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Seed germination and seedling vigor of Dalle Khursani (*Capsicum annum* L.) as influenced by organic seed treatments, maturity stages and storage duration

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Abstract

This study evaluated the effect of organic seed treatments, seed maturity stages, and storage duration on germination and seedling vigor of Dalle Khursani (*Capsicum annum* L.). Seeds were subjected to different organic treatments including cow urine, cow dung slurry, combined treatments, water soaking, and control. Germination parameters and seedling vigor indices were recorded. Maximum germination (84%) was observed in 24 hour water soaking, followed by 24 hour cow dung slurry (82%). Among different stages of maturity, seeds harvested at the turning stage exhibited the highest germination (70.4%) compared to red and mature green stages. Storage duration significantly influenced seed viability, with one-year-old seeds showing 63% germination, while seeds stored for more than two years showed drastic reduction or complete loss of viability. The results indicate that appropriate organic treatments and optimal maturity stage can significantly enhance germination and seed performance, while prolonged storage adversely affects viability.

Keywords: Dalle Khursani, maturity stages, organic, seed priming, seed vigor, storage

1. Introduction

Dalle Khursani (*Capsicum annum* L.) is one of the most important cash crops as well as GI crop of Sikkim, highly valued for its intense pungency, unique flavour, and significant contribution to farmers' income. The successful establishment of a healthy crop stand largely depends on rapid, uniform seed germination and vigorous seedling growth. However, germination performance in chilli is often influenced by several factors, including seed maturity, storage duration, and pre-sowing treatments (Bewley and Black, 1994) [2]. In addition, intrinsic seed characteristics such as seed coat thickness, capsaicin content, and the presence of germination-inhibiting compounds may restrict water uptake and gaseous exchange, thereby delaying germination and reducing seedling vigor. Environmental factors such as temperature, moisture availability, and relative humidity further affect the physiological and biochemical processes associated with seed germination and seedling establishment (Saini *et al.*, 2021; Tu *et al.*, 2022) [19, 25]. Consequently, delayed and non-uniform seedling emergence often results in poor field establishment, reduced seedling vigor, uneven crop growth, and ultimately lower fruit yield and quality (Demir and Okcu, 2004; E'rahim *et al.*, 2021) [4, 5].

Seed priming has emerged as an effective and economical technique for enhancing germination performance and seedling vigor. It involves controlled hydration of seeds using water or nutrient-rich solutions followed by re-drying to their original moisture content, allowing the initiation of pre-germinative metabolic activities without radicle protrusion (McDonald, 1999) [14]. Priming activates several physiological and biochemical processes, including enzyme synthesis, repair of cellular membranes, mobilization of stored food reserves, and enhancement of antioxidant defense systems, which collectively improve germination rate, germination uniformity, and stress tolerance during early seedling growth. Numerous studies in chilli and other vegetable crops have demonstrated the positive effects of seed priming on germination, seedling vigor, and subsequent crop performance. Farmers cultivating Dalle Khursani in Sikkim frequently encounter problems of delayed germination and poor seedling uniformity, which adversely affect nursery management and

crop productivity. Furthermore, as Sikkim is recognized as India's first fully organic state, the use of synthetic chemical seed treatments is limited, necessitating the development of environmentally friendly and sustainable alternatives. Despite the economic importance of Dalle Khursani, limited information is available regarding the influence of seed maturity stages and storage duration on its germination behavior and seed quality. Moreover, no systematic study has been conducted to evaluate the effectiveness of organic seed-priming treatments in improving germination and seedling establishment of Dalle Khursani. Therefore, the present investigation was undertaken to assess the effects of different seed maturity stages, storage periods, and organic priming treatments on seed germination, seedling vigor, and storability of Dalle Khursani, with the objective of developing low-cost, sustainable, and farmer-friendly technologies for enhancing crop establishment and productivity.

2. Materials and Methods

2.1 Experimental Site and Plant Material

The present investigation was carried out in the laboratory of ICAR Research Complex for NEH Region, Sikkim Centre, Tadong, Sikkim, India. Seeds of Dalle Khursani (*Capsicum annum* L.) were collected from healthy and disease-free plants grown under organic farming conditions which were subjected to different experiments.

2.2 Seed Priming Treatments

Untreated seeds of Dalle Khursani were cleaned, shade-dried, and subjected to different organic priming treatments. It consisted of eight treatments viz., T₁: Cow urine (20%) soaking for 12 h; T₂: Cow urine (20%) soaking for 24 h; T₃: Cow dung slurry (1:1) soaking for 12 hr; T₄: Cow dung slurry (1:1) soaking for 24 h; T₅: Cow urine (20%) + cow dung slurry soaking for 12 h; T₆: Cow urine (20%) + cow dung slurry soaking for 24 h; T₇: Water soaking for 12 h; T₈: Water soaking for 24 h; T₉: Control. Unprimed seeds were used as control. Each treatment consisted of three replications. After completion of the soaking period, seeds were removed from the solutions, washed with distilled water where necessary then surface dried, and shade dried back to their original moisture content before conducting germination tests. Fifty seeds per replication were used for the germination studies as per ISTA (ISTA, 2024) [10] procedure. Observations on germination percentage (%), time taken for germination, time taken for 50% germination, uniformity of germination, mean germination time, shoot length, root length, seedling dry weight, seed vigor index (SVI I) and (SVI II). Radical emergence was recorded daily for 14 and 21 consecutive days to calculate mean germination time.

2.3 Germination parameters

Germination percentage

Germination percentage (GP) was calculated by dividing the number of germinated seeds by the total number of seeds, multiplied by 100 (Tanveer *et al.*, 2010) [24]. It is expressed in%.

$GP = \frac{S}{T} \times 100$, wherein, S= no of germinated seeds and T= No of total seeds.

Time to Initiation of Germination (T_i)

Time required for the appearance of the first germinated

seed was recorded and expressed in hours.

Time to 50% Germination (T₅₀)

The time taken to achieve 50% of the final germination was calculated according to the method described by Coolbear *et al.* (1984) [3]:

Uniformity of Germination (T₉₀-T₁₀)

Uniformity of germination was calculated as the difference between the time required for 90% and 10% of total germination

Mean Germination Time (MGT)

Mean germination times (MGT) was calculated according to the following equation:

$$MGT = \frac{\sum(n \times d)}{N}$$

Where, n is the number of seeds germinated on each day, d is the number of days counted from the beginning of germination and N is the total number of seeds germinated at the termination of the experiment.

2.4 Seedling Growth Parameters

At the final count, ten normal seedlings were randomly selected from each replication for measurement of seedling growth attributes.

Shoot Length and Root Length

Shoot length was measured from the collar region to the tip of the shoot, while root length was measured from the collar region to the tip of the primary root using a ruler and expressed in centimeters (cm).

Seedling Dry Weight

Selected seedlings were dried in a hot-air oven at 60°C for 24 h until constant weight was attained. Dry weight was recorded using an analytical balance and expressed in grams per seedling.

Seedling Vigour Indices

Seedling vigour indices were calculated following Abdul-Baki and Anderson (1973) [1].

Seedling Vigour Index-I (SVI-I)

$SVI-I = \text{Germination percentage (\%)} \times \text{mean seedling length (cm)}$

Where, seedling length = shoot length + root length

Seedling Vigour Index-II (SVI-II)

$SVI-II = \text{Germination (\%)} \times \text{Seedling Dry Weight (g)}$

2.5 Evaluation of Seed Viability under Storage

To assess the effect of storage duration on seed viability, seeds collected during four different years (2021, 2022, 2023, and 2024) were subjected to standard germination testing. Germination percentage was recorded and compared among storage periods to determine the retention of seed viability during storage.

2.6 Effect of Fruit Maturity Stage on Seed Germination

Seeds extracted from fruits harvested at mature green, turning, and red-ripe stages were evaluated for germination percentage using the standard germination procedure. The

effect of physiological maturity on seed quality was assessed by comparing germination percentages among maturity stages.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using SPSS software. Treatment means were compared using Duncan's Multiple Range Test (DMRT) or Least Significant Difference (LSD) at 5% level of significance. Results are presented as mean $\pm$ Standard error (SE).

3. Results and Discussion

3.1 Effect of different pre-sowing treatments on germination parameters

From the Table 1, it is clear that there were significant variations in germination behavior among the different pre-sowing treatments. Germination percentage ranged from 52.0% to 84.0%. The highest germination was recorded in seeds soaked in water for 24 h (84.0%), which was statistically at par with 24 h cow dung slurry treatment (82.0%), 24 h cow urine treatment (76.0%), and 24 h cow urine + cow dung slurry treatment (76.0%). The lowest germination percentage was observed in seeds treated with 12 h cow urine soaking (52.0%), indicating that shorter-duration cow urine treatment adversely affected germination. The initiation of germination (T_1) was

significantly influenced by the treatments. Seeds soaked in cow dung slurry for 24 h initiated germination earliest (56.89 h), followed by the combination treatment of cow urine + cow dung slurry treatment for 24 h (66.79 h). In contrast, the longest time to germination initiation was recorded in 12 h cow urine-soaked seeds (205.26 h), demonstrating delayed germination under this treatment. Although differences in time to attain 50% germination (T_{50}) were not statistically significant, numerical variation was evident among treatments. The shortest T_{50} was recorded in T_6 (162.76 h), followed by T_8 (185.91 h) and T_4 (189.51 h), whereas the highest T_{50} was observed in T_1 (284.21 h). Similarly, uniformity of germination (T_{90-10}) showed no significant differences among most treatments; however, T_4 and the control exhibited comparatively higher values, indicating a wider spread of germination over time. Mean germination time (MGT) differed significantly among treatments. The lowest MGT, indicating faster overall germination, was observed in T_6 (133.02 h), followed by T_3 (142.86 h) and T_4 (154.92 h). Conversely, T_1 exhibited the highest MGT (249.46 h), reflecting slower germination. Prolonged soaking treatments, particularly 24 h water treatment, 24h cow dung slurry and 24 h cow urine + cow dung slurry enhanced germination performance by increasing germination percentage and reducing the time required for germination.

Table 1: Effect of different organic treatments on seed germination parameters of Dalle Khursani

Treatment	Germination (%)	Time to initiate germination, T_1 (h)	Time to achieve 50% germination, T_{50} (h)	Uniformity of germination (T_{90-10}) (h)	Mean Germination Time
T_1 : 12 hrs cow urine (20%) soaking	52.00 $\pm$ 3.0 ^d	205.26 $\pm$ 40.4 ^a	284.21 $\pm$ 4.7 ^a	46.44 $\pm$ 26.2 ^b	249.46 $\pm$ 21.7 ^a
T_2 : 24 hrs cow urine (20%) soaking	76.00 $\pm$ 2.0 ^{ab}	104.32 $\pm$ 3.1 ^{bc}	214.88 $\pm$ 9.0 ^a	120.40 $\pm$ 4.0 ^{ab}	190.68 $\pm$ 3.6 ^{bc}
T_3 : 12 hrs cowdung slurry (1:1)	60.00 $\pm$ 6.0 ^{cd}	78.60 $\pm$ 3.3 ^{bc}	244.22 $\pm$ 120.7 ^a	124.33 $\pm$ 86.9 ^{ab}	142.86 $\pm$ 26.7 ^{cd}
T_4 : 24 hrs cowdung slurry (1:1)	82.00 $\pm$ 2.0 ^a	56.89 $\pm$ 0.3 ^c	189.51 $\pm$ 21.5 ^a	186.61 $\pm$ 32.7 ^a	154.92 $\pm$ 2.8 ^{bcd}
T_5 : 12 hrs urine (20%) + cowdung slurry	72.00 $\pm$ 2.0 ^{abc}	104.36 $\pm$ 37.9 ^{bc}	211.16 $\pm$ 50.5 ^a	114.43 $\pm$ 1.6 ^{ab}	182.31 $\pm$ 33.0 ^{bcd}
T_6 : 24 hrs urine (20%) + cowdung slurry	76.00 $\pm$ 6.0 ^{ab}	66.79 $\pm$ 18.1 ^{bc}	162.76 $\pm$ 43.3 ^a	115.53 $\pm$ 81.6 ^{ab}	133.02 $\pm$ 16.6 ^d
T_7 : 12 hrs water soaking	73.00 $\pm$ 1.0 ^{abc}	76.94 $\pm$ 19.9 ^{bc}	197.89 $\pm$ 35.5 ^a	135.10 $\pm$ 13.7 ^{ab}	165.05 $\pm$ 23.0 ^{bcd}
T_8 : 24 hrs water soaking	84.00 $\pm$ 4.0 ^a	82.40 $\pm$ 0.4 ^{bc}	185.91 $\pm$ 8.0 ^a	126.87 $\pm$ 18.4 ^{ab}	170.60 $\pm$ 6.16 ^{bcd}
Control	66.00 $\pm$ 4.0 ^{bc}	75.92 $\pm$ 3.9 ^{bc}	250.76 $\pm$ 37.3 ^a	185.61 $\pm$ 20.7 ^a	170.89 $\pm$ 1.4 ^{bcd}

Table 1 contd.: Effect of different organic treatments on seed germination (seedling growth and vigor) of Dalle Khursani

Treatments	Shoot Length (cm)	Root length (cm)	Seedling dry weight	SVI I	SV II
T_1 : 12 hrs cow urine (20%) soaking	1.04 $\pm$ 0.5 ^b	1.35 $\pm$ 0.2 ^b	0.05 $\pm$ 0.013 ^{ab}	120.02 $\pm$ 48.9 ^c	2.26 $\pm$ 0.50 ^c
T_2 : 24 hrs cow urine (20%) soaking	2.22 $\pm$ 0.1 ^{ab}	2.97 $\pm$ 0.1 ^a	0.04 $\pm$ 0.002 ^{ab}	394.55 $\pm$ 28.9 ^{ab}	3.26 $\pm$ 0.07 ^{abc}
T_3 : 12 hrs cowdung slurry (1:1)	2.57 $\pm$ 0.5 ^a	2.92 $\pm$ 0.7 ^a	0.05 $\pm$ 0.002 ^{ab}	336.39 $\pm$ 105.8 ^{ab}	3.16 $\pm$ 0.41 ^{abc}
T_4 : 24 hrs cowdung slurry (1:1)	2.76 $\pm$ 0.6 ^a	2.58 $\pm$ 0.4 ^a	0.05 $\pm$ 0.005 ^{ab}	435.06 $\pm$ 71.3 ^{ab}	3.72 $\pm$ 0.36 ^{ab}
T_5 : 12 hrs urine (20%) + cowdung slurry	1.90 $\pm$ 0.2 ^{ab}	2.90 $\pm$ 0.1 ^a	0.04 $\pm$ 0.005 ^b	346.26 $\pm$ 33.3 ^{ab}	2.73 $\pm$ 0.28 ^{bc}
T_6 : 24 hrs urine (20%) + cowdung slurry	2.80 $\pm$ 0.4 ^a	2.91 $\pm$ 0.1 ^a	0.05 $\pm$ 0.002 ^{ab}	436.91 $\pm$ 76.4 ^{ab}	3.54 $\pm$ 0.39 ^{ab}
T_7 : 12 hrs water soaking	2.05 $\pm$ 0.4 ^{ab}	2.32 $\pm$ 0.4 ^{ab}	0.05 $\pm$ 0.004 ^{ab}	319.14 $\pm$ 67.1 ^{ab}	3.47 $\pm$ 0.30 ^{ab}
T_8 : 24 hrs water soaking	2.38 $\pm$ 0.2 ^a	3.23 $\pm$ 0.1 ^a	0.05 $\pm$ 0.002 ^{ab}	469.76 $\pm$ 5.6 ^a	3.79 $\pm$ 0.35 ^{ab}
Control	2.08 $\pm$ 0.2 ^{ab}	2.29 $\pm$ 0.1 ^{ab}	0.05 $\pm$ 0.006 ^{ab}	287.34 $\pm$ 4.7 ^{abc}	3.18 $\pm$ 0.24 ^{abc}

3.2 Effect of different pre-sowing treatments on seedling growth and vigor

Seedling growth parameters were markedly influenced by the pre-sowing treatments (Table 1 contd). Shoot length varied from 1.04 to 2.80 cm. The maximum shoot length was recorded in seeds treated with 24 h cow urine + cow dung slurry (2.80 cm), followed closely by 24 h cow dung slurry (2.76 cm) and 12 h cow dung slurry (2.57 cm). The shortest shoots were observed in T_1 (1.04 cm), which was significantly inferior to most other treatments. Root length also differed significantly among treatments. The longest roots were produced by seeds soaked in water for 24 h (3.23 cm), followed by 24 h cow urine treatment (2.97 cm), 12 h

cow dung slurry (2.92 cm), and 24 h cow urine + cow dung slurry (2.91 cm). The shortest roots were observed in T_1 (1.35 cm), indicating poor seedling development under this treatment. Seedling dry weight showed limited variation among treatments and remained statistically similar. Values ranged between 0.04 and 0.05 g, suggesting that the treatments exerted minimal influence on dry matter accumulation during the early seedling stage. Significant differences were observed in Seedling Vigor Index-I (SVI-I). The highest SVI-I was recorded in T_8 (469.76), followed by T_6 (436.91) and T_4 (435.06), indicating superior seedling establishment and growth under these treatments. The lowest vigor index was observed in T_1 (120.02),

corresponding to its poor germination and seedling growth performance. Seedling Vigor Index-II (SVI-II) varied from 2.26 to 3.79, with the highest value recorded in T₈ (3.79), followed by T₄ (3.72) and T₆ (3.54). The lowest value was observed in T₁ (2.26).

Hydro priming or water soaked seeds recorded highest germination percentage which was statistically at par with 24 h cow dung slurry treatment, 24 h cow urine treatment, and 24 h cow urine + cow dung slurry treatment. Similar result has been reported by Kumar *et al.*, 2024 in ajwain. The enhanced germination can be attributed to optimal water uptake, which activates enzymatic pathways and metabolic processes critical for seed vigor (Farooq *et al.*, 2006; Patra *et al.*, 2016) [7, 17]. Combination of cowdung slurry and cow urine was the best treatment for other germination parameters. The higher germination in cowdung slurry and cattle urine treatment may be attributed due to the presence of growth promoting substances (auxin) in the cattle cowdung. Similar report were observed by Shirol *et al.*, (2005) [21] and Shinde and Malshe (2015) [20]. The use of bio-regulators in enhancing seed germination and seedling vigour is well known (Pampanna Y and Sulkeri, 2001) [16]. Presence of biologically active substances, microbes, weak acids of some bioregulators like cow dung resulted in enhanced germination in *Calophyllum inophyllum* and *Melia azadirachta* (Sujatha and Manjappa, 2015) [22].

3.3 Effect of seed storage duration on germination percentage

From the Figure 1, it clearly indicates that the germination of seed was markedly influenced by the duration of seed storage. Germination percentage declined progressively with increasing storage period, indicating a substantial loss of seed viability over time. Seeds stored for one year exhibited the highest germination percentage (63%), followed by seeds stored for two years (42%). In contrast, seeds stored for three years as well as seeds stored for four

years failed to germinate recording 0% germination. These results demonstrate a strong negative relationship between storage duration and seed viability. A reduction of approximately 33.3% in germination was observed when storage duration increased from one year to two years. Furthermore, complete loss of germination in seeds stored for three and four years suggests severe deterioration of seed quality during prolonged storage. The decline in germination may be attributed to ageing-related physiological and biochemical changes, including membrane degradation, reduced enzyme activity, depletion of food reserves, and accumulation of oxidative damage.

Seed quality and storage environment are among the most important determinants of seed longevity, viability, and vigor during storage. Seeds possessing high physiological quality generally exhibit faster and more uniform germination, resulting in superior seedling establishment, enhanced growth performance, and improved crop productivity (Kibar and Yücesan, 2021) [9]. However, prolonged storage often leads to progressive deterioration of seed quality, manifested by reductions in germination percentage and germination rate, particularly when seeds are subsequently exposed to abiotic stresses (Jiang *et al.*, 2021) [11]. This decline is largely attributed to the excessive accumulation of reactive oxygen species (ROS) during storage (Moncaleano-Escandon *et al.*, 2013; Lozano-Isla *et al.*, 2018) [15, 13]. The overproduction of ROS induces oxidative stress, a primary factor responsible for seed ageing and reduced longevity. Oxidative damage affects essential cellular components, including lipids, proteins, and nucleic acids, leading to membrane disruption, impaired metabolic activity, and loss of cellular integrity (Groot *et al.*, 2012; Zhou *et al.*, 2019) [8, 26]. Lipid per oxidation, often indicated by increased malondialdehyde (MDA) accumulation, serves as a key marker of membrane deterioration during seed ageing. Reduction in germination capacity might also be associated with decreased antioxidant enzyme activity. Similar has been reported by Liu *et al.*, 2015.

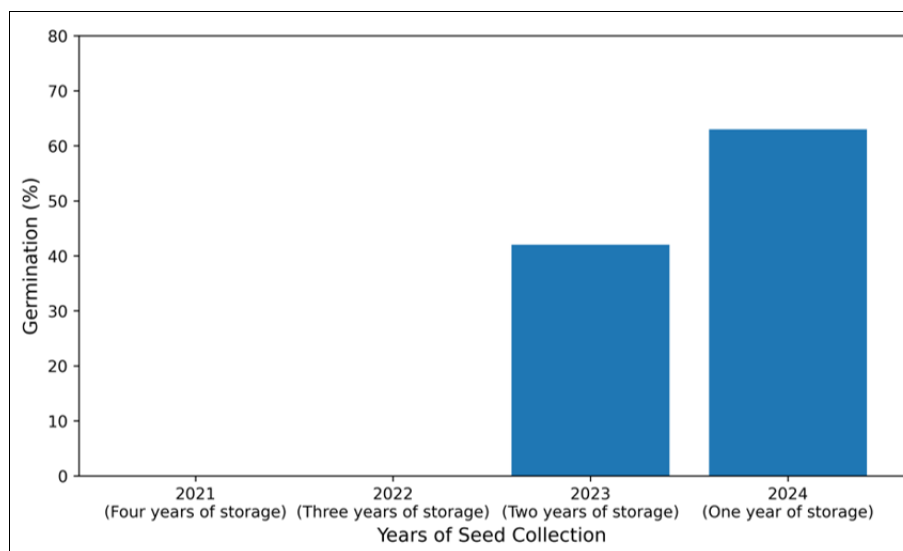


Fig 1: Seed germination of Dalle Khursani harvested during different years for viability test

3.4 Effect of fruit maturity stage on seed germination

Seed germination was significantly influenced by the stage of fruit maturity at the time of seed extraction (Figure 2). Among the different maturity stages evaluated, seeds obtained from fruits at the turning stage exhibited the

highest germination percentage (70.4%), indicating superior physiological maturity and viability. Seeds extracted from mature green fruits recorded a moderate germination percentage of 51.6%, while those obtained from fully red fruits showed the lowest germination (46.4%). The

comparatively lower germination observed in mature green fruits may be attributed to incomplete seed development. Seeds harvested from fruits at the turning stage possess optimal physiological development, resulting in enhanced germination performance. Compared with seeds from red

fruits, turning-stage seeds showed an increase of approximately 24% in germination, highlighting the importance of selecting the appropriate fruit maturity stage for seed collection. Similar results have been reported by Takac *et al.*, 2014.

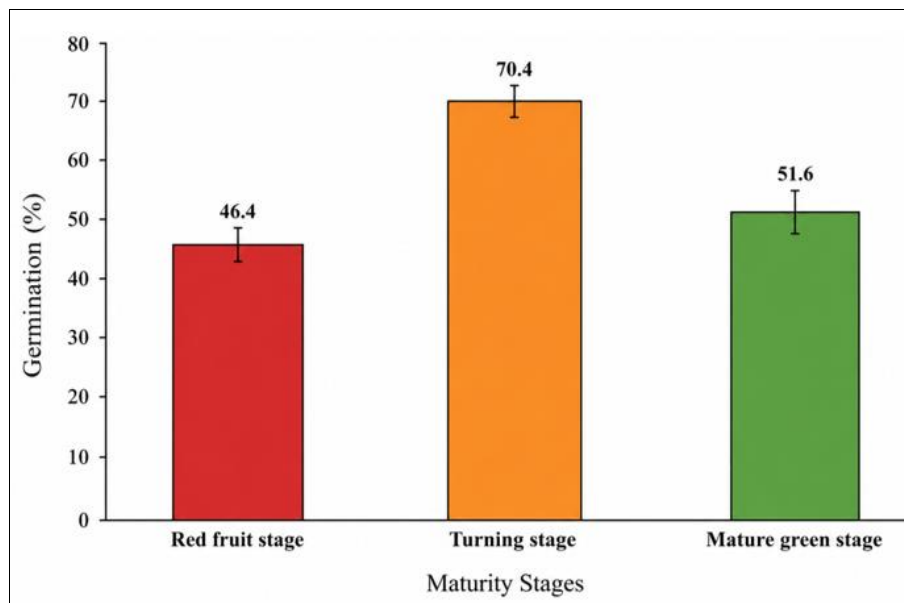


Fig 2: Germination percentage of Dalle Khursani at different stages of maturity

4. Conclusion

It can be concluded that pre-sowing seed treatments significantly influenced germination behavior, seedling growth, and seed vigor of *Dalle Khursani*. Among the treatments evaluated, soaking seeds in water for 24 hr, cow dung slurry for 24 hr, and cow urine (20%) combined with cow dung slurry for 24 h markedly enhanced germination percentage, seedling development, and vigor compared with the untreated control. Treatment of seeds in 24hrs water (hydropriming) was particularly effective in improving germination percentage, root length, and seedling vigour indices, whereas combination of 24hr cow urine (20%) and cowdung slurry significantly reduced mean germination time and promoted shoot growth. In contrast, soaking seeds in cow urine for 12 hr adversely affected germination and seedling performance, indicating possible inhibitory effects at shorter exposure conditions.

The study further revealed that the turning stage is the most appropriate maturity stage for seed collection, as seeds harvested at this stage exhibited superior germination performance and overall seed quality. Therefore, harvesting fruits at the turning stage is recommended for efficient seed propagation and nursery production. In addition, seed storage duration had a pronounced effect on seed viability, with germination remaining relatively stable up to one year of storage, followed by a substantial decline thereafter. Consequently, the use of freshly harvested or one-year-old seeds is recommended to ensure better germination and establishment, whereas seeds stored for more than two years may not be suitable for propagation due to significant loss of viability and vigor.

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