

## HERMETIC STORAGE MAINTAINS SEED QUALITY AND MINIMIZES THE SEED VIGOR LOSSES IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

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### Abstract

A uniform and vigorous stand establishment is essential for profitable cotton production. Poor-quality seed and postharvest cotton storage losses play a major role in crop stand failure. Hermetic storage uses sealed, airtight units to control moisture and restrict gas exchanges, maintaining initial moisture levels and controlling pests by limiting oxygen. In this study, cotton seeds with initial moisture levels of 6% and 8% were stored for six months under conventional and hermetic conditions. Conventional storage led to a rise in moisture content from 6.0% to 6.8% and from 8.0% to 8.9%, while hermetic storage showed minimal increases. Germination rates exceeded 90% after three months but declined after six months, notably in seeds with 8% initial moisture stored conventionally. Free fatty acid (FFA) and malondialdehyde (MDA) content increased over time conventionally, whereas seeds with 6% initial moisture stored hermetically showed lower FFA (0.44% to 0.49%) and MDA (0.55 to 0.62 nmole g<sup>-1</sup> DW) accumulation. Total soluble sugars rose slightly in seeds under hermetic conditions at 6% initial moisture and displayed improved vigor (75.10% to 77.30%) and quicker germination compared to conventionally stored seeds. After six months, cotton seeds with 8% initial moisture in conventional storage exhibited higher electrical conductivity (EC), whereas seeds with 6% initial moisture stored hermetically showed minimal EC changes. Hermetic technology has resulted in minimizing quality losses by reducing metabolic activities. It is safe, pesticide-free, and a sustainable storage technology suitable for seeds, particularly in hot, humid climates. It can ensure food security by limiting insect and fungi infestation and reducing quantity and quality degradation in stored cotton.

**Key words:** Cottonseed, Conventional, Hermetic storage, Germination speed, Seed viability.

### Introduction

Postharvest crop management is a major challenge, especially in developing countries. Handling and storage of grains and seeds is very important to protect seeds from spoilage (Kumar & Kalita, 2017). The purpose of seed storing is to keep the seeds in good physical and physiological condition from the time of harvest to the time of sowing. Seed moisture is an important factor during seed storage that affects seed quality because seed survival depends on moisture content (Dadlani *et al.*, 2023). Understanding the optimal seed types for storage is crucial to extend storage lifespan and prevent contamination by storage fungi (Bradford *et al.*, 2020). While weight loss in seeds is easily quantifiable, assessing changes in seed vigor and quality during storage is a complex task, particularly in determining suitability for consumption by humans or animals or subsequent crop planting (Ekpa *et al.*, 2019). Viability, germination percentage, and vigor stand as pivotal considerations for seed quality (Shaban, 2013). Elevated seed moisture content during storage compromises seed quality, escalating the rate of seed respiration, and generating heat that can detrimentally impact germination rates. For instance, maize seeds stored with higher moisture content (15%) exhibited diminished viability, vigor, and germination rates, alongside increased fungal growth and substantial loss in dry matter (Afzal *et al.*, 2017). Agricultural produce, especially seeds, is commonly stored by farmers in polypropylene and jute bags to shield them from erratic climatic conditions. However, these materials are prone to deterioration when exposed to fluctuating humidity, temperature, and precipitation, impacting seed quality (Bewley & Black, 2013; Afzal *et al.*, 2017). Consequently, poor seed quality often compels farmers to sell their produce at lower prices shortly after harvesting (Tefera *et al.*, 2011). Inadequate seed storage can result in

two-fold damage: physical and nutritional. Physical damage manifests as seed weight and color deterioration, while nutritional loss diminishes seed vigor (Boxall, 2002). These impacts are influenced by various factors: abiotic elements like temperature, humidity, and moisture, and biotic factors such as insect and fungal infestation, all significantly impacting seed quality (Abedin *et al.*, 2012).

In regions like Pakistan, where cotton harvesting commences early in the morning amidst lingering dew, the picked cotton often retains higher moisture levels. This excess moisture leads to physical losses during the ginning process and subsequent quality decline during storage (Afzal *et al.*, 2020). Traditional storage methods inadequately manage ambient humidity variations, resulting in reduced cotton seeds' vigor and viability (Afzal *et al.*, 2020). Conversely, cotton harvested in dry, hot weather is prone to physical damage during ginning, leading to quality deterioration during processing and storage. Notably, insect pests contribute to about 30-40% of post-harvest seed losses, while environmental factors, including humidity, account for 25% (Abass *et al.*, 2014). Among these factors, ambient humidity plays a significant role in seed deterioration and vigor (Hill *et al.*, 2007; Bradford *et al.*, 2020). The critical linkage lies in recognizing the impact of these environmental and processing factors on seed quality. Excessive moisture content in seeds and high environmental humidity accelerate seed spoilage, particularly when stored in traditional bags like cloth or jute (Wagacha & Muthomi, 2008).

Seeds commonly find themselves within traditional porous packaging like jute or cloth bags, allowing even dried seeds to absorb moisture under high ambient relative humidity, leading to a decline in seed vigor (Rajendran *et al.*, 2005). Effectively controlling grain moisture and pest activity in stored seeds is achieved through sealed, airtight, or hermetic storage systems. Hermetic storage employs an airtight barrier that curtails moisture reabsorption,

maintaining consistent seed moisture content by preventing external oxygen ingress. This prevents ongoing biological activity in seeds by utilizing the available oxygen, effectively controlling insect infestations. Materials such as super bags, comprised of high-grade polyethylene with barrier layers, exemplify hermetic packaging. These bags exhibit minimal water vapor transmission ( $\leq 5 \text{ g m}^{-2} \text{ day}^{-1}$ ) and oxygen entry ( $\leq 4 \text{ cc m}^{-2} \text{ day}^{-1}$ ), ensuring safe seed storage (Bakhtavar *et al.*, 2019). Hermetic materials resist abrupt changes in seed moisture content caused by environmental shifts due to their barrier layers (Odjo *et al.*, 2022). Seeds stored in super bags display enhanced quality and maximal germination rates (Ben *et al.*, 2006). Similarly, hermetic bags have proven effective in reducing cowpea seed losses compared to woven plastic bags during storage (Baoua *et al.*, 2013). Improperly stored seeds are highly prone to infestation by insects, rodents, and fungi, hastening seed deterioration processes (Afzal *et al.*, 2017). Prior research emphasizes that maintaining seeds in dry conditions within hermetic storage prolongs their shelf life significantly (Afzal *et al.*, 2020).

This study addresses a significant knowledge gap in seed storage practices by scrutinizing the drawbacks of conventional porous packaging, particularly jute or cloth bags, which allow moisture absorption in dried seeds, adversely affecting seed vigor and quality. The novelty of this research lies in advocating for hermetic storage systems as an innovative alternative. Utilizing airtight barriers, hermetic storage prevents moisture reabsorption, maintains consistent seed moisture content, and presents an efficient approach to control biological activity and preserve seed quality. Our hypothesis posits that hermetic storage, with its minimal moisture transmission rates, will mitigate seed deterioration, leading to enhanced quality, reduced infestation, and prolonged longevity compared to traditional packaging. The aims of this study include evaluating the impact of storage methods on seed vigor, assessing the efficacy of hermetic materials, and investigating the extent to which hermetic storage mitigates infestation, ultimately providing empirical evidence supporting the hypothesis.

$$\text{Amount of water required} = \left[ \frac{100 - \text{Initial seed moisture content}}{100 - \text{Final seed moisture content}} \times 100 \right] - \text{Seed weight}$$

Thus, required seed moisture contents were kept maintained i.e., 6% and 8% and then the seed was stored in hermetic and conventional bags for 06 months at room temperature.

**Data collection:** To monitor the storage moisture and temperature, the Data Loggers® of Centor Thai group Thailand were used. Fluctuations in humidity and temperature were observed throughout the storage period. The temperature was initially low till January and then gradually started to rise in the following months.

Germination and quality were checked by taking 100 seeds from each treatment every month.

The following observations were recorded during the study.

As mentioned earlier, seed moisture was measured every month. For this, we used the gravitational method in which 5 g of seeds were taken from each treatment, and their weight was noted and later ground in Petri dishes.

## Material and Methods

**Experimental site and design:** Cyto-179 the cotton crop variety was sown in three locations of the cotton core area, CRS-Khan Pur, CCRI-Multan, and CRI-Faisalabad. The crop was grown with standard documented practices and manually picked the seed cotton. The present storage research experiment was planned in the Biotechnology Laboratory of Central Cotton Research Institute, Multan for two consecutive years from December 2017 to May 2018 and from December 2018 to May 2019 i.e. the seed of each season was stored for 6 months. Each treatment (bag) contained 5 kg of seed and treatments were randomized by following a completely randomized design (CRD) with a factorial arrangement in triplicate.

**Treatment plan:** Treatments of experiments were seed moisture content ( $M_1 = 6\%$ ,  $M_2 = 8\%$ ) and storage methods ( $S_1 =$  conventional storage,  $S_2 =$  hermetic storage). Cloth bags were used for conventional storage. After ginning, the initial seed moisture content of the seed and its germination capacity were tested.

**Seed storage conditions:** To optimize the seed's moisture content to the desired level, seeds were spread on the cement floor for five to six hours for two days. After drying the seed for two days, a sample of 5 g of seed from each treatment was taken and its moisture content was determined whether the required moisture level had reached or not. After testing the seed, it was found that the moisture content came down to 6% which was suitable for one of our treatments ( $M_1 = 6\%$ ) but much less than the other treatment ( $M_2 = 8\%$ ).

In such a situation, some quantity of water was sprinkled on the seed to get the required moisture content. By following the equation given below, the amount of water was calculated.

After determining the initial weight, the seeds taken from both treatments were oven-dried at  $103^\circ\text{C}$ . After 17 h in the oven, the weight of the seed was again noted. Thus, the seed moisture content was calculated by putting both the known weights into the equation.

$$\text{Seed moisture contents (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

To determine the seed germination (%), 100 seeds from each treatment were taken and placed on plates between what man filter paper and left at  $25^\circ\text{C}$ . The seed germination was noted daily and the final germination was determined with the help of the equation.

$$\text{Germination \% age} = \frac{\text{Number of final germinated seed}}{\text{Total seed sown}} \times 100$$

Similarly, the germination index was determined with the help of the following equation.

$$\text{Germination index} = \frac{\text{No. of germinated seed at first count}}{\text{No. of days to first count}} + \frac{\text{No. of germinated seed at final count}}{\text{No. of days to final count}}$$

Seed vigor refers to the ability of the seed to germinate under various field conditions and produce normal and healthy seedlings. Two tests are used to determine seed vigor i.e. the direct and indirect test. The direct test, just like field conditions is developed in the lab, and seed vigor and seedling performance is tested. The cold test is a common example of a direct method to test the vigor of a seed where seed germination is checked by keeping it at a low temperature. In our experiment, 50 seeds were taken from each treatment and placed on petri plates at 18.1°C. After germination, the seedling growth was observed for 12 days. For data recording seedling length (10 seedlings) from each treatment was measured and seedlings showing a length of 1.5 inches or greater were considered vigorous seedlings. On the other hand, indirect tests assess the seed parameters that may affect its performance in the field. Respiration rate and electrical conductivity tests are used as indirect seed vigor tests.

For speed of germination, the germinated seeds were counted daily and finally, the speed of germination was determined with the help of equation.

$$\text{Speed of germination} = \left( \sum \frac{N_1}{D_1} + \frac{N_2 - n_1}{D_2} + \frac{N_n - N_{n-1}}{D_n} \right)$$

The N shows germinated seeds and the D represents days.

For shoot, root length, and fresh and dry weight, fifty seeds from each treatment were sown in a petri plate and the seedlings were allowed to grow for 20 days. After 20 days, seedlings were weighed, and fresh and dry weights were noted. Similarly, the leaves of the same seedlings also were taken for leaf area measurement and then the dry weight of the leaves was noted. Similarly, for seedlings growth rate, 50 seeds taken from each treatment were sown in a pot comprising sand, and seedlings were allowed to grow for 30 days. After 30 days, the stems of the seedlings were separated from the roots, dried and their weight was taken to calculate the seedling growth rate.

**Biochemical attributes:** For free fatty acid profile (FFA) measurement, Soxhlet is a very good and widespread method for extracting bioactive compounds from solid materials. Twenty grams of dried and ground seeds were placed in Whatman filter paper No 42 in a disposable thimble, soaked with 200 ml of n-hexane, and then left at 70°C. The solvent in the flask was heated, evaporated, and collected in the condenser. This process continued till all the solvent evaporated and collected in the condenser and distillation flask filled with condensed solvent. The oil extracted during the Soxhlet extraction apparatus was considered as total oil contents and the extraction process continued for 08 h. The solvent in the flask was evaporated while the oil remained in the flask so that it to be used for further analysis. After some further processes such as the addition of 15 mL n-hexane and 75 mL isopropanol, free fatty acids (%) were determined by the following equation.

$$\text{Free fatty acid (\%)} = \frac{2.82 \times \text{volume of 0.1 N NaOH in mL}}{\text{Weight of oil (g)}} \times 100$$

The basic principle for the electrical conductivity (EC) test is the deterioration of the cells inside the seed which results in poor quality seed. By keeping the seed at a low temperature, it is determined through electrolyte leakage how much of the seed has deteriorated. For EC, 50 seeds were chosen from each replication and their weight was noted. Then two beakers of 250 ml were filled with water and 50 seeds from each treatment were placed inside these beakers. The beakers were kept for 24 h at room temperature (20 ± 2°C). After 24 h, the water in these beakers was tested for EC and then conductivity was determined with the help of equation.

$$\text{Conductivity} = \frac{\text{Conductivity reading} - \text{Background reading}}{\text{Weight of replicate}} \times 100$$

### Statistical analysis

Statistical analysis was performed using Statistics software (version 8.1) and data were represented as the mean of squares. A two-way analysis of variance (ANOVA) was performed to evaluate statistical differences among treatments. The differences between individual means were compared using Tukey's test at p<0.05.

### Results

**Germination and moisture content:** Seed moisture content was stable for two months under standard storage settings, then rose with time to a peak after six months (Figs. 1 and 2). After six (6) months of storage, the moisture content of seeds with an initial moisture content of 6 and 8% grew to 6.8 and 8.9%, respectively. Seed moisture content increased somewhat, but not enough to be statistically significant, when kept in a hermetic environment. Every month, the cotton seeds in storage were examined to determine whether any had sprouted (Figs. 1 and 2). After three months of storage, conventional and hermetic cotton seeds had germination rates above 90% (Fig. 2).

Cotton seed germination dropped dramatically after 3 months of storage, and after six (6) months of storage, germination was greater in seeds with an initial moisture level of 6% than in cotton seeds with 8% initial moisture content. The seeds with six (6) and eight (8) percent initial moisture content grew at a greater rate (82-87%) when kept under hermetic circumstances as opposed to the more commonplace conventional storage (78-83% germination).

**Free fatty acids (%):** With time, the amount of free fatty acids (FFAs) in cotton seeds increased significantly (Fig. 3), but the lowest FFA content was found when seeds with six (6) percent an initial moisture content were stored in hermetic conditions (0.44% in 2017–2018 and 0.49% in 2018–2019), followed by seeds with an initial moisture content of 8%. Contrarily, seeds with an initial moisture content of 8% and stored in conventional conditions showed higher Free Fatty Acid content (1.50% in 2017–2018 and 1.55% in 2018–2019), while seeds with an initial moisture content of 6% and stored in conventional conditions had statistically identical free fatty acid (FFA) content as seen in seeds stored in hermetic conditions with an 8% initial

moisture content. Similarly, cotton's malondialdehyde (MDA) level rose with time (Fig. 3). The least MDA content was found in cotton seed that had 6% initial moisture content and was stored in hermetic conditions (0.6 nmole  $g^{-1}$  DW in 2017-2018 and 0.5 nmole  $g^{-1}$  DW in 2018-2019), which was followed by seed that had 8% initial moisture content and was stored in hermetic condition. Maximum MDA concentration was found in cotton seeds with an initial moisture content of 8% that were kept according to standard procedures (1.14 nmole  $g^{-1}$  DW in 2017-2018 and 1.34 nmole  $g^{-1}$  DW in 2018-2019). Similar to this, after six months of storage, cotton seed kept under hermetic conditions and with a 6 percent initial moisture content showed increased total soluble sugars (TSS) (4.5  $mg\ g^{-1}$  seed in 2017-2018 and 4.50  $mg\ g^{-1}$  seed in 2018-2019). (Figure 3). On the other hand, the minimal TSS of cotton seed with initial moisture contents of 6% and 8% that was kept under normal circumstances was reported (1.41  $mg\ g^{-1}$  seed in 2017-2018 and 1.4  $mg\ g^{-1}$  seed in 2018-2019).

**Germination and seed vigor (%):** Seed vigor and speed of germination were influenced by storage conditions

(Fig. 4). The germination rates of cotton seed kept under hermetic circumstances were significantly higher than those stored in conventional settings (14.19 and 14.67%, respectively) in both 2017–18 and 2018–19. (69.13% in 2017-2018 and 65.11% in 2018-2019). In 2018-19, cotton seeds with a 6% beginning moisture content and kept in hermetic circumstances had better vigor (76.29%), whereas seeds with an 8% starting moisture content and stored in hermetic conditions had considerably lower vigor (72.4% and 82.20%). Seeds with 8% initial moisture content and kept in standard settings presented the lowest levels of seed vigor (63.8% in 2017-2018 and 61.7% in 2018-2019). Cotton seeds with an initial moisture content of 6% in hermetic conditions exhibited the fastest germination rates (14.59 and 15.16% per day, respectively, in 2017-2018 and 2018-2019), followed by cotton seeds with 6% initial moisture content in conventional and hermetic conditions, congruently (Fig. 4). Cotton seeds with an initial moisture content of 8% and kept in typical settings had the slowest germination rates (13.28 in 2017-2018 and 13.63 in 2018-2019).

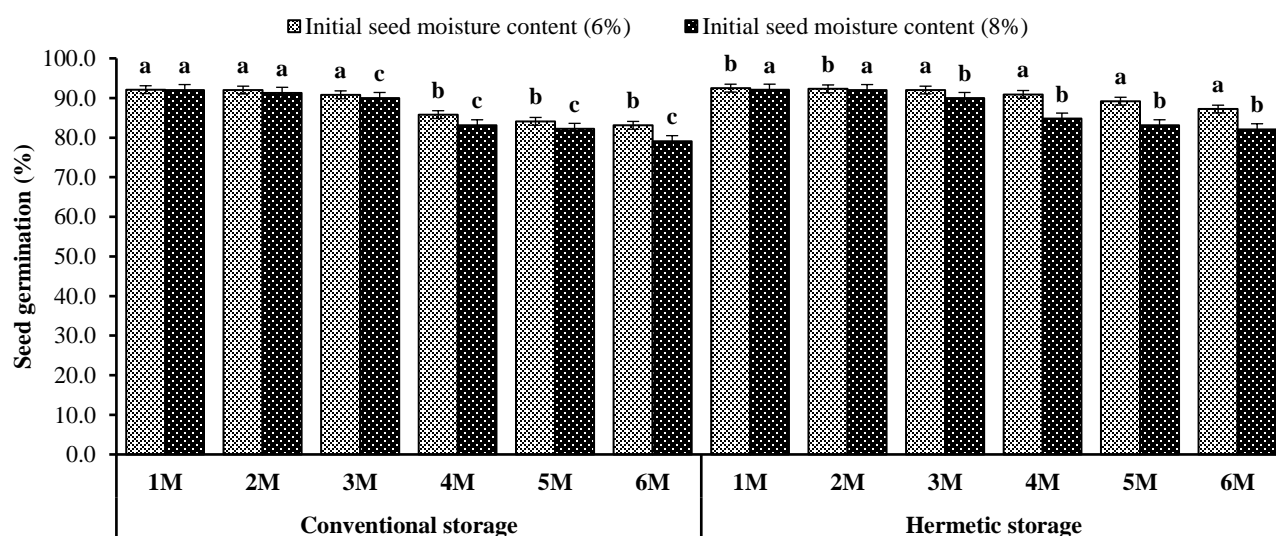


Fig. 1. Effect of seed moisture content and storage conditions during 2017-18. Bars are an average of 3 replicates  $\pm$  SE compared by using Tukey's test. Different bar letters are showing significant change at  $p \leq 0.05$ .

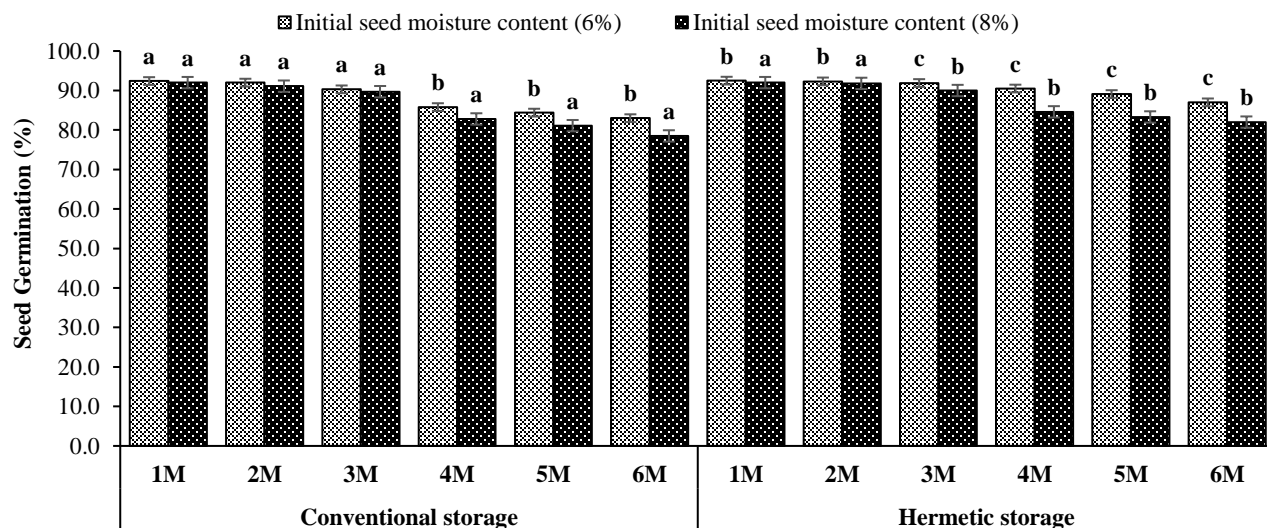


Fig. 2. Effect of seed moisture content and seed storage conditions during 2018-19. Bars are an average of 3 replicates  $\pm$  SE compared by using Tukey's test. Different bar letters are showing significant change at  $p \leq 0.05$ .

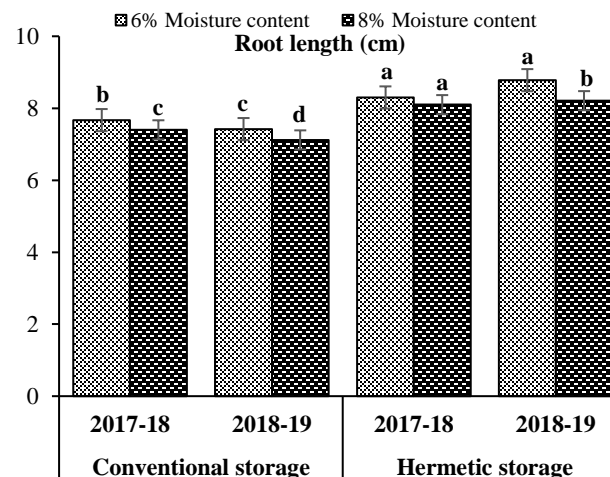
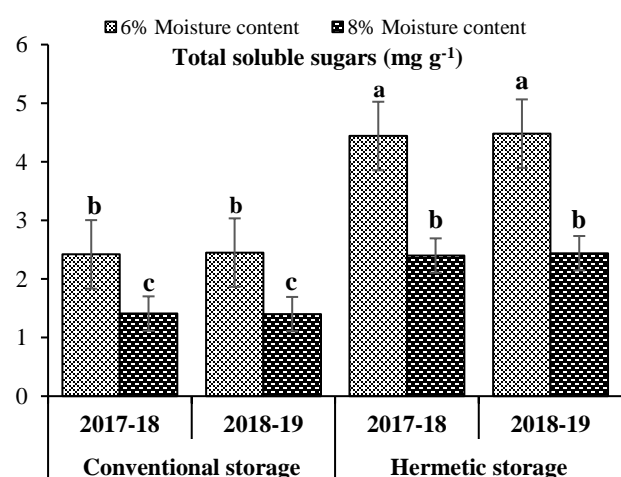
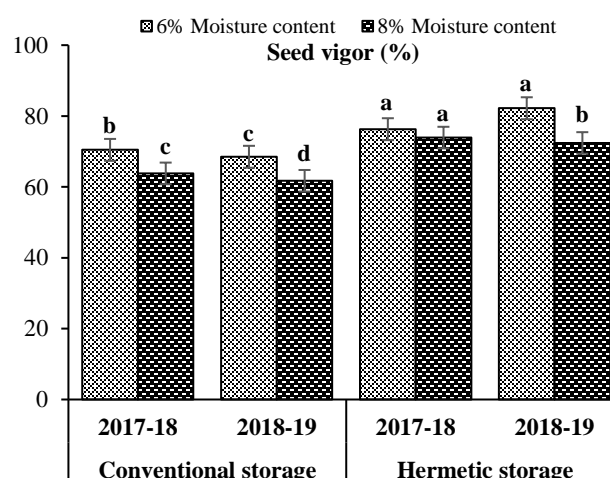
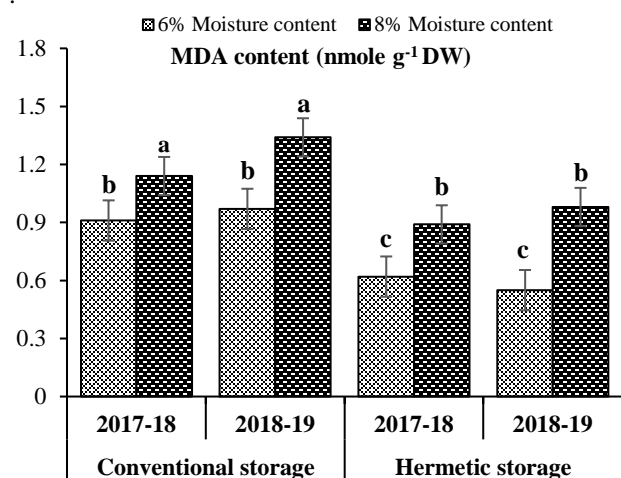
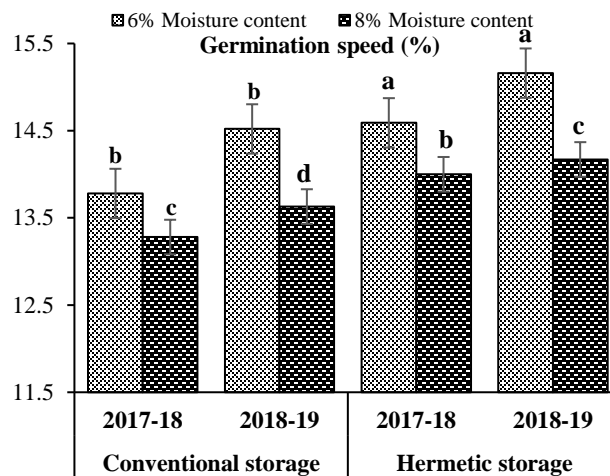
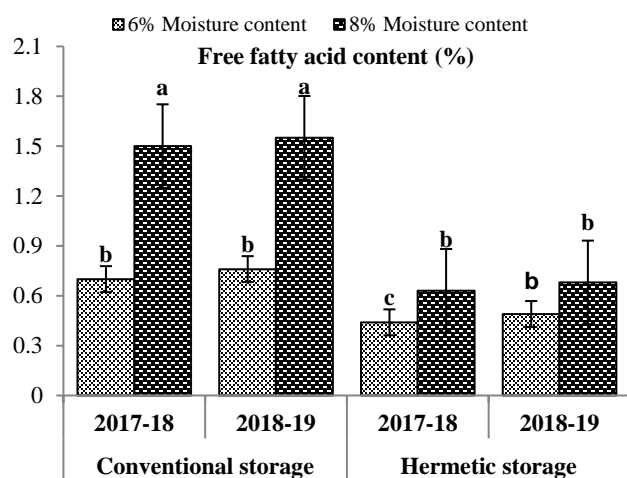


Fig. 3. Impact of Storage conditions and seed moisture content on FFA, MDA content, and Total Soluble sugars of cotton seeds. Bars are an average of 3 replicates  $\pm$  SE compared by using Tukey's test. Different bar letters are showing significant change at  $p \leq 0.05$ .

**Shoot and root length (cm):** When cotton seed was held for six months with an initial moisture level of 8%, the plant's shoot and root length reduced (Fig. 4). Cotton seed maintained under hermetic circumstances showed increased shoot (24.25 cm in 2017-18 and 25.07 cm in 2018-19) and root length (8.30 cm in 2017-18 and 8.78 cm in 2018-19). Seeds that were

Fig. 4. Impact of seed storage conditions and seed moisture content on Germination Speed, Seed vigor, Shoot Length, and Root Length of cotton seedlings. Bars are an average of 3 replicates  $\pm$  SE compared by using Tukey's test. Different bar letters are showing significant change at  $p \leq 0.05$ .

initially moistened to 6% had longer shoots after traditional storage than seeds that were first moistened to 8%. When cotton seeds were first moistened to 8% and kept in standard circumstances, the minimum shoot length was 22.67 cm in 2017-2018 and 23.33 cm in 2018-2019. The root length was 7.40 cm in 2017-2018 and 7.12 cm in 2018-2019.

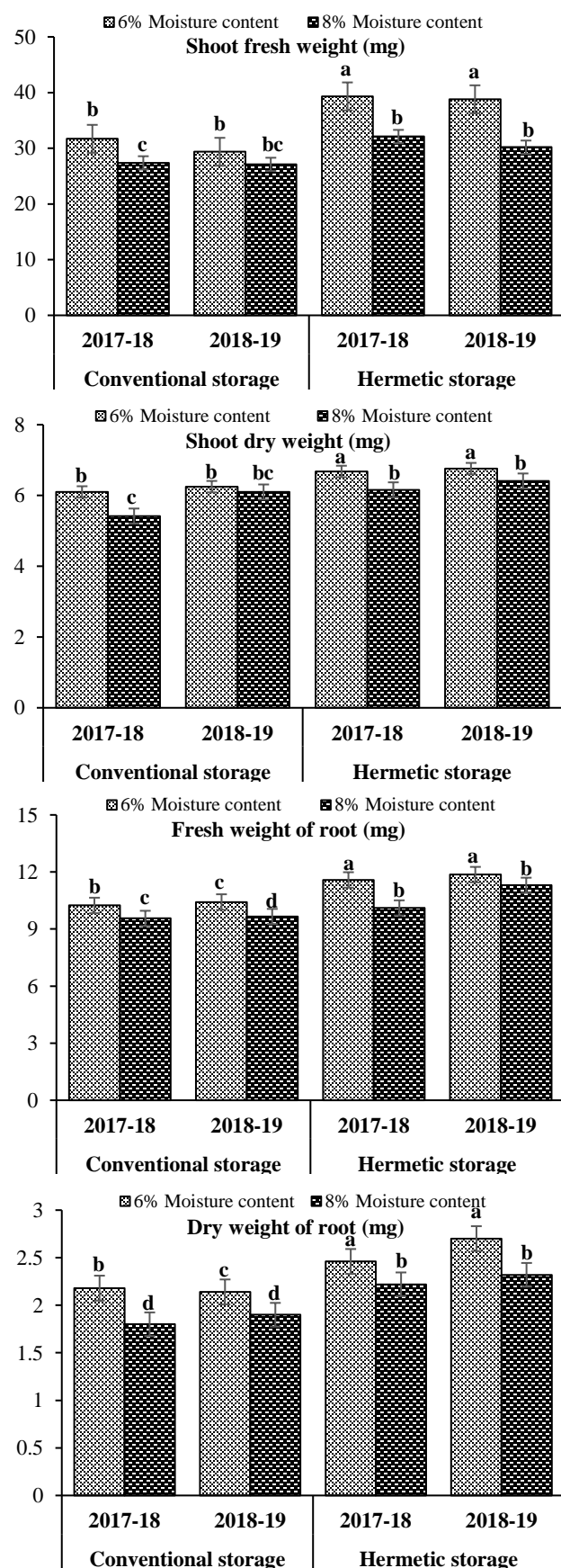


Fig. 5. Impact of seed storage conditions and seed moisture content on fresh and dry shoot weight (mg), root fresh and dry weight (mg) of cotton seedlings. Bars are an average of 3 replicates  $\pm$  SE compared by using Tukey's test. Different bar letters are showing significant change at  $p \leq 0.05$ .

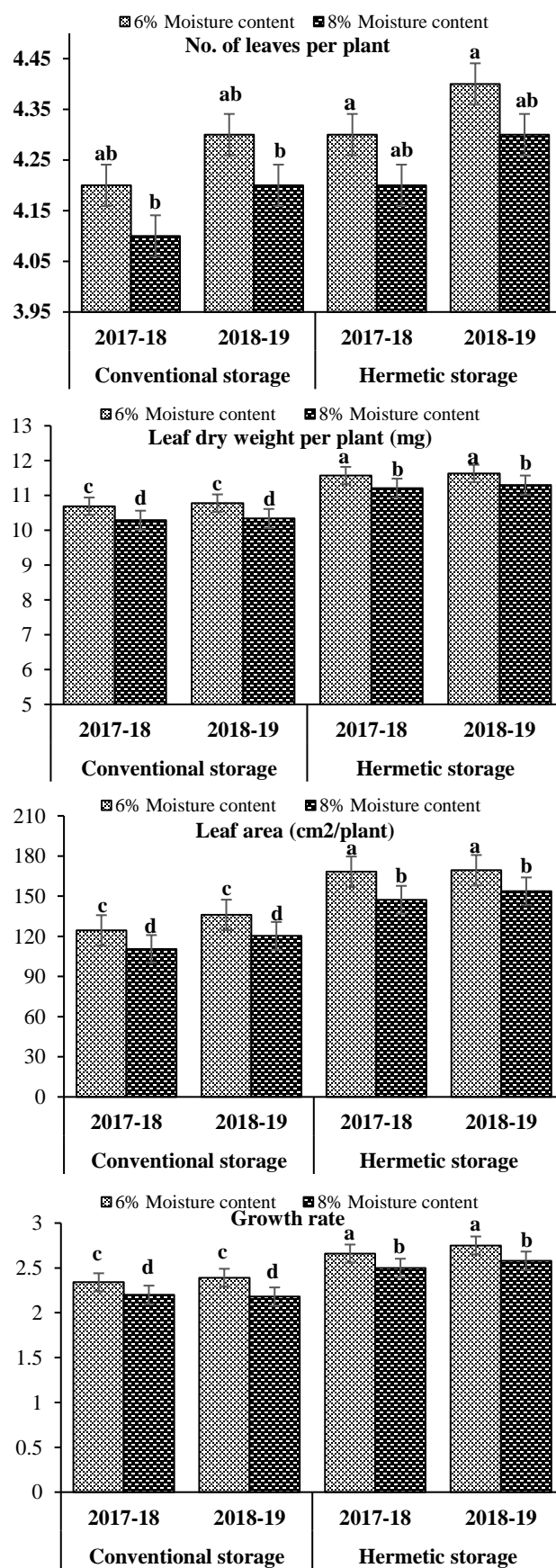


Fig. 6. Impact of storage conditions and seed moisture content on Leaves dry weight, Leaf area, Number of leaves plant<sup>-1</sup>, and growth rate of cotton seedlings. Bars are an average of 3 replicates  $\pm$  SE compared by using Tukey's test. Different bar letters are showing significant change at  $p \leq 0.05$ .

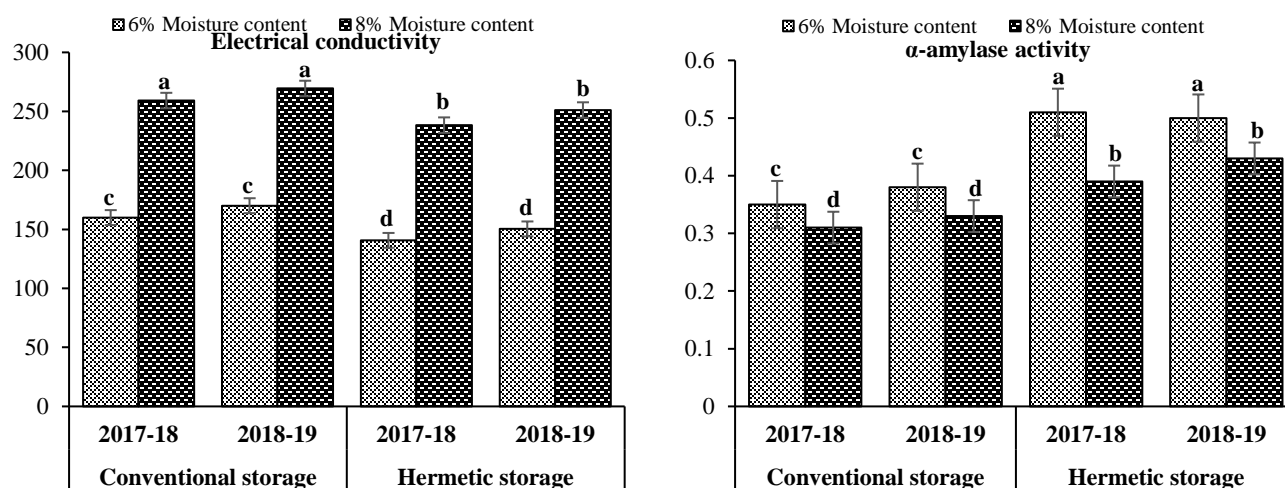


Fig. 7. Impact of storage conditions and seed moisture content on electrical conductivity and  $\alpha$ -amylase activity ( $\text{IU mg}^{-1}$  seed) of cotton seeds. Bars are an average of 3 replicates  $\pm$  SE compared by using Tukey's test. Different bar letters are showing significant change at  $p \leq 0.05$ .

**Shoot and root fresh weight (mg):** Greater shoot fresh weight (39.31 mg in 2017-2018 and 38.79 mg in 2018-2019) and fresh root weight (11.58 mg in 2017-2018 and 11.86 mg in 2018-2019) of cotton seedlings was observed when cotton seed had 6% and 8% initial moisture content under hermetic conditions (Fig. 5). Minimum fresh shoot weight (29.53 mm in 2017-2018 and 28.73 mg in 2018-2019) and fresh root weight (9.56 mg in 2017-2018 and 9.66 mg in 2018-2019) of seedlings was recorded when cotton seeds were stored in conventional conditions with 8% initial moisture content of seeds. The same trend was recorded for shoot and root dry weight (Fig. 5).

Seedlings grown from cotton seeds with a 6% initial moisture content in hermetic conditions recorded maximum values for leaf number (4.3 leaves  $\text{plant}^{-1}$  in 2017-2018 and 4.4 leaves  $\text{plant}^{-1}$  in 2018-2019), leaf dry weight (11.6 mg  $\text{plant}^{-1}$  in 2017-2018 and 11.63 mg  $\text{plant}^{-1}$  in 2018-2019), and leaf area of plant (163.2  $\text{cm}^2 \text{plant}^{-1}$  in 2017-2018 and 169.3  $\text{cm}^2 \text{plant}^{-1}$  in 2018-2019) (Fig. 6). However, the number of leaves produced by seedlings developed from seeds maintained in standard settings (6% moisture content) and hermetic conditions (8% moisture content) were statistically equivalent. Cotton seedlings grown from seeds harvested from conventional storage with an initial moisture content of 8% produced the fewest leaves per plant (4.1 leaves  $\text{plant}^{-1}$  in 2017-2018 and 4.2 leaves  $\text{plant}^{-1}$  in 2018-2019), the least dry weight of cotton leaves (10.29 mg  $\text{plant}^{-1}$  in 2017-2018 and 10.34 mg  $\text{plant}^{-1}$  in 2018-2019), and the smallest leaf area of plant (10.3  $\text{cm}^2 \text{plant}^{-1}$  in 2017-2018 and 10.4  $\text{cm}^2 \text{plant}^{-1}$  in 2018-2019). The plant's pace followed a similar pattern (Fig. 6). Seedlings developed from seeds maintained in hermetic circumstances with an initial moisture level of 6% grew faster than those grown from cotton seeds with an initial moisture content of 8% (2.7  $\text{mg day}^{-1}$  in 2017-2018 and 2.8  $\text{mg day}^{-1}$  in 2018-2019). Seedlings developed from seeds maintained in typical settings for six months and having an initial moisture content of 8% grew at a minimum rate of 2.20  $\text{mg day}^{-1}$  in 2017-2018 and 2.18  $\text{mg day}^{-1}$  in 2018-2019.

**Electrical conductivity (EC) and  $\alpha$ -amylase activity of cotton seed:** After storage for six months, cotton seed was tested for EC (Fig. 7), which revealed that when the initial

moisture content of the seed was 8%, the EC was higher ( $248.8 \mu\text{S cm}^{-1} \text{g}^{-1}$  in 2017-2018 and  $260.3 \text{ S cm}^{-1} \text{g}^{-1}$  in 2018-2019, and when it was 6%, the EC was lower ( $150.3 \mu\text{S cm}^{-1} \text{g}^{-1}$  in 2017-2018 and  $160.4 \mu\text{S cm}^{-1} \text{g}^{-1}$  in 2018-2019). Cotton seeds with a 6% starting moisture content and kept in hermetic circumstances, then cotton seeds with a 6% initial moisture content and stored in conventional settings, both exhibited the lowest EC ( $140.7 \mu\text{S cm}^{-1} \text{g}^{-1}$  in 2017-2018 and  $150.4 \mu\text{S cm}^{-1} \text{g}^{-1}$  in 2018-2019). Cotton seeds with an initial moisture level of 8% during storage and kept in conventional settings had the highest EC ( $259.2 \mu\text{S cm}^{-1} \text{g}^{-1}$  in 2017-2018 and  $269.4 \mu\text{S cm}^{-1} \text{g}^{-1}$  in 2018-2019), followed by seeds with an initial moisture content of 8% and stored in hermetic conditions. The greatest  $\alpha$ -amylase activity was seen in cotton seeds maintained in hermetic settings with a 6% initial moisture content ( $0.52 \text{ IU mg}^{-1}$  seed in 2017-2018 and  $0.51 \text{ IU mg}^{-1}$  seed in 2018-2019), followed by cotton seeds in hermetic conditions with an 8% initial moisture content (Fig. 7). During both research years, cotton seeds kept under conventional settings with a starting moisture level of 6% had less  $\alpha$ -amylase activity as compared to seeds stored under hermetic conditions with an initial moisture content of 8%. When cotton seeds were kept under standard circumstances with an initial moisture content of 8%, the lowest level of  $\alpha$ -amylase activity was observed ( $0.31 \text{ IU mg}^{-1}$  seed in 2017-2018 and  $0.33 \text{ IU mg}^{-1}$  seed in 2018-2019).

## Discussion

The primary cause of seed quality decline is excess moisture content, but proper drying and storage can sustain seed quality (Afzal, 2018; Bakhtavar *et al.*, 2019). This study used conventional and hermetic storage methods to store seeds with initial moisture contents of 6% and 8% for six months. Results at three and six months revealed that cotton seeds stored conventionally experienced increased moisture content, while those under hermetic conditions showed no significant rise. Cotton seeds are hygroscopic and absorb or release moisture until equilibrium with the surroundings (Afzal *et al.*, 2017; Bradford *et al.*, 2020). Hermetic storage prevented moisture ingress, unlike traditional cloth or jute bags susceptible to moisture

absorption during high humidity (Retinassababady & Ramanadane, 2014; Afzal *et al.*, 2017). Hermetic storage maintains seed integrity, preventing spoilage and enhancing germination potential (Afzal *et al.*, 2019, Kamran *et al.*, 2020). Similar findings were reported in quinoa seeds packed hermetically, showing no moisture increase (Bakhtavar *et al.*, 2019). Thus, hermetic storage is an effective method (Bakhtavar *et al.*, 2019). Cotton seeds with 8% initial moisture, stored conventionally, exhibited slower and less vigorous germination compared to 6% moisture seeds under hermetic conditions. In conventional storage, increased seed moisture and oxygen intake led to seed deterioration, impacting germination and growth (Harrington & Kozlowski, 1972, Tubbs *et al.*, 2016). Furthermore, seeds in porous material showed lower germination and viability compared to hermetic storage in corn, wheat, and quinoa (Afzal *et al.*, 2019; Bakhtavar & Afzal, 2020). Reactive oxygen species (ROS) produced due to excess moisture negatively impact cotton seed viability and germination (Bailey, 2004). High relative humidity promotes seed growth, while oxygen exposure leads to seed degradation. Hermetic storage limits respiration, maintaining original moisture content and restricting biological activity (Tripathi & Lawande, 2014). Biochemical indicators like free fatty acids (FFA) suggest seed aging rates (Staus & Hopper, 1983). Elevated FFA content in conventionally stored seeds indicates aging, impacting mitochondrial function and lipid peroxidation (Wilson & McDonald, 1986; Levina *et al.*, 1999). Lipid peroxidation damages membranes, proteins, and DNA, contributing to seed deterioration (Wilson & McDonald, 1986; Matthews *et al.*, 2002). Leachate electrical conductivity (EC) measurement indicates seed decay (Murthy *et al.*, 2003, Afzal *et al.*, 2019). Seeds in conventional storage with 8% initial moisture exhibited higher EC, correlating with increased deterioration and reduced vigor (Murthy *et al.*, 2003; Afzal *et al.*, 2019). Malondialdehyde (MDA) levels, a marker for degradation, were higher in conventionally stored cotton seeds due to moisture-induced lipid peroxidation (Bailey, 2004; Groot *et al.*, 2015). Hermetic storage mitigates seed degradation by preserving low MDA concentrations, enhancing seed vitality (Afzal *et al.*, 2017; Bakhtavar *et al.*, 2019; Bakhtavar & Afzal, 2020). Additionally, reduced  $\alpha$ -amylase activity and soluble sugars in conventionally stored seeds indicate compromised seed health (Pérez-García & González-Benito, 2006; Marques *et al.*, 2014). High seed moisture levels deplete starch stores, impacting seed growth (Murthy *et al.*, 2003). Conclusively, traditional storage conditions with increased seed moisture cause biochemical alterations, affecting cotton seed quality adversely. Hermetic storage maintains seed viability, ensuring better germination and growth, reflecting positively in field conditions (Ellis & Roberts, 1980; Ghassemi-Golezani *et al.*, 2010; Kamran *et al.*, 2020).

## Conclusions

In conclusion, the current study showed the quality of cotton seed is reduced by increasing moisture content in conventional storage conditions. On the other hand, hermetic storage-maintained seed quality and minimized quality losses by reducing metabolic activities. It is an environment-friendly and sustainable technology that

maintains the initial levels of moisture and controls pests by the lack of oxygen. This study provided additional evidence of the effectiveness of hermetic storage technologies in minimizing quantitative and qualitative losses in smallholder farming systems. Additional studies may focus on how to promote these technologies in rural areas while identifying key aspects of the success of postharvest interventions.

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