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BIOCHEMICAL CHANGES IN RELATION TO SEED QUALITY IN ACCELERATED AGED COTTON SEED VARIETIES IN DIFFERENT CONTAINERS

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Abstract

The effect of storage container and storage time period was studied in four varieties of cotton. The seeds were stored in three different containers and exposed to varying relative humidity 45%, 60%, 75% and 90% RH for 60 days. The seeds were analyzed for electrical conductivity of leachates, dehydrogenase enzyme activity and peroxidase enzyme activity. The leakage of electrolytes increased with increase in storage time period and relative humidity, whereas the specific activity of both dehydrogenase and peroxidase enzyme decreased with increase in relative humidity and storage time period in all four varieties.

Key words : Cotton seed, electrical conductivity, dehydrogenase enzyme and peroxidase enzyme.

Introduction

All seeds undergo ageing process during long-term storage, which leads to deterioration in seed quality, especially in the humid tropical regions. However, the rate of seed deterioration can vary among various plant species, cultivars and storage conditions (Rao *et al*., 1996). The exact cause of seed viability loss are still unknown, but the viability is affected by pre-harvest climatic conditions, seed type, seed structure, seed health, temperature, relative humidity and seed moisture content (Abba and Lovato, 1999). One important factor governing the rate of viability loss is lipid peroxidation in consequence of formation of an increased amount of free oxygen radicals (Wilson and McDoland (1986). The major cause of membrane disruption are increase in free fatty acid level and free radicals produced by lipid peroxidation (Grilli *et al*., 1995). A protective mechanism that could scavenge the harmful peroxidatively produced free radicals within the seed to keep these deleterious compounds to a minimum have been reported in sunflower and soybean (Sung, 1996). Loycrajjou *et al*. (2008) reported that ageing induced deterioration increase the extent of protein oxidation thus inducing loss of functional properties of proteins and enzymes. Biochemical and physiological deterioration during seed ageing has been studied mostly under accelerated ageing conditions using high temperature and high seed water content (Hsu *et al*., 2003).

Cotton (*Gossypium hirsutum* L.) seed is one of the most sensitive agronomic seeds where significant deterioration occurs after just one year of storage. Cottonseed like other oil seeds is more prone to deterioration due to high oil content. To study the physiological and biochemical changes in seeds during ageing, accelerated ageing has been widely used. In accelerated ageing, the seeds are self-aged by subjecting them to high relative humidity (>90%) and temperature (≥40°C). The seeds so aged are compared for morphological, physiological, biochemical and genetic changes with control ones. The present study has been envisaged to study the biochemical aspects of seed deterioration in cottonseeds during accelerated ageing.

Materials and Methods

The present research work has been carried out in the laboratories of Department of Botany, S.G.N. Khalsa (P.G.) College and Seed Testing Laboratory, Sriganganagar (Rajasthan), India. Seed material comprised of four varieties of cotton *viz*., RG-8, RG-18, RS-810 and RS-2013, having germination above minimum seed certification standards was collected from

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Agricultural Research Station, Sriganganagar. The seeds were surface sterilized with 0.01% mercuric chloride solution for 5 minutes and then washed thoroughly and repeatedly with distilled water before drying. The seeds were then kept in three different containers, *viz*. aluminium bag, polythene bag and craftpaper bag, further under humid conditions of 45%, 60%, 75% and 90%. Relative humidity was maintained with potassium hydroxide (Solomon, 1951).

Electrolyte leakage

To determine electrical conductivity (EC) in the seed leachates, 50 seeds were taken out from each container of all dessicators. Samples of 25 seeds were weighed and taken in beaker in duplicate and soaked in 50ml distilled water in a beaker for 24h at 30°C temperature. At the end of specified period, the contents of beaker were gently stirred and liquid filtered into another beaker. The filtrate was used for knowing electrical conductivity which was measured by conductivitymeter and reported in mhos/cm/gm.

Dehydrogenase enzyme

The dehydrogenase enzyme activity was assayed by the method as described by Singh and Govind (1999). 15-20 seeds were grounded finely and 200mg flour of seed was soaked in 5ml of 0.5% tetrazolium solution with pH 7.0 at 38° C for 2 hours. Then, it was centrifuged at 10,000 rpm for 3 minutes and the supernatant was poured off. The formazan was extracted with 10 ml acetone for 16 hours followed by the centrifugation and absorbance of the solution was determined in calorimeter at 520nm. Increase in 1.0 Optical Density was considered equivalent to 1 unit of dehydrogenase activity.

Peroxidase enzyme

The peroxidase enzyme activity was assayed by the method as described by Matkovich *et al*. (1989). 15-20 seeds were grounded finely and extracted in 25ml of phosphate buffer. The extract was centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was poured off. Zero the spectrophotometer at 500 nm using the blank solution. To the supernatant (0.5 ml), add 2ml dilute hydrogen peroxide, 1 ml guaiacol, 2 ml phosphate buffer. Increase in 0.1 Optical Density was considered equivalent to 1 unit of peroxidase activity. All statistical analysis were done following (Chandel, 2004).

Results and Discussion

The Electrical Conductivity of seed leachates increased in all four varieties with increase in relative humidity during accelerated ageing in all the containers. These results are similar to the results obtained in seeds of cotton by Presley (1958). Minimum leakage of electrolytes 126 micromhos/cm/gm were observed at 45% RH in seeds of variety RG-8 and this conductivity of electrolyte was 2.2% more as compared to the seeds stored as respective control. Tables 1, 2, 3, 4 illustrated that the effect of containers was also significant on leakage of electrolyte in all four varieties. Polythene bag was best storage container followed by aluminium bag and craftpaper bag, respectively. Minimum electrical conductivity of leachates 139 micromhos/cm/gm was shown by the seeds of variety RG-8 stored in polythene bags, whereas maximum values 224 micromhos/cm/gm was shown by the seeds of variety RS-2013 when stored in craftpaper bag. He observed that seed of high viability soaked in water resulted in high resistance (low conductivity) readings and that the resistance reading decreased as the relative humidity and temperature increased. Effect of container was also significant on leakage of electrolyte in all four varieties. Polythene bag was best storage container followed by aluminium bag and craftpaper bag respectively. Minimum electrical conductivity of leachates 139 micromhos/cm/gm was shown by the seeds of variety RG-8 stored in polythene bags, whereas maximum values 224 micromhos/cm/gm was shown by the seeds of variety RS-2013 when stored in craftpaper bag. Membrane disruption is one of the main reasons of seed deterioration as a result, seed cells are not able to retain their normal physical condition and functioning, which in turn resulted in increased leaching of electrolytes. Our results are similar to the results obtained by Pandey (1989), who found that the fluctuating seed moisture during storage in paperbags might be responsible for faster seed deterioration through its effect on cell membrane, which in turn resulted in high leakage of solutes from seeds. Effect of storage time period was significant on leakage of electrolytes in all four varieties. Leakage of electrolytes increased with increase in storage time period. After 60 days of storage, minimum electrical conductivity of electrolytes 215 micromhos/cm/gm was observed in seeds of variety RG-8, but increased by 62.4% with respect to 15 days control 81.2 micromhos/cm/gm under the effect of storage time period.

Specific activity of dehydrogenase enzyme decreased significantly in all four varieties of cotton with increase in relative humidity. Maximum dehydrogenase activity 10.73 µg/mg protein was observed at 45% RH in seeds of variety RG-18 and enzyme activity was 1% less compared to the control. As this enzyme is used to access the viability of the seeds, this trend indicate that there was a decrease in viability of seed. Agboola and Etejere (1991) established that high humidity during storage has also been found

Table 1 : Effect of container, storage time period and relative humidity on seeds of variety RG-8 on electrical conductivity of seed leachates.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**81.2**	86.8	98.4	107.8	109.2	**100.55**
	30	**99.6**	102.4	105.3	112.2	117.8	**109.43**
	45	**113.4**	117.7	122.4	132.75	133.85	**126.68**
	60	**220.8**	224.7	232.2	247.5	253.4	**239.45**
	Mean	**128.75**	**132.90**	**139.58**	**150.06**	**153.56**	**144.03**
Polythene bag	**15**	**78.3**	81.2	84.2	88.6	96.6	**87.65**
	30	**92.5**	96.2	107.5	110.7	125.4	**109.95**
	45	**109.7**	112.3	125.4	132.8	147.6	**129.53**
	60	**213.8**	215.7	224.6	235.2	239.4	**228.73**
	Mean	**123.575**	**126.35**	**135.43**	**141.83**	**152.25**	**138.96**
Craftpaper bag	**15**	**84.4**	87.4	96.6	98.6	108.9	**97.88**
	30	**101.8**	103.3	109.3	117.5	131.8	**115.48**
	45	**135.5**	143.2	155.6	172.7	180.6	**163.03**
	60	**227.8**	236.8	242.8	249.2	265.7	**248.63**
	Mean	**137.38**	**142.68**	**151.08**	**159.50**	**171.75**	**156.25**
Grand Mean		**129.90**	**133.98**	**142.03**	**150.46**	**159.19**	**146.41**
CD-.05	**C- 3.5**	**C*T-5.7**	**C*T*Tr-21.2**			**C**	**Container**
	T-7.3	**C*Tr-9.8**				**T**	**Time (days)**
	Tr-4.2	**T*Tr-12.5**				**Tr**	**Humidity**

Table 2 : Effect of container, storage time period and relative humidity on seeds of variety RG-18 on electrical conductivity of seed leachates.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**88.7**	92.5	95.7	99.15	102.8	**97.54**
	30	**102.4**	108.7	115.6	122.8	132.9	**120.00**
	45	**119.2**	122.5	129.4	132.9	154.7	**134.88**
	60	**226.6**	235.8	239.8	242.85	268.4	**246.71**
	Mean	**134.225**	**139.875**	**145.125**	**149.425**	**164.7**	**149.78**
Polythene bag	**15**	**81.7**	84.8	88.2	96.4	99.95	**92.34**
	30	**99.2**	102.5	108.9	117.8	121.8	**112.75**
	45	**112.8**	116.8	119.6	125.6	141.7	**125.93**
	60	**220.3**	225.6	229.8	241.15	254.4	**237.74**
	Mean	**128.5**	**132.425**	**136.625**	**145.2375**	**154.4625**	**142.19**
Craftpaper bag	**15**	**92.8**	96.2	98.5	103.5	108.7	**101.73**
	30	**107.8**	109.8	113.8	118.9	123.8	**116.58**
	45	**122.4**	129.7	132.5	145.6	153.4	**140.30**
	60	**232.3**	242.8	251.6	262.8	275.8	**258.25**
	Mean	**138.825**	**144.625**	**149.1**	**157.7**	**165.425**	**154.21**
Grand Mean		**133.85**	**138.98**	**143.62**	**150.79**	**161.5291667**	**148.73**
CD-.05	**C- 11.2**	**C*T-28.5**	**C*T*Tr-100.5**			**C**	**Container**
	T-25.3	**C*Tr-45.7**				**T**	**Time (days)**
	Tr-16.5	**T*Tr-59.1**				**Tr**	**Humidity**

Table 3 : Effect of container, storage time period and relative humidity on seeds of variety RG-810 on electrical conductivity of seed leachates.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**103.6**	107.8	109.6	112.7	115.8	**111.48**
	30	**141.8**	149.7	167.6	174.9	115.8	**152.00**
	45	**195.7**	201.4	225.4	236.7	242.6	**226.525**
	60	**258.2**	262.8	275.7	287.9	294.2	**280.15**
	Mean	**174.825**	**180.425**	**194.575**	**203.05**	**192.1**	**192.54**
Polythene bag	**15**	**102.2**	104.4	105.3	107.2	109.8	**106.68**
	30	**132.9**	134.5	136.8	142.7	149.5	**140.88**
	45	**183.7**	192.7	192.9	197.4	201.6	**196.15**
	60	**247.8**	251.7	255.2	261.2	267.8	**258.98**
	Mean	**166.65**	**170.825**	**172.55**	**177.125**	**182.175**	**175.67**
Craftpaper bag	**15**	**107.5**	108.5	110.75	119.2	121.8	**115.06**
	30	**148.6**	149.8	158.7	175.8	184.8	**167.28**
	45	**203.4**	209.6	209.9	227.8	248.2	**223.88**
	60	**265.9**	271.8	276.8	289.9	297.2	**283.93**
	Mean	**181.35**	**184.925**	**189.0375**	**203.175**	**213**	**197.53**
Grand Mean		**174.28**	**178.73**	**185.39**	**194.45**	**195.76**	**186.59**
CD-.05	**C- 14.5**	**C*T-7.1**	**C*T*Tr-21.7**			**C**	**Container**
	T-17.9	**C*Tr-12.7**				**T**	**Time (days)**
	Tr-15.7	**T*Tr-14.2**				**Tr**	**Humidity**

Table 4 : Effect of container, storage time period and relative humidity on seeds of variety RG-2013 on electrical conductivity of seed leachates.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**102.7**	104.5	105.9	107.8	109.1	**106.83**
	30	**148.8**	149.1	151.6	154.8	159.7	**153.80**
	45	**262.8**	264.8	265.2	269.7	272.3	**268.00**
	60	**374.4**	375.6	377.2	381.7	384.8	**379.83**
	Mean	**222.175**	**223.5**	**224.975**	**228.5**	**231.475**	**227.11**
Polythene bag	**15**	**99.8**	103.4	104.2	106.3	108.5	**105.60**
	30	**127.4**	128.4	129.6	132.6	138.8	**132.35**
	45	**259.7**	261.8	263.8	265.7	268.7	**265.00**
	60	**363.5**	364.7	365.9	371.8	374.8	**369.30**
	Mean	**212.6**	**214.575**	**215.875**	**219.1**	**222.7**	**218.06**
Craftpaper bag	**15**	**104.4**	106.8	108.7	109.7	112.6	**109.45**
	30	**152.7**	153.8	155.7	156.4	158.9	**156.20**
	45	**264.9**	265.3	267.8	271.4	273.4	**269.48**
	60	**375.8**	376.9	379.8	382.8	387.3	**381.70**
	Mean	**224.45**	**225.7**	**228**	**230.075**	**233.05**	**229.21**
Grand Mean		**219.74**	**221.26**	**222.95**	**225.89**	**229.08**	**224.79**
CD-.05	**C- 12.3**	**C*T-16.1**	**C*T*Tr-32.7**			**C**	**Container**
	T-25.1	**C*Tr-29.4**				**T**	**Time (days)**
	Tr-13.7	**T*Tr-14.7**				**Tr**	**Humidity**

Table 5: Effect of container, storage time period and relative humidity on seeds of variety RG-8 on dehydrogenase enzyme activity.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**13.68**	13.47	13.35	13.09	12.72	**13.16**
	30	**11.49**	11.47	11.21	10.92	9.86	**10.87**
	45	**9.72**	9.71	9.44	9.24	8.93	**9.33**
	60	**7.44**	7.42	7.29	6.95	6.27	**6.98**
	Mean	**10.58**	**10.52**	**10.32**	**10.05**	**9.45**	**10.08**
Polythene bag	**15**	**13.83**	13.67	13.48	13.15	12.85	**13.29**
	30	**11.51**	11.51	11.37	10.98	10.71	**11.14**
	45	**9.83**	9.83	9.45	9.05	8.95	**9.32**
	60	**7.58**	7.58	7.57	7.25	7.15	**7.39**
	Mean	**10.69**	**10.65**	**10.47**	**10.11**	**9.92**	**10.28**
Craftpaper bag	**15**	**13.35**	13.26	13.05	12.81	11.05	**12.54**
	30	**11.21**	11.19	10.85	10.73	8.76	**10.38**
	45	**9.27**	9.22	9.02	8.95	8.15	**8.84**
	60	**7.26**	7.15	6.85	6.62	6.10	**6.68**
	Mean	**10.27**	**10.21**	**9.94**	**9.78**	**8.52**	**9.61**
Grand Mean		**10.51**	**10.46**	**10.24**	**9.98**	**9.29**	**9.99**
CD-.05	**C- 3.5**	**C*T-5.7**	**C*T*Tr-21.2**			**C**	**Container**
	T-7.3	**C*Tr-9.8**				**T**	**Time (Days)**
	Tr-4.2	**T*Tr-12.5**				**Tr**	**Humidity**

Table 6 : Effect of container, storage time period and relative humidity on seeds of variety RG-18 on dehydrogenase enzyme activity.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**13.89**	13.88	13.71	13.45	12.75	**13.45**
	30	**11.85**	11.85	11.75	11.29	11.05	**11.49**
	45	**9.86**	9.85	9.68	9.36	9.17	**9.52**
	60	**7.69**	7.68	7.62	7.56	7.42	**7.57**
	Mean	**10.82**	**10.82**	**10.69**	**10.42**	**10.10**	**10.50**
Polythene bag	**15**	**14.65**	14.25	14.13	13.86	13.49	**13.93**
	30	**11.99**	11.99	11.76	11.25	11.09	**11.52**
	45	**9.89**	9.88	9.73	9.35	9.27	**9.56**
	60	**7.87**	7.87	7.67	7.59	7.46	**7.65**
	Mean	**11.10**	**11.00**	**10.82**	**10.51**	**10.33**	**10.67**
Craftpaper bag	**15**	**13.55**	13.54	13.48	13.28	12.65	**13.24**
	30	**11.67**	11.67	11.55	11.20	11.00	**11.36**
	45	**9.54**	9.54	9.50	9.29	9.15	**9.37**
	60	**7.44**	7.44	7.26	7.16	7.05	**7.23**
	Mean	**10.55**	**10.55**	**10.45**	**10.23**	**9.96**	**10.30**
Grand Mean		**10.82**	**10.79**	**10.65**	**10.39**	**10.13**	**10.49**
CD-.05	**C- 11.2**	**C*T-28.5**				**C**	**Container**
	T-25.3	**C*Tr-45.7**	**C*T*Tr-100.5**			**T**	**Time (Days)**
	Tr-16.5	**T*Tr-59.1**				**Tr**	**Humidity**

Table 7 : Effect of container, storage time period and relative humidity on seeds of variety RG-810 on dehydrogenase enzyme activity.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**13.80**	13.79	13.62	13.55	13.35	**13.58**
	30	**11.53**	11.53	11.45	11.31	11.20	**11.37**
	45	**9.57**	9.57	9.28	9.15	9.06	**9.27**
	60	**7.30**	7.29	7.26	7.13	7.11	**7.20**
	Mean	**10.55**	**10.55**	**10.40**	**10.29**	**10.18**	**10.35**
Polythene bag	**15**	**13.81**	13.80	13.77	13.64	13.46	**13.67**
	30	**11.65**	11.64	11.56	11.45	11.29	**11.49**
	45	**9.68**	9.68	9.47	9.27	9.17	**9.40**
	60	**7.68**	7.67	7.43	7.31	7.25	**7.42**
	Mean	**10.71**	**10.70**	**10.56**	**10.42**	**10.29**	**10.49**
Craftpaper bag	**15**	**13.75**	13.74	13.59	13.45	13.34	**13.53**
	30	**11.27**	11.27	11.19	11.09	10.95	**11.13**
	45	**9.35**	9.35	9.25	9.11	9.04	**9.19**
	60	**7.12**	7.11	7.05	6.95	6.81	**6.98**
	Mean	**10.37**	**10.37**	**10.27**	**10.15**	**10.04**	**10.21**
Grand Mean		**10.54**	**10.54**	**10.41**	**10.28**	**10.17**	**10.41**
CD-.05	**C- 14.5**	**C*T-7.1**	**C*T*Tr-21.7**			**C**	**Container**
	T-17.9	**C*Tr-12.7**				**T**	**Time (days)**
	Tr-15.7	**T*Tr-14.2**				**Tr**	**Humidity**

Table 8 : Effect of container, storage time period and relative humidity on seeds of variety RG-2013 on dehydrogenase enzyme activity.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**13.82**	13.81	13.75	13.55	13.43	**13.64**
	30	**11.42**	11.42	11.36	11.21	11.13	**11.28**
	45	**9.46**	9.46	9.39	9.32	9.21	**9.35**
	60	**8.48**	8.48	8.27	8.22	8.15	**8.28**
	Mean	**10.80**	**10.79**	**10.69**	**10.58**	**10.48**	**10.64**
Polythene bag	**15**	**13.87**	13.87	13.79	13.64	13.27	**13.64**
	30	**11.55**	11.55	11.49	11.35	11.25	**11.41**
	45	**9.59**	9.59	9.47	9.39	9.28	**9.43**
	60	**8.65**	8.65	8.55	8.37	8.21	**8.45**
	Mean	**10.92**	**10.92**	**10.83**	**10.69**	**10.50**	**10.73**
Craftpaper bag	**15**	**13.77**	13.76	13.65	13.41	13.21	**13.51**
	30	**11.25**	11.25	11.15	11.07	10.85	**11.08**
	45	**9.28**	9.28	9.15	9.08	8.92	**9.11**
	60	**7.35**	7.35	7.25	7.15	7.05	**7.20**
	Mean	**10.41**	**10.41**	**10.30**	**10.18**	**10.01**	**10.22**
Grand Mean		**10.71**	**10.71**	**10.61**	**10.48**	**10.33**	**10.53**
CD-.05	**C- 12.3**	**C*T-16.1**	**C*T*Tr-32.7**			**C**	**Container**
	T-25.1	**C*Tr-29.4**				**T**	**Time (days)**
	Tr-13.7	**T*Tr-14.7**				**Tr**	**Humidity**

Table 9 : Effect of container, storage time period and relative humidity on seeds of variety RG-8 on peroxidase enzyme activity.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**74.6**	73.4	66.5	62.5	58.2	**65.15**
	30	**68.3**	66.9	64.7	56.7	54.9	**60.80**
	45	**64.7**	62.9	56	53.5	52.9	**56.33**
	60	**60.4**	58.5	54.2	52.1	44.8	**52.40**
	Mean	**67**	**65.43**	**60.35**	**56.20**	**52.70**	**58.67**
Polythene bag	**15**	**77.4**	74.5	67.5	63.6	59.43	**66.26**
	30	**71.4**	68.5	65.7	60.2	56.5	**62.73**
	45	**68.7**	66.7	62.8	58.8	54.9	**60.80**
	60	**65.5**	59.4	54.2	52.4	51.17	**54.29**
	Mean	**70.75**	**67.28**	**62.55**	**58.75**	**55.50**	**61.02**
Craftpaper bag	**15**	**72.8**	69.1	63.7	59.3	55.8	**61.98**
	30	**65.3**	63.3	60.15	55.4	52.3	**57.79**
	45	**58.3**	55.3	53.3	51.3	50.4	**52.58**
	60	**53.7**	47.5	45.3	43.5	41.8	**44.53**
	Mean	**62.53**	**58.80**	**55.61**	**52.38**	**50.08**	**54.22**
Grand Mean		**66.76**	**63.83**	**59.50**	**55.78**	**52.76**	**57.97**
CD-.05	**C- 3.5**	**C*T-5.7**	**C*T*Tr-21.2**			**C**	**Container**
	T-7.3	**C*Tr-9.8**				**T**	**Time (days)**

Table 10 : Effect of container, storage time period and relative humidity on seeds of variety RG-18 on peroxidase enzyme activity.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**81.5**	78.2	77.1	73.5	71.2	**75.00**
	30	**74.8**	73.4	72.1	68.9	67.8	**70.55**
	45	**69.5**	68.9	67.5	63.3	58.6	**64.58**
	60	**64.6**	60.7	59.7	56.4	52.17	**57.24**
	Mean	**72.60**	**70.30**	**69.10**	**65.53**	**62.44**	**66.84**
Polythene bag	**15**	**83.6**	79.4	76.45	72.1	71.98	**74.98**
	30	**78.2**	76.5	73.2	69.3	65	**71.00**
	45	**72.6**	71	68.2	67.2	62.8	**67.30**
	60	**67.6**	66.8	64.5	63.5	61.4	**64.05**
	Mean	**75.50**	**73.43**	**70.59**	**68.03**	**65.30**	**69.33**
Craftpaper bag	**15**	**79.4**	76.6	75.3	70.1	69.9	**72.98**
	30	**73.1**	71.5	67.1	66.8	65.4	**67.70**
	45	**67.4**	65.2	63.4	62.2	56.8	**61.90**
	60	**63.4**	59.5	58.3	55.7	52	**56.38**
	Mean	**70.83**	**68.20**	**66.03**	**63.70**	**61.03**	**64.74**
Grand Mean		**72.98**	**70.64**	**68.57**	**65.75**	**62.92**	**66.97**
CD-.05	**C- 11.2**	**C*T-28.5**	**C*T*Tr-100.5**			**C**	**Container**
	T-25.3	**C*Tr-45.7**				**T**	**Time (days)**
	Tr-16.5	**T*Tr-59.1**				**Tr**	**Humidity**

Table 11 : Effect of container, storage time period and relative humidity on seeds of variety RG-810 on peroxidase enzyme activity.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**84.9**	83.4	82.7	79.4	76.3	**80.45**
	30	**75.6**	73	71.8	69.1	68.9	**70.70**
	45	**69.4**	66.5	65.6	64.7	63.5	**65.08**
	60	**65.5**	64.6	63.7	62.6	61.7	**63.15**
	Mean	**73.85**	**71.875**	**70.95**	**68.95**	**67.6**	**69.84**
Polythene bag	**15**	**87.5**	86.3	84.6	82.8	79.7	**83.35**
	30	**78.7**	76.3	74.5	72.1	69.5	**73.10**
	45	**71.3**	70.3	69.6	68.3	65.5	**68.43**
	60	**68.7**	65.8	64.7	62.8	61.8	**63.78**
	Mean	**76.55**	**74.68**	**73.35**	**71.50**	**69.13**	**72.16**
Craftpaper bag	**15**	**81.77**	80.5	79.6	77.8	75.59	**78.37**
	30	**74.5**	72.1	70.4	68.9	67.6	**69.75**
	45	**67.8**	65.9	64.7	64.5	62.5	**64.40**
	60	**62.4**	61.5	59.6	58.6	57.4	**59.28**
	Mean	**71.62**	**70.00**	**68.58**	**67.45**	**65.77**	**67.95**
Grand Mean		**74.01**	**72.18**	**70.96**	**69.30**	**67.50**	**70.74**
CD-.05	**C- 14.5**	**C*T-7.1**	**C*T*Tr-21.7**			**C**	**Container**
	T-17.9	**C*Tr-12.7**				**T**	**Time (days)**
	Tr-15.7	**T*Tr-14.2**				**Tr**	**Humidity**

Table 12 : Effect of container, storage time period and relative humidity on seeds of variety RG-2013 on peroxidase enzyme activity.

Container	Time period	Control	Relative humidity				Mean
			45%	60%	75%	90%	
Aluminium bag	**15**	**75.4**	72.8	71.6	70.8	68.7	**70.98**
	30	**68.7**	65.1	64.3	62.7	60.5	**63.15**
	45	**64.6**	62.8	61.4	59.4	57.6	**60.30**
	60	**60.7**	58.2	57.8	55.7	53.7	**56.35**
	Mean	**67.35**	**64.73**	**63.78**	**62.15**	**60.13**	**62.69**
Polythene bag	**15**	**78.6**	75.6	73.7	72.4	71.2	**73.23**
	30	**71.7**	68.7	67.4	65.5	63.4	**66.25**
	45	**68.4**	65.6	64.7	62.8	61.4	**63.63**
	60	**62.9**	60.1	59.6	58.1	57.2	**58.75**
	Mean	**70.40**	**67.50**	**66.35**	**64.70**	**63.30**	**65.46**
Craftpaper bag	**15**	**72.9**	71.6	69.4	67.3	65.2	**68.38**
	30	**64.7**	63.4	62.1	61.4	59.8	**61.68**
	45	**61.5**	61.1	60.7	58.2	56.2	**59.05**
	60	**41.6**	40.7	36.4	38.2	36.7	**38.00**
	Mean	**60.18**	**59.20**	**57.15**	**56.28**	**54.48**	**56.78**
Grand Mean		**65.98**	**63.81**	**62.43**	**61.04**	**59.30**	**61.64**
CD-.05	**C- 12.3**	**C*T-16.1**	**C*T*Tr-32.7**			**C**	**Container**
	T-25.1	**C*Tr-29.4**				**T**	**Time (days)**
	Tr-13.7	**T*Tr-14.7**				**Tr**	**Humidity**

among other factors to reduce substrate for early enzyme activities and therefore leading to loss of viability. The loss of activity of dehydrogenase enzyme parallels the loss in seed viability and vigour. Seeds stored in polythene bags retained more enzyme activity over other two containers, whereas minimum enzyme activity 8.52 µg/mg protein was recorded in craftpaper bags, when treated with 90% RH and enzyme activity was 17% less compared to respective control. Our results are in accordance with Bhattacharya *et al*. (1983), who established that with increase in storage period, dehydrogenase activity was weakened in air tight storage but fall rapidly in seeds stored in non-airtight storage containers. Maximum dehydrogenase activity was observed after 15 days of storage and it decreased gradually as the storage period prolonged in all varieties. After 60 days of storage, maximum dehydrogenase activity 8.65 µg/mg protein was noticed for seeds of variety RS-2013 stored in polythene bags at 45% RH. This trend shows that dehydrogenase activity decreased with increase in relative humidity and storage time period in all the containers in all four varieties of cotton. Effect of time period was much more pronounced than the effect of relative humidity. A decline in respiratory enzymes like dehydrogenase in seeds may contribute to respiratory failure. Since the production of some respiratory enzymes and isozymes is an indication of embryo viability, the inability of the seed to correct these enzymes deficiencies incurred during development or storage can be associated with loss of seeds viability.

Specific activity of peroxidase enzyme decreased significantly in all four varieties of cotton with increase in relative humidity. Maximum peroxidase activity 74.7 µg/mg protein was observed at 45% RH in seeds of variety RS-810. As this enzyme is involved in removing several free-radicals produced within the seeds, this trend indicates that there was a decrease in protective mechanism of enzyme activity. These results are in agreement with most of the previous reports (Sung and Jeng, 1994; Pukacka and Ratajczak, 2005) observed that the loss of viability of beech seeds during storage at different temperature and relative humidity levels is closely related to reactive oxygen species produced in seed tissues and the peroxidase enzyme. Polythene bags were the best storage containers among the three containers. Maximum enzyme activity 72 µg/mg protein was shown by the seeds of RS-810 stored in polythene bags, whereas minimum enzyme activity 54 µg/mg protein was shown by the seeds of variety RG-8 stored in craftpaper bags (Rao *et al*., 2006) found that fresh onion seeds stored in aluminium laminated pouches have minimum accumulation of free peroxide radicals and enhanced the peroxidase activity. The peroxidase activity increased upto 15 days *i.e*. maximum. However, with further increase in storage time period, the peroxidase activity decreased gradually. After 60 days maximum peroxidase activity 64 µg/mg protein was noticed for seeds of variety RG-18 stored in polythene bags with 45% RH. The protective mechanism involving several free radicals and peroxide-scavenging enzymes like peroxidase have been evaluated within the mechanism of seed ageing (Hsu *et al*., 2003). These results indicated that with increased accelerated ageing, the seeds were unable to host the scavenging enzymes like peroxidase and respiratory enzymes like dehydrogenase. Increased electrolyte leakage with ageing confirmed the inferior quality of the aged cotton seeds.

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