PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp4): 01–08
https://doi.org/10.14719/pst.5035

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RESEARCH ARTICLE

# Impact of packaging and storage conditions on marigold seed quality: Physiological and biochemical perspectives

**MP Nourin[1], R Vigneshwari[2*], R Umarani[3], V Manonmani[1], M Ganga[4], D Thirusendura Selvi[1] & S Kavitha[5]**

[1] Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[2] Agricultural Research Station, Bhavanisagar, Tamil Nadu Agricultural University, Erode 638 451, Tamil Nadu, India

[3] Seed Centre, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[4] Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[5] Centre for Students Welfare, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Email: rv77@tnau.ac.in

OPEN ACCESS

**ARTICLE HISTORY**
Received: 12 September 2024
Accepted: 13 October 2024
Available online
Version 1.0 : 25 November 2024

Check for updates

**Additional information**
**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Nourin MP, Vigneshwari R, Umarani R, Manonmani V, Ganga M, Selvi DT, Kavitha S. Impact of packaging and storage conditions on marigold seed quality: Physiological and biochemical perspectives. Plant Science Today.2024;11(sp4):01-08. https:/doi.org/10.14719/pst.5035

## Abstract

Marigold is an important ornamental crop highly valued for its aesthetic appeal and versatile applications in horticulture, medicine and environmental sustainability. However, declining seed viability and vigour present significant challenges in marigold seed preservation. This research investigated the influence of various storage conditions and packaging materials on the germination and vigour traits of the Pusa Narangi Gainda variety. The marigold seeds were kept in various packaging and storage conditions: cloth bags ($T_0$), aluminium foil pack in ambient condition ($T_1$), aluminium foil vacuum pack in ambient condition ($T_2$), aluminium foil pack at 5 °C ($T_3$) and aluminium foil vacuum pack at 5 °C ($T_4$). The results suggest that storing seeds in aluminium foil vacuum pack at 5 °C positively impacted germination and biochemical parameters like catalase and peroxidase activity. These findings underscore the importance of optimal storage conditions for enhancing seed longevity and vigour in marigold.

## Keywords

marigold; packaging container; seed viability; storage conditions; vigour index

## Introduction

Marigold is a significant annual flowering crop in the Asteraceae family, widely cultivated for commercial purposes. Among the 33 different *Tagetes* species, *Tagetes erecta* (African Marigold) and *Tagetes patula* (French Marigold) are the most frequently cultivated species and native to Mexico and South Africa respectively (1). Marigold flowers are integral to garland making and landscape gardening (2), while marigold oil, known for its insecticidal and medicinal properties, is highly esteemed in the cosmetics and fragrance industries (3). Marigold is also a salient commercial source of the natural carotenoid used for food, animal feed and chemical and pharmaceutical industries (4). India's leading marigold-producing states are West Bengal, Gujarat, Madhya Pradesh, Karnataka and Andhra Pradesh (5) .

The demand for African marigolds is increasing, because of their multipurpose use as loose flowers, ornamental flowers, medicinal properties and other industrial uses (6). However, one of the biggest bottlenecks in marigold production is the loss of seed viability and vigour during postharvest storage (7, 8). Although genetic makeup determines the quality of seeds, appropriate seed storage significantly affects seed vigour (9). Environmental conditions, seed treatments and packaging methods are the main factors which influence the seed quality during storage (10). Inadequate storage practices lead to diminished seed longevity and viability, emphasizing the importance of optimizing storage conditions (11). While earlier research has examined how moisture and temperature affect seed viability, limited investigations have been conducted on the combined impact of vacuum packaging and low-temperature storage on marigold seeds. This study aims to bridge this gap by evaluating the biochemical and physiological responses of marigold seeds to various storage conditions. Improper post-harvest handling of marigold seeds can negatively impact seed quality, germination rates and vigour.

## Materials

The research was conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, during 2023-24, to evaluate how different storage containers and environments affect the quality parameters of marigold seeds. Marigold seeds of the variety Pusa Narangi Gainda were purchased from IARI, New Delhi. Cloth bags, aluminium foil packs and aluminium foil vacuum packs were obtained from the Department of Seed Science and Technology, TNAU, Coimbatore. Upon achieving a moisture content of 6 % through drying, the required quantities of seeds were stored in different containers as per the following treatments:

$T_0$: Storage of seeds in cloth bag (white cotton cloth bag of 6 x 5 inches dimension) in ambient condition

$T_1$: Storage of seeds in aluminium foil pack in ambient condition

$T_2$: Storage of seeds in aluminium foil vacuum pack in ambient condition

$T_3$: Storage of seeds in aluminium foil pack at 5 °C

$T_4$: Storage of seeds in aluminium foil vacuum pack at 5 °C

## Methods

Aluminium foil packs were heat sealed by using table top heat-sealing machine (8 inches) and for vacuum packing, a tabletop vacuum packaging machine was used whereas the cloth bags were machine stitched with an electric sewing machine (Fig. 1). The storage durations were labelled as $P_0$ through $P_6$, corresponding to the initial month up to 6 months. The data on the germination %, speed of germination, shoot length, root length, vigour index, moisture content, electrical conductivity, catalase and peroxidase activity of seeds were recorded at monthly intervals during storage.

### Speed of germination (Maguire, 1962):

Four replicates of 25 seeds each were germinated in the top-of-paper method. The number of seeds germinated was counted from the 2$^{nd}$ day onwards up to the 14$^{th}$ day. From the number of seeds germinated on each counting day, the speed of germination was computed by adopting the formula.


**Fig. 1.** Marigold seeds stored in different containers.

$$\frac{X_1}{Y_1} + \frac{X_2}{Y_2} + \cdots\cdots\cdots + \frac{X_n - (X_{n-1})}{Y_n}$$

$X_1$ - Number of seeds germinated on the first observation day

$X_2$ - Number of seeds germinated on the second observation day

$X_n$ - Number of seeds germinated on the nth observation day

$Y_1$ - Number of days from sowing to first observation day

$Y_2$ - Number of days from sowing to second observation day

$Y_n$ - Number of days from sowing to nth observation day

### Germination %:

The germination test was performed in a sand medium using 4 x 100 seeds in a germination room illuminated with fluorescent light maintained at 25 ± 2 °C and 95 ± 5 % RH. After 14 days, the seedlings were evaluated and the normal seedlings were counted and expressed in %.

### Root length (cm):

Ten normal seedlings from the standard germination test were randomly selected and the root length was measured from the collar region to the tip of the primary root and the average was expressed in cm.

### Shoot length (cm):

Ten normal seedlings from the standard germination test were randomly selected and the length between the collar and tip of the primary shoot was measured as shoot length and average expressed in cm.

### Vigour index:

Vigour index (VI) was computed from the germination % and the dry matter production following the formula suggested by Abdul-Baki and Anderson (1973) and expressed in whole numbers.

$$VI = \text{Germination \%} \times \text{Seedling length}$$

### Moisture content (%):

The seeds were placed in a weighing bottle and kept in a hot air oven maintained at 103 ± 2 °C. After 16 h of drying, they were cooled in a desiccator for 30 min. The moisture content of the seeds was calculated by using the following formula.

$$\text{Moisture content (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

where, M1- the weight of weighing bottle alone

M2- weight of bottle + sample before drying

M3- weight of bottle + sample after drying

### Electrical conductivity (dS/m):

Four replicates of 25 seeds each were taken at random from each treatment. Seeds were initially rinsed with deionised water and then soaked in 25 mL of deionised water for 6 h at room temperature (25 ± 3 °C). The seed steep water was decanted and referred to as seed leachate. The electrical conductivity of seed leachate was measured in a digital conductivity meter with a cell constant of one and expressed as dS $m^{-1}$.

### Catalase activity (µg/min/g) (13):

500 mg of the pre-germinated seed sample was homogenized with 5 mL of extraction buffer (0.1 M phosphate buffer, $p^H$ of 7.5, containing 0.5 mM EDTA) in cold condition to make the enzyme extract. The collected enzyme extract was centrifuged at 15000 rpm for 20 min at 5 °C and only the supernatant was collected and used as an enzyme. Add 3 mL of 50 mM potassium phosphate buffer (PH 7.5) and 12.5 mM hydrogen peroxide (0.5 mL of 75 mM $H_2O_2$) with 50 µL of enzyme extract and then make up the volume up to 3 mL using distilled water. Absorbance at 240 nm was absorbed for 1 min after the reaction was started after adding substrate $H_2O_2$. Catalase activity in the sample was calculated based on the decomposition of the amount of $H_2O_2$. The initial and final values of $H_2O_2$ were calculated and compared with a standard curve drawn with the known concentration of $H_2O_2$. By this, the catalase activity of the seed sample was calculated and expressed in µg of reduced $H_2O_2$/g/min.

### Peroxidase activity ($mg^{-1}$ protein $min^{-1}$) (14):

500 mg of pre-germinated seed samples were homogenized with 5 mL of 0.25 M Tris buffer ($p^H$ 6.0) to extract the enzymes. The mixture was then centrifuged at 10000 rpm for 10 min at 5 $^0$C. Add 0.4 mL of enzyme extract, 0.5 mL of 1 % $H_2O_2$ and 0.5 mL of 0.5 % aqueous pyrogallol solution and incubate at 25 $^0$C for 10 min. The reaction was stopped by adding 0.5 mL of 5 % $H_2SO_4$ after 10 min. At zero time and 10 min later, the OD value of the solution was determined using an Optima UV-Vis spectrophotometer and peroxidase activity was expressed in units $mg^{-1}$ protein $min^{-1}$.

The experimental design used is Factorial Completely Randomized Design. The physical and physiological parameters were evaluated (12). The seeds were subjected to biochemical analysis *viz.,* electrical conductivity, catalase (13) and peroxidase (14) for the estimation of enzyme activity.

### Statistical analysis:

Data from several studies were evaluated using approach for the 'F' test of significance (15). Before analysis, the % data were converted, if needed, to angular (Arc-sine) values. At the 5 % (P=0.05) probability level, the crucial difference (CD) was computed and "NS" stands for non-significant "F" values.

## Results

The results of this study indicated that marigold seeds stored in aluminium foil vacuum packs at 5 °C ($T_4$) exhibited better seed quality characteristics than those stored in other conditions.

### Germination %:

The seeds stored in cloth bags ($T_0$) exhibited the most significant decline in germination %, from the initial value of 88 to 51 %. The seeds stored in vacuum packs at 5 °C showed the highest germination % (72 %) after 6 months of storage. The overall mean germination % for all temperature conditions and containers showed a downward trend (Table 1).

### Speed of germination:

The speed of germination, an important indicator of seed vigour, showed a general decline across all treatments during the storage period. Speed of germination was 7.41 at the onset of the experiment before seed storage. Storage of seeds in cloth bags ($T_0$) led to a notable reduction in the speed of germination from 7.41 to 5.05 (a decrease of 32 % in speed of germination; Table 2) after 6 months of storage period, whereas aluminium foil vacuum pack at 5 °C ($T_4$) maintained the highest speed of germination (7.09) after 6 months of storage.

### Root length (cm) and Shoot length (cm):

A notable decline in both root length and shoot length (Table 3 and Table 4) was also observed, which was linked to the type of storage containers used during the storage period. Specifically, utilization of vacuum aluminium packs resulted

**Table 1.** Impact of treatments and storage duration on germination percentage in marigold

| Treatments /Period | $P_0$ | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | Mean |
|---|---|---|---|---|---|---|---|---|
| $T_0$ | 88<br>(70.20) | 85<br>(67.49) | 82<br>(65.40) | 72<br>(58.50) | 66<br>(54.75) | 54<br>(47.68) | 51<br>(45.76) | **72**<br>**(58.54)** |
| $T_1$ | 88<br>(70.20) | 86<br>(68.60) | 83<br>(65.94) | 74<br>(59.35) | 68<br>(55.55) | 62<br>(51.95) | 60<br>(50.77) | **75**<br>**(60.34)** |
| $T_2$ | 88<br>(70.20) | 87<br>(69.21) | 84<br>(66.45) | 77<br>(61.60) | 70<br>(57.22) | 68<br>(55.61) | 63<br>(52.76) | **77**<br>**(61.87)** |
| $T_3$ | 88<br>(70.20) | 87<br>(69.16) | 84<br>(66.96) | 78<br>(62.04) | 72<br>(58.57) | 70<br>(56.88) | 70<br>(56.86) | **79**<br>**(62.96)** |
| $T_4$ | 88<br>(70.20) | 87<br>(69.57) | 86<br>(68.06) | 82<br>(64.92) | 76<br>(61.03) | 76<br>(61.15) | 72<br>(58.09) | **81**<br>**(64.72)** |
| **Mean** | **88**<br>**(70.20)** | **87**<br>**(68.81)** | **84**<br>**(66.56)** | **77**<br>**(61.28)** | **71**<br>**(57.43)** | **66**<br>**(54.66)** | **63**<br>**(52.85)** | **77**<br>**(61.68)** |
| | **P** | **T** | **P x T** | | | | | |
| **SEd** | **1.31** | **1.12** | **2.93** | | | | | |
| **CD (P=0.05)** | **2.61** | **2.21** | **5.85** | | | | | |

$T_0$ :Storage of seeds in cloth bag in ambient condition $T_1$ :Storage of seeds in aluminium foil pack in ambient condition $T_2$ : Storage of seeds in aluminium foil vacuum pack in ambient condition $T_3$ : Storage of seeds in aluminium foil pack at 5$^o$C $T_4$ : Storage of seeds in aluminium foil vacuum pack at 5$^o$C The storage durations were labelled as $P_0$ through $P_6$, corresponding to the initial month up to six months.

**Table 2.** Impact of treatments and storage duration on speed of germination in marigold

| Treatments/ Period of storage | $P_0$ | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | Mean |
|---|---|---|---|---|---|---|---|---|
| **$T_0$** | 7.41 | 7.25 | 6.41 | 6.30 | 6.07 | 5.36 | 5.05 | **6.26** |
| **$T_1$** | 7.41 | 7.28 | 7.11 | 6.75 | 6.72 | 6.19 | 5.83 | **6.75** |
| **$T_2$** | 7.41 | 7.33 | 7.16 | 7.30 | 6.83 | 6.25 | 6.11 | **6.91** |
| **$T_3$** | 7.41 | 7.35 | 7.27 | 7.23 | 7.19 | 6.82 | 6.57 | **7.12** |
| **$T_4$** | 7.41 | 7.41 | 7.39 | 7.36 | 7.22 | 7.11 | 7.09 | **7.28** |
| **Mean** | **7.41** | **7.32** | **7.07** | **6.98** | **6.81** | **6.34** | **6.13** | **6.87** |
| | **P** | **T** | **P x T** | | | | | |
| **SEd** | **0.12** | **0.11** | **0.27** | | | | | |
| **CD (P=0.05)** | **0.24** | **0.21** | **0.55** | | | | | |

$T_0$ : Storage of seeds in cloth bag in ambient condition $T_1$ :Storage of seeds in aluminium foil pack in ambient condition $T_2$ : Storage of seeds in aluminium foil vacuum pack in ambient condition $T_3$ : Storage of seeds in aluminium foil pack at 5ºC $T_4$ : Storage of seeds in aluminium foil vacuum pack at 5ºC The storage durations were labelled as $P_0$ through $P_6$, corresponding to the initial month up to six months.

**Table 3**. Impact of treatments and storage duration on root length (cm) in marigold

| Treatments/ Period of storage | $P_0$ | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | Mean |
|---|---|---|---|---|---|---|---|---|
| **$T_0$** | 5.00 | 4.60 | 4.06 | 3.93 | 3.66 | 3.50 | 3.20 | **3.99** |
| **$T_1$** | 5.00 | 4.70 | 4.30 | 4.13 | 3.76 | 3.60 | 3.50 | **4.14** |
| **$T_2$** | 5.00 | 4.73 | 4.36 | 4.23 | 3.96 | 3.76 | 3.60 | **4.24** |
| **$T_3$** | 5.00 | 4.83 | 4.50 | 4.40 | 4.20 | 3.86 | 3.76 | **4.36** |
| **$T_4$** | 5.00 | 4.90 | 4.73 | 4.46 | 4.33 | 4.03 | 3.96 | **4.49** |
| **Mean** | **5.00** | **4.75** | **4.39** | **4.23** | **3.99** | **3.75** | **3.61** | **4.25** |
| | **P** | **T** | **P x T** | | | | | |
| **SEd** | **0.099** | **0.084** | **0.223** | | | | | |
| **CD (P=0.05)** | **0.199** | **0.168** | **NS** | | | | | |

$T_0$ : Storage of seeds in cloth bag in ambient condition $T_1$ : Storage of seeds in aluminium foil pack in ambient condition $T_2$ : Storage of seeds in aluminium foil vacuum pack in ambient condition $T_3$ : Storage of seeds in aluminium foil pack at 5ºC $T_4$ : Storage of seeds in aluminium foil vacuum pack at 5ºC The storage durations were labelled as $P_0$ through $P_6$, corresponding to the initial month up to six months.

**Table 4.** Impact of treatments and storage duration on shoot length (cm) in marigold

| Treatments/ Period of storage | $P_0$ | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | Mean |
|---|---|---|---|---|---|---|---|---|
| **$T_0$** | 6.03 | 5.23 | 4.80 | 4.33 | 4.06 | 3.90 | 3.86 | **4.60** |
| **$T_1$** | 6.03 | 5.26 | 5.00 | 4.60 | 4.33 | 4.16 | 3.93 | **4.76** |
| **$T_2$** | 6.03 | 5.50 | 5.36 | 5.00 | 4.70 | 4.40 | 4.23 | **5.03** |
| **$T_3$** | 6.03 | 5.70 | 5.53 | 5.16 | 4.76 | 4.53 | 4.40 | **5.16** |
| **$T_4$** | 6.03 | 5.93 | 5.83 | 5.60 | 5.46 | 5.33 | 5.30 | **5.64** |
| **Mean** | **6.03** | **5.53** | **5.31** | **4.94** | **4.66** | **4.46** | **4.35** | **5.04** |
| | **P** | **T** | **P x T** | | | | | |
| **SEd** | **0.094** | **0.079** | **0.210** | | | | | |
| **CD (P=0.05)** | **0.187** | **0.158** | **NS** | | | | | |

$T_0$ : Storage of seeds in cloth bag in ambient condition $T_1$ : Storage of seeds in aluminium foil pack in ambient condition $T_2$ : Storage of seeds in aluminium foil vacuum pack in ambient condition $T_3$ : Storage of seeds in aluminium foil pack at 5ºC $T_4$ : Storage of seeds in aluminium foil vacuum pack at 5ºC The storage durations were labelled as $P_0$ through $P_6$, corresponding to the initial month up to six months.

in a comparatively lesser decrease in shoot and root length compared to cloth bags. At the end of the 6-month storage period, vacuum packed seeds stored at 5 °C ($T_4$) recorded significantly longer shoot and root length (5.30 cm and 3.96 cm) over all other treatments, followed by aluminium foil pack at 5 °C (3.76 cm and 4.40 cm). The shortest root length and shoot length (3.20 cm and 3.86 cm) were noted from cloth bags stored at ambient temperature ($T_0$) after 6 months. However, the interaction between treatment and storage duration was not significant for these parameters.

## Vigour index:

The seedling vigour index of marigold also declined as the storage period lengthened. The decline in vigour was markedly greater in seeds kept in cloth bags at ambient conditions, with a 59 % decrease, while the loss of vigour remained relatively stable for seeds stored in vacuum packs at 5 °C (Fig. 2). The highest mean vigour index was recorded in the seeds preserved in aluminium foil vacuum packs at 5 °C (830) followed by aluminium foil pack at 5 °C (759). Seeds stored in cloth bags recorded the lowest vigour index (396) after the storage period. The data revealed a significant correlation between storage conditions and period on vigour index and seed germinability, both individually and in combination.

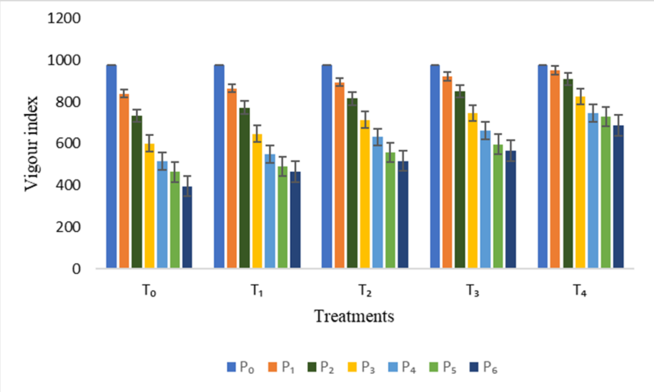


**Fig. 2.** Impact of treatments and storage duration on the vigour index in marigold seeds.

## Moisture content (%):

The seeds are hygroscopic and attain equilibrium moisture content by gaining or losing moisture content, depending on the nature of the containers in which they are stored. While seeds placed in aluminium foil packs and aluminium foil vacuum packs had stable moisture content for the whole 6 months storage period, seeds stored in cloth bags experienced a linear increase in moisture content (Table 5). The highest mean moisture content was observed in seeds kept in cloth bags (7.05 %), whereas all other storage treatments exhibited similar moisture levels.

## Electrical conductivity (dS/m):

The degree of seed deterioration and loss in seed vigour can be estimated from the electrical conductivity of the seed leachates. Less vigorous seeds release more solutes, which results in a higher E.C. value. In marigold, a high extent of seed deterioration was observed in cloth bags ($T_0$) (55 % increase in E.C). After six months of storage, the seeds stored within aluminium foil vacuum packs at 5 °C had the lowest electrical conductivity (0.1267), followed by aluminium foil pack at 5 °C (0.1290) (Table 6).

## Catalase activity (µg/g/min):

The activity of enzymes that scavenge free radicals, such as catalase and peroxidase, exhibited a direct proportion to reductions in germination % and an inverse relationship with the period of storage. The initial catalase activity was 5.67 µg/g/min. However, as the storage duration increased, a general decline in catalase activity was observed. Seeds stored in vacuum pack at 5 °C ($T_4$) consistently maintained the highest catalase activity throughout the storage period. The mean catalase activity (4.09 µg/g/min) of the seeds stored in vacuum pack at 5 °C was 1.9 times higher than that of seeds stored in the cloth bags (2.15 µg/g/min) (Fig. 3).

**Table 5.** Impact of treatments and storage duration on moisture content (%) in marigold

| Treatments/ Period of storage | $P_0$ | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | Mean |
|---|---|---|---|---|---|---|---|---|
| $T_0$ | 6.24 (14.46) | 6.31 (14.54) | 6.82 (15.14) | 7.34 (15.72) | 7.41 (15.80) | 7.48 (15.88) | 7.72 (16.13) | **7.05 (15.38)** |
| $T_1$ | 6.24 (14.46) | 6.25 (14.51) | 6.26 (14.54) | 6.28 (14.57) | 6.28 (14.61) | 6.29 (14.65) | 6.29 (14.67) | **6.27 (14.57)** |
| $T_2$ | 6.24 (14.46) | 6.24 (14.47) | 6.25 (14.49) | 6.26 (14.49) | 6.27 (14.50) | 6.27 (14.51) | 6.27 (14.53) | **6.26 (14.49)** |
| $T_3$ | 6.24 (14.46) | 6.24 (14.47) | 6.26 (14.49) | 6.26 (14.51) | 6.26 (14.56) | 6.27 (14.58) | 6.28 (14.62) | **6.26 (14.53)** |
| $T_4$ | 6.24 (14.46) | 6.24 (14.46) | 6.25 (14.48) | 6.25 (14.48) | 6.26 (14.49) | 6.27 (14.50) | 6.27 (14.50) | **6.26 (14.48)** |
| **Mean** | **6.24 (14.46)** | **6.26 (14.49)** | **6.37 (14.63)** | **6.48 (14.75)** | **6.49 (14.79)** | **6.52 (14.82)** | **6.57 (14.89)** | **6.42 (14.69)** |
| | **P** | **T** | **P x T** | | | | | |
| **SEd** | **0.022** | **0.018** | **0.048** | | | | | |
| **CD (P=0.05)** | **0.043** | **0.036** | **0.096** | | | | | |

$T_0$ : Storage of seeds in cloth bag in ambient condition $T_1$ : Storage of seeds in aluminium foil pack in ambient condition $T_2$ : Storage of seeds in aluminium foil vacuum pack in ambient condition $T_3$ : Storage of seeds in aluminium foil pack at 5°C $T_4$ : Storage of seeds in aluminium foil vacuum pack at 5°C The storage durations were labelled as $P_0$ through $P_6$, corresponding to the initial month up to six months.

**Table 6.** Impact of treatments and storage duration on electrical conductivity ($dSm^{-1}$) in marigold

| Treatments/ Period of storage | $P_0$ | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | Mean |
|---|---|---|---|---|---|---|---|---|
| $T_0$ | 0.0713 | 0.0960 | 0.1207 | 0.1373 | 0.1477 | 0.1537 | 0.1603 | **0.1267** |
| $T_1$ | 0.0713 | 0.0877 | 0.1047 | 0.1197 | 0.1300 | 0.1317 | 0.1347 | **0.1114** |
| $T_2$ | 0.0713 | 0.0863 | 0.0970 | 0.1183 | 0.1273 | 0.1290 | 0.1303 | **0.1085** |
| $T_3$ | 0.0713 | 0.0840 | 0.0927 | 0.1127 | 0.1243 | 0.1263 | 0.1290 | **0.1058** |
| $T_4$ | 0.0713 | 0.0820 | 0.0853 | 0.1100 | 0.1193 | 0.1237 | 0.1267 | **0.1026** |
| **Mean** | **0.0713** | **0.0872** | **0.1001** | **0.1196** | **0.1297** | **0.1329** | **0.1362** | **0.1110** |
| | **P** | **T** | **P x T** | | | | | |
| **SEd** | **0.0015** | **0.0013** | **0.0034** | | | | | |
| **CD (P=0.05)** | **0.0030** | **0.0025** | **0.0067** | | | | | |

$T_0$ : Storage of seeds in cloth bag in ambient condition $T_1$ : Storage of seeds in aluminium foil pack in ambient condition $T_2$ : Storage of seeds in aluminium foil vacuum pack in ambient condition $T_3$ : Storage of seeds in aluminium foil pack at 5°C $T_4$ : Storage of seeds in aluminium foil vacuum pack at 5°C The storage durations were labelled as $P_0$ through $P_6$, corresponding to the initial month up to six months.

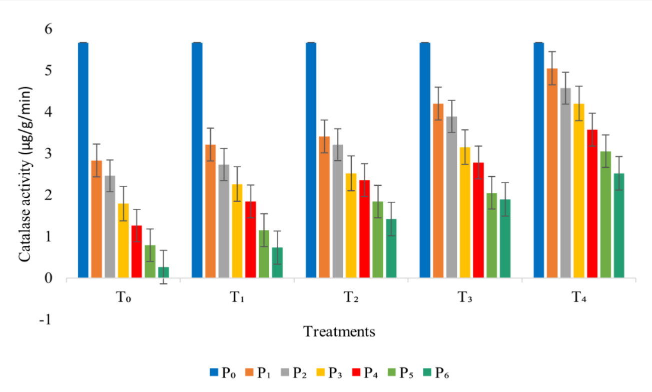


**Fig.3.** Impact of treatments and storage duration on catalase activity ( µg /g/min) in marigold seeds.

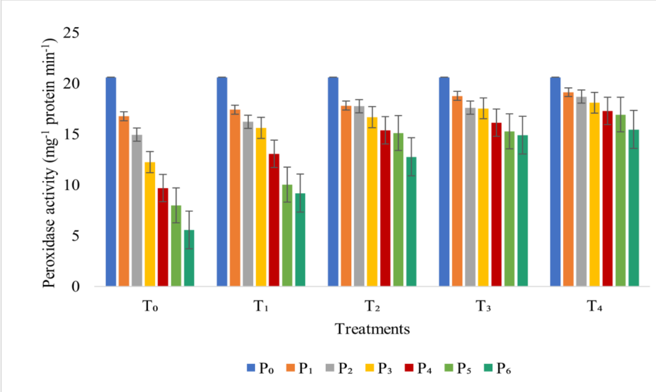


**Fig. 4.** Impact of treatments and storage duration on peroxidase activity ($mg^{-1}$ protein $min^{-1}$) in marigold seeds.

### Peroxidase activity ($mg^{-1}$ protein $min^{-1}$):

Peroxidase activity also demonstrated a trend similar to that of the catalase activity. Seeds stored in vacuum pack at 5 °C again showed the best retention of enzyme activity, with the highest peroxidase activity (15.47 $mg^{-1}$ protein $min^{-1}$) at the end of the 6$^{th}$ month. In contrast, seeds stored in cloth bags exhibited the most rapid decline, with peroxidase activity dropping to 5.57 $mg^{-1}$ protein $min^{-1}$by the 6$^{th}$ month (Fig. 4). The preservation of both catalase and peroxidase activities suggests that storage of seeds in vacuum pack at 5 °C provides the most favourable conditions for maintaining enzyme stability in marigold seeds during storage.

## Discussion

The primary objective of seed storage is to preserve planting materials for the upcoming season without compromising their viability and vigour (16). The decline in seed quality can be slowed down by maintaining optimal storage conditions, including temperature, moisture content and storage duration (17). This study was designed to assess the impact of storage environments and packaging materials on the physical, physiological and biochemical characteristics of marigold (*Tagetes erecta*) variety Pusa Narangi Gainda seeds over 6 months.

The findings of the study indicated that the seeds kept in the aluminium foil vacuum pack at 5 °C ($T_4$), outperformed all other storage treatments. The improved seed quality in this treatment can be ascribed to the combined benefits of low-temperature storage and the protective barrier provided by the aluminium foil vacuum pack. Storage at a low temperature (5 °C) has been demonstrated to effectively slow down the deterioration of seed quality and extend the longevity of the seeds by slowing down metabolic processes, reducing seed respiration and minimizing the activities of hydrolytic enzymes responsible for seed ageing, as previously noted (18-20). Additionally, the aluminium foil vacuum pack provided an airtight and moisture-proof environment, preventing the ingress of atmospheric oxygen and moisture, the known accelerants of seed deterioration (21).When the seeds are stored in permeable containers, the internal humidity fluctuates with the surrounding conditions, leading to variations in water content until the seeds reach a hygroscopic equilibrium, ultimately reducing seed vigour and viability (22). The moisture proof nature of aluminium foil pouches and vacuum packs was particularly beneficial in extending the seed germination and vigour, which is consistent with the findings of (23). The combination of low-temperature storage and vacuum packaging effectively preserves seed vigour, likely by reducing metabolic activity and oxidative stress (24).

The observed reductions in germination %, speed of germination and vigour index over time are due to natural ageing processes that cause the loss of organic solutes from ongoing respiratory activity, even under controlled temperature and humidity conditions (25). Moreover, the decline in germination as the storage period progresses can be attributed to the reduction in food reserves, including carbohydrates, starch, proteins and oil (26). These findings align with earlier studies on various crops, including tomato (27) groundnut (28) and soybean (29).

From our results, the highest EC levels were reported in the marigold seeds kept in the cloth bags. Lipid peroxidation of seeds during storage may result in ionic homeostasis loss and an impairment of cell membrane integrity which is the primary cause of the increase in EC of seeds (30). Vacuum packaging helps to reduce lipid peroxidation, thus extending the longevity of seeds (31). Comparable results were reported in Soyabean (32) and Onion (33).

The decrease in catalase and peroxidase activities during extended seed storage treatments can be attributed to elevated ROS production, which causes oxidative stress (34, 35). Prior research has demonstrated that mitochondria's susceptibility to oxidative stress might result in a drop in the antioxidant enzyme activity (36, 37). As ageing progresses, the inner membrane of mitochondria undergoes vesiculation, the disappearance of cristae occurs and ATP synthase dissociates, resulting in a diminished supply of ATP to the cells (38). Therefore, as seeds age, a physiological imbalance develops due to heightened reactive oxygen species (ROS) production and a weakened antioxidant system, which renders the seeds less viable (39).

The parallel decline in germination parameters, enzyme activities and seedling growth metrics across treatments further supports the strong interrelationship between these factors (40). The consistently superior performance of $T_4$ (aluminium foil vacuum pack at 5 °C) across all measured parameters suggests that this treatment provides optimal conditions for preserving marigold seed vigour and viability during storage, likely by reducing metabolic activity and oxidative stress (41, 42). In contrast, seeds stored in cloth bags ($T_0$) exhibited the poorest performance as the huge pore size of the cloth bags offered open access to the water vapours, which were rapidly absorbed by the seeds and eventually raised seed moisture content (43).

The results of this study reinforce the importance of appropriate packaging and storage conditions for maintaining seed vigour and viability during storage, consistent with earlier research (44, 45). These insights are particularly significant for the marigold seed industry, as they inform the development of optimized storage protocols that can enhance seed quality, reduce post-harvest losses and improve overall crop productivity.

## Conclusion

This study demonstrates that storage conditions significantly impact marigold seed viability, enzyme activities and subsequent seedling growth over time. The combination of low temperature (5 °C) and vacuum packaging ($T_4$) appears to be the most effective method for maintaining seed quality during storage. These findings have important implications for the commercial storage of marigold seeds and potentially other ornamental species. Future research could explore the molecular mechanisms underlying the observed effects, such as changes in gene expression patterns related to antioxidant systems and stress responses during seed storage.

## Acknowledgements

We are deeply grateful to Tamil Nadu Agricultural University (TNAU), Coimbatore, for providing financial support throughout the entire research process and during the preparation of this manuscript.

## Authors' contributions

MP conducted the experiments and prepared the original draft of the manuscript. RV contributed to the conceptualisation of the study. RU and VM supervised the work, drafted and reviewed the manuscript. MG participated in sequence alignment and manuscript editing. DT and SK were responsible for visualisation. All authors have read and approved the final version of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** The authors declare that they have no conflict of interest.

**Ethical issues:** "None".

**Did you use generative AI to write this manuscript?** No

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