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Full Length Article

Cotton Seed Quality and Storage Physiology in Relation to Harvest and Post-harvest Storage Techniques

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Abstract

Cotton seed mainly loses its viability and vigour during harvesting and storage. This study was designed to evaluate the effect of picking time and post-harvest storage techniques on germination and vigour of cotton seed. In the first phase of experiment, initial seed quality (seed moisture content, germination and vigour) was determined at four different picking times i.e. 15 August, 15 September, 15 October and 15 November. In the second phase, seed from different picking (Factor A) was dried to 8% moisture contents and stored for six months by different storage techniques i.e., Ginned cotton seed and Un-Ginned seed cotton (Factor B) in different packaging materials i.e. hermetic and jute bags (Factor C). Maximum moisture (17.36%) was recorded from seed cotton picked on 15th September. September picking gave the best quality seed having maximum thousand seed weight (106.13 g) and germination percentage (67.25%). In storage experiment, highest seed vigour index- I (3256.9) and II (29.43), total soluble sugar contents (2.86 mmol g^{-1} FW), catalase (2.78 U g^{-1} FW) and peroxidase (1.72 U g^{-1} FW) was measured from September picked cotton seed stored in hermetic bags after ginning. Highest malondialdehyde contents (3.01 nmol g^{-1} FW) were measured in August picked un-ginned seed under conventional storage. Maximum seed germination (65%) and lowest seed deterioration was observed in September picked seed stored in hermetic bags after ginning. In conclusion, cotton seed picked during the month of September after drying (maximum 8%) should be ginned and stored in hermetic bags to retain maximum viability for seasonal storage under ambient conditions.

Keywords: Germination; Phenology; Picking time; Storage; Vigour; Antioxidants

Introduction

Cotton (*Gossypium hirsutum* L.) is the major fiber and industrial crop in the world and famous as "white gold". According to Global Climate Risk Index, Pakistan is among the top 10 countries most vulnerable to climate change (Eckstein *et al*. 2021). Crucial factors which are limiting the cotton production in Pakistan are cotton leaf curl disease, heat stress, higher input charges (insecticide, fertilizer, crop seed, pesticide, etc.), higher insect pest attack, low seed germination, seed adulteration and marketing problems (Malik and Ahsan 2016). Low seed germination in cotton is the result of poor harvesting and postharvest management. Dormancy due to biochemical inhibitor such as (Kloth and Turley 2010) or due to physical prevention of moisture uptake by seed coat prevent seed germination in freshly harvested cotton seed.

Picking time in cotton is the most critical stage and varying quality of cotton seed is obtained at different pickings. Late picking leads to dead seeds affecting weight and quality of seed (Abbas *et al.* 2010). Pre-harvest and post-maturation conditions lead to seed deterioration and even one week of exposure to rainy weather results in 20 to 30% loss in the viability of cotton seed (Dayal *et al*. 2017). Humid weather and heavy rainfall are harmful for cotton particularly at boll-opening and picking stage. Seed ageing due to delay in harvesting is the result of membrane degradation, reduced seed minerals, amino acids and seed leachates leakage (War *et al*. 2020). Picking from immature bolls affects the value of the lint and the seed germination (Soomro *et al*. 2004).

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During storage, quality of seed and viability is affected by storage technique (Lakshmi *et al.* 2021). Membrane integrity of aged seed is lost by phospholipid damage that increased leakage of electrolytes and other substances from cells (Zamani *et al.* 2010) resulting in high electrical conductivity of seed leachates. The major symptom of lipid oxidation during seed deterioration is high levels of malondialdehydes (Corbineau 2024). Catalyze activity (CAT) is particularly important in oily seeds as it reduces H_2O_2 produced by β-oxidation of fatty acids (Bailly 2004). Peroxidase plays a crucial role in seed metabolism by using hydrogen peroxide as an acceptor, perhaps promoting defense mechanisms and reducing quality loss (Ushimaru *et al*. 2001). Aged seeds of various plant species including cotton have lower total soluble sugar contents (Salam *et al*. 2018). Seed viability is highly correlated with soluble sugars amounts (Abdellaoui *et al*. 2013). These biochemical and physiological indicators have been studied in this study to assess seed quality subjected to various seed storage techniques.

Choice of packaging material directly impacts seed moisture and subsequent seed quality during storage. Conventional packaging materials are porous in nature and even dried seeds can gain moisture under high ambient relative humidity (Bakhtavar *et al*. 2019). Hermetic packing has made a significant contribution to safe, pesticide-free seed storage for a variety of seeds particularly in hot and humid regions (Bakhtavar and Afzal 2020). Hermetic Super Bag has very low oxygen permeation rate (4 $cc/m^2/day$), water vapor transmission rate (5 g m^{-2} day^{-1}). The present study was conducted with the objective to test that picking time, storage technique and packaging materials impact the quality of cotton seed. Moreover, the physiological and biochemical basis for seed deterioration have been explored.

Materials and Methods

The experiment was carried out during kharif season, 2023 at Cotton Research Institute, Multan, Pakistan. Sowing of cotton variety FH-333 was done on April 27, 2023. The net plot size was 6 m × 4.5 m with three replications. The treatments of the first experiment include cotton picking at four different times (15 August, 15 September, 15 October and 15 November) from same plots. The meteorological data of the experimental duration is presented in Fig. 1.

After picking seed cotton was dried for 2 to 3 days to attain the required moisture content (8%). After drying, ginned and un-ginned seed was packed in different storage materials i.e. hermetic and conventional (Jute) storge bags. Thousand seed weight was determined only for 1st phase of experiment. Seed moisture contents, electrical conductivity of seed leachates and seed germination parameters were determined for both phases. Vigour indices, CAT, POD, MDA and total soluble sugars were recorded for the storage phase only.

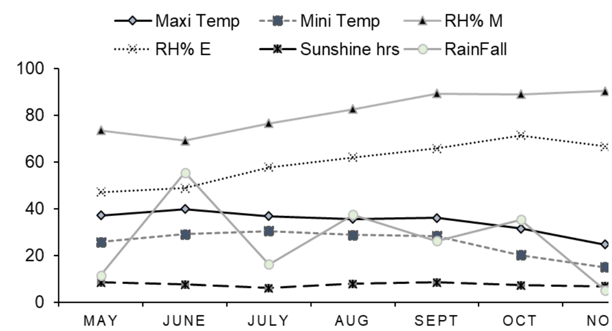

Fig. 1: Weather data collected during crop period (May-Nov) for the year 2023

Seed moisture

Seed moisture percentage was determined by ISTA low constant oven dry method using following equation:

$$\text{Seed moisture (\%)} \frac{\text{Loss of weight}}{\text{Original weight}} \times 100$$

1000-seed weight

Thousand seeds were counted from produce of each picking time and weight was recorded with digital weight balance and expressed in grams.

Seed germination

For seed germination, 400 seeds in 4 replicates were sown in sand media having pH within 6-7.5 and conductivity no more than 40 millisiemens per meter. The seed germinator was set at 25ºC and sand media containing seed was placed in seed germinator. Final count was recorded on 12th day (ISTA 2020). Normal seedlings were expressed as percent germination.

Electrical conductivity testing

From each replication, 50 standard seeds were counted, weighed and soaked in a 500 (mL) beaker holding 250 (mL) of distilled water and retained at 20°C for 24 h. The electrical conductivity of seed leachates was calculated by taking conductivity reading with a conductivity meter and expressed as $\mu S\ cm^{-1}\ g^{-1}$.

$$EC = \frac{\text{Conductivity reading} - \text{background reading}}{\text{weight of replicate (g)}}$$

Vigor indices

The vigor index I and II were measured as follows:

Vigor Index - I = Average seedling length (mm) × Germination (%)

Vigour Index - II = Average seedling dry weight (mg) × Germination (%).

The seedling length of 10 normal seedlings were measured and average was taken to calculate vigor index -I. Seedings were dried in drying oven at 80°C for three days and dry weight was recorded. Dry weight was multiplied with germination percentage to calculate vigour index –II.

Determination of catalase (CAT) activity

The CAT activity was determined by following (Beers and Sizer 1952) with slight modification. Cotton seeds were ground through pestle and mortars in fine powder and 100 mg seed powder was moistened in 5 mL of phosphate buffer and then incubated at 70°C for 30 min. The reaction mixture consisted of 0.5 mL H_2O_2 (40 mM), 2 mL of sodium phosphate buffer (50 Mm, pH 7.0) and 0.5 mL enzyme extract in a total volume of 3.0 mL. The decomposition of H_2O_2 was measured by a reduction in absorbance at 240 nm by using a spectrophotometer.

Determination of peroxidase (POD) activity

The POD activity was determined by guaiacol oxidation method (Chance and Maehly 1955) with slight modifications. Seeds (100 mg) were grounded in powder form and moistened in 5 mL of sodium phosphate buffer then incubated at 70°C for 30 min. The POD reaction solution (total 3 mL) containing 1 mL of sodium acetate buffer (50 mM, pH 5.0), 0.9 ml of H_2O_2 (40 mM), 1 mL of guaiacol (20 mM) and 0.1 mL enzyme extract. The reaction mixture containing sodium acetate as replacement of enzyme solution was used as blank. Absorbance was taken at 470 nm with spectrophotometer to calculate POD.

Determination of malondialdehyde (MDA)

For the determination of MDA, 1 g seed powder was homogenized in 8 ml of 10% TCA solution. This reaction mixture was centrifuged at 10,000 rpm for 15 min. Supernatant (2 mL) was collected and mixed with 2 mL of 0.6% TBA prepared in 20% TCA solution. Reaction mixture was incubated for 20-25 min in a water bath set at 100°C. Mixture was cooled and centrifuged for 5 min. Supernatant was collected and absorbance was taken at 600, 532 and 450 nm. Following equation was used to calculate the MDA contents:

$$MDA = 6.45(A_{532} - A_{600}) - 0.56A_{450}$$

Determination of total soluble sugars

Soluble sugar was determined following the phenol sulfuric acid method according to (Roby and White 1987). 100 mg of seeds were ground in fine powder and moistened in 5 mL of methanol (80%) then incubated at 70°C for 30 min. The extract was centrifuged at 5,000 rpm for 10 min. To 1 mL of supernatant, 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid, were added. Absorbance was taken by a spectrophotometer at 490 nm. Soluble sugar concentrations were expressed as mmol g^{-1} seed FW.

Statistical analysis

The first phase of experiment was conducted in Randomized Complete Block Design (RCBD) whereas second phase of experiment was completed by using Completely Randomized Design (CRD) with factorial arrangement. Statistical analysis of recorded parameters was done by applying Fisher's Analysis of Variance technique using software "Statistics 10.1". Treatment means compared through LSD test at 5% probability level.

Results

Phase 1

Seed moisture: Harvesting at various stages showed significant variation in seed moisture percent. Maximum seed cotton moisture (17.36%) was recorded when picking was done on 15th of September compared to August, October and November picking time having (13.23%, 13.35% and 16.15%) respectively (Table 1).
1000-seed weight: Significant difference ($p \leq 0.05$) was observed in 1000-seed weight of cotton at different picking time (Table 1). Seed cotton picked on 15 September gave highest 1000-seed weight (106.13 g) followed by seed cotton picked on 15th of October. The lowest 1000-seed weight (83.88 g) was recorded when picking was done on 15 August.
Seed germination: Seed from September picking showed maximum germination (67.25%) whereas August picking had least seed germination (46.25%). Order of seed germination for various picking was September>October>November>August (Table 1).
Electrical conductivity: Electrical conductivity (EC) (µS $cm^{-1}g^{-1}$) of cotton seed was found statistically significant at ($p \leq 0.05$) in different picking times (Table 1). Seed from September Picking showed minimum electrical conductivity (27.25). Seed picked on October showed EC (30.11) followed by November picking seed (33.53). Results showed that August picked seed had maximum EC (49.77) compared to other picking times.

Phase 2

Cotton seed moisture after storage: Maximum moisture (9.37%) was recorded from un-ginned seed of August picking stored in conventional bags. Minimum seed moisture (7.07%) was recorded for the ginned seed of September picking stored in hermetic bags (Table 2). In both ginned and unginned storage techniques, conventional storage showed higher seed moisture as compared to hermetic storage.
Electrical conductivity of seed leachates after storage: Maximum electrical conductivity of seed leachates (66.11) was

Table 1: Effect of picking time on different seed quality attributes of cotton seed like (seed cotton moisture (%), 1000 seed weight (g), EC (µS $cm^{-1}g^{-1}$) and Seed germination (%)

Picking time	Seed cotton moisture (%)	1000 seed weight (g)	EC (µS $cm^{-1}g^{-1}$)	Seed germination (%)
15^{th} of August	13.23 b	83.88 c	49.77 a	46.25 c
15^{th} of September	17.36 a	106.13 a	27.26 d	67.25 a
15^{th} of October	13.35 b	96.72 b	30.11 c	55.00 b
15^{th} of November	16.15 a	93.60 b	33.53 b	53.25 b
LSD value at 5%	1.33	4.96	2.69	6.22

Treatment means not sharing same letters are significantly different at *p≤ 0.05*

Table 2: Effect of picking time on different seed quality attributes of cotton seed after storage seed germination, cotton seed moisture and EC (µS $cm^{-1}g^{-1}$)

	Picking	Seed Germination (%)				Cotton seed moisture (%)				EC (µS $cm^{-1}g^{-1}$)			
		Ginned Storage		Un-Ginned Storage		Ginned Storage		Un-Ginned Storage		Ginned Storage		Un-Ginned Storage	
		Hermetic Storage	Conventional storage	Hermetic Storage	Conventional Storage	Hermetic Storage	Conventional Storage	Hermetic Storage	Conventional Storage	Hermetic Storage	Conventional Storage	Hermetic Storage	Conventional Storage
Picking time	August picking	44.33 fg	42.00 gh	42.00 gh	40.33 h	7.54 f	8.44 de	8.88 b	9.37 a	56.57 a-d	63.20 ab	66.11 a	61.50 abc
	September picking	65.00 a	62.00 ab	62.33 ab	60.33 b	7.07 g	8.20 e	8.59 cd	9.15 a	37.50 i	39.60 hi	42.98 f-i	40.89 ghi
	October picking	53.00 c	52.00 cd	51.00 cd	50.33 cd	7.18 g	8.26 e	8.65 bcd	9.19 a	3.84 e-i	40.12 ghi	51.80 c-f	48.76 d-h
	November picking	49.67 de	47.00 ef	46.33 f	45.00 fg	7.46 f	8.33 e	8.69 bc	9.30 a	48.76 d h	48.92 d-h	54.17 b-e	50.29 d-g
LSD value at 5%		Storage technique × Storage materials × Picking time =3.166				Storage technique × Storage materials × Picking time = 0.249				Storage technique × Storage materials × Picking time = 10.67			

Treatment means not sharing same letters are significantly different at *p≤ 0.05*

Table 3: Effect of picking time on different seed quality attributes of cotton seed after storage (Vigour index- I and Vigour index- II)

	Picking	Vigour index- I				Vigour index- II			
		Ginned Storage		Un-Ginned Storage		Ginned Storage		Un-Ginned Storage	
		Hermetic storage	Conventional storage	Hermetic storage	Conventional storage	Hermetic storage	Conventional storage	Hermetic storage	Conventional storage
Picking time	August picking	1404.7 fg	1275.7 gh	1251.9 gh	1073.0 h	13.76 e-h	12.42 ghi	11.25 i	11.84 hi
	September picking	3256.9 a	3099.3 ab	3072.8 ab	2865.5 b	29.43 a	25.61 b	25.74 b	23.46 b
	October picking	2412.1 c	2277.1c	2179.7 c	1896.3 d	20.96 c	17.47 d	17.45 d	13.59 f-i
	November picking	1717.8 de	1492.8 efg	1550.7 ef	1355.3 fg	16.09 de	14.93 ef	14.81 efg	12.35 ghi
LSD value at 5%		Storage technique × Storage materials × Picking time = 268.35				Storage technique × Storage materials × Picking time = 2.493			

Treatment means not sharing same letters are significantly different at *p≤ 0.05*

recorded from the un-ginned seeds of August picking that were stored in jute bags. Minimum (37.50) electrical conductivity (Table 2) was measured from the seed of September picking that were stored in hermetic bags after ginning.

Seed germination after storage: Cotton seed of September picking stored in hermetic bags in ginned conditions had maximum (65%) germination (Table 2). Minimum seed germination (40%) was recorded for the seeds of August picking stored in jute without ginning.

Vigor index-I of stored seed: Highest vigour index-I (3256.9) was calculated for September picked seed stored in hermetic bags in ginned form. In un-ginned form, seeds of August picking stored in conventional bags had lowest (1073.0) seed vigour index-I (Table 3).

Vigour index- II in stored seed: Highest seed vigour index- II (29.43) was recorded from ginned seed of September picking stored in hermetic bags. Lowest (11.25) vigour index- II (Table 3) was calculated for the un-ginned seed of August picking stored in conventional bags.

CAT activity in stored seed: Interactive effect of picking time, storage techniques and storage materials on catalase activity was statistically significant at ($p \leq 0.05$) September picking seed after ginning stored in hermetic bags gave maximum catalase activity (2.78 U g^{-1} FW). Minimum catalase activity (2.53 U g^{-1} FW) was observed in August picked un-ginned seed stored in conventional bags (Fig. 2).

POD activity in stored seed: The interactive effect of picking time, storage techniques and packaging materials on peroxidase activity was statistically significant at ($p \leq 0.05$). Seed of September picking stored in hermetic bags in ginned conditions gave highest peroxidase activity (1.72 U g^{-1} FW) values. The lowest values for peroxidase activity (1.24 U g^{-1} FW) were given by seed of August picking, when it was stored in conventional bags in un-ginned condition (Fig. 3).

MDA contents in stored seed: Picking time, storage techniques and packaging materials significantly affected

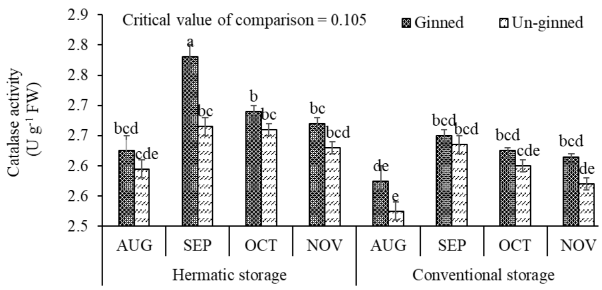

Fig. 2: Changes in activities of catalase measured from the seeds stored using different storage techniques and packaging materials, Means ± SE

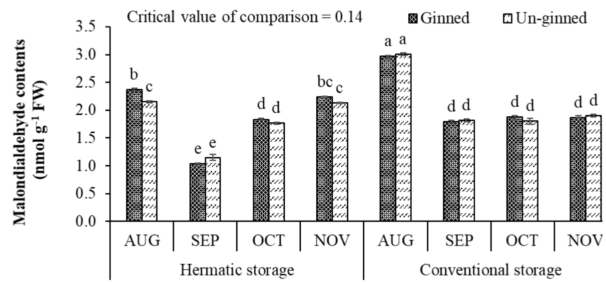

Fig. 4: Malondialdehyde (MDA) contents measured from the seeds stored using different storage techniques and packaging materials. Means ± SE

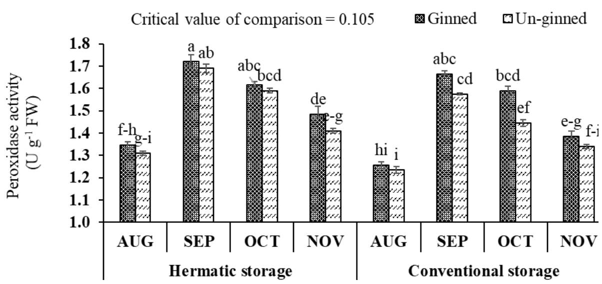

Fig. 3: Changes in activities of peroxidase measured from the seeds stored using different storage techniques and packaging materials, Means ± SE

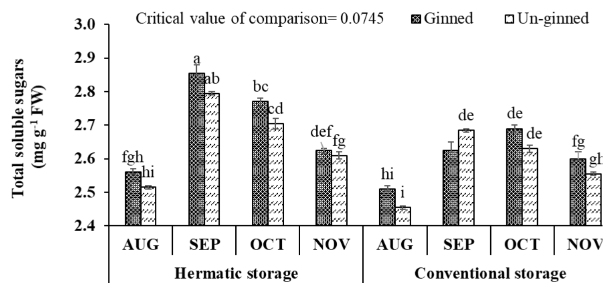

Fig. 5: Total soluble sugars contents measured from the seeds stored using different storage techniques and packaging materials. Means ± SE

MDA content in cotton seed. Un-ginned (3.01 nmol g^{-1} FW) and ginned seed (2.97 nmol g^{-1} FW) from August picking stored in conventional packaging had the highest MDA contents when stored in conventional bags. Lowest MDA contents (1.04 nmol g^{-1} FW) were found in the ginned seed of September picking store in hermetic storage (Fig. 4).

Total soluble sugars in stored seed: Highest total soluble sugar contents (2.85 mmol g^{-1} FW) were found in ginned seed of September picking stored in hermetic bags. The lowest total soluble sugar contents (2.46 mmol g^{-1} FW) were measured from unginned seed of August picking stored in conventional bags (Fig. 5).

Discussion

Cotton seed from different pickings showed (Table 1) significant variation in moisture contents of seed lots. The various picking times had an effect on physiological quality of the cotton seeds. Rainfall during September before the picking increased the seed moisture contents.

Maximum seed weight in September picking might be due to better source sink relationship. Recorded weather data indicates that maximum sunshine hours, better window of minimum and maximum temperature was recorded during the month of September (Fig. 1). Seed at physiological maturity attain maximum dry weight and quality by accumulation of food reserves (Dayal *et al.* 2017). Weather data of this study indicated that average monthly temperature and rainfall in August was higher as compared to September (Fig. 1). High temperature reduces source and sink activities. To improve cotton seed yields, balanced source-sink relationships are needed by improving photo assimilate production and transport to seed (Qin *et al.* 2023).

Better seed germination was due to cumulative effect of higher percentage of mature and healthy seeds and higher seed weight compared with other three pickings. Due to high temperature and erratic rainfalls (Fig. 1) seed development was affected and seeds were not fully matured in August picking. October and November picked seeds showed less germination. This may also be due to non-ideal weather conditions for boll and seed development later in the season (Dayal *et al*. 2014).

Electrical conductivity of seed leachates was less in the picking of 15th September, indicating proper maturity and reduced damage to cellular membranes of seed tissues. Delay in harvesting, ageing processes and damage to membranes increased minerals and amino acids leaching out of the seed and gradually increased the electrical conductivity of seed leachates (War *et al*. 2020).

The initial seed quality before storage, seed moisture content, temperature and humidity during the storage period all have a significant impact on final seed viability (Aqil 2020). High moisture content causes decline in seed viability during storage and losses of stored crops due to fungal spoilage and insect pests. Seed storage life of orthodox seeds is reduced by one half for each 1% increase in moisture contents or 10°F increase in storage temperature (Harrington 1972). Hermetic storage bags resist any significant change in seed moisture. Dried seeds stored in hermetic bags are unlikely to get moisture from the surrounding air of high relative humidity.

During deterioration, vigour is the first component of seed quality, which is lost followed by a loss of germination capacity and viability (Trawatha *et al*. 1995). The decline in germination as the storage progressed, was due to seed aging and depletion food reserves (Deepika *et al*. 2014).

Electrical conductivity test is commonly used as an indicator for integrity of cellular membrane and seed vigour (Bewley *et al*. 2013). In our study, electrical conductivity of seed leachates increase in jute bags. Lipid peroxidation is the main process that contributes to deterioration and seed aging and subsequently triggers disruption of the membrane (Goel and Sheoran 2003). The membrane integrity is lost by phospholipids damage that results in increased membrane permeability and leakage of electrolytes and other substances (Zamani *et al*. 2010).

Catalase activity in seeds could be used as a criterion for determining the ability of seeds to tolerate oxidative stress (Prodanovic *et al*. 2007). Catalases are involved in the decomposition of hydrogen peroxide in cells (Muniz *et al*. 2007). The lowest catalase activity was found in August picking when un-ginned seed was stored in conventional bags.

Peroxidase plays an important role in seed metabolism, potentially enhancing protective mechanisms and preventing loss of seed quality (Ushimaru *et al*. 2001). In conventional storage, seed absorbs more moisture contents from surroundings and deterioration of seed occurs which ultimately reduced the ability of seed germination. Reduced peroxidase activities are reported in aged rice seed (Zhou *et al*. 2002).

The reduced activity of antioxidant enzymes hampered ROS scavenging and accelerated lipid peroxidation. Increased malondialdehyde content was closely linked to a decrease in germinability (Goel and Sheoran 2003). Increased lipid peroxidation level (indicated by the high MDA content) was associated with deterioration of cotton seeds. Our results also showed that as the MDA contents (Fig. 4) increased during storage, germination of seed also decreased.

Total soluble sugars in cotton seed decreased as storage time increased. This is due to aging related limitations in respiratory substrates (Sharma *et al*. 2013). September picked seed stored in hermetic bags showed (Fig. 5) highest total soluble sugars contents. However, the decrease in total soluble sugar was more pronounced in the seed stored in jute bag which could be due to the high temperature and relative humidity during storage which accelerated the depletion of carbohydrate reserves and increased respiratory metabolism (Salam *et al*. 2018).

Conclusion

The picking time of 15th September showed highest seed germination, vigour, maximum seed weight. September picked cotton seed should be dried to at least 8% seed moisture for hermetic storage after ginning to retain maximum viability after 6-month storage. Seed germination, catalases, peroxidases activities and total soluble sugars contents were higher with low malondialdehyde contents and EC of seed leachates in hermetically stored ginned seed of September picking indicating reduced rate of seed deterioration.

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Author Contribution

Muhammad Nadeem: Conduct experiment, acquisition, analysis and interpretation of data, writing original draft, revision of manuscript; Abdul Ghaffar; Muhammad Amir Bakhtavar; Shahid Iqbal, Wazir Ahmed, Muhammad Iqbal: Conception and design of the work, provision of resources, supervision of the work, review of manuscript, approved the version to be published; Muhammad Najeeb Rasool: Data analysis, visualization and review of manuscript.

Conflicts of Interest

The authors declare that they have no known conflict of interests that could have appeared to influence the work reported in this paper.

Data Availability

Data will be available from corresponding author upon request.

Ethics Approval

Not applicable to this paper.

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