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IMPACT OF SEED STORAGE CONTAINERS ON SEED BIOCHEMICAL STRUCTURES AND SEED QUALITY ATTRIBUTES IN CHICKPEA (*CICER ARIETINUM*) SEED

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ABSTRACT

In present study, the different reactive phenotypic and biochemical parameters of seedlings showed the shaping of deterioration pattern under diverse environs in which the innovation of ideal situation for improving the seed storability as well as seed quality was our fundamental objective. The treatment effect was prominent at later stages due to deterioration of storability was maximum. In comparison to normal, the effect of T4 provided the highest peak followed by T7. Considering the treatments, the parameters related to seedling clearly pointed out an understandable pattern of seed deterioration. In most cases, the 12 Month of storage was the turning point for initiating the rapid rate of deterioration. A significant discrepancy showed prominence in between treatments and control with progression of storage duration where the treatments, T4 and T7 created a separate group in contrast to other treatments and control.

INTRODUCTION

Lentil (*Lens culinaris*) is important annual grain legume crop cultivated on around 4 million hectares area (Andrews and McKenzie, 2007). It is an excellent source of protein, carbohydrates, vitamins and other non-nutritional components (Urbano*et al.,* 2007). It is categorized into Red and green types based on cotyledon and seed coat colour and around 75% of world production is constituted by red lentils (McNeil *et al*., 2007). The foremost aim of farmers for seed storage is to maintain good extent of seeds which can give rapid, uniform and vigorous seedlings from seedbed after the sowing. In eastern India, lentil seeds has to pass a lot of environmental inconsistency during pre and post-harvesting stages due to very high relative humidity of rainy season. Therefore, the excellent seed also cannot express fully during sowing time due to their deterioration at stored period. The deterioration of stored seeds is influenced by numerous factors *viz*., physical (temperature and relative humidity), biological (storage microflora) and mechanical (storage conditions, methods and duration). According to Bradford (2004) during seed deterioration lipid peroxidation and free radicle production are occurs which damages the cell membrane's physical integrity resulting into loss of cell compartmentalization and expulsion of solutes. Peroxidation of unsaturated fatty acids is considered to be one of the main reasons for loss of storability, which occurs due to decreased levels of antioxidants, reduced activity of free radical and peroxide scavenging enzymes, and increased malondialdehyde content (Bailly et al., 1996 ;Hsu and Sung, 1997).Seed deterioration is also associated with metabolic changes*viz.*,reduction in carbohydrates and protein content. The carbohydrate and protein reduction with ageing may results in insufficient respiratory substrates during seed germination which can be seen more clearly after the rootlet emergence (Bernal-Lugo and Leopold, 1992; Cruz-Perez *et al*., 2003). Standardization of appropriate seed packaging and duration could ensure satisfactory planting quality of lentil seeds at the time of sowing. In the present study, experiments were conducted tofind out possible ways to improve storability of lentil seedswith respect to seed storage container.

MATERIAL AND METHODS

Study site The experiment was conducted at Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India (22.94° N altitude, 88.533° E longitude and 9.75m MSL) during 2013-14. Climate in this region is classified as humid region and received 200-400cm rainfall annually.

Preparation of seed materials: The experiment was documented considering two varieties of Lentil i.e. B77 (V1) and WBL58 (V2) in the year 2016. The variable storage environments for retaining the seed quality were - Ambient condition at room as T1 (control); CO_2 incubator (1% CO_2 + normal O_2) with 32±1°C as T2; Seed Drier with 34±1°C as T3; Plastic container (700 gauge) as T4; Cotton Bag as T5; Earthen pot as T6 and Polythene packet (40 micron) as T7. The harvested lentil

seed was stored in various environments to judge the seed storing competence and these were assessed 0M, 6M and 12 months respectively in comparison to harvest stage. **Seed quality evaluation:** Germination assays were performed according to theprocedure described by ISTA (2012) by placing Petri plates in germination (20-22 °C, relative humidity 90 ± 2 %) (100 sterilized seeds per petri plate in four replicates). Germination was scored as radicle growth of 2mm or more and counting of normal and abnormal seedlings were taken place after the 10th day of germination test (final count) followed by calculation of germination%. After the germination test, seed vigour index were measured and calculated according to the procedure laid down (ISTA, 2012).**Biochemical analysis:** The reducing sugar of α-amylase was determined by the dinitrosalicylic acid (DNSA) method (*Miller 1959*). One unit of the αamylase activity is defined as the amount of enzyme that releases the amount of reducing sugar equivalent to 1 µmol of reducing sugar under the assay conditions.Peroxidase activity (POX) was measured by the procedure given by Rao*et al*., (1996), through monitoring the formation of tetraguaiacol (ε=26.6 mM-1 cm-1) from guaiacol. The specific activity of enzyme was expressed as µmol/min/mg protein tetraguaiacol formed.Protein content of the enzyme extract is generally estimated by lowry's methods, which is based on biuret reaction of protein with alkaline cupric tartrate. The product formed is cu^{2}+- protein complex, measurement absorbance at 555nm(Lowry, 1951).Conductivity tests have also been applied to detect vigour differences in many other grain legumes and indeed some other species (ISTA, 1995)**Root surface area** The Root Image Analyser, WIN RHIZO (PRO BASIC STD4800) was utilizing for this comprehensive study on seedling roots considering the previous observations of Pierret*et al.* (2013). The scanning procedures were done by using the flat-bed scanners (Epson Expression/STD 1600 scanner). **Statistical analysis:** To assess the statistical significance of the varieties and their interaction effects with storage duration, data obtained in the study was subjected to two-way analysis of variance (Cochran and Cox, 1957). The percentages data were transformed to *arc sin* value before the analysis and least significant difference was calculated from the error mean sum of squares to test the significance between two means.The study was related to storage device and vigour index, biochemical, root surface area of crucial for seedlings establishment in field condition.

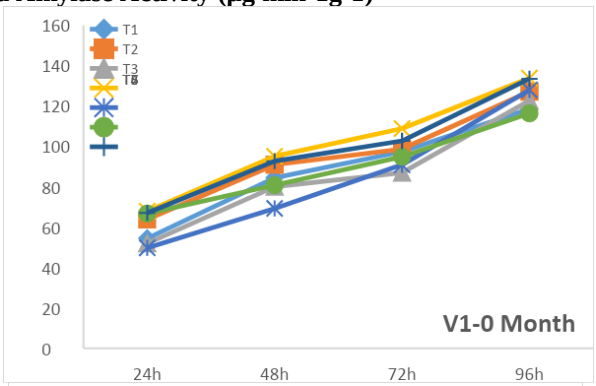
Table 1:

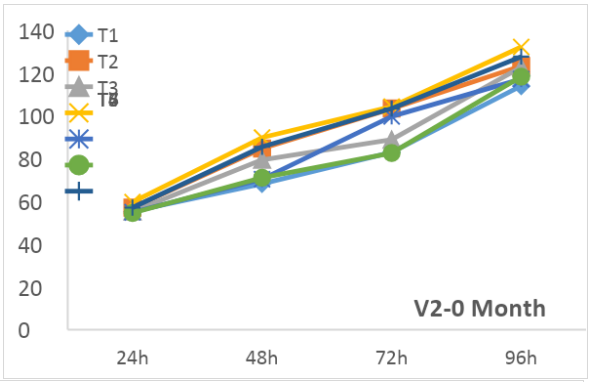
Treatments	Germination %		Vigour Index		Root surface area (cm^2)	
	Mean		Mean		Mean	
	V1	V2	V1	V2	V1	V2
T_1	83.90	84.58	1936.93	2055.44	41.48	46.20
T_2	86.40	86.47	2115.43	2200.67	46.80	49.56
T_3	85.83	85.17	2069.88	2030.94	43.15	48.18
T_4	90.22	89.97	2282.36	2248.42	51.98	52.85
T_5	85.92	84.97	2009.13	2088.81	43.62	45.35
T_6	82.69	83.37	1895.47	1996.13	42.53	47.57
T_7	84.52	84.75	2109.27	2160.14	46.99	50.92
SEm±	*0.47*	*0.49*	*9.42*	*10.24*	*0.21*	*0.25*
CD (1%)	*1.73*	*1.82*	*34.71*	*36.48*	*0.77*	*0.82*
F.SIg	*S*	*S*	*S*	*S*	*S*	*S*

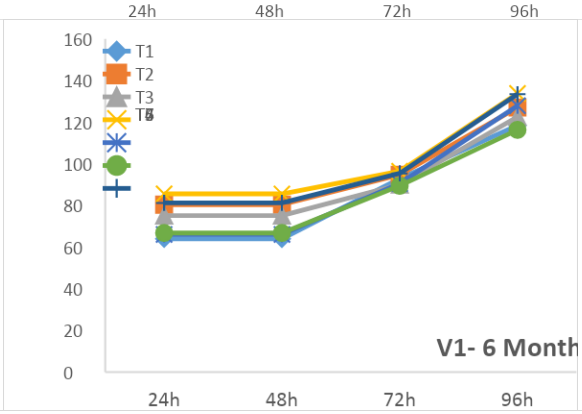
Table 2:

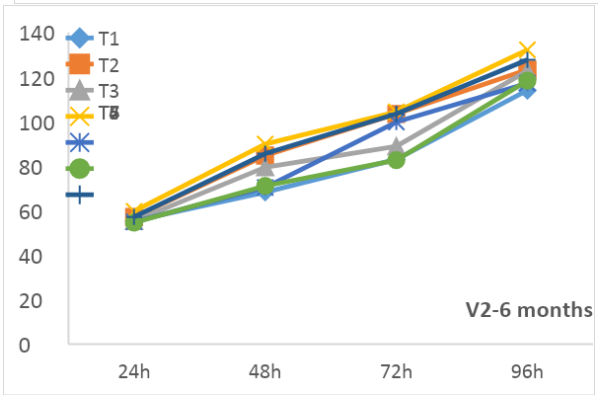
Month	Germination %		Vigour Index		Root surface area (cm^2)	
	Mean		Mean		Mean	
	V1	V2	V1	V2	V1	V2
0M	94.71	95.31	2645.60	2650.65	55.80	58.90
6M	91.0	89.33	2229.05	2270.75	46.98	50.28
12M	71.21	72.19	1304.70	1413.14	32.88	36.80
SEm±	0.44	0.46	8.72	7.35	0.19	0.22
CD (1%)	1.61	1.64	32.13	33.16	0.72	0.76
F.SIg	S	S	S	**S**	S	S
Treatment X Months	**T X M**		**T X M**		**T X M**	
SEm±	1.15	1.13	23.08	24.37	0.51	0.58
CD (1%)	4.25	4.27	85.01	89.11	1.89	1.94
F.SIg	S	S	S	S	S	S

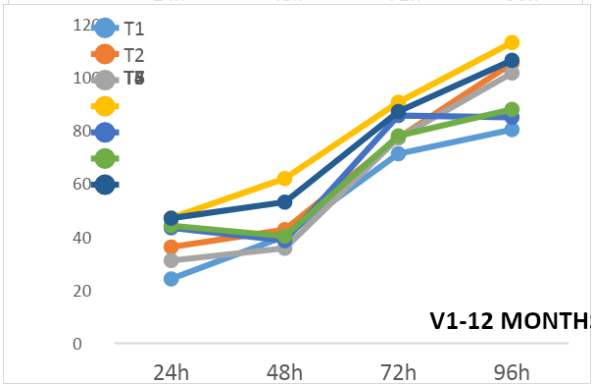
Alfa Amylase Activity (µg min-1g-1)

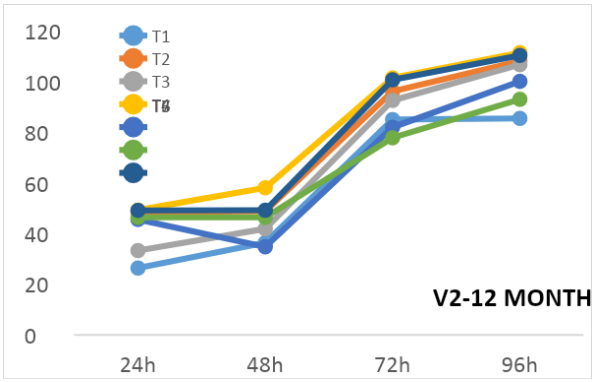




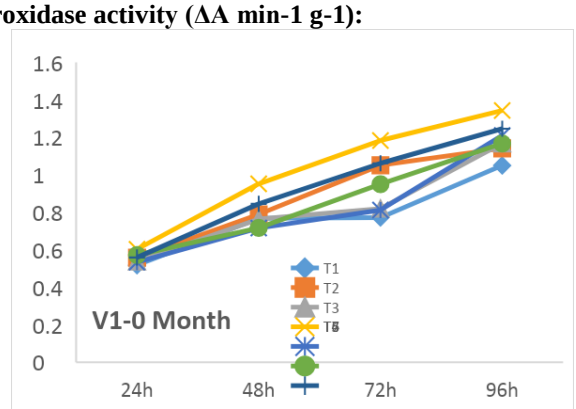


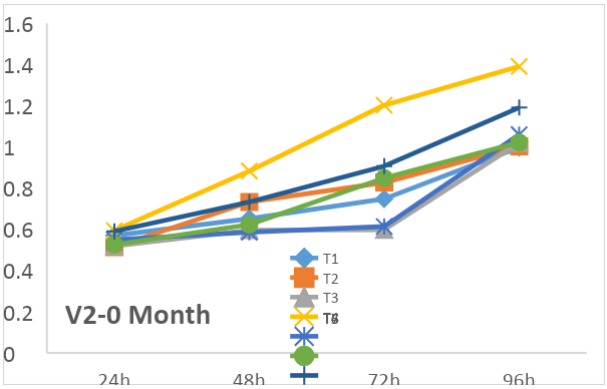


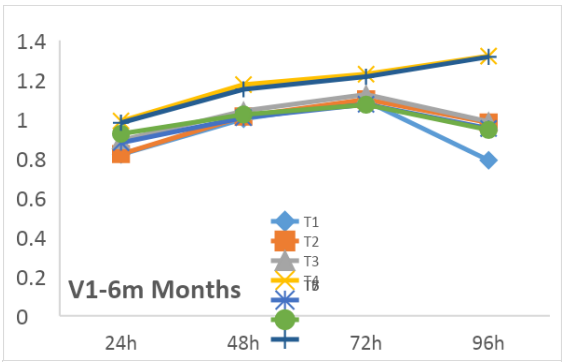


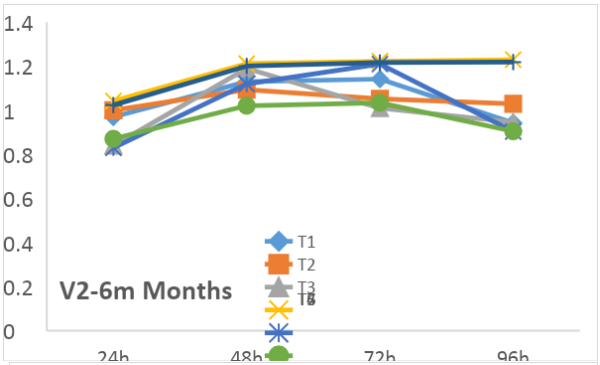


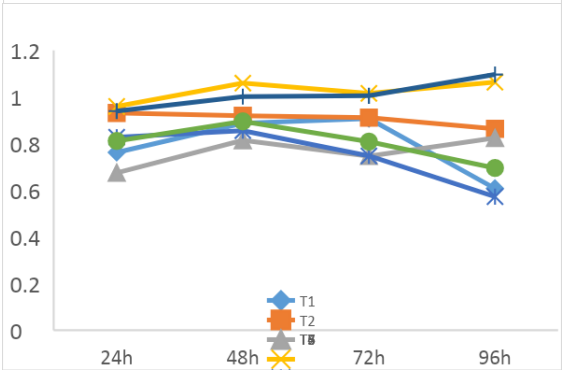
Peroxidase activity (ΔA min-1 g-1):

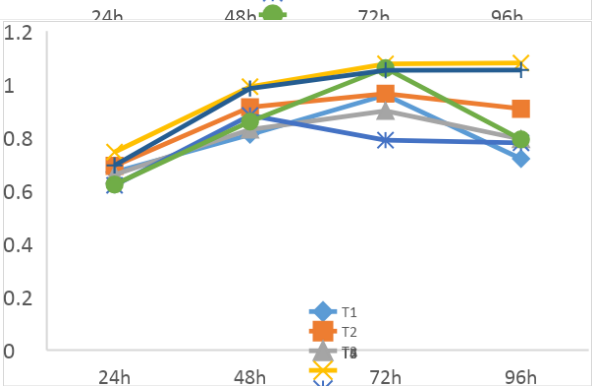






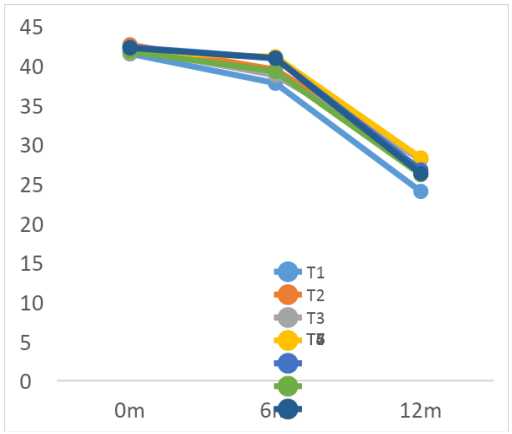




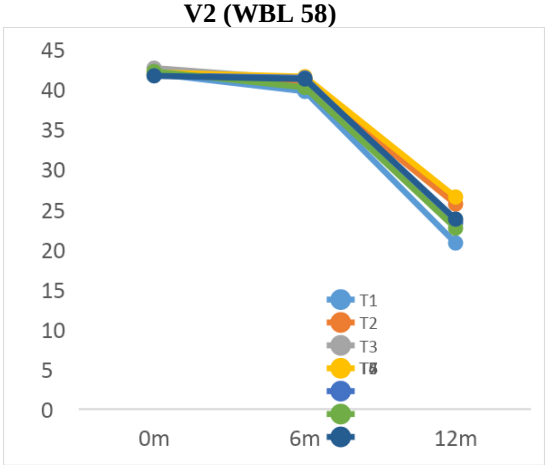


Protein (mg g-1)

V1 (B77)

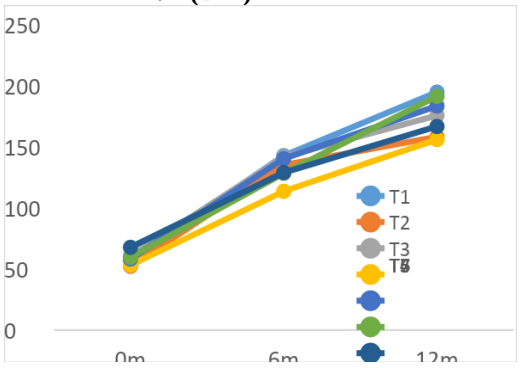


V2 (WBL 58)

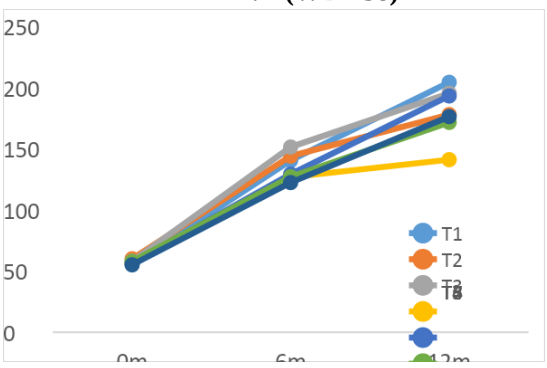


Electrical Conductivity (µS m-1)

V1 (B77)



V2 (WBL 58)



RESULT AND DISCUSSION

Seed qualityGermination %The different storage durations have direct impact on seed deterioration which was reported as declined in seed germination in all treatments as well as genotypes over the time. Among the varieties WBL-58 (24.25%) showed lower declined in seed germination (from 2 months to 12 months)

compared to BL-77 (24.81%). The higher germination was recorded in T4 treatment (90.22 & 89.97%) after 12 months of storage than other treatments in BL-77 and WBL-58 genotypes respectively. The lowest seed germination was recorded in earthen pot and control treatment in both genotypes after 12 months of storage.

Vigour Index-I The different storage durations have direct impact on seed deterioration which was reported as declined in vigour index in all treatments as well as genotypes over the time. Among the varieties BL-77 (2282.36) showed lower declined in Vigour index (storage duration) compared to WBL (2248.42). The higher Vigour index was recorded in T4 treatment (2282.36) after 12 months of storage than other treatments in BL-77 and WBL-58 genotypes respectively. The lowest seed vigour index was recorded in earthen pot and control treatment in both genotypes after 12 months of storage. **Root surface area (cm^2)** Mean treatment effects on total root Surface areaindicated a considerable variation among diverse treatments, where T4 (plastic container) confirmed best effect followed by P7 (polythene packet). V2 (WBL 58) showed superior effect over V1 (B77) in non-significant manner. The different storage durations also showed a significant declination in all steps however its rate was high up in 6 M followed by 12 M. The superiority of T4 was detected in both V1 and V2in consideration of significant demarcationn though the values of T7 indicted better among the rest treatments. Similarly, the rate of deterioration was maximum in 3M in genotype V1 while 12M was highest in V2.**Biochemical evaluation**: **Amylase:** The biochemical activity of seed at the beginning of germination process indicated the potentiality liable for development of better seedling establishment. The α-amylase was analyzed for different duration of seed imbibition and found that the maximum activity was measured in V_2 (WBL 58) compare to V_1 (B77). In both varieties the α-amylase activity was reduced as the period of storage increased from 2 to 12 months. The enzymatic activity was also influenced due to seed imbibition and the maximum activity was reported at 96 hrs of imbibition in all aged seeds. The maximum α-amylase activity (139.07& 138.73 µg $min^{-1}g^{-1}$) were recorded in V_2 variety under plastic container (T4) and plastic packet at 96 hrs seed imbibition respectively. Whereas, the minimum activity was recorded in B77 under control treatment at 2 months of storage and 24 hrs seed imbibition. **Peroxidase:** The activity of Peroxidase was an important indicator for seed quality through elimination of antagonistic effect during seed germination process supportive to develop the high vigour seedling. Similar to amylase, peroxidase activity was also followed same trend and the maximum activity was recorded in WBL 58 than B77. The enzymatic activity in fresh seeds was increased as the duration of seed imbibition prolonged in both the genotypes. The aged seeds of both genotypes showed increasing trend for peroxidase activity from 24-72 hrs of seed imbibition. However, the activity was reduced after 72 hrs in all treatments except T_4 and T_7 for seed stored under 6 & 12 months storage. The maximum activity was recorded for seed stored under plastic container (1.391ΔA min-1 g-1) followed by plastic packet (1.191ΔA min-1 g-1) in case of 2 months stored seeds of variety B77 at 96 hrs after imbibition. While the minimum peroxidase activity was reported in control seed throughout the storage period in both the variety.**Protein:** The fraction of soluble protein may be imperative at preliminary level which is helpful to accelerate cell division and differentiation during seed germination. The protein activity was analyzed in aged seeds and it was reported that the activity was gradually decreased over the storage period and minimum protein activity was reported in 12 months aged seeds compared to freshly harvested seed in both the varieties. After 12 months of ageing the maximum activity was observed in T_4 (28.24 mg g-1) followed by T_2 (28.15) and T_3 (28.08) while, minimum was recorded in control treatment (23.99 mg g-1) in case of variety B77. Similarly, in WBL58 genotype the maximum protein activity was reported in T_4 (26.53) whereas, minimum was recorded in T_1 (20.78) after 12 months of storage period. **Electrical Conductivity:**The electrical conductivity was measured for determining the quality of seed which has a vital role in indication of seed storability. The greater value specified the maximum amount of leach out material from the seed liable to decline the quality. The lower value was encouraging in the same way by maintaining membrane integrity which has observed in different treatment pattern under a significant manner. In both B77 and WBL 58 genotypes seed stored in plastic containers (T_4) showed lower seed leachates (155.97 &141.35 µSm^{-1}) than T_1(194.75 & 204.7) respectively after 12 months of storage.

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