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Seed physiological and biochemical parameters of cotton *as* influenced by different packaging materials and storage conditions

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Abstract
A lab experiment was carried out to study that seed physiological and biochemical parameters of cotton *as* influenced by different packaging materials and storage conditions. cotton seeds were stored in different packaging materials *viz*., cloth bags and aluminium bags and vacuum packed bags stored at room temperature (25±2 ºC) and cold storage (4±1 ºC) for a period of 18 months. The treatments having six combinations and consisting of different containers *viz*., cloth bags, aluminium bags and vacuum packed bags were replicated four times in both cold and ambient storage conditions in completely randomized design. The results of the study revealed that the least fluctuations in all seed biochemical parameters *viz.*, oil content and enzyme activity such as α-amylase, lipase and protease and seed physiological parameters such as mineral content (Cu, Mn, Zn and Mn), moisture content and electrical conductivity values were recorded in vacuum packed seeds than cloth, aluminium bags for cotton seeds stored under cold storage compared to room temperature throughout the storage period. Among the containers, the seeds stored in vacuum packed bags maintained the seed biochemical and seed physiological parameters with least deterioration compared to seeds stored in cloth bags and aluminium bags.

Practical application: Farmers, traders normally pack the seeds of various crops in either polythene bags, gunny bags or cloth bags before being used for propagation in the next season. Many seeds loose viability during the storage due to their sensitivity to oxidation and variation in moisture content during the storage period. It has been found that storing the crop seeds under vacuum packed bags enhance the shelf life while maintaining the quality. Since the seed is an essential input in agriculture, it is utmost necessary to maintain the viability and Vigour of the seed. Many a times, it so happens that the good quality seed is not available to the farmers in time due to various reasons, the average productivity of most of the crop plants has gone down considerably in the last one decade and one of the reasons for such decline is the poor quality of seeds being used by the farmers. Vacuum packaging has been found to be superior technology in preserving the seed quality i.e. physiological and biochemical aspects of different field crops.

Keywords: moisture content, electrical conductivity, mineral content, biochemical parameters, oil content, cotton, vacuum packaging and cold storage

1. Introduction

In the chain of seed production, storage of seeds assumes a greater importance as the "Seed saved is seed produced" an old adage holds well even today. Seed is the foundation of agriculture for enhancing crop production. But the availability of quality seed is the main constraint to crop production in developing country like India. The use of quality seed can contribute significantly to increased grain yield as well as to increased availability of every day's food intake. Seed viability is a major factor in crop establishment and subsequent productivity in many parts of the world. Losses in seed quality occur during field weathering, harvesting and storage. Seeds get damaged if they are exposed to high temperature and high humidity. In agriculture; seed is a vehicle to deliver almost all agro-based technological innovations so that the farmers can exploit the genetic potential of new varieties. The availability, access and use of seeds of adaptable varieties are, therefore, the major determinants to attain the efficiency and productivity of other packages like irrigation, fertilizers and pesticides. This is one of the vital keys to increase crop production, enhance food security and alleviate rural poverty in the developing countries. In storage, the viability and vigour of the seeds not only vary from genera to genera and variety to variety, but it is also regulated by many physico-chemical factors like moisture content, atmospheric relative humidity, temperature, initial seed quality, physical and chemical composition of seed, gaseous exchange, storage structure, packaging materials *etc*., [15]. To combat these factors effectively, storing seeds in vapour proof containers like polythene bag, aluminium foils, tins or any sealed containers is found to be more useful in maintaining the desired quality of seeds for longer period [23], unlike those stored in moisture pervious containers like cloth bag and cloth bag [59].

Indian cotton industry is considered as the centre of finest textiles industry in the world. The total area under this crop in the world is 101.71 m ha and the productivity is 680 kg per ha [3]. India has a pride of place in the global cotton scenario with the highest cotton growing area of 111.42 lakh ha with a total production of 3339.10 lakh bales of (170 kg) and productivity of 599 kg per ha [3]. In Karnataka, the area under cotton cultivation is 5.47 lakh ha with a production of 10.00 lakh bales and an average productivity of 392 kg lint per ha [4]. In spite of larger area under the crop, the yield of cotton per hectare is considerably low both at state and national levels, due to several factors *viz*., use of low quality seeds, larger cotton area under rainfed situation, pest and disease incidence *etc*. Among these non-availability of high quality seeds in adequate quantity seems to be the major factor which contributes for enhanced cotton yield in our country.

In cotton, upon storage, many enzymatic changes, oxidation and respiration occur. If the viability and vigor is not maintained properly during storage period, it will be difficult to sell it as a seed material for the next season. Post-harvest storage life of cotton largely depends on the genotypes, treatment, packaging material and storage conditions. In storage, viability and vigour of the seeds is regulated by many physico-chemical factors as the seed is hygroscopic in nature, seed quality is affected by variation in moisture content, relative humidity and temperature [7]. To combat these factors, it is better to store the seeds in moisture vapour proof containers like polythene bag, aluminium foil, tin or any sealed container to maintain the quality for longer period. Research concerning these aspects is very meagre. Keeping these aspects in the view and considering their importance in maintaining viability for longer period the present investigation was carried out.

2. Materials and Methods

2.1 seed materials and storage

A storage experiment was carried out for a period of 18 months at Department of Crop Physiology, University of Agricultural Sciences, Dharwad. Freshly harvested cotton seeds (Sahana) were dried under sun and stored under different storage conditions and containers. The temperature maintained in the cold storage was around (4 °C ± 1 °C) and relative humidity was 85 to 90 per cent throughout the storage period while, for ambient storage, bags were stored in the laboratory at room temperature (25±2 °C). Cotton seeds were packed in 10 g in vacuum packed bags (The machine used for vacuum packaging of different seeds was OLPACK 501/V manufactured by Interprise–brussels S.A., bruxtainer division N, Belgium) and & aluminium bags while 500 g into cloth bags. After packaging of all the seeds in different containers, 50% bags were stored properly in the iron racks without stacking so that all the bags were uniformly exposed to the particular treatment condition; while 50% bags were stored under cold storage. The treatments having six combinations and consisting of different containers viz., cloth bags, aluminium bags and vacuum packed bags were replicated four times in both cold and ambient storage conditions in completely randomized design.

The observations on various seed physiological parameters *viz*., electrical conductivity [47], and the per cent moisture were obtained by using MB 45 Halogen Moisture Analyzer from Ohaus, USA, while estimation of mineral content i.e. Copper (Cu), Zinc (Zn), Iron (Fe) and Manganese (Mn) contents in seed were estimated using Atomic Absorption Spectrophotometer (AAS-4141, Electronic Corporation of India Ltd.) and determination of all the biochemical parameters i.e. α-amylase activity, lipase enzyme activity, protease enzyme activity and oil content was estimated by methods devised by [8, 28, 48, 51] respectively at bimonthly interval upto18 months. The analysis and interpretation of the experimental data was done as suggested by [46], with level of significance used as P = 0.01.

3. Results and Discussion

3.1 Influence on seed physiological parameters

3.1.1 Moisture content (%)

The moisture content (%) of cotton seeds presented in Table 1 indicated significant differences between the treatments at all the stages of storage upto 18 months except at the initial stages (i.e., 0 months). In general, these was no change in the moisture content of vacuum packed as well as aluminium packed seeds during storage for 18 months however there was a slight decline in the moisture content with progress in the storage time. More fluctuations were seen in the moisture content of cotton seeds stored in cloth bags, irrespective of storage at room temperature or cold storage throughout the storage period. All through the 18 months of storage, the maximum moisture content (12.00%) was observed in cloth bags at cold storage followed by cloth bags at room temperature (9.91%). Lower moisture content (8.26 and 8.25%) was observed in aluminium packed seeds followed by vacuum packed seeds (8.24 and 8.23%) at cold storage and room temperature, respectively. Polythene containers at cold storage recorded higher values of moisture content at all the stages of storage even upto 18 months as compared to containers stored at room temperature. These results are in agreement with the findings of [14], in shelled peanuts, [38] in wheat, [56] in groundnut kernels, [47, 13, 52], in chilli powder, [54] in whole chilli and [34] in soybean storability for longer period.

3.1.2 Electrical conductivity (dSm^{-1})

The electrical conductivity (dSm^{-1}) of cotton seeds differed significantly between the treatments at all the stages of storage period (Table 2). The increase in electrical conductivity of vacuum packed seeds was much lesser and slower than in aluminium packed seeds at all the stages of storage. At 18 months of storage, seeds stored in vacuum packed bags recorded significantly lower values of electrical conductivity (0.545 and 0.548 d Sm^{-1}) at cold storage and room temperature as compared to aluminium bags (0.635 and 0.665 d Sm^{-1}) while, maximum values of electrical conductivity (0.692 and 0.728 d Sm^{-1}) was recorded in cloth bags, respectively. It is clear from the results that the vacuum packed seeds could maintain lower electrical conductivity as compared to aluminium bags followed by cloth bags during the storage period. The values of electrical conductivity in aluminium packed seeds at room temperature were onpar with cloth bag seeds stored at cold storage throughout the storage period. The values of electrical conductivity, in all the containers stored at cold storage shown lesser than those stored at room temperature during the storage period. Similar results were obtained by [50] in rice, [43] in brinjal seeds, [41] in groundnut, [62] in soybean, [40] in sunflower, [53] in chilli and [6] in maize seeds.

3.2 Mineral's content

3.2.1 Copper content (Cu, ppm)

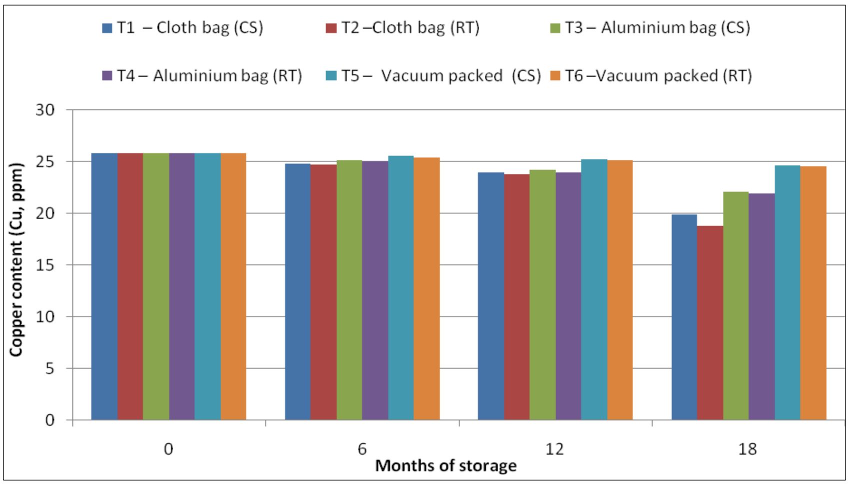

Fig 1: Influence of packaging and storage conditions on copper content (Cu, ppm) at different time intervals of storage in cotton seeds

Copper content (ppm) of cotton seeds differed significantly between treatments and there was a gradual decline with progress in storage period (Fig., 1). The decline in copper content of vacuum packed seeds was minimum compared to aluminium bags followed by cloth bags throughout the storage period stored either under room temperature or cold storage. The copper content of vacuum packed did not differ significantly among themselves when at stored cold storage or room temperature but cloth bag and aluminium bag seeds differed significantly with each other from 10 months onwards and until upto 18 months of storage. At 18 months of storage, maximum reduction in copper content (from 14.07 to 13.88 ppm) was recorded in cloth bag seeds followed by aluminium bag seeds (from 15.18 to 14.83 ppm) and minimum reduction was found in vacuum packed seeds (from 18.70 to 18.64 ppm) when kept under either cold storage or room temperature.

3.2.2 Iron content (Fe, ppm)

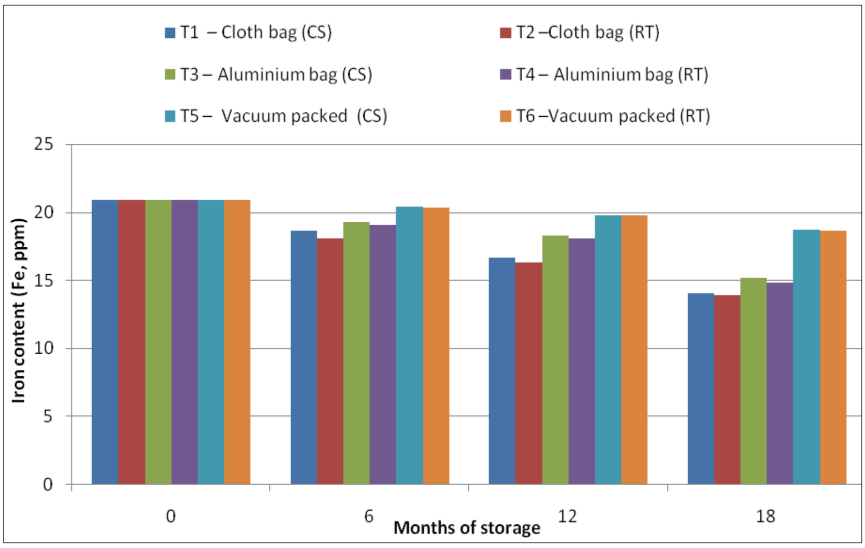

Fig 2: Influence of packaging and storage conditions on iron content (Fe, ppm) at different time intervals of storage in cotton seeds

The data related to the iron content (ppm) of cotton seeds differed significantly between treatments recorded with progress in storage period (Fig., 2). The iron content of vacuum packed seeds did not differ significantly among themselves stored when cold storage or room temperature but cloth bags seeds and aluminium bag seeds differed significantly with each other upto 18 months of storage. At the end of storage i.e., 18 months, the maximum reduction in iron content (from 39.93 to 38.60 ppm) was recorded in cloth bag seeds followed by aluminium bag seeds from (44.33 to 42.67 ppm) and minimum reduction was found in vacuum packed seeds (from 49.35 to 49.23 ppm). When kept under cold storage or room temperature. The reduction in shoot length of vacuum packed seed was almost nil and maintained higher iron content throughout the storage period over all other treatments. The iron content of vacuum packed seeds kept under cold storage was onpar with seeds stored at room temperature throughout the storage period.

3.2.3 Zinc content (Zn, ppm)

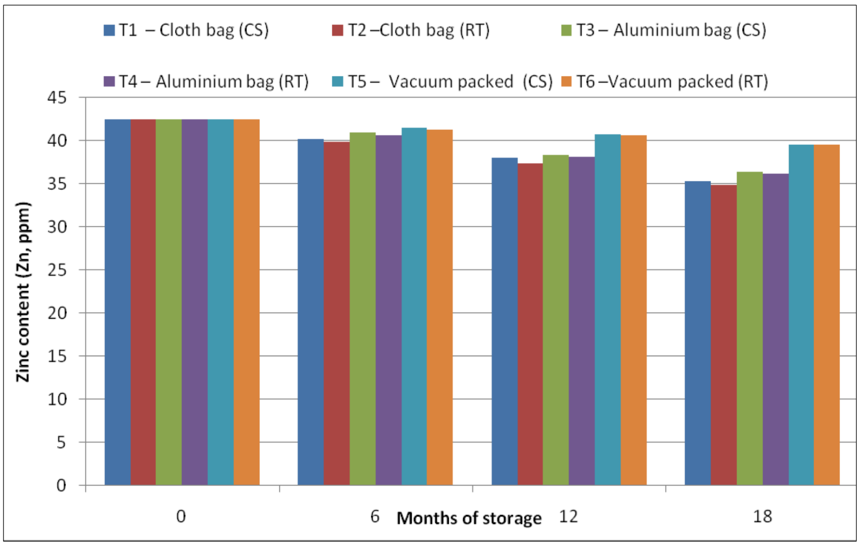

Fig 3: Influence of packaging and storage conditions on zinc content (Zn, ppm) at different time intervals of storage in cotton seeds

Zinc content (ppm) of cotton seeds differed significantly between the treatments and reduced from 4 months onwards until upto 18 months of storage in cloth bag seeds (Fig. 3). At the end of storage periods, the vacuum packed bag seeds recorded significantly higher values of zinc content (39.52 and 39.50 ppm) when kept at cold storage and room temperature compared to aluminium bags (36.37 and 36.16 ppm) as well as cloth bags (35.27 and 34.86 ppm), respectively. It is clear from the results that the vacuum packed seeds could maintain higher zinc content compared to aluminium bag seeds followed by cloth bag seeds throughout the storage period.

3.2.4 Manganese content (Mn, ppm)

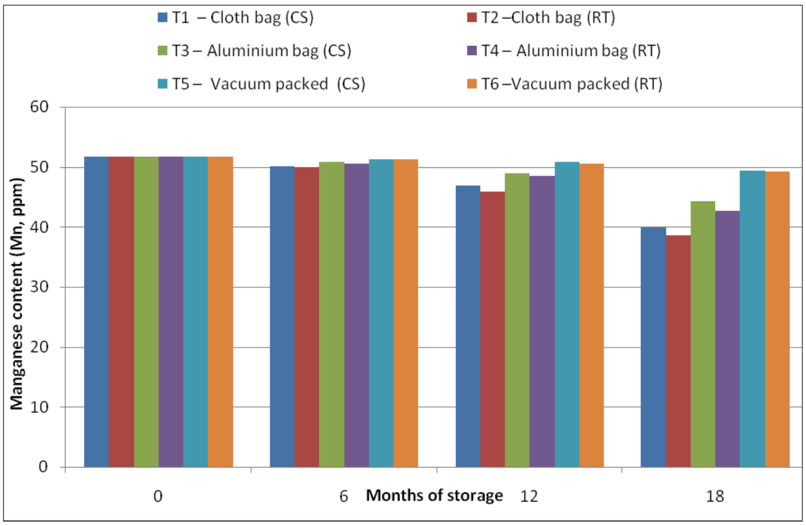

Fig 4: Influence of packaging and storage conditions on manganese content (Mn, ppm) at different time intervals of storage in cotton seeds

The data on manganese content (ppm) of cotton seeds as influenced by different packaging materials and storage condition significantly differences among the treatment during storage (Fig. 4). By the end of 18 months of storage, the maximum reduction in manganese content (from 19.93 to 18.80 ppm) was recorded in cloth bags followed by aluminium bag seeds (From 22.13 to 21.92 ppm) and minimum reduction was found in vacuum packed seeds (from 24.65 to 24.58 ppm) when kept either under cold storage or room temperature.

It is clear from the above results for all the mineral's content that the seeds stored in cloth bags under cold storage were on par with the aluminium bags stored under cold storage similarly the cloth bags stored at room temperature was also on par with aluminium bags stored at room temperature. The vacuum packed seeds could maintain higher mineral's content values throughout the storage period. The vacuum packed seeds could maintain higher manganese content values throughout the storage period. Mineral elements like zinc, copper, iron and manganese are very crucial for plant growth and human health, and play a key role in various physiological and biochemical processes. Results pertaining to mineral contents during study including copper, iron, zinc and manganese of soybean seeds showed gradual decrease with an advancement of storage period at all the stages of storage. Among the containers, the decrease in mineral content was very less in vacuum packed bags compared to aluminium, and cloth bags throughout the storage period under both ambient and cold storage. Variation in the mineral contents between the packaging materials could be attributed to redistribution of mineral elements in seeds and possible microbial contamination [10]. In the aluminium bags mineral content values were higher than cloth bags, but it was lower than vacuum packed bags. Same results were obtained by [20] in groundnut, [21] in melon seeds, [5] in white yam [17] in cocoyam chips, [37, 45] in chilli and [64] in greenbeans.

Higher reduction of iron content in aluminium and cloth bags may be attributed to its sensitivity to temperature [29] reported that copper, iron, zinc and manganese contents in both conventional and vacuum packed bags were affected significantly and decreased with an increased storage period of 10 months. At 18 months of storage, vacuum packed bags recorded significantly higher values of copper, iron, zinc and manganese compared to aluminium, while significantly lower values were found in cloth bags stored under both ambient and cold storage. Similarly, [5] also reported a decrease in Zn, Cu and Fe of cocoyam and white yam stored for six months. Higher mineral contents during cold storage compared to ambient storage could be attributed due to lower internal physiological and biochemical processes in the seed there by prolonging the shelf life of seeds during storage. Higher micronutrients in vacuum packed bags compared to cloth bags, may be due to decrease in ash content [29]. Similar findings were also reported in shelled melon seeds [21], in millet seeds [20], in cocoyam chips.

3.3. Hydrolytic enzymes activity

3.3.1 α-amylase activity (mg maltose released/min)

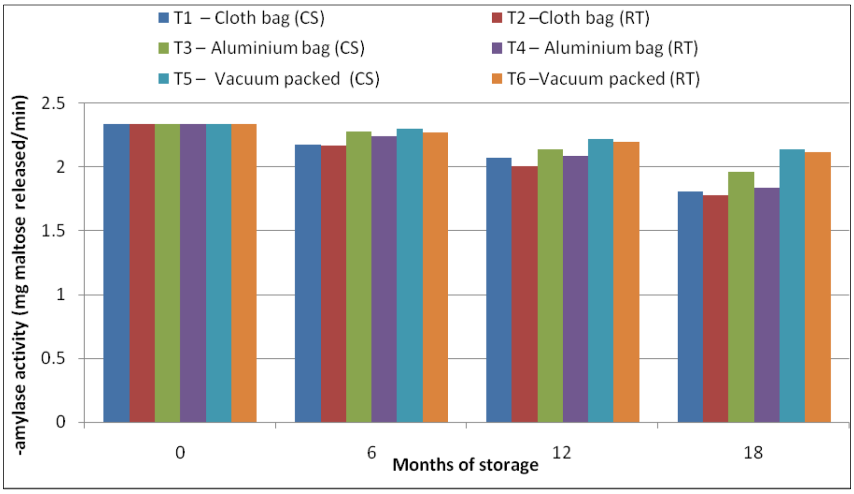

Fig 5: Influence of packaging and storage conditions on α-amylase activity (mg maltose released/min) at different time intervals of storage in cotton seeds

α-amylase activity (mg maltose released/min) of cotton seeds differed significantly between treatments and there was a gradual decline with progress in storage period (Fig., 5). The decline in α-amylase activity of vacuum packed seeds was minimum as compared to aluminium bag seeds followed by cloth bag seeds throughout the storage period stored under room temperature or cold storage. At the end of storage i.e., 18 months the maximum reduction in α-amylase activity from 1.81 to 1.78 mg maltose released/min was recorded in cloth bag seeds followed by aluminium bag seeds (from 1.96 to 1.84 mg maltose released/min) and minimum reduction was found in vacuum packed seeds (from 2.14 to 2.12 mg maltose released/min) kept either under cold storage or room temperature. The α-amylase activity values of aluminium packed seeds superior over cloth bag seeds. The α-amylase activity values of vacuum packed seeds kept under cold storage was with the seeds stored at room temperature throughout the storage period. Similar observations were also reported in wheat seeds [11] and in naturally aged gram, chickpea and wheat seeds [2], and in mustard [60].

3.3.2 Lipase activity (meq. free fatty acid/min/g)

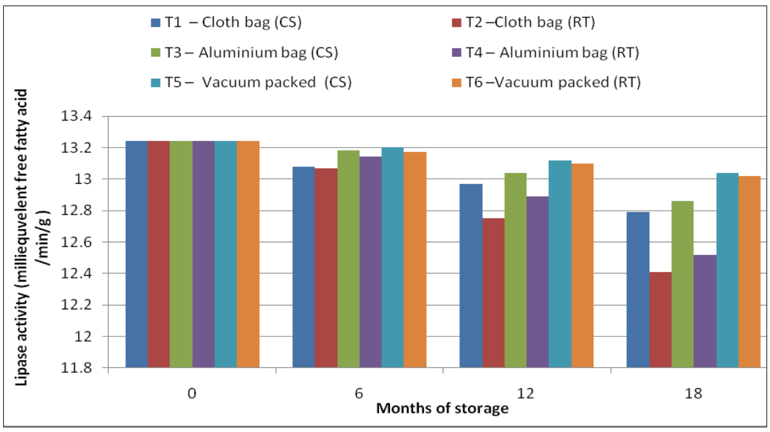

Fig 6: Influence of packaging and storage conditions on lipase activity (Milliequvelent free fatty acid/min/g) at different time intervals of storage in cotton seeds

Lipase activity (meq. free fatty acid/min/g) of cotton seeds differed significantly between treatments and there was gradual decline with an advancement in storage period (Fig., 6). The reduction was greater in cloth bag as well as aluminium packed seeds from the early stage to up to 18 months of storage, stored under room temperature. At the end of storage periods, i.e., 18 months vacuum packed seeds stored under cold storage and room temperature recorded significantly higher values of lipase activity (13.04 and 13.02 meq. free fatty acid/min/g) compared to aluminium bags (12.86 and 12.52 meq. free fatty acid/min/g) as well as cloth bags (12.79 and 12.41 meq. free fatty acid/min/g), respectively. It is clear from the results that the vacuum packed seeds could maintain higher lipase activity compared to aluminium bags followed by cloth bags throughout the storage period. Our results are in good agreement with results of [15, 12, 33] in wheat and [18] in cucurbitaceous seeds.

3.3.3 Protease activity (mg amino acid released/min/ml)

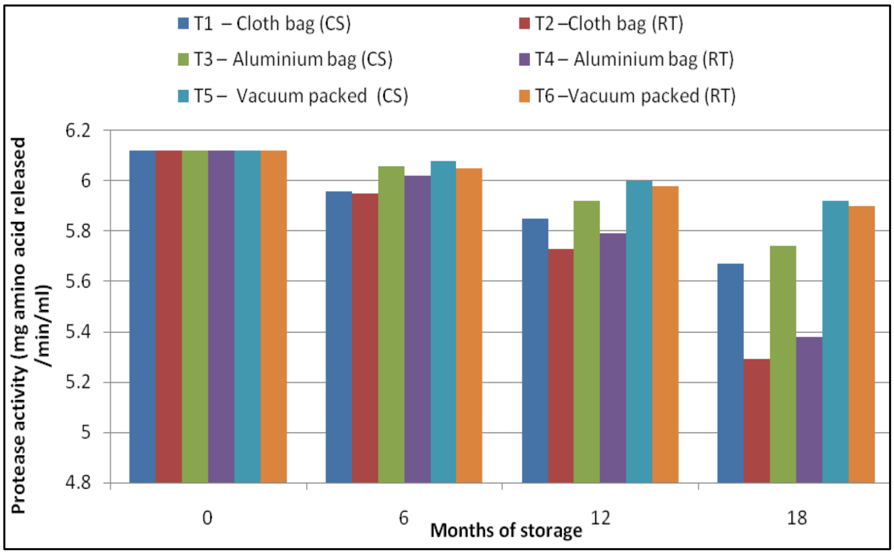

Fig 7: Influence of packaging and storage conditions on protease activity (mg amino acid released/min/ml) at different time intervals of storage in cotton seeds

Protease activity (mg amino acid released/min/ml) of cotton seeds differed significantly between treatments during storage period (Fig., 7). The decline in protease activity of vacuum packed seeds was very minimum and a slow decline with an advancement in storage period. While, cloth bags as well as aluminium bag seeds was found to reduce great from the initial stage to 18 months of storage under room temperature. At the end of storage period, vacuum packed seeds recorded significantly higher protease activity (5.92 and 5.90 mg amino acid released/min/ml) kept at cold storage or room temperature compared to aluminium bags (5.74 and 5.38 mg amino acid released/min/ml) as well as cloth bags (5.67 and 5.29 mg amino acid released/min/ml), respectively. It is clear from the results that the vacuum packed seeds could maintain higher protease activity compared to aluminium bags followed by cloth bags throughout the storage period. Similar results were observed by [11] in wheat, [61] in radish, [44] in sunflower. Same results of lipase activity were also observed in germinated seeds of castorbean [42, 57] in bajara & sorghum and the African bean [19].

Loss of viability of seeds has been correlated to enzymatic activity [1] reported that the activity of respiratory and associated enzymes *viz.*, peroxidase, glutamic acid oxidase and catalase, activity of hydrolytic enzymes *viz., α-amylase, lipase and* proteases are associated with the degradation of organelles membranes, nucleoproteins, etc. In crop seeds, the development of amylase activity constitutes an important event in germination. During germination of seeds, a massive breakdown of the reserve substances begin with the help of amylolytic, proteolytic and lipolytic enzymes and the products are transported to the growing seedlings for their development. The remaining small amounts of protein represents enzymes concerned with metabolic processes during seed development and germination [35]. Among the storage conditions, ambient storage recorded higher enzyme activities compared to cold storage. This may be due to higher temperature and higher metabolic activity under ambient storage. These findings are in agreement with those of [63, 24] in castor, [31] in bush bean, and [58] in wheat, [25] in sorghum and [39] in quinoa, [31] in oat seeds, [24, 55] in mungbean.

3.4 Oil content (%)

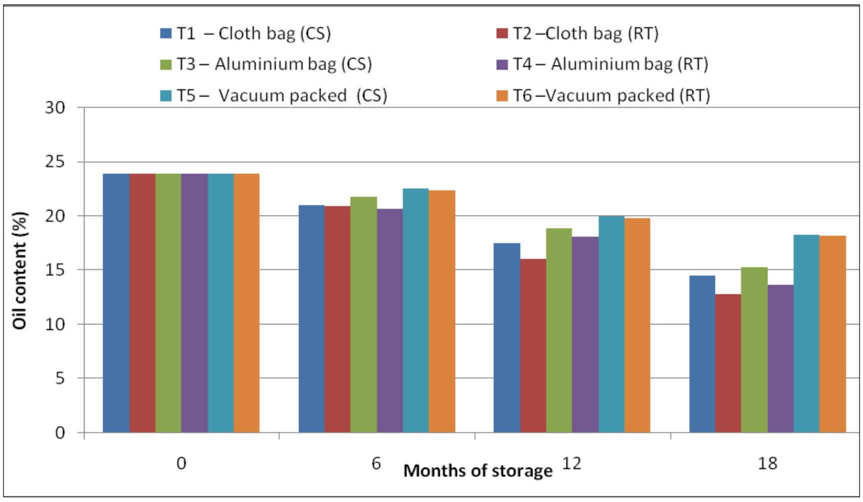

Fig 8: Influence of packaging and storage conditions on oil content (%) at different time intervals of storage in cotton seeds

The oil content (%) of cotton seeds showed significant differences between treatments (Fig. 8). The reduction in oil content was observed from 6 months onwards and until upto 18 months of storage in cloth bag seeds at room temperature. Significantly higher values of oil content (18.3 and 18.2%) when kept at cold storage and room temperature compared to aluminium bags (15.3 and 13.7%) as well as cloth bags (14.5 and 12.8%), respectively. It is clear from the results that the vacuum packed seeds could maintained higher oil content compared to aluminium bags followed by cloth bags throughout the storage period. In oil seed crops, such as cotton, auto-oxidation of lipids and increase of free fatty acids during storage period are the main reasons for rapid deterioration of the oilseeds [32] reported that storage condition of oil seeds before industrial extraction might influence the quality of the crude oil. Similarly, [39] also showed that storage time and storage temperature had significant effect on free fatty acid content in Quinoa (*Chenopodium quinoa*) seed oil. Different longevity of seed storage as well as storage conditions exerts significant influence on oil content. Similar results were also noticed by [49] in groundnut, [9, 30] in soybean.

Table 1: Influence of packaging and storage conditions on moisture content (MC, %) at different time intervals of storage in cotton seeds

Treatments	Storage period (months)									
	0	2	4	6	8	10	12	14	16	18
T_1 – Cloth bag (CS)	8.30	12.03	12.08	12.05	12.10	12.09	12.07	12.03	12.01	12.00
T_2 –Cloth bag (RT)	8.30	10.24	10.22	10.09	10.07	10.92	10.88	10.68	9.95	9.91
T_3 – Aluminium bag (CS)	8.30	8.73	8.71	8.63	8.59	8.51	8.50	8.40	8.27	8.26
T_4 – Aluminium bag (RT)	8.30	8.56	8.69	8.59	8.46	8.40	8.31	8.28	8.26	8.25
T_5 – Vacuum packed (CS)	8.30	8.35	8.36	8.37	8.35	8.34	8.32	8.27	8.26	8.24
T_6 –Vacuum packed (RT)	8.30	8.32	8.34	8.31	8.30	8.29	8.28	8.26	8.25	8.23
S.Em (±)	0.15	0.11	0.08	0.03	0.04	0.03	0.02	0.03	0.01	0.04
C. D. (1%)	NS	0.33	0.23	0.08	0.12	0.08	0.06	0.09	0.03	0.11

NS = Non-significant CS = Cold storage RT = Room temperature

Table 2: Influence of packaging and storage conditions on electrical conductivity (EC, dSm^{-1}) at different time intervals of storage in cotton seeds

Treatments	Storage period (months)									
	0	2	4	6	8	10	12	14	16	18
T_1–Cloth bag (CS)	0.519	0.522	0.529	0.538	0.550	0.568	0.584	0.614	0.649	0.692
T_2–Cloth bag (RT)	0.518	0.524	0.534	0.553	0.561	0.589	0.616	0.635	0.695	0.728
T_3–Aluminium bag (CS)	0.518	0.516	0.527	0.530	0.539	0.553	0.572	0.586	0.608	0.635
T_4–Aluminium bag (RT)	0.518	0.526	0.532	0.538	0.548	0.565	0.584	0.598	0.625	0.665
T_5–Vacuum packed (CS)	0.518	0.519	0.522	0.524	0.528	0.531	0.534	0.537	0.540	0.545
T_6–Vacuum packed (RT)	0.518	0.520	0.523	0.526	0.530	0.533	0.536	0.539	0.543	0.548
S.Em (±)	0.001	0.002	0.001	0.001	0.001	0.001	0.0016	0.001	0.002	0.001
C. D. (1%)	NS	NS	0.002	0.003	0.002	0.003	0.0048	0.003	0.005	0.003

NS = Non-significant CS = Cold storage RT = Room temperature

4. Conclusions

Seed physiological and biochemical parameters deterioration is an inexorable and an irreversible process. The quality and viability of cotton seeds are subjected to variations during storage conditions and it has been found that the life span of seeds depends on moisture content of the seeds, relative humidity, temperature, light and oxygen content under which the seeds are stored. It has been found in the present study that it is possible to extend the shelf life of cotton seeds up to 18 months without deterioration in seed biochemical parameters *viz.*, enzyme activity such as α-amylase, lipase, protease and oil content and seed physiological parameters such as mineral content (Cu, Mn, Zn and Mn), moisture content and electrical conductivity by storing them under vacuum packaging. Since seed is an important input in agriculture which determines not only the production but also the productivity, it is essential to maintain the seed quality as well as seed vigor for better crop yield potential.

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