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EFFECT OF HYDROPHOBIC POLYMER COATINGS ON PHYSIOLOGICAL PERFORMANCE AND BIOCHEMICAL STABILITY OF ONION SEEDS DURING STORAGE

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ABSTRACT
Onion (Allium cepa L.) seeds exhibit rapid deterioration during storage, resulting in reduced viability and vigour due to moisture fluctuations and oxidative damage. The present study evaluated the effects of hydrophobic polymer coatings on the physiological and biochemical quality of onion seeds (cv. Arka Kalyan) during six months of storage under ambient and cold (10 ± 2°C) conditions. Six polymer formulations, comprising chitosan (1 and 2%), sodium alginate (2%), methyl cellulose (3 and 4 ml) and methyl vinyl acetate (4 ml), were evaluated against an uncoated control in a factorial completely randomized design with three replications. Among the treatments, 2% chitosan consistently maintained superior seed quality, recording lower moisture content (7.89 and 7.39%), higher germination energy (64.00 and 65.00%) and lower electrical conductivity (33.00 µS cm^{-1} g^{-1}) under ambient and cold storage, respectively. It also enhanced dehydrogenase and catalase activities, indicating improved membrane stability. Thus, 2% chitosan coating offers potential for improving onion seed storability.

KEYWORDS: Hydrophobic polymer coating; Chitosan; Onion seed; Seed longevity; Membrane integrity; Antioxidant enzymes

1. INTRODUCTION
The availability of physiologically sound seeds is a prerequisite for achieving uniform crop establishment and sustainable agricultural production. Onion (*Allium cepa* L.), an economically important vegetable crop, contributes substantially to global food and horticultural systems. India ranks second in global onion production after China. During 2024–25, the global area under onion cultivation was reported to be approximately 5.84 million hectares, with a production of 111.2 million tonnes (Anonymous, 2024). Despite its importance, onion seed production and distribution systems face a significant challenge in maintaining seed viability and vigour during storage. The inherently limited storability of onion seeds, coupled with fluctuations in storage temperature and relative humidity, accelerates physiological deterioration and reduces their planting value (Deepa *et al.*, 2013).
Seed ageing involves a complex sequence of physiological, biochemical and structural changes that progressively impair cellular integrity. Membrane deterioration is a critical component of this process, as disruption of membrane organization increases permeability and promotes the loss of essential cellular constituents. Oxidative stress plays a central role in this deterioration through the excessive accumulation of reactive oxygen species (ROS), which induce lipid peroxidation and oxidative modification of cellular macromolecules (McDonald, 1999; Xing *et al.*, 2025). Increasing moisture availability and temperature may accelerate these processes, leading to impaired cellular metabolism, reduced dehydrogenase activity and altered antioxidant defence mechanisms. Catalase and peroxidase activities are particularly relevant indicators of the seed's capacity to regulate oxidative stress during storage (Brar *et al.*, 2019). In addition, structural characteristics of the onion seed coat, including the region surrounding the hilum, may contribute to its susceptibility to moisture penetration and deterioration (Mohamed-Yasseen *et al.*, 1996). Collectively, these processes result in reduced germination, impaired seedling growth and shortened storage longevity.
An effective approach to improving seed longevity involves modifying the seed surface to regulate its interaction with the storage environment. Polymer-based film coating provides an opportunity to achieve this through the formation of a continuous layer around individual seeds. Depending on their physicochemical properties, polymer films can influence moisture absorption, water-vapour transmission and gas exchange, thereby potentially moderating storage-induced deterioration (Taylor *et al.*, 1998). The application of biodegradable polymers is particularly relevant for developing environmentally compatible seed enhancement technologies. Chitosan, sodium

alginate and methyl cellulose are promising film-forming materials with potential applications in seed coating. Among these, chitosan has attracted considerable interest owing to its film-forming characteristics and reported effects on biochemical stability, lipid peroxidation and electrolyte leakage in stored seeds (Winarti *et al.*, 2023).

Previous studies have explored polymer-based treatments for improving onion seed germination and regulating water uptake (Kavak and Eser, 2009). However, the potential of different polymer formulations to maintain seed physiological performance and biochemical stability throughout extended storage remains insufficiently investigated. In particular, the relationship between coating formulation, storage temperature, membrane permeability and antioxidant enzyme activity requires further elucidation. A comparative evaluation under contrasting storage environments is therefore essential to determine whether polymer coatings provide consistent protection against seed deterioration.

Against this background, the present study investigated six polymer-based coating formulations, selected through preliminary standardization, along with an uncoated control, to evaluate their influence on the physiological and biochemical quality of onion (*Allium cepa* L.) cv. Arka Kalyan seeds during six months of storage under ambient and low-temperature (10 ± 2°C) conditions. The investigation further assessed treatment × storage environment interactions to understand the differential responses of seed-quality attributes and identify an effective coating formulation for improving onion seed storability.

2. MATERIALS AND METHODS

The present investigation was conducted during 2025–26 in the laboratories of the Department of Seed Science and Technology, Gandhi Krishi Vignana Kendra (GKVK), University of Agricultural Sciences, Bengaluru, Karnataka, India. Freshly harvested seeds of onion (*Allium cepa* L.) cv. Arka Kalyan were procured from the ICAR–Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru. The study was undertaken to assess the effectiveness of different polymer-based coating formulations in preserving the physiological and biochemical quality of onion seeds during storage. Six coating treatments, together with an untreated control, were evaluated using an initial seed quantity of 1 kg per treatment. The treatment combinations were designated as follows: T_1, chitosan (1%); T_2, chitosan (2%); T_3, sodium alginate (2%); T_4, methyl cellulose (provisionally 2%, applied at 3 ml kg^{-1} of seed); T_5, methyl cellulose (applied at 4 ml kg^{-1} of seed); T_6, methyl vinyl acetate (applied at 4 ml kg^{-1} of seed); and T_7, uncoated control.

2.1. Preparation of coating formulations and seed treatment

Chitosan (low-to-medium molecular weight; degree of deacetylation >75%) was prepared at concentrations of 1 and 2% (w/v). The respective quantities of chitosan (1 and 2 g) were dissolved separately in 100 ml of 1% (v/v) acetic acid and continuously stirred for 3–5 h at room temperature to obtain homogeneous coating solutions. Sodium alginate (2%, w/v) was prepared by dissolving 2 g in 100 ml of distilled water under continuous stirring for 1–2 h. To facilitate ionic cross-linking and stabilization of the alginate film, the coated seeds were subsequently immersed in 0.1 M calcium chloride ($CaCl_2$) solution for 30–60 s. Methyl cellulose and methyl vinyl acetate formulations were applied at the respective rates of 3 and 4 ml kg^{-1} of seed. The individual formulations were uniformly distributed over the seed surface using a laboratory-scale rotary pan seed coater. Following coating, the treated seeds were shade-dried to remove excess surface moisture.

2.2. Storage conditions and experimental design

The coated seed lots and untreated control were separately packed in 480-gauge plastic pouches and stored under two distinct storage environments: ambient conditions (S_1) and low-temperature storage at 10 ± 2°C (S_2). Seed-quality attributes were evaluated at monthly intervals throughout the six-month storage period or until germination declined below the minimum seed certification standard of 70%. The experiment was established using a factorial completely randomized design (FCRD), with seven coating treatments, two storage environments and three replications, constituting 42 treatment–storage experimental units.

2.3. Evaluation of physiological seed-quality attributes

2.3.1. Seed moisture content

Seed moisture content was determined using the high-constant-temperature oven method. Approximately 2 g of seed sample was dried at 130 ± 2°C for 4 h. Moisture content was calculated on a wet-weight basis and expressed as a percentage, following ISTA (2025).

2.3.2. Germination energy

Germination energy was assessed by recording the percentage of seeds that germinated within 48 h, following the procedure described by Wang and Sang (2008).

$$\text{Germination Energy (GE)} = \frac{n}{N} \times 100$$

Where, n = number of seed germinated after the appointed time (48 hours); N = total number of seed kept for the test.

2.3.3. Seedling vitality and growth parameters

Twenty-five seeds were placed on moistened paper towel/filter paper in Petri dishes/trays and kept in a germination chamber at 25 ± 2° (Lingyun *et al.,* 2017). The germination index (GI) so obtained was used to compute vitality index:

Vitality index (VI) = GI x S

Where, GI = germination index;

S = average weight of 10 fresh seedlings.

Seedling growth was further assessed by measuring root and shoot lengths separately in ten randomly selected normal seedlings at the final germination count (12^{th} day).

2.4. Determination of biochemical attributes and membrane integrity

2.4.1. Membrane injury index (MII)

Membrane injury index (MII) was determined following the procedure described by Blum and Ebercon (1981), using the relative electrical conductivity of seed leachates measured before and after complete thermal disruption of cellular membranes. The conductivity ratio was used to quantify the extent of membrane injury.

2.4.2. Electrical conductivity (EC)

Electrical conductivity was independently determined as an indicator of seed membrane permeability. For this purpose, 100 seeds were soaked in 25 ml of distilled water for 24 h at 25 ± 1°C. The conductivity of the resulting leachate was measured using a conductivity meter and expressed as µS cm^{-1} g^{-1} of seed, following ISTA (2025).

2.4.3. Dehydrogenase activity

Dehydrogenase activity was quantified using the tetrazolium reduction assay. Embryonic axes isolated from ten seeds were incubated in 0.5% tetrazolium chloride solution for 4 h at 25 ± 1°C under dark conditions. The resulting formazan was extracted using 2-methoxyethanol, and its absorbance was measured at 480 nm using a spectrophotometer (Perl et al., 1978).

2.4.4. Antioxidant enzyme activities

Peroxidase activity was determined using a guaiacol-based colorimetric assay in the presence of hydrogen peroxide and phosphate buffer. The enzymatic reaction was monitored spectrophotometrically at 436 nm, following Sadasivam and Manickam (2008). Catalase activity was estimated using the ammonium molybdate–hydrogen peroxide method, with absorbance measured at 405 nm (Goth, 1991). Enzyme activity was expressed in the appropriate units based on the respective assay calculations.

2.4.5. Total soluble sugar content

Total soluble sugar content was estimated using the phenol–sulphuric acid method (Dubois et al., 1951). The absorbance of the developed colour was recorded at 490 nm, and sugar concentration was expressed as µg ml^{-1} of extract.

2.4.6. Total soluble protein content

Total soluble protein content was determined by the Lowry method (Lowry et al., 1951), using acetone-defatted seed powder extracted in 0.1 M sodium phosphate buffer (pH 7.0). Absorbance was measured at 660 nm, and protein content was expressed as µg g^{-1} of seed material.

2.5. Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) appropriate for a two-factor factorial completely randomized design. The main effects of coating treatment (T) and storage environment (S), together with their interaction (T × S), were examined to determine their influence on seed physiological and biochemical attributes. Treatment means were compared using the critical difference (CD) test at the 5% probability level, wherever the corresponding F-test indicated significant differences. Standard errors of the mean (SEm ±) and critical differences were calculated and presented to facilitate statistical interpretation of treatment effects.

3. RESULTS

3.1. Physiological changes associated with seed moisture and germination performance

Seed moisture content exhibited a gradual increase during storage under both environments, with a greater accumulation under ambient storage (S_1) than under cold storage (S_2) (Figure 1). The overall mean increased from 7.51 to 8.45% under S_1, compared with 7.55 to 7.90% under S_2. Significant differences between storage environments became apparent from the fifth month. Among the coating treatments, 2% chitosan (T_2) consistently maintained the lowest moisture content, whereas the uncoated control (T_7) exhibited the highest. The treatment × storage interaction remained non-significant throughout the study.

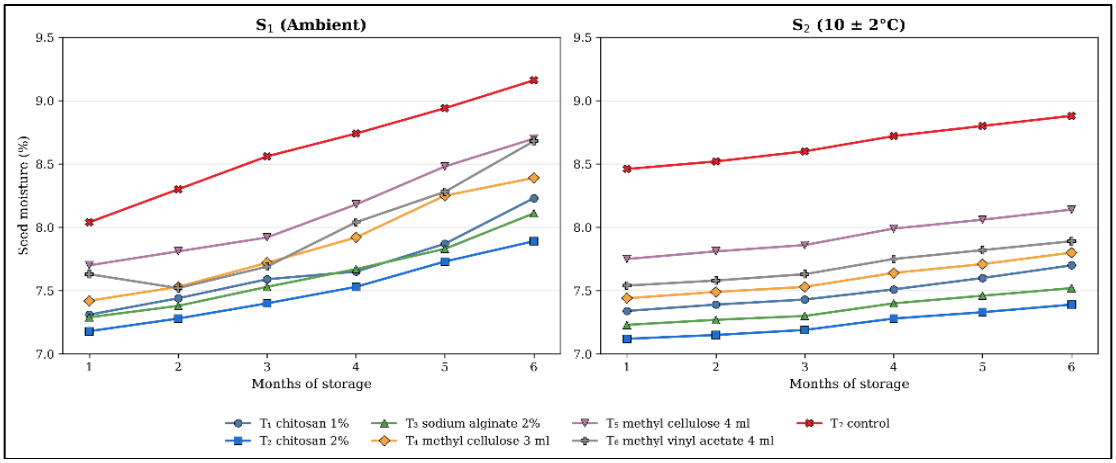


Figure 1. Effect of hydrophobic polymer coatings on seed moisture content (%) of onion seeds during storage under ambient storage and 10 ± 2°C

In contrast to the increasing moisture content, germination energy declined progressively with storage duration (Figure 2). The overall mean decreased from 77.57 to 55.14% under S_1 and from 79.57 to 60.19% under S_2.

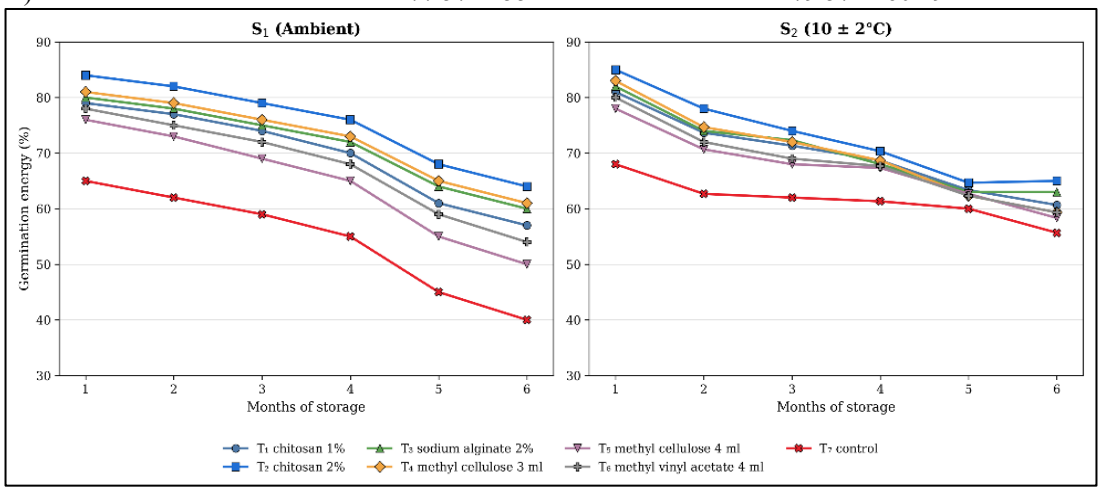


Figure 2. Effect of hydrophobic polymer coatings on germination energy (%) of onion seeds during storage under ambient storage and 10 ± 2°C

T_2 consistently recorded the highest germination energy, retaining 64.00 and 65.00% under ambient and cold storage, respectively, after six months. The corresponding values in T_7 were 40.00 and 55.67%. The treatment × storage interaction became significant from the third month onwards.

3.2. Changes in seedling vigour and growth characteristics

The vitality index declined progressively under both storage environments, with a more pronounced reduction under ambient conditions (Table 1).

Table 1. Effect of hydrophobic polymer coating and storage condition on vitality index of onion seeds during storage

Treatments	$M_1 S_1$	$M_1 S_2$	$M_2 S_1$	$M_2 S_2$	$M_3 S_1$	$M_3 S_2$	$M_4 S_1$	$M_4 S_2$	$M_5 S_1$	$M_5 S_2$	$M_6 S_1$	$M_6 S_2$
T_1	1.62	1.83	1.58	1.81	1.53	1.79	1.47	1.76	1.40	1.73	1.31	1.69
T_2	2.00	2.02	1.96	2.00	1.90	1.98	1.83	1.95	1.74	1.91	1.64	1.87
T_3	1.66	1.88	1.62	1.87	1.57	1.85	1.51	1.82	1.43	1.79	1.34	1.75
T_4	1.62	1.63	1.57	1.62	1.51	1.60	1.45	1.58	1.37	1.55	1.28	1.52
T_5	1.33	1.58	1.28	1.57	1.22	1.55	1.15	1.52	1.07	1.49	0.98	1.45

Treatments	$M_1 S_1$	$M_1 S_2$	$M_2 S_1$	$M_2 S_2$	$M_3 S_1$	$M_3 S_2$	$M_4 S_1$	$M_4 S_2$	$M_5 S_1$	$M_5 S_2$	$M_6 S_1$	$M_6 S_2$
T_6	1.57	1.60	1.52	1.59	1.46	1.57	1.39	1.55	1.31	1.52	1.22	1.48
T_7	1.31	1.38	1.25	1.37	1.18	1.35	1.10	1.32	1.01	1.29	0.91	1.21
Mean	**1.59**	**1.70**	**1.54**	**1.69**	**1.48**	**1.67**	**1.41**	**1.64**	**1.33**	**1.61**	**1.24**	**1.57**
Source	**M_1**		**M_2**		**M_3**		**M_4**		**M_5**		**M_6**	
	SE.m±	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**
T	**0.005**	**0.015**	**0.005**	**0.015**	**0.005**	**0.016**	**0.005**	**0.015**	**0.006**	**0.017**	**0.006**	**0.018**
S	**0.003**	**0.008**	**0.003**	**0.008**	**0.003**	**0.009**	**0.003**	**0.008**	**0.003**	**0.009**	**0.003**	**0.009**
T × S	**0.007**	**0.021**	**0.007**	**0.022**	**0.008**	**0.022**	**0.007**	**0.022**	**0.008**	**0.024**	**0.009**	**0.025**

T_1: chitosan @ 1%; T_2: chitosan @ 2%; T_3: sodium alginate @ 2%; T_4: methyl cellulose @ 3 ml; T_5: methyl cellulose @ 4 ml; T_6: methyl vinyl acetate @ 4 ml; T_7: control (uncoated); S_1: ambient; S_2: 10 ± 2°C; M_1 to M_6: first to sixth month after storage CD at 5%; NS: non-significant.

The overall mean decreased from 1.59 to 1.24 under S_1 and from 1.70 to 1.57 under S_2. Significant treatment, storage and interaction effects were observed throughout the experimental period. T_2 maintained the highest vitality index, whereas T_7 consistently exhibited the lowest values. A comparable pattern was observed for seedling growth. Root length decreased from 5.49 to 4.35 cm under S_1 and from 5.74 to 5.47 cm under S_2 (Table 2).

Table 2. Effect of hydrophobic polymer coating and storage condition on root length (cm) of onion seeds during storage

Treatments	$M_1 S_1$	$M_1 S_2$	$M_2 S_1$	$M_2 S_2$	$M_3 S_1$	$M_3 S_2$	$M_4 S_1$	$M_4 S_2$	$M_5 S_1$	$M_5 S_2$	$M_6 S_1$	$M_6 S_2$
T_1	5.69	5.87	5.53	5.85	5.33	5.82	5.12	5.75	4.87	5.68	4.61	5.59
T_2	5.87	6.47	5.72	6.44	5.57	6.40	5.37	6.35	5.12	6.28	4.88	6.18
T_3	5.84	6.14	5.72	6.12	5.54	6.08	5.32	6.02	5.07	5.96	4.82	5.87
T_4	5.69	5.65	5.52	5.64	5.32	5.63	5.07	5.55	4.82	5.48	4.55	5.41
T_5	5.08	5.24	4.89	5.21	4.66	5.17	4.41	5.12	4.12	5.03	3.82	4.97
T_6	5.27	5.62	5.07	5.59	4.89	5.54	4.65	5.52	4.41	5.43	4.12	5.34
T_7	5.01	5.19	4.80	5.16	4.55	5.12	4.27	5.07	3.97	5.00	3.65	4.92
Mean	**5.49**	**5.74**	**5.32**	**5.72**	**5.12**	**5.68**	**4.89**	**5.63**	**4.63**	**5.55**	**4.35**	**5.47**
Source	**M_1**		**M_2**		**M_3**		**M_4**		**M_5**		**M_6**	
	SE.m±	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**
T	**0.005**	**0.015**	**0.005**	**0.016**	**0.006**	**0.018**	**0.006**	**0.018**	**0.006**	**0.017**	**0.005**	**0.015**
S	**0.003**	**0.008**	**0.003**	**0.008**	**0.003**	**0.01**	**0.003**	**0.009**	**0.003**	**0.009**	**0.003**	**0.008**
T × S	**0.007**	**0.021**	**0.008**	**0.022**	**0.009**	**0.025**	**0.009**	**0.025**	**0.008**	**0.024**	**0.007**	**0.021**

T_1: chitosan @ 1%; T_2: chitosan @ 2%; T_3: sodium alginate @ 2%; T_4: methyl cellulose @ 3 ml; T_5: methyl cellulose @ 4 ml; T_6: methyl vinyl acetate @ 4 ml; T_7: control (uncoated); S_1: ambient; S_2: 10 ± 2°C; M_1 to M_6: first to sixth month after storage CD at 5%; NS: non-significant.

T_2 maintained the longest roots, with final values of 4.88 and 6.18 cm, respectively. Similarly, shoot length decreased from 8.17 to 6.59 cm under S_1 and from 8.40 to 8.09 cm under S_2 (Table 3). The corresponding final shoot lengths in T_2 were 7.23 and 8.96 cm. In both parameters, T_7 recorded the lowest values. Treatment, storage and interaction effects were significant throughout storage.

Table 3. Effect of hydrophobic polymer coating and storage condition on shoot length (cm) of onion seeds during storage

Treatments	$M_1 S_1$	$M_1 S_2$	$M_2 S_1$	$M_2 S_2$	$M_3 S_1$	$M_3 S_2$	$M_4 S_1$	$M_4 S_2$	$M_5 S_1$	$M_5 S_2$	$M_6 S_1$	$M_6 S_2$
T_1	8.43	8.81	8.20	8.76	7.92	8.72	7.61	8.66	7.23	8.57	6.87	8.47
T_2	8.67	9.30	8.45	9.26	8.22	9.21	7.92	9.14	7.57	9.07	7.23	8.96
T_3	8.45	9.01	8.22	8.96	7.96	8.91	7.66	8.85	7.36	8.77	6.97	8.67
T_4	8.33	8.17	8.07	8.14	7.80	8.12	7.48	8.04	7.14	7.97	6.77	7.88
T_5	7.61	7.83	7.35	7.82	7.03	7.76	6.69	7.72	6.32	7.63	5.93	7.57
T_6	8.15	7.94	7.92	7.90	7.65	7.86	7.34	7.80	7.02	7.73	6.62	7.65
T_7	7.56	7.73	7.27	7.68	6.94	7.64	6.47	7.57	6.12	7.50	5.76	7.41
Mean	**8.17**	**8.40**	**7.93**	**8.36**	**7.65**	**8.32**	**7.31**	**8.25**	**6.96**	**8.18**	**6.59**	**8.09**
Source	**M_1**		**M_2**		**M_3**		**M_4**		**M_5**		**M_6**	

	SE.m±	CD	SE.m±	CD	SE.m±	CD	SE.m±	CD	SE.m±	CD	SE.m±	CD
T	**0.005**	**0.015**	**0.005**	**0.015**	**0.005**	**0.015**	**0.017**	**0.05**	**0.006**	**0.02**	**0.006**	**0.017**
S	**0.003**	**0.008**	**0.003**	**0.008**	**0.003**	**0.008**	**0.009**	**0.03**	**0.003**	**0.01**	**0.003**	**0.009**
T × S	**0.007**	**0.021**	**0.007**	**0.021**	**0.007**	**0.021**	**0.024**	**0.07**	**0.008**	**0.02**	**0.008**	**0.024**

T_1: chitosan @ 1%; T_2: chitosan @ 2%; T_3: sodium alginate @ 2%; T_4: methyl cellulose @ 3 ml; T_5: methyl cellulose @ 4 ml; T_6: methyl vinyl acetate @ 4 ml; T_7: control (uncoated); S_1: ambient; S_2: 10 ± 2°C; M_1 to M_6: first to sixth month after storage CD at 5%; NS: non-significant.

3.3. Changes in membrane integrity and electrolyte leakage

Membrane injury index increased progressively during storage, indicating an increasing degree of membrane injury with storage duration (Table 4).

Table 4. Effect of hydrophobic polymer coating and storage condition on membrane injury index of onion seeds during storage

Treatments	M_1 S_1	M_1 S_2	M_2 S_1	M_2 S_2	M_3 S_1	M_3 S_2	M_4 S_1	M_4 S_2	M_5 S_1	M_5 S_2	M_6 S_1	M_6 S_2
T_1	0.77	0.72	0.80	0.73	0.83	0.75	0.86	0.77	0.89	0.79	0.91	0.81
T_2	0.74	0.68	0.77	0.69	0.80	0.71	0.83	0.73	0.86	0.75	0.88	0.77
T_3	0.75	0.70	0.78	0.71	0.81	0.73	0.84	0.75	0.87	0.77	0.89	0.79
T_4	0.79	0.74	0.82	0.75	0.85	0.77	0.88	0.79	0.91	0.81	0.93	0.83
T_5	0.82	0.78	0.85	0.79	0.88	0.81	0.91	0.83	0.93	0.85	0.95	0.87
T_6	0.81	0.76	0.84	0.77	0.87	0.79	0.90	0.81	0.92	0.83	0.94	0.85
T_7	0.84	0.80	0.87	0.81	0.90	0.83	0.93	0.85	0.95	0.87	0.97	0.89
Mean	**0.79**	**0.74**	**0.82**	**0.75**	**0.85**	**0.77**	**0.88**	**0.79**	**0.90**	**0.81**	**0.92**	**0.83**
Source	**M_1**		**M_2**		**M_3**		**M_4**		**M_5**		**M_6**	
	SE.m±	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**
T	**0.004**	**0.012**	**0.004**	**0.012**	**0.004**	**0.012**	**0.004**	**0.012**	**0.004**	**0.012**	**0.004**	**0.012**
S	**0.002**	**0.006**	**0.002**	**0.006**	**0.002**	**0.006**	**0.002**	**0.006**	**0.002**	**0.006**	**0.002**	**0.006**
T × S	**0.006**	**NS**	**0.006**	**NS**	**0.006**	**NS**	**0.006**	**NS**	**0.006**	**NS**	**0.006**	**NS**

T_1: chitosan @ 1%; T_2: chitosan @ 2%; T_3: sodium alginate @ 2%; T_4: methyl cellulose @ 3 ml; T_5: methyl cellulose @ 4 ml; T_6: methyl vinyl acetate @ 4 ml; T_7: control (uncoated); S_1: ambient; S_2: 10 ± 2°C; M_1 to M_6: first to sixth month after storage CD at 5%; NS: non-significant.

The overall mean increased from 0.79 to 0.92 under S_1 and from 0.74 to 0.83 under S_2. T_2 consistently maintained the lowest index, whereas T_7 exhibited the highest values. Treatment and storage effects were significant throughout, while their interaction remained non-significant.

Electrical conductivity exhibited a corresponding progressive increase (Figure 3). The overall mean increased from 28.24 to 39.86 µS cm^{-1} g^{-1} under S_1 and from 26.10 to 30.90 µS cm^{-1} g^{-1} under S_2. T_2 maintained the lowest EC values at the end of storage (33.00 and 27.00 µS cm^{-1} g^{-1}), whereas T_7 recorded the highest (52.00 and 39.00 µS cm^{-1} g^{-1}). The treatment × storage interaction was significant during the first, fifth and sixth months.

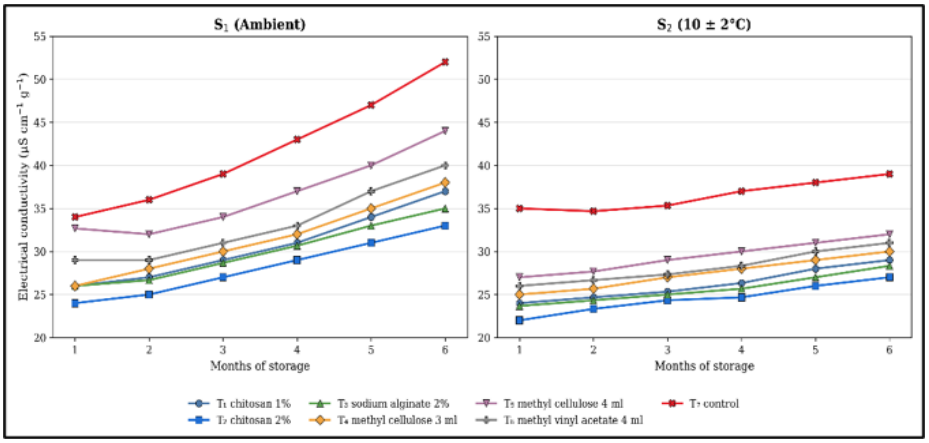


Figure 3. Effect of hydrophobic polymer coatings on electrical conductivity (µS cm^{-1} g^{-1}) of onion seeds during storage under ambient storage and 10 ± 2°C

3.4. Changes in metabolic enzyme activity

Total dehydrogenase activity declined progressively under both storage environments (Table 5).

Table 5. Effect of hydrophobic polymer coating and storage condition on total dehydrogenase activity (A_{480}) of onion seeds during storage

Treatments	M1 S_1	M1 S_2	M2 S_1	M2 S_2	M3 S_1	M3 S_2	M4 S_1	M4 S_2	M5 S_1	M5 S_2	M6 S_1	M6 S_2
T_1	0.280	0.310	0.280	0.300	0.270	0.300	0.250	0.290	0.240	0.280	0.220	0.270
T_2	0.350	0.380	0.340	0.380	0.330	0.370	0.310	0.363	0.290	0.350	0.270	0.340
T_3	0.250	0.350	0.240	0.343	0.230	0.340	0.220	0.330	0.210	0.310	0.200	0.310
T_4	0.250	0.250	0.230	0.243	0.220	0.237	0.210	0.240	0.200	0.230	0.190	0.220
T_5	0.183	0.210	0.170	0.193	0.160	0.190	0.150	0.190	0.140	0.190	0.130	0.170
T_6	0.210	0.230	0.200	0.233	0.190	0.220	0.180	0.223	0.170	0.220	0.160	0.210
T_7	0.150	0.160	0.110	0.163	0.103	0.160	0.103	0.160	0.080	0.150	0.070	0.140
Mean	**0.240**	**0.270**	**0.220**	**0.270**	**0.210**	**0.260**	**0.200**	**0.260**	**0.190**	**0.250**	**0.180**	**0.240**
Source	**M1**		**M2**		**M3**		**M4**		**M5**		**M6**	
	SE.m±	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**	**SE.m±**	**CD**
T	**0.004**	**0.012**	**0.004**	**0.01**	**0.004**	**0.012**	**0.004**	**0.012**	**0.004**	**0.012**	**0.004**	**0.012**
S	**0.002**	**0.007**	**0.002**	**0.006**	**0.002**	**0.006**	**0.002**	**0.006**	**0.002**	**0.006**	**0.002**	**0.006**
T × S	**0.006**	**0.018**	**0.005**	**0.015**	**0.006**	**0.017**	**0.006**	**0.017**	**0.006**	**0.017**	**0.006**	**0.017**

T_1: chitosan @ 1%; T_2: chitosan @ 2%; T_3: sodium alginate @ 2%; T_4: methyl cellulose @ 3 ml; T_5: methyl cellulose @ 4 ml; T_6: methyl vinyl acetate @ 4 ml; T_7: control (uncoated); S_1: ambient; S_2: 10 ± 2°C; M_1 to M_6: first to sixth month after storage CD at 5%; NS: non-significant.

T_2 consistently maintained the highest activity, with final values of 0.270 and 0.340 A_{480}, respectively. In contrast, T_7 recorded the lowest activity (0.070 and 0.140 A_{480}). Treatment, storage and their interaction significantly influenced dehydrogenase activity throughout the study. T_3 also exhibited comparatively higher activity than T_1 under cold storage.

3.5. Changes in antioxidant enzyme activities

Peroxidase activity declined progressively during storage, with a greater reduction under ambient conditions (Figure 4). The overall mean decreased from 0.557 to 0.447 ΔA_{436} min^{-1} g^{-1} under S_1 and from 0.577 to 0.527 under S_2. T_2 consistently maintained the highest activity, whereas T_7 recorded the lowest. Treatment and storage effects were significant throughout, while their interaction was non-significant.

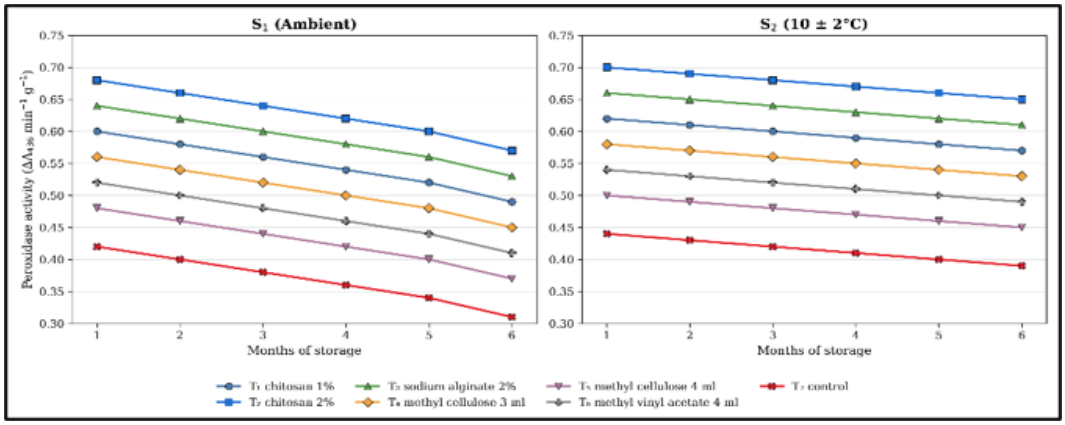


Figure 4. Effect of hydrophobic polymer coatings on peroxidase activity (ΔA_{436} min^{-1} g^{-1}) of onion seeds during storage under ambient storage and 10 ± 2°C

Catalase activity also declined progressively during storage (Figure 5). The overall mean decreased from 1.350 to 1.050 mM H_2O_2 decomposed min^{-1} under S_1 and from 1.450 to 1.310 under S_2.

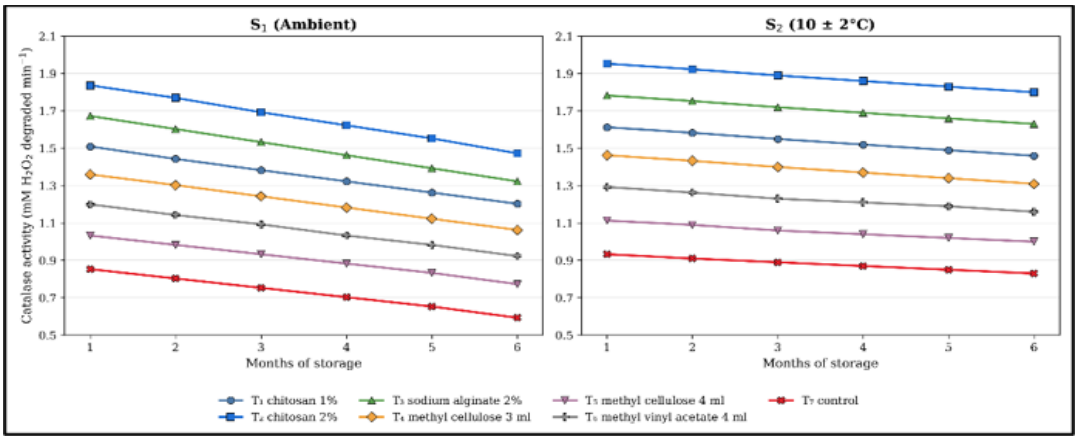


Figure 5. Effect of hydrophobic polymer coatings on catalase activity (mM H_2O_2 degraded min^{-1}) of onion seeds during storage under ambient storage and 10 ± 2°C

T_2 maintained the highest activity, recording 1.473 and 1.800 mM H_2O_2 decomposed min^{-1} after six months under ambient and cold storage, respectively. The corresponding values in T_7 were 0.593 and 0.830. Treatment and storage effects were significant throughout, while their interaction became significant from the second month onwards.

3.6. Changes in soluble sugar leakage and protein content

Total soluble sugar concentration in seed leachate increased progressively during storage, with a greater increase under ambient conditions (Figure 6). The overall mean increased from 36.71 to 83.71 µg ml^{-1} under S_1 and from 34.71 to 60.86 µg ml^{-1} under S_2. T_2 consistently recorded lower soluble sugar leakage, with a final concentration of 60.0 µg ml^{-1}, whereas T_7 recorded the highest value of 86.0 µg ml^{-1}. Treatment and storage effects were significant throughout, while their interaction became significant during the fifth and sixth months.

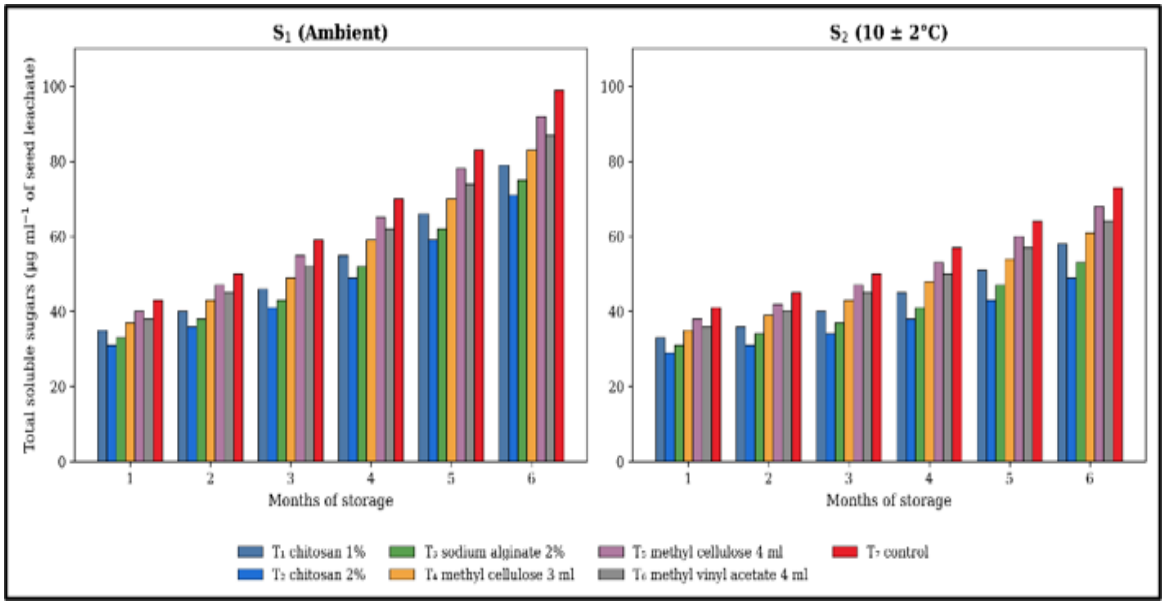


Figure 6. Effect of hydrophobic polymer coatings on total soluble sugars (µg ml^{-1} of seed leachate) of onion seeds during storage under ambient storage and 10 ± 2°C

Total soluble protein content, conversely, decreased progressively during storage (Figure 7). The overall mean declined from 95.43 to 85.43 µg g^{-1} under S_1 and from 98.43 to 88.43 µg g^{-1} under S_2.

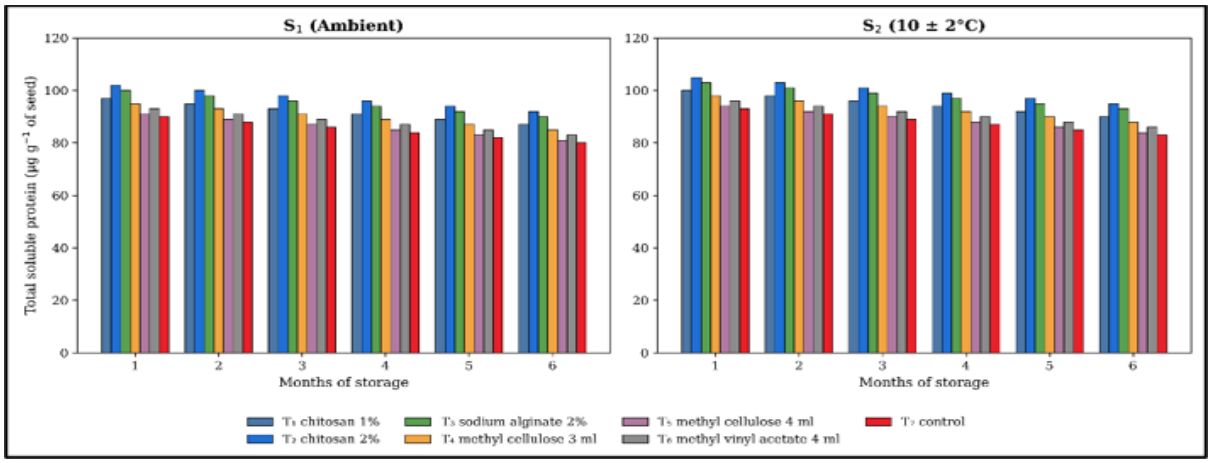


Fig. 7. Effect of hydrophobic polymer coatings on total soluble protein (µg g⁻¹ of seed) of onion seeds during storage under ambient storage and 10 ± 2°C

T_2 maintained the highest protein concentration after six months (93.5 µg g^{-1}), whereas T_7 recorded the lowest (81.5 µg g^{-1}). Treatment and storage effects were significant throughout the experimental period, while the treatment × storage interaction remained non-significant.

Overall, the physiological and biochemical observations consistently identified T_2 (2% chitosan) as the treatment associated with the highest retention of seed quality during storage, while the uncoated control exhibited comparatively greater deterioration under both storage environments.

4. DISCUSSION

The findings of the present investigation demonstrate that polymer coating treatments and storage environment jointly influenced the physiological and biochemical quality of onion seeds during storage. Across the evaluated parameters, 2% chitosan coating (T_2) showed the most consistent favourable response, whereas the uncoated control (T_7) generally exhibited greater deterioration. The comparatively better retention of seed quality under cold storage further highlights the importance of combining suitable coating formulations with appropriate storage conditions.

4.1. Seed moisture regulation and storage stability

The progressive increase in seed moisture content, particularly under ambient conditions, indicates the influence of storage environment on seed moisture equilibrium. T_2 consistently maintained comparatively lower moisture content, while T_7 recorded higher values. This response may be related to the film-forming characteristics of chitosan and its influence on moisture exchange at the seed surface. However, the specific contribution of coating permeability requires direct evaluation. The non-significant treatment × storage interaction indicates that the relative effects of the coating treatments on seed moisture content remained consistent across both environments. These observations are broadly consistent with Parolin *et al.* (2026) and Ranganathan and Groot (2023).

4.2. Retention of germination capacity and physiological vigour

The decline in germination energy and vitality index during storage reflects progressive deterioration of seed physiological vigour. The comparatively greater reduction under ambient conditions indicates that the storage environment accelerated the loss of germination performance. T_2 consistently maintained higher values for both parameters, demonstrating better retention of early germination capacity and seedling vitality. The significant treatment × storage interaction observed for germination energy from the third month onwards and for vitality index throughout the storage period indicates that the magnitude of treatment response was influenced by storage conditions. Since vitality index incorporates both germination-related performance and seedling growth, its maintenance provides complementary evidence of better physiological quality. These findings are consistent with Perl *et al.* (1978) and Thirusendura Selvi and Saraswathy (2018).

4.3. Preservation of seedling growth potential

The superior performance of T_2 was further reflected in root and shoot development. Both parameters declined progressively during storage, with greater reductions under ambient conditions. The consistently higher root and shoot lengths in T_2 indicate better retention of seedling establishment potential, whereas the shorter seedlings recorded in T_7 suggest greater deterioration of the physiological processes supporting early growth. The significant treatment × storage interaction demonstrates that seedling growth responses were influenced by the combined effects of coating treatment and storage environment. This pattern is consistent with the association between seed

ageing, membrane deterioration and impaired seedling development discussed by McDonald (1999) and Xing *et al.* (2025).

4.4. Membrane deterioration and electrolyte leakage

Membrane-related parameters provided additional evidence of treatment-associated differences in seed deterioration. The progressive increase in membrane injury index and electrical conductivity indicates declining membrane integrity and increasing permeability during storage. Both parameters showed comparatively favourable values in T_2, while T_7 exhibited greater deterioration. The lower EC and membrane injury index under cold storage further demonstrate the importance of temperature management in maintaining cellular integrity. Although the treatment × storage interaction was non-significant for membrane injury index, it was significant for EC during the first, fifth and sixth months. This difference indicates that the two indicators responded differently to the combined treatment and storage effects. Nevertheless, their overall treatment trends support comparatively better membrane stability in chitosan-coated seeds. Similar relationships between membrane integrity, electrolyte leakage and seed deterioration have been reported by Goel *et al.* (2003), Hwang and Sung (1991) and Ghaderifar *et al.* (2021).

4.5. Maintenance of respiratory metabolic activity

The biochemical indicators further supported the physiological responses. Dehydrogenase activity declined during storage, indicating a reduction in the measured metabolic competence of the seeds. T_2 maintained higher activity than T_7, suggesting better retention of respiratory metabolic activity. However, the comparatively higher activity of sodium alginate-coated seeds (T_3) than 1% chitosan-coated seeds (T_1) under cold storage, together with their different relative performance under ambient conditions, indicates that the response was formulation- and environment-dependent. The significant treatment × storage interaction supports this interpretation. These observations are consistent with the use of dehydrogenase activity as an indicator of seed physiological quality, as discussed by Satya Srii and Nagarajappa (2024) and Brar *et al.* (2019).

4.6. Antioxidant enzyme responses during storage

A similar treatment response was observed for the measured antioxidant enzyme activities. Peroxidase and catalase activities declined during storage, with comparatively higher values maintained under cold storage. T_2 consistently retained higher activities than the uncoated control, indicating better maintenance of the measured enzyme activities. The non-significant treatment × storage interaction for peroxidase suggests consistent relative treatment responses across the two environments, whereas the significant interaction for catalase from the second month onwards indicates environment-dependent treatment effects. At six months, catalase activity in T_2 remained substantially higher than in T_7 under both storage conditions. These findings suggest that chitosan coating was associated with better retention of antioxidant enzyme activity during storage. Nevertheless, because reactive oxygen species and lipid peroxidation were not directly measured, the results should not be interpreted as direct evidence of reduced oxidative damage. The role of antioxidant enzyme systems in seed ageing has been discussed by Brar *et al.* (2019) and Goel *et al.* (2003).

4.7. Soluble metabolite leakage and protein retention

Changes in soluble sugar concentration and total soluble protein further reflected the biochemical changes associated with storage. The progressive increase in soluble sugar concentration in seed leachate, particularly under ambient conditions, suggests enhanced leakage of soluble constituents as storage progressed. T_2 recorded lower sugar concentrations, whereas T_7 showed higher values, indicating comparatively lower solute leakage in chitosan-coated seeds. The significant treatment × storage interaction during the fifth and sixth months suggests that treatment differences became more pronounced during the later storage period. This response is consistent with the membrane deterioration mechanism described by McDonald (1999). However, this interpretation is valid only if the soluble sugar assay was performed using seed leachate rather than an extract of seed tissue.

In contrast, total soluble protein content declined progressively during storage, with comparatively greater retention under cold conditions. T_2 maintained 93.5 µg g^{-1} after six months, compared with 81.5 µg g^{-1} in the uncoated control. The non-significant treatment × storage interaction indicates that the relative treatment response for protein retention remained consistent across both storage environments. The greater retention of soluble protein in chitosan-coated seeds is broadly consistent with the findings of Winarti *et al.* (2023), who reported improved protein retention in chitosan-coated soybean seeds.

Overall, the consistent superiority of 2% chitosan coating across several physiological and biochemical indicators suggests that it was the most promising formulation among those evaluated for maintaining onion seed quality during the six-month storage period. Its favourable performance was evident in germination energy, seedling vitality, root and shoot development, membrane-related indicators, dehydrogenase activity, antioxidant enzyme activities and soluble protein retention. The comparatively better performance under cold storage further indicates that polymer coating should be considered a complementary seed-quality management strategy rather than a substitute for appropriate storage conditions. These findings provide a basis for further evaluation of chitosan-based seed coatings under longer storage durations and different packaging and environmental conditions.

5. CONCLUSION

The present investigation revealed that polymer coating treatments influenced the physiological and biochemical quality of onion seeds during six months of storage, with 2% chitosan (T_2) exhibiting the most consistent favourable response among the treatments evaluated. Seeds treated with 2% chitosan maintained comparatively lower moisture content, higher germination energy and seedling vitality, greater root and shoot lengths, and better membrane integrity, as indicated by lower electrical conductivity and membrane injury index. The treatment also favoured the retention of dehydrogenase, catalase and peroxidase activities, together with higher total soluble protein content and lower soluble sugar concentration in the seed leachate. These beneficial responses were observed under both ambient and cold storage conditions, with comparatively better overall seed quality maintained under cold storage (10 ± 2°C). Significant treatment × storage interactions for selected physiological and biochemical parameters further indicated that the magnitude of the coating effects varied depending on the storage environment. Collectively, the findings identify 2% chitosan as a promising polymer coating formulation for improving the retention of onion seed quality during storage. Its application, particularly in combination with appropriate storage conditions, may provide a useful complementary approach for enhancing onion seed storability. Further studies involving direct assessment of coating permeability, oxidative stress-related biochemical markers and extended storage periods are necessary to elucidate the underlying mechanisms and establish the commercial applicability of this treatment.

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Author Contributions

G. R. Gagana, S.R.: Undertook experimental investigations, data recording, and preliminary evaluation of results.; Radha, B. N.: Formulated the research concept, designed the methodological framework, and provided comprehensive technical guidance.; K. Vishwanath: Extended laboratory assistance for conducting the experimental work. Krishnamma P. N.: Provided technical assistance for laboratory analysis.

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Consent to participate

All authors consented to participate in the article (Radha BN -on behalf of all coauthors).

Consent to publish

All authors gave their consent for the article to be published if it was accepted (Radha BN, on behalf of all coauthors). Disclosure statement: No potential conflict of interest was reported by the authors.

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