids at the end of their maturity cycle, either directly from the field or through bruchids migrating from infested seeds in nearby granaries or seed godowns.

Traditional production techniques are usually followed in pulses and post-harvest losses accounts for 20–25% (Maneepun, 2003). During a period of six months storage, 50 to 60 per cent damage is noticed due to insects (Caswellet, 1973). Sharma (1984) reported that infestation due to *Callosobruchus chinensis* in various pulses was 68, 56, 49, and 52 percent in cowpea, chickpea, pigeon pea, and greengram, respectively during a period of six months storage. The rate of seed deterioration could be minimized to a certain extent either by storing it in controlled environmental conditions or by imposing certain seed treatments before storage. Seed treatment serves as the best alternative strategy to maintain the seed quality, since controlled conditions are highly expensive. Synthetic insecticides and fumigants are often used in storage to combat pests, but their widespread use in the field and storage has resulted in a slew of issues, including insecticide resistance, poisonous contaminants in food crops, waste and rising application prices (Kumar *et al.*, 2013). Physical methods like X- rays, electron beams, gamma rays, etc. can be used as an alternative for fumigation in agricultural commodities against insect pests. These physical radiations accelerate the production of reactive oxygen species in pests that impair multiple cellular pathway processes (Pyror, 1986). Irradiation of cereals and legumes has emerged as a new technology to combat the problems caused by the storage pests and helps to maintain its longevity in storage.

Chitosan are biologically active compounds and can be used to protect the crop plants against pests and diseases. The use of chitosan biopolymers as bio-stimulants in agriculture would help to minimize the amount of fertilizers and plant protection chemicals used in agriculture, as well as elicit more safe and sustainable organic agriculture (Pichyangkuraa and Chadchawanb, 2015). Chitosan has strong pesticidal activity in some plant species. Chitosan's insecticidal properties were demonstrated against cotton leafworm *Spodoptera littoralis*, *Helicoverpa armigera*, *Aphis gossypii*, and many stored pests.

With this background, the present study was formulated to enhance the storage life of cowpea seeds with the objective of standardization of gamma doses for irradiation and concentration of chitosan for seed coating for enhancing the storage life of grain cowpea (*Vigna unguiculata* (L.) Walp.).

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Pulses are the major source of human nutrition containing high amount of proteins, carbohydrates, vitamins complex and minerals. It also contains many amino acids like cysteine, tryptophan, methionine, threonine and lysine (Saxena *et al.,* 2010). It is widely cultivated in the tropics and subtropics of Asia, Africa, Central and South America, and parts of southern Europe and the USA. Pulses form an essential dietary component in South Asia, and their cultivation improves soil health.

About 70 different insect pests that attack stored seeds have been identified in pulses. Among them, pulse beetle is of economic importance as they develop and multiply rapidly causing heavy loss. Protecting seeds from the pulse beetle during storage is a major concern for growers. Various control measures including toxic chemicals and fumigants have been used extensively. But these measures posed serious problems like residual toxicity, acute and chronic toxicity, environmental pollution and development of resistance. Though, numerous technologies have been developed for the control of pulse beetle in storage; however, none of them serves the purpose completely.

The literature in the aspects of nature of the pest, damage caused by the pest, gamma irradiation and chitosan seed coating for enhancing the storage life is reviewed in this section.

2.1 MAJOR CHALLENGES IN COWPEA SEED STORAGE

The requirement of cowpea has been increasing over decades but there was a deficit in cowpea production in comparison with the demand by the growing population. In addition to the shortage of production, the stored product was also affected by various abiotic and biotic factors.

Among them, damage by insect pests are of economic concern as they contribute to nearly 10- 50 per cent of damage and damage loss. Apart from them, mites, rodents, birds, and microbes also cause great loss in storage. Generally, the infestation is carried over from damaged field crops to the storehouses and continues to spread (Upadhyay and Ahmad, 2011). Seeds stored in farmer's houses and godowns serve as suitable habitat for bruchids growth and development. Among the various pests, the pulse beetle adversely affects the stored seeds and greatly contributes to reduction of the economic produce of cowpea.

The extent of damage caused by pulse beetle depends upon the preference and differs from host to host. Lack of knowledge, poor and insufficient storage facilities and adverse environmental conditions are the major reasons for post-harvest losses. The losses during storage were a ssessed to 25- 50 per cent in which damage by pulse beetle contributed about 5-10 percent. But this varies depending on pulse varieties, storage conditions, processing and geographical locations (Lal and Gujar, 2007).

2.2 PULSE BEETLE *Callosobruchus sp.*

Pulse beetle are small sized insects, (1.0-6.0 mm) belonging to the family Bruchidae of order Coleoptera. These are minor pests in the field which become a major pest during the storage of seeds (Ofuya and Bamigbola, 1991). Nearly 117 different species of bruchids belonging to 11 genera are found in India (Jat *et al.*, 2013). The predominant species of *Callosobruchus* found in India are *C. chinensis*, *C. maculatus* and *C. analis* (Dias and Yadav, 1988). Seed damage by pulse beetle commences in the field and continues its infestation in the storage also.

Adult beetles are oval brownish, elongated with cream, brownish black markings on its body. Grubs are 'C' shaped, legless, cream coloured with 3-4 mm long. They can complete four generations within a year under optimum storage environment. Female beetle has shorter life span compared to male beetle. Life span of adult females ranged from 9 to 12 days with an average of 9.6±1.14 days. Adult males had life span of 9 to 14 days with an average of 11.0±1.87 days (Varma and Anandhi, 2010).

The incubation period of *Callosobruchus chinensis* on different pulses ranged between 4.00 to 6.00 days with longest in redgram (5±0.79 days) and shortest in greengram (4±0.21 days). Similarly, longest larval period was observed in moth bean (14±0.80 days) and shortest in chickpea (12±0.35 days).

Among different pulses the pupal period and adult longevity varies between 7 to 10 days and 7 to 20 days respectively (Hosamani *et al.,* 2018).

Pulse seed damage is extensive, both qualitatively and quantitatively. The pulse beetle on chickpea caused a 55-69 per cent drop in seed weight and a 45-66 percent drop in protein content. It completes its entire immature life cycle in individual seeds that in turn results in reduction in germination potential, market value and nutritional value of produce.

Pulse beetle infestation caused a 100 percent loss of pulse seeds (Gujar and Yadav, 1978). Infestation by bruchids starts in the field by laying eggs on maturing pods. The grubs on hatching bore into the seeds and feed the inner content leaving the pod empty. After the pupal stage, the adult beetle emerges from the grain thereby causing circular holes in it (Ali *et al.,* 2004; Atwal and Dhaliwal 2005; Koona and Koona 2006; Swapan, 2016).

The bruchid grubs feed on internal endosperm content that leads to damage of grains along with reduction in nutritional value and loss in germination capacity (Roy *et al.,* 2014). Grubs feed the epithelium of the pod and remain hidden inside the germinating seeds (Credland and Wright, 1990). When the infested seeds are stored, the insect growth and population increases, resulting in complete loss of seeds within six months (Maina *et al.,* 2011; Sujatha *et al.,* 2015).

Srinivasan *et al.* (2010) estimated that pulse beetle, *Callosobruchus sp* causes nearly 100 percent post-harvest seed losses during severe stages of infestation and is considered as the most destructive pest of pulses during storage. Rustammni *et al.* (1985) reported that storage losses caused by pulse beetles in blackgram, chickpea and gardenpea were about 56.3, 46.7 and 50.9 per cent respectively.

Pulse beetle infestation caused about 12.5 per cent losses in warehouse storage (Rahman, 1971). Raghavendra and Loganathan (2017) reported that *Callosobruchus maculatus* larvae caused 100 per cent infestation of cowpea seeds within a month of exposure in pigeon pea seeds. According to Sharma *et al.* (2013), bruchids in different pulses caused post-harvest loss of 30-40 per cent within six months of storage and it reaches 100 percent when the seeds are untreated. *Callosobruchus maculatus* infestation results in 90 per cent yield loss in black gram under storage conditions (Soundararajan *et al.,* 2012).

Four holes per seed caused 100 percent loss in seed germination due to bruchid attack. Based on the seed genotype, morphological and biochemical features, *C. maculatus* and *C. chinensis* both caused seed yield loss of about 7-73 per cent in green gram seeds (Sarwar, 2012). Venkatesham *et al.* (2015) reported that mean weight loss percentage and seed damage was about 48.73 and 99.3 percent after 120 days which was higher than 4.19 and 7.86 per cent after 30 days of storage.

Bhatnagar *et al.* (2001) revealed that 88.1 per cent of cowpea seeds were infested during four months storage by *C. maculatus*. Sadozai *et al.* (2003) found 79.55 and 11.54 per cent seeds were damaged in pea and gram during three months storage by *C. maculatus*.

Anandhi *et al.* (2008) investigated population growth, grain damage and other factors caused by pulse beetle in chickpea for 30 to 180 days and observed that the mean population of pulse beetle was 648.3 after the release of adult beetles in five pairs in 250 g of chickpea.

2.3 IMPORTANCE OF GAMMA IRRADIATION

Kovacs and Keresztes (2002) reported that gamma radiation is the most intense and most penetrating electromagnetic radiation with energy levels ranging from 10 to several hundred-kilo electron volts.

Gamma rays serves as an effective means of decontaminants and disinfectants of agricultural and food products (Loaharanu, 1994). Irradiation by use of gamma source Co 60 or Cs 135, X rays or high energy electrons provides an alternative to chemical treatment that leads to inherent problems like residues and environmental pollution (Farkas, 1998).

Irradiation with gamma rays helps in the preservation and sterilization of cereal grain and food (Mokobia and Anomohanran, 2005). Nowadays, irradiation being a preservative method enhances the hygienic qualities and shelf life of processed foods and raw materials (Tresina and Mohan, 2011).

2.3.1 Gamma irradiation against storage pests

Irradiation can potentially eliminate insect pests of stored grains as well as field crops. It is an eco-friendly technology for insect pest management, without causing any induced residual effect and radioactivity. Although, many stored pests (especially Coleoptera) could be controlled with lower gamma rays, a gamma dose of 500 Gy could stop the reproduction of all stored product pests.

Irradiation with gamma doses 200 Gy, 300 Gy and 500 Gy showed 100 per cent mortality of bruchids. Also, it was noted that gamma irradiation had no impact on seed viability (Enu and Enu, 2014; Bhalla *et al.,* 2008). Gamma doses of 800, 900, and 1000 Gy were effective in maintaining the viability of cowpea seeds by causing mortality of pulse beetle *C. maculatus.* This ionizing radiation method may be implemented as part of the integrated pest management system on stored cowpea (Echereobia *et al.,* 2014). When *Callosobruchus chinensis* was exposed to 200–600 Gy gamma doses, complete sterility of both male and female adults was noticed (Chiluwal *et al.,* 2019).

On exposure of eggs and larvae of the grain weevil, *Sitophilus granaries* to gamma doses of 10-500 Gy, it was noted that larvae were unable to mature into adults at 30-500 Gy, whereas pupal and adult stages showed complete sterility at 70 Gy and hence a dose of 70 Gy was adequate for complete sterility of old adults (Aldryhim and Adam, 1999).

Bhalla *et al.,* (2008) observed 100 percent sterility of adult beetles on exposure of bruchid infested greengram seeds with 100 Gy gamma rays. Darfour *et al.,* (2012) reported that 100 per cent mortality of adult beetles was noticed on exposure of *C. maculatus* with 750 Gy gamma doses. Gamma dose of 1200 Gy resulted in total mortality of cigarette beetle, *Lasioderma serricorne* within a short period of eight days (Kumar *et al.,* 2017).

Abbas *et al.* (2011) found that exposure of Indian meal moth pupae to 650 Gy for five days prevented the emergence of adults. Complete egg hatching and inhibition of larval growth was observed when *Plodia interpunctella was* exposed to 350 Gy gamma radiations (Ayvaz *et al.,* 2008). Sujeetha *et al.* (2020) reported that complete mortality of eggs, larvae, pupae ad adults were noticed at gamma doses of 400 Gy, 650 Gy and 850 Gy.

Gamma radiation of 25-1200 Gy highly suppressed the pests like grain weevil, Mediterranean flour moth, cigarette beetle, medfly, onion fly, fall armyworm, tobacco budworm, African cotton leafworm in both field and storage conditions (Timbadiya *et al.,* 2018). Eggs and young larvae of Mediterranean flour moth (*Ephestia kuehniella*) exposed to 200 Gy gamma rays showed an inhibition in adult emergence and 100 per cent malformation in the first instar larvae (Ayvaz and Tuncbilek, 2006).

Female *Tribolium castaneum* was exposed to 6000 Gy gamma radiation, complete inhibition of pheromone was observed (Abdu *et al.*, 1985). Tandon *et al.* (2009) proved that gamma rays of 70 Gy and above were required to control the larvae and adult beetles of *Tribolium castaneum.*

The eggs of the tobacco budworm, *Helicoverpa assulta,* exposed to gamma rays 100 Gy resulted in hatching of 19.88 per cent of the eggs followed by death of all the larval and pupal stages whereas 1.52 per cent adults were emerged when five to six days pupae were exposed to gamma irradiation (Park *et al.,* 2015).

Arthur *et al.* (2016) observed that the final instar larvae of the fall armyworm, *Spodoptera frugiperda,* exposed with gamma doses of 200 Gy showed lowered pupation rate (30 per cent) and adult emergence (10 per cent). Gamma doses of 200 Gy could be also used as phytosanitary measures against larvae and adults of fall armyworm.

Hammad *et al.* (2020) reported that no adults were emerged from eggs and larvae exposed with 450 Gy and 650 Gy gamma doses. It was also observed that 650 Gy was effective against cowpea weevil and hence required for quarantine and phytosanitary security.

2.3.2 Influence of gamma rays on germination parameters

Gamma irradiation of wheat seeds with 0.05, 0.1, 0.5, 1.0 and 3.0 kGy showed a decrease in germination percentage with an increase in dosage (Linko and Milner, 1960). Radiation-induced changes in respiration of radish, wheat, corn and sorghum seeds were observed during germination and subsequent seedling development (Woodstock and Justice, 1967).

Exposure of castor seeds to 4000 Gy gamma rays increased RNA and protein synthesis during the early stages of seed germination (Kuzin *et al.,* 1975; Kuzin *et al.,* 1976)

Soni *et al.* (2014) experimented with rice seed and found that 200 Gy gamma dose enhanced the seed germination parameters, biochemical properties in various storage containers like Grainpro bags and HDPE bags.

With a rise in gamma-ray dosage, there was a decrease in seed germination in rice. Gamma radiation of above 300 Gy leads to severe physiological effects on seedling height, percentage seedling survival and tiller formation whereas below 300 Gy did not affect germination (Harding *et al.,* 2012). Higher gamma rays of 2000 Gy increased seed germination, biochemical and physiological properties of sorghum seeds (Meena *et al.,* 2016).

Selim and El-Banna, (2001) proposed that gamma doses of 5- 50 Gy can be used for preservation of pea seeds and stimulating growth and germination of seed thereby enhancing its yield and quality. They also reported that gamma dose of 100, 150 and 200 Gy as inhibitory doses and higher doses (250-400 Gy) as lethal doses.

2.3.2.1 Germination percentage

Exposure of dry carrot seeds to 100 and 500 Gy resulted in increased seed germination, whereas further higher doses lead to reduction in leaf size and delay in germination (Al-Safadi and Simon, 1996).

Rao and Suvartha (2006) reported that tomato seeds exposed to an irradiation level of 30 kGy enhanced the germination percentage. Ariraman *et al.* (2014) observed a reduction in germination percentage, seedling length and seedling vigour index of pigeonpea seeds with increase in gamma doses of 50 kGy.

Hell and Silveria (1974) reported that *Phaseolus vulgaris* seed germination was found to be decreased when exposed to 800Gy gamma radiation.

A decline in seed germination percentage was reported with an increase in gamma doses of three rice varieties (Kim *et al.*, 1970). Cheng *et al.* (2010) reported that at lower doses of 10 Gy to 30 Gy there was an increase in the emergence percentage of minitubers of potato, whereas no emergence was noticed at the high dose of 60 Gy.

Lactuca sativa showed an increase in germination percentage and germination index when treated with 30 Gy and a decrease in vegetative growth like root and shoot length at 70 Gy (Marcu *et al.*, 2013). The stimulatory effect on germination was noticed when *Lathyrus chrysanthus* was exposed to radiation doses of 100 and 150 Gy (Beyaz *et al.*, 2016).

Bashir *et al.* (2013) reported that lower gamma doses exhibited less biological damage and higher doses showed reduction in germination and survival percentage of fenugreek. Minisi *et al.* (2013) concluded that higher gamma doses lower seed germination and survival percentage.

2.3.2.2 Speed of germination

Rice seedlings showed increased speed of germination on exposure to 100 Gy gamma rays but further increase inhibited germination of seedlings (Maity *et al.*, 2005). Kabuli type of chickpea was more affected by gamma irradiation than desi types (Toker *et al.*, 2005).

Two different genotypes of wheat were exposed to gamma radiation 100 to 400 Gy, lowest germination percentage was observed in 300 Gy. Gamma doses above

200 Gy showed reduction in Mean Germination Time (MGT), root length, shoot length, shoot and root dry weight (Borzouei *et al.*, 2010).

An increase in speed of germination was observed in tomato and okra seeds when they are exposed to 100 Gy and 200 Gy gamma rays (Nargis, 1995; Kumar and Mishra, 2006). Gamma doses of 25 Gy showed an increase in speed of germination by 0.95 in *Pterocarpus sp* (Akshatha and Chandrasekar, 2013)

2.3.2.3 Seedling length

The impact of mutagens on the physiological system were predominantly responsible for the loss in shoot and root length (Gaul, 1970). Gamma rays of 500 Gy resulted in 50 per cent reduction of seedling length in laboratory study and 50 per cent reduction in survival of seedlings during field studies in ragi. It was revealed that there was an increase in the deleterious effects of gamma irradiation at regular intervals and the LD_{50} dosage was located near to the dose of 500 Gy (Rajendra *et al.*, 2017).

Gamma rays of 800 Gy showed reduction in shoot length of amaranthus seedlings (Aynehband and Afsharinafar, 2012). Reduction in shoot length (5.1 and 5.9 cm) was observed bengal gram and black gram exposed with 1000 Gy gamma rays at nine months of storage (Pranesh *et al.*, 2019).

Uma and Salimath (2001) reported a drastic reduction seedling length at higher gamma doses of 1000-6000 Gy. Reduction in sprout length up to 20.4 per cent and 58.8 per cent were observed in soybean seeds on irradiation with 100 Gy and 300 Gy respectively (Yun *et al.*, 2013). Seedling length was the highest on the fifth day of observation in okra seeds treated with 50, 100, 150, and 200 Gy gamma radiations (Jaipo *et al.*, 2019).

2.3.2.4 Seedling dry weight

Gamma ray doses of 100 Gy resulted in 25 per cent increase in dry weight in wheat whereas 200, 300, and 400 Gy resulted in decrease in dry weight compared to control (Borzouei *et al.*, 2010). Gamma irradiated seeds showed reduction in seedling fresh and dry weight due to decline in moisture content or plant growth as a result of radiation stress (Majeed *et al.,* 2010).

2.3.2.5 Seedling vigour index

A decrease in seed germination of french beans was observed at 800 Gy gamma rays (Hell and Silveira, 1974). Chandrashekar *et al.* (2013) documented that *Terminalia arjuna* showed an increase in germination speed and vigor index at 30 Gy gamma irradiation.

Plant vigor and grain productivity could be improved by gamma irradiation of 3-7 Gy (Singh and Datta, 2010). Chandrashekar (2015) found that seedling vigor index showed two-fold increase at 50 Gy compared to control in *Canarium strictum.*

Inhibition of physiological and biological processes such as enzyme activity required for seed germination leads to reduced germination under various mutagenic treatments (Kurobane *et al.,* 1979).

Blackgram seeds were irradiated at five different gamma doses 150, 200, 250, 300, 350 Gy. Germination percentage was reduced to 50 per cent at 250 Gy. Other phenotypic traits like plant height, number of pods per plant, pod length, number of primary branches and number of seeds per pod showed reduction at doses above 250 Gy. This reduction may be attributed to the chromosomal damage or physiological disturbance of the plant cells caused by mutation effect (Ramya *et al.,* 2014).

Morphological traits such as germination percentage, plant height, root length, shoot and root dry weight of long bean seedlings were reduced at higher gamma-ray doses of 800 Gy (Kon *et al.,* 2007).

Reduction in morphological parameters such as height of the plant, number of branches and clusters per plant, number of leaves and pods per plant, 100 seed weight and yield of seed was observed at 500 Gy in M_1 generation of cowpea (Girija and Dhanavel, 2013).

Gamma doses of 10 Gy showed increase in 1000 kernel weight and harvest index of canola (Rahimi and Bahrani, 2011). Reduction in physiological traits on exposure to gamma rays was due to sudden destruction of growth inhibitors and metabolic changes (Ariraman *et al.,* 2014).

According to Tshilenge-Lukanda *et al.* (2013) lower dose of gamma rays (100 Gy) can increase the pod yield of groundnut and other morpho-agronomic parameters, particularly for the JL24 groundnut variety.

Bonde *et al.* (2020) reported that maximum reduction in root length, shoot length and total seedling length was observed at gamma doses of 700 Gy in greengram

2.4 CHITOSAN AND ITS EFFECTS ON PLANT SYSTEM

Chitosan, a carbohydrate biopolymer consisting of N-acetyl-D glucosamine and D-glucosamine units obtained from insect's cuticle, shells of crustacean, and cell wall of fungus. It is biocompatible, biodegradable, non-toxic to both plants and animals with LD_{50} to mice >16kg^{-1} (Singla and Chawla, 2001).

The two important factors like the degree of N- acetylation and molecular weight have a great impact on biological phenomenon like defense mecahnisms. (Rabea *et al.,* 2003; Badawy, 2010). Chitosan stimulates the defensive mechanisms, seedling growth and action various enzymes like glucanases and chitinases (Hien, 2004). It enhances the excretion of resistant enzymes and monitors the plant immune system. It also increases the plant resistance ability against insects and diseases (Doares *et al.,* 1995).

Chitosan is only soluble in mild organic acids like acetic acid, lactic acid, benzoic or succinic acids. After dissolving in acids, chitosan can be cast as films or combined with natural or synthetic polymers. Nanoparticles coated with chitosan have positive charge on its surface that improves the suspension stability (Dammak *et al.,* 2017).

Chitosan improves the uptake and availability of nutrients and water by regulating osmotic pressure of the cell and thereby promotes plant growth (Guan *et al.,* 2009). Chitosan improves the innate defensive mechanisms of plants (Fondevilla and Rubiales, 2012) and antimicrobial properties (Rabea *et al.,* 2009).

Rice seeds treated with chitosan increased the seedling quality, panicle number and grain yield by 1.9 per cent to 4.2 per cent (Lu *et al.,* 2002). Boonlertnirun *et al.* (2008) reported that seed treatment of chitosan with 80 ppm along with four times soil application increased the overall plant growth and yield of rice.

2.4.1 Seed treatment with chitosan

Zeng *et al.* (2012) proposed that chitosan forms semi- permeable film on the seed surface of soybean and promotes seed germination by maintaining and absorbing soil water. Chitosan also helps to repel insects by stimulating the plants to produce specific antibodies.

Dzung *et al.* (2002) reported that germination, growth and yield of soybean could be enhanced by chitosan treatment. Chitosan also regulates plant response against several abiotic stresses like salt stress (Qing-Zhong, 2002; Dzung *et al.,* 2011).

Chitosan-treated wheat and rice seeds showed yield increase of 5 to 20 per cent over non-treated seeds (Freepons, 2020).

Chitosan nanoparticles enhanced the germination and growth of seedlings at a very low concentration of 5μg mL^{-1} due to higher adsorption on wheat seed surface compared to chitosan 50μg mL^{-1}. It also enhanced auxin-related gene expression, increased biosynthesis and transport of IAA thereby increasing its concentration in roots and shoots (Li *et al.,* 2019).

Burrows *et al.* (2007) observed that 0.5 percent HCl demineralized chitosan treated seeds recorded the highest germination percentage (90%) in peanut, while chitosan demineralized with 1 per cent HCl and 5 per cent CH_3COOH slightly improved the average number of leaves by 82.7 and 68.6 per cent, as well as plant height by 58.45 and 48.92 per cent, respectively.

Seeds primed with 0.2 per cent chitosan solution showed increase in germination per cent of 80 per cent where it was only 52 per cent in control. Maximum root length and dry weight was observed in seeds treated with 0.5 per cent chitosan (Batool and Asghar, 2013).

2.4.2 Effect of chitosan in pest management

Insecticidal activities of chitosan ethyl carbamate and chitosan diethyl phosphate at different concentrations were evaluated against the green peach aphid and compared with imidacloprid (Cabrera *et al.*, 2002). Chitosan diethyl phosphate at 0.5 per cent showed greater aphid mortality compared to imidachloprid.

Zhang *et al.* (2003) observed that cole leaves sprayed with 0.3 per cent chitosan solution resulted in 40 and 72 per cent mortality of *Helicoverpa armigera and Plutella xylostella* at 72 Hours After Treatment (HAT). It was also reported that chitosan application on flowers at 6 to 60 g L^{-1} showed 93 to 99 percent mortality of mealy plum aphid, *Hyalopterus pruni.*

Said *et al.* (2011) revealed the presence of disorganized, elongated and disintegrated midgut epithelia in the third instar larvae of *Galleria melleonella* L. fed with an artificial diet amended with chitosan. Badway and El-Aswad (2012) evaluated chitosan of different molecular weights 2.27 x 10^5, 3.60 x 10^5, 5.97 x 10^5, and 9.47 x10^5 g mol^{-1} along with various metal complexes like silver, copper, nickel and mercury. It was revealed that artificial diet incorporated with chitosan 2.27 x 10^5 g mol^{-1} with complexes Ni and Hg @ 4 g kg^{-1} resulted in maximum growth inhibition, feeding inhibition and mortality in third instar larvae of *Spodoptera litura.*

Bharani *et al.* (2014) investigated the insecticidal activity of Beauvericin (Csnp- Bv) loaded with chitosan nanoparticles for the control of *Spodoptera litura* and found that there was 100 per cent mortality of larvae treated 1.0, 0.01, 0.001 mg concentrations in the first and second instars. This formulation also highly reduced pupal period and rate of adult emergence.

Zeng *et al.* (2012) concluded that increased concentration of chitosan from one to five per cent increased the antifeedant rate of artificial diet fed to black cut worm *Agrotis ipsilon* Hufnagel, soybean aphid *Aphis gossypii* and pod borer *Maruca vitrata* F. It was also reported that chitosan also acts as a signal molecule to the plant. The highest antifeedant effect was observed in *Maruca vitrata* (87.24 per cent) followed by *Agrotis ipsilon* (82.89 per cent) and *Aphis gossypii* (80.21 per cent).

Sahab *et al.* (2015) were the first to report the impact of chitosan on coleopteran pests. They observed that artificial diet containing 12.5 parts chitosan (CS) -g- polyacrylic acid (PAA) nanoparticles reduced the mean number of eggs per female in cowpea weevil, *Callosobruchus maculatus* from 95.3 to 10.9 *in vitro* and from 94.3 to 19.9 in storage compare to control. The percentage of weevil development also showed 71.7 per cent decrease compared to control. *Callosobruchus chinensis* also showed similar decrease in fecundity, from 96.3 to 21.9 per cent in the laboratory and 91.3 to 21.1 per cent in storage. The percentage of insect growth also showed 73.0 per cent decrease compared to control. It was also reported that a diet containing 12.5 parts chitosan reduced *A. gossypii* fecundity from 97.3 to 20.9 and 90.3 to 28.9 in laboratory and semi-field conditions and 77.8 per cent decrease in larval weight.

2.4.3 Influence of chitosan treatment on germination parameters

Seed treatment of chitosan with 1 percent and foliar spray of 0.5 per cent showed a significant increase in growth, 1000 seed test weight and yield of chilli (Akter *et al.*, 2018).

Maize seed treatment with chitosan showed no effect on germination at low temperatures but enhanced germination at optimum temperature and environmental conditions (Guan *et al.*, 2009).

Improvement in germination percentage, root and shoot length, photosynthesis rate, stomatal conductance and root activity was observed in wheat seeds treated with oligochitosan (Lian-Ju *et al.*, 2014)

2.4.3.1 Germination percentage

Zeng *et al.* (2012) reported that soybean seed coated with 5 per cent chitosan showed enhanced germination of seeds (90%) and no significant difference among seeds treated with 4 and 5 per cent chitosan solution. He also revealed that 5 per cent chitosan increased the yield up to 20 per cent compared to control.

Increased percentage of germination and hypocotyl length was found in the seeds primed with 3g L^{-1} chitosan compared to control. Highest radicle length and seedling weight in seeds primed with 6g L^{-1} chitosan was reported (Al- Tawaha and Al- Ghzawi, 2013).

Chitosan coated groundnut seeds showed improved seed germination percentage, activity of lipase enzyme and auxin (Indole acetic acid) (Zhou *et al.*, 2002). Soaking maize seeds in chitosan solution increased the germination percentage (Guan *et al.*, 2009).

2.4.3.2 Speed of germination

Chitosan coated seeds showed improvement in seedling growth and development of wheat seeds compared to control (Zeng and Luo, 2012). Sen and Mandal (2016) concluded that 0.1 per cent chitosan at 5 per cent moisture level and 0.2 per cent at 10 per cent moisture levels served as an ideal elicitor for improving the speed of germination and synchronize the emergence of seedlings. Also, chitosan alleviated the detrimental effect of salinity upto 6 dSm^{-1}. Guan et al. (2009) revealed that seeds primed with chitosan showed reduction in mean germination time and increased speed of germination.

2.4.3.3 Seedling length and dry weight

An increase in germination rate, hypocotyl length, and radicle in rapeseed were noticed when seeds were soaked with chitosan (Sui *et al.*, 2002). Treatment with chitosan solution of 493 kDa improved the overall growth and quality of soybean sprouts (No *et al.*, 2003). Cho *et al.* (2008) concluded that chitosan seed treatment with 476 kDa improved the total dry weight, width and length of hypocotyl of sunflower seeds.

Sheikha and Al- Malki (2011) found that chitosan at 0.5 per cent increased the root length of cowpea by 32.78 per cent. Chitosan at 2.5 per cent increased fresh shoot weight by 6.76 per cent and root weight by 8.13 per cent.

2.4.3.4 Seedling vigour index

Shao *et al.* (2005) suggested that seed priming with chitosan solutions improved the rate of germination, germination percentage, chlorophyll content, shoot length and seedling vigor of maize. Priming of pearl millet seeds with chitosan at 2.5g kg^{-1} increased the germination percentage (99%) and seedling vigor (1782) of seeds (Manjunatha *et al.*, 2008).

Sui *et al.* (2002) observed that rapeseed coated with small molecular weight chitosan had a positive effect on seedling growth, root length and germination index. In maize chitosan treatment enhanced the activity of hydrolytic enzymes like α-amylase and protease and helps in the rapid mobilization of food reserves and its degradation and ultimately increased the germination and vigor of seedlings (Saharan *et al.*, 2016).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The project entitled "Irradiation and seed coating for enhancing storage life of grain cowpea (*Vigna unguiculata* (L.) Walp.)" was conducted in the Department of Seed Science and Technology, College of Agriculture, Vellayani, Thiruvananthapuram during 2020-2021 to assess the storage potential of cowpea seeds through gamma irradiation and chitosan seed coating and also to study the morphological changes that occurs due to gamma irradiation. The materials used and the methods adopted for the study is described in this chapter.

3.1 EXPERIMENT DETAILS

3.1.1 Location and climate

The study was conducted in the Department of Seed Science and Technology, College of Agriculture, Vellayani, Thiruvanandhapuram located at 8°5' N latitude and 76°9'E longitude and at an altitude of 29 m above MSL.

3.1.2 Experimental material

The study was conducted using the seeds of grain cowpea variety Kanakamony released by KAU. Seeds were procured from Onattukara Regional Research Station, Kayamkulam

3.2 IRRADIATION OF COWPEA SEEDS WITH GAMMA RAYS

3.2.1 Design and layout

Design: Completely Randomized Block Design (CRD)

Treatments: 6

Replications: 3

Table 1: Different doses of gamma rays

Treatments	Gamma doses
T_1	100 Gy
T_2	200 Gy
T_3	300 Gy
T_4	400 Gy
T_5	500 Gy
T_6	Control

3.2.2 Imposition of treatments

3.2.2.1 Gamma irradiation instruments and source

For the present study, the gamma chamber -5000 was used as irradiation source which was installed at Indian Institute of Horticultural Research, Bangalore.

3.2.2.2 Specifications of gamma chamber - 5000

The gamma chamber-5000 is a small self-shielded cobalt-60 gamma irradiation chamber with a 5000 cc irradiation volume. The irradiation seed material can be placed in a sample chamber in a vertical drawer inside the lead flask, and the treatment doses can be adjusted accordingly. This drawer is moved up and down using a system motorised drive, allowing for exact positioning of the sample chamber in the radiation field's centre.

The Cobalt-60 sources are double-encased in stainless steel pencils (resistant to corrosion) and subjected to rigorous testing in accordance with international guidelines. In the vertical drawer, eight millimeter diameter access holes are provided for the entry of service sleeves for gases, thermocouples, and other items. There is also a device for rotating or swirling samples during irradiation. Cobalt-60 source is surrounded by the lead barrier which is sufficient to maintain the radiation field within permissible limits. The time it takes for radiation to reach a sample varies depending on the dose. Following the application of irradiation, a vertical drawer rises from which samples can be taken.

Table 2: Specifications of gamma chamber - 5000

Maximum Co- 60 source capacity	518 TBq (14000 Ci)
Dose rate at maximum capacity	~ 9 kGy/hr (0.9 Mega Rad/hr) at the center of sample chamber
Dose rate uniformity	+25% or better radially; -25% or better axially
Irradiation volume	5000cc approx.
Size of sample chamber	17.2cm (dia) X 20.5cm (ht)
Shielding material	Lead & stainless steel
Weight of the unit	5600 kg. approx.
Size of unit	125cm (l) X 106.5cm (w) X 150cm (ht)
Timer range	6 seconds onwards

3.2.3.3 Procedure for imposition of gamma irradiation

For imposition of gamma irradiation treatments, 800 g of seeds were used for each treatment. The seeds were filled in the sample chamber and lid was then closed. Then the required doses were set as per the treatments. The duration taken for irradiation was adjusted automatically as per the dose det. Generally low doses require less time exposure when compared to high doses.

3.2.4 Seed storage

Seeds after treatment were packed in sealed polythene bags and stored for six months.

3.2.5 Observations

The below mentioned germination parameters and pulse beetle infestation assessment was taken at monthly intervals for six months. Morphological abnormalities arising due to irradiation was also analyzed at field level experiment.

3.2.5.1 Pulse beetle infestation assessment

3.2.5.1.1 Percentage seed damage

The percent seed damage was determined by collecting a sample of 100 seeds from each three replications of each treatment at monthly intervals. The damaged seed were separated from the total seed taken and counted. The seed having one or more holes were counted and considered as damaged seed. Based on the data obtained from the samples examined, the percentage seed damage was calculated by the procedure described by Adams and Schulten (1978) and expressed in percentage.

$$\text{Percentage seed damage} = \frac{\text{Number of damaged seeds}}{\text{Total number of seeds taken}} \times 100$$

3.2.5.1.2 Seed weight loss percentage

The percentage seed weight loss was assessed by taking a random sample of 100 cowpea seeds from all replicates of each treatment. Seed weight loss percentage was calculated by using the formula given by Adams and Schulten (1978) and expressed in percentage.

$$\text{Seed weight loss percentage} = \frac{U\,(Nd) - D\,(Nu)}{U\,(Nu+Nd)} \times 100$$

Nd- Number of damaged seeds

Nu- Number of undamaged seeds

D- Weight of damaged seeds

U- Weight of undamaged seeds

3.2.5.1.3 Number of eggs per 100 seeds

Hundred seeds were taken randomly from each replication of all treatments and number of eggs laid on those seeds was counted.

3.2.5.1.4 Number of damaged seeds, weight of damaged seeds, number of undamaged seeds, and weight of undamaged seeds

Hundred seeds were randomly taken and all these parameters were calculated for all treatments and were compared with the untreated control.

3.2.5.2 Germination parameters

3.2.5.2.1 Germination percentage (%)

The germination test was carried out with 100 seeds in four replications with rolled paper towel method as prescribed by ISTA. Normal healthy seeds were taken for germination test. The numbers of normal seedlings in each replication were counted on 8^{th} day for cowpea and the mean germination was calculated and expressed in percentage (ISTA, 2013).

3.2.5.2.2 Speed of germination

Germination for each day was counted and recorded upto 8^{th} day and expressed in percentage.

The speed of germination was calculated by employing the following formula suggested by Maguire (1962),

$$\text{Speed of germination} = X_1 / Y_1 + X_2 / Y_2 + \ldots\ldots\ldots\ldots\ldots + X_n - X_{n-1} / Y_n$$

Where X_n = percent germination on n^{th} day

Y_n = number of days from sowing to n^{th} count

3.2.5.2.3 Seedling shoot length

In each treatment, ten normal seedlings were randomly selected on eighth day. The shoot length was measured from the base of the primary leaf to the base of the hypocotyls and the mean was calculated and expressed in cm.

3.2.5.2.4 Seedling root length

The root length for normal seedlings selected for shoot length was calculated and mean is expressed in cm.

3.2.5.2.5 Seedling dry weight (g)

Ten normal seedlings were selected from each treatment and air dried for six hours and then in hot air oven at 60° C for 48 h and was cooled at room temperature for 45 minutes, then the dry weight of seedlings were recorded and expressed in g.

3.2.5.2.6 Seedling vigor index I

The seedling vigor index was calculated by adopting the formula suggested by Abdul- Baki and Anderson (1973).

$$\text{Seedling Vigour index I} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

3.2.5.2.7 Seedling vigor index II

The seedling vigor index II was computed by adopting the formula suggested by Abdul- Baki and Anderson (1973).

$$\text{Seedling Vigour index II} = \text{Germination (\%)} \times \text{Seedling dry weight (g)}$$

Plate 1: Field preparation

Plate 2: Formation of ridges and furrows

Plate 3: Flowering stage of cowpea plants

Plate 4: Pod filling stage of cowpea plants

Plate 5: General view of experimental area for field evaluation

3.2.5.3 Field evaluation of irradiated seeds for morphological parameters

3.2.5.3.1 Design and layout

Design : Randomized Block Design (RBD)

Treatment : 6

Replications : 3.

3.2.5.3.2 Planting material

Irradiated seeds were selected randomly from each treatment and sown in field to evaluate morphological parameters arising due to gamma irradiation. Seeds were sown with the spacing of 30 × 15 cm.

3.2.5.3.3 Morphological parameters

3.2.5.3.3.1 Germination percentage

Fifty numbers of randomly selected seeds from each treatment were sown in a well prepared field at 3 cm depth. The germination percentage was expressed in percentage.

$$\text{Germination percentage} = \frac{\text{Total number of seedlings emerged}}{\text{Total number of seeds sown}} \times 100$$

3.2.5.3.2 Plant height

It was measured from ground level to the top most fully opened leaf at harvest stage. Height of plant was recorded in cm.

3.2.5.3.3 Number of pods per plant

The total numbers of pods from all selected plants were counted manually from all the treatments.

3.2.5.3.4 Number of seeds per pod

The total number of seeds from randomly selected pods in all treatments was counted.

3.2.5.3.5 100 seed weight

Hundred seeds were randomly taken from each treatment and its weight was calculated using electronic balance.

3.2.5.3.6 Morphological abnormalities

All the plants were analyzed from vegetative stage until harvest for various morphological abnormalities arising due to irradiation and compared with the untreated control.

3.3 SEED COATING OF COWPEA SEEDS WITH CHITOSAN

3.3.1 Design and Layout

The experiment was carried out in Completely Randomized Block design (CRD) with 11 treatments in three replications.

3.3.1.1 Preparation of chitosan solution and seed coating

Chitosan powder was dissolved at 1, 2, 3, 4 and 5 per cent (w/v) into aqueous solution of 1 per cent acetic acid (w/v). 50 g of cowpea seeds were taken in three replications in each treatment and mixed with 1 mL for 1:50 ratio and 5 mL for 1:10 ratio. Seeds were taken in plastic tray and mixed with chitosan solutions at different concentrations and shade dried for 8 hours.

3.3.2 Seed storage

Seeds after treatment were packed in sealed polythene bags and stored for six months.

1 % chitosan

2 % chitosan

3 % chitosan

4 % chitosan

5 % chitosan

Plate 6: Different concentrations of chitosan solution prepared chitosan powder

Table 3: Different concentrations of chitosan solution

Treatment	Chitosan doses
T_1	1 % @ 1 mL 50 g^{-1} of seed
T_2	1 % @ 5mL 50 g^{-1} of seed
T_3	2 % @ 1mL 50 g^{-1} of seed
T_4	2 % @ 5mL 50 g^{-1} of seed
T_5	3 % @ 1mL 50 g^{-1} of seed
T_6	3 % @ 5mL 50 g^{-1} of seed
T_7	4 % @ 1mL 50 g^{-1} of seed
T_8	4 % @ 5mL 50 g^{-1} of seed
T_9	5 % @ 1mL 50 g^{-1} of seed
T_{10}	5 % @ 5mL 50 g^{-1} of seed
T_{11}	Control

3.3.1 Observations

Germination parameters and pulse beetle damage assessment were recorded at monthly intervals for a period of six months.

3.3.3.1 Pulse beetle infestation assessment

3.3.3.1.1 Percentage seed damage

The percent seed damage was determined by collecting a sample of 100 seeds from each three replications of each treatment at monthly intervals. The damaged seed were separated from the total seed taken and counted. The seed having one or more holes were counted and considered as damaged seed. Based on the data obtained from the samples examined the percent seed damage was calculated by the formula described by Adams and Schulten (1978) and expressed in percentage.

$$\text{Percentage seed damage} = \frac{\text{Number of pulse beetle damaged seeds}}{\text{Total number of seeds taken}} \times 100$$

3.3.3.1.2 Seed weight loss percentage (%)

The percentage seed weight loss was assessed by taking a random sample of 100 cowpea seeds from all replicates of each treatment. Seed weight loss percentage was calculated by using the formula given by Adams and Schulten (1978) and expressed in percentage.

$$\text{Seed weight loss percentage} = \frac{U\,(Nd) - D\,(Nu)}{U\,(Nu+Nd)} \times 100$$

Nd- Number of damaged seeds

Nu- Number of undamaged seeds

D- Weight of damaged seeds

U- Weight of undamaged seeds

3.3.3.1.3 Number of eggs per 100 seeds

Hundred seeds were randomly selected from each replication of all treatments and number of eggs laid on those seeds was counted

3.3.3.1.4 Number of damaged seeds, weight of damaged seeds, number of undamaged seeds, and weight of undamaged seeds

Hundred seeds were randomly taken and all these parameters were calculated for all treatments and were compared with the untreated control.

3.3.3.2 Germination parameters

3.3.3.2.1 Germination percentage

The germination test was carried out with 100 seeds in four replications with rolled paper towel method as prescribed by ISTA. Normal healthy seeds were taken for germination test. The germination test was conducted at room temperature and a germination period of 8 days adopted throughout the study. The numbers of normal seedlings in each replication were counted on 8th day for cowpea and the mean germination was calculated and expressed in percentage (ISTA, 2013)

3.3.3.2.2 Speed of germination

Germination for each day was counted and recorded upto 8th day and expressed in percentage. The speed of germination was calculated by employing the following formula suggested by Maguire (1962),

$$\text{Speed of germination} = X_1 / Y_1 + X_2 / Y_2 + \ldots\ldots\ldots\ldots\ldots + X_n - X_{n-1} / Y_n$$

Where X_n = percent germination on nth day

Y_n = number of days from sowing to nth count

3.3.3.2.3 Seedling shoot length

In each treatment, ten normal seedlings were randomly selected on eighth day. The shoot length was measured from the base of the primary leaf to the base of the hypocotyls and the mean was calculated and expressed in cm.

3.3.3.2.4 Seedling root length

The root length for normal seedlings selected for shoot length was calculated and mean is expressed in cm.

3.3.3.2.5 Seedling dry weight

Ten normal seedlings were selected from each treatment and air dried for six hours and then in hot air oven at 60° C for 48 h and was cooled at room temperature for 45 minutes, then the dry weight of seedlings were recorded and expressed in g.

3.3.3.2.6 Seedling vigor index I

The seedling vigor index was calculated by adopting the formula suggested by Abdul- Baki and Anderson (1973)

Seedling Vigour index I = Germination (%) × Seedling length (cm)

3.3.3.2.7 Seedling vigor index II

The seedling vigor index II was computed by adopting the formula suggested by Abdul- Baki and Anderson (1973)

Seedling Vigour index II = Germination (%) × Seedling dry weight (g)

3.4 STATISTICAL ANALYSIS

The collected data were statistically analyzed using Analysis of Variance Technique (ANOVA) under Completely Randomized Block Design (CRD) for storage studies and Randomized Block Design (RBD) for field studies. WASP and OPSTAT software were used for obtaining mean, Standard Error (SE) and Critical Difference.

RESULTS

4. RESULTS

The present investigation entitled "Irradiation and seed coating for enhancing storage life of grain cowpea (*Vigna unguiculata* (L.) Walp.) was carried out to standardize the gamma doses and chitosan concentration with optimum quantity for increasing the storage life of cowpea seeds. This experiment was carried out in the Department of Seed Science and Technology, College of Agriculture, Vellayani during 2019-21. Various parameters were studied in both the experiments for a period of six months during storage. The data obtained during the course of investigation were statistically analyzed and the results are presented with suitable tables.

4.1 IRRADIATION OF COWPEA SEEDS WITH GAMMA RAYS

The study was carried out with different gamma doses of 100, 200, 300, 400 and 500 Gy and stored samples were analysed for the following parameters and the results are given in the following headings

4.1.1 Pulse beetle infestation assessment

4.1.2 Seed germination parameters

4.1.3 Morphological parameters

4.1.1 Pulse beetle infestation assessment:

The impact of different gamma doses on pulse beetle damage during storage period is furnished below:

4.1.1.1 Percentage seed damage (%)

The result of influence of different gamma doses on percentage seed damage during storage period is presented in the Table 4.

All the gamma doses used were significantly superior to control in reducing infestation of cowpea seeds by pulse beetle during storage. Seed infestation was not observed in treatments T_1 (100 Gy) for up to three months of storage and T_2 (200 Gy) for up to five months of storage. No seed damage was observed in treatment T_3 (300

Gy), T_4 (400 Gy) and T_5 (500Gy) throughout the storage period. In contrast, seed infestation was observed from first month up to six months in control.

At the end of storage period, percentage seed damage was highest in Control (56.33 %) which was then followed by 100 Gy (2.667%) and 200 Gy (0.667%) whereas no seed damage was recorded in treatment 300 Gy, 400 Gy and 500Gy throughout the storage period of six months.

4.1.1.2 Seed weight loss percentage (%)

The result of influence of different gamma doses on seed weight loss percentage during storage period is presented in the Table 5.

Seed weight loss percentage was not observed in treatments T_1 (100 Gy) for up to three months of storage and T_2 (200 Gy) for up to five months of storage. No seed weight loss was observed in treatment T_3 (300 Gy), T_4 (400 Gy) and T_5 (500Gy) throughout the storage period. However, weight loss was observed from first month up to six months in control.

At the end of storage period of six months, seed weight loss percentage was highest in Control (28.182 %) which was then followed by 100 Gy (0.995 %) and 200 Gy (0.290 %) whereas no weight loss was recorded in treatment 300 Gy, 400 Gy and 500 Gy throughout the storage period.

4.1.1.3 Number of eggs per 100 seeds (nos)

The result of the experiment on the impact of different gamma doses on number of eggs per 100 seeds is presented in the Table 6.

The results indicated that all the treatments were significantly superior to control in inhibiting egg laying by the female beetle. No eggs were laid in seeds exposed to gamma rays of T_3 (300 Gy), T_4 (400 Gy) and T_5 (500 Gy) throughout the storage period. However oviposition was observed in control from the first month of storage onwards. The number of eggs laid progressively increased in control over the period of study.

At the end of storage period, highest number of eggs was laid in control with the number of eggs ranging from 0.333 from the first month to 78.333 in the sixth month of storage. It was followed by T_1 (100 Gy) with the number of eggs ranging from 0.00 – 4.333 and T_2 (200 Gy) with 0.00-1.667 from first to six months of storage.

4.1.1.4 Number of damaged and undamaged seeds (nos)

'There were significant differences between treatments in the mean number of seeds damaged and undamaged by pulse beetle (Table 7).

There was no seed damage in the seeds treated with T_3 (300 Gy), T_4 (400 Gy) and T_5 (500 Gy) throughout the storage period. Damaged seeds were not observed in treatments T_1 (100 Gy) for up to three months of storage and T_2 (200 Gy) for up to five months of storage. At the end of storage period of six months, number of damaged seeds was highest (56.333) in control which is followed by 100 Gy (3.00) and 200 Gy (0.667) whereas no damaged seeds were recorded in treatment 300 Gy, 400 Gy, 500Gy throughout the storage period of six month

4.1.1.5 Weight of damaged and undamaged seeds (g)

The mean weight of damaged and undamaged seeds was significantly different from forth month of storage (Table 8).

There was no weight loss in the seeds treated with T_3 (300 Gy), T_4 (400 Gy) and T_5 (500 Gy) throughout the storage period. Weight loss was not observed in treatments T_1 (100 Gy) for up to three months of storage and T_2 (200 Gy) for up to five months of storage. At the end of storage period of six months, highest mean weight of damaged seeds (4.163 g) was found in control which is followed by 100 Gy (0.180 g) and 200 Gy (0.040 g) whereas no weight loss was recorded in treatment 300 Gy, 400 Gy, 500Gy throughout the storage period of six month.

Table 4: Effect of gamma doses on percentage seed damage (%) of cowpea seeds for six months of storage

Treatment	Percentage seed damage (%)					
	Months After Storage (MAS)					
	1	2	3	4	5	6
T_1 (100 Gy)	0.000 (1.00)**	0.000 (1.00)	0.000 (1.00)	0.333 (1.138)	0.667 (1.244)	2.667 (1.816)
T_2 (200 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.667 (1.244)
T_3 (300 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_4 (400 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_5 (500 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_6 (Control)	0.333 (1.138)	0.667 (1.244)	2.000 (1.656)	5.667 (2.499)	16.333 (4.096)	56.333 (7.558)
SE (m)	0.05	0.10	0.14	0.19	0.23	0.24
CD (5%)	NS*	NS	NS	0.609	0.738	0.754

*NS- Non- significant, **Values in parenthesis are square root transferred values

Table 5: Effect of gamma doses on seed weight loss percentage (%) of cowpea seeds for six months of storage

Treatment	Seed weight loss percentage (%)					
	Months After Storage (MAS)					
	1	2	3	4	5	6
T_1 (100 Gy)	0.00 (1.00)**	0.000 (1.00)	0.000 (1.00)	0.292^b (1.123)	0.226^b (1.098)	0.995^b (1.373)
T_2 (200 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.290^b (1.122)
T_3 (300 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_4 (400 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_5 (500 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_6 (Control)	0.176 (1.079)	0.226 (1.098)	0.748 (1.303)	2.840^a (1.862)	6.261^a (2.649)	28.182^a (5.395)
SE (m)	0.03	0.04	0.06	0.18	0.14	0.13
CD (5%)	NS*	NS	NS	NS	0.463	0.414

*NS- Non- significant, **Values in parenthesis are square root transferred values.

The values in the same column with the same alphabet as superscript are not significantly different.

Table 6: Effect of gamma doses on number of eggs per 100 seeds (nos) of cowpea seeds for six months of storage

Treatment	Number of eggs per 100 seeds (nos)					
	Months After Storage (MAS)					
	1	2	3	4	5	6
T_1 (100 Gy)	0.000 (1.00)**	0.000 (1.00)	0.000 (1.00)	0.667^{b} (1.244)	1.333^{b} (.1.471)	4.333^{b} (2.150)
T_2 (200 Gy)	0.000 (1.00)	0.000 (1.00)	0.333 (1.138)	0.000 (1.00)	0.000 (1.00)	1.667^{bc} (1.577)
T_3 (300 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_4 (400 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_5 (500 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_6 (Control)	0.333 (1.138)	1.000 (1.328)	3.000 (1.882)	9.333^{a} (3.150)	33.333^{a} (5.785)	78.333^{a} (8.903)
SE (m)	0.05	0.08	0.20	0.25	0.29	0.28
CD (5%)	NS*	NS	NS	NS	0.916	0.878

*NS- Non- significant, **Values in parenthesis are square root transferred values,

The values in the same column with the same alphabet as superscript are not significantly different.

Table 7: Effect of gamma doses on number of damaged and undamaged (nos) of cowpea seeds for six months of storage

Treatment	Number of damaged (D) and undamaged seeds (U) (nos)											
	Months After Storage (MAS)											
	1		2		3		4		5		6	
	D	U	D	U	D	U	D	U	D	U	D	U
T_1 (100 Gy)	0.00 (1.00)**	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0 (10.05)	0.33^b (1.138)	99.66^a (10.032)	0.667^b (1.244)	99.33^a (10.017)	3.00^b (1.995)	97.00^a (9.899)
T_2 (200 Gy)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0 (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)	0.667^c (1.244)	99.33^a (10.017)
T_3 (300 Gy)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0 (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)
T_4 (400 Gy)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0 (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)
T_5 (500 Gy)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0 (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)	0.00 (1.00)	100.0^a (10.05)
T_6 (Control)	0.33 (1.138)	33.00^b (4.00)	0.67 (1.24)	32.66^b (3.989)	2.00 (1.656)	98.00 (9.950)	5.60^a (2.492)	94.33^b (9.762)	16.333^a (4.096)	83.67^b (9.195)	56.333^a (7.558)	43.67^b (6.661)
SE (m)	0.05	1.22	0.14	1.21	0.14	0.02	0.96	0.04	0.23	0.10	0.20	0.16
CD (5%)	NS*	3.816	NS	3.794	NS	NS	2.998	0.154	0.738	0.316	0.646	0.499

*NS- Non-significant, **Values in parenthesis are square root transferred values.

The values in the same column with the same alphabet as superscripts are not significantly different.

Table 8: Effect of gamma doses on weight of damaged and undamaged seeds (g) of different treatments for six months of storage

Treatment	Weight of damaged (D) and undamaged seeds(U) (g)											
	Months After Storage (MAS)											
	1		2		3		4		5		6	
	D	U	D	U	D	U	D	U	D	U	D	U
T_1 (100 Gy)	0.00 (1.00)**	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.013^b (1.007)	10.507^a (3.392)	0.047^b (1.023)	10.473^a (3.387)	0.180^b (1.085)	10.340^a (3.367)
T_2 (200 Gy)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520^a (3.394)	0.00 (1.00)	10.520^a (3.394)	0.040^b (1.019)	10.480^a (3.388)
T_3 (300 Gy)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520^a (3.394)	0.00 (1.00)	10.520^a (3.394)	0.00 (1.00)	10.520^a (3.394)
T_4 (400 Gy)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520^a (3.394)	0.00 (1.00)	10.520^a (3.394)	0.00 (1.00)	10.520^a (3.394)
T_5 (500 Gy)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520 (3.394)	0.00 (1.00)	10.520^a (3.394)	0.00 (1.00)	10.520^a (3.394)	0.00 (1.00)	10.520^a (3.394)
T_6 (Control)	0.017 (1.008)	10.503 (3.392)	0.047 (1.023)	10.473 (3.387)	0.133 (1.063)	10.387 (3.374)	0.440^a (1.194)	10.080^b (3.328)	1.143^a (1.456)	9.377^b (3.221)	4.163^a (2.266)	6.357^b (2.708)
SE (m)	0.003	0.001	0.009	0.003	0.061	0.005	0.034	0.012	0.044	0.020	0.054	0.044
CD (5%)	NS*	NS	NS	NS	NS	NS	0.105	0.038	0.137	0.063	0.167	0.137

*NS- Non- significant, **Values in parenthesis are square root transferred values,

The values in the same column with the same alphabet as superscripts are not significantly different

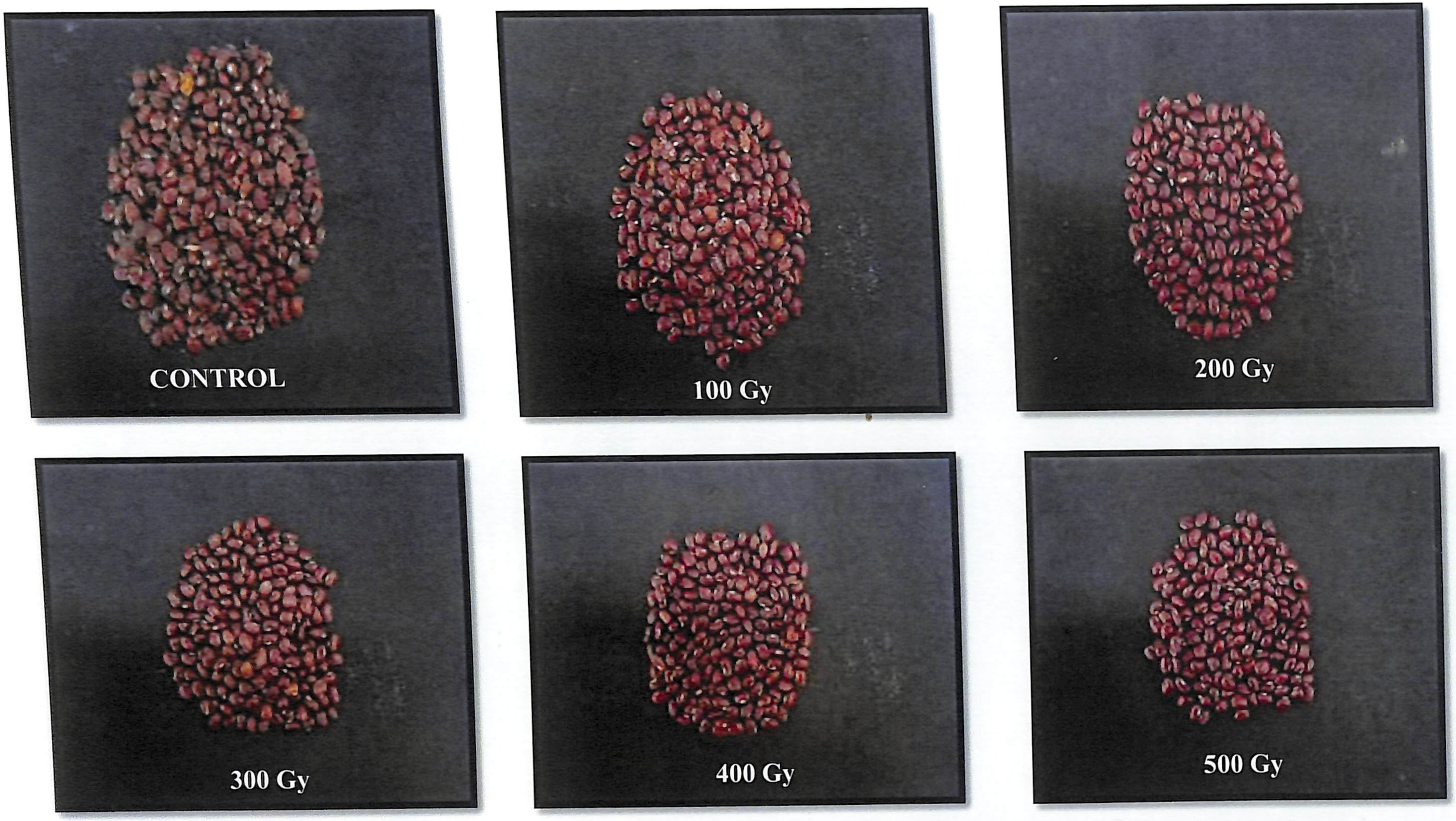


Plate 7: Comparison of pulse beetle infestation in the seed lots treated with different doses of gamma rays

4.1.2 Seed germination parameters

The different seed germination parameters were taken for a period of six months of storage.

4.1.2.1 Germination percentage (%)

At the end of six-month storage, T_2 (200 Gy) recorded highest germination per cent (84.33%), which was on par with T_1 (100 Gy) and T_6 (Control) with germination per cent of 81.80 per cent and 80.66 per cent. Also, T_5 (500 Gy) recorded the lowest germination per cent (26.33%) (Table 9). The mean germination percentage of various gamma doses ranged between 88.91% (200 Gy) and 78.81% (500 Gy).

4.1.2.2 Speed of germination

The speed of germination during storage period as recorded to be the highest in T_2 (200 Gy) *i.e.*, 32.13. The mean germination speed of seeds after various gamma dose treatments ranged between 34.45 (100 Gy) and 27.67 (500 Gy). This was found to be on par with T_1 (100 Gy, 32.10) and T_6 (Control, 31.40). The slowest germination was recorded for the treatment T_5 (500 Gy) given in Table 10.

4.1.2.3 Seedling shoot length (cm)

The mean seedling shoot length under various gamma doses varied form 13.25 cm (100 Gy) to 9.90 cm (500 Gy). At the end of storage period (sixth month), T_2 (200 Gy) recorded the highest seedling shoot length (11.83 cm), which was on par with T_6 (Control, 11.56 cm) and T_1 (100 Gy, 11.50 cm). The least seedling shoot length (9.90 cm) was recorded in T_5 (500 Gy) (Table 11).

4.1.2.4 Seedling root length (cm)

The mean seedling root length under various gamma doses varied between 15.02 cm and 8.09 cm (Table 12). On studying the seedling shoot length after six months of storage, the treatment T_1 (100 Gy) recorded the highest seedling root length(13.84 cm) which was on par with T_2 (200 Gy, 13.43 cm) and T_6 (Control, 12.45 cm). T_5 (500 Gy) showed the minimum seedling root length of 7.23 cm.

4.1.2.5 Seedling dry weight (g)

At the end of six-month storage, T_2 (200 Gy) recorded seedling dry weight (0.703 g), which was on par with T_1 (100 Gy), T_6 (Control) and T_3 (300 Gy) with 0.593 g, 0.687 g and 0.641 g (Table 13). Also, T_5 (500 Gy) recorded the lowest seedling dry weight (0.549 g) among the treated seeds as well as control seeds (Table 6). The mean seedling dry weight of various gamma doses varied between 0.747 (200 Gy) and 0.634 g (500 Gy).

4.1.2.6 Seedling vigour index I

The impact of various treatments on seedling vigour index I is given in Table 14. At the end of sixth month, highest seedling vigour index I (2130.49) was observed in T_2 (200 Gy) which was on par with T_1 (100 Gy) with 2074.57 and T_6 (Control) with 1939.24. The lowest value (1146.47) was exhibited by T_5 (500 Gy). The mean vigour index I of the seedlings over the storage period ranged between 2502.29 (200 Gy) and 1403.98 (500 Gy).

4.1.2.7 Seedling vigour index II

The impact of different gamma doses on seedling vigour index II is given in Table 15. At the end of storage period of six months, highest seedling vigour index II (59.28) was recorded in T_2 (200 Gy) which was on par with T_1 (100 Gy) with 56.73 and T_6 (control) with 55.52. The lowest value (39.86) was observed in T_5 (500 Gy). The mean vigour index II of seedlings during the storage period ranged between T_2 (66.49) and T_5 (49.56).

Table 9: Effect of gamma doses on germination percentage (%) of cowpea seeds for six months of storage

Treatment	Germination percentage (%)						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1 (100 Gy)	91.20^{a} (9.60)**	91.10^{a} (9.59)	89.00^{a} (9.48)	87.80 (9.42)	87.33^{a} (9.39)	81.80^{ab} (9.09)	88.03 (9.38)
T_2 (200 Gy)	91.70^{a} (9.62)	90.60^{ab} (9.57)	90.40^{a} (9.56)	89.50 (9.51)	86.90^{ab} (9.37)	84.33^{a} (9.23)	88.91 (9.43)
T_3 (300 Gy)	89.20^{ab} (9.49)	86.90^{ab} (9.37)	86.30^{ab} (9.34)	85.60 (9.30)	85.73^{ab} (9.31)	78.19^{bc} (8.89)	85.32 (9.24)
T_4 (400 Gy)	85.70^{bc} (9.31)	85.80^{b} (9.31)	83.00^{bc} (9.16)	81.30 (9.06)	80.23^{bc} (9.01)	76.79^{cd} (8.81)	82.43 (9.08)
T_5 (500 Gy)	81.39^{c} (9.07)	80.27^{c} (9.01)	79.39^{c} (8.96)	78.39 (8.90)	74.83^{c} (8.70)	72.60^{d} (8.57)	78.81 (8.88)
T_6 (Control)	90.50^{ab} (9.56)	89.40^{ab} (9.50)	88.19^{a} (9.44)	87.10 (9.38)	85.70^{ab} (9.30)	80.66^{abc} (9.03)	86.93 (9.32)
SE (m)	0.08	0.08	0.07	0.17	0.12	0.08	
CD (5%)	0.272	0.258	0.227	NS*	0.383	0.269	

*NS- Non- significant, **Values in parenthesis are square root transferred values,

The values in the same column with the same alphabet as superscripts are not significantly different.

Table 10: Effect of gamma doses on speed of germination of cowpea seeds for six months of storage

Treatments	Speed of germination						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1 (100 Gy)	36.73^a	35.43^a	35.33^a	33.60^a	33.53^a	32.10^a	34.45
T_2 (200 Gy)	35.40^b	35.40^a	35.20^a	33.70^a	33.40^a	32.13^a	34.21
T_3 (300 Gy)	31.60^c	31.20^b	31.10^b	30.43^b	29.60^b	28.40^b	30.39
T_4 (400 Gy)	31.50^c	30.83^b	29.63^c	28.43^c	28.00^c	27.60^b	29.33
T_5 (500 Gy)	29.53^d	28.10^c	28.03^c	27.53^c	26.50^d	26.33^c	27.67
T_6 (Control)	36.60^a	35.10^a	34.20^a	33.73^a	32.80^a	31.40^a	33.97
SE (m)	0.25	0.30	0.34	0.39	0.32	0.40	
CD (5%)	0.793	0.954	1.077	1.236	1.010	1.250	

The values in the same column with the same alphabet as superscripts are not significantly different.

Table 11: Effect of gamma doses on seedling shoot length (cm) of cowpea seeds for a period of six months

Treatment	Seedling Shoot length (cm)						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1 (100 Gy)	14.16^{ab}	14.16^{a}	13.83^{a}	13.16^{a}	12.73^{a}	11.50^{ab}	13.25
T_2 (200 Gy)	14.83^{a}	14.06^{a}	13.63^{a}	12.23^{ab}	11.93^{a}	11.83^{a}	13.08
T_3 (300 Gy)	13.43^{bc}	11.96^{bc}	11.56^{bc}	11.36^{b}	10.80^{b}	10.30^{bc}	11.56
T_4 (400 Gy)	12.66^{cd}	11.26^{cd}	10.63^{cd}	10.16^{c}	10.03^{b}	9.53^{cd}	10.71
T_5 (500 Gy)	11.70^{d}	10.63^{d}	10.03^{d}	9.33^{c}	9.13^{c}	8.56^{d}	9.90
T_6 (Control)	12.83^{cd}	12.63^{ab}	12.60^{ab}	12.50^{a}	12.33^{a}	11.56^{ab}	12.40
SE (m)	0.43	0.43	0.44	0.30	0.32	0.41	
CD (5%)	1.345	1.342	1.392	0.962	1.00	1.298	

The values in the same column with the same alphabet as superscripts are not significantly different.

Table 12: Effect of gamma doses on seedling root length (cm) of cowpea seeds for six months of storage

Treatment	Seedling root length (cm)						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1 (100 Gy)	16.00^{a}	16.27^{a}	15.63^{a}	14.27^{b}	14.10^{a}	13.84^{a}	15.01
T_2 (200 Gy)	15.97^{a}	15.50^{a}	15.50^{a}	15.40^{a}	14.35^{a}	13.43^{ab}	15.02
T_3 (300 Gy)	10.87^{b}	10.63^{b}	10.87^{b}	10.50^{c}	9.73^{b}	9.17^{c}	10.26
T_4 (400 Gy)	10.00^{bc}	9.83^{b}	9.50^{c}	9.27^{d}	9.13^{b}	8.46^{c}	9.37
T_5 (500 Gy)	8.93^{c}	8.57^{c}	8.20^{d}	8.03^{e}	7.57^{c}	7.23^{d}	8.09
T_6 (Control)	15.86^{a}	15.57^{a}	15.07^{a}	14.33^{a}	14.30^{a}	12.45^{b}	14.60
SE (m)	0.39	0.32	0.36	0.32	0.44	0.35	
CD (5%)	1.323	1.022	1.135	1.016	1.378	1.096	

The values in the same column with the same alphabet as superscripts are not significantly different.

Table 13: Effect of gamma doses on seedling dry weight (g) of cowpea seeds for six months storage

Treatment	Seedling dry weight (g)						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1 (100 Gy)	0.775	0.750^{a}	0.744^{ab}	0.735^{a}	0.702^{a}	0.693^{a}	0.726
T_2 (200 Gy)	0.783	0.765^{a}	0.759^{a}	0.747^{a}	0.725^{a}	0.703^{a}	0.747
T_3 (300 Gy)	0.736	0.724^{ab}	0.721^{bcd}	0.721^{a}	0.694^{a}	0.641^{ab}	0.706
T_4 (400 Gy)	0.704	0.701^{b}	0.700^{cd}	0.672^{b}	0.603^{b}	0.597^{bc}	0.663
T_5 (500 Gy)	0.693	0.693^{b}	0.693^{d}	0.604^{c}	0.576^{b}	0.549^{c}	0.634
T_6 (Control)	0.737	0.734^{ab}	0.728^{abc}	0.727^{a}	0.699^{a}	0.687^{a}	0.718
SE (m)	0.02	0.03	0.03	0.01	0.02	0.02	
CD (5%)	*NS	0.018	0.013	0.032	0.076	0.066	

*NS- Non- significant

The values in the same column with the same alphabet as superscripts are not significantly different

Table 14: Effect of gamma doses on seedling vigor index I of cowpea seeds for six months of storage

Treatment	Seedling vigour index I						
	Months After Storage (MAS)						
	M1	M2	M3	M4	M5	M6	Mean
T_1 (100 Gy)	2752.23^{a} (52.46)**	2771.56^{a} (52.66)	2622.31^{a} (51.20)	2408.79^{a} (49.06)	2344.17^{a} (48.42)	2074.57^{a} (45.53)	2495.61 (49.96)
T_2 (200 Gy)	2823.33^{a} (53.17)	2677.85^{ab} (51.76)	2635.00^{a} (51.33)	2470.26^{a} (49.70)	2277.82^{a} (47.71)	2130.49^{a} (46.17)	2502.29 (50.02)
T_3 (300 Gy)	2168.93^{b} (46.56)	1963.32^{c} (44.32)	1935.13^{b} (44.00)	1869.07^{b} (43.24)	1760.52^{b} (41.96)	1522.68^{b} (39.03)	1869.94 (43.24)
T_4 (400 Gy)	1942.31^{c} (44.08)	1809.70^{d} (42.53)	1672.02^{c} (40.89)	1579.01^{c} (39.79)	1539.83^{c} (39.21)	1383.03^{b} (37.19)	1654.32 (40.67)
T_5 (500 Gy)	1677.31^{d} (40.97)	1541.25^{e} (39.27)	1447.06^{d} (38.05)	1362.79^{d} (36.90)	1248.98^{d} (35.34)	1146.47^{c} (33.87)	1403.98 (37.47)
T_6 (Control)	2598.82^{a} (50.97)	2521.47^{b} (50.22)	2438.30^{a} (49.37)	2336.43^{a} (48.33)	2281.56^{a} (47.75)	1939.24^{a} (44.02)	2352.64 (48.50)
SE (m)	0.70	0.53	0.63	0.79	0.88	0.72	
CD (5%)	2.201	1.653	1.985	2.486	2.759	2.264	

**Values in parenthesis are square root transferred values

The values in the same column with the same alphabet as superscripts are not significantly different.

Table 15: Effect of gamma doses on seedling vigour index II of cowpea seeds for six months of storage

Treatment	Seedling vigour index II						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1 (100 Gy)	70.74^{a} (8.46)**	68.30^{a} (8.32)	66.14^{ab} (8.19)	64.50^{a} (8.09)	61.33^{a} (7.89)	56.73^{ab} (7.59)	64.62 (8.04)
T_2 (200 Gy)	71.85^{a} (8.53)	69.32^{a} (8.38)	68.56^{a} (8.34)	66.84^{a} (8.23)	63.08^{a} (8.00)	59.28^{a} (7.76)	66.49 (8.15)
T_3 (300 Gy)	65.64^{ab} (8.16)	62.92^{bc} (7.99)	62.24^{c} (7.95)	61.70^{ab} (7.92)	59.58^{a} (7.79)	50.14^{bc} (7.15)	60.37 (7.77)
T_4 (400 Gy)	60.29^{bc} (7.83)	60.09^{cd} (7.82)	58.07^{d} (7.69)	54.68^{bc} (7.45)	48.60^{b} (7.03)	45.89^{cd} (6.84)	54.60 (7.39)
T_5 (500 Gy)	56.37^{c} (7.57)	55.67^{d} (7.53)	55.04^{e} (7.49)	47.37^{c} (6.95)	43.03^{b} (6.63)	39.86^{d} (6.39)	49.56 (7.04)
T_6 (Control)	66.69^{ab} (8.26)	65.57^{ab} (8.16)	64.25^{bc} (8.07)	63.40^{a} (8.02)	60.05^{a} (7.80)	55.52^{ab} (7.51)	62.58 (7.91)
SE (m)	0.14	0.09	0.06	0.16	0.21	0.16	
CD (5%)	0.441	0.308	0.201	0.527	0.662	0.513	

**Values in parenthesis are square root transferred values

The values in the same column with the same alphabet as superscripts are not significantly different.

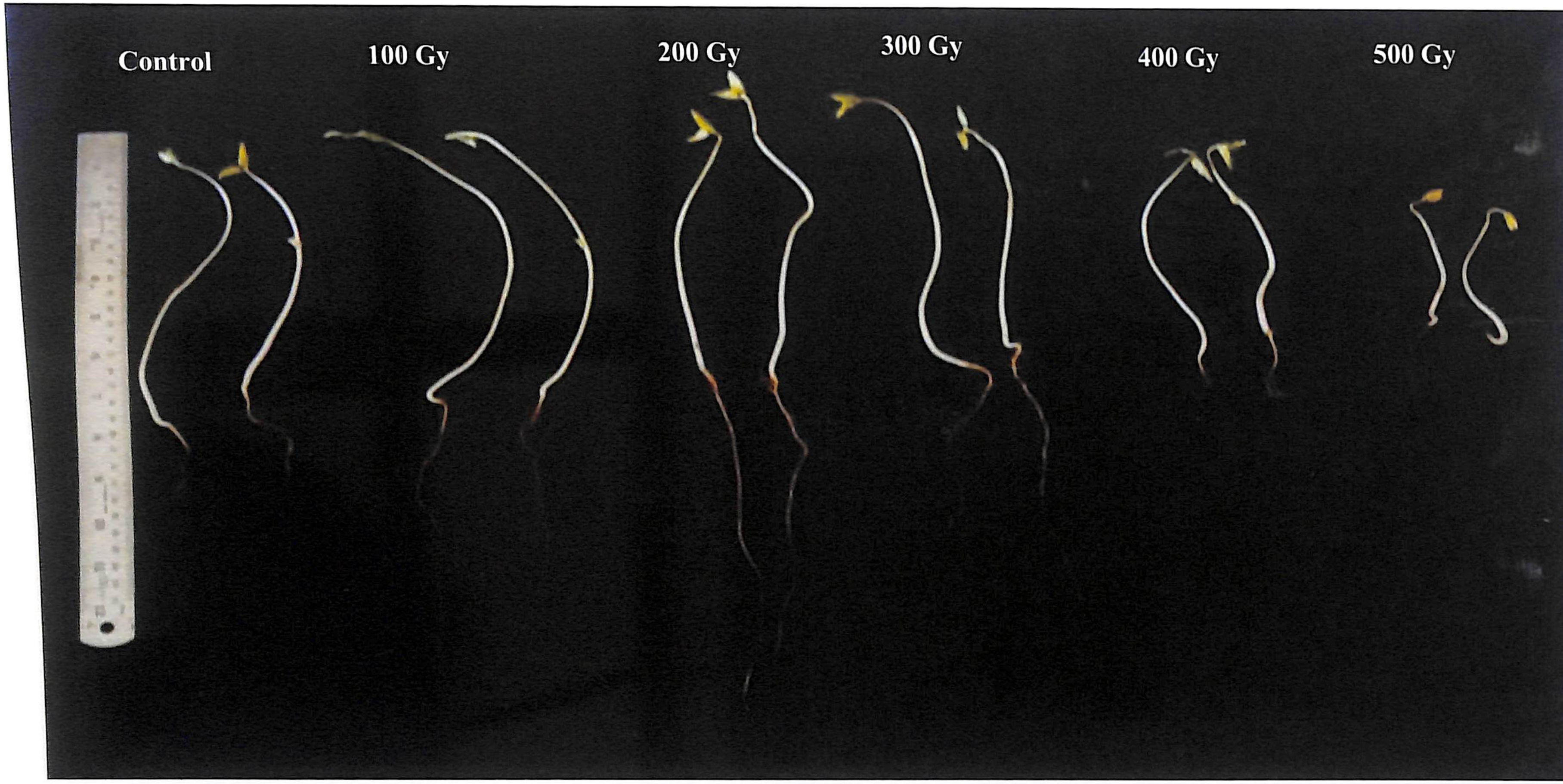


Plate 8: Comparison of seedling parameters in the seed lots treated with different doses of gamma rays at the end of six months storage

4.1.3 Field evaluation of treated seeds for observing morphological parameters

The study was carried out with 50 seeds in each treatment (100, 200, 300, 400, 500 Gy and Control) which were sown in the field with the spacing of 30 × 15 cm and different morphological parameters abnormalities arising due to irradiation were analyzed and observations are presented in Table 16.

4.1.3.1 Germination percentage (%)

Gamma irradiation did not appear to delay the germination of seeds at low doses like 100 Gy, 200 Gy and 300 Gy. At higher doses i.e., 400 Gy and 500 Gy, a slight delay was noticed. The treatment (T_5) 500 Gy exhibited a low germination percentage of 62% followed by (T_4) 400 Gy with 76 %, and (T_3) 300 Gy with 84 %. Treatment (T_2) 200 Gy, (T_1) 100 Gy recorded germination percentage of 96% and 96 % and control with 96% respectively.

4.1.3.2 Plant height (cm)

The height of the plants was recorded on fortieth day of sowing and the data given in the Table 16. The plant height values showed a slight reduction with increasing doses of gamma rays, compared with that of the control. Highest gamma dose of 500 Gy (T_5) resulted in decline in plant height (35.99cm). There was no significant difference among the mean values of control, 100 Gy, 200 and 300 Gy which lies between 39.80cm - 40.00cm.

4.1.3.3 Number of pods plant^{-1} (nos)

The data on number of pods plant $^{-1}$ is given in the Table 16. The mean number of pods plant^{-1} ranged between 15.20 nos in (T_5) 500 Gy to 16.67 nos in (T_2) 200 Gy as compared to the (T_6) control (16.37 nos). The highest mean was recorded in (T_2) 200 Gy with 16.67 nos and the lowest in (T_5) 500 Gy with 15.20 nos. The means for this character were higher in lower gamma doses compared to control and decreased with increase in gamma doses.

4.1.3.4 Number of seeds pod^{-1} (nos)

The mean for this character was highest in (T_3) 300 Gy (15.27 nos), followed by (T_2) 200 Gy (15.25 nos) and lowest in treatment (T_5) 500 Gy (15.08 nos) among all the treatments.

4.1.3.5 100 seed test weight (g)

The variation in 100 seed test weight was recorded and presented in Table 16. Less variation in means of the character was observed in all the treatments and there were no significant differences among the treatments. The mean for 100 seed test weight ranged between 10.53 g (200 Gy) to 10.48 g (500 Gy).

4.1.3.6 Morphological abnormalities

No morphological abnormalities was recorded in control and lower gamma doses, whereas few crinkled leaves were found in higher doses like (T_4) 400 Gy and (T_5) 500 Gy in the early days. However, these plants recovered and produced normal leaflets afterwards.

Table 16: Morphological parameters of the plants from different treatments under field condition

Treatment	Germination percentage (%)	Plant height (cm)	Number of pods $plant^{-1}$	Number of seeds pod^{-1}	100 seed test weight (g)
T_1 (100 Gy)	96.0^a (9.849)**	39.92^a	16.48^b	15.24^a	15.20
T_2 (200 Gy)	96.0^a (9.849)	39.91^a	16.67^a	15.25^a	10.53
T_3 (300 Gy)	84.0^b (9.218)	39.96^a	16.39^b	15.27^a	10.52
T_4 (400 Gy)	76.0^c (8.774)	38.52^b	16.39^b	15.10^b	10.49
T_5 (500 Gy)	62.0^d (7.937)	35.99^b	15.20^d	15.08^c	10.48
T_6 (Control)	96.0^a (9.849)	39.95^c	16.37^b	15.20^a	10.52
SE (m)	0.077	0.03	0.03	0.02	0.01
CD (5%)	0.245	0.123	0.117	0.069	NS*

*NS- Non- significant, **Values in parenthesis are square root transferred values

The values in the same column with the same alphabet as superscripts are not significantly different

500 Gy crinkled leaves

400 Gy crinkled leaves

Plate 9: Effect of gamma rays on morphological traits of cowpea plants in field condition

4.2 SEED COATING OF COWPEA SEEDS WITH CHITOSAN

The study was conducted with different concentrations of chitosan 1 %, 2 %, 3 %, 4 % and 5 % each at 2 different doses as 1 ml and 5 ml for 50 g of seeds and stored seeds were analyzed for different parameters and results are presented in the following headings

4.2.1 Pulse beetle infestation assessment

4.2.2 Germination parameters

4.2.1 Pulse beetle damage

The impact of chitosan seed coating on damage by pulse damage during storage period is furnished below

4.2.1.1 Percentage seed damage (%)

All the chitosan doses used were significantly superior to control in reducing infestation of cowpea seeds during storage (Table 17). Seed infestation was not observed in treatments 1 % @ 1mL 50 g^{-1}, 1 % @ 5 mL 50 g^{-1}, 2 % @ 1 mL 50 g^{-1} of seed for up to four months of storage. No seed damage was observed in treatment 4 % @ 1 mL 50 g^{-1} of seed, 4 % @ 5 mL 50 g^{-1}, 5 % @ 1 mL 50 g^{-1} of seed and 5 % @ 5 mL 50 g^{-1} of seed throughout the storage period. In contrast, seed infestation was observed from first month up to six months in control. At the end of storage period, percentage seed damage was highest in control (56.33%), 1 % @ 1 mL 50 g^{-1} of seed (7.667%) and no seed damage was observed in 4 % @ 1 mL 50 g^{-1} of seed, 4 % @ 5 mL 50 g^{-1}, 5 % @ 1 mL 50 g^{-1} and 5 % @ 5 mL 50 g^{-1} of seed.

4.2.1.2 Seed weight loss percentage (%)

The impact of chitosan seed coating on seed weight loss percentage during storage period is presented in the Table 18. No seed weight loss was observed in treatment 4 % @ 1 mL 50 g^{-1} of seed, 4 % @ 5 mL 50 g^{-1}, 5 % @ 1 mL 50 g^{-1} of seed and 5 % @ 5 mL 50 g^{-1} of seed throughout the storage period. In contrast, seed weight loss was observed from first month up to six months in control. At the end of storage period of six months, seed weight loss percentage was high in Control (28.182%), whereas no weight loss was recorded in treatment 4 % @1 1mL 50 g^{-1}, 4 % @5 mL 50 g^{-1}, 5 % @1 mL 50 g^{-1} and 5 % @ 5mL 50 g^{-1} of seed.

4.2.1.3 Number of eggs 100 $seeds^{-1}$ (nos)

The result indicated that all the treatments were significantly superior to control in inhibiting egg laying by the female beetle (Table 19). No eggs were laid in seeds treated with protectants and stored for four months. However, oviposition was observed in treatments 1 % @ 1 mL 50 g^{-1}, 1 % @ 5 mL 50 g^{-1}, 2 % @ 1 mL 50 g^{-1} , 2 % @ 5 mL 50 g^{-1} ,and 3 % @ 1 mL 50 g^{-1} from fourth month onwards. The number of eggs laid increasingly progressively over the period of study. In contrast, no eggs were laid in seeds treated with chitosan at 4% and 5 % till the end of storage period. Highest number of eggs was observed in control, which varied from 0.333 in first month to 78.333 in the sixth month of storage.

4.2.1.4 Number of damaged and undamaged seeds (nos)

There was significant differences between treatments in the mean number of seeds damaged and undamaged by pulse beetle (Table 20) There was no seed damage in the seeds treated with 4 % and 5 % chitosan solution. At the end of storage period of six months, number of damaged seeds was highest in control (56.333) which is then followed by 1% 1mL 50 g^{-1} (7.667), 1 % 5mL 50g^{-1} (4.333) and 2 % 1 mL 50 g^{-1} of seed (4.333) and no seed damage was found in 4 % 1 mL 50 g^{-1}, 4 % 5 mL 50 g^{-1}, 5 % 1mL 50 g^{-1}, and 5 % 5mL 50 g^{-1} of seed.

4.2.1.5 Weight of damaged and undamaged seeds (g)

The mean weight of damaged and undamaged seeds differed significantly from fourth month of storage (Table 21). There was no weight loss in the seeds treated with 4 % and 5 % chitosan solution. At the end of storage period of six months, highest weight of damaged seeds were found in control (4.163 g) which is then followed by 1% 1mL 50 g^{-1} (0.450 g), 1 % 5mL 50g^{-1} (0.247 g) and 2 % 1 mL 50 g^{-1} (0.267 g) and no weight loss was found in 4 % 1mL 50g^{-1}, 4 % 5 mL 50 g^{-1}, 5 % 1mL 50 g^{-1}, and 5 % 5mL 50 g^{-1} of seed.

Table 17: Effect of chitosan seed coating on percentage seed damage (%) of cowpea seeds for six months of storage

Treatment	Percentage seed damage (%) Months After Storage (MAS)					
	1	2	3	4	5	6
T_1(1% @ 1 mL 50 g^{-1})	0.000 (1.00)**	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	2.333^{b} (1.794)	7.667^{b} (2.922)
T_2(1% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	1.000^{bc} (1.382)	4.333^{b} (2.266)
T_3(2% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	1.333^{bc} (1.471)	4.333^{b} (2.231)
T_4(2% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.667^{c} (1.244)
T_5(3% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.333 (1.138)	1.667^{c} (1.483)
T_6(3% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.333^{c} (1.138)
T_7(4% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_8(4% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_9(5% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_{10}(5% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_{11}(control)	0.333 (1.138)	0.667 (1.244)	2.000 (1.656)	5.667 (2.499)	16.333^{a} (4.096)	56.333^{a} (7.758)
SE (m)	0.04	0.07	0.10	0.13	0.20	0.26
CD (5%)	NS*	NS	0.319	0.408	0.618	0.778

*NS- Non- significant, **Values in parenthesis are square root transferred values.

The values in the same column with the same alphabet as superscripts are not significantly different

Table 18: Effect of chitosan seed coating on seed weight loss percentage (%) of cowpea seeds for six months of storage

Treatment	Seed weight loss percentage (%) Months After Storage (MAS)					
	1	2	3	4	5	6
T_1 (1% @ 1 mL 50 g^{-1})	0.000 (1.00)**	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	1.337^{b} (1.505)	3.551^{b} (2.127)
T_2 (1% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.592^{bc} (1.245)	2.147^{b} (1.745)
T_3 (2% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.834^{bc} (1.324)	1.859^{b} (1.659)
T_4 (2% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.353^{bc} (1.145)
T_5 (3% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.176^{c} (1.079)	0.833^{bc} (1.290)
T_6 (3% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.207^{c} (1.091)
T_7 (4% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_8 (4% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_9 (5% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_{10} (5% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_{11} (control)	0.176 (1.079)	0.226 (1.098)	0.748 (1.303)	2.840 (1.862)	6.261^{a} (2.649)	28.182^{a} (5.395)
SE (m)	0.02	0.03	0.04	0.13	0.14	0.15
CD (5%)	NS*	NS	0.142	0.384	0.424	0.460

*NS- Non- significant, **Values in parenthesis are square root transferred values.

The values in the same column with the same alphabet as superscripts are not significantly different.

Table 19: Effect of chitosan seed coating on number of eggs 100 seeds^{-1} of cowpea seeds for six months of storage

Treatment	Number of eggs 100 seeds^{-1}					
	Months After Storage (MAS)					
	1	2	3	4	5	6
T_1(1% @ 1 mL 50 g^{-1})	0.000 (1.00)**	0.000 (1.00)	0.000 (1.00)	0.667^{b} (1.276)	3.667^{b} (2.157)	9.333^{b} (3.199)
T_2(1% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.333^{b} (1.138)	2.333^{bc} (1.821)	6.667^{b} (2.733)
T_3(2% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.333^{b} (1.138)	1.333^{cd} (1.520)	5.333^{bc} (2.491)
T_4(2% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.333^{d} (1.138)	2.000^{cd} (1.621)
T_5(3% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.667^{d} (1.244)	3.000^{de} (1.853)
T_6(3% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	1.333^{de} (1.471)
T_7(4% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_8(4% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_9(5% @ 1 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_{10}(5% @ 5 mL 50 g^{-1})	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_{11}(control)	0.333 (1.138)	1.000^{a} (1.328)	3.000^{a} (1.882)	9.333^{a} (3.150)	33.333^{a} (5.785)	78.333^{a} (8.903)
SE (m)	0.04	0.06	0.14	0.15	0.22	0.26
CD (5%)	NS*	0.189	0.426	0.457	0.653	0.795

*NS- Non- significant, **Values in parenthesis are square root transferred values.

The values in the same column with the same alphabet as superscripts are not significantly different

Table 20: Effect of chitosan seed coating on number of damaged (D) and undamaged seeds (U) (nos) for six months of storage

Treatment	Number of damaged and undamaged seeds (nos)											
	Months After Storage (MAS)											
	1		2		3		4		5		6	
	D	U	D	U	D	U	D	U	D	U	D	U
T_1(1% @ 1 mL 50g^{-1})	0.000 (1.00)**	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	2.333^{b} (1.794)	97.667^{a} (9.933)	7.667^{b} (2.922)	92.333^{b} (9.660)
T_2(1% @ 5 mL 50g^{-1})	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	1.000^{bc} (1.382)	99.000^{a} (9.983)	4.333^{b} (2.266)	95.667^{ab} (9.831)
T_3(2% @ 1 mL 50g^{-1})	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	1.333^{bc} (1.471)	98.667^{a} (10.05)	4.333^{b} (2.231)	95.667^{ab} (9.831)
T_4(2% @ 5 mL 50g^{-1})	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.667^{c} (1.244)	99.333^{ab} (10.017)
T_5(3% @ 1 mL 50g^{-1})	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.333^{c} (1.138)	99.667^{a} (10.03)	1.667^{c} (1.483)	98.333^{ab} (9.966)
T_6(3% @ 5 mL 50g^{-1})	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.333^{c} (1.138)	99.667^{ab} (10.03)
T_7(4% @ 1 mL 50g^{-1})	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)
T_8(4% @ 5 mL 50g^{-1})	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)
T_9(5% @ 1 mL 50g^{-1})	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)
T_{10}(5% @ 5 mL 5g^{-1})	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.50)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)	0.000 (1.00)	100.0^{a} (10.05)
T_{11} (control)	0.333 (1.138)	33.0^{b} (4.00)	0.667 (1.244)	32.667^{b} (3.989)	2.000 (1.656)	98.00^{b} (9.950)	5.667 (2.492)	94.333^{b} (9.762)	16.333^{a} (4.096)	83.667^{b} (9.195)	56.333^{a} (7.558)	43.667^{c} (6.661)
SE (m)	0.10	9.95	0.20	9.84	0.34	0.34	0.70	0.70	1.41	1.41	1.81	1.81
CD (5%)	NS*	29.370	NS	29.074	NS	1.028	NS	2.077	4.185	4.185	5.348	5.348

*NS- Non- significant, **Values in parenthesis are square root transferred values.

The values in the same column with the same alphabet as superscripts are not significantly different

Table 21: Effect of chitosan seed coating on weight of damaged (D) and undamaged seeds (U) (g) for six months of storage

Treatment	Weight of damaged and undamaged seeds (g)											
	Months After Storage (MAS)											
	1		2		3		4		5		6	
	D	U	D	U	D	U	D	U	D	U	D	U
T_1(1% @1 mL $50g^{-1}$)	0.000 (1.00)**	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.107^{b} (1.052)	10.413^{a} (3.378)	0.450^{b} (1.202)	10.070^{a} (3.327)
T_2(1% @5 mL $50g^{-1}$)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.52^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.043^{bc} (1.021)	10.477^{a} (3.388)	0.247^{bc} (1.116)	10.273^{a} (3.358)
T_3(2% @1 mL $50g^{-1}$)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.52^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.053^{bc} (1.026)	10.467^{a} (3.386)	0.267^{bc} (1.123)	10.253^{a} (3.354)
T_4(2% @5 mL $50g^{-1}$)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.52^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.033^{c} (1.016)	10.487^{a} (3.389)
T_5(3% @1 mL $50g^{-1}$)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.017^{c} (1.008)	10.503^{a} (3.392)	0.090^{c} (1.042)	10.430^{a} (3.381)
T_6(3% @5 mL $50g^{-1}$)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.013^{c} (1.007)	10.507^{a} (3.392)
T_7(4% @1 mL $50g^{-1}$)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)
T_8(4% @5 mL $50g^{-1}$)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)
T_9(5% @1 mL $50g^{-1}$)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)
T_{10}(5% @5 mL $50g^{-1}$)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520 (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)	0.000 (1.00)	10.520^{a} (3.394)
T_{11}(control)	0.017 (1.008)	10.503 (3.392)	0.047 (1.023)	10.473 (3.387)	0.133 (1.063)	10.387^{b} (3.374)	0.440 (1.194)	10.080^{b} (3.328)	1.143^{a} (1.456)	9.377^{b} (3.221)	4.163^{a} (2.266)	6.357^{b} (2.708)
SE (m)	0.005	0.005	0.014	0.014	0.024	0.02	0.05	0.17	0.09	0.09	0.18	0.18
CD (5%)	*NS	NS	NS	NS	NS	0.072	NS	0.059	0.284	0.284	0.536	0.536

*NS- Non- significant, **Values in parenthesis are square root transferred values.

The values in the same column with the same alphabet as superscripts are not significantly different.

T_{11}

T_1

T_2

T_3

T_4

T_5

T_6

T_7

T_8

T_9

T_{10}

Plate 10: Comparison of chitosan treatment on pulse beetle infestation

T_1: 1% @ 1 mL 50 g^{-1} T_2: 1% @ 5 mL 50 g^{-1} T_3: 2% @ 1 mL 50 g^{-1} T_4: 2% @ 5 mL 50 g^{-1} T_5: 3% @ 1 mL 50 g^{-1} T_6: 3% @ 5 mL 50 g^{-1}
T_7: 4% @ 1mL 50 g^{-1} T_8: 4% @ 5mL 50 g^{-1} T_9: 5% @ 1mL 50 g^{-1} T_{10}: 5% @ 5mL 50 g^{-1} T_{11}: Control

4.2.2 Seed germination parameters

The different seed germination parameters were taken for a period of six months of storage and observations were taken at monthly intervals

4.2.2.1 Germination percentage (%)

The variations in seed germination percentage due to chitosan treatment are given Table 22. At the end of storage period of six months, highest seed germination per cent was found in (T_{10}) 5 % @ 5 mL 50 g^{-1} of seed (89.37 %) which was on par with (T_9) 5 % @ 1 mL 50 g^{-1} of seed (88.43 %), and (T_8) 4 % @ 5 mL 50 g^{-1} of seed (87.53 %). The least value (80.67 %) was observed in control (T_{11}). The mean germination per cent of various treatments at the end of storage period ranged between 86.93 % (control) and 91.88 % (5 % @ 5 mL 50 g^{-1}).

4.2.2.2 Speed of germination

The variations in speed of germination (Table 23) due to chitosan were found throughout the storage period. All the treatments were found to be superior over control. At the end of storage period of six months, highest speed of germination (36.83) was found in (T_{10}) 5 % @ 5 mL 50 g^{-1} of seed which was on par with (T_8) 4 % @ 5 mL 50 g^{-1} of seed (36.82) and the least value (31.40) was recorded in (T_{11}) control. The mean speed of germination of various treatments at the end of storage period varied between 39.13 (5 % @ 5 mL 50 g^{-1}) and 33.97 (control).

4.2.2.3 Seedling shoot length (cm)

The impact of various seed treatments on seedling shoot length is furnished in Table 24. At the end of sixth month storage period, seedling shoot length (14.90 cm) was highest in seeds treated with (T_{10}) 5 % @ 5 mL 50 g^{-1} of seed which was on par with (T_8) 4 % @ 5 mL 50 g^{-1} of seed (14.70 cm), (T_9) 5 % @ 1 mL 50 g^{-1} of seed (14.23 cm), and(T_7) 4 % @ 1mL 50 g^{-1} of seed (14.20 cm). The least value (11.50 cm) was recorded in (T_{11}) control. The mean shoot length value of various seed treatments varied between 12.41 cm (control) and 15.96 cm (5 % @ 5 mL 50 g^{-1}) at the end of storage period.

4.2.2.4 Seedling root length (cm)

The effects of various seed treatments on seedling root length are furnished in Table 25. At the end of six months storage period, seedling root length (17.53 cm) was highest in seeds treated with (T_{10}) 5 % @ 5 mL 50 g^{-1} of seed and lowest (12.45 cm) in control (T_{10}). The mean root length value of various seed treatments varied between 14.59 cm (control) and 18.25 cm (5 % @ 5 mL 50 g^{-1}) at the end of storage period.

4.2.2.5 Seedling dry weight (g)

At the end of sixth month, seedling dry weight (0.747 g) was highest in seeds treated with (T_{10}) 5 % @ 5 mL 50 g^{-1} of seed which was on par with (T_8) 4 % @ 5 mL 50 g^{-1} of seed with 0.740 g, (T_9) 5 % @ 1 mL 50 g^{-1} with 0.738 g, (T_6) 3 % @ 5 mL 50 g^{-1} with 0.729 g, (T_7) 4 % @ 1 mL 50 g^{-1} with 0.726 g, and (T_5) 3 % @ 1 mL 50 g^{-1} with 0.724 g. The lowest value (0.687 g) was recorded in (T_{10}) control. The mean seedling dry weight value of various seed treatments varied between 0.719g (control) and 0.724g (5 % @ 5 mL 50 g^{-1}) at the end of storage period (Table 26).

4.2.2.6 Seedling vigour index I

At the end of storage, highest seedling vigour index I values (2898.28) were observed in (T_{10}) 5 % @ 5 mL 50 g^{-1} of seed which was on par with (T_8) 4 % @ 5 mL 50 g^{-1} of seed (2719.75). The lowest value (1939.24) was exhibited by Control (T_{11}). The mean seedling vigour index I of the seedlings over the storage period varied between 2362.63 (control) and 3144.84 (5 % @ 5 mL 50 g^{-1}) (Table 27).

4.2.2.7 Seedling vigour index II

The impact of various treatments on seedling vigour index II is given in Table 28. At the end of storage, highest vigour index II values (66.79) were observed in (T_{10}) 5 % @ 5 mL 50 g^{-1} of seed (66.79) which was on par with (T_9) 5 % @ 1 mL 50 g^{-1} of seed (65.26), and (T_8) 4 % @ 5 mL 50 g^{-1} of seed (64.81). The lowest value (55.52) was shown by Control (T_{11}). The mean vigour index II of the seedlings over the storage period varied between 55.52 (control) and 66.79 (in 5 % @ 5 mL 50 g^{-1}).

Table 22: Effect of chitosan seed coating on germination percentage (%) of cowpea seeds for six months of storage

Treatment	Germination percentage (%)						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1(1% @ 1 mL 50 g^{-1})	91.80^{cd} (9.63)**	90.50 (9.57)	88.50^{e} (9.46)	87.13 (9.39)	86.40 (9.35)	82.40^{fg} (9.13)	87.29 (9.34)
T_2(1% @ 5 mL 50 g^{-1})	92.23^{bcd} (9.66)	91.20 (9.60)	88.90^{de} (9.48)	87.23 (9.39)	86.37 (9.35)	83.13^{efg} (9.17)	88.18 (9.39)
T_3(2% @ 1 mL 50 g^{-1})	92.30^{bcd} (9.66)	91.30 (9.61)	89.23^{de} (9.50)	88.03 (9.44)	87.53 (9.41)	84.43^{def} (9.24)	88.30 (9.40)
T_4(2% @ 5 mL 50 g^{-1})	92.53^{bc} (9.67)	91.80 (9.63)	89.30^{de} (9.50)	88.13 (9.44)	87.83 (9.42)	85.30^{cde} (9.29)	89.15 (9.44)
T_5(3% @ 1 mL 50 g^{-1})	92.43^{bcd} (9.67)	92.10 (9.65)	89.13^{de} (9.49)	89.50 (9.51)	88.30 (9.45)	85.80^{cd} (9.32)	89.54 (9.46)
T_6(3% @ 5 mL 50 g^{-1})	93.43^{abc} (9.71)	92.40 (9.66)	89.73^{cde} (9.52)	89.10 (9.49)	87.90 (9.43)	86.30^{bcd} (9.34)	89.76 (9.47)
T_7(4% @ 1 mL 50 g^{-1})	93.60^{abc} (9.73)	92.40 (9.66)	90.43^{cd} (9.56)	89.43 (9.51)	88.53 (9.46)	86.83^{bcd} (9.37)	90.20 (9.50)
T_8(4% @ 5 mL 50 g^{-1})	94.10^{ab} (9.75)	92.20 (9.65)	91.20^{bc} (9.60)	90.37 (9.56)	88.90 (9.48)	87.53^{abc} (9.41)	90.72 (9.52)
T_9(5% @ 1 mL 50 g^{-1})	94.13^{ab} (9.75)	93.93 (9.74)	92.70^{ab} (9.68)	90.40 (9.56)	89.13 (9.49)	88.43^{ab} (9.46)	91.40 (9.56)
T_{10}(5% @ 5 mL 50 g^{-1})	94.60^{a} (9.78)	94.13 (9.75)	93.10^{a} (9.70)	90.70 (9.58)	89.40 (9.51)	89.37^{a} (9.51)	91.88 (9.59)
T_{11} (control)	90.50^{d} (9.57)	89.40 (9.51)	88.19^{e} (9.44)	87.10 (9.38)	85.70 (9.31)	80.67^{g} (9.03)	86.93 (9.32)
SE (m)	0.03	0.05	0.03	0.05	0.05	0.04	
CD (5%)	0.104	NS*	0.095	NS	NS	0.139	

*NS- Non- significant, **Values in parenthesis are square root transferred values.

The values in the same column with the same alphabet as superscripts are not significantly different

Table 23: Effect of chitosan seed coating on speed of germination of cowpea seeds for six months of storage

Treatment	Speed of germination						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1 (1% @ 1ml $50g^{-1}$)	36.60^{de}	36.13^{de}	35.13^{e}	34.90^{e}	33.40^{e}	32.40^{fg}	34.76
T_2 (1% @ 1ml $50g^{-1}$)	36.63^{d}	36.40^{d}	35.80^{e}	34.90^{e}	33.90^{e}	33.53^{de}	35.19
T_3 (2% @ 1ml $50g^{-1}$)	36.70^{d}	36.30^{d}	37.13^{e}	35.23^{e}	34.13^{e}	32.63^{ef}	35.35
T_4 (2% @ 1ml $50g^{-1}$)	36.90^{d}	36.50^{d}	36.40^{de}	35.63^{de}	34.60^{cd}	33.90^{cd}	35.66
T_5 (3% @ 1ml $50g^{-1}$)	36.70^{c}	37.83^{c}	37.50^{cd}	36.73^{cd}	35.73^{bcd}	34.60^{bcd}	35.51
T_6 (3% @ 1ml $50g^{-1}$)	40.30^{bc}	38.43^{bc}	38.40^{bc}	37.83^{bc}	35.84^{bc}	34.83^{bc}	37.60
T_7 (4% @ 1ml $50g^{-1}$)	40.13^{ab}	39.60^{ab}	39.10^{ab}	38.20^{ab}	36.93^{b}	35.30^{b}	38.21
T_8 (4% @ 1ml $50g^{-1}$)	40.80^{ab}	39.63^{ab}	39.50^{ab}	38.60^{ab}	37.13^{a}	36.83^{a}	38.75
T_9 (5% @ 1ml $50g^{-1}$)	40.20^{a}	40.20^{a}	39.93^{ab}	38.40^{a}	37.50^{a}	36.50^{a}	38.79
T_{10} (5% @ 1ml $50g^{-1}$)	40.80^{a}	40.70^{a}	40.53^{a}	39.13^{a}	36.80^{a}	36.83^{a}	39.13
T_{11} (control)	36.60^{e}	35.10^{e}	34.20^{f}	33.73^{f}	32.80^{g}	31.40^{g}	33.97
SE (m)	0.22	0.40	0.34	0.38	0.39	0.38	
CD (5%)	0.677	1.198	1.011	1.144	1.163	1.140	

The values in the same column with the same alphabet as superscripts are not significantly different

Table 24: Effect of chitosan seed coating on seedling shoot length (cm) of cowpea seeds for six months of storage

Treatment	Seedling shoot length(cm)						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1(1% @ 1 mL 50 g^{-1})	14.37^{de}	14.00^{d}	13.90^{e}	13.50^{fg}	12.80^{de}	12.13^{ef}	13.45
T_2(1% @ 5 mL 50 g^{-1})	14.00^{e}	13.80^{d}	13.50^{e}	13.47^{g}	12.97^{de}	12.47^{ef}	13.37
T_3(2% @ 1 mL 50 g^{-1})	15.10^{cd}	14.60^{cd}	14.30^{de}	13.90^{efg}	12.80^{de}	12.30^{ef}	13.83
T_4(2% @ 5 mL 50 g^{-1})	15.30^{bcd}	14.60^{cd}	14.80^{cd}	14.17^{def}	13.40^{cde}	12.70^{de}	14.16
T_5(3% @ 1 mL 50 g^{-1})	15.50^{bc}	15.30^{bc}	15.30^{bc}	14.57^{cde}	13.53^{cde}	13.17^{cde}	14.56
T_6(3% @ 5 mL 50 g^{-1})	16.70^{a}	16.10^{ab}	15.00^{cd}	14.77^{bcd}	13.93^{bcd}	13.70^{bcd}	15.03
T_7(4% @ 1 mL 50 g^{-1})	16.00^{abc}	15.70^{b}	15.50^{bc}	15.20^{abc}	14.33^{abc}	14.20^{abc}	15.16
T_8(4% @ 5 mL 50 g^{-1})	16.70^{a}	16.10^{ab}	15.90^{ab}	15.33^{ab}	14.90^{ab}	14.70^{ab}	15.61
T_9(5% @ 1 mL 50 g^{-1})	15.90^{abc}	15.60^{b}	15.40^{bc}	15.37^{ab}	14.53^{abc}	14.23^{abc}	15.12
T_{10}(5% @ 5 mL 50 g^{-1})	16.20^{ab}	16.90^{a}	16.70^{a}	15.80^{a}	15.23^{a}	14.90^{a}	15.96
T_{11}(control)	12.83^{f}	12.63^{e}	12.60^{f}	12.50^{h}	12.33^{e}	11.57^{f}	12.41
SE (m)	0.34	0.28	0.27	0.23	0.42	0.37	
CD (5%)	1.026	0.831	0.812	0.695	1.259	1.106	

The values in the same column with the same alphabet as superscripts are not significantly different

Table 25: Effect of chitosan seed coating on seedling root length (cm) of cowpea seeds for six months of storage

Treatment	Seedling root length(cm)						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1 (1% @ 1 mL 50 g^{-1})	16.20^{ef}	15.80^{f}	15.50^{de}	15.23^{def}	15.13^{cf}	14.20^{f}	15.34
T_2 (1% @ 5 mL 50 g^{-1})	16.30^{ef}	15.93^{f}	15.50^{de}	15.10^{ef}	15.23^{ef}	14.60^{ef}	15.39
T_3 (2% @ 1 mL 50 g^{-1})	16.30^{ef}	16.20^{ef}	15.73^{de}	15.53^{cde}	15.13^{ef}	14.80^{def}	15.62
T_4 (2% @ 5 mL 50 g^{-1})	16.83^{de}	16.30^{ef}	15.83^{de}	15.83^{bcde}	15.40^{de}	15.20^{cdef}	15.90
T_5 (3% @ 1 mL 50 g^{-1})	17.23^{cd}	16.43^{def}	16.10^{de}	16.00^{bcde}	15.90^{cde}	15.53^{bcde}	16.20
T_6 (3% @ 5 mL 50 g^{-1})	17.53^{bcd}	16.93^{cde}	16.23^{d}	16.20^{bcd}	16.10^{cde}	15.83^{bcd}	16.47
T_7 (4% @ 1 mL 50 g^{-1})	17.80^{bc}	17.30^{bcd}	16.53^{cd}	16.40^{bc}	16.23^{bcd}	16.10^{bc}	16.68
T_8 (4% @ 5 mL 50 g^{-1})	18.23^{ab}	17.80^{abc}	17.40^{bc}	16.80^{b}	16.53^{bc}	16.37^{b}	17.14
T_9 (5% @ 1 mL 50 g^{-1})	18.20^{ab}	18.10^{ab}	17.93^{ab}	17.90^{a}	17.20^{ab}	16.23^{bc}	17.59
T_{10} (5% @ 5 mL 50 g^{-1})	18.80^{a}	18.53^{a}	18.53^{a}	18.23^{a}	17.90^{a}	17.53^{a}	18.25
T_{11} (control)	15.87^{f}	15.57^{f}	15.07^{e}	14.33^{f}	14.30^{f}	12.45^{g}	14.59
SE m	0.30	0.33	0.36	0.34	0.33	0.35	
CD (5%)	0.901	1.000	1.077	1.029	0.995	1.049	

The values in the same column with the same alphabet as superscripts are not significantly different

Table 26: Effect of chitosan seed coating on seedling dry weight (g) of cowpea seeds for six months of storage

Treatment	Seedling dry weight (g)						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1(1% @ 1 mL 50 g^{-1})	0.738^{e}	0.733^{e}	0.729^{e}	0.727^{d}	0.718^{cd}	0.701^{ef}	0.724
T_2(1% @ 5 mL 50 g^{-1})	0.742^{de}	0.735^{de}	0.729^{e}	0.728^{d}	0.719^{cd}	0.703^{def}	0.729
T_3(2% @ 1 mL 50 g^{-1})	0.746^{de}	0.739^{cde}	0.731^{de}	0.731^{cd}	0.723^{bc}	0.710^{cde}	0.73
T_4(2% @ 5 mL 50 g^{-1})	0.754^{cde}	0.743^{cde}	0.734^{cde}	0.733^{cd}	0.728^{bc}	0.715^{bcde}	0.735
T_5(3% @ 1 mL 50 g^{-1})	0.762^{bcd}	0.754^{bcd}	0.748^{bcd}	0.739^{bcd}	0.726^{bc}	0.724^{abcde}	0.742
T_6(3% @ 5 mL 50 g^{-1})	0.769^{abc}	0.756^{abc}	0.749^{bc}	0.743^{bcd}	0.734^{bc}	0.729^{abc}	0.747
T_7(4% @ 1 mL 50 g^{-1})	0.774^{abc}	0.763^{ab}	0.754^{ab}	0.745^{bcd}	0.733^{bc}	0.726^{abcd}	0.749
T_8(4% @ 5 mL 50 g^{-1})	0.778^{ab}	0.768^{ab}	0.762^{ab}	0.749^{abc}	0.742^{ab}	0.740^{a}	0.757
T_9(5% @ 1 mL 50 g^{-1})	0.784^{a}	0.771^{ab}	0.759^{ab}	0.753^{ab}	0.740^{abc}	0.738^{ab}	0.758
T_{10}(5% @ 5 mL 50 g^{-1})	0.785^{a}	0.775^{a}	0.768^{a}	0.766^{a}	0.758^{a}	0.747^{a}	0.767
T_{11}(control)	0.737^{e}	0.734^{e}	0.728^{e}	0.727^{d}	0.699^{d}	0.687^{f}	0.719
SE (m)	0.007	0.007	0.006	0.006	0.008	0.008	
CD (5%)	0.021	0.020	0.017	0.019	0.024	0.023	

The values in the same column with the same alphabet as superscripts are not significantly different

Table 27: Effect of chitosan seed coating on seedling vigour index I of cowpea seeds for six months of storage

Treatment	Seedling vigour index I						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1 (1% @ 1 mL 50 g^{-1})	$2,805.83^{d}$ (52.98)**	$2,697.72^{e}$ (51.94)	$2,601.99^{ef}$ (51.02)	$2,503.94^{g}$ (50.04)	$2,422.81^{gh}$ (49.22)	$2,169.48^{f}$ (46.58)	2533.63 (50.34)
T_2 (1% @ 5 mL 50 g^{-1})	$2,794.94^{d}$ (52.88)	$2,712.65^{e}$ (52.08)	$2,578.26^{f}$ (50.78)	$2,492.15^{g}$ (49.93)	$2,435.71^{fgh}$ (49.36)	$2,250.06^{ef}$ (47.44)	2543.96 (50.44)
T_3 (2% @ 1 mL 50 g^{-1})	$2,898.13^{cd}$ (53.84)	$2,811.37^{de}$ (53.03)	$2,679.73^{def}$ (51.78)	$2,590.76^{fg}$ (50.91)	$2,445.26^{fg}$ (49.46)	$2,288.31^{ef}$ (47.84)	2618.93 (51.18)
T_4 (2% @ 5 mL 50 g^{-1})	$2,973.49^{c}$ (54.53)	$2,836.98^{de}$ (53.27)	$2,736.02^{de}$ (52.31)	$2,643.75^{efg}$ (51.43)	$2,529.44^{efg}$ (50.30)	$2,379.90^{de}$ (48.78)	2683.26 (51.80)
T_5 (3% @ 1 mL 50 g^{-1})	$3,025.90^{bc}$ (55.01)	$2,922.70^{cd}$ (54.07)	$2,799.06^{cd}$ (52.91)	$2,736.32^{def}$ (52.31)	$2,598.83^{def}$ (50.99)	$2,462.49^{cd}$ (49.63)	2757.55 (52.51)
T_6 (3% @ 5 mL 50 g^{-1})	$3,198.51^{a}$ (56.56)	$3,050.66^{bc}$ (55.24)	$2,802.83^{cd}$ (52.95)	$2,759.43^{cde}$ (52.54)	$2,639.25^{cde}$ (51.38)	$2,549.13^{bcd}$ (50.48)	2803.30 (52.95)
T_7 (4% @ 1 mL 50 g^{-1})	$3,163.69^{ab}$ (56.24)	$3,049.41^{bc}$ (55.23)	$2,896.48^{c}$ (53.83)	$2,826.32^{cd}$ (53.16)	$2,706.82^{bcd}$ (52.02)	$2,631.12^{bc}$ (51.30)	2878.97 (53.66)
T_8 (4% @ 5 mL 50 g^{-1})	$3,287.42^{a}$ (57.34)	$3,125.27^{b}$ (55.91)	$3,036.83^{b}$ (55.11)	$2,903.84^{bc}$ (53.89)	$2,794.49^{abc}$ (52.87)	$2,719.75^{ab}$ (52.15)	2977.93 (54.87)
T_9 (5% @ 1 mL 50 g^{-1})	$3,210.28^{a}$ (56.66)	$3,165.78^{b}$ (56.26)	$3,090.04^{b}$ (55.60)	$3,007.41^{ab}$ (54.85)	$2,828.58^{ab}$ (53.19)	$2,694.08^{b}$ (51.91)	2999.36 (54.77)
T_{10} (5% @ 5 mL 50 g^{-1})	$3,311.21^{a}$ (57.55)	$3,335.48^{a}$ (57.76)	$3,280.56^{a}$ (57.28)	$3,087.09^{a}$ (55.57)	$2,962.4^{a}$ (54.43)	$2,898.28^{a}$ (53.84)	3144.84 (56.28)
T_{11} (control)	$2,598.82^{e}$ (50.97)	$2,521.47^{f}$ (50.22)	$2,438.30^{g}$ (49.39)	$2,336.43^{h}$ (48.33)	$2,281.53^{h}$ (47.76)	$1,939.24^{g}$ (44.02)	2352.63 (48.50)
SE (m)	0.51	0.51	0.44	0.51	0.55	1.74	
CD (5%)	1.515	1.509	1.296	1.526	0.636	0.590	

**Values in parenthesis are square root transferred values.

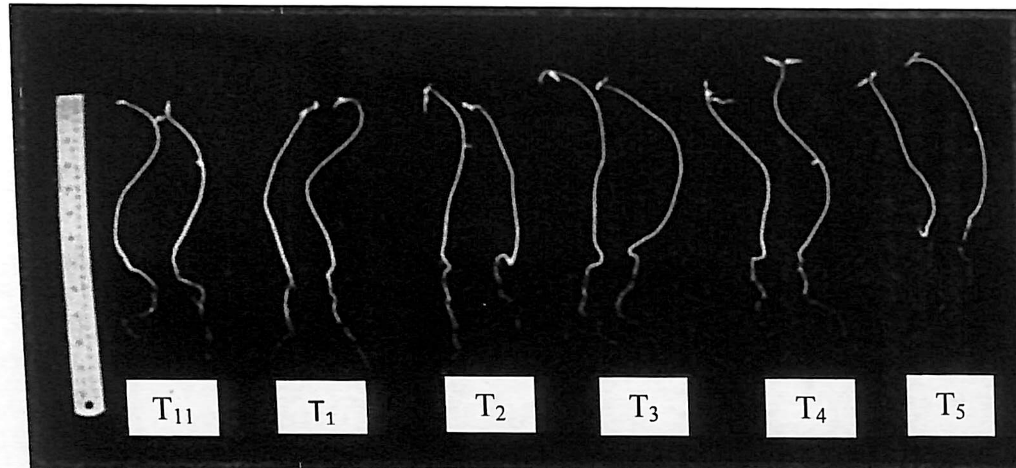
The values in the same column with the same alphabet as superscripts are not significantly different

Table 28: Effect of chitosan seed coating on seedling vigour index II of cowpea seeds for a period of six months

Treatment	Seedling vigour index II						
	Months After Storage (MAS)						
	1	2	3	4	5	6	Mean
T_1(1% @ 1 mL 50 g^{-1})	67.78^{fg} (8.29)**	66.33^{fg} (8.21)	64.52^{f} (8.09)	63.37^{e} (8.02)	62.07^{bc} (7.94)	57.76^{de} (7.67)	63.64 (7.98)
T_2(1% @ 5 mL 50 g^{-1})	68.47^{efg} (8.33)	66.99^{fg} (8.25)	64.81^{ef} (8.11)	63.51^{e} (8.03)	62.10^{bc} (7.94)	58.44^{de} (7.71)	64.05 (8.00)
T_3(2% @ 1 mL 50 g^{-1})	68.86^{efg} (8.36)	67.46^{efg} (8.27)	65.23^{def} (8.14)	64.35^{bc} (8.08)	63.28^{bc} (8.02)	59.95^{cd} (7.81)	64.86 (8.05)
T_4(2% @ 5 mL 50 g^{-1})	69.80^{def} (8.41)	68.21^{def} (8.32)	65.57^{def} (8.16)	64.60^{bc} (8.10)	63.97^{abc} (8.06)	61.02^{cd} (7.87)	65.52 (8.09)
T_5(3% @ 1 mL 50 g^{-1})	70.44^{cde} (8.45)	69.44^{cde} (8.39)	66.67^{cde} (8.23)	66.14^{abc} (8.19)	64.11^{ab} (8.07)	62.15^{bc} (7.95)	66.49 (8.15)
T_6(3% @ 5 mL 50 g^{-1})	71.85^{bcd} (8.35)	69.86^{cd} (8.42)	67.21^{cd} (8.26)	66.20^{abc} (8.20)	64.52^{ab} (8.09)	62.94^{bc} (7.99)	67.10 (8.19)
T_7(4% @ 1 mL 50 g^{-1})	72.45^{abc} (8.57)	70.50^{bc} (8.46)	68.19^{bc} (8.32)	66.63^{abc} (8.22)	64.90^{ab} (8.12)	63.04^{bc} (8.00)	67.62 (8.22)
T_8(4% @ 5 mL 50 g^{-1})	73.21^{ab} (8.61)	70.81^{bc} (8.47)	69.50^{ab} (8.40)	67.68^{ab} (8.29)	65.96^{ab} (8.18)	64.81^{ab} (8.11)	68.66 (8.29)
T_9(5% @ 1 mL 50 g^{-1})	73.83^{ab} (8.65)	72.45^{ab} (8.57)	70.39^{a} (8.45)	68.07^{a} (8.31)	65.96^{ab} (8.18)	65.26^{ab} (8.14)	69.83 (8.36)
T_{10}(5% @ 5 mL 50 g^{-1})	74.29^{a} (8.68)	72.98^{a} (8.60)	71.53^{a} (8.52)	69.51^{a} (8.40)	67.77^{a} (8.29)	66.79^{a} (8.23)	70.48 (8.40)
T_{11} (control)	66.69^{g} (8.22)	65.57^{g} (8.16)	64.25^{f} (8.08)	63.41^{e} (8.02)	60.05^{c} (7.80)	55.52^{e} (7.51)	62.58 (7.91)
SE (m)	0.05	0.04	0.04	0.07	0.08	0.07	
CD (5%)	0.137	0.127	0.130	0.210	0.259	0.225	

**Values in parenthesis are square root transferred values.

The values in the same column with the same alphabet as superscripts are not significantly different



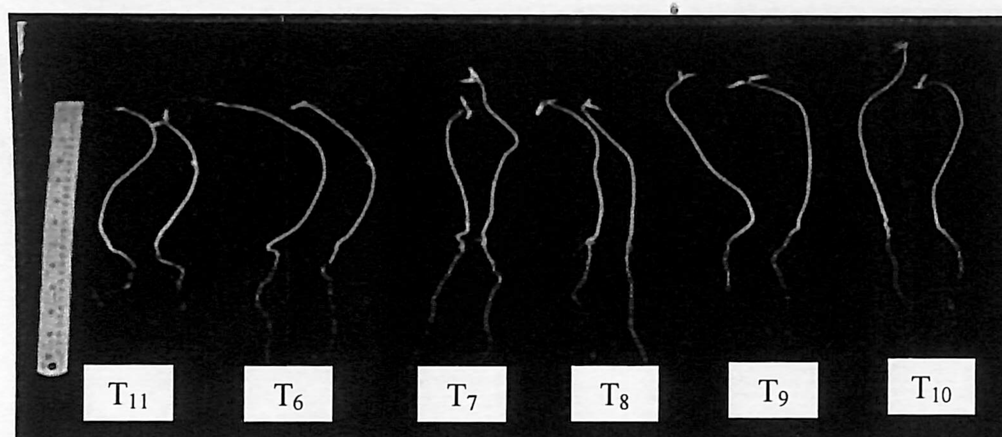


Plate 11: Comparison of seedling parameters in the seed lots coated with different concentations of chitosan at the end of six months of storage

DISCUSSION

5. DISCUSSION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a major pulse crop providing a major source of protein for human nutrition. It has a unique capability of adaptation in the drier regions where other pulse crop does not perform well. However, to get the required yield, it is a prerequisite to have an optimum quantity of good quality seed. Despite the crop, seed viability and quality has a considerable effect on seedling growth, development and yield. One of the most significant constraints in increasing productivity is seed storage. Main internal and external factors that affect seed longevity are the variety of seeds, initial seed quality, storage conditions, moisture content, insects, pests, and fungi. Besides this, seed aging is the most common phenomenon in which all the vital metabolism occurs culminating in the end of life of seed. Aging involves the sequential deterioration of many systems within tissues.

Maintenance of seed quality during storage highly depends on the storage environment. Seeds become more susceptible to infestation and infection when the storage environment is poor. It highly accelerates the aging process and leads to seed death within a short period of time. Storage of seeds at optimum temperature or by using appropriate seed treatments, the rate of aging and deterioration of seed can be delayed.

Among the major constraints, the pulse beetle is the most serious pest. Most of its infestation starts from the field and continues in the storage. Several techniques have been adopted namely, physical, chemical, and mechanical, but all of which have certain limitations at the farmer's level. Fumigants are effective for their control; however traditional structures are not airtight to impose such treatments. Inappropriate pesticide application affects the food chain (Rajendran, 2003). Many pesticides are restricted globally because of their residues in foodstuffs and the high occurrence of pesticide resistance. None of the techniques had proved to be effective in controlling infestation, infection, and ultimately the longevity of seeds.

Under such conditions, a new technique is required to overcome such challenges during seed storage. Gamma irradiation serves as an alternative as there is no development of insect resistance and absence of residues. In comparison with other physical treatments, gamma irradiation is quick, convenient, and more effective because of its penetration power (Delia *et al.*, 2013). However, its functional changes in the plant system highly depend on the duration and strength of its exposure.

Natural products like chitosan also serve as an alternative to pesticides as it reduces the negative effect on human health. It also gained considerable interest in various fields because of its unique properties like biodegradability, non-toxicity, and antimicrobial activity.

So in view of its importance, an investigation was carried out to standardize the dose of gamma rays and concentration of chitosan for seed coating for enhancing the storage life of grain cowpea. The observations and results from the experiments are discussed below.

5.1 IRRADIATION OF COWPEA SEEDS WITH GAMMA RAYS

. The objective of the study was to standardize the dose of gamma rays for enhancing the storage life of seeds. Cowpea seeds (Variety: Kanakamony) were irradiated at gamma unit in IIHR, Bangalore, was stored for a period of six months. During storage, different observations were taken such as pulse beetle infestation parameters and germination parameters monthly for a period of six months. Irradiated seeds were also grown in the field to check any morphological abnormalities arising due to irradiation.

5.1.1 Pulse beetle infestation assessment

In the study, pulse beetle (*Callosobruchus sp*) infestation was the only pest found infesting the seeds. No other stored pest incidence was noticed throughout the storage period. Pulse beetle damage and insect eggs were not noticed in all the treatments from the initial to three months of storage. However, in control, the infestation was noticed from the first month and increased up to six months of storage. In treatment T_1 (100 Gy), and T_2 (200 Gy) significant infestation and insect eggs were noticed from the fourth and fifth months onwards. Although insect eggs and infestation were noticed in T1 and T_2, their percentage decreased with an increase in gamma dose.

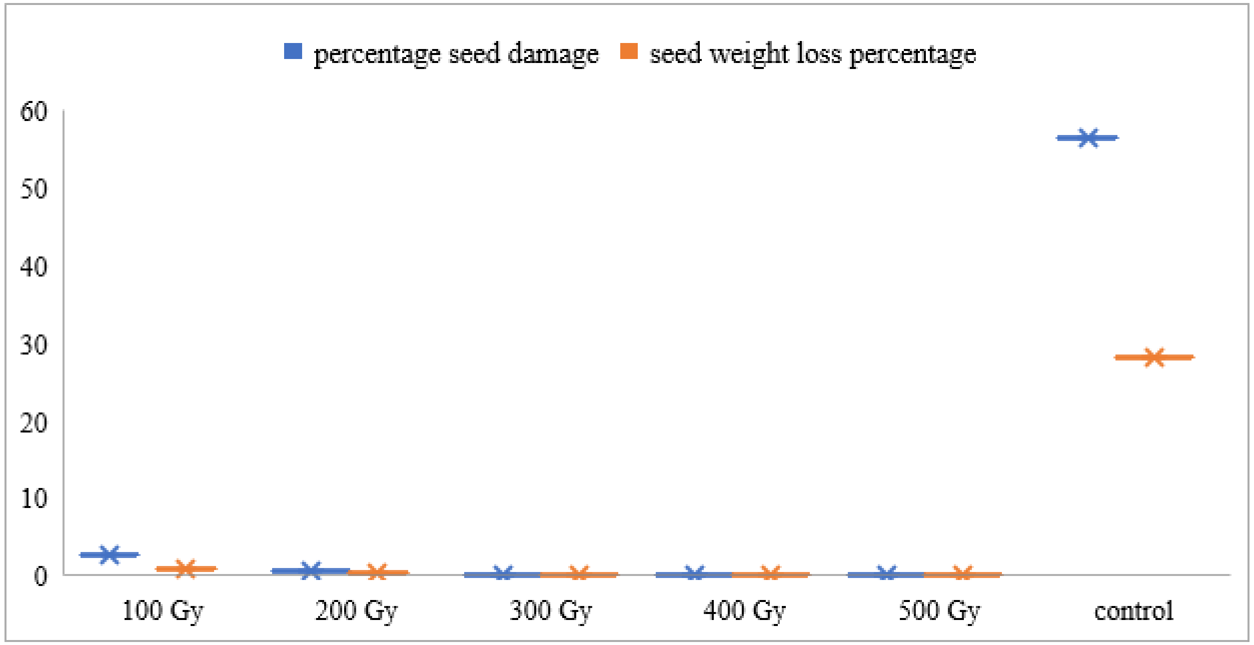


Fig 1: Effect of gamma doses on pulse beetle infestation at the end of six months of storage

Whereas, in treatments T_3, T_4, and T_5 (300 Gy, 400 Gy, and 500 Gy), the insect eggs and damage were not noticed till the end of the sixth month. Gamma irradiation proved to be an alternative for pulse beetle control and these results were confirmed with the investigations carried out by Dongre *et al.* (1997), Ahmed *et al.* (2003) and Tripathi *et al.* (2015). A comparison of the effect of gamma doses on pulse beetle infestation is given in Figure 1.

Exposing the seeds to gamma radiation (100 Gy to 500 Gy) decreased the percentage seed damage, seed weight loss percentage, number of eggs per 100 seeds, number of damaged seeds, and weight of damaged seeds. In the same line, Darfour *et al.* (2012) concluded that gamma rays of 250 Gy lead to 100 per cent mortality of *C. maculatus* within 8 days of irradiation. Exposure of green gram seeds infested with pulse beetle with different gamma doses ranging from 100 Gy to 500 Gy showed varying effects and 100 Gy was found to have a sterilizing effect on adult beetles (Bhalla *et al.*, 2008).

Similarly, *Tribolium confusum* irradiated with 800 Gy gamma doses showed 100 per cent mortality at 7 days after exposure (Kovacs and Kiss, 1985). Molin (2001) suggested that lower doses causes sterility or malformed insects whereas higher doses induce complete mortality of beetles. Pulse beetle eggs were the most vulnerable stage and all other life stages of beetles showed complete mortality with increase in gamma doses (Supawan *et al.*, 2005). The results are in line with the findings of Enu and Enu (2014) who revealed that 300 Gy and 500 Gy gamma doses showed 100 per cent mortality of *Sitophilus zeamais* and *Callosobruchus maculatus*. Exposure of *C. chinensis* with 800 Gy showed 100 per cent pupal mortality (Bhuiya *et al.*, 1985). No adult emergence was observed on the treatment of *Sitophilus granarius* eggs at 50-100 Gy (Brown *et al.*, 1972).

Earlier findings confirmed the present investigations which state that gamma irradiation acts as a disinfecting agent and leads to pulse beetle mortality in storage.

5.1.2 Germination parameters

Seed germination parameters showed a decline in untreated seeds due to seed aging. However, gamma irradiation at lower doses could reduce this decline due to aging. At six months of storage, the highest seed germination percentage was found in 200 Gy (84.33 per cent). Reduction in germination parameters was noticed with increase in storage period of rice (Kumar *et al.* 2004; Selvaraju and Krishnaswamy, 2005). Susmitha and Rai (2017) reported that a decrease in germination potential was due to the aging process that consequently leads to depletion of food reserves and seed deterioration. A comparison of different treatments on germination percentage is given in Figure 2.

The result of the present study revealed gamma irradiation at lower doses increases the seed germination over the control but irradiation with higher doses reduced the percentage of germination. Lower doses of gamma rays had stimulatory effect on germination by RNA activation and protein synthesis. A lower dose of gamma rays had a stimulatory effect on germination due to better oxygen uptake and dehydrogenase enzyme activity that provides metabolites to the embryo and thereby increases the metabolic activity. The decline in seed germination percentage at higher doses might be attributed to the high cell membrane permeability that progressively results in a high loss of leachates (Krishnaswamy and Seshu, 1989). The presence of non-volatile growth inhibitors also reduces germination percentage in gamma-irradiated seeds (Rajarajeshwari, 2011). Increased gamma doses may cause injury in seeds that leads to a decline in germination percentage. Decline in cowpea germination was observed with advancement in storage period at irradiation dosage of 10- 60 Kr (Uma and Salimath, 2001).

Speed of germination was also highest in seeds treated with lower doses (100 Gy and 200 Gy). But with higher doses, there was a trend for reduction in the speed of germination. The results are in accordance with earlier literature which showed two-fold increases in the speed of germination of *Terminalia arjuna* seeds compared to control when exposed to gamma rays of 100 Gy (Chandrashekar *et al.*, 2013). An increase in speed of germination was observed when tomato and okra seeds were exposed to 100 Gy and 200 Gy (Nargis, 1995; Kumar and Mishra 2004). Early reports of Akshatha and Chandrasekar (2013) also support these findings. The lower dose of gamma rays (25 Gy) imposed a significant increase in the speed of germination in *Pterocarpus sp.*

In the present study, shoot length and root length reduced significantly with higher doses of gamma irradiation. Similar findings were reported by Pranesh *et al.* (2019) in which a reduction in shoot length (5.1 and 5.9 cm) was observed at 1000 Gy gamma rays in Bengal gram and black gram at the end of nine months of storage. Gamma rays of 800 Gy had a pronounced effect on the shoot length of amaranthus seeds with maximum reduction (Aynehband and Afsharinafar, 2012). They also reported that poor shoot growth might be attributed due to injury to the seeds.

Marcu *et al.* (2013) observed that higher gamma doses beyond 100 Gy reduced the root length by 71 per cent in maize. Aynehband and Afsharinafar (2012) reported that an inverse relationship was found between root length and gamma doses. Uma and Salimath (2001) reported a drastic reduction in shoot and root length at higher doses of 10 -60 Kr.

Reduction in mitotic activity of meristematic tissues might be the reason for the reduction in shoot and root growth at higher gamma doses (Khalil *et al.*, 1986). Similar findings were reported that high gamma doses greatly affect the synthesis of protein (Xiuzher, 1994) reduces the production of growth hormones like IAA (Chandorkar and Clark, 1986) inhibits leaf-gas exchange, reduces water exchange, and growth enzymes activity (Stoeva *et al.*, 2001). The least dose of 200 Gy was the most effective dose for all types of chickpeas as it improved the germination parameters (Toker *et al.*, 2005). Seedling length at 50, 100, 150, and 200 Gy was the highest on the fifth day of observation in Okra (Jaipo *et al.*, 2019).

In the study, seedling dry weight also reduced with increase in gamma doses. Reduction in dry weight of seedlings might be attributed to reduced seedling length that has a direct correlation with the dry weight and ultimately with seedling vigour index. The results are contradictory with Borzouei *et al.* (2010) who reported that gamma doses of 100 Gy resulted in a 25 per cent increase in dry weight whereas 200, 300, and 400 Gy resulted in a decrease in dry weight compared to control. The reduced seedling weight might be attributed to reduced growth or moisture content in the plant due to radiation stress (Majeed *et al.*, 2010).

Seedling vigour index I and II of cowpea progressively decreased with the advancement in the storage period. Akshatha and Chandrasekar (2013) found that seedling vigour index increased at a lower dose of 25 and 50 Gy in *Pterocarpus sp.* The increase in seedling vigour could be due to increased germination percentage, shoot length, root length and dry weight at lower doses which have a positive correlation with vigour index. A comparison between different treatments on seedling vigour index I & II is given in Figure 3 & 4.

This result is supported by Chandrashekar (2015) who found that seedling vigour index showed a two-fold increase at 50 Gy compared to control in *Canarium strictum*. Improvement in growth parameters may be due to enhanced photosynthesis that leads to an increase in carbohydrate content.

All the above literature supported our results which recorded the highest seed germination attributes like germination percentage, speed of germination, shoot and root length, dry weight, and seedling vigour index at lower gamma doses (100 and 200 Gy) whereas the lowest was recorded at higher doses like 300 Gy, 400 Gy, and 500 Gy.

A field experiment was conducted to evaluate the morphological parameters of the plants from the irradiated seeds. Fifty plants from each treatment were observed from the germination stage until harvest for any abnormalities. At the field level also, germination percentage of irradiated seeds decreased with increased doses. The mean values of all other morphological characters were observed and found that there was a reduction in plant height, number of pods plant^{-1}, number of seeds pod^{-1}, and 100 seed test weight in higher doses compared with control. Few crinkled leaves were observed in 400 Gy and 500 Gy at earlier stages which was then recovered later. Lower doses (100 Gy and 200 Gy) did not show any significant changes from the control showing that these doses did not produce any change in the genetic makeup of the seeds.

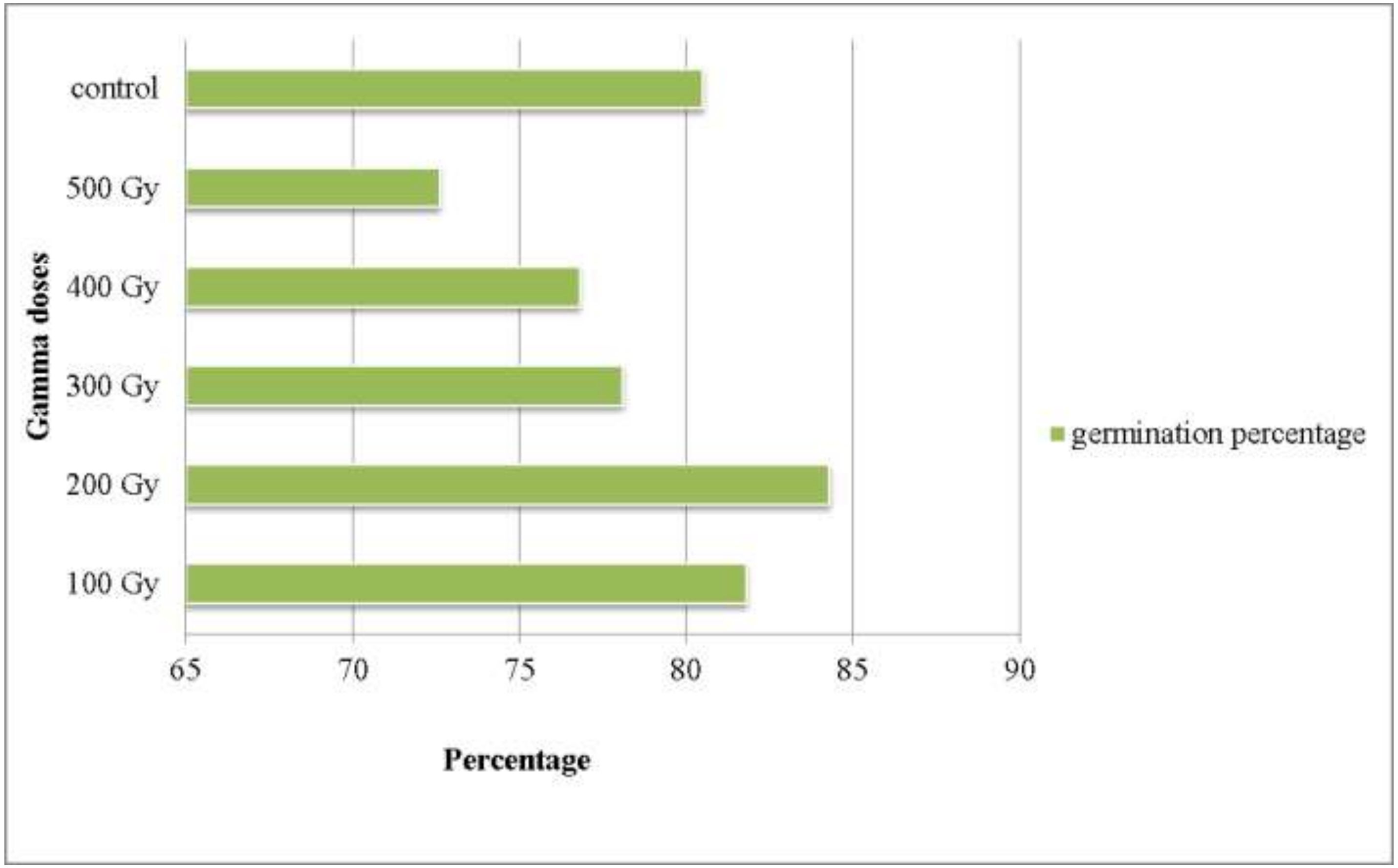


Fig 2: Effect of gamma doses on germination percentage at the end of six months of storage

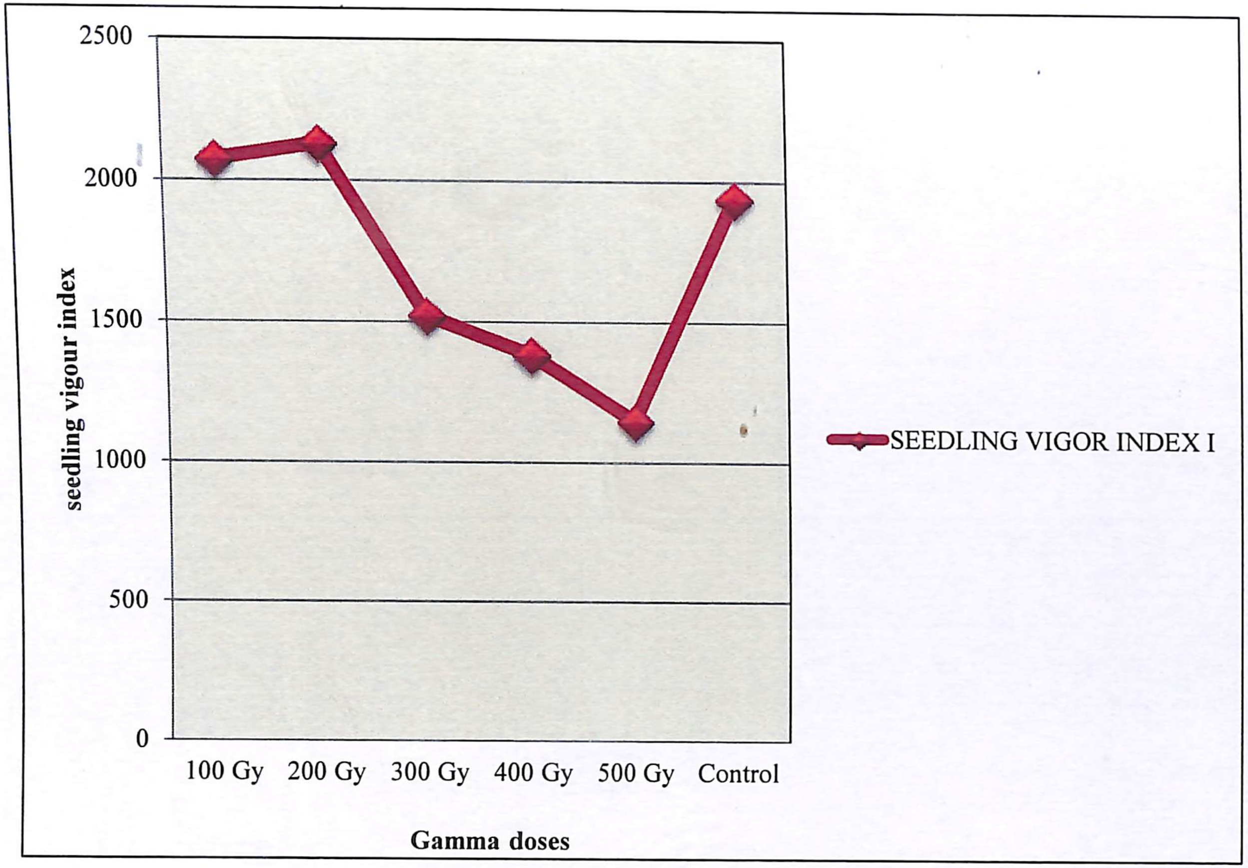


Fig 3: Effect of gamma doses on seedling vigour index I at the end of six months of storage

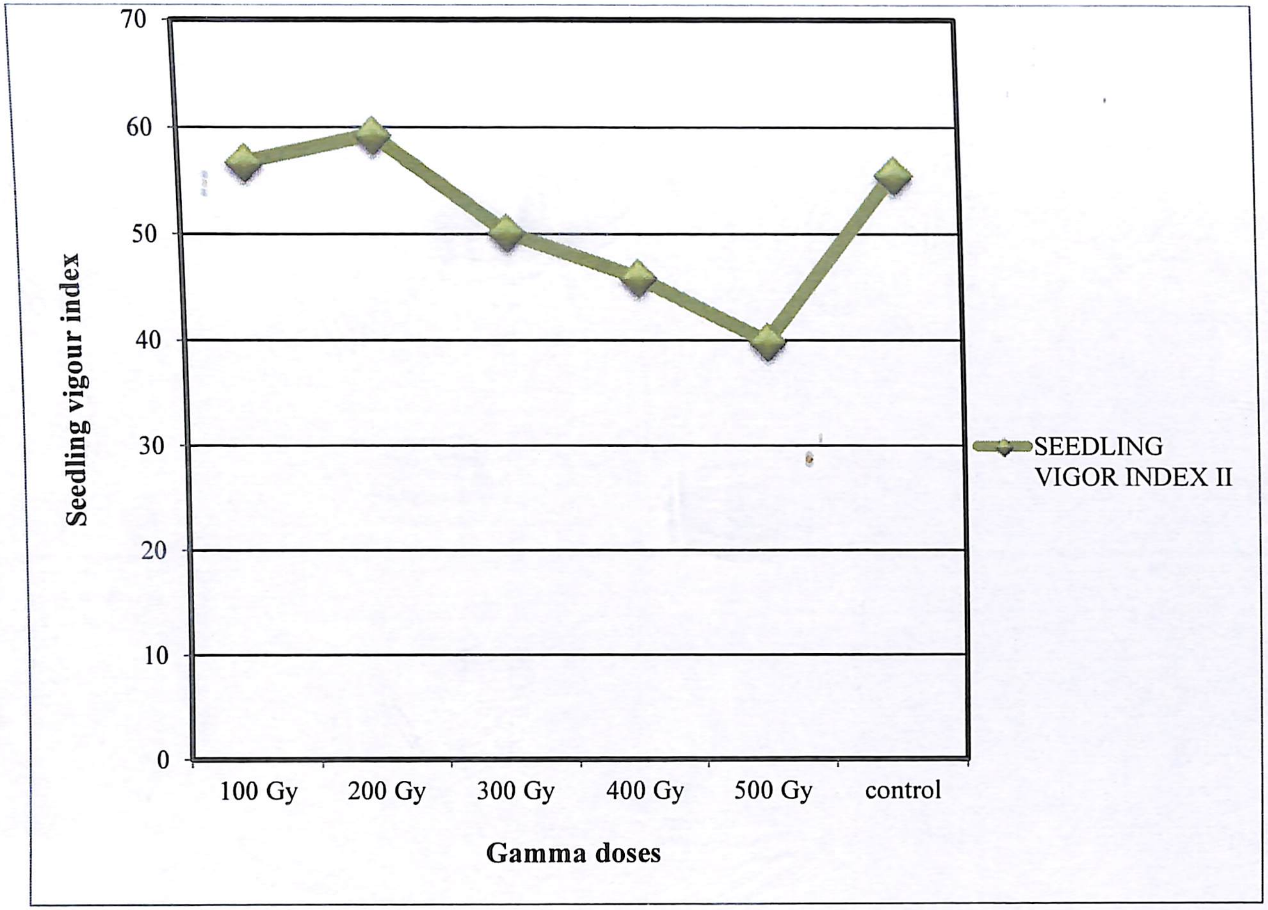


Fig 4: Effect of gamma doses on seedling vigour index II at the end of six months

High gamma doses ranging from 350-500 Gy decreased germination percentage of chickpea (Hameed *et al.,* 2008). A greater reduction in seed germination was reported at 25 kGy. This effect might be attributed to damage to the initially dividing cells (Ariraman *et al.,* 2014). Mudibu *et al.* (2011) reported that an increase in the number of pods per plant was observed in three varieties of soybean irradiated with 200 Gy and 400 Gy. Delayed flowering may happen at higher gamma doses. An increase in 1000 kernel weight and harvest index of canola was found in seeds treated with 100 Gy gamma rays (Rahimi and Bahrani, 2011). Sometimes the reduction in physiological traits may be due to sudden destruction of growth inhibitors and metabolic changes (Ariraman *et al.,* 2014).

5.2 SEED COATING OF COWPEA SEEDS WITH CHITOSAN

Chitosan is a biopolymer obtained from shells of crabs, lobsters and shrimps. It is derived from chitin by deacetylation process. Cowpea seeds were coated with different concentration of chitosan (1%, 2 %, 3 %, 4 % and 5%) each with 2 different doses as 1 mL and 5 mL $50g^{-1}$ of seed. Coated seeds were shade dried and stored for 6 months. The objective of this study is to standardize the concentration of chitosan in order to enhance the storage life and to know the positive effects of seed coating on germination and seedling traits of cowpea. During storage, different observations were taken such as germination parameters, pulse beetle infestation monthly for a period of six months.

5.2.1 Pulse beetle infestation assessment

In the present study, pulse beetle damage and insect eggs were not noticed in all the treatments from the initial to four months of storage. However, in control, the infestation was noticed from the first month and increased up to six months of storage. In treatment T_1 (1 % @ 1mL $50g^{-1}$), T_2 (1% @ 5mL $50g^{-1}$), T_3 (2% @ 1mL $50g^{-1}$) and T_5 (3% @ 1mL $50g^{-1}$) significant infestation and insect eggs were noticed from fifth month onwards. Although insect eggs and infestation were noticed in these treatments their percentage decreased with an increase in concentration and quantity used. Whereas, in treatments T_4, and T_6 (2% @ 5mL $50g^{-1}$ and 3% @ 5mL $50g^{-1}$) infestation was noticed from the sixth month onwards. In treatments T_7 (4% @ 1mL

50g^{-1}), T_8 (4% @ 5mL 50g^{-1}), T_9 (5% @ 1mL 50g^{-1}) and T_{10} (5% @ 5mL 50g^{-1}) the insect eggs and damage were not noticed till the end of the sixth month and it was in line with the earlier literature. Chitosan seed treatment proves to be an alternative for pulse beetle control and these results were confirmed with the investigations carried out by Rajkumar *et al.* (2020), Sahab *et al.* (2015). A comparison of different treatments on pulse beetle infestation is given in Figure 5.

Nearly, 77.8 per cent decrease in the mean number of eggs female^{-1} of Soybean aphid *Aphis gossypii* was observed with the treatment of nano chitosan with insects compared to control. The percentage of nano chitosan treated *Callosobruchus maculatus* and *Callosobruchus chinensis* showed a decrease in insect growth of 71.7 per cent and a 73 per cent decrease compared to control. Also, insecticidal activity of chitosan was exhibited at the concentration of 600-600mg L^{-1} (Sahab *et al.*, 2015).

Chitosan solution at 3g L^{-1} had a better insecticidal activity of 72 per cent against *Plutella xylostella* compared to 1200 mg L^{-1}. Similarly, chitosan at 3 mg/l exhibited 38.4 per cent mortality of *Helicoverpa armigera* after 24 hours, and 40 per cent mortality at 72 hours. Higher insecticidal activity of 70-80 per cent was reported against *Aphis gossypii, Metopolophium dirhodum, and Rhopalosiphum padi* (Zhang *et al.*, 2003).

Chitosan also exhibits high insecticidal activity against *Hyalopterus pruni* with 90-93 per cent mortality. Said *et al.* (2011) revealed the presence of disorganized, elongated, and disintegrated midgut epithelia in the third instar larvae of *Galleria melleonella* L. fed with an artificial diet amended with chitosan.

Bharani *et al.* (2014) investigated the insecticidal activity of chitosan nanoparticles integrated with Beauvericin (Csnp- Bv) formulation on *Spodopters litura* and found that there was 100per cent mortality of larvae when handled with 1.0, 0.01, 0.001 mg concentrations in the first and second instars and that the per cent mortality decreased with scale, reaching 24, 11.2, and 3.0 per cent in the sixth instars, respectively.

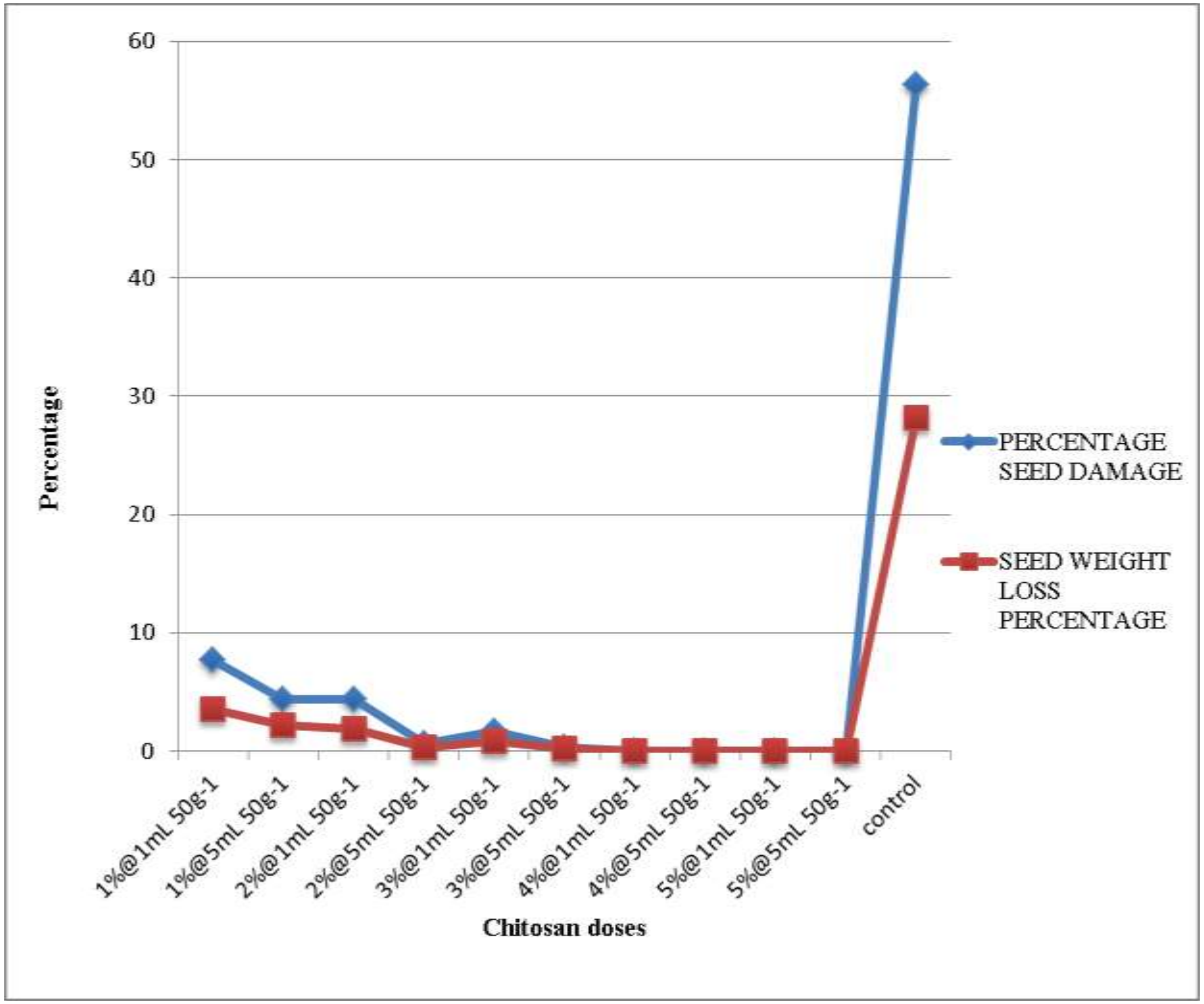


Fig 5: Effect of chitosan seed coating on pulse beetle infestation at six months of storage

5.2.2 Germination parameters

Chitosan improved the seed germination at all concentrations compared to control. After six months of storage, seed coated with chitosan at (T_{10}) 5 % @ 5 mL $50g^{-1}$ recorded the highest seed germination percentage (89.37 %) compared to control (80.67 %). The results are in accordance with Zeng *et al.* (2012) who reported that increased seed germination was observed in the concentration of 5 per cent. Seed coating with chitosan enhances the seed germination as it had an excellent film-forming property that forms semi-permeable film on the surface of seeds that helps to absorb soil moisture and maintain seed moisture (Zeng *et al.*, 2012). It also helps the seeds from corrupting by cutting off excess soil moisture. Increased seed germination percentage was observed in the seeds primed with 3g L^{-1} chitosan compared to control (Al- Tawaha and Al- Ghzawi, 2013). Improvement in germination is due to increased anti-oxidant activity at the time of seed germination. Soaking maize seeds in chitosan solution increased germination percentage. The increase in germination parameters could be attributed to improved enzyme activities of nitrogen metabolism by chitosan (Shao *et al.*, 2005). A comparison of different treatments on germination percentage is given in Figure 6.

In the study, highest speed of germination (36.83) was recorded in treatment (T_{10}) 5 % @ 5 mL $50g^{-1}$ compared to control (31.40). It was found that all the treatments were superior to control from the first month of storage onwards. Seed germination percentage was significantly increased in seeds coated with chitosan compared to control (Zeng and Luo, 2012).

Seedling shoot and root length were highest in the seeds coated with 5 per cent and 4 per cent chitosan. Similar findings were observed by Zeng *et al.* (2012) who reported that root and shoot were longer, thicker, and well developed in chitosan-coated seeds compared to non-coated seeds. Chitosan reduced transpiration rate by improving root length that helps to alleviate stress conditions because of its hydrophilic nature (Bittelli *et al.*, 2001). Seedling dry weight, seedling vigour index I and II were highest in the seeds coated with high concentrations of chitosan.

Different concentrations of chitosan from lower to higher (1 % to 5 %) have different degrees of improvement in germination parameters compared to control. A comparison of different treatments on seedling vigour index I is given in Figure 7.

Treatment of chickpea seeds with chitosan nanoparticles showed improvement in seed germination, root and shoot length, seed vigour index, and seedling vegetative biomass. It also resulted in the formation of more lateral roots in chickpea. He found that chitosan-treated seeds showed 100 per cent germination whereas it was only 92 per cent in control (Sathiyabama and Parthasarathy, 2016). The application of chitosan improved the shoot and root length of rice plants (Vasudevan *et al.*, 2002). A comparison of different treatments on seedling vigour index II is given in Figure 8.

Chitosan has a positive correlation with plant growth-promoting processes like nutrient absorption, cell division, protein synthesis, and cell elongation (Amin *et al.*, 2007). Priming of seeds with chitosan solution increased germination parameters like germination percentage, rate of germination, seedling length, and vigour index in *Carum copticum* seeds (Batool and Asghar, 2013). Chitosan treatment of maize enhanced the synthesis of hydrolytic enzymes like protease and α-amylase that helps in the rapid mobilization of food reserves and its degradation, which ultimately increased germination and vigour of seedlings (Saharan *et al.*, 2016). Variations in response to germination parameters of seeds may be due to biopolymer concentration and quantity used for seed coating.

In this study Gamma irradiation proved to be an effective method for controlling pulse beetle infestation during storage in grain cowpea. Higher doses protected the seeds completely without any infestation at the end of six months. However the treatment with these dozes affected the germination parameters negatively and doses 400 &500 Gy produced some abnormalities in the progeny also. Irradiation at 300 Gy could protect the seeds in storage with no infestation upto six months but the seedling parameters showed reduction compared to control. Morphological parameters of the progeny from the seeds treated with this dose did not show significant variation from the control except for seed germination in the field.

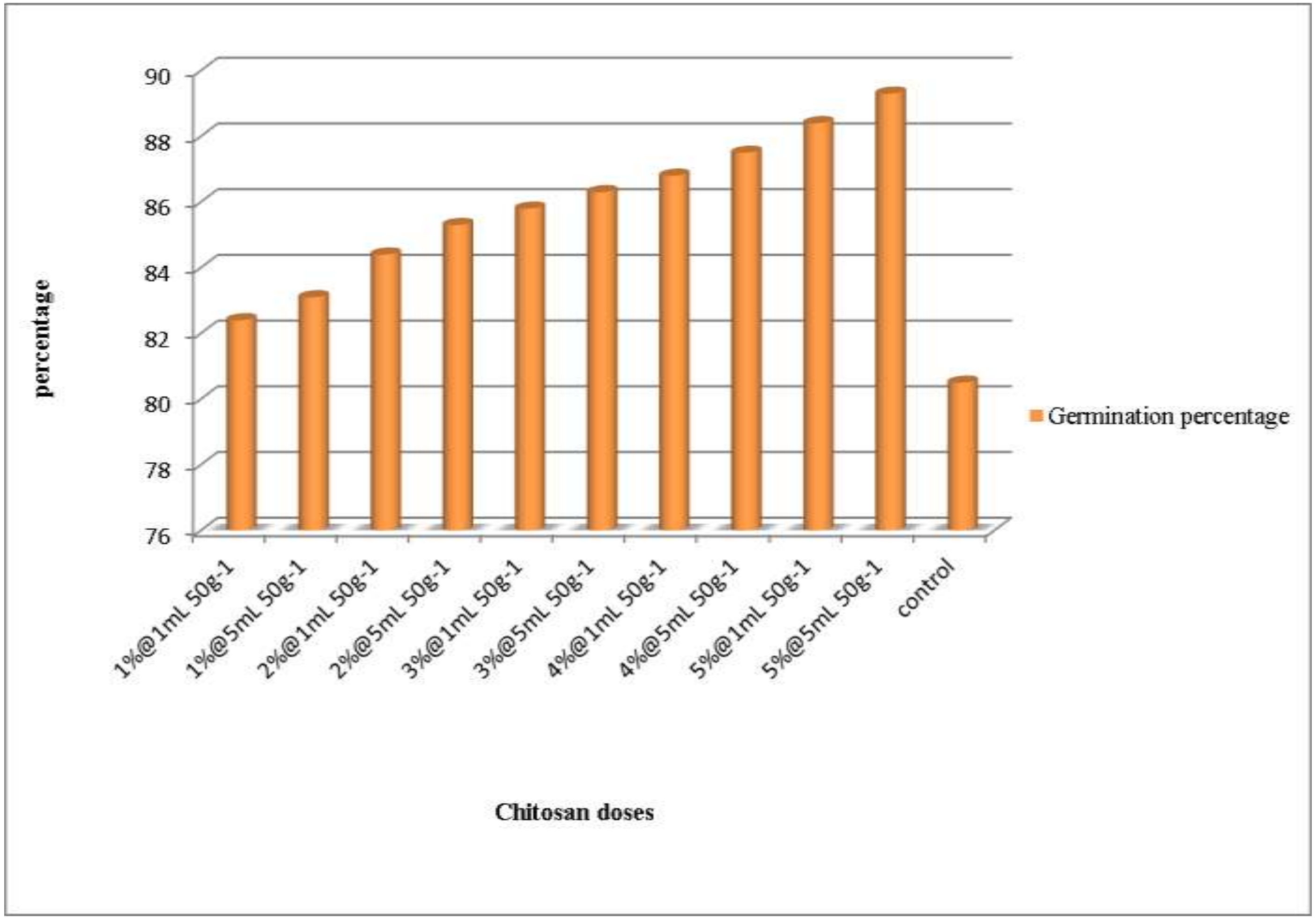
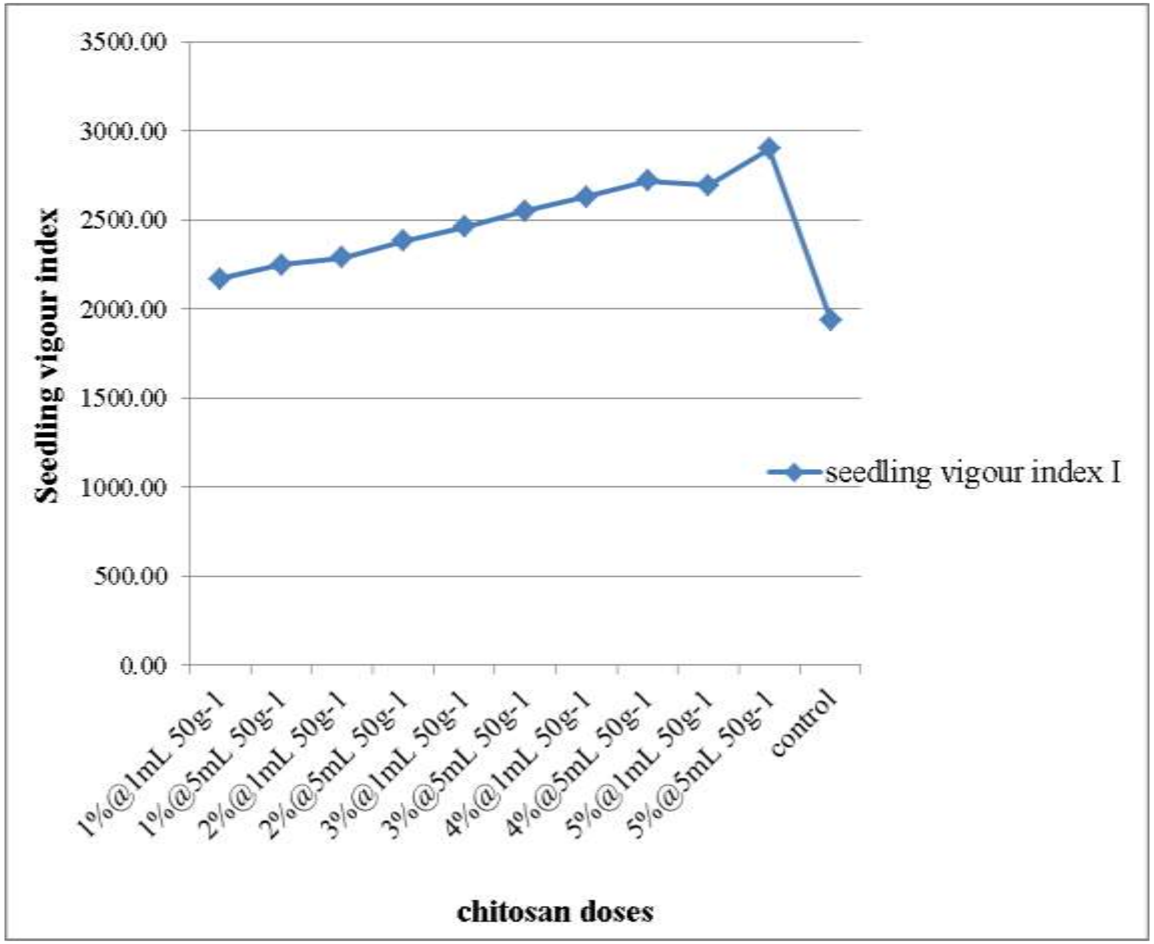


Fig 6: Effect of chitosan seed coating on germination percentage at six months of storage



` **Fig 7: Effect of chitosan seed coating on seedling vigour index I at six months of storage**

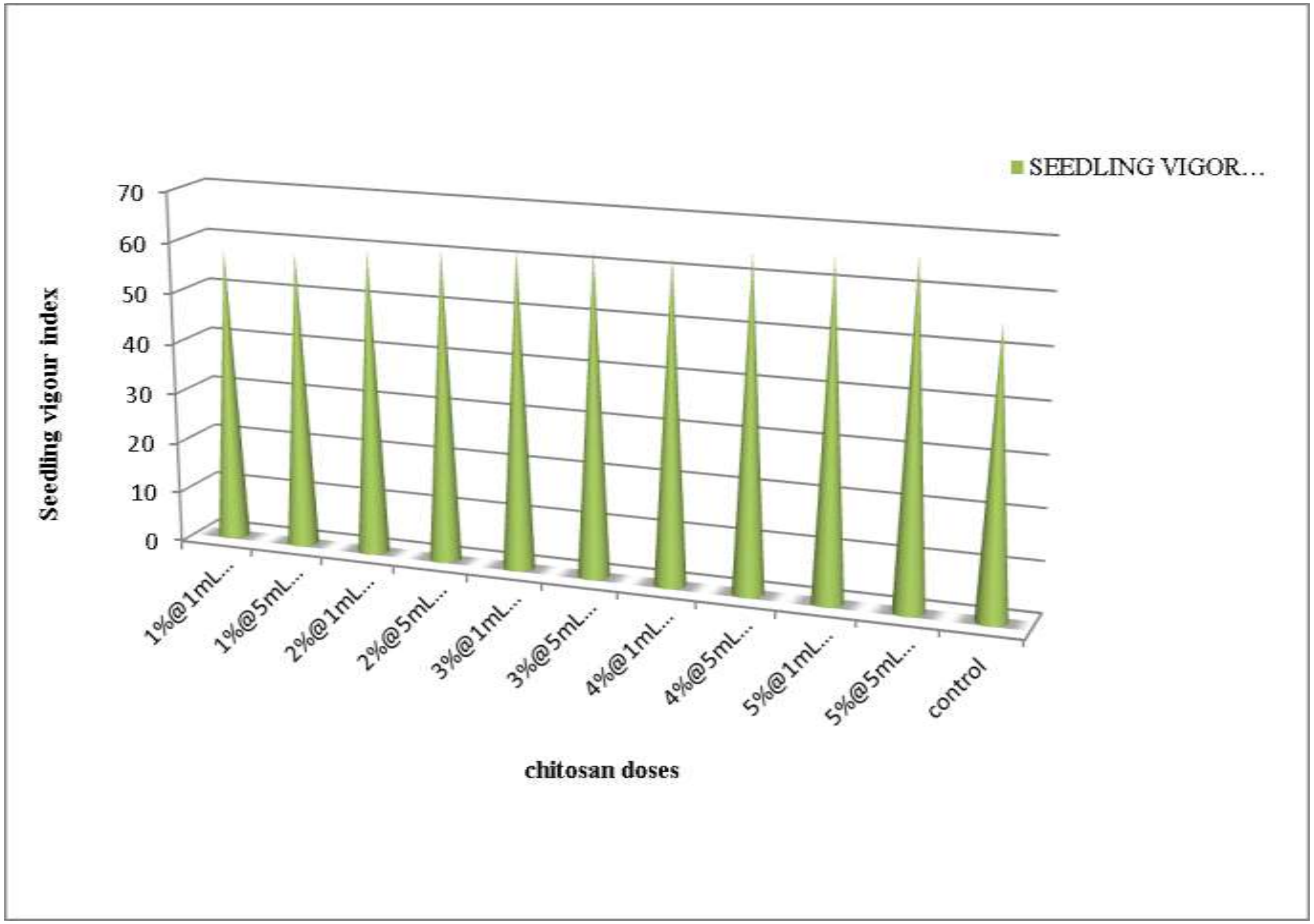


Fig 8: Effect of chitosan seed coating on seedling vigour index II at six months of storage

Gamma radiation at 200 Gy registered a higher value for seed germination parameters and with very low compared to control upto six months. So Gamma irradiation at 200Gy and 300 Gy can be recommended for safe storage of grain cowpea seeds.

Chitosan at 5% @ 5mL $50g^{-1}$ exhibited higher values for seed germination parameters and showed no pulse beetle infestation till the end of the storage period of six months. Chitosan below 5% improved seed germination but was effective in controlling pulse beetle for short period of time only. Chitosan treatment at 5% @ 5mL $50g^{-1}$ can be recommended for safe storage of grain cowpea seeds. Both gamma irradiation and chitosan seed coating maintained the longevity of seeds during storage and both were effective in controlling the storage pests. Seed coating with chitosan had an additional advantage of improvement of seed germination parameters. Both these treatments are ecofriendly and can be used without any harm to the environment. Since Gamma irradiation requires special facilities of treatment plant chitosan coating will be a better technology for small scale farmers.

SUMMARY

6. SUMMARY

The study entitled "Irradiation and seed coating for enhancing storage life of grain cowpea (*Vigna unguiculata* (L.) Walp.)" was carried out to standardize the dose of gamma rays for irradiation and concentration of chitosan for seed coating for enhancing the storage life of grain cowpea. The experiment was carried out in the Department of Seed Science and Technology, College of Agriculture, Vellayani during 2020-2021. The first experiment was irradiation of cowpea seeds with gamma rays and the second experiment was seed coating of cowpea seeds with chitosan and the seeds were stored in polythene bags for a period of six months. Both the experiments were conducted in Completely Randomized Block Design (CRD) with three replications.

The seeds were irradiated with five different doses (100 Gy to 500 Gy) at Indian Institute of Horticultural Research (IIHR), Bangalore and seeds were stored in polythene bags. Another set of seeds were coated with chitosan at five different concentrations (1 % to 5 %) at two different quantities for each concentration as 1mL $50g^{-1}$ of seeds and 5mL $50g^{-1}$ of seeds. Coated seeds were then shade dried and packed in polythene bags. All the treated seeds were then stored for six months along with control. Observations on germination parameters and pulse beetle infestation were recorded in both experiments at monthly intervals for six months. The salient finding of this study is summarized below.

Among the different gamma doses, insect eggs and pulse beetle infestation were not noticed in all the treatments from the initial to three months of storage. In treatments 300 Gy, 400 Gy and 500 Gy, insect eggs and pulse beetle infestation were not noticed till the end of six months. However, in control, the seed damage percentage was noticed from the first month onwards and increased up to 56.333% at the end of six months of storage. In treatment T_1 (100 Gy) and T_2 (200 Gy), it was only 2.667 per cent and 0.667 per cent at the end of the sixth month. Seed weight loss percentage in control at sixth month was 28.182 per cent. The number of eggs 100 $seeds^{-1}$ in control on the sixth month was 78.333 nos. In treatment T_1 (100 Gy) and T_2 (200 Gy), it was 4.333 nos and 1.667 nos at the end of the sixth month. The number of damaged seeds in control, T_1 (100 Gy) and T_2 (200 Gy) in the sixth month was 56.333 nos, 3.00 nos, and 0.667 nos. The weight of damaged seeds in control, T_1 (100 Gy) and T_2 (200 Gy) was 4.163 g, 0.180 g and 0.040 g. Gamma rays at all doses were effective in controlling pulse beetle infestation.

Germination parameters were studied in the undamaged seeds to study the effect of irradiation in seed aging. Among the different doses of irradiation, T_2 (200 Gy) significantly recorded the highest seed germination percentage (84.33%). Lowest germination percentage was observed in T_5 (500 Gy) of 72.60 per cent at six months of storage. Speed of germination, seedling shoot length and seedling dry weight was highest at T_2 (200Gy) with 32.13, 11.83 cm and 0.703 g and lowest in T_5 (500 Gy) with 26.33, 8.56 cm, 0.549 g. Seedling root length was highest at T_1 (100 Gy) with 13.84 cm and lowest in T_5 (500 Gy) with 7.23 cm. Seedling vigour index I and II were maximum at T_2 (200 Gy) with 2130.49 and 59.28 and minimum at T_5 (500 Gy) at six months of storage. All the germination parameters showed increased value at lower doses of gamma rays 100 Gy and 200 Gy and declined at higher doses 300 Gy, 400 Gy, and 500 Gy compared to control. Increase in germination parameters may be due to stimulatory effect on RNA activation and protein synthesis.

At the field level, morphological evaluation of irradiated seeds was carried out to observe any abnormalities occurring due to mutation. All the plants from each treatment were observed separately for various traits. At gamma doses 300 Gy, 400 Gy and 500 Gy, there was reduction in germination percentage, plant height, number of pods plant^{-1}, number of seeds pod^{-1} and 100 seed test weight compared to control. Progressive decrease in all morphological parameters was observed with increase in doses of above 200 Gy. Few crinkled leaves were observed in 400 Gy and 500 Gy at earlier stages which was then recovered later

The seeds were coated with chitosan at five different concentrations (1 % to 5 %) in two different quantities for each concentration as 1mL 50g^{-1} of seeds and 5mL 50g^{-1} of seeds and pulse beetle infestation was observed. Among the different chitosan treatments, insect eggs and pulse beetle infestation were not noticed in all the chitosan coated seeds from the initial to four months of storage. Seeds stored without chitosan coating (control) reported highest percentage seed damage (56.33 %), seed weight loss percentage (28.182 %), number of eggs 100 seeds^{-1} (78.333), number of damaged seeds (56.333) and weight of damaged seeds (4.163g). In treatments T_7 (4% @ 1mL 50g^{-1}), T_8 (4% @ 5mL 50g^{-1}), T_9 (5% @ 1mL 50g^{-1}), T_{10} (5% @ 5mL 50g^{-1}) the insect eggs and damage were not noticed till the end of the sixth month. In treatment T_1 (1% 1mL 50g^{-1}), T_2 (1% @ 5mL 50g^{-1}), T_3 (2% @ 1mL 50g^{-1}) and T_5 (3% @ 1mL 50g^{-1}) significant infestation and insect eggs were noticed from fifth month onwards. In treatment T_4 (2% 5mL 50g^{-1}) and T_6 (3% @ 5mL 50g^{-1}) significant bruchids infestation and insect eggs were noticed at the sixth month only. Although insect eggs and infestation were noticed in these treatments, their percentage decreased with an increase in concentration and quantity used.

The undamaged seeds in the control and the coated seeds were assessed for germination parameters at monthly intervals for six months to assess the effect of seed coating on the seed germination parameters. Among the different treatments of chitosan, highest seed germination percentage of 89.37 per cent was observed in T_{10} (5 % @ 5 mL 50g^{-1}). The treatment T_{10} (5 % @ 5 mL 50g^{-1}) produced the maximum speed of germination (36.83), seedling shoot length (14.90 cm), seedling root length (17.53 cm), and seedling dry weight (0.747 g). The minimum germination percentage (80.67 %), speed of germination (31.40), seedling shoot length (11.56 cm), seedling root length (12.45 cm) and seedling dry weight (0.687 g) was observed in control. Seedling vigour index I and II was maximum at T_{10} (5 % @ 5 mL 50g^{-1}) with 2898.28 and 66.79 and minimum in control (1939.24 and 55.52). Different concentrations of chitosan from lower to higher (1% to 5%) showed improvement in germination parameters compared to control.

In this study Gamma irradiation proved to be an effective method for controlling pulse beetle infestation during storage in grain cowpea. Higher doses protected the seeds completely without any infestation at the end of six months. However the treatment with these dozes affected the germination parameters negatively and doses 400 &500 Gy produced some abnormalities in the progeny also. Irradiation at 300 Gy could protect the seeds in storage with no infestation upto six months but the seedling parameters showed reduction compared to control.

Morphological parameters of the progeny from the seeds treated with this dose did not show significant variation from the control except for seed germination in the field. Gamma radiation at 200 Gy registered a higher value for seed germination parameters and with very low compared to control upto six months. So Gamma irradiation at 200 Gy and 300 Gy can be recommended for safe storage of grain cowpea seeds.

Chitosan at 5% @ 5mL $50g^{-1}$ exhibited higher values for seed germination parameters and showed no pulse beetle infestation till the end of the storage period of six months. Chitosan below 5% improved seed germination but was effective in controlling pulse beetle for short period of time only. Chitosan treatment at 5% @5mL50g can be recommended for safe storage of grain cowpea seeds. Both gamma irradiation and chitosan seed coating maintained the longevity of seeds during storage and both were effective in controlling the storage pests. Seed coating with chitosan had an additional advantage of improvement of seed germination parameters. Both these treatments are ecofriendly and can be used without any harm to the environment. Since Gamma irradiation requires special facilities of treatment plant chitosan coating will be a better technology for small scale farmers.

REFERENCES

7. REFERENCES

Abbas, H., Nouraddin, S., Reza, Z. H., Iraj, B., Mohammad, B., Hasan, Z., Hossein, A. M., and Hadi, F. 2011. Effect of gamma radiation on different stages of Indian meal moth *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae). *Afr. J. Biotechnol.* 10(20): 4259-4264.

Abdu, R. M., Abdel-Kader, M. M., Hussein, M. A., and Abdel-Rahman, H. A. 1985. Biological effects of gamma radiation on stored product insects. 4-radiation effects on sex pheromone production and perception by the rust-red flour beetle. *Tribolium castaneum* (herbst). *Katar Univ. Sci. Bull.* 5: 279-286.

Abdul-Baki, A. A. and Anderson, J. D. 1973. Physiological and biochemical deterioration of seed. In: Kozlowski, T.T., Seed Biology (2nd Ed.). Academic Press, New York, London, pp. 283-315.

Adams, J. M. and Schulten, G. G. M. 1978. Losses caused by insects, mites and microorganisms. In: *Post-Harvest Grain Loss Assessment Methods.* American Association of Cereal Chemists, St. Paul, Minnesota. USA. pp. 98-100.

Ahmed, K. S., Itino, T., and Ichikawa, T. 2003. Duration of developmental stages of *Callosobruchus chinensis* L.(Coleoptera: Bruchidae) on azuki bean and the effects of neem and sesame oils at different stages of their development. *Pak. J. Biol. Sci. 6*(10): 932-335.

Akshatha, C. K. and Chandrashekar, K. R. 2013. Effect of gamma irradiation on germination growth and biochemical parameters of *Pterocarpus santalinus*, an endangered species of Eastern Ghats. *Eur. J. Exp. Bio.* 3:.266-70.

Akter, J., Jannat, R., Hossain, M. M., Ahmed, J. U., and Rubayet, M. T. 2018. Chitosan for plant growth promotion and disease suppression against anthracnose in chilli. *Int. J. Environ. Agric. Biotechnol.* 3(3): 806-816.

Aldryhim, Y. N. and Adam, E. E. 1999. Efficacy of gamma irradiation against *Sitophilus granarius* (L.)(Coleoptera: Curculionidae). *J. Stored Products Res.* 35(3): 225-232.

Ali, S. M., Mahgoub, S. M., Hamed, M. S., and Gharib, M. S. A. 2004. Infestation potential of *Callasobruchus chinensis* and *C. maculatus* on certain broad bean seed varieties. *Egypt J. Agric. Res.* 82 (3): 1127-1135.

Al-Safadi, B. and Simon, P. W. 1996. Gamma irradiation-induced variation in carrots (*Daucus carota* L.). *J. Am. Soc. for Hortic. Sci.* 121(4): 599-603.

Al-Tawaha, A. R. M. and Al-Ghzawi, A. L. A. 2013. Effect of chitosan coating on seed germination and salt tolerance of lentil (*Lens culinaris* L.). *Res. Crops.* 14(2): 489-491.

Amin, A. A., Rashad EL-Sh M., and EL-Abagy H. M. H., 2007. Physiological effect of indole-3-butyric acid and salicylic acid on growth, yield and chemical constituents of onion plants. *J. Appl. Sci. Res.* 3: 1554-1563.

Anandhi, P., Varma, S., and Sarvanan, L. 2008. Estimation of losses and evaluation of different storage containers against pulse beetle, *Callosobruchus chinensis* (Linnaeus) in bengal gram. *J. of Insect Sci.* 2(1): 40-43.

Ariraman, M., Gnanamurthy, S., Dhanavel, D., Bharathi, T., and Murugan, S. 2014. Mutagenic effect on seed germination, seedling growth and seedling survival of Pigeon pea (*Cajanus cajan* (L.) Millsp). *Int. Lett. Nat. Sci.* 16: 41-49.

Arthur, V., Arthur, P. B., and Machi, A. R. 2016. Pupation, adult emergence, and F1 egg hatch after irradiation of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) last instars. *Florida Entomol.* 99(6): 59-61.

Atwal, A. S. and Dhaliwal, G. S. 2005. Agricultural Pests of South Asia and their Management. Kalyani Publishers, New Delhi, 505p.

Aynehband, A. and Afsharinafar, K. 2012. Effect of gamma irradiation on germination characters of amaranth seeds. *Eur. J. Exp. Biol.* 2(4): 995-999.

Ayvaz, A. and Tuncbilek, A. S. 2006. Effects of gamma radiation on life stages of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *J. Pest Sci.* 79(4): 215-222.

Ayvaz, A., Albayrak, S., and Karaborklu, S. 2008. Gamma radiation sensitivity of the eggs, larvae and pupae of Indian meal moth *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae). *Pest Manag. Sci. formerly Pesticide Sci.* 64(5): 505-512.

Badawy, M. E. 2010. Structure and antimicrobial activity relationship of quaternary N-alkyl chitosan derivatives against some plant pathogens. *J. Appl. Polym. Sci.* 117(2): 960-969.

Badway, M. E. I. and El-Aswad. A. F. 2012. Insecticidal Activity of Chitosans of Different Molecular Weights and Chitosan-metal Complexes against Cotton Leaf worm *Spodoptera littoralis* and Oleander Aphid *Aphis nerii. Plant Protect. Sci.* 48(3): 131–141.

Bal, S. S. 1976. Magnitude and types of seed storage needs in India. *Seed Res.* 4: 1-5.

Bashir. S., Wani, A. A., and Nawchoo, I. A. 2013. Mutagenic sensitivity of gamma rays, EMS and sodium azide in *Trigonella foenum-graecum* L. *Sci. Res. Reporter*. 3(1): 20–26.

Batool, M. and Asghar, R. 2013. Seed priming with chitosan improves the germination and growth performance of ajowan (*Carum copticum*) under salt stress. *Eurasia J. Biosci.* 7: 69–76.

Beyaz, R., Kahramanogullari, C. T., Yildiz, C., Darcin, E. S., and Yildiz, M. 2016. The effect of gamma radiation on seed germination and seedling growth of *Lathyrus chrysanthus* Boiss. under in vitro conditions. *J. Environ. Radioactivity*. 162:129-133.

Bhalla, S., Gupta, K., Lal, B., Kapur, M. L., and Khetarpal, R. K. 2008. Efficacy of various non-chemical methods against pulse beetle, *Callosobruchus maculatus* (Fab.). In: Endure International Conference. *Diversifying Crop Protection,* October 2008, La Grande- Motte, France, pp 12-15.

Bharani, R. A., Namasivayam, S. K. R., and Shankar, S. S. 2014. Biocompatible chitosan nanoparticles incorporated pesticidal protein beauvericin (csnp-bv) preparation for the improved pesticidal activity against major groundnut defoliator I (Fab.)(Lepidoptera; Noctuidae). *Int. Chem. Tech. Res*. 6(12): 5007-5012.

Bhatnagar, A., Bhadauria, N. S., and Jakhmola, S. S. 2001. Efficacy of vegetable oils against pulse beetle, *Callosobruchus maculatus* (Fab.) on cowpea. *Indian J. Entomol.* 63(3): 237- 239.

Bhuiya, A. D., Ahmed, M., Rezaur, R., Seal, D. R., Nahar, G., Islam, M. M., and Islam, M. S. 1985. Insect disinfestation of pulses by irradiation. In: *Radiation disinfestation of food and agricultural products,* Dhaka, 18(7).

Bittelli, M., Flury, M., Campbell, G. S., and Nichols, E. J. 2001. Reduction of transpiration through foliar application of chitosan. *Agric. For. Meteorol.* 107(3): 167-175.

Bonde, P.J., Thorat, B.S., and Gimhavnekar, V.J. 2020. Effect of Gamma Radiation on Germination and Seedling Parameters of Mung Bean (*Vigna radiata*). *Int. J. Curr. Microbiol. Appl. Sci.* 11: 1582–1587

Boonlertnirun, S., Boonraung, C., and Suvanasara, R. 2008. Application of chitosan in rice production. *J. Metals Mater. Minerals*. 18(2): 47-52

Borzouei, A., Kafi, M., Khazaei, H., Naseriyan, B., and Majdabadi, A. 2010. Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings. *Pak. J. Bot.* 42(4): 2281-2290.

Brown, G. A., Brower, J. H., and Tilton, E. W., 1972. Gamma radiation effects on *Sitophilus zeamais* and *S. granarius*. *J. Econ. Entomol.* 65(1): 203-205.

Burrows, F., Louime, C., Abazinge, M., and Onokpise, O. 2007. Extraction and evaluation of chitosan from crab exoskeleton as a seed fungicide and plant growth enhancer. *American-Eurasian J. Agric. Environ. Sci.* 2(2): 103-111.

Cabrera, G., Casals, P., Cardenas, G., Taboada, E., and Neira, P. 2002 Biodegradable chitosan derivatives with potential agriculture uses. In: Proceedings 10th

IUPAC International Congress on the Chemistry of Crop Protection, Basel, 2002; 227p

Caswellet, G. H. 1973. The storage of cowpea. *Samaru Agriculture Newsletter*, Nigeria. 15: 20-73.

Chandorkar, K. R. and Clark, G. M. 1986. Physiological and morphological responses of Pinus strobus L. and Pinus sylvestris L. seedlings subjected to low-level continuous gamma irradiation at a radioactive waste disposal area. *Environ. Exp. Bot. 26*(3): 259-270.

Chandrashekar, K. R. 2015. Gamma rays accelerated seed germination and physiological attributes in *Canarium strictum* Roxb. In: *Proceedings of the NAARRI international conference on state of the art radiation processing-radiation processing in a changing world: Book of abstracts and souvenir*, Mumbai, India, 41p.

Chandrashekar, K. R., Somashekarappa, H. M., and Souframanien, J. 2013. Effect of gamma irradiation on germination, growth, and biochemical parameters of *Terminalia arjuna* Roxb. *Radiat. Prot. Enviro.* 36(1): 38.

Cheng, L., Yang, H., Lin, B., Wang, Y., Li, W., Wang, D., and Zhang, F. 2010. Effect of gamma-ray radiation on physiological, morphological characters and chromosome aberrations of minitubers in *Solanum tuberosum* L. *Int. J. Radiat. Biol.* 86(9): 791-799.

Chiluwal, K., Kim, J., Bae, S. D., Roh, G. H., Park, H. J., and Park, C. G. 2019. Effect of gamma irradiation on fecundity, sterility, and female sex pheromone production of *Callosobruchus chinensis* (Coleoptera: Bruchidae). *J. Econ. Entomol.* 112(11): 156-163.

Cho, M. H., No, H. K., and Prinyawiwatkul, W. 2008. Chitosan treatments affect growth and selected quality of sunflower sprouts. *J. Food Sci.* 73: 570-577.

Credland, P. F. and Wright, A. W. 1990. Oviposition deterrents of *Callosobruchus maculatus* (Coleoptera: Bruchidae). Physiol. Entomol. 15: 285-298.

Dammak, I., Bittante, A. M. Q. B., Lourenco, R. V., and do Amaral Sobral, P. J. 2017. Properties of gelatin-based films incorporated with chitosan-coated microparticles charged with rutin. *Int. J. Biol. Macromolecules*. 101: 643-652.

Darfour, B., Ocloo, F. C. K., and Wilson, D. D. 2012. Effects of irradiation on the cowpea weevil *(Callosobruchus maculates* F.) and moisture sorption isotherm of cowpea seed (*Vigna unguiculata* L. Walp). *Arthropods*. 1(1): 4.

Delia, M., Grigore, D., Constatntin, C., and Victoria, C. 2013. Gamma radiation effects on seed germination and pigment content, and ESR study of induced free radicals in maize (Zea mays). *J. Biol. Phys.* 39: 625-634.

Dias, C. A. R. and Yadav, T. D. 1988. Incidence of pulse beetles in different legume seeds. *Indian J. Entomol.* 50(4): 457-461.

Doares, S. H., Syrovets, T., Weiler, E. W., and Ryan, C. A. 1995. Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proc. Natl Acad. Sci.* 92(10): 4095-4098.

Dongre, T. K., Harwalkar, M. R., Nene, S. P., and Padwal-Desai, S. R. 1997. Radio-sensitivity of different developmental stages of pulse beetle *(Callosobruchus maculatus). J. Food Sci. Technol. (Mysore).* 34(5): 413-415.

Dzung, N. A., Khanh, V. T. P., and Dzung, T. T. 2011. Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. *Carbohyd. Polym.* 84(2): 751-755.

Dzung, N. A., Thang, N. T., Suchiva, V. K., Chandrkrachang, S., Methacanon, P., and Peter, M. G. 2002. Effects of oligoglucosamine prepared by enzyme degradation on the growth of soybean. *Adv. Chitin Sci.* 5: 463-467.

Echereobia, C. O., Asawalam, E. F., Emeasor, K. C., Nwana, I. E., and Sahayaraj, K. 2014. Efficacy of gamma irradiation for the control of cowpea bruchid,(*Callosbruchus maculatus* F.). *J. Sustain. Agric. Environ.* 15(1): 69-79.

Enu, R. and Enu, P. 2014. Sterilization of grains using ionizing radiation: the case in Ghana. *Eur. Sci. J.* 10(6): 117-136.

Farkas, J. 1998. Irradiation as a method for decontaminating food: a review. *Int. J. food Microbiol.* 44(3): 189-204.

Fondevilla, S. and Rubiales, D. 2012. Powdery mildew control in pea. A review. *Agron. Sustain. Dev.* 32(2): 401-409.

Freepons, D. 2020. *Applications of chitin and chitosan* (2nd Ed.). CRC Press, 12p

Gaul, H. 1970. Plant injury and lethality. *Plant injury and lethality.* 102: 85-90

Girija, M. and Dhanavel, D. 2013. Effect of Gamma rays on quantitative traits of Cowpea in M1 generation. *Int. J. Res. Biol. Sci.* 3(2): 84-87.

Guan, Y. J., Hu, J., Wang, X. J., and Shao, C. X. 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J. Zhejiang Univ. Sci.* 10(6): 427-433.

Gujar, G. T. Yadav T. D. 1978. Feeding on *Callosobruchus chinensis* L. in Green gram. Indian J. Entomol. 40(2): 108-112.

Hameed, A., Shah, T. M., Atta, B. M., Haq, M. A., and Sayed, H. I. N. A. 2008. Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. *Pakist. J. Bot.* 40(3): 1033-1041.

Hammad, A., Gabarty, A., and Zinhoum, R. A. 2020. Assessment irradiation effects on different development stages of *Callosobruchus maculatus* and on chemical, physical and microbiological quality of cowpea seeds. *Bull. Entomol. Res.* 110(4): 497-505.

Harding, S. S., Johnson, S. D., Taylor, D. R., Dixon, C. A., and Turay, M. Y. 2012. Effect of gamma rays on seed germination, seedling height, survival percentage and tiller production in some rice varieties cultivated in Sierra Leone. *J. Exp. Agric. Int.* 247-255.

Hell, K. G. and Silveira, M. 1974. Imbibition and germination of gamma irradiation *Phaseolus vulgaris* seeds. *Field Crop Abst.* 38(6): 300.

Hien, N. Q. 2004. Radiation degradation of chitosan and some biological effects. *Radiat. Processing Polysaccharides*. 1422: 67.

Hosamani, G. B., Jagginavar, S. B., and Karabhantanal, S. S. 2018. Biology of pulse beetle *Callosobruchus chinensis* on different pulses. *J. Entomol. Zool. Stud.* 6(4): 1898-1900.

ISTA [International Seed Testing Association]. 2013. International rules for seed testing. *Seed Sci. Technol.* 27: 25-30.

Jaipo, N., Kosiwikul, M., Panpuang, N., and Prakrajang, K. 2019. Low dose gamma radiation effects on seed germination and seedling growth of cucumber and okra. *J. Phys. Conf. Ser.* 1380(1): 012-106.

Jat, N. R., Rana, B. S., and Jat, S. K. 2013. Estimation of losses due to pulse beetle in chickpea. *Bioscan* 8(3): 861-863.

Khalil, S. J., Rehman, S., Afridi, K., Jan, M. T. 1986. Damage induced by gamma radiation in morphological and chemical characteristics of barley. *Sarhad J. Agric.* 2: 45- 54.

Kim, H. S., Choi, Y. R., Kim, S. K., and Ham, I. J. 1970. Studies on the Preservation of Korean Rice by Gamma-irradiation (III) on disinfection of rice by gamma-ray irradiation. *Korean J. Food Sci. Technol.* 2(1): 113-120.

Kon, E., Ahmed, O. H., Saamin, S., and Majid, N. M. 2007. Gamma radiosensitivity study on long bean *(Vigna sesquipedalis)*. *Am. J. Appl. Sci.* 4(12): 1090-1093.

Koona, P. and Koona, O. E. S. 2006. Protectant effect of *Erigeron floribundus* (Asteraceae) against damage to stored legume seeds by infesting bruchids in the Western highlands of Cameroon. *Res. J. Agric. Biol. Sci.* 2: 303-306.

Kovacs, E. and Keresztes, A. 2002. Effect of gamma and UV-B/C radiation on plant cells. *Micron.* 33(2): 199-210.

Kovacs, E. and Kiss, I. 1985. Disinfestation of wheat germ, wheat, and dried mushrooms by irradiation. In: J.H. Moy (eds), *Radiation Disinfestation of Food and Agricultural Product.* University of Hawaii Press, America, pp 189-198。

Krishnaswamy, V. and Seshu, D. V. 1989. Seed germination rate and associated characters in rice. *Crops Sci.* 29: 904-908.

Kumar, A. and Mishra, M. N. 2004. Effect of Gamma Rays EMS and NMU on Germination, Seedling Vigour, Pollen Viability and Plant survival in M1 and M2 Generation of Okra (*Abelmoschus esculentus* L. Moench). *Adv. Plant Sci.* 17: 295-298.

Kumar, A. and Mishra, M. N. 2006. Mutation frequency and chlorophyll mutations by gamma-irradiation and ethyl methane sulphonate treatment in okra (*Abelmoschus esculentus* L. Moench). *Int. J. Pl. Sci.* 1(1): 101-103.

Kumar, K. R., Reddy, C. N., Lakshmi, K. V., Rameash, K., Keshavulu, K., and Rajeswari, B. 2017. Management of cigarette beetle (*Lasioderma serricorne* Fabricius) in turmeric (*Curcuma longa* Linnaeus) by using of gamma radiation. *J. Entomol. Zool. Stud.* 5(3): 1723-1727.

Kumar, K. V. S., Savitri, H., and Reddy, M. B. 2004. Effects of fungicides on storability of rice hybrids and their parental lines. In: *27th ISTA Congress Seed Symposium,* Budapest, Hungary, pp.83.

Kumar, S., Sharma, A. K., Rawat, S. S., Jain, D. K., and Ghosh, S. 2013. Use of pesticides in agriculture and livestock animals and its impact on environment of India. *Asian J. Environ. Sci.* 8(1): 51-57.

Kurobane, I., Yamaguchi, H., Sander, C., and Nilan, R. 1979. The effects of gamma irradiation on the production and secretion of enzymes and enzymatic activities in barley. *Environ. Exp. Bot.* 19: 75-84

Kuzin, A. M., Vagabova, M. E., and Primak-Mirolyubov, V. N. 1975. Molecular mechanisms of ionizing radiation stimulating effect on seeds. Activation of RNA synthesis. *Radiobiologiya*. 15(5): 747-750.

Kuzin, A. M., Vagabova, M. E., and Revin, A. F. 1976. Molecular mechanisms of stimulating action of an ionizing radiation on seeds. *Radiobiologiya*. 16(2): 259-261.

Lal, R. R. and Gujar, T. 2007. *Post-Harvest management of Pulses*. Indian Institute of Pulses Research, Kanpur, 208: 024.

Li, R., He, J., Xie, H., Wang, W., Bose, S.K., Sun, Y., Hu, J., and Yin, H. 2019. Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *Int. J. Biol. Macromolecules*. 126: 91-100.

Lian-Ju, M., Yue-Ying, L., Lan-Lan, W., Xue-Mei, L., Liu, T. and Bu, N., 2014. Germination and physiological response of wheat (Triticum aestivum) to pre-soaking with oligochitosan. *Int. J. Agric. Biol.* 16(4).

Linko, P. and Milner, M. 1960. Treatment of wheat with ionizing radiations. V. Effect of gamma radiation on some enzyme systems. *Cereal Chem. 37: 223-227.*

Loaharanu, P. 1994. Food irradiation in developing countries: a practical alternative. *IAEA Bull.* 36: 30–35.

Lu, J., Zhang, C., Hou, G., Zhang, J., Wan, C., Shen, G., Zhang, J., Zhou, H., Zhu, Y., and Hou, T. 2002. The biological effects of chitosan on rice growth. *Acta Agric.* 18(4): 31-34.

Maguire, J. D. 1962. Speed f germination aid in selection and evaluation for seedling emergence and vigour. *Crop Sci.* 2: 176-177.

Maina, Y. T., Dauda, Z., and Degri, M. M. 2011. Effects of different levels of infestation and storage durations on the development of *Callosobruchus maculatus* (Fab.) in stored cowpea *Vigna unguiculata* (L.) Walpers. *Prod. Agric. Technol.* 7(1): 49 - 54.

Maity, J. P., Mishra, D., Chakraborty, A., Saha, A., Santra, S. C., and Chanda, S. 2005. Modulation of some quantitative and qualitative characteristics in rice (*Oryza sativa* L.) and mung (*Phaseolus mungo* L.) by ionizing radiation. *Radiat. Phys. Chem.* 74(5): 391-394.

Majeed, A. and Muhammad, Z. 2010. Gamma irradiation effects on some growth parameters of *Lepidium sativum* L. *World J. Fungal and Plant Biol.* 1(1): 8-11.

Maneepun, S. 2003. Traditional processing and utilization of legumes. *Processing and utilization of legumes*, pp.9-14.

Manjunatha, G., Roopa, K. S., Prashanth, G. N., and Shekar Shetty, H. 2008. Chitosan enhances disease resistance in pearl millet against downy mildew caused by *Sclerospora graminicola* and defence-related enzyme activation. *Pest Manag. Sci.: formerly Pesticide Sci.* 64(12): 1250-1257.

Marcu, D., Cristea, V., and Daraban, L. 2013. Dose-dependent effects of gamma radiation on lettuce (*Lactuca sativa var. capitata*) seedlings. *Int. J. Radiat. Biol.* 89(3): 219-223

Meena, R. S., Tonapi, V. A., Bhat, B. and Rani, K.J. 2016. Effect of Gamma irradiation on seed quality, insect infestation and fungal infection in Sorghum (*Sorghum bicolor* L. Moench). *J. of Progressive Agric.* 7(2): 111-117.

Minisi, F. A., El-mahrouk, M. E., Rida, M. E. F., and Nasr, M. N. 2013. Effects of gamma radiation on germination, growth characteristics and morphological variations of *Moluccella laevis* L. *American-Eurasian J. Agric. Environ. Sci.* 13: 696–704.

Mokobia, C. E. and Anomohanran, O. 2005. The effect of gamma irradiation on the germination and growth of certain Nigerian agricultural crops. *J. Radiological Prot.* 25(2): 181.

Molin, R. A. 2001. *Food Irradiation: Principles and Applications*. John Wiley and Sons, New York, 469 p.

Mudibu, J., Nkongolo, K. K. C., Mehes-Smith, M., and Kalonji-Mbuyi, A. 2011. Genetic analysis of a soybean genetic pool using ISSR marker: effect of gamma radiation on genetic variability. *Int. J. Plant Breed. Genet.* 5(3): 235-245.

Nargis, S. 1995. Influence of pelleting, magnetic treatment and radiation on performance of differentiality aged seeds in tomato (*Lycopersicon esculentum*) cv PKM-1.Department of Seed Science and Technology, TNAU, 100-110pp.

No, H. K., Lee, K. S., Kim, I. D., Park, M. J., Kim, S. D., and Meyers, S. P. 2003. Chitosan treatment affects yield, ascorbic acid content, and hardness of soybean sprouts. *J. Food Sci.* 68(2): 680-685.

Ofuya, T. I. and Bamigbola, K. A. 1991. Damage potentials, growth and development of seed beetle, *Callosobruchus maculatus* (Fabricius)(Coleoptera: Bruchidae) on some tropical legumes. *Trop. Agric. (Trinidad)*, 68: 33-36.

Park, J. S., Lee, J. Y., Jeong, S. Y., Ahn, S. J., and Kim, I. 2015. Effects of gamma radiation on different developmental stages of the oriental tobacco budworm, *Helicoverpa assulta* (Lepidoptera: N octuidae). *Entomol. Res.* 45(2): 110-115.

Pichyangkuraa, R. and Chadchawanb, S. 2015. Biostimulant activity of chitosan in horticulture. *Sci. Hortic.* 196: 49-65

Pranesh, P., Doddagoudar, S. R., Vasudevan, S. N., Shakunthala, N. M., and Aswathanarayana, D. S. 2019. Influence of Gamma Irradiation and Seed Treatment Chemicals on Seed Longevity of Bengal Gram (*Cicer arietinum* L.) and Black Gram (*Vigna mungo* L.). *Int. J. Curr. Microbiol. Appl. Sci.* 8(12): 2866–2881.

Pyror, W. A. 1986. Oxy-radical and related species, their formation, life and reactions. *Ann Rev Physiol.* 48: 657-661.

Qing-Zhong, R. S. L. X. 2002. Effects of Chitosan Coating on Seed Germination and Salt-tolerance of Seedlings in Hybrid Rice (*Oryza sativa* L.). *Acta Agron. Sinica.* 6: 14.

Rabea, E. I., Badawy, M. E. T., Stevens, C. V., Smagghe, G., and Steurbaut, W. 2003. Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules*. 4(6): 1457-1465.

Rabea, E. I., Badawy, M. E., Steurbaut, W., and Stevens, C. V. 2009. In vitro assessment of N-(benzyl) chitosan derivatives against some plant pathogenic bacteria and fungi. *Eur. Polym. J.* 45(1): 237-245.

Raghavendra, D. and Loganathan, M. 2017. Effect of pulse beetle (*Callosobruchus maculatus* L.) Infestation on quality of pigeon pea seed. *Int. J. Pure Appl. Biosci.* 5(6): 976-980.

Rahimi, M.M. and Bahrani, A. 2011. Effect of gamma irradiation on qualitative and quantitative characteristics of canola *(Brassica napus* L.*). Middle-East J. Sci. Res.* 8(2): 519-525.

Rahman S. 1971. Study on the morphology and biology of *Callosobruchus chinensis* Linn. *C. analis* Fab. and extent of damage to pulses. M. Sc thesis. Dhaka University. 199p

Rajarajeshwari. 2011. Studies on the effect of seed UV irradiation on growth, seed yield, quality and storability of chickpea genotypes. M.Sc. (Ag) thesis, University of Agricultural Sciences, Raichur, 65p.

Rajendra, A. A., Magan, P. D., and Vishnu, S. S. 2017. Effect of gamma radiation on germination and seedling parameter of finger millet (*Eleusine coracana* L. Gaertn.). *IJCS*. 5(4): 1978-1982.

Rajendran, S. 2003. Environment and health aspects of pesticides use in Indian agriculture. *Proceeding of the Third International Conference on Environment and Health,* India, 15-17: 353-373 December 2003.

Rajkumar, V., Gunasekaran, C., Dharmaraj, J., Chinnaraj, P., Paul, C. A., and Kanithachristy, I. 2020. Structural characterization of chitosan nanoparticle loaded with *Piper nigrum* essential oil for biological efficacy against the stored grain pest control. *Pesticide Biochem. Physiol.* 166: 104566.

Ramya, B., Nallathambi, G., and Ram, S. G. 2014. The effect of mutagens on M1 population of black gram (*Vigno mungo* L. Hepper). *Afr. J. Biotechnol.* 13(8): 951-956.

Rao, P. K. and Suvartha, C. 2006. Effect of Gamma Rays on In vivo and In vitro Seed Germination, Seedling Height and Survival Percentage of *Lycopersicon esculentum* cv. Pusa. *Adv. Plant Sci.* 19(2): 335.

Roy, B., Amin, M. R., Jalal, S., Kwon, Y. J., and Suh, S. J. 2014. Evaluation of common cocklebur *Xanthium strumarium* leaf extract as post-harvest grain protectant of black gram against pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae) and isolation of crude compound. *Entomol. Res.* 44(6): 254-261.

Rustammni, M. A., Naqvi, S. M. S. H., Munchi, G. H., Abrol, G. H., 1985. Relative resistance/susceptibility of different pulses against pulse beetle *Callosobruchus chinensis* L. *Bangladesh J. Entomol.* 6: 13-21.

Sadozai, A., Naeem, M., Shah, M., and Ali, A. 2003. Host preference of pulse beetle *Callosobruchus maculatus* in different legumes. *Sarhad J. Agric.* 19(4): 557-561.

Sahab, A. F., Waly, A. I., Sabbour, M. M., and Nawar, L. S. 2015. Synthesis, antifungal and insecticidal potential of Chitosan (CS)-g-poly (acrylic acid)(PAA) nanoparticles against some seed borne fungi and insects of soybean. *Int. J. Chem. Tech Res.* 8(2): 589-598.

Saharan, V., Kumaraswamy, R. V., Choudhary, R. C., Kumari, S., Pal, A., Raliya, R., and Biswas, P. 2016. Cu-chitosan nanoparticle mediated sustainable approach to enhance seedling growth in maize by mobilizing reserved food. *J. Agric. Food Chem.* 64(31): .6148-6155.

Said, S. M., El-Sayed, S. M., Farid, H. E .A., and Abozid, M. M. 2011. Insecticidal effect of chitosan prepared by different chemical processing sequences against *Galleria melleonella*. *J. Pharma. Sci.* 43: 123-32.

Sarwar, M. 2012.Assessment of resistance to the attack of bean beetle Callosobruchus maculatus (Fabricius) in chickpea genotypes on the basis of various parameters during storage. *Songklanakarin J. Sci. Technol.* 3 (3): 287-291.

Sathiyabama, M. and Parthasarathy, R. 2016. Biological preparation of chitosan nanoparticles and its in vitro antifungal efficacy against some phytopathogenic fungi. *Carbohyd. Polym.* 151: 321-325.

Saxena, R. K., Prathima, C., Saxena, K. B., Hoisington, D. A., Singh, N. K., and Varshney, R. K. 2010. Novel SSR markers for polymorphism detection in pigeonpea (*Cajanus spp.*). *Plant breed.* 129(2): 142-148.

Selim, A. H. and El-Banna, E. N. 2001. Ionizing irradiation effects on germination, growth, some physiological and biochemical aspects and yield of pea, Pisum sativum L. plants. *Mansoura Univ. J. Agric. Sci. Egypt.* 26(3): 1697-1719

Selvaraju, P. and Krishnaswamy, V. 2005. Improved storage by halogen mixture and polylined gunny bag in rice. *Seed Res.* 33(2): 229-231.

Sen, S. K. and Mandal, P. 2016. Solid matrix priming with chitosan enhances seed germination and seedling invigoration in mung bean under salinity stress. *J. Cent. Eur. Agric.* 17(3): 749-762.

Shao, C. X., Hu, J., Song, W. J., and Hu, W. M. 2005. Effects of seed priming with chitosan solutions of different acidity on seed germination and physiological characteristics of maize seedling. *J. Zhejiang Univ. (Agric. Life Sci.)*. 31(6): 705-708.

Sharma, R., Devi, R., Kumar Sharma, R., and Mehia, J. C. 2013. Efficacy of some botanicals against pulse beetle, *Callosobruchus chinensis* (L.) in chickpea. *Legume Res: An Int. J.* 36(2).

Sharma, S. S. 1984. Review of Literature on the loss caused by *Callosobruchus spp.* (Coleoptera: Bruchidae) during storage of pulses. Bull. Grain Technol. 22: 62-71.

Sheikha, S. A. and Al-Malki, F. M. 2011. Growth and chlorophyll responses of bean plants to the chitosan applications. *Eur. J. Sci. Res.* 50(1): 124-134.

Singh, B. and Datta, P. S. 2010. Gamma irradiation to improve plant vigour, grain development, and yield attributes of wheat. *Radiat. Phys. Chem.* 79(2): 139-143.

Singla, A. K. and Chawla, M. 2001. Chitosan: Some pharmaceutical and biological aspects-an update. *J. Pharmacy Pharmacol.* 53(8): 1047-1067.

Soni, K. A., Sreedhar, M., Vanisree, S. and Keshavulu, K., 2014. Influence of gamma radiation and packaging materials on germination parameters in aromatic rice variety Sugandha Samba during storage. *The J. Res. PJTSAU.* 42(3).

Soundararajan, R. P., Chitra, N., Geetha, S., and Poorani, J. 2012. Biological control of bruchid *Callosobruchus maculatus* (F.) in blackgram. *J. Biopesticides.* 5: 192.

Srinivasan, T., Duraimurugan, P., Singh, S.K., and Chattopadhyay, C. 2010. Bruchids infestation in pulses and its management. *Indian Farming.* 60: 13-16.

Stoeva, N., Zlatev. Z., and Bineva, Z. 2001. Physiological response of beans (*Phaseolus vulgaris* L.) to gamma-radiation contamination, II. Water-exchange, respiration and peroxidase activity. *J. Environ. Prot. Eco.* 2: 304-308.

Sui, X. Y., Zhou, Z. L., and Zhang, W. Q. 2002. Effect of Chitosan as Seed Coatings on Seed Germination and Seedling Growth and Several Physiological and Biochemical Indexes in Rapeseeds. *Plant Physiol. Commun.* 38(3): 225-227.

Sujatha, K., Ramamoorthy, K., and Sivasubramaniam, K. 2015. Efficacy of some indigenous plant products against pulse beetle *Callosobruchus spp* on cowpea (*Vigna unguiculata*. L. Hepper.) seeds. *Agric. Sci. Digest-A Res. J.* 35(1): 74-77.

Sujeetha, A. R., Girish, A. G., Sankarganesh, E., Sridhar, M., Ghosh, S. K., Hadapad, A. P., Hire, R. S., and Venugopalan, V. P. 2020. Effect of gamma radiation on pulse beetle, *Callosobruchus chinensis* (l.)(coleoptera: bruchidae) with different storage conditions. *J. Exp. Zool. India*. 23(1): 569-574.

Supawan, J., Hormchan, P., Sutantawong, M., and Wongpiyasatid, A. 2005. Effects of Gamma Radiation on Azuki Bean Weevil, *Callosobruchus chinensis* (L.). *Agric. Nat. Resour*. 39(2): 206-215.

Susmitha, P. and Rai, P. K. 2017. Studies on effects of seed treatments, polymer coating and packaging materials on seed quality parameters of stored cowpea (*Vigna unguiculata*) seeds. *J. Pharmacognosy Phytochem*. 6(3): 677-681.

Swapan, T. K. 2016. Bruchid resistance in food legumes-an overview. *Res. J. Biotechnol.* 11: 7

Tandon, S., Singh, A., and Kanaujia, S. 2009. Effect of gamma radiation on growth and development of rust red flour beetle *Tribolium castaneum* (Herbst.). *J. Plant Prot. Res.* 49(3).

Timbadiya, B., Sisodiya, D., and Sharma, A. 2018. Gamma radiation: an important tool for pest management in agriculture. *Trends Biosci*. 47(11): 4347-4349.

Toker, C., Uzcn, B., Canci, H., and Ceylan, F. O. 2005. Effects of gamma irradiation on the shoot length of Cicer seeds. *Radiat Phys Chem*. 73: 365-7.

Tresina, P. S. and Mohan, V. R. 2011. Effect of gamma irradiation on physicochemical properties, proximate composition, vitamins and antinutritional factors of the tribal pulse *Vigna unguiculata subsp. unguiculata*. *Int. J. Food Sci. Technol.* 46(8): 1739-1746.

Tripathi, K., Chauhan, S. K., Gore, P. G., Prasad, T. V., Srinivasan, K., and Bhalla, S. 2015. Screening of cowpea [*Vigna unguiculata* (L.) Walp.] accessions against pulse-beetle, *Callosobruchus chinensis* (L.). *Legume Res. Int. J.* 38(5): 675-680.

Tshilenge-Lukanda, L., Kalonji-Mbuyi, A., Nkongolo, K. K. C., and Kizungu, R. V. 2013. Effect of gamma irradiation on morpho-agronomic characteristics of groundnut (*Arachis hypogaea* L.). *Am. J. Plant Sci.* 4(11): 2186-2192.

Uma, M. S. and Salimath, P. M. 2001. Effect of ionizing radiations on germination and emergence of cowpea seeds. *Karnataka J. Agric. Sci* 14(4): 1063-1064.

Upadhyay, R. K. and Ahmad, S. 2011. Management strategies for control of stored grain insect pests in farmer stores and public ware houses. *World J. Agric. Sci.* 7(5): 527-549.

Varma, S. and Anandhi, P. 2010. Biology of pulse beetle (*Callosobruchus chinensis* Linn., Coleoptera: Bruchidae) and their management through botanicals on stored mung grains in Allahabad region. *Legume Res.* 33: 38-41.

Vasudevan, P., Reddy, M. S., Kavitha, S., Velusamy, P., Paulraj, R. S., Purushothaman, S. M., Brindha Priyadarisini, V., Bharathkumar, S., Kloepper, J. W., and Gnanamanickam, S. S. 2002. Role of biological preparations in enhancement of rice seedling growth and grain yield. *Curr. Sci.* 83(9): 1140-1143.

Venkatesham, V., Meena, R. S., and Laichattiwar, M. A. 2015. Assessment of losses due to pulse beetle *Callosobruchus chinensis* l. in chickpea. *Ecol. Environ. Conserv.* 21(2): 759-761.

Woodstock, L. W. and Justice, O. L. 1967. Radiation-induced changes in respiration of corn, wheat, sorghum and radish seeds during initial stages of germination in relation to subsequent seedling growth. *Radiat. Bot.* 7(2): 129-136.

Xiuzher, L. 1994. Effect of irradiation on protein content of wheat crop. *J. Nucl. Agric. Sci. China.* 15: 53–55

Yun, J., Li, X., Fan, X., Li, W., and Jiang, Y. 2013. Growth and quality of soybean sprouts (*Glycine max* L. Merrill) as affected by gamma irradiation. *Radiat. Phys. Chem.* 82: 106-111.

Zeng, D. and Luo, X. 2012. Physiological effects of chitosan coating on wheat growth and activities of protective enzyme with drought tolerance. *Open J. Soil Sci.* 2(3): 282.

Zeng, D., Luo, X., and Tu, R. 2012. Application of bioactive coatings based on chitosan for soybean seed protection. *Int. J. Carbohyd. Chem.* 5p.

Zhang, M. I., Tan, T., Yuan, H., and Rui, C. 2003. Insecticidal and fungicidal activities of chitosan and oligo-chitosan. *J. Bioactive Compatible Polym.* 18(5): 391-400.

Zhou, Y. G., Yang, Y.D., Qi, Y. G., Zhang, Z. M., Wang, X. J., and Hu, X. J. 2002. Effects of chitosan on some physiological activity in germinating seed of peanut. *J. Peanut Sci.* 31 (1): 22-25.

IRRADIATION AND SEED COATING FOR ENHANCING STORAGE LIFE OF GRAIN COWPEA (*Vigna unguiculata* (L.) Walp.)

by

JAYASHRI S

(2019-11-037)

ABSTRACT

of the thesis submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2021

ABSTRACT

The present study entitled "Irradiation and seed coating for enhancing storage life of grain cowpea (*Vigna unguiculata* (L.) Walp.)" was carried out in the Department of Seed Science and Technology, College of Agriculture, Vellayani during 2020-2021, with an objective to standardize the dose of gamma rays for irradiation and concentration of chitosan for seed coating for enhancing the storage life of grain cowpea. The study was divided into two experiments which were conducted in Completely Randomized Block Design (CRD) with three replications. In the first experiment, the seeds were irradiated with five different doses (100 Gy to 500 Gy) at Indian Institute of Horticultural Research (IIHR), Bangalore. Another set of seeds were coated with chitosan at five different concentrations (1 % to 5 %) at two different quantities for each concentration as 1mL $50g^{-1}$ of seeds and 5mL $50g^{-1}$ of seeds. Coated seeds were then shade dried and packed in polythene bags and stored for six months along with control.

In the first experiment, the cowpea seeds irradiated with 300 Gy, 400 Gy and 500 Gy gamma rays were not affected by pulse beetle infestation till the end of six months of storage. However, in control, the seed damage was observed which varied from 0.333% in first month to 56.333% in sixth month of storage with a seed weight loss of 28.182 per cent. The damage percentage recorded was 2.667 percent and 0.667 per cent in treatment T_1 (100 Gy) and T_2 (200 Gy) respectively in the sixth month of storage. Thus the gamma ray irradiation in all doses proved to be effective in controlling pulse beetle infestation. Germination parameters were studied in the undamaged seeds to study the effect of irradiation in seed aging. Among the different doses of gamma irradiation, T_2 (200 Gy) recorded the highest seed germination percentage (84.33%), speed of germination (32.13 days), seedling shoot length (11.83 cm), seedling dry weight (0.703 g) and seedling vigour index I (2130.49) and II (59.29). All the germination parameters showed increased value at lower doses of gamma rays (100 Gy and 200 Gy) and declined at higher doses (300 Gy, 400 Gy, and 500 Gy) compared to control. Morphological evaluation of gamma irradiated seeds grown in field showed that the morphological parameters did not vary significantly from the control in treatments with gamma doses 100 Gy and 200 Gy. Gamma

irradiation at 300 Gy also did not show variation in morphological parameters compared to control except for field germination percentage. But progressive decrease in all morphological parameters was observed for the treatments with gamma doses 400 Gy and 500 Gy. Reduction in germination percentage, plant height, number of pods $plant^{-1}$, number of seeds pod^{-1} and 100 seed test weight was observed when compared to control.

In the second experiment, among the different chitosan treatments, no seeds were observed with insects upto four months of storage. Although insect eggs and infestation were noticed in treatments such as T_1 (1% 1 ml $50g^{-1}$), T_2 (1% @ 5 ml $50g^{-1}$), T_3 (2% @ 1 ml $50g^{-1}$) and T_5 (3% @ 1 ml $50g^{-1}$) at the end of storage period, the percentage of infestation decreased with an increase in concentration and quantity of chitosan used. The grain cowpea seeds coated with different concentrations of chitosan from lower to higher (1% to 5%) have different degrees of improvement in germination parameters compared to control. Among the different treatments of chitosan, T_{10} (5 % @ 5 ml $50g^{-1}$) recorded the highest seed germination percentage (89.37 %), speed of germination (36.83), seedling shoot length (14.90 cm), seedling root length (17.53 cm), seedling dry weight (0.747 g), seedling vigour index I (2898.28) and II (66.79).

In this study Gamma irradiation proved to be an effective method for controlling pulse beetle infestation during storage in grain cowpea. However the treatment with higher doses 400 Gy and 500 Gy affected the germination parameters negatively and produced some abnormalities in the progeny. Thus, the gamma irradiation at 200Gy and 300 Gy can be recommended for safe storage of grain cowpea seeds. Chitosan at 5% @ 5 ml $50g^{-1}$ exhibited higher values for seed germination parameters and showed no pulse beetle infestation till the end of the storage period of six months. Chitosan treatment at 5% @ 5 ml $50g^{-1}$ can be recommended for safe storage of grain cowpea seeds. Gamma irradiation and chitosan seed coating are eco-friendly methods in enhancing the storage life of grain cowpea and were effective in controlling the storage pests. Since gamma irradiation requires special facilities for seed treatment, chitosan seed coating will be a better technology for small scale farmers.

സംഗ്രഹം

"ധാന്യ പയറിന്റെ (വിഗ്ന അങ്കികുലാറ്റ (എൽ.) വാൾപ്പ്.) സംഭരണ ആയുസ്സ് വർധിപ്പിക്കുന്നതിനുള്ള വികിരണവും വിത്ത് പൂശും" എന്ന വിഷയത്തിൽ ഒരു പഠനം 2020-2021 കാലയളവിൽ തിരുവനന്തപുരത്തെ വെള്ളായണി കാർഷിക കോളേജിലെ സീഡ് സയൻസ് ആൻഡ് ടെക്നോളജി വിഭാഗത്തിൽ വെച്ച് നടത്തുകയുണ്ടായി. പല അളവിൽ വിത്ത് വികിരണം ചെയ്യുകയും കൈറ്റോസിൻ ഉപയോഗിച് വിത്ത് പൂശുകയും ചെയ്യുക വഴി ധാന്യ പയറുകളുടെ സംഭരണ ആയുസ്സ് വർദ്ധിപ്പിക്കുക എന്നതാണ് പഠന ലക്ഷ്യം. രണ്ട് പരീക്ഷണങ്ങളായി വിഭജിച്ച ഈ പഠനം, മൂന്ന് പകർപ്പുകളോടെ കംപ്ലീറ്റ്ലി റാൻഡമൈസ്ഡ് ബ്ലോക്ക് ഡിസൈനിൽ (സിആർഡി) ആണ് നടത്തിയത് .ആദ്യ പരീക്ഷണത്തിൽ, ബാംഗ്ലൂരിലെ ഇന്ത്യൻ ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഹോർട്ടികൾച്ചറൽ റിസർച്ചിൽ (IIHR) ഗാമ രശ്മികൾ അഞ്ച് വ്യത്യസ്ത ഡോസുകൾ (100 Gy മുതൽ 500 Gy വരെ) ഉപയോഗിച്ച് വിത്ത് വികിരണം ചെയ്തു. 1 മില്ലിലിറ്റർ 50 ഗ്രാം വിത്തുകൾ, 5 മില്ലിലിറ്റർ 50 ഗ്രാം വിത്തുകൾ എന്നിങ്ങനെ രണ്ട് വ്യത്യസ്ത സാന്ദ്രതകളിൽ (1% മുതൽ 5% വരെ) മറ്റൊരു കൂട്ടം വിത്തുകൾ കൈറ്റോസിൻ ഉപയോഗിച് പൂശി. പൂശിയ വിത്തുകൾ തണലിൽ ഉണക്കി പോളിത്തീൻ ബാഗുകളിൽ പാക്ക് ചെയ്തു 6 മാസം സംഭരിച്ചു.

ആദ്യ പരീക്ഷണത്തിൽ, 300 Gy, 400 Gy, 500 Gy ഗാമാ രശ്മികൾ ഉപയോഗിച്ച് വികിരണം ചെയ്ത പയർ വിത്തുകൾ ആറ് മാസത്തെ സംഭരണം കഴിയുന്നതു വരെ പൾസ് വണ്ടുകളുടെ ആക്രമണം ബാധിച്ചില്ല. എന്നാൽ വികിരണം ചെയ്യാത്ത വിത്തുകളിൽ ആദ്യ മാസത്തിൽ 0.333% മുതൽ ആറാം മാസത്തിൽ 56.333% വരെ നാശവും 28.182% ഭാരവും കുറഞ്ഞതായി നിരീക്ഷിക്കപെട്ടു. അങ്ങനെ ഗാമ വികിരണം ചെയുന്നത് വിത്തുകളിൽ വണ്ടുകളുടെ ആക്രമണം നിയന്ത്രിക്കുന്നതിൽ ഫലപ്രദം ആണെന്ന് തെളിഞ്ഞു. വികിരണത്തിന്റെ ഫലത്തെ കുറിച് പഠിക്കുവാൻ കേടുപാടുകൾ സംഭവിക്കാത്ത വിത്തുകളിൽ മുളയ്ക്കുന്നതിനുള്ള പരാമീറ്ററുകൾ പഠിച്ചു. ഗാമാ വികിരണത്തിന്റെ വിവിധ ഡോസുകളിൽ, T_2 (200 Gy) ഏറ്റവും ഉയർന്ന

വിത്ത് മുളയ്ക്കൽ ശതമാനം (84.33%), മുളയ്ക്കുന്നതിന്റെ വേഗത (32.13 ദിവസം), തൈകളുടെ ഷൂട്ട് നീളം (11.83 സെന്റീമീറ്റർ), തൈകളുടെ ഉണങ്ങിയ ഭാരം (0.703 ഗ്രാം), തൈകളുടെ വീര്യ സൂചിക I (2130.49), II (59.29) എന്നിവ രേഖപ്പെടുത്തി. എല്ലാ മുളപ്പിക്കൽ പാരാമീറ്ററുകളും ഗാമാ കിരണങ്ങളുടെ കുറഞ്ഞ ഡോസുകളിൽ (100 Gy, 200 Gy) വർദ്ധിച്ച മൂല്യം കാണിക്കുകയും നിയന്ത്രണവുമായി താരതമ്യപ്പെടുത്തുമ്പോൾ ഉയർന്ന ഡോസുകളിൽ (300 Gy, 400 Gy, 500 Gy) കുറയുകയും ചെയ്തു. 100 Gy, 200 Gy ഗാമാ ഡോസുകൾ ഉപയോഗിച്ചുള്ള ട്രീറ്റ്മെന്റുകളിലെ നിയന്ത്രണത്തിൽ രൂപാന്തര പരാമീറ്ററുകളിൽ കാര്യമായി വ്യത്യാസപ്പെട്ടിട്ടില്ലെന്ന് വയലിൽ വളർത്തിയ ഗാമാ വികിര ണം ചെയ്ത വിത്തുകളുടെ വിലയിരുത്തൽ കാണിച്ചു. 300 Gy-ലെ ഗാമാ വികിരണം, ഫീൽഡ് മുളയ്ക്കൽ ശതമാനം ഒഴികെ നിയന്ത്രണവുമായി താരതമ്യപ്പെടുത്തുമ്പോൾ രൂപാന്തര പരാമീറ്ററുകളിൽ വ്യത്യാസം കാണിച്ചിട്ടില്ല. എന്നാൽ 400 Gy, 500 Gy എന്നീ ഗാമാ ഡോസുകൾ ഉപയോഗിച്ചുള്ള ട്രീറ്റ്മെന്റിൽ എല്ലാ രൂപാന്തര പാരാമീറ്ററുകളിലും കുറവ് രേഖപ്പെടുത്തി. നിയന്ത്രണവുമായി താരതമ്യപ്പെടുത്തുമ്പോൾ മുളയ്ക്കുന്ന ശതമാനം, ചെടിയുടെ ഉയരം, കായ്കളുടെ എണ്ണം, ഒരു പോഡിലെ വിത്തുകൾ വിത്തുകളുടെ ടെസ്റ്റ് വെയ്ഗ്റ് എന്നിവയിൽ കുറവുണ്ടായി.

രണ്ടാമത്തെ പരീക്ഷണത്തിൽ, വ്യത്യസ്ത കൈറ്റോസിൻ ട്രീട്മെന്റുകളിൽ നാല് മാസത്തെ സംഭരണം വരെ വിത്തുകളോടൊപ്പം പ്രാണികളൊന്നും കണ്ടില്ല. T_1 (1% 1 മില്ലിലിറ്റർ 50 ഗ്രാം), T_2 (1% 5 മില്ലിലിറ്റർ 50 ഗ്രാം), T_3 (2% 1 മില്ലിലിറ്റർ 50 ഗ്രാം), T_5 (3% 1 മില്ലിലിറ്റർ 50 ഗ്രാം) തുടങ്ങിയ ട്രീട്മെന്റുകളിൽ പ്രാണികളുടെ മുട്ടയും ആക്രമണവും ശ്രദ്ധയിൽപ്പെട്ടെങ്കിലും സംഭരണ കാലയളവിന്റെ അവസാനത്തിൽ, ഉപയോഗിച്ച ചിറ്റോസന്റെ സാന്ദ്രതയിലും അളവിലും വർദ്ധനവുണ്ടായതോടെ കീടബാധയുടെ ശതമാനവും കുറഞ്ഞതായി നിരീക്ഷിക്കപെട്ടു. വ്യത്യസ്ത സാന്ദ്രതയിലുള്ള കൈറ്റോസിൻ പൊതിഞ്ഞ ധാന്യ പയർ വിത്തുകൾക്ക് നിയന്ത്രണവുമായി താരതമ്യം ചെയ്യുമ്പോൾ മുളയ്ക്കൽ പരാമീറ്ററുകളിൽ വ്യത്യാസം കണ്ടെത്തി.

കൈറ്റോസിന്റെ വിവിധ ട്രീട്മെന്റുകളിൽ, T_{10} (5 % 5 മില്ലിലിറ്റർ 50 ഗ്രാം) ഏറ്റവും ഉയർന്ന വിത്ത് മുളയ്ക്കൽ ശതമാനം (89.37 %), മുളയ്ക്കുന്നതിന്റെ വേഗത (36.83), തൈകളുടെ ചിനപ്പുപൊട്ടലിന്റെ നീളം (14.90 സെന്റിമീറ്റർ), തൈകളുടെ വേരിന്റെ നീളം (17.53 സെന്റിമീറ്റർ), തൈകൾ ഉണങ്ങിയ ഭാരം (0.747 ഗ്രാം), തൈകളുടെ വീര്യ സൂചിക I (2898.28), II (66.79) എന്നിവ രേഖപ്പെടുത്തി.

ഈ പഠനത്തിൽ, ഗാമാ വികിരണം ചെയ്ത ധാന്യം പയർ സംഭരിക്കുമ്പോൾ പൾസ് വണ്ടുകളുടെ ആക്രമണം നിയന്ത്രിക്കുന്നതിനുള്ള ഫലപ്രദമായ മാർഗ്ഗമാണെന്ന് തെളിയിക്കപ്പെട്ടു. എന്നിരുന്നാലും, 400 Gy, 500 Gy എന്നി ഉയർന്ന ഡോസുകൾ ഉപയോഗിച്ചുള്ള ട്രീറ്റ്മെന്റ് മുളയ്ക്കുന്ന പാരാമീറ്ററുകളെ പ്രതികൂലമായി ബാധിക്കുകയും മുളപ്പിച്ച തൈകളിൽ ചില അസാധാരണതങ്ങൾ ഉണ്ടാക്കുകയും ചെയ്തു. അതിനാൽ, ധാന്യ പയർ വിത്തുകൾ സുരക്ഷിതമായി സൂക്ഷിക്കുന്നതിന് 200Gy, 300 Gy ഗാമാ വികിരണം ശുപാർശ ചെയ്യാവുന്നതാണ്. കൈറ്റോസിൻ 5% @ 5 മില്ലിലിറ്റർ 50 ഗ്രാം വിത്ത് മുളയ്ക്കുന്നതിനുള്ള പാരാമീറ്ററുകൾക്ക് ഉയർന്ന മൂല്യങ്ങൾ കാണിക്കുകയും ആറ് മാസത്തെ സംഭരണ കാലയളവ് അവസാനിക്കുന്നതുവരെ പൾസ് വണ്ടുകളുടെ ആക്രമണം കാണിക്കുകയും ചെയ്തില്ല. ധാന്യ പയർ വിത്തുകൾ സുരക്ഷിതമായി സൂക്ഷിക്കാൻ 5% @ 5 മില്ലിലിറ്റർ 50 ഗ്രാം എന്ന തോതിൽ കൈറ്റോസിൻ ട്രീറ്റ്മെന്റ് ശുപാർശ ചെയ്യാം. ഗാമാ വികിരണവും കൈറ്റോസിൻ വിത്ത് പൂശലും ധാന്യ പയറിന്റെ സംഭരണ ആയുസ്സ് വർദ്ധിപ്പിക്കുന്നതിനുള്ള പരിസ്ഥിതി സൗഹൃദ രീതികളാണ്, സംഭരണ കീടങ്ങളെ നിയന്ത്രിക്കുന്നതിൽ ഇത് ഫലപ്രദമാണ്. ഗാമാ വികിരണം ഉപയോഗിച്ചുള്ള വിത്ത് സംസ്കരണത്തിന് പ്രത്യേക സൗകര്യങ്ങൾ ആവശ്യമായതിനാൽ, ചെറുകിട കർഷകർക്ക് കൈറ്റോസിൻ വിത്ത് പൂശുന്നത് മികച്ച സാങ്കേതികവിദ്യയായിരിക്കും.