

NOTE ON SEED GERMINATION OF TWO ENDEMIC SPECIES FROM WESTERN GHATS, INDIA

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ABSTRACT

Improving seed germination in species with hard seed coats requires the use of suitable pretreatment techniques. The present study examines the effectiveness of different pretreatments on the germination of two endemic species from the western ghats, *Eriolaena quinquelocularis* (Malvaceae) and *Ziziphus williamii* (Rhamnaceae). Both species have a hard seed testa, which is a major factor causing delayed and poor germination. In this study, the effects of hot water treatment, water soaking, sulphuric acid (H₂SO₄) treatment, and mechanical scarification were tested and compared with an untreated control to determine the most effective method for promoting germination.

Keywords: Endemic; *Eriolaena quinquelocularis*; Pretreatment; Germination; Western Ghats; *Ziziphus williamii*

INTRODUCTION

Climate change is causing major shifts in environmental conditions, which in turn influence seed-based plant reproduction. Seed production and germination are closely connected processes that are strongly controlled by environmental cues regulating dormancy and germination. This regulation is essential for plant reproductive success, enabling species to persist during unfavourable periods (Graeber *et al.*, 2012; Long *et al.*, 2015). Disruptions in germination phenology can alter plant community composition, affecting species diversity and ecosystem stability (Walck *et al.*, 2011). Therefore, understanding seed dormancy and germination in endemic species is crucial for biodiversity conservation and long-term ecosystem resilience. The present study investigates the germination behaviour of two endemic species, *Eriolaena quinquelocularis* (Wight & Arn.) Drury and *Ziziphus williamii* Bhandari & Bhansali, to gain insights into their adaptive responses to changing environmental conditions.

The genus *Eriolaena* DC. (Malvaceae) includes nine species distributed across India, Southeast Asia, and southern China. Among these, *E. lushingtonii*, *E. quinquelocularis*, and *E. candollei* are endemic to India (Chandramohan *et al.*, 2020). *Eriolaena quinquelocularis* (Wight & Arn.) Drury is a rare small tree with greyish-white bark, reaching 8–10 m in height (Figure 1). It occurs mainly in moist to dry deciduous forests, thriving in open slopes. The species shows anemochorous (wind) and entomophilous (insect) dispersal mechanisms (Raju *et al.*, 2013). Locally known as “Bothi,” it holds medicinal value, with the root paste used to treat excessive menstrual bleeding (Natarajan & Paulsen, 2000). The stem bark fibres are also used for rope making (Reddy, 2006). According to the NGCPR Heritarium database (2024), the species is endemic to India and occurs sparsely along the Western Ghats, including Rajasthan, Maharashtra, Karnataka, Tamil Nadu, and Kerala. The genus *Ziziphus* (Rhamnaceae) is distributed across the tropical and subtropical Old World and southern tropical America (POWO, 2024). Species of this genus are medicinally important and widely used in traditional remedies, with propagation commonly achieved through budding or grafting. However, the hard endocarp poses a major barrier to seed germination and rootstock production

(Aboutalebi *et al.*, 2012). *Ziziphus williamii* Bhandari & Bhansali is a rare tree endemic to the Western Ghats, India (Figure 2), where the hard endocarp severely restricts germination. Despite their ecological importance, studies on the germination and dormancy of these species are limited. This study investigates seed dormancy and pre-sowing treatments to improve germination. Understanding the germination requirements and environmental responses of *Eriolaena quinquelocularis* and *Ziziphus williamii* is crucial for effective conservation and sustainable management. Based on a thorough literature survey, this is likely the first report on the germination and ex-situ conservation of these two species.

MATERIALS AND METHODS

Seed Collection and Processing of *E. quinquelocularis*:

Mature capsules were collected from two distinct locations within Tryambakeshwar tehsil in Nashik district, Maharashtra. The first site, *Harsul* in Tryambakeshwar tehsil, has a deciduous landscape. A key landmark, Khairai Fort (20°08'51"N, 73°23'56"E), covers about 90 ha at 690 m elevation, where the species commonly occurs at higher altitudes. The second site, Santosha Hill in Belgaon Dhaga, about 11 km from Nashik, supports a population of *Eriolaena quinquelocularis*. The hill attains an elevation of 980 m and covers an area of approximately 100 ha, offering favourable habitat conditions for the species. The capsules dehisced loculicidally, and those partially open were considered mature and suitable for seed collection. After collection, capsules were air-dried for 2–3 days in a well-ventilated area. The seeds were then manually extracted, cleaned, and damaged or infested seeds were discarded. Finally, the clean seeds were stored in airtight containers with ash to prevent insect attack and kept under ambient conditions until further analysis.

Seed Collection and Processing of *Z. williamii*:

Ripen fruits were collected from Nashik district, Maharashtra, India (20°00'43"N, 73°47'47"E) between mid-March and early May, after naturally falling to the ground, indicating full ripeness. Healthy fruits were selected for the study. The pulp was removed by washing fruits 4–5 times under running water, followed by 2 days of sun-drying and 2 days of shade-drying. Due to the hard fruit coat, seeds were manually extracted using minimal-pressure hand tools to avoid damage. The cleaned seeds were shade-dried for one day and then stored at room temperature in airtight glass containers.

Pretreatments:

Pretreatments were designed to overcome seed dormancy and enhance germination efficiency.

Seeds of *Eriolaena quinquelocularis* were extracted from capsules and subjected to multiple pre-sowing treatments (Table 1), with untreated seeds used as control.

Water soaking: Seeds were soaked in distilled water at room temperature for 48 h, with water changed every 12 h to remove leachates.

Hot water treatment: Seeds were immersed in hot water for 5 min, followed by overnight soaking in distilled water.

Acid scarification: Seeds were treated with concentrated sulfuric acid for 5, 15, or 30 min, followed by overnight soaking in distilled water.

Seeds of *Ziziphus williamii* were subjected to pretreatments selected based on their seed morphology and hard seed coat characteristics (Table 1).

Seed cracking: This method involves carefully applying mechanical force to weaken the hard seed coat without damaging the embryo, improving water absorption, gas exchange, and promoting faster, uniform germination.

Acid treatment: Seeds were immersed in concentrated sulfuric acid (H₂SO₄) for 60 or 90 min to break the hard coat, then thoroughly washed under running water before sowing.

Germination conditions:

The experiment was conducted using a completely randomized design (CRD). Germination was monitored daily for 30 days for both species. Radicle emergence of ≥ 2 mm was considered successful germination,

and observations were recorded at regular intervals. Seeds were sown in sterilized cocopeat, and substrate moisture was maintained through regular watering to provide optimal germination conditions.

Assessment of Seed Viability:

Seed viability was evaluated using the Tetrazolium (Tz) test (Basu *et al.*, 2013).

Eriolaena quinquelocularis seeds were scarified and soaked overnight, then embryos carefully extracted and immersed in 1% Tz solution at pH 7 in the dark for 12 h.

Ziziphus williamii seeds were separated from the endocarp and soaked for 24 h, followed by removal of seed coats and cotyledons to expose the embryo. The embryos were then immersed in 1% Tz solution at pH 7 for 24 h in the dark.

Seed embryo and cotyledon viability was determined by observing the staining patterns.

Germination indices:

1. Germination Percentage (GP) = $(\text{Number of seeds Germinated} / \text{Total seeds sown}) \times 100$.

2. Mean Germination Time (MGT) = $\sum (n \times t) / \sum n$

Where n is the number of seeds germinated on day t .

3. Seedling Vigour Index (SVI) = $GP \times \text{Total seedling length (mm)}$.

RESULTS AND DISCUSSION

Phenological characteristics:

Eriolaena quinquelocularis peak flowering and fruits from April to July, while *Ziziphus williamii* from November to December and fruits from March to April, representing the primary reproductive phase.

Fruit and seed morphological characteristics were observed to provide insights into the reproductive biology and germination patterns of both species (Table 2).

Eriolaena quinquelocularis: Fruits are capsules with a rough woody texture, measuring 4-5 cm. young fruits are green, turning woody brown at maturity with low water content, making it hard. Capsules contain numerous small seeds (0.6-0.7 cm), ovoid-obovate, rough, which are green when young and black and hard when mature with an impermeable testa. Seeds are endospermous and exhibit epigeal germination.

Ziziphus williamii: Fruits type is drupe, ovoid and fleshy, measuring 2.3 ± 0.11 cm, with a smooth texture. Young fruits are light yellow-green, maturing to red-brown. Fruits are edible ripen in April-May are edible. The tetrazolium test on freshly extracted seeds indicated maximum viability. Germination was monitored daily for 30 days, and significant differences ($p < 0.05$) were observed among pre-sowing treatments for both species. For *Eriolaena quinquelocularis*, the highest germination percentage (GP, 98%) was recorded in seeds treated with concentrated sulfuric acid for 30 min (T5) (Figure 3). Hot water-treated seeds showed 53% GP, while seeds treated with sulfuric acid for 15 min (T4) germinated at 47%. The control group (T0) exhibited only 15.55% GP, and seeds soaked in water for 48 h (T1) showed 38.88%. For *Ziziphus williamii*, the highest GP (88.88%) was achieved with seed cracking (T1), followed by 82.22% (T3) and 62.22% (T2). The control group (T0) had the lowest germination (33.33%) (Figure 4). Mean germination time (MGT) was significantly affected by treatments for *Eriolaena quinquelocularis*. Seeds treated with sulfuric acid for 30 and 15 minutes germinated fastest, with MGTs of 7 and 8 days, respectively (Table 3). Control seeds required 18 days, similar to water-soaked seeds (17 days), while hot water-treated seeds had an MGT of 9 days. For *Ziziphus williamii*, acid treatments for 90 and 60 minutes notably reduced MGT, whereas the control (T0) had the longest MGT of 21.33 days. Seedling growth: Treatment T1 produced the highest total seedling length (TSL) and seedling vigour index (SVI), followed by T2 and T3. Untreated seeds showed the shortest TSL and lowest SVI. The results demonstrate that pre-sowing treatments markedly affect germination and seedling establishment in both species. The hard, impermeable seed coats serve as a natural mechanism for delayed germination, which may confer an adaptive advantage under variable environmental conditions. For ex-situ conservation, the application of appropriate pretreatments is crucial to enhance germination success. In *Eriolaena quinquelocularis*, the highest germination percentage was obtained following treatment with concentrated sulphuric acid for 30 minutes, emphasizing its role in enhancing water uptake and activating metabolic processes necessary for germination. Previous studies have similarly

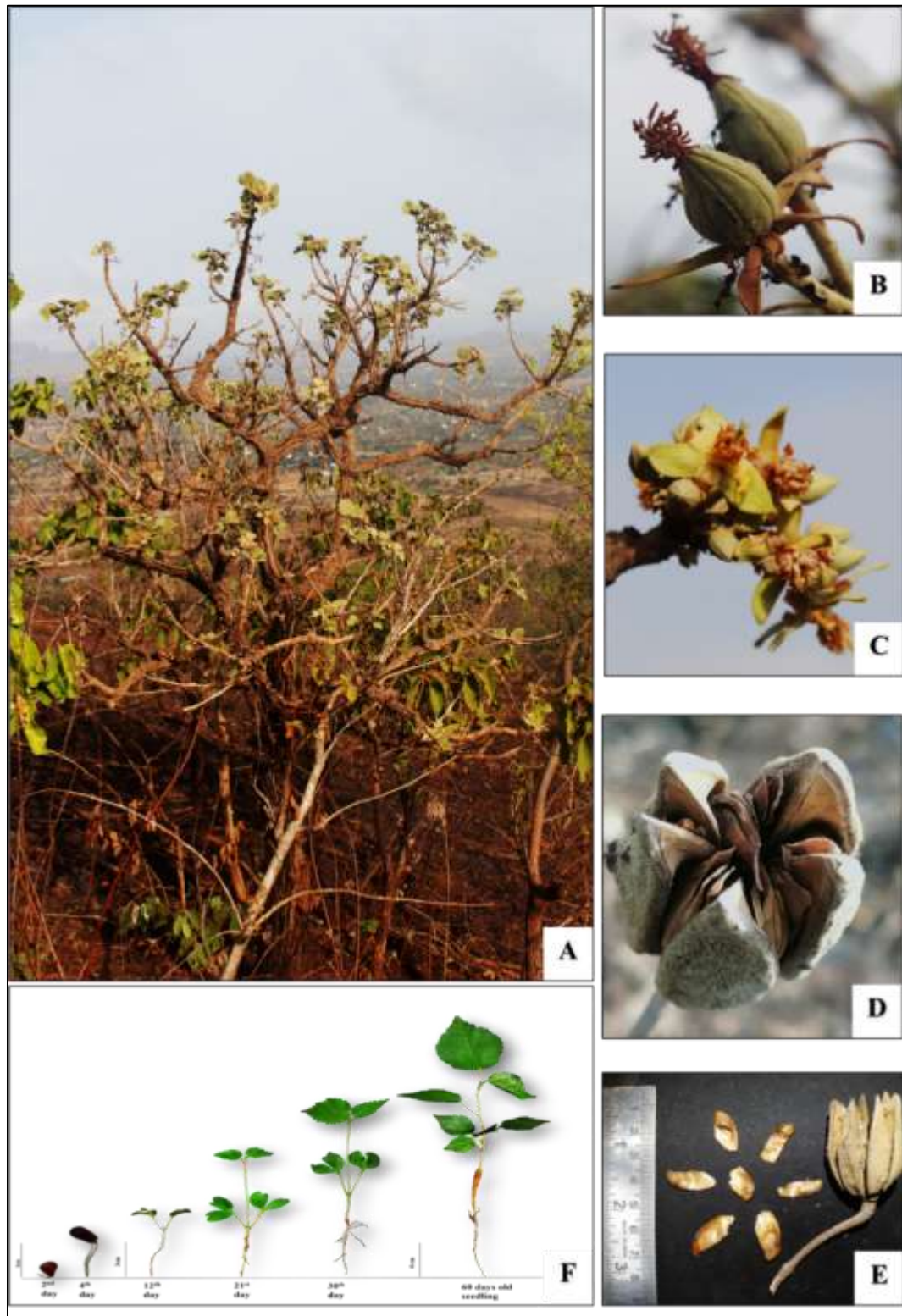


Figure 1: *Eriolaena quinquelocularis* A: habit; B: buds; C: open flowers; D: mature capsule; E: capsule & seeds; F: seedling development stages

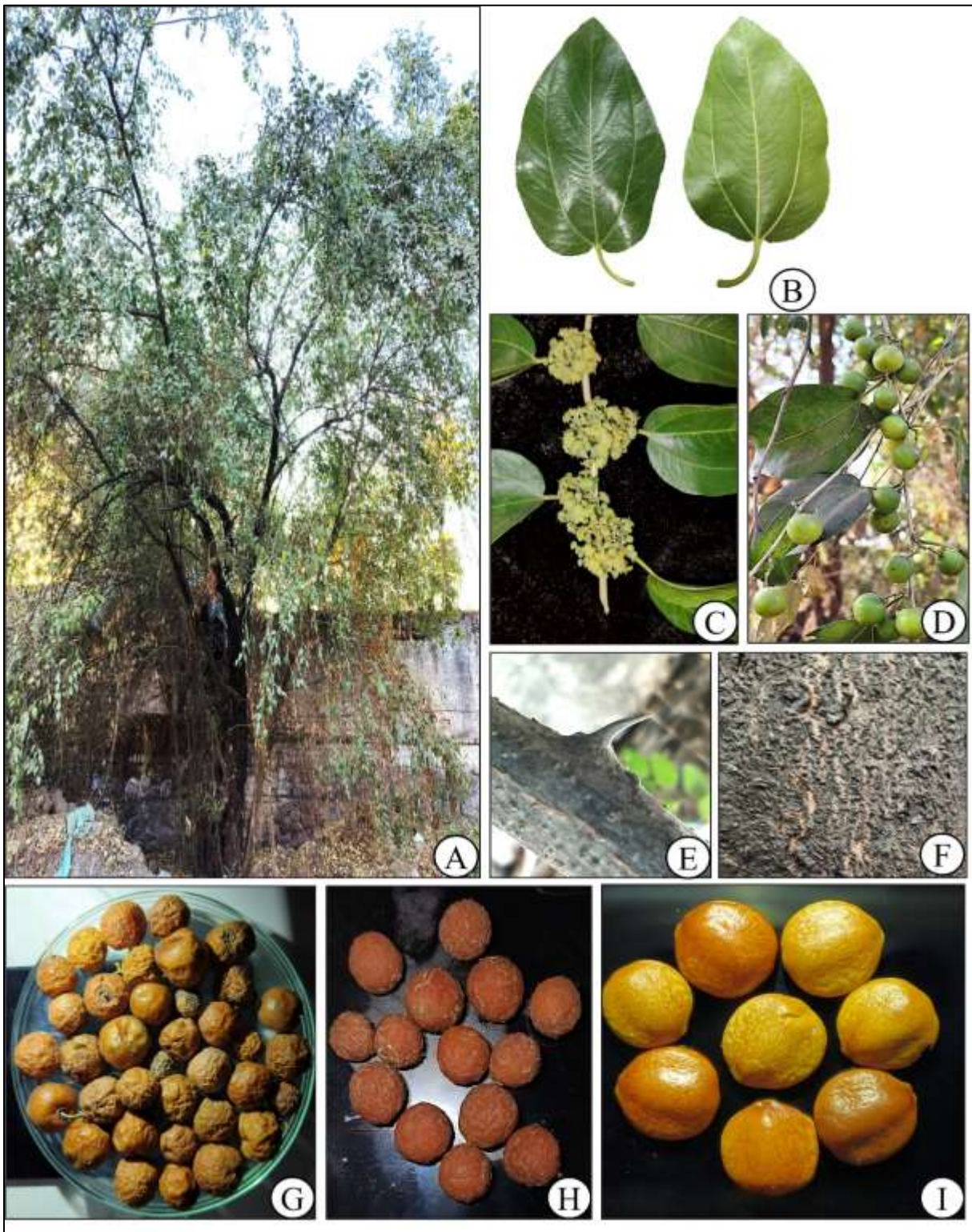


Figure 2. *Ziziphus williamii*: A-habit; B- leaves (both surface); C- flowers; D- immature drupes; E- spines; F-bark; G-ripped drupes; H-fruits (pulp removed); I-seeds

Table 1: Pretreatments

S.No.	Pretreatments	Time intervals (min/hrs)
<i>Eriolaena quinquelocularis</i>		
1	T0 (Control)	-
2	T1 (Water soaking)	48h
3	T2 (Hot water treatment)	5 min
4	T3 (conc. H ₂ SO ₄)	5 min
5	T4 (conc. H ₂ SO ₄)	15 min
6	T5 (conc. H ₂ SO ₄)	30 min
<i>Ziziphus williamii</i>		
1	T0 (Control)	-
2	T1 (Seed cracked followed by water soaking)	48h
3	T2 (conc. H ₂ SO ₄)	60 min
4	T3 (conc. H ₂ SO ₄)	90 min

reported that acid treatments effectively break dormancy and enhance germination. Sulphuric acid acts by increasing seed coat permeability, thereby promoting germination in species with hard or physically dormant seeds (Khalofah, 2022). Studies by Ansari *et al.*, (2016) and Dawar *et al.*, (2024) also reported sulfuric acid as the most effective treatment for breaking dormancy and enhancing permeability. However, prolonged acid exposure can damage seeds, increase mortality and cause abnormal seedlings (Khalofah, 2022). Mean germination time (MGT) was notably reduced in seeds treated with concentrated sulfuric acid, followed by hot water treatment, indicating that the seed coat is largely impermeable to water. Pretreatments, especially acid scarification, help rapid hydration, promoting faster germination under favourable conditions. Hardness and low permeability are mainly caused by hydrophobic compounds such as lignin and wax, which reduce water and oxygen uptake, leading to low germination rates in many Malvaceae species (Bakker, 2001). Hot water treatment also improved mean germination time (MGT), though less effectively than acid treatment, indicating that hot water softens the seed coat, promoting faster hydration and germination. Similar results were reported by Tadros *et al.* (2011), who found that soaking *Leucaena leucocephala* seeds in 70°C water for 20 min effectively broke dormancy. Hot water treatment is widely used to overcome dormancy in hard-coated seeds, typically soaking them at 40–100°C for durations depending on species and seed coat thickness (Tadros *et al.*, 2011). Wang *et al.* (2011) also reported that soaking wild *Vigna* seeds in 80°C water for 3–6 min effectively broke seed coat dormancy. The hard fruit surface may help in moisture retention and protection against pests. Acid scarification (T6 and T7) moderately enhanced germination by softening the seed coat, improving water uptake and radicle emergence, and reducing the time to first emergence compared to the control. Similarly, Saied *et al.* (2008) reported a decrease in first emergence days in *Ziziphus spina-christi* following acid treatment.

Table 2: Maturity indices of *E. quinquelocularis* & *Z. williamii*

<i>Eriolaena quinquelocularis</i> (Wight & Arn.) Drury		
Character	Variables	Observation
Fruit	Type	capsule
	Colour(young)	green
	Colour(mature)	woody brown
	Water content	less at maturity
	hardness	hard
	Size (cm)	4-5
	shape	cone shaped
	texture	rough woody
	Ripen	march-april
	Edible	-
Seed	Colour(young)	green
	Colour(mature)	black
	No. of seeds per Capsule	numerous
	Hardness	hard
	Size (mm)	6-7
	Shape	ovoid-obovate
	Texture	rough
	Testa	hard, impermeable
	Endospermous	yes
	Germination	epigeal
<i>Ziziphus williamii</i> Bhandari & Bhansali		
Fruit	Type	drupe
	Colour(young)	light yellow-green
	Colour(mature)	red-brown
	Water content	high at maturity
	hardness	fleshy
	Size (cm)	3-4
	shape	globose
	texture	smooth
	Ripen	april-may
	Edible	yes
Seed	Colour(young)	Light brown
	Colour(mature)	dark brown
	No. of seeds per Drupe	two, one sometime
	Hardness	hard
	Size (mm)	8-9
	Shape	abaxial side flat, somewhat circular
	Texture	smooth
	Testa	hard, impermeable
	Endosperm	yes
	Germination	epigeal

Table 3: Observation table

<i>Eriolaena quinquelocularis</i>								
Treatment	No of seeds sown (30 per replica)	No of seeds germinated	Germination percent age (%)	Mean Germination Time (Days)	Mean root length (mm)	Mean shoot length (mm)	Total seedling length (mm)	Seedling vigour index
T0 (Control)	90	14	15.55	18.28	58.66	29.66	88.32	1373.37
T1 (Water soaking)	90	35	38.88	17.85	63	32.53	95.53	3714.20
T2 (Hot water treatment)	90	48	53.33	9.86	65	33.6	98.6	5258.33
T3 (conc. H ₂ SO ₄)- 5min	90	22	24.44	15.45	63.8	33.6	97.46	2381.92
T4 (conc. H ₂ SO ₄)- 15min	90	43	47.77	8.12	68.66	36	104.66	4999.60
T5 (conc. H ₂ SO ₄)- 30 min	90	89	98.88	7.72	65.3	37.06	102.36	10121.35
<i>Ziziphus williamii</i>								
T0 (Control)	45	15	33.33	21.33	66	84.6	150.6	5019.498
T1 (Seed Cracked followed by 48 h soaking)	45	40	88.88	8.8	123.4	109.8	233.2	20726.816
T2 (conc. H ₂ SO ₄) 60min	45	28	62.22	7.76	94.6	106.4	201	12506.22
T3 (conc. H ₂ SO ₄) 90min	45	37	82.22	5.81	97.2	92	189.2	15556.024

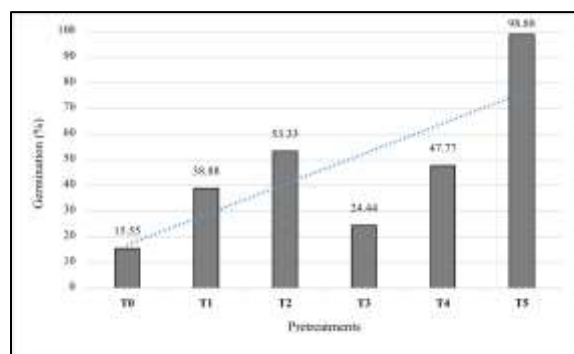


Figure 3: Effect of Pretreatment on Germination of *E. quinquelocularis*

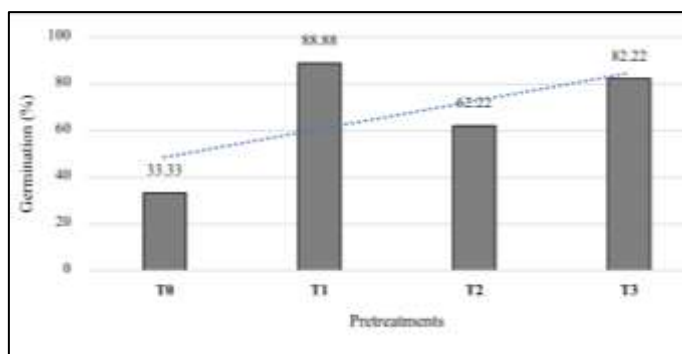


Figure 4: Effect of Pretreatment on Germination of *Z. williamii*

CONCLUSION

This study highlights the importance of appropriate seed pretreatments for improving germination and seedling performance, particularly for ex-situ conservation. Acid scarification and hot water treatments markedly enhanced germination in *Eriolaena quinquelocularis*, whereas *Ziziphus williamii* responded better to seed cracking, water soaking, or removal of the hard seed coat. Both species exhibit hard, water-impermeable seed coats, necessitating scarification for successful germination. Cocopeat proved to be an effective substrate for early seedling growth, and healthy saplings were successfully raised for restoration in natural habitats. These findings emphasize the critical role of pre-sowing treatments in promoting germination and seedling development, offering practical guidance for agroforestry and conservation initiatives.

ACKNOWLEDGEMENTS

RSJ, KNB, SSK, SGA and KVCG are thankful to principals of their respective college for necessary facilities. RSJ is thankful to Mahatma Jyotiba Phule Research Fellowship (Mahajyoti_2022) for financial assistance. SSK thanks Savitribai Phule Pune University (SPPU), Pune, for the ASPIRE Research Mentorship Grant.

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