INFLUENCE OF MODIFIED ATMOSPHERIC STORAGE CONDITIONS ON LONGEVITY OF PIGEONPEA (*Cajanus cajan* L.) SEEDS

MANJUNATHA, B.

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE, RAICHUR

UNIVERSITY OF AGRICULTURAL SCIENCES

RAICHUR – 584 104

JUNE, 2013

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Thesis submitted to the
University of Agricultural Sciences, Raichur
In partial fulfillment of the requirement for the
Degree of

Master of Science (Agriculture)

in

SEED SCIENCE AND TECHNOLOGY

By

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CERTIFICATE

This is to certify that the thesis entitled **"INFLUENCE OF MODIFIED ATMOSPHERIC STORAGE CONDITIONS ON LONGEVITY OF PIGEONPEA (*Cajanus cajan* L.) SEEDS"** submitted by **Mr. MANJUNATHA, B.** in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **SEED SCIENCE AND TECHNOLOGY,** College of Agriculture, Raichur, University of Agricultural Sciences, Raichur, is a record of research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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Affectionately Dedicated
To

My beloved Parents
Sri. Buddareddy and Smt. Eswaramma

ACKNOWLEDGEMENT

With ever regardful memories……

It is a matter of pleasure to glance back and recall the path one traverses during the days of hard work and pre-perseverance. It is still great at this juncture to recall all the faces and spirit in the form of teachers, friends, near and dear ones. I would consider this work nothing more than incomplete without attending to the task of acknowledging the overwhelming help I received during this endeavor of mine.

It is very difficult to express one's feelings in words but formality demands to do so the extent possible. I feel the inadequacy of diction in expressing my sincere heartfelt gratitude to **Dr. S.N. VASUDEVAN,** *Professor & Head, Department of Seed Science and Technology, College of Agriculture, Raichur and the Chairman of my advisory committee. His level of guidance, lively encouragement, constructive criticism and generous assistance at every stage of my research work, is behind measure, in fact it was he who motivated me to work on this aspect and any credit goes to him, his keen observations in detecting errors and correcting manuscript which consumed his valuable time and efforts is far beyond the call of duty. His kind and understanding nature has been overwhelming and unforgettable throughout my life.*

`My diction doesn't seem too rich enough to provide suitable words to articulate my sincere and heartfelt gratitude to **Dr. Basave Gowda,** *Special Officer (Seeds), UAS, Raichur and the member of my advisory committee who has given fruitful advice and also a constant encouragement throughout my venture of this study for which I am greatly indebted to him.*

I avail this opportunity to express sincere thanks to my advisory committee consisting scientists like **Dr. Udaykumar Nidoni,** *Associate Professor & Head, Department of Processing and Food Engineering, College of Agricultural Engineering, UAS, Raichur and* **Dr. A.G. Sreenivas,** *Associate Professor, Department of Agricultural Entomology, College of Agriculture, Raichur, for giving valuable suggestions and serving as member of my advisory committee and also I cannot forget helpful and honest personalities,* **Dr. N.M. Shakuntala,** *Associate Professor of Seed Sciences and Technology, College of Agriculture, Raichur,* **Dr. S. B. Patil** *Assistant Professor of Seed Science and Technology, College of Agriculture, Bheemarayangaudi,*

Dr. S.R. Doddagoudar, *Assistant Professor of Seed Sciences and Technology, College of Agriculture, Raichur,* **Dr. Sangetha I. Macha**, *Assistant Professor of Seed Science and Technology, College of Agriculture, Raichur and* **Mr. Rakesh C. Mathad** *Assistant Professor of Seed Sciences and Technology, College of Agriculture, Raichur, for providing necessary and valuable guidance during my study period.*

I wish to record my thanks to all staff and non-staff of the Department of Seed Sciences and Technology, for their help in course of my research work and being supported by Sri. Ramaswamy, Smt. Parvatamma, Anjaneya and others.

How miserable this part of life would have been without the company of my senior friends and all UG and PG classmates and junior friends of course a long chain I remember always.

One last word; since it is practically impossible to list all contributions to my work, it seems proper to issue a blanket of thanks for those who helped me directly and indirectly during the course of study.

Raichur

June, 2013 **(MANJUNATHA, B.)**

CONTENTS

LIST OF ABBREVIATIONS

Abbreviation		Expansion
g	:	Gram
kg	:	Kilogram
cm	:	Centimeter
m	:	Meter
%	:	Per cent
NS	:	Non significant
RH	:	Relative humidity
S.Em	:	Standard Error of mean
C.D.	:	Critical Difference
Fig.	:	Figure
MAS	:	Months after storage
MASC	:	Modified atmospheric storage conditions
ppm	:	Parts per million
mg	:	Milligram

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Introduction

I. INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.)] is an important pulse crop in India. It is also known as red gram, Arhar and tur. Pigeonpea is grown worldwide on 4.67 m ha area with an annual production of 3.30 mt in about 50 countries (FAO, 2010). India is the largest producer and consumer of pigeonpea with an annual production of 2.46 million tonnes, followed by Myanmar (0.72 mt), Malawi (0.18 mt) and Kenya (0.10 mt). In sub-Sahara Africa (Kenya, Malawi, Tanzania, Uganda, and Mozambique) long duration pigeonpea constitute an important component of rainfed agriculture.

In India, it is one of the important grain legume and occupies second position in area and production next to chickpea. It is mainly grown in the states of Maharashtra, Karnataka, Uttar Pradesh, Madhya Pradesh and Gujarat. It is grown on an area of 4.04 m ha with an annual production of 2.65 million tonnes with a productivity of 656 kg per ha. Its area, production and productivity trends in India in last five decades showed that there was about 2 per cent increase in area per year but the yield levels were stagnated around 600-700 kg per ha. (Anon., 2012)

In Karnataka, pigeonpea occupies second place in area (0.77 mha) and ranks second in production (0.36 mt) with a productivity of 466 kg per ha (Anon., 2012). Gulbarga is very important potential district in the country for extensive cultivation of pigeonpea. It is also grown in Bidar, Bijapur, Dharwad, Bellary and Belgaum districts of northern Karnataka. Average productivity of pigeonpea in Karnataka accounts for 700 kg/ ha and its potential yield marked up to 3.5 tons per hectare (Anon., 2012).

Pigeonpea is one of the major pulse crops, endowed with several unique characteristics. It contains about 23.6 per cent protein, which is almost three times that of cereals. Pigeonpea supplies a major share of protein requirement for the vegetarian population of the country. Every person on an average is required to consume 70 to 80 g of pulse per day in order to maintain good health and physique. The crop is consumed as whole seeds, dehulled or as flour. In the Caribbean region, people consume the seeds as green (immature) pods. The combination of dal-chawal (pulse-rice) or dal-roti (pulse-wheat bread) is an integral part of any average Indian diet. The plants woody stems are valuable as firewood, thatch, and fencing and as substrate to culture the lac producing insects. The leaves are an important source of organic matter and nitrogen; adding as much as 40 kg nitrogen per hectare to the soil. It is particularly rich in lysine, riboflavin,

thiamine, niacin and iron. Nutritional value of edible portion per 100 g of red gram is 335 cal. of Energy, 22.3 g Protein, 1.7 g Fat, 7.3 mg Ca, 5.8 mg Fe, 0.45 mg Thiamin, 0.19 mg Riboflavin, 2.9 mg Niacin, and 132 mg Vit.A value. It has good nutritive value although, it contains considerable amount of antinutritional polyphenolic compounds which inhibit the digestive enzymes trypsin, chymotrypsin and amylase.

The production of quality seed not only involves technical skill, finance, etc. but the produced seeds need to be properly protected until next planting time. Hence, the adage "seed saved is seed produced" assumes paramount importance as the seeds are more prone for various storage factors *viz*., temperature, seed moisture content, relative humidity (RH), containers, initial seed quality, several storage pests etc which cause 10-100 per cent qualitative and quantitative loss during storage.

Proper storage of seed in seed production programme is an important activity in order to maintain its quality parameters like viability, vigour and seed health until it is sown. Now a days seed consumer, tiller of the land demand for high quality seeds. Supplying high quality seeds can be achieved by an appropriate postharvest storage technology. A major cause of stored seed degradation and deterioration are insect pests and microorganisms. Many studies have shown that modified atmosphere of elevated carbon dioxide and depleted oxygen is effective method against insect and microorganisms during storage. Modified atmospheric storage is one of the seed and food preservation methods that maintain the natural quality of food products in addition to extending the storage life. Modified atmosphere (MA) reduces the respiration rate of seeds and activity of insects or microorganisms in seeds.

After the pigeonpea seeds are harvested, controlling quality deterioration becomes more difficult because the seeds are much more prone to attack by more than 200 species of insect pests causing enormous losses. The losses have been estimated to vary from 46.6 to 63.6 per cent, both in field and as well as in storage condition. Better methods and techniques are needed to improve conditions and environments that cause quality deterioration. A low-oxygen atmosphere system for handling of pigeonpea seeds appears to have potential for improving storage practices.

The only technology that retains the special capacity of fumigation for in-situ treatment of stored commodities, as well as offering a similar diversity of application technologies, is the modified atmosphere (MA) method. Modified atmosphere offer an

alternative that is safe and environmentally benign to the use of conventional residue-producing chemical fumigants for controlling insect pests attacking stored grains, oilseeds, pulses, processed commodities and packaged foods. Although the economics involved in the application of MA prevent their full replacement of conventional fumigants, novel approaches to the use of MA indicates their suitability for niche applications.

Disinfestations of stored seeds using modified atmospheric storage (MA) involves the alteration of the natural storage gases such as carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2), to render the atmosphere in the stores lethal to pests. The MA includes neither alteration of the storage atmosphere by addition of toxic gases such as phosphine or methyl bromide, nor regulation or alteration of the atmospheric water content. The MA may be achieved in several ways: by adding gaseous or solid CO_2, by adding a gas of low O_2 content (e.g. pure N_2 or output from a hydrocarbon burner) or by allowing metabolic processes within an airtight storage to remove O_2, usually with associated release of CO_2. Such atmospheres are referred to as 'high-CO_2', 'low-O_2', and 'hermetic storage' atmospheres, respectively. They are collectively known as 'modified atmospheres' (Banks and Fields, 1995). Modified atmosphere storage of seeds is a suitable alternative to the use of chemical fumigants and contact insecticides that are known to leave carcinogenic residues in the treated product (Bailey and Banks, 1980; Shejbal and Boislambert, 1988).

The most important component in MA is carbon dioxide which is a non-flame, colorless and odorless gas, about 1.5 times as heavy as air. Carbon dioxide can be supplied from an external source to a silo using either gas producer from a liquid supplied in pressurized cylinders or from solid "dry ice". Solid "dry ice" is useful source of CO_2 because changes directly form solid to a gas. Its can be supplied as blocks, crushed ice or pellets. Blocks are useful to make up gas loss during treatment due to their slower release. Crushed ice or pellets rapidly change to a gas and are best for initial gas addition (Graver, 2004). Jayas and Jeyamkondan (2002) reported that there are some different methods of introducing CO_2 as "dry ice" in the seeds mass in silo: (i) introducing of dry ice under the perforate floor or in the perforated duct, (ii) introducing of dry ice on the top surface of the seeds covered with a carbon dioxide impermeable sheet, (iii) introduction of equal amounts of dry ice on the top surface under the sheet and in the perforated duct, (iv) introducing of dry ice through a 10 cm – diameter perforated tube installed vertically

in the center of the seeds bulk, (v) introduction of one-quarter of the dry ice on the top surface under the sheet and the remaining three-quarter in an insulated box placed under the sheet. The fourth method gave the most uniform CO_2 concentration in the seeds mass and used the least amount of carbon dioxide to maintain the desired CO_2 concentration. However, installation of 10 cm–diameter perforated tube would be very difficult, therefore the last method was recommended for practical use (Alagusundaram *et al*., 1995).

The effectiveness of modified atmosphere for controlling various stored products pests depends on the temperature and moisture content of the seeds, species and storage life of pests, gaseous composition and uniformity of gas distribution and exposure time of the MA treatment (Łukasiewicz *et al*., 1999). However, the research study on prolonging viability of pigeonpea seeds using modified atmospheric storage is scanty.

Hence, the present investigation was planned to study the influence of modified atmospheric storage conditions on longevity of pigeonpea seeds with following objectives;

1. To study the effect of different gases on seed longevity of pigeonpea seeds
2. To study the biochemical changes during storage

Review of Literature

II. REVIEW OF LITERATURE

Review of literature pertaining to inf1uence of modified atmospheric storage conditions on seed quality of pigeonpea seeds and biochemical changes during storage is presented in this chapter. Reviews pertaining to modified atmospheric storage of pigeonpea seeds are limited. Hence, literatures available on other crops are included to support the present investigations.

2.1 Influence of modified atmospheric storage conditions on seed quality

Kondo *et al*. (1927, 1929, 1930 and 1934) found that both rough and hulled rice can be stored sealed with carbon dioxide or air for up to four years with little loss of viability provided the seed moisture content is less than 13 per cent.

Guillaumin (1928) reported that soybeans in open storage for nearly six years lost viability, whereas seeds sealed in an atmosphere free of oxygen germinated 92 per cent and those under vacuum had 100 per cent viability.

Sampietro (1931) observed that rice seeds with either 5 or 13 per cent moisture lost all viability when sealed in carbon dioxide, air, or under a partial vacuum.

Barton (1939) reported that dandelion seeds retained their viability about equally well when sealed in partial vacuum or in air, except seeds containing 7.9 per cent moisture seemed to deteriorate more rapidly in a partial vacuum.

Sayre (1940) observed that corn seeds with 18 per cent moisture content stored at 30°C in oxygen died within three years and the germination of the seeds in carbon dioxide and nitrogen dropped noticeably. At low temperatures corn seeds with 18 per cent moisture sealed in carbon dioxide and nitrogen had good germination for 5 years.

Simpson (1953) reported that low moisture (7%) cotton seeds retained their initial viability when sealed in air, oxygen, carbon dioxide, or nitrogen and stored at 21°and 32 °C. Seeds with 13 per cent moisture dropped to one-half to two-thirds of the original germination under all storage conditions.

Harrison and Mc Leish (1954) and Harrison (1956) revealed that lettuce seeds sealed in carbon dioxide retained their viability better at room temperature than did similar seeds sealed in air, storage in carbon dioxide revealed differences in longevity between cultivars.

Kaloyereas (1955) opined that deterioration of high moisture (20.80%) paddy seeds at 30°C can be delayed for a few weeks by sealing them in an atmosphere of carbon dioxide mixed with1000 ppm of ethylene oxide.

Goodsell *et al.* (1955) reported that the average pooled germination of the two corn hybrids in the three storage gases was still 95 per cent or better after 5 years for the seeds at 8,10 and 12 per cent moisture at 18°C and 4°C and those containing 3 and 10 per cent moisture at 16°C. Seeds containing 12 or 14 per cent moisture at 16°C and those with 8 and 10 per cent moisture at 29°C deteriorated rapidly after one year. Corn seeds containing 12 to 14 per cent moisture were practically all dead after one-half to one year, regardless of the surrounding gas.

Davies (1956) noticed that seeds of both red and white clover stored under vacuum and in nitrogen were short lived than those stored unsealed.

Peterson *et al*. (1956) revealed that loss of viability of high moisture wheat seeds can be delayed for several days by sealing under either 50 or 75 per cent of carbon dioxide.

Evans (1957) observed that the Red clover seeds containing 10.3 per cent moisture when sealed with carbon dioxide lost all viability in 23 years, but when calcium chloride was used with the carbon dioxide, only about one-third of the viability was lost.

Glass *et al.* (1959) revealed that loss of viability of high moisture wheat seeds can be delayed for several days by sealing under either 50 or 75 per cent of carbon dioxide or nitrogen.

Isely *et al.* (1960) observed that cabbage seeds stored equally well when sealed in air, nitrogen, or a partial vacuum.

Bass *et al.* (1962) opined that vacuum, carbon dioxide, nitrogen, helium, and argon storage had no advantage over sealed-in-air storage for lettuce seeds during two years.

Bass *et al.* (1963) concluded that sorghum seeds after second year of storage retained significantly higher germination when sealed under a partial vacuum than when sealed in air, carbon dioxide, nitrogen, argon or helium.

Roberts and Abdalla (1968) reported that for pea seeds the period of safe storage decreased as the oxygen concentration in the storage atmosphere increased from O to 21 per cent.

Roberts and Abdulla (1968b) concluded that higher the oxygen content of the storage environment, the shorter the viability period for barley seeds. The deleterious effects of oxygen were most pronounced at higher seed moisture levels.

Jay (1980) reported that high CO_2 atmospheres generally killed insects faster than high N_2 atmospheres, and CO_2 concentration of 60 per cent could be allowed to fluctuate as low as 35 per cent to get good insect control. In addition, absorption of CO_2 by grain or oilseeds might make it more effective against species whose immature stages feed inside the kernel.

Slay *et al*. (1985) evaluated low-oxygen atmosphere method of storing peanut seeds and observed that the seed lots exposed to low oxygen had better germination than control.

Doijode (1993) observed that percentage of germination in chilli cv. Sel-1 seeds was greater in the vaccum-stored seeds than in the untreated control. Storage of chilli seeds under partial vaccum was beneficial for preserving viability for three years under ambient conditions.

Storage of mustard seeds in three different controlled environments studied by Neeta singh *et al.* (1993) revealed that the mean germination period was maximum when seeds were stored at 25 ^{o}C and minimum at 10 ^{o}C.

Gass *et al.* (1998) studied the effect of different gaseous atmosphere and seed moisture content on viability of rye seeds in long-term storage experiment. After 10 years of storage seeds kept in vacuum or neutral gases preserved their viability at a level close to 80% of germinability, whereas in seeds stored with air, viability decreased to about 30 per cent. Investigation of two levels of seed moisture content did not show significant effect on seed viability during the first 10 years of storage, either in vacuum or in air. However, after 15 years of storage, in the case of seed kept in vacuum, samples with lower seed moisture content showed higher viability.

Alvindia *et al.* (1999) conducted an experiment on modified atmosphere storage of bagged maize outdoors using flexible liners and reported that insect infestation was completely prevented in the CO_2 – enriched cubes while, few live insects were noticed in the hermetic cubes. Insect-damaged kernels and weight loss were minimized. Grain moisture content remained stable after three months although mould growth was noted at the top surface of the stacks. Grain quality was preserved and seed germination was not affected.

Viggiano *et al.* (2000) studied the papaya (*Carica papaya* L.) seed storage in relation to the degree of humidity, type of packing and storage atmosphere. Seeds were dried to three moisture contents (7.2, 9.3 and 11.3%), packed in flexible aluminium or paper bags, and stored in the laboratory (27^0C and 83 % RH), in a chamber (20 ^{o}C and 69% RH) or in a cold chamber (10^oC and 63% RH). The seeds were subjected to moisture content evaluations (bimonthly), and germination and vigour tests (first germination count test and accelerated ageing). Dormancy breaking in seeds was observed after two months' storage, regardless of the atmosphere, packing and moisture content of seeds.

A storage study on germination and viability of soybean and sorghum seeds by Rathi *et al*. (2000) revealed that an atmosphere containing high CO_2 does not have a detrimental effect on seeds as much as high storage temperatures and high moisture content and they concluded that control atmosphere storage could be a potent alternative for commercial long term storage of seeds.

Borem *et al*. (2001) studied seed quality of bean (*Phaseolus vulgaris* L.) in storage with modified atmosphere equipment. The bean seed moisture content, germination, vigour, and insect infestation were kept constant during the whole storage period when seeds were maintained in a cold chamber at 5 ^{o}C. However, these characteristics changed when seeds were stored at ambient temperature using the modified atmosphere equipment without protection or wrapped in aluminium foil.

Khan *et al.* (2002) studied the role of moisture content and controlled atmosphere in *Citrus* seed storage. The survival of *Citrus* seeds was examined under different moisture contents and storage atmospheres. Controlled atmosphere did not have any consistent effect in maintaining *Citrus* seed viability.

Piriz Carillo *et al.* (2003) reported that germination capacities of seeds of *Araucaria anguistifolia* were found different, depending on the films used, refrigeration temperatures and storage times tested. Germination capacities were best kept during storage at 0 ^{o}C.

Doijode (2003) revealed that percentage of cabbage seed germination was reduced rapidly in modified atmosphere storage, reduction being least in seeds stored in carbon dioxide after three years of storage. The studies suggests that storage of cabbage seeds especially in carbon dioxide is effective and inexpensive for short-term preservation of seed quality and would prove beneficial in absence of cold storage facility.

Target gas concentrations for insect toxicity are three per cent or less of oxygen and or 60 per cent or more of carbon dioxide. Thus, one type of controlled atmosphere would be addition of CO_2 to levels above 60 per cent for 24 hours or more, or flushing an exposed space with an inert gas such as nitrogen to displace O_2 below three per cent. A low oxygen atmosphere can also be achieved and maintained by applying vacuum, or low pressure, to an infested commodity in a gas-tight chamber so that all the atmospheric gases decrease, including oxygen (Mbata *et al.*, 2004).

Bera *et al*. (2004) reported that storage of wheat seeds in CO_2 rich atmosphere irrespective of concentrations and periods showed no adverse effect on germinability, vigour and no change in the dehydrogenase activity and malondialdehyde contents.

Barzali *et al*. (2005) observed that rye seed with 5.5 per cent moisture content kept at -15, 0 and 10^0C in hermetically sealed glass filled with air, CO_2, N_2, and under vacuum showed highest germinability and viability at 15^oC followed by 10 ^{o}C and 0 ^{o}C and also highest under vacuum and N_2 as compared to air and CO_2.

Arvanitoyannis *et al.* (2005) studied the effect of grafting and modified atmosphere packaging (MAP) on eggplant quality parameters during storage and reported that Vitamin C was negatively affected by grafting it storage, while MAP prolonged the shelf life. Although pH was not affected by grafting but was positively affected by MAP. Flesh firmness was negatively affected by grafting and reduced over storage, but positively affected by MAP. Sensory analysis showed higher ratings of fruits from ungrafted plants for sweetness, acceptance and hardness whereas, no difference was detected for overall acceptance. Fruits stored under MAP were better maintained compared with those stored in air.

Gang Ji *et al*. (2005) conducted an experiment on delayed modified atmosphere packaging of fresh cut romaine lettuce and its effect on quality maintenance and shelf-life. They reported that the modified atmospheres obtained with 16.6 pmol/s/Pa oxygen transmission rate (OTR) film increased discoloration when present, and generally had less off odour development and CO_2 injury compared to MAP with 8.0 pmol/s/Pa OTR film. Delayed packaging affected overall quality of fresh cut lettuce packaged in both the films. A 12 hour delayed packaging into packages prepared from 8.0 OTR film maintained quality by inhibiting CO_2 injury, off-odour development and tissue electrolyte leakage.

However, an 8 hour delayed packaging into packages prepared from 16.6 OTR film was better at maintaining the quality of fresh cut lettuce at 5^{o}C for 14 days and delayed packaging may be an alternative method to optimize or balance package O_2 suboptimal OTR film packaging conditions.

Iconomou *et al.* (2006) studied the cereal characterstics as affected by controlled atmospheric storage conditions and reported that grains of corn and wheat stored under controlled atmospheric conditions of 2% and 8% O_2 for 12 and 6 months periods, respectively and the storage under high nitrogen atmospheric conditions kept the flour acidity stable for all the storage period and enhanced the germination ability of grains and finally inhibition of the existing entomological and microbial counts.

Doijode (2006) observed that garden pea (*Pisum sativum* L.) seeds of *cv* Green Pearl were maintained high seed quality in terms of viability and vigour preserved with carbon dioxide storage followed by nitrogen. It is beneficial to pack well-dried seeds along with carbon dioxide in laminated aluminium foil pouches for the maintenance of high viability till next growing or for short-term conservation of seed germplasm. The modified atmosphere storage is effective for preservation of genetic diversity and useful especially in absence of cold storage facility.

Conyers and Bell (2007) evaluated N_2 based Modified Atmosphere storage with elevated CO_2 (10–20%) at 20^{o} and 25°C with 75 per cent and 85 per cent RH at each temperature. When CO_2 was increased to 10 per cent or 20 per cent, reducing O_2 to five per cent was sufficient to eliminate emergence of *Sitophilus granarius* (L.) at 20°C, but few individuals emerged at 25˚C. For *C. ferrugineus* there was 95 per cent reduction with five per cent O_2 plus 20 per cent CO_2 at 20°C, but not at 25°C.

Bera *et al.* (2008*)* reported that under modified atmospheric storage (up to 80% CO_2) of paddy seed with 11 per cent moisture content could be stored safely at least up to 12 months without much reduction in seed viability.

Yogeesha *et al.* (2008) studied the effect of seed moisture, packaging materials and storage temperature on seed viability of papaya seeds, and concluded that seeds could be stored without any decline in viability and vigour for 24 months at 15^{0}C irrespective of variety, packaging materials and seed moisture. Among the packaging materials the highest germination and vigour was observed in seeds packed in polylined aluminium pouches.

Nasar – Abbas *et al.* (2008) observed that nitrogen was effective in reducing colour darkening by an appreciable level, whereas storage in oxygen accelerated the colour darkening process. Ethylene had some effect whereas, the other MAP treatments were ineffective in reducing colour darkening in faba beans and the analytical studies revealed that tannin concentration was negatively correlated with colour darkening in faba bean. Air, vacuum and ethylene treated samples showed similar changes in phenolic constituents after 12 months storage but samples flushed with CO_2 and especially those flushed with O_2 had much higher losses in phenolic constituents demonstrating that colour darkening is likely to be due to oxidative transformation of phenolic contents. Flushing with N_2, which reduced colour darkening and tannin losses, would be useful in maintaining quality and improving market opportunities and acceptance during long-term storage of faba beans.

Adetumbi *et al.* (2009) studied the effect of storage materials and environments on drying and germination quality of maize (*Zea mays* L) seed. Results showed that maize seed deterioration was not absolutely avoided over time regardless of the storage materials used, but the rate was reduced if adequate attention was given to storage environment, such as per cent relative humidity, temperature and initial moisture per cent of the seed.

Li Peng Xia *et al.* (2009) reported the effects of modified atmosphere packaging on changes of physiology and quality of pine nut at ambient temperature. The results showed that storage condition of low O_2, high CO_2 correspondingly were achieved by 30 micro m purple PVC bag and 30 micro m white PVC bag. The 30 micro m purple Poly Vinyl Chloride bag and 30 micro m white PVC bag were effective in slowing down respiratory rate, reduce the losses of soluble total sugars, maintain better-grade per cent of pine nut kernels, avoid the decrease of fat content and iodine value, and restrain the increase of acid value and peroxide value compared with 40 micro m Polyethylene bag. The results of the study confirmed that 30 micro m PVC bags were beneficial in terms of quality wherein storage quality of unshelled pine nut was better than that of shelled pine nut with the same modified atmosphere packaging.

Shehata *et al.* (2009) reported that cowpea seeds treated with gas mixture containing 80% CO_2 + 4% O_2 + 16% N_2 showed the lowest infestation and weight loss percentage as well as highest germination percentage and total crude protein. Untreated seeds showed

the highest infestation and weight loss percentage, lowest germination percentage and total crude protein.

Tatipata (2009) conducted an experiment on the effect of seed moisture content, packaging material and storage period on mitochondrial inner membrane of soybean seed and found that soybean seeds stored in aluminium foil bags recorded high phospholipids, protein content of mitochondrial inner membrane, germination, coefficient velocity of germination and delayed seed deterioration compared to polyethylene and wheat bags.

Mustafa *et al*. (2010) determined the effects of different modified atmosphere packaging applications for prolonging the shelf life of dill leaves and observed that Modified atmosphere packaging based on low density polyethylene was fixed as the most successful application on dill leaves and pursued by modified atmosphere packaging based on polyvinylchloride (PVC). Moreover, 0-1°C storage temperature was more effective as keeping the quality of the crop. The effects of MAP based on both PVC and LDPE reduced at 4-5°C storage temperature. As a result, MAP based on LDPE at 0-1°C storage temperature was confirmed as the best method for prolonging the shelf life of dill leaves with keeping the quality.

Shivappa (2011) studied the effect of modified atmospheric storage on storability of onion and observed that, the seeds exposed to gaseous combination of 60% N_2 + 40% CO_2 + 0% O_2 and stored in aluminum foil pouch maintained better quality in terms of germination and vigour up to the end of ten months of storage followed by vacuum storage.

Shrishail (2011) studied the effect of modified atmospheric storage on storability of groundnut and reported that, the seed kernels exposed to gaseous combination of 60% N_2 + 40% CO_2 + 0%O_2 and stored in polythene bag maintained better quality in terms of germination and vigour up to the end of ten months of storage followed by vacuum storage.

Xihong Li *et al*. (2011) observed that 1-methylcyclopropene (1-MCP) (650 ppb) and modified atmosphere packaging (MAP) reduced chilling injury symptoms which were correlated with decreased electrolyte leakage and malondialdehyde content. The combination of 1-MCP and MAP further reduced chilling injury. Atomic force microscope (AFM) images showed that, the surface of the sweet peppers with 1-MCP and MAP treatments were smoother than of the control samples. The activities of Superoxide

dismutase (SOD), Catalase (CAT) and Peroxidase (POD) decreased during the first 15 days of storage followed by an increase during the later period of storage. Treatment with 1-MCP, MAP alone and in combination frequently reduced the activities of those enzymes during storage. These results suggested that, combination of 1-MCP treatment and MAP is a promising treatment for reducing chilling injuries of peppers stored at 4°C.

2.2 Biochemical changes during storage of seeds

It has long been known that electrical conductivity of solutions in which plant tissues are bathed, showed increase with tissue age (Abdul-Baki and Anderson, 1972).

Seed protein content could be related to vigour in three ways 1) by the direct relationship of seed size, quantity of proteins, 2) by the stability of the protein synthesis system, 3) by the stability of protein transformation (Abdul-Baki, 1980).

Powell (1986) they considered that reduction in enzymatic activity, respiration and macromolecules synthesis are associated with initial deterioration of membrane system.

In French bean after two years of storage, both vigour and viability were reduced as evidenced as percentage increase in leakage of different solutes into soak water over control value of electrolytes by 93%, dehydrogenase by 74%, sugars by 184% and proteins by 215% (Pandey, 1989).

In seeds, protein synthesis is essential for germination to be completed and for the radicle to emerge. A decline in protein synthesis occurs with increase in age in variety of tissues and organisms. Induction of 2 new proteins (15 and 43KD), subsequent to accelerated ageing as a result of increase in their corresponding mRNA was reported in sunflower seeds (Xavir *et al.*, 1990).

Basavarajappa *et al.* (1991) reported that when seeds were exposed to high temperature, an elevation in the enzyme kinetics probably caused a degradation/ denaturation of some enzymes, protein pattern changed with accelerated ageing (100% RH) at 41^{o}C after 72 and 96 hours, but with low temperature at 25^{o}C there was not much change in enzyme and protein pattern. There was a decrease in total seed protein after ageing; these results were associated with an increase in the free amino acids concentration, suggesting degradation of protein macromolecules in maize seeds.

All the biochemical pathways require enzymes to catalyse reactions within the cell. The relationship of an enzymatic activity with seed ageing has been well documented. Many enzymes remain active after all viability is lost; however, the dehydrogenases are one group of enzymes that were shown to be directly related with loss of viability. Membrane reorganization is slower (or) may be prevented as a consequence of ageing and cell death (Taylor, 1997) therefore; cell membrane integrity may be tested directly (or) indirectly as a means to predict seed quality.

Machado *et al*. (2001) reported that equal quantities of protein were loaded on to the gels and electrophoresis carried out for the seeds subjected to 24, 48, 72 and 96 hrs of artificial ageing at 41^{o}C, protein pattern were changed after 72 hours of artificial ageing and naturally aged seeds of French bean showed alteration after two years of storage. The seeds exposed to high relative humidity, but not to high temperature there was no change in protein electrophoretic patterns at any time showing that increased moisture is not enough to damage seed or its structures, as this happen in naturally ageing.

Gurusinghe *et al.* (2002) used the seeds of tomato which were subjected to osmopriming with PEG and subsequently dried at 37 or 40^{o}C for 2 to 4 hours to study the banding pattern of total soluble seed proteins using SDS-PAGE. They observed the induction of heat shock protein (hsp 70), the abundance of Bip (78kD binding protein) and class I small hsp' in primed seeds subjected to post priming treatment that could involve in the extension of seed longevity.

Measurements of electrical conductivity (EC) of seed soak water (4 x 100 seeds, each in 40 ml deionised water for 17 and 24 h), both initially and for seeds after controlled deterioration (CD) of 24 h at 20% mc, were negatively and significantly correlated with total germination after CD, and seedling emergence in transplant modules for 13 seed lots of the cabbage cultivar Yalova 1 (Mathews *et al.,* 2009).

Smruti Das *et al*. (2010) revealed that rice seeds stored under high temperature (45^{o}C) and humidity (100%) for 15 days, which facilitated Accelerated ageing deterioration. Under treated conditions, seeds of different wild rice species showed decrease in percent germination and concentrations of proteins and starch but increase in conductivity of leachate and content of sugar. The SDS PAGE analysis of seed proteins showed that not only the total number of bands, but also their intensity interms of thickness differed for each species during storage.

Material and Methods

III. MATERIAL AND METHODS

Storage experiment was conducted in the laboratory of Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur during 2012-13 to find out the influence of modified atmospheric storage conditions on longevity of pigeonpea seeds.

The details of materials used and methods followed during the course of investigation are described in this chapter.

3.1 General description

3.1.1 Experimental site

The laboratory experiments were conducted in the laboratory of Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur from July 2012 to April 2013 and the packaging of the pigeonpea seeds was done in the Department of processing and Food Engineering, College of Agricultural Engineering, UAS, Raichur with the modified atmosphere packaging equipment (MAP).

3.1.2 Location

Geographically the Raichur is situated in the transitional tract (Zone-2) of Karnataka State at 16° 15' North latitude and 77° 20' East longitude with an altitude of 389 meter above mean sea level.

3.1.3 Weather data during storage period

The meteorological data from 2012 to 2013 were recorded at the Meteorological Observatory of Main Agricultural Research Station, UAS, Raichur and are presented in Table 1.

3.1.4 Seed source

The seeds of pigeonpea cultivar BSMR 736 produced in *kharif* 2011-12 were obtained from the Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur immediately after harvest. The seeds were dried under sun for two to three days to bring the seed moisture to 7.55% level and Seed quality parameters before storage were recorded (Table 2).

3.1.5 Description of the variety

BSMR-736 is a pigeonpea variety with indeterminate spreading plant type, it grows up to a height of about 175 to 190 cm, bears yellow flowers, pods are green at early stage and later purple streak will appear and produce 3 to 4 brown seeds in each pod and it is moderately resistant to sterility mosaic and wilt disease. It yields about 12-14 q per ha in rain fed condition while, in irrigated condition 18-20 q/ha yield can be obtained.

3.2 Experimental details

The experiment consisted of 14 treatment combinations and details of the treatments are furnished below.

Treatments	Concentrations of gas combinations
$\mathbf{T_1}$	Control
$\mathbf{T_2}$	70% N_2 : 20% O_2 : 10% CO_2
$\mathbf{T_3}$	60% N_2 : 20% O_2 : 20% CO_2
$\mathbf{T_4}$	40% N_2 : 20% O_2 : 40% CO_2
$\mathbf{T_5}$	20% N_2 : 20% O_2 : 60% CO_2
$\mathbf{T_6}$	80% N_2 : 10% O_2 : 10% CO_2
$\mathbf{T_7}$	70% N_2 : 10% O_2 : 20% CO_2
$\mathbf{T_8}$	50% N_2 : 10% O_2 : 40% CO_2
$\mathbf{T_9}$	30% N_2 : 10% O_2 : 60% CO_2
$\mathbf{T_{10}}$	90% N_2 : 00% O_2 : 10% CO_2
$\mathbf{T_{11}}$	80% N_2 : 00% O_2 : 20% CO_2
$\mathbf{T_{12}}$	60% N_2 : 00% O_2 : 40% CO_2
$\mathbf{T_{13}}$	40% N_2 : 00% O_2 : 60% CO_2
$\mathbf{T_{14}}$	Vacuum

Packaging material: 700 gauge polyethylene bag

Design: Complete Randomized Design (CRD) with 3 Replications

Storage period: 10 months (July 2012 to April 2013)

3.2.1 Method of modified atmosphere packaging

Polyethylene bags of gauge 700 measuring 40 cm length and 25 cm breadth were used for packing purpose. In these bags, one kg of pigeonpea seeds were packed along with the gases like carbon dioxide, nitrogen and oxygen in different concentrations according to the treatments. Firstly, the valves of the gas cylinders were opened and were released at a pressure of 7 kg/cm^2 and the different combinations of carbon dioxide, nitrogen and oxygen were mixed in the mixing chamber. According to the treatments given, gas flow rate was controlled in the buffer tank which was directly connected to the packaging unit. The pigeonpea seeds of 1 kg were packed using the packaging machine by evacuiking the air, then flushing with the gases of required combinations followed by sealing automatically (Plate 7).

Composition of the gas *i.e.*, O_2 and CO_2 gas concentrations inside the package was checked by Check mate head space gas analyser (Plate 5) with the help of septum which prevents leakage of gas from polyethylene bag.

3.2.2 Procedure to use MAP instrument (Plate 6)

The cylinders containing Carbon dioxide (CO_2), Oxygen (O_2) and Nitrogen (N_2) gas (Plate 1) were checked for the pressure and the pressure of the gases was adjusted by following the steps detailed below.

a) The top dial in the mixing chamber was adjusted to the required CO_2 gas concentration and the value of X (mentioned below the upper dial) was noted then adjusted the bottom dial by calculating the value of N_2/X (Plate 2).

Where,

N_2 = Nitrogen gas concentration

X = Number below the upper dial

b) The desired gas concentrations were checked by using check mate gas analyzer. Through the gas sampling port the gases were allowed to pass through needle and the gas concentration in gas mixing chamber was recorded (Plate 2).

c) If the required gas concentration was not achieved then dialer was tuned to get the exact gas concentration. The sampling port was closed the gas present in the buffer tank was evacuated.

Plate 1: Gas cylinders with mixing chamber

Plate 2: Gas mixing chamber

d)

e)

Plate 3: Buffer tank for mixed gas

Plate 4: Packaging unit

Plate 5: Checkmate head space gas analyzer

Plate 6: Modified Atmosphere Packaging experimental setup

d) Buffer tank (Plate 3) needed to be evacuated to achieve required gas concentration. The gas was supplied through tube to modified atmosphere packing unit for packaging of seeds.

In Packaging Unit (Plate 4) the heat level of sealing adjusted to 2.0 to 2.5 to achieve proper sealing. The packaging material (polyethylene, 700 gauge) was kept in a packaging unit in which the vacuum was created by evacuating air present in the packing material and then filled the required gas concentration from buffer tank and sealed.

3.3 Observations

3.3.1 Germination

Germination test was conducted with four replicates of 100 seeds each in the paper (between papers) medium in the walk-in germination room. The germination room was maintained at 25 ± 1°C temperature and 90 ± 2% RH. At the end of sixth day of placing the seeds, the number of normal seedlings in each replication was counted and the germination was calculated and expressed in percentage (ISTA, 2013).

3.3.2 Root length (cm)

From the germination test, ten normal seedlings were selected randomly in each treatment from all the replication on 6^{th} day. The root length of each seedling was measured from collar region to the tip of primary root and the average root length was expressed in centimeter.

3.3.3 Shoot length (cm)

Ten normal seedlings used for root length measurement were also used for the measurement of shoot length. The shoot length was measured from the base of primary leaf to collar region and mean shoot length was expressed in centimeter

3.3.4 Seedling length (cm)

From the germination test, ten normal seedlings were selected randomly from each treatment on the day of final count. The seedling length was measured from tip of shoot to root tip and the mean length was calculated and expressed as seedling length in centimeters.

3.3.5 Seedling Vigour index-I (SVI-I)

The seedling vigour index was computed using the formula suggested by Abdul-Baki and Anderson (1973) and expressed as whole number.

Vigour index I = Germination percentage x Mean length of seedlings (cm)

3.3.6 Seedling Vigour index -II (SVI-II)

The seedling vigour index- II was computed multiplying germination percentage with seedlings dry weight and expressed as whole number.

Vigour index-II = Germination (%) × Dry weight of seedlings (mg)

3.3.7 Dry weight of seedling (mg)

Ten normal seedlings used for measuring seedling length were taken in butter paper and dried in a hot-air oven maintained at 80^{o} C temperature for 24 hours. Then ten seedlings were removed and allowed to cool in a desiccator for 30 minutes before weighing in an electronic balance. The average weight was calculated and expressed as seedling dry weight in milligrams.

3.3.8 Field emergence (%)

One hundred seeds were sown in four replicates on a well-prepared seedbed and adequate soil moisture was maintained by regular watering. Field emergence count was taken on 12^{th} day after sowing and the percentage was calculated taking into account the emergence of normal seedlings.

3.3.9 Speed of germination

Seeds were germinated on paper medium with four replications of 100 seed each. The number of seeds germinated was recorded daily up to the day of final count. The speed of germination was calculated by using the formula suggested by Maguire (1962).

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots\ldots + \frac{X_n (X_n\text{-}1)}{Y_n}$$

Where,

X_n : Number of seeds germinated at n^{th} count

Y_n : Number of days from sowing to n^{th} count

3.3.10 Hundred seed weight (g)

Hundred seeds were counted randomly and weighed up to two decimal places. The mean weight of three replications was calculated and expressed in grams.

3.3.11 Seed health test: Pathogen infection by Blotter method

Storage fungi present on seeds were detected using blotter method as prescribed in ISTA guidelines. Ten seeds were placed equidistantly on three layered moistened blotter taken in sterilized petriplate. Each treatment was replicated four times. They were incubated at 20^{o}C for seven days with alternate cycle of 12 hour near ultra violet (NUV) range and for remaining 12 hour in dark. On eighth day, the plates were examined under stereo binocular microscope for the presence of seed borne fungi. The number of infected seeds were counted and expressed in percentage (ISTA, 2013).

3.3.12 Per cent insect damage

About 100 seeds from each replication were drawn at random from each treatment. The damage of seeds attributed to pulse beetle (*Callosobruchus chinensis* L.) was recorded by observing the infested and uninfested seeds manually. The damaged seeds with holes, eggs or both were counted as infested seeds and expressed in percentage on number basis.

$$\text{Per cent insect damage} = \frac{\text{Number of damaged seeds}}{\text{Total number of seeds observed}} \times 100$$

3.3.13 Moisture content (%)

Two replicates of five grams of seed material were taken for determining the moisture content using low constant method. The powdered seed material was placed in a weighed moisture cup. After removing the lid, moisture cups were placed in hot air oven maintained at 103 ± 2^{o}C for 16 ± 1 hour and the contents were allowed to dry. Then, the contents were weighed in an electronic balance along with moisture cup and lid. The moisture content was worked out using the following formula and expressed as percentage (ISTA, 2013).

$$\text{Moisture content (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where,

M_1 : Weight of the moisture cup alone

M_2 : Weight of the moisture cup + sample before drying

M_3 : Weight of the moisture cup + sample after drying

3.4 Biochemical parameters

3.4.1 Dehydrogenase activity

Representative seeds (25) from each treatment were taken and preconditioned by soaking in water overnight at room temperature. Seeds were taken at random and the embryos were excised. The embryos were steeped in 0.25 per cent solution of 2, 3, 5-triphenyl tetrazolium chloride solution and kept in dark for two hours at 40 ^{o}C for staining. The stained seeds were thoroughly washed with water and then soaked in 10 ml of 2 methoxy ethanol (methyl cellosolve) and kept overnight for extracting the red colour formazan. The intensity of red colour was measured using ELICO UV-VIS spectrophotometer (model SC-159) using blue filter (470 nm) and methyl cellosolve as the blank. The OD value obtained was reported as dehydrogenase activity (Kittock and Law, 1968).

3.4.2 Electrical conductivity (dSm^{-1})

Five grams of seeds in four replicates were soaked with acetone for half a minute and thoroughly washed in distilled water for five times. Then the seeds were soaked in 25 ml distilled water and kept in an incubator maintained at 25 ± 1^{o}C for 24 hours. The seed leachate was collected and volume was made up to 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in the digital conductivity bridge (ELICO) with a cell constant of 1.0 and the mean value expressed in deci Simons per meter.

3.4.3 Protein content

Seed samples from each treatment were ground into fine powder. The seed samples were analyzed for protein content by Lowry's method and protein content was finally expressed in mg per 100 g seed sample by referring to standard graph.

3.4.4 Electrophoresis analysis of soluble seed protein

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of total soluble seed protein was carried out by using 12 per cent polyacrylamide gel according to the method prescribed by Laemeli (l970) with slight modifications. The electrophoresis was done in vertical slab gel of 16 cm x 14 cm x 1 mm dimension. The detailed procedure followed is as follows:

(a) Sample preparation

The seed sample was crushed by pestle and mortar. The ground seed sample was put into eppendorf tubes for defatting. The seed powder was vigorously mixed with 2 ml CMA (Chloroform, Methanol and Acetone) (1:2:1 ratio) solution for 20 min at room temperature. The step was repeated 3-4 times. To the defatted seed material, 1.5 ml of extraction buffer (Tris glycine 25 mM, pH 8.5) was added and agitated thoroughly and kept at 4^{o} C overnight for protein extraction. Then the suspension was centrifuged under refrigeration at 10,000 rpm for 10 min and the clear supernatant was collected. This protein extract was dissolved in an equal amount of working buffer and kept in boiling water at 90 ^{o}C for 10 minutes, cooled and again centrifuged at 10,000 rpm for 5 minutes and the supernatant was collected and used for loading on to the gel.

(b) Preparation of gel for electrophoresis

i) Resolving gel (12.5%) was prepared by mixing 10.41 ml of 30 per cent acrylamide solution, 6.25 ml of resolving gel buffer, 7.7 ml of water, 0.5 ml of 10 per cent SDS, 0.5 ml of 10 per cent APS and 0.02 ml TEMED and quickly poured into the gel plates leaving a margin of 2.0 to 3.0 cm on upper side of the gel. Overlaid with water and left for polymerization for about 30 minutes.

ii) Stacking gel (5%) was prepared by mixing 1.0 ml 30 per cent acrylamide, 1.5 ml of stacking gel buffer, 3.4 ml distilled water, 0.07 ml 10 % APS and 0.02 ml TEMED. Top of the resolving gel was thoroughly cleaned before pouring the stacking gel, then the stacking gel solution was poured on to the top of the resolving gel solution and immediately a comb was inserted to form the wells of 1.5 cm depth taking care not to trap the air bubbles underneath the comb. The gel was allowed to polymerize for 30 minutes, then the comb was removed carefully and the wells were rinsed with distilled water.

iii) Tank buffer: 3 g of Tris +14 g of Glycine +1g of SDS was added per litre of double distilled water.

(c) Electrophoresis

The upper and lower reservoirs of electrophoretic unit were filled with electrode buffer. Then 20 µl of protein extract was loaded into the wells of stacking gel by layering them under electrode buffer using micropipette. A current of 1.5 mA per well was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 mA

per well. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel, which took six to eight hours.

(d) Staining

Coomasic blue (0.5%) was prepared by dissolving 1 g of coomasie brilliant blue R-250 in 100 ml methanol, 20 ml acetic acid and 80 ml distilled water. After dissolving of coomasic blue, it was filtered through Wattman No. 1 filter paper and used for staining.

The gel was immersed in coomasic blue solution overnight under room temperature then; stained gel was destained, using destaining solution which was prepared by mixing 227 ml of methanol (45%), 46 ml acetic acid (10%) and 227 ml of distilled water, until the bands were clearly visible. After destaining, photographs of the gel were taken. The zymogram of the gel was traced on OHP sheet.

(e) Evaluation and documentation

The electrophoregrams of the gel were prepared by measuring the distance of each band from the point of loading. The Rm value was calculated as given below:

$$Rm = \frac{\text{Distance travelled by the protein}}{\text{Distance travelled by the tracking dye}}$$

Bands were numbered on the basis of increasing Rm values; the relative intensities and mobilization of protein bands and presence or absence of specific bands or combination of different bands were analyzed.

3.5 Statistical analysis

The data collected from the experiments were analyzed statistically by the procedure prescribed by Sundararaj *et al*. (1972). Critical difference were calculated at 5% level wherever 'F' test was significant. The data on percentage of germination, field emergence and seed infection were transferred into arcsine square root percentage values and transferred data were used for statistical analysis (Snedecor and Cochran, 1967). Absolute control treatment was compared with rest of the treatment by following ANOVA: statistical analysis.

Table 1. Monthly meteorological data for the year 2012-13 and mean of the last 81 years (1931-2012) at Main Agricultural Research Station, Raichur

Month	Rainfall (mm)		Temperature (^{O}C)				Relative Humidity (%)	
			Maximum		Minimum			
	1931-2012	2012-2013	1931-2012	2012-2013	1931-2012	2012-2013	1931-2012	2012-2013
April	12.81	41.00	39.9	38.5	24.37	23.9	52.31	25
May	42.00	02.00	39.71	39.9	25.24	25.5	59.19	23
June	113.30	57.90	35.30	36.2	23.26	23.8	78.169	36
July	74.03	110.60	33.39	32.4	22.54	22.0	76.56	54
August	72.73	50.00	32.87	32.8	22.44	21.7	79.57	50
September	180.43	126.00	32.18	31.9	22.2	20.7	81.49	55
October	61.96	44.60	31.53	32.2	20.48	18.3	78.38	47
November	20.87	36.80	31.31	31.3	19.06	17.0	78.23	48
December	3.95	0.00	30.50	32.0	16.17	15.6	75.10	34
January	1.62	0.00	31.30	34.6	16.73	15.8	76.33	34
February	1.07	0.00	32.53	34.2	18.45	18.1	61.44	25
March	43.21	0.00	36.53	37.7	22.53	19.9	55.33	19
Total	**627.98**	**468.90**						

Table 2. Seed quality parameters of pigeonpea cultivar (BSMR-736) before storage

Sl. No.	Seed quality parameters	Initial level
1.	Germination (%)	94
2.	Root length (cm)	18.25
3.	Shoot length (cm)	16.20
4.	Seedling length (cm)	34.45
5.	Seedling vigour index-I	3238
6.	Seedling vigour index-II	8234
7.	Seedling dry weight (mg) / seedling	87.60
8.	Field emergence (%)	90
9.	Speed of germination	26
10.	Test weight (g)	11.02
11.	Per cent insect damage	0
12.	Seed infection (%)	0
13.	Moisture content (%)	7.55
14.	Dehydrogenase enzyme activity (OD value)	0.802
15.	Electric conductivity (dSm^{-1})	0.513
16.	Protein content (%)	20.90

Vacuum Packaging

Normal Packaging

Plate 7: Vacuum and normal packaging of pigeonpea seeds

Experimental Results

IV. EXPERIMENTAL RESULTS

A laboratory experiment was conducted to study the "Influence of modified atmospheric storage conditions on longevity of pigeonpea seeds".

The experiment consisted of 14 treatments *viz.*, (T_1-Control, T_2-(70% N_2+20% O_2 +10% CO_2), T_3-(60% N_2+20% O_2+20% CO_2), T_4-(40% N_2+20% O_2+40% CO_2), T_5-(20% N_2+20% O_2 + 60% CO_2),T_6- (80% N_2+10% O_2 +10% CO_2), T_7-(70% N_2+10% O_2 +20% CO_2), T_8-(50% N_2 +10% O_2+40% CO_2),T_9-(30% N_2+10% O_2+60% CO_2), T_{10}-(90% N_2+00% O_2+10% CO_2),T_{11}-(80% N_2+00% O_2+20% CO_2),T_{12}- (60% N_2+00% O_2+40% CO_2), T_{13}-(40% N_2+00% O_2+ 60% CO_2), T_{14}-Vacuum), in 700 gauge polyethylene bag. The experiment was carried out in completely randomised design in three replications. The laboratory experiment results are presented in this chapter.

4.1 Influence of modified atmospheric storage conditions on seed quality and longevity of pigeonpea

4.1.1 Seed germination (%)

The results of germination percentage as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 3.

With the advancement of storage period, the mean germination percentage declined from 94.00 per cent at the initial stage to 69.30 per cent at the end of ten months of storage period.

Germination percentage due to MASC differed significantly in all the months of storage except for the second month of storage. The seeds exposed to treatment T_{13}: (40% N_2 + 0% O_2 +60% CO_2) recorded maximum germination of 78.30 per cent after ten months of storage period followed by T_{12} (77.87%) and T_{14} (74.53 %). Only T_{12} and T_{13} treatments maintained above the minimum germination (75%) as per Indian Minimum Seed Certification Standards. The lowest germination of 60.13 per cent was noticed in T_1-control after ten months of storage.

4.1.2 Root length (cm)

The data on root length as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 4.

The mean root length decreased from 18.25 cm at the initial stage to 14.66 cm after ten months of storage.

Table 3. Influence of modified atmospheric storage conditions on germination (%) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	88.00 (69.94)*	82.67 (65.43)	72.33 (58.30)	68.35 (55.79)	60.13 (50.87)
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	89.03 (71.04)	83.73 (66.28)	76.00 (60.71)	74.67 (59.84)	64.00 (53.18)
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	89.33 (71.03)	84.17 (66.59)	76.67 (61.16)	76.02 (60.71)	64.73 (53.61)
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	89.67 (71.35)	84.67 (67.02)	79.00 (62.76)	76.33 (60.93)	65.07 (53.82)
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	89.93 (71.84)	84.78 (67.11)	79.50 (63.11)	76.67 (61.17)	66.33 (54.57)
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	91.00 (72.87)	85.25 (67.46)	80.00 (63.47)	77.83 (61.95)	67.57 (55.32)
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	91.17 (72.87)	85.34 (67.52)	80.34 (63.72)	78.17 (62.18)	68.40 (55.84)
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	91.70 (73.49)	86.90 (68.84)	82.67 (65.43)	79.00 (62.76)	69.00 (56.20)
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	92.00 (73.90)	87.03 (68.96)	83.41 (66.00)	79.36 (63.01)	70.17 (56.92)
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	92.17 (74.01)	88.33 (70.09)	84.67 (67.05)	81.40 (64.49)	71.13 (57.53)
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	92.33 (74.13)	89.02 (70.95)	85.33 (67.57)	82.35 (65.20)	73.03 (58.75)
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	92.67 (74.55)	90.23 (71.82)	87.33 (69.21)	85.00 (67.25)	77.87 (61.97)
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	93.16 (74.87)	92.17 (73.79)	88.00 (69.78)	86.36 (68.37)	78.30 (62.27)
T_{14} : Vacuum	92.40 (74.04)	89.10 (70.75)	86.83 (68.76)	84.17 (66.59)	74.53 (59.72)
Mean	**91.03**	**86.67**	**81.57**	**78.97**	**69.30**
S.Em±	1.870	0.913	0.640	0.441	0.757
CD (5%)	NS	2.645	1.854	1.441	2.194

NS – Non significant

* Figures in the parenthesis indicate arc sign transformed values

Table 4. Influence of modified atmospheric storage conditions on root length (cm) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	17.27	16.50	16.12	15.50	14.16
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	17.29	16.83	16.23	15.60	14.38
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	17.31	16.85	16.25	15.77	14.41
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	17.33	16.87	16.33	15.80	14.44
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	17.35	16.92	16.40	15.97	14.49
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	17.37	17.03	16.54	16.00	14.52
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	17.38	17.05	16.63	16.03	14.63
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	17.40	17.06	16.68	16.06	14.60
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	17.43	17.08	16.72	16.08	14.67
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	17.53	17.14	16.83	16.10	14.77
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	17.57	17.16	16.89	16.14	14.85
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	17.73	17.36	16.94	16.19	14.88
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	17.80	17.51	17.03	16.31	15.67
T_{14} : Vacuum	17.61	17.33	16.91	16.16	14.87
Mean	**17.45**	**17.04**	**16.60**	**15.97**	**14.66**
S.Em±	0.200	0.096	0.164	0.116	0.222
CD (5%)	NS	0.280	0.477	0.337	0.644

NS – Non significant

Root length due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) recorded higher root length of (15.67 cm) after ten months of storage period followed by T_{12} (14.88 cm) and T_{14} (14.87 cm). Markedly lower root length of (14.16 cm) was recorded in T_1-control after ten months of storage.

4.1.3 Shoot length (cm)

The data on shoot length as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 5.

The mean shoot length decreased from 16.20 cm at the initial stage to 11.21cm after ten months storage.

Shoot length due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) recorded higher shoot length of 11.46 cm after ten months of storage period followed by T_{12} (11.41 cm) and T_{14} (11.40 cm). Markedly lower shoot length of 10.30 cm was recorded in T_1-control after ten months of storage.

4.1.4 Seedling length

The data on seedling length as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 6.

The mean seedling length decreased from 34.45 cm at the initial stage to 25.88 cm after ten months of storage.

Seedling length due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}: (60% N_2 + 0% O_2 + 40% CO_2) recorded higher seedling length of (27.13 cm) after ten months of storage period followed by T_{12} (26.30 cm) and T_{14} (26.27 cm). Markedly lower seedling length of (24.46 cm) was recorded in T_1-control after ten months of storage.

4.1.5 Seedling vigour index-I

The data on seedling vigour index-I as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 7.

The mean seedling vigour index-I decreased from 3238 at the initial stage to 1797 after ten months of storage.

Table 5. Influence of modified atmospheric storage conditions on shoot length (cm) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	15.81	14.40	13.70	12.40	10.30
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	15.85	14.87	14.02	13.22	11.10
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	15.87	14.89	14.03	13.35	11.16
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	15.88	14.90	14.43	13.42	11.18
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	15.90	14.93	14.47	13.44	11.20
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	15.95	15.05	14.55	13.54	11.25
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	15.96	15.08	14.56	13.59	11.26
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	15.97	15.10	14.58	13.62	11.30
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	15.99	15.17	14.60	13.65	11.32
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	16.00	15.19	14.72	13.66	11.34
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	16.01	15.26	14.78	13.69	11.35
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	16.05	15.31	14.93	13.73	11.41
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	16.08	15.60	14.95	13.80	11.46
T_{14} : Vacuum	16.03	15.21	14.92	13.71	11.40
Mean	**15.95**	**15.06**	**14.51**	**13.48**	**11.21**
S.Em±	0.171	0.175	0.141	0.079	0.069
CD (5%)	NS	0.508	0.408	0.230	0.202

NS – Non significant

Table 6. Influence of modified atmospheric storage conditions on seedling length (cm) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	33.08	30.90	29.82	27.90	24.46
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	33.13	31.70	30.26	28.82	25.49
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	33.18	31.74	30.28	29.13	25.57
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	33.22	31.77	30.76	29.22	25.62
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	33.25	31.85	30.88	29.41	25.70
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	33.32	32.08	31.09	29.54	25.77
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	33.34	32.13	31.19	29.62	25.89
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	33.37	32.16	31.26	29.68	25.90
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	33.43	32.25	31.32	29.73	25.99
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	33.53	32.33	31.55	29.76	26.11
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	33.58	32.42	31.67	29.83	26.20
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	33.79	32.66	31.88	29.92	26.30
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	33.88	33.11	31.98	30.11	27.13
T_{14} : Vacuum	33.64	32.54	31.83	29.87	26.27
Mean	**33.40**	**32.11**	**31.12**	**29.46**	**25.88**
S.Em±	0.258	0.224	0.235	0.157	0.230
CD (5%)	NS	0.650	0.681	0.456	0.666

NS – Non significant

Table 7. Influence of modified atmospheric storage conditions on seedling vigour index-I of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	2909	2554	2157	1907	1471
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	2952	2655	2299	2153	1630
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	2964	2672	2322	2214	1656
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	2979	2690	2430	2230	1668
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	2991	2700	2455	2255	1705
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	3032	2735	2487	2299	1741
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	3040	2742	2506	2315	1771
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	3059	2795	2584	2345	1787
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	3074	2807	2613	2359	1824
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	3091	2855	2671	2423	1858
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	3100	2886	2703	2456	1914
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	3131	2947	2784	2543	2048
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	3156	3051	2814	2601	2124
T_{14} : Vacuum	3109	2900	2764	2514	1958
Mean	**3042**	**2785**	**2542**	**2330**	**1797**
S.Em±	66.87	37.10	31.09	25.75	38.01
CD (5%)	NS	107.48	90.07	74.60	110.12

NS – Non significant

Seedling vigour index-I due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) recorded higher seedling vigour index-I of 2124 after ten months of storage period followed by T_{12}- 2048 and T_{14}- 1958. Markedly lower seedling vigour index-I of 1471 was recorded in T_1-control after ten months of storage.

4.1.6 Seedling vigour index-II

The data on seedling vigour index-II as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 8.

The mean seedling vigour index-II decreased from 8234 at the initial stage to 5472 after ten months of storage.

Seedling vigour index-II due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) recorded higher seedling vigour index-II of 6271 after ten months of storage period followed by T_{12}- 6233 and T_{14}- 5965. Markedly lower seedling vigour index-II of 4611 was recorded in T_1-control after ten months of storage.

4.1.7 Seedling dry weight (mg)

The data on seedling dry weight as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 9.

The mean seedling dry weight decreased from 87.60 mg at the initial stage to 78.88 mg after ten months of storage.

Seedling dry weight due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}:(40%N_2+0%O_2+60%CO_2) recorded higher seedling dry weight 80.09 mg after ten months of storage period followed by T_{12} (80.05 mg) and T_{14} (80.04 mg). Markedly lower seedling dry weight of (76.67 mg) was recorded in T_1-control after ten months of storage.

4.1.8 Field emergence (%)

The data on field emergence as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 10.The mean field emergence decreased from 90% at the initial stage to 60.45% after ten months of storage.

Table 8. Influence of modified atmospheric storage conditions on seedling vigour index-II of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	7569	6778	5787	5361	4611
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	7660	7004	6236	6025	4994
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	7687	7046	6313	6136	5054
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	7719	7098	6511	6165	5089
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	7744	7121	6578	6203	5192
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	7838	7165	6650	6302	5291
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	7856	7179	6680	6334	5365
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	7905	7322	6874	6408	5423
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	7932	7337	6938	6438	5597
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	7950	7449	7044	6608	5682
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	7967	7515	7100	6692	5843
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	7997	7627	7273	6909	6233
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	8043	7794	7346	7023	6271
T_{14} : Vacuum	7973	7529	7227	6841	5965
Mean	**7846**	**7283**	**6754**	**6389**	**5472**
S.Em±	163.98	87.92	79.96	63.96	117.58
CD (5%)	NS	254.72	231.66	185.29	340.63

NS – Non significant

Table 9. Influence of modified atmospheric storage conditions on seedling dry weight (mg/seedling) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	86.00	82.00	80.00	78.43	76.67
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	86.04	83.65	82.04	80.69	78.00
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	86.05	83.71	82.33	80.73	78.08
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	86.08	83.83	82.42	80.76	78.15
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	86.10	84.00	82.75	80.90	78.27
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	86.13	84.05	83.12	80.97	78.33
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	86.18	84.13	83.15	81.03	78.40
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	86.20	84.25	83.16	81.11	78.60
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	86.22	84.30	83.18	81.13	79.77
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	86.26	84.33	83.20	81.18	79.88
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	86.28	84.41	83.21	81.26	80.01
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	86.31	84.53	83.28	81.29	80.05
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	86.33	84.56	83.47	81.32	80.09
T_{14} : Vacuum	86.29	84.51	83.23	81.27	80.04
Mean	**86.18**	**84.02**	**82.75**	**80.86**	**78.88**
S.Em±	0.175	0.282	0.389	0.394	0.617
CD (5%)	NS	0.817	1.128	1.144	1.787

NS – Non significant

Table 10. Influence of modified atmospheric storage conditions on field emergence (%) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	83.02 (65.70) *	76.00 (60.70)	67.20 (55.09)	61.10 (51.44)	54.20 (47.43)
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	84.06 (66.51)	77.62 (61.80)	72.30 (58.28)	64.49 (53.45)	57.37 (49.26)
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	84.43 (66.80)	77.78 (61.91)	72.91 (58.67)	64.63 (53.53)	57.71 (49.46)
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	84.56 (66.93)	78.54 (62.44)	73.33 (58.95)	64.86 (53.68)	58.40 (49.86)
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	84.77 (67.06)	78.60 (62.48)	74.00 (59.38)	65.28 (53.94)	58.50 (49.92)
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	84.86 (67.14)	78.84 (62.65)	75.29 (60.23)	65.51 (54.67)	58.60 (49.98)
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	85.00 (67.26)	79.00 (62.76)	76.01 (60.70)	66.76 (54.82)	58.70 (50.04)
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	85.33 (67.53)	79.10 (62.83)	80.48 (63.82)	68.10 (55.64)	59.37 (50.43)
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	85.41 (67.58)	79.24 (62.93)	81.23 (64.36)	68.47 (55.87)	59.80 (50.68)
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	85.48 (67.64)	79.32 (62.98)	82.20 (65.08)	69.53 (56.53)	61.00 (51.38)
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	85.60 (67.64)	79.50 (63.11)	82.58 (65.36)	70.31 (57.02)	63.29 (52.74)
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	85.78 (67.92)	84.33 (66.72)	83.33 (65.95)	73.33 (58.95)	66.40 (54.60)
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	86.56 (68.53)	84.67 (66.99)	83.60 (66.16)	76.67 (61.15)	67.30 (55.15)
T_{14} : Vacuum	85.68 (67.82)	84.27 (66.88)	82.67 (65.43)	73.13 (58.81)	65.70 (54.18)
Mean	**85.03**	**79.77**	**77.65**	**68.012**	**60.45**
S.Em±	0.520	0.299	0.464	0.498	0.323
CD (5%)	NS	0.868	1.345	1.444	0.937

NS – Non significant* Figures in the parenthesis indicate arc sign transformed values

Field emergence due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13} :(40% N_2 + 0% O_2 + 60% CO_2) recorded higher field emergence (67.30%) after ten months of storage period followed by T_{12} (66.40%) and T_{14} (65.70%). Markedly lower field emergence of (54.20%) was recorded in T_1-control after ten months of storage.

4.1.9 Speed of germination

The data on speed of germination as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 11.

The mean speed of germination decreased from 26 at the initial stage to 19.53 after ten months of storage.

Speed of germination due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) recorded higher Speed of germination of 20.04 after ten months of storage period followed by T_{12} 19.97 and T_{14} 19.93. Markedly lower Speed of germination 17.97 was recorded in T_1-control after ten months of storage.

4.1.10 Test weight (g)

The data on test weight as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 12.

Test weight declined with the advancement of storage period. The mean test weight decreased from 11.02 g at the initial stage to 9.90 g after ten months of storage.

Test weight due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) recorded higher test weight of (10.16 g) after ten months of storage period followed by T_{12} (10.12 g) and T_{14} (10.08 g). Markedly lower test weight of (9.29 g) was recorded in T_1-control after ten months of storage.

4.1.11 Seed infection (%)

The data on seed infection as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 13.

Seed infection Increased with the advancement of storage period. The mean seed infection (%) increased from (0%) at the initial stage to (13.03%) after ten months of storage.

Table 11. Influence of modified atmospheric storage conditions on speed of germination of pigeonpea seeds

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	23.80	22.67	20.57	19.21	17.97
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	24.39	22.72	21.61	20.27	19.18
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	24.42	22.74	21.68	20.34	19.34
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	24.50	22.76	21.71	20.42	19.38
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	24.55	22.80	21.75	20.51	19.43
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	24.63	22.81	22.77	20.60	19.58
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	24.70	22.92	22.82	20.72	19.63
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	24.83	22.99	21.88	20.88	19.69
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	24.86	23.06	21.91	20.92	19.71
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	24.93	23.28	22.18	20.94	19.81
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	24.95	23.38	22.26	21.01	19.86
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	25.00	23.57	22.34	21.07	19.97
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	25.02	23.65	22.40	21.10	20.04
T_{14} : Vacuum	24.98	23.39	22.29	21.04	19.93
Mean	**24.68**	**23.05**	**22.01**	**20.64**	**19.53**
S.Em±	0.257	0.194	0.119	0.175	0.178
CD (5%)	NS	0.562	0.346	0.507	0.518

NS – Non significant

Table 12. Influence of modified atmospheric storage conditions on test weight (g) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	10.20	10.14	10.09	9.85	9.29
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	10.30	10.28	10.19	9.97	9.57
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	10.35	10.33	10.28	10.00	9.76
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	10.43	10.37	10.34	10.02	9.89
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	10.45	10.39	10.37	10.05	9.84
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	10.48	10.40	10.39	10.08	9.91
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	10.52	10.44	10.35	10.10	9.96
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	10.57	10.47	10.41	10.12	9.99
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	10.63	10.50	10.40	10.13	10.00
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	10.66	10.53	10.42	10.15	10.03
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	10.72	10.55	10.44	10.17	10.05
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	10.75	10.65	10.51	10.25	10.12
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	10.76	10.69	10.53	10.28	10.16
T_{14} : Vacuum	10.74	10.59	10.45	10.24	10.08
Mean	**10.54**	**10.45**	**10.37**	**10.10**	**9.90**
S.Em±	0.191	0.097	0.071	0.044	0.133
CD (5%)	NS	0.283	0.207	0.130	0.387

NS – Non significant

Table 13. Influence of modified atmospheric storage conditions on seed infection (%) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	3.02 (10.01) *	6.35 (14.60)	11.36 (19.71)	14.70 (22.56)	18.01 (25.12)
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	2.73 (9.51)	5.80 (13.94)	9.69 (18.15)	11.30 (19.65)	14.59 (22.47)
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	2.67 (9.39)	5.63 (13.72)	9.53 (17.99)	11.05 (19.42)	14.50 (22.39)
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	2.54 (9.15)	5.47 (13.53)	9.47 (17.93)	10.96 (19.34)	14.33 (22.26)
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	2.46 (9.03)	5.24 (13.24)	9.19 (17.65)	10.87 (19.25)	13.83 (21.83)
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	2.31 (8.71)	4.64 (12.44)	8.81 (17.27)	10.80 (19.20)	13.67 (21.70)
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	1.98 (8.08)	4.13 (11.73)	8.56 (17.02)	10.62 (19.02)	13.57 (21.63)
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	1.91 (7.95)	3.35 (10.53)	8.13 (16.58)	10.46 (18.88)	12.78 (20.96)
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	1.86 (7.83)	3.10 (10.15)	8.08 (16.53)	10.38 (18.81)	12.54 (20.75)
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	1.41 (6.82)	1.96 (8.05)	5.29 (13.30)	8.08 (16.52)	11.98 (20.26)
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	1.12 (6.08)	1.91 (7.95)	5.25 (13.25)	8.04 (16.48)	11.90 (20.19)
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	0.89 (5.42)	0.82 (5.20)	4.18 (11.80)	7.51 (15.91)	10.23 (18.66)
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	0.00 (0.00)	0.80 (5.11)	4.14 (11.75)	7.48 (15.88)	10.15 (18.59)
T_{14} : Vacuum	0.93 (5.54)	0.87 (5.36)	4.21 (11.85)	7.68 (16.09)	10.43 (18.82)
Mean	**1.84**	**3.57**	**7.56**	**9.99**	**13.03**
S.Em±	0.250	0.233	0.139	0.136	0.322
CD (5%)	0.724	0.675	0.404	0.394	0.934

* Figures in the parenthesis indicate arc sign transformed values

Seed infection (%) due to MASC differed significantly in all the months of storage. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) showed lowest seed infection of (10.15%) after ten months of storage period followed by T_{12} (10.23%) and T_{14} (10.43%). Markedly highest seed infection of (18.01%) was recorded in T_1- control after ten months of storage.

4.1.12 Per cent insect damage

The data on per cent insect damage as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 14.

Per cent insect damage Increased with the advancement of storage period. The mean insect damage increased from 0 per cent at the initial stage to 25.25 per cent after ten months of storage

Per cent insect damage due to MASC differed significantly in all the months of storage. The seeds exposed to T_{12}: (60% N_2 + 0% O_2 + 40% CO_2) and T_{13} - (40% N_2 + 00% O_2+ 60% CO_2) recorded zero per cent insect damage after ten months of storage period followed by T_{14} (5.5) per cent and T_{11} (5.8) per cent. Markedly highest insect damage (60 %) was observed in T_1-control after ten months of storage.

4.1.13 Moisture content (%)

The data on moisture content as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 15.

The mean moisture content increased from 7.55 per cent at the initial stage to 8.85 per cent after ten months of storage.

Among the modified atmospheric storage conditions there was no significant difference in all the months of storage except ten months after storage. However, the seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) recorded lowest moisture content (8.68%) after ten months of storage period followed by T_{12} (8.71%) and T_{14} (8.72%). Markedly highest seed moisture content (9.04%) was recorded in T_1-control after ten months of storage.

4.2 Biochemical parameters

4.2.1 Dehydrogenase enzyme activity (OD value)

The data on dehydrogenase activity as influenced Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 16 and Plate 8.

Table 14. Influence of modified atmospheric storage conditions on per cent insect damage of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	8.0	14.0	24.3	41.3	60.0
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	6.5	12.5	22.1	38.7	57.0
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	6.3	12.0	21.7	38.3	56.2
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	5.9	11.8	20.2	37.0	55.5
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	5.7	11.6	19.7	36.7	54.7
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	3.0	6.3	7.6	10.0	14.0
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	2.5	5.5	6.7	9.5	13.3
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	2.2	5.1	6.4	9.2	13.0
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	2.0	4.8	6.2	8.2	12.3
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	1.0	1.9	3.2	4.4	6.2
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	1.0	1.7	2.6	4.0	5.8
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	0.0	0.0	0.0	0.0	0.0
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	0.0	0.0	0.0	0.0	0.0
T_{14} : Vacuum	1.0	1.4	2.3	3.8	5.5
Mean	**3.22**	**6.33**	**10.21**	**17.21**	**25.25**
S.Em±	0.335	0.723	0.515	0.992	0.907
CD (5%)	0.973	2.096	1.494	2.876	2.629

Table 15. Influence of modified atmospheric storage conditions on moisture content (%) pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	8.00	8.13	8.41	8.71	9.04
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	7.98	8.11	8.40	8.70	9.00
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	7.97	8.10	8.40	8.70	8.98
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	7.97	8.10	8.40	8.69	8.95
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	7.96	8.09	8.39	8.69	8.91
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	7.96	8.09	8.39	8.68	8.87
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	7.95	8.08	8.38	8.68	8.85
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	7.95	8.08	8.38	8.67	8.83
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	7.94	8.07	8.38	8.67	8.81
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	7.94	8.07	8.37	8.66	8.79
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	7.94	8.07	8.37	8.66	8.77
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	7.92	8.06	8.36	8.65	8.71
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	7.91	8.05	8.35	8.64	8.68
T_{14} : Vacuum	7.93	8.06	8.37	8.66	8.72
Mean	**7.95**	**8.08**	**8.38**	**8.67**	**8.85**
S.Em±	0.016	0.023	0.023	0.014	0.040
CD (5%)	NS	NS	NS	NS	0.117

NS – Non significant

Dehydrogenase activity declined with the advancement of storage period. The mean dehydrogenase activity decreased from 0.802 OD value at the initial stage to 0.240 OD value after ten months of storage.

Dehydrogenase activity due to MASC differed significantly in all the months of storage. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) recorded higher OD value of 0.276 after ten months of storage period followed by T_{12} - 0.271 and T_{14} - 0.269. Markedly lower Dehydrogenase activity 0.211 OD value was recorded in T_1-control after ten months of storage.

4.2.2 Electrical conductivity of seed leachates (dSm^{-1})

The data on electrical conductivity of seed leachates (EC) as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 17.

Electrical conductivity of seed leachates (EC) increased with the advancement of storage period. The mean value of seed leachates (EC) increased from 0.513 dSm^{-1} at the beginning to 2.083 dSm^{-1} after ten months of storage.

Electrical conductivity of seed leachates (EC) due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) showed lower EC value (2.029) after ten months of storage period which was preceded by T_{12} (2.042), T_{14} (2.045), T_{11} (2.057) and T_{10} (2.059) whereas, higher EC value noticed in T_1-control (2.207) after ten months of storage.

4.2.3 Protein content (%)

The data on protein content as influenced by Modified Atmospheric Storage Conditions (MASC) during storage are presented in Table 18.

Seed protein declined with the advancement of storage period. The mean protein content decreased from 20.90% at the initial stage to 19.03% after ten months of storage.

Protein content due to MASC differed significantly in all the months of storage except for the second month. The seeds exposed to T_{13}: (40% N_2 + 0% O_2 + 60% CO_2) recorded higher Protein content of 19.33% after ten months of storage period followed by T_{12}- 19.26% and T_{14}-19.19%. Markedly lower protein content of 18.13 % was recorded in T_1-control after ten months of storage.

Table 16. Influence of modified atmospheric storage conditions on total dehydrogenase activity (OD value) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	0.691	0.572	0.455	0.357	0.211
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	0.700	0.603	0.485	0.367	0.220
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	0.706	0.616	0.490	0.371	0.223
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	0.719	0.627	0.492	0.379	0.226
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	0.755	0.630	0.494	0.386	0.229
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	0.761	0.639	0.498	0.387	0.232
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	0.768	0.645	0.504	0.394	0.234
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	0.764	0.657	0.516	0.398	0.237
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	0.765	0.660	0.524	0.401	0.240
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	0.766	0.662	0.549	0.405	0.245
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	0.768	0.664	0.551	0.408	0.250
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	0.772	0.671	0.556	0.429	0.271
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	0.777	0.677	0.561	0.432	0.276
T_{14} : Vacuum	0.770	0.669	0.553	0.425	0.269
Mean	**0.748**	**0.642**	**0.516**	**0.395**	**0.240**
S.Em±	0.010	0.014	0.007	0.006	0.006
CD (5%)	0.031	0.043	0.022	0.019	0.019

NS – Non significant

Table 17. Influence of modified atmospheric storage conditions on electrical conductivity (dSm^{-1}) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	0.639	0.906	1.272	1.861	2.207
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	0.632	0.877	1.266	1.796	2.153
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	0.627	0.859	1.263	1.792	2.106
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	0.623	0.853	1.257	1.777	2.098
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	0.620	0.850	1.233	1.771	2.084
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	0.619	0.811	1.221	1.767	2.077
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	0.615	0.801	1.216	1.759	2.072
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	0.613	0.791	1.192	1.741	2.070
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	0.610	0.789	1.187	1.739	2.062
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	0.609	0.786	1.183	1.735	2.059
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	0.607	0.785	1.182	1.733	2.057
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	0.598	0.768	1.170	1.726	2.042
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	0.597	0.765	1.165	1.721	2.029
T_{14} : Vacuum	0.602	0.772	1.180	1.728	2.045
Mean	**0.615**	**0.815**	**1.213**	**1.760**	**2.083**
S.Em±	0.011	0.013	0.016	0.018	0.024
CD (5%)	NS	0.037	0.049	0.053	0.072

NS – Non significant

Table 18. Influence of modified atmospheric storage conditions on protein content (%) of pigeonpea seeds during storage

Treatments	Months of storage (July-2012 to April 2013)				
	2	**4**	**6**	**8**	**10**
T_1 : Control	20.03	19.92	19.72	18.23	18.13
T_2 : 70% N_2 + 20% O_2 + 10% CO_2	20.06	19.98	19.79	19.00	18.93
T_3 : 60% N_2 + 20% O_2 + 20% CO_2	20.11	20.02	19.84	19.20	18.98
T_4 : 40% N_2 + 20% O_2 + 40% CO_2	20.13	20.04	19.86	19.33	19.00
T_5 : 20% N_2 + 20% O_2 + 60% CO_2	20.17	20.07	19.88	19.36	19.02
T_6 : 80% N_2 + 10% O_2 + 10% CO_2	20.22	20.08	19.93	19.44	19.07
T_7 : 70% N_2 + 10% O_2 + 20% CO_2	20.25	20.10	19.94	19.50	19.08
T_8 : 50% N_2 + 10% O_2 + 40% CO_2	20.28	20.12	19.96	19.52	19.10
T_9 : 30% N_2 + 10% O_2 + 60% CO_2	20.29	20.13	19.98	19.54	19.12
T_{10} : 90% N_2 + 00% O_2 + 10% CO_2	20.30	20.14	20.00	19.56	19.14
T_{11} : 80% N_2 + 00% O_2 + 20% CO_2	20.32	20.16	20.01	19.58	19.16
T_{12} : 60% N_2 + 00% O_2 + 40% CO_2	20.43	20.22	20.05	19.63	19.26
T_{13} : 40% N_2 + 00% O_2 + 60% CO_2	20.59	20.35	20.08	19.65	19.33
T_{14} : Vacuum	20.34	20.20	19.99	19.60	19.19
Mean	**20.25**	**20.10**	**19.93**	**19.36**	**19.03**
S.Em±	0.406	0.050	0.037	0.246	0.121
CD (5%)	NS	0.147	0.108	0.714	0.352

NS – Non significant

4.3 Analysis of Protein zymogram after ten months of seed storage

Protein profiles observed after ten months of Modified Atmospheric Storage Conditions of seeds are presented in Table 19, Plate 9 and Fig 1. Seeds exposed to 0% O_2 and vacuum condition altogether 8 protein bands were present in each treatment. When seeds exposed to (10% O_2) altogether 6 protein bands were present. But 2 and 5 bands were absent, remaining 4 bands were present in each treatment. In case of (20% of O_2) altogether 5 bands were present but 2, 3 and 5 bands were absent and remaining 5 bands were present in each treatment. In case of control altogether 4 bands were present but 1, 2, 3 and 5 bands were absent.

Table 19. Analysis of protein bands in SDS PAGE after ten months of modified atmospheric storage conditions of pigeonpea seeds

Band number	Treatments													
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}
1	-	+	+	+	+	+	+	+	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-	-	-	+	+	+	+
3	-	-	-	-	-	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	-	-	-	-	-	-	-	-	-	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+

T_1 - Control

T_2 - 70% N_2 + 20% O_2 + 10% CO_2

T_3 - 60% N_2 + 20% O_2 + 20% CO_2

T_4 - 40% N_2 + 20% O_2 + 40% CO_2

T_5 - 20% N_2 + 20% O_2 + 60% CO_2

T_6 - 80% N_2 + 10% O_2 + 10% CO_2

T_7 - 70% N_2 + 10% O_2 + 20% CO_2

T_8 - 50% N_2 + 10% O_2 + 40% CO_2

T_9 - 30% N_2 + 10% O_2 + 60% CO_2

T_{10} -90% N_2 + 00% O_2 + 10% CO_2

T_{11} - 80% N_2 + 00% O_2 + 20% CO_2

T_{12} - 60% N_2 + 00% O_2 + 40% CO_2

T_{13} - 40% N_2 + 00% O_2 + 60% CO_2

T_{14} - Vacuum

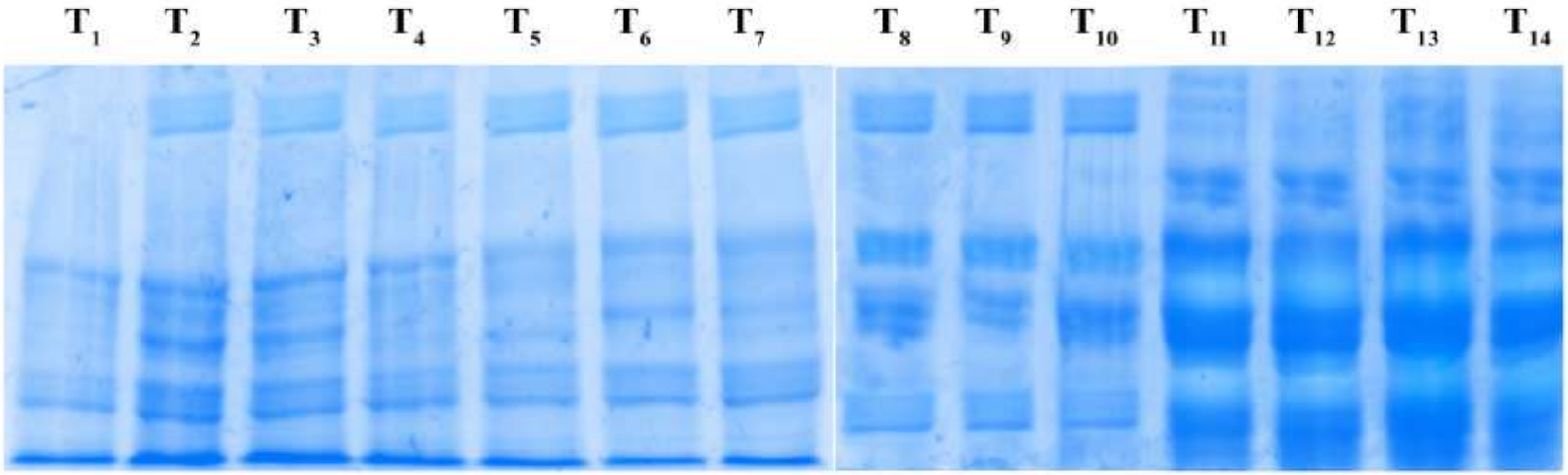

Plate 9: Effect of modified atmospheric storage conditions on Protein profile of pigeonpea seeds after 10 months of storage

T_1-Control
T_2-70% N_2+20%O_2+10% CO_2
T_3-60% N_2+20%O_2+20% CO_2
T_4-40% N_2+20%O_2+40% CO_2
T_5-20% N_2+20%O_2+60% CO_2
T_6-80%N_2+10%O_2+10% CO_2
T_7-70%N_2+10%O_2+20% CO_2
T_8-50%N_2+10%O_2+40% CO_2
T_9-30%N_2+10%O_2+60% CO_2
T_{10}-90%N_2+00%O_2+10% CO_2
T_{11}-80% N_2+00% O_2+20% CO_2
T_{12}-60% N_2+00% O_2+40% CO_2
T_{13}-40% N_2+00% O_2+60% CO_2
T_{14}-Vacuum

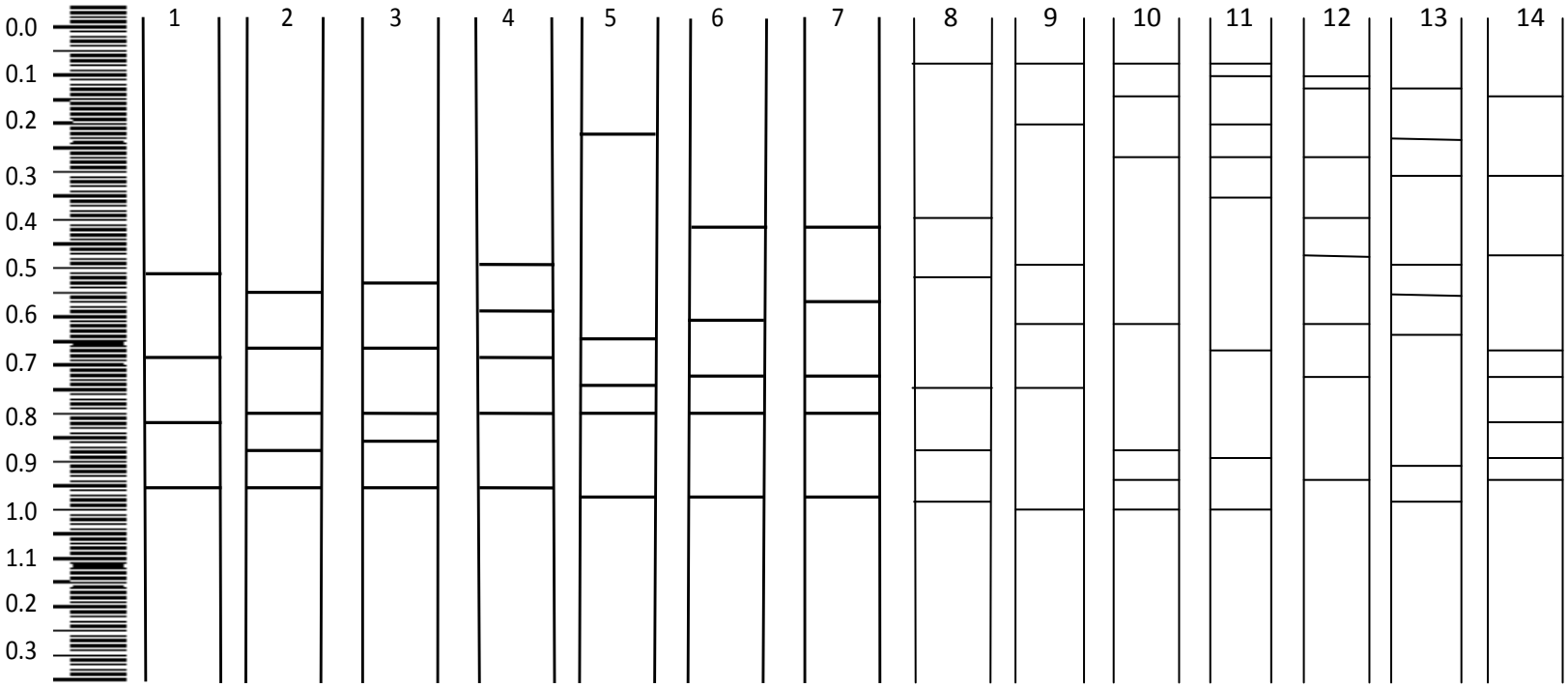

Fig.1.Zymogram of total soluble proteins of pigeonpea seeds at different treatments of modified atmospheric storage conditions in SDS denaturing gel

Discussion

V. DISCUSSION

Pigeonpea is an important pulse crop in India for its rich source of protein. There is a sizeable quantitative and qualitative loss of pigeonpea seeds due to adverse effect of several biotic and abiotic factors during storage. The maintenance of high quality in seed during storage is of greater importance. Hence, it is appropriate to give due emphasis to reduce qualitative as well as quantitative losses of pigeonpea seeds during storage.

After the pigeonpea seeds are harvested, controlling quality deterioration becomes more difficult because the seeds are much more prone to attack by more than 200 species of insect pests causing enormous losses. The losses have been estimated to vary from 46.6 to 63.6 per cent, both in field and as well as in storage condition. Better methods and techniques are needed to improve conditions and environments that cause quality deterioration. A low-oxygen atmosphere system for handling of pigeonpea seeds appears to have potential for improving storage practices.

Therefore, an alternative to toxic residue building chemical fumigants to protect stored seed from insect pest infestations and also to slow down seed deterioration is Modified Atmospheric Storage. Alternatively high carbon dioxide treatment is residue free and approved by Environmental Protection Agency, USA. Carbon dioxide treated seeds are also accepted in the organic market.

Keeping this in view, an experiment was conducted in order to know the seed storability of pigeonpea cultivar BSMR-736 by subjecting to different modified atmospheric storage conditions with different combination of gases like nitrogen, carbon dioxide and oxygen at different concentrations. The Experiment consisted of 14 testaments *viz.*, (T_1 - Control, T_2 - (70% N_2+20% O_2 +10% CO_2), T_3 - (60% N_2 + 20% O_2 + 20% CO_2), T_4 - (40% N_2 +20% O_2 + 40% CO_2), T_5 - (20% N_2 + 20% O_2 + 60% CO_2), T_6 - (80% N_2 + 10% O_2 + 10% CO_2), T_7 - (70% N_2 + 10% O_2 + 20% CO_2), T_8 - (50% N_2 + 10% O_2 + 40% CO_2), T_9 - (30% N_2 + 10% O_2 + 60% CO_2), T_{10} - (90% N_2 + 00% O_2 + 10% CO_2), T_{11} - (80% N_2 + 00% O_2 + 20% CO_2), T_{12} - (60% N_2 + 00% O_2 + 40% CO_2), T_{13} -(40% N_2 + 00% O_2+ 60% CO_2), T_{14}-Vacuum).

The seeds exposed to these gas combinations were stored in the 700 gauge polyethylene bags for 10 months under ambient condition of Raichur from July 2012 to

April 2013 and the experiment was carried out in completely randomised design in three replications and observations on various seed quality parameters were recorded bimonthly.

The results of different seed quality parameters recorded from this experiment are discussed here under.

5.1 Influence of modified atmospheric storage conditions on seed quality and longevity of pigeonpea

In the present study the modified atmospheric storage conditions exhibited significant effect on seed germination of pigeonpea seeds. The seeds which were stored with the gaseous combination of 40% N_2 + 0% O_2 + 60% CO_2 showed better germination throughout the storage period followed by the gaseous combination of 60% N_2 + 0% O_2 + 40% CO_2 and seeds stored under vacuum condition.

The germination percentage was significantly higher (78.30%) in T_{13} (40% N_2 + 0% O_2 + 60% CO_2) followed by T_{12} (60% N_2 + 0% O_2 + 40% CO_2) i.e. 77.87%, T_{14}- Vacuum 74.53% and T_{11}- (80% N_2 + 0% O_2 + 20% CO_2) which was about 73.03% at the end of ten months of storage period.

Modified atmosphere storage of seeds devoid of oxygen showed retention of higher seed viability for an appreciable period. Both seed viability and vigour were well preserved with modified atmospheric storage particularly with carbon dioxide and vacuum condition.

The probable reason for differences in longevity of seeds in the modified atmospheric storage conditions might be due to the variation in the gas concentrations, where the treatments T_{13} , T_{12} having gas combination of higher CO_2 with zero percentage of oxygen concentration *i.e.*, low oxygen atmosphere and also the seeds stored under vacuum condition showed better germination. Germination was reported to decrease in peas with increase in oxygen level (Roberts and Abdalla, 1968). Under the vacuum condition seed quality could be preserved even under higher temperature as reported by Barzalli *et al*. (2005).

In general, ageing is manifested by the decrease of metabolic activity and an increase of catabolic processes (Gorecki *et al*., 1996). In particular, an oxidative stress might be reduced in O_2 - free storage atmospheres (Justice and Bass, 1978; Wilson

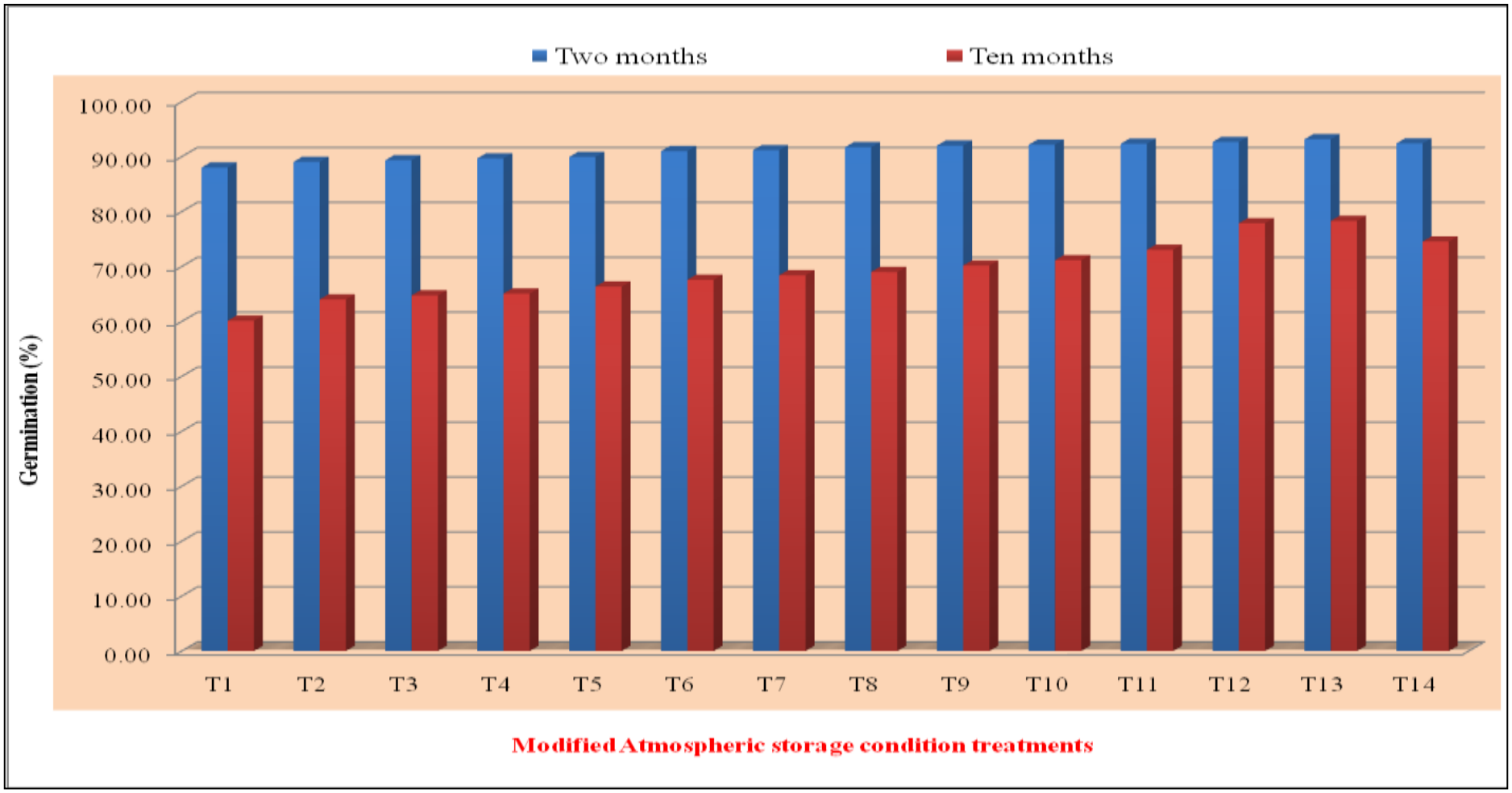

Fig. 2. Influence of modified atmospheric storage conditions on germination (%) of pigeonpea seeds during storage

McDonald, 1986 and Benson, 1990). It should be noted that seed deterioration during storage could result in marked changes in the content and activity of enzymes capable for degrading the stored reserves (Priestley, 1986; Smith and Berjak, 1995 and Walters, 1998). In the present investigation it was observed that the dehydrogenase activity in the seeds was maximum and hence, better maintenances of seed quality in modified atmospheric storage condition compared to control. Another reason for seed ageing may be the accumulation of deleterious effects on membranes due to oxidative damages to fatty acids and proteins denaturation as a result of maillard reactions (Narayana Murthy and Sun, 2000). The advantage of higher seed reserve utilization efficiency in seeds stored in vacuum, provide energy for a faster growing rate of the seedlings. In the present study also maximum speed of germination (20.04, 19.97 and 19.93) was noticed in the treatment T_{13} (40% N_2 + 0% O_2 + 60% CO_2), T_{12} - (60% N_2 + 0% O_2 + 40% CO_2) and T_{14} Vacuum respectively. The similar results were also reported by Guillaumin (1928), Rathi *et al*. (2000), Bera *et al.* (2004) and Bera *et al*. (2008).

Another advantage of low oxygen method is that the moisture content of pigeonpea seed stored in the container does not change very much. A similar study by Slay *et al.* (1985) also indicate that up to 20% less storage space was required for the low oxygen method. The moisture content of seed plays a major role in determination of seed storability (Copeland and Mcdonald, 1995).

The root length and shoot length of pigeonpea seeds were decreased gradually with the advancement in storage period. However, highest root length was recorded in T_{13} - (40% N_2 + 0% O_2 + 60% CO_2) (15.67 cm) followed by T_{12} - (60% N_2 + 0% O_2 + 40% CO_2) (14.88 cm), while lowest root length was recorded in T_1 control (14.16 cm) at the end of ten months of storage period.

At the end of ten months of storage period, highest shoot length was recorded in T_{13} - (40% N_2 + 0% O_2 + 60% CO_2) (11.46 cm) followed by T_{12} - (60% N_2 + 0% O_2 + 40% CO_2) (11.41 cm), while lowest shoot length was recorded (10.30 cm) in T_1 control.

The decline in root and shoot length might be due to the damage caused by fungi and insects and also toxic metabolites which might have hindered the seedling growth. Similar findings were reported in onion seeds by Shivappa (2011) and Shrishail (2011) in groundnut. Deterioration in seed quality associated with decrease in root and shoot length with the passage of time had been confirmed by earlier workers in many crops.

The significant difference due to modified atmospheric storage conditions on seedling length was recorded throughout the storage period except in the second month. At the end of ten months of storage period, highest seedling length was recorded in T_{13} (40% N_2 + 0% O_2 + 60% CO_2) (27.13 cm) followed by T_{12} - (60% N_2 + 0% O_2 + 40% CO_2) (26.30 cm), while the lowest seedling length was recorded in T_1 (control) (24.46 cm). This gradual decline in seedling length might be due to damage caused by fungi and insects and also toxic metabolites which might have hindered the seedling growth.

In the present study, highest vigour index-I and vigour index-II was recorded in T_{13} - (40% N_2 + 0% O_2 + 60 % CO_2) (2124) (6271) followed by T_{12} - (60 % N_2 + 0% O_2 + 40% CO_2) (2048) (6233), while lowest vigour index was recorded in T_1 control (1471) (4611) respectively at the end ten months of storage period. Gradual decline in seed vigour index was noticed due to age induced decline in germination, decrease in dry matter accumulation in seedling and decrease in seedling length.

The significant difference due to modified atmospheric storage conditions on seedling dry weight was recorded throughout the storage period except in the second month. At the end of ten months of storage period, highest seedling dry weight was recorded in T_{13} - (40% N_2 + 0% O_2 + 60% CO_2) (80.09 mg) followed by T_{12} - (60% N_2 + 0% O_2 + 40% CO_2) (80.05 mg), while the lowest seedling dry weight was recorded in T_1 (control) (76.67 mg). This gradual decline in seedling dry weight may be due to deterioration of seed, decreased in the germination percentage and root and shoot length. Seedling length, vigour index-I, vigour index-II and seedling dry weight these findings were also reported by Shivappa (2011) in onion and Shrishail (2011) in groundnut.

Significantly highest field emergence was recorded at fourth month to the end of tenth month of storage period. At the end of ten months of storage period highest field emergence was recorded in T_{13} (40% N_2 + 0% O_2 + 60% CO_2) (67.30%) followed by T_{12} - (60% N_2 + 0% O_2 + 40% CO_2) (66.40%) and T_{14} - Vacuum (65.70), while lowest Field emergence was recorded in T_1 control (54.20%). The decrease in field emergence may be due to deteriorative changes in cell and cell organelles and germination capacity of seed under natural soil condition. Where the treatments having gas combination of higher CO_2 with zero percentage of oxygen level showed better field emergence.

At the end of ten months of storage period highest test weight was recorded in T_{13} (40% N_2 + 0% O_2 + 60% CO_2) 10.16 g followed by T_{12} - (60% N_2 + 0% O_2 + 40% CO_2)

Fig.3.Influence of modified atmospheric storage conditions on field emergence (%) of pigeonpea seeds during storage

10.12 g while the lowest test weight was recorded in T_1 control (9.29 g). The decrease in the test weight was observed as the storage period increased. This may be due to infestation of the insects, which normally feed both internally and externally and also due to activity of the fungi the reduction in food storage occur in the seed as the ageing process is advanced. Shehata *et al.* (2009) also noticed lowest infestation and weight loss percentage in cowpea seeds exposed to gas mixture of 80% CO_2 + 4% O_2 + 16% N_2.

The germination (78.30%), shoot length (11.46 cm), root length (15.65 cm), seedling length (27.13 cm), vigour index-I (2124), vigour index-II (6271) seedling dry weight (80.09 mg), speed of germination (20.04) dehydrogenase activity (0.276 OD value), were significantly higher with lower moisture content (8.70%), EC value (2.029 dSm^{-1}) and seed infection (10.15%) in seeds stored under treatment T_{13} - (40% N_2 + 0% O_2 + 60% CO_2) when compared with other treatments. whereas, all these parameters were lowest in seeds stored in control *i.e*. without modified atmospheric storage conditions (Fig. 2-6).

Regarding the effect of the modified atmospheric storage conditions the lowest insect damage (0) per cent was noticed in the treatments T_{13} and T_{12} after ten months of storage. The reduction in insect activity might be due to high CO_2 content which is relatively more toxic for all developmental stages of *Callosobruchus maculatas* than low CO_2 content (Hashem *et al*., 1993, 1995 and Hashem and Reichmuth, 1994).

The atmosphere containing low concentration of oxygen was more toxic in the shorter exposure period (Hashem *et al.,* 1993; Hashem and Reichmuth, 1996; Hashem, 2000, Neeson and Banks, 2000). Another important issue related to insect disinfestation is the lack of oxygen, which is called anoxia. Lack of oxygen is a major reason for insect mortality; it increases the acid level in the form of lactic acid and causes poisoning (Mbata and Reichmuth, 1996).

Similarly, lowest seed infection was recorded in T_{13} (40% N_2 + 0% O_2 + 60% CO_2) 10.15% followed by T_{12} - (60% N_2 + 0% O_2 + 40% CO_2) 10.23 % .while, the highest seed infection was recorded in T_1 control (18.01%) at the end of ten months of storage period. These findings were also reported by Shivappa (2011) in onion and Shrishail (2011) in groundnut.

The lowest moisture content of seeds was recorded in T_{13} (40% N_2 + 0% O_2 + 60% CO_2) 8.68% followed by T_{12} -(60% N_2 + 0% O_2 + 40% CO_2) 8.71% .while, the highest

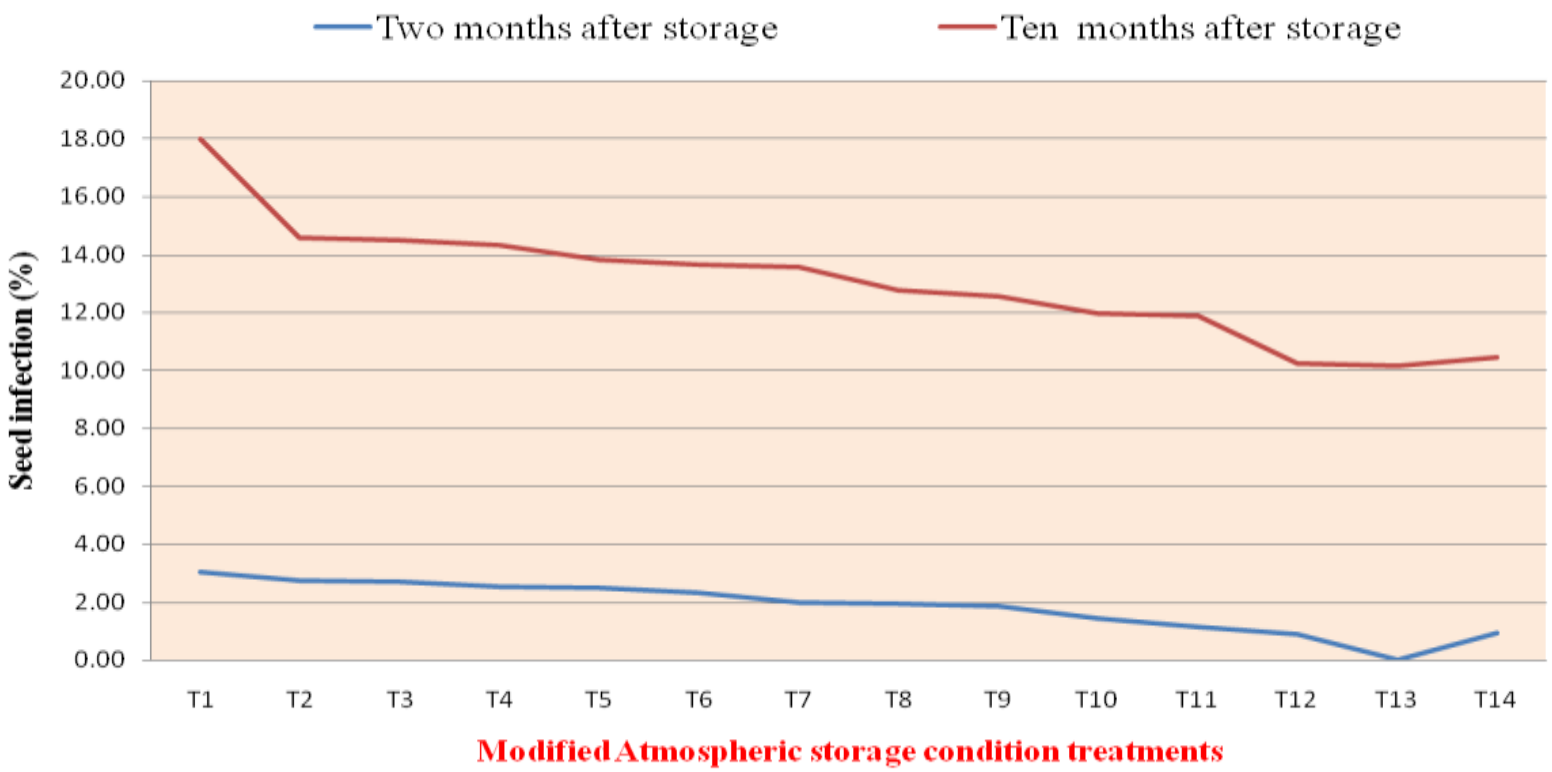

Fig.6.Influence of modified atmospheric storage conditions on seed infection (%) of pigeonpea seeds during storage

moisture content of seeds was recorded in T_1 control (9.04%) at the end of ten months of storage period. These findings were also reported by Shivappa (2011) in onion and Shrishail (2011) in groundnut.

5.2 Biochemical parameters

The dehydrogenase enzyme activity is a good stable metabolic marker to estimate the degree of vigour in seeds (Saxena *et al*., 1987) and have positive association with vigour and viability of seeds (Rurdrapal and Basu, 1970; Halder and Gupta, 1982 and Kharluki, 1983)

The significant difference due to modified atmospheric storage conditions on dehydrogenase activity was recorded throughout the storage period. At the end of ten months of storage period, highest dehydrogenase activity was recorded in T_{13} (40% N_2 + 0% O_2 + 60% CO_2) 0.276 followed by T_{12}-(60% N_2 + 0% O_2 + 40% CO_2) 0.271, while the lowest dehydrogenase activity was recorded in T_1 (control) 0.211. This gradual decline in dehydrogenase activity may be due to reduction in vigour level of seed as the deterioration occurs rapidly over a period of time under uncontrolled condition.

At the end of ten months of storage period, lowest electrical conductivity of seed leachate was recorded in T_{13} (40% N_2 + 0% O_2 + 60% CO_2) (2.029 dSm^{-1}), which was preceded by T_{12} - (60% N_2 + 0% O_2 + 40% CO_2) (2.042 dSm^{-1}) and this might be due to the better maintenance of membrane integrity while, the highest electrical conductivity was recorded in T_1 control (2.207 dSm^{-1}). Increase in electrical conductivity as the storage period advanced this may be due to increased membrane permeability and decreased integrity of seed coat resulted in excess release of electrolytes which caused higher electrical conductivity.

Higher protein content of seed has been found to favour maintenance of vigour and viability during storage (Hewrton, 1994 and Ching and Schoolcraft, 1968). In present investigation, at the end of ten months of storage period highest protein content was recorded in T_{13} (40% N_2 + 0% O_2+ 60% CO_2) 19.33% followed by T_{12} - (60% N_2 + 0% O_2 + 40% CO_2) 19.26% while the lowest protein content was recorded in T_1 control (18.13%). However, decrease in the protein content was observed in all the treatments as the storage period advanced. Similar findings were also observed by Shehata *et al*. (2009) in cowpea seeds exposed to higher CO_2 gas concentration.

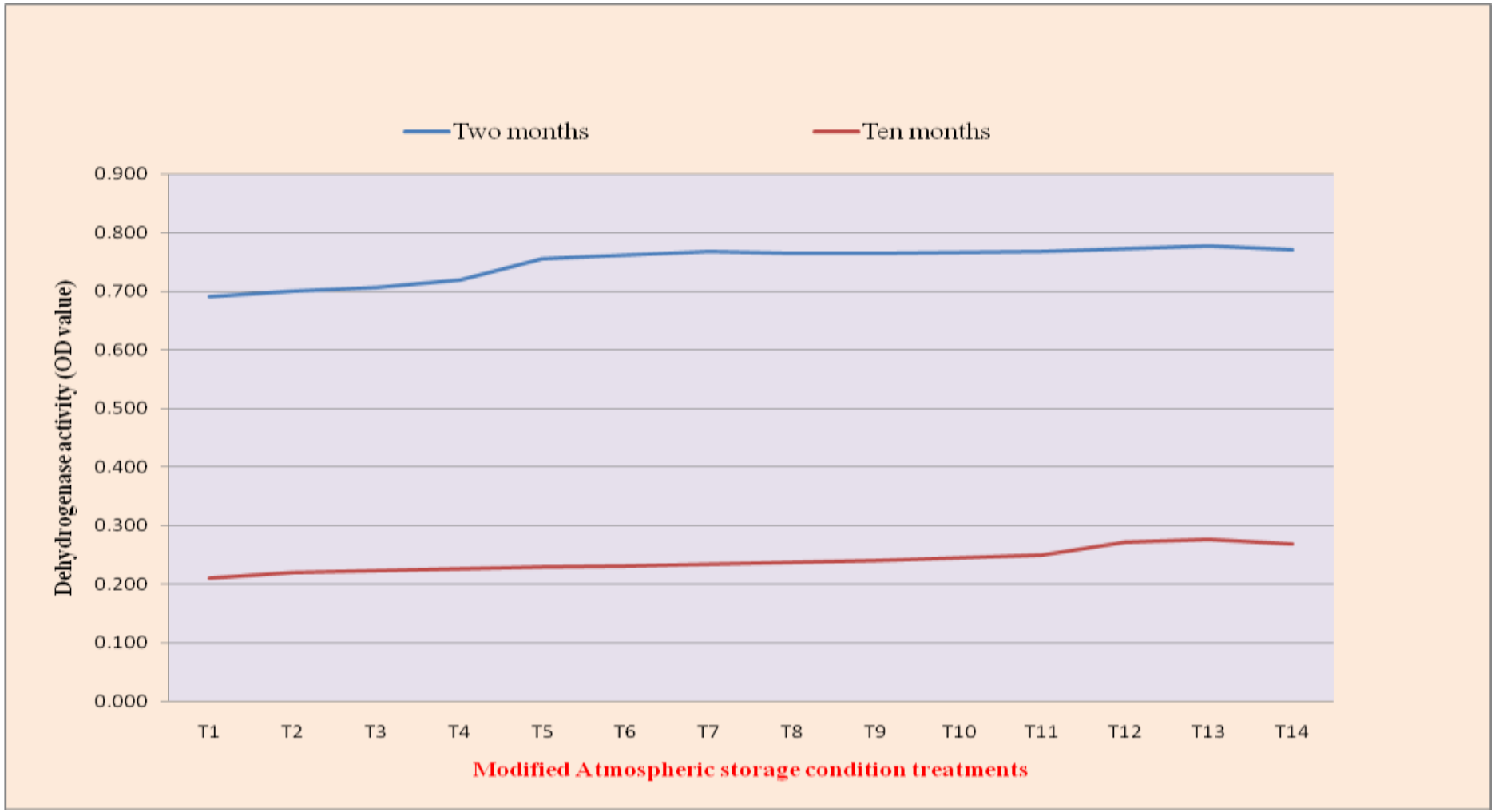

Fig. 4 Influence of modified atmospheric storage conditions on dehydrogenase activity (OD value) of pigeonpea seeds during storage

Alterations in protein profile due to effect of gases were noticed in the present investigation. Seeds exposed to 0%, 10% and 20% O_2 showed deviation from control protein profile in terms of number and intensity. Seeds in control exhibited lesser number of protein bands compared to seeds exposed to 0% O_2. In control the numbers of bands were less due to maximum seed deterioration as a result band number 1, 2, 3 and 5 (Rm value 0.165, 0.337, 0.486 and 0.711 respectively) were not noticed in control. Whereas, band number 2 and 5 (Rm value 0.337 and 0.711 respectively) were not noticed when seeds exposed to 10% O_2. Band number 2, 3 and 5 (Rm value 0.337, 0.486 and 0.711 respectively) were not noticed when seeds exposed to 20% O_2. However, higher polymorphism due to gas treatments was observed with reference to the intensity of bands rather than number of bands. Electrophoretic variations in proteins can be used to assay the amount of deterioration of seeds during storage. Machado *et al.* (2001) reported the changes in banding pattern of protein profiles of naturally and artificially aged French bean seeds and Smruti Das *et al*. (2010) revealed that the SDS PAGE analysis of seed proteins showed not only the total number of bands but, also their intensity in terms of thickness differed for each species during storage.

Practical utility of the results

Based on the results of present study, following findings are of practical application for better storage of pigeonpea seed.

1. Pigeonpea seeds packed with the gaseous combination of higher concentration of carbon dioxide and zero percentage of oxygen enhances storability up to ten months with higher seed quality parameters.

2. Pigeonpea seeds can also be packed under vacuum condition for maintenance of better seed quality up to eight months especially of nucleus and breeder seeds.

3. Storing pigeonpea seeds with gaseous combination of higher concentration of carbon dioxide and zero percentage of oxygen and packing in 700 gauge polyethylene bag may be recommended for better maintenance of all the seed quality parameters for longer period.

4. The modified atmosphere storage of pigeonpea seeds is a simple, easily adaptable and cost effective process, particularly beneficial in the absence of cold storage facility for the preservation of seed quality.

5. Insect pests can be managed without any residual effect.
6. Modified atmospheric storage can be replaced with fumigants as it is eco-friendly.

Future line of work

1. The effect of modified atmospheric storage condition with different gaseous combinations of CO_2:O_2:N_2 and other gases for storability of pigeonpea seeds may be studied.
2. Changes in molecular aspects during storage may be studied.
3. Similar studies can also be undertaken in other crops.

SummaryandConclusions

VI. SUMMARY AND CONCLUSIONS

Investigations on the "Influence of modified atmospheric storage conditions on longevity of pigeonpea seeds" were under taken during 2012 to 2013 in the Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur. The results obtained are summarized in this chapter.

The experiment consisted of 14 treatments *viz*., (T_1-Control, T_2-(70% N_2 + 20% O_2 + 10% CO_2), T_3-(60% N_2 + 20% O_2 + 20% CO_2), T_4-(40% N_2 + 20% O_2 + 40% CO_2), T_5-(20% N_2 + 20% O_2 + 60% CO_2), T_6-(80% N_2 + 10% O_2 + 10% CO_2), T_7- (70% N_2 + 10% O_2 + 20% CO_2), T_8 - (50% N_2 + 10% O_2 + 40% CO_2), T_9-(30% N_2 + 10% O_2 + 60% CO_2), T_{10} - (90% N_2 + 00% O_2 + 10% CO_2), T_{11} - (80% N_2 + 00% O_2 + 20% CO_2), T_{12}-(60% N_2 + 00% O_2 + 40% CO_2), T_{13} -(40% N_2 + 00% O_2 + 60% CO_2), T_{14}-Vacuum).

The experiment was carried out as per the completely randomised design with three replications. The required quantity of pigeonpea seeds were exposed to the modified atmospheric storage conditions according to the treatments and were packed in 700 gauge polythene bags. The observations on various seed quality parameters were recorded bimonthly.

The pigeonpea seeds packed with gaseous combinations of T_{13} - (40% N_2 + 0% O_2 + 60% CO_2) recorded the higher values on seed germination (78.30%), shoot length (11.46 cm), root length (15.67 cm), seedling length (27.13 cm), seedling vigour index-I (2124), seedling vigour index-II (6271), seedling dry weight (80.09 mg/seedling), field emergence (67.30%) speed of germination (20.04), dehydrogenase enzyme activity (0.276 OD value), at the end of tenth months of storage period.

The above treatment combinations recorded lower seed moisture content, electrical conductivity of seed leachate, seed infection and per cent insect damage throughout the storage period and at the end of storage they were 8.70 per cent, 2.029 dSm^{-1}, 10.15 per cent, and 0 per cent respectively. The next best treatment combination of 60 % N_2 + 0% O_2 + 40% CO_2 (8.71%, 2.042 dSm^{-1}, 10.23% and 0 per cent respectively) followed by vacuum storage (8.72%, 2.045 dSm^{-1}, 10.43% and 5.5 per cent respectively) also recorded the lowest values for the above parameters. Whereas, these parameters were higher in seeds without exposure (control) to modified atmosphere storage conditions (8.77%, 2.207 dSm^{1}, 18.01% and 60 per cent respectively).

The seed protein content was highest (19.33 %) when exposed to 40% N_2 + 0% O_2 + 60% CO_2 and lowest was recorded in T_1 - Control (8.13%) after ten months of storage. Studies on protein profiles showed alteration in their number and intensity due to deterioration in seeds during storage.

The treatments T_{13} - (40% N_2 + 0% O_2 + 60% CO_2) and T_{12}-(60% N_2 + 0% O_2 + 40% CO_2) maintained highest germination of 78.30 and 77.87 percentage respectively up to 10 months of storage with 18.17 % and 17.74 % increase over the control. While, T_0 (control) retained standard germination percentage (75%) up to four months only. Treatment T_{13} on par with $T_{12,}$ cost of modified atmospheric packaging was calculated for T_{13} and T_{12} treatments, Rupees 26 and 23 required per quintal of seed pacaking respectively, it includes only gas cost (Appendix).

From the present investigation following, conclusions have been drawn:

- Pigeonpea seeds packed with gaseous combination of higher concentration of carbon dioxide (60%) and zero percentage of oxygen enhanced longevity up to 10 months with higher seed quality parameters.

- Pigeonpea seeds packed with gaseous combination of higher concentration of carbon dioxide and zero percentage of oxygen reduced the pathogen infection and insect damage.

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**Originals not seen*

Appendix

APPENDIX

- **Cost of modified atmospheric packaging**

  Size of the packet 40x25 cm

  Water capacity of the packet =3528 cm^3

  Cost of CO_2 gas cylinder (30 kg) =1500`, per kg 50`

  Cost of N_2 cylinder (30 kg) =1200`, per kg 47`

  Volume of CO_2 `92/m^3

  Volume of N_2 `47/m^3

  Density of CO_2 =1.842 kg/m^3

  Density of N_2 =1.165 kg/m^3

- **Cost of modified atmospheric packaging for 40% N_2 +00% O_{2+} 60% CO_2**
- Per packet `0.26

  Per quintal `26

- **Cost of modified atmospheric packaging for 60% N_2 +00% O_{2+} 40% CO_2**
- Per packet `0.23

  Per quintal `23

INFLUENCE OF MODIFIED ATMOSPHERIC STORAGE CONDITIONS ON LONGEVITY OF PIGEONPEA (*Cajanus cajan* L.) SEEDS

MANJUNATHA, B. **2013** **Dr. S.N. VASUDEVAN**
Major Advisor

ABSTRACT

Laboratory experiment was conducted at the Department of Seed Science and Technology, University of Agricultural Sciences, Raichur during 2012-2013 to study the influence of modified atmospheric storage conditions on longevity of pigeonpea (*Cajanus cajan* L.) seeds. The seeds were exposed to various gaseous combinations *Viz*., T_1-Control, T_2-(70 % N_2 + 20 % O_2 + 10 % CO_2), T_3-(60 % N_2 + 20 % O_2 + 20 % CO_2), T_4-(40 % N_2 + 20 % O_2 + 40 % CO_2), T_5-(20 % N_2 + 20 % O_2 + 60 % CO_2), T_6-(80 % N_2 + 10 % O_2 + 10 % CO_2), T_7-(70 % N_2 + 10 % O_2 + 20 % CO_2), T_8-(50 % N_2 + 10 % O_2 + 40 % CO_2), T_9-(30 % N_2 + 10 % O_2 + 60 % CO_2), T_{10}-(90 % N_2 + 00 % O_2 + 10 % CO_2),T_{11}-(80 % N_2 + 00 % O_2 + 20 % CO_2),T_{12}- (60 % N_2 + 00 % O_2 + 40 % CO_2), T_{13}-(40 % N_2 + 00 % O_2 + 60 % CO_2), T_{14}-Vacuum. The results revealed that, the seeds stored in 700 gauge polyethylene bag with gaseous combination of 40 % N_2 + 00 % O_2 + 60 % CO_2 showed better seed quality parameters *Viz.*, germination (78.30 %), root length (15.67 cm), shoot length (11.46 cm), seedling length (27.13 cm), seedling vigour index-I (2124), seedling vigour index-II (6271), seedling dry weight (80.09 mg), field emergence (67.30 %), speed of germination (20.04), test weight(10.16), dehydrogenase enzyme activity (0.276 OD value), protein content (19.33 %) and less seed leachate (2.029 dSm^{-1}), moisture content (8.70 %), seed infection (10.15 %) and insect damage (zero %) at the end of tenth month of storage compared to other treatments**.** Whereas, in control prescribed germination of 75 % was noticed up to four months only. Further, protein profiles showed alteration in their number of bands.