



Assessment of Seed Physiological Parameters in *Gymnema sylvestre* R.Br. for Optimizing Propagation and Storage Strategies

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Authors' contributions

This work was carried out in collaboration among all authors. Author MKV conceptualized the study, designed the experiments, supervised the research activities, and finalized the manuscript. Author DM conducted the germination trials, viability testing, and data collection. Author NS contributed to the methodological refinement, data interpretation, and critical manuscript revision. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated seed viability, water imbibition behavior, and desiccation tolerance of *Gymnema sylvestre* R.Br. to evaluate its potential for propagation and long-term storage. Tetrazolium (TZ) staining tests at varying concentrations (0.25%, 0.5%, and 1%) and durations

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(1 to 24 hours) revealed that 0.5% TZ consistently yielded 100% viability across all durations, making it the most effective treatment. Statistical analysis confirmed that TZ concentration, staining duration, and their interaction significantly influenced viability outcomes ($p < 0.001$). The seeds exhibited a clear triphasic water imbibition pattern, with fresh weight increasing by 158.79% at 30 hours before plateauing, indicative of typical orthodox seed hydration. Germination trials demonstrated that no pre-treatment was necessary, as seeds showed high viability without signs of physical or physiological dormancy. Desiccation trials, followed by storage at -20°C , further confirmed the orthodox nature of the seeds. Germination improved from 89.26% in fresh seeds to 90.83% after desiccation and 95.38% after three months of cold storage. These results establish *G. sylvestre* seeds as viable, desiccation-tolerant, and suitable for direct sowing and long-term conservation under low-temperature storage conditions.

Keywords: Seed germination; seed viability; tetrazolium test; seed storage; water imbibition; orthodox seeds; medicinal plants; seed physiology; propagation techniques.

1. INTRODUCTION

More than 80% of the global population relies on medicinal plants for primary healthcare needs. With the increasing demand for herbal products, the trade volume of plant-based raw materials has grown significantly. Currently, around 90% of raw drugs used in traditional medicine systems such as Ayurveda, Siddha, Unani, and Homeopathy are collected from the wild, of which nearly 70% involves destructive harvesting (Gowthami et al., 2021). Among the vulnerable medicinal plants impacted by unsustainable harvesting is *Gymnema sylvestre* R. Br., a perennial woody climber from the family Apocynaceae (formerly Asclepiadaceae), widely known as “Gudmar” or “Madhunashini”—literally, “sugar destroyer”—for its well-documented antidiabetic properties.

Native to tropical and subtropical forests of India, Sri Lanka, Southeast Asia, and parts of Africa and Australia, *G. sylvestre* inhabits dry deciduous forests up to 600 m elevation (Pandey, 2012). The leaves, when chewed, temporarily suppress the sensation of sweetness and have long been used in traditional formulations such as *Mahavisagarbha Taila*, *Ayaskrti*, and *Nyagrodhadi Churna* to manage diabetes, asthma, hepatosplenomegaly, and other ailments (Anonymous, 2003; Nadkarni, 1993). Ethnobotanical accounts and modern studies confirm its diverse therapeutic applications, including digestive, anti-inflammatory, antihelminthic, cardioprotective, and hepatoprotective effects (Saneja et al., 2010).

Pharmacologically, the activity of *G. sylvestre* is largely attributed to gymnemic acids—oleanane-type triterpenoid saponins with hypoglycemic,

insulinotropic, and antihyperlipidemic properties (Tiwari et al., 2014). Other active constituents, such as gymnemagenin, flavonoids, polyphenols, and anthraquinones, contribute to its antioxidant, antimicrobial, and anti-inflammatory actions (Zhao et al., 2017). It is a key ingredient in several polyherbal antidiabetic formulations, including IME-9 and BGR-34, which have been clinically validated (Paliwal, Kathori, & Upadhyay, 2009; Kanetkar, Singhal, & Kamat, 2007). Recent reviews further document its role in managing Type 2 diabetes via β -cell stimulation and insulin regulation, although potential hepatotoxicity has also been reported (Jamadagni et al 2021; Karwa et al. 2022).

Recent studies have reinforced the therapeutic potential of *Gymnema sylvestre*, a traditional medicinal plant renowned for its antidiabetic, antihyperlipidemic, and anti-craving properties. A clinical trial by Martinez et al. (2020) demonstrated that supplementation with *G. sylvestre* in combination with zinc and chromium significantly reduced fasting blood glucose and HbA1c levels in prediabetic individuals, with no reported hepatic or renal toxicity. Preclinical investigations have further confirmed its efficacy in restoring cerebral microvascular architecture in diabetic rat models (Sandeck et al., 2021) and improving lipid profiles in animals fed a high-fat diet (Singh et al., 2017). Moreover, advanced metabolomic profiling and molecular docking studies have identified gymnemic acid IV and gymnestrogenin as key bioactive triterpenoid saponins with notable antifungal activity, particularly through the inhibition of ergosterol biosynthesis and fungal growth (Neel et al., 2025). In addition, *G. sylvestre* has shown promising effects in managing polycystic ovarian syndrome (PCOS), where it improved endocrine parameters and ovarian morphology via

modulation of gene expression (Vora et al., 2023). Collectively, these findings underscore the broad-spectrum pharmacological profile of *Gymnema sylvestre*, encouraging further clinical validation and integration into therapeutic strategies.

Despite its growing commercial demand, large-scale cultivation of *G. sylvestre* remains limited, primarily due to inadequate knowledge of its reproductive biology. While vegetative propagation is commonly practiced (Pandey, 2012), seed-based propagation remains underutilized due to uncertain germination behavior and lack of standardized storage protocols. Understanding the physiological and biochemical traits of seeds—such as viability, dormancy, and desiccation tolerance—is essential for optimizing propagation and conservation. Tetrazolium (TZ) staining provides a rapid method for evaluating seed viability, while water imbibition analysis reveals key hydration phases critical for germination. Assessing desiccation tolerance and cold storage response is fundamental for classifying seed storage behavior as orthodox, intermediate, or recalcitrant (Hong and Ellis, 1996).

Given the species' ecological and economic value, and the urgent need for sustainable utilization, this study aims to comprehensively evaluate the seed physiology of *G. sylvestre*, focusing on viability testing, imbibition dynamics, dormancy status, desiccation tolerance, and long-term storage potential. The findings will inform both ex situ conservation efforts and efficient nursery practices for its large-scale cultivation.

2. MATERIALS AND METHODOLOGY

Study Area: The research was conducted at Jabalpur, Madhya Pradesh, India (21°17' to 26°52' N latitude; 74°08' to 82°49' E longitude), characterized by a subtropical climate with distinct seasons: hot summers (April–June), monsoon rains (July–September), and cool winters (October–February). The region receives an average annual rainfall of ~1370 mm, with temperatures ranging from 10°C in winter to 48°C in summer. *Gymnema sylvestre* exhibits flowering from October to January, followed by fruiting between March and April. The maturation of fruits and seeds generally occurs during April to May.

Seed Extraction and Processing: Mature brown pods of *Gymnema sylvestre* are collected

manually (April 2024) during the fruiting season by plucking. The harvested pods are sun-dried to promote natural splitting. Once dehiscent, seeds are extracted, cleaned by removing feather-like structures, and prepared for sowing or storage.

Seed Viability Testing: Seed viability was assessed using the tetrazolium (TZ) test following ISTA protocols. Three concentrations of 2,3,5-triphenyl tetrazolium chloride solution (1.0%, 0.5%, and 0.25%) were standardized. A random sample of 100 seeds per replication was soaked in water for hydration, and the seed coat was removed to facilitate TZ solution penetration. Seeds were incubated in the TZ solution in the dark for up to 24 hours, with observations recorded at 1, 2, 4, and 24 hours. Viability was expressed as the percentage of viable seeds.

Water Imbibition Test: Fresh seeds were weighed and soaked in distilled water at room temperature. At specific intervals, seeds were removed, surface moisture was blotted off, and seeds were reweighed. The increase in fresh weight over time was used to analyse seed water uptake patterns.

Germination Evaluation: Seeds of both *H. pubescens* and *W. tinctoria* did not exhibit physical or physiological dormancy, and hence, no pre-treatment was required. Prior to sowing, seeds were surface-sterilized using 5% Captain solution for five minutes. Three replicates of 25 seeds were placed in sterilized sand trays lined with moist filter paper. The trays were incubated in a seed germinator at 25°C under a 16/8 h light/dark cycle (Silva et al., 2004). Germination was monitored daily for four weeks, with radicle emergence of 1 cm as the criterion for germination. The following parameters were recorded:

1. Final Germination Percentage (FGP) (Scott et al. 1984)

$$FGP = G / T \times 100$$

Where G = No. of germinated seeds and T = No. of seeds sown

2. Mean germination time (MGT) (Orchard, 1977)

$$MGT = Gt \times Dt / G$$

Where Gt = No. of germinated seeds at day-t, Dt = No. of days at "t" from the day of sowing and G = Total no. of germinated seeds.

3. Root Length (cm): Measured as the average length of 10 primary roots.
4. Shoot Length (cm): Measured as the average height of the 10-seedling shoot.
5. Seedling vigour index $I = G\% \times \text{Seedling length (cm)}$ (Abdul-Baki and Anderson 1973).

Seed Desiccation Trials: Seeds were dried using silica gel at a 1:1 weight ratio (silica gel to seeds). Moisture content was measured periodically until it dropped below 5%. Seeds were then vacuum-packed for storage trials, and some seeds were reserved for germination assessments.

Moisture Content Determination: Seed moisture content was determined using the oven-drying method at $103 \pm 2^\circ\text{C}$ for 17 ± 1 hours following ISTA (2023) guidelines. Four replicates per species were used, with moisture expressed as a percentage on a fresh weight basis.

Seed Storage Behavior: Seed storage behavior was evaluated using the simplified screening scheme of Hong and Ellis (1996). Seeds were

vacuum-sealed in three-layer aluminium foil pouches and stored at -20°C in cold storage.

Statistical Analysis: All data were subjected to ANOVA to identify significant differences among treatments at a 5% significance level ($p \leq 0.05$). Critical Difference (C.D.), Standard Error of mean (SEm), and Coefficient of Variation (C.V.) were calculated to assess variability.

3. RESULTS AND DISCUSSION

Seed Carpology: The fruits of *Gymnema sylvestre* appear as paired, linear follicles (Fig. 1.) that are lanceolate and cylindrical (terete) in shape, measuring up to 7.5 cm in length and approximately 0.8 cm in diameter. They are dark green in color, often swollen at the center, and typically mature between March and May. The seeds are flat, winged, and equipped with silky hairs that aid in wind dispersal. Each seed measures about 10×5 mm, with some reaching up to 1.3 cm in length. The average seed length ranges from 0.8120 to 1.134 cm, while the width varies between 0.2110 and 0.3220 cm. Approximately 60,000 to 70,000 seeds are present per kilogram.



Fig. 1. Fruit and seed carpology of *Gymnema Sylvestris* (A) Follicles (B) Seed with silky hairs (C) Microscopic Seed Image (Scale 2900.00 µm)

Table 1. Impact of TZ Concentration and Staining Duration on Viability Percentage of *Gymnema sylvestre*

Staining duration (hrs.)	TZ Conc. (%)			Mean
	1	0.5	0.25	
1	71.33	96.00	95.23	87.52
2	95.33	100.00	98.33	97.89
4	100.00	100.00	100.00	100.00
24	100.00	100.00	100.00	100.00
Mean	91.67	99.00	98.39	
Factors	C.D.	SE(d)	SE(m)	F-Calculated
TZ Conc.	1.67	0.81	0.57	109.81*
Staining duration	1.45	0.70	0.49	68.02*
TZ Conc. X Staining duration	2.90	1.40	0.99	46.56*

Table 2. TZ staining patterns of viable (1) and non-viable seeds (2-6) in *Gymnema sylvestre*

S. No.	Topographic description	Topographic pattern
1	Embryonal axis and cotyledons completely stained	
2	Embryonal axis & cotyledon unstained radicle stained	
3	25% Embryonal axis and cotyledons stained	
4	Embryonal axis and cotyledons unstained, radicle tip partially stained	
5	Embryonal axis and cotyledons stained lightly	
6	Embryonal axis with cotyledons unstained	

3.1 Seed Viability

The viability of *Gymnema sylvestre* seeds was significantly influenced by Tetrazolium (TZ) concentration and staining duration. At 1-hour, lower TZ concentrations performed better, with viability reaching 96.00% at 0.5% and 95.23% at 0.25%, compared to 71.33% at 1%. At 2 hours, viability improved across all treatments, peaking at 100% for 0.5%, followed by 98.33% (0.25%) and 95.33% (1%). All treatments achieved 100% viability at 4 and 24 hours, confirming complete staining efficiency. Mean viability was highest at 0.5% TZ (99.00%), followed by 0.25% (98.39%) and 1% (91.67%). ANOVA revealed significant effects of TZ concentration ($F = 109.81^*$), staining duration ($F = 68.02^*$), and their interaction ($F = 46.56^*$). These results suggest

that 0.5% or 0.25% TZ for 2–4 hours are optimal for accurate, efficient viability assessment. Viable seeds showed complete staining of the embryonal axis and cotyledons, while non-viable seeds displayed partial or no staining in various seed parts, including the radicle and cotyledons.

Water Imbibition: The water imbibition curve (Fig. 2) of *Gymnema sylvestre* seeds illustrates the increase in seed fresh weight (%) over time as the seeds absorb water. Initially, at 0 hours, there is no change in seed weight. By 2 hours, the seeds exhibit a 23.44% increase in fresh weight, with a standard error (SE) of 0.68, indicating the onset of water uptake. This trend continues steadily, reaching 35.16% at 4 hours (SE = 1.02). A rapid imbibition phase occurs between 6 to 12 hours, with seed fresh weight

increasing significantly—72.01% at 6 hours, 81.10% at 8 hours, 90.66% at 10 hours, and peaking at 110.71% at 12 hours. The increasing SE values during this phase indicate some variability in water absorption among seeds. Beyond 12 hours, the rate of water absorption slows, reaching 121.81% at 24 hours and 131.68% at 27 hours. The highest recorded increase is 158.79% at 30 hours, after which a slight decline is observed—158.29% at 33 hours and 157.02% at 36 hours—indicating that the seeds may have reached saturation or experienced minor water loss. This pattern

suggests a triphasic imbibition process typical of orthodox seeds, with an initial rapid water uptake, a slower plateau phase, and a final stabilization, preparing the seeds for germination.

Seed pre-treatment: *Gymnema sylvestre* seeds did not exhibit physical or physiological dormancy, and therefore no pre-treatment was required prior to sowing. Preliminary germination tests confirmed the viability of untreated seeds, validating their readiness for direct propagation without additional interventions (Fig. 3, Fig. 4).

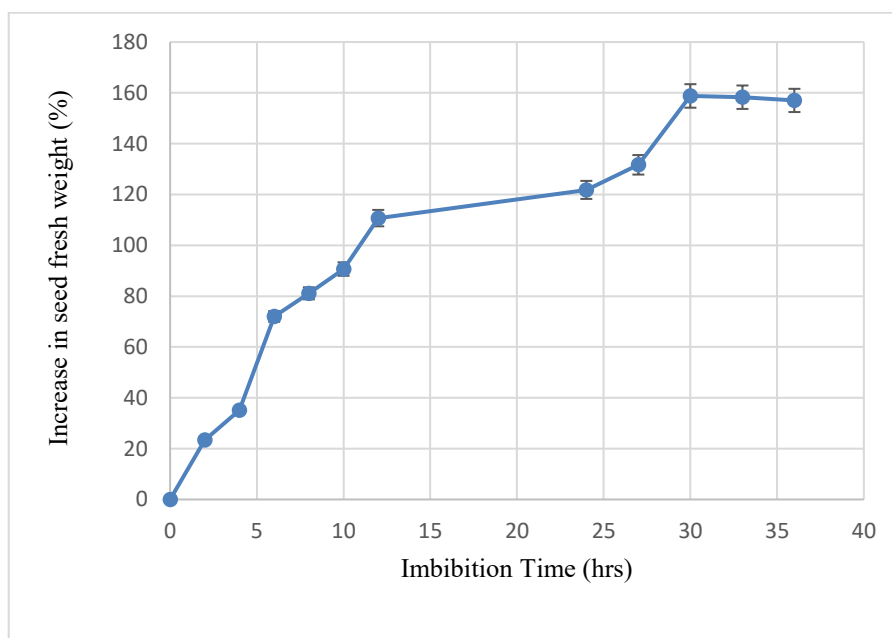


Fig. 2. Water Imbibition curve of *Gymnema sylvestre* seeds



Fig. 3. Germination pattern of *Gymnema sylvestre* seeds



Fig. 4. Seed germination of *Gymnema sylvestre*

Table 3. Different desiccation stages and respective germination percentage after desiccation

Initial seed Moisture Content%	Initial seed germination % Mean±SE	Desiccated safe moisture content %	Germination% Mean±SE	3 months after -20 °C storage germination % Mean±SE	Seed Storage Categories
9.60	89.26±3.57	5.36	90.83±3.63	95.38±3.82	Orthodox

Standardization of Seed Storability Behaviour:

Desiccation trials were conducted to assess the seed storability of *Gymnema sylvestre* using silica gel in desiccators. The initial seed moisture content was 9.60%, with a germination rate of 89.26±3.57%. After desiccation to safe moisture content of 5.36%, the germination percentage increased to 90.83±3.63%. Seeds stored at -20°C for three months maintained good viability, with a germination rate of 95.38±3.82%. These findings suggest that *Gymnema sylvestre* seeds are classified under the orthodox storage category, as they demonstrated high storability and maintained viability after prolonged storage under controlled conditions. In conclusion, the seeds of *Gymnema sylvestre* exhibit good storability under orthodox conditions, with improved germination following desiccation and low-temperature storage.

The viability assessment of *Gymnema sylvestre* seeds using tetrazolium (TZ) staining proved to be a reliable and effective method for rapidly identifying viable seeds. The differential response across concentrations and durations revealed that a 0.5% TZ solution provided optimal staining, consistently resulting in 100%

viability across all exposure durations, with clear visualization of living tissues in both the embryonal axis and cotyledons. The 0.25% concentration followed closely with a mean viability of 99.13%, while 1% TZ, although slightly less effective overall (91.88%), achieved full staining at extended durations (12–24 hours). These results demonstrate that the tetrazolium test is sensitive and accurate for *G. sylvestre*, in agreement with similar viability studies conducted on other medicinal and forest species. ANOVA results confirmed that TZ concentration, staining duration, and their interaction were all statistically significant factors influencing viability ($F = 24.80, 21.20, \text{ and } 16.49$ respectively; $p < 0.001$), reinforcing the need to optimize staining parameters for accurate viability estimation.

The water imbibition behavior of *G. sylvestre* seeds followed the classic triphasic curve typical of orthodox seeds. The first phase (Phase I), characterized by a slow but steady water uptake, reached 35.16% by 4 hours. This was followed by a rapid hydration phase (Phase II), peaking at 110.71% between 6 to 12 hours—indicative of the reactivation of metabolic processes required for germination. The third phase (Phase III), between 12 and 36 hours, showed a plateau

(158.79% at 30 hours), suggesting that the seeds had achieved full hydration and readiness for radicle protrusion. These findings align closely with those of Arunakumara and Subasinghe (2015), who observed that cold water soaking significantly enhanced seed germination in *G. sylvestre* by facilitating effective moisture absorption and dormancy release. Their work reported an increase in germination rate from 28.5% to 42.5% after 24-hour cold water treatment, emphasizing the role of imbibition in enhancing propagation success.

Germination trials in the present study demonstrated that untreated seeds of *G. sylvestre* do not exhibit any significant physical or physiological dormancy. This observation is particularly important as it simplifies propagation protocols for nursery and conservation programs. Similar findings were observed by Vijay et al. (2024, 2025a, 2025b) in species such as *Flacourtia indica* and *Commiphora wightii*, where seed size, maturity stage, and pre-treatment methods played significant roles in germination behavior and propagation success. These insights collectively suggest that for *G. sylvestre*, the straightforward nature of germination under optimal moisture conditions makes it a suitable candidate for both conservation and commercial cultivation.

The desiccation tolerance and storage behavior further affirmed the orthodox nature of *G. sylvestre* seeds. Post-harvest drying marginally improved germination from 89.26% to 90.83%, while cold storage at -20°C for three months led to a significant increase in germination (95.38%), demonstrating excellent storability and physiological stability under dry and low-temperature conditions. These results reflect possible alleviation of latent dormancy and preservation of metabolic integrity, which is crucial for long-term ex situ conservation and seed banking. This agrees with the findings of Chinapolaiah et al. (2019), who emphasized that moisture content critically determines seed viability during conservation. They reported substantial variation in germplasm quality across regions and recommended rigorous selection and storage protocols to maintain viability during long-term storage.

Despite the relatively good seed viability and storability observed in experimental settings, field propagation using seeds remains inconsistent. This is supported by the Tamil Nadu Agricultural University (TNAU, 2022), which highlights low

natural germination and recommends vegetative propagation methods as more reliable alternatives for farmers and conservationists. Their guidelines recommend propagation through hardwood cuttings, treated with rooting hormones such as IBA (500 ppm), particularly during favorable months (March or July), which resulted in up to 52.5% rooting success. Pandey (2012) also endorsed this dual approach—combining seed propagation with vegetative methods—to maximize success in large-scale cultivation programs. He further noted that optimal field establishment was achieved at 50×50 cm spacing using 4000 kg FYM/ha, producing up to 1.5 tonnes/ha of dry leaves over a two-year cycle with biannual harvests in June and October.

In addition to traditional methods, in vitro propagation using synthetic seed technology has gained attention as a sustainable alternative for *G. sylvestre* conservation. Murthy et al. (2018) successfully developed synthetic seeds by encapsulating nodal segments in alginate beads and storing them under controlled conditions, achieving high regeneration frequency and genetic stability as confirmed by ISSR markers. This approach not only circumvents issues related to seed desiccation and viability loss but also enables year-round propagation with minimal genetic variation, thereby supporting long-term germplasm preservation strategies.

In conclusion, the results of this study collectively suggest that *Gymnema sylvestre* seeds possess high innate viability, a typical orthodox seed imbibition pattern, no dormancy constraints, and excellent desiccation and cold storage tolerance. These attributes make them ideal for both propagation and seed banking. However, environmental variability and seed quality differences necessitate careful pre-sowing management. While seed-based propagation is feasible, integrating vegetative and biotechnological methods can enhance large-scale cultivation and conservation outcomes. These findings complement and reinforce earlier research (Pandey, 2012; Chinapolaiah et al., 2019; TNAU, 2022; Murthy et al., 2018), supporting a multi-pronged approach to the sustainable utilization and ex situ conservation of this valuable medicinal species.

4. CONCLUSION

This study provides a comprehensive physiological assessment of *Gymnema sylvestre*

seeds, establishing key parameters for their propagation and ex situ conservation. Tetrazolium (TZ) viability testing identified 0.5% TZ concentration as the most effective across all durations, consistently yielding 100% viability. Statistical analyses confirmed significant effects of TZ concentration, staining duration, and their interaction, emphasizing the need for standardized testing protocols. Water imbibition studies revealed a characteristic triphasic hydration curve, confirming the orthodox nature of the seeds and their readiness for germination. Germination trials further demonstrated that seeds are non-dormant and suitable for direct sowing without pre-treatment. Desiccation and cold storage trials validated the seeds' storability, with germination improving from 89.26% in fresh seeds to 95.38% after storage at -20°C , confirming their desiccation tolerance and long-term viability. Collectively, these findings affirm that *G. sylvestre* seeds are viable, non-dormant, and amenable to storage—supporting their use in large-scale cultivation and conservation programs.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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