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EFFECT OF SEED MOISTURE CONTENT ON GERMINATION AND SEEDLING PERFORMANCE OF *Hippophae rhamnoides* L.

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Abstract: Wild plant resources play an important role in the livelihoods of marginal communities who lives in the forest fringes. One such species which highly contributes to the livelihood of Himalayan regions is *Hippophae rhamnoides* L. The present study helps to determine the safe moisture content of seeds for storage without deteriorating its germination capacity. In this study silica gel was used forseed desiccation. Theseed germination and vigour was studied at 4 different seed moisture level i.e., 10%, 8%, 6% and 4%. The study revealed that Germination percentage and seed vigour was highest for SMC 10% i.e. 87% ± 15.90 and584.20 respectivelyand decreased with reduction in seed moisture content. Lowest germination percentage and seed vigour was recorded at SMC 4% i.e. 54 ± 6.92 and 345.6 respectively. The morphometric study revealed that length, width and thickness of *Hippophae rhamnoides* L. seeds ranged from3.76 – 4.10 mm, 2.02 – 2.14 mm and 1.46 – 1.63 mm respectively. The insights derived from the study can aid in designing appropriate seed storage protocols for the conservation of this lesser-known multipurpose plant species of Indian Himalayan Region.

Keywords: *Hippophae rhamnoides,* Sea buckthorn, Germination, Seed moisture content, Vigour.

1. INTRODUCTION

Hippophae rhamnoides L., commonly known as sea buckthorn, is a versatile yet underutilized plant species belonging to the family Elaeagnaceae. Native to the higher Himalayan regions, this species holds significant ecological and economic potential, particularly for hill communities (Dhyani et al., 2007; Rongsen, 1992). Wild plant resources

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play a crucial role in the livelihoods of rural populations worldwide, especially among marginalized socioeconomic groups, including indigenous, tribal, and forest-fringe communities. For centuries, plants have served as sources of food, medicine, fiber, fuel, and other essential commodities (Maikhuri et al., 1994).

The Indian Himalayan Region (IHR) is globally recognized for its unique and socio-economically valuable floristic diversity (Samant et al., 1998; Pant &Samant, 2007). Among these plant resources, H. rhamnoides has garnered considerable attention due to its exceptional medicinal and ecological significance. Every part of this plant possesses bioactive properties, making it highly valuable for pharmaceutical and nutraceutical applications. Adapted to extreme climatic conditions, *H. rhamnoides* thrives in temperatures ranging from -45°C to +43°C and is predominantly found along riverbanks and sun-exposed slopes (Dhyani et al., 2007). It is primarily wind-pollinated, as its flowers lack nectar and do not attract insect pollinators (Li & Beveridge, 2003) and exhibit epigeal germination (Bhowmik et al., 2013).

After harvest, seeds undergo numerous metabolic changes that result in altered viability and germination status. Storage-dependent seed physiological changes are strictly species-specific, and knowing them has clear implications for species propagation and multiplication (Sharma *et al.,* 2006). Preserving seeds from a good seed year may benefits farmers and cultivators of this particular species. Given the rarity of *H. rhamnoides* and its high seed cost, proper seed storage is essential for meeting increasing cultivation demands. Seed moisture content and storage conditions play a crucial role in maintaining seed viability. However, limited information is available on desiccation protocols for medium- to long-term storage of *H. Rhamnoides* seeds. Considering the necessity of exploring desiccation strategies, this study is based on the hypothesis that *H. Rhamnoides* seeds will exhibit differential responses depending on seed moisture content. Based on this premise, the study aims to:

- Examine the morphological characteristics of *H. rhamnoides* seeds.
- Assess seed germination and seedling performance under varying seed moisture content levels.

2. MATERIALS AND METHODS

2.1 Study area

The source of seed for our research is Udaipur range of Lahaul and Spiti district of Himachal Pradesh. Udaipur is located in the western most portion of the Lahaul Valley. Rainfall occurs primarily from May to mid-September and ranges from 10 mm to 300 mm. From November to April, late winter colds bring substantial snowfall (120-400 cm) and temperatures as low as -19°C (Kumar *et al*., 2014). The landscape's harsh environmental circumstances, such as low temperature and rainfall, high wind speed, and uneven ground topography, leads to adaptation of very less species in this area among which *Hippophae rhamnoides* L. is well known. (Wagnon *et al.*, 2007).

2.2 Morphological study of seed

Various dimensions of seeds were measured in this study, and the mean value of each dimension was calculated. In this experiment, the seed length, seed thickness, and seed width are measured by Vernier Calliper from an ungraded seed batch, and their mean value was determined individually. Seed index (100 seed weight) was also measured.

2.3 Seed germination at different moisture content

Seed moisture content was checked by hot air oven method by partially crushing the seeds (1g) in four replications, taking the fresh weight of these seeds and then putting them in oven at temperature 103 ± 2℃ for 17 hours. After 17 hours dry weight of all the four replications was taken and Moisture percentage (according to ISTA, 2010) was calculated using the formula

$$\text{MC \%} = \frac{Fresh\ weight\ of\ seeds - dry\ weigth\ of\ seeds}{Fresh\ weigth\ of\ seeds} \times 100$$

Moisture content Desiccation formula (ISTA, 2010)

$$Target\ weight = \frac{100 - initial\ moisture\ content}{100 - desired\ moisture\ content} \times Total\ seed\ weight$$

Desiccation was done by putting seeds in a muslin cloth bag which was further put in silica gel and put in an air tight container. Reduction of weight from Initial total seed weight to the target weight was recorded every day.

Seed moisture content was desiccated from 10% to 8%, 8% to 6% and 6% to 4%. Target seed weight for MC 10%, 8%, 6% and 4% was 14.29g, 13.90g, 13.41g and 12.40g respectively.

Once the seed weight reached the target weight, seeds were sown in substrate Quartzite sand in 4 replications having 25seeds in each replication and kept in seed germinator with temperature 25±1°C. Germination status was recorded every day.

Treatments

Treatments	**Name of treatments**
T1	MC 10 %
T2	MC 8%
T3	MC 6%
T4	MC 4%

2.4 Seed germination test

Germination tests were performed in a complete randomized design with four replications of 25 seeds. Seeds of moisture content 10%, 8%, 6% and 4 % were sown in Petri dish containing quartzite sand as the substrate. Germination was recorded for a limited period of 21 days. Seedling length (shoot length + root length) of the seedlings was recorded after 21 days.

2.5 Germination percentage (G%)

The goal of assessing seed germinative quality is to determine the percentage of seeds in a given lot that are likely to produce seedlings. The percentage of germination was obtained by counting the number of seeds that germinated in a particular sample.

$$\textbf{Germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

2.6 Germination energy (%)

Germination energy is a measure of germination speed and thus an indicator of seed and seedling vigour. When germination reached its apex, germination energy was estimated as a percentage of the total number of seeds that had germinated.

$$\textbf{Germination energy (\%)} = \frac{\text{Number of seeds germinated upto time of peak germinaton}}{\text{Total number of seeds sown}} \times 100$$

2.7 Mean daily germination (MDG)

The cumulative germination percentage of seeds at the conclusion of the test period was divided by the number of days from sowing to the end of the test, or total days to get the mean daily germination.

2.8 Peak value (PV)

The maximum mean daily germination achieved at any point during the test period was used to compute the peak value (Czabator, 1962).

2.9 Germination value (GV)

Germination value is a measure that combines seed germination speed and completeness. Germination counts were recorded and calculated on a daily basis.

$GV = PV \times MDG$

2.10 Germination Index (GI)

The germination index was derived by dividing the total number of seeds germinated at the end of the experiment by the time required for 50% germination.

2.11 Seed vigour index (S.V.I.)

The germination percentage and seedling length of the same seed lot are used to compute this. The seed vigour index is determined by multiplying the percentage of germination by the length of the seedling. The seed lot with the highest seed vigour index is considered to be more vigorous (Abdul-baki and Anderson, 1973).

S.V. I. = Germination percentage × seedling length (i.e., root + shoot length)

2.12 Assessment of seedlings

The effect of treatment was assessed periodically through germination and initial growth performance of the seedlings in petri dish. Five seedlings from each treatment were randomly selected to estimate the seedling length (i.e., root and shoot length). Data were statistically analysed for studying the morphological growth variation for each treatment.

2.13 Statistical analysis

Since all these experiments were conducted in a homogenous laboratory condition, hence Completely Randomized Design (CRD) was used, and data was statistically analyzed using WASP 2.0software of ICAR (Indian Council of Agriculture Research).

3. RESULTS AND DISCUSSION

3.1 Morphological study of seed

Table 1: Seed parameters of *Hippophaerhamnoides* L.

Replications	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Seed Index/100 Seed weight (g)
R1 (mean ± S.D)	3.76 ± 0.37	2.02 ± 0.28	1.63 ± 0.26	0.93 ± 0.004
R2 (mean ± S.D)	3.79 ± 0.41	2.06 ± 0.18	1.46 ± 0.23	0.93 ± 0.005
R3 (mean ± S.D)	3.94 ± 0.42	2.09 ± 0.23	1.56 ± 0.25	0.93 ± 0.006
R4 (mean ±S. D)	4.10 ± 0.43	2.14 ± 0.16	1.62 ± 0.19	0.94 ± 0.008
Mean ± SEm	3.89 ± 0.40	2.07 ± 0.21	1.56 ± 0.23	0.93 ± 0.005
Range	3.76 – 4.10	2.02 – 2.14	1.46 – 1.63	0.93 – 0.94

Seed parameters can be considered essential traits for the early selection of seed sources (Bharti *et al.,* 2024). The morphological parameters of seeds analyzed in this study included seed length, seed width, and seed thickness (mm). As presented in Table 1, the average seed length, width, and thickness were observed within the range of 3.76–4.10 mm, 2.02–2.14 mm, and 1.46–1.63 mm, respectively. The Seed Index (g) was recorded as 0.93 ± 0.005, with a range of 0.93–0.94 g. The results were consistent to the study conducted by (Aras *et al.,* 2007), which concluded length and width of *Hippophae rhamnoides* L. ranged from 2.86 to 6.77 mm and 1.13 to 3.54mm.

Figure 1. Seeds of *Hippophae rhamnoides* L.

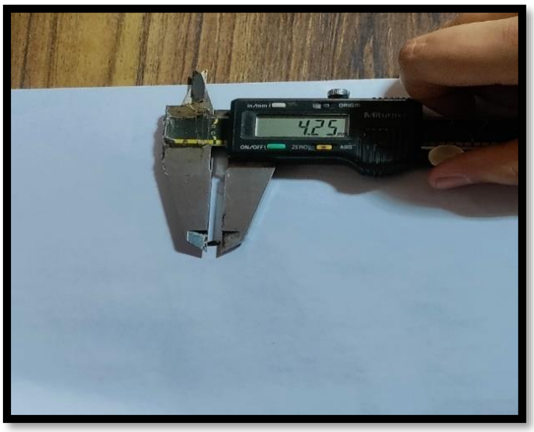

Figure 2. Measurement of seed morphological parameters of *Hippophae rhamnoides* L.

3.2 Germination Percentage (G%) and Germination Energy (GE%)

The desiccation of seed moisture content significantly influenced germination percentage and germination energy. The highest germination percentage (87% ± 15.09) was observed in T1 (MC 10%), followed by T2 (MC 8%) and T3 (MC 6%) with germination percentages of 84% ± 3.26 and 82% ± 2.30, respectively. The lowest germination percentage (54% ± 6.92) was recorded in T4 (MC 4%), indicating a sharp decline at lower moisture content levels.

Germination energy followed a similar trend, with T1 (MC 10%) exhibiting the highest GE% (67%), followed by T3 (MC 6%) and T2 (MC 8%) with GE% values of 54% and 49%, respectively. The lowest germination energy (38%) was recorded for T4 (MC 4%).

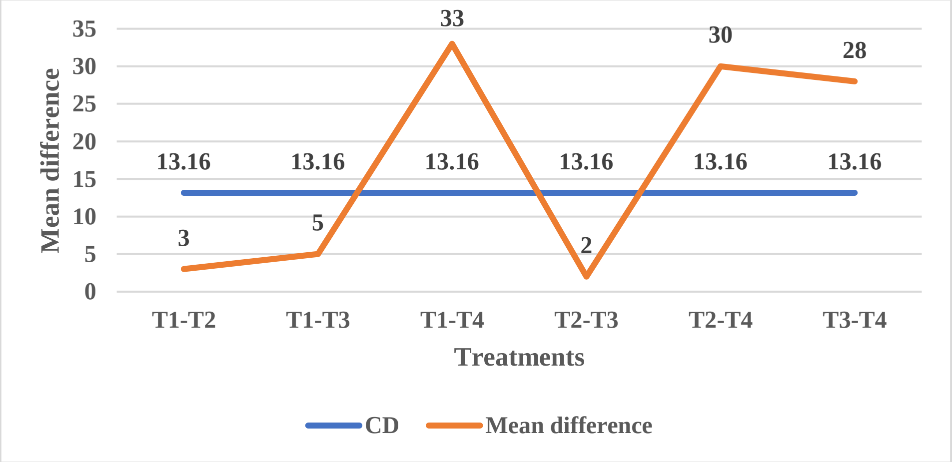

Figure 3. Graph showing Critical difference and mean difference between treatments

Statistical analysis indicated that T1, T2, and T3 were not significantly different, while T4 was significantly different from all three (T1, T2, and T3), as the mean difference values (33%, 30%, and 28%) exceeded the critical difference (CD) value. This suggests that extreme desiccation below MC 6% significantly reduces germination capacity, likely due to increased seed dormancy or damage to metabolic processes essential for germination (Bewley & Black, 1994; Walters et al., 2005).

3.3 Mean Germination Time (MGT)

Mean germination time (MGT) was found to be highest in T4 (MC 4%) at 13.94 days, indicating delayed germination. T1 (MC 10%) followed with 11.96 days, while T2 (MC 8%) and T3 (MC 6%) recorded significantly lower MGT values of 6.96 and 4.97 days, respectively. Lower MGT is often associated with increased metabolic efficiency and better seed viability, which corresponds with earlier studies suggesting that moderate moisture retention enhances seed germination kinetics (Bradford, 1990).

3.4 Mean Daily Germination (MDG)

The highest mean daily germination (MDG) was observed in T3 (MC 6%) at 7.16, followed by T2 (MC 8%) and T1 (MC 10%) with values of 5.14 and 3.80, respectively. The lowest MDG (1.85) was recorded for T4 (MC 4%). The higher MDG in seeds with MC 6% suggests an optimal balance of moisture retention, which facilitates uniform and rapid germination (Ellis & Roberts, 1980).

3.5 Peak Value (PV)

T3 (MC 6%) exhibited the highest peak value (9), followed by T2 (MC 8%) at 7 and T1 (MC 10%) at 4.46. The lowest peak value (2.11) was recorded for T4 (MC 4%). Peak value is a key indicator of germination uniformity, with higher values signifying a concentrated period of germination activity, which has been associated with improved seedling establishment (Ranal & Santana, 2006).

3.6 Germination Value (GV)

Germination value (GV) was highest in T3 (MC 6%) at 64.47, followed by T2 (MC 8%) at 38.03 and T1 (MC 10%) at 16.97. The lowest GV (3.90) was recorded for T4 (MC 4%). The highest GV in T3 indicates superior seed performance and suggests that moderate desiccation (MC 6%) optimizes germination efficiency (Copeland & McDonald, 2001).

3.7 Germination Index (GI)

The germination index (GI) followed a similar trend, with T3 (MC 6%) showing the highest value (22.57), followed by T2 (MC 8%) at 15.17 and T1 (MC 10%) at 8.94. The lowest GI (3.64) was recorded for T4 (MC 4%). This pattern further supports the observation that moderate desiccation enhances seed vigor, likely by reducing excess moisture that could lead to microbial contamination or hypoxic conditions (Almansouri et al., 2001).

3.8 Seed Vigour Index (SVI)

Seed vigour index (SVI) was highest in T1 (MC 10%) at 584.20, followed by T2 (MC 8%) at 516.6 and T3 (MC 6%) at 475.6. The lowest SVI (345.6) was observed in T4 (MC 4%). Higher SVI values indicate better seedling performance and establishment potential, highlighting the importance of maintaining adequate moisture content for seed viability (Finch-Savage & Bassel, 2016).

3.9 Seedling Length

Seedling length was highest in T1 (MC 10%) at 6.71 ± 1.26 cm, followed by T2 (MC 8%) at 6.15 ± 0.79 cm and T3 (MC 6%) at 5.83 ± 0.92 cm. The lowest seedling length (5.64 ± 0.88 cm) was observed in T4 (MC 4%). Seedling length is a crucial parameter indicating post-germination vigor and establishment, with reductions at lower moisture levels likely due to metabolic constraints imposed by desiccation stress (Bewley, 1997).

Figure 4. Measuring length of T1 seedlings.

Figure 5. Measuring length of T2 seedlings.

Figure 6. Measuring length of T3 seedlings.

Figure 7. Measuring length of T4 seedlings.

4. CONCLUSION

The results indicate that moderate desiccation (MC 6%) maximizes several key germination parameters, including MDG, PV, GV, and GI. However, the highest seed vigor and seedling length were observed in seeds with MC 10%, suggesting that maintaining slightly higher moisture content enhances overall seedling performance. Conversely, extreme desiccation (MC 4%) significantly reduces germination percentage, energy, and vigor, likely due to increased metabolic and physiological stress. Furthermore, the findings confirm that *Hippophae rhamnoides* L. seeds exhibit orthodox storage behaviour. These results highlight the critical role of seed moisture content in optimizing germination and seedling establishment, providing valuable insights for seed conservation and propagation strategies.

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